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Modulation of Type I Allergy by Environmental Anthropogenic Factors and the Endocannabinoid System

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1 Abstract and Zusammenfassung

1.1 Abstract

It has been repeatedly shown that the prevalence of atopic diseases (e.g. asthma) has increased and still is increasing worldwide especially in the industrialized countries. Factors such as geneteic predisposition, environmental air pollutions, or the decline of infectious diseases might contribute to this phenomenon.

Diesel exhaust particles (DEP) which are the most important contributor to environmental airborne pollution have been described as potent adjuvant in the induction and maintenance of allergic diseases. The exact mechanisms by which particles enhance allergic immune responses are still a matter of debate. Dendritic cells are antigen-presenting cells which prime and polarize naive T cells and therefore are pivotal in the initiation of an immune response. Furthermore, they are the first line of defence in the early phase of immune reaction. Thus, it is likely that environmental pollutants interact with this cell type. Further, it is known that dendritic cells not only activate T cells but also interact with B cells. As adjuvant effects on sensitization phase are mostly due to increased IgE response it might be that particles interact with B cells function to produce IgE.
Thus, murine bone marrow-derived dendritic cells (BMDC) and human monocyte-derived dendritic cells (MoDC) were exposed to different kinds of particles or particle-associated organic compounds in the absence or presence of maturation stimuli such as lipopolysachharide (LPS) or polyinosinic: polycytidylic acid (poly(I:C)). Cellular maturation, viability, and cytokine production of these cells was analyzed. Furthermore, the functionality of particle-exposed murine BMDC to suppress B cell isotype switching to IgE was monitored in the murine system.
Only highly polluted DEP but not particle-associated organic compounds, carbon black or less polluted DEP modulated dendritic cell phenotype. These particles significantly suppressed LPS-induced interleukin (IL-)12p70 production in murine and human DC, whereas cell surface marker expression was altered only in human MoDC. Thus, the immunomodulatory capacity of particles depend on particle characteristics, cell type, and origin. Interestingly, SRM-exposure alters DC function as SRM-exposed immature BMDC lost the ability to suppress IgE isotype switch in B cells.
Taken together, this reveals that highly polluted particles not only interfere with dendritic cell maturation but also with dendritic cell function, thus suggesting a role in Th2 immune deviation.

In a second project, the role of the endocannabinoid system (ECS) was studied in a murine model

of type I hypersensitivity. The ECS consists of two G-protein-coupled receptors, cannabinoid (CB) CB1 and CB2 receptor and the endogenously produced ligands (endocannabinoids) which activate these receptors. In addition, exogenous ligands, namely cannabinoids such as the Δ^9-tetrahydrocannabinol (THC) can bind to these receptors. They are of interest because they do not only have psychoactive but also immunomodulatory effects. The intrinsic role of the ECS is still under discussion, however, it is known that the ECS not only plays a role in physiological processes, but even in immune regulation.

It has been previously shown, that the ECS plays a role for contact hypersensitivity. This thesis investigated the role of the ECS, in particular of the cannabinoid receptors CB1 and CB2 on allergic airway inflammation. To this end, a murine model for allergic airway inflammation was used. This model is composed of two phases: in the sensitization phase mice were sensitized with ovalbumin (OVA); during elicitation phase mice were challenged with OVA-aerosol. Two issues were investigated: 1.) The role of an impairment of the ECS was studied using CB receptor double-knockout mice (DKO) lacking both CB receptors. 2.) The study the influence of the activation of ECS wildtype mice were treated with THC during the respective phases.

The impairment of the ECS using CB receptor DKO mice led to an increased total cell count of cells in the bronchoalveolar lavage (BAL) as well as cellular infiltration into lung tissue and increased hyperplasia of mucus cells in the bronchia in DKO mice in comparison to WT mice, but with a similar cell type pattern in differential cell count. Interestingly, although DKO mice had an exacerbated allergic inflammation of the lung, they did not show qualitative changes in immune response towards Th1 or Th2 as analyses of cytokine profile was not altered in DKO compared to WT mice. Moreover, immunoglobulin levels in serum, analyzed over the time period of the experiment, remained on the same levels in WT and DKO mice.

The activation of the ECS using THC-treatment dramatically reduced airway inflammation in wildtype mice irrespective of application time. Differential cell count of BAL cells showed an decrease in the absolute numbers of macrophages and eosinophils. This leads to a shift in the percentage distribution of cell types towards the fraction of macrophages. Furthermore, humoral immune response was significantly suppressed in mice which received THC during sensitization phase.

Taken together, these data indicate a potential protective role of the activated endocannabinoid system not only in contact allergy but also in type I hypersensitivity. Thus, compounds activating the ECS might function as promising drugs to use as therapeutic in asthmatics. However, drugs which do not exert psychotropic effects would be desirable. Further investigations focusing on the contribution of the two respective receptors to these effects and the identification of an potent candidate for the use as therapeutic are warranted.

1.2 Zusammenfassung

Es ist bekannt, dass die Prävalenz von allergischen Erkrankungen wie Asthma bronchiale ständig steigt. Verschiedene Faktoren, wie z.B. genetische Veranlagung, die Abnahme von Infektionserkrankungen, oder auch Veränderungen des Lebensstils einschließlich einer zunehmenden Umweltverschmutzung scheinen dies zu beeinflussen.

Dieselpartikel (DEP), die den Hauptanteil an der Luftverschmutzung darstellen, konnten bereits als potentes Adjuvans bei der Auslösung und Erhaltung von allergischen Erkrankungen identifiziert werden. Der exakte Mechanismus wodurch diese Partikel die allergische Immunantwort verstärken sind allerdings noch nicht geklärt. Dendritische Zellen sind antigenpräsentierende Zellen und sind unerlässlich bei der Initiierung der Immunantwort, da sie in der Lage sind, naive T Zellen zu polarisieren. Desweiteren stellen sie eine frühe Phase der Immunabwehr in der Lunge dar. Es ist demnach sehr wahrscheinlich, dass Umweltpartikel mit diesen Zellen interagieren und sie beeinflussen. Es ist weiterhin bekannt, dass dendritische Zellen auch mit B Zellen interagieren. Da adjuvante Effekte auf die Sensibilisierungsphase meist mit erhöhten IgE-Werten einhergehen, wird diskutiert, dass Partikel diese IgE-Produktion der B Zellen beeinflussen.
In dieser Arbeit wurden murine dendritische Zellen, die aus Knochenmarkzellen generiert wurden (BMDC), und humane dendritische Zellen, die von Monozyten stammen, verschiedenen Partikeln oder Partikel-assoziierten organischen Substanzen ausgesetzt. Vitalität, Zellreifung und Zytokin-Produktion der Zellen wurde analysiert. Desweiteren wurde die Funktionalität der murinen BMDC und ihre Fähigkeit den Isotypenswitch in B Zellen zu beeinflussen untersucht.
Es konnte festgestellt werden, dass nur stark verschmutzte Partikel, nicht aber Partikel-assoziierte organische Substanzen, Kohlenstoffkerne oder kaum verschmutzte Partikel phänotypische Veränderungen der dendritischen Zellen hervorriefen. Die Behandlung mit diesen Partikeln führte vor allem zu einer verminderten Interleukin (IL-)12p70-Sekretion in murinen und humanden dendritischen Zellen. Die Expression von Oberflächenmarkern war allerdings nur in humanen Zellen verändert. Die immunmodulierende Wirkung von Partikeln schien demnach nicht nur von den Partikeleigenschaften, sondern auch von Zelltyp bzw. deren Herkunft abzuhängen.
Desweiteren führte die Exposition von murinen BMDC mit verschmutzten Partikeln zu einer veränderten Funktionaliät der Zellen. Unreife, mit Partikel behandelte Zellen haben die Fähigkeit verloren, den Isotypenswitch zu IgE in B Zellen zu unterdrücken.
Zusammengefasst zeigen diese Ergebnisse, dass verschmutzte Partikel nicht nur die Reifung von dendritischen Zellen beeinflussen können, sondern auch deren Funktion. Somit könnten sie bei der Einleitung einer Immunantwort diese in Richtung einer Th2-Antwort lenken.

In einem zweiten Teil wurde der Einfluss des Endocannabinoidsystems (ECS) auf die Typ I Allergie untersucht. Das ECS besteht aus zwei G-Protein-gekoppelten Rezeptoren (CB1- und CB2-Rezeptor) und den endogen produzierten Liganden (Endocannabinoide), die die genannten Rezep-

toren aktivieren können. Über die intrinsiche Rolle des ECS wird derzeit noch diskutiert. Es ist jedoch bekannt, dass das ECS auch an der Immunregulation beteiligt ist. Zusätzlich können exogene Liganden, die Cannabinoide wie z.B. das Δ^9-tetrahydrocannabinol (THC), an die Rezeptoren binden und das ECS beeinflussen. Diese Substanzgruppe ist von Interesse, da sie nicht nur psychoaktive, sondern auch immunmodulatorische Wirkung haben soll.
Es konnte bereits gezeigt werden, dass das ECS eine Rolle in der Kontaktallergie (Typ IV Allergie) spielt. In vorliegender Doktorarbeit wurde untersucht, ob das ECS auch eine Rolle bei der Typ I Allergie (Soforttyp) spielt. Hierfür wurde ein murines Modell für die Typ I Hypersenstivität verwendet, das aus zwei Phasen besteht: der Sensibilisierungsphase, in der die Mäuse mit Ovalbumin (OVA) sensibilisiert wurden und der anschließenden Effektor-Phase in der mit einem OVA-Aerosol provoziert wurde. Es wurden zwei Strategien verfolgt: 1.) Der Einfluss einer Hemmung des ECS wurde durch die Verwendung von Doppelknockout-Mäusen, die keinen der beiden CB-Rezeptoren besitzen (DKO-Mäuse) untersucht. 2.) Um den Einfluss der Aktivierung des ECS auf die Allergieentstehung zu untersuchen, wurden Mäuse mit dem Agonisten THC in den einzelnen Phasen behandelt.
Bei Hemmung des ECS zeigte sich eine erhöhte Allergen-induzierte Zellinfiltration in den Lunge der DKO-Mäuse im Vergleich zu den entsprechenden Wildtyp-Tieren. Die differentielle Zellzählung bestätigte, dass die Erhöung der Gesamtzellzahl auf eine Erhöhung aller Zelltypen zurückführen war, so dass die prozentuale Zellverteilung beider Gruppen ähnlich war. Desweiteren ergaben histopahtologische Färbungen von Lungenschnitten, dass DKO-Mäuse vermehrt Zellinfiltrationen in das Lungengewebe sowie eine verstärkte Hyperplasie der Mukuszellen der Bronchien aufzeigten. Die humorale Immunantwort war hiervon nicht betroffen, da die Immunglobulinspiegel in den Seren der DKO-Mäuse sich nicht von denen der Wildtyp-Mäuse unterschieden.
Die Aktivierung des ECS durch THC-Gabe in Wildtyp-Tieren führte zu einer signifikant verminderten Zellinfiltration in die Lunge, unabhängig in welcher Phase THC verabreicht wurde. Die differentielle Zellzählung des Lungeninflitrats ergab, dass die abolute Zahl der Makrophagen und Eosinophilen abnahm, was zu einer Verschiebung der prozentualen Zellverteilung zugunsten der Makrophagen führte.
Da die Hemmung des ECS durch Rezeptor-Knockout eine verstärkte Allergen-induzierte Entzündungsreaktion der Lunge herrvorrief, die Aktivierung des ECS durch Liganden hingegen eine Verbesserung der allergischen Symptome bewirkte, hat das ECS scheinbar ein protektive Wirkung auf die Typ I Allergie. Demnach könnten Verbindungen, die das ECS aktivieren als vielversprechende Therapeutika in der Behandlung von Asthmatikern fungieren.

2 Introduction

2.1 Allergy – An Immunological Reaction

The word ‚allergy' was formulated by Clemens von Pirquet who described his observations in the ‚Münchner Medizinische Wochenschrift' in 1906. He described the phenomenon that the exposure of foreign agents (e.g. vaccinia or horse anti-serum) could produce either immunity or hypersensitivity. He chose the term allergy which means allos ('other') and ergon ('work'), because the same agents was able to induce an altered reactivity (Kay, 2006). The first recorded case of allergy, however, dates back in 1819, by Charles Blackely who observed that 'hay-fever epidemic' was associated with the movement of people from the countryside into the cities (Blackley, 1873). Allergies have been described to increase over the past decades especially in industrialized countries. The reason for this rapid increase in the incidence of allergic diseases is discussed critically with controversial opinions. Several factors such as genetic predisposition, environmental air pollutions, lifestyle changes, or the decline of infectious diseases might contribute (Karol, 2002).

2.1.1 Mechanisms of Allergy (Atopy)

Today, the word allergy is mostly used to describe hypersensitivity mechanisms, particularly the IgE-mediated allergic diseases (atopy), and not an uncommitted biological response as von Pirquet suggested.

The different forms of allergic reactions were classified by their pathomechanism by Gell and Coombs (Gell and Coombs, 1963) who have differentiated four distinct types of hypersensitivity, namely type I, II, III, and IV. This has been extended by Roitt and Ring (Ring, 1998; Roitt, 1984) who included type V and VI reactions. The presented study is limited to the type I hypersensitivity which includes the classical allergic immediate type reaction such as allergic rhinitis, allergic asthma, insect venom allergy, and anaphylaxis. Immediate hypersensitivity reactions consist of an immediate reaction (within minutes), dominated by vascular and smooth muscle responses to mediators followed by a delayed late-phase reaction which contribute significantly to the immunopathology of an allergic response. The late phase reaction is characterized by inflammation of leukocytes (including neutrophils, eosinophils, basophils, and Th2 cells) and develops 2 to 24 hours later (see Figure 1).

As allergic disorders are immunologic reactions there is a communication between the innate and the adaptive immunity. All these reactions commonly follow a typical sequence of events which can be divided into a sensitization phase and the elicitation phase. In brief, the initial exposure to an antigen leads to an antigen-specific activation of T helper type 2 (Th2) cells mostly by activation of naïve CD4 T cells through dendritic cells. Th2 cells then stimulate B cells to differente into

plasma cells and produce IgE antibodies. These IgE antibodies then bind to Fc receptors of mast cells resulting in the release of mediators from the mast cells by re-exposure to the antigen. This causes the subsequent pathologic reactions of early allergic response. The late phase of allergic response is due to infiltration of eosinophilic cells, Th2 cells, and mast cells (see Figure 1).

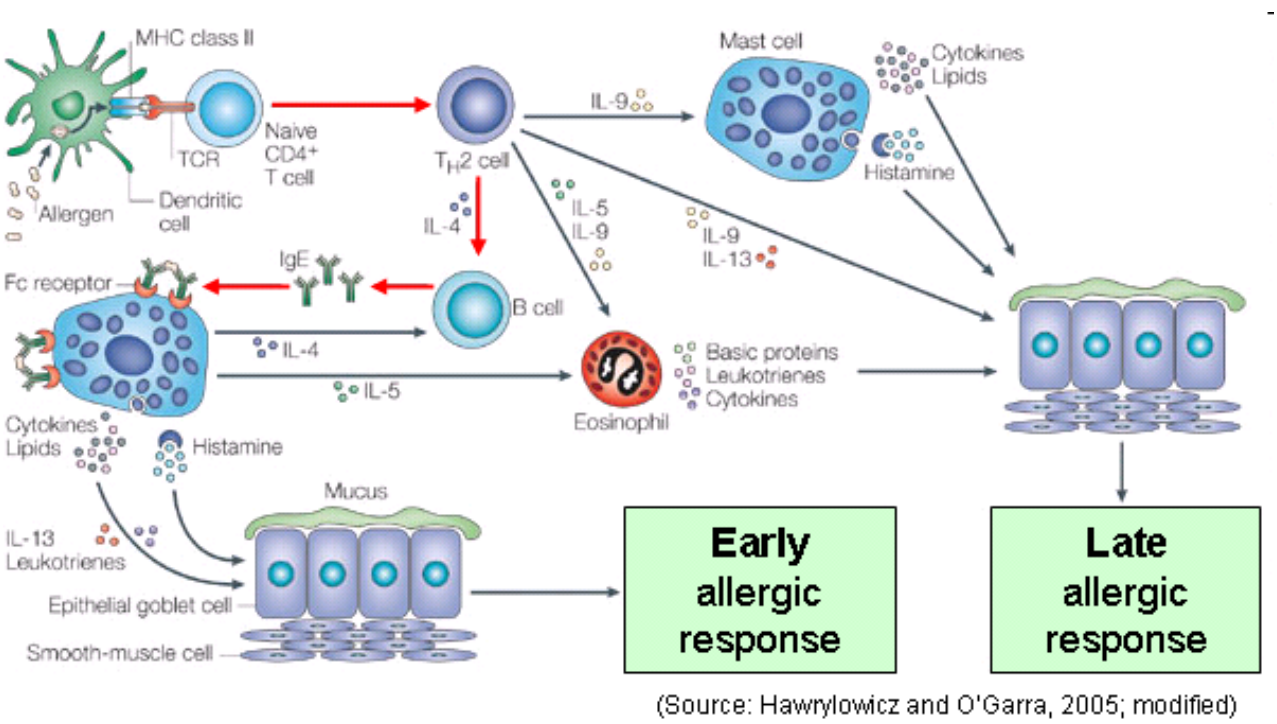


Figure 1: Allergic mechanism

Induction of a Th2 Response

The activation of $CD4^+$ helper T cells of the Th2 subset and their secretion of Th2-like cytokines such as interleukin (IL-)4, IL-5, and IL-13 is required e.g. for stimulating B cells to undergo class switch recombination (CSR), differentiate into plasma cells, and produce antibodies, in particular IgE. Dendritic cells (DC) play an important role in the efficient stimulation of T cells (Banchereau and Steinman, 1998). Immature dendritic cells which can be found throughout all tissues take up antigens and undergo maturation through the nuclear factor $\varkappa$B (NF-$\varkappa$B) pathway by receiving certain danger signals such as the pathogen-associated molecular patterns (PAMP), various cytokines, or tissue factors (TF) (Chain, 2003). As a result, they mature and up-regulate co-stimulatory molecules while migrating to lymphoid tissues where they are able to specifically activate T cells in the T cell regions by providing a number of sequential signals (see Figure 2 and Figure 3).

The first signal is the interaction of the T cell receptor (TCR) with the specific antigenic peptide presented by major histocompatibility class II (MHC II) on the surface of dendritic cells. This determines the antigen specificity of T cells (see Figure 2). The second signal is a co-signal which can either be positive (co-stimulation) or negative (co-inhibition), including the molecules CD80 (B-1) and CD86 (B7-2) on dendritic cells and CD28 (stimulatory) or CTLA-4 (inhibitory) on T cells (see Figure 2 for T cell activation) (Baxter and Hodgkin, 2002; Chen, 2004).

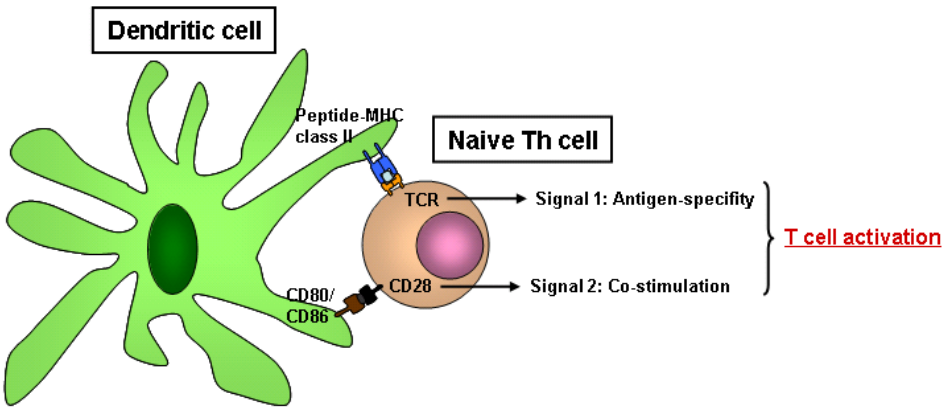


Figure 2: T cell activation by dendritic cells

With the third signal, dendritic cells determine the functional polarization of $CD4^+$ T cells towards Th1, Th2, or Treg cells. Different PAMP and danger signals first polarize dendritic cells which further polarize T cells. Thus, they can be divided into type 1, type 2, and regulatory-type PAMPs. The type 1 PAMPs are the most extensively studied and include TLR agonists such as LPS. LPS binds to TLR4 and induces IL-12 production in dendritic cells and therefore polarizes T cells towards a Th1 phenotype (Boonstra et al., 2003; Langenkamp et al., 2000). CCR2 ligand, histamine, and PGE_2 belong to the type 2 PAMPs which stimulate dendritic cells to secrete Th2-polarizing factors, e.g. CCL2, hence polarizing naive Th cells into Th2 cells (see Figure 3). The existence of a fourth signal which stimulates T cells to produce homing receptors that are tissue specific is poorly characterized (Campbell and Butcher, 2002).

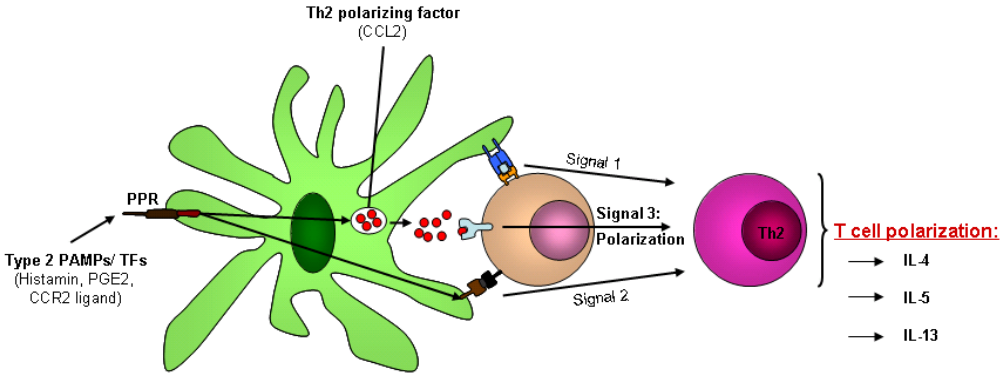


Figure 3: Polarization of T cells

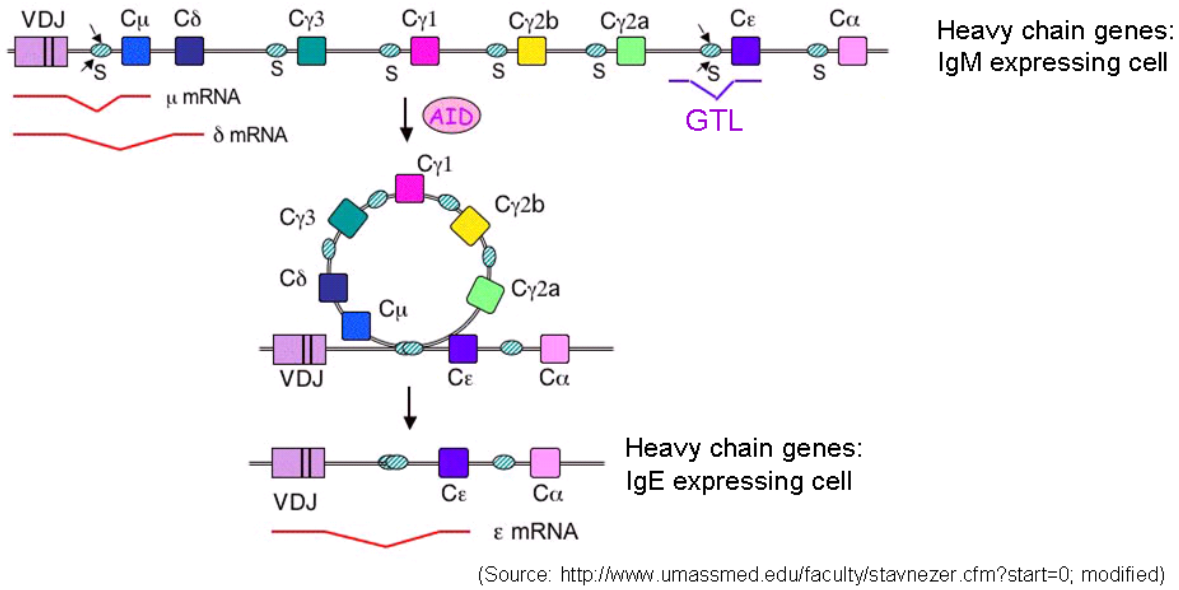
These differentiated Th2 cells do not only promote B cells to differentiate into plasma cells and switching to IgE, but are also involved in other reactions of the immediate hypersensitivity reaction (see Figure 1). For example, Th2 cells produce IL-5 and IL-13. IL-5 activates eosinophils and IL-13 stimulates epithelial cells to secrete increased amounts of mucus. Moreover, Th2 cells accumulate at sites of immediate hypersensitivity reaction and therefore contribute to the inflammation of the late-phase reaction.

Activation of B Cells and Class Switch Recombination (CSR) to IgE

Th2 cells activate B cells specific for allergens in a T cell dependent manner by stimulation with CD40 ligand and the secretion of IL-4 which enhances the transcription of Cε germline transcripts (Cε GTLs) and activation-induced cytidine deaminase (AID), the rearrangement of the IgE genomic locus, and the production of IgE antibodies (Geha et al., 2003).

The phenomenon of isotype switching allows a single B cell clone to differentiate into plama cells and produce antibodies with the same fine specificity but different effector functions which are determined by the heavy chain isotype. The arrangement of the Ig heavy chain genes in the germline normally consists of an array of variable (V_H) genes, diversity (D) genes, joining (J_H) genes, and genes encoding for the constant portion of the Ig heavy chains (C_H), each flanked with a switch region (S) containing repetitive DNA sequences, except the Cδ. The IgE gene locus is illustrated in Figure 4.

The differentiation of B cells and production of a functional IgE molecule requires two DNA rearrangement events. In general, differentiation into a mature B cell involves the recombination of individual heavy-chain V_H, D, and J_H exons to form a $V_H(D)J_H$ segment that encodes for the antigen-binding domain of the Ig molecule and lies in proximity of Cμ. Transcription and alternative splicing result in IgM and IgD mRNA which are then translated into IgM and IgD proteins leading to IgM^+IgD^+ B cells. The second rearrangement involves isotypes switching by deletional class switch recombination (CSR) involving Sμ and Sε resulting in a B plasma cell expressing IgE and excision of the genomic DNA from Sμ to the Sε sequence (Figure 4). RNA splice isoforms with the M1 and M2 exons encode membrane IgE. Since the $V_H(D)J_H$ segment is conserved, the antigen specificity of the Ig remains unaltered.



(Source: http://www.umassmed.edu/faculty/stavnezer.cfm?start=0; modified)

Figure 4: Class switch recombination to IgE

In order to undergo isotype switching to IgE, B cells require two distinct signals (Vercelli 1989, Vercelli 1990) which are delivered by T cells through a complex series of interactions (see Figure 5) (Clark and Ledbetter, 1994). Antigen-specific B cells present the antigen in contex of MHC class

II on the surface which is recognized by the TCR complex of an antigen-specific polarized Th2 cell. This leads, first to the secretion of cytokines, in particular IL-4 and/ or IL-13, and second, to the expression of the CD40 ligand (CD40L, CD154) by T cells.
IL-4 binds to the IL-4 receptor α-chain (IL-4Rα) on B cells which delivers the first signal for isotype switching by the recruitment and activation of the tyrosine kinases Janus-activated kinase (JAK)-1. The activation of JAK3 is delivered through IL-4Rγc. The activated JAKs phoshporylate tyrosine residues of IL-4Rα, which act as a signal transducer and activator of transcription 6 (STAT6). The transient expression of CD40L which belongs to the TNF-superfamily on T cells engages CD40 which is constitutively expressed on B cells (Banchereau et al., 1994) and leads to the oligomerization of CD40 on B cells. This triggers signaling by the tumour-necrosis factor receptor-associated factors (TNFR)-associated factors (TRAFs) (Pullen et al., 1999) to nuclear translocation of the transcription factors nuclear factor-κB (NF-κB) and activator protein 1 (AP-1). The synergism of STAT6 and NF-κB activates the transcription of the Iε promotor and leads to the production of Cε GLTs and AID (Iciek et al., 1997; Messner et al., 1997). The exact role of the germline transcript is presently unclear, but it is a prerequisite of CSR to IgE (Gauchat et al., 1990; Jung et al., 1993).

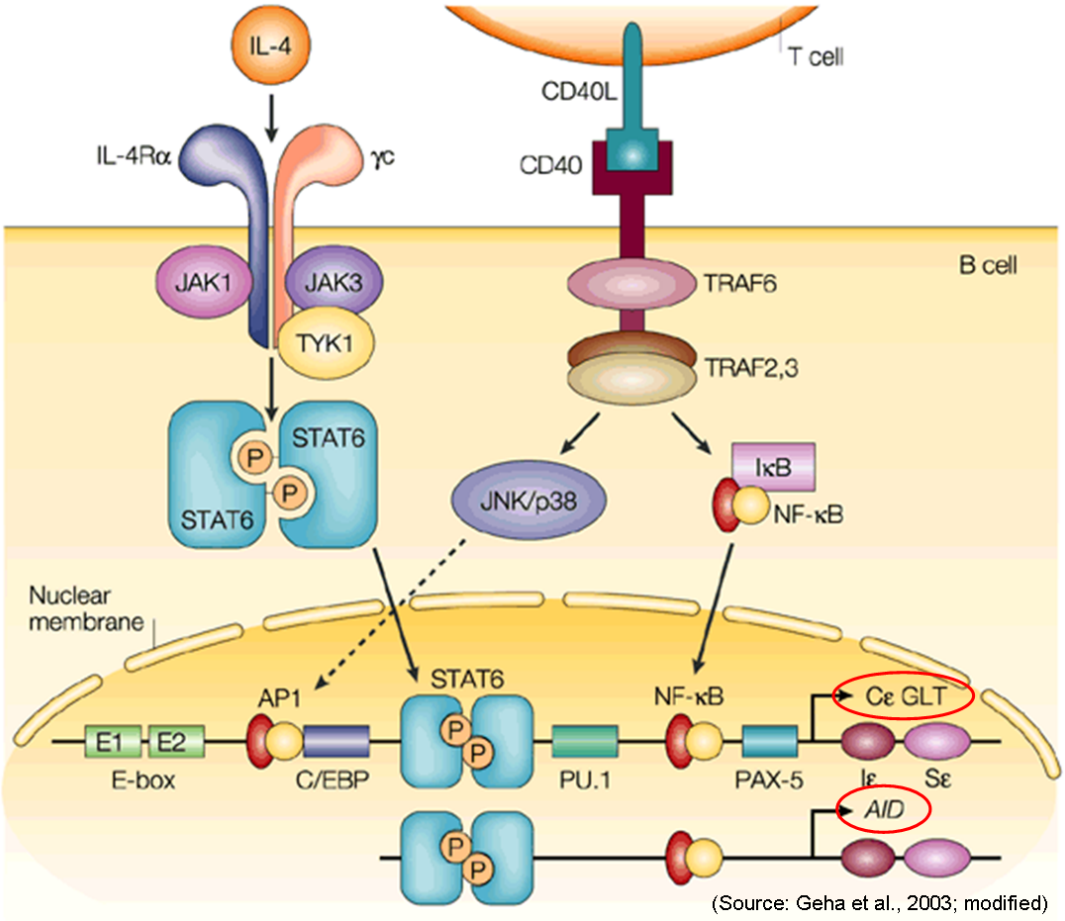


Figure 5: Activation of Cε GTL and AID transcription in B cells

Activation-induced cytidine deaminase (AID) targets single-stranded (ss) DNA that is produced during transcription and substitutes U for C. Base-excision repair results in dsDNA break and repair of these breaks in the Sμ and Sε regions by the joining of non-homologous ends results in Sμ and Sε deletional switch recombination (see Figure 4) (Chaudhuri et al., 2003). The intervening DNA is circularized and deleted. This rearrangement brings the $V_H(D)J_H$ segments encoding the antigen-binding site into proximity of the Cε-encoding segments resulting in the transcription of a mRNA that encodes for a full ε heavy chain (see Figure 4).

Just to mention, the existence of several T cell independent pathways for inducing IgE class switching in the presence of IL-4 have been described. These include the corticosteroid hydrocortisones (Jabara et al., 1991), the induction of IgE-specific CSR by B cell activation factor belonging to the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) (Litinskiy et al., 2002), or Epstein-Barr virus infection (Jabara et al., 1990). Furthermore, DC themselves can have major effects on B cell differentiation, proliferation, and immunoglobulin production by secretion of e.g. IL-12 after stimulation by DC40L on activated T cells (Dubois et al., 1997).

Mast Cell Activation and Mediator Release

Allergen-specific IgE produced by B cells circulates throughout the body and finally binds to a high-affinity Fc receptor specific for ε heavy chain (Fcε RI) on tissue mast cells. Additionally, circulating basophils and eosinophils are able to bind IgE. Mast cells, basophils, and eosinophils are the effector cells of immediate hypersensitivity reactions (Wedemeyer et al., 2000). It is also known that dendritic cells and monocytes in humans also express Fcε RI.

In sensitized individuals subsequent exposures to allergen which binds to IgE on mast cells leads to cross-linking of Fc receptors and activation of the cells by initiating a signaling cascade involving tyrosinase kinases. Activation results in mediator release of IgE-sensitized mast cells by degranulation, which is in turn responsible for some of the subsequent pathologic reactions. The mediators, which are pre-formed as well as newly synthesized, include histamine, leukotrienes, and cytokines. They promote vascular permeability, smooth muscle contraction, and mucus production. Chemokines (e.g. eotaxin and MCP-5) released by mast cells and other cell types in combination of adhesion molecule interactions direct the recruitment of inflammatory cells that contribute to the late allergic response (see Figure 1). This is characterized by an influx of eosinophils and Th2 cells, both expressing receptors for many of the same chemokines (Sallusto et al., 1997). Eosinophils release an array of pro-inflammatory mediators upon activation by e.g. IL-5, including leukotrienes and basic proteins, and they might be an important source of IL-3, IL-13, and granulocyte/macrophage colony-stimulating factor.

2.1.2 A Murine Model for Type I Hypersensitivity

As the mechanism of allergen-induced airway inflammation cannot be studied in humans, animal models have been generated mimicking human allergic airway inflammation. A high degree of analogy between the murine and human immune system makes the mouse the most powerful mammalian system for studying the genetics of allergy.

The presented study used an allergic airway disease model, a classical manifestation of type I hypersensitivity which includes a sensitization and elicitation phase. After the animals were sensitized with a model allergen, namely ovalbumin (OVA), airway inflammation can be induced by exposure of these OVA-sensitized mice to an OVA-aerosol (elicitation phase).

2.2 Role of Anthropogenic Factors in Allergy

It has been repeatedly shown that the prevalence of atopic diseases (e.g. asthma) has increased and still is increasing worldwide (Ring et al., 2001). As already mentioned, there are several factors which might contribute to this observation. Genetic factors clearly play a role in the development of atopic diseases, but they alone seem not to be the sole factor for the increased prevalence, because the increase occurred only within the last 40 years, mainly in industrialized countries (Hopkin, 1997). Lifestyle factors such as decreased exercise or outdoor activities are more likely to be responsible for this increase. One discussed factor is the decline in infectious diseases especially during childhood also known as the 'hygiene hypothesis' in industrialized countries. This proposes that sensitization may be prevented by infections occurring in early childhood (Serafini, 1997; Strachan, 2000). However, the most likely factor contributing to the increased prevalence of atopic diseases are environmental conditions, such as changes in outdoor as well as in indoor environments. There is great evidence from epidemiologic studies for a strong association of airborne particulates and the development of allergic sensitization. These epidemiologic studies (Diaz-Sanchez et al., 2003; Pope, 2000; Riedl and Diaz-Sanchez, 2005) which can only show an association between the level of air pollution and incidence of allergic diseases are underlined by experimental animal studies. They show that particles may play an important adjuvant role in the induction of the allergic response by e.g. showing increased total and allergen-specific IgE antibody response (Heo et al., 2001; Nel et al., 1998). These studies have been further extended in human studies (Diaz-Sanchez, 1997; Diaz-Sanchez et al., 1994; Diaz-Sanchez et al., 1997; Fujieda et al., 1998; Takenaka et al., 1995).

2.2.1 Particulate Matter

Air pollutants can be distinguished between primary and secondary, indoor and outdoor, or gaseous and particulate pollutants (Bernstein et al., 2004). The presented study focuses on the ambient particulate matters as primary combustion products.
There are a variety of natural and man-made sources for particular matter, e.g. combustion of wood and fossil fuels (Ormstad et al., 1997; Seaton et al., 1995). The composition of the particulate air pollutions from different areas may vary enormously. The most important contributor to particulate matter is the combustion of diesel fuels by diesel engines, creating diesel exhaust particles (DEP)/ diesel particulate matter (DPM). These DEP are different in their composition when produced with different engines, engine load, and type of diesel fuel, and therefore are different in the biologic effects they exert (Sjogren et al., 1996). DEP consist of an inert carbon core surrounded with a variety of elemental and organic carbon species (e.g. polyaromatic hydrocarbons) (Barfknecht et al., 1982; Lovik et al., 1997; Nel et al., 1998). These adsorbed organic compounds can be volatile or semivolatile organic species (e.g. polycyclic aromatic hydrocarbons (PAH), nitro-PAH, quinones), transition metals (iron, nickel, vanadium, copper), ions (sulphate, nitrate, acidity), reactive gases (ozone, peroxides, aldehydes), materials of biologic origin (endotoxin, bacteria, viruses, animal

and plant debris, pollens), and minerals (quartz, asbestos, soil dust). One of the major chemical components of DEP are PAH which contain 3 to 5 benzene rings and are products of incomplete combustion processes. The most common PAH in DEP are listed in Table 1 (Barfknecht et al., 1982).

Particular matter (PM) is usually classified by their size (aerodynamic diameter) with all particles with less than 10 μm defined as PM_{10} which are often referred to as inhalable particles. PM_{10} can be further subdivided into 3 categories, with coarse PM (2.5-10 μm = $PM_{2.5\text{-}10}$), fine PM (0.1-2.5 μm = $PM_{2.5}$), and ultrafine PM ($<$0.1 μm = $PM_{0.1}$) (Granum and Lovik, 2002). Most of the DEP exist as fine or ultrafine particles which are primarily formed during the combustion. But as they form agglomerates, DEP mostly exist in varying sizes. As DEP exist mainly as ultrafine or fine particles they are able to remain in the air for a long time and can be easily deposited in the lung. There is discussion that smaller particles have greater biologic effects, because they have a greater relative surface and therefore can carry more chemicals (Li et al., 2002a; Oberdorster and Utell, 2002). Furthermore, smaller particles might be more respirable and hence toxic.

Table 1: Polycyclic aromatic hydrocarbons in DEP

Compound	Organic extract (%)
Phenanthrene (0, C2, C3, and C4 methylated)	52
Fluorenes (0, C2, C3, and C4 methylated)	15
Naphthalenes	13
Fluoranthrenes	10
Pyrenes (including benopyrene)	10

(modified from Barfknecht et al., 1982)

2.2.2 Mode of Action

The exact mechanisms by which air pollutants influence the development of allergic disease are complex and still not well understood (Diaz-Sanchez and Riedl, 2005). It is unclear whether particles by themselves exert these effects or whether particle-bound chemicals are responsible. This is reviewed by Granum and Lovik (Granum and Lovik, 2002). Supposingly, both the particulate core and the surrounding compounds have the ability to initiate and increase the allergic reaction (Heo et al., 2001; Lovik et al., 1997).

There are studies linking the pro-inflammatory and adjuvant effects to the chemicals that surround the carbon core of the diesel particles rather than the core itself (Baulig et al., 2003; Li et al., 2000). Moreover, there is evidence that PAH extracted from DEP (e.g. pyrene or phenanthrene) can effect allergic immune responses. The upregulation of IL-4 production (Bommel et al., 2000), increased IgE production in mice injected intraperitoneally with ovalbumin (Suzuki et al., 1993; Takenaka et al., 1995; Tsien et al., 1997) or induction of increased inflammation (Fahy et al.,

1999; Terada et al., 1999) was already reported.
In contrast, some studies show an adjuvant effect for the particle core on specific IgE production in mice (Granum et al., 2001a; Granum et al., 2001b) or enhanced local inflammatory response (Finkelstein et al., 1997; Granum et al., 2001b; Lovik et al., 1997).
However, it is likely that there is a combined effect between these two factors because both components contribute to the adjuvant effect. There are studies indicating that the adjuvant effect of DEP is due to the particle core itself, but with an additional effect from the adsorbed chemicals (Heo et al., 2001; Lovik et al., 1997).
One must be aware that most of the studies used either single particulate matter (Diaz-Sanchez et al., 1999; Fujieda et al., 1998; Granum et al., 2001a; Imrich et al., 2000; Lovik et al., 1997; van Zijverden et al., 2000), mixtures of different particle types (Li et al., 1997; Ormstad, 2000; Takafuji et al., 1989), or solutions with organic extracts from particles (Bommel et al., 2000; Dreher et al., 1997; Fahy et al., 1999; Lambert et al., 2000; Takenaka et al., 1995; Tsien et al., 1997). In real-life situation, however, particle compositions vary and therefore have different effects depending on the compounds bound.

One discussed mechanism by which DEP exert their effects is the production of reactive oxygen species (ROS) upon exposure. This causes inflammation by initiating redox-sensitive signaling pathways such as mitogen activated protein kinase and the nuclear factor $\varkappa$B cascade (Li et al., 2003). ROS, such as superoxide, hydrogen peroxide, and hydroxyl radical, are reactive with proteins, lipids, and DNA, leading to cellular damage. There are some *in vitro* studies, demonstrating the involvement of oxidative stress in mediating the adjuvant effects of DEP, e.g. after exposure of macrophages or bronchial epithelial cells to DEP or DEP extracts (Hiura et al., 1999; Li et al., 2002b). Moreover, murine *in vivo* studies showed a clear effect of ROS generation after DEP exposure (Lim et al., 1998; Takano et al., 1997).
As DEP are a very heterogeneous mixture with hundreds of chemicals present, it is very likely that other mechanisms occur. And even the role of DEP as carriers for antigens (e.g. pollens) has to be considered (Knox et al., 1997). Finally, it should be mentioned that endotoxin is virtually ubiquitous, and can influence inflammatory and adjuvant activity caused by particles (Ning et al., 2000).

2.2.3 Particles in Allergic Immune Responses

As allergic reactions are divided into two phases, the sensitization and the elicitation phase, the question arises when do particles exert their effects. Human exposure studies, animal models, and cellular studies were accomplished to investigate the specific effects of particles.

There are several experimental studies in human and in mice showing that particles augment the allergic immune response by acting during sensitization phase (Diaz-Sanchez et al., 1999; Granum et al., 2000; Granum et al., 2001b; Heo et al., 2001; Lovik et al., 1997; Takafuji et al., 1989). Diaz-Sanchez et al. showed in an experimental human study that DEP may interfere with the

sensitization phase of the allergic reaction. He could induce a de novo mucosal IgE response to the neoantigen keyhole limpet hemocyanin (KLH) (Diaz-Sanchez et al., 1999). This effect that particles induce allergic sensitization could also been shown in an experimental mouse model (Granum et al., 2000). There are a variety of studies showing an increased allergic response when particles are present either before or together with the allergen (Granum et al., 2001b; Heo et al., 2001; Takafuji et al., 1989).
One of the first *in vivo* studies of Muranaka in mice showed the potential adjuvant effects of DEP, finding an enhanced production of allergic antibodies (IgE) in mice after i.p. injection of DEP along with allergen (Muranaka et al., 1986). This effect seems to be independent from the route of application, e.g. intranasal or other applications resulted in similar effects (Fujimaki et al., 1994; Fujimaki et al., 1997; Ichinose et al., 1997; Miyabara et al., 1998; Nel et al., 1998; Takafuji et al., 1987; Takano et al., 1997). In addition, DEP induced increased airway eosinophilic inflammation, goblet cell hyperplasia, and airway hyperreactivity (AHR) in murine models of asthma (Ichinose et al., 2002; Ohta et al., 1999; Sagai et al., 1996).

