

Production of IL-12 by Peyer patch–dendritic cells is critical for the resistance to food allergy

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Background: Dendritic cells (DCs) play a pivotal role in antigen presentation and regulation of immune responses; however, their involvement in food allergy remains to be fully understood.

Objective: Our aim was to investigate T_H1–T_H2 reciprocal regulation of DCs' function in the gut and systemic immune system and its effect on food allergy in mice with different susceptibility to food allergy.

Methods: Freshly isolated CD11c⁺B220⁺DCs from peanut-sensitized allergy-susceptible C3H/HeJ and allergy-resistant Balb/c mice were cultured to determine levels of IL-12p70 produced in the presence of cytokines, including IL-4. Systemic levels of IL-12 were assessed *in vivo* after antigen challenge with or without IL-4. Targeted oral delivery of microencapsulated neutralizing anti-IL-12 antibody to Peyer patches (PPs) was performed in Balb/c before administration of each sensitizing dose.

Results: Peyer patch–DCs but not splenic DCs from sensitized C3H/HeJ but not Balb/c mice produced less IL-4–dependent IL-12p70. *In vivo* data confirmed this was restricted to the gut immune system, and it was not linked to reduced expression of IL-4 receptor or the lack of functional Toll-like receptor 4; instead, IL-4 failed to inhibit IL-10 production by PP-DCs, a pathway critically involved in IL-4–dependent production of IL-12p70. Finally, neutralization of IL-12 within PPs by specific antibody during antigen presentation significantly increased Balb/c susceptibility to food allergy.

Conclusion: Reciprocal T_H1–T_H2 control of DCs' function within the inductive site of the gut immune system is altered in food allergy.

Clinical implications: Production of IL-12p70 by PP-DCs during antigen presentation is critical for the development of food allergy. (*J Allergy Clin Immunol* 2007;120:659–65.)

Key words: Dendritic cell, food allergy, IL-12, IL-4, Peyer patch

Abbreviations used

CPE:	Crude peanut extract
CT:	Cholera toxin
DC:	Dendritic cell
FAE:	Follicle-associated epithelium
FITC:	Fluorescein isothiocyanate
IL-4R:	IL-4 receptor
PLA:	Poly(lactic-acid)
PP:	Peyer patch
Sp:	Spleen
TLR:	Toll-like receptor

Until recently, because of ethical problems in performing studies on the immunologic mechanisms underlying sensitization to food in human beings and the lack of suitable animal models, little was known about the role of dendritic cells (DCs), and of gut-derived DCs in particular, in sensitization and maintenance of IgE-mediated reactions to food.¹ The establishment of a mouse model of food allergy that mimics IgE-mediated type I hypersensitivity reactions in human beings² provided us with a powerful tool to investigate the genesis and progression of this disease. Indeed, its use enabled us to establish that DCs are key to the production of food allergen–specific IgE. First, adoptive transfer of DCs from allergic mice induced allergen-specific IgE in naive syngeneic recipients in the absence of allergen challenge.³ Second, T-cell–mediated DC apoptosis, a downregulatory mechanism particularly effective in the gut,⁴ was impaired in allergic mice, showing that the finely balanced DC–T-cell interplay is altered in food allergy.⁵ The finding that 2 different mouse strains displayed differential susceptibility to food allergy⁶ offered us the opportunity to investigate further regulatory features of DCs that might be accountable for it. It was reported that strain-specific susceptibility was linked to differential T_H1–T_H2 responses, with splenocytes from cow's milk–sensitized and peanut-sensitized allergy-susceptible C3H/HeJ mice showing increased IL-4 and IL-10 secretion whereas those from allergy-resistant Balb/c showed significantly increased production of IFN- γ . Recently, a direct link between the lack of functional Toll-like receptor (TLR)–4 and strain-specific susceptibility to food allergy was proposed.⁷ However, the interplay between T_H1 and T_H2 cytokines in the regulation of DCs function remained to be determined. Within this scenario, the production of bioactive IL-12 by DCs was of particular interest both for its strict regulation by

