

ORIGINAL ARTICLE

The role of intestinal dendritic cells subsets in the establishment of food allergy

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Summary

Background Food allergy affects approximately 6% of children and is the leading cause of hospitalization for anaphylactic reactions in westernized countries. Crucial in the establishment of allergy is the activation of dendritic cells (DC) leading to T helper 2-mediated responses.

Objective We, therefore, investigated whether changes in DC subsets precede the establishment of food allergy, and which DC subsets have functional relevance during allergic sensitization in a mouse model.

Methods Changes in DC populations in the intestine were analysed after exposure to cholera toxin alone and in combination with peanut extract (PE) as an allergen. To study the functional role of DC subsets in relation to food allergy, we used expansion of DC *in vivo* by treatment with Flt3L.

Results Sensitization to PE in this mouse model was accompanied by a shift in DC subsets in intestinal tissues towards more CD11b⁺DC and less CD103⁺DC. No significant changes in the plasmacytoid DC (pDC) numbers were observed. Flt3L treatment, resulting in the expansion of all DC subtypes, inhibited allergic manifestations in our model, including Th2 cytokine production, PE-specific IgE and PE-induced mast cell degranulation. pDC depletion reversed Flt3L-induced inhibition of IgE responses and mast cell degranulation.

Conclusions and Clinical Relevance The establishment of food allergy is accompanied by profound changes in DC subsets in the intestine towards more inflammatory CD11b⁺DC. In addition, expansion of DC numbers by Flt3L, in particular pDC, inhibits the establishment of allergic manifestations in the intestine. These findings are of relevance for understanding the role of DC subsets early during the process of allergic sensitization, and may lead to new therapeutic or prophylactic opportunities to prevent food allergy.

Keywords dendritic cell, food allergy, intestine, mouse model, peanut allergy

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Introduction

Currently, food allergy poses a major health problem in the western world, affecting 6–8% of children and 1–4% of adults [1]. Knowledge of allergic manifestations has increased over the years, but the underlining mechanisms responsible for allergic sensitization to food proteins remain elusive. The decision between active immunity, including sensitization to allergens, or tolerance, is crucial at mucosal surfaces such as the intestine. Here, the dendritic cell (DC) will play a crucial role, as these cells

are the most potent antigen-presenting cells, steering T cells into activation or regulation. Different subsets of DC have been described in the intestine of mice based on the expression of CD11c, CD11b, CD8 α , mPDCA and recently CD103 and CX₃CR1 [2]. Expression of these markers led to the description of three subsets of DC in intestinal tissues: CD11c⁺CD11b⁺conventional ('myeloid') DC (cDC), CD11c⁺CD103⁺DC (CD103⁺ DC) and CD11c⁺B220⁺ mPDCA⁺ plasmacytoid DC (pDC) [3]. These subsets have their own functional specialization, but exogenous factors or inflammatory conditions could alter their phenotype. For

instance, CD103 DC can migrate from the intestinal mucosa to mesenteric lymph nodes (MLN), produce retinoic acid and TGF- β and stimulate retinoic acid receptor signalling in responding T cells, thereby inducing Foxp3⁺ regulatory T cells involved in tolerance induction [4]. However, the exact role of different DC subsets in the process of sensitization to food proteins *in vivo* has not been fully elucidated.

Animal models contribute to the insight into the mechanisms of sensitization to food proteins. In mouse models, the mucosal adjuvant cholera toxin (CT) is generally used to induce allergic sensitization to co-administered proteins in mice, while feeding the protein alone induces oral tolerance [5]. CT is widely used to investigate antigen-specific IgE characterized sensitization to proteins via the oral route [6]. Allergic sensitization induced by CT is accompanied by up-regulation of MHCII and co-stimulatory molecules on monocyte- and bone marrow-derived DC, resulting in DC that preferably prime naive CD4 T cells to a Th2 phenotype [7]. Furthermore, these matured DC express functional chemokine receptors CCR7 and CXCR4, involved in the migration of DC subpopulations from the intestine to the MLN and from the sub-epithelial dome to T cell areas of the Peyer's patches (PP) [8–10]. Although induction of DC activation and maturation may indeed be of major importance to allergic sensitization, it has been shown that induction of oral tolerance to food proteins also involves maturation and migration of intestinal DC [11]. This indicates that more complicated processes are involved in allergic sensitization in the intestine.

The present study focuses on the changes in DC populations in the intestine and intestinal draining lymph nodes immediately after exposure to CT alone and in combination with peanut extract (PE) as an allergen. Here, we show that induction of food allergy results in profound effects on the number of DC subsets. To study the functional role of DC subsets in relation to food allergy, we used expansion of DC *in vivo* by treatment with Flt3L, a growth factor for DC. This ameliorated the food-allergic response, probably by expansion of pDC. Therefore, the present findings are of relevance in understanding the role of DC subsets early during the process of allergic sensitization, and may lead to new therapeutic or prophylactic opportunities to prevent food allergy.

Material and methods

Mice

Five-week-old specific pathogen-free female C3H/HeO_uJ mice were purchased from Charles River (Lyon, France). All mice were housed under specific pathogen-free conditions within the animal care facility at the Utrecht

University. Experiments were approved by the Animal Experiments Committee of the Utrecht University.