Furthermore, epidemiologic and experimental studies, human as well as murine, indicate that particles can excaberate the elicitation phase of an allergic reaction (Diaz-Sanchez et al., 2000; Diaz-Sanchez et al., 1997; Fujieda et al., 1998). In subjects challenged nasally with DEP, DEP increased IgE isotype switching *in vivo* resulting in increased total IgE levels (Diaz-Sanchez, 1997; Diaz-Sanchez et al., 1994; Fujieda et al., 1998). Moreover, DEP may act together with allergen on mast cells and lymphocytes to induce IL-4 and IgE production, as well as in the absence of allergen on the secretion of chemokines from epithelial and mononuclear cells and thus increase inflammation (Diaz-Sanchez et al., 2000).

A series of *in vitro* studies in humans and mice have shown that multiple cell types may be influenced by exposure to DEP or DEP-derived chemicals, e.g. epithelial and mononuclear cells, neutrophils, lymphocytes, and mast cells (Nel et al., 1998). Upon stimulation with DEP or chemicals, they may release pro-inflammatory mediators, chemotactic cytokines, and adhesion molecules and therefore be responsible for the *in vivo* observed phenomenon (Riedl and Diaz-Sanchez, 2005).
In addition to enhanced numbers of IgE-secreting B lymphocytes in the nasal mucosa after intranasal challenge with DEP (Diaz-Sanchez et al., 1994) and increased IgE production in human tonsillar and peripheral blood B lymphocytes (Takenaka et al., 1995), increased IgE production in human B cells cultured in the presence of DEP-derived chemicals could be observed (Tsien et al., 1997). However, whether these effects are due to direct or indirect affecting B cells remains to be answered. There are various publications focusing on the effects of DEP on T cells, however it is not clear whether this occurs via direct effects on T cells or indirectly by targeting antigen presenting cell (APC) such as macrophages or dendritic cells. As APC interact with T cells and direct cytokine production they play an important role in the induction of an immunological reaction. There is good evidence that DEP and associated chemicals can directly affect macrophages and enhance their role as APC. For example, DEP as well as DEP extracts can induce *in vitro* IL-1 production in rat pulmonary alveolar macrophages (Yang et al., 1997).

In addition to the effects on immune cells, DEP can also act on other mucosal cell types. DEP and benzo(a)pyrene can stimulate cytokine secretion (IL-8 and GM-CSF) in human epithelial cells (Ohtoshi et al., 1998; Terada et al., 1999). IL-8 and TNF-α in turn can up-regulate GM-CSF and IL-8 by airway epithelial cells. IL-8 belongs to the CXC family of chemotactic or chemokines and is important in the chemotaxis of mononuclear cells, activation of eosinophils, mediator release from basophils or mast cells, and augmentation of T cell proliferation and cytokine production.

These observations are summarized in several reviews (Granum and Lovik, 2002; Nel et al., 1998; Peterson and Saxon, 1996; Riedl and Diaz-Sanchez, 2005; Salvi and Holgate, 1999).

2.2.4 Diesel, Dendritic Cells, and Immunoglobulins

DC are pivotal in the initiation of an immune response (Mellman and Steinman, 2001). They are professional APC thus playing an important role in the presentation of antigens and engagement of naïve T cells which in turn leads to T cell polarization. The polarization of T cells is determined by the maturation state of DC and the polarizing signals expressed by DC (Kalinski et al., 1999; Kapsenberg, 2003; Steinman, 2003) which is determined by the additional signals inducing a mature DC response e.g. the presence of microbial stimuli (Kalinski et al., 1999). Furthermore, DC can have major effects on B cell differentiation, proliferation, and immunoglobulin production by secretion of soluble factors, e.g. IL-12, or through induction of CD40L on activated T cells (Dubois et al., 1997). Moreover, DC directly suppress IgE production from B cells stimulated with anti-CD40 mAb and IL-4, whereas DC enhance IgG1 CSR as previously reported. Thus DC might play a potential role as an IgE-negative regulator *in vivo* (Litinskiy et al., 2002; Obayashi et al., 2007)

This work focuses on the modulatory capacities of particles and particle-associated compounds in the development of allergic diseases at a very early stage. To address this question we investigated the effects of various particles and organic compounds on phenotypic maturation of murine and human DC. Furthermore, the capability of SRM-exposed murine DC to interact with and influence cells of the innate immune system such as B cells was analyzed. The data demonstrate that highly polluted diesel exhaust particles but not particle-associated organic compounds, less polluted DEP or carbon core modulating DC function by influencing the maturation of DC as well as affecting DC capability to interact with CSR to IgE in B cells.

Several types of diesel exhaust particles were used in this thesis: a standardised reference material, which is a typical heavy-duty diesel engine particulate emission of the early 1980s, was used as a model particle (SRM), two diesel exhaust particles which were produced by combustion of Renault diesel engines with and without catalytic devices (C-Euro3 and Euro3, respectively), and the commercially available carbon black (CB) sample Printex G. To analyze the effects of particle-associated organic compounds phenanthrene, EPA-PAH, and AERex samples were used in this thesis. AERex is an organic extract of urban aerosol which was prepared from airborne particular matter. EPA-

PAH contains the 16 priority PAH which were considered by the environmental protection agency as potentially harmful. Phenanthrene is the most frequent polycyclic aromatic hydrocarbon on DEP and some studies showed an adjuvant effect of phenanthrene on the allergic immune reaction (Schober et al., 2007; Takenaka et al., 1995).

2.3 The Endocannabinoid System (ECS)

The endocannabinoid system (ECS) is a physiologic system which consists of the cannabinoid receptors, their endogenous ligands (the endocannabinoids), the proteins for their synthesis and inactivation, as well as the intracellular signaling pathways affected by endocannabinoids. Its function is still discussed; besides the function as regulator within the central nervous system, a role in cell to cell communication within the immune system, and in the regulation of cell proliferation and cell death has been discussed. Thus, the ECS is a complex endogenous signaling system that influences multiple metabolic pathways.

Although the system was not known as the ECS per se, the first report on the therapeutic properties of cannabinoids goes back to the year 200 before Christ (Di Marzo, 2006). Cannabis research on toxicology and the psychotrophic effects began in the 19^{th} century which gradually evolved into cannabinoid research after the identification of the active principles of cannabis, namely delta-9-tetrahydrocannabinol (Δ^9-TCH). This finding allowed the chemical modification of this natural product and the identification of specific binding sites and molecular targets for the cannabinnoids (Mechoulam, 2000). Another step forward was the cloning of the first cannabinoid receptor which was the beginning of endocannabinoid research. Today, the endocannabinoid research develops into the field of drug development; drugs such as exogenous cannabinoid receptor agonists or antagonists or drugs which modulate the biosynthesis or degradation of endocannabinoids (Di Marzo, 2006).

2.3.1 Cannabinoid Receptors and Signaling

One of the early hypothesis about the mechanism of action of Δ^9-TCH was that it perturbed neuronal cell membranes, resulting in non-selective interference of neurotransmission (Hillard et al., 1985). This theory was disproved by the identification of the cannabinoid (CB) receptors. There are at least two types of CB receptors expressed in mammalian tissues (Howlett et al., 2002). First, the CB1 receptor which was identified by Howlett and co-workers (Devane et al., 1988) and cloned by Matsuda in 1990 (Matsuda et al., 1990), and second, the CB2 receptor which was isolated and cloned by Munro in 1993 (Munro et al., 1993).

CB1 receptors are expressed predominately at central and peripheral nerve terminals where they mediate inhibition of transmitter release (Devane et al., 1988; Mackie, 2005). Additionally, CB1 receptors are located in a wide range of tissues such as adipose tissue, liver, muscle, the gastrointestinal tract, and pancreas (Bouaboula et al., 1993). CB1 receptors are also expressed in some immune cells, however their expression is lower than for CB2 receptors (Howlett et al., 2002).
The CB2 receptor is less widely expressed than the CB1 receptor (Whiteside et al., 2007). CB2 receptors are found mainly in immune-related organs or tissues such as the tonsils, spleen, thymus, and bone marrow with particular high expression levels on B cells and natural killer cells (Bouaboula

et al., 1993; Howlett et al., 2002, Kaminski et al., 1992). Moreover, CB2 receptors are also expressed in neurons, but at much lower concentrations than the CB1 receptor. The role of the CB2 receptors on neurons is not yet understood. It is known that the level of CB2 receptors often increases after a pathological stimulus (reviewed in Howlett et al., 2002).
Interestingly, the tissue distribution of CB1 and CB2 accounts for the well-known psychotropic and peripheral effects of cannabinoids (Klein et al., 1998b; Klein et al., 2003).

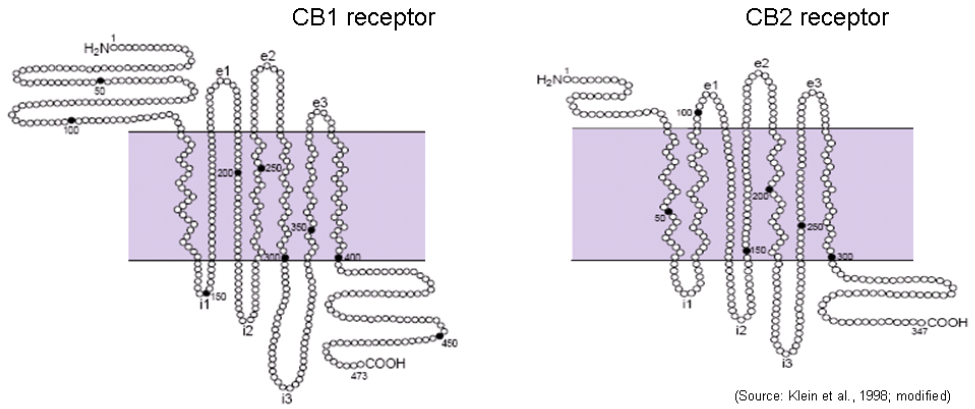


Figure 6: Mouse cannabinoid receptors CB1 and CB2

Both CB receptors belong to the rhodopsin subfamily of G protein-coupled receptors. They consist of a single polypeptide chain which has seven transmembrane spanning regions with an extracellular, glycosylated N-terminus and an intracellular C-terminus, as illustrated in Figure 6. CB1 receptor has longer extra- and intracellular tails than CB. CB1 is 66% similar to CB2 at aminoacid level, however, the transmembrane regions show a 78% similarity. There are three loops at the extracellular as well as intracellular side; e1-e3 are extracellular loops, while i1-i3 are the respective intacellular loops (Klein et al., 1998).

CB receptors are linked to $G_{i/o}$ proteins with a difference between CB1 and CB2 receptors in their selective interaction with G_i and G_o proteins (Glass and Northup, 1999). Activation of CB1 receptor results in interaction with both G_i and G_o proteins, CB2 receptor interacts efficiently only with G_i proteins. Moreover, the nature of the agonist presents different potency to stimulate coupling of CB1 or CB2 receptors to G proteins (Demuth and Molleman, 2006). G proteins are heterotrimeric (α, β, γ subunits) GTP-binding proteins. In its inactive state, the α subunit of G proteins binds GDP. When stimulated, GDP is substituted for GTP which results in the activation of the α subunit and $\beta\gamma$ complex. Different signaling pathways are affected after CB receptor stimulation of G proteins (see Figure 6):
Both receptors are coupled via $G_{i/o}$ proteins negatively to adenylyl cyclase (AC) and cAMP (Howlett and Fleming, 1984). The inhibition of AC and cAMP leads to inactivation of the protein kinase A (PKA) phosphorylation pathway which includes phophorylation of several transcription factors. The PKA-dependent signaling cascade has major importance for gene regulation in immune cells, and the role for CB receptors in modulation of cAMP-dependent immune response was shown

e.g. for IL-2 gene transcription (Novak et al., 1990). Thus, cannabinoid receptor stimulation can interfere and antagonize with the early events in immune cell activation.
G_i proteins also link CB receptors positively to the mitogen-activated protein kinase (MAPK) signaling cascade (Howlett, 2005). Anandamide and Δ^9-TCH were shown to stimulate MAP kinase activity and to increase phosphorylation of the arachidonate-specific cytoplasmic phospholipase A2 (Wartmann et al., 1995). But cannabinol demonstrated inhibitory effects on ERK MAP kinases in stimulated mouse splenocytes (Faubert and Kaminski, 2000).
CB1 receptors are also coupled through Gs proteins to activate adenylyl cyclase (Howlett et al., 2002). This shows that the regulation of AC as well as MAP kinases by cannabinoids is complex and may differ depending on cell type, agonist, and experimental conditions.
Additionally, CB1 receptor stimulation of $G_{i/o}$ proteins is directly coupled to inhibition of voltage-activated $Ca2^+$ channels (Mu et al., 1999) and to stimulation of inwardly rectifying K^+ channels in neurons with both inhibiting neurotransmitter release.

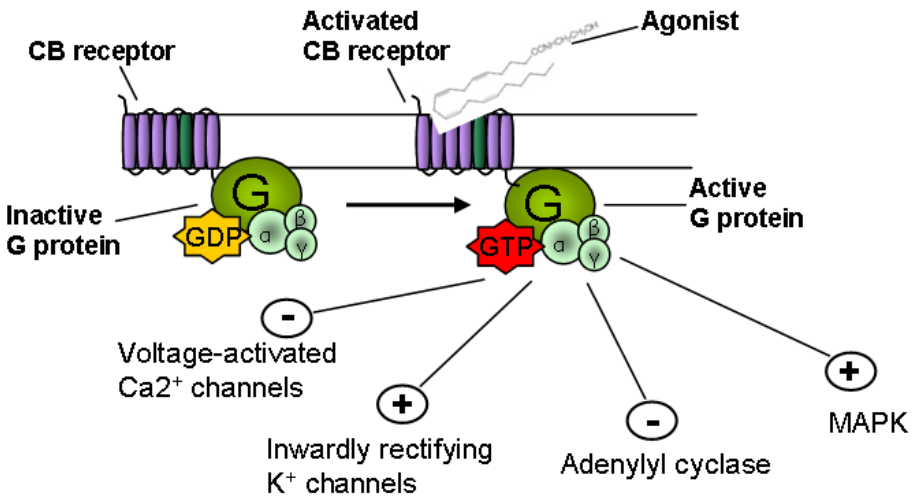


Figure 7: Activation of G protein

Furthermore, it is known that CB1 as well as CB2 receptors possess constitutive activity. This could be shown by increased constitutive MAP kinase-activating properties *in vitro* (Bouaboula et al., 1997). Finally, cannabinoid receptors were shown to decrease the availability of $G_{i/o}$ proteins for other G-protein-linked receptors thus decreasing the strength of diverse stimuli. This may influence cellular homeostasis and lead to impairment of the immune response.

One should keep in mind that effects of cannabinoids were observed which could not be explained by the involvement of CB1 or CB2 receptors (Wiley and Martin, 2002). Thus, the involvement of other pharmacological targets was investigated (Begg et al., 2005; Mackie and Stella, 2006). Up to now it remains elusive whether there are more receptors belonging to the cannabinoid receptor group. The transient receptor potential vanilloid type 1 (TRPV1) receptor and the orphan receptor GPR55 are under discussion (Pertwee, 2006, 2007).

2.3.2 Ligands of Cannabinoid Receptors: Agonists and Antagonists

There are CB receptor agonists which show a certain receptor selectivity (CB1-selective, CB2-selective, or non-selective) and different affinities. These agonists can be divided into different chemical classes (Figure 8): the classical cannabinoids (e.g. Δ^9-TCH synthesized by Cannabis sativa), synthetic non-classical cannabinoids (e.g. CP55,940), aminoalkylindoles (e.g. R-(+)-WIN55212), and eicosanoids which derive from the endocannabinoids (e.g. arachidonolylethaolamide (AEA) also known as anandamide or 2-arachidonoyl glycerol (2-AG)) (Pertwee, 2006). Another classification can be done by their source, e.g. endogenous versus exogenous agonists. AEA and 2-AG are the two most prominent endocannabinoids which are all derived from lipid precursors. Endocannabinoids are not stored in synaptic vesicles as classical neurotransmitters are, but are synthesized on demand after an action potential triggered the opening of calcium channels. Endocannabinoids are released from postsynaptic cells and exert their action by binding to and activating of presynaptic CB receptors. This reverse path is referred to as retrograde signaling. Endocannabinoids are removed by a yet unknown tissue uptake processes, and are finally metabolized rapidly. Thus, they act locally at the sites of synthesis (De Petrocellis et al., 2004; Wilson and Nicoll, 2001).

It is important to note that CB receptor agonists have pharmacological differences. As mentioned, CB receptor agonists can differ in CB1 and CB2 potency and efficacy, and in the extent to which they are CB1 or CB2 receptor selective.

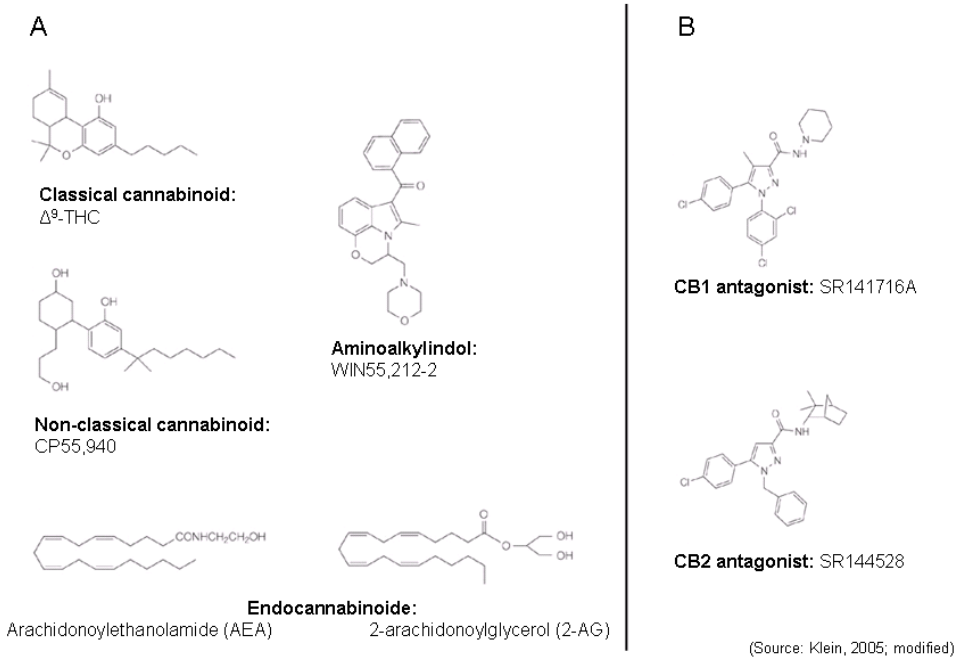


Figure 8: Cannabinoid receptor ligands: (A) Agonists and (B) Antagonists

The identification of CB receptors was followed by the development of CB1- and CB2-selective receptor antagonists (Howlett et al., 2002; Muccioli, 2007). Among these is the CB1 receptor antagonist SR141716A (Rimonabant), which was discovered in 1994 at Sanofi pharmaceutical

company (Rinaldi-Carmona et al., 1994). As most of the CB1 receptor antagonists are not neutral, SR141716A not only blocks the effects from agonists, but due to the inverse agonistic action SR141716A inhibits constitutively active CB1 receptors (MacLennan et al., 1998; Pertwee, 2005). CB1 receptors signal even without an added agonist because they exist in this constitutively active form. This makes the interpretation of *in vivo* effects difficult, because it is not clear whether these are due to blockade or to an inverse agonistic action.

The first CB2 receptor antagonist, SR144528, was also synthesized by Sanofi researchers (Rinaldi-Carmona et al., 1998), and is also an inverse agonist. Because CB2 receptors are present in many types of immune cells, the function of CB2 antagonists as anti-inflammatory and anti-allergic drug is discussed.

2.3.3 Effects of Cannabinoids on Immune Cells

The ECS itself regulates many functions throughout the body such as movement, memory, immune regulation, appetite, and pain (De Petrocellis et al., 2004). The role of the ECS in immune regulation, health, and disease is still not clarified. In general, imbalances in the ECS results in various diseases, e.g. inflammation. Preparations of *Cannabis sativa* were used long time ago in Asian medicine to reduce the severity of pain, inflammation, and asthma. However, the negative effect of marijuana smoke on defence mechanisms against bacterial and viral infections were also investigated a long time after (Klein et al., 1998a). The increased development of an inflammatory response to an infection is largely due to the release of pro-inflammatory cytokines and other proteins that can be modulated by cannabinoids (Di Marzo et al., 1996).

Cannabis use was associated with an increase in the percentage of $CD4^+$ T cells in peripheral blood while T cell proliferation varied depending on parameters such as the amount of drug exposure (Wallace et al., 1988). This impaired host defence by cannabinoids was also shown in experimental animal studies. However, it was not known which cells are targeted by cannabinoids, and whether it directly or indirectly affect their function. Macrophages and T cells are suggested as primary target cells (Friedman et al., 1995; Klein et al., 1998a). *In vitro* studies with human and mouse cells demonstrate that cannabinoids can modulate T and B cell proliferation, antibody production, and cytotoxic activity. Mostly, inhibition of T cell proliferation was demonstrated. However, these THC effects were biphasic, with lower drug doses increasing proliferation (Derocq et al., 1995; Luo et al., 1992) and higher doses suppressing the response (Klein et al., 1998b). Several studies on B cells *in vivo* (Kaminski et al., 1994; Nahas and Osserman, 1991; Schatz et al., 1993) and *in vitro* (Kaminski et al., 1994) showed the inhibitory effects of cannabinoids on antibody formation at micromolar concentrations.

Many studies on cytokine secretion have shown that cannabinoids suppress the pro-inflammatory cytokine and chemokine production. These studies indicate that cannabinoid treatment directs the cytokine network away from cell-mediate immunity by somehow suppressing Th1 cell activity

(e.g. IL-12, IFNγ, TNFα) (Blanchard et al., 1986; Zheng et al., 1992), and towards humoral immunity through the overproduction of Th2-type cytokines or regulatory cytokines (e.g. TGF-β, IL-10) (Klein, 2005; Klein et al., 1998b; Zhu et al., 2000). These immunomodulatory properties of cannabinoids are mediated partially through cannabinoid-receptor-dependent as well as –independent mechanisms (e.g. through vanilloid receptors) (Breivogel et al., 2001), thus demonstrating multiple mechanisms of cannabinoid activity depending on the nature of the pro-inflammatory stimulus, target cell, or the cannabinoid.

Macrophages are the first line of defence against bacterial infection. Studies on macrophages showed that a variety of functions are modulated, mostly impaired, by cannabinoids. But the effective drug concentrations are high relative to *in vivo* doses, so extrapolation to human or murine *in vivo* studies is difficult (Klein et al., 1998b).

In addition, activation of immune cells by LPS or other stimuli also modulates the expression of the cannabinoid receptors CB1 and CB2 by these cells, although findings from separate studies are inconsistent, with some reports showing up-regulation (Klein et al., 1995) and others showing down-regulation (Carlisle et al., 2002; Lee et al., 2001).

2.3.4 The ECS in Allergic Airway Inflammation

The effects of cannabinoids on the immune system are broad and involve responses associated with innate, humoral, and cell-mediated immunity. But the role or involvement of CB1 and CB2 receptors is still not solved, as different studies show different responses, but all agree that T cells are sensitive to modulation by cannabinoids. As the secretion of Th2 like cytokines plays a key role in the development of asthma, the question arises whether cannabinoids can modulate the allergic inflammation.

It was already shown that cannabinoid-treatment attenuated the ovalbumin-induced allergic airway response in A/J mice by inhibiting the expression of critical T cell cytokines and the associated inflammatory response (Jan et al., 2003). Furthermore, it could be shown that the lack of the endocannabinoid system leads to an exacerbated allergic inflammation in a model for cutaneous contact hypersensitivity (Karsak et al., 2007).

Based on these facts, the presented study was conducted to examine the role of the endocannabinoid system, especially their receptors, in the development of allergic airway diseases in a murine model of type I hypersensitivity, and moreover, the possible effects of CB receptor agonist such as THC were investigated. Several key immunologic as well as pathologic features associated with the allergen-induced allergic airway response were investigated.

3 Material

3.1 Cells

All cells were obtained and cultured under sterile conditions.

Table 2: Cells

Cells	Reference / Source
Murine GM-CSF clone (hypoxanthine-aminopterin-thymidine-sensitive Ag8653 myeloma cell line transfected with murine GM-CSF cDNA isolated from a T cell clone by PCR and inserted into the vector BCMGSNeo)	(Zal et al., 1994)
Murine bone marrow-derived dendritic cells (BMDC)	BALB/c
Murine splenic B cells	BALB/c
Human monocyte-derived dendritic cells (MoDC)	Human volunteers (atopic and non-atopic)

3.2 Animals

All mice were housed under specified pathogen-free (SPF) conditions in individual ventilated cages (IVC) at the animal care facility at the Helmholtz Zentrum München. They were housed in a 12 hours light/dark-cycle and had water and mouse chow *ad libitum*. Mice were used at 8-12 weeks of age. The experimental procedures used in this thesis were approved by the District Government of Upper Bavaria.

Table 3: Animals

Animals	Reference
BALB/c	Charles River Laboratories International, Inc.
C57BL/6	Charles River Laboratories International, Inc.
$Cnr1^{-/-}/Cnr2^{-/-}$ cannabinoid receptor double knock-out (DKO)	Prof. Dr. Andreas Zimmer, University of Bonn, Germany (Buckley et al., 2000; Zimmer et al., 1999)

3.3 Environmental Compounds

Different organic compounds as well as particles were used for exposure of murine and human cells (see Table 4).

Table 4: Overview – environmental compounds

Compound	Characterization	Manufacturer
Phenanthrene	single PAH	Sigma-Aldrich (Deisenhofen, Germany)
EPA-PAH	comprises the 16 U.S. EPA priority pollutants	Labor Dr. Ehrenstorfer GmbH, Reference Material for Residue Analysis (Augsburg, Germany)
AERex	organic extracts of urban aerosol from airborne particular matter	Dr. Matuschek, Institute of Ecological Chemistry, Helmholtz Zentrum München (Neuherberg, Munich)
SRM	Standard reference material of DPM/ DEP	National Institute of Standards & Technology (NIST, Gaithersburg, MD, USA)
C-Euro3 / Euro3	DEP prepared with or without catalytic device	CERTAM (Rouen, France)
CB	Printex G particles as carbon black	Evonik Degussa GmbH (Essen, Germany)

3.3.1 Particle-associated Organic Compounds

Different particle-associated organic compounds were used. Both single substances and mixtures of substances, which contain a variety of polycyclic aromatic hydrocarbons (PAH).

Phenanthrene

Phenanthrene was used as a representative of PAH. Phenanthrene was dissolved in DMSO at a concentration of 10 mM. This stock was stored in a dark glass container at 4°C.

EPA-PAH

The EPA-PAH standard constitutes a mixture of the priority 16 PAH (see Table 5) determined by the U.S. Environmental Protection Agency (EPA) each at a concentration of 100 mg/l in DMSO. This stock was stored in a dark glass container at 4°C.

Table 5: Composition of EPA-PAH standard

Composition of EPA-PAH standard		
Acenaphthene	Benzo*[g,h,i]*perylene	Indeno*[1,2,3-cd]*pyrene
Acenaphthylene	Benzo*[a]*pyrene	Naphthalene
Anthracene	Chrysene	Phenanthrene
Benz*[a]*anthracen	Dibenz*[a,h]*anthracene	Pyrene
Benzo*[b]*fluoranthene	Fluoranthene	
Benzo*[k]*fluoranthene	Fluorene	

AERex

Different organic extracts of urban aerosol (AERex) containing various PAH concentrations were used. AERex samples were kindly provided by Dr. Matuschek, Institute of Ecological Chemistry, Helmholtz Zentrum München, who did all the sampling of airborne particular matter (PM), preparation of PM extracts, and HPLC analysis to determine the particle-associated PAH-contents (Schober et al., 2006). In order to compare different AERex samples, each PM extract was dissolved in 0.1 ml DMSO which was equalized to 100%. This stock was stored in a dark glass container at 4°C.

The samples were 1 day-samples (AERex I, II, III, and IV) which were collected in July 2006 with a sampler using a PM2.5 head. Table 7 shows sampling details and in Table 6 PAH contents in AERex samples are listed.

Table 6: HPLC analysis of particle-associated PAH contents in AERex samples

	AERex I [pg/μl]	AERex II [pg/μl]	AERex III [pg/μ]	AERex IV [pg/μl]
Acenaphthene*	13.66	2.55	4.53	n.d.
Acenaphthylene*	-	-	-	-
Anthracene*	48.93	36.97	19.09	15.06
Benz(a)anthracen*	13.34	11.96	10.74	8.96
Benzo(b)fluoranthene*	15.49	6.30	n.d.	n.d.
Benzo(k)fluoranthene*	5.63	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene*	n.d.	n.d.	n.d.	n.d.
Benzo(a)pyrene*	n.d.	n.d.	n.d.	n.d.
Chrysene*	41.30	24.73	18.16	14.35
Dibenz(a,h)anthracene*	n.d.	n.d.	n.d.	n.d.
Fluoranthene*	712.32	469.65	217.76	201.63
Fluorene*	47.52	23.66	15.49	11.10
Indeno(1,2,3-cd)pyrene*	n.d.	n.d.	n.d.	n.d.
Naphthalene*	157.18	102.36	59.95	95.41
Phenanthrene*	483.92	307.23	161.61	111.87
Pyrene*	491.96	290.04	164.82	105.93

Note. Analysis of particle-associated PAH contents by HPLC (pg per μL extract). Solvent was DMSO. n.d., not detected. -, not determined. * U.S. EPA priority pollutant.

Table 7: Sampling of organic extracts of urban aerosol (AERex)

Sample	Date	Total Time	Total Air Volume
AERex I	20.06.2006	21.81 hrs	1011,6 m^3
AERex II	23.06.2006	22.13 hrs	1029,3 m^3
AERex III	26.06.2006	31.96 hrs	1539,5 m^3
AERex IV	29.06.2006	14.01 hrs	1118,2 m^3

3.3.2 Particles

SRM – Standard Reference Material 1650a

The Standard Reference Material 1650a (SRM) was used as a model particle of DEP. SRM was obtained from the National Institute for Standards and Technology (NIST). These particles were stored at 4°C in a dark glass.

Particles used for the preparation of SRM 1650a were DPM which were obtained in 1983 through the Coordinating Research Council, Inc., Atlanta, GA. The sample is typical of heavy-duty diesel engine particulate emissions of the early 1980s.

Data from HPLC analysis of particle-associated PAH are shown in Table 8 which were done by Dr. Matuschek, Institute of Ecological Chemistry, Helmholtz Zentrum München. For detailed information of certified values of PAH distribution which also provided for this particle sample see Certificate of Analysis, SRM 1650a Diesel Particulate Matter, NIST, Gaithersburg, MD, (07 November 2000) which is provided online at www.nist.gov.

Certified and reference values for SRM 1650a are listed in Table 9. Specific particle surface area was assessed by the Brunauer-Emmett-Teller (BET) method. The BET method calculates the specific surface area from the quantity of a particular gas being absorbed in multimolecular layers on the surface of the respective particle (Brunauer et al., 1938).

Euro3/ C-Euro3

Euro3 and C-Euro3 were DEP samples which were collected in a particle trap from an Euro3 regulation compliant supercharged diesel engine operated with desulfured gazole without and with oxidation catalyst placed between the turbocharger and the diesel particle filter. These particles represent less polluted particles. HPLC analyses for PAH contents on Euro3 and C-Euro3 were done by Dr. Matuschek, Institute of Ecological Chemistry, Helmholtz Zentrum München (see Table 8).

Carbon black (CB)

As a source for ultrafine carbonaceous particles (carbon black; CB) Printex G was used. They are commercially available from Evonik/Degussa GmbH and are used as carbon black particles for various technical applications. These particles were stored at 4°C in a dark glass. Reference values for Printex G are shown in Table 9.

Table 8: HPLC analysis of particle-associated PAH

	SRM [pg/mg]	C-Euro3 [pg/mg]	Euro3 [pg/mg]
Acenaphthene*	n.d.	15.32	31.57
Acenaphthylene	-	-	-
Anthracene*	1,390	32.56	198.03
Benz*[a]*anthracene*	6,330	57.19	58.10
Benzo*[b]*fluoranthene*	8,810	48.23	38.01
Benzo*[k]*fluoranthene*	2,640	7.94	9.31
Benzo*[g,h,i]*perylene*	6,500	4.72	11.07
Benzo*[a]*pyrene*	1,330	12.05	20.05
Benzo*[e]*pyrene*	7,440	62.22	81.01
Chrysene*	14,500	160.24	138.97
Dibenzo*[a,h]*anthracene*	750	4.52	5.42
Fluoranthene*	49,900	238.56	501.13
Fluorene*	780	31.35	113.42
Indeno*[1,2,3-cd]*pyrene*	5,620	n.d.	n.d.
Naphthalene*	13.430	3,599	8,074
Phenanthrene*	68.400	425.36	1,963.53
Pyrene	47,500	300.26	1,495.39

Note. Analysis of particle-associated PAH contents by HPLC (pg per mg particles). n.d., not detected. -, not determined. * U.S. EPA priority pollutant.

Table 9: Reference values for SRM and Printex G

Properties	SRM	Printex G
Particle diameter	-	51 nm
Mean diameter: 50% of the volume is less	17.1 ± 0.1 µm	-
Surface area (BET)*	108 m^2/g	30 m^2/g
Total extractable mass / Volatiles	20.2 ± 0.4 %	0.9 %

Note. *Specific surface area determined by mulit-point N_2 adsorption BET (Brunauer-Emmet-Teller) method.

3.4 Chemicals, Biochemicals, and Kits

Table 10: Chemicals

Chemical	Formula	Manufacturer
Ammonium chloride	NH_4Cl	Sigma-Aldrich
ß-mercaptoethanol	C_2H_6OS	Merck
BSA		Sigma-Aldrich
Citric acid monohydrate, ACS reagent	$HOC(COOH)(CH_2\ COOH)_2 \bullet H_2O$	Sigma-Aldrich
Cremophor EL®		Fluka
Diethylether	$(C_2H_5)_2O$	Merck
Disodiumcarbonate	Na_2CO_3	Sigma-Aldrich
Disodiumhydrogenphosphate	Na_2HPO_4- dibasic	Sigma-Aldrich
Dimethylsulfoxide (DMSO)	$(CH_3)_2SO$	Merck
Entellan®		Merck
Eosin Y, alcoholic	$C_{20}H_6Br_4Na_2O_5$	Thermo Scientific
Ethanol absolut	C_2H_5OH	Merck
Ethylenediaminetetra acetic acid (EDTA)	$C_{10}H_{16}N_2O_8$	Sigma-Aldrich, Gibco
Ficoll		
Formaldehyde	CH_2O	Staub&Co
Heparin-Natrium-250000	$C_{12}H_{19}NO_{20}S_3$	Ratiopharm GmbH
Hematoxylin	$C_{16}H_{14}O_6$	
Hydrochloric acid	HCl	Merck
Imject® Alum	$Al(OH_3)$	Pierce
Ionomycin calcium salt	$C_{41}H_{70}CaO_9$	Sigma-Aldrich
Ketamine for animals	$C_{13}H_{16}ClNO$	WDT
Paraffin - PARAPLAST		McCormick™ Sc.
Pertex		Medite
Potassium chloride	KCl	Merck
Potassium hydrogenphosphate	KH_2PO_4	Merck
Potassium hydroxide	KOH	Merck
Propidium iodide	$C_{27}H_{34}I_2N_4$	
Schiffs reagent		Merck
Sodium azide	NaN_3	Roth
Sodium chloride	NaCl	Merck
Sodium hydrogencarbonate	$NaHCO_3$	Merck
Sulfuric acid	H_2SO_4	Merck
Tetramethylbenzidin (TMB)	$C_{16}H_{20}N_2$	Fluka
TRISma® base	$NH_2C(CH_2OH)_3$	Sigma-Aldrich
Trypan blue	$C_{34}H_{24}N_6O_{14}S_4\ Na_4$	Sigma-Aldrich
TWEEN®20	$C_{58}H_{114}O_{26}$	Sigma-Aldrich
Xylazin for animals	$C_{12}H_{16}N_2S$	aniMedica
Xylene		Hedinger

Table 11: Biochemicals for in vitro and in vivo use

Reagent	Concentration	Manufacturer
anti-mouse CD40 (purified)	5 μg/ml	eBioscience
LPS	0.1 to 1 μg/ml	InvivoGen
OVA V	2 mg/ml	Sigma-Aldrich
poly(I:C)	20 μg/ml	InvivoGen
Recombinant human IL-4	500 U/ml	PeproTech
Recombinant human GM-CSF	500 U/ml	Promo Cell
Recombinant murine IL-4	100 ng/ml	R&D Systems
Recombinant murine GM-CSF	50 ng/ml	PeproTech
SR 141716A (SR1)	1 mg per kg bw	Sanofi Aventis
SR 144528 (SR2)	1 mg per kg bw	Sanofi Aventis
Δ^9-THC (THC)	50 mg per kg bw	Sigma-Aldrich

Table 12: Kits

Kit		Manufacturer
Mouse Regulatory T Cell Staining Kit	- PE anti-mouse/rat Foxp3 (FJK16s) - PE Rat IgG2a isotype control - Affinity purified anti-mouse CD16/32 (FcBlock) - Flow Cytometry Staining Buffer - Fixation/Permeabilization Concentrate (4x): dilute with Fixation/Permeabilization Diluent - Fixation/Permeabilization Diluent - Permeabilization Buffer (10x)	eBioscience
Diff Quick Staining Set	- Stain solution I: 1.22 g/l Eosin in phosphate buffer - Stain solution II: 1.1 g/l Thiazin-dye in phosphate buffer - Fixation solution: 2 mg/l Fast Green in methanol	Medion Diagnostics
Limulus Amebocyte Lysate (LAL) QCL-1000	- Lysate - Endotoxin - LAL Reagent Water	Lonza
ZytoChem-Plus AP Polymer-Kit	- BlockingSolution - PostBlock - AP-Polymer	Zytomed
Permanent AP Red Kit	- Permanent AP Red Buffer - Permanent AP Red Chromogen $\rightarrow$2.5 ml & 1 Drop	Zytomed
PAS Staining Kit	- Periodic acid 0.5%, aqueous - Schiff's reagent	Merck

3.5 Antibodies

Table 13: Antibodies for FACS

anti-mouse	Conjugate	Conc. ($\rightarrow$ 10 μl)	Clone	Isotype	Manufacturer
CD4	FITC	5 μg/ml	GK1.5	Rat IgG2b, $\varkappa$	BD Pharmingen™
CD4	PE	2 μg/ml	RM4-5	Rat IgG2a, $\varkappa$	BD Pharmingen™
CD8a (Ly-2)	PE	2 μg/ml	53-6.7	Rat IgG2a, $\varkappa$	BD Pharmingen™
CD11c	APC	10 μg/ml	HL3	Ar ham IgG1,λ	BD Pharmingen™
CD16/CD32	purified	1 μg/ml	2.4G2	Rat IgG2b, $\varkappa$	BD Pharmingen™
CD25	Biotin	10 μg/ml	7D4	Rat IgM	BD Pharmingen™
CD45R/B220	PE	2 μg/ml	RA3-6B2	Rat IgG2a, $\varkappa$	BD Pharmingen™
CD80	PE	2 μg/ml	16-10A1	Ar ham IgG2b $\varkappa$	BD Pharmingen™
CD86 (B7-2)	FITC	5 μg/ml	GL1	Rat IgG2a, $\varkappa$	BD Pharmingen™
I-A^d/I-E^d MHC II	FITC	5 μg/ml	2G9	Rat IgG2a, $\varkappa$	BD Pharmingen™
FoxP3	PE	5 μg/ml	FJK16s	Rat IgG2a	eBioscience
Streptavidin	APC	5 μg/ml	-	-	BD Pharmingen™
anti-human					
Goat anti-mouse IgG (γ chain spec.) human adsorbed	FITC	2 μg/ml	-	Anti-goat	Southern Biotech
Fcε RI	-	0.1 μg/ml	CRA1	IgG2b	Cosmo Bio Co.
CD80	PE	25 μg/ml	L307.4	Mouse IgG1, $\varkappa$	BD Pharmingen™
CD83	PE	25 μg/ml	HB15e	Mouse IgG1, $\varkappa$	BD Pharmingen™
CD86	PE	25 μg/ml	2331 (FUN-1)	Mouse IgG1, $\varkappa$	BD Pharmingen™
CD1a	APC	25 μg/ml	HI149	Mouse IgG1, $\varkappa$	BD Pharmingen™
HLA-ABC	FITC	25 μg/ml	G46-2.6	Mouse IgG1, $\varkappa$	BD Pharmingen™
HLA-DR	FITC	25 μg/ml	G46-6	Mouse IgG2a,$\varkappa$	BD Pharmingen™

Table 14: Antibodies for MACS

anti-mouse	Conjugate	Conc. ($\rightarrow$ 10^7 cells)	Clone	Manufacturer
CD16/CD32	purified	10 μg/ml ($\rightarrow$ 40 μl)	2.4G2	BD Pharmingen™
CD11c	Biotin	5 μg/ml ($\rightarrow$ 40 μl)	HL3	BD Pharmingen™
CD45R/B220	PE	2 μg/ml ($\rightarrow$ 40 μl)	RA3-6B2	BD Pharmingen™
Anti-PE MicroBeads	-	1:20 ($\rightarrow$ 100 μl)	-	Miltenyi Biotec
Anti-Biotin MicroBeads	-	1:20 ($\rightarrow$ 100 μl)	-	Miltenyi Biotec

Table 15: Antibodies for immunohistochemistry

anti-mouse	Isotype	Usage	Manufacturer
p-CB1 (Ser 316)	Rabbit polyclonal affinity purified antibody (IgG)	1:1000 = 0.2 μg/ml	Santa Cruz Biotech., Inc.
CB2 (H-60)	Rabbit polyclonal affinity purified antibody (IgG)	1:1000 = 0.2 μg/ml	Santa Cruz Biotech., Inc.
MCP2 (Biotin)	Rabbit polyclonal (IgG)	0.5 μg/ml	abcam

Table 16: Antibodies and proteins for ELISA

Murine cytokine	Use	Description	Concentration [pg/ml]	Manufacturer
	Capture	Anti-mouse monoclonal antibody	1:250	BD OptEIA™
	Detection	Biotin anti-mouse monoclonal antibody	1:250	
IFNγ	Standard	Recombinant mouse IFNγ	2000-15.6 pg/ml	
IL-4		Recombinant mouse IL-4	500-3.95 pg/ml	
IL-5		Recombinant mouse IL-5	1000-7.8 pg/ml	
IL-6		Recombinant mouse IL-6	1000-7.8 pg/ml	
IL-10		Recombinant mouse IL-10	2000-15.6 pg/ml	
IL-12p70		Recombinant mouse IL12p70	8000-15.6 pg/ml	
TNF		Recombinant mouse TNF	1000-3.95 pg/ml	
IL-13	Capture	Anti-mouse IL-13	1:250	Biosource
	Detection	Anti-mouse IL-13 Biotin	1:250	
	Standard	Recombinant mouse IL-13	1000-3.9 pg/ml	
Human cytokine				
	Capture	Anti-human monoclonal antibody	1:250	BD OptEIA™
	Detection	Biotin anti-human monoclonal antibody	1:250	
IL-6	Standard	Recombinant human IL-6	2000-31.3 pg/ml	
IL-10		Recombinant human IL-10	500-7.8 pg/ml	
IL-12p70		Recombinant human IL12p70	2000-31.3 pg/ml	
TNF		Recombinant human TNF	500-7.8 pg/ml	
	Enzyme	Streptavidin-horseradish peroxidase conjugate (SAv-HRP)	1:250	BD OptEIA™/ Biosource
Ig				
total IgE	Capture	Sheep anti-mouse IgE (IgG Fraction)	10 μg/ml	The Binding Site
	Detection	Anti-mouse IgE Biotin	1.25 μg/ml	BD Pharmingen™
	Standard	Purified mouse IgE	250-0.98 ng/ml	BD Pharmingen™
total IgG	Capture	Goat anti-mouse IgG-affinity purified	10 μg/ml	Bethyl Laboratories
	Detection	Goat anti-mouse IgG-HRP conjugate	0.01 μg/ml	
	Standard	Mouse Reference Serum	500-7.8 ng/ml	
total IgM	Capture	Purified anti-mouse IgM	2 μg/ml	BD Pharmingen™
	Detection	HRP rat anti-mouse IgM	0.5 μg/ml	BD Pharmingen™
	Standard	Mouse IgM,$\varkappa$ (Clone MOPC 104E)	500-7.8 ng/ml	Sigma-Aldrich
OVA-IgE	Capture	OVA V	10 μg/ml	Sigma-Aldrich
	Detection	Anti-mouse IgE Biotin	1.25 μg/ml	BD Pharmingen™
	Standard	Mouse Anti-ovalbumin IgE	1000-3.9 ng/ml	Biozol
OVA-IgG1	Capture	OVA V	1 μg/ml	Sigma-Aldrich
	Detection	Anti-mouse IgG1 Biotin	1.25 μg/ml	BD Pharmingen™
	Standard	Monoclonal Anti-chicken Egg-Albumin-clone OVA 14	250-0.49 ng/ml	Sigma-Aldrich
OVA-IgG2a	Capture	OVA V	1 µg/ml	Sigma-Aldrich
	Detection	Anti-mouse IgG2a Biotin	1.25 μg/ml	BD Pharmingen™
	Standard	Serum from sensitized C57BL/6 mice	1:20 – 1:10240	Own laboratory
	Enzyme	Streptavidin-peroxidase conjugate	1 ng/ml	Calbiochem

