

An *in vitro* model of allergen-dependent IgE synthesis by human B lymphocytes: comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite)

R. E. O'HEHIR, V. BAL,* D. QUINT,† R. MOQBEL, A. B. KAY, E. D. ZANDERS† & J. R. LAMB*

Department of Allergy and Clinical Immunology, National Heart and Lung Institute, Brompton Hospital,

*MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, London and †Glaxo Group Research, Greenford, Middlesex

Accepted for publication 27 November 1988

SUMMARY

An allergen-dependent *in vitro* model of immunoglobulin E (IgE) synthesis by human B cells is reported. Using this model, it is demonstrated that polyclonal T cells and CD4⁺ *Dermatophagoides* spp. (house dust mite)-specific T-cell clones derived from an atopic, house dust mite (HDM)-allergic individual are able to support IgE synthesis by autologous B cells. The helper activity was interleukin-4 (IL-4) dependent as only cloned T cells expressing detectable mRNA for IL-4 were able to induce IgE synthesis without the addition of exogenous IL-4. Peripheral and cloned T cells reactive with HDM could also be identified from a non-atopic individual but neither population was able to support IgE production even in the presence of exogenous IL-4.

INTRODUCTION

Immunoglobulin E (IgE) is responsible for allergic sensitivity in atopic individuals (Ishizaka, 1984). Although it is evident that IgE synthesis is regulated in part by interleukin-4 (IL-4), which in the mouse is secreted by a particular subset of helper T cells (Howard & Paul, 1983; Coffman *et al.*, 1986; Mosmann & Coffman, 1987), basic differences between atopic and non-atopic individuals in the regulation of IgE synthesis have not been resolved. This has been hindered by the absence of suitable allergen-dependent systems for *in vitro* IgE production by autologous B cells (Leung & Geha, 1987). Much of the available information has been derived from the analysis of pathological variants associated with hyper-IgE production (Geha *et al.*, 1981; Buckley & Fiscus, 1975) or from the use of alloreactive (Lanzavecchia & Parodi, 1984; Umetsu *et al.*, 1985) and autoreactive (Leung, Young & Geha, 1986) T-cell clones. Furthermore, polyclonal B-cell activators have failed to induce IgE synthesis in peripheral blood B cells of both non-atopic and allergic donors, thereby failing to provide suitable models for the analysis of IgE regulation (Saryan, Leung & Geha, 1983; Fiser & Buckley, 1979).

In this report, an *in vitro* model of IgE synthesis using autologous B cells is described for the most common mite species inducing allergy (*Dermatophagoides* spp.; house dust mite, HDM) and, using allergen-specific polyclonal T cells and clones from an atopic and a non-atopic individual, the differences in IgE regulation are investigated.

MATERIALS AND METHODS

Antigen preparations

Lyophilized extracts of *Dermatophagoides farinae* (*D. farinae*), *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), *Parietaria judaica*, Timothy grass and mixed grass pollen were kindly provided by Pharmacia (Uppsala, Sweden).

Isolation of human T cell clones

A panel of house dust mite-specific CD4⁺ T-cell clones was isolated from the peripheral blood of an atopic individual with perennial rhinitis and a non-atopic healthy subject, using the limiting dilution cloning method described previously (O'Hehir *et al.*, 1987). For cloning, viable cells (0.3 cells/well) were resuspended in complete medium (RPMI-1640 supplemented with 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin) containing 5% screened human A⁺ serum and plated in Microtest II trays together with irradiated (2500 rads) autologous peripheral blood mononuclear cells (PBMC; 1×10^6 /ml), *D. farinae* (10^3 BU/ml) and interleukin-2 (IL-2; Lymphocult T, 10% v/v, Biotest Folex, Frankfurt, FRG). Before use in assay systems the clones were rested for 6–8 days after the last addition of filler cells and antigen.

Abbreviations: Bu, biological unit; *D. farinae*, *Dermatophagoides farinae*, IgE, immunoglobulin E; IL-4, interleukin-4; PBMC, Peripheral blood mononuclear cells; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis.

Correspondence: Dr R. E. O'Hehir, Dept. of Allergy & Clinical Immunology, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, U.K.

