T Cell Reactivity with Allergoids: Influence of the Type of APC

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The use of allergoids for allergen-specific immunotherapy has been established for many years. The characteristic features of these chemically modified allergens are their strongly reduced IgE binding activity compared with the native form and the retained immunogenicity. T cell reactivity of chemically modified allergens is documented in animals, but in humans indirect evidence of reactivity has been concluded from the induction of allergen-specific IgG during immunotherapy. Direct evidence of T cell reactivity was obtained recently using isolated human T cells. To obtain further insight into the mechanism of action of allergoids, we compared the Ag-presenting capacity of different APC types, including DC and macrophages, generated from $CD14^+$ precursor cells from the blood of grass pollen allergic subjects, autologous PBMC, and B cells. These APC were used in experiments together with Phl p 5-specific T cell clones under stimulation with grass pollen allergen extract, rPhl p 5b, and the respective allergoids. Using DC and macrophages, allergoids exhibited a pronounced and reproducible T cell-stimulating capacity. Responses were superior to those with PBMC, and isolated B cells failed to present allergoids. Considerable IL-12 production was observed only when using the DC for Ag presentation of both allergens and allergoids. The amount of IL-10 in supernatants was dependent on the phenotype of the respective T cell clone. High IL-10 production was associated with suppressed IL-12 production from the DC in most cases. In conclusion, the reactivity of Th cells with allergoids is dependent on the type of the APC. ***The Journal of Immunology,*** **2000, 165: 1807–1815.**

Allergoids have been established for allergen-specific immunotherapy for several years (1, 2). Their characteristic feature is minimal IgE-binding activity but retained immunogenicity, documented in both animals and humans (3–10). Few data are available that directly show the T cell-stimulating capacity of allergoids. Most studies concluded from the observation of the induction of allergen- as well as allergoid-specific Igs that allergoids are immunogenic (1–3, 5, 6, 11, 12) because the synthesis of IgG is a T cell-controlled process. A shift of the cytokine profile toward Th1 during treatment with an OVA allergoid was shown in mice (4). In humans effective allergoid therapy was evident by significant reduction of the IL-5 production in nasal secretions accompanied by increased IFN-γ production (13), indicating the involvement of T cells in this beneficial effect.

In a previous study we showed that the majority of Phl p 5-specific T cell clones were stimulated by the allergoid when using autologous irradiated PBMC for Ag presentation, although in comparison with the native allergen the magnitude of proliferation was often lower (14). When comparing autologous PBMC with cultivated DC for Ag presentation, a considerable increase in the proliferation could be observed with both allergen and allergoid, and the magnitude of proliferation varied with the individual T cell clone (TCC)[2] (14). The reduced reactivity of allergoids with autologous PBMC for Ag presentation was attributed to the reduced IgE reactivity (14), because a high proportion of these cells (e.g., B cells) use Ig or Fc receptors for Ag recognition and uptake (15–18).

PBMC are not the target APC for specific immunotherapy via s.c. injections. In the skin the most potent APC are DC, which are essentially important for the initiation of primary T cell-mediated immune responses to foreign Ags (19). These DC are considered immature DC, with a strong Ag-capturing and processing ability, but low T cell-stimulating capacity (20). After Ag uptake they migrate under the influence of inflammatory agents via afferent lymph or blood to secondary lymphoid organs. During this migration they develop into mature DC, which have lost the ability to capture Ag, but have acquired increased capacity to stimulate T cells (20). DC derived from separated blood monocytes and cultured under the influence of GM-CSF and IL-4 possess the phenotype and functional characteristics of immature dendritic cells (21, 22). Another potent APC population in nonlymphoid tissues is the macrophages, which are also very effective for priming naive T cells (23, 24).

Several methods have been described recently for the generation of DC from progenitors in human blood under cultivation with GM-CSF and IL-4 (25–28). The generation of macrophages from hemopoietic progenitors requires different culture conditions, such as growing on hydrophobic Teflon membranes (29) with or without the presence of cytokines such as GM-CSF or M-CSF (30–32).

In this study we compared the ability of Phl p 5-specific T cell clones to respond to allergen and allergoid when presentation was performed by different kinds of APC, including irradiated autologous PBMC, DC, macrophages (Mϕ), and B cells.

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[2] Abbreviations used in this paper: TCC, T cell clone; DC, dendritic cell; DC_{imm}, immature DC; Mϕ, macrophages; PLA_2, phospholipase A_2; PNU, protein nitrogen units.

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In view of the fact that IL-12 is known to be involved in the induction of Th1 responses (24, 33, 34) and that DC and Mϕ are the principal sources of this cytokine, we looked for IL-12 production in the supernatants of the stimulated TCC and APC. IL-10 concentrations were also measured, since this cytokine is recognized to counteract IL-12 production (35–37) and is produced in progressively larger amounts by T cells of the peripheral blood from patients during the course of specific immunotherapy with bee venom (38).

Our results demonstrate that the grass pollen allergoids retain a high specific T cell-stimulating capacity, in particular when they are presented by professional APC such as DC or Mϕ. Thus, allergoids appear well suited for allergen specific immunotherapy through their ability to target T cells, but without provoking all the side effects mediated by IgE reactivity.

Materials and Methods

Media and reagents

A commercially produced grass pollen extract (Allergopharma, Reinbek, Germany), derived from pollen from *Phleum pratense*, *Lolium perenne*, *Holcus lanatus*, *Dactylis glomerata*, *Poa pratensis*, and *Festuca pratensis* supplied by Allergon (Engelholm, Sweden), was used. The production procedure involved the extraction in Coca's solution (5 g of NaCl and 2.5 g of $NaHCO_3$/L) at 4°C for 16 h, centrifugation, diafiltration (M_r cutoff, 5 kDa), and lyophilization. Allergoids were produced from molecular characterized and standardized extracts by treatment with formaldehyde according to the method described by Marsh et al. (7). The source of Phl p 5 and rPhl p 5b had been described previously (14, 39).

For the cultivation of DC and Mϕ the cytokines GM-CSF, IL-4, TNF-α, IL-6, IL-1β, and IFN-γ were obtained from BioSource (Ratingen, Germany), M-CSF was obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany), IL-2 from Strathmann Biotech (Hannover, Germany), and PGE_2 from Sigma (Deisenhofen, Germany).

T cell clones

The generation of Phl p 5-specific T cell clones from grass pollen allergic individuals was performed as previously described (14, 40).

Isolation of CD14- and CD19-positive cells

Isolation of PBMC from the blood of the respective allergic donor was performed with Lymphoprep medium (density, 1.077; Life Technologies, Eggenstein, Germany) in Leucosep tubes (Greiner, Frickenhausen, Germany) according to the instructions of the manufacturers.

$CD14^+$ cells were obtained by magnetic cell sorting using positive selection with CD14-MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and MiniMACS Separation Columns type MS (Miltenyi Biotec). The separation of the $CD14^+$ cells was performed in MACS buffer consisting of PBS, pH 7.2, supplemented with 0.5% BSA fraction V (Merck, Darmstadt, Germany) and 2 mM EDTA according to the instructions of the manufacturer. The effluent was collected for subsequent separation of B cells with CD19-MicroBeads (Miltenyi Biotec). These isolation procedures generally yielded >90% pure monocytes or B cells, respectively.

