

Regulatory mechanisms of *in vitro* human IgE synthesis*

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The IgE antibody system is among the most sophisticated of the known immune defense mechanisms. Although its specific function is still unknown, this system probably protects the host against offending agents encountered via the respiratory and gastrointestinal tracts. Unlike other immunoglobulin classes, IgE molecules bind specifically and avidly to receptors localized on the surface of tissue mast cells and circulating basophils. These cells are capable of producing and releasing several potent mediator molecules. The interaction of the antigen with a small number of IgE molecules is already sufficient to initiate a protective reaction for which the system was designed. However, because of its unusual amplification power, the IgE antibody system is highly susceptible to perturbations resulting in overproduction of IgE which determines the appearance of allergic manifestations.

The highest IgE levels (excluding IgE myelomas) have been found in patients with hyper-IgE (Buckley's) syndrome, where cellular immune defects coexist with susceptibility to *Staphylococcus aureus* infections (1). Very high IgE serum levels are characteristic of patients infected by parasites, which interestingly induce the production of a large excess of parasite-specific and -nonspecific IgE. Also primary im-

mune deficiencies of the T cell system, such as Di George's (9), Nezelof (3), and Wiskott-Aldrich (16) syndromes, or secondary immune deficiencies, such as Hodgkin's disease (11), AIDS (8) and bone marrow transplant patients during the acute phase of GVHD (10), frequently show abnormally high serum levels of IgE. The human condition most commonly associated with increased IgE antibody production is atopy, the familial allergic disorder of immediate-type hypersensitivity to allergens.

For these reasons, knowledge of the mechanisms regulating the IgE antibody response is of primary importance for understanding the possible alterations responsible for IgE-mediated allergic disorders in humans.

T cell clone-induced IgE synthesis

The first unambiguous demonstration of *in vitro*-induced IgE synthesis in normal B cells was provided by the use of alloreactive human T cell clones (TCC) (14). The alloreactive TCC we used was able to induce proliferation of B cells derived from the original donor of the stimulating cells, with the production of considerable amounts of all immunoglobulin classes, including IgE. In addition, this clone induced IgE synthesis even in B cell cultures from other normal or atopic individuals, provided that they shared the appropriate alloantigen (14). Successful induction of IgE synthesis *in vitro* by the use of selected autoreactive T cell clones has

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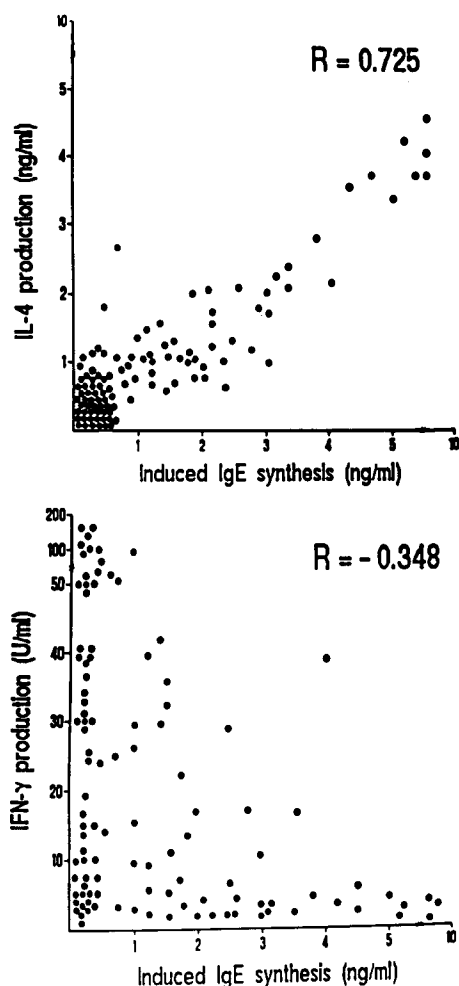


Fig. 1. Ability of TCC to induce IgE synthesis vs their ability to produce IL-4 or IFN- γ . Each clone (1×10^6 /ml) was cultured for 24 h with PHA (1% vol/vol) and its SUP assayed for content in IL-4 by an ELISA and in IFN- γ by a calibrated RIA. T cell blasts (0.4×10^6 /ml) were cultured for 10 days with B cells from 2 different donors and SUP from these cultures was assayed for IgE content. Mean IgE values of duplicate determinations performed on B cell cultures were plotted against mean values of duplicate IL-4 determinations (upper panel) and mean values of duplicate IFN- γ amounts (lower panel).

