 doi:10.20944/preprints202207.0222.v1

Article

DEPs Induce Local IgE Class Switching Independent of Their Ability to Stimulate iBALT

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Abstract: Background. Diesel exhaust particles (DEPs) have a great impact on general increase of atopic diseases worldwide. However, it is still unknown whether DEPs induce systemic B-cell IgE class switching in secondary lymphoid organs or locally in lungs, in inducible bronchial-associated lymphoid tissue (iBALT). The aim of this work was to identify the exact site of DEPs mediated B-cell IgE class switching and pro-allergic antibodies production. Methods. We immunized BALB/c mice with different OVA doses (0.3 and 30 μg) intranasally in the presence and absence of two types of DEPs, SRM1650B and SRM2786. We used low (30 μg) and high (150 μg) DEPs doses in our study. Results. Only high DEP dose induced IgE production regardless of particle type. Local IgE class switching was stimulated upon treatment with both types of particles with both low and high antigen doses. Despite the similar ability of two standard DEP samples to stimulate IgE production, their ability to induce iBALT formation and growing, was markedly different upon co-administration together with low antigen doses. Conclusion. DEPs induced local IgE class switching takes place in pre-existing iBALTs, independently of de novo iBALT formation, at least in the case of SRM1650B co-administrated with low antigen doses.

Keywords: diesel particulate matter; antibody production; tertiary lymphoid structures; local Ig class switch; antigen doses; lungs

1. Introduction

One of the main hypotheses explaining the increasing of prevalence of different IgE-mediated pathologies such as asthma, allergic rhinitis and atopic dermatitis considers air pollution as a major cause for this phenomenon [1-3]. The main type of pollutants which is linked to allergy and asthma development is particulate matter, especially, diesel exhaust particles (DEPs) [2,3]. These particles are by-products of diesel fuel combustion and consist of carbonaceous core with adsorbed polycyclic aromatic hydrocarbons (PAH) [4]. These organic compounds possess pro-inflammatory properties [3,5,6]. As shown by several research groups, the DEPs concentration in ambient air is strongly associated with increasing prevalence of asthma cases [2,3,7,8]. Administration of these particles in mouse allergy model leads to exacerbation of allergic inflammation and pro-allergic antibody production [5,9-14].

Recently it was clearly shown that intratracheal instillation of monodisperse Alum or Silica particles caused iBALT formation and local IgE production in C57BL/6 mice [15]. Though DEPs induced allergic inflammation has been studied in detail [5,9-14] the exact location of DEPs induced B-cell class switching and IgE production was not identified yet. Although the research data confirmed that DEPs induced local lymphoid tissue formation in mouse lungs upon instillation [16], it remains unclear whether IgE production could be

 doi:10.20944/preprints202207.0222.v1

functionally associated with these structures. In contrast to monodisperse Alum or Silica, DEPs are multi-component polydisperse in nature.4The toxic effects of DEPs on living cells are mostly linked to oxidative and genotoxic stress induced by PAHs, especially, by adsorbed benzo(a)pyrene (BaP) although the induction of cell death cannot be excluded [3,13,17-20]. It is noteworthy that despite the inability of the bulk DEPs to penetrate lung epithelium and to enter systematic circulation, fine type of DEPs with less than 0,1 μm in diameter can do this [2,3]. Upon DEPs accumulation in lung tissue, PAHs are able to desorb from carbon core and enter the blood or lymphatic system. Therefore, DEPs accumulated in lung tissue may have not only local but also systemic effects on effect on the immunity.

The properties of DEPs vary significantly depending on their specific source as well as atmospheric conditions and the time they spent in the air [2,13]. For example, DEPs SRM1650B collected directly from heat exchangers of diesel engine have 0,18 μm mean diameter when disaggregated by ultrasound but when aggregated their resulting size exceeded 1-10 μm [21]. DEPs SRM2786 collected from the air of urban areas in Central Europe have average particle diameter 2,8 μm without preliminary disaggregation (and due to this fact they are considered as fine particulate matter), with 10% of particles having size less that 0,91 μm and 90% having size less than 6,9 μm [22]. In the other hand the ability to enter the lungs after inhalation is inversely proportional to its size. It is widely accepted that particles with size less than 2,5 μm have high ability to penetrate lung tissue after intranasal inhalation. Particles with size less than 0,1 μm can penetrate epithelial barriers [2,3]. SRM2786 DEP sample despite the fact that these particles were coined as "fine" almost does not contain such particles. In contrast in DEP1650B sample 10% of particles have size less than 0,12 μm according to its analysis by NIST [21,22].

In our previous work we found that low but not high antigen doses induce rapid local B-cell IgE class switching accompanied by minimal IgG1 production in fat-associated lymphoid clusters rather than regional lymph nodes [23]. However the properties of iBALT may be significant differ from fat-associated lymphoid structures. So, it is not clearly whether low or high antigen doses co-administrated with DEPs would stimulate IgE class switching.

The aim of this work was to clarify (a) whether DEPs induce local (in lung tissue) or systemic (in lymph nodes) IgE class switching and IgE production when introduced with either low or high antigen doses, and (b) whether local IgE class switching is accompanied by iBALT induction. We also compared the effect of two type of DEPs SRM1650B and SRM2786 on local and systemic immune response.

2. Materials and Methods

2.1. Animals

Female BALB/c mice 5-6 weeks were purchased from the Scientific Center of Biomedical Technologies (Andreevka, Russia). Mice were fed *ad libitum* and were kept in 12 hours light dark cycle. All the animal experiments were performed according to the IACUUC protocol number 350 approved by the local committee of IBCh RAS on 21.06.2020.

2.2. Immunization and sample collection

Mice were immunized for 8 consecutive weeks. Mice received saline as a control or OVA (Sigma Aldrich, Darmstadt, Germany) in low (0.3 μg) or high (30 μg) doses alone or with DEPs SRM1650B (DEP1) or SRM2786 (DEP2) (NIST, USA 3 times a week for the first 2 weeks and 2 times a week in the remained 6 weeks. Low (30 μg/mice) or high (150 μg/mice) doses of DEPs were used. Before usage in protocol DEPs samples were sonicated for 15 minutes in saline with 2% of normal mouse serum. Immunization was performed by i.n. route under isoflurane (Baxter) anesthesia in 50 μl saline contained 2% of normal mouse serum taken from the same mice before the experiment. Normal mouse serum was added to immunization solutions to prevent DEPs aggregation [13].

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After 8 weeks of immunization blood were taken from suborbital sinusto estimate specific antibody titers. Blood samples were incubated for 20 minutes at +37ºC followed by centrifugationat 600g to obtain serum samples which were stored at -20ºC prior to use.

The next day mice were anesthetized with isoflurane and sacrificed by cervical dislocation after the manipulations; lung tissue and regional lymph nodes were collected and homogenized in ExtractRNA to obtain samples for gene expression measurement. For CXCL13 quantification by ELISA lungs were homogenized in PBS containing 1% Triton-X100. For H&E histology lungs were filled with 4% PFA via trachea dissected from the thorax and placed into 4% PFA.

After immunization with different antigen and DEPs doses, some mice were challenged with 250 μg of OVA 3 times a week for 2 weeks to evaluate lung allergic inflammation intensity in different groups. Mice were euthanized,lavage fluid was taken as described previously by L.V. Hoecke et al. [24]. Briefly, Bronchoalveolar lavage (BAL) was collected by applying 0,8 mL of ice-cold PBS using an 18G cannula (Abbocath, ICU Medical, USA) through the trachea to the airways twice. The total BAL volume was approximately 1,3mL. BALs were centrifuged at 600g and cell pellets were resuspended in 0,2 ml PBS.

2.3. ELISA

To estimate specific IgE, IgG1, IgG2a and IgA production we performed ELISA as described previously [23]. We used HRP labeled anti-mouse IgE (clone 23G3, Abcam) in 1:1000 dilution, biotin anti-mouse IgG1 (clone RMG1-1, BioLegend), anti-mouse IgG2a (clone RMG2a-62, BioLegend) or anti-mouse IgA (clone RMA-1, BioLegend) in 1:5000 dilution. In the case of biotinylated primary antibodiesStreptavidin-HRP was used as a secondary conjugate in 1:7000 dilution. CXCL13 (BCA1) concentration in lung homogenates was measured with mouse BCA1 matched antibody pair kit (Abcam, ab218172) according to manufacturer instructions.

2.4. Gene expression measurements

qPCR was performed as described previously using standard phenol-chloroform extraction, DNAse I (EN0521, ThermoScientific) treatment and standard first strand cDNA synthesis and amplification protocols with OT M-MuLV-RH kit (R01-250, BioLabMix, Novosibirsk, Russia) and BioMaster HS-qPCR kit (MH020-2040, BioLabMix, Novosibirsk, Russia) [23]. The following primers and probe sets were used. GAPDH F: GGAGAGTGTTTCCTCGTCCC; GAPDH R: ACTGTGCCGTTGAATTTGCC; GAPDH probe: /6-FAM/-CGCCTGGTCACCAGGGCTGCCATTTGCAGT-/BHQ-1/; HPRT F: CAGTCCCAGCGTCGTGATTA; HPRT R: TCCAGCAGGTCAGCAAAGAA; HPRT probe: /6-FAM/-TGGGAGGCCATCACATTGTGGCCCTCTGTGTG-/BHQ-1/; germline ε F: CCCACTTTTAGCTGAGGGCA; germline ε R: CTGGTTAAGGGCAGCTGTGA; germline ε probe: /6-FAM/-CGCCTGGGAGCCTGCACAGGGGGC-/BHQ-1/; circular μ-ε F: CCCACTTTTAGCTGAGGGCA; circular μ-ε R: CGAGGGGGAAGACATTTGGG; circular μ-ε probe: /6-FAM/-CGCCTGGGAGCCTGCACAGGGGGC-/BHQ-1/; circular γ1-ε F: AGATTCACAACGCCTGGGAG; circular γ1-ε R: GTCACTGTCACTGGCTCAGG; circular γ1-ε probe: /6-FAM/-CCACTGGCCCCTGGATCTGCTGCCCA-/BHQ-1/; germline γ1 F: AGAACCAAGGAAGCTGAGCC; germline γ1 R: AGTTTGGGCAGCAGATCCAG; germline γ1 probe: /6-FAM/-AGGGGAGTGGGCGGGGAGGCCA-/BHQ-1/; postswitch ε F: CCAGTCCACATGCTCTGTGT; postswitch ε R: AGCGTGGGGAACTGGTTAAG; postswitch ε probe: /6-FAM/-TGGGGTCCCCAGAGCCCTGCTCCTGT-/BHQ-1/; postswitch γ1 F: CCTCTGGCCCTGCTTATTGT; postswitch γ1 R: GTCACTGTCACTGGCTCAGG; postswitch γ1 probe: /6-FAM/-CCACTGGCCCCTGGATCTGCTGCCCA-/BHQ-1/; Cd19 F: GAGGCACGTGAAGGTCATTG; Cd19 R: TTGAAGAATCTCCTGGCGGG; Cd19 probe: /6-FAM/-AGCAGTGTGGCTCTGGCTGTTGAGAACTGGTG-/BHQ-1/; Bcl6 F: CACTATAGGGCGGCGAGC; Bcl6 R: TGCCTT-

