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Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production

(interleukin 4/interferon γ/IgE)

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ABSTRACT **We have established a large panel of T-cell clones (TCCs) specific for *Dermatophagoides pteronyssinus* and *Lolium perenne* group I grass pollen allergens (total, 61) and for tetanus toxoid and protein purified derivative bacterial antigens (total, 38) from the peripheral blood of two atopic individuals and then analyzed their ability to produce interleukin 4 (IL-4), IL-5, and interferon γ (IFN-γ). Upon stimulation with phorbol 12-myristate 13-acetate plus anti-CD3 antibody, the great majority of TCCs specific for bacterial components was able to produce both IL-4 and IFN-γ, whereas most *D. pteronyssinus*- and *L. perenne* group I-specific TCCs produced IL-4, but no, or limited, IFN-γ. Moreover, the mean amounts of IL-4 and IFN-γ released by allergen-specific TCCs were significantly higher and lower, respectively, than the mean amounts produced by TCCs specific for bacterial components. Under the same experimental conditions, virtually all allergen-specific TCCs, but only one-third of tested TCCs specific for bacterial components, expressed IL-5 RNA and secreted IL-5 in their supernatants. Eighteen TCCs (nine specific for allergens and nine specific for bacterial components) were also assessed for their ability to induce IgE synthesis by autologous B cells in response to stimulation with the specific antigen. Under these experimental conditions, all allergen-specific TCCs, but only one-third of TCCs specific for bacterial components that produced IL-4 but no, or little, IFN-γ induced the synthesis of detectable amounts of IgE. The demonstration that most allergen-specific helper T cells in atopic individuals are able to produce high amounts of IL-4 (and IL-5), but no IFN-γ, may explain why allergens induce production of IgE antibodies and increase eosinophils.**

The interaction between environmental allergens and the immune system is critical to the development of specific human allergy. This interaction is presumably initiated by uptake and presentation of allergen by major histocompatibility complex (MHC) class II-positive accessory cells to allergen-specific helper T lymphocytes. Several significant associations have been observed between particular MHC haplotypes and responsiveness toward different purified allergens (1, 2). Activated helper T cells then induce B lymphocytes to produce allergen-specific antibodies of the IgE class. However, in individuals genetically determined to recognize allergenic epitopes, the origin of a preferential IgE antibody response is still unclear. Earlier studies in rodents suggested that IgE production could be regulated not only by antigen-specific helper and suppressor T cells (3, 4), but also through isotype-specific factors showing affinity for IgE (the so-called IgE-binding factors) (5). More recently, a further pathway of IgE regulation, essentially based on the reciprocal activity of interleukin 4 (IL-4) and interferon γ (IFN-γ) has been disclosed in both mice (6, 7) and humans (8–11).

Therefore, we wondered whether helper T cells specific for allergens may differ from helper T cells specific for other antigens for their phenotype of cytokine secretion. To explore such a possibility, we have investigated the profile of lymphokines produced by allergen-specific human T-cell clones (TCCs) or by human TCCs specific for microbial components established from the same atopic donors. We found that TCCs specific for bacterial components can usually produce both IL-4 and IFN-γ, whereas the majority of allergen-specific TCCs are able to produce IL-4 and IL-5, but no, or little, IFN-γ.

MATERIALS AND METHODS

Reagents. Protein purified derivative (PPD) of *Mycobacterium tuberculosis* and purified tetanus toxoid were obtained from Istituto Sieroterapico e Vaccinogeno Sclavo (Siena, Italy). *Dermatophagoides pteronyssinus* extract (prepared from isolated mite bodies) was obtained from Lofarma Allergeni (Milan). Purified group I allergen of *Lolium perenne* was prepared as reported (12). Phytohemagglutinin (PHA) was purchased from GIBCO and phorbol 12-myristate 13-acetate (PMA) was from Sigma. OKT3 (anti-CD3), OKT4a (anti-CD4), OKT8 (anti-CD8), and OKM1 (anti-CD14) monoclonal antibodies (mAbs) were purchased from Ortho Pharmaceuticals and B1 (anti-CD20) was from Kontron (Zurich). IFN-γ, IL-4, and IL-5 oligonucleotide probes were purchased from Amgen Biologicals. The anti-IL-5 (TRK 5) mAb was kindly provided by R. L. Coffman (DNAX).