Treatment protocols

Peanuts were kindly provided by Intersnack Nederland BV and PE was prepared as described previously [12]. CT was obtained from List Biological Laboratories Inc. (Campbell, CA, USA). To investigate the role of intestinal DCs during sensitization, mice ($n = 3$ –4) were orally exposed to phosphate-buffered saline (PBS), CT (15 μ g) or PE (6 mg) mixed with CT on days 0, 1 and 2 and sacrificed at day 3. To expand DC *in vivo*, mice were treated with 10 μ g human Flt3L (kindly provided by Amgen, Seattle, WA, USA) for 9 consecutive days [13]. To subsequently deplete pDC in this protocol, mice were treated with the pDC-depleting antibody 120G8 on days 1, 3, 6 and 8 of Flt3L treatment, as described before [13]. All other mice received PBS as a control to Flt3L treatment and rat IgG (MOPC-21, Sigma-Aldrich, St Louis, MO, USA) as a control to 120G8 administration. To elicit oral sensitization towards PE, mice ($n = 8$) were orally gavaged with PE plus CT for 3 consecutive days, and dosing was repeated every week for 4 weeks (exposure on days 0, 1, 2, 7, 14, 21 and 28). Control groups received PBS plus CT. All mice received a challenge of 12 mg PE on day 35, followed by blood sampling after 45 min and were sacrificed 1 day later.

Isolation of intestinal lymphocytes

Intra-epithelial lymphocyte (IEL) cell fractions and lamina propria lymphocyte (LPL) cell fractions were isolated as described previously [14]. In brief, the small intestine was removed from mice, and faecal contents were flushed with HB5S (Invitrogen, Breda, the Netherlands). After removing PP, the small intestine was cut into 1 cm pieces and shaken vigorously in HB5S/EDTA. The mixture was passed through a filter and remaining tissue was used to isolate LPL, whereas the flow trough was washed once, followed by isolation of IEL lymphocytes using a Percoll gradient (GE Healthcare, Eindhoven, the Netherlands). For LPL isolation, the remaining tissue was shaken once again in HB5S/EDTA, washed and digested with collagenase/DNAse [Roche (Woerden, the Netherlands)/Sigma (Zundert, the Netherlands)]. Cells were collected via a 100 μ m filter and tissue digestion was repeated. Both cell fractions were pooled and washed once. Thereafter, LPL cells were further enriched by a Percoll gradient. The collected PP were shaken in HB5S/EDTA, washed once, cut into small fragments and digested at 37 °C with collagenase/DNAse (Roche/Sigma). Cells were passed through a 70 μ m gauze to obtain single cells. Lymphocytes from MLN and spleen were obtained by squeezing the organs through a 70 μ m gauze, after which single cells were washed once. To remove erythrocytes in the spleen, cells were incubated with lysis buffer (containing NH₄Cl, KHCO₃ and Na₂EDTA) for 1 min, and washed once in PBS.

Measurement of peanut extract-specific antibodies and mMCP-I in serum

PE-specific IgG1, IgG2a and IgE in serum were measured by ELISA as described previously [12]. Serum was collected within 45 min after an oral challenge and mMCP1 was determined using a specific ELISA kit (Moredun Scientific Ltd., Midlothian, Scotland). ELISA was performed according to the instructions of the manufacturer.

Cell culture and cytokine measurement

Spleens were isolated from all mice on day 36. Spleen single cell suspensions (3.75×10^5 per well) were incubated in RPMI1640 supplemented with pen/strep and 10% fetal calf serum in the presence or absence of 100 µg/ml PE in 96-well plates for 96 h at 37 °C, 5% CO₂, after which culture supernatants were harvested and stored at -20 °C until analysis. In the culture supernatants, the levels of IFN-γ, IL-4, IL-5 and IL-13 were determined using commercially available sandwich ELISA (e-Bioscience, Vienna, Austria), according to the instructions of the manufacturers.

Flow cytometry

For flow cytometric analysis, cells were pre-incubated with anti-CD16/CD32 (2.4G2) to block FcR, stained with fluorescent-labelled antibodies and stored in 0.4% formaldehyde until acquisition. The following antibodies were used: B220, CD11b, CD11c, CD103 (e-Bioscience) and mPDCA-1 (Miltenyi, Leiden, the Netherlands). Data were acquired by means of FACSCalibur (Becton Dickinson, Breda, the Netherlands) and analysis was performed using Weasel (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia).

Statistical analysis

Data are presented as means ± standard error of the mean (SEM) and analysed using GraphPad Prism software. Before testing, data on antibody levels were logarithmically transformed to obtain a normal distribution. Normally distributed continuous variable comparisons were performed using a one-way ANOVA, followed by Bonferroni as a *post hoc* test. $P < 0.05$ was considered significant.