3.6 Buffers and Solutions, Supplements and Media

Table 17: Buffers and solutions

Solution	Composition
Anaesthezia	100 mg ketamine / 0.5% xylazin → 6 ml PBS → i.p. injection: 100 μl per 10 g bodyweight
Assay diluent	10% FCS (heat incactivated) → 1x PBS
Coating buffer A (IFNγ, IL-4, IL-5, IL-6, IL-13, Ig)	0.1 M sodium carbonate: 8.4 g $NaHCO_3$ / 3.56 g Na_2CO_3 → q.s. to 1 l / pH 9.5
Coating buffer B (IL-10, IL-12, TNF)	0.2 M sodium phosphate: 11.8 g Na_2HPO_4 / 16.1 g NaH_2PO_4 → q.s. to 1 l / pH 6.5
Eosin Y solution	stock solution – 1%: 10 g eosin / 200 ml aqua dest. / 800 ml 95% ethanol working solution – 25%: 250 ml Eosin Y stock solution 750 ml 80% ethanol / 5 ml acial acetic acid (concentrated)
FACS buffer	2% FCS (heat inactivated) / 0.02% NaN_3 → 1x PBS
4% Formalin	Formaldehyde [37-40%] → aqua dest.
Haematoxylin solution (Mayer)	50 g alum / 1 g hematoxylin / 0.2 g sodium iodide → q.s. to 1 l
MACS buffer	0.5% FCS (heat inactivated) / 2 mM EDTA → 1x PBS
OVA for provocation [1%]	1% OVA V → 1x PBS (12 ml per challenge)
OVA for sensitization [1 or 10 μg]	2 mg Imject®Alum / 1 or 10 µg OVA V / in 200 μl 1x PBS → rotate for 30 min at room temperature
PBS (10x)	80 g NaCl / 11.6 g Na_2HPO_4 / 2 g KH_2PO_4 / 2 g KCl → q.s. to 1 l /pH 7.4

→ to be continued

Table 17 continued

Solution	Composition
PBS-Tween	0.05% Tween®-20 → 1x PBS
PBS/1%BSA	1% BSA → 1x PBS
Red blood cell (RBC) lysis buffer (10x)	ACK buffer: 1.5 M NH_4Cl / 10 mM $NaHCO_3$ / 10 mM disodium EDTA → q.s. to 1 l / pH 7.4 / steril filter
Sodium citrate buffer	10 mM sodium citrate buffer: 2.94 g trisodiumcitrat → q.s. 1 l / pH to 6.0
Stopp solution	2 N sulphuric acid: 180 ml aqua dest. / 30 ml H_2SO_4 [96%]
Substrate buffer	0.2 M citrate buffer: 42.05 g citic acid monohydrate → q.s. to 1 l /pH 3.95
TMB stock	24 mg TMB / 500 μl ethanol absolute / 500 μl DMSO
TMB buffer	0.06% H_2O_2 / 1% TMB stock → substrate buffer
TRIS buffer (10x)	500 mM TRISma® base → q.s. to 1 l / pH 7.4
TRIS-Tween	0.05% Tween® -20 → 1x TRIS Buffer [50 mM]
TRIS/1%BSA or 3%BSA	1% or 3% BSA → 1x TRIS Buffer [50 mM]
Trypanblue	10% trypanblue stock → PBS

Table 18: Supplements and media

Medium	Composition in RPMI1640	Manufacturer
D-PBS		Gibco
HBSS		Gibco
BAL medium	RPMI1640 (+ L-Glutamine)	Gibco
	5% FCS (heat inactivated)	PAA
FI medium	RPMI1640 (+ L-Glutamine)	Gibco
	10% FBS (heat inactivated) (EU Research Grade Serum)	Perbio
	2 mM L-Glutamine	Gibco
	0.1 mg/ml Gentamycin	Gibco
huR10 medium	FI medium	
	500 U/ml recombinant human IL-4	R&D Systems
	500 U/ml recombinant human GM-CSF	PromoCell
R10 medium	RPMI1640 (+ L-Glutamine)	Gibco
	10% FCS (heat inactivated)	PAA
	2 mM Glutamine	Gibco
	1,000 U/ml Penicilin	Gibco
	1,000 μg/ml Streptomycin	Gibco
	50 μM ß-Mercaptoethanol	Sigma
R10-complete medium	R10 medium	
	1 mM MEM Sodium Pyruvate	Gibco
	0.1 mM MEM Non Essential Amino Acids	Gibco
	20 mM HEPES	Gibco

3.7 Laboratory Equipment and Consumables

Table 19: Laboratory equipment

Equipment	**Type**	Manufacturer
Centrifuge	Megafuge1.0R	Heraeus
	Laborfuge 400R	Heraeus
	Biofuge pico	Heraeus
CO_2 incubator	IR-Sensor	Sanyo
Compressor		600-4 BS
Cyto centrifuge	Cytospin3	Shandon
Drying chamber	UT 6060	Heraeus
Embedding machine	EG 1150C	Leica Co.
Flowcytometer	FACSCalibur^{TM}	BD Bioscience
Freezer (-20°C)	GS 5203-11	Liebherr
Freezer (-80°C)	KLT 4785	Kryosafe
Fridge (4°C)	UKS 5000-10	Liebherr
Hood	HS 15	Heraeus
Immunostainer	TechMate Horizon Automated Immunostainer	Dako
Microscope	Axiovert 40CFL	Zeiss
	MS 5, Stereomicroscope	Leica
	Leica DM LB (with Leica DC 300F Camera)	Leica Microsysteme LTD.
Microtome	HM355S	Microm
Mixer	Reax2	
Nebulizer		Pari Boy
Ph meter	CG 841	Schott
Neubaur counting chamber	Haemacytometer - CE doooelt	Assistent
Photometer	Mulitskan Ascent V1.24	Thermo Fisher
Pipettes	Reference (10, 20, 100, 200, 1000 μl)	Eppendorf AG
	Multipette plus	Eppendorf AG
	Transferpipette-12 (2-200 μl)	Brand
Pipettor	accu-jet	Brand
Scale	PB 303 DR	Mettler
	SBC 31	SCALTEC
Sonication bath	Sonorex R52	Bandelin
Tissue processor		Shadon Co.
Tissue stainer	COT	medite
Tissue drying oven	TDO50	medite
Vortex		MS 1 Minishaker
Vario MACS®		Miltenyi Biotec
Water bath	1083	GFL
	PSI	Medizintechnik Grünewald
Water purification device	MilliQ 1, EASYpure UV	Millipore

Table 20: Consumables

Material	**Type**	Manufacturer
Canula	Sterican G-17, G-20	Braun
Cell culture tissues	6,12,24,96-well plate; Multidish 6, Polystyren	Nunclon™Δ
	96-well; Micro WellTM Maxi Sorp U-Form	Nunc-Immuno™
	92 mm dish; polystyrene	Nunclon™Δ
	T75 flasks	Nunclon™Δ
Cell strainer	70 μm	
Combitips® plus	0.5, 1, 5 ml	Eppendorf AG
Coverslip	24x60 mm	Menzel-Glaser
FACS tubes	Microtubes; 1.2 ml Loose NS	Alpha laboratories
Glass slide	50 Elka	Hecht-assistant
Gloves	Aloecare	Meditrade
MACS® columns	25 LS	Miltenyi Biotec
Pipettes	1, 2, 5, 10, 25 ml	Greiner bio-one
Sealing tape		Nunc
Tips	10, 200, 300, 1000 μl	Eppendorf AG
Safelock tubes	0.5, 1.5, 2.0 ml	Eppendorf AG
Syringes	Omnifix®-F (1, 5, 10, 20 ml)	Braun
	Luer Lock (10, 20 ml)	BD
Sterilfilter units	Millex 0.22 μm	Millipore
	Filtropur V50.02	Sarstedt
Tissue embedding cassette		Simport
Tubes for plasma collection	LI 1000 A	KABE
– Lithium heparin		Labortechnik
Tubes	15, 50 ml	Sarstedt

3.8 Software

Table 21: Software

Software		Manufacturer
Ascent Software	Version 2.6	Thermo Labsystems Oy
CellQuest™ Pro	Version 4.0.2	BD Bioscience
Leica IM1000		Leica
Microsoft Office	Professional Edition 2003	Microsoft Corporation, 2003
SigmaPlot for Windows	Version 11.0	Systat Software, Inc.

4 Methods

4.1 General Techniques

4.1.1 Preparation of Particles

Particles (SRM, C-Euro3, Euro3, and CB) were suspended in PBS by repeated vortexing and sonification (10 min) at a concentration of 1 mg/ml. These particles were further dilutet in media and added to cultures to achieve final concentrations in the range of 1 to 100 μg/ml. Because ultrafine particles forms agglomerates in suspension despite vigorous mixing, cells were exposed to agglomerates of particles and not to the single fine particles.

4.1.2 Determination of Endotoxin: LAL Assay

LPS was quantified by Limulus Amebocyte Lysate (LAL) assay according to the manufacturer. It is important to be consistent in the order of reagent addition from well to well or row to row, and in the rate of pipetting.
In brief, plates were pre-equilibrated to 37°C $\pm$ 1°C in a heating block adapter, and then 50 μl of sample or standard were added into the appropriate well. Each series of determinations included a blank (containing 50 μl of LAL Reagent Water) plus the four endotoxin standards run in duplicate. Fifty microliters of LAL were added for 10 min at 37°C, then 100 μl of substrate solution (prewarmed to 37°C $\pm$ 1°C) was added for 6 min, finally 100 μl of stop reagent was added, and the absorbance was measured at 405-410 nm.
Phenanthrene, EPA-PAH, AERex samples, SR1, and SR2 were provided in DMSO.THC was provided in ethanol. OVA V was tested in PBS. Particles were suspended in PBS as described above. A dilution series was tested.

4.2 Cell Culture

BMDC were cultured in R10 medium and B cells were cultured in R10-complete medium. Human MoDC were first cultured in FI medium and than in huR10 medium. All cells were incubated at 37°C in 5% CO_2.

All washing steps were performed by centrifugation at 1,200 rpm for 10 min at 4°C except otherwise stated. After cells were centrifuged, the supernatant was aspirated, and the cell pellet loosened by stroking the tube.

4.2.1 Cell Yield Evaluation

Cells were washed once and an aliquot volume was mixed 1:10 (or 1:2) in 4% trypan blue solution. trypan blue negative cells were counted as viable under the microscope in a Neubauer chamber. All four quadrants were counted and final cell yield calculation was done with the average cell yield of two counts: Cell yield = Average count/4 * Volume * Dilution factor * 10^4

4.2.2 Magnetic Adsorped Cell Sorting (MACS)

The MACS technology with a positive selection strategy was used for enrichment of B220-positive B cells in splenocytes and CD11c-positive dendritic cells in BMDC cultures. Therefore, cells were stained with a PE- or Biotin-conjugated primary antibody and then magnetically labelled with anti-Biotin or anti-PE MicroBeads.
In brief, after the cell number of a single-cell suspension was determined and cells were washed with MACS buffer, 10^7 total cells were resuspended in 40 μl MACS buffer, mixed well and incubated with 10 μg/ml Fc-Block for 10 min at 4-8°C. Then, the respective antibody was added in an appropriate concentraion (see Table 14) and incubated for 30 min at 4-8°C. Cells were washed with MACS buffer by adding 10-20x labelling volume and centrifuged. Then 10^7 total cells were incubated with the respective MicroBeads in MACS buffer and incubated for an additional 15 min at 4-8°C. Cells were again washed with MACS buffer and resuspended in 500 μl MACS buffer per 10^8 total cells. Magnetic separation was performed by applying up to 10^8 magnetically labelled cells per LS column placed in the magnetic field of a VarioMACS. The column was rinsed with 2 ml MACS buffer and then cell suspension was applied. The column was washed with 3x3 ml MACS buffer. The entire effluent, which displays the unwanted cell fraction, was discarded. Retained cells were eluted outside the magnetic field as magnetically labelled (CD11c-positive BMDC or B220-positive B cells). Cell yield of enriched cells was determined as described and purity of cell enrichment was evaluated by direct flow cytometry as described.

4.2.3 Isolation and Cultivation of Murine Primary Cells

Murine Bone Marrow-derived Dendritic Cells (BMDC)

Femurs and tibiae of female, 8–12 weeks old BALB/c mice were removed and purified from the

surrounding muscle tissue. Thereafter, intact bones were left in 70% ethanol for 1 min for disinfection and transferred to R10 medium. Then, both ends were cut with scissors and the marrow flushed with R10 medium using a syringe (G17 needle for femur or G20 needle for tibiae). After one wash in R10 medium, erythrocytes were depleted by using RBC lysis buffer (10 ml per mouse) which was stopped by the addition of an equal amount of R10 medium. Cells were centrifuged and resuspended in R10 medium for determination of cell yield. About 4–5x10^7 leukocytes were obtained per mouse.

The method for differentiation of DC from unfractionated mouse bone marrow (BM) cells in the presence of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) was adapted from previous publications (Ando et al., 2008; Inaba et al., 1992a; Inaba et al., 1992b; Lutz et al., 1999). As source for GM-CSF the culture supernatant from a cell line which secrets murine GM-CSF was used. At day 0, BM cells were seeded at 3x10^6 cells per 92 mm dish in 10 ml R10 medium containing 10% (v/v) GM-CSF supernatant and were cultured at standard conditions for 8 days. At day 3, another 10 ml R10 medium containing GM-CSF supernatant were added to the plates to reach a final concentration of 10% (v/v). At day 6, half of the medium was replaced by 10 ml of fresh R10 medium and GM-CSF supernatant with a final concentration of 10% (v/v) GM-CSF. At day 8, non-adherent immature BMDC (imBMDC) were harvested carefully and for expression of the surface markers by flow cytometry as described. In brief, the DC marker CD11c, co-stimulatory molecules (CD86, CD40, and CD80), and the major histocompability complex II (MHC II) were investigated as decribed. For stimulation experiments, this BMDC cells were either used directly or enriched for CD11c expressing cells using the MACS technology.

Splenocyte Preparation

Spleens were dissected as described. The splenic capsule was squeezed through a cell strainer with a 70 μm nylon mesh into a 50 ml tube to yield a single cell suspension (splenocytes). After centrifugation, 10 ml RBC lysis buffer was added at room temperature and the cells were gently resuspended for 5 min. Cells were washed with R10-complete medium and resuspended with 10 ml R10-complete medium. Cell yield evaluation was performed with trypan blue exclusion as described.

B Cell Isolation

Splenocytes were isolated as described above and were used for isolation of B220-positive B cells by MACS technology as described.

4.2.4 Isolation and Cultivation of Human Primary Cells

Human Monocyte-derived Dendritic Cells (MoDC)

Monocyte-derived dendritic cells were generated from peripheral blood monocytes (PBMC) after isolation of PBMC by Ficoll density gradient centrifugation of heparinised blood from volunteers.

In brief, sterile blood was obtained into heparinised syringes and then mixed with 2-4x the volume of PBS in a 50 ml tube. About 15 ml Ficoll was prepared in a new 50 ml tube and 35 ml blood-PBS-mixture was carefully layered on top. This was centrifuged at 2100 rpm for 20 min with brake off. Resultant layers are approximately from top to bottom: Plasma – platelets - PBMCs – Ficoll – red blood cells (with granulocytes). Most of the plasma was pipetted off, the mononuclear cell layer was carefully aspirated, transferred to a new 50 ml tube, washed twice, and resuspended in 30 ml FI medium. The viability of cells was determined by trypan blue exclusion as described.
PBMC were seeded in 2-4x10^7 cells per T75 bottle in 10 ml FI medium. This was incubated for 1-2 hrs to allow adherence of monocytes. Supernatant was discarded and adherent cells were washed once or twice with 5-10 ml HBSS to remove non-adherent cells. Adherent monocytes were incubated with 10 ml huR10 medium containing recombinant human IL-4 [500 U/ml] and GM-CSF [500 U/ml]. On day 2, 4, and 6, half of the medium was replaced by fresh huR10 containing IL-4 and GM-CSF.
On day 7, immature monocyte-derived dendritic cells (MoDC) were harvested and could be used for further stimulation assays. Cells were subsequently characterised by flow cytometry for expression of surface markers as described. In brief, within the CD1a-positive cells the expression of Fcε RI, CD80, CD83, CD86, HLA-ABC, and HLA-DR was .

4.2.5 Cell Lines: muGM-CSF Clone

The muGM-CSF clone is a cell line which grows in suspension. These cells were cultured in R10 medium at standard cell culture conditions. Exponentially growing cells were used to harvest the supernatant which contains murine GM-CSF. This supernatant was sterilfiltered and stored in aliquots at -20°C till further use.

4.2.6 Stimulation and Exposure of Cells

BMDC

On day 8, BMDC or CD11c-enriched BMDC were used for exposure to different compounds or particles. In brief, cells were plated at 1.5x10^6 cells per well into a 6-well plate in a total of 1.6 ml R10 medium. For complete maturation, LPS at a concentration of 100 ng/ml was added to the cells. Furthermore, cells were exposed to increasing concentrations of different anthropogenic compounds or whole particles for 20 hrs. After 20 hrs (day 9), cell supernatant was harvestet and stored for further analysis of cytokine levels by ELISA as decribed. Cells were collected and assessed for flow cytometric analysis of surface markers as well as viability of cells as described. When CD11c-enriched cells were used for co-culture experiments with B cells, cell yield of each stimulation condition was determined by trypan blue exclusion as described.

B Cells

Naïve B220-positive B cells were cultured either alone or co-cultured with CD11c-enriched BMDC. In brief, 100,000 B cells were plated into each well of a flat 96-well plate in a total of 200 μl R10-complete medium. For induction of class switch recombination, B cells were stimulated with purified anti-CD40 at 5 μg/ml and recombinant mouse IL-4 at 100 ng/ml. In co-culture experiments, 100,000 CD11c-enriched BMDC were added to B cells. After 8 days of co-culture, supernantants were harvested and for immunoglobulin levels by ELISA.

MoDC

For stimulation of MoDC, cells were collected on day 7, counted and plated at 100,000 cells per well into a 96-well plate in a total of 0.2 ml huR10 medium. For complete maturation, LPS at 1 μg/ml or polyIC at 20 μg/ml was added to the cells. Furthermore, cells were exposed to increasing concentrations of phenanthrene, DMSO, or SRM. After 24 hrs (day 8), cell supernatants were harvestet which were further for cytokine levels by ELISA. Cells were collected and assessed for flow cytometric analysis of surface markers and viability of cells.

4.3 Animal Handling

4.3.1 General in vivo Handling

Intraperitoneal injection: I.p. injection was done in the lower left or right quadrant of the abdomen, avoiding the abdominal midline. Injection was done with moderate pressure and speed.

Anesthesia of mice: To anesthetize mice for up to 30 min i.p. injection of a Ketamine/Xylazin mixture was used. One hundred microliters per 10 g bodyweight were admistered. For short term anesthesia of mice, ether was used.

Euthanasia: After anesthesia was induced by usual means, euthanasia was completed by cervical dislocation. As the induction of general anesthesia followed by death without regaining consciousness, this is acceptable. Mice which were used for BAL analysis were given an overdose administration of injectable anesthetic.

Blood collection: Blood collection was done by the puncture of the orbital sinus or plexus of mice. About 200 μl was collected from anesthetized mice in two weeks intervals from alternate

sides. Blood samples were allowed to rest for 1 hr. Afterwards, blood samples were centrifuged for 7 min at 12,000 rpm and supernatant was transferred to a new tube. Serum samples were stored at -20°C for further analysis of serum immunoglobulin levels by ELISA.

Splenectomy: Mice were first anesthetized and then sacrificed by cervical dislocation. Mice were placed on their right sides and the fur was doused with 70% ethanol. Using sterilized surgical scissors, the skin but not the peritoneal wall on the left side of the mouse was cut open. With a fresh pair of sterilized surgical scissors, an incision was cut in the exposed peritoneal wall. The spleen was grasped using sterilized medium forceps and pulled through the incision in the peritoneal wall. Spleens were left in cold PBS or medium till further use.

4.3.2 Sensitization Protocol and Study Design

4.3.2.1 Sensitization Protocol

Mice (8 to 12-week-old females) were sensitized with OVA/alum by the interaperitoneal route four times and challenged by exposure to aerosolized OVA three times every other day using a standard protocol (Figure 9) unless otherwise specified.
In brief, mice were sensitized with either 1 or 10 μg OVA, 2 mg alum in 200 μl PBS on days 0, 7, 14, and 28. Non-sensitized mice received intraperitoneal injection of saline. At the end of the sensitization period, mice underwent allergen challenge via the airways by nebulization of 12 ml 1% OVA in PBS for 20 min in a chamber (14.5x23x22 cm) with not more than 15 mice were in one box. This was performed 3 times every other day. This procedure induces allergic inflammation in the airways which can be evaluated by BAL performed 24 hrs after the last OVA exposure.

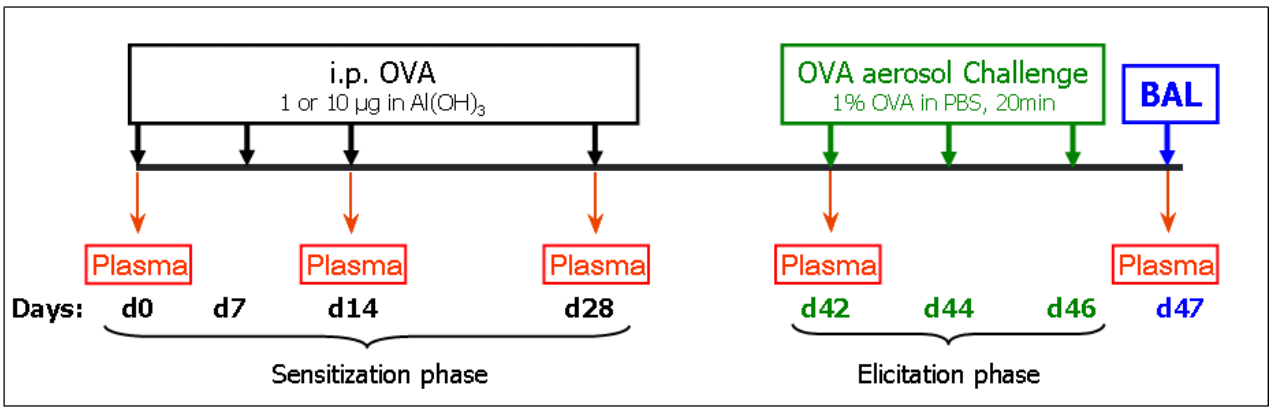


Figure 9: Sensitization protocol

4.3.2.2 Study Design

To analyze the role of cannabinoid receptors on allergic airway inflammation we sensitized $Cnr1^{-/-}$/ $Cnr2^{-/-}$ cannabinoid receptor double-knockout mice (DKO) and C57BL/6 wildtype (WT) mice as inidicated in the basic sensitization scheme shown in Figure 9. Non-sensitized control mice were used in every study to determine baseline levels.
In studies using the cannabinoid receptor antagonists, these were administered i.p. with 1 mg per kg bodyweight 30 min prior to each sensitization in sensitization phase, each challenge in elicitation phase, or in both phases. Antagonists which provided in DMSO were mixed with Chremophor and than with PBS (1:1:98). Control mice were treated with vehicle (VH: 1% DMSO, 1% Cremophor in saline).
To analyze the effect of Δ^9-Tetrahydrocannabinol (THC) on allergic airway inflammation, 50 mg per kg bodyweight THC was administered i.p. daily for three consecutive days before each sensitization in sensitization phase, before each challenge in elicitaion phase, or in both phases. THC provided in ethanol was mixed with Cremophor and then in PBS (1:1:18). Control mice were treated with vehicle (VH: 5% Ethanol, 5% Cremophor in saline).

4.3.3 Bronchoalveolar Lavage (BAL) and Total Cell Count

After euthanization, the chest was opened, the trachea was cannulated, and BAL was performed. In brief, BAL was performed five times with 0.8 ml PBS which was instilled into the lungs. The thorax of the mouse was massaged and the PBS was harvested by gentle aspiration. The BAL fluid (BALF) of the first instillation was collected separately and supernatants were stored after centrifugation at 1,000 rpm for 10 min at −20°C for further analysis of cytokine levels by ELISA. Cells from all five washes were pooled, centrifuged, and resuspended in BAL medium in an appropriate volume (about 2 ml for sensitized animals and 0.5 ml for control groups) for quantification of total cell yield in a 1:2 dilution in trypan blue. If erythrocytes were present in the sample, pelleted cells were treated with RBC lysis buffer, washed, centrifuged, and again resuspenden in BAL medium. BAL cells were further used for cytospin preparation and/or flow cytometric analysis.

4.4 Histology

4.4.1 Cytospins and Differential Cell Count

At least two cytospins were prepared from each sample of the BAL cells. About 50,000 cells were spun onto slides with a cytospin centrifuge in a total volume of 150 μl at 400 rpm for 10 min at an acceleration 'medium'. After slides were dried completely, they were fixed and stained with Diff-Quick according to the manufacturer's instruction. In short, cytospins were fixed for 90 sec in 'Fixative Solution', stained in 'Stain Solution I' for 1 min, then in 'Stain Solution II' for 2 min, and finally rinsed with distilled water. Every staining was controlled for its qualitiy and staining procedure was adapted if required. Slides were dried overnight and mounted in Entelan.
A minimum of 400 leukocytes per sample were counted by using standard morphological criteria. Macrophages, lymphocytes, as well as eosinophil and neutrophil granulocytes can be distinguished. The total number of eosinophils, macrophages, and lymphocytes were calculated as follows: (total number of nucleated cells within airway) /100 x (percentage of cell type within airway). The percentage of cell types found within the BAL fluid was calculated as follows: (number of cell type) x 100 /(total number of cells within cytospin).

4.4.2 Lung Preparation and Sectioning

After BAL, the lung was excised, and divided if required. One lobe was frozen in liquid nitrogen, and the second lobe was fixed for histopathological examination in 4% formalin at 4°C.
Fixed lungs were sectioned sagitally and cut into small pieces. The tissues were dehydrated through increasing concentrations of ethyl alcohol 70%, 80%, 90%, and 100%, cleared in xylene, infiltrated in paraffin by an automatic tissue processor, and embedded in paraffin wax with an embedding machine. Tissue blocks can be stored at room temperature for years. Three- to four-micron sections of the lung tissues were cut from the paraffin blocks with a microtome and were placed onto a glass slide. These slides with paraffin sections were placed in a 65°C oven for 28 min (so the wax just starts to melt) to bond the tissue to the glass. Slides can be stored overnight at room temperature before using for an appropriate staining. The slides containing paraffin sections were placed in a slide holder (glass or metal). First, sections were deparaffinised by leaving slides in xylene (4x2 min). An excess of xylene was removed before going into ethanol. For rehydration descending alcohol series were used: 2x2 min in 100% ethanol, 2x2 min in 96% ethanol, 1x2 min in 70% ethanol, and 1x2 min in deionised H_2O.

4.4.3 Hematoxylin and Eosin: H&E Staining

An excess of water was removed from slides with deparaffined section before going into Mayer's hematoxylin. For staining with hematoxylin, slides were left in hematoxylin solution for 3x2 min, rinsed first with deionised water and then with tap water for 2 min. An excess of water was removed from slide holder before going into eosin. For staining with eosin, slides were left in eosin for 2 min. A dehydration step followed with 3x2 min in 100% ethanol. An excess of ethanol was removed before going into xylene. Slides were cleared in xylene 3x2 min. The slides can be stored in xylene overnight. Afterwards slides were covered with coverslips using mounting medium. Slides were dried overnight.

4.4.4 Periodic Acid Schiff's: PAS Staining

For PAS-Staining, deparaffined tissue section were first left in periodic acid solution for 5 min and then rinsed with distilled water very carefully. Next, slides were placed in Schiff's reagent (at room temperature) for 15 min, washed in warm tap water for 10 min, counterstained in Mayer's hematoxylin for 5 min, and again washed in tap water for 5 min. Slides were then dehydrated in ascending alcohol series (5 min in 70% ethanol, 5 min in 96% ethanol, 5 min in 100% ethanol), cleared with xylene and finally coverslips were mounted onto slides using mounting medium. Slides were dried overnight.

4.5 Immunological Methods

4.5.1 Flow Cytometry (FACS)

Multiple combinations of differently labelled surface markers were used to characterise each cell sample. Unstained cells were used to determine levels of auto fluorescence. When staining with multiple markers with different fluorochromes, each fluorochrome was run individually (with substituted isotype matched antibodies). All samples were assessed in a FACSCalibur™ and the data were with CellQuest™ Pro software.

4.5.1.1 Surface Staining

An appropriate amount of cells of each experimental preparation was transferred to FACS tubes (2x10^5 to 1x10^6 cells per tube), washed in 500 μl FACS buffer, resuspended in 10 μl FACS buffer

containing 1 μg/ml anti-CD16/32, and incubated on ice for 10 min. This antibody binds a common epitope in both CD16 (FcγRIII) and CD32 (FcγRII) and is used to prevent nonspecific binding. For surface staining, cells were labelled for 30 min at 4°C in 10 μl of a flourochrome-, or biotin-conjugated antibody or the appropriate isotype control in an appropriate dilution (see Table 13). About 500 μl FACS buffer was used to wash cells. After staining with biotin-conjugated antibodies, cells were incubated with a flourochrome-labelled streptavidin for another 15 min at 4°C and washed again. Cells were resuspended in 200 μl FACS buffer for analysis. In order to discriminate between living and dead cells, 10 µl of 0.1 μg/ml propidium iodide was added just before analysis.

4.5.1.2 Intracellular Staining of Mouse T Regulatory Cells

The anti-mouse/rat Foxp3 Staining Set from eBioscience was used with slight modifications. In brief, about 1x10^6 prepared splenocytes were added to each FACS tube. Surface molecules were stained as usual (see above) by using surface antibodies CD4-FITC and CD25-Biotin or indicated isotypes from BD Pharmingen. After surface staining, cells were washed twice with 500 μl FACS Buffer, resuspended in 1 ml freshly prepared Fixation/Permiabilization working solution, mixed well, and incubated at 4°C for 30 min to 18 hrs in the dark. Cells were washed twice with 1 ml Permeabilization Buffer and blocked with 1 μg/ml Fc-Block in Permeabilization Buffer at 4°C for 15 min. Without washing 100 μl diluted PE anti-mouse FoxP3 antibody or istotype control diluted in Permeablization Buffer was incubated at 4°C for 30 min in the dark. Cells were washed once with Permeabilization Buffer and resuspended in 200 μl FACS buffer.

4.5.2 Enzyme Linked Immunosorbent Assay (ELISA)

For all ELISA techniques 96-well Micro Well™ Maxi Sorp plates were used. All incubation steps were performed with sealed plates to avoid evaporation. Analyses were performed on a Multiskan Ascent 1.24 with Ascent Software. The optical density (OD) for each well was measured with a microplate reader set to 450 nm. A standard curve was run with each plate.

4.5.2.1 Cytokine ELISA (Murine and Human)

IL-4/-5/-6/-10/-12, IFNγ, and TNF ELISA

For detection of cytokines the BD OptEIA™ Kits were used as listed in Table 16 with slight modifications. In brief, the appropriate purified anti-cytokine capture antibody was diluted either in coating buffer A or B, 50 μl were added per well (100 μl for IL-4) and incubated overnight at 4°C. The plates were washed 3 times with PBS-Tween and 100 μl Assay diluent per well was added for 1-2 hrs to prevent non-specific binding (200 μl for IL-4). Again, plates were washed three washes with PBS-Tween. Fifty microliters of the appropriate standards or samples diluted in Assay diluent were added per well (100 μl for IL-4). After incubation for 2 hrs at room temperature, plates were

washed 5 times with PBS-Tween. The appropriate biotinylated anti-cytokine detection antibody and streptavidin-HRP were diluted as indicated in Assay Diluent and 50 μl were added to each well (100 μl for IL-4). Plates were incubated for 1 hr at room temperature, followed by 8 washes with PBS-Tween. TMB buffer was added with 50 μl per well (100 μl for IL-4), incubated at room temperature in the dark (5-30 min) to allow colour development. Incubation was stopped by adding 25 μl 1 N H_2SO_4 per well (50 μl for IL-4).

IL-13 ELISA

When using the IL-13 kit from Biosource, the detection antibody was directly added to the samples with 25 μl per well. After the plates were washed 5 times, streptavidin-HRP was added with 50 μl per well for 30 min.

4.5.2.2 Immunoglobulin ELISA

Total IgE

ELISA plates were coated with 50 μl coating antibody per well diluted in coating buffer A. After an incubation step of 2 hrs at room temperature or overnight at 4°C, plates were washed three times with TRIS-Tween and blocked with 200 μl TRIS/3%BSA per well for 2 hrs at room temperature. Again, plates were washed three times with TRIS-Tween. Fifty microliters per well of standards or samples diluted in TRIS/3%BSA were added in duplicates and incubated overnight at 4°C. After four washings with TRIS-Tween, 50 μl/well biotinylated detection antibody diluted in TRIS/3%BSA were added, and the plates were incubated for 2 hrs at room temperature. After four washes with TRIS-Tween, plates were incubated for 30 min with 50 μl/well streptavidin-horseradish peroxidise diluted in TRIS/3%BSA. Plates were then washed eight times with TRIS-Tween. Colour development was done by the addition of 50 μl TMB buffer per well and incubation at room temperature in the dark. Colour development was stopped with 25 μl/well 1 N sulfuric acid and absorbance was measured at 450 nm using an ELISA plate reader.

Total IgM

ELISA plates were coated overnight with 50 μl coating antibody per well diluted in PBS. Without washing, plates were directly blocked with 200 μl PBS/1%BSA per well for 1 hr at room temperature and then washed three times with PBS-Tween. Then 50 μl of standards or samples diluted in PBS/1%BSA were added per well in duplicates and incubated for 1 hr at room temperature. After three washes with PBS-Tween, 50 μl/well biotinylated detection antibody diluted in PBS/1%BSA were added, and the plates were incubated for 1 hr at room temperature. After five washes with PBS-Tween, colour development with TMB buffer and sulfuric acid was done as described above.

Total IgG

ELISA plates were coated with 50 μl coating antibody per well diluted in coating-buffer A for 1 hr at room temperature. Plates were washed three times with TRIS-Tween and blocked with 100 μl TRIS/1%BSA per well for 30 min at room temperature. Again, plates were washed three times with TRIS-Tween. Then 50 μl of standards or samples diluted in TRIS/1%BSA were added per well in duplicates and incubated for 1 hr at room temperature. After five washes with TRIS-Washing, 50 μl/well biotinylated detection antibody diluted in TRIS/1%BSA were added, and the plates were incubated for 1 hr at room temperature. After five washes with TRIS-Tween, colour development with TMB buffer and sulfuric acid was done as described above.

OVA-specific IgE, IgG1, and IgG2a

ELISA plates were coated overnight with 50 μl OVA V diluted as described in coating buffer A. After 2 hrs incubation at room temperature or overnight at 4°C, plates were washed three times with TRIS-Tween and blocked with 200 μl TRIS/3%BSA per well for 2 hrs at room temperature. Plates were washed three times with TRIS-Tween. Fifty microlitres of standards or serum samples (dilution series) diluted in TRIS/3%BSA were added per well in duplicates for 2 hrs at room temperature. After four washings with TRIS-Tween, 50 μl/well of the respective biotinylated detection antibody was added in TRIS/3%BSA and plates were incubated for 2 hrs at room temperature. After four washings with TRIS-Tween, plates were incubated for 30 min with 50 μl/well streptavidin-horseradish peroxidise diluted in TRIS/3%BSA. Plates were washed eight times with TRIS-Tween, then colour development with TMB buffer and sulfuric acid was done as described above.

4.6 Data-Analysis and Statistics

Calculation of data was done with Microsoft Excel or SigmaPlot. To compare two independent groups the paired two-tailed student's *t*-test was used.

Data of *in vitro* cell culture experiments are shown as the summarized data of several experiments and expressed as the arithmetic means $\pm$ SEM. For some experiments, the data from different experiments were calculated as the percentage aberration of exposed cells to control cells (equalized to 100%). The raw data values of these experiments are listed in the Appendix. A paired two-tailed student's *t*-test was performed to compare differences of exposed samples to controls. A p-value of $\leq$0.05 was considered statistically significant (*p$<$0.05; **p$<$0.01).

For statistical evaluation of *in vivo* studies the mean $\pm$ SEM was determined for each study group in the individual experiments. A paired two-tailed student's *t*-test was performed to compare study groups with controls. A p-value of $\leq$0.05 was considered statistically significant (*p$<$0.05; **p$<$0.01).

5 Results

5.1 Immunomodulation of Allergic Immune Response by Anthropogenic Factors in vitro

One discussed mechanism which might contribute to the effect of the adjuvant capacity of DEP on allergic immune response is via direct modulation of DC maturation by anthropogenic compounds. We therefore investigated whether anthropogenic compounds directly modulate the phenotype and functional maturation of different cell types such as murine or human DC. We used different particle preparation as well as organic compounds in our *in vitro* system. We analyzed exposed cells for their phenotype. Furthermore, we studied whether the exposure of murine DC results in an altered manner of the DC to interact with other cells of the adaptive immune system such as B cells, thus having an effect on DC function.

5.1.1 Direct Exposure of Murine Bone Marrow-derived Dendritic Cells (BMDC)

BMDC were exposed to both increasing concentrations of particle-associated organic compounds and particles in the absence or presence of LPS. After exposure, cell viability, phenotypic analysis, and cytokine secretion into the cell supernatant of immature BMDC (imBMDC) and mature (mBMDC)was analyzed.

Viability of BMDC

In order to exclude toxic effects of particle-associated organic compounds as well as particles, exposed cells were stained with propidium iodide and assessed for flow cytometric analyses to determine the viability. Figure 10 shows viablecells as percentage of propidium iodide negative cells of total cells. Data is expressed as mean $\pm$ SEM of several experiments. None of the tested compounds used in the experiments decreased the viability of BMDC at the concentration ranges used.

Surface Marker Expression of BMDC

Further, the surface expression of maturation markers was monitored by flow cytometry. Markers such as the co-stimulatory molecules CD40, CD80, and CD86 as well as the expression of the MHC class II molecule I-A^d (MHC II) within the $CD11c^+$ population of immature and mature BMDC were analyzed. Data is shown as the mean $\pm$ SEM of the mean fluorescence intensity (MFI) of the indicated markers. In general, expression of these markers in immature BMDC was

very low. As shown in Figure 11, exposure to any of the particle-associated organic compounds or particles failed to modulate the expression of these markers in medium-treated imBMDC (red line). The ability of imBMDC to respond to engagement of TLR4 was confirmed by stimulation with LPS. As expected, after LPS-stimulation, mBMDC showed a mature phenotype (blue line) with an increased expression of all maturation markers compared to the level of medium-treated imBMDC. To examine the potential modulatory effect of particle-associated orangic compounds or particles on the maturation process, BMDC were co-incubated with LPS and the indicated compounds or particles, respectively. As shown in Figure 11 (blue lines), none of these markers showed altered expression after simultaneous exposure to LPS and the respective substance.

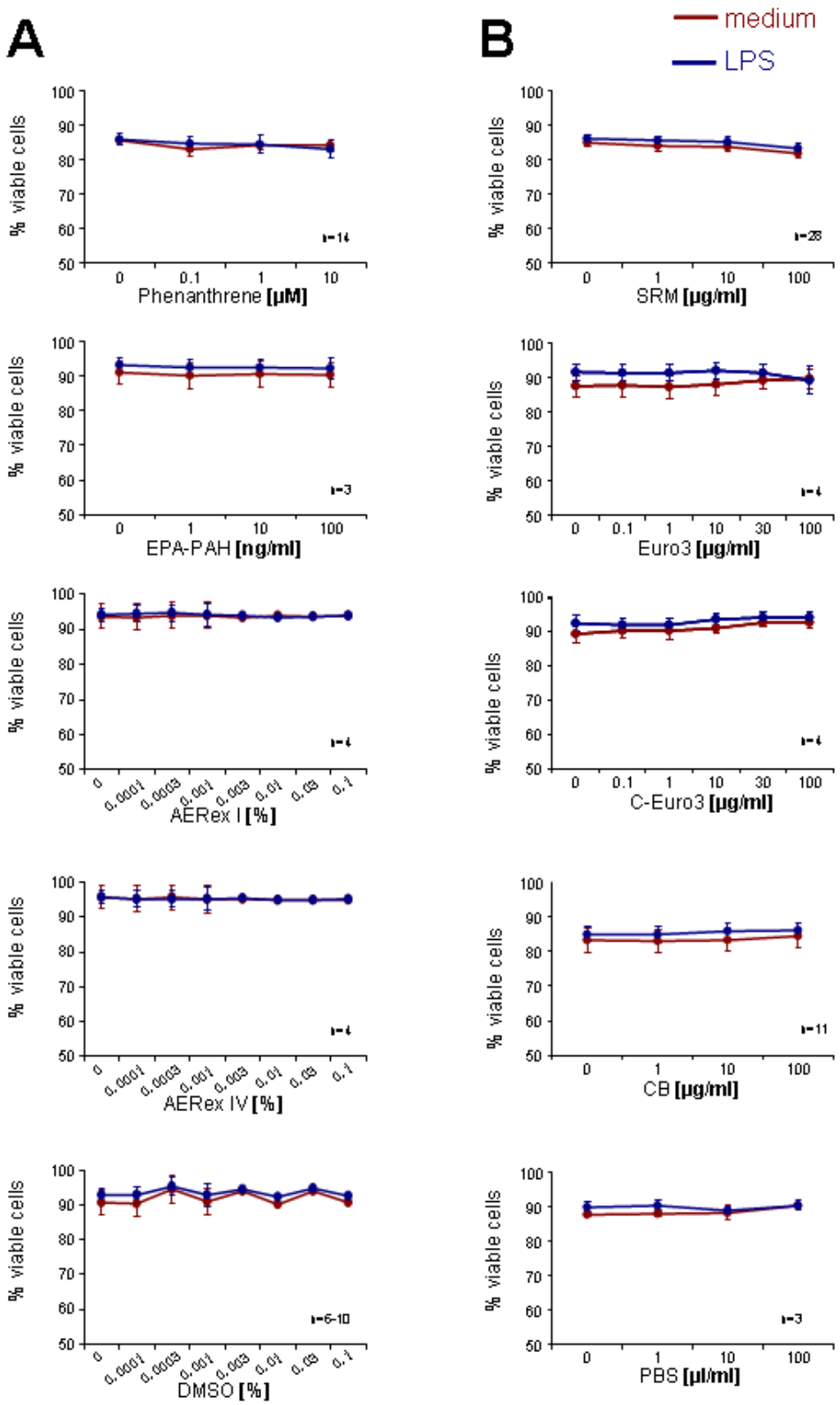
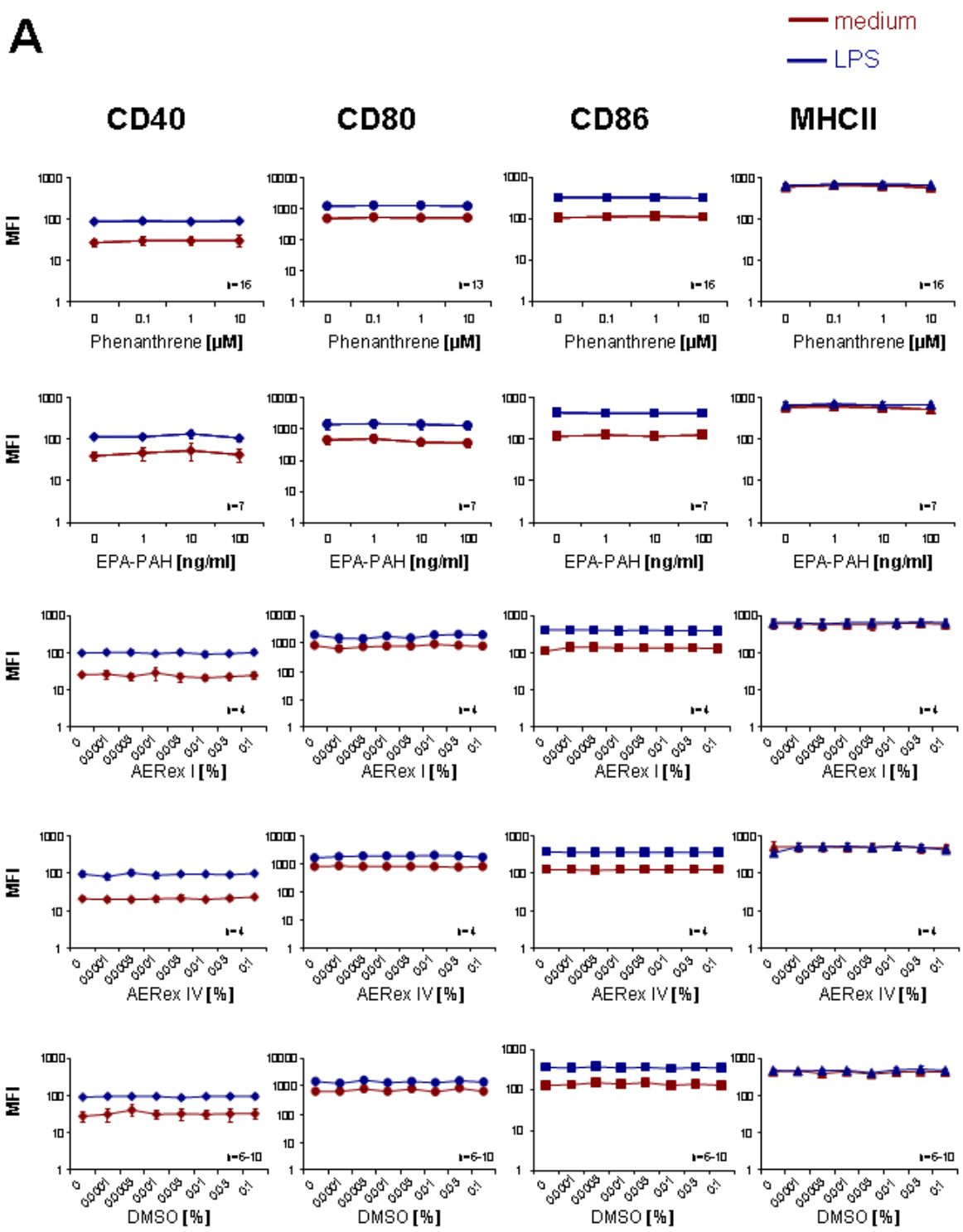


Figure 10: Viability of BMDC exposed to particle-associated compounds or particles

BMDC were exposed to increasing concentrations of **(A)** different particle-associated organic compounds (phenanthrene, EPA-PAH, AERex I, AERex IV, or DMSO as solvent control) or **(B)** particles (SRM, Euro3, C-Euro3, CB, or PBS as solvent control) as indicated. BMDC were left untreated (imBMDC; red line) or LPS-stimulated (mBMDC; blue line). Viability of cells was determined by propidium iodide staining and flow cytometric measurements. No toxic effects of indicated agents were observed. The mean ± SEM of viable cells was calculated as percentage of total cells.