Proliferation assays

Cloned T cells (5×10^4 /ml) were stimulated with *D. farinae* and mixed grass pollen at different concentrations in the presence of autologous irradiated PBMC (1.25×10^5 /ml). After 60 hr incubation, tritiated methyl thymidine ($[^3\text{H}]\text{TdR}$; 1 μCi /well, Amersham International, Amersham, Bucks) was added and the culture harvested onto glass-fibre filters 8–16 hr later. Proliferation, as correlated with $[^3\text{H}]\text{TdR}$ incorporation, was measured by liquid scintillation spectroscopy. For polyclonal T-cell responses, PBMC (2.5×10^5 /ml) were stimulated with antigen for 6 days. The results are expressed as mean counts per minute (c.p.m.) for triplicate cultures.

Culture conditions for in vitro antibody production

Erythrocyte rosette negative cells (E^-) containing B cells and monocytes (<2% contaminating E^+ cells) were isolated using aminoethylisothiuronium bromide hydrobromide (AET)-treated sheep erythrocytes and centrifugation on Percoll (Pharmacia) gradients (Lamb *et al.*, 1983). Cloned T cells (2.5×10^5 /ml) or E^+ cells (1.5×10^6 /ml) were cultured with *D. farinae* or mixed grass pollen (5×10^3 BU/ml) and autologous E^- cells (2.5×10^5 /ml) in complete medium containing 10% fetal calf serum (FCS), with or without the addition of recombinant IL-4 (100 U/ml; Genzyme, Boston, MA). The supernatants were harvested at 10–12 days and the total levels of IgE and IgG determined by enzyme-linked immunosorbent assays (ELISA).

Determination of immunoglobulin synthesis

Total IgE and IgG were measured using solid-phase ELISA as described in detail elsewhere (Larche *et al.*, 1988). Briefly, for IgG, plates were coated with goat anti-human IgG (1:500; Miles Scientific, Slough, Bucks), blocked with 1% bovine serum albumin (BSA) and then incubated with the test samples. For detection of IgG, alkaline phosphatase-labelled goat anti-human IgG (1:500 dilution; Sigma, Poole, Dorset) conjugate was used and absorbance measured at 405 nm. For IgE, mouse anti-human IgE monoclonal antibody (1:500; Serotec, Kidlington, Oxon) was used to coat plates and biotinylated goat anti-human IgE (1:250; Vector, Peterborough, Cambs) was used as a second antibody followed by streptavidin biotinylated-horseradish peroxidase conjugate. Absorbance was measured at 450 nm. For both IgG and IgE assays standard curves were performed. The limits of detection were 8–1000 ng/ml and 50–12,000 pg/ml for IgG and IgE, respectively. IgG or IgM at concentrations of 50 μg /well were not detected in the IgE assay.

Dot blot analysis of T-cell clones

T-cell clones were activated on solid-phase anti-CD3 antibody (OKT3; Ortho Pharmaceutical Corp., Raritan, NJ) for 6 hr in the presence of 5% IL-2, collected, washed and resuspended in phosphate-buffered saline (PBS) containing cycloheximide (50 μg /ml) and vanadyl ribonucleoside complex (10 mM). Selective mRNA immobilization on the nylon membrane (Hybond-N, Amersham) was as described elsewhere (Bresser, Doering & Gillespie, 1983). Briefly, cells were lysed using pronase digestion followed by detergent treatment; the lysate was saturated with sodium iodide and put onto the membrane. After washing, the membrane was exposed to UV light before storage. Prehybridization and hybridization were carried out at 42° in 50% formamide and $6 \times \text{SSC}$ ($20 \times \text{SSC} = 3 \text{ M NaCl} + 0.3 \text{ M Na citrate}$). Human IL-2 cDNA (Taniguchi *et al.*, 1983) was

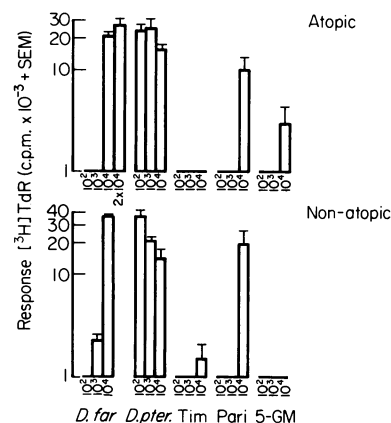


Figure 1. Antigen specificity of PBMC from an atopic and a non-atopic individual. PBMC (2.5×10^5 /ml) were cultured with a panel of soluble aeroallergens at different concentrations. Proliferation was determined by $[^3\text{H}]\text{TdR}$ incorporation after 6 days. Background responses of PBMC to medium in the absence of antigen were less than 115 c.p.m.

labelled by random priming using a DNA-labelling kit (Boehringer Mannheim, FRG), and for IL-4 two partially overlapping reverse and complementary oligonucleotides (Yokota *et al.*, 1986)

- (i) 5'-GTGCGACTGCACAGCAGTTCCACAGGCACA-3'
- (ii) 3'-GTGTCCGTGTTTCGTCGACTAGGCTAAGGAC-5'

were annealed and filled in using $^{32}\text{PdCTP}$ (Amersham) and three unlabelled dNTPs in the presence of Klenow enzyme.