Results

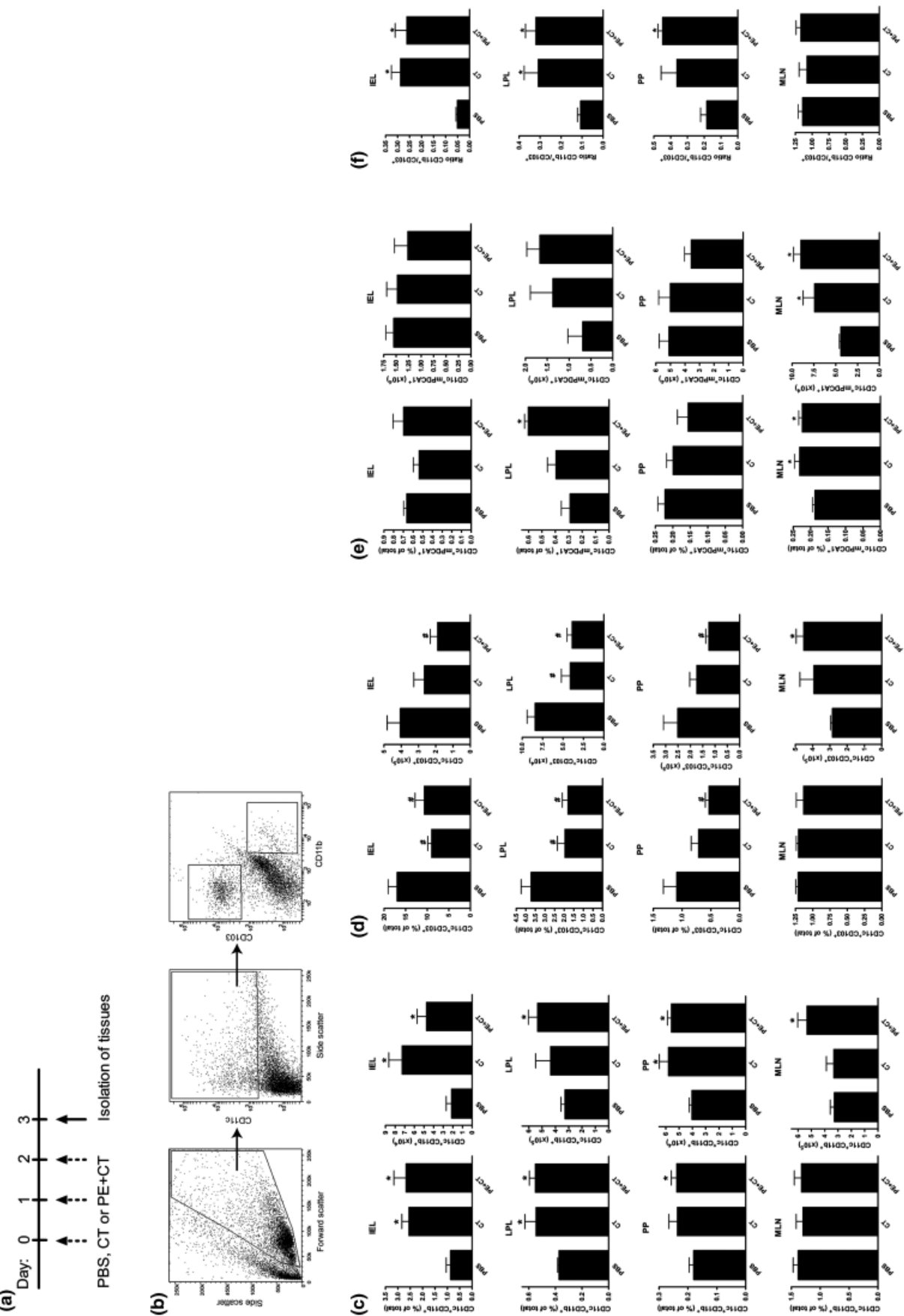
Sensitization to peanut increases conventional CD11b⁺ DC but reduces CD103⁺ DC

We first studied whether CT-induced peanut sensitization in our model was accompanied by changes in DC subsets in intestinal tissues (Fig. 1a). For this purpose, IEL, LPL cell fractions and PP and MLN were analysed by flow cytometry (Fig. 1b). Intra-gastric administration of CT, with or without PE, induced a significant increase in the percentage of CD11c⁺CD11b⁺cDC in the IEL, LPL and PP (Fig. 1c). This was reflected in the absolute number of these cells in these fractions. In addition, an increase in the number of cDC in the MLN was observed. In contrast, CT or PE with CT caused a decrease in the percentage and absolute number of CD11c⁺CD103⁺ mucosal DC in the IEL, LPL and PP but not in the MLN, where an increase in the absolute number of these cells was observed (Fig. 1d). Together, CT or PE+CT administration shifted the absolute ratio of cDC vs. CD103⁺DC towards more cDC and less CD103⁺DC (Fig. 1f) in the intestinal tissues but not in the MLN. In addition, the percentage and number of CD11c⁺mPDCA⁺pDC were increased in the MLN and LPL, while no changes were observed in the other intestinal fractions (Fig. 1e). We did not observe any effect of PE administration alone on the earlier mentioned cell populations (data not shown).