A
medium
LPS
CD40
CD80
CD86
MHCII
MFI
Phenanthrene [µM]
EPA-PAH [ng/ml]
AERex I [%]
AERex IV [%]
DMSO [%]

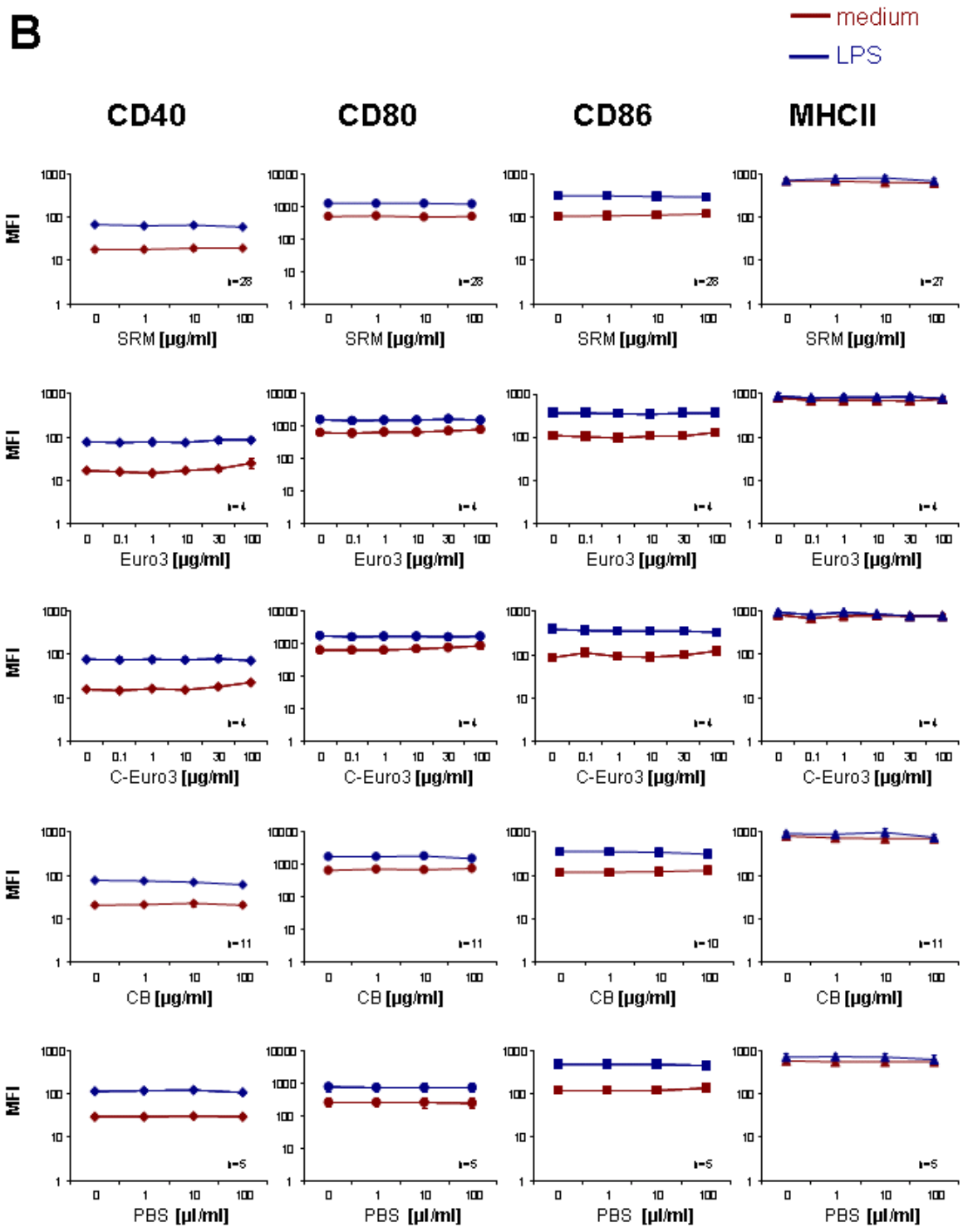


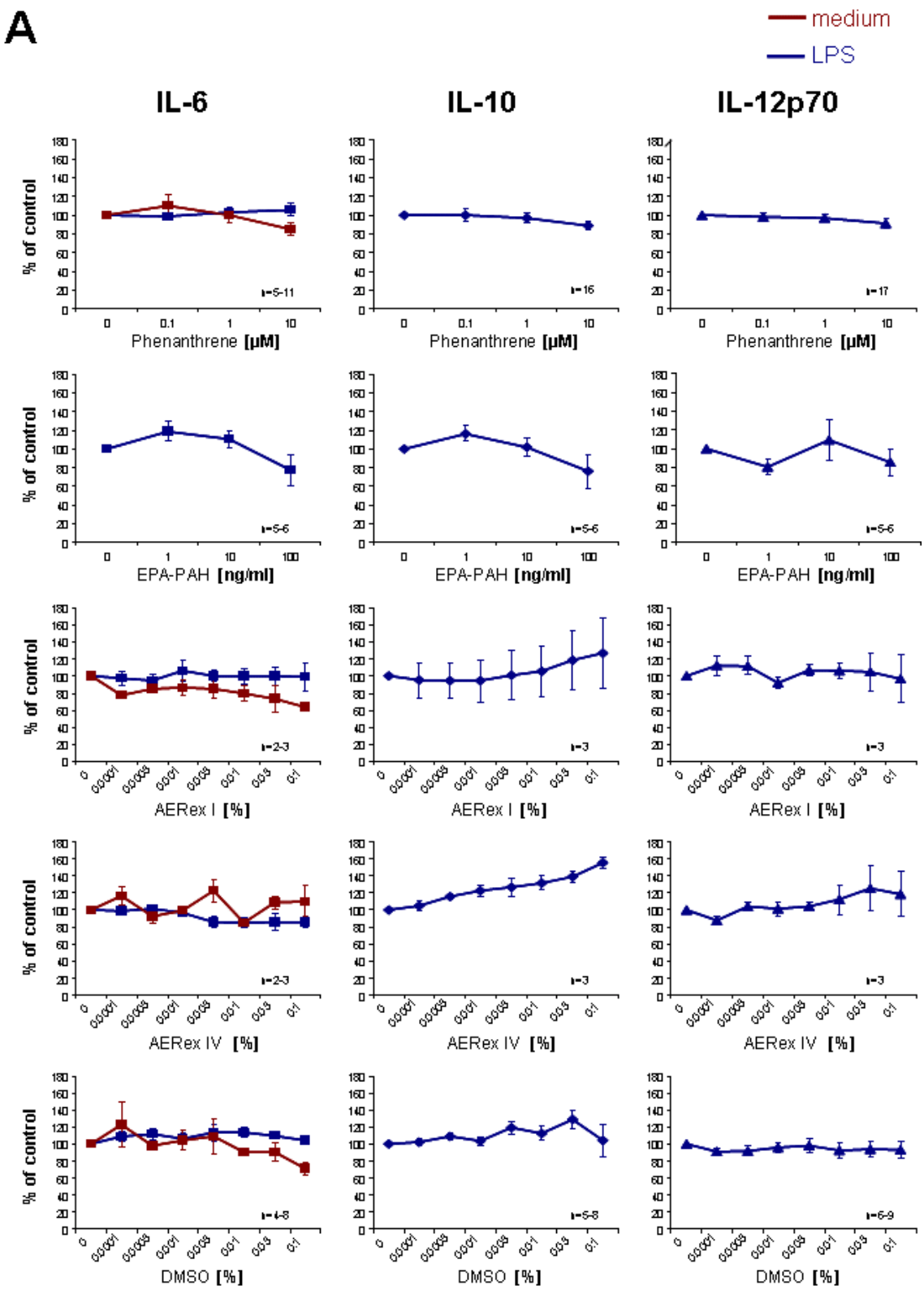
Figure 11: Surface marker expression on BMDC exposed to particle-associated organic compounds or particles

BMDC were treated with increasing concentrations of **(A)** different particle-associated organic compounds (phenanthrene, EPA-PAH, AERex I, AERex IV, or DMSO as solvent control) or **(B)** particles (SRM, CB, Euro3, C-Euro3, or PBS as solvent control) as described. Exposure was performed on untreated immature BMDC (imBMDC; red line) or LPS-stimulated mature BMDC (mBMDC; blue line). Phenotypic maturation of BMDC was evaluated by flow cytometric analysis of cell surface markers CD40, CD80, CD86, and MHCII within the CD11c-positive population. No significant changes after exposure were detectable. Expression of surface markers was determined as the mean fluorescence intensity (MFI). The mean ± SEM is shown.

Cytokine Secretion of BMDC

Furthermore, cell supernatants of BMDC exposed to particle-associated organic compounds or particles were used to measure cytokine levels for IL-6, IL-10, and IL-12p70. Figure 12 shows the mean ± SEM of the percentage of cytokine secretion compared to non-exposed control BMDC (medium-treated or LPS-stimulated). The range of each cytokine in the culture supernatants is shown as mean ± SEM in Table 1 in [pg/ml].

Culture supernatants of medium-treated imBMDC did not contain measurable amounts of IL-10 and IL-12p70. Only IL-6 was detectable in these supernatants (Figure 12, red line). Supernatants from LPS-treated mBMDC cultures contained measurable amounts of all three cytokines mentioned above (Figure 12, blue line).
IL-6 secretion of BMDC with or without stimulation of LPS was not affected significantly by any of the particle-associated organic compounds at indicated concentrations (Figure 12A). Within medium-treated imBMDC, high concentrations of particles (SRM, Euro3, C-Euro3, or CB) led to an increase in IL-6 secretion (Figure 12B). Further, particle-associated organic compounds also failed to exert an effect on LPS-induced IL-10 and IL-12p70 cytokine secretion (Figure 12A). In contrast, after SRM-exposure, LPS-induced IL-12p70 cytokine secretion was significantly decreased in a dose-dependent way. Further, IL-6 secretion was reduced, also not dose-dependently. However, other particles than SRM did not exert any alteration of cytokine secretion (Figure 12B).


A
medium
LPS
IL-6
IL-10
IL-12p70
% of control
Phenanthrene [µM]
EPA-PAH [ng/ml]
AERex I [%]
AERex IV [%]
DMSO [%]

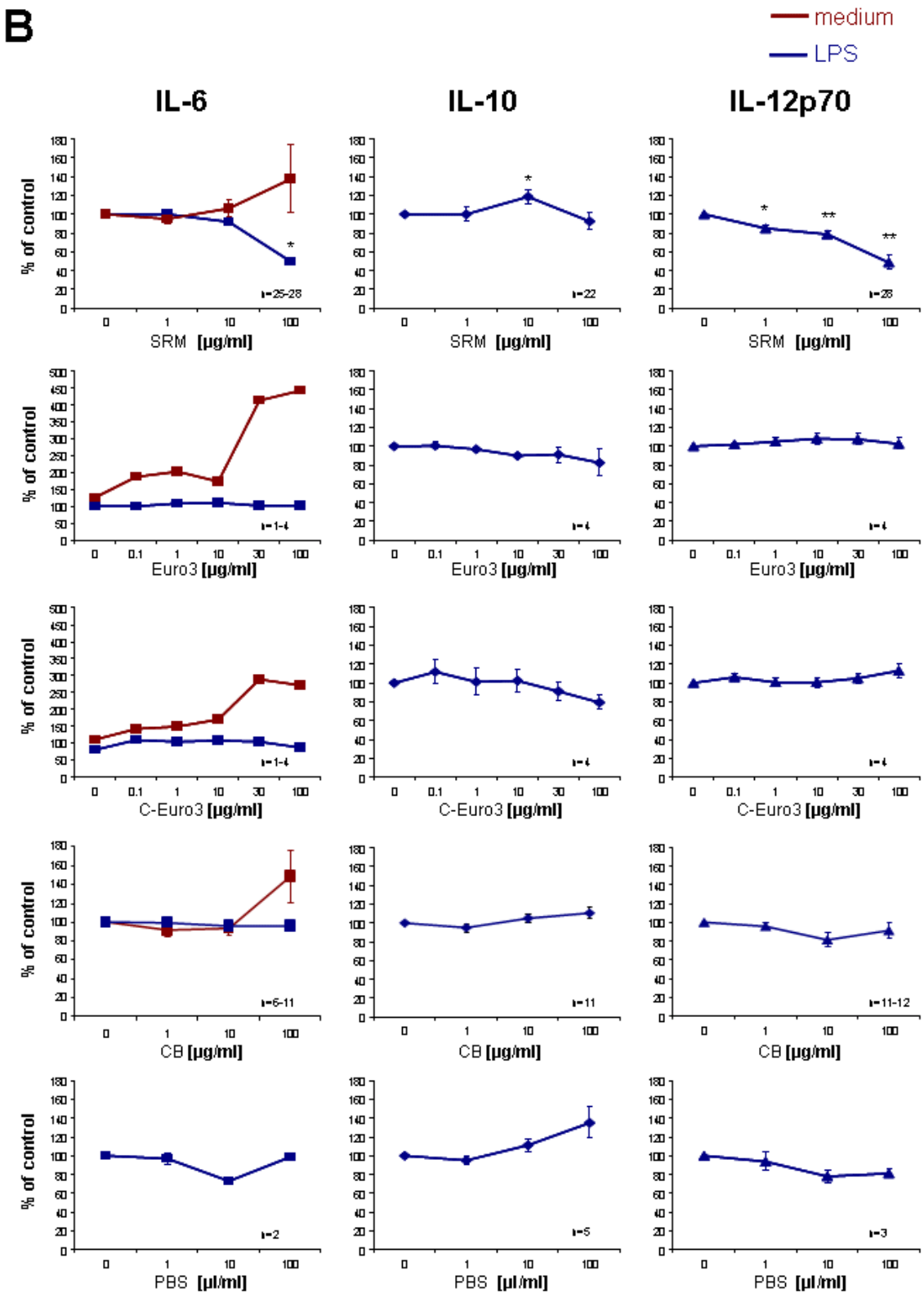


Figure 12: Cytokine secretion of BMDC exposed to particle-associated organic compounds or particles
BMDC were treated with increasing concentrations of **(A)** different particle-associated organic compounds (phenanthrene, EPA-PAH, AERex I, AERex IV, or DMSO as solvent control) or **(B)** particles (SRM, Euro3, C-Euro3, CB, or PBS as solvent control) as described. Exposure was performed on medium-treated imBMDC (red line) or LPS-treated mBMDC (blue line). Cytokine secretion of IL-6, IL-10, and IL-12p70 into cell culture supernatant was evaluated by ELISA. A significant and dose-dependent reduction of LPS-induced IL-12p70 was seen in SRM-exposed cells, and a reduction in LPS-induced IL-6 for the highest concentration. The mean percentage of medium- or LPS-induced cytokine production ± SEM is shown. Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to control BMDC.

5.1.2 Effects of SRM-exposed BMDC on IgE-Switching in B Cells

B cells were obtained from splenocyte preparation from BALB/c mice by sorting with the MACS technology as described. Obtained cells were >95% $B220^+$ (data not shown).
For co-culture experiments, BMDC were enriched for CD11c expression by MACS technology. Purity of enriched $CD11c^+$ BMDC was up to 95% (data not shown) as described. Medium-treated imBMDC or LPS-stimuluated mBMDC were used for co-culture experiments with B cells.

CSR to IgE in B Cells and Suppression by BMDC

Under basal conditions, cultured $B220^+$ B cells did not proliferate or secrete any IgE after 8 days of culture (data not shown). Class switch recombination (CSR) could be induced by stimulating $B220^+$ B cells with anti-CD40 and IL-4. In this setting IgE, IgM, and IgG secretion of B cells into the cell culture supernatant was induced (Figure 13. Absolute immunoglobulin levels of B cell cultures are listed in Table 24 as mean ± SEM in [ng/ml].

We employed a co-culture system with $B220^+$ B cells and CD11c-enriched BMDC to analyze the effects of BMDC on B cell CSR. Anti-CD40/IL-4-stimulated B cells were co-cultured with either imBMDC or mBMDC. Figure 13 shows the immunoglobulin secretion by anti-CD40/IL-4-stimulated and co-cultured B cells as mean ± SEM as percentage of immunoglobulin secretion compared to only stimulated control B cells. BMDC suppressed IgE secretion in B cells which was only significant for imBMDC (see Figure 13). Additionally, the secretion of IgM was significantly decreased, whereas the IgG-secretion was strongly increased when B cells were co-cultured with imBMDC as well as mBMDC. mBMDC showed a stronger effect in the upregulation of IgG secretion than imBMDCs. These results show that BMDC, immature as well as mature, can directly affect immunoglobulin subset production by B cells.

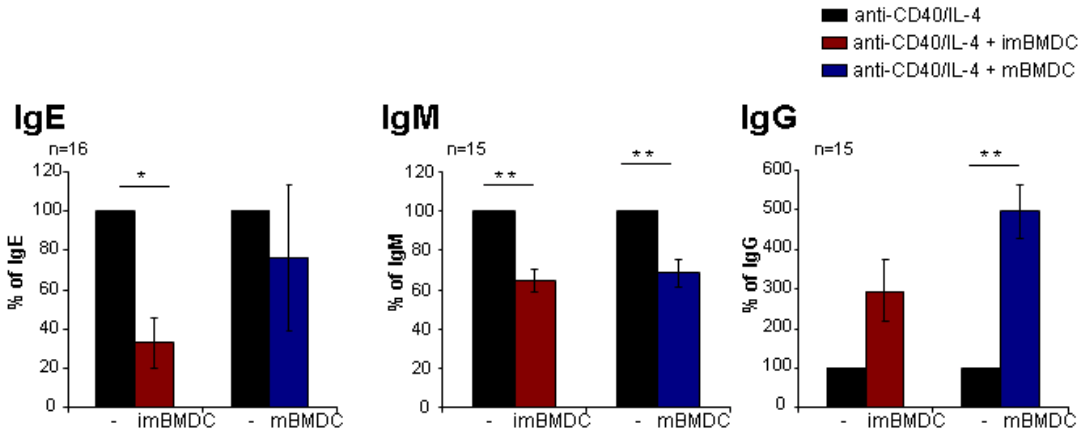


Figure 13: Effects of BMDC on CSR in B cells
B cells were either only anti-CD40/IL-4 stimulated (black bars) or additionally co-cultured with CD11c-enriched imBMDC (red bars) or mBMDC (blue bars) for 8 days. Immunoglobulin secretion into cell supernatants was measured by ELISA. Immature as well as mature BMDC were able to suppress the induced IgE-switching in B cells. Data are presented as percentage (mean ± SEM) of control immunoglobulin secretion by only stimulated B cells. Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to stimulated control B cells.

SRM-Exposure Disables BMDC to Suppress CSR to IgE in B Cells

We further investigated the effect of SRM-exposed BMDC on IgE-switching in B cells. To address this question, we co-cultured B cells with SRM-exposed imBMDC or mBMDC. Figure 14 shows the percentage of immunoglobulin secretion of B cells co-cultured with BMDC pre-exposed to increasing concentration of SRM compared to control B cells stimulated with anti-CD40/IL-4. Absolute immunoglobulin levels of B cell co-cultures are listed in Table 14 as means ± SEM in [ng/ml].

As can be seen in Figure 14A, levels of IgE in cell culture supernatants strongly increased in co-cultures when imBMDC were exposed to increasing concentration of SRM. This indicates that imBMDC exposed to SRM lost their ability to suppress IgE isotype switching in B cell in a dose-dependent manner. Additionally, IgM secretion increased in co-cultures with imBMDC exposed to SRM dose-dependently. Although suppression of IgE-switching and hence IgE secretion was much more reversed than IgM secretion, none did reach control levels. In contrast IgG secretion was not altered when imBMDC were exposed to SRM.

Unlike to the effect of SRM-exposed imBMDC, SRM-exposed mBMDC were not able to reverse the suppression of IgE-switching in B cells when exposed to SRM (Figure 14B). IgM secretion was not altered in co-culture with SRM-exposed mBMDC. Moreover, SRM-exposed mBMDC down-regulated the IgG secretion of B cells which was induced by co-culturing stimulated B cells with mBMDC. This down-regulation was dose-dependent and significant for all SRM concentrations used.

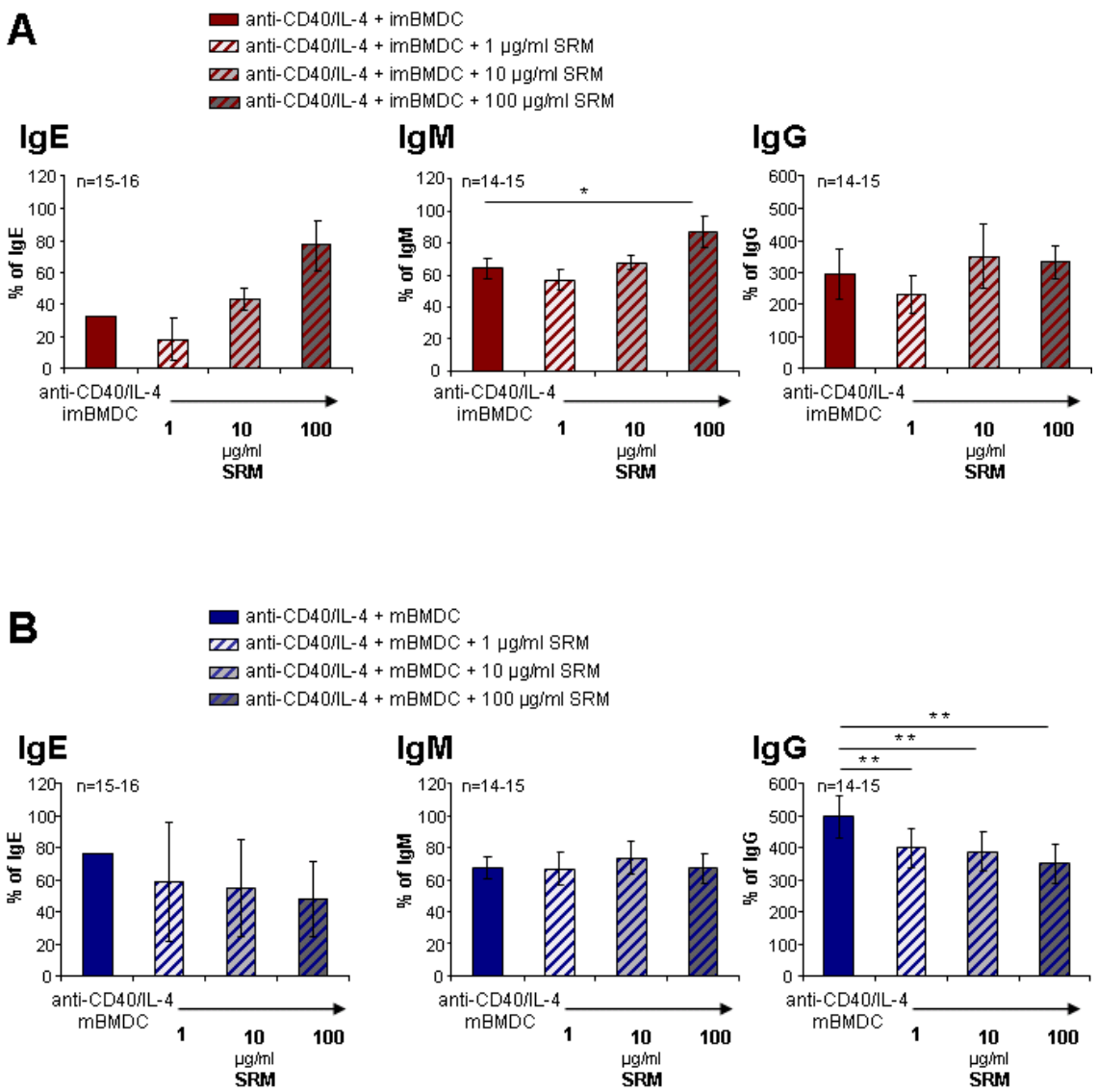


Figure 14: Effects of SRM-exposed BMDC on immunoglobulin secretion in B cells

B cells were anti-CD40/IL-4 stimulated and co-cultured for 8 days with **(A)** imBMDC (red bars) or **(B)** mBMDC (blue bars) pre-exposed to increasing concentrations of SRM. Immunoglobulin secretion into cell supernatants was measured by ELISA. ImBMDC pre-exposed to SRM were able to reverse the suppression of IgE-switching, although not significantly. Data are presented as percentage (mean ± SEM) of control immunoglobulin production by stimulated B cells. Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to stimulated control B cells.

5.1.3 Direct Exposure of Human Monocyte-derived Dendritic Cells (MoDC)

We also examined the potential adjuvant effect of phenanthrene and the highly polluted diesel particle sample SRM on human monocyte-derived dendritic cells (MoDC). Immature MoDC of different donors were exposed to various concentrations of these agents in the absence or presence of LPS or poly(I:C) as described. After exposure, cell viability, phenotypic analysis, and cytokine secretion into cell supernatant was analyzed.

Viability of MoDC

Viability of MoDC after exposue to phenanthrene or SRM was determined by propidium iodide staining and flow cytometric analyses to exclude toxic effects. In Figure 15 viability of MoDC exposed to either phenanthrene or SRM is shown as percentage of viable cells of total cells (means ± SEM). In the concentration ranges used, neither phenanthrene nor SRM did decrease the viability of MoDC. Further, the solvent DMSO did not induce toxic effect leading to decreased viability of MoDC (data not shown).

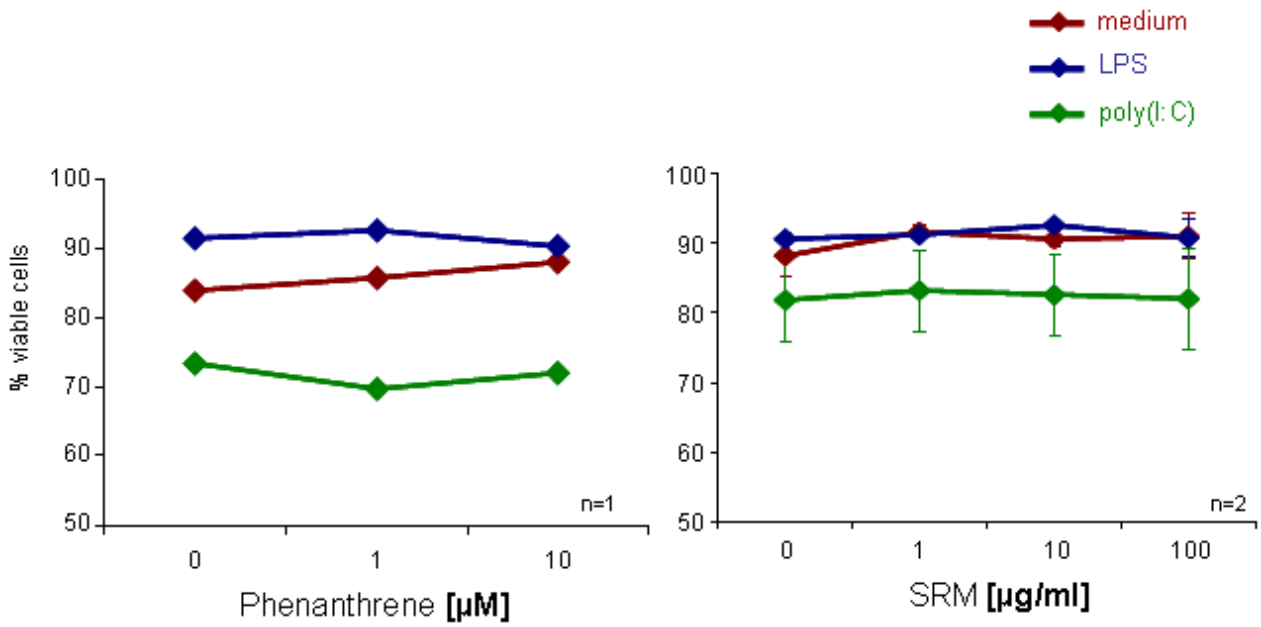
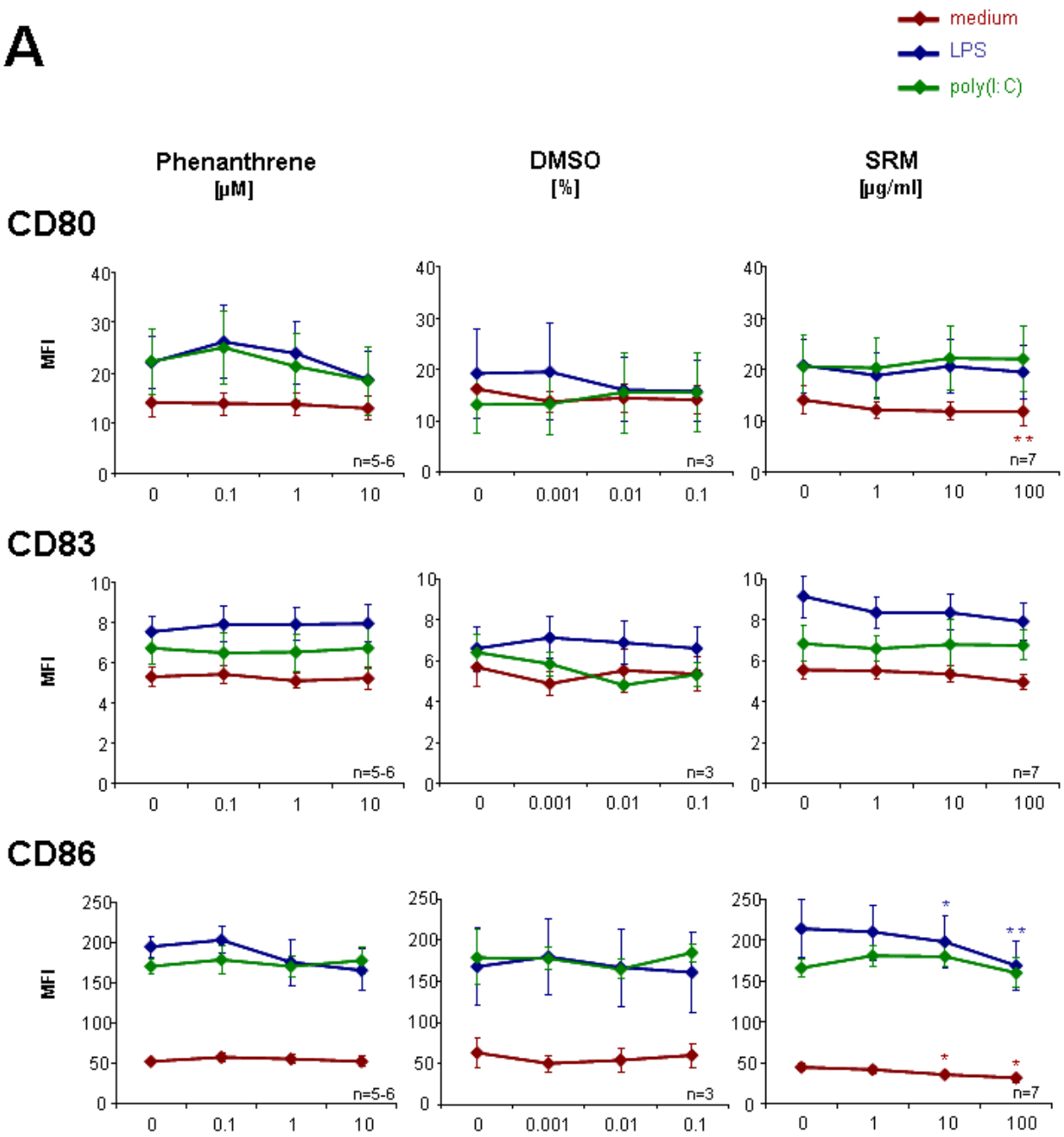


Figure 15: Viability of MoDC exposed to phenanthrene or SRM
MoDC of different donors were medium-treated (medium; red line) or stimulated with LPS (blue line) or poly(I:C) (green line) for 24 hrs and exposed to phenanthrene or SRM as indicated. Viability of cells was determined by propidium iodide staining and flow cytometric measurements. No toxic effects of indicated agents were observed. The mean ± SEM of propidium iodid negative cells was calculated as percentage of total cells.

Surface Marker Expression of MoDC

The expression ofmaturation markers CD80, CD83, CD86, as well as Fcε RI, HLA-ABC, and HLA-DR on human MoDC were examined using flow cytometry (see Figure 16). Data is shown as mean ± SEM of mean fluorescence intensity (MFI). The expression level of medium-treated MoDC (red line) and the level of up-regulation by stimulation with LPS (blue line) or poly(I:C) (green line) depended on the donor. In general, LPS or poly(I:C) stimulation up-regulated the levels of the three DC maturation markers (Figure 15A), whereas Fcε RI, HLA-ABC, and HLA-DR (Figure 16B) were influenced differently.



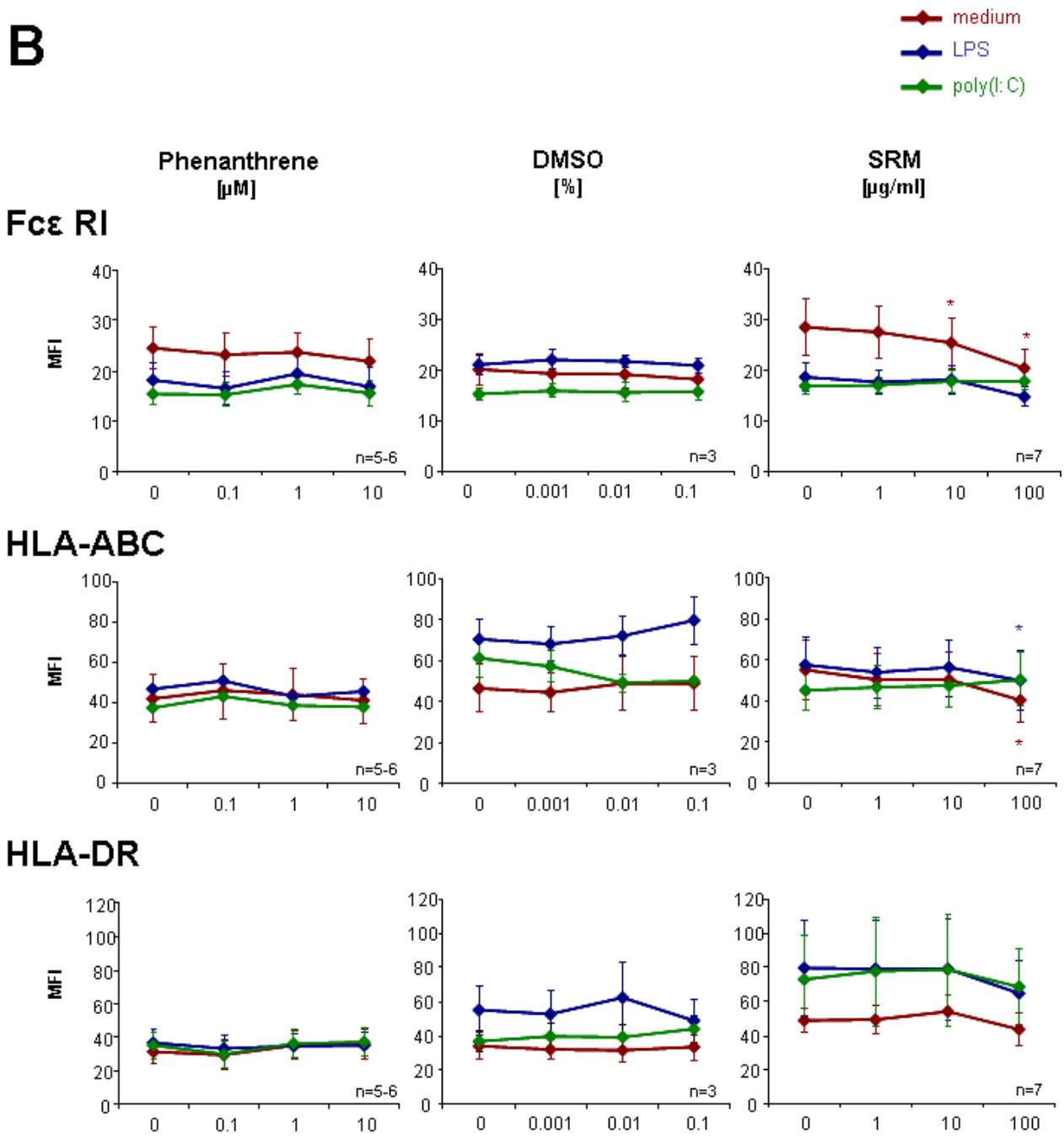


Figure 16: Surface marker expression on MoDC exposed to phenanthrene or SRM

MoDC of different donors were stimulated with LPS (blue line) or poly(I:C) (green line) or left untreated (medium; red line) for 24 hrs. Additionally, cells were exposed to phenanthrene, DMSO, or SRM as indicated. Cells were recovered and stained with mAbs against **(A)** CD80, CD83, CD86 and **(B)** Fcε RI, HLA-ABC, HLA-DR. Marker expression was analyzed by flow cytometry. Only exposure of SRM was able to down regulate some of these markers. Data are expressed as mean fluorescence intensity (MFI) ± SEM. Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to non-exposed control MoDC.

As shown in the first two lanes of Figure16, none of the markers yielded altered expression after exposure of MoDC to phenanthrene or the solvent DMSO alone. The expression profiles of SRM-exposed MoDC are shown in the last lane. SRM-exposure of medium-treated MoDC down-regulated the expression of the maturation markers CD80 and CD83, and the expression of Fcε RI and HLA-ABC, which was statistically significant only for 10 and/or 100 μg/ml. The co-exposure of SRM and LPS led to a significant down-regulation only in CD86 and HLA-ABC compared to only LPS-stimulated MoDC. CD86 expression was down-regulated in a dose-dependent way, but expression of HLA-ABC was only affected at the highest SRM concentration. Simultaneous exposure of SRM and poly(I:C) did not significantly suppress the expression of any of these markers.

Cytokine Secretion of MoDC

The cytokine secretion by MoDC after 24 hrs of culture was examined by ELISA. We measured the cytokine secretion by MoDC incubated to medium (red line) or additionally stimulated with LPS (blue line) or poly(I:C) (green line). Figure 17 shows the percentage of cytokine secretion compared to non-exposed control MoDC (medium-, LPS-, or poly(I:C)-induced). The range of each cytokine in the culture supernatants is shown mean $\pm$ SEM in Table 26 [pg/ml].
Culture supernatants of medium-incubated MoDC without stimulation mostly did not contain measurable amounts of cytokines. Stimulation with LPS or poly(I:C) however increased the secretion of these cytokines.
Phenanthrene and the solvent control DMSO did not change the secretion of any of these cytokines in a significant or dose-dependent way. IL-12p70 production was decreased by SRM when MoDC were stimulated with LPS or poly(I:C) in a dose-dependent fashion. The suppression of LPS-induced IL-12p70 secretion was significant for the two highest SRM concentrations, whereas the poly(I:C)-induced IL-12p70 secretion was only significantly suppressed in the highest SRM concentration . Under the same conditions, IL-6, IL-10, and TNF secretion followed a similar trend, but these levels were not significantly different compared to control levels.