Generation of DC

$CD14^+$ cells were cultivated under serum-free conditions in Ultraculture Medium (BioWhittaker, Verviers, Belgium) supplemented with 2 mM Glutamax I (Life Technologies), antibiotic-antimycotic solution (Sigma), and 20 μM mercaptoethanol (Life Technologies) together with IL-4 (200 ng/well) and GM-CSF (60 ng/well). $CD14^+$ cells (3×10^6) were seeded in a total volume of 1 ml in a 24-well culture plate (Nunc, Wiesbaden, Germany). Every second day half of the medium was removed and substituted with fresh medium containing both cytokines in equal amounts, as described above. After 7–10 days of culture the cells were harvested and used as APC for autologous TCC in proliferation assays, an aliquot of the cells was prepared for characterization by FACS analysis. These cells were considered immature DC. To confirm that the cultivated cells were of the DC phenotype, maturation was induced by incubating the immature DC after day 7 for an additional 3 days in a cocktail of different cytokines according to the method described by Jonuleit et al. (41) using TNF-α (10 ng/ml), IL-6 (10 ng/ml), IL-1β (10 ng/ml), and PGE_2 (1 μg/ml).

Generation of Mϕ

Numerous culture conditions for the generation of Mϕ from $CD14^+$ blood monocytes are described in the literature; therefore, several different media were tested in preliminary trials. Basic medium was either Ultraculture (BioWhittaker) or IMDM (Life Technologies). The medium was used either with or without the cytokines and serum or with the addition of GM-CSF or M-CSF and 2% AB-Serum (Sigma) or 10% FCS (Life Technologies). The medium leading to the highest yield of viable cells with Mϕ-like properties was Ultraculture medium supplemented with 2 mM Glutamax I, antibiotic-antimycotic solution, and 20 μM mercaptoethanol, as described above, with addition of 2% AB serum and 30 ng/ml M-CSF. $CD14^+$ cells (5×10^6) were seeded in a total volume of 1 ml in a 24-well culture plate for suspension cultures (Greiner, Frickenhausen, Germany). Every second or third day half of the medium was removed and substituted with fresh medium, adding M-CSF on days 3 and 5. After 7–10 days of culture the cells were harvested and used as APC for autologous TCC in proliferation assays. An aliquot of the cells was prepared for characterization by FACS analysis. Activated Mϕ were obtained by adding 10 ng/ml IFN-γ for the last 24 h of cultivation.

Proliferation assay

For the proliferation assay the cells of a TCC were seeded at 2×10^4 T cells/well of a 96-well culture plate in triplicate with the Ags in optimal stimulation doses (100 PNU/ml of grass pollen allergen and allergoid, 10 μg of Phl p 5/ml). The different autologous APC were used in the following amounts per well: 5×10^4 irradiated (30 Gy) PBMC, 5×10^3 DC or Mϕ, and 5×10^4 B cells. These numbers of APC were found to be optimal for the stimulation of 2×10^4 T cells/well. After 48 h in the incubator (37°C, humidified atmosphere, 5% CO_2) 100 μl of supernatant was removed, and equal supernatants were pooled and frozen at −20°C until assayed for cytokines. Immediately after removal of half the medium it was supplemented with fresh medium and 1 μCi [^{3}H]thymidine (Amersham-Pharmacia, Braunschweig, Germany)/well and further incubated for 16 h. Cells were then harvested on Microbeta filter mats with a 96-well cell harvester (Wallac ADL, Freiburg, Germany) and prepared for measurement in a Microbeta scintillation counter (Wallac ADL) as described previously (14).

Cytokine measurement

The cytokines IL-12 (p40 and p70) and in some cases IL-12p70 and IL-10 were determined in culture supernatants of stimulated TCC/APC using Cytoscreen ELISA kits (BioSource, Ratingen, Germany) according to the instructions of the manufacturer. The supernatants were obtained after a 48-h incubation period.

FACS analysis

Cell surface marker expression of the cultivated cells was evaluated by double-immunofluorescence staining using the following FITC- or PE-labeled mAbs for FACS analysis: CD14 (clone M5E2), CD16 (3G8), CD86 (2331 FUN-1), and CD11c (B-ly6) from PharMingen (Hamburg, Germany); CD80 (MAB104), CD-83 (HB15A), and HLA-DR (Immu357) from Coulter-Immunotech (Hamburg, Germany); and CD 123 (9F5) from Becton Dickinson (Heidelberg, Germany). The unlabeled mAbs Max I and Max III (42) were supplied by Prof. R. Andreesen (University of Regensburg, Regensburg, Germany). Isotype controls were IgG1 (107.3), IgG2a (G155-178), and IgG2b (27-35) obtained from PharMingen. For secondary isotype control we used a mouse mAb 2F9 of the IgG1 isotype with specificity for Par o 1 (43) and FITC-labeled goat-anti-mouse IgG1 (Coulter-Immunotech).

FACS analysis was performed with a FACScalibur (Becton Dickinson, Heidelberg, Germany). Marker expression was evaluated as the percentage of positive cells among DC or Mϕ defined by forward/side scatter characteristics.

Statistics

For statistical evaluation the Wilcoxon signed rank test (software: Jandel SigmaStat, version 2.0; Jandel, San Ramon, CA) was used to compare the stimulation indexes obtained with different batches of grass pollen extracts and allergoids for a single T cell clone and autologous DC from identical cultivation batches. The Wilcoxon signed rank test was further used for evaluation of the stimulation indexes obtained with allergen and allergoid using a panel of T cell clones and DC or Mϕ as APC, respectively.

Table I. *Flow cytometric analysis of monocyte derived immature DC, mature DC, and nonactivated and activated Mϕ*[a]

Marker	APC			
	DC_{imm}	DC_{mat}	Mϕ	$M\phi_{act}$
CD 11c	93 ± 5	93 ± 3	95 ± 5	95 ± 6
CD 14	10 ± 10	12 ± 12	92 ± 15	92 ± 10
CD 16	3 ± 10	4 ± 8	47 ± 29	32 ± 19
CD 64	2 ± 3	4 ± 5	46 ± 23	91 ± 6
CD 80	9 ± 12	39 ± 25	2 ± 4	5 ± 6
CD 83	0	41 ± 20	0	0
CD 86	51 ± 18	84 ± 11	36 ± 28	55 ± 20
CD 123	24 ± 16	66 ± 18	2 ± 3	0
HLA-DR	77 ± 20	83 ± 10	75 ± 18	90 ± 5
Max 1	41 ± 28	35 ± 29	67 ± 28	67 ± 29
Max 3	13 ± 10	20 ± 22	68 ± 27	71 ± 19

[a] Results are expressed as the mean ± SD of at least 10 experiments with cells from different subjects.

Results

Characterization of cultured DC and Mϕ by FACS analysis

During the culture process toward Mϕ the $CD14^+$ cells increased in size. They grew nonadherant due to the hydrophobic culture wells. The yield of cells for the generation of Mϕ was generally higher compared with the generation of DC. DC grew nonadherant in small clusters and exhibited a veiled and dendritic morphology (observations from light microscopy; data not shown). DC and Mϕ were characterized by specific surface markers, as determined by FACS analysis (Table I). The estimation of the percentage of positive cells is based on at least 10 experiments.

As expected for immature DC the expression of the monocyte/Mϕ marker CD14 was down-regulated, whereas it was found in large quantity on Mϕ. Furthermore, these DC_{imm} lacked CD83 expression, the typical marker for mature DC that was up-regulated when maturation of the DC was induced by the incubation with the cytokine cocktail; it was not found on the Mϕ. The IL-3α receptor CD123 was found exclusively on the DC and was up-regulated upon induction of maturation. Low and high affinity receptors for IgG (CD16 and CD64, respectively) were found only on the Mϕ, with a strong up-regulation of the latter upon activation by IFN-γ, whereas the DC lacked these markers. Costimulatory molecule CD86 was found on both cell types and was up-regulated upon facilitation of maturation of DC or activation of Mϕ. CD80 was expressed to a lesser extent and was found only on the DC. MHC II molecule HLA-DR was expressed on the majority of the cells and further up-regulated upon maturation/activation. Max 1 and Max 3 were expressed to a higher degree on Mϕ than on DC_{imm}. Maturation or activation did not change the degree of expression of Max 1 and Max 3. All T cell stimulation experiments with DC and Mϕ described in this paper refer to DC_{imm} and non-IFN-γ-activated Mϕ.