Treatment with Flt3L in vivo induces an increase in the number of CD11b⁺ DC, CD103⁺ DC and mPDCA⁺ pDC

We next investigated the functional role of the previously mentioned DC subtypes by expanding the DC numbers by treatment of mice with the DC growth factor Flt3L (Fig. 2a). Analysis of PP, MLN and spleen showed that Flt3L treatment indeed induced a dramatic expansion of CD11c⁺CD11b⁺cDC, CD11c⁺CD103⁺DC and CD11c⁺mPDCA⁺pDC (Fig. 2b). While the increase in cDC was approximately 10-fold, the other populations increased threefold. Although not determined in detail, this increase was also absolute as the spleens of Flt3L-treated mice were significantly enlarged. To further investigate the role of pDC during this expansion, mice were treated with the pDC-depleting antibody 120G8. This antibody depleted or prevented the increase in pDC but did not affect other DC

Fig. 1. Analysis of dendritic cell (DC) subsets in the intestine during allergic sensitization. C3H/HeO_uJ mice ($n = 4$) were exposed to phosphate-buffered saline (PBS), peanut extract (PE), cholera toxin (CT) and PE+CT for 3 consecutive days and then cells were isolated from intraepithelial (IEL), lamina propria lymphocyte (LPL) fractions, Peyer's patches (PP) and mesenteric lymph nodes (MLN) and were analysed using flow cytometry (a). Cells were gated based on the FSC-SSC pattern and CD11c staining and subsequently analysed based on CD11b expression (conventional DC) or CD103 expression (mucosal DC). Pictures show the representative dotplots of DC analysis in PP of a PBS-treated mouse (b). The percentage (first column) and absolute number (second column) of CD11c⁺CD11b⁺DC (c) CD11c⁺CD103⁺DC (d) or CD11c⁺B220⁺mPDCA⁺pDC (e) and the ratio of the absolute number of CD11b⁺DC vs. CD103⁺DC are shown (f). All data are represented as mean ± SEM. * $P < 0.05$ compared with control.