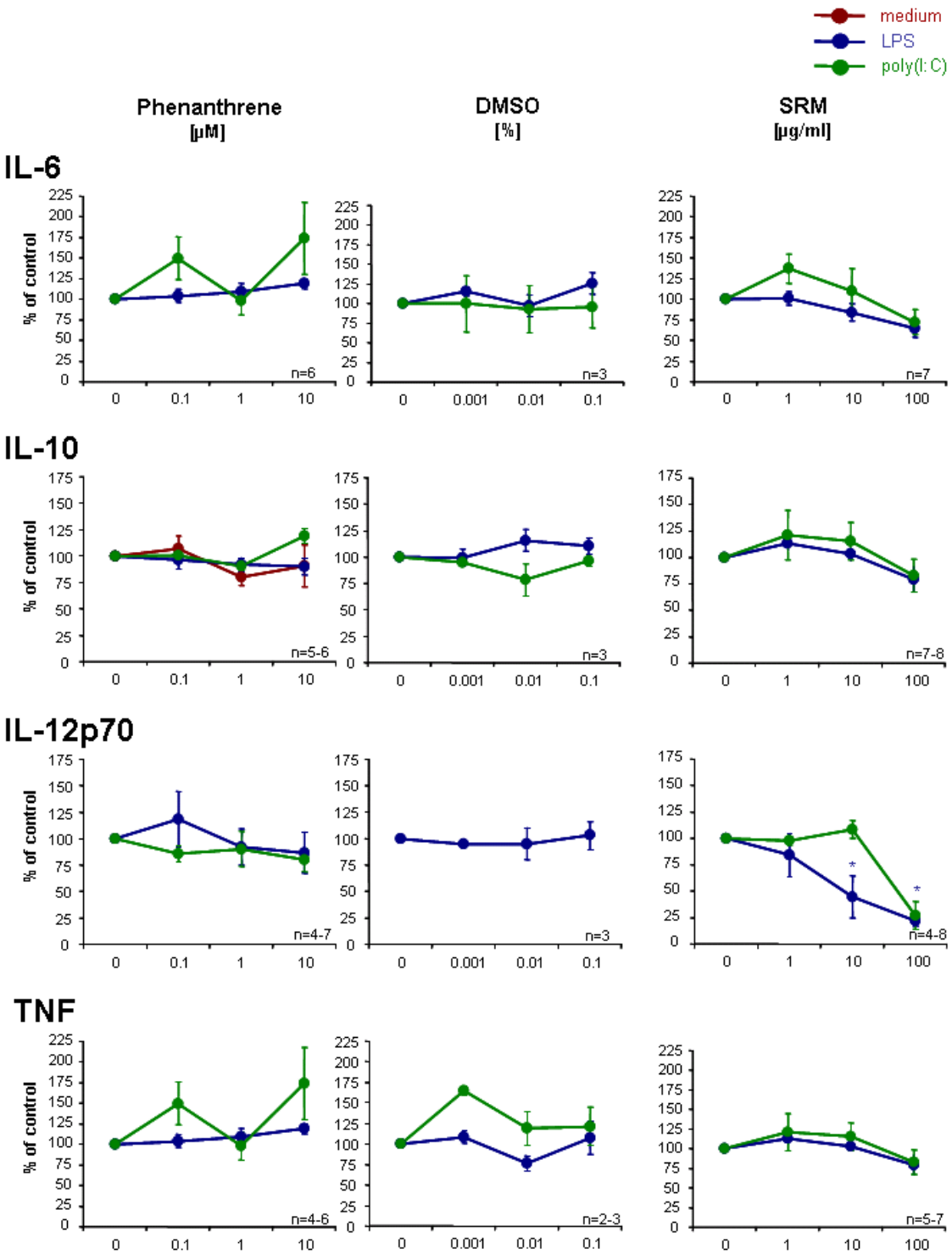


Figure 17: Cytokine levels of MoDC exposed to phenanthrene or SRM

MoDC of different donors were medium-treated (medium; red line), stimulated with LPS (blue line) or poly(I:C) (green line) for 24 hrs. Cells were additionally exposed to phenanthrene, DMSO, or SRM as indicated. Culture supernatants were recovered and cytokine secretion was measured by ELISA. A significant and dose-dependent decrease in IL-12p70 secretion was only detected after cells were exposed to SRM. The mean percentage of medium-, LPS-, or poly(I:C)-induced secretion ± SEM is shown. Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to non-exposed control cells.

5.2 Role of the Endocannabinoid System in a Murine Model of Allergic Airway Inflammation

To study the role of the endocannabinoid system in the development of allergic airway diseases, we used C57BL/6 wildtype mice (WT) or Cnr1$^{-/-}$/Cnr2$^{-/-}$ cannabinoid receptor double knock-out mice (DKO) in the standard protocol. Two main issues were investigated: The role of 1) the impairment of the ECS and 2) the activation of the ECS in the development of allergic airway inflammation.

5.2.1 Impairment of the ECS

The main issue when analyzing the role of the impairment of the ECS on allergic airway inflammation was to study the impairment of the CB receptor. Two approaches were used two achieve this. First, DKO and WT mice were OVA-sensitized and OVA-challenged according to the basic sensitization scheme to analyze the role of a total knock-out of both cannabinoid receptors over the whole time of the experiment. Second, pharmacological blockade of CB1 and/or CB2 receptors was used to study the involvment of the single receptors during the different phases of the esperiment.

5.2.1.1 Mice Lacking Cannabinoid Receptors: DKO mice

In this experiment, DKO and WT mice were sensitized with 1 μg ovalbumin according to the basic sensitization scheme and the allergic airway inflammation was analyzed.

Cells in Bronchoalveolar Lavage (BAL) Fluid

One day after the last challenge, mice were sacrificed, the airways were lavaged, and BAL cells were analyzed. Total and differential cell counts for numbers of macrophages, neutrophil and eosinophil granulocytes as well as lymphocytes in BAL fluid (BALF) from non-sensitized and OVA-sensitized (1 μg) WT and DKO mice after OVA-aerosol challenge are shown in Figure 18, respectively. Data is shown as the mean $\pm$ SEM from one experiment with 4 to 6 mice per study group.
OVA-sensitized mice showed a significantly increased total cell number compared to non-sensitized mice (see Figure 18A) indicating that antigen challenge caused a strong airway inflammation in the lung. Interestingly, the total cell number in OVA-sensitized DKO mice was higher than in WT mice, although not significant. The percentage aberration in total cell counts compared to control group and the p-value is shown in Table 27.
Absolute numbers of differential cell counts are shown in Figure 18B. The increase in total cell counts in OVA-sensitized mice was reflected in the cellular composition of the BAL fluid. Compared with non-sensitized animals, allergen challenge in OVA-sensitized animals led to an increase in almost all leukocyte populations especially in eosinophils. The higher total cell count in OVA-sensitized DKO mice compared to OVA-sensitized WT was also reflected by higher cell counts in

all leukocyte subsets.

The percentage distributions of differential cell counts within different study groups are shown in Figure 18C. BAL cells from non-sensitized mice were mainly macrophages with a very low percentage of neutrophils, eosinophils, and lymphocytes. In OVA-sensitized mice the fraction of macrophages decreased and the fraction of eosinophils and neutrophils increased, while the population of lymphocytes was not changed. However, the percentage distribution of differential cell counts from OVA-sensitized DKO mice was not different from WT mice.

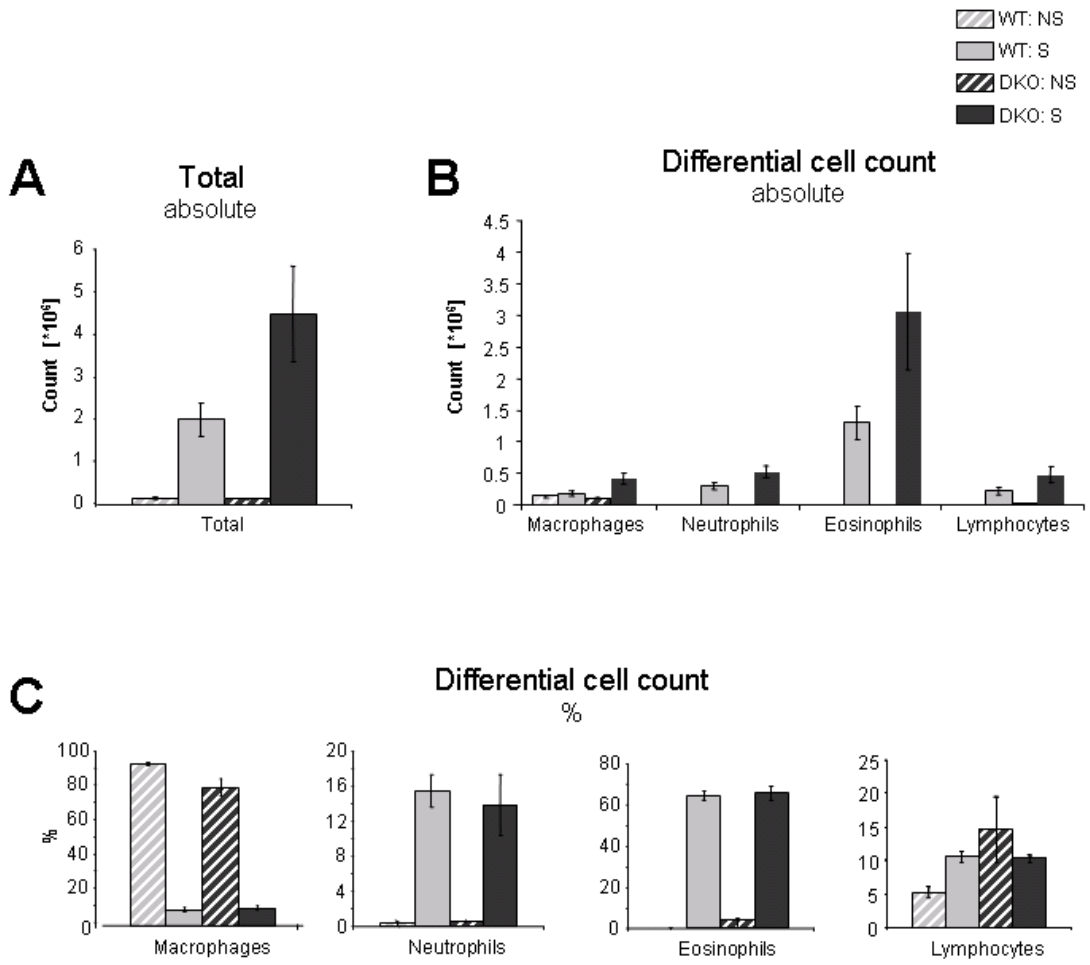


Figure 18: BAL cell counts in DKO mice

Non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars) and OVA-sensitized (1 μg) WT (WT:S, grey bars) and DKO mice (DKO:S, black bars) were evaluated 24 hrs after OVA-aerosol challenge for total and differential cell counts as described. Absolute numbers of **(A)** total cell count and **(B)** differential cell count and **(C)** the percentage distribution of differential cell count was calculated. OVA-sensitized DKO mice showed slightly higher total cell counts compared to WT mice, but a similar cell pattern. Results are expressed as mean ± SEM (n=4-6/ group).

Histology of Lung Inflammation

Inflammatory cell recruitment into the lungs of non-sensitized or OVA-sensitized and challenged mice was further studied by histological analysis. Representative lung sections which were stained with hematoxylin–eosin (H&E) are shown in Figure 19. Lungs were considered inflamed if eosinophilic and lymphocytic infiltration around bronchioles and vessels, as well as goblet-cell hyperplasia and smooth muscle thickening could be detected in H&E stined tissues. In non-sensitized and OVA-challenged mice, the pulmonary histology was normal demonstrating no inflammation at all or just minimal inflammation foci. OVA-sensitized WT and DKO mice challenged with OVA showed an increased lung inflammation with peribronchial infiltrates of inflammatory cells which consisted of eosinophils and mononuclear cells. Moreover, thickening of the airway epithelia was seen in OVA-sensitized mice. OVA-sensitized DKO mice showed a slightly higher OVA-induced inflammatory cell influx and thickening of the airway epithelia than OVA-sensitized WT mice.

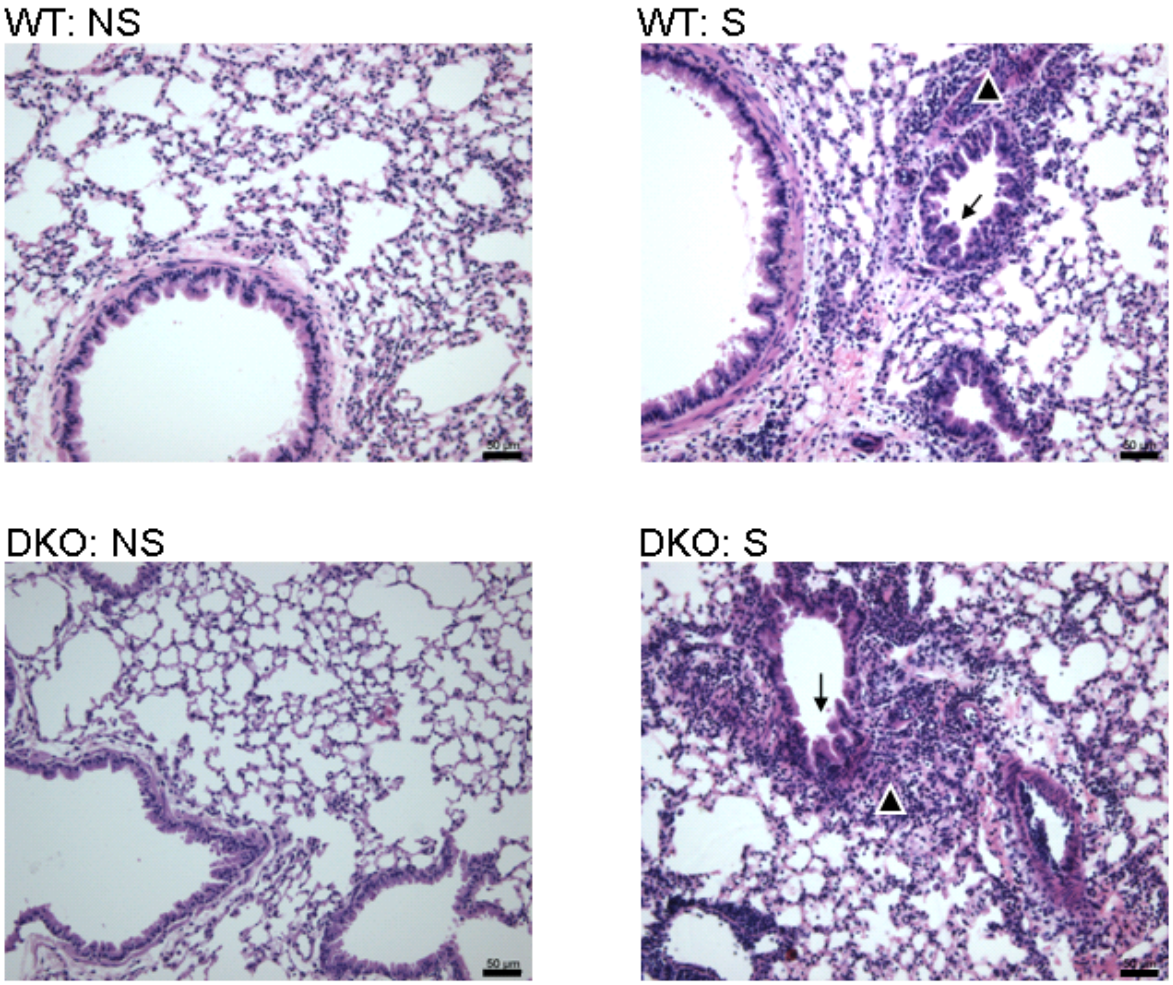


Figure 19: Hematoxylin-eosin (H&E) staining of lung samples of DKO mice
Representative lung sections from non-sensitized (NS) or OVA-sensitized (S) WT and DKO mice were stained with hematoxylin–eosin (H&E) to determine peribronchial inflammation. Tissue was examined by light microscopy. Inflammatory infiltrate and mucous cell hyperplasia are indicated by arrowheads and arrows, respectively. Histopathologic analysis of lung tissue showed lung inflammation in OVA-sensitized mice with slightly higher peribronchial cell infiltrates in DKO mice. Original magnification 20x; scale bar, 50 μm.

Mucus overproduction and secretion into bronchiolar lumen was detected by staining with periodic acid Schiff (PAS). Histological evaluation of PAS stained lung samples from non-sensitized WT and DKO mice showed no mucous production at all. In contrast, OVA-sensitized WT and DKO mice showed inflammatory infiltrate and mucus hypersecretion at 24 hrs after the last OVA-aerosol challenge as shown in a representative sample which was counterstained with hematoxylin (see Figure 20). Again, OVA-sensitized DKO mice exhibited slightly more PAS-positive mucus-containing epithelial cells than OVA-sensitized WT mice.

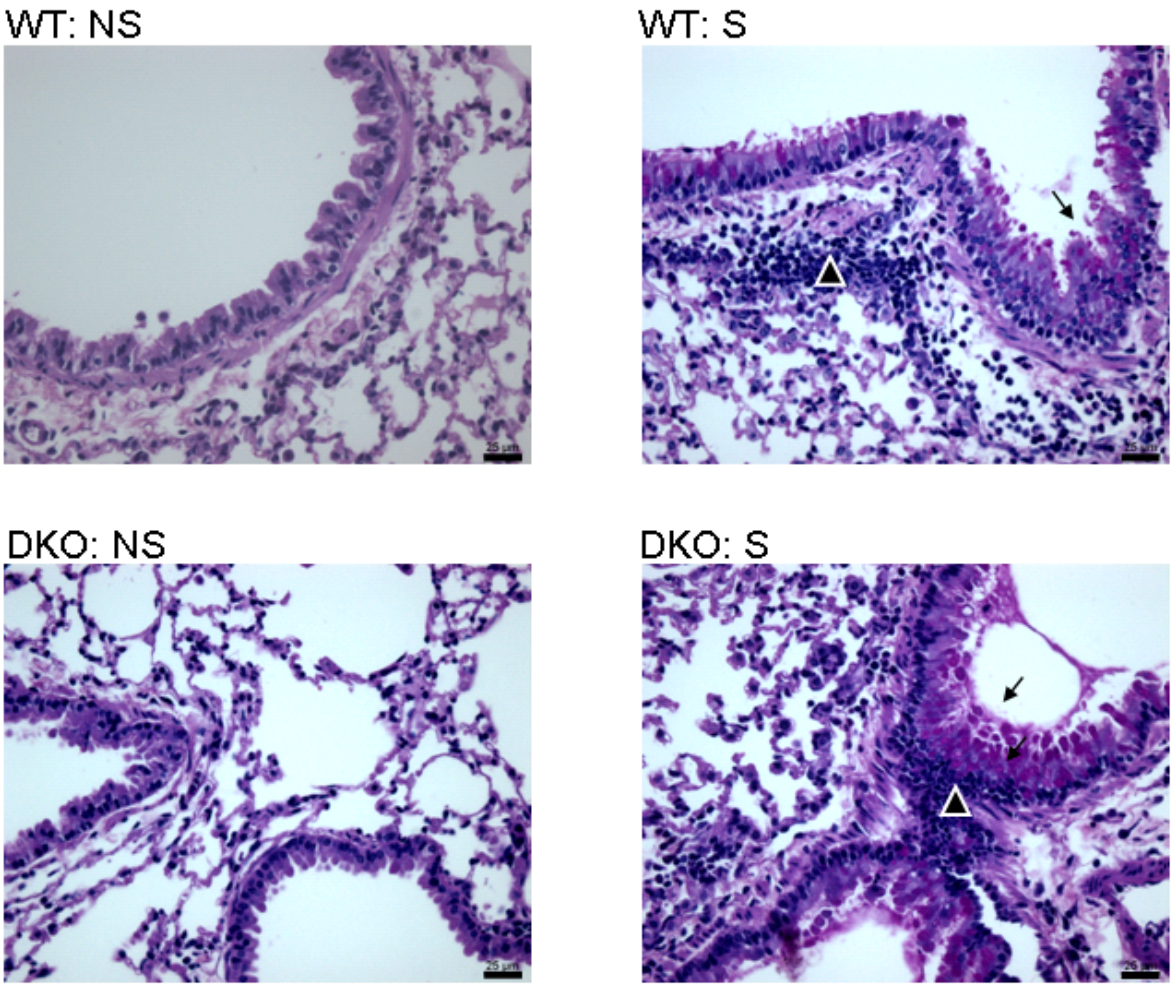


Figure 20: Periodic acid Schiff (PAS) staining of lung samples of DKO mice
Representative lung sections from non-sensitized (NS) OVA-sensitized (S) WT and DKO mice were stained with periodic acid Schiff (PAS) to determine PAS-positive mucus-containing cells. Tissue was examined by light microscopy. Inflammatory infiltrate and mucus hypersecretion are indicated by arrows and arrowheads, respectively. Histopathologic analysis of PAS-stained lung tissue showed a slight increase in mucus production in DKO mice. Original magnification 40x; scale bar, 25 μm.

Cytokine Profile in BALF

The cytokine pattern in the BAL fluids was analyzed from non-sensitized and OVA-sensitized WT and DKO mice. Data is shown as the mean ± SEM from one experiment with 4 to 6 mice per study group. As shown in Figure 21 no significant changes in cytokine levels were observed in OVA-sensitized DKO mice compared to WT mice. IL-4 level remained about the same in all study groups. Only a tendency for reduced IL-5 and IL-13 secretion could be detected in DKO mice. Other cytokines, namely IL-10, IL-12, and IFNγ were under detection limit.

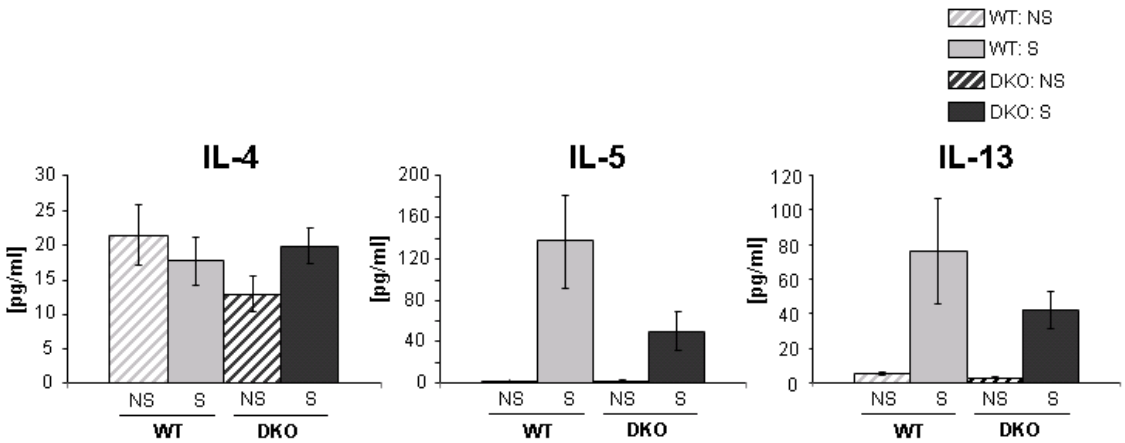


Figure 21: BALF cytokine profile in DKO mice
The presence of IL-4, IL-5, and IL-13 in BAL fluids from non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars) and OVA-sensitized (1 μg) WT (WT:S, grey bars) and DKO mice (DKO:S, black bars) was analyzed by ELISA. There were no significant changes in cytokine levels in lungs of different study groups. Results are expressed as mean ± SEM of one experiment (n=4-6/ group).

Immunoglobulin Levels in Serum

Serum samples which were obtained over the time period of the experiment were analyzed for levels of immunoglobulins (Ig) (see Figure 22). Levels of OVA-specific IgG1, total IgE, and OVA-specific IgE were determined at different time points during sensitization at day 0, 14, and 28, before challenge on day 42, and after challenge on day 47. Non-sensitized mice exerted no major changes in immunoglobulin levels which were mostly under detection. OVA-sensitized mice, however, showed increases in all three immunoglobulin levels, especially on days 42 and 47.

As shown in Figure 22A, levels of OVA-specific IgG1 increased in OVA-sensitized WT as well as DKO mice. However, DKO mice have a slightly higher increase in OVA-specific IgG1, although not significant.

The levels of total IgE mice also increased in both, OVA-sensitized WT and DKO mice. However, WT mice have a slightly higher increase in total IgE, although not significant.

Levels of OVA-IgE were very low, and could be detected only before and after challenge on days 42 and 47 (see Figure 22C). Again, OVA-sensitized WT and DKO mice showed similar levels of OVA-specific IgE.

Taken together, no significant alteration of immunoglobulin levels in different study groups could be detected.

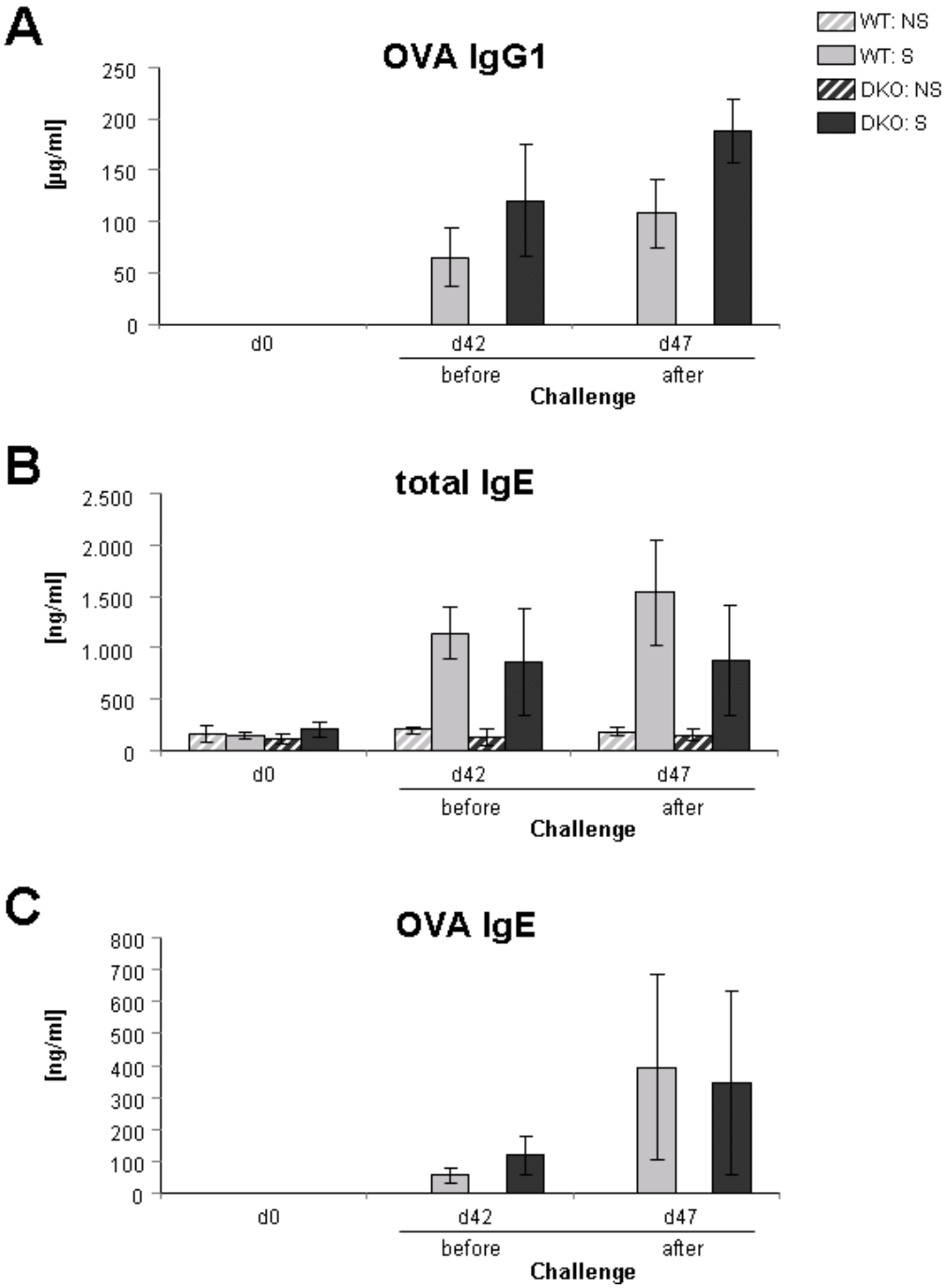


Figure 22: Serum immunoglobulin levels in DKO mice

Serum from non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars) and OVA-sensitized (1 μg) WT (WT:S, grey bars) and DKO mice (DKO:S, black bars) were analyzed for immunoglobulin (Ig) levels by ELISA. At indicated days (d0, d42, d47), blood samples were collected, serum was obtained, and **(A)** OVA-specific IgG1, **(B)** total IgE, and **(C)** OVA-specific IgE was determined. No significant changes in different study groups were detected. Data are expressed as the means ± SEM of one experiment (n=4-6/ group).

5.2.1.2 Selective Pharmacological Blockade of Cannabinoid Receptors

In the second approach, the influence of the single cannabinoid receptors in the different phases of allergic airway inflammation was analyzed by using different OVA-sensitized (10 μg) study groups: DKO mice, C57BL/6 wildtype (WT) mice, WT mice treated with cannabinoid receptor antagonists, recpectively. The antagonists used were SR141716A (SR1) for CB1 receptor and SR144528 (SR2) for CB2 receptor which were used either alone or in combination. Antagonists were administered prior to each sensitization during sensitization phase (s), each challenge during elicitation phase (e), or during both, sensitization and elicitation phase (s+e) as described. Control mice, which did not receive antagonists were treated with vehicle (VH: 1% DMSO, 1% Cremophor in saline) in during all phases. The dose of 1 mg per kg bodyweight antagonists was selected for these studies according to previous reports.

Cells in Bronchoalveolar Lavage (BAL) Fluid

BAL fluid (BALF) from non-sensitized and OVA-sensitized (10 Second, pharmacological blockade of CB1 and/or CB2 receptors was used to study the involvment of the single receptors during the different phases of the esperiment. g) WT and DKO mice after OVA-aerosol challenge were analyzed for total as well as differential cell counts. Data shown in Figure 23 is calculated as the mean $\pm$ SEM from one experiment with 8 mice per study group and 1-3 mice per non-sensitized control group.

As expected, total cell counts in non-sensitized mice were very low, showing no signs of inflammation. In contrast, OVA-sensitized mice showed a significantly increased total cell numbers (see Figure 23A). With this OVA-dose, the total cell number in OVA-sensitized WT and DKO mice was similar. Moreover, the total cell counts of different study groups, namely OVA-sensitized WT mice which were treated with antagonists at different time points were not significantly altered compared to OVA-sensitized WT mice. The percentage aberrations of total cell counts and p-values compared to control group are shown in Table 28.

Absolute numbers of differential cell counts are shown in Figure 23B. The increase in total cell counts in OVA-sensitized mice was reflected by an increase in all leukocyte populations. There were no significant differences in different study groups and indicated cell types, except for the neutrophil counts. A significant up-regulation of absolute neutrophil count in DKO mice and WT treated with SR1 in the elicitation phase.

The percentage distributions of differential cell counts within different study groups are shown in Figure 23C. BAL cells from non-sensitized mice are normaly mainly macrophages with a very low percentage of neutrophils, eosinophils, and lymphocytes. Here we could detect high levels of eosinophils in the non-sensitized DKO group and therefore a lower percentage of macrophages. The non-sensitized WT group showed very high numbers of lymphocytes. These are groups with one mouse per group and therefore the counts cannot be seen as average. In OVA-sensitized mice the fraction of macrophages decreased whereas the fraction of eosinophils increased. The population

of eosinophils was very high in this setting compared to other experiments. However, there were no major changes in different study groups in the percentage distribution of these cell subsets.

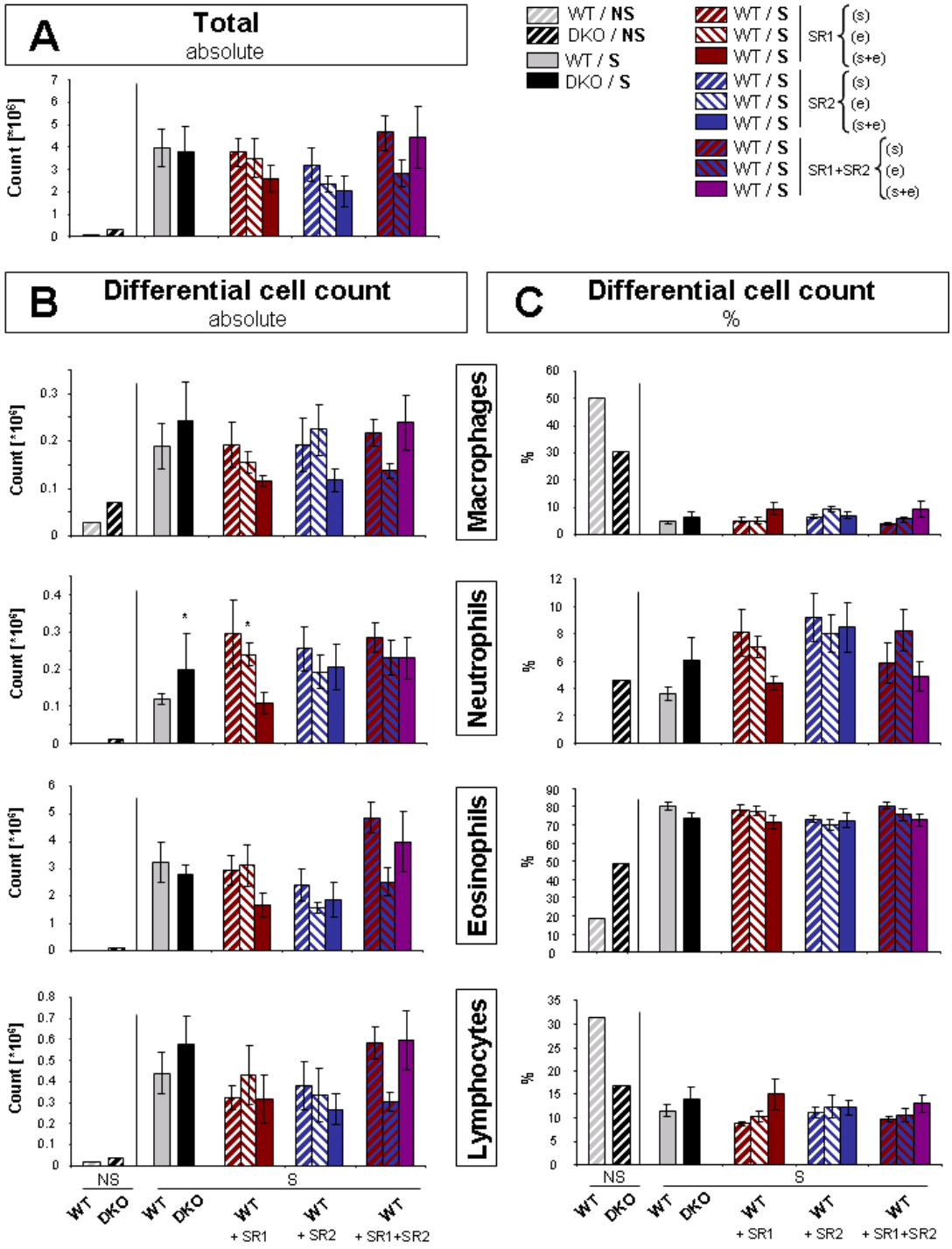


Figure 23: BAL cell counts after pharmacological blockade of CB receptors
Non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars), OVA-sensitized (10 μg) WT (WT:S, grey bars) and DKO mice (DKO:S, black bars), and and OVA-sensitized SR1-treated (red, as indicated), SR2-treated (blue, as indicated), or SR1/SR2-treated (purple, as indicated) WT mice were evaluated 24 hrs after OVA-aerosol challenge for total and differential cell counts as described. Absolute numbers of **(A)** total cell count and **(B)** differential cell count and **(C)** the percentage distribution of differential cell count was calculated. Results are expressed as mean ± SEM (n=8/ study group; n=1 per NS group). Statistical significance: *p<0.05 by paired student's *t*-test compared to OVA-sensitized WT mice.

Cytokine Profile in BALF

The cytokine pattern in the BAL fluids was analyzed from OVA-sensitized WT and DKO mice. Data is shown as the mean ± SEM from one experiment with 8 mice per study group. All cytokine levels of non-sensitized animals as well as levels for IL-10, IL-12, and IFNγ in OVA-sensitized mice were under detection. As shown in Figure 24 no significant changes in cytokine levels were observed in different study groups.

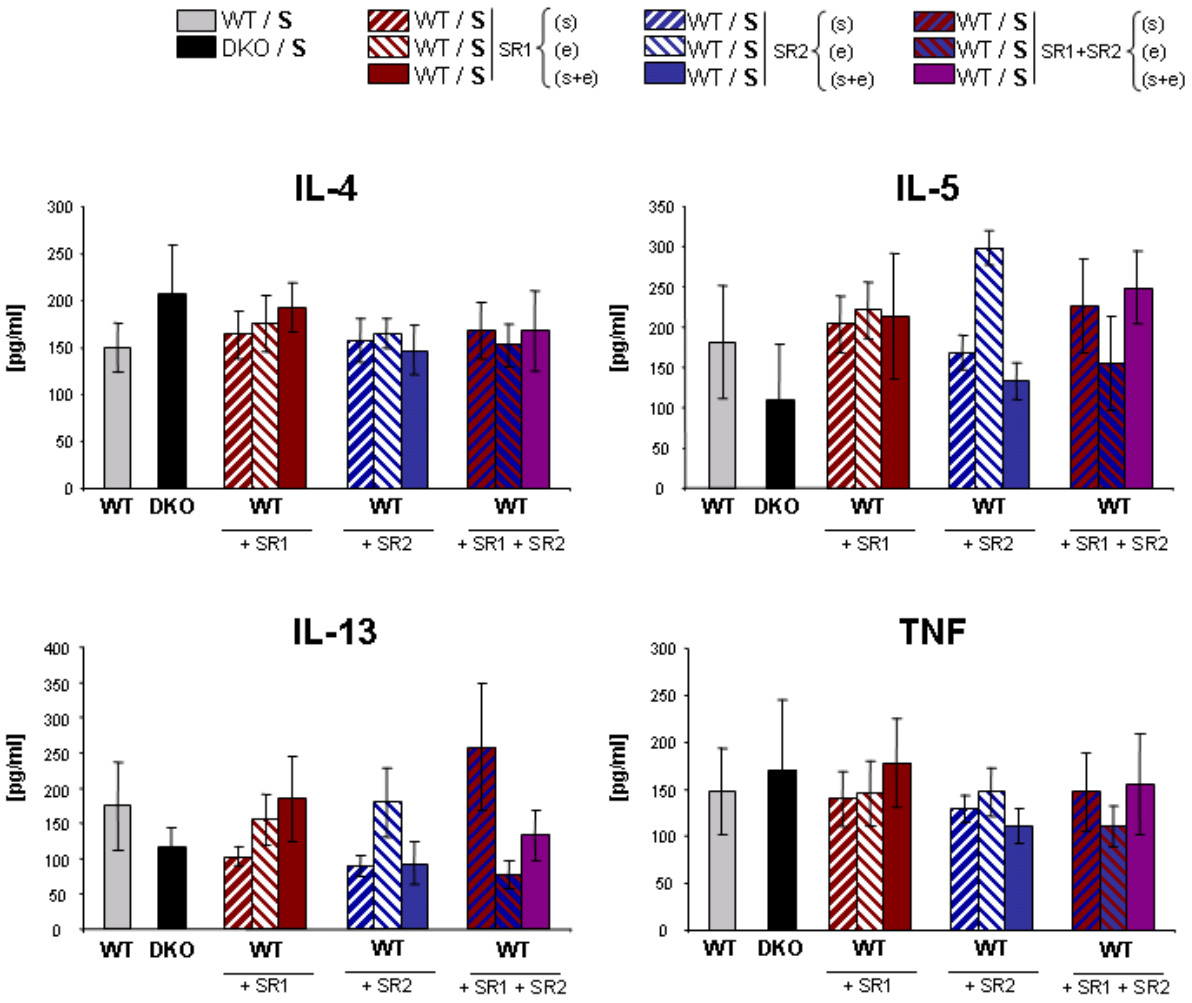


Figure 24: BALF cytokine profile after pharmacological blockade of CB receptors
The presence of IL-4, IL-5, IL-13, and TNF in BAL fluids from OVA-sensitized (10 μg) WT (WT:S, grey) and DKO mice (DKO:S, black), and OVA-sensitized SR1-treated (red, as indicated), SR2-treated (blue, as indicated), or SR1/SR2-treated (purple, as indicated) WT mice was analyzed by ELISA. There were no significant changes in cytokine levels in lungs of different study groups. Results are expressed as mean ± SEM of one experiment (n=8/ group).

Splenocytes Populations

Splenocytes were prepared as described and assessed either for analysis of $CD4^+$ and $CD8^+$ cells, or intracellular expression of FoxP3 within the CD4/CD25-positive cells by flow cytometric analysis. Data of CD4/CD8 expression are shown in Figure 25A and are calculated as percentage ofrom total cells. The percentage of $CD4^+$ and $CD8^+$ cells in non-sensitized and OVA-sensitized WT and DKO mice was similar. Moreover, OVA-sensitized WT mice which were treated with the antagonists SR1 and SR2 in sensitization and elicitation phase showed no alteration in the number of CD4- or CD8-positive cells. Thus, the ratio of CD4/CD8 cells was similar in all study groups.
Data of FoxP3 expressing cells are shown in Figure 25B and are calculated as percentage within the CD4/CD25-positive population. The percentage of FoxP3 positive cells in non-sensitized and OVA-sensitized WT and DKO mice was similar. Treatment of WT mice with both antagonists in both phases did not alter the expression of FoxP3 in these mice.

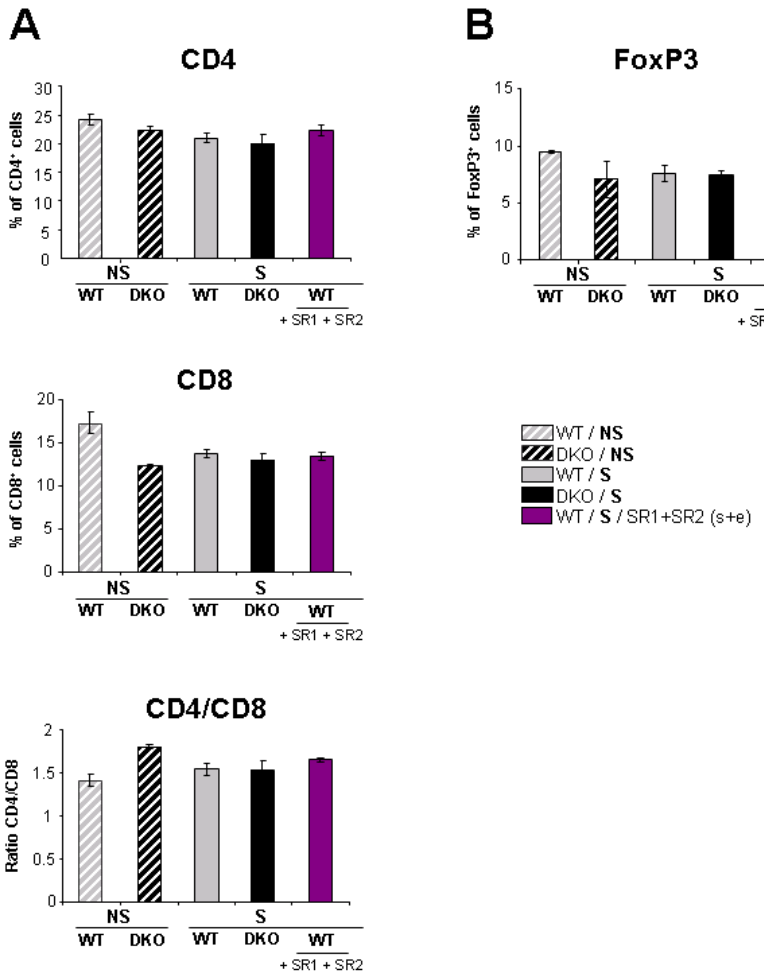


Figure 25: Splenocyte characterization after pharmacological blockade of CB receptors
Splenocytes from non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars), OVA-sensitized (10 μg) WT (WT:S, grey bars), DKO (DKO:S, black bars), and OVA-sensitized antagonist-treated (WT/S/SR1+SR2(s+e), purble bar) WT mice were analyzed for **(A)** expression CD4 and CD8 and **(B)** intracellular expression of FoxP3 in CD4/CD25 positive cells. The expression of CD4/CD8 or FoxP3 was not altered compared to OVA-sensitized WT mice. Results are expressed as mean ± SEM of one experiment (n=8/ group).

Immunoglobulin Levels in Serum

Blood was taken at different time points during experiment and serum samples were analyzed for levels of immunoglobulins (Ig) (see Figure 26). Levels of OVA-specific IgG1, total IgE, and OVA-specific IgE were determined at different time points during sensitization at day 0, 14, and 28, before challenge on day 42, and after challenge on day 47. While non-sensitized mice mostly showed no detectable amounts of immunoglobulins, OVA-sensitized mice, showed increases in all three immunoglobulin levels, especially on days 42 and 47.

As shown in Figure 26A, levels of OVA-specific IgG1 increased in OVA-sensitized mice till day 42 and remained equal afterwards. OVA-sensitized DKO mice and WT mice treated with indicated antagonists showed no significantly altered levels of OVA-specific IgG1.

The levels of total IgE in OVA-sensitized mice were under detection till day 42, but could be measured in samples of days 42 and 47 (see Figure 26B). Again, there were no significant changes in different study groups compared to OVA-sensitized WT mice.

Levels of OVA-specific IgE were mostly under detection limit and could only be detected after challenge on day 47 (see Figure 26C). At this time point, OVA-sensitized WT and DKO mice showed comparable levels of OVA-specific IgE which were not altered in OVA-sensitized WT mice treated with antagonists.