Autoradiography from immunoblots of *D. farinae*

Mite body extract of *D. farinae* was separated by SDS-PAGE and transferred to nitrocellulose. The strips were probed with sera from the mite-allergic clone donor and from the non-allergic clone donor and autoradiographed to detect binding of IgE as described previously (O'Hehir *et al.*, 1987). Briefly, after blocking non-specific binding with 2% human serum albumin in Tris buffer (pH 7.5), dilutions of the sera (1:2 and 1:5) were allowed to react over the nitrocellulose sheets for 16 hr. After washing, they were incubated for a further 16 hr with ^{125}I anti-IgE (Pharmacia; 100 μl /approximately 10^4 c.p.m.). The washed, dried sheets were exposed to X-OMAT L Kodak film with Du Pont Quanta III intensifying screens at -70° for 48 hr and then developed.

RESULTS

Antigen specificity of polyclonal and monoclonal T cells isolated from an atopic and a non-atopic individual

At the polyclonal level, the response of peripheral T cells from an atopic individual with perennial rhinitis and a non-atopic donor to *D. farinae* extract was comparable (Fig. 1). T cells from both individuals proliferated in a dose-dependent manner when stimulated with soluble *D. farinae* extract. Furthermore, proliferative responses were observed to the closely related member of the same genus, *D. pteronyssinus*, and to *Parietaria judaica* compared with the medium control.

Cloned T cells derived from the same individuals (clones DE26 and DE9 from the atopic subject and clone DF1 from the

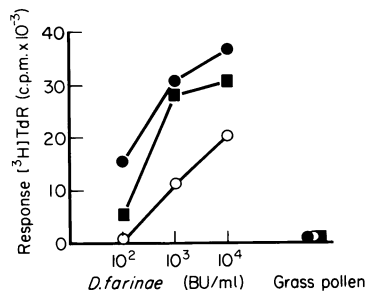


Figure 2. Cloned T cells ($5 \times 10^4/\text{ml}$) were cultured with *D. farinae* and mixed grass pollen over a concentration range in the presence of autologous, irradiated PBMC ($1.25 \times 10^5/\text{ml}$). Proliferation was determined by [^3H]TdR incorporation at 72 hr. Dose-response curves for *D. farinae* are shown; DE26 (open circles), DE9 (closed circles) and DF1 (closed squares). Only the maximum proliferative response to mixed grass pollen is plotted. Background responses to accessory cells in the absence of antigen were less than 275 c.p.m. and in all cases the percentage SEM was less than 25%.

Table 1. *D. farinae*-induced immunoglobulin synthesis by polyclonal and cloned T cells from an allergic individual

	T cells	E ⁻	Antigen	IL-4	IgE pg/ml	IgG ng/ml
(a)	DE26	+	—	—	0	48
	DE26	+	<i>D. farinae</i>	—	252	565
	DE26	+	Grass pollen	—	0	30
	DE26	+	<i>D. farinae</i>	+	682	452
(b)	DE9	+	—	—	0	0
	DE9	+	<i>D. farinae</i>	—	0	73
	DE9	+	Grass pollen	—	0	0
	DE9	+	<i>D. farinae</i>	+	595	2843
(c)	E ⁺	+	<i>D. farinae</i>	—	90	12
	—	+	<i>D. farinae</i>	—	0	0
	—	+	Grass pollen	—	0	0
	—	+	<i>D. farinae</i>	+	41	11
	E ⁺	+	<i>D. farinae</i>	+	245	1335
	E ⁺	+	—	+	40	0

T-cell clones DE26 (a) and DE9 (b) were isolated from the same atopic individual. IgE and IgG synthesis by E⁻ cells (c) cultured with IL-4 and antigen without added T cells are shown. Polyclonal T-cell help provided by E⁺ cells (c) was also determined. Background IgG and IgE synthesis by E⁻ cells cultured with antigen have been subtracted from the test groups. These were as follows: For (a), IgG ≤ 15 ng/ml, IgE ≤ 98 pg/ml; (b), IgG ≤ 15 ng/ml, IgE ≤ 90 pg/ml; (c), IgG ≤ 14 ng/ml, IgE ≤ 47 pg/ml.