DC are highly effective APC for allergoid presentation to T cells

When comparing DC with autologous PBMC in terms of their capacities to present allergen- or allergoid-derived peptides to Phl p 5-specific TCC, proliferation was markedly higher when using DC, especially with the allergoid (14). A panel of 22 Phl p 5-reactive TCC derived from seven different allergic patients was stimulated with grass pollen extract and allergoid and with rPhl p 5b and the corresponding allergoid in the presence of DC as APC. The resulting median values of the whole panel are shown in Table II. Due to their specificity, some clones showed a higher response with the whole grass pollen preparations, other clones showed a higher response with the Phl p 5b isoform used. The degree of reactivity with allergoid compared with the native allergen also varied with the specificity of the TCC, but all TCC investigated showed pronounced reactivity with the two kinds of allergoid when presented by DC. Furthermore, the strength of the proliferative response was dependent on the condition of the respective TCC; with the same preparation of DC it was possible to achieve a stimulation index of about 200 for one clone, whereas another TCC from the same donor responded with a stimulation index of only 5–10.

To make sure that the reactivity of a particular TCC with one batch of allergoid is not a chance observation but an inherent feature of the TCC we investigated the responses of several TCC with different batches of allergoid made from different batches of grass pollen extract ($n = 10$). All batches of allergoid and the corresponding allergen showed very similar reactivity patterns (Fig. 1). The differences between allergen and allergoid reactivities of individual clones were often significant (Fig. 1), but comparison of the median values of the whole panel of TCC revealed no significant differences (Table II).

Table II. *Comparison of the median values of stimulation indices obtained with a panel of TCC after stimulation with grass pollen allergen or rPh1 p 5b and the corresponding allergoids and Ag presentation by DC_{imm} or Mϕ*

APC	Allergen Source	Median SI[a] Allergen	Median SI Allergoid	*p*	No. of TCC Investigated
DC_{imm}	Grass pollen	9.80	8.65	0.060	22
	rPh1 p 5b	9.30	14.30	0.561	15
Mϕ	Grass pollen	7.30	4.00	0.089	17
	rPh1 p 5b	11.10	11.50	0.266	13

[a] SI, stimulation index.

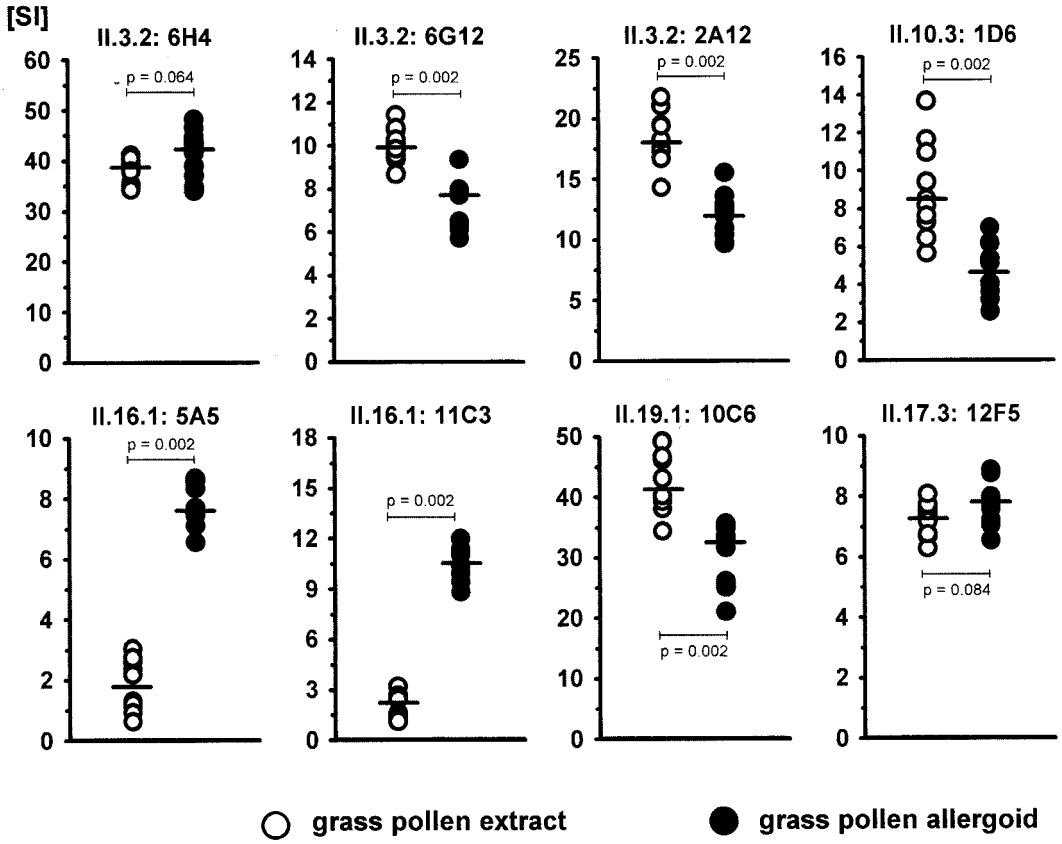

FIGURE 1. Proliferation of Phl p 5-specific TCC from different patients after stimulation with different batches ($n = 10$) of grass pollen extract and allergoid using DC for Ag presentation. The concentration of both allergen and allergoid was 100 PNU/ml. Horizontal bars mark the median values. Values for p were obtained with the Wilcoxon signed rank test.

Mϕ are superior to PBMC in presenting allergoids to T cells

Mϕ cultured from $CD14^+$ cells were tested for their T cell-stimulating properties as APC compared with those of the irradiated autologous PBMC. Higher proliferations were obtained when Mϕ instead of PBMC were used for Ag presentation (Fig. 2), especially with the allergoids. Again, when comparing the median values of allergen and allergoid reactivity of the panel of TCC, the allergen and allergoid did not differ significantly (Table II). All TCC gave a clear response with both allergoids.

B cells fail to present allergoid to T cells

B cells isolated from PBMC were used for Ag presentation and compared with PBMC, DC, or Mϕ when possible. In an experiment using TCC II.19.10C6 (Fig. 3) B cells mediated a similar high proliferation as DC when presenting the major allergen rPhl p 5b, whereas the other allergen preparations, especially the allergoid, were less active with B cells. Using Mϕ, all allergen preparations except rPhl p 5b yielded a much higher proliferation of TCC, and DC were superior for presenting all allergen preparations.

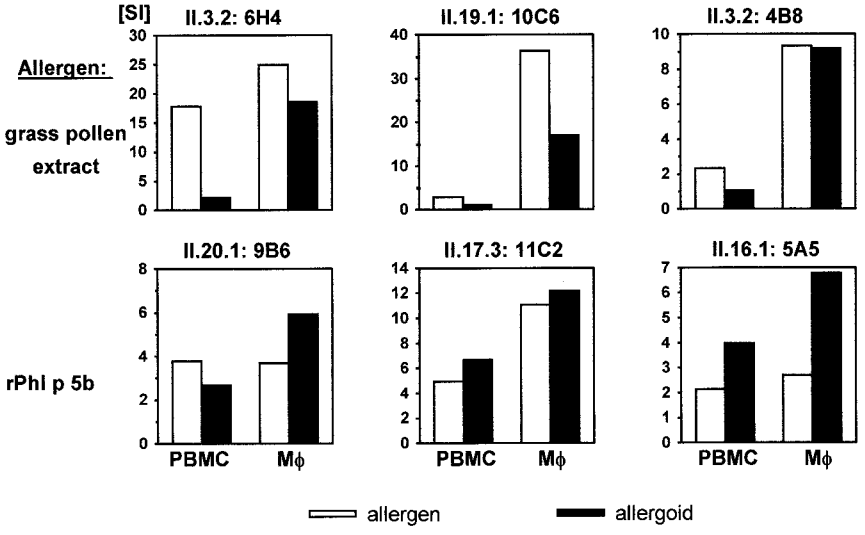

FIGURE 2. Comparison of the proliferative response (stimulation index (SI)) of some TCC with grass pollen allergen and allergoid or rPhl p 5b and rPhl p 5b allergoid obtained with different populations of APC: autologous PBMC (5×10^4 PBMC and 2×10^4 T cells/well) or Mϕ (5×10^3 Mϕ and 2×10^4 T cells/well).