Patients. Peripheral blood mononuclear cells (PBMCs) were obtained from two allergic donors. The first donor was a 23-year-old man with extrinsic asthma and immediate type cutaneous hypersensitivity to *D. pteronyssinus* extract. He had been vaccinated 1 year before with tetanus toxoid and showed a 1:256 serum titer of anti-tetanus toxoid antibodies (as measured by hemagglutination inhibition assay), as well as a delayed type cutaneous hypersensitivity to tetanus toxoid. The second donor was a 22-year-old man with a history of seasonal rhinitis and immediate type cutaneous hypersensitivity to *L. perenne* and to other grass pollens. He had been vaccinated with bacillus Calmette–Guérin and showed delayed type cutaneous hypersensitivity to PPD.

Generation of Allergen-Specific TCCs. Antigen- or allergen-specific T-cell lines were obtained by a technique described in detail (13). Briefly, 0.5 × 10^6 PBMCs per ml from the two donors were stimulated with antigens (tetanus toxoid and PPD; 1 μg/ml) or allergens (*D. pteronyssinus*, 10 μg/ml; *L.*

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Abbreviations: IFN-γ, interferon γ; IL, interleukin; rIL, recombinant IL; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; PPD, protein purified derivative; TCC, T-cell clone.

perenne group I, 10 μg/ml) in RPMI 1640 medium (Seromed, Berlin) supplemented with 2 mM L-glutamine (GIBCO), 20 μM 2-mercaptoethanol, 100 units of penicillin per ml, 50 μg of streptomycin per ml (complete medium), and 5% human AB^+ serum in 24-well flat-bottomed culture plates (3524; Costar) for 5 days. Subsequently, recombinant IL-2 (rIL-2) (25 units/ml; Biogen, Geneva) was added and kept in culture for an additional 7 days. Viable T-cell blasts were then separated by a Ficoll/Hypaque density gradient and antigen or allergen specificity of T-cell lines was assessed before the cloning procedure. T-cell blasts were seeded at 0.3 cell per well in 96-well round-bottomed plates (Nunclon; Nunc) in the presence of 10^5 irradiated (5000 rads) allogeneic spleen mononuclear cells as feeder cells, 1% (vol/vol) PHA, and rIL-2 (20 units/ml) in complete medium supplemented with 10% heat-inactivated fetal calf serum (FCS; HyClone). Growing microcultures were then expanded at weekly intervals with 10^5 irradiated feeder cells and rIL-2. To expand established clones, T-cell blasts were restimulated every 3 weeks with 0.1% PHA and irradiated allogeneic feeder cells. The TCC phenotype was examined on a FACStar analyzer (Becton Dickinson) using fluorescein isothiocyanate- or phycoerythrin-conjugated anti-CD3, anti-CD4, anti-CD8 mAb. The specificity of T-cell lines or TCCs was assessed as described (13). Briefly, 2×10^4 T-cell blasts were incubated in triplicate 200-μl cultures in the presence of 10^5 irradiated (5000 rads) autologous PBMCs plus the appropriate antigen (tetanus toxoid or PPD, 0.5 μg/ml) or allergen (*D. pteronyssinus* or *L. perenne* group I, 5 μg/ml) in 96-well round-bottomed microtiter plates for 48 hr at 37°C, in a humified atmosphere of 5% CO_2/95% air. After pulsing for 16 hr with 0.5 μCi of [^{3}H]thymidine per well (1 Ci = 37 GBq) (Amersham), radionuclide uptake was measured by scintillation counting. When the stimulation index (ratio between the mean cpm obtained in triplicate cultures with autologous irradiated PBMCs plus antigen and the mean cpm obtained in triplicate cultures containing PBMCs alone) was >10, responses were considered positive.

Quantitation of IL-4, IL-5, and IFN-γ in TCC Supernatants. Viable T-cell blasts of antigen- or allergen-specific TCCs were extensively washed and incubated at 10^6 cells per ml in the presence of 10^6 autologous irradiated mononuclear cells as antigen presenting cells and appropriate antigen (tetanus toxoid and PPD; 2 μg/ml) or allergen (*D. pteronyssinus* and *L. perenne* group I; 10 μg/ml) for 72 hr in complete medium supplemented with 10% heat-inactivated FCS at 37°C. TCCs were also stimulated for 24 hr with PMA (10 ng/ml) and mAb anti-CD3 (50 ng/ml) to achieve maximal stimulation. Cultures were then centrifuged and supernatants were collected, filtered through a 0.22-μm filter, and then stored in aliquots at −70°C until used. For the measurement of IFN-γ and IL-4 in TCC supernatants, the IMRX IFN-γ RIA (Centocor, Malvern, PA) and Quantikine Immunoassay (R & D Systems, Minneapolis), respectively, were used according to the manufacturer's instructions.