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GCTTCACAGTCCAA; Bcl6 probe: /6-FAM/-CCCCTGCTGCGGAGCAATGGTAAA-GCCCGC-/BHQ-1/; Ebi2 F: CAAACACGGACTGCCACAAC; Ebi2 R: CAATGACAAC-CAAGGCCAGC; Ebi2 probe: /6-FAM/-TCTCTATGCCCACCACAGCACAGCCAGGGT-/BHQ-1/; Tnfa F: TCTTCTCAAAATTCGAGTGACAAGC; Tnfa R: GA-TAGCAAATCGGCTGACGGT; Tnfa probe: /6-FAM/-AGGCTGCCCCGACTAC-GTGCTCCTCACCCA-/BHQ-1/; Ifna1 F: TGCCCAGCAGATCAAGAAGG; Ifna1 R: TCAGGGGAAATTCCTGCACC; Ifna1 probe: /6-FAM/-CCTG-CAAGGCTGTCTGATGCAGCAGGTGGG-/BHQ-1/; IL4 F: CCATATCCAC-GGATGCGACA; IL4 R: AAGCACCTTGGAAGCCCTAC; IL4 probe: /6-FAM/-AGGGAC-GCCATGCACGGAGATGGATGTGCC-/BHQ-1/; IL13 F: GTGTCTCTCCCTCTGACCCT; IL13 R: TCTGGGTCCTGTAGATGGCA; IL13 probe: /6-FAM/-CCGCTGGCGGGTTCTGTGTAGCCCTGGATT-/BHQ-1/; Ifng F: TCAGGCCATCAG-CAACAACA; Ifng R: CTTCCTGAGGCTGGATTCCG; Ifng probe: /6-FAM/-AG-GTCAACAACCCACAGGTCCAGCGCCAAG-/BHQ-1/. All primers and probes were designed by NIH Primer BLAST. Expression of genes and transcripts of interest as well as excision circles were normalized to geometric mean expression of GAPDH and HPRT in respective samples.

2.5. Flow cytometry

Allergic airway inflammation manifestation was estimated by the presence of myeloid cells in lavage. Before cell staining cell concentration in lavages was quantified under the light microscope. Cells in 0,2 ml PBS were stained by following anti-mouse antibodies from BioLegend: SiglecF-BV421 (clone 17007L), CD11c-FITC (clone N418), F4/80-PE (clone BM8), Gr-1-PerCP (clone RB6-8C5), CD11b-PECy7 (clone M1/70), MHCII (I-A/I-E)-APC (clone M5.114.15.2). The incubation was performed for 1 hour at +4ºC. Live/dead cell discrimination was performed by Zombie Aqua (BioLegend) according to manufacturer instructions. The analysis was performed on the MACS Quant Tyto Cytometer (MiltenyiBiotech, Germany). Cell concentration in lavages was quantified by multiplication of the percent of cell population of interest in live cells on the total cell concentration in samples.

Eosinophils were estimated as SiglecF+CD11c- cells; alveolar macrophages as SiglecF+CD11c+F4/80+ cells; Neutrophils as SiglecF-CD11c-CD11b+Gr-1+ cells according to slightly modified protocols used by other research groups [24, 25]. The overall gating strategy is shown on Figure S1. Although according to this strategy interstitial macrophages, monocytes and dendritic cells were also present in lavage samples their quantities were much lower.

2.6. Histology analysis of lung tissue

Lung tissue samples were subjected to H&E histology. After filling with 4% PFA and dissecting from the thorax lung samples were cut under -20ºC on 20 μm thick sections and were stained by H&E staining kit (ab245880, Abcam) according to manufacturer instructions. Microscopic images with different magnifications were obtained. In order to to estimate the impact of i.n. administrated antigen and DEPs on iBALT induction and growing, we quantified the relative area as ratio Total area of all histological features/ Total area of iBALT structuresoccupied by iBALTs in these sections.

2.7. Statistics

Group mean and standard deviations were quantified in each case. The ANOVA test with correction by multiple comparisons was used to evaluate the significance of the difference between groups. Each experiment was performed 2-3 times. Differences with $p<0.05$ was considered as significant.

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3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. DEPs induce pro-allergic antibodies formation

It is generally accepted that DEPs induce pro-allergic antibodies formation and subsequent development of asthma in human clinics and in laboratory animal asthma models [5-14]. In this work, we utilized two types of DEPs. SRM1650B (DEP1) originated directly from the heat exchangers of diesel engine possess 0.18 μm mean diameter when ultrasonically disintegrated and 1-10 μm upon aggregation [21]. SRM2786 (DEP2) collected from the air of urban areas had mean particle diameter 2,8 μm in disaggregated state [22]. These particles are more stable than DEP1 which are more prone to aggregation. It is widely accepted that particles with size less than 2,5 μm have high ability to penetrate lung tissue after intranasal inhalation. Particles with size less than 0,1 μm can penetrate epithelial barriers [2,3]. Our results show that both DEPs induced IgE antibody formation in comparable levels when administered in high but not in low doses. This effect was independent of co-administered antigen doses. High doses of DEPs in combined with low (Figure 1a, b) or high antigen doses (Figure S1a, b) in comparison to OVA alone induced formation of specific and total IgE in mice after prolonged administration. Low doses of DEPs did not induce IgE antibody formation in comparison with either saline treated mice or mice treated with OVA alone.

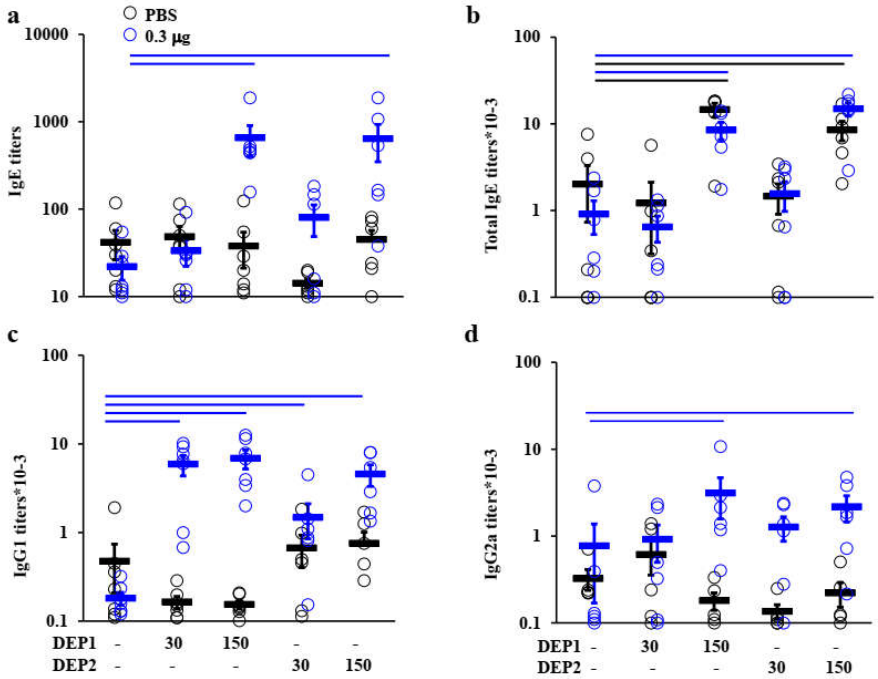

Figure 1. Antibody production in response to low OVA doses, DEPs, or their combination. BALB/c mice were immunized 2-3 times a week for 8 weeks (total 18 immunizations) via i.n. route by 0.3 μg OVA alone or with different DEPs types at 30 or 150 μg. The titers of OVA specific IgE (a), IgG1 (c), IgG2a (d) or total IgE (b) were measured. Statistical differences of groups immunized by antigen with particles vs OVA control are shown with blue bars, and statistical differences of groups immunized by particles alone vs saline control are shown by black bars ($p<0.05$). The graphs show the data from one representative experiment out of three independent experiments. Six mice were included in each experimental group.

DEPs also induce specific IgG1 formation in mice when co-administered with the low (0.3 μg) OVA dose and the titer of the specific IgG1 was higher upon co-administration

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with the high (30 μg) OVA dose (Figure 1c, Figure S1c). In all cases both, high and low doses of DEP1 and DEP2 stimulated IgG1 formation with the exception of low DEP2 doses when combined with low antigen doses (Figure 1c). We also measured levels of specific IgG2a associated with type 1 immune response [26] and IgA in immunized mice. The elevation of IgA accompanies the suppression of the IgE response apparently due to immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells [27]. Both DEP1 and DEP2 doses promoted IgG2a formation upon administration with high antigen dose (Figure S1d). Only high doses of DEPs were able to promote IgG2a production upon immunization with low OVA dose (Figure 1d). On the contrary, the effects of particles on IgA production were remarkably different. The induction of specific IgA production was observed only upon administration of DEP2 but not DEP1, together with high antigen dose. Both low and high doses of DEP2 had a statistically significant effect on IgA titers (Figure S2).