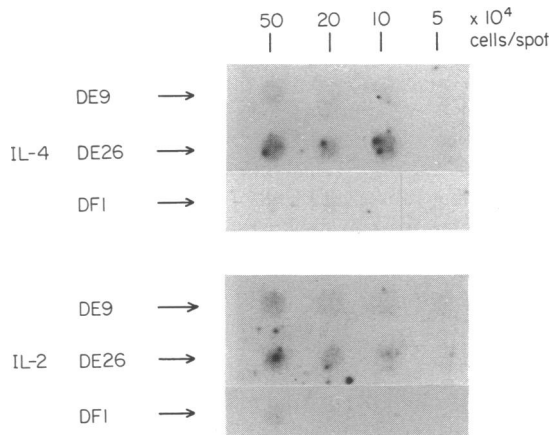


Figure 3. Dot blot analysis of mRNA for IL-2 and IL-4 in T-cell clones. Cloned T lymphocytes, activated for 6 hr with solid-phase anti-CD3, were probed using human IL-2 as cDNA and IL-4 oligonucleotides. Comparable mRNA for actin was present in all clones.

non-atopic subject) proliferated strongly in a dose-dependent fashion in response to the inducing allergen, *D. farinae*, but failed to recognize the unrelated allergen, mixed grass pollen (Fig. 2).

Dot blot analysis of mRNA for IL-2 and IL-4 in T-cell clones

The levels of IL-2- and IL-4-specific mRNA were compared for the T-cell clones DE9 and DE26 from the atopic and DF1 from the non-atopic individual 6 hr after activation with insolubilized anti-CD3 antibody (OKT3). Dot blot analysis of clones DE9 and DE26 revealed the presence of comparable levels of IL-2 transcripts (positive at 10×10^4 cell equivalents/spot; Fig. 3) whereas clone DF1 was positive at 50×10^4 cell equivalents/spot. DE26 also gave positive hybridization for IL-4 mRNA (10×10^4 cell equivalents/spot) as opposed to DE9, which was only weakly positive when mRNA from five-fold the number of cell-equivalents/spot was probed, and DF1, which had no detectable IL-4-specific mRNA.

Ability of *Dermatophagoides* spp.-specific T-cell clones to support IgE and IgG synthesis in vitro

Clone DE26 isolated from the atopic subject with detectable mRNA levels for IL-4 was able to collaborate with autologous E⁻ cells in the presence of the specific *D. farinae* allergen to support IgE synthesis. This was enhanced three-fold by the addition of exogenous IL-4 at the initiation of cultures (Table 1a). Clone DE9 with barely detectable IL-4 transcripts helped IgE synthesis only when exogenous IL-4 was added to the cultures (Table 1b). Similarly, allergen-dependent IgG synthesis mediated by T-cell clones DE26 and DE9 was also enhanced in the presence of exogenous IL-4. The failure of the autologous E⁻ cells cultured with antigen and IL-4 to stimulate marked IgE synthesis demonstrated the requirement for T cells in the system (Table 1c). The presence of antigen was required since E⁺ and E⁻ cells co-cultured with IL-4 were unable to induce IgE synthesis. Peripheral T cells (E⁺) from the atopic donor when cultured with autologous E⁻ cells and *D. farinae* induced the synthesis of IgE that was enhanced by the addition of IL-4 (Table 1c).

Cloned CD4⁺ T cells (DF1) from the non-atopic donor, which had no detectable mRNA for IL-4 (Fig. 3), were unable to induce IgE synthesis even in the presence of exogenous IL-4 (Table 2a), although able to support IgG secretion. Total IgG synthesis was also enhanced by the additional IL-4 added to the cultures. Similarly, stimulation of E⁻ cells from the non-atopic individual with allergen and peripheral T cells failed to induce

Table 2. *D. farinae*-induced immunoglobulin synthesis by polyclonal and cloned T cells from a non-atopic individual

	T cells	E ⁻	Antigen	IL-4	IgE pg/ml	IgG ng/ml
(a)	DF1	+	—	—	0	0
	DF1	+	<i>D. farinae</i>	—	0	290
	DF1	+	Grass pollen	—	0	0
	DF1	+	<i>D. farinae</i>	+	0	420
(b)	E ⁺	+	—	—	0	0
	E ⁺	+	<i>D. farinae</i>	—	0	122
	—	+	<i>D. farinae</i>	+	20	190
	E ⁺	+	—	+	40	200
	E ⁺	+	<i>D. farinae</i>	+	55	360
(c)	E ⁺ allo	+	—	—	93	26
	E ⁺ allo	+	—	+	575	2385