For the quantitation of IL-5 in TCC supernatants, a biological assay with the murine line LyH7.B13 (kind gift of R. Palacios, Basel) was used (14). Briefly, TCC supernatants were added at different concentrations to 8×10^3 LyH7.B13 cells and cultured for 24 hr. After a 6-hr pulse with 0.5 μCi of [^{3}H]thymidine per well (Amersham), radionuclide uptake was measured by scintillation counting. A semiquantitative estimate of IL-5 content was obtained by comparing the results obtained in parallel cell cultures in the presence of known concentrations of human recombinant IL-5 (Amersham). In all experiments, LyH.B13 cells did not proliferate in the presence of human rIL-2, rIL-3, rIL-4, rIL-6, and rIFN-γ. The proliferative response induced by rIL-5 and TCC supernatants was consistently abrogated by anti-human IL-5 mAb (1 μg/ml).

Values of the cytokine content 5 SD over those of control supernatant derived from irradiated feeder cells alone were regarded as positive.

IFN-γ, IL-4, and IL-5 Cytoplasmic RNA Expression in TCCs. Cellular RNA was obtained from 20 TCCs (9 antigen- and 11 allergen-specific TCCs) stimulated with PMA plus anti-CD3 antibody according to White and Bancroft (15). Briefly, 1×10^6 T-cell blasts were washed extensively with protein-free phosphate-buffered saline (PBS) (pH 7.2) and pelleted by centrifugation (12,000 × *g*) in a sterile 1.5-ml tube (4224; Eppendorf). After resuspension in 10 mM Tris·HCl, pH 7.0/1 mM EDTA, cells were lysed by 2-fold addition of 5 μl of 5% Nonidet P-40 (Boehringer Mannheim), with mixing on ice in between additions. After nuclei were pelleted (15,000 × *g* for 15 min), 50 μl of supernatant was transferred into a sterile 1.5-ml tube containing 30 μl of 20× NaCl/Cit (1× = 0.15 M NaCl/0.015 M trisodium citrate) plus 20 μl of 37% formaldehyde (Boehringer Mannheim). The mixture was then incubated at 60°C for 15 min and stored at −70°C. For analysis, 5–20 μl of each sample was serially diluted twice with 15× NaCl/Cit in a 96-well microtiter plate and 100 μl of each dilution was applied with suction to a 4-mm-diameter spot on a nylon sheet (Hybond N+; Amersham) supported on a Whatman paper sheet with a 96-hole Biodot apparatus (Bio-Rad). Each undiluted sample was also treated with 10 units of RNase A (Boehringer Mannheim) as a control. Prehybridization of the nylon membrane; preparation by the 5′-end-labeling technique of the ^{32}P-labeled IFN-γ, IL-4, and IL-5 oligonucleotide probes (specific activity, $1–2 \times 10^8$ cpm/μg); hybridization; and autoradiography were performed as described (16).

Assay for Induction of IgE, IgG, and IgM by TCCs. Enriched B-cell suspensions were prepared from peripheral blood of the TCC donors as described (9, 10). They usually contained 50–70% B cells, 10–15% monocytes, and <1% T cells, as judged by cytofluorographic analysis with anti-CD20, anti-CD14, anti-CD3 mAb and are referred to as B cells for simplicity. The culture system used for induction of immunoglobulin synthesis was performed in duplicate tubes containing 4×10^5 B cells and 2×10^5 autologous clonal T cells in the presence of the appropriate antigen (tetanus toxoid and PPD; 2 μg/ml) or allergen (*D. pteronyssinus* and *L. perenne* group I; 10 μg/ml) in 1 ml of 10% FCS-containing medium. After 10 days, supernatants were collected and assayed for IgE, IgG, and IgM content, as described in detail elsewhere (9, 10).