3.2. DEPs stimulate allergic inflammation

We next evaluated whether DEP mediated antigen specific humoral immune response resulted in antigen-specific sensitization and subsequent asthma development at antigen challenging dose. We focused on the effects of DEPs dose 150 μg/mice since only this particular amount stimulated the formation of pro-allergic antibodies. Despite the comparable levels of specific IgE induced by both high and low OVA doses co-administrated together with DEPs, the significantly higher titers of IgG1 and IgG2a which accompanied IgE production were observed at OVA high doses (Figure 1). In the steady state, the allergen-specific IgE antibodies are bound to FcεRI receptors on the surface mast cell, and allergen-induced FceRI ligation initiates pro-inflammatory signaling cascades and release of anaphylactic mediators. Meanwhile, IgG1 and IgG2a mediate PAF-dependent processes in macrophages [28]. Apparently, the administration of challenging OVA dose can promote a multiple cellular immune response (flow cytometry gating strategy is shown in Figure S3 and representative contour plots in Figure S4). The effects of DEP1 on antigen-dependent neutrophils accumulation in BAL were comparable for mice pre-immunized with low and high doses of OVA (Figure 2b, e). Accumulation of eosinophils (gating strategy is shown in Figure S3) were higher in mice immunized by low OVA dose (0.3 μg) (Figure 2a). However it should be mentioned that eosinophils accumulation is induced in the presence of DEP1 and high antigen doses in comparison with mice immunized with high antigen doses alone (Figure 2d). Alveolar macrophages content in BALs wascomparable in high and low dose groups after short high dose challenge and was also enhanced by DEPs (Figure 2c, f). It is also interesting that long term DEPs administration alone per se potentiated macrophages accumulation upon short high-dose challenge. In the absence of the particles low dose of antigen per sepotentiated macrophages accumulation and a high dose per se potentiates neutrophils accumulation (Figure 2 e, f). Data for DEP2 were similar. In summary, for the same DEPs the allergic inflammation in low and high OVA dose groups were different. Compared to high doses, low antigen doses induced allergic immune response which is more dependent on eosinophils, though these differences were not very crucial.

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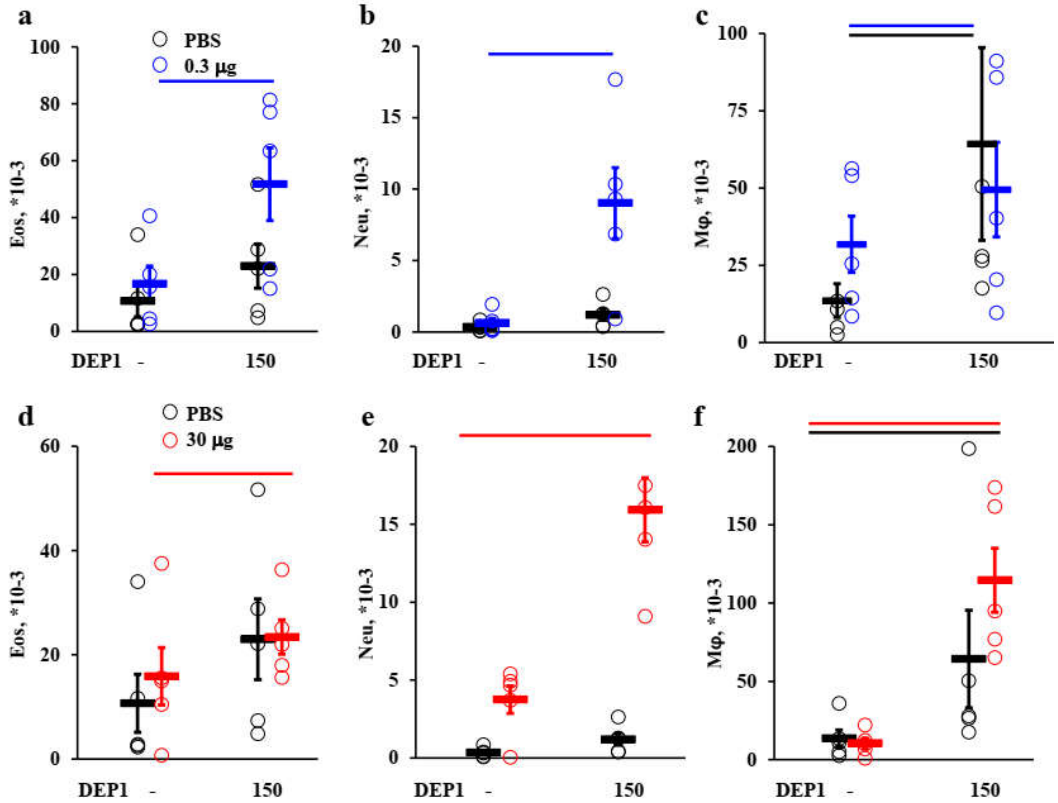

Figure 2. Cell response to DEPs. BALB/c mice were immunized by OVA low, 0.3 μg (a-c) or high, 30 μg (d-f) doses alone or with 150 μg of DEP1. After this mice were challenged 3 times a week for 2 weeks by 250 μg OVA and sacrificed. Lavages were taken and Eosinophils (Eo), Neutrophils (Neu) and Alveolar Macrophages (Mϕ) cell numbers were quantified by flow cytometry. Representative data from one out of three independent experiments. Significant differences vs PBS are shown with black bars; vs OVA control – with blue ones ($p<0.05$). Representative data from 3 independent experiments.

3.3. DEPs induced local and system Ig class switching

To answer the question whether and where the B-cell class switch exactly occurs,we have measured expression of germline ε and germline γ1 transcripts, as well as circular transcripts corresponding to direct and sequential class switch to IgE [29]. Because of its rapid degradation in living proliferating cells, DNA excision circles may serve as indicators of sites where the B-cell class switch occurs. Despite the fact that both type of particles induced IgE production in comparable levels, their properties in relation to stimulation of local or system isotype switch were slightly different. DEP1 induced both local (in lung tissue) and systemic (in regional lymph nodes) isotype switching to IgE (germline ε) when administrated with low antigen doses (Figure 3 a, e). DEP1 administrated with high antigen doses stimulated only local IgE class switching (Figure 3 a). Only local IgE class switching was induced by DEPs independent of their types with both low and high antigen doses (Figure 3 a). In the DEP1 treated low antigen group, both direct (μ-ε) and sequential (γ1-ε) mechanisms mediated the local IgE class switch (Figure A5 a, b). However, only direct was observed in the high antigen dose group (Figure A5 a). In lymph nodes, DEP1 and low OVA doses stimulated B-cell IgE class switching only by the direct mechanism (Figure A5 c). DEP2 induced both local and systemic IgE class switching both in low and high antigens groups (Figure 3 a, e). Similarly to DEP1, DEP2 significantly induced local direct and sequential IgE class switching in the low dose group (Figure A5 a, b). Both types of IgE switching mechanisms were induced in regional lymph nodes after DEP2 administration with low antigen doses (Figure A5 c, d). Both types of particles accelerated the IgG1 class switching (germline γ1 expression) in lung tissue after low antigen doses administration (Figure 3 b). In lymph nodes, DEPs did not accelerate IgG1 class

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switching in response to low OVA dose (Figure 3f). In high antigen group, DEP1 but not DEP2, exerted positive effects on IgG1 class switching in combination with low antigen doses (Figure 3 f). We do not exclude the possibility that during long-term immunization at conventional conditions certain minor non-relevant environmental antigens could enter lung tissue with experimentally administrated DEPs. This may explain the expression of certain B cell class switch markers even in the absence of OVA (Figure 3).

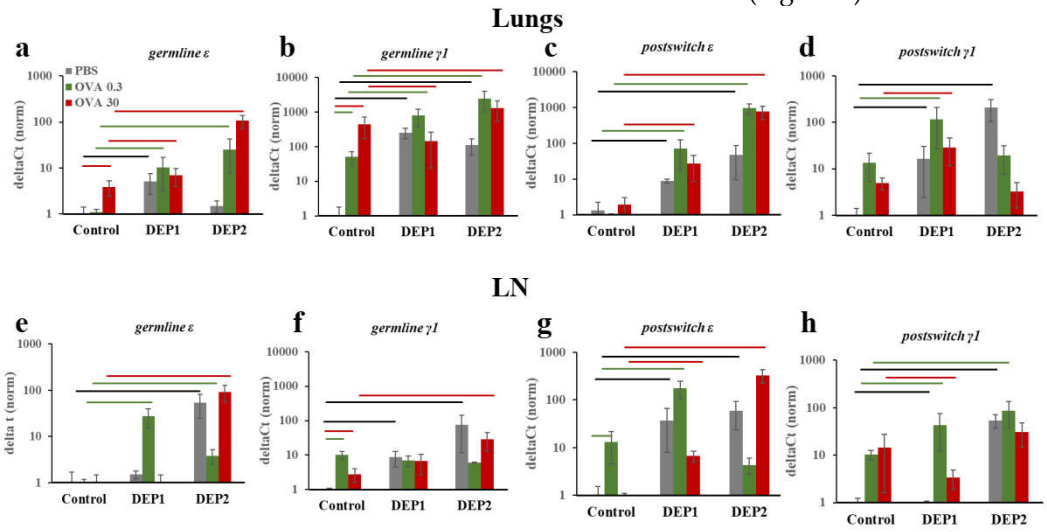

Figure 3. Expression of transcripts corresponding to B-cell Ig class switching and antibody production in lung tissue and regional lymph nodes. BALB/c mice were immunized 2-3 times a week for 8 weeks (total 18 immunizations) via i.n. route without antigen (PBS), with low (0.3 μg) OVA dose or high (30 μg) OVA dose without particles (control) or with either DEP1 or DEP2. After immunization mice were sacrificed. Expression of transcripts corresponding to IgE (germline ε) (a, e), IgG1 (germline γ1) (b, f) class switching, as well as transcripts corresponding to mature IgE (postswitch ε) (c, g) and IgG1 (postswitch γ1) (d, h) producing cells were measured in lung tissue (a-d) and regional lymph nodes (e-h). Black bars indicate significant ($p<0.05$) differences between mice immunized without antigen with different DEPs and respective control without DEPs; green bars indicate significant differences between mice immunized by low OVA dose with or without DEPs and respective control groups; red bars indicate significant differences between mice immunized by high OVA dose with or without DEPs and respective control groups. Representative data from 3 independent experiments.

It is known that tissue resident B-cells that locally encounter antigen tend to migrate in regional lymph nodes [30] where the most antibody production eventually occurs. However, both type of particles induced accumulation of postswitch ε transcripts not only in regional lymph nodes but also in lung tissue (Figure 3 c, g). Only for lung tissue this effect was independent of the antigen dose co-administrated with DEPs and the type of DEPs (Figure 3 c). Low antigen doses induced postswitch γ1 transcripts which indicated IgG1 production in the lungs and lymph nodes (Figure 3 d, h). The effects of DEP2 on the expression of postswitch γ1 transcripts were insignificant in the lungs (Figure 3 d). DEP1 stimulated the accumulation of IgG1 producing cells in lungs independent of antigen doses and DEP2 stimulated the accumulation of IgG1 producing cells mostly in lymph nodes respectively (Figure 3 d, h).