T-cell clone DF1 (A) was isolated from a healthy, non-atopic individual. IgE and IgG synthesis by E⁻ cells cultured with IL-4 and antigen without added T cells is shown (b). Polyclonal T cell help provided by E⁺ cells (b) and cloned allogeneic E⁺ cells (c) are shown. Background immunoglobulin synthesis by E⁻ cells cultured with antigen have been subtracted from the test groups. These were as follows: For (a), IgG ≤ 70 ng/ml, IgE ≤ 190 pg/ml; for (b) and (c), IgG ≤ 15 ng/ml, IgE ≤ 94 pg/ml.

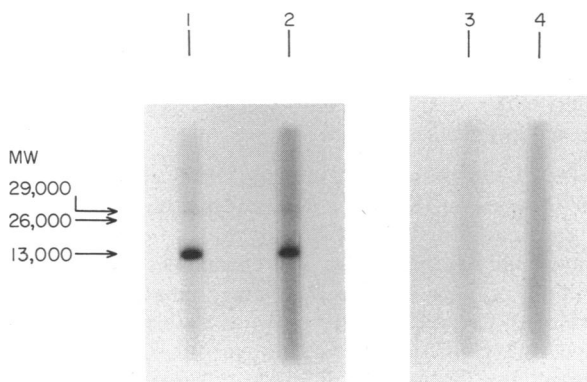


Figure 4. Autoradiographs of Western blots showing the IgE response of sera to *D. farinae*. Mite body extract of *D. farinae* was separated by SDS-PAGE and transferred to nitrocellulose. The strips were probed with sera from the mite allergic clone donor (1:2 dilution, lane 1; 1:5 dilution, lane 2) and from the non-atopic clone donor (1:2 dilution, lane 3; 1:5 dilution, lane 4) and autoradiographed to detect binding of IgE.

IgE secretion, although IgG was produced, albeit at low amounts (Table 2b). The addition of exogenous IL-4 to these cultures did not markedly enhance IgE synthesis above that observed in the controls (Table 2b).

The stimulation of B-cell enriched populations from the non-atopic subject with cloned allogeneic T cells confirmed their capacity for the synthesis of IgE (Table 2c). This response was strongly enhanced in the presence of exogenous IL-4 for both IgE and IgG synthesis.

Reactivity pattern of specific IgE to immunoblots of *D. farinae*

Autoradiographs using serum from the allergic donor of clones DE9 and DE26 showed strong IgE binding to the *D. farinae* immunoblots (Fig. 4). A distinct band was evident at 13,000 MW, which corresponds to the *Der f* II allergen of *D. farinae* that has recently been recognized as a major allergen. Fainter bands were also evident at 26,000 and 29,000 MW, corresponding to the *Der f* I and *Der f* III allergens.

The autoradiographs using serum from the non-atopic clone donor showed no evidence of specific IgE directed at any of the determinants of *D. farinae*, consistent with the lack of allergic symptoms, negative prick skin tests and negative radioallergosorbent testing (RAST; data not shown).

DISCUSSION

The inability to isolate antigen-specific T cells combined with the absence of an allergen-dependent *in vitro* model of antibody synthesis has restricted the investigation of differences in T-cell regulation of IgE induction in atopic and non-atopic individuals. In this report it is demonstrated that the T-cell repertoires of an atopic and a normal subject both include cells capable of recognizing house dust mite allergen, and that HDM-specific T-cell clones can be isolated from each donor. Furthermore, differences in the functional activity of these T-cell clones and polyclonal E⁺ cells were examined in an *in vitro* model of allergen-dependent IgE synthesis.

Analysis of murine T-cell clones suggests that there are two non-overlapping subsets of CD4⁺ T cells (Th1 and Th2) that differ in their lymphokine production and functional activities (Mosmann & Coffman, 1987). The Th2 cells, which produce IL-4 but not IL-2 or IFN- γ , are potent helpers for B cell IgE synthesis (Howard & Paul, 1983; Coffman *et al.*, 1986). Therefore, the HDM-specific T-cell clones described here were analysed for their ability both to produce IL-4, as determined by dot blot analysis for mRNA, and to support IgE synthesis. The T-cell clones from the atopic individual differed markedly in their levels of IL-4 transcripts, whereas no mRNA for IL-4 could be detected in the cloned T cells (DF1) derived from the non-atopic subject. In contrast, all the clones had detectable levels of IL-2 transcripts. Thus, the dichotomy of lymphokine production observed for murine T-cell clones appears not to apply so broadly to human T-cell clones. From the heterogeneity of T cells as regards their ability to secrete lymphokines (Del Prete *et al.*, 1988), caution must be exercised in extrapolating from the analysis of these clones to the intact T-cell repertoire of the individuals tested in this study. However, the functional characteristics observed at the polyclonal T-cell level would support these observations.