RESULTS

A total of 43 *D. pteronyssinus*-specific, 18 *L. perenne* group I-specific, 28 tetanus toxoid-specific, and 10 PPD-specific TCCs were established from two atopic donors showing immediate type cutaneous hypersensitivity to *D. pteronyssinus* extract or *L. perenne* group I and delayed cutaneous hypersensitivity to tetanus toxoid or PPD, respectively. All TCCs were assessed for their ability to produce IL-4 and IFN-γ in response to stimulation with PMA plus anti-CD3 mAb. In parallel experiments, we have indeed shown that under these conditions, even in the absence of anti-CD3 cross-linkage, production of cytokines is higher than that achieved by any other T-cell stimulant (data not shown). The results of these experiments are summarized in Table 1. Twenty-four tetanus toxoid-specific TCCs, derived from the first donor, produced both IL-4 and IFN-γ, 2 produced only IL-4, and 2 produced only IFN-γ. In contrast, only 23 of 43 *D. pteronyssinus*-specific TCCs produced both IL-4 and IFN-γ, 2 produced only IFN-γ, and 18 produced only IL-4. All 10 PPD-specific TCCs derived from the second atopic donor produced IFN-γ and 9 of them produced IL-4 as well. In contrast, 16 of 18 *L. perenne* group I-specific TCCs

Table 1. Production of IL-4 and IFN-γ by allergen-specific TCCs and TCCs specific for bacterial antigens in response to stimulation with PMA plus anti-CD3 antibody

T-cell donor	Antigen specificity	No. of antigen-specific TCCs	No. of TCCs producing cytokine			Mean (± SE) cytokine content in supernatant of TCCs producing both IL-4 and IFN-γ	
			IL-4 + IFN-γ	IFN-γ	IL-4	IFN-γ, units/ml	IL-4, pg/ml
F.S.	Tetanus toxoid	28	24	2	2	139 ± 13 (NS)	3392 ± 655 (NS)
	D. pteronyssinus	43	23	2	18	109 ± 22 (NS)	4396 ± 874 (NS)
A.M.	PPD	10	9	1	0	174 ± 24*	1143 ± 424†
	L. perenne group I	18	7	2	9	78 ± 24*	5919 ± 2110†

Clonal T blasts were extensively washed, counted, and incubated for 24 hr at 37°C in the presence of PMA (10 ng/ml) plus anti-CD3 mAb (50 ng/ml) at 10^6 cells per ml. IFN-γ and IL-4 content in TCC supernatant was evaluated by commercial RIA and ELISA, respectively, as described in *Materials and Methods*. NS, not significant.
*$P < 0.005$.
†$P < 0.025$.

produced IL-4, but only 9 of them were able to produce detectable amounts of IFN-γ. On the whole, tetanus toxoid- and PPD-specific TCCs produced higher amounts of IFN-γ ($P < 0.01$ and $P < 0.025$, respectively) and lower amounts of IL-4 ($P < 0.025$ and $P < 0.1$, respectively) than *D. pteronyssinus*- and *L. perenne* group I-specific TCCs, respectively. When IL-4 and IFN-γ concentrations released by TCCs producing both lymphokines were compared, no statistically significant differences in the mean amounts of IL-4 and IFN-γ produced by *D. pteronyssinus*- and tetanus toxoid-specific TCCs were found. However, *L. perenne* group I-specific TCCs produced significantly higher amounts of IL-4 ($P < 0.025$) and significantly lower amounts of IFN-γ ($P < 0.005$) than PPD-specific TCCs (Table 1). In 3 *L. perenne* group I-specific and in 2 PPD-specific TCCs, the expression of IL-4 and IFN-γ RNA upon PMA plus anti-CD3 stimulation was also examined. As shown in Fig. 1, IL-4, but not IFN-γ, RNA was found in all 3 *L. perenne* group I-specific TCCs. In contrast, both PPD-specific TCCs expressed IFN-γ RNA, but one of them failed to express IL-4 RNA.

The ability of five tetanus toxoid-specific, five *D. pteronyssinus*-specific, four *L. perenne* group I-specific, and four PPD-specific TCCs to produce IL-4 and IFN-γ upon stimulation with the specific antigen was also investigated. In addition, the same clones were assessed for their ability to provide a helper function for IgM, IgG, and IgE synthesis in autologous B cells in the presence of tetanus toxoid, *D. pteronyssinus*, *L. perenne* group I, and PPD, respectively. All five *D. pteronyssinus*-specific TCCs produced IL-4, but not IFN-γ, in response to stimulation with *D. pteronyssinus* and showed the ability to induce IgE synthesis in autologous B cells. In contrast, all five tetanus toxoid-specific TCCs produced IFN-γ in response to antigen stimulation and three of them produced IL-4 as well. The ability to induce IgE synthesis in autologous B cells was restricted to two clones producing IL-4 and limited amounts of IFN-γ (Table 2). Under the same experimental conditions, all *D. pteronyssinus*- or tetanus toxoid-specific TCCs were able to stimulate the synthesis of both IgM and IgG in autologous B cells