also been reported (15). Unfortunately, such *in vitro* models, although very elegant, can be applied only to a limited number of B cell donors. To overcome these difficulties, more recently we assessed the ability to induce IgE synthesis *in vitro* of TCC obtained by stimulation of single T cells with phytohemagglutinin (PHA), fol-

lowed by repeated addition of interleukin 2 (IL-2). To date, the activity of a total of 643 TCC so obtained from the peripheral blood or tonsils of 12 nonatopic donors has been evaluated. Following activation with PHA, 117 (18%) TCC were found to provide helper function for IgE - and for IgG and IgM - synthesis in B cells from all donors tested (4, 12). Furthermore, stimulation of clonal T cells with anti-CD3 (OKT3) monoclonal antibody (mAb) developed a helper activity for IgE synthesis comparable to that displayed by the same TCC following activation with PHA (12). From these data, we concluded that the helper function of these clones on IgE synthesis was mediated by a direct activation of the CD3 molecular complex. Thus, by developing an *in vitro* system of IgE production which by-passes both antigen specificity and MHC-antigen restriction, we achieved a model suitable for extensive investigations on IgE regulatory mechanisms in humans.

IL-4 is the essential mediator for the in vitro IgE synthesis

After we were successful in establishing TCC providing helper function for IgE synthesis, attempts were made to generate clonal soluble factor(s) able to induce or enhance IgE production. Upon stimulation with PHA for 24 h, supernatants (SUPs) of TCC derived from normal individuals and selected for their IgE helper function (TCC SUPs), were found to induce IgE synthesis in both normal and atopic B cells showing low or undetectable spontaneous IgE production *in vitro*. In contrast, TCC SUPs derived from PHA-stimulated TCC unable to provide helper function for IgE synthesis consistently failed to elicit or enhance production of IgE (7). Partial physicochemical characterization of the factor(s) in TCC SUPs providing helper function for IgE showed that it apparently had a mw between 10 and 50 kD and did not bind to immobilized IgE. Interestingly, the IgE helper activity of TCC SUPs was strongly inhibited by the addition to B cell cultures of human recombinant interferon-gamma (IFN- γ) (7).

It has recently been shown that interleukin 4 (IL-4), a pleiotropic lymphokine previously described as B cell stimulatory factor 1 (BSF-1), is a strong stimulant of IgG1 and IgE secretion by mouse B cells treated with lipopolysaccharide (LPS) (3). In this system IFN- γ induces LPS-stimulated B cells to develop into IgG2a-secreting cells, and is able to inhibit the switching to IgG1 and IgE induced by IL-4. The recent availability of human recombinant IL-4, and the use of an appropriate immunoenzymatic assay to measure it in biological fluids, allowed us to test the possibility that such a lymphokine was indeed, at least in part, responsible for the IgE helper activity of our TCC SUPs. To do this, the ability to induce IgE synthesis *in vitro* in human B cells of 109 CD4 TCC was compared to the ability of produce IL-2, IL-4 and IFN- γ in the SUPs following 24-h stimulation with PHA. A significant positive correlation ($r = 0.725$) was found between the ability of TCC to induce IgE synthesis and the ability to release IL-4. In contrast, there was an inverse relationship ($r = -0.348$) between the IgE helper activity of TCC and the ability to release IFN- γ . In contrast, no statistical correlation between the ability to induce IgE synthesis and to produce IL-2 was observed (5).

The ability to induce IgE synthesis in B cells of SUPs from 71 of these CD4 TCC was also investigated. Twenty-nine SUPs (all derived

from TCC active in IgE synthesis) induced production of substantial amounts of IgE in target B cells. There was a correlation between the amount of IgE synthesized by B cells in response to these SUPs and their IL-4 content. An even higher correlation ($r = 0.94$) was found between the IgE synthesis induced by these SUPs and the ratio between the amount of IL-4 and IFN- γ present in the same SUPs (Fig. 1) (5).

Like IL-4-containing SUPs, recombinant IL-4 (rIL-4) was also found to be able to induce IgE production in B cells from both atopic and nonatopic donors (5). More importantly, the addition to B cell cultures of anti-IL-4 antibody virtually abolished not only the IgE synthesis induced by rIL-4, but also that stimulated by active TCC and their SUPs (5). In contrast, the IgG synthesis induced by TCC SUPs was not – or was only slightly – inhibited by the presence in culture of anti-IL-4 antibody (5). Taken together, these data indicate that, in humans as in mice, IL-4 and IFN- γ play an important and reciprocal role in the regulation of IgE synthesis.