Investigation of the functional activity of the HDM-specific T-cell clones revealed that clone DE26, with marked IL-4 mRNA levels when co-cultured with autologous E⁻ cells, and *D. farinae* induced IgE synthesis that was enhanced by the addition of exogenous IL-4. In contrast, clone DE9, also isolated from the atopic individual but with minimal levels of IL-4 transcripts, supported IgE synthesis only in the presence of exogenous IL-4. The T-cell dependence of IgE induction was demonstrated by the failure of E⁻ cells to synthesize IgE when cultured with only IL-4 and *D. farinae*. Similarly, the necessity for antigen is demonstrated as E⁺ and E⁻ cells co-cultured with IL-4 alone failed to induce IgE synthesis, and this suggests that

antigen rather than IL-4 activates the T cells to provide B-cell help. Further support for this is that the T-cell clones are unresponsive to IL-4 stimulation (data not shown). The observation that peripheral E⁺ cells from the atopic individual were capable of supporting allergen-dependent IgE synthesis implies that the T-cell clones were not unique cells but representative of those in the polyclonal population. The relatively low levels of IgE induced by polyclonal T cells reflects the low numbers of HDM-immune T cells circulating in peripheral blood (Halvorsen, Bosnes & Thorsby, 1986). These findings are consistent with the concept that B cells from atopic individuals are able to synthesize IgE when the appropriate T-cell help is provided.

In contrast, both polyclonal and cloned CD4⁺ T cells reactive with HDM from the non-allergic subject were unable to induce allergen-dependent IgE synthesis even following the addition of exogenous IL-4. The presence and absence of *D. farinae*-specific IgE in the sera of the atopic and non-atopic individuals, respectively, was confirmed by immunoblotting and autoradiography and suggests that the *in vitro* findings reflect the *in vivo* status of these subjects. The stimulation of B cells from the non-atopic donor with cloned allogeneic T cells confirmed their capacity to synthesize IgE. It is well documented that non-atopic individuals are capable of generating an IgE response following parasitic infection (Nutman *et al.*, 1985) and allostimulation (Umetsu *et al.*, 1985). The failure of clone DF1, or of the peripheral E⁺ cells from the non-atopic subject, to induce IgE synthesis cannot be explained solely by its inability to secrete IL-4 for the addition of exogenous IL-4 did not overcome the defect. Additional regulatory mechanisms may be required (Maggi *et al.*, 1988) or there may be a defect in the IL-4 receptor. It has been suggested that the inability of T cells lacking IL-4 to support IgE synthesis is partly due to the production of IFN- γ (Mosmann & Coffman, 1987; Coffman & Carty, 1986; Snapper & Paul, 1987). Nevertheless, in the presence of anti-IFN- γ antibody and IL-4, clone DF1 was unable to provide appropriate help although marked enhancement of IgE synthesis was observed under similar culture conditions with the clone DE9 (data not shown). Furthermore, the observation that cloned CD4⁺ HDM-specific T cells from the non-atopic individual fail to support IgE synthesis contradicts the hypothesis that failure of IgE production in non-allergic subjects results solely from inhibition by regulatory (CD8⁺ suppressor) T cells. Nevertheless, it must be emphasized that population studies are required to resolve differences unequivocally in the regulatory mechanisms of both atopic and non-atopic individuals in their responses to allergen.

The absence of allergen-triggered IgE in the non-atopic system may result from the absence of specific B cells which can act as highly efficient antigen-presenting cells concentrating antigen via Ig receptors (Lanzavecchia, 1985) which are already clonally expanded in the atopic individual. Alternatively, the failure to synthesize IgE could be due to a defect in isotype switching (Cebra, Komisar & Schweitzer, 1984; Radbruch *et al.*, 1986; Gearhart, Sigal & Klinman, 1975) in the non-atopic subject. However, the induction of IgE synthesis by allostimulation makes this explanation unlikely. Interestingly, both subjects were able to synthesize IgG in response to *D. farinae* stimulation.