3.4. Hystology analisis of iBALTs in lungs of mice after antigen and (or) DEPs administration

Due to lung localization of both antigen dose-independent B cell switching and DEP-mediated dose-independent accumulation of IgE producing cells, we next focused on local B class switching in details. The local immune response usually depends on tertiary lymphoid structures such as iBALTs in lungs [31]. Therefore, we decided to examine if iBALT development and growing in lung tissue was accelerated by DEPs. Despite the fact that both type of DEPs in high doses induced local IgE class switching and IgE production

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when administrated with low antigen doses, only high doses of DEP2 induced iBALT formation per se and in combination with 0,3 μg OVA (Figure 4a). Both types of DEPs, however, within the entire range of concentrations stimulated iBALT formation in response to the high dose of antigen (Figure 4b). Despite the facts that (1) low OVA doses in combination with DEP2 high dose potentiated iBALT formation and growing in lungs (Figure 4 a) and (2) high OVA doses administrated with both doses of DEP1 and high dose of DEP2 triggerred iBALT formation (Figure 4 b), low OVA doses in combination with high DEP1 dose though induced IgE production (Figure 1) did not triggered iBALT formation (Figure 4a-b). From these facts it becomes clear that in some groups, where iBALT formation and growing were significantly triggered, local IgE production was absent or insignificant. In the other hand, in group immunized with low antigen dose and high DEP1 dose local B-cell IgE class switching was induced but significant iBALT growing was not detected. Representative histological of lung tissues images show that the sites of DEP accumulation, especially of DEP1 which was prone to form larger aggregates, did not always coincide with either sites of de novo iBALT formation or with the sites of immune cell infiltrates (Figure 4 c, Figure 5).

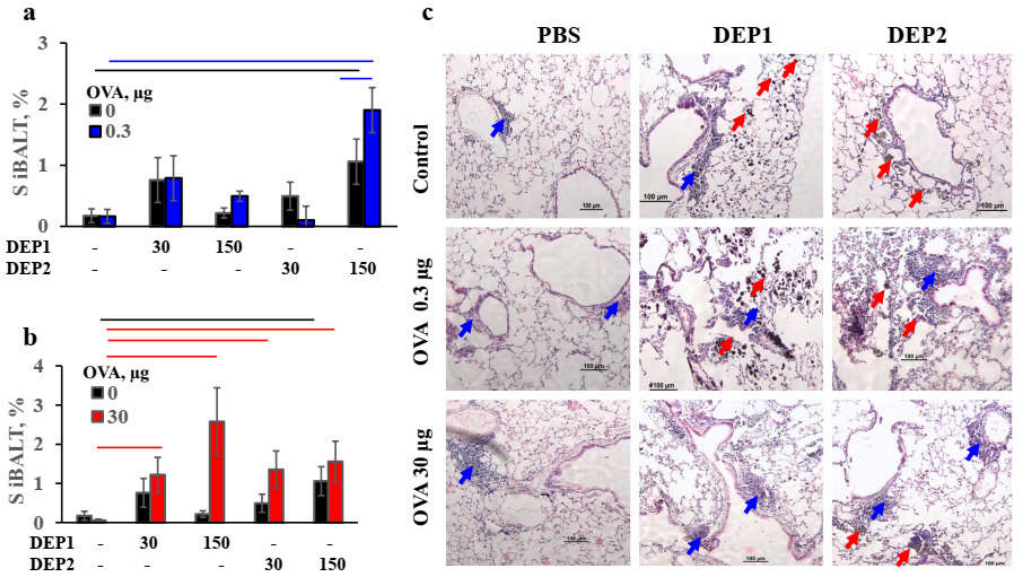

Figure 4. Histological analysis of lung tissue after prolonged antigen and DEPs administration. BALB/c mice were immunized 2-3 times a week for 8 weeks (total 18 immunizations) via i.n. route with indicated doses of antigen and 150 μg of DP1 or DP2. Following immunization, mice were sacrificed and lungs were isolated and stained for histological analysis (H&E). Relative square of iBALT on histological sections of mice immunized by PBS and low antigen doses with or without DEPs (a) or with high antigen doses (b), and representative histological images (100X) (c) are shown. Red arrows correspond to DEPs, blue arrows show iBALTs. Black bars show significant differences between groups immunized with particles alone and PBS control ($p<0.05$). Blue and red bars show differences between mice immunized with low (blue) or high (red) OVA doses together with DEPs and mice immunized with OVA alone. The graphs show the data from one representative experiment out of three independent experiments.. Six mice were included in each experimental group.

3.4. Stimulation of cytokines and CXCL13 chemokine expression in response to antigen and (or) DEPs administration

It is curious, why two closely related DEPs exerted different effects on iBALT formation while stimulating similar pro-allergic humoral response. It is known that CXCL13 produced by stromal cells in response to TNFα and LTα based interactions with

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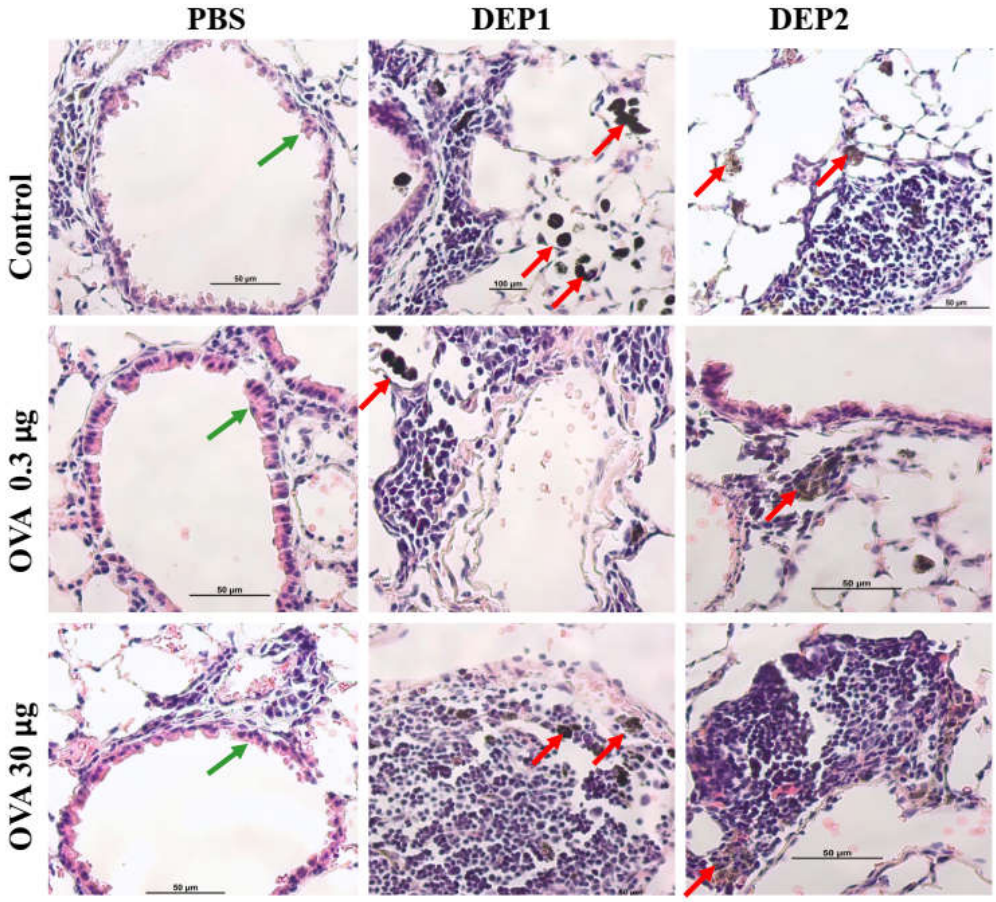

Figure 5. Histological H&E images in high magnification (200X) of lung tissue. BALB/c mice were immunized as indicated in Fig. 4, the lungs were processed for H&E staining. Red arrows show DEPs, green arrows correspond to iBALTs.

B-cells is crucially involved in initiation of the lymphoid lymphoid tissue orchestration and lymphoid neogenesis [31-33]. To evaluate the ability of two type of DEPs to stimulate CXCL13 production, we measured the levels of chemokine production in lung homogenates of immunized mice. Indeed in contrast to DEP1, DEP2 in combination with low OVA dose weakly but significantly triggered CXCL13 production. DEP1 mediated the increase in CXCL13 production only in the high antigen dose group (Figure 6a).

It is unlikely that the different ability of DEP1 and DEP2 to potentiate iBALT formation and growing in lung tissue in combination with low antigen doses was linked to different ability to induce expression of iBALT promoting pro-inflammatory cytokines. Generally it is accepted that TNFa contributes to iBALT formation [32]. High antigen doses induced TNFα gene expression in lung tissue without DEPs and with two type of DEPs as well. In the low antigen dose group, the induction on TNFa gene expression was observed only upon treatment with DEPs regardless their type (Figure 6b). It was shown recently that type I interferones are also capable to induce iBALT formation [34]. Both types of DEPs induced IFNα1 expression independently of the co-administered antigen dose. Antigen alone did not induce IFNα1 expression either in low or high dose (Figure 6c).

Local IgE class switching and IgE production must depend on certain cytokines production, IL-4 particularly [1], regardless the presence or absence of iBALT formation. Indeed both types of DEPs stimulated IL-4 expression in lung tissue independently of the antigen dose (Figure 6d). Although the expression of IL-13 in mice immunized with DEPs in combination with antigen was not significantly higher compared to saline immunized mice, it was significantly higher in comparison to mice immunized with antigen alone irrespective of DEPs type and OVA dose (Figure 6e). At the same time, we could not detect

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the induction of IFNγ expression by DEPs in mouse lungs (Figure 6f). Therefore, both types of DEPs stimulated local cytokine production associated with B-cell IgE class switching.