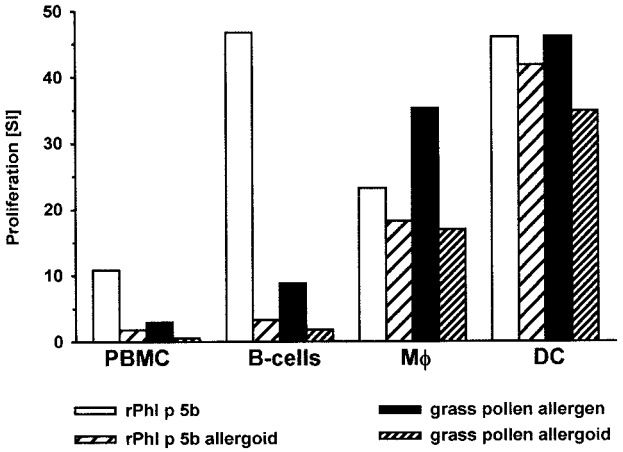

FIGURE 3. Comparison of the proliferative response (stimulation index (SI)) of TCC II.19: 10C6 with rPhl p 5b and rPhl p 5b allergoid or grass pollen allergen and allergoid (100 PNU/ml of each) obtained with different populations of APC: PBMC (5×10^4 cells/well), B cells (5×10^4 cells/well), and Mϕ or DC (5×10^3 cells/well), all autologous APC. T cells were used in an amount of 2×10^4 cells/well.

In another experiment DC and B cells were compared for Ag presentation to five TCC of different phenotypes from the same donor. Whereas DC brought about a strong stimulation of all five TCC with both allergen and allergoid (Fig. 4), B cells were effective for Ag presentation only for the allergen and only for three of the clones. These clones belong to the Th2 phenotype as deduced from their cytokine spectrum (data not shown). In contrast, the nonresponding TCC exhibit Th1 (6H4) and Th0 (5D6) phenotypes, respectively.

IL-12 production during APC/T cell interaction

IL-12 is recognized as a key cytokine for the induction of Th1 responses, and DC and Mϕ are the main sources of this cytokine. IL-12 (p40 plus p70) was detectable in large amounts in supernatants, where the Ag presentation was performed by DC (Fig. 5). The amount of IL-12 did not parallel the proliferation; larger differences in the proliferation obtained with the allergen were not accompanied by a similar course in IL-12 production and often led to similar IL-12 levels. The IL-12 production was dependent on activated T cells because neither the control stimulation (T cells and DC, but no allergen) nor nonrelevant major allergens (for example, cat dander or birch pollen extract) nor the DC and allergen without specific T cells led to IL-12 production (data not shown). Using PBMC (Fig. 5), isolated B cells (Fig. 4), or Mϕ (Fig. 6), very little or no IL-12 was detectable.

IL-10 production during APC/T cell interaction

IL-10 was measured in parallel to IL-12 in some supernatants upon Ag stimulation of TCC and APC. Very little or no IL-10 was obtained in supernatants of TCC and B cells for Ag presentation (Fig. 4) except for one TCC (3D2). In contrast, IL-10 was found in supernatants from the interaction of Ag-activated TCC with DC or Mϕ for Ag presentation in varying amounts (Figs. 4 and 6). The amount of IL-10 from the interaction of APC with a certain TCC varies with the type of the APC (Fig. 6); some interactions resulted

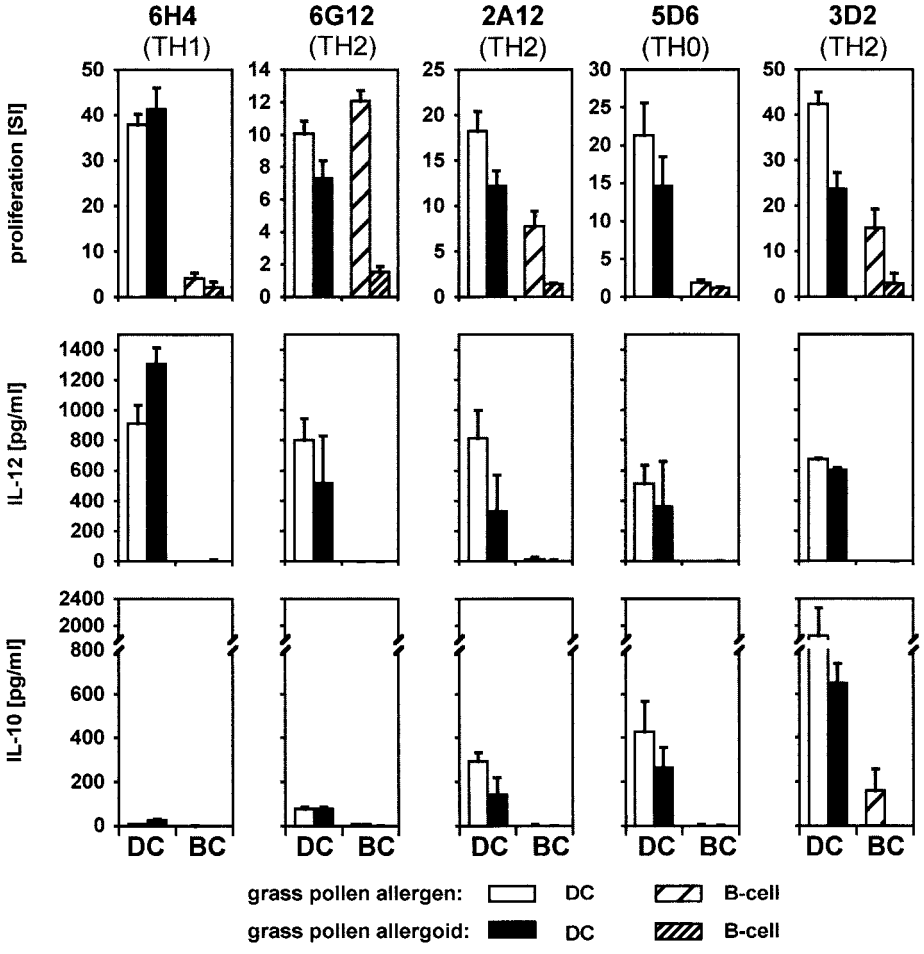

FIGURE 4. Comparison of the proliferative response (stimulation index (SI)) and the production of IL-12 (p40 plus p70) and IL-10 from Phl p 5-specific TCC of different phenotypes and the same atopic subject after stimulation with grass pollen allergen and allergoid (100 PNU/ml) together with autologous DC or B cells for Ag presentation. The ratio of T cells to APC is given in Fig. 3. The numbers of the different grass pollen allergen and allergoid preparations tested were 10–11 for DC and 4–5 for B cells. The phenotype of the TCC is indicated in parentheses.