Role of T cells, IL-2 and IL-6 in IL-4 induced in vitro IgE synthesis

The main information coming from our work in the human system is consistent with that de-

Table 1

Requirement of untreated or mitomycin C-treated autologous T cells for IL-4-dependent IgE synthesis by tonsillar B cells

Cells added in culture	Induced IgE synthesis (ng/ml)*			
	Exp. 1		Exp. 2	
	Medium	IL-4	Medium	IL-4
Nil	< 0.3	< 0.3	< 0.3	< 0.3
Autologous untreated T cells (3.2×10^4)	< 0.3	2.6	< 0.3	3.9
Autologous untreated T cells (12×10^4)	< 0.3	4.6	< 0.3	6.2
Autologous untreated T cells (50×10^4)	< 0.3	7.9	< 0.3	8.2
Autologous untreated T cells (200×10^4)	0.4	3.7	< 0.3	2.1
Autologous Mitomycin C-treated T cells (3.2×10^4)	< 0.3	0.8	< 0.3	1.5
Autologous Mitomycin C-treated T cells (12×10^4)	< 0.3	2.4	< 0.3	3.4
Autologous Mitomycin C-treated T cells (50×10^4)	< 0.3	11.8	0.5	6.9
Autologous Mitomycin C-treated T cells (200×10^4)	< 0.3	12.5	0.4	10.3

* Highly purified B cells (4×10^4) were cultured alone or in the presence of different T cell concentrations and an optimal dose of IL-4 (200 U/ml).

Table 2

T-B cell contact is required for the IL-4-dependent IgE synthesis *in vitro*

Culture conditions in double chamber*		Induced IgE synthesis (ng/ml)**	
External chamber	Internal chamber	Exp. 1	Exp. 2
B cells	IL-4	< 0.3	0.9
B cells + IL-4	T cells	0.4	0.8
T cells + IL-4	B cells	< 0.3	0.9
B cells + T cells	IL-4	6.5	11.8

* B cells (4×10^5 /ml) were cultured in the presence of IL-4 (200 U/ml) and of autologous T cells (0.4×10^6 /ml) as described in the table.

** After 10 days supernatants from both compartments of the same well were pooled and assayed for IgE content.

rived from the murine model. However, at least two substantial differences seem to exist between humans and mice. The first important difference between the mouse and human systems arose when we asked whether other cell types besides B cells were required in showing that rIL-4, which was active on unfractionated tonsillar mononuclear cells, was consistently unable to induce IgE synthesis in highly purified B cells from the same tonsils (5). On the other hand, readdition of appropriate concentrations of both untreated and mitomycin C-treated autologous T cells enabled tonsillar B cells to synthesize IgE in response to rIL-4 (Table 1). Taken together, our results suggest that the presence in culture of T cells is necessary for IL-4-mediated IgE synthesis in human B cells.

At least two possible explanations may be provided for these findings.

The first possibility, which is now being investigated in our laboratory, is that a T-B cell-to-cell contact is necessary for IL-4-induced IgE synthesis. mAbs specific for several surface antigens of T and B cells (anti-CD4, anti-CD20, anti-ClassII, anti-CD23, etc.) were able to inhibit IgE synthesis induced by IL-4 in a dose-related fashion (data not shown). In addition, when cultures were performed with T and B cells separated by a membrane filter, no pro-

duction of IgE antibody was seen (Table 2) when IL-4 was added to the chamber containing either T cells or B cells. Furthermore, virtually all TCC, without any previous activation, were able to induce high levels of IgE synthesis in the presence of IL-4, despite their own recognition (Table 3). These data suggest that T-B cell contact plays a crucial role in the induction of IgE synthesis *in vitro*, even though the mechanism of cell-to-cell recognition has yet to be elucidated. Lastly, sequential addition of T cells and IL-4 in culture has shown that T cells are needed in the first hours of the culture, whereas the maximum activation of IgE synthesis was obtained when IL-4 was added on day 4 (data not shown), confirming that this molecule is active after T-B cell contact.

Another possibility is that T cell derived lymphokines other than IL-4 are also involved in the induction of IgE synthesis. To explore this possibility we tested the effect on IgE synthesis stimulated by TCC