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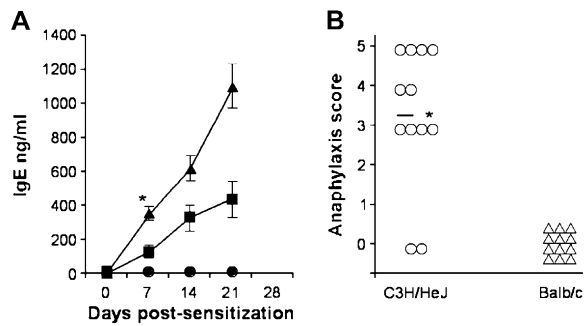


FIG 1. Serum levels of allergen-specific IgE antibody (**A**) in peanut-sensitized C3H/HeJ (▲) and Balb/c (■). ●, Nonsensitized mice of both strains. *Beginning of significant difference ($P < .01$). Mice were sensitized on days 0 and 7 and challenged with allergen (peanut) on day 21. Severity of anaphylactic responses was assessed in both strains (**B**). *Significant difference ($P < .001$).

IFN- γ and IL-4⁸ and for its role in shaping balanced T_H1-T_H2 responses.⁹

Here we report that Peyer patch (PP)-DCs but not spleen (Sp)-DCs from C3H/HeJ showed a decline in the ability to produce IL-12p70 in the presence of IL-4, the most powerful enhancer of IL-12 production by DCs in both human beings and mice.⁸ In contrast, neither PP-derived nor Sp-derived DCs from Balb/c mice showed sensitization-associated change of levels of IL-12p70 produced in the presence of IL-4. The critical role played by IL-12 at the time of antigen presentation within the PPs was ultimately confirmed by the observation that specific targeting of neutralizing anti-IL-12 antibody via poly(lactic-acid) (PLA) microspheres markedly increased Balb/c susceptibility to food allergy. These data taken together would suggest that T_H2(IL-4)-dependent production of IL-12 by PP-DCs plays a critical role in the development of food allergy.

METHODS

Mouse model of food allergy

Female C3H/HeJ, congenic C3H/FeOuJ and Balb/c mice, 5 weeks old, were maintained in an access-restricted room in conventional conditions throughout the experiments. Experiments were conducted under the guidelines of the Animal Act (1986) of the United Kingdom and the current Italian law. Ground whole peanuts were used as antigen, and crude peanut extract (CPE) was prepared as described in detail¹⁰; levels of LPS were determined by a standard Limulus test (Sigma, Poole, United Kingdom [UK]). Mice were sensitized according to an established protocol.² Briefly, mice received intragastric administration of 5 mg/mouse (near equivalent of 1 mg peanut protein) of ground whole peanut together with 10 μ g/mouse of cholera toxin (CT) (Calbiochem, Beeston, UK) in PBS on days 0 and 7. Control groups were administered the same dose of CPE, CT, or PBS alone. DCs were isolated from spleen and PPs of sensitized and control mice 24 hours after the delivery of the second sensitizing (or control) dose (day 8 postinitial sensitization). Additional groups of mice were challenged by intragastric gavage on week 3 after the initial sensitizing dose with CPE (10 mg mouse divided into 2 doses at 30-minute intervals), and percentage of mice that developed type I hypersensitivity reaction was assessed by using a scoring

system previously described.^{2,3,5} Serum levels of peanut-specific IgE antibody were determined by ELISA in all groups of sensitized and control mice as described in detail previously.³ Plasma levels of histamine were detected by using an enzyme immunoassay kit (Immunotech Inc, Marseille, France).

Preparation of DCs

CD11c⁺B220⁻ DCs from spleen and PPs were isolated by flow cytometry as described in detail elsewhere.^{3,5} Phenotypic analysis was performed by flow cytometry as described⁴ using fluorescein isothiocyanate (FITC)-labeled antibody anti-Ia, CD80, CD40, CD11b (BD Biosciences, Oxford, UK), CD86 (AMS Biotechnology, Oxford, UK), CD8 α (Ebioscience, San Diego, Calif), DEC205 (NLCD-145), and intercellular adhesion molecule-1 (Pharmingen, Oxford, UK). Also, expression of IL-4 receptor (IL-4R) in CD11c⁺B220⁻ DCs was assessed by flow cytometry using a primary rat mAb and phycoerythrin-conjugated antirat secondary antibody (both from R&D Systems, Minneapolis, Minn). Fourteen to 16 mice/group were used.