If the T-cell clones studied here recognize different determinants within the allergen and the T-cell recognition of a particular epitope within a protein can influence immune

function (Adorini *et al.*, 1977) then this could also offer an explanation for the inability of DF1 to induce IgE synthesis. This is supported by the observation that only the peripheral T cells of HDM allergic subjects respond to affinity-purified *Der p* I allergen (Rawle *et al.*, 1984). Furthermore, as T-cell antigen recognition depends upon the binding of peptide antigen to MHC proteins, the haplotype of the individuals will influence the epitopes recognized and the immune response induced (Rothbard *et al.*, 1988). Indeed, this is supported by the observation that immune responsiveness to *Amb a* VI in allergic individuals, as assessed by the presence of specific IgE in the serum, is HLA-DR5 associated (Marsh *et al.*, 1987).

The experimental model described here has allowed analysis of qualitative differences in helper activity for allergen-dependent *in vitro* IgE synthesis mediated by *Dermatophagoides* spp.-specific polyclonal and monoclonal T cells derived from an atopic and a non-atopic individual. Knowledge of mechanisms involved in such IgE regulation is required to facilitate the development of effective therapeutic regimens for allergic diseases.

ACKNOWLEDGMENTS

Dr Robyn O'Hehir is the recipient of a Sir Robert Menzies Medical Scholarship. This work was supported by the Menzies Trust and the Clinical Research Committee of the National Heart and Chest Hospitals, London. The authors are grateful to Drs I. Goldsmith and T. Taniguchi for the gifts of the cDNA probes and to the Department of Microbiology, National Heart & Lung Institute, Brompton Hospital, London, for the generous use of facilities. We thank Drs M. D. Chapman, A. Cooke, R. Lechler and M. Owen for helpful scientific discussion.

REFERENCES

- ADORINI L., HARVEY M.A., HILLER A. & SERCARZ E.E. (1979) The fine specificity of regulatory T cells. II. Suppressor and helper T cells are induced by different regions of the hen egg-white lysozyme (HEL) in a genetically non-responder mouse strain. *J. exp. Med.* **150**, 293.
- BRESSER J., DOERING J. & GILLESPIE D. (1983) Laboratory Methods. Quick blot: selective mRNA or DNA immobilisation from whole cells. *DNA*, **2**, 243.
- BUCKLEY R.H. & FISCUS S.A. (1975) Serum IgD and IgE concentration in immunodeficiency diseases. *J. clin. Invest.* **55**, 157.
- CEBRA J., KOMISAR J. & SCHWEITZER P. (1984) C μ isotype 'switching' during normal B lymphocyte development. *Ann. Rev. Immunol.* **2**, 493.
- COFFMAN R.L. & CARTY J. (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J. Immunol.* **136**, 949.
- COFFMAN R.L., O'HARA J., BOND M.W., CARTY J., ZLOTNICK E. & PAUL W.E. (1986) B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* **136**, 4538.
- DEL PRETE G., MAGGI E., PARRONCHI P., CHRETIEN I., TIRI A., MACCHIA D., RICCI M., BANCHEREAU J., DE VRIES J. & ROMAGNANI S. (1988) IL-4 is an essential factor for the IgE synthesis induced *in vitro* by human T cell clones and their supernatants. *J. Immunol.* **140**, 4193.
- FISER P.M. & BUCKLEY R.H. (1979) Human IgE biosynthesis *in vitro*: studies with atopic and normal blood mononuclear cells and subpopulations. *J. Immunol.* **123**, 1788.
- GEARHART P.J., SIGAL N.H. & KLINMAN N.R. (1975) Production of antibodies of identical idiotype but diverse immunoglobulin classes by cells derived from a single stimulated B cell. *Proc. natl. Acad. Sci. U.S.A.* **72**, 1707.