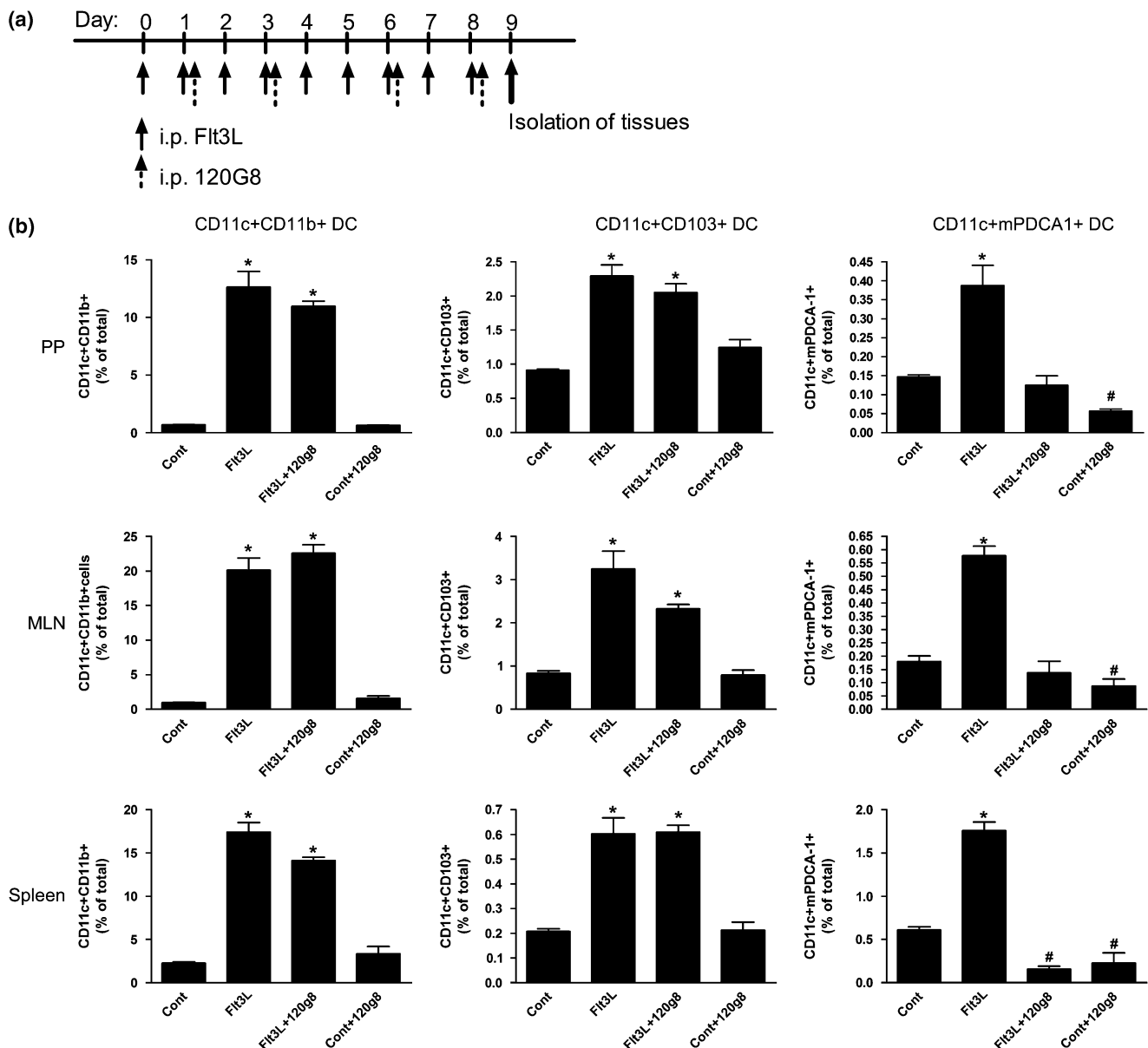


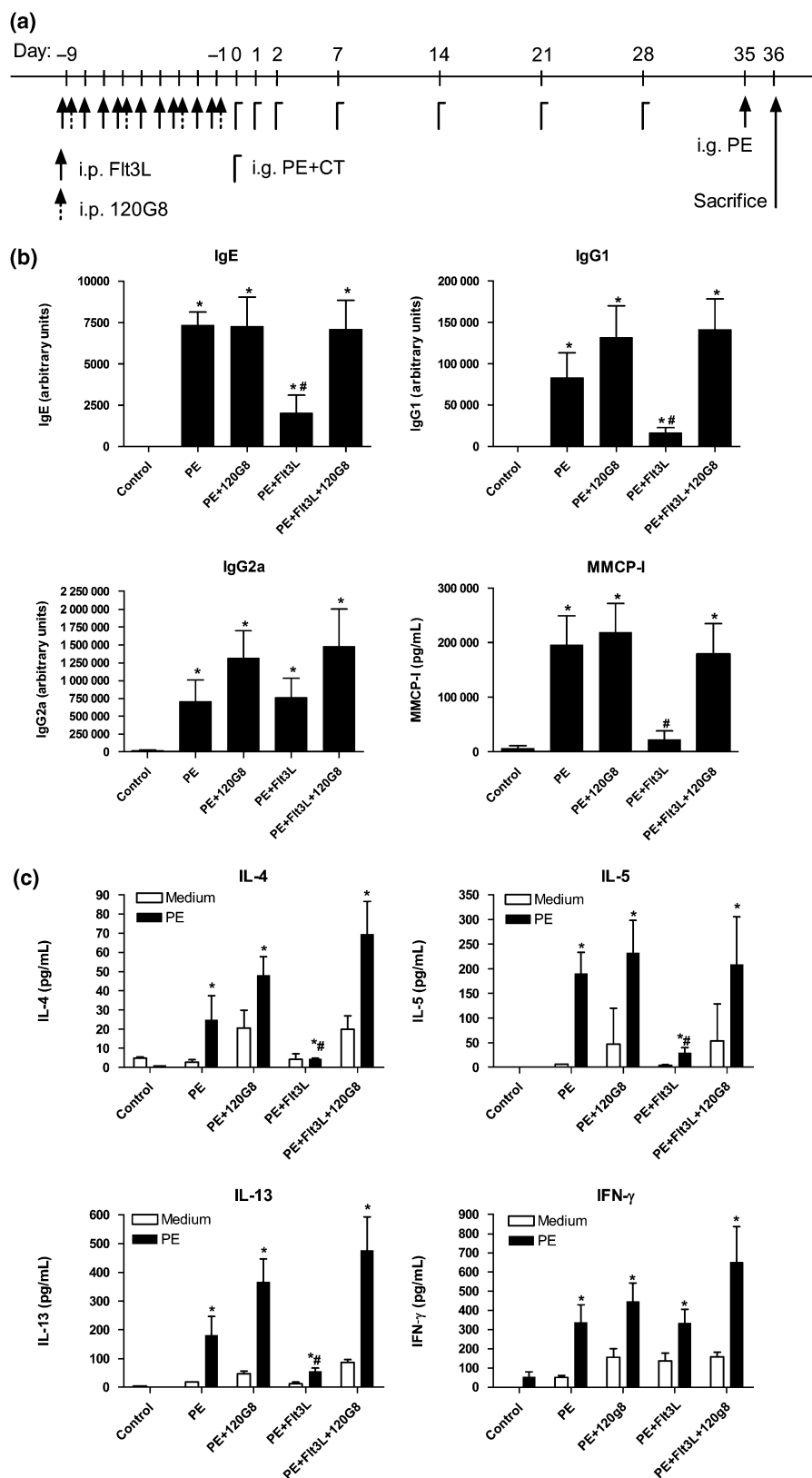
Fig. 2. Expansion of dendritic cells (DC) in vivo by Flt3L treatment. C3H/HeOJ mice ($n = 4$) were exposed to phosphate-buffered saline (PBS), Flt3L, the pDC depleting antibody 120G8 or Flt3L and 120G8 for 9 consecutive days as described in Material and methods (a). Cells were isolated from Peyer's patches (PP), mesenteric lymph nodes (MLN) and spleen and were analysed using flow cytometry as described before. The percentage of CD11c⁺CD103⁺, CD11c⁺CD11b⁺ or CD11c⁺mPDCA1⁺DC are shown (b). Data are represented as mean $\pm$ SEM. */ $\#P < 0.05$ compared with control.

subsets (Fig. 2b) or other lymphocytes such as T or B cells [13], data not shown). In summary, this protocol provides an opportunity to explore whether increased cDC, CD103⁺DC or pDC numbers could modulate allergic sensitization.