Stimulation of DCs for lymphokine production

Spleen-derived and PP-derived DCs (10^5 /well) were cultured in triplicate in 96-well round-bottomed plates and challenged with antigenic stimuli with the ability to induce high levels of IL-12⁸ such as anti-CD40 (10-20 μ g/mL) and CpG¹¹ (250 nmol/L) in the presence of various concentrations of T_H1 (IFN- γ), T_H2 (IL-4), and GM-CSF. In some cases, CPE (1-10 μ g/mL) was added to DC cultures. In all cell cultures, various concentrations of polymixin B (Sigma-Aldrich, Poole, UK) were also used to rule out possible stimulation as a result of low levels LPS contamination in the recombinant cytokines. After 22 to 24 hours, culture supernatants were collected and stored at -80°C until used. Levels of IL-12p70 were determined by ELISA (R&D Systems). Levels of IL-10 produced by DCs after *in vitro* challenge intraperitoneally with CpG in the presence or absence of IL-4 were also assessed by ELISA (R&D Systems).

In vivo production of IL-12p70/40

Sensitized and control mice of both strains were challenged intraperitoneally with CpG (10 nmol) with or without IL-4 (0.5 μ g)⁸ or PBS. Mice were bled after 4 to 6 hours, sera were collected, and levels of IL-12p70/40 were determined by ELISA.

Targeting of microencapsulated anti-IL-12 antibody to PPs

Antimouse IL-12 antibody was prepared by immunizing rabbits with 200 μ g/mL recombinant mouse IL-12 subcutaneously in adjuvant (Titermax Gold; Sigma-Aldrich) and then boosted with 100 μ g IL-12 at various intervals, and IgG fractions of preimmune and immune sera were purified by chromatography on protein-G Sepharose (Pharmacia, Milton Keynes, UK). The anti-IL-12 antibody completely neutralized 200 pg/mL mouse recombinant IL-12p70 in the phorbol myristate acetate proliferation assay¹² at 0.5 μ g/mL, and although it showed low cross-reactivity with p40, it did not have any effect on IL-23-mediated production of IL-17 by splenocytes¹³ *in vitro* (see this article's Fig E1 in the Online Repository at www.jacionline.org). To target anti-IL-12 antibody to intestinal PP, we prepared anti-IL-12 antibody-loaded PLA-microspheres produced by a double-emulsion technique.¹⁴ FITC-labeled anti-IL-12 antibody was used for the preparation of several batches of microparticles. Briefly, anti-IL-12 antibody (30-50 mg/mL) and IgG fraction from preimmune sera were mixed with 500 mg PLA (Polysciences, Northampton, UK). The mixture was sonicated on ice and added to

TABLE I. *In vitro* production of IL-12p70 by splenic and PP-derived DCs from sensitized and nonsensitized C3H/HeJ and Balb/c mice†