- GEHA R.S., REINHERZ E.L., LEUNG D., MCKEE K.T., SCHLOSSMAN S.F. & ROSEN F.S. (1981) Deficiency of suppressor T cells in the hyper-immunoglobulinaemia E syndrome. *J. clin. Invest.* **68**, 783.
- HALVORSEN R., BOSNES V. & THORSBY E. (1986) T cell responses to a *Dermatophagoides farinae* allergen preparation in allergics and healthy controls. *Int. Arch. Allergy Appl. Immunol.* **80**, 62.
- HOWARD M. & PAUL W.E. (1983) Regulation of B cell growth and differentiation by soluble factors. *Ann. Rev. Immunol.* **1**, 307.
- ISHIZAKA K. (1984) Regulation of IgE synthesis. *Ann. Rev. Immunol.* **2**, 159.
- LAMB J.R., SKIDMORE B.J., GREEN N., CHILLER J. & FELDMANN M. (1983) Induction of tolerance in influenza-virus immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. *J. exp. Med.* **157**, 1434.
- LANZAVECCHIA A. (1985) Antigen-specific interaction between T and B cells. *Nature (Lond.)*, **314**, 537.
- LANZAVECCHIA A. & PARODI B. (1984) *In vitro* stimulation of IgE production at a single precursor level by human alloreactive T helper clones. *Clin. exp. Immunol.* **55**, 197.
- LARCHE M., LAMB J.R., O'HEHIR R.E., IMAMI-SHITA N., ZANDERS E.D., QUINT D., MOQBEL R. & RITTER M.A. (1988) Functional evidence for a monoclonal antibody that binds to the human interleukin-4 receptor. *Immunology*, **65**, 617.
- LEUNG D.Y.M. & GEHA R.S. (1987) Regulation of the human IgE antibody response. *Intern. Rev. Immunol.* **2**, 75.
- LEUNG D.Y.M., YOUNG M.C. & GEHA R.S. (1986) Induction of IgG1 and IgE synthesis in normal B cells by autoreactive T cell clones. *J. Immunol.* **136**, 2851.
- MAGGI E., DEL PRETE G.F., TIRI A., MACCHIA D., PARRONCHI P., RICCI M. & ROMAGNANI S. (1988) T cell clones providing helper function for IgE synthesis release soluble factor(s) that induce IgE production in human B cells: possible role for interleukin 4 (IL-4). *Clin. exp. Immunol.* **73**, 57.
- MARSH D.G., FREIDHOFF L.R., ERHLICHKATZKY E., BIAS W.B. & ROEBBER M. (1987) Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb A VI* (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics*, **26**, 230.
- MOSMANN T.R. & COFFMAN R.L. (1987) Two types of mouse helper T-cell clone; implications for immune regulation. *Immunol. Today*, **8**, 223.
- NUTMAN T.B., VOLKMAN D.J., HUSSAIN R., FAUCI A.S. & OTTESEN E.A. (1985) Filarial parasite-specific T cell lines: Induction of IgE synthesis. *J. Immunol.* **134**, 1178.
- O'HEHIR R.E., YOUNG D.B., KAY A.B. & LAMB J.R. (1987) Cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite): a comparison of T- and B-cell antigen recognition. *Immunology*, **62**, 635.
- RADBRUCH A., BURGER C., KLEIN S. & MULLER W. (1986) Control of immunoglobulin class switch recombination. *Immunol. Rev.* **89**, 69.
- RAWLE F.C., MITCHELL E.B. & PLATTS-MILLS T.A.E. (1984) T cell responses to the major allergen from the house dust mite, *Dermatophagoides pteronyssinus*, antigen PI: comparison of patients with asthma, atopic dermatitis and perennial rhinitis. *J. Immunol.* **133**, 195.
- ROTHBARD J.B., LECHLER R.I., HOWLAND K., BAL V., ECKELS D.D., SEKALY R., LONG E.O., TAYLOR W.R. & LAMB J.R. (1988) Structural model of HLA-DR1 restricted T cell antigen recognition. *Cell*, **52**, 515.
- SARYAN J.A., LEUNG D.Y.M. & GEHA R.S. (1983) Induction of human IgE synthesis by a factor derived from T cells of patients with hyper IgE states. *J. Immunol.* **130**, 242.
- SNAPPER C.M. & PAUL W.E. (1987) B cell stimulatory factor-1 (interleukin-4) prepares resting murine B cells to secrete IgG1 upon subsequent stimulation with bacterial lipopolysaccharide. *Science*, **236**, 944.
- TANIGUCHI T., MATSUI H., FUJITA T., TAKAOKA C., KASHIMA H., YOSHIMOTO R. & HAMURO J. (1983) Structure and expression of a cloned cDNA for human interleukin-2. *Nature (Lond.)*, **302**, 305.
- UMETSU D.T., LEUNG D.Y.M., SIRAGANIAN R., JABARA H.H. & GEHA R.H. (1985) Differential requirements of B cells from normal and allergic subjects for the induction IgE synthesis by an alloreactive T cell clone. *J. exp. Med.* **162**, 202.
- YOKOTA R., OTSUKA T., MOSMANN T., BANCHEREAU J., DEFANCE T., BLANCHARD D., DEVRIES J.E., LEE F. & ARAI K. (1986) Isolation and characterisation of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell stimulating activities. *Proc. natl. Sci. Acad. U.S.A.* **83**, 5894.