Expansion of dendritic cells attenuates allergic sensitization by peanut

Hereafter, we examined whether selective DC expansion by *in vivo* Flt3L treatment and pDC depletion affected peanut-allergic responses (Fig. 3a). After sensitization by

oral exposure to PE and CT, mice developed a strong induction of peanut-specific IgE, IgG1 and IgG2a (Fig. 3b). In addition, mice displayed mast cell degranulation upon an oral challenge with peanut, as measured by increased levels of mMCP1 in the serum. To investigate PE-specific T cell responses, splenocytes were stimulated with PE. PE sensitization and challenge induced Th2 and Th1 cytokines including IL-4, IL-5, IL-13 and IFN- γ production after restimulation *ex vivo* (Fig. 3c). Expansion of DC by Flt3L before exposure to PE inhibited these allergic manifestations in our model, including PE-specific IgE, PE-induced mast cell degranulation and Th2 cytokine



production (Fig. 3b–c). Th1-associated IgG2a and IFN- γ production were unaffected by Flt3L-induced DC expansion. Interestingly, pDC depletion during Flt3L-induced DC expansion reversed the inhibition of Th2 cytokine production, IgE responses and mast cell degranulation completely. In contrast, pDC depletion by 120G8 before PE sensitization did not affect sensitization in our model, most likely as we did not deplete these cells during the sensitization phase in our model. These data show that the expansion of DC numbers by Flt3L inhibits the establishment of allergic manifestations in the intestine that was mediated via the expansion of pDC.

Discussion

The mucosal adjuvant CT induces allergic sensitization to co-administered proteins in mice, while feeding the protein alone induces tolerance. In this way, CT can be used as a tool to model the induction of food allergy. CT is not likely to be involved in the pathogenesis of food allergy itself, but other bacterial, viral [15] or non-bacterial stimuli [16] might act via the same route and mechanism. For instance, the more common enterotoxin SEB administered with antigen impaired oral tolerance and allowed allergic sensitization to ovalbumin orally [17]. In addition to others [8, 18], we found that sensitization to peanut causes an increase in conventional Th2-priming CD11c⁺CD11b⁺DC in the intestinal compartments IEL, LPL in PP to various extents. It is very likely that the number of these cDC increased because of migration; however, it is unknown what could have triggered this, as we and others do not observe any toxicity, pathology or inflammation in the intestine after CT exposure.

The present data show that in addition, sensitization to peanut causes a decrease of immunoregulatory mucosal CD103⁺DC [4] in various intestinal locations (IEL, LPL and PP). This observed change in intestinal DC subset distribution to a more inflammatory DC subtype favours immune activation. It has been shown that exposure to CT results in the migration of DC to MLN and in subsequent T cell activation [8]. It remains to be investigated whether CT affects DC distribution *in vivo* directly or indirectly, for instance by stimulating the production of Th2-skewing thymic stromal lymphopoietin (TSLP) by intestinal epithelial cells (IEC) [5, 8]. Several other reports associated an increase in cDC with an increase in inflammation and Th2-mediated disease [19, 20] at mucosal surfaces. Earlier

reports confirmed that the CT-induced increase in MLN DC numbers was associated with an increased expression of OX40L and Jagged 2 expression on these DC, leading to Th2 skewing *ex vivo* [8].

Our data show that the effects of CT on DC subset distribution may contribute to its adjuvant capacity. Recently, lamina propria-derived CD103⁺DC were described to have the capacity to induce the induction of CD25⁺Foxp3⁺regulatory T cells via a mechanism involving TGF- β and retinoic acid [4, 21]. Interestingly, during sensitization to peanut in our model, we observed that the CD103⁺DC subset was decreased in the LP as well, while absolute numbers increased in the MLN. This implicates that upon an inflammatory stimulus, such as CT, CD103⁺DC migrated to the MLN, as suggested before [22, 23]. In contrast, Flt3L treatment increased the CD103⁺DC numbers in all intestinal tissues, but this increase was not able to counterbalance the increase of pro-inflammatory CD11b⁺DC. Unfortunately, due to the lack of available reagents, it is not possible to manipulate CD103⁺DC. This leaves the exact role of this subset of DC under physiological conditions *in vivo*, including the establishment of allergic disease, to be elucidated.

The earlier mentioned studies [8, 9, 18] were not able to study the functional role of individual DC subsets during allergic sensitization in the intestine *in vivo*. Therefore, in order to better define the exact role of earlier mentioned DC subsets, we expanded the number of DC *in vivo* with the haemopoietic growth factor Flt3L. To investigate the role of cDC vs. pDC, we also used a depleting antibody for pDC in the presence or absence of Flt3L. This endogenous expansion of DC is advantageous over methods using the transfer of *in vitro* cultured DC subsets as *in vitro* manipulation and transfer into mice might change the intrinsic functional properties of DC.