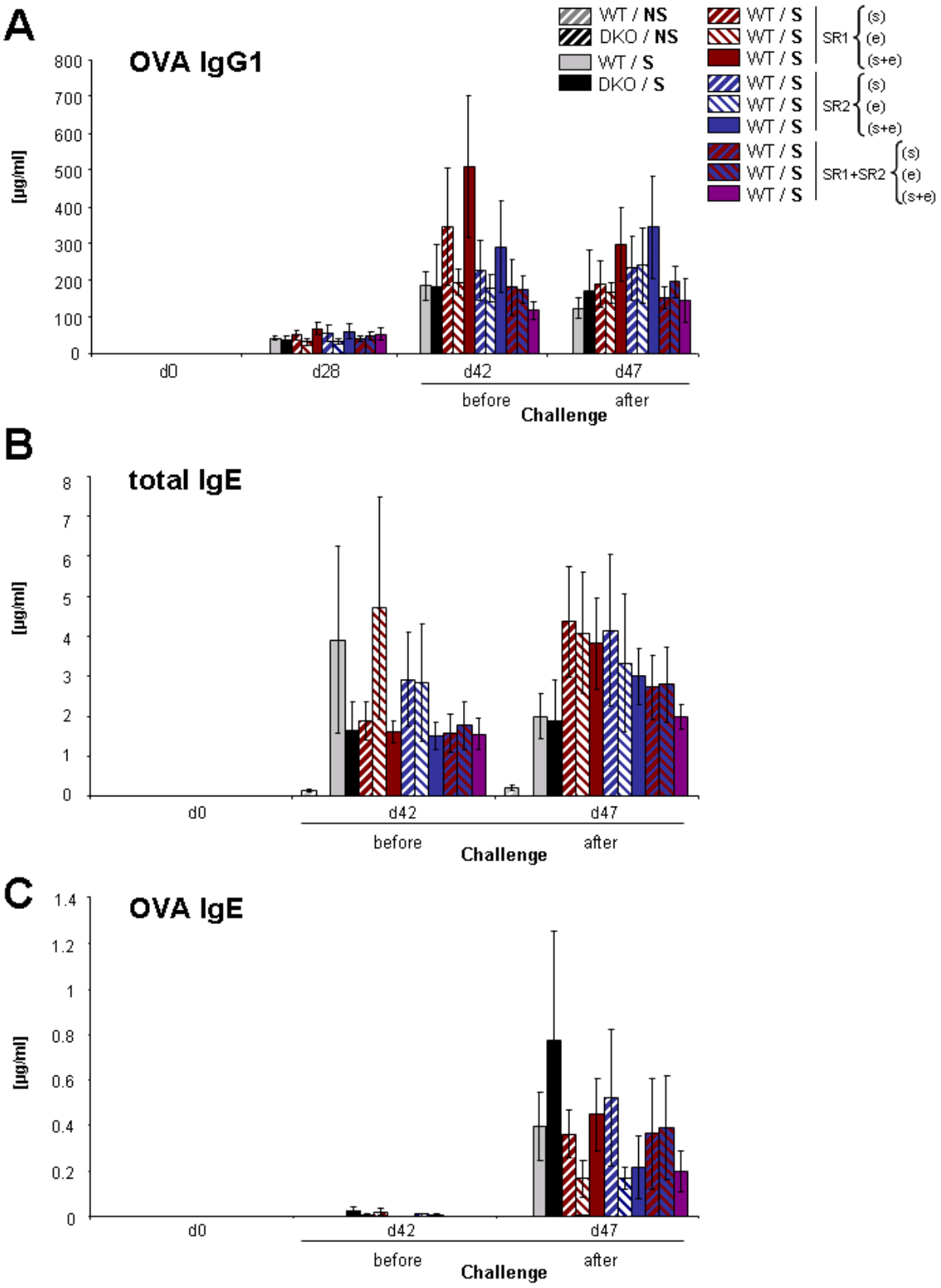


Figure 26: Serum immunoglobulin levels after pharmacological blockade of CB receptors
Serum samples from non-sensitized WT (WT:NS, hatched grey bars) and DKO mice (DKO:NS, hatched black bars), OVA-sensitized (10 μg) WT (WT:S, grey bars) and DKO mice (DKO:S, black bars), and OVA-sensitized antagonist-treated (as indicated) WT mice were analyzed for immunoglobulin (Ig) levels by ELISA. At indicated days (d0, d28, d42, and d47), blood samples were collected, serum was obtained, and levels of **(A)** OVA-specific IgG1, **(B)** total IgE, and **(C)** OVA-specific IgE was determined. No significant changes in different study groups were detected. Data are expressed as the means ± SEM of one experiment (n=8/ group).

5.2.2 Activation of the ECS

In the next approach, the influence on allergic airway inflammation after the activation of the ECS by the ligand Δ^9-Tetrahydrocannabinol (THC) was analyzed. Therefor, C57BL/6 wildtype (WT) mice were sensitized with 10 μg OVA/alum as described. Additionally, mice were treated with THC at different time points: THC was administered either daily for three consecutive days prior to each sensitization during sensitization phase (THC(s)), or once one day prior to each challenge during elicitation phase (THC(e)), or during both, sensitization and elicitation phase (THC(s+e)) with 50 mg per kg bodyweight. The dose of 50 mg/kg THC was selected for these studies according to previous reports (Jan et al., 2003). Control mice were treated with vehicle (VH: 5% Ethanol, 5% Cremophor in saline).

Cells in Bronchoalveolar Lavage (BAL)

Cells in the BAL fluid from different study groups were analyzed 24 hrs after OVA-aerosol challenge are shown in Figure 27, respectively. Total as well as differential cell counts for macrophages, neutrophils, eosinophils, and lymphocytes were determined.

Non-sensitized but OVA-challenged mice showed very low total cell numbers, indicating that no inflammation occurred. However, OVA-sensitized mice showed a significantly increased total cell number compared to non-sensitized mice (see Figure 27A). This means that aerosol challenge in sensitized animals led to an airway inflammation in the lung. However, treatment of OVA-sensitized mice with THC partially reduced the total number of inflammatory cells in BAL depending on time points of administration. When mice received THC only during sensitization phase but not during elicitation phase, total cell count was reduced compared to control group even though not significant. OVA-sensitized mice treated with THC only during elicitation phase or during both phases showed a significant reduction of total BAL cell count compared to OVA-sensitized control group. The percentage aberrations of total cell counts and p-values compared to control group are shown in Table 29.

Absolute numbers after differential cell counts are shown in Figure 27B. The increase in total cell counts in OVA-sensitized mice was mainly due to an increase in macrophages and eosinophils. Although neutrophil and lymphocyte numbers were higher in OVA-sensitized mice compared with non-sensitized animals these subsets constitute just small populations. However, THC-treatment resulted in a significant altered cell type pattern compared to vehicle-treated OVA-sensitized control mice. The absolute counts of macrophages and eosinophils in BAL fluid samples were generally lower in THC-treated mice than in OVA-sensitized control mice, but still higher than in non-sensitized mice. Macrophage numbers decreased significantly in all THC-treated groups with the most prominent effects in mice treated during both phases. THC-treatment in either sensitization or elicitation phase resulted in a reduction of eosinophil infiltration into the lungs although not significant. This reduction was highly significant after THC-treatment during both phases. Absolute neutrophil cell count was only significantly reduced in mice treated with THC during sensitization and elicitation phase. However, treatment with THC did not affect lymphocyte numbers.

The percentage distributions of differential cell counts within different study groups are shown in Figure 27C. BAL cells from non-sensitized mice were mainly macrophages with a very low percentage of neutrophils, eosinophils, and lymphocytes. In OVA-sensitized mice the fraction of macrophages decreased and the fraction of eosinophils increased. However, after treatment with THC during sensitization, elicitation, or both phases, the cell pattern in differential cell counts shifts back to a higher percentage of macrophages and a lower percentage of eosinophils. This was most prominent for mice treated with THC in both phases. In this group the fraction of neutrophils was also highly reduced, without changes in lymphocyte fraction.

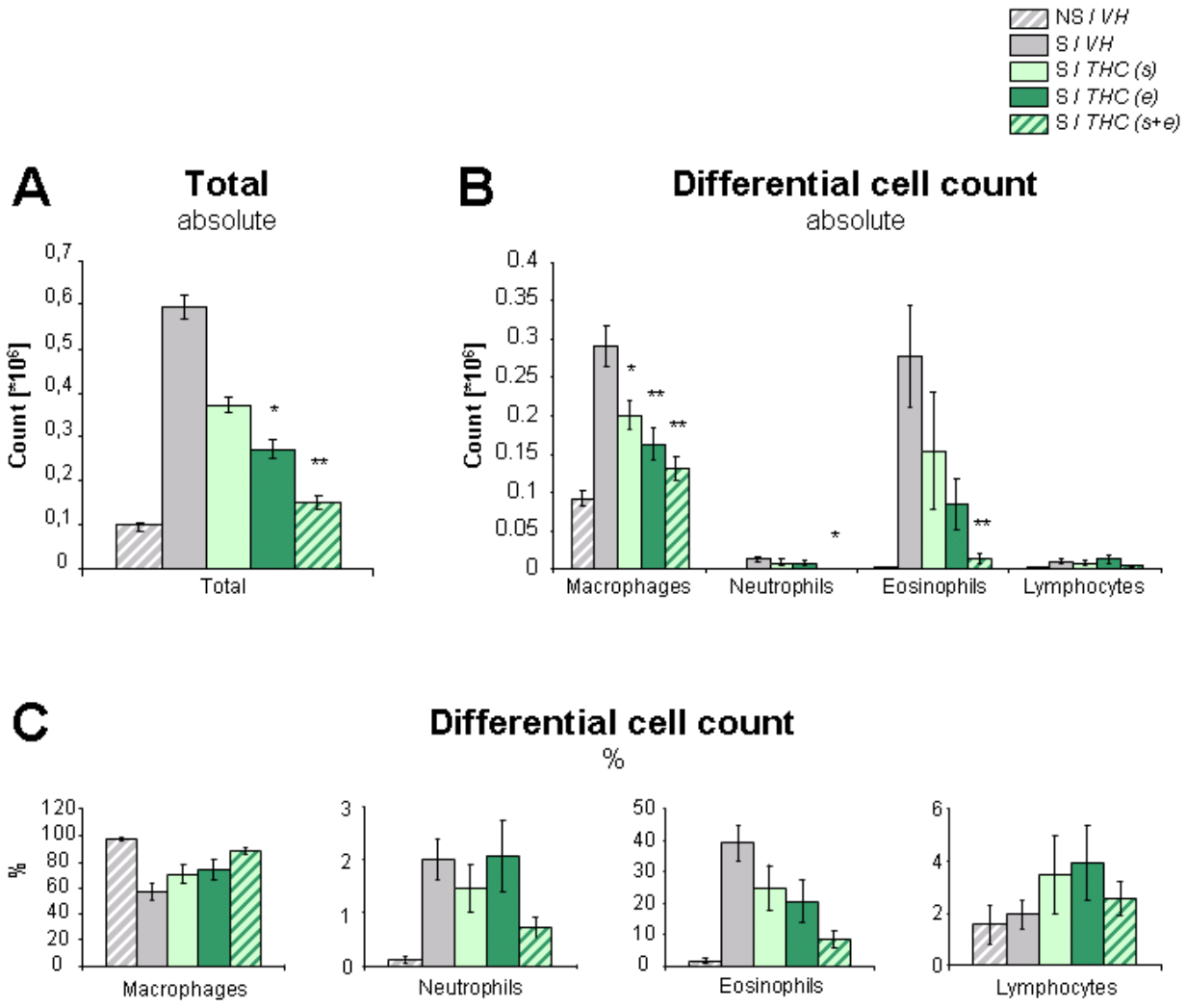


Figure 27: BAL cell counts in mice after THC-treatment

Non-sensitized mice (NS/VH, hatched grey bars), OVA-sensitized (S/VH, grey bars), and OVA-sensitized THC-treated mice (as indicated) (S/THC, green) were evaluated 24 hrs after OVA-aerosol challenge for total and differential cell counts as described. Absolute numbers of **(A)** total cell count and **(B)** differential cell count and **(C)** percentage distribution of differential cell count was calculated. The increase in total cells as well as increased eosinophilia in OVA-sensitized mice which were mostly macrophages and eosinophils was reduced by THC-treatment. Results are expressed as mean ± SEM of one experiment (n=10/ group). Statisical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to S/VH control mice.

Cytokine Profile in BALF

BAL fluids from OVA-sensitized and challenged mice were analyzed for the cytokine pattern. The cytokines IL-13, IL-4, IL-5, as well as IFNγ and IL-10 were analyzed in by ELISA. Data is shown as the mean ± SEM from one experiment with 10 mice per study group. As shown in Figure 28 no significant changes in cytokine levels were observed in OVA-sensitized mice compared to mice treated with THC as indicated.

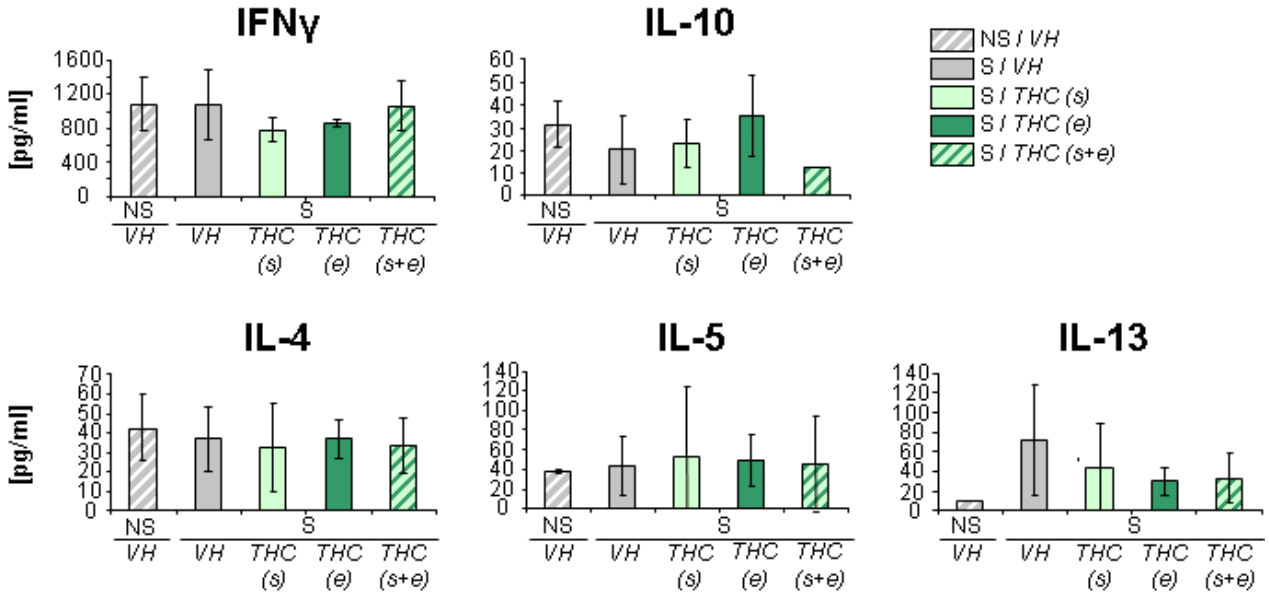


Figure 28: Th2 cytokine response in BAL fluid after THC-treatment
The presence of IFNγ, IL-10, IL-4, IL-5, and IL-13 in BAL fluids from non-sensitized (NS/VH; hatched grey bars), OVA-sensitized (S/VH; grey bars), and OVA-sensitized THC-treated mice (as indicated) (S/THC, green) was analyzed by ELISA. There were no changes in cytokine levels in lungs of different study groups. Results are expressed as mean ± SEM of one experiment (n=10/ group).

Splenocyte Populations

Furthermore, splenocyte preparations were assessed for their expression of CD4 and CD8 and intracellular expression of FoxP3 within the CD4/CD25-positive population by flow cytometric analysis.

Data of CD4/CD8 expression are shown in Figure 29A and are displayed as percentage of total cells. The percentage of $CD4^+$ and $CD8^+$ cells in non-sensitized and OVA-sensitized mice was similar. However, OVA-sensitized THC-treated mice showed an altered amount of $CD4^+$ cells, but a constant number of $CD8^+$ cells. This resulted in a shifted ratio of CD4/CD8 cells which was elevated after THC-treatment either during sensitization or elicitation phase. However, mice treated with THC in both phases showed a reduced number of $CD4^+$ cells and therefore a lower CD4/CD8 ratio.

Data of FoxP3 expressing cells are shown in Figure 29B and are displayed as percentage within the CD4/CD25-positive population. The percentage of FoxP3 positive cells in non-sensitized and

OVA-sensitized mice was similar. THC-treatment of mice did not alter the expression of FoxP3 in these mice.

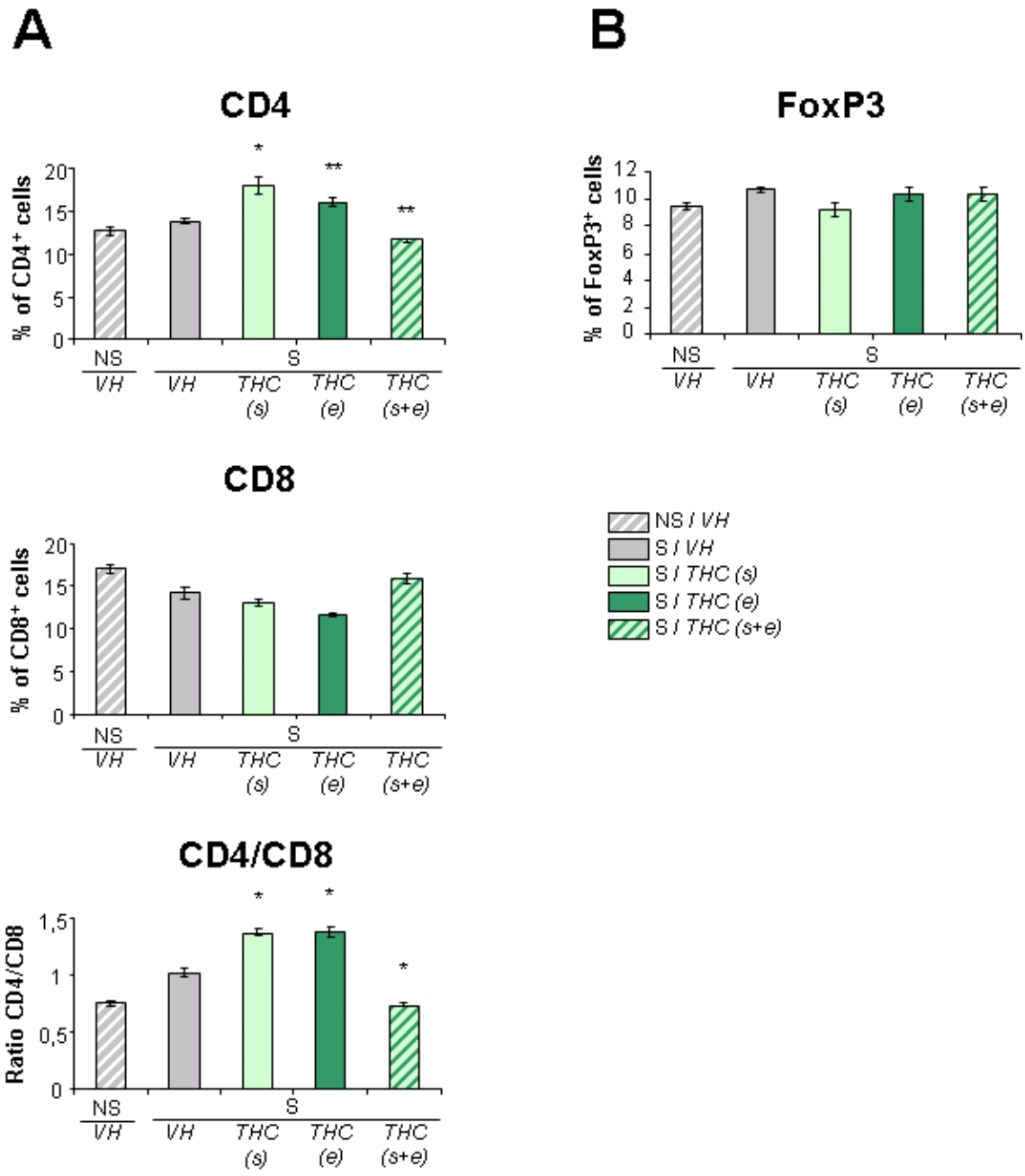


Figure 29: Surface CD4/CD8 expression and intracellular FoxP3 levels in splenocytes after THC-treatment

Splenocytes from non-sensitized mice (NS/VH, hatched grey bars), OVA-sensitized (S/VH, grey bars), and OVA-sensitized THC-treated mice (as indicated) (S/THC, green) were analyzed for **(A)** expression CD4 and CD8 and **(B)** intracellular expression of FoxP3 within CD4/CD25 positive cells. The expression of CD4/CD8 in THC-treated mice was significantly altered compared to control mice. Results are expressed as mean ± SEM of one experiment (n=6/ group). Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to S/VH control mice.

Immunoglobulin Levels in Serum

Serum samples were obtained at different time points during the experiment and were analyzed for levels of immunoglobulins (Ig) (see Figure 30). Levels of OVA-specific IgG1, total IgE, and OVA-specific IgE were determined during sensitization phase at day 0, 14, and 28, before challenge on day 42, and after challenge on day 47. Non-sensitized mice did not show an increase in any of the mentioned immunoglobulins. OVA-sensitized mice, however, showed increases in all three immunoglobulin levels, especially on days 42 and 47.

As shown in Figure 30A, levels of OVA-IgG1 increased in OVA-sensitized mice untill day 42 and remained constant afterwards. Interestingly, THC-treatment attenuated the increase of OVA-IgG1 levels which was significant when mice were treated either in sensitization phase or in both phases. The levels of total IgE in OVA-sensitized mice increased over the entire experiment and could be detected already at day 14. This increase was suppressed in mice receiving THC either during sensitization or during both phases (see Figure 30B). Levels of OVA-IgE were mostly under detection and could only be detected after challenge on day 47 (see Figure 30C).

Again, the increase of OVA-IgE in OVA-sensitized mice was significantly reduced in mice treated with THC during sensitization phase. THC-treatment only during elicitation phase showed no effect on OVA-IgE levels, whereas the administration during both phases led to a highly significant reduction of OVA-IgE levels in serum.

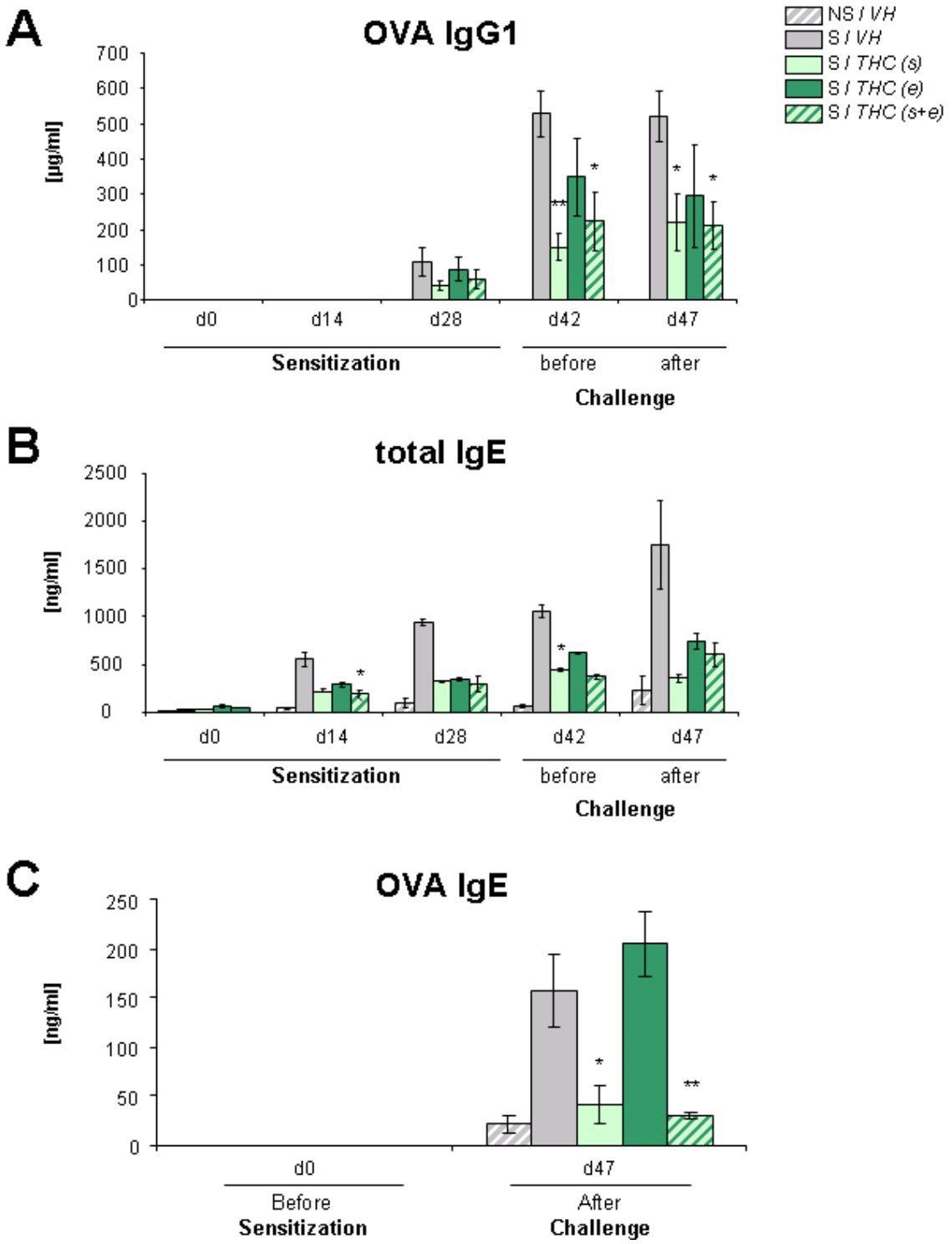


Figure 30: Serum immunoglobulin levels after THC-treatment

Serum from non-sensitized (NS/VH, hatched grey bars), OVA-sensitized (S/VH, grey bars), and OVA-sensitized THC-treated mice (as indicated) (S/THC, green) were analyzed for immunoglobulin (Ig) levels by ELISA. At indicated days (d0, d28, d42, and d47), blood samples were collected, serum was obtained, and levels of **(A)** OVA-specific IgG1, **(B)** total IgE, and **(C)** OVA-specific IgE was determined. A reduction of all immunoglobulin levels could be observed in mice treated with THC in sensitization phase. Data are expressed as the means ± SEM of one experiment (n=10/ group). Statistical significance: *$p<0.05$ and **$p<0.01$ by paired student's *t*-test compared to S/VH control mice.

6 Discussion

6.1 Immunomodulation of Allergic Immune Response by Anthropogenic Factors in vitro

The subject of the first part of the thesis is that the concomitant exposure to inhalable highly polluted 'dirty' DEP (SRM) but not particle-associated organic compounds, less polluted 'clean' DEP (Euro3 or C-Euro3), or ultrafine carbon black (CB) particles modulates DC maturation in the murine and human system. In addition, SRM-exposed murine BMDC lost the capability to suppress IgE production and class switching in B cells. Thus, the presented work suggests that highly polluted particles not only interfere with DC maturation but also with DC function arguing for a role in Th2 immune deviation.

6.1.1 Importance of Particle Characteristics and in vivo Relevance

DEP consist of a carbonaceous particle core which is surrounded by elemental and organic carbon species (Lovik et al., 1997; Nel et al., 1998). There are many studies discussing the role of physical particle properties (e.g. size, surface area), the particle itself, and particle-bound chemicals on the allergic immune response. Therefore, several particle samples have been assessed with different properties concerning particle size and chemical load. SRM, Euro-3, and C-Euro3 are all DEP as they are derived from combustion of a diesel engine. They vary in their chemical composition as they are derived from different engines. HPLC analysis of PAH contents were accomplished to determine the level of pollution. According to SRM particles which are highly polluted, C-Euro3 and Euro3 are less polluted. Printex G comprises carbon black (CB) which contain only low amounts of organic compounds compared to DEP and therefore are used as a model particle for the carbon core of DEP. These particles are commercially available synthetic particles. No particles of other size or from other sources were included in this thesis.

The most common definition of PM is PM_{10} which are coarse particles with an aerodynamic diameter less than 10 μm. These are often referred to as inhalable particles. However, several studies indicate that the level of $PM_{2.5}$ can be more closely related to health effects than PM_{10} (Peters et al., 1997; Schwartz and Neas, 2000; Schwartz et al., 1999). Further, the $PM_{0.1}$ fraction is getting more into the focus of research (Penttinen et al., 2001). There is evidence that the number concentration of these ultrafine particles is slightly increasing (Cyrys et al., 2003). Particle size determines in what region of the airways the material will be deposited during inhalation. Moreover, small molecules have an increased retention time in the lungs because they are not that efficiently removed from the lungs (Gehr et al., 1993). Further, ultrafine particles are shown to

penetrate the epithelial barrier compared to fine particles (Oberdorster et al., 1992) thus more particles reach the interstitial space of the lung. In contrast, particles with a larger surface area may interact to a higher extent with alveolar cells than smaller particle do. This may lead to a stronger cellular response. Alessandrini et al. showed that allergen-sensitized mice are more susceptible in their allergic response to ultrafine particle inhalation by using a well-established mouse model of OVA-induced allergic inflammation of the lung (Alessandrini et al., 2006). Further, they showed that OVA-sensitized mice had significantly increased ultrafine particle deposition compared to non-sensitized mice (Alessandrini et al., 2008).
It is difficult to determine whether the number or the surface area of particles contribute to the observed effects as they are closely related to each other. It is important to keep in mind that there can be a large number of small particles in ambient air having important biological effects but hardly contribute to the total mass.
Smaller particles obviously have a higher proinflammatory potency than larger specimens at comparable doses. Moreover, there may be other reasons for the increased susceptibility of allergic individuals, e.g. changes in long-term clearance kinetics or biologic response to particles (Alessandrini et al., 2008).

There are several studies indicating that adsorbed chemical substances can have adjuvant effects on the allergic immune response (Fahy et al., 1999; Heo et al., 2001; Takenaka et al., 1995; Tsien et al., 1997). One of the major chemical substances found on DEP are PAH (Lovik et al., 1997; Nel et al., 1998) with phenanthrene representing about 52% of these PAH (Barfknecht et al., 1982). It was already shown that PAH modulates allergic immune response by e.g. upregulated IL-4 production (Bommel et al., 2000), increased IgE production (Takenaka et al., 1995; Tsien et al., 1997), or induced inflammation (Fahy et al., 1999; Terada et al., 1999). Several organic compounds were assessed for their modulatory capacity on murine BMDC and human MoDC. Among the organic compounds, phenanthrene is a representative of a single substance, whereas EPA-PAH and AERex are complex mixtures. The EPA-PAH sample is composed of the priority 16 PAH of the EPA. AERex samples are organic extracts of urban aerosol (AERex) from airborne particular matter containing various PAH concentrations.
Although several studies showed that DEP organic extracts or PAH influence allergic immune response, this study could not detect modulatory effects of phenanthrene, EPA-PAH, or the extracts form urban aerosol in the employed system. Moreover, carbon black samples did not show any effect on DC function. However, highly polluted particle such as the SRM sample does modulate DC phenotype or function, whereas less polluted particles failed to do so. CB contains elemental carbon with very low amounts of extractable organic (about 1%), whereas SRM contain about 20% of extractable organics. When the HPLC analyses of PAH on Euro3 and C-Euro3 samples are compared with the SRM sample it is obvious that C-Euro3 and Euro3 are less polluted than SRM regarding the amount of PAH. This indicates that the particle per se may induce the basic effect which can be further modulated or increased by adsorbed chemical substances.

The question arises whether the particle concentrations used in this study represent relevant particle

doses when exposed under real-life conditions. This is extensively discussed by the group of Nel (Li et al., 2003). They performed an *in vivo* dosimetric evaluation of total PM and $PM_{2.5}$ deposition in an exposed adult and found that the biological relevant tissue culture concentration of DEP ranges from 0.2 to 20 $\mu g/cm^2$. This calculation is based on the fact that inhaled particles are not symmetrically distributed throughout this region but tend to accumulate at so-called hotspots of deposition (Phalen et al., 2006) which are at airway bifurcation points. To allow comparison, it is necessary to convert particle concentrations, calculated as mass per unit volume (mg/ml), into particle mass per unitary surface area in the culture dish. In this thesis maximal concentration of 100 μg/ml were applied. This concentration is equal to about 15 $\mu g/cm^2$ after converting. Thus, our concentration falls well within this range. This calculation only embraces weight measurements without determination of physical characteristics such as number concentration, size distribution, and the total surface area. To compare effects from e.g. ambient particulate matter from different sources weight measurements should be extended by the number of sized particles per volume unit of air.

Further, the question arises whether the *in vitro* exposure to particles is realistic. *In vitro* exposures are normally performed in liquid cultures under standard cell culture conditions. However, this type of exposure does not reflect the real situation of exposure regarding particle application. In this approach, particles are added as a suspension to the culture medium. This differs basically from deposition of airborne particles, as the particles' physical properties might change during resuspension. However, this approach is the most common way to evaluate the influence of particles (Nel et al., 2006; Oberdorster et al., 2005). Studies on the interaction between cells and particles at the air-liquid interface would provide a more realistic setup regarding the application of particles (Maier et al., 2008).
In vitro studies are commonly used to analyze cell specific mechanisms, however, results from such *in vitro* exposure studies should be considered carefully. Especially when transmitting to real life *in vivo* situations. These single cell studies are mostly monocellular systems or at the most co-culture system containing two to three cell types. But intercellular communication and signaling *in vivo* implies a lot more cell types. Thus, the use of animal studies focusing on specific mechanisms are indispensable (Alessandrini et al., 2006; Alessandrini et al., 2008; Alessandrini et al., 2009).

As endotoxin is ubiquitous in the environment it might be reasonable to speculate that particle preparations activate pattern recognition receptors via contaminant microbial products (Daniels et al., 2000; Monn and Becker, 1999; Ning et al., 2000). No endotoxin was detectable within all the organic compounds, however, particle preparation showed detectable amounts of endotoxin. In murine alveolar macrophages it was shown that trace amounts of endotoxin on particle preparations showed biological activity. This activity and the endotoxin content was mostly associated with the particle fraction not the water-soluble fraction (Ning et al., 2000). To exclude effects on maturation which are due to contaminating endotoxin in our system, identical doses of particle-contaminating LPS on imBMDC were tested. These concentrations had no effects on BMDC surface marker expression or cytokine secretion (data not shown). This leads to the suggestion that endotoxin

contamination does not influence this testing. However, this can only be an indication as LPS origin might differ in particle samples. Further, synergistic interaction between the trace amounts of endotoxin and other proinflammatory parts of the particles cannot be excluded.

Further, the possibility that particles interfere with our ELISA system can be excluded as only IL-6 and IL-12p70 secretion was decreased after SRM-exposure whereas the secretion of IL-10 remained constant. This was further confirmed by spiking experiments for IL-12p70 measurements (data not shown) which showed no interference of particle to detect recombinant IL-12 in the samples.

6.1.2 Cellular Effects of Particle Exposure

Several *in vitro* studies reveal an effect of particles on a variety of cell types such as macrophages, airway epithelial cells, T cells, and B cells (Nel et al., 1998). Although macrophages and epithelial cells are clearly affected by particles, these cells are not directly involved in antigen-specific immune response. But the effects on these cells might contribute to an important mechanism occurring during the provocation phase which is the particle-mediated induction of nonspecific inflammatory response (Granum and Lovik, 2002). For example, Bayram et al. showed that exposure of cultured human bronchial epithelial cells to DEP extracts leads to an increased synthesis and release of proinflammatory mediators, cytokines, and adhesion molecules (Bayram et al., 1998). Further, it was shown that increased GM-CSF secretion of human bronchial epithelial cells upon DEP exposure mediates DC maturation (Bleck et al., 2006). This supports the idea that nonspecific inflammatory effects of particles may add to the allergic inflammation.

However, DC not only contribute to the adaptive immune system but DC are one of the first line of defence as they are abundant throughout the respiratory epithelium (Holt and Stumbles, 2000; Reibman et al., 2003) where they recognize inhaled agents (McWilliam et al., 1994). Thus, it is likely that environmental pollutants deliver their adjuvant signals through the interaction with those immature DC which are generated from circulating precursors, a process which is enhanced by environmental stimuli (McWilliam et al., 1994). Furthermore, it has been already demonstrated for intestinal DC that these mucosal DC sample through the surface of this epithelium (Rescigno et al., 2001). Upon activation, DC produce and secrete IL-12, up-regulate co-stimulatory molecules which leads to altered DC morphology, phenotype, and the ability to act as an APC (Winzler et al., 1997) which in turn induce T cell response either in Th1 or Th2 direction (Agnello et al., 2003).

There are few studies suggesting a modulatory capacity of particles on DC, e.g. it has been demonstrated that DEP can suppress IL-12 production of DC in humans (Ohtani et al., 2005; Williams et al., 2007). Others did not find a direct effect of DEP on DC function but suggested that DEP induce antigen-independent DC maturation via epithelial cell-DC interactions which are mediated by GM-CSF (Bleck et al., 2006). Regarding the expression of surface molecules in DEP-exposed cells an up-regulation in immature (Williams et al., 2007) as well as mature cells (Verstraelen et al., 2005) was observed by several groups.

This thesis shows that highly polluted particles (SRM) inhibit IL-12p70 secretion upon stimulation with LPS and thus inhibiting DC maturation, conditions that favour Th2 responses and allergic inflammation (Reider et al., 2002; Trinchieri, 2003). This leads to the suggestion that the cytokine milieu induced by particle-exposure directs toward a Th2 response. Moreover, lower levels of LPS-induced IL-6 in supernatants of cells exposed to SRM compared to levels after stimulating BMDC with LPS were observed. IL-6 is a cytokine which is multifunctional and also promotes T cell activation (Ettmayer et al., 2006). Interestingly, no effect on IL-10 secretion after particle-exposure was detectable. IL-10 is believed to inhibit Th1 cytokine production and would favour a Treg cell response (Fiorentino et al., 1989; Fiorentino et al., 1991).

Several studies have shown that particle-associated organic compounds, mainly PAH, have a broad spectrum of action on many different cells involved in the inflammatory reaction. One of these studies showed decreased LPS-induced cytokine production of dendritic cells after incubation with pro-oxidative DEP chemicals which is most likely due to induced oxidative stress in DC (Chan et al., 2006), whereas another study showed that DEP-PAH modulated the chemokine pathway of DC by up-regulating IL-8 and RANTES and down-regulating MCP-1 (Fahy et al., 1999). Finally it was shown, that exposure to benzo(*a*)pyrene inhibited *in vitro* functional differentiation and maturation of blood monocyte-derived DC (Laupeze et al., 2002). However, in this study no effects were detected of the tested compounds on DC function.

Analyzing human MoDC, similar results were observed. No effects were measured after MoDC exposure to phenanthrene. However, cytokine secretion, namely IL-6 and IL-12p70 of LPS-stimulated MoDC was decreased after SRM-exposure (Figure 4). Furthermore, SRM-exposed immature MoDC showed a lower expression of CD80, CD86, Fcε RI, and HLA-ABC (Figure 3). Additionally, MoDC matured in the prescence of SRM also displayed an altered phenotype, e.g. reduced up-regulation of surface markers such as CD86 and HLA-ABC. However, neither cytokine secretion nor surface marker expression of poly(I:C)-stimulated MoDC were influenced by SRM-exposure.

The adjuvant activity of particles in allergen-indcued IgE responses has been shown for the first time long ago in a murine *in vitro* study (Muranaka et al., 1986). This is in line with other studies showing that the exposure of mice to the antigen together with particles results amongst other symptoms in higher IgE and IgG titers (Fujimaki et al., 1994; Fujimaki et al., 1997; Heo et al., 2001; Takafuji et al., 1987; Takano et al., 1997). These studies were extended in human *in vivo* studies which showed enhanced specific IgE production in allergic patients after combined exposure to allergen and particles (Diaz-Sanchez et al., 1997). This was due to an increased level of productive but not germline epsilon mRNA transcripts, therefore acting on postswitched IgE-producing cells (Diaz-Sanchez et al., 1994). Furthermore, it was shown that DEP could induce a de novo mucosal IgE response to the neoantigen KLH thus facilitating the sensitization to this allergen in already allergic patients (Diaz-Sanchez et al., 1999). Thus, one can speculate that DEP did not alter the immune response to KLH by polarizing toward a Th2 like response but just increased the normal immune response in these allergic patients. However, there are animal studies with strains of mice which are not favoured to develop an IgE response (Granum et al., 2000), thus supporting the idea

that particles may enhance allergic sensitization.

Additionally, for the first time, IgE production in naïve B cells which were stimulated to undergo CSR and co-cultured with BMDC was analyzed. After these stimulated naïve B cells were co-cultured with BMDC they show decreased IgE levels. This is in line with studies from Obayashi et al. (Obayashi et al., 2007) although in this thesis IgE production was much more suppressed by imBMDC than by mBMDC. *In vivo* it might be necessary that DC which migrate to peripheral lymph nodes control IgE production (Hemmi et al., 2001) indicating that the clearance of IgE-positive cells which have down-regulated IgE expression is a possible mechanism how IgE production is regulated *in vivo* (Lam et al., 1997). Interestingly, in co-cultures of naïve B cells and DPM-exposed BMDC, the IgE production was increased which most probably results from a decreased capability of SRM-exposed imBMDC to suppress IgE production. Thus SRM-exposed imBMDC lost their ability to function as regulator. However, this could not be observed in SRM-exposed mBMDC. It has been previously reported that the suppression of IgE production at a protein level mainly involves direct cell-to-cell contact (Obayashi et al., 2007). However, the surface molecules which might contribute to the suppression of IgE expression are not identified yet.

Moreover, B cell isotype switching in the human system and the influence of co-culturing human B cells with MoDC was investigated. However, this setting did not give similar results as observed in the murine system. The most likely explanation for this might be the different type of DC induced *in vitro* between mice and men in which murine BMDC might represent a more realistic counterpart of lymph node bound DC which are most probably the cells interacting on B cell immunoglobulin production by direct contact. Moreover, BMDC display DC mainly from the myeloid origin and lung DC in the mouse are mainly of myeloid origin, however, MoDC are not the subclass of DC which are representative in the airway (Laupeze et al., 2002).

6.1.3 Further Considerations

Mechanisms – Role of ROS and Oxidative Stress?

To clarify the exact mechanism of particle effects was not subject of this thesis. Only speculation about the underlying mechanism, how these effects are mediated in murine and human DC *in vitro* can be made. It is most likely a result from reactive oxygen species (ROS) which are generated after exposure to particles and which is followed by the generation of oxidative stress within the exposed cells as showed by others (Ohtani et al., 2005; Whitekus et al., 2002). ROS (e.g. superoxide, hydrogen peroxide, and hydroxyl radical) are reactive with proteins, lipids, and DNA, leading to cellular damage. Oxidative stress is defined as a depletion of reduced glutathione in exchange for an increase in oxidized glutathione (Rahman et al., 1999). Thus, DEP might increase oxidative stress directly through the induction of ROS and indirectly through the resultant enhanced inflammation, which thus generates additional ROS and further inflammation (Riedl and Diaz-Sanchez, 2005). Very low levels of oxidative stress are regulated by the cell's cytoprotective defense. However,

at higher levels of ROS these defense mechanisms do not work any more resulting in oxidative stress. Phase 2 drug metabolizing and antioxidant enzymes comprise these defense mechanisms. If these enzymes are overwhelmed, ROS activate redox-sensitive transcription factors such as MAPK, activator protein (AP)-1 and NF-χB which in turn regulate the expression of e.g. proinflammatory cytokines, chemokines, and adhesion molecules. However, very high levels of oxidative stress result in cell apoptosis or necrosis.

It is suggested that ROS production is induced by the chemicals associated to DEP, rather than the carbon core (Baulig et al., 2003). This supports the data, as no effects were detectable of less polluted particles and carbon black on DC. However, highly polluted DC led to an altered phenotype of DC, although a more attenuated rather than activated effect was observed. Moreover, studies investigating ROS production after stimulation with DEP are mostly conducted in macrophages, neturophils, eosinophils, and epithelial cells. Interestingly, a study showed that the glutathione levels in murine APC determine the outcome of Th1/Th2 cytokine response through the level of IL-12 production (Peterson et al., 1998). However, studies on ROS production on enriched DC are few.