Treatment	Strain	NS	CpG	αCD40
Spleen-DCs IL-12p70 pg/mL				
PBS	Balb/c	<10	4520 ± 50	1690 ± 62
Peanut	Balb/c	—	4540 ± 46	1780 ± 74
CT	Balb/c	—	4010 ± 62	1620 ± 40
CT + peanut	Balb/c	—	4390 ± 50	1590 ± 55
CT ± peanut-(CPE)	Balb/c	—	4210 ± 65	1500 ± 35
PBS	C3H/HeJ	<10	3800 ± 60	1510 ± 45
Peanut	C3H/HeJ	—	3850 ± 55	1480 ± 40
CT	C3H/HeJ	—	3300 ± 38	1420 ± 35
CT + peanut	C3H/HeJ	—	3290 ± 45	1390 ± 70
CT ± peanut-(CPE)	C3H/HeJ	—	3390 ± 30	1285 ± 50
PP-DCs IL-12p70 pg/ml				
PBS	Balb/c	<10	4250 ± 80	1210 ± 50
Peanut	Balb/c	—	4240 ± 62	1180 ± 60
CT	Balb/c	—	4060 ± 90	1400 ± 42
CT + peanut	Balb/c	—	3750 ± 55	1360 ± 40
CT + peanut-(CPE)	Balb/c	—	3600 ± 70	1350 ± 20
PBS	C3H/HeJ	<10	4150 ± 40	1340 ± 35
Peanut	C3H/HeJ	—	3800 ± 30	1290 ± 30
CT	C3H/HeJ	—	3680 ± 25	1220 ± 20
CT + peanut	C3H/HeJ	—	900 ± 22*	300 ± 9*
CT + peanut-(CPE)	C3H/HeJ	—	840 ± 45**	320 ± 15**

NS, Nonstimulated.

†Freshly isolated CD11c⁺/B220⁺ DCs from spleen and PP from control, nonsensitized groups (PBS, peanut, and CT) and sensitized (CT + peanut) Balb/c and C3H/HeJ mice were cultured in presence of a lymphokine cocktail (IFN-γ, 2 ng/mL; GM-CSF, 100 U/mL; IL-4, 100 U/mL) and challenged with either CpG or anti-CD40 stimuli for 22 to 24 hours. The supernatants were assayed for IL-12p70 by ELISA. *Significant reduction compared with controls (*P* < .01). In some cultures, CPE (1-10 μg/mL) was added to DC cultures from sensitized mice, CT + peanut-(CPE). Addition of CPE did not alter IL-12 production.**

1% poly(vinyl alcohol) (Polysciences) in water, vortexed, and added to 100 mL 0.3% poly(vinyl alcohol) under stirring conditions for 5 hours. Microparticles were then recovered by centrifugation and lyophilization. The majority of microparticles ranged between 1 and 10 μm and released 30% to 40% of the antibody within 4 to 6 hours. The biological activity of the released anti-IL-12 antibody was assessed by the phorbol myristate acetate proliferation assay as described. Delivery of microparticles was performed by oral gavage twice before (5 and 2 hours) and once after (4 hours) the administration of each sensitizing dose. Transport of FITC-labeled microparticles across the follicle-associated epithelium (FAE) of PPs was monitored by fluorescence microscopy at various intervals after oral gavage on frozen tissue sections (7-8 μm).

Statistics

Statistical comparison was made by using the Wilcoxon rank test. *P* values were considered significant at ≤.05.

RESULTS

IgE responses and anaphylactic reactions

Recently, the notion that Balb/c mice, in contrast with C3H/HeJ, did not undergo a type I reaction after oral administration of peanut with CT⁶ has been questioned.¹⁵ We first determined levels of peanut-specific serum IgE antibody (Fig 1, A) and anaphylactic responses (Fig 1, B) in both mouse strains. Both strains produced IgE antibody, although the response was significantly higher in

C3H/HeJ mice. Ten of 12 C3H/HeJ mice (83%) displayed symptoms of strong anaphylactic response, whereas none of the Balb/c mice did so. Plasma levels of histamine reached 11,599 ± 1500 nmol/L and 585 ± 40 nmol/L in sensitized C3H/HeJ and Balb/c, respectively. Thus, under these experimental conditions, the C3H/HeJ strain showed greater susceptibility to food allergy than Balb/c mice.