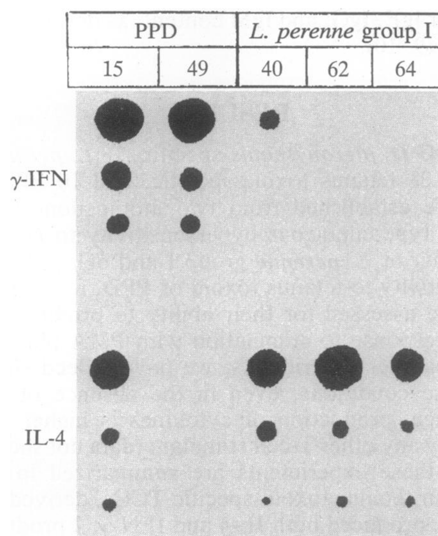

FIG. 1. Expression of IL-4 and IFN-γ cytoplasmic RNA in three *L. perenne* group I-specific and two PPD-specific TCCs stimulated with PMA plus anti-CD3 antibody. Cytoplasmic RNA was prepared from 24-hr-stimulated TCCs as described in *Materials and Methods*. The undiluted sample contained RNA derived from 10^6 T-cell blasts.

Table 2. Production of IL-4 and IFN-γ and induction of IgE in autologous B cells by allergen-specific TCCs and by TCCs specific for bacterial components in response to stimulation with specific antigen

TCC	Antigen specificity	Cytokine production*		IgE synthesis by autologous B cells,† ng/ml
		IL-4, pg/ml	IFN-γ, unit(s)/ml	
None	—	—	—	0.2
FS 47	*D. pteronyssinus*	793	2	2.3
FS 35	*D. pteronyssinus*	325	1	4.1
FS 18	*D. pteronyssinus*	436	1	3.3
FS 14	*D. pteronyssinus*	3223	2	15.1
FS 21	*D. pteronyssinus*	4125	3	9.8
FS 4	Tetanus toxoid	10	85	0.2
FS 3	Tetanus toxoid	1186	12	14.9
FS 8	Tetanus toxoid	460	10	9.4
FS 10	Tetanus toxoid	10	137	0.2
FS 17	Tetanus toxoid	1298	46	0.6
None	—	—	—	0.3
AM 41	*L. perenne* group I	2052	2	6.2
AM 15	*L. perenne* group I	6272	9	7.4
AM 65	*L. perenne* group I	4320	4	2.7
AM 57	*L. perenne* group I	5440	5	4.1
AM 29	PPD	1000	28	2.9
AM 16	PPD	19	236	0.9
AM 17	PPD	112	225	0.3
AM 50	PPD	415	240	0.3

Antigens were used at the following concentrations: *D. pteronyssinus*, 10 μg/ml; tetanus toxoid, 2 μg/ml; *L. perenne* group I, 10 μg/ml; PPD, 2 μg/ml.
*Clonal T blasts were extensively washed, counted, and incubated for 72 hr at 37°C in the presence of specific antigens at 10^6 cells per ml. IL-4 and IFN-γ content in TCC supernatant was evaluated by commercial ELISA and RIA, respectively.
†T blasts (2×10^5) were cultured in duplicate with 4×10^5 autologous B cells per ml for 10 days in the presence of specific antigens. IgE content in culture supernatant was evaluated by RIA.

irrespective of their pattern of IL-4/IFN-γ production (data not shown). Likewise, all *L. perenne* group I-specific TCCs produced IL-4, but no or limited amounts of IFN-γ, in response to the specific antigen and induced IgE synthesis by autologous B cells, whereas only 1 of 4 PPD-specific TCCs that produced IL-4 and low amounts of IFN-γ was able to induce the production of IgE (Table 2).

In 4 *D. pteronyssinus*-, 11 *L. perenne* group I-, 5 tetanus toxoid- and 4 PPD-specific TCCs the expression of cytoplasmic IL-5 RNA and secretion of IL-5 were also assessed. Fourteen of 15 allergen-specific TCCs expressed IL-5 RNA and secreted IL-5. In contrast, IL-5 RNA expression and secretion were observed in only 3 of 9 TCCs specific for bacterial antigens (Table 3). Fig. 2 shows the results obtained by testing *D. pteronyssinus*- and some tetanus toxoid-specific TCCs for IL-5 RNA expression.