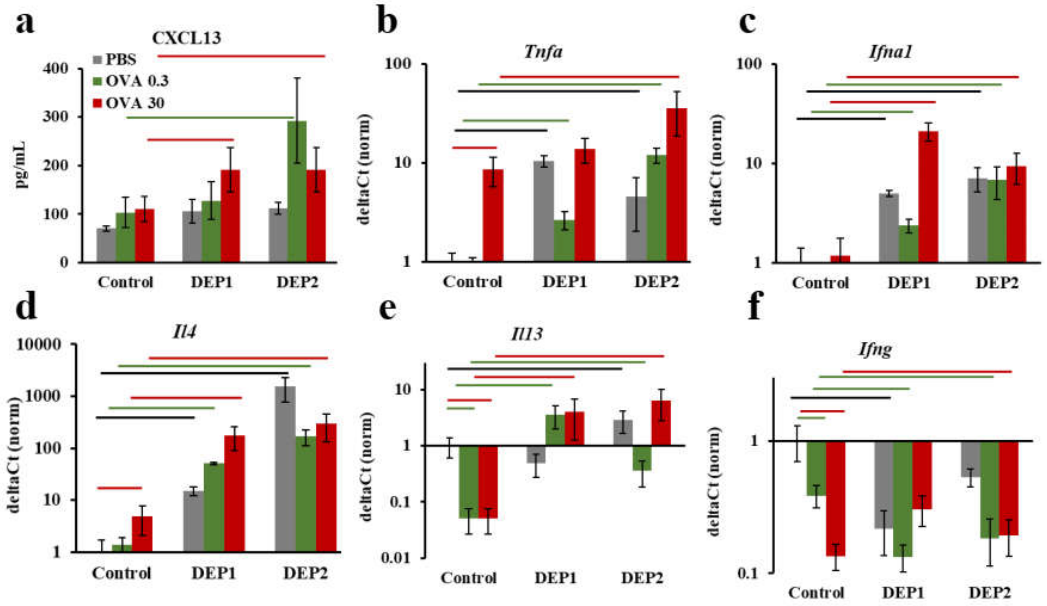

Figure 6. Production of iBALT-inducing chemokine and expression of cytokines in lung tissue of immunized mice. BALB/c mice were immunized as described in Fig. 4. Control mice were immunized in the absence of DEPs. The production of CXCL13 was measured in lung homogenates (a), expression of iBALT-promoting cytokines Tnfa (b) and Ifna1 (c), type 2-immune response promoting cytokines Il4 (d) and Il13 (e), and type 1 immune response promoting cytokine Ifng (f) in the lung tissue. Black bars indicate significant ($p<0.05$) differences between mice immunized without antigen with different DEPs and respective control without DEPs; green bars indicate significant differences between mice immunized by low OVA dose with or without DEPs and respective control groups; red bars indicate significant differences between mice immunized by high OVA dose with or without DEPs and respective control groups. The graphs show the data from one representative experiment out of three independent experiments. Six mice were included in each experimental group.

Despite the different impact of two types of DEPs on iBALT formation, both of them induced B-cell accumulation in the lungs,as can be judged by Cd19 expression, and this effect was slightly dependent on antigen dose (Figure 7a). High antigen doses also induced germinal centers formation in lung tissue (Bcl6 expression which is germinal center marker gene [35]). DEP1 does not stimulate their formation in the low antigen dose group. DEP2 rather than DEP1 in combination with low antigen doses stimulated germinal centers formation in lungs (Figure 7b). At the same time both types of particles triggered the expression of Ebi2, the extrafollicular foci formation marker [35], and this effect was independent of the co-administered antigen dose (Figure 7c).

4. Discussion

The ability of particulate matter arises from either industrial sources or incomplete combustion of diesel and petroleum fuel to induce respiratory allergy and asthma has been shown previously in many research works [1-14]. However, in most of the works, only one type of particles was used. It is known that the ability of particulate matter to enter the lung and to trigger local and systemic inflammation depends on the size of the particles. In our work, we use two type of DEPs DEP1 and DEP2. The first particles originate directly from heat fuel exchangers of diesel engine; they are more polydisperse in nature and tend to form large aggregates despite of their small size *per se* [21]. The second particles were collected from the air [22] and in fact may represent a mixture of particles derived from industrial sources and from incomplete fuel combustion. In the other hand,

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they may represent a fraction of first ones, which were not prone to aggregate. Due to this fact they are carried in the air for a long time.

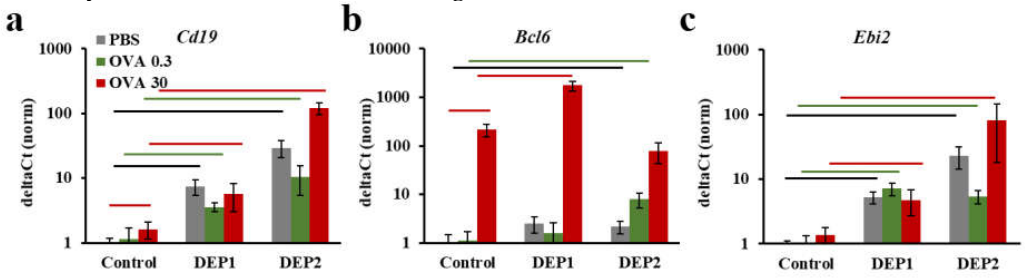

Figure 7. Expression of B-cell linked genes in lung tissue. BALB/c mice were immunized as described in Fig. 4. Control mice were immunized in the absence of DEPs. Expression of markers for B-cell relative number (Cd19; a), B-cell extrafollicular activation (Ebi2; b) or germinal center formation (Bcl6; c) is shown. Black bars indicate significant ($p<0.05$) differences between mice immunized without antigen with different DEPs and respective control without DEPs; green bars indicate significant differences between mice immunized by low OVA dose with or without DEPs and respective control groups; red bars indicate significant differences between mice immunized by high OVA dose with or without DEPs and respective control groups. The graphs show the data from one representative experiment out of three independent experiments. . Six mice were included in each experimental group.

Despite such differences these type of particles stimulated humoral immune response in comparable levels. Although only DEP2 stimulated IgA formation, the resulting IgA titers were not very high in comparison with IgG titers and this phenomena may not cause very marked effects in relation to allergic inflammation. In relation to IgE, the effect of these particles on IgG1 and even IgG2a production were very similar. While in some cases IgG1 and IgG2a production were stimulated by relatively low DEPs doses, IgE production is stimulated only by high DEPs doses (150 μg/mice-immunization, or about 15 mg/kg weekly). Although such DEPs amounts usually do not penetrate into human organism even in heavily polluted industrial or urban areas, it should be noted that DEPs tend to accumulate in human organism for a long time and, therefore, to achieve high doses [2,3]. Our data are in agreement with research indicating that only high DEPs doses potentiate strong allergic inflammation [12] but in contradiction with other where even low DEPs doses triggers type 2 immune response [14]. However in the latter work allergen extract contained additional stimuli but not pure protein was used as an allergen [14]. The fact that only high DEPs doses induce IgE production and asthma development may indicate that such substances are actually account for asthma and allergy development per se only in regions with heavy industrial and road traffic burden and mostly in people of middle or elderly ages or in people initially predisposed to atopic diseases. Although in most of European and North American countries fuel and engine standards presume very low if any amount of particulate matter after combustion compared with analogues standards 30-35 years ago [36] one can conclude that soon this type of air pollution will cease to be the leader case of increasing asthma prevalence. Unfortunately in developing countries of Asia and Africa where diesel fuel is widely used as well as a fuel based on coal and wood and the ecological rules are not so hard this problem is very actual [37].

We have previously shown that upon administration of antigen in fat tissue with tertiary lymphoid structures low but not high antigen doses mainly induce IgE production [23]. However, the immune response upon administration of antigen in lung tissue may be substantially different from that initiates after administration in subcutaneous fat due to different properties of either tertiary lymphoid structures in lungs and fat tissue or different rate and ways of antigen delivery to secondary lymphoid organs from these two sites. Indeed, in the present work DEPs stimulate IgE synthesis in response to both low and high OVA doses. This fact indirectly shows that the impact of DEPs on IgE response is not linked with germinal centers induction because this process is prohibited when an-

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tigen is administered in low doses. This was than directly confirmed. Though allergic inflammation after the high dose challenge in mice was more eosinophilic in nature if mice were immunized previously for a long time with DEPs and low antigen dose, co-administration of high antigen dose and DEPs followed by challenge also triggers eosinophils accumulation in the lungs. Accumulation of neutrophils was antigen-specific and comparable in both cases. So antigen dose administrated with DEPs for a long time make a little if any difference in the resulting picture of potentiated local allergic inflammation.

DEPs stimulate both local and system IgE class switching when administrated with low antigen doses. But in the case of SRM1650B we observed only local IgE class switching after their co-administration with high antigen dose, and in the case of SRM2786 only local IgE producing cells accumulation after their co-administration with high antigen dose. Despite the fact that PAH which can desorbs from particles and enter the system circulation upon particulate matter accumulation in the lungs could *per se* stimulate B-cell, T-cell and dendritic cell activation, based on our data we can suppose that high antigen doses induce higher levels of iBALT development accompanied by accumulation of antigen-presenting cells (APCs) mostly in these structures. When accumulate mostly in iBALT these cells weekly transport particles and antigen to lymph nodes. In contrast, when low antigen doses were administered, migration of these cells towards iBALT may not be so strong and some APCs instead of this could transport particles and antigens to lymph nodes. As it were, B-cells from iBALT *per se* could migrate in some cases from tissue to lymph nodes and indeed even in the group where high OVA dose and high SRM1650B dose were administrated to mice and only local IgE switching could be observed, IgE producing cells accumulation was detected in both sites. However, in this work we decide to focus on local processes.

From our previous works, we concluded that in young allergic patients [38] and in laboratory animals [23] IgE class switching occurs mainly by direct mechanism. The results from this work came into contradiction with this fact because we observe accumulation of both type of circular transcripts corresponding to IgE class switching from IgM and from IgG1 as well. This fact is in agreement with some results shown that both type of B-cell IgE class switching could be observed in allergic patients [39]. Even in animal models, this switching may occur via either direct [40] or sequential mechanism [41] dependent on the model used. However, it should be mentioned that when co-administrated with high antigen doses when intensive iBALT growing is observed DEPs induce only direct IgE class switching. This situation is closely related to the clinical cases from patients with asthma or chronic allergic rhinitis at the later stages of disease development when high antigen doses accumulate in iBALT or nasal polyps for a long time. So direct IgE class switch may be more important also further studies are needed to answer this question. The lack of significant effect of DEPs on the expression of *germline* and *postswitch γ1* transcripts in some cases may reflect the fact that in the case of IgG1 DEPs stimulate its production without incresing of the IgG1 class switch *per se* but by accelerating of the differentiation of IgG1 expressing B-cells to the final stages of IgG1-producing plasma cell. It is also possible that the effect of DEPs on IgG1 class switching and accumulation of IgG1 producing cells is more strong and significant in the early stages of the process.