IL-4-dependent production of IL-12p70 is impaired in PP-derived DCs from C3H/HeJ but not Balb/c mice

We tested the ability of IFN-γ, GM-CSF, and IL-4 to increase the production of IL12p70 by both splenic and PP-derived DCs from Balb/c and C3H/HeJ mice. Sp-DCs and PP-DCs (Table I) from sensitized (CT + peanut) Balb/c produced similar amounts of IL-12p70 compared with control (PBS, CT, or peanut alone), nonsensitized mice when challenged with antigenic stimuli in the presence of all these cytokines. The pattern was different in C3H/HeJ mice, and a significant reduction was seen in PP-derived DCs from sensitized (CT + peanut) mice. Decline of IL-4-mediated IL-12p70 production was restricted to PP-DCs, and no differences were observed in Sp-DCs. Addition of CPE to DC cultures did not have any effects on the production of IL-12p70. Next, we titrated the effects of each cytokine on the production of IL-12p70

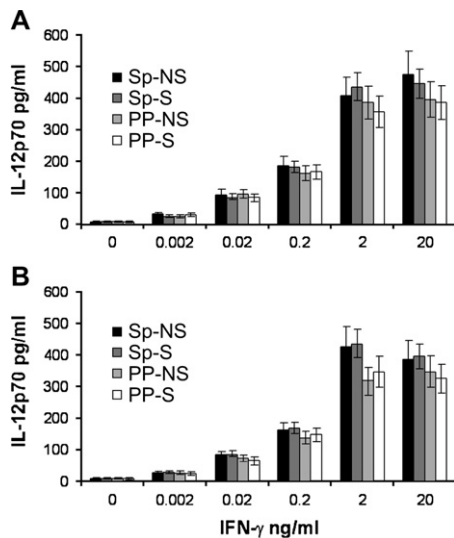


FIG 2. Effects of IFN- γ on the production of IL-12p70 by Sp-DCs and PP-DCs from Balb/c (A) and C3H/HeJ (B) mice. DCs were cultured with CpG and increasing concentrations of IFN- γ . Levels of IL-12p70 in the supernatants were determined by ELISA. Means \pm SDs of 3 independent experiments. NS, Nonsensitized; S, sensitized.

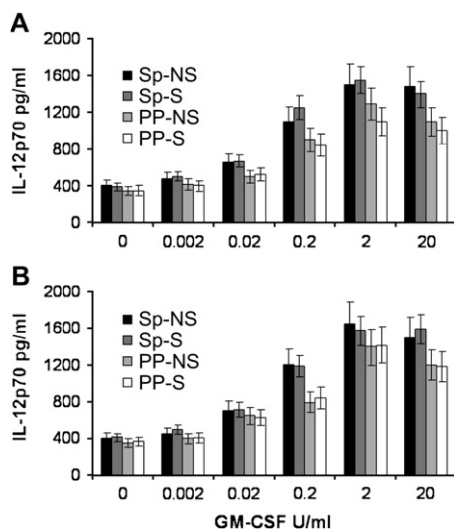


FIG 3. Effects of GM-CSF on the production of IL-12p70 by Sp-DCs and PP-DCs from Balb/c (A) and C3H/HeJ (B) mice. DCs were cultured with CpG and increasing concentrations of GM-CSF. Levels of IL-12p70 in the supernatants were determined by ELISA. Means \pm SDs of 2 independent experiments. NS, Nonsensitized; S, sensitized.

by DCs. Mice orally immunized with peanut alone were used as a nonsensitized control group. Fig 2, A, shows that the presence of IFN- γ induced a significant increase in the production of IL-12p70 by CpG-challenged Sp-DCs and PP-DCs from both nonsensitized and sensitized Balb/c (Fig 2, A) and C3H/HeJ (Fig 2, B). A similar pattern was seen with GM-CSF (in the presence of IFN- γ) in both systemic and PP-derived DCs from Balb/c (Fig 3, A) and C3H/HeJ mice (Fig 3, B). On the other hand, IL-4