DISCUSSION

Coffman and coworkers (17) have recently proposed a major subdivision of mouse helper TCCs based on differences in their pattern of cytokine production. Clones belonging to the subset designated Th1 synthesized and secreted IL-2 and IFN-γ, but not IL-4 or IL-5 upon activation, whereas clones of the Th2 subset produced IL-4 and IL-5, but not IL-2 or IFN-γ. Studies on cytokine production profiles of human TCCs derived from different lymphoid sources concluded that this classification is not valid for human T cells (10, 18). However, in certain pathological conditions, helper T cells exhibiting Th1- or Th2-like profiles of cytokine production can accumulate in target organs. For example, the majority of $CD4^+$ T cells infiltrating the thyroid gland in patients with autoimmune thyroid diseases develop *in vitro* into TCCs able to produce IFN-γ, but not IL-4 (19), whereas most T cells infiltrating the conjunctiva of patients with vernal conjunctivitis develop into TCCs producing high amounts of IL-4 but no, or limited, IFN-γ (20). Furthermore, higher proportions of IL-4-producing and lower proportions of IFN-γ-producing TCCs have been established from the peripheral blood of patients with hyper-IgE syndrome, helminthic infestations, or severe atopy than from the peripheral blood of normal individuals (21). All these TCCs were established by stimulating single T cells with PHA and, therefore, the antigens they react to remain unknown. More recently, Wierenga *et al.* (22) have reported a clear-cut dichotomy in the production of IL-4 and IFN-γ between some *D. pteronyssinus*-specific and tetanus toxoid-specific or *Candida*-specific TCCs established from the peripheral blood of two allergic donors. The results of the present study partially confirm and extend these findings. The majority of TCCs specific for bacterial antigens, such as tetanus toxoid and PPD, derived from two atopic individuals, were able to produce both IL-4 and IFN-γ, but most of them did not produce IL-5 even upon maximal stimulation with PMA plus anti-CD3 antibody. By contrast, TCCs specific for house dust mite and grass pollen allergens, derived from the same individuals, were inducible to the production of higher amounts of IL-4 and lower amounts of IFN-γ in comparison with TCCs specific for bacterial components. In addition, virtually all allergen-specific IL-4-producing TCCs, like the Th2 helper TCCs described in mice (23), also produced IL-5, whereas only a few tetanus toxoid- and PPD-specific TCCs were inducible to the production of IL-5. The compartmentalization of Th2-like cells in the allergen-specific T-cell repertoire of allergic patients may account, at least in part, for both the increased proportion of IL-4-, and the reduced proportion of IFN-γ-producing TCCs observed in patients with severe atopy (21). Obviously, it is possible that in atopics even helper T cells specific for bacterial antigens are inducible to higher IL-4 production than in nonatopics. To test this possibility, the profile of cytokine production by high numbers of TCCs specific for PPD or tetanus toxoid obtained from both atopic and nonatopic donors should be compared.

The finding that allergen-specific TCCs usually produce high amounts of IL-4 but no, or limited amounts of, IFN-γ may account for the *in vivo* production of IgE antibodies in response to allergen stimulation. On the other hand, the demonstration that the great majority of TCCs specific for bacterial components are inducible to the production of high concentrations of IFN-γ may explain why, despite the ability of the same TCCs to produce IL-4, bacterial infections usually do not result in the production of IgE antibodies. It has indeed clearly shown that IL-4 is the essential mediator for IgE synthesis, whereas IFN-γ exerts a negative regulatory role on the production of this immunoglobulin class (8, 9). Accordingly, the results of this study indicate that only allergen-specific TCCs or TCCs specific for bacterial components producing IL-4 but no, or limited amounts of, IFN-γ can induce the synthesis of IgE. However, a relationship

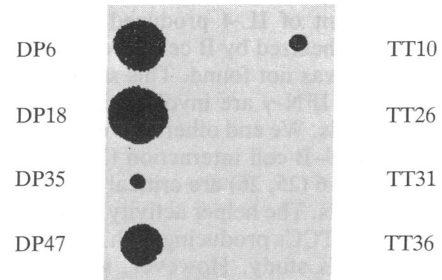

FIG. 2. Expression of IL-5 RNA in four *D. pteronyssinus* (DP)-specific and four tetanus toxoid (TT)-specific TCCs stimulated with PMA plus anti-CD3 antibody. For details, see the legend to Fig. 1 and *Materials and Methods*.