It is tempting to speculate that the different abilities of two types of particulate matter to induce iBALT formation and growing after accumulation in lung tissue is due to their different tendencies to form relatively large (>10 μm) aggregates. Despite the fact, that prior to administration to mice DEPs were sonicated, lung microphotographs from H&E histology shows that large aggregates of particles were present in lung tissue especially in the case of SRM1650B. According to its characteristic by NIST SRM2786 despite their larger size prior to aggregation are less prone to form large aggregates [22]. Large aggregates slowly diffuse through the epithelium and are hardly phagocytized by APCs. So despite their ability to damage airway epithelium they are hardly delivered into the lung tissue with small soluble antigens and for this reason their ability to induce iBALT could be limited. In the other hand SRM2786 as particles which were carried in the air for a long

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time may have a different chemical composition from SRM1650B and this fact could be responsible for such difference.

We do not however focus on this difference too closely because both type of particles induce comparable local IgE class switching and overall IgE production. We found however that this difference is linked with their different abilities to stimulate initial CXCL13 production and germinal center response when particles co-administrated with low antigen doses. It is likely that particles from the fine type of particulate matter SRM2786 adsorb co-administrated antigen on their surface and then penetrate the epithelial barrier with it. When these particles accumulate in tissue adsorbed antigen serves as a stronger signal for B- and T-cells than soluble one [42]. Certainly, SRM1650B also can adsorb a proportion of OVA on their surface but when they form large aggregates they hardly pass the epithelial barrier and the proportion of antigen adsorbed on their surface remained in alveoli or bronchi space out of reach for B- and T-cells. The other possibility is that specific chemicals in present in SRM2786 but absent in SRM1650B serve as an additional trigger for CXCL13 production.

Despite these discrepancies both type of particulate matter trigger IgE production. So the main conclusion from this work is that local IgE production in response to particulate matter in independent from the ability of this particulate matter to induce iBALT formation and growing. Common but not different properties for this two type of DEPs must be responsible for local B-cell IgE class switch induction. Therefore, it is very important to mention that both type of DEPs induce local *Ebi2* expression which is an indicator of extrafollicular foci formation [35]. Both type of DEPs induce local *TNFα*, *IFNα1* expression, as well as *IL*-4 and *IL*-13 expression, but the expression of *IFNγ* is not induced. From these data it becomes clear that DEPs induce local IgE production by specific stimulation of type 2 immune response and this is in agreement with most previously works [5,11,12,14]. Though it was shown that DEPs [13] or their carbon core [5] also capable to induce IFNγ production, we do not observe induction of *IFNγ* expression in mouse lungs in our work. The expression of IL-13 was induced by DEPs not so strong as IL-4 expression and in the first case only differences between groups immunized with antigen and DEPs versus antigen alone immunized mice but not between DEPs and saline immunized mice were significant. This may indicate that DEPs create such polarization of the immune response by activating cells that express mostly IL-4 but not IL-13. Conventional T-helpers 2 express both cytokines [43] but most T-follicular helpers [44] with the exception of the small subset [45] express only *IL*-4 but not *IL*-13. In our case, however IgE production does not always followed by germinal centers induction as was judged by *Bcl6* expression which is the germinal center master regulator [46]. DEPs may stimulate basophils and mast cells directly [2,3,47,48] and it is accepted that these type of cells express mostly IL-4 and less *IL*-13. We hope that further works will clarify the participation of different cytokine producing cells in our model.

In the case of SRM1650B induction of *TNFα* and *IFNα1* expression when these particles were co-administrated with low antigen dose does not lead to iBALT induction and growing notwithstanding that these cytokines have iBALT promoting activity [31,32,34]. Despite this fact, TNFα may be important for IgE class switching because of its ability to activate or inhibit B-cells and specifically B-cell Ig class switching depending on particular conditions [49,50] independent of its ability to induce tertiary lymphoid structures development. We could not conclude if IFNα1 has some role in local B-cell class switching if its expression did not results in iBALT induction. But when expressed in the absence of type II interferon (*IFNγ*) its induction could serve as an indicator of tissue DNA damage by PAHs from DEPs because after DNA damage STING-TBK1 signaling stimulates its expression [51].

It is also interesting that local germinal center induction was not required for IgE production stimulation by SRM1650B and low antigen doses. It is in contradiction with some results on animal models where high affinity and clinically relevant IgE production requires B-cell germinal center response [41]. But previously it was established that IgE switched B-cells are relatively rare in germinal centers and disappear from them upon

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their full maturation [40] so IgE switching may occur in very small and early stage of germinal center presence of which could not be clearly found by qPCR. Instead of this strong extrafollicular response induction was always accompanied DEPs induced local B-cell IgE class switching and this is in agreement with some clinical data where it was shown that local IgE production is mostly linked with extrafollicular B-cell response [52,53].

From our work, it becomes clear that local IgE production surprisingly does not always required *de novo* iBALT formation and growing. It does not mean however, that iBALT *per se* were not required for IgE B-cell class switching. Because iBALTs were present even in naïve mice despite their low numbers and small sizes it is likely that even small and rare iBALTs are sufficient to mount local specific IgE production. Because of according to some data fully matured germinal centers hampered IgE class switching [38] and larger iBALTs contained such germinal centers it could be that exactly small size iBALTs represent a place where IgE class switching actually occurs. Indeed according some data large iBALTs if they were formed prior to allergen administration to mice do not enhance but even dampen allergic inflammation as well as total IgE production though in that case iBALT was induced by LPS but not by DEPs [54]. DEPs even do not induce iBALTs growing induce extrafollicular B-cell activation and CD19-expressing B-cell accumulation in the lungs. One interesting hypothesis is that IgE class switching may occur in B-cells which situated beyond iBALTs in lung tissue in B-cell aggregates and infiltrates. B-cells entering non-immune organs do not always form tertiary lymphoid structures though in such cases they represent cells recirculating from secondary lymphoid organs to this peripheral tissues [55]. Poorly organized B-cell aggregates were also found in some cases in peripheral tissues [56]. The recent work shows that in immunized mice IgE producing B-cells forms such aggregates though they were found in spleen but not in tissues and the authors suggest that IgE-producing plasma cells form such aggregates after IgE switching due to the appearance of specific receptors on their surface [57]. However it is tempting to speculate that specific FcγRII/III on the surface of IgE plasma cells if does not trigger IgE class switching alone may serve as prosurvival signals for recently switched IgE+ B-cells because inhibition of such aggregates formation leads to reduced serum IgE levels in mice. If so stimuli that enhance B-cell accumulation in tissue, rapid proliferation and differentiation in antibody producing cells may lead to such aggregates formation where B-cells receive pro-survival signals even beyond the organized tertiary lymphoid clusters structures. Further works needed to estimate if one of these hypothesis is a true. Due to the paucity of IgE+ cells in the overall B-cell population and the expression of CD23 that binds exogenous IgE from serum [58] it is badly hard if even possible to answer this question by using convential immunohistochemistry. However if IgE+ cells indeed formed locally or in regional lymph nodes but outside B-cell follicles of these structures, and even outside tissue tertiary lymphoid clusters or outside B-cell zones of lymph nodes, their formation must be insensitive to specific inhibitors of signaling pathways and cytokines responsible for tertiary lymphoid clusters and B-cell follicles formation and must be sensitive for specific inhibitors of pathways responsible for B-cell aggregates formation.

5. Conclusions

Overall, DEPs can induce local as well as system B-cell IgE class switching which leads to allergic immune response formation. However, local but not system IgE class switch in response to DEPs could be observed independent of antigen dose and type of particles. This local IgE class switch occurs mainly by direct mechanisms also low antigen doses can stimulate sequential switch as well. Only high DEP doses induce IgE production and so DEPs may be responsible for atopic diseases development only when present constantly in very high concentration in the air when someone dwell in regions with high road traffic burden or in allergy prone individuals. Local DEPs induced B-cell IgE class switching independent of their ability to induce expression of CXCL13, germinal center development and *de novo* iBALT formation and growing. Small initially presented iBALTs

in lungs or other structures may be responsible for IgE production in this case. DEP induced local B-cell IgE class switching was accompanied by extrafollicular B-cell activation and induction of type 2 immune response cytokines expression in the absence of type 1 immune response activation.

Abbreviations: DEPs – Diesel exhaust particles; DEP1 – SRM1650b standardized type of diesel exhaust particles; DEP2 – SRM2786 standardized type of diesel exhaust particles; iBALT – Inducible bronchial-associated lymphoid tissue; SRM – Standard reference material.