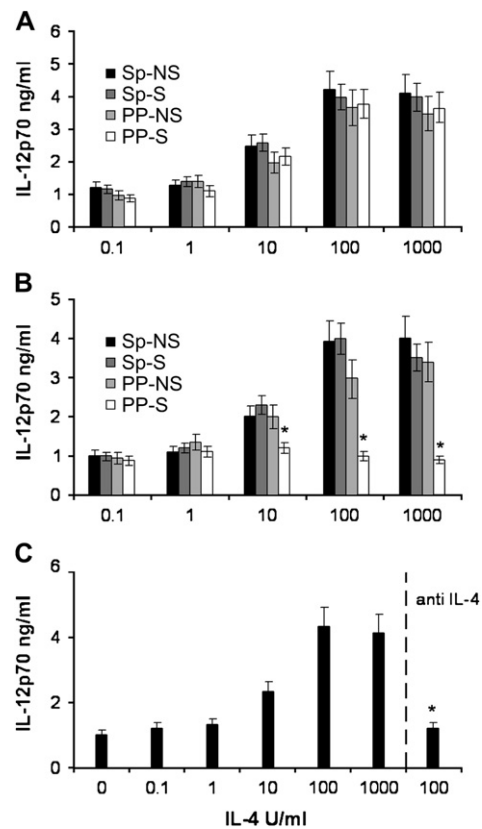
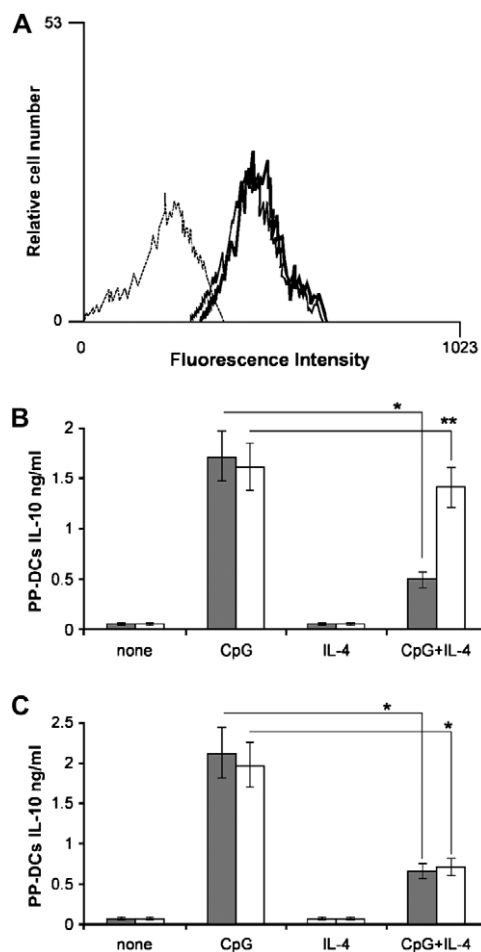


FIG 4. Effects of IL-4 on the production of IL-12p70 by Sp-DCs and PP-DCs from Balb/c (A) and C3H/HeJ (B). Significant reduction ($*P < .001$) was seen only in PP-DCs from sensitized C3H/HeJ mice. The effect of IL-4 was abolished by addition of anti-IL-4 antibody (C, anti-IL-4 panel) to PP-DCs cultured with 100 U/mL IL-4. Means \pm SDs of 3 independent experiments. NS, Nonsensitized; S, sensitized.

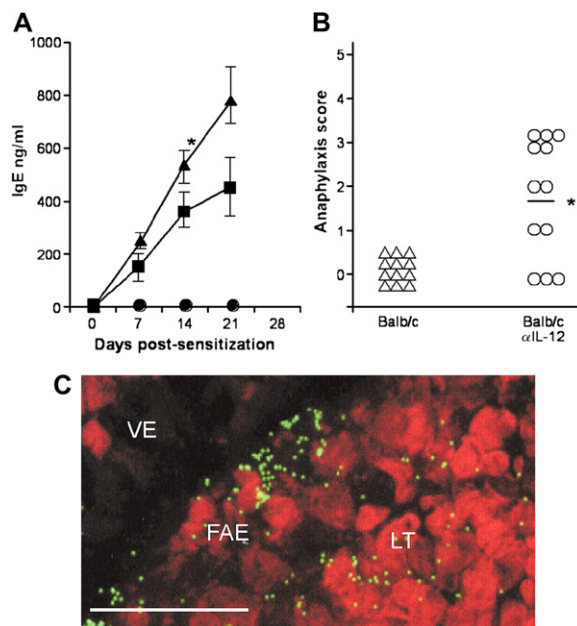
(in the presence of IFN- γ and GM-CSF) failed to promote further the production of IL-12p70 by PP-DCs from allergy-susceptible C3H/HeJ (Fig 4, B) but not Balb/c mice (Fig 4, A). The specificity of IL-4 was confirmed by adding anti-IL-4 antibody to the cultures (Fig 4, C). A pattern similar to that of C3H/HeJ mice was observed in congenic (TLR4⁺) C3H/FeOuJ mice (see this article's Fig E2 in the Online Repository at www.jacionline.org), suggesting that genetic susceptibility to food allergy does not depend solely on the presence of a functional LPS receptor. Furthermore, *in vivo* experiments showed that systemic levels of IL-4-dependent IL-12p70/p40 were slightly higher in Balb/c mice but did not differ between sensitized and nonsensitized mice of both strains (see this article's Table E1 in the Online Repository at www.jacionline.org).