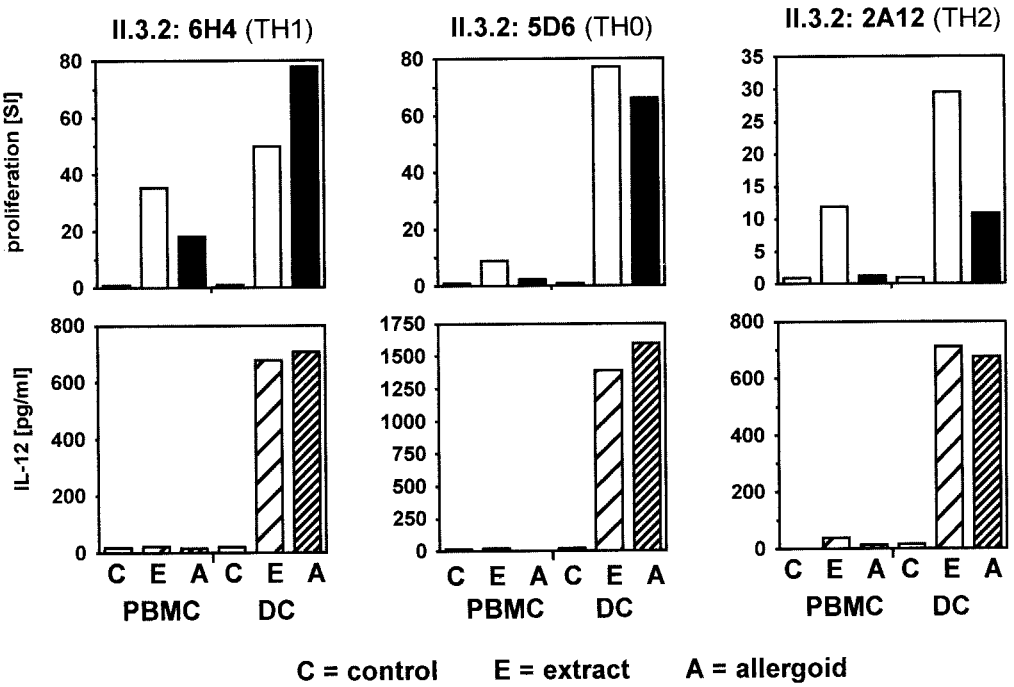

FIGURE 5. Proliferation (stimulation index (SI)) of TCC stimulated with grass pollen allergen and allergoid using autologous PBMC or DC for Ag presentation and IL-12 detection (p40 + p70) in culture supernatants of stimulated cells. Control stimulations (C) were performed without Ag. Stimulations were performed with grass pollen extract (E) or the respective allergoid (A) in concentrations of 100 PNU/ml. The phenotype of the TCC is indicated in parentheses.

in considerably higher IL-10 production when DC performed the Ag presentation (with TCC 1D6 and 12F5), and other interactions gave higher IL-10 production when Mϕ acted as APC (with TCC 11C3 and 6G12). The experiments did not show whether the source of IL-10 was the TCC or the APC.

The Th phenotype directs the cytokine profile of DC

Comparing Mϕ and DC as APC with two TCC from the same donor (II.17.1), we found a higher concentration of IL-12 in the supernatants of DC and one of the TCC (1D8), whereas the interaction of the APC with the other TCC (12F5) resulted in reduced IL-12 production (Fig. 6). In the supernatants of the latter, considerable production of IL-10 became obvious, whereas the interaction with TCC 1D8 resulted in little IL-10 production. The Mϕ produce very little IL-12 upon interaction with the TCC and Ag, whereas IL-10 was obtained in larger amounts with TCC 12F5 (Fig. 6). Both TCC were formerly characterized as Th0 clones, but regarding their interaction with DC, TCC 1D8 led to an IL-12-dominated milieu, whereas TCC 12F5 led to an IL-10-dominated milieu.

Comparing the IL-12 and IL-10 production in supernatants of the TCC from Fig. 4 when Ag is presented by either DC or B cells, pronounced IL-12 and IL-10 production was only found with DC for Ag presentation. A strong induction of proliferation of the Th2-like clone 6G12 by B cells was not accompanied by IL-12 production of these APC. The interaction of the DC with TCC 6H4, which exhibits a Th1 phenotype, yielded low levels of IL-10 and very high levels of IL-12. In contrast, the interaction of the DC with TCC 6G12, 2A12, 3D2, and 5D6, which share a Th2- or Th0-like phenotype, respectively, gave higher IL-10 production and lower IL-12 production compared with TCC 6H4 (Fig. 4). Thus, the phenotype of a TCC directs the cytokine profile of the DC. The interaction of DC with TCC of Th2-like character resulted in lower IL-12 and higher IL-10 production, whereas the interaction of DC with Th1-like TCC led to higher IL-12 and lower IL-10 production.

Discussion

Our data reveal a strong and reproducible T cell-stimulating capacity of allergoids by direct stimulation of allergen-specific T cells in vitro. The results clearly show that the stimulation of Th cells by the allergoid is dependent on the type of the APC. Immature DC and Mϕ generated from $CD14^+$ precursors of blood cells are highly effective cells for the presentation of allergoids, whereas PBMC and B cells are less effective.

The cultivated DC possess the phenotypical and morphological characteristics of DC (data for the latter not shown) (20, 41, 44, 45): no or low expression of CD14; the absence of low affinity (CD16) and high affinity (CD64) IgG receptors; expression of CD123, which has been described as a suitable marker for the isolation of DC from mononuclear cells (44); and especially strong expression of costimulatory molecules CD86 and MHC-II. CD83, as a typical marker of mature DC (45), and CD80 were up-regulated upon induction of maturation. In contrast to DC, the Mϕ showed, as expected (20, 30, 46), strong expression of CD14; no DC markers such as CD123 and CD83; considerable expression of CD16, CD64, and the costimulatory molecules CD86 and MHC II; as well as high expression of the Mϕ-associated markers Max 1 and Max 3. Max 1 and Max 3 molecules were described as lineage-restricted differentiation Ags of mature Mϕ (42). We also detected Max 1 on DC, an observation that has not previously been documented in the literature.

There are few reports concerning the direct stimulation of T cells with allergoids. In a previous study we showed a clear T cell reactivity of allergoids by direct stimulation of PBMC from allergic subjects and by stimulation of T cell lines and clones using the conventionally taken autologous PBMC for Ag presentation (14). In this system the stimulating capacity of the allergoid was often lower compared with that of the native allergen. Stimulation of PBMC from house dust mite allergic and nonallergic subjects with house dust mite allergen as well as allergoid showed similar T