Table 3. Expression of IL-5 RNA and secretion of IL-5 by allergen-specific TCCs and TCCs specific for bacterial antigens in response to stimulation with PMA plus anti-CD3 mAb

TCC	Antigen specificity	IL-5 RNA expression	IL-5 production, units(s)/ml
AM 40	*L. perenne* group I	−	<0.5
AM 62	*L. perenne* group I	+	1.8
AM 64	*L. perenne* group I	+	23.3
AM 7	*L. perenne* group I	+	87.2
AM 23	*L. perenne* group I	+	30.0
AM 33	*L. perenne* group I	+	17.5
AM 65	*L. perenne* group I	+	15.2
AM 30	*L. perenne* group I	ND	67.2
AM 41	*L. perenne* group I	ND	37.0
AM 15	*L. perenne* group I	ND	62.6
AM 5	*L. perenne* group I	ND	41.4
FS 6	*D. pteronyssinus*	+	65.8
FS 18	*D. pteronyssinus*	+	21.6
FS 35	*D. pteronyssinus*	+	39.4
FS 47	*D. pteronyssinus*	+	33.7
AM 49	PPD	−	<0.5
AM 16	PPD	+	21.9
AM 31	PPD	−	<0.5
AM 36	PPD	−	<0.5
FS 11	Tetanus toxoid	+	21.2
FS 28	Tetanus toxoid	−	<0.5
FS 62	Tetanus toxoid	+	5.1
FS 10	Tetanus toxoid	−	<0.5
FS 26	Tetanus toxoid	−	<0.5

Cytoplasmic RNA was obtained by treatment of 10^6 T-cell blasts with Nonidet P-40 and hybridized with ^{32}P-labeled IL-5 oligonucleotide probe. Secreted IL-5 was measured in TCC supernatant by a biological assay. ND, not determined.

between the amount of IL-4 produced by TCCs and the amount of IgE synthesized by B cells even in the absence of IFN-γ production was not found. This suggests that factors other than IL-4 or IFN-γ are involved in the regulation of human IgE synthesis. We and others have indeed shown that both physical T-cell–B-cell interaction (11, 24) and production of IL-2 and IL-6 (25, 26) are critical for IL-4-dependent human IgE synthesis. The helper activity for IgE synthesis of all antigen-specific TCCs producing both IL-4 and IFN-γ was not assessed in this study. However, we have previously shown that supernatants of TCCs containing both cytokines are able to induce IgE synthesis only when the balance between their IL-4 and IFN-γ content is largely in favor of the former (9).

The reason why most allergen-specific TCCs behave as Th2-like helper cells is unclear. At present, there is no evidence to determine whether this is due to (*i*) a difference in the structure of allergen versus antigen, (*ii*) the way and route of immunization, or (*iii*) microenvironmental factors preferentially driving naive T cells to differentiate into a Th2-like pathway. It is well known that the same allergen when injected subcutaneously during specific immunotherapy prevalently elicits an IgG response (27), suggesting that the microenvironment in which the antigen is lodged may be an important factor in the type of Th cell that is activated. The assessment of the lymphokine production profile of allergen-specific TCCs before and after specific immunotherapy might help to clarify this point. The possible role of other inductive factors, such as hormones and cytokines, should also be explored. Recently, it has been shown that in mice resting T cells will produce IL-2 and IFN-γ rather than IL-4 upon primary activation by antigens. The IL-4-producing T cells in naive mice are cells that are already activated by environmental antigens and required the presence of IL-2 and IL-4 (28). It is likely that cell types other than T lymphocytes are responsible for the supply of IL-4 required by T cells to shift toward the Th2 subset after antigen recognition (29). Thus, we suggest that in atopic individuals chronically or seasonally repeated *in vivo* exposure to limited concentrations of allergens, as well as still unknown inductive microenvironmental influences, might concur to induce the preferential development of naive T cells into the IL-4- and IL-5-producing (Th2-like) helper subset. These cells are responsible both for production of IgE antibodies and for accumulation of eosinophils that characterize allergic inflammation.