We observed that expansion of DC by Flt3L strongly decreased sensitization to peanut, as shown by lower levels of peanut-specific IgG1 and IgE and Th2 cytokine production. The reduction in mast cell degranulation could be explained by the significant reduction in IgE we observed. Less IgE in the circulation would lead to less mast cell-bound IgE, leading to decreased mast cell activation upon a second encounter with the allergen. The control of immunization by intestinal DC was demonstrated previously by Viney et al. [11], who showed that Flt3L treatment expanded DC numbers in the intestine, resulting in enhanced oral tolerance to ovalbumin. We

Fig. 3. Expansion of dendritic cells (DC) *in vivo* by Flt3L treatment lowers food-allergic responses. C3H/HeO/J Mice were exposed to PE+CT during a 4-week period, as described in Material and methods. Before sensitization, the indicated groups received Flt3L and/or 120G8 (a). PE-specific IgG1, IgG2a and IgE levels in the serum of mice were determined by ELISA and mMCP1 levels in C3H/HeO/J mice were determined in blood samples that were collected within 45 min after an oral PE challenge (b). Splenocytes were cultured for 96 h in the presence of PE or medium alone, and cytokine production in the supernatant was determined by means of specific ELISA (c). Data are represented as mean $\pm$ SEM of eight mice. * $P < 0.05$ with control. [‡] $P < 0.05$ compared with the PE-sensitized group. PE, peanut extract; CT, cholera toxin.

now append these experiments, using a depleting antibody for pDC. Although the combination of this antibody with Flt3L treatment did not deplete all pDC, it significantly reduced pDC numbers while not affecting the increase in cDC and CD103⁺DC numbers. Surprisingly, this selective expansion of cDC, CD103⁺DC and pDC vs. expansion of cDC and CD103⁺DC alone showed that the protective effect of Flt3L treatment on establishment of food allergy is clearly dependent on expansion of pDC. Our data are in line with other experiments in a mucosal setting, where expansion of DC and pDC in particular [13, 24] was protective in pulmonary inflammation induced by respiratory syncytial virus or ovalbumin allergen. In addition, we (data not shown) and several other studies observed no functional change in pDC or cDC after Flt3L treatment [11, 25, 26]. Therefore, the effects of Flt3L *in vivo* are caused by an increase in pDC number but not by a change in the functional/phenotypic characteristics of pDC.

It seems that the expansion of cDC by Flt3L does not influence the adaptive immune response in our and other models or it is possible that pDC have the ability to prevent a detrimental effect of cDC expansion. While the increase in cDC was approximately 10-fold, the increase in other populations was smaller, approximately three-fold. However, the following functional data using a pDC-depleting antibody suggest that even a relatively small (three-fold) increase in pDC number can have a strong functional and regulatory effect. We observed an increase in the number of pDC in the MLN during allergic sensitization. Recently, it was shown that only MHCII-expressing pDC could alleviate T cell-mediated autoimmunity [27]. This would mean that pDC inhibit T cell responses in an antigen-specific manner. It has been suggested that the protective effect of Flt3L and expansion of pDC is

mediated via programmed death ligand-1 (PDL-1) and induction of regulatory T cells [28, 29]. Whether this also happens during allergic sensitization and or after (plasmacytoid) DC expansion in this model is not clear. We did not observe differences in PDL-1 or PD-1 expression on pDC or cDC during administration of CT or PE and CT and administration of Flt3L (data not shown). In addition, in contrast to other studies [29], together with Kool et al. [24], we did not find changes in the percentage and number of FoxP3⁺regulatory T cells after sensitization and/or Flt3L treatment (data not shown). Possibly, a T_{reg}-independent mechanism is responsible for the observed effects of pDC expansion. Interestingly, Kool et al. [24] suggested that pDC keep cDC immature and depleting pDC resulted in more mature cDC.

In conclusion, in these studies, we show for the first time that allergic sensitization in the intestine is accompanied by changes in DC subset numbers. Most profound was a relative increase in the number of CD11b⁺DC, explaining in part the Th2 polarizing signal CT provides *in vivo* [30]. In addition, expansion of different DC subsets showed the pro-allergenic role of CD11b⁺DC but also showed that pDC expansion was able to significantly lower the food-allergic response. The latter could provide a basis for a therapy based on enhancing pDC functions. For instance, treatment with CpG motifs or ODN, both strong ligands for pDC, has already proven to be possibly beneficial in the treatment of allergic disease [31] and food allergy [32] in particular.

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References

- 1 Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2010; 125:S116–25.
- 2 Iwasaki A. Mucosal dendritic cells. *Annu Rev Immunol* 2007; 25:381–418.
- 3 Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* 2008; 8:435–46.
- 4 Coombes JL, Siddiqui KR, Arancibia-Carcamo CV *et al.* A functionally specialized population of mucosal CD103⁺DCs induces Foxp3⁺regulatory T cells via a TGF- β and retinoic acid-dependent mechanism. *J Exp Med* 2007; 204:1757–64.
- 5 Berin MC, Mayer L. Immunophysiology of experimental food allergy. *Mucosal Immunol* 2009; 2:24–32.
- 6 Knippels LM, van Wijk F, Penninks AH. Food allergy: what do we learn from animal models? *Curr Opin Allergy Clin Immunol* 2004; 4:205–9.
- 7 Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. *Eur J Immunol* 2000; 30:2394–403.
- 8 Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* 2008; 180:4441–50.
- 9 Anosova NG, Chabot S, Shreedhar V, Borawski JA, Dickinson BL, Neutra MR. Cholera toxin, *E. coli* heat-labile toxin, and non-toxic derivatives induce dendritic cell migration into the follicle-associated epithelium of Peyer's patches. *Mucosal Immunol* 2008; 1:59–67.
- 10 Shreedhar VK, Kelsall BL, Neutra MR. Cholera toxin induces migration of dendritic cells from the subepithelial dome region to T- and B-cell areas of Peyer's patches. *Infect Immun* 2003; 71:504–9.
- 11 Viney JL, Mowat AM, O'Malley JM, Williamson E, Fanger NA. Expanding dendritic cells in vivo enhances the induction of oral tolerance. *J Immunol* 1998; 160:5815–25.
- 12 van Wijk F, Nierkens S, de Jong W *et al.* The CD28/CTLA-4-B7 signaling pathway is involved in both allergic sensitization and tolerance induction to orally administered peanut proteins. *J Immunol* 2007; 178:6894–900.

- 13 Smit JJ, Lindell DM, Boon L, Kool M, Lambrecht BN, Lukacs NW. The balance between plasmacytoid DC versus conventional DC determines pulmonary immunity to virus infections. *PLoS One* 2008; 3:e1720.
- 14 Bol-Schoenmakers M, Marcondes Rezende M, Bleumink R *et al.* Regulation by intestinal gammadelta T cells during establishment of food allergic sensitization in mice. *Allergy* 2011; 66:331–40.
- 15 Fecek RJ, Marcondes Rezende M, Busch R, Hassing I, Pieters R, Cuff CF. Enteric reovirus infection stimulates peanut-specific IgG2a responses in a mouse food allergy model. *Immunobiology* 2010; 215:941–8.
- 16 Bol-Schoenmakers M, Bleumink R, Rezende MM *et al.* Diclofenac enhances allergic responses in a mouse peanut allergy model. *Clin Exp Allergy* 2011; 41:424–33.
- 17 Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009; 123:231, e4.
- 18 Anjuere F, Luci C, Lebens M *et al.* In vivo adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin. *J Immunol* 2004; 173:5103–11.
- 19 Smit JJ, Rudd BD, Lukacs NW. Plasmacytoid dendritic cells inhibit pulmonary immunopathology and promote clearance of respiratory syncytial virus. *J Exp Med* 2006; 203:1153–9.
- 20 de Heer HJ, Hammad H, Soullie T *et al.* Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004; 200:89–98.
- 21 Sun JB, Raghavan S, Sjoling A, Lundin S, Holmgren J. Oral tolerance induction with antigen conjugated to cholera toxin B subunit generates both Foxp3⁺CD25⁺ and Foxp3[−]CD25[−] CD4⁺ regulatory T cells. *J Immunol* 2006; 177:7634–44.
- 22 Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Forster R, Agace WW. Functional specialization of gut CD103⁺ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 2005; 202:1063–73.
- 23 Schulz O, Jaensson E, Persson EK *et al.* Intestinal CD103⁺, but not CX3CR1⁺, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* 2009; 206:3101–14.
- 24 Kool M, van Nimwegen M, Willart MA *et al.* An anti-inflammatory role for plasmacytoid dendritic cells in allergic airway inflammation. *J Immunol* 2009; 183:1074–82.
- 25 Castellaneta A, Abe M, Morelli AE, Thomson AW. Identification and characterization of intestinal Peyer's patch interferon-alpha producing (plasmacytoid) dendritic cells. *Hum Immunol* 2004; 65:104–13.
- 26 Kingham TP, Chaudhry UI, Plitas G, Katz SC, Raab J, DeMatteo RP. Murine liver plasmacytoid dendritic cells become potent immunostimulatory cells after Flt-3 ligand expansion. *Hepatology* 2007; 45:445–54.
- 27 Irla M, Kupfer N, Suter T *et al.* MHC class II-restricted antigen presentation by plasmacytoid dendritic cells inhibits T cell-mediated autoimmunity. *J Exp Med* 2010; 207:1891–905.
- 28 Tokita D, Mazariegos GV, Zahorchak AF *et al.* High PD-L1/CD86 ratio on plasmacytoid dendritic cells correlates with elevated T-regulatory cells in liver transplant tolerance. *Transplantation* 2008; 85:369–77.
- 29 McGee HS, Edwan JH, Agrawal DK. Flt3-L increases CD4⁺CD25⁺Foxp3⁺ICOS⁺ cells in the lungs of cockroach-sensitized and -challenged mice. *Am J Respir Cell Mol Biol* 2010; 42:331–40.
- 30 Marinaro M, Staats HF, Hiroi T *et al.* Mucosal adjuvant effect of cholera toxin in mice results from induction of T helper 2 (Th2) cells and IL-4. *J Immunol* 1995; 155:4621–9.
- 31 Broide DH. Immunomodulation of allergic disease. *Annu Rev Med* 2009; 60:279–91.
- 32 Zhu FG, Kandimalla ER, Yu D, Agrawal S. Oral administration of a synthetic agonist of Toll-like receptor 9 potentially modulates peanut-induced allergy in mice. *J Allergy Clin Immunol* 2007; 120:631–7.