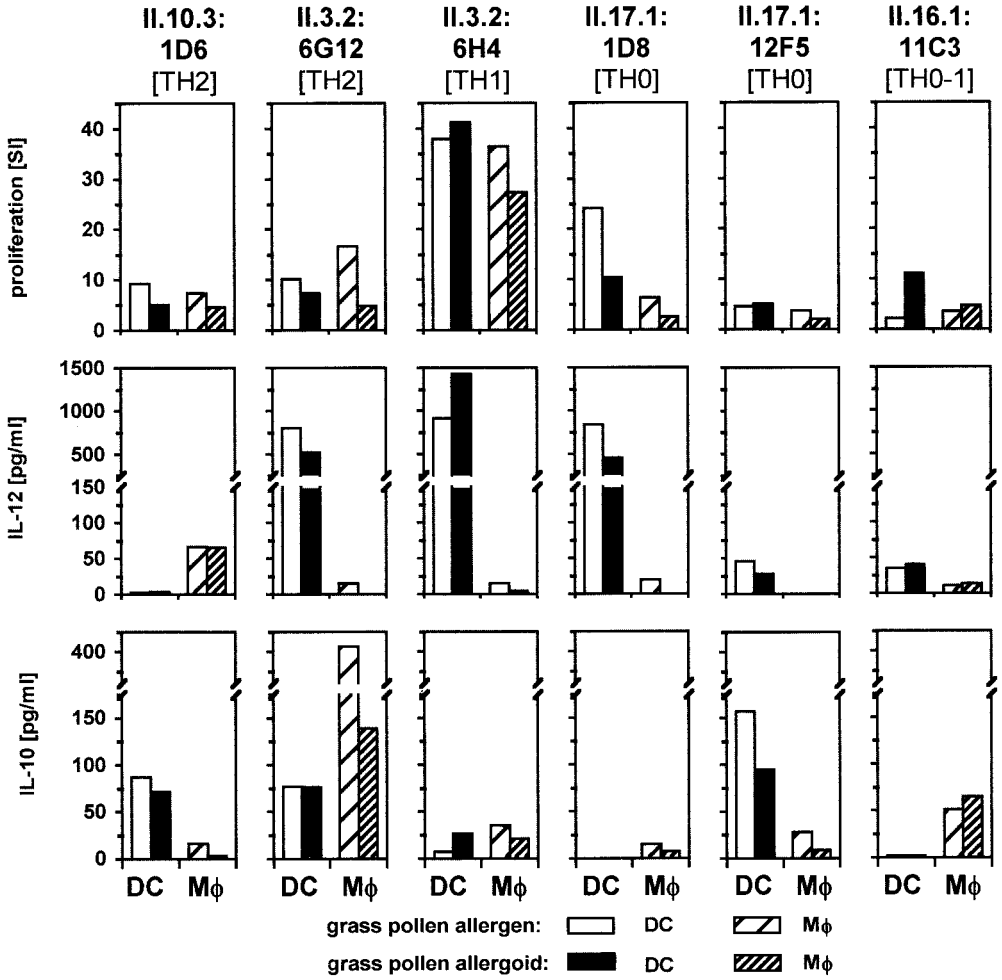

FIGURE 6. Comparison of the proliferative responses and the production of IL-12 (p40 plus p70) and IL-10 from Phl p 5-specific TCC of different phenotypes and different atopic subjects after stimulation with grass pollen allergen and allergoid (100 PNU/ml) together with either DC or Mϕ as APC. Stimulation of TCC from the same patient (II.3 and II.17) were performed with the same autologous APC preparations.

cell-stimulating properties (47). Dormann et al. (48) compared native Bet v 1 and maleic anhydride- and formaldehyde-modified preparations of Bet v 1 for their stimulating capacity with a panel of Bet v 1-specific TCC using autologous PBMC for Ag presentation. Both the native allergen and the maleic anhydride-modified Bet v 1 revealed strong IgE reactivity as well as strong T cell-stimulating capacity, whereas the formaldehyde-modified allergen showed both reduced IgE and T cell reactivity. PBMC are not the target APC in s.c. injection immunotherapy, and therefore we investigated the reactivity of allergoids with APC that more closely resemble those acting under in vivo conditions. Using pure populations of APC, including DC, Mϕ, and B cells, we found the differences in their abilities to present allergoids. The differences may be attributable to the particular Ag uptake mechanisms used by these cells; DC and Mϕ possess the capacity to ingest Ag by macropinocytosis or phagocytosis (20, 22, 49–51). Although all TCC investigated showed a pronounced reactivity with the allergoid using DC or Mϕ for Ag presentation, the magnitude of reactivity obtained by the allergoid compared with the allergen varied. As discussed previously (14), these differences might be attributable to formaldehyde-modified T cell peptides of the allergoid. The chemical modification may alter the affinity of the peptide for the TCR, and the possible outcome is higher, similar, or lower stimulating capacity of the allergoid, dependent on the specificity of the TCC. Observations from several model systems studying altered peptide ligands support this hypothesis (52, 53). It is also probable that the allergoid-derived peptides differ from the peptides of the native material in their peptide-flanking residues, leading to peptides with more agonistic or antagonistic features, as recently described for an immunodominant epitope of hen egg lysozyme (54).

Furthermore, the lower proliferation to allergoid compared with the allergen sometimes observed with some TCC may be a result of the hindered Ag uptake via Fcϵ or Fcγ receptors due to destroyed B cell epitopes, since this mechanism is also used by these cells (20, 22, 55–57). Both receptors for IgE, the high affinity receptor FcϵRI and the low affinity receptor FcϵRII, were found on monocyte-derived DC (58).

The weak proliferation of appropriate TCC induced by the allergoid, in contrast to the allergen, as a result of Ag presentation by B cells is indirect evidence that Ab-mediated Ag uptake mechanisms fail to work for allergoids. It is supposed that this is the main reason for the observed lower stimulating capacity of PBMC-mediated Ag presentation (14). The observation of van Neerven et al. (17) that serum IgE facilitates allergen presentation to Th cells by a factor of >100 supports this hypothesis. These authors showed that this process is mediated by the low affinity IgE receptor CD23. Ag uptake via allergen-specific IgE bound to FcϵRII enables all B cells bearing this receptor, even those that are not specific for the

allergen, to efficiently take up and present allergen-derived peptides to Th cells, leading to an up-regulation of the allergic response. Our results using B cells for Ag presentation and TCC of different phenotypes (Th2 and Th1) confirm the observations of other authors using murine (59, 60) or human TCC (61), showing that B cells are very effective in presenting native allergen to Th2 clones, but fail to stimulate Th1 clones. Therefore, the destruction of Ab-binding epitopes as a result of chemical modification hinders the Ag presentation by B cells. Consequently, there will be no activation of the elevated number of existing Th2 cells of the allergic subject via this pathway, and therefore no further IL-4 and IL-5 production, which would further amplify the allergic reaction.

In contrast to B cells, DC and Mϕ present Ag to Th1, Th2, and Th0 clones equally well. Concerning DC, these results contrast to a degree with those of Ria et al. (62), who observed Th2 cell-mediated inhibition of IL-12 production from DC. We observed considerable IL-12 production even with Th2-like TCC (Fig. 6). When investigating the simultaneous production of IL-10 and IL-12 resulting from the interaction of different TCC with the same DC preparation, we obtained higher IL-12 concentrations in supernatants from TCC that do not produce or initiate IL-10 production.

The FcϵRI has been found on DC (55, 56). It seems not to be important for priming immune responses because it requires Abs of high affinity, which are themselves the result of an immune response. Thus, the FcϵRI is thought to be involved in the amplification of ongoing immune responses, such as allergy (32, 63). The outcome of T cell stimulation might vary if the presentation by DC were to be performed with varying contributions from FcϵRI (55). Triggering of the receptors is supposed to lead to a release of different cytokines by these DC with the capacity to skew the immune response (55). The absence of IgE epitopes on allergoids prevents FcϵRI-mediated uptake or triggering of these APC. Therefore, allergoids are not able to amplify allergic reactions via IgE/Fcϵ receptor-dependent mechanisms.

The IL-12 production of the APC was investigated because this cytokine is known to be a main factor in the induction of Th1 responses from naive T cells (24, 33, 34) and even in the suppression of ongoing Th2 responses (23). The physiological function of IL-12p40 in vivo is still unclear (64). An IL-12p70 antagonistic function has been described, but under certain conditions IL-12p40 acts similarly to IL-12p70, and it has been shown to act as a chemoattractant for macrophages (64). Our results show that allergen as well as allergoid presentation to allergen-specific TCC by DC led to a pronounced IL-12 production, which was not the case with control stimulations or stimulation with nonrelevant major allergen. Therefore, both allergen and allergoid preparations possess the capacity to prime naive T cells in the direction of Th1 responses or any other mechanism that is exerted by IL-12p70 and IL-12p40. Different TCC influenced the levels of IL-12 production to various degrees, and significant IL-10 production was associated with lower IL-12 production. These results clearly indicate that the phenotype of an Ag-specific TCC might influence the surrounding cytokine milieu of the DC in which priming occurs. The interaction of DC with allergen-specific Th2-like cells resulted in less IL-12 and higher IL-10 production than the interaction with Th1-like cells. Despite the fact that both allergen and allergoid induced strong TCC proliferative responses in the presence of Mϕ, the latter failed to produce IL-12. Recently, it was shown that M-CSF-generated Mϕ are deficient in IL-12 production (p70 and p40) even after stimulation with LPS (65).