Thus, as DEP are very heterogeneous in their composition, it is very likely that other mechanisms are present after DEP exposure. It was reported that DEP inhibited the LPS-induced IFNγ-production in a murine model (Finkelman et al., 2004). Further, one study suggests that DEP exposure in DC induces the Nrf2 pathway which then down-regulates DC maturation and LPS-induced IL-12 secretion (Chan et al., 2006). As this system a down-regulation of LPS-induced IL-12 secretion after exposure to highly polluted particles was observed, this might be the mechanism behind.

Influence of Genes – Genetic Predisposition?

Further, one should keep in mind that the effects of particles on allergic immune responses differ between individuals. Certain individuals have a genetic predisposition to react to particles. There are several studies identifying candidate susceptibility genes that may be important for the expression of asthma and allergic diseases (Barnes and Marsh, 1998), as well as genes that may be important for the effect of air pollution. This might concern a very heterogeneous class of genes including genes involved in cytokine response in general, or genes involved in the cellular response to particles or particle-associated components.

Furthermore, gene-environment interactions might be important. As mentioned earlier, there may be a synergism between the endotoxin bound to particles and other proinflammatory components of particles (Ning et al., 2000). Thus polymorphisms in genes associated with the transmission of the LPS signal such as CD14 and Tlr4 can affect the expression of allergic responses (Koppelman et al., 2001). This group showed the association of a polymorphism in the CD14 gene and the expression of a more severe allergic reaction. CD14 does not directly transmit the signal across the plasma membrane but CD14 acts cooperatively with Tlr4 by focusing LPS on the cell surface, whereas Tlr4 act as the signal transducer into the cell (Beutler, 2000).

Further, individuals who are unable to generate suitable antioxidant defenses might have an in-

creased risk of DEP-induced airway inflammation. Individuals with polymorphisms in phase 2 enzymes such as the glutathione-S-transferases (GSTs) have previously been shown to be associated with asthma (Gilliland et al., 2002; Spiteri et al., 2000). As already shown these patients have enhanced nasal allergic responses to DEP (Gilliland et al., 2004).

'Western' Lifestyle - The Hygiene Hypothesis

The connection between allergic sensitization and lifestyle is well known as hygiene hypothesis. This includes that improved hygiene is suggested to withdraw environmental stimuli from the developing immune system which would turn the immune system toward a Th1-like response (Strachan, 2000). Thus the hygiene hypothesis includes that infections occurring during early childhood and stimulating a Th1 response deviated the immune system away from a Th2 response thus preventing sensitization. This hypothesis is underlined by epidemiologic studies which additionally suggest that the degree of atopy is inversely related to social class, family size, and birth order (Busse and Lemanske, 2001). Further, vaccination against certain infections has been suggested to promote atopic senisitzation by preventing the microbial stimulation that up-regulate Th1-like responses (Warner and Warner, 2000). The greater exposure to endotoxin together with the colonization of the gut flora with Lactobacilli is thought to be responsible for the lower number of asthma among farm children (Nafstad et al., 2000). However, both epidemiologic studies and human challenge studies showed that endotoxin can exacerbate asthma and allergic inflammation (Gereda et al., 2000a; Gereda et al., 2000b).

6.1.4 Conclusion

It is well accepted that environmental factors seem to play an important role in the increased prevalence of allergic disorders. Ambient air pollution may influence sensitization as well as provocation phases of allergic immune responses. It is known that the adjuvant effects on sensitization phase are mostly due to increased IgE response. Thus not only the effects of particle-exposure on DC phenotype but also on DC functionality to interact on B cells switching to IgE were investigated.

In conclusion, particle-exposed DC exhibit a very heterogeneous immunological activation depending on particle characteristics, cell type, origin, and incubation conditions. Although no altered phenotype after particle-exposure of DC in the murine system was observed, MoDC showed slightly modified surface marker expression. Interestingly, these imBMDC lost their functionality to inhibit IgE class switching in B cells. In contrast, concomitant exposure of LPS-stimulated DC with particles, triggers a cellular response that interferes with DC maturation as these DC exhibit a more immature state with reduced levels of IL-12p70 and IL-6 when compared to fully LPS-matured DC. This lack in inflammatory cytokine production might favour a Th2-like immune response, although no effects were detectable when the co-culturing mBMDC with B cells. Taken together, particle exposure leads to a loss of DC function, thus particles rather have immunoinhibitory than

immunostimulatory effects. Future studies of the molecular and cellular mechanism of particles at the level of DC activation and interaction with B cells are warranted.

This thesis shows that the effects only occurred after exposure to highly polluted DEP. Further particle-associated components such as chemical substances, allergens, endotoxin which additionally might influence the modulatory effects of these particles to direct the immune response towards a Th1- or Th2-like response have to be identified. If the substances or classes of substances which contribute to the enhanced allergic immune reaction can be identified, an appropriate step can be made to decrease the emission of the responsible compounds. It might be reasonable to reduce the amount of total emissions or change fuel composition.

6.2 Role of the Endocannabnioid System (ECS) in a Murine Model of Allergic Airway Inflammation

The second part of this thesis shows that the endocannabinoid system seems to play a role not only in physiological processes, but even has an attenuating function during the induction of allergic immune response. The down-regulation of endocannabinoid signaling by ablation of the CB receptors led to an augmented allergen-induced airway inflammation in a murine model. Further, the activation of the system by administration of THC, a ligand which binds to both CB receptors, ameliorated the allergic immune reaction. Thus, indicating a protective role for the ECS in the maintenance of allergic airway diseases.

6.2.1 The ECS in Allergic Immune Responses

The ECS is a complex endogenous signaling system that influences multiple metabolic pathways. Its ubiquitous expression indicates a vital role in normal physiology (De Petrocellis et al., 2004). The ECS is belived to regulate energy balance and behaviours such as food intake, fear, and anxiety, and to modulate lipid and glucose metabolism (Cota et al., 2003; Di Marzo and Matias, 2005). The functions of the ECS in physiological processes especially in immune function are less well studied (Correa et al., 2005).

It seems that endocannabinoids play a role in innate immunity. Besides the release of cytokines and chemokines, immune cells such as lymphocytes, DC, and macrophages from humans and animals which are stimulated by e.g. bacteria produce endocannabinoids, sch as AEA and 2-AG (Di Marzo et al., 1996). These endocannabinoids seem to be involved in cellular migration by functioning as chemotactic agent for leukocytes (Maestroni, 2004; Oka et al., 2004). In addition, these activated cells up-regulate the expression of both types of CB receptors. However, these findings are inconsistent, with some studies showing up-regulation (Klein et al., 1995) and some showing down-regulation of CB receptor expression (Carlisle et al., 2002; Lee et al., 2001; Matias et al., 2002).

Moreover, there is even less information about the influence of the ECS in the development of allergic disease. Recently, the role of the ECS during allergic contact dermatitis was studies by the group of Zimmer (Karsak et al., 2007). They showed in a murine model of cutaneous contact hypersensitivity (CHS) that mice lacking both CB receptors (DKO) display a marked increase in allergic inflammation (Karsak et al., 2007) compared to WT mice using the contact allergen 2,4-dinitrofluorobenzene (DNFB). Allergic contact dermatitis is a T cell-mediated allergic reaction which mounts a specific cutaneous allergic response after repeated allergen contact (Knop et al., 1982) and belongs to the type IV hypersensitivity. Based on the observation that the ECS plays an important role in T cell-mediated allergic immune response, this thesis was conducted to study the role of the ECS in an IgE-dependent allergic disease in a murine model of type I hypersensitivity.

Using the low-dose protocol with 1 μg OVA for sensitization an increased lung infiltration in DKO mice compared to WT mice could be observed. This higher infiltration of total cell count was due to an increase in all cell types counted, namely macrophages, neutrophils, eosinophils, and lymphocytes. The higher infiltration of inflammatory cells into the lung in our mouse model of allergic airway inflammation is in concordance with an increased infiltration of the skin with granulocytes in the CHS model (Karsak et al., 2007). The increase of airway inflammation in DKO mice was associated with histopathological changes in the bronchiolar airways. Analysis of the lung tissues following hematoxylin-eosin (H&E) staining showed elevated cellular infiltration into lung tissue in DKO mice in comparison to the WT mice. Likewise gene deficient mice showed increased hyperplasia of mucus cells in the bronchi.

For the CHS model it is suggested that the ECS regulates allergic inflammation through a modulation of the chemokine system, in particular up-regulation of the monocyte chemotactic protein 2 (MCP-2)/chemokine (C-C motif) ligand 8 (CCL8) (Karsak et al., 2007). This means that the exposure to contact allergens lead to the activation of the ECS and the production of endocannabinoids which reduces the allergic response through regulating the chemokine production. Th2 cytokines secreted by T cells play a key role in the induction of allergic airway inflammation, thus cytokine secretion in BAL fluid allows discrimination of a Th1/Th2 biased response. IL-4 and IL-13 are required for IgE production and goblet cell hyperplasia and IL-5 is essential for the development of tissue eosinophilia. IFNγ is a typical cytokine for a Th1 immune response and IL-10 seems necessary for a shift towards a T regulatory response. Despite the increased cell infiltration in their lungs, DKO mice did not show an altered or exacerbated cytokine profile compared to WT mice. However, chemokine levels were not determined. To further investigate whether DKO mice exhibit different cytokine pattern, re-stimulated splenocytes and lymph node cells were analyzed for cytokine secretion as well as intracellular cytokine production. Similar to the findings of BAL cytokines, no altered expression of these cytokines was found in cells of the spleen or lymph node of DKO mice compared to WT mice (data not shown).

Further, allergic airway response in WT mice treated with the antagonists SR1 and/or SR2 which selectively bind to CB1 receptor or CB2 receptor were investigated. Further, kinetic studies were included by applying the antagonists either only during sensitization or elicitation or during both phases. This setting allowed the investigation of the role of the single CB receptors in the regulation of the airway inflammation and additionally to determine the time frame in which they might exert their effects.

There was no significant regulation pattern in airway inflammation nor in immunoglobulin production detectable within this setting. Even the increased lung inflammation of DKO observed in the low-dose protocol with 1 μg OVA could not be seen in the high-dose protocol with 10 μg OVA. Despite the fact that not all experiments are conclusive, as the experiment with 10 μg OVA did not supported these findings, it appears that in addition to the role in CHS that the ECS has an attenuating function in allergic airway inflammation. This might be due to the already high level of inflammation in this single experiment. About 80% of the cells found in the BAL were eosinophils

compared to about 40-60% eosinophils in other experiments. However, the total cell numbers in these two experiments remained similar. This tremendous increase in eosinophil numbers might be one possible reason why no further augmentation of lung inflammation could be observed in DKO mice. The reason for the variability between experiments is unclear but is most likely due to the variability which often occurs in *in vivo* models. Further, if the dose of antagonists does not produce observable defects, one cannot conclude that there are no effects, since the dose may simply have been too low to produce an effect. Thus, the evaluation whether administration of antagonist were without significant changes is not possible because it is not clear whether the use of the higher OVA dose or the lack of specific effects are the reason therefor.

6.2.2 THC in Allergic Airway Inflammation

THC Effects in a Murine Model

Cannabinoids have been characterized as immunomodulators. They generally suppress immune responses, however some studies show an enhancing effect (Friedman et al., 1995). Nevertheless, it is well-known that cannabinoids exert many differential effects on the immune system including responses associated with innate, humoral, and cell-mediated immunity. However, data concerning the role of cannabinoids in allergic disease are low. Earlier, it was already demonstrated that cannabinoid treatment attenuated bronchoconstriction associated with asthma (Tashkin et al., 1974; Tashkin et al., 1975). The study of Jan et al. showed that cannabinoid treatment attenuated allergic airway response in A/J mice (Jan et al., 2003). Further, THC was shown to reduce allergic inflammation in an animal model of CHS (Karsak et al., 2007). This, together with the results obtained from CB receptor deficient mice leads to the suggestion that the activation of the ECS by e.g. administration of cannabinoids might reduce allergic airway inflammation in WT mice.

Thus, in a further experiment the effects of the activation of the ECS in allergic airway inflammation were examined. Therefore, the plant-derived cannabinoid THC was applied to WT mice. THC activates both the CB1 and CB2 receptor because it binds non-selectively to both CB receptors. However, the affinity of THC to bind to CB2 receptor is less than to CB1 receptor. Further, as a partial (low-efficacy) agonist, THC can behave either as an agonist or antagonist at CB2 receptors (Howlett et al., 2002; Pertwee, 2002). Kinetic studies were carried out to investigate which phase of the allergic airway response is most susceptible to THC effects.

This thesis clearly demonstrates that administration of THC significantly diminished eosinophilic airway inflammation as well as serum allergen-specific IgE and IgG1 as well as total IgE levels in a mouse model of asthma.

An increase in total cell number of BAL cells was observed in OVA-sensitized mice compared to that from non-sensitized control mice. This increase was due to an increase in total cell number mainly of macrophages and eosinophils and in part of neutrophils. This prominent leukocytic infiltration of eosinophils, lymphocystes, and neutrophils is a typical characterization of late-phase allergic

reaction (Cohn et al., 2004; Kay, 2001).
A decrease in total numbers of eosinophils and macrophages was observed by differential cell staining from cytospin preparations of BAL cells in mice treated with THC during sensitization phase, elicitation phase, or both phases, respectively. Further, the percentage contribution of cell types adjusts to that of a non-sensitized animal. Thus, treatment of mice with THC leads to an alleviated inflammation of airways. Greatest effects on inhibition of lung inflammation were seen when THC was administered during sensitization and elicitation phase, thus it seems like that both phases of the allergic immune response are susceptible to THC effects.

Although eosinophils are key cells in asthma, their proliferation and inflitration appear to be controlled by T cells. This T cell hypothesis of asthma suggests that the disease is regulated by specialized activated T memory cells which 'home' to the lung after an appropriate allergen exposure. No significant alterations in lymphocyte numbers could be seen in the BAL within different study groups. However, only total lymphocyte count was determined in this study. Another group showed an increase in numbers of activated CD4 (expressing e.g. CD25) and CD8 T cells in both BAL fluid and blood from asthmatic patients compared with normal healthy non-atopic control subjects (Walker et al., 1991). Thus, the consistence of T cell populations in the spleen could be advantageous. The ratio of CD4/CD8 cells was altered after THC treatment which was due to altered numbers of CD4 positive cells. However, there was no consistent up- or down-regulation. Further, the induction of T cells of the regulatory type was analyzed. As no changes in the percentage of CD4/CD25/FoxP3-expressing cells were detectable, it might be assumed that Tregs do not play a pivotal role in the effects mediated through THC treatment.

Several other studies already showed that cannabinoids modulate immune response by regulating cytokine production. Many studies have shown that cannabinoids mostly suppress the production of cytokines in the innate and adaptive immue responses (Berdyshev, 2000; Klein et al., 2000; Klein et al., 1998). However, cannabinoids have also been shown to increase the production of cytokines (e.g. TNF, IL-1, IL-6, IL-10). Thus cannabinoids might either suppress or enhance the production of pro-inflammatory agents. This might depend on the stimulus itself, the type of cannabinoid, or the applied dosage of cannabinoid as many of the effects mediated by cannabinoids are biphasic, e.g. increased activity with acute or smaller doses, decreased activity with larger doses or chronic use. One study demonstrated that cannabinoids inhibit the regulation of cytokines including IL-2, and the Th2-like cytokines IL-4, IL-5, and IL-13 by inhibiting DNA binding of the nuclear factor of activated T cells (NFAT) (Jan et al., 2002; Yea et al., 2000). Moreover, the study from Jan et al. showed that cannabinoid treatment effectively attenuated OVA-induced Th2 cytokine (IL-2, IL-4, IL-5, and IL-13) steady-state mRNA expression in lung tissue (Jan et al., 2003).

Further, it is known that increased eosinophil infiltration is driven by over-expression of chemokines as well as Th2 cytokines, such as IL-4, IL-5, and IL-13, which leads to an exacerbated airway inflammation. Although the expression and production of Th2 cytokines are important for IgE-production, airway inflammation, and induction of mucous hypersecretion by goblet cells, no effects of THC-administration onto cytokine levels in BAL fluids could be observed. Cytokine levels were similarly

in response to THC exposures compared to controls.

Considerations on THC in vivo

The pharmacokinetics of a substance relate to the kinetics of its absorption, distribution in the body, metabolism, and excretion. The most important parameters are clearance, volume distribution, bioavailability, and half-life. The route of THC-administration might affect its efficacy as it will influence these parameters (Adams and Martin, 1996; Agurell et al., 1986; Brenneisen et al., 1996).

Several animal studies use the i.p. injection of THC, however, little information about absorption and bioavailability of THC after i.p. injection is available. It is well-known that the distribution of a compound throughout the body is similar after i.v. and i.p. administration. The onset of the responses to i.v. THC occurred within 10 min and lasted for approximately 2 hrs (Rosenkrantz et al., 1973). Therefore, one can assume that the absorption and bioavailability of THC are similar when administered i.p. Bioavailability of the lipophilic THC was increased by application in an oil suspension (Agurell et al., 1986; Brenneisen et al., 1996).

THC is mostly plasma protein bound, but because of its lipophilicity it is rapidly distributed into tissues, especially into fatty tissues which is reflected in a large volume of distribution (about 10 l/kg) (Baselt and Carvey, 1995). Elimination of THC mainly occurs by metabolism, including allylic oxidation, epoxidation, aliphatic oxidation, decarboxylation, and conjugation. THC has an active metabolite, 11-hydroxy-THC, which has activity comparable to the parent compound. Further, enterohepatic recirculation of THC metabolites may occur, as metabolites are excreted in the urine and feces (Baselt and Carvey, 1995). The elimination of THC can be described by a 2-compartment model with an α-half-life of about 4 hours and a β-half-life of about 25-36 hours (Hunt and Jones, 1980; Lemberger et al., 1971; Ohlsson et al., 1982).

The doses of THC are applicable to humans following appropriate extrapolation for host mass / surface ratios (Cabral and Dove Pettit, 1998). The mouse is less susceptible to THC than humans. This difference can be expressed on the basis of drug dose which means that 50 mg per kg body-weight in the mouse corresponds with to a dose of 5.1 mg per kg in man assuming equivalency on the basis of mg per m (Klaassen and Doull, 1980). In humans THC intake as much as 15 mg per kg per day has been observed (Tennant and Groesbeck, 1972). Thus, we assume that the repeatedly i.p. administration of THC is sufficient to reach and retain effective THC plasma levels.

Mechanism of THC Effects

THC functions by activating the CB1 and CB2 receptors which are normally engaged by endo-cannabinoids. These CB receptor-dependent mechanisms most probably involve G-protein signaling, as CB receptors are G-protein-coupled receptors. Thus, their ligation during immune stimulation probably leads to regulation of gene products that are required for immune-cell function. Further, it is known that G_i/G_o proteins are differentially activated depending on the type of receptor and the nature of the agonist. While after activation the CB1 receptor interacts with both G_i and G_o proteins, the CB2 receptor interacts efficiently only with G_i protein. Further, in some systems and

under some conditions, CB1 receptor activation might lead to interaction with Gs-proteins (Demuth and Molleman, 2006). This might in part explain why different ligands exert different physiological responses.

The activation of CB receptors affects several major signaling pathways. The activation of CB1 receptor leads to $G_{i/o}$ -protein coupled inhibition of adenylate cyclase followed by a decrease of intracellular cAMP, and stimulation of MAP kinases. cAMP regulates PKA-dependent signaling cascade which is important for gene regulation in immune cells. The expression secretion of IL-2 after forskolin-stimulation was inhibited by THC in the murine T-cell line EL4.IL-2 (Condie et al., 1996). Moreover, THC and cannabinol inhibited cAMP-responsive genes (Herring et al., 1998; Koh et al., 1997). However, 2-AG did not influence cAMP response in mouse splenocytes (Ouyang et al., 1998).

However, it should be considered that beside the CB receptor-dependent effects cannabinoids might act through CB receptor-independent ways. These CB receptor-independent mechanisms might include the interaction with vanilloid receptors which are known to bind endocannabinoids (Veldhuis et al., 2003) or the binding to a yet uncharacterized receptor (Breivogel et al., 2001). In addition, effects on lipid-raft structure and function (Biswas et al., 2003) which are known to be important for immune cell function might be involved (Vogt et al., 2002). Thus cannabinoids might have multiple mechanisms of activity (Jan et al., 2002; Zygmunt et al., 2002). This issue can be investigated by applying THC or other ligands for CB receptors in CB receptor-deficient mice. Moreover, further studies to investigate the involvement of the single CB receptors are warranted. This can be accomplished by using ligands which are either CB1 receptor-selective or CB2 receptor-selective. The use of CB2-selective ligands would be advantageous because they maintain its immunomodulatory activity without psychotropic activity.

6.2.3 Experimental Setup of the Mouse Model: Considerations

Allergic airway inflammation belongs to the type I hypersensitivity (Gell and Coombs, 1963) which comprises the classical allergic immediate type reaction. The mechanism involves activation of mast cells and basophils after IgE-dependent cross-linking of Fcε RI receptors on the cell. Mast cells and eosinophils are thought to be the main effector cells producing soluble factors which are important during recruitment of inflammatory cells or are implicated in pathological changes by the release of mediators such as histamine, respectively (Adelroth, 1998).

A murine model of allergic airway disease was used in this hypothesis-driven approach to investigate the role of the ECS in the establishment of an allergen-induced immune response.

There is controversy over the clinical relevance of the use of animal models; however, animal models are of high interest because they allow the manipulation of variables, e.g. gene knock-outs that are not possible to be studied in humans. Further, the mouse can be sensitized to a number of different antigens, there is a large number of commercial available species-specific reagents, and

dose-response evaluations can be performed. In addition, the murine immune system has been relatively well characterized.

A well-established murine model of allergic airway disease which includes a systemic sensitization phase with OVA/alum and a challenge phase with OVA-aerosol was used in this thesis (Alessandrini et al., 2006; Jakob et al., 2006). One can question the use of an adjuvant during sensitization phase, the way of sensitization, and the dosage of allergen. All these parameters modulate the allergen-induced airway inflammation (Jakob et al., 2006).

Adjuvants already prime the type of immune response and do not resemble the natural exposure of allergens. The absorption of allergen to aluminium hydroxide (alum) leads to Th2-dominated antibody production (Jakob et al., 2006). Alum acts mainly by forming depots which allows a slow release of antigen and thus provides a longer interaction between antigen, APC and lymphocytes (Edelman, 1980; Warren and Chedid, 1988).

Further, systemic sensitization does not reflect the real situation of sensitization against an aeroallergen. A model of airway sensitization in the absence of adjuvant would be more natural mode of sensitization to airborne allergens. However, this leads to a low allergen-specific IgE and limited accumulation of eosinophils in the lung (Hamelmann et al., 1997) as already shown in A/J mice which are known to be a high responder strain. In this thesis, C57BL/6 mice which are known to be low-responder were used, thus the application of a systemic sensitization with OVA is reasonable. The genetic background of the mice is an important factor which determines the outcome of the specific immune response. It is well known that the allergen-specific and total IgE as well as eosinophil counts in the BAL is strain-dependent as the response in A/J mice and BALB/c mice is much higher than in C57BL/6 mice (Ewart et al., 2000; Jakob et al., 2006). The usage of an adjuvant with a systemic sensitization protocol with C57BL/6 mice is necessary to mount an adequate airway inflammation and allows the investigation of substances or mechanisms that modulate this immune response.

Further, duration and allergen-dosage are important factors which might influence the immune response. Sensitization with very high (200 μg) OVA doses over a short period of time did not mount eosinophilia or OVA-specific antibody production in BALB/c mice (Jakob et al., 2006). However, sensitization with intermediate or even low doses of OVA (1 μg) and additional OVA aerosol exposures led to a pronounced eosinophilia. As C57BL/6 mice are known to be low-responder, we used a protocol with 4 repetitive injections of either 1 μg or 10 μg OVA followed by 3 OVA-aerosol challenges every other day. The protocol which uses 10 μg OVA for sensitization is known to mount a stable allergen-induced airway inflammation as well as a humoral immune response. The dose of 1 μg OVA in sensitization was further used in this study for sensitization of C57BL/6 mice is actually a common dose for sensitization of BALB/c mice.

6.2.4 Conclusion

This thesis shows for the first time in a murine model of type I hypersensitivity that the ECS seems to have an attenuating role in the development of an allergen-induced airway inflammation. CB receptor-deficient mice showed a higher airway inflammation compared to WT mice in the low-dose protocol. This was due to an increase in all cell subpopulation as the percentage distribution was not altered in these two study groups. Thus, these results show that the ECS plays a protective role not only in contact dermatitis (Karsak et al., 2007) but also in the induction of an allergic airway response.

Moreover, this study demonstrates that the treatment of C57BL/6 low responder mice with THC nearly abolished airway inflammation irrespective of the time point of application. The profound increase of total cell numbers in the airways of OVA-sensitized mice which was largely due to the increased infiltration of macrophages and eosinophils could be partially reversed by THC treatment of mice. However, allergen-specific IgE production was decreased only when THC was applied during sensitization phase. Similar results were obtained by others in A/J mice which are high responder mice for allergic airway inflammation (Jan et al., 2003).

Together, these results suggest that agonistic ligation of the CB receptors which activate the ECS may have a suppressive effect on the development of some features of allergic asthma. Thus cannabinoid-based drugs might be a promising tool as immunotherapeutic compound in allergic diseases such as asthma. However, cannabinoids which do not exert psychotropic effects are desirable. In regard to the therapeutic potential of cannabinoids, further investigations using other dosing, application routes, or time points of THC administration are clearly needed.

7 References

Adams, I.B., Martin, B.R., 1996. Cannabis: pharmacology and toxicology in animals and humans. Addiction 91, 1585-1614.

Adelroth, E., 1998. How to measure airway inflammation: bronchoalveolar lavage and airway biopsies. Can Respir J 5 Suppl A, 18A-21A.

Agnello, D., Lankford, C.S., Bream, J., Morinobu, A., Gadina, M., O'Shea, J.J., Frucht, D.M., 2003. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol 23, 147-161.

Agurell, S., Halldin, M., Lindgren, J.E., Ohlsson, A., Widman, M., Gillespie, H., Hollister, L., 1986. Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man. Pharmacol Rev 38, 21-43.

Alessandrini, F., Beck-Speier, I., Krappmann, D., Weichenmeier, I., Takenaka, S., Karg, E., Kloo, B., Schulz, H., Jakob, T., Mempel, M., Behrendt, H., 2009. Role of oxidative stress in ultrafine particle-induced exacerbation of allergic lung inflammation. Am J Respir Crit Care Med 179, 984-991.

Alessandrini, F., Schulz, H., Takenaka, S., Lentner, B., Karg, E., Behrendt, H., Jakob, T., 2006. Effects of ultrafine carbon particle inhalation on allergic inflammation of the lung. J Allergy Clin Immunol 117, 824-830.

Alessandrini, F., Semmler-Behnke, M., Jakob, T., Schulz, H., Behrendt, H., Kreyling, W., 2008. Total and regional deposition of ultrafine particles in a mouse model of allergic inflammation of the lung. Inhal Toxicol 20, 585-593.

Ando, K., Maeda, J., Inaji, M., Okauchi, T., Obayashi, S., Higuchi, M., Suhara, T., Tanioka, Y., 2008. Neurobehavioral protection by single dose l-deprenyl against MPTP-induced parkinsonism in common marmosets. Psychopharmacology (Berl) 195, 509-516.

Banchereau, J., Bazan, F., Blanchard, D., Briere, F., Galizzi, J.P., van Kooten, C., Liu, Y.J., Rousset, F., Saeland, S., 1994. The CD40 antigen and its ligand. Annu Rev Immunol 12, 881-922.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245-252.

Barfknecht, T.R., Hites, R.A., Cavaliers, E.L., Thilly, W.G., 1982. Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. Dev Toxicol Environ Sci 10, 277-294.

Barnes, K.C., Marsh, D.G., 1998. The genetics and complexity of allergy and asthma. Immunol Today 19, 325-332.

Baselt, R.C., Carvey, R.H., 1995. Disposition of Toxic Drugs and Chemicals in Man 4th ed. Chemical Toxicology Institute, Foster City, CA.

Baulig, A., Sourdeval, M., Meyer, M., Marano, F., Baeza-Squiban, A., 2003. Biological effects of atmospheric particles on human bronchial epithelial cells. Comparison with diesel exhaust particles. Toxicol In Vitro 17, 567-573.

Baxter, A.G., Hodgkin, P.D., 2002. Activation rules: the two-signal theories of immune activation. Nat Rev Immunol 2, 439-446.

Bayram, H., Devalia, J.L., Sapsford, R.J., Ohtoshi, T., Miyabara, Y., Sagai, M., Davies, R.J., 1998. The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. Am J Respir Cell Mol Biol 18, 441-448.

Begg, M., Pacher, P., Batkai, S., Osei-Hyiaman, D., Offertaler, L., Mo, F.M., Liu, J., Kunos, G., 2005. Evidence for novel cannabinoid receptors. Pharmacol Ther 106, 133-145.

Berdyshev, E.V., 2000. Cannabinoid receptors and the regulation of immune response. Chem Phys Lipids 108, 169-190.

Bernstein, J.A., Alexis, N., Barnes, C., Bernstein, I.L., Nel, A., Peden, D., Diaz-Sanchez, D., Tarlo, S.M., Williams, P.B., 2004. Health effects of air pollution. J Allergy Clin Immunol 114, 1116-1123.

Beutler, B., 2000. Tlr4: central component of the sole mammalian LPS sensor. Curr Opin Immunol 12, 20-26.

Biswas, K.K., Sarker, K.P., Abeyama, K., Kawahara, K., Iino, S., Otsubo, Y., Saigo, K., Izumi, H., Hashiguchi, T., Yamakuchi, M., Yamaji, K., Endo, R., Suzuki, K., Imaizumi, H., Maruyama, I., 2003. Membrane cholesterol but not putative receptors mediates anandamide-induced hepatocyte apoptosis. Hepatology 38, 1167-1177.

Blackley, C.H., 1873. Experimental researches on the causes and nature of Catarrhus Aestivus (Hay-fever and Hay-asthma). London, Baillière Tindall and Cox.

Blanchard, D.K., Newton, C., Klein, T.W., Stewart, W.E., 2nd, Friedman, H., 1986. In vitro and in vivo suppressive effects of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. Int J Immunopharmacol 8, 819-824.

Bleck, B., Tse, D.B., Jaspers, I., Curotto de Lafaille, M.A., Reibman, J., 2006. Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation. J Immunol 176, 7431-7437.

Bommel, H., Li-Weber, M., Serfling, E., Duschl, A., 2000. The environmental pollutant pyrene induces the production of IL-4. J Allergy Clin Immunol 105, 796-802.

Boonstra, A., Asselin-Paturel, C., Gilliet, M., Crain, C., Trinchieri, G., Liu, Y.J., O'Garra, A., 2003. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. J Exp Med 197, 101-109.

Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.P., Le Fur, G., Casellas, P., 1997. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem 272, 22330-22339.

Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., Le Fur, G., Casellas, P., 1993. Cannabinoid-receptor expression in human leukocytes. Eur J Biochem 214, 173-180.

Breivogel, C.S., Griffin, G., Di Marzo, V., Martin, B.R., 2001. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 60, 155-163.

Brenneisen, R., Egli, A., Elsohly, M.A., Henn, V., Spiess, Y., 1996. The effect of orally and rectally administered delta 9-tetrahydrocannabinol on spasticity: a pilot study with 2 patients. Int J Clin Pharmacol Ther 34, 446-452.

Buckley, N.E., McCoy, K.L., Mezey, E., Bonner, T., Zimmer, A., Felder, C.C., Glass, M., 2000. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. Eur J Pharmacol 396, 141-149.

Busse, W.W., Lemanske, R.F., Jr., 2001. Asthma. N Engl J Med 344, 350-362.

Cabral, G.A., Dove Pettit, D.A., 1998. Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. J Neuroimmunol 83, 116-123.

Campbell, D.J., Butcher, E.C., 2002. Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. J Exp Med 195, 135-141.

Carlisle, S.J., Marciano-Cabral, F., Staab, A., Ludwick, C., Cabral, G.A., 2002. Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol 2, 69-82.

Chain, B.M., 2003. Current issues in antigen presentation–focus on the dendritic cell. Immunol Lett 89, 237-241.

Chan, R.C., Wang, M., Li, N., Yanagawa, Y., Onoe, K., Lee, J.J., Nel, A.E., 2006. Pro-oxidative diesel exhaust particle chemicals inhibit LPS-induced dendritic cell responses involved in T-helper differentiation. J Allergy Clin Immunol 118, 455-465.

Chaudhuri, J., Tian, M., Khuong, C., Chua, K., Pinaud, E., Alt, F.W., 2003. Transcription-targeted DNA deamination by the AID antibody diversification enzyme. Nature 422, 726-730.

Chen, L., 2004. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol 4, 336-347.

Clark, E.A., Ledbetter, J.A., 1994. How B and T cells talk to each other. Nature 367, 425-428.

Cohn, L., Elias, J.A., Chupp, G.L., 2004. Asthma: mechanisms of disease persistence and progression. Annu Rev Immunol 22, 789-815.

Condie, R., Herring, A., Koh, W.S., Lee, M., Kaminski, N.E., 1996. Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4.IL-2. J Biol Chem 271, 13175-13183.

Correa, F., Mestre, L., Molina-Holgado, E., Arevalo-Martin, A., Docagne, F., Romero, E., Molina-Holgado, F., Borrell, J., Guaza, C., 2005. The role of cannabinoid system on immune modulation: therapeutic implications on CNS inflammation. Mini Rev Med Chem 5, 671-675.

Cota, D., Marsicano, G., Tschop, M., Grubler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thone-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C., Pasquali, R., Lutz, B., Stalla, G.K., Pagotto, U., 2003. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112, 423-431.

Cyrys, J., Stolzel, M., Heinrich, J., Kreyling, W.G., Menzel, N., Wittmaack, K., Tuch, T., Wichmann, H.E., 2003. Elemental composition and sources of fine and ultrafine ambient particles in Erfurt, Germany. Sci Total Environ 305, 143-156.

Daniels, A.U., Barnes, F.H., Charlebois, S.J., Smith, R.A., 2000. Macrophage cytokine response to particles and lipopolysaccharide in vitro. J Biomed Mater Res 49, 469-478.

De Petrocellis, L., Cascio, M.G., Di Marzo, V., 2004. The endocannabinoid system: a general view and latest additions. Br J Pharmacol 141, 765-774.

Demuth, D.G., Molleman, A., 2006. Cannabinoid signalling. Life Sci 78, 549-563.

Derocq, J.M., Segui, M., Marchand, J., Le Fur, G., Casellas, P., 1995. Cannabinoids enhance human B-cell growth at low nanomolar concentrations. FEBS Lett 369, 177-182.

Devane, W.A., Dysarz, F.A., 3rd, Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34, 605-613.

Di Marzo, V., 2006. A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists. Trends Pharmacol Sci 27, 134-140.

Di Marzo, V., De Petrocellis, L., Sepe, N., Buono, A., 1996. Biosynthesis of anandamide and related acylethanolamides in mouse J774 macrophages and N18 neuroblastoma cells. Biochem J 316 (Pt 3), 977-984.

Di Marzo, V., Matias, I., 2005. Endocannabinoid control of food intake and energy balance. Nat Neurosci 8, 585-589.

Diaz-Sanchez, D., 1997. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. Allergy 52, 52-56; discussion 57-58.

Diaz-Sanchez, D., Dotson, A.R., Takenaka, H., Saxon, A., 1994. Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. J Clin Invest 94, 1417-1425.

Diaz-Sanchez, D., Garcia, M.P., Wang, M., Jyrala, M., Saxon, A., 1999. Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. J Allergy Clin Immunol 104, 1183-1188.

Diaz-Sanchez, D., Penichet-Garcia, M., Saxon, A., 2000. Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. J Allergy Clin Immunol 106, 1140-1146.

Diaz-Sanchez, D., Proietti, L., Polosa, R., 2003. Diesel fumes and the rising prevalence of atopy: an urban legend? Curr Allergy Asthma Rep 3, 146-152.

Diaz-Sanchez, D., Riedl, M., 2005. Diesel effects on human health: a question of stress? Am J Physiol Lung Cell Mol Physiol 289, L722-723.

Diaz-Sanchez, D., Tsien, A., Fleming, J., Saxon, A., 1997. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol 158, 2406-2413.

Dreher, K.L., Jaskot, R.H., Lehmann, J.R., Richards, J.H., McGee, J.K., Ghio, A.J., Costa, D.L., 1997. Soluble transition metals mediate residual oil fly ash induced acute lung injury. J Toxicol Environ Health 50, 285-305.

Dubois, B., Vanbervliet, B., Fayette, J., Massacrier, C., Van Kooten, C., Briere, F., Banchereau, J., Caux, C., 1997. Dendritic cells enhance growth and differentiation of CD40-activated B lymphocytes. J Exp Med 185, 941-951.

Edelman, R., 1980. Vaccine adjuvants. Rev Infect Dis 2, 370-383.

Ettmayer, P., Mayer, P., Kalthoff, F., Neruda, W., Harrer, N., Hartmann, G., Epstein, M.M., Brinkmann, V., Heusser, C., Woisetschlager, M., 2006. A novel low molecular weight inhibitor of dendritic cells and B cells blocks allergic inflammation. Am J Respir Crit Care Med 173, 599-606.

Ewart, S.L., Kuperman, D., Schadt, E., Tankersley, C., Grupe, A., Shubitowski, D.M., Peltz, G., Wills-Karp, M., 2000. Quantitative trait loci controlling allergen-induced airway hyperresponsiveness in inbred mice. Am J Respir Cell Mol Biol 23, 537-545.

Fahy, O., Tsicopoulos, A., Hammad, H., Pestel, J., Tonnel, A.B., Wallaert, B., 1999. Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. J Allergy Clin Immunol 103, 1115-1124.

Faubert, B.L., Kaminski, N.E., 2000. AP-1 activity is negatively regulated by cannabinol through inhibition of its protein components, c-fos and c-jun. J Leukoc Biol 67, 259-266.

Finkelman, F.D., Yang, M., Orekhova, T., Clyne, E., Bernstein, J., Whitekus, M., Diaz-Sanchez, D., Morris, S.C., 2004. Diesel exhaust particles suppress in vivo IFN-gamma production by inhibiting cytokine effects on NK and NKT cells. J Immunol 172, 3808-3813.

Finkelstein, J.N., Johnston, C., Barrett, T., Oberdorster, G., 1997. Particulate-cell interactions and pulmonary cytokine expression. Environ Health Perspect 105 Suppl 5, 1179-1182.

Fiorentino, D.F., Bond, M.W., Mosmann, T.R., 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170, 2081-2095.

Fiorentino, D.F., Zlotnik, A., Vieira, P., Mosmann, T.R., Howard, M., Moore, K.W., O'Garra, A., 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 146, 3444-3451.

Friedman, H., Klein, T.W., Newton, C., Daaka, Y., 1995. Marijuana, receptors and immunomodulation. Adv Exp Med Biol 373, 103-113.

Fujieda, S., Diaz-Sanchez, D., Saxon, A., 1998. Combined nasal challenge with diesel exhaust particles and allergen induces In vivo IgE isotype switching. Am J Respir Cell Mol Biol 19, 507-512.

Fujimaki, H., Nohara, O., Ichinose, T., Watanabe, N., Saito, S., 1994. IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particulates and antigen. Toxicology 92, 261-268.

Fujimaki, H., Saneyoshi, K., Shiraishi, F., Imai, T., Endo, T., 1997. Inhalation of diesel exhaust enhances antigen-specific IgE antibody production in mice. Toxicology 116, 227-233.

Gauchat, J.F., Lebman, D.A., Coffman, R.L., Gascan, H., de Vries, J.E., 1990. Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. J Exp Med 172, 463-473.

Geha, R.S., Jabara, H.H., Brodeur, S.R., 2003. The regulation of immunoglobulin E class-switch recombination. Nat Rev Immunol 3, 721-732.

Gehr, P., Geiser, M., Im Hof, V., Schurch, S., Waber, U., Baumann, M., 1993. Surfactant and inhaled particles in the conducting airways: structural, stereological, and biophysical aspects. Microsc Res Tech 26, 423-436.

Gell, P.G.H., Coombs, R.R.A., 1963. The classification of allergic reactions underlying diesease. Clinical Aspects of Immunology, Blackwell Science, Oxford, 217-237.

Gereda, J.E., Leung, D.Y., Liu, A.H., 2000a. Levels of environmental endotoxin and prevalence of atopic disease. JAMA 284, 1652-1653.

Gereda, J.E., Leung, D.Y., Thatayatikom, A., Streib, J.E., Price, M.R., Klinnert, M.D., Liu, A.H., 2000b. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 355, 1680-1683.

Gilliland, F.D., Gauderman, W.J., Vora, H., Rappaport, E., Dubeau, L., 2002. Effects of glutathione-S-transferase M1, T1, and P1 on childhood lung function growth. Am J Respir Crit Care Med 166, 710-716.

Gilliland, F.D., Li, Y.F., Saxon, A., Diaz-Sanchez, D., 2004. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. Lancet 363, 119-125.

Glass, M., Northup, J.K., 1999. Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. Mol Pharmacol 56, 1362-1369.

Granum, B., Gaarder, P.I., Eikeset, A., Stensby, B.A., Lovik, M., 2000. The adjuvant effect of particles-importance of genetic background and pre-sensitisation. Int Arch Allergy Immunol 122, 167-173.

Granum, B., Gaarder, P.I., Groeng, E., Leikvold, R., Namork, E., Lovik, M., 2001a. Fine particles of widely different composition have an adjuvant effect on the production of allergen-specific antibodies. Toxicol Lett 118, 171-181.

Granum, B., Gaarder, P.I., Lovik, M., 2001b. IgE adjuvant effect caused by particles - immediate and delayed effects. Toxicology 156, 149-159.

Granum, B., Lovik, M., 2002. The effect of particles on allergic immune responses. Toxicol Sci 65, 7-17.

Hamelmann, E., Oshiba, A., Loader, J., Larsen, G.L., Gleich, G., Lee, J., Gelfand, E.W., 1997. Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. Am J Respir Crit Care Med 155, 819-825.

Hemmi, H., Yoshino, M., Yamazaki, H., Naito, M., Iyoda, T., Omatsu, Y., Shimoyama, S., Letterio, J.J., Nakabayashi, T., Tagaya, H., Yamane, T., Ogawa, M., Nishikawa, S., Ryoke, K., Inaba, K., Hayashi, S., Kunisada, T., 2001. Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor-beta1-dependent cells. Int Immunol 13, 695-704.

Heo, Y., Saxon, A., Hankinson, O., 2001. Effect of diesel exhaust particles and their components on the allergen-specific IgE and IgG1 response in mice. Toxicology 159, 143-158.

Herring, A.C., Koh, W.S., Kaminski, N.E., 1998. Inhibition of the cyclic AMP signaling cascade and nuclear factor binding to CRE and kappaB elements by cannabinol, a minimally CNS-active cannabinoid. Biochem Pharmacol 55, 1013-1023.

Hillard, C.J., Harris, R.A., Bloom, A.S., 1985. Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: fluorescence studies. J Pharmacol Exp Ther 232, 579-588.

Hiura, T.S., Kaszubowski, M.P., Li, N., Nel, A.E., 1999. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 163, 5582-5591.

Holt, P.G., Stumbles, P.A., 2000. Characterization of dendritic cell populations in the respiratory tract. J Aerosol Med 13, 361-367.

Hopkin, J.M., 1997. Mechanisms of enhanced prevalence of asthma and atopy in developed countries. Curr Opin Immunol 9, 788-792.

Howlett, A.C., 2005. Cannabinoid receptor signaling. Handb Exp Pharmacol, 53-79.

Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., Pertwee, R.G., 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54, 161-202.

Howlett, A.C., Fleming, R.M., 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol 26, 532-538.

Hunt, C.A., Jones, R.T., 1980. Tolerance and disposition of tetrahydrocannabinol in man. J Pharmacol Exp Ther 215, 35-44.

Ichinose, T., Takano, H., Miyabara, Y., Sadakaneo, K., Sagai, M., Shibamoto, T., 2002. Enhancement of antigen-induced eosinophilic inflammation in the airways of mast-cell deficient mice by diesel exhaust particles. Toxicology 180, 293-301.

Ichinose, T., Takano, H., Miyabara, Y., Yanagisawa, R., Sagai, M., 1997. Murine strain differences in allergic airway inflammation and immunoglobulin production by a combination of antigen and diesel exhaust particles. Toxicology 122, 183-192.

Iciek, L.A., Delphin, S.A., Stavnezer, J., 1997. CD40 cross-linking induces Ig epsilon germline transcripts in B cells via activation of NF-kappaB: synergy with IL-4 induction. J Immunol 158, 4769-4779.