Supplementary Materials: Figure S1: Antibody production in response to high OVA doses, DPs, or their combination. BALB/c mice were immunized 2-3 times a week for 8 weeks (total 18 immunizations) via i.n. route by 30 μgOVA alone or at the presence of 30 or 150 μg DEPs as indicated.. The titers of OVA specific IgE (a), IgG1 (c), IgG2a (d) or total IgE (b) were measured. Red bars show statistical differences between groups immunized with antigen and particles and antigen alone. Statistical differences of groups immunized by particles alone vs saline control are shown by black bars ($p<0.05$).The graphs show the data from one representative experiment out of three independent experiments. Six mice were included in each experimental group. **Figure S2**: BALB/c mice were immunized with 0.3 μg (a) or 30 μgOVA (b) alone or in combination with 30 or 150 μg of DEPs. The titers of OVA specific IgA were measured. Statistical differences of groups immunized with high antigen doses in combination with particles vs.OVA control are shown by red bars ($p<0.05$). The graphs show the data from one representative experiment out of three independent experiments. Six mice were included in each experimental group. **Figure S3**: I – Cells; II – Single cells; III – Living cells; IV – CD11lowc-SiglecF+ Eosinophils; V – CD11c+SiglecF+ cells; VI – SiglecF- cells; VII – CD11c+SiglecF+F4/80+ Alveolar Macrophages; VIII – CD11c+SiglecF- Dendritic Dells; IX – CD11c- not Alveolar Macrophages not Eosinophils not Dendritic Cells; X – Gr-1+CD11b+CD11c-SiglecF- Neutrophils; XI – not Neutrophils not Alveolar Macrophages not Eosinophils not Dendritic cells; XII – F4/80+CD11b+SiglecF-CD11c- Interstitial Macrophages; XIII – F4/80-CD11b+SiglecF-CD11c- monocytes. **Figure S4**: Representative flow cytometry contour plots and gating of Eosinophils (a), Macrophages (a, b), and Neutrophils (c). **Figure S5**: BALB/c mice were immunized 2-3 times a week for 8 weeks (total 18 immunizations) via i.n. route without antigen (PBS), with low (0.3 μg) OVA dose or high (30 μg) OVA dose without particles (control) or with either DEP1 or DEP2. After immunization mice were sacrificed. Expression of transcripts corresponding to direct IgE class switching (circular μ-ε) (a, c) or sequential IgE class switching (circular γ1-ε) (b, d) class switching were measured in lung tissue (a, b) and regional lymph nodes (c, d). Black bars indicate significant ($p<0.05$) differences between mice immunized without antigen with different DEPs and respective control without DEPs; green bars indicate significant differences between mice immunized by low OVA dose with or without DEPs and respective control groups; red bars indicate significant differences between mice immunized by high OVA dose with or without DEPs and respective control groups. Representative data from 3 independent experiments.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used Conceptualization, D.B.Ch. and G.V.F.; methodology, D.B.Ch., M.V.K., M.A.Sh.; formal analysis, G.V.F., E.I.K.; investigation, D.B.Ch., O.D.K., M.V.K., D.S.T., M.A.Sh.; resources, D.B.Ch., E.I.K.; data curation, D.B.Ch., O.D.K.; writing—original draft preparation, D.B.Ch., E.I.K.; writing—review and editing, G.V.F.; supervision, G.V.F.; project administration, D.B.Ch., E.I.K.; funding acquisition, D.B.Ch., E.I.K. All authors have read and agreed to the published version of the manuscript.

Funding: The reported study was funded by RFBR according to the research projects number 19-05-50064 and 20-015-00360. The funders had no role in the study design, data collection and analysis, decision to publish, or the preparation of the manuscript.

Institutional Review Board Statement: The animal study protocol was approved by IACUUC protocol number 350 approved by the local committee of IBCh RAS on 21.06.2020.

Data Availability Statement: The data presented in this study are available in figures within the article and in Supplementary File S1.

Acknowledgments: We thank the senior scientist of the Laboratory of Cell Interactions of IBCh RAS Svirshchevskaya E.V. for providing a room and basal equipment for this work.

Conflicts of Interest: The authors declare no conflict of interest.

 doi:10.20944/preprints202207.0222.v1

References

1. Poulsen, L.K., Hummelshoj, L. Triggers of IgE class switching and allergy development. *Ann. Med.* **2007**; 39(6): 440-456.
2. Balmes, R.J. How does diesel exhaust impact asthma? *Thorax*. **2011**; 66(1): 4-6.
3. Pandya, R.J., Solomon, G., Kinner, A., Balmes, R.J. Diesel Exhaust and Asthma: Hypotheses and molecular mechanisms of action. *Envir. Health Persp*. **2002**; 110(S1): 103-112.
4. Wang, X., Wang, Y., Bai, Y., Wang, P., Zhao, Y. An overview of physical and chemical features of diesel exhaust particles. *Journal of Energy Institute*. **2018**; 92(6): 1864-1888.
5. Yanagisava, R., Takano, H., Inoue, K.-I., Sadakane, K., Yoshino S., Yamaki, K., Yoshikawa, T., Hayakawa, K. Components of diesel exhaust particles differentially affect Th1/Th2 response in a murine model of allergic airway inflammation. *Clin. Exp. Allergy*; **2006**; 36: 386-395.
6. Bonvallot, V., Baeza-Squilban, A., Baulig, A., Boland, S., Muzeau, F., Barouki, R., Marano, F. Organic Compounds from Diesel Exhaust Particles Elicit a Proinflammatory Response in Human Airway Epithelial Cells and Induce Cytochrome p450 1A1 Expression. *Am. J. Respir. Cell Mol. Biol.* **2001**; 25(4): 515-521.
7. Pania, S., Brown, J.L., Frew, A.J. The role of pollutants in allergic sensitization and the development of asthma. *Allergy*. **2002**; 57: 1111-1117.
8. Brant, E.B., Biagini Myers, J.M., Acciani, T.H., Ryan, P.H., Sivaprasad, U., Ruff, B., LeMasters, G.K., Bernstein, D.I., Lockey, J.E., LeCras, D.T., Hershey, G.K.K. Exposure to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses, promoting asthma susceptibility. *J. Allergy Clin. Immunol.* **2015**; 136(2): 295-303.e7.
9. Brandt, E.B., Bolcas, P.E., Ruff, B.P., Hershej, G.K.K. IL33 contributes to diesel pollution-mediated increase in experimental asthma severity. *Allergy*. **2020**; 75(9): 2254-2266.
10. Simon, D.A., Munoz, X., Gomez-Oloves, S., de Homdedeu M., Untoria, M.-D. Effects of diesel exhaust particle exposure on a murine model of asthma due to soybean. *PLoS One*. **2017**; 12(6): e0179569.
11. Brandt, E.B., Kovalic, M.B., Lee, G.B., Gibson, M.A., Acciani, T.H., LeCras, T.D., Ryan, P.H., Budelsky, A.L., Hershey, G.K.K. Diesel exhaust particle induction of IL-17A contributes to severe asthma. *J. Allergy Clin. Immunol*. **2013**; 132: 1194-1204.
12. Kim, B.-G., Lee, P.-H., Lee, S.-H., Kim, Y.E., Shin, M.Y., Kang, Y., Bae, S.H., Kim, M.J., Rhim, T., Park, C.S., Jang, A.S. Long-Term Effects of Diesel Exhaust Particles on Airway Inflammation and Remodeling in a Mouse Model. *Allergy Asthma Immunol. Res.* **2016**; 8(3): 246-256.
13. Park, E.-J., Roh, J., Kim, S.N., Choi, S. Biological Responses to Diesel Exhaust Particles (DEPs) Depend on the Physicochemical Properties of the DEPs. *PLoS One*. **2011**; 6(10): e26749.
14. De Grove, K.C., Provoost, S., Hendricks, R.W., McKenzie, A.N.J., Seys, L.J.M., Smitha Kumar, S., Tania Maes, T., Brusselle, G.G., 1, Joos, G.F. Dysregulation of type 2 innate lymphoid cells and TH2 cells impairs pollutant-induced allergic airway responses. *J. Allergy Clin. Immunol*. **2017**; 139(1): 246-257.e4.
15. Kuroda, E., Ozasa, K., Temizoz, B., Ohata, K., Christine X Koo, C.X., Kanuma, T., Kusakabe, T., Kobari, S., Horie, M., Morimoto, Y., Nakajima, S., Kabashima, K., Ziegler, S.F., Iwakura, Y., Ise, W., Kurosaki, T., Nagatake, T., Kunisawa, J., Takemura, N., Uematsu, S., Hayashi, M., Aoshi, T., Kobiyama, K., Coban, C., Ishii, K.J. Inhaled Fine Particles Induce Alveolar Macrophage Death and Interleukin-1a Release to Promote Inducible Bronchus-Associated Lymphoid Tissue Formation. *Immunity*. **2016**; 45: 1299-1310.
16. Hiramatsu, K., Azuma, A., Kudoh, S., Desaki, M., Takizawa, H., Sugawara, I. Inhalation of diesel exhaust for three months affects major cytokine expression and induces bronchus-associated lymphoid tissue formation in murine lungs. *Exp. Lung Res*. **2003**; 29(8): 607-622.
17. Wang, J., Huang, J., Wang, L., Chen, C., Yang, D., Jin, M., 1, Bai, C., Song, Y. Urban particulate matter triggers lung inflammation via the ROS-MAPK-NF-κB signaling. *J. Thorac. Dis*. **2017**; 9(11): 4398-4412.
18. Janzen, K., Roursgaard, M., Desler, C., Loft S., Rasmussen, L.J., Moller, P. Oxidative damage to DNA by diesel exhaust particle exposure in co-cultures of human lung epithelial cells and macrophages. *Mutagenesis*. **2012**; 27(6): 693-701.
19. Golokhvast, K., Vitkina, T., Gvozdenko, T., Kolosov, V., Yankova, V., Kondratieva, E., Gorkavaya, A., Nazarenko, A., Chaika, V., Romanova, T., Karabtsov, A., Perelman, J., Kiku, P., Tsatsakis, A. Impact of Atmospheric Microparticles on the Development of Oxidative Stress in Healthy City/Industrial Seaport Residents. *Oxid. Med. Cell Longev*. **2015**; 2015: 412173.
20. Matsuo, M., Shimada, T., Uenishi, R., Sasaki, N., Sagai, M. Diesel exhaust particle-induced cell death of cultured normal human bronchial epithelial. *Biol. Pharm. Bull*. **2003**; 26(4): 438-447.
21. Gonzales, C.A., Chocuette, S.J. National Institute of Standards and Technology. *Certificate of Analysis. Standart reference material 1650B. Diesel particulate matter*. Gaithersburg. Certificate Issue Date 07 July 2021.
22. Gonzales, C.A., Chocuette, S.J. National Institute of Standards and Technology. *Certificate of Analysis. Standart reference material 2786. Fine Atmospheric Particulate Matter (Mean Particle Diameter <4 μm)*. Gaithersburg. Certificate Issue Date 02 July 2021.
23. Chudakov, D.B., Rysantsev, D.Yu., Tsaregorodtseva, D.S., Kotsareva, O.D., Fattakhova, G.V., Svirshchevskaya, E.V. Tertiary lymphoid structure related B-cell IgEisotype switching and secondary lymphoid organ linked IgE production in mouse allergy model. *BMC Immunol*. **2020**; 21: 45.
24. Hoecke, L.V., Job, E.R., Saelens, X., Roose, K. Bronchoalveolar Lavage of Murine Lungs to Analyze Inflammatory Cell Infiltration. *JoVe*. **2017**; 123: E55398.
25. Stevens, W.W., Kim, T.S., Pujanauski, L.M., Hao, X., Braciale, T.J. Detection and quantitation of eosinophils in the murine respiratory tract by flow cytometry. *J. Immunol. Methods*. **2007**; 327(1-2): 63-74.