IL-12 deficiency is linked to lack of IL-4-dependent suppression of IL-10 production by DCs and not to reduced expression of IL-4R

The possibility that impaired production of IL-12 by PP-DCs in response to IL-4 was a result of reduced



expression of IL-4R in this cell population was investigated. Flow cytometry analysis showed that PP-DCs from both sensitized and nonsensitized C3H/HeJ did not display any variation in IL-4R (Fig 5, A). Second, because it is known that IL-4-dependent production of IL-12p70 by DCs is strictly linked to a signal transducer and activator of transcription (STAT) 6-mediated suppression of IL-10,¹⁶ we decided to determine levels of IL-10 in PP-DCs from nonsensitized and sensitized C3H/HeJ (Fig 5, B) and Balb/c (Fig 5, C) mice challenged with CpG in the presence or absence of IL-4.¹⁶ We observed that PP-DCs from nonsensitized C3H/HeJ mice (Fig 5, B, black bars) challenged with CpG plus IL-4 showed a significant reduction of IL-10 levels. The pattern was different in PP-DCs from sensitized mice (Fig 5, B, white bars). In this case, we observed only a minor decline of IL-10 production. No difference in IL-4-mediated suppression of IL-10 production was seen in sensitized (Fig 5, C, black



bars) and nonsensitized (Fig 5, C, white bars) allergy-resistant Balb/c mice. These data suggested that in PPs of sensitized C3H/HeJ mice, IL-4 has lost the ability to induce the production of IL-12 by failing to instruct DCs to produce less IL-10.

Targeting of anti-IL-12 antibody to PPs increased Balb/c susceptibility to food allergy

IL-12-deficient mice displayed a series of abnormal immunologic features, including aberrant B-cell responses¹⁷; thus, we devised a strategy to neutralize IL-12 within the PPs of immunologically intact Balb/c mice. Targeting of fluorescent biodegradable PLA-microparticles to the FAE of PPs was monitored at various intervals (1 hour in Fig 6, C) by fluorescence microscopy. Neutralization of IL-12 significantly increased the susceptibility of Balb/c mice to food allergy. Plasma levels of IgE increased in Balb/c treated with anti-IL-12 antibody (Fig 6, A) compared with mice treated with microencapsulated preimmune sera. The difference in IgE levels was significant at day 14, and it increased further at day 21. Seventy-five percent of mice treated with anti-IL-12 antibody showed symptoms of allergic reaction (Fig 6, B). Symptoms ranged between low (score 1, scratching and rubbing around the nose and head; 2 mice of 12) to medium/high intensity (score 3, wheezing, labored respiration, signs of cyanosis around the mouth and tail; 5 mice of 12). Plasma levels of histamine reached 8955 ± 390

nmol/L in anti-IL-12 treated mice compared with 485 ± 55 nmol/L in sham-treated Balb/c.

DISCUSSION

We demonstrated that T_H2 (IL-4)-controlled production of the T_H1 cytokine IL-12p70 by DCs was impaired in the PPs of sensitized allergy-susceptible C3H/HeJ but not in allergy-resistant Balb/c mice and that the lack of IL-12 at the time of antigen presentation is critical for the development of food allergy. Decline of IL-4-controlled production of IL-12p70 was restricted to PPs, whereas splenic DCs were not affected by the allergic status.