Imrich, A., Ning, Y., Kobzik, L., 2000. Insoluble components of concentrated air particles mediate alveolar macrophage responses in vitro. Toxicol Appl Pharmacol 167, 140-150.

Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., Steinman, R.M., 1992a. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med 176, 1693-1702.

Inaba, K., Steinman, R.M., Pack, M.W., Aya, H., Inaba, M., Sudo, T., Wolpe, S., Schuler, G., 1992b. Identification of proliferating dendritic cell precursors in mouse blood. J Exp Med 175, 1157-1167.

Jabara, H.H., Ahern, D.J., Vercelli, D., Geha, R.S., 1991. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. J Immunol 147, 1557-1560.

Jabara, H.H., Schneider, L.C., Shapira, S.K., Alfieri, C., Moody, C.T., Kieff, E., Geha, R.S., Vercelli, D., 1990. Induction of germ-line and mature C epsilon transcripts in human B cells stimulated with rIL-4 and EBV. J Immunol 145, 3468-3473.

Jakob, T., Alessandrini, F., Gutermuth, J., Köllisch, G., Javaheri, A., Aguilar, A., Mempel, M., Ring, J., Ollert, M., Behrendt, H., 2006. Phenotyping Allergy in the Laboratory Mouse. In: Hrabe de Angelis, M., Chambon, P., Brown, S. (Eds.) Standards of Mouse Model Phenotyping. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 253-281.

Jan, T.R., Farraj, A.K., Harkema, J.R., Kaminski, N.E., 2003. Attenuation of the ovalbumin-induced allergic airway response by cannabinoid treatment in A/J mice. Toxicol Appl Pharmacol

188, 24-35.

Jan, T.R., Rao, G.K., Kaminski, N.E., 2002. Cannabinol enhancement of interleukin-2 (IL-2) expression by T cells is associated with an increase in IL-2 distal nuclear factor of activated T cell activity. Mol Pharmacol 61, 446-454.

Jung, S., Rajewsky, K., Radbruch, A., 1993. Shutdown of class switch recombination by deletion of a switch region control element. Science 259, 984-987.

Kalinski, P., Hilkens, C.M., Wierenga, E.A., Kapsenberg, M.L., 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today 20, 561-567.

Kaminski, N.E., Koh, W.S., Yang, K.H., Lee, M., Kessler, F.K., 1994. Suppression of the humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G-protein coupled mechanism. Biochem Pharmacol 48, 1899-1908.

Kapsenberg, M.L., 2003. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol 3, 984-993.

Karol, M.H., 2002. Respiratory allergy: what are the uncertainties? Toxicology 181-182, 305-310.

Karsak, M., Gaffal, E., Date, R., Wang-Eckhardt, L., Rehnelt, J., Petrosino, S., Starowicz, K., Steuder, R., Schlicker, E., Cravatt, B., Mechoulam, R., Buettner, R., Werner, S., Di Marzo, V., Tuting, T., Zimmer, A., 2007. Attenuation of allergic contact dermatitis through the endocannabinoid system. Science 316, 1494-1497.

Kay, A.B., 2001. Allergy and allergic diseases. First of two parts. N Engl J Med 344, 30-37.

Kay, A.B., 2006. 100 years of 'Allergy': can von Pirquet's word be rescued? Clin Exp Allergy 36, 555-559.

Klaassen, C.D., Doull, J., 1980. Evaluation of safety. Toxicologic evaluation. In: Doull, J., Klaassen, C.D., Amdur, M.O. (Eds.) Toxicology: The Basic Science of Poisons. Macmillan, New York, p. 11.

Klein, T.W., 2005. Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat Rev Immunol 5, 400-411.

Klein, T.W., Friedman, H., Specter, S., 1998a. Marijuana, immunity and infection. J Neuroimmunol 83, 102-115.

Klein, T.W., Lane, B., Newton, C.A., Friedman, H., 2000. The cannabinoid system and cytokine network. Proc Soc Exp Biol Med 225, 1-8.

Klein, T.W., Newton, C., Friedman, H., 1998b. Cannabinoid receptors and immunity. Immunol Today 19, 373-381.

Klein, T.W., Newton, C., Larsen, K., Lu, L., Perkins, I., Nong, L., Friedman, H., 2003. The cannabinoid system and immune modulation. J Leukoc Biol 74, 486-496.

Klein, T.W., Newton, C., Zhu, W., Daaka, Y., Friedman, H., 1995. delta 9-Tetrahydrocannabinol, cytokines, and immunity to Legionella pneumophila. Proc Soc Exp Biol Med 209, 205-212.

Knop, J., Stremmer, R., Neumann, C., De Maeyer, E., Macher, E., 1982. Interferon inhibits the suppressor T cell response of delayed-type hypersensitivity. Nature 296, 757-759.

Knox, R.B., Suphioglu, C., Taylor, P., Desai, R., Watson, H.C., Peng, J.L., Bursill, L.A., 1997. Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. Clin Exp Allergy 27, 246-251.

Koh, W.S., Crawford, R.B., Kaminski, N.E., 1997. Inhibition of protein kinase A and cyclic AMP response element (CRE)-specific transcription factor binding by delta9-tetrahydrocannabinol (delta9-THC): a putative mechanism of cannabinoid-induced immune modulation. Biochem Pharmacol 53, 1477-1484.

Koppelman, G.H., Reijmerink, N.E., Colin Stine, O., Howard, T.D., Whittaker, P.A., Meyers, D.A., Postma, D.S., Bleecker, E.R., 2001. Association of a promoter polymorphism of the CD14 gene and atopy. Am J Respir Crit Care Med 163, 965-969.

Lam, K.P., Kuhn, R., Rajewsky, K., 1997. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell 90, 1073-1083.

Lambert, A.L., Dong, W., Selgrade, M.K., Gilmour, M.I., 2000. Enhanced allergic sensitization by residual oil fly ash particles is mediated by soluble metal constituents. Toxicol Appl Pharmacol 165, 84-93.

Langenkamp, A., Messi, M., Lanzavecchia, A., Sallusto, F., 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. Nat Immunol 1, 311-316.

Laupeze, B., Amiot, L., Sparfel, L., Le Ferrec, E., Fauchet, R., Fardel, O., 2002. Polycyclic aromatic hydrocarbons affect functional differentiation and maturation of human monocyte-derived dendritic cells. J Immunol 168, 2652-2658.

Lee, S.F., Newton, C., Widen, R., Friedman, H., Klein, T.W., 2001. Differential expression of cannabinoid CB(2) receptor mRNA in mouse immune cell subpopulations and following B cell stimulation. Eur J Pharmacol 423, 235-241.

Lemberger, L., Tamarkin, N.R., Axelrod, J., Kopin, I.J., 1971. Delta-9-tetrahydrocannabinol: metabolism and disposition in long-term marihuana smokers. Science 173, 72-74.

Li, N., Hao, M., Phalen, R.F., Hinds, W.C., Nel, A.E., 2003a. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin Immunol 109, 250-265.

Li, N., Kim, S., Wang, M., Froines, J., Sioutas, C., Nel, A., 2002a. Use of a stratified oxidative stress model to study the biological effects of ambient concentrated and diesel exhaust particulate matter. Inhal Toxicol 14, 459-486.

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J., Nel, A., 2003b. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111, 455-460.

Li, N., Venkatesan, M.I., Miguel, A., Kaplan, R., Gujuluva, C., Alam, J., Nel, A., 2000. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. J Immunol 165, 3393-3401.

Li, N., Wang, M., Oberley, T.D., Sempf, J.M., Nel, A.E., 2002b. Comparison of the pro-oxidative and proinflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages. J Immunol 169, 4531-4541.

Li, X.Y., Gilmour, P.S., Donaldson, K., MacNee, W., 1997. In vivo and in vitro proinflammatory effects of particulate air pollution (PM10). Environ Health Perspect 105 Suppl 5, 1279-1283.

Lim, H.B., Ichinose, T., Miyabara, Y., Takano, H., Kumagai, Y., Shimojyo, N., Devalia, J.L., Sagai, M., 1998. Involvement of superoxide and nitric oxide on airway inflammation and hyperresponsiveness induced by diesel exhaust particles in mice. Free Radic Biol Med 25, 635-644.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., Cerutti, A., 2002. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 3, 822-829.

Lovik, M., Hogseth, A.K., Gaarder, P.I., Hagemann, R., Eide, I., 1997. Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. Toxicology 121, 165-178.

Luo, Y.D., Patel, M.K., Wiederhold, M.D., Ou, D.W., 1992. Effects of cannabinoids and cocaine on the mitogen-induced transformations of lymphocytes of human and mouse origins. Int J Immunopharmacol 14, 49-56.

Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rossner, S., Koch, F., Romani, N., Schuler, G., 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J Immunol Methods 223, 77-92.

Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol, 299-325.

Mackie, K., Stella, N., 2006. Cannabinoid receptors and endocannabinoids: evidence for new players. AAPS J 8, E298-306.

MacLennan, S.J., Reynen, P.H., Kwan, J., Bonhaus, D.W., 1998. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. Br J Pharmacol 124, 619-622.

Maestroni, G.J., 2004. The endogenous cannabinoid 2-arachidonoyl glycerol as in vivo chemoattractant for dendritic cells and adjuvant for Th1 response to a soluble protein. FASEB J 18, 1914-1916.

Maier, K.L., Alessandrini, F., Beck-Speier, I., Hofer, T.P., Diabate, S., Bitterle, E., Stoger, T., Jakob, T., Behrendt, H., Horsch, M., Beckers, J., Ziesenis, A., Hultner, L., Frankenberger, M., Krauss-Etschmann, S., Schulz, H., 2008. Health effects of ambient particulate matter–biological

mechanisms and inflammatory responses to in vitro and in vivo particle exposures. Inhal Toxicol 20, 319-337.

Matias, I., Pochard, P., Orlando, P., Salzet, M., Pestel, J., Di Marzo, V., 2002. Presence and regulation of the endocannabinoid system in human dendritic cells. Eur J Biochem 269, 3771-3778.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561-564.

McWilliam, A.S., Nelson, D., Thomas, J.A., Holt, P.G., 1994. Rapid dendritic cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. J Exp Med 179, 1331-1336.

Mechoulam, R., 2000. Looking back at Cannabis research. Curr Pharm Des 6, 1313-1322.

Mellman, I., Steinman, R.M., 2001. Dendritic cells: specialized and regulated antigen processing machines. Cell 106, 255-258.

Messner, B., Stutz, A.M., Albrecht, B., Peiritsch, S., Woisetschlager, M., 1997. Cooperation of binding sites for STAT6 and NF kappa B/rel in the IL-4-induced up-regulation of the human IgE germline promoter. J Immunol 159, 3330-3337.

Miyabara, Y., Ichinose, T., Takano, H., Lim, H.B., Sagai, M., 1998. Effects of diesel exhaust on allergic airway inflammation in mice. J Allergy Clin Immunol 102, 805-812.

Monn, C., Becker, S., 1999. Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM2.5) and coarse particles (PM10-2.5) in outdoor and indoor air. Toxicol Appl Pharmacol 155, 245-252.

Mu, J., Zhuang, S.Y., Kirby, M.T., Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoid receptors differentially modulate potassium A and D currents in hippocampal neurons in culture. J Pharmacol Exp Ther 291, 893-902.

Muccioli, G.G., 2007. Blocking the cannabinoid receptors: drug candidates and therapeutic promises. Chem Biodivers 4, 1805-1827.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61-65.

Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., Tokiwa, H., 1986. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol 77, 616-623.

Nafstad, P., Magnus, P., Jaakkola, J.J., 2000. Early respiratory infections and childhood asthma. Pediatrics 106, E38.

Nahas, G.G., Osserman, E.F., 1991. Altered serum immunoglobulin concentration in chronic marijuana smokers. Adv Exp Med Biol 288, 25-32.

Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. Science 311, 622-627.

Nel, A.E., Diaz-Sanchez, D., Ng, D., Hiura, T., Saxon, A., 1998. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. J Allergy Clin Immunol 102, 539-554.

Ning, Y., Imrich, A., Goldsmith, C.A., Qin, G., Kobzik, L., 2000. Alveolar macrophage cytokine production in response to air particles in vitro: role of endotoxin. J Toxicol Environ Health A 59, 165-180.

Novak, T.J., Chen, D., Rothenberg, E.V., 1990. Interleukin-1 synergy with phosphoinositide pathway agonists for induction of interleukin-2 gene expression: molecular basis of costimulation. Mol Cell Biol 10, 6325-6334.

Obayashi, K., Doi, T., Koyasu, S., 2007. Dendritic cells suppress IgE production in B cells. Int Immunol 19, 217-226.

Oberdorster, G., Ferin, J., Gelein, R., Soderholm, S.C., Finkelstein, J., 1992. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. Environ Health Perspect 97, 193-199.

Oberdorster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., Yang, H., 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2, 8.

Oberdorster, G., Utell, M.J., 2002. Ultrafine particles in the urban air: to the respiratory tract–and beyond? Environ Health Perspect 110, A440-441.

Ohlsson, A., Lindgren, J.E., Wahlen, A., Agurell, S., Hollister, L.E., Gillespie, H.K., 1982. Single dose kinetics of deuterium labelled delta 1-tetrahydrocannabinol in heavy and light cannabis users. Biomed Mass Spectrom 9, 6-10.

Ohta, K., Yamashita, N., Tajima, M., Miyasaka, T., Nakano, J., Nakajima, M., Ishii, A., Horiuchi, T., Mano, K., Miyamoto, T., 1999. Diesel exhaust particulate induces airway hyperresponsiveness in a murine model: essential role of GM-CSF. J Allergy Clin Immunol 104, 1024-1030.

Ohtani, T., Nakagawa, S., Kurosawa, M., Mizuashi, M., Ozawa, M., Aiba, S., 2005. Cellular basis of the role of diesel exhaust particles in inducing Th2-dominant response. J Immunol 174, 2412-2419.

Ohtoshi, T., Takizawa, H., Okazaki, H., Kawasaki, S., Takeuchi, N., Ohta, K., Ito, K., 1998. Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation in vitro. J Allergy Clin Immunol 101, 778-785.

Oka, S., Ikeda, S., Kishimoto, S., Gokoh, M., Yanagimoto, S., Waku, K., Sugiura, T., 2004. 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces the migration of EoL-1 human eosinophilic leukemia cells and human peripheral blood eosinophils. J Leukoc Biol 76, 1002-1009.

Ormstad, H., 2000. Suspended particulate matter in indoor air: adjuvants and allergen carriers. Toxicology 152, 53-68.

Ormstad, H., Gaarder, P.I., Johansen, B.V., 1997. Quantification and characterisation of suspended particulate matter in indoor air. Sci Total Environ 193, 185-196.

Ouyang, Y., Hwang, S.G., Han, S.H., Kaminski, N.E., 1998. Suppression of interleukin-2 by the putative endogenous cannabinoid 2-arachidonyl-glycerol is mediated through down-regulation of the nuclear factor of activated T cells. Mol Pharmacol 53, 676-683.

Penttinen, P., Timonen, K.L., Tiittanen, P., Mirme, A., Ruuskanen, J., Pekkanen, J., 2001. Ultrafine particles in urban air and respiratory health among adult asthmatics. Eur Respir J 17, 428-435.

Pertwee, R., 2002. Sites and mechnisms of action. . In: Grotenhermen, F., E., R. (Eds.) Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential. Haworth Press, Binghamton (NY), pp. pp 73-88.

Pertwee, R.G., 2005. Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. Life Sci 76, 1307-1324.

Pertwee, R.G., 2006. The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond) 30 Suppl 1, S13-18.

Pertwee, R.G., 2007. GPR55: a new member of the cannabinoid receptor clan? Br J Pharmacol 152, 984-986.

Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J., Heyder, J., 1997. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med 155, 1376-1383.

Peterson, B., Saxon, A., 1996. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. Ann Allergy Asthma Immunol 77, 263-268; quiz 269-270.

Peterson, J.D., Herzenberg, L.A., Vasquez, K., Waltenbaugh, C., 1998. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci U S A 95, 3071-3076.

Phalen, R.F., Oldham, M.J., Nel, A.E., 2006. Tracheobronchial particle dose considerations for in vitro toxicology studies. Toxicol Sci 92, 126-132.

Pope, C.A., 3rd, 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? Environ Health Perspect 108 Suppl 4, 713-723.

Pullen, S.S., Labadia, M.E., Ingraham, R.H., McWhirter, S.M., Everdeen, D.S., Alber, T., Crute, J.J., Kehry, M.R., 1999. High-affinity interactions of tumor necrosis factor receptor-associated factors (TRAFs) and CD40 require TRAF trimerization and CD40 multimerization. Biochemistry 38, 10168-10177.

Rahman, Q., Abidi, P., Afaq, F., Schiffmann, D., Mossman, B.T., Kamp, D.W., Athar, M., 1999. Glutathione redox system in oxidative lung injury. Crit Rev Toxicol 29, 543-568.

Reibman, J., Hsu, Y., Chen, L.C., Bleck, B., Gordon, T., 2003. Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. Am J Respir Cell Mol Biol 28, 648-654.

Reider, N., Reider, D., Ebner, S., Holzmann, S., Herold, M., Fritsch, P., Romani, N., 2002. Dendritic cells contribute to the development of atopy by an insufficiency in IL-12 production. J Allergy Clin Immunol 109, 89-95.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., Ricciardi-Castagnoli, P., 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2, 361-367.

Riedl, M., Diaz-Sanchez, D., 2005. Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 115, 221-228; quiz 229.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., et al., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350, 240-244.

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G.L., 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 284, 644-650.

Ring, J., 1998. Angewandte Allergologie, MMV Medizin Verlag, München.

Ring, J., Kramer, U., Schafer, T., Behrendt, H., 2001. Why are allergies increasing? Curr Opin Immunol 13, 701-708.

Roitt, I.M., 1984. Essential Immunology, Blackwell Science, Oxford.

Rosenkrantz, H., Irwin, A.H., Braude, M.C., 1973. Inhalation, Parenteral and Oral LD50 Values of delta-9-Tetrahydrocannabinol in Fischer Rats. Toxicol Appl Pharmacol 28, 18-27.

Sagai, M., Furuyama, A., Ichinose, T., 1996. Biological effects of diesel exhaust particles (DEP). III. Pathogenesis of asthma like symptoms in mice. Free Radic Biol Med 21, 199-209.

Sallusto, F., Mackay, C.R., Lanzavecchia, A., 1997. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 277, 2005-2007.

Salvi, S., Holgate, S.T., 1999. Mechanisms of particulate matter toxicity. Clin Exp Allergy 29, 1187-1194.

Schatz, A.R., Koh, W.S., Kaminski, N.E., 1993. Delta 9-tetrahydrocannabinol selectively inhibits T-cell dependent humoral immune responses through direct inhibition of accessory T-cell function. Immunopharmacology 26, 129-137.

Schober, W., Belloni, B., Lubitz, S., Eberlein-Konig, B., Bohn, P., Saritas, Y., Lintelmann, J., Matuschek, G., Behrendt, H., Buters, J., 2006. Organic extracts of urban aerosol ($<$ or $=$PM2.5) enhance rBet v 1-induced upregulation of CD63 in basophils from birch pollen-allergic individuals. Toxicol Sci 90, 377-384.

Schober, W., Lubitz, S., Belloni, B., Gebauer, G., Lintelmann, J., Matuschek, G., Weichenmeier, I., Eberlein-Konig, B., Buters, J., Behrendt, H., 2007. Environmental polycyclic aromatic hydro-

carbons (PAHs) enhance allergic inflammation by acting on human basophils. Inhal Toxicol 19 Suppl 1, 151-156.

Schwartz, J., Neas, L.M., 2000. Fine particles are more strongly associated than coarse particles with acute respiratory health effects in schoolchildren. Epidemiology 11, 6-10.

Schwartz, J., Norris, G., Larson, T., Sheppard, L., Claiborne, C., Koenig, J., 1999. Episodes of high coarse particle concentrations are not associated with increased mortality. Environ Health Perspect 107, 339-342.

Seaton, A., MacNee, W., Donaldson, K., Godden, D., 1995. Particulate air pollution and acute health effects. Lancet 345, 176-178.

Serafini, U., 1997. Do infections protect against asthma and atopy? Allergy 52, 955-957.

Sjogren, M., Li, H., Banner, C., Rafter, J., Westerholm, R., Rannug, U., 1996. Influence of physical and chemical characteristics of diesel fuels and exhaust emissions on biological effects of particle extracts: a multivariate statistical analysis of ten diesel fuels. Chem Res Toxicol 9, 197-207.

Spiteri, M.A., Bianco, A., Strange, R.C., Fryer, A.A., 2000. Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation. Allergy 55 Suppl 61, 15-20.

Steinman, R.M., 2003. The control of immunity and tolerance by dendritic cell. Pathol Biol (Paris) 51, 59-60.

Strachan, D.P., 2000. The role of environmental factors in asthma. Br Med Bull 56, 865-882.

Suzuki, T., Kanoh, T., Kanbayashi, M., Todome, Y., Ohkuni, H., 1993. The adjuvant activity of pyrene in diesel exhaust on IgE antibody production in mice. Arerugi 42, 963-968.

Takafuji, S., Suzuki, S., Koizumi, K., Tadokoro, K., Miyamoto, T., Ikemori, R., Muranaka, M., 1987. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. J Allergy Clin Immunol 79, 639-645.

Takafuji, S., Suzuki, S., Koizumi, K., Tadokoro, K., Ohashi, H., Muranaka, M., Miyamoto, T., 1989. Enhancing effect of suspended particulate matter on the IgE antibody production in mice. Int Arch Allergy Appl Immunol 90, 1-7.

Takano, H., Yoshikawa, T., Ichinose, T., Miyabara, Y., Imaoka, K., Sagai, M., 1997. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. Am J Respir Crit Care Med 156, 36-42.

Takenaka, H., Zhang, K., Diaz-Sanchez, D., Tsien, A., Saxon, A., 1995. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. J Allergy Clin Immunol 95, 103-115.

Tashkin, D.P., Shapiro, B.J., Frank, I.M., 1974. Acute effects of smoked marijuana and oral delta9-tetrahydrocannabinol on specific airway conductance in asthmatic subjects. Am Rev Respir Dis 109, 420-428.

Tashkin, D.P., Shapiro, B.J., Lee, Y.E., Harper, C.E., 1975. Effects of smoked marijuana in experimentally induced asthma. Am Rev Respir Dis 112, 377-386.

Tennant, F.S., Jr., Groesbeck, C.J., 1972. Psychiatric effects of hashish. Arch Gen Psychiatry 27, 133-136.

Terada, N., Hamano, N., Maesako, K.I., Hiruma, K., Hohki, G., Suzuki, K., Ishikawa, K., Konno, A., 1999. Diesel exhaust particulates upregulate histamine receptor mRNA and increase histamine-induced IL-8 and GM-CSF production in nasal epithelial cells and endothelial cells. Clin Exp Allergy 29, 52-59.

Trinchieri, G., 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3, 133-146.

Tsien, A., Diaz-Sanchez, D., Ma, J., Saxon, A., 1997. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. Toxicol Appl Pharmacol 142, 256-263.

van Zijverden, M., van der Pijl, A., Bol, M., van Pinxteren, F.A., de Haar, C., Penninks, A.H., van Loveren, H., Pieters, R., 2000. Diesel exhaust, carbon black, and silica particles display distinct Th1/Th2 modulating activity. Toxicol Appl Pharmacol 168, 131-139.

Veldhuis, W.B., van der Stelt, M., Wadman, M.W., van Zadelhoff, G., Maccarrone, M., Fezza, F., Veldink, G.A., Vliegenthart, J.F., Bar, P.R., Nicolay, K., Di Marzo, V., 2003. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J Neurosci 23, 4127-4133.

Verstraelen, S., Van Den Heuvel, R., Nelissen, I., Witters, H., Verheyen, G., Schoeters, G., 2005. Flow cytometric characterisation of antigen presenting dendritic cells after in vitro exposure to diesel exhaust particles. Toxicol In Vitro 19, 903-907.

Vogt, A.B., Spindeldreher, S., Kropshofer, H., 2002. Clustering of MHC-peptide complexes prior to their engagement in the immunological synapse: lipid raft and tetraspan microdomains. Immunol Rev 189, 136-151.

Walker, C., Kaegi, M.K., Braun, P., Blaser, K., 1991. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J Allergy Clin Immunol 88, 935-942.

Wallace, J.M., Tashkin, D.P., Oishi, J.S., Barbers, R.G., 1988. Peripheral blood lymphocyte subpopulations and mitogen responsiveness in tobacco and marijuana smokers. J Psychoactive Drugs 20, 9-14.

Warner, J.A., Warner, J.O., 2000. Early life events in allergic sensitisation. Br Med Bull 56, 883-893.

Warren, H.S., Chedid, L.A., 1988. Future prospects for vaccine adjuvants. Crit Rev Immunol 8, 83-101.

Wartmann, M., Campbell, D., Subramanian, A., Burstein, S.H., Davis, R.J., 1995. The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. FEBS Lett 359, 133-136.

Wedemeyer, J., Tsai, M., Galli, S.J., 2000. Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 12, 624-631.

Whitekus, M.J., Li, N., Zhang, M., Wang, M., Horwitz, M.A., Nelson, S.K., Horwitz, L.D., Brechun, N., Diaz-Sanchez, D., Nel, A.E., 2002. Thiol antioxidants inhibit the adjuvant effects of aerosolized diesel exhaust particles in a murine model for ovalbumin sensitization. J Immunol 168, 2560-2567.

Whiteside, G.T., Lee, G.P., Valenzano, K.J., 2007. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. Curr Med Chem 14, 917-936.

Wiley, J.L., Martin, B.R., 2002. Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chem Phys Lipids 121, 57-63.

Williams, M.A., Porter, M., Horton, M., Guo, J., Roman, J., Williams, D., Breysse, P., Georas, S.N., 2007. Ambient particulate matter directs nonclassic dendritic cell activation and a mixed TH1/TH2-like cytokine response by naive CD4+ T cells. J Allergy Clin Immunol 119, 488-497.

Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410, 588-592.

Winzler, C., Rovere, P., Rescigno, M., Granucci, F., Penna, G., Adorini, L., Zimmermann, V.S., Davoust, J., Ricciardi-Castagnoli, P., 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. J Exp Med 185, 317-328.

Yang, H.M., Ma, J.Y., Castranova, V., Ma, J.K., 1997. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor-alpha from rat alveolar macrophages. Exp Lung Res 23, 269-284.

Yea, S.S., Yang, K.H., Kaminski, N.E., 2000. Role of nuclear factor of activated T-cells and activator protein-1 in the inhibition of interleukin-2 gene transcription by cannabinol in EL4 T-cells. J Pharmacol Exp Ther 292, 597-605.

Zal, T., Volkmann, A., Stockinger, B., 1994. Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. J Exp Med 180, 2089-2099.

Zheng, Z.M., Specter, S., Friedman, H., 1992. Inhibition by delta-9-tetrahydrocannabinol of tumor necrosis factor alpha production by mouse and human macrophages. Int J Immunopharmacol 14, 1445-1452.

Zhu, L.X., Sharma, S., Stolina, M., Gardner, B., Roth, M.D., Tashkin, D.P., Dubinett, S.M., 2000. Delta-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. J Immunol 165, 373-380.

Zimmer, A., Zimmer, A.M., Hohmann, A.G., Herkenham, M., Bonner, T.I., 1999. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A 96, 5780-5785.

Zygmunt, P.M., Andersson, D.A., Hogestatt, E.D., 2002. Delta 9-tetrahydrocannabinol and cannabinol activate capsaicin-sensitive sensory nerves via a CB1 and CB2 cannabinoid receptor-independent mechanism. J Neurosci 22, 4720-4727.

8 Appendix

8.1 Abbreviations

Abbreviation	Explanation
2-AG	2-arachidonoyl glycerol
AC	adenylyl cyclase
AEA	arachidonoylethanolamide = anandamide
AERex	organic extracts of urban aerosol
ag	antigen
AHR	airway hyperreactivity
AID	activation-induced cytidine deaminase
APC	antigen presenting cell
APC	allophycocyanin
APRIL	a proliferation-induced ligand
aqua. dest	distilled water
BAFF	B cell activating factor
BMDC	bone marrow-derived dendritic cells
BSA	bovine serum albumin
CB	cannabinoid
CCL	C-chemokine ligand
CCR	chemokine (C-C motif) receptor
CD	cluster of differentiation
C-Euro3	DEP prepared with catalytic device
CHS	contact hypersensitivity
CSR	class switch recombination
CTLA	cytotoxic T-lymphocyte antigen
DC	dendritic cell
DEP	diesel exhaust particles
DKO	double knock-out
DMSO	dimethylsulfoxid
DNA'	deoxyribonucleic acid
DNFB	2,4-dinitrofluorobenzene
DPBS	Dulbecco's phosphate buffered saline
DPM	diesel particulate matter
ECS	endocannabinoid system

Abbreviation	Explanation
EDTA	Ethylendiamintetraacetat
ELISA	enzyme-linked immunoabsorbent assay
EPA	environmental protection agency
ERK	extracellular signal-regulated kinases
Euro3	DEP prepared without catalytic device
FACS	fluorescent activated cell sorting
FBS	fetal bovine serum
FCS	fetal calf serum
FCS	forward scatter
Fcε RI	high affinity Fcε receptor I
Fi medium	first incubation medium
FITC	flouresence isothiocyanat
FoxP3	Forkhead box P3
GM-CSF	granulocyte macrophage colony-stimulating factor
GTL	germline transcripts
HBSS	Hank's balanced salt solution
HE	hematoxylin-eosin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HLA	human leucocyte antigen
huR10	human DC medium
i.p.	intraperetoneal
IFN	interferon
Ig	immunoglobulin
IL	interleukin
imBMDC	immature BMDC
JAK	Janus-activated kinase
kg	kilogram
KLH	keyhole limpet hemocyanin
LAL	limulus amebocyte lysate

Abbreviation	Explanation
LPS	lipopolysaccharide
M	molar
mAb	monoclonal antibody
MACS	magnetic activated cell sorting
MAPK	mitogen-activated protein kinase
mBMDC	mature BMDC
MCP	monocyte chemotactic protein
MHC II	major histocompativility class II
N	normal
NF-xB	nuclear factor xB
OD	optical densitiy
OVA	ovalbumin
PAH	polycyclic aromatic hydrocarbons
PAMP	pathogen-associated molecular patterns
PAS	perodic acid schiff's
PBMC	peripheral blood monocyte
PBS	phosphate buffered saline
PE	phycoerythrin
PGE2	prostaglandin E2
Phe	phenanthrene
PI	propidium iodide
PKA	protein kinase A
PM	particulate matter
poly(I:C)	polyinosinic:polycytidylic acid
R10	standard medium
R10	mouse DC medium
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	rounds per minute
RPMI	Roswell Park Memorial Institute
Sav-HPR	
SD	standard deviation
SEM	standard error of mean

Abbreviation	Explanation
SPF	specified pathogen free
SR141716A	CB1 receptor antagonist: (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride)
SR144528	CB2 receptor antagonist: (N-{(1S)-endo-1,3,3-trimethyl bicyclo[2.2.1 heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide})
SRM	standard reference material
SSC	sideward scatter
STAT	signal transducer and activator of transcription
TCR	T cell receptor
TF	tissue factor
TGF	transforming growth factor
Th1	T helper type 1 cells
Th2	T helper type 2 cells
THC	dela-9-tetrahydrocannabinol
TLR	toll like receptor
TMB	tetramethylbenzidin
TNF	tumour necroses factor
TNFR	TNF receptor-associated factor
TRAF	TNFR-associated factor
Treg	regulatory T cells
TRIS	trishydroxymethylaminomethane
TRPV1	transient receptor potential vanilloid type 1
U	units
wt	wildtype

8.2 Supplementary Data

Table 22: Endotoxin contents

	LPS [pg/ml]		LPS [pg/ml]
Phenanthrene#	n.d.	SRM*	270
EPA-PAH#	n.d.	Euro3*	4300
AERex I#	n.d.	C-Euro3*	3300
AERex IV#	n.d.	CB*	380

Note. LPS was quantified by Limulus Amebocyte Lysate (LAL) assay according to the manufacturer. n.d., not detected. Solvent was # DMSO or * PBS and samples were further diluted in R10 medium.

Table 23: Absolute cytokine levels from BMDC cultures

Absolute cytokine levels Mean ± SEM [pg/ml]									
	IL-6			IL-10			IL-12		
A									
Medium									
± Phenanthrene	194.24	±	80.07		n.d.			n.d.	
± EPA-PAH		n.d.			n.d.			n.d.	
± AERex I	234.24	±	53.15		n.d.			n.d.	
± AERex IV	99.35	±	28.34		n.d.			n.d.	
± DMSO	132.52	±	32.33		n.d.			n.d.	
LPS									
± Phenanthrene	54,303.40	±	12,270.05	88.38	±	7.76	1,534.73	±	274.58
± EPA-PAH	13,174.46	±	2,630.35	51.13	±	6.66	450.35	±	133.29
± AERex I	32,033.33	±	5,281.48	200.61	±	110.90	496.23	±	125.27
± AERex IV	39,650.00	±	2,626.94	176.28	±	86.56	1,102.19	±	300.79
± DMSO	25,667.97	±	4,031.44	449.29	±	201.24	5,110.97	±	3,235.67
B									
Medium									
± DPM	141.67	±	19.04		n.d.			n.d.	
± Euro3	126.97				n.d.			n.d.	
± C-Euro3	110.91				n.d.			n.d.	
± CB	172.98	±	29.29		n.d.			n.d.	
LPS									
± DPM	101,487.7	±	36,035.81	92.23	±	13.98	1,466.24	±	190.90
± Euro3	23,950	±	3,833.81	81.41	±	13.06	913.5	±	204.54
± C-Euro3	25,650	±	3,781.28	83.62	±	11.05	798.14	±	146.31
± CB	120,397.67	±	51,175.80	97.52	±	13.43	1,461.9	±	323.66
± PBS	80.22	±	8.76	625.49	±	70.3	986.84	±	93.93

Note. Medium-treated or LPS-stimulated BMDC were exposed to (A) particle-associated organic compounds or (B) particles as indicated. The secretion of cytokines into the supernatant was analyzed by ELISA. Data are presented as absolute values as mean ± SEM in [pg/ml]. These data represent reference values for the calculation of percentage-induced cytokine secretion of medium-treated or LPS-stimulated BMDC.

Table 24: Absolute immunoglobulin levels from stimulated B cell cultures

Absolute immunoglobulin levels Mean ± SEM [ng/ml]									
	IgE			**IgM**			**IgG**		
anti-CD40/IL-4	**2,899.61**	±	**3,815.43**	**2,222.72**	±	**1,085.51**	**27.88**	±	**48.76**
+ imBMDC	371.98 *	±	680.03	1,329.03 **	±	585.21	49.73	±	58.35
+ mBMDC	817.84	±	1,202.72	1,292.99 **	±	464.20	111.28 **	±	145.28

Note. B cells were either stimulated with anti-CD40/IL-4 or stimulated and co-cultured with imBMDC or mBMDC as described. The secretion of immunoglobulins into the supernatant was analyzed by ELISA. Data are presented as absolute values and given as mean ± SEM in [ng/ml]. These data represent reference values for the calculation of percentage abberation compared to only stimulated B cells. Statistical significance: *p<0.05 and **<0.01 by paired student's *t*-test compared to stiumlated control B cells.

Table 25: Absolute immunoglobulin levels in stimulated B cells co-cultured with SRM-exposed BMDC

Absolute immunoglobulin levels Mean ± SEM [ng/ml]									
	IgE			**IgM**			**IgG**		
A) + imBMDC									
-	**371.98**	±	**680.03**	**1329.03**	±	**585.21**	**49.73**	±	**58.35**
+ 1 μg/ml SRM	236.96	±	342.27	1159.911	±	440.8587	41.32	±	54.93
+ 10 μg/ml SRM	1,480.53	±	3,327.52	1369.113	±	463.1016	59.89	±	70.41
+ 100 μg/ml SRM	2,560.44	±	6,703.92	1616.914	±	496.569	95.42	±	155.44
B) + mBMDC									
-	**817.84**	±	**1,202.72**	**1,292.99**	±	**464.20**	**111.28**	±	**145.28**
+ 1 μg/ml SRM	716.01	±	907.38	1,202.67	±	281.71	100.52	±	139.83
+ 10 μg/ml SRM	1,379.17	±	2,728.05	1,305.31	±	434.17	91.87	±	133.24
+ 100 μg/ml SRM	1,170.79	±	2,676.67	1,240.08	±	660.67	83.19	±	131.83

Note. B cells were stimulated with anti-CD40/IL-4 and co-cultured with (A) imBMDC or (B) mBMDC which were pre-exposed to SRM at indicated concentrations. The secretion of immunoglobulins into the supernatant was analyzed by ELISA. Data are presented as absolute values and given as mean ± SEM in [ng/ml].

Table 26: Absolute cytokine levels of MoDC cultures

Absolute cytokine levels Mean ± SEM [pg/ml]												
	IL-6			**IL-10**			**IL-12**			**TNFa**		
Medium												
± Pheanthrene	n.d.			25.83	±	5.52	n.d.			n.d.		
± DMSO	n.d.			n.d.			n.d.			n.d.		
± SRM	n.d.			18.08	±	3.55	n.d.			n.d.		
LPS												
± Pheanthrene	56,503	±	22,097	3,922	±	1,918	2,153	±	599	3,352	±	1,150
± DMSO	73,063	±	29,009	4,074	±	2,953	2,093	±	560,2	34,571	±	25,053.
± SRM	62,140	±	20,865	2,912	±	1,349	1,999	±	692,5	4,217.	±	1,421
poly(I:C)												
± Pheanthrene	19,754	±	5,281	420.56	±	297.08	46,776	±	21,255	5,455	±	2,749
± DMSO	87,539	±	48,345	81.60	±	25.01	18,500			26,308	±	18,590
± SRM	18,169	±	6,678	373.85	±	259.35	38,383	±	21,590	6,282	±	2,651

Note. Meidum-treated, LPS-, or poly(I:C)-stimulated BMDC were exposed to phenanthrene, DMSO, or SRM as indicated. The secretion of cytokines into the supernatant was analyzed by ELISA. Data are presented as absolute values as mean ± SEM in [pg/ml]. These data represent reference values for the medium-/ LPS- or poly(I:C)-induced cytokine secretion after exposure of cells.

Table 27: Percentage aberation of total cell counts in WT and DKO mice

	[%]	**p-value (to S/VH)**
WT/NS	7.35	0.012*
WT/S	**100**	-
DKO/NS	6.92	0.012*
DKO/S	223.38	0.28

Note. Percentage abberation of total cell counts compared to OVA-sensitized wt control group (WT/S). Statistical significance: *$p<0.05$ by paired student's *t*-test compared to control group (mean; n=4-6/ group).

Table 28: Percentage aberation of total cell counts

	[%]	p-value (to S/VH)
WT/NS	1.59	
DKO/NS	5.75	
WT/S	**100**	-
DKO/S	95.96	0.72
WT/S/SR1(s)	94.91	0.84
WT/S/SR1(e)	88.52	0.75
WT/S/SR1(s+e)	65.51	0.10
WT/S/SR2(s)	81.17	0.59
WT/S/SR2(e)	59.36	0.18
WT/S/SR2(s+e)	51.24	0.19
WT/S/SR1+SR2(s)	117.1	0.62
WT/S/SR1+SR2(e)	71.0	0.37
WT/S/SR1+SR2(s+e)	112.1	0.70

Note. Percentage abberation of total cell counts compared to OVA-sensitized wt control group (WT/S). Statistical significance: *p<0.05 by paired student's *t*-test compared to control group (mean; n=8/ group).

Table 29: Percentage aberation of total cell counts in WT treated with THC

	[%]	p-value (to S/VH)
NS/VH	16.37	0.002 *
S/VH	**100**	-
S/THC(s)	62.8	0.138
S/THC(e)	47.7	0.021*
S/THC(s+e)	25.53	0.001*

Note. Percentage abberation of total cell counts compared to OVA-sensitized wt control group (S/VH). Statistical significance: *p<0.05 by paired student's *t*-test compared to control group (mean; n=10/ group).

8.3 Publications

8.3.1 Presentations

A. Braun, T. Greiner, A. Zimmer, A. Zimmer, H. Behrendt, M. Mempel
Cannabinoid receptor deficiency increases allergen-induced airway inflammation in a murine model
Oral presentation and poster presentation: XXVIII. EAACI 2009, Warsaw, Poland
Allergy 2009: 64 (Suppl. 90): 54-55

A. Braun, T. Greiner, A. Zimmer, A. Zimmer, H. Behrendt, M. Mempel
Increased allergen-induced airway inflammation in cannabinoid receptor deficient mice
Oral presentation: 21. Mainzer Allergie-Workshop, Mainz
Allergo Journal 2009: 19/1: 54

A. Braun, T. Greiner, A. Zimmer, A. Zimmer, H. Behrendt, M. Mempel
Deficiency in cannabinoid receptors results in an increased allergen-induced airway inflammation in a murine model
Poster presentation: 6th Symposium on Environmental Allergy and Allergotoxicology: Climate Change and Allergy, Munich, Neuherberg

A. Braun, M. Bewersdorff, J.T.M. Buters, T. Jakob, H. Behrendt, M. Mempel
Particle-treatment of dendritic cells reverses the suppression of IgE-switch in murine B cells in vitro
Poster presentation: 6th Symposium on Environmental Allergy and Allergotoxicology: Climate Change and Allergy, Munich, Neuherberg

A. Braun, M. Bewersdorff, J.T.M. Buters, T. Jakob, H. Behrendt, M. Mempel
Suppression of IgE isotype switch in murine B cells by dendritic cells can be reversed by diesel particles
Poster presentation: XXVII. EAACI 2008, Barcelona, Spain
Allergy 2008: 63 (Suppl. 88): 90

A. Braun, M. Bewersdorff, J.T.M. Buters, T. Jakob, H. Behrendt, M. Mempel
Diesel particles reverse dendritic cell-mediated suppression of IgE isotype switch in murine B cells
Oral presentation: 20. Mainzer Allergie-Workshop, Mainz
Allergo Journal 2008: 17/1: 39

A. Braun, M. Bewersdorff, J.T.M. Buters, T. Jakob, H. Behrendt, M. Mempel
Modulation of dendritic cell-mediated suppression of IgE isotype switch in murine B cells in vitro by diesel particles
Poster presentation: 6th EAACI-GA2LEN Davos Meeting, Pichl, Austria

A. Braun, M. Bewersdorff, J. Gutermuth, W. Schober, M. Mempel, H. Behrendt, J.T.M. Buters, T. Jakob
Effects of anthropogenic environmental compounds on murine dendritic cells in vitro
Oral presentation: 19. Mainzer Allergie-Workshop, Mainz
Allergo Journal 2007: 16/1: 35

A. Braun, M. Bewersdorff, J. Gutermuth, W. Schober, H. Behrendt, J.T.M. Buters, T. Jakob
Effects of phenanthrene on phenotypical and functional maturation of murine dendritic cells in vitro
Poster presentation: XXVI. EAACI 2006, Wien, Austria

8.3.2 Publications

A. Braun, M. Bewersdorff, J. Lintelmann, G. Matuschek, T. Jakob, M. Göttlicher, W. Schober, J.T.M. Buters, H. Behrendt, M. Mempel
Differential impact of diesel particle composition on pro-allergic dendritic cell function
Toxicological Sciences 2009: doi: 10.1093/toxsci/kfp239

S. Gilles, J. Huss-Marp, A. Braun, J. Gutermuth, C. Blume, W. Schober
Environmental Allergy, München 2009: Ein neuer Blick auf die Mechanismen der Allergie; 6th Symposium on Environmental Allergy and Allergotoxicology: Climate Change and Allergy.
Allergo Journal 2009: 18/4: 244-248

A. Braun, C. Blume, S. Gilles, J. Gutermuth, J. Huss-Marp, W. Schober
Environmental Allergy, München 2009: 10 Jahre ZAUM – Zentrum Allergie und Umwelt; 6th Symposium on Environmental Allergy and Allergotoxicology: Climate Change and Allergy.
Allergo Journal 2009: 18/3: 174-179

J. Gutermuth, G. Köllisch, M. Bewersdorff, A. Braun, F. Alessandrini, T. Jakob
Immunology highlights at high altitude: review of the fourth EAACI-GA2LEN Davos Meeting
Allergy 2006: 61: 1197–1199

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8.5 Curriculum Vitae

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