26. Firacative, C., Gressler, A.F., Schubert, K., Schulze, B., Müller, U., Brombacher, F., von Bergen, M., Alber, G. Identification of T helper (Th)1- and Th2-associated antigens of Cryptococcus neoformans in a murine model of pulmonary infection. *Sci Rep.* **2018**; 8: 2681.
27. Fujita, H., Soyka, M.B., Akdis, M., Akdis, C.A. Mechanisms of allergen-specific immunotherapy. *Clin. Transl. Allergy*. **2012**; 2: 2.
28. Strait, R.T., Morris, S.C., Yang, M., Qu, X.-W., Finkelman, F.D. Pathways of anaphylaxis in the mouse. *J. Allergy Clin. Immunol.* **2002**; 109(4): 658-668.
29. Xu, Z., Zan, H., Pone, E.J., Mai, T., Casali, P. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. *Nat. Rev. Immunol*. **2012**; 12: 517-531.
30. Rayamajhi, M., Delgado, C., Condon, T.V., Riches, D.W., Lenz, L.L. Lung B cells promote early pathogen dissemination and hasten death from inhalation anthrax. *Mucosal Immunol*. **2012**; 5: 444-454.
31. Foo, S.Y., Philips, S. Regulation of inducible BALT formation and contribution to immunity and pathology. *Mucosal Immunol*. **2010**; 3: 537-544.
32. Benezech, C., Liu, N.-T., Walker, J.A., Kruglov, A.A., Loo, Y., Nakamura, K., Zhang, Y., Nayar, S., Jones, L.H., Flores-Langarica, A., McIntosh, A., Marshall, J., Barone, F., Besra, G., Miles, K., Allen, J.E., Gray, M., Kollias, G., Cunningham, A.F., Withers, D.R., Toellner, K.M., Jones, N.D., Veldhoen, M., Nedospasov, S.A., McKenzie, A.N.J., Caamaño, J.H. Inflammation-induced formation of fat-associated lymphoid clusters. *Nat. Immunol*. **2015**; 16: 819-828.
33. Ngo, V.N., Korner, H., Gunn, M.D., K N Schmidt, Riminton, D.S.,, Cooper, M.D., Browning, J.L., Sedgwick, J.D., Cyster, J.G. Lymphotoxin α/β and Tumor Necrosis Factor Are Required for Stromal Cell Expression of Homing Chemokines in B and T Cell Areas of the Spleen. *J. Exp. Med*. **1999**; 189(2): 403-412.
34. Denton, A.E., Innocentin, S., Carr, E.J., Bradford, B.M., Lafouresse, F., Mabbott, N.A., Mörbe, U., Ludewig, B., Groom, J.R., Good-Jacobson, K.L., Linterman, M.A. Type I interferon induces CXCL13 to support ectopic germinal center formation. *J. Exp. Med*. **2019**; 216(3): 621-637.
35. Enoksson, S.L., Grasset, E.K., Hagglof, T., Mattsson, N., Kaiser, Y., Gabrielsson, S., McGaha, T.L., Scheynius, A., C I Karlsson, M.C.I. The inflammatory cytokine IL-18 induces self-reactive innate antibody responses regulated by natural killer T cells. *Proc. Natl. Acad. Sci*. **2011**; 108(51): E1399-1407.
36. Fiebig, M., Wiartalla, A., Holderbaum, B., Keisow, S. Particulate emissions from diesel engines: correlation between engine technology and emissions. *J. Occup. Med. Toxicol*. **2014**; 9:6.
37. Mannucci, P.M., Franchini, M. Health Effects of Ambient Air Pollution in Developing Countries. *Int. J. Environ. Res. Pub. Health*. **2017**; 14(9): 1048.
38. Svirshchevskaya, E., Fattakhova, G., Khlgatyan, S., Chudakov, D., Kashirina, E., Ryazantsev, D., Kotsareva, O., Zavriev, S. Direct versus sequential immunoglobulin switch in allergy and antiviral responses. *Clin. Immunol*. **2016**; 170: 31-38.
39. Takhar, P., Corrigan, C.J., Smuthwaite, L., O'Connor, B.J., Durham, S.R., Lee, T.H., Gould, H.J. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. *J. Allergy Clin. Immunol*. **2007**; 119(1): 213-218.
40. Talay, O., Yan, D., Brightbill, H.D., Straney, E.E.M., Zhou, M., Ladi, E., Lee, W.P., Egen, J.G., Austin, C.D., Xu, M., Wu, L.C. IgE+ memory B cells and plasma cells generated through a germinal-center pathway. *Nat. Immunol.* **2012**; 13: 394-404.
41. He, J.-S., Subramanian, S., Narang, V., Srinivasan, K., Saunders, S.P., Carbajo, D., Wen-Shan, T., Hamadee, N.H., Lum, J., Lee, A., Chen, J., Poidinger, M., Zolezzi, F., Lafaille, J.J., de Lafaille, M.A.C. et al. IgG1 memory B cells keep the memory of IgE responses. *Nat. Commun*. **2017**; 8: 641.
42. Fox, C.B., Kramer, R.M., Balmes, L., Dowling, Q.M., Vedvick, T.S. Working together: interactions between vaccine antigens and adjuvants. *Ther. Adv. Vaccines*. **2013**; 1(1): 7-20.
43. Bao, K., Reinhardt, L.R. The differential expression of IL-4 and IL-13 and its impact on type-2 Immunity. *Cytokine*. **2015**; 75(1): 25-37.
44. Liang, H.-E., Reinhardt, L.R., Bando, J.K., Sullivan, B.M., Ho, I.-C., Locksley, R.M. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat. Immunol*. 2012; 13: 58-66.
45. Gowthaman, U., Chen, J.S., Zhang, B., Flynn, W.F., Lu, Y., Song, W., Joseph, J., Gertie, J.A., Xu, L., Collet M.A., Grassmann, J.D.S., Simoneau, T., Chiang, D., Berin, M.C., Craft, J.E., Weinstein, J.S., Williams, A., Eisenbarth, S.C. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science*. **2019**; 365(6456): doi:10.1126/science.aaw6433.
46. Basso, K., Dalla-Favera, R. BCL6: master regulator of the germinal center reaction and key oncogene in B cell lymphomagenesis. *Adv. Immunol*. **2010**; 105: 193-210.
47. Diaz-Sanchez, D., Penichet-Garcia, M., Saxon, A. Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. *J. Allergy Clin. Immunol.* **2000**; 106(6): 11401146.
48. Devouassoux, G., Saxon, A., Metcalfe, D.D., Prussin, C., Colomb, M.G., Brambilla, C., Diaz-Sanchez, D. Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils. *J. Allergy Clin. Immunol.* **2002**; 109(5): 847-853.
49. Boussiotis, V.A., Nadler, L.M., Strominger, J.L., Goldfield, A.E. Tumor necrosis factor alpha is an autocrine growth factor for normal human B cells. *Proc. Natl. Acad. Sci*. **1994**; 91(15): 7007-7011.
50. Frasca, D., Romero, M., Diaz, A., Alter-Wolf, S., Ratliff, M., Landin, A.M., Riley, R.L., Blomberg, B.B. A molecular mechanism for TNF-α-mediated down-regulation of B cell responses. *J. Immunol*. **2002**; 188(1): 279-286.
51. Hartlova, A., Erttmann, S.F., Raffi, F.A.M., Schmalz, A.M., Resch, U., Anugula, S., Lienenklaus, S., Nilsson, L.M., Andrea Kröger, A., Nilsson, J.A., Ek, T., Weiss, S., O'Gekara, N. DNA Damage Primes the Type I Interferon System via the Cytosolic DNA Sensor STING to Promote Anti-Microbial Innate Immunity. *Immunity*. **2015**; 42(2): 332-343.

52. Corrado, A., Ramonell, R.P., Woodruff, M.C., Tipton, C., Wise, S., Levy, J., DelGaudio, J,, Kuruvilla, M.E,, Magliocca, K.R., Tomar, D., Garimalla, S., Scharer, C.D., Boss, J.M., Wu, H., Gumber, S., Fucile, C., Gibson, G., Rosenberg, A., Sanz, I., Lee, F.E. Extrafollicular IgD+ B cells generate IgE antibody secreting cells in the nasal mucosa. *Mucosal Immunol*. **2021**; 14: 1144-1159.
53. Feldman, S., Kasjanski, R., Poposki, J., Hernandez, D., Chen, J.N., Norton, J.E., Suh, L., Carter, R.G., Stevens, W.W., Peters, A.T., Kern, R.C., Conley, D.B., Tan, B.K., Shintani-Smith, S., Welch, K.C., Grammer, L.C., Harris, K.E., Kato, A., Schleimer, R.P., Hulse, K.E. Chronic Airway Inflammation Provides a Unique Environment for B Cell Activation and Antibody Production. *Clin. Exp. Allergy*. **2017**; 47(4): 457-466.
54. Hwang, J.Y., Silva-Sanchez, A., Carragher, D.M., de la Luz Garcia-Hernandez, M., Rangel-Moreno, J., Randall, T.D. Inducible Bronchus–Associated Lymphoid Tissue (iBALT) Attenuates Pulmonary Pathology in a Mouse Model of Allergic Airway Disease. *Front. Immunol*. **2020**; 11: 570661.
55. Inman, C.F., Murray, T.Z., Bailey, M., Cose, S. Most B cells in non-lymphoid tissues are naïve. *Immunol. Cell Biol*. **2012**; 90(2): 235-242.
56. Schropp, V., Rohde, J., Rovituso, D.M., Jabari, S., Bharti, R., Kuerten, S. Contribution of LTi and TH17 cells to B cell aggregate formation in the central nervous system in a mouse model of multiple sclerosis. *J. Neuroinflamm*. **2019**; 16: 111.
57. Hikosara, M., Murata, A., Yoshino, M., Hayashi, S.-I. Correlation between cell aggregation and antibody production in IgE-producing plasma cells. *Biochem. Biophys. Rep*. **2017**; 10: 224-231.
58. Aranda, C.J., de Lafaille, M.A.C. The Secret Life of IgE-Producing Cells. *Immunity*. **2019**; 50: 285-287.