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1. March, D. G., Meyers, D. A., Freidhoff, F. R., Kautzky, E. E., Roebber, M., Norman, P. S., Hsu, S. H. & Bias, W. B. (1982) *J. Exp. Med.* **155**, 1452–1463.
2. Roebber, M., Klapper, D. G., Goodfriend, L., Bias, W. B., Hsu, S. H. & March, D. G. (1985) *J. Immunol.* **134**, 3062–3069.
3. Uede, T. & Ishizaka, K. (1982) *J. Immunol.* **129**, 1391–1397.
4. Suemura, M., Shiho, O., Deguchi, H., Yamamura, Y., Bottcher, I. & Kishimoto, T. (1981) *J. Immunol.* **127**, 465–470.
5. Ishizaka, K. & Ishizaka, T. (1989) in *Fundamental Immunology*, ed. Paul, W. P. (Raven, New York), pp. 867–888.
6. Coffman, R. L. & Carty, J. (1986) *J. Immunol.* **136**, 949–954.
7. Finkelman, F. D., Katona, I. M., Urban, J. F., Snapper, C. M., Ohara, J. & Paul, W. E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9675–9678.
8. Pene, J. F., Rousset, F., Briere, F., Chretien, I., Bonnefoy, J., Spits, H., Yokota, T., Arai, N., Arai, K., Banchereau, J. & de Vries, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6880–6884.
9. Del Prete, G. F., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Ricci, M., Banchereau, J., de Vries, J. & Romagnani, S. (1988) *J. Immunol.* **141**, 4193–4198.
10. Maggi, E., Del Prete, G. F., Macchia, D., Parronchi, P., Tiri, A., Chretien, I., Ricci, M. & Romagnani, S. (1988) *Eur. J. Immunol.* **18**, 1045–1050.
11. Vercelli, D., Jabara, H. H., Arai, K. & Geha, R. S. (1989) *J. Exp. Med.* **169**, 1295–1303.
12. Ansari, A. A., Kihara, T. K. & March, D. G. (1987) *J. Immunol.* **139**, 4034–4041.
13. Lanzavecchia, A., Santini, P., Maggi, E., Del Prete, G. F., Falagiani, P., Romagnani, S. & Ferrarini, M. (1983) *Clin. Exp. Immunol.* **52**, 21–28.
14. Fattah, D., Quint, D. J., Proudfoot, A., O'Malley, R., Zanders, E. D. & Champion, B. R. (1990) *Cytokines* **2**, 112–121.
15. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572.
16. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 1, p. 7.53.
17. Mossman, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. (1986) *J. Immunol.* **136**, 2348–2357.
18. Noma, T., Nakakubo, H., Sugita, M., Kumagai, S., Maeda, M., Shimizu, A. & Honjo, T. (1989) *J. Exp. Med.* **169**, 1853–1858.
19. Del Prete, G. F., Tiri, A., Mariotti, S., Parronchi, P., Pinchera, A., Romagnani, S. & Ricci, M. (1989) *Clin. Exp. Rheum.* **7**, 41–45.
20. Maggi, E., Biswas, P., Del Prete, G. F., Parronchi, P., Macchia, D., Simonelli, C., Emmi, L., De Carli, M., Tiri, A., Ricci, M. & Romagnani, S. (1991) *J. Immunol.* **146**, 1169–1174.
21. Romagnani, S., Del Prete, G. F., Maggi, E., Parronchi, P., Tiri, A., Macchia, D., Giudizi, M. G., Almerigogna, F. & Ricci, M. (1989) *Clin. Immunol. Immunopathol.* **50**, S13–S23.
22. Wierenga, E. A., Snoek, M., de Groot, C., Chretien, I., Bos, J. D., Jansen, H. M. & Kapsenberg, M. L. (1990) *J. Immunol.* **144**, 4651–4656.
23. Cherwinski, H. M., Shumacher, J. H., Brown, K. D. & Mosmann, T. R. (1987) *J. Exp. Med.* **166**, 1229–1234.
24. Parronchi, P., Tiri, A., Macchia, D., Biswas, P., Simonelli, C., Maggi, E., Del Prete, G. F., Ricci, M. & Romagnani, S. (1990) *J. Immunol.* **144**, 2102–2108.
25. Maggi, E., Del Prete, G. F., Parronchi, P., Tiri, A., Macchia, D., Biswas, P., Simonelli, C., Ricci, M. & Romagnani, S. (1989) *Immunology* **68**, 300–306.
26. Vercelli, D., Jabara, H. H., Arai, K.-I., Yokota, T. & Geha, R. S. (1989) *Eur. J. Immunol.* **19**, 1419–1424.
27. Norman, P. S. (1980) *J. Allergy Clin. Immunol.* **65**, 87–95.
28. Swain, S. L., McKenzie, D. T., Weinberg, A. D. & Hancock, W. (1988) *J. Immunol.* **141**, 3445–3455.
29. Ben-Sasson, S. Z., Le Gros, G., Conrad, D. H., Finkelman, F. D. & Paul, W. E. (1990) *J. Immunol.* **145**, 1127–1136.