The characteristic response pattern of a particular TCC was found to be a reproducible feature of different allergoid production batches. However, differences in the responses of TCC to allergen and allergoid may reflect the specificity of the TCC and suggest that the outcome of processing of allergen and allergoid might be different, leading to similar, but not always the same, peptides for presentation. Similar conclusions were drawn by Akdis et al. (18) for nonrefolded and refolded PLA_2 from bee venom, which were shown to have a strong influence on the type of APC that acts for presentation. Only the refolded PLA_2 led to effective Ag presentation by B cells, whereas the refolded as well as nonrefolded PLA_2 was bound and processed equally well by monocytes. In mice, Yang et al. (4) demonstrated the preferential induction of Th1 responses in vivo by a chemically modified Ag (glutaraldehyde-polymerized OVA).

In conclusion, the data reveal that allergoids have a strong T cell-stimulating capacity and demonstrate the advantage of the allergoid principle for use in specific immunotherapy. The strongly reduced ability of allergoids to react with IgE will prevent not only mediator release from mast cells and basophils, but also the uptake via IgE on FcϵRI and FcϵRII receptors of APC, which is supposed to result in an amplification of the allergic response. The observations suggest that the adoption of the allergoid principle in the development of genetically modified allergens with reduced IgE reactivity but retained T cell reactivity (40) will provide a promising basis for the development of newer therapeutic vaccines for allergen-specific immunotherapy.

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References

1. Norman, P. S., M. D. Lawrence, M. Lichtenstein, A. Kagey-Sobotka, and D. G. Marsh. 1982. Controlled evaluation of allergoid in immunotherapy of ragweed hay fever. *J. Allergy Clin. Immunol. 70:248.*
2. Bousquet, J., A. Hejjaoui, W. Skassa-Brociek, B. Guérin, H. J. Maasch, H. Dhivert, and F. B. Michel. 1987. Double-blind placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J. Allergy Clin. Immunol. 80:591*.
3. Hayglass, K. T., and W. P. Stefura. 1991. Antigen-specific inhibition of ongoing murine IgE immune responses. II. Inhibition of IgE responses induced by treatment with glutaraldehyde-modified allergens is paralleled by reciprocal increases in IgG2a synthesis. *J. Immunol. 147:2455*.
4. Yang, X., R. S. Gieni, T. R. Mosmann, K. T. Hayglass. 1993. Chemically modified antigen preferentially elicits induction of Th1-like cytokine synthesis patterns in vivo. *J. Exp. Med. 178:349*.
5. Haddad, Z. H., D. G. Marsh, and D. H. Campbell. 1972. Studies on "allergoids" prepared from naturally occurring allergens. II. Evaluation of allergenicity and assay of antigenicity of formalinized mixed grass pollen extracts. *J. Allergy Clin. Immunol. 49:197*.
6. Marsh, D. G., L. M. N. Lichtenstein, and D. H. Campbell. 1970. Studies on "allergoids" prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalized rye group I component. *Immunology 18:705*.
7. Marsh, D.G. 1971. Preparation and properties of "allergoids" derived from native pollen allergens by mild formalin treatment. *Int. Arch. Allergy Appl. Immunol. 41:199.*
8. Maasch, H. J., W. Geissler, R. Wahl, G. Winter, and J. Maass. 1982. Untersuchungen an Allergenen und Allergoiden aus 6 Gräserpollen. *Allergologie 5:83.*
9. Puttonen, E., H. J. Maasch, and L. Pilström. 1982. Studies on allergen and allergoid preparations from purified Timothy (*Phleum pratense*) pollen extracts. I. Physicochemical characteristics and binding to allergen-specific human IgE. *Int. Arch. Allergy Appl. Immunol. 68:1*.
10. Takatsu, K., K. Ishizaka, and T. P. King. 1975. Immunogenic properties of modified antigen E. III. Effect of repeated injections of modified antigen on immunocompetent cells specific for native antigen. *J. Immunol. 66:336*.
11. Baumgarten, C., G. Kunkel, and R. Rudolph. 1982. Grass pollen allergoid: a four year follow up study. *Respiration 46:97.*
12. Loidolt, D., and H. J. Maasch. 1987. Hyposensibilisierung bei Pollinose: Vergleichende klinische und immunologische Untersuchungen mit Allergen-Depot und Allergoid-Depot. *Allergologie 5:176.*

13. Klimek, L., D. Dormann, E. R. Jarman, O. Cromwell, H. Riechelmann, and A. B. Reske-Kunz. 1999. Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis. *Clin. Exp. Allergy 29:1326.*
14. Kahlert, H., H.-T. Stüwe, O. Cromwell, and H. Fiebig. 1999. Reactivity of T-cells with grass pollen extract and allergoid. *Int. Arch. Allergy Immunol. 120:146.*
15. Maurer, D., C. Ebner, B. Reininger, E. Fiebiger, D. Kraft, J.-P. Kinet, and G. Stingl. 1995. The high affinity IgE receptor (FcϵRI) mediates IgE-dependent allergen presentation. *J. Immunol. 154:6285.*
16. van der Heijden, F. L., R. J. J. van Neerven, M. van Katwijk, J. D. Bos, and M. L. Kapsenberg. 1993. Serum-IgE facilitated allergen presentation in atopic disease. *J. Immunol. 150:3643.*
17. van Neerven, R. J. J., T. Wikborg, G. Lund, B. Jacobsen, A. Brinch-Nielsen, J. Arnved, and H. Ipsen. 1999. Blocking antibodies induced by specific allergy vaccination prevent the activation of $CD4^+$ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol. 163:2944.*
18. Akdis, C. A., T. Blesken, D. Wymann, M. Akdis, and K. Blaser. 1998. Differential regulation of human cytokine patterns and IgE and IgG4 responses by conformational antigen variants. *Eur. J. Immunol. 28:914.*
19. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, D.-S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive $CD4^+$ T cells. *J. Immunol. 154:5071.*
20. Cella, M., F. Sallusto, and A. Lanzavecchia. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol. 9:1220.*
21. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med. 179:1109.*
22. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med. 182:389.*
23. Desmedt, M., P. Rottiers, H. Dooms, W. Fiers, and J. Grooten. 1998. Macrophages induce cellular immunity by activating Th1 cell responses and suppressing Th2 cell responses. *J. Immunol. 160:5300.*
24. Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of Th1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science 260:547.*
25. Bender, A., M. Sapp, G. Schuler, R. M. Steinman, and N. Bhardwaj. 1996. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J. Immunol. Methods 196:121.*
26. Romani, N., D. Reider, M. Heuer, S. Ebner, E. Kämpgen, B. Eibl, D. Niederwieser, and G. Schuler. 1996. Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. *J. Immunol. Methods 196:137.*
27. Zhou, L.-J., and T. F. Tedder. 1996. $CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells. *Proc. Natl. Acad. Sci. USA 93: 2588.*
28. Pickl, W. F., O. Majdic, P. Kohl, J. Stöckl, E. Riedl, C. Scheinecker, C. Bello-Fernandez, and W. Knapp. 1996. Molecular and functional characteristics of dendritic cells generated from highly purified $CD14^+$ peripheral blood monocytes. *J. Immunol. 157:3850.*
29. Andreesen, R., J. Picht, and G. W. Löhr. 1988. Primary cultures of human blood-born macrophages grown on hydrophobic Teflon membranes. *J. Immunol. Methods 56:295.*
30. Lopez, M., C. Martinache, S. Canepa, M. Chokri, F. Scotto, and J. Bartholeyns. 1993. Autologous lymphocytes prevent the death of monocytes in culture and promote, as do GM-CSF, IL-3 and M-CSF, their differentiation into macrophages. *J. Immunol. Methods 159:29.*
31. Toujas, L., J.-G. Delcros, E. Diez, N. Gervois, G. Semana, G. Corradin, and F. Jotereau. 1997. Human monocyte-derived macrophages and dendritic cells are comparably effective in vitro in presenting HLA class I-restricted exogenous peptides. *Immunology 91:635.*
32. Schuler, G., B. Thurner, and N. Romani. 1997. Dendritic cells: from ignored cells to major players in T-cell-mediated immunity. *Int. Arch. Allergy Immunol. 112:317.*
33. Trinchieri, G. 1993. Interleukin-12 and its role in the generation of Th1 cells. *Immunol. Today 14:335.*
34. McDyer, J. F., C.-Y. Wu, and R. A. Seder. 1998. The regulation of IL-12: its role in infectious, autoimmune, and allergic diseases. *J. Allergy Clin. Immunol. 102:11.*
35. Borish, L. 1998. IL-10: evolving concepts. *J. Allergy Clin. Immunol. 97:719.*
36. Kalinski, P., I. H. N. Schuitemaker, C. M. U. Hilkens, and M. L. Kapsenberg. 1998. Prostaglandin E_2 induces the final maturation of IL-12-deficient $CD1a^+CD83^+$ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J. Immunol. 161:2804.*
37. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kämpgen, N. Romani, and G. Schuler. 1996 High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD 40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med. 184:741.*
38. Akdis, C. A., and K. Blaser. 1999. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J. 13:603.*
39. Bufe, A., G. Schramm, M. B. Keown, M. Schlaak, and W.-M. Becker. 1995. Major allergen Phl p 5b in timothy grass is a novel pollen Rnase. *FEBS Lett. 363:6.*
40. Schramm, G., H. Kahlert, R. Suck, B. Weber, H.-T. Stüwe, W.-D. Müller, A. Bufe, W.-M. Becker, M. Schlaak, O. Cromwell, et al. 1999. "Allergen engineering:" variants of the timothy grass pollen allergen Phl p 5b with reduced IgE-binding capacity but conserved T-cell reactivity. *J. Immunol. 162:2406.*
41. Jonuleit, H., U. Kühn, G. Müller, K. Steinbrink, L. Paragnik, E. Schmitt, J. Knop, and A. H. Enk. 1997. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *Eur. J. Immunol. 27:3135.*
42. Andreesen, R., K. J. Bross, J. Osterholz, and F. Emmerich. 1986. Human macrophage maturation and heterogeneity: analysis with a newly generated set of monoclonal antibodies to differentiation antigens. *Blood 67:1257.*
43. Kahlert, H., B. Weber, M. Teppke, R. Wahl, O. Cromwell, and H. Fiebig. 1996. Characterization of major allergens of *Parietaria officinalis*. *Int. Arch. Allergy Immunol. 109:141.*
44. Olweus, J., A. BitMansour, R. Warnke, P. A. Thompson, J. Carballido, L. J. Picker, and F. Lund-Johansen. 1997. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc. Natl. Acad. Sci. USA 94:12551.*
45. Zhou, L.-J., and T. F. Tedder. 1995. Blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J. Immunol. 154:3821.*
46. Santin, A. D., P. L. Hermonat, A. Ravaggi, M. Chiriva-Internati, M. J. Cannon, J. C. Hiserodt, S. Pecorelli, and G. P. Parham. 1999. Expression of surface antigens during the differentiation of human dendritic cells vs macrophages from blood monocytes in vitro. *Immunobiology 200:187.*
47. Weber, B., H.-T. Stüwe, A. Verhoef, J. R. Lamb, H. Fiebig, and O. Cromwell. 1997. An house dust mite allergoid that retains T-cell reactivity but has strongly reduced allergenicity. *Allergy Clin. Immunol. Int. 9(Suppl. 4):10.*
48. Dormann, D., C. Ebner, E. R. Jarman, E. Montermann, D. Kraft, and A. B. Reske-Kunz. 1998. Responses of human birch pollen allergen-reactive T cells to chemically modified allergens (allergoids). *Clin. Exp. Allergy 28:1374.*
49. Lanzavecchia, A. 1996. Mechanism of antigen uptake for presentation. *Curr. Opin. Immunol. 8:348.*
50. Reis e Sousa, C., P. D. Stahl, and J. M. Austyn. 1993. Phagocytosis of an antigen by Langerhans cells in vitro. *J. Exp. Med. 178:509.*
51. Aderem, A., and D. M. Underhill. 1999. Mechanism of phagocytosis in macrophages. *Annu. Rev. Immunol. 17:593.*
52. Sloane-Lancaster, J., B. D. Evavold, and P. M. Allen. 1993. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature 363:156.*
53. Matsuoka, T., H. Kohrogi, M. Ando, Y. Nishimura, and S. Matsushita. 1996. Altered TCR ligands affect antigen-presenting cell responses: up-regulation of IL-12 by an analogue peptide. *J. Immunol. 157:4837.*
54. Carson, R. T., D. D. Desai, K. M. Vignali, and D. A. A. Vignali. 1999. Immunoregulation of Th cells by naturally processed peptide antagonists. *J. Immunol. 162:1.*
55. Bieber, T. 1997. FcϵRI-expressing antigen-presenting cells: new players in the atopic game. *Immunol. Today 18:211.*
56. Bieber, T. 1997. Fcϵ RI on human epidermal Langerhans cells: an old receptor with new structure and functions. *Int. Arch. Allergy Immunol. 113:30.*
57. Stingl, G., and D. Maurer. 1997. IgE-mediated allergen presentation via FcϵRI on antigen-presenting cells. *Int. Arch. Allergy Immunol. 113:24.*
58. van den Heuvel, M. M., D. D. C. Vanhee, P. E. Postmus, E. C. M. Hoefsmit, and R. H. J. Beelen. 1998. Functional and phenotypic differences of monocyte-derived dendritic cells from allergic and nonallergic patients. *J. Allergy Clin. Immunol. 101:90.*
59. Chang, T.-L., C. M. Shea, S. Urioste, R. C. Thompson, W. H. Boom, and A. K. Abboa. 1990. Heterogeneity of helper/inducer T lymphocytes. III. Responses of IL-2 and IL-4 producing (Th1 and Th2) clones to antigens presented by different accessory cells. *J. Immunol. 145:2803.*
60. Gajewski, T. F., M. Pinnas, T. Wong, and F. W. Filch. 1991. Murine Th1 and Th2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J. Immunol. 145:1750.*
61. Beck, L., R. Roth, and H. L. Spiegelberg. 1996. Comparison of monocytes and B cells for activation of human T helper cell subsets. *Clin. Immunol. Immunopathol. 78:56.*
62. Ria, F., G. Penna, and L. Adorini. 1998. Th1 cells induce and Th2 inhibit antigen-dependent IL-12 secretion by dendritic cells. *Eur. J. Immunol. 28:2003.*
63. Novak, N., J. Haberstock, E. Geiger, and T. Bieber. 1999. Dendritic cells in allergy. *Allergy 54:792.*
64. Ha, S. J., C. H. Lee, S. B. Lee, C. M. Kim, K. L. Jang, H. S. Shin, and Y. C. Sung. 1999. A novel function of IL-12p40 as a chemotactic molecule for macrophages. *J. Immunol. 163:2902.*
65. Smith, W., M. Feldmann, and M. Londei. 1998. Human macrophages induced in vitro by macrophage colony-stimulating factor are deficient in IL-12 production. *Eur. J. Immunol. 28:2498.*