Differential regulatory features between PP-derived and Sp-derived DCs have been previously observed in regard to their ability to polarize T-cell responses¹⁸ and susceptibility to antigen-specific T-cell-mediated apoptosis.⁴ Although the molecular basis of this differential behavior remains to be determined, it is now clear that the local microenvironment plays a major role in shaping DCs properties, with the gut epithelium in particular being of paramount importance.¹⁹ In physiologic conditions, the gut epithelium favors the induction of DC (DC2) with the ability to polarize T_H2 responses via the secretion of soluble mediators such as thymic stromal lymphopoietin, a local mechanism that prevents the onset of T_H1 -mediated pathologies of the gastrointestinal tract. Thus, although the role of the gut epithelium in allergy is still unknown, it is possible that allergy-associated regulatory properties of intestinal DCs might be linked to alteration in intestinal epithelial cell-DC cross-talk. The combination of properties of allergenic proteins and a further gut epithelium-mediated shift toward a T_H2 -dominated environment would create the optimal scenario for an allergic type of response to take place. Another feature of PP-DCs is their higher susceptibility to T-cell-mediated apoptosis compared with splenic DCs⁴; however, it was recently reported that a large proportion of DCs from allergic mice escaped this downregulatory mechanism.⁵ The ability to survive T-cell-mediated apoptosis might be linked to a reduced production of IL-12p70 in allergy. Although substantial experimental evidence exists to support the notion of DC1 and DC2 lineages, it has also been suggested that the kinetics of DC activation might have an effect on the DC1-DC2 dichotomy.²⁰ When challenged *in vitro*, myeloid DCs induced a T_H1 response; however, when these became exhausted, they stopped producing IL-12p70 and favored T_H2 cells. Thus, it is plausible that the survival of allergen-loaded DCs might result in an increase of circulating, exhausted DCs that have lost the ability to produce IL-12p70 in response to IL-4 and induce T_H2 responses. Indeed, PP-DCs from sensitized C3H/HeJ mice showed an increased ability to induce the specific T-cell immunoglobulin mucin-domain containing (Tim-2), a specific T_H2 cell surface marker,²¹ on naive $CD4^+CD62L^+CD44^{low}$ T cells after allogeneic stimulation (Nicoletti et al, unpublished data, December 2006).

Therefore, it would be interesting to study to what extent the increased resistance to apoptosis affected IL-4-dependent production of IL-12p70 by PP-DCs.

IL-4 promotes IL-12p70 production by DCs by suppressing the production of IL-10 by DCs themselves.¹⁶ Antigenic challenge of PP-DCs from sensitized C3H/HeJ but not Balb/c in the presence of IL-4 failed to inhibit the production of IL-10. IL-4-mediated suppression of DCs' production of IL-10 is STAT6-dependent; thus, our study would suggest that alteration of signal transduction pathways may be ultimately responsible for abnormal immune responses in allergy. Furthermore, we observed that both levels of IgE and severity of anaphylactic responses were lower, but not significantly different, in $TLR4^+$ C3H/HeOuJ compared with congenic $TLR4^-$ C3H/HeJ mice. These data confirmed and extended previous observations that the lack of a functional $TLR4^+$ along with other factors²² plays a role in determining individual susceptibility to food allergy. Finally, the finding that IL-4-dependent production of IL-12p70 by PP-DCs is impaired in food allergy provides a mechanistic explanation for the observation that oral delivery of liposome-encapsulated IL-12 suppressed allergic reactions in the same animal model used here.²³ Although further investigation on the potential of IL-12 as therapeutic agent in food allergy is required, this latter observation would suggest that delivery of IL-12 to PPs by an appropriate delivery vector, such as M-cell-specific recombinant bacteria²⁴ or biodegradable microparticles,²⁵ may represent an effective strategy to restore adequate levels of IL-12p70 within the intestinal immune system of individuals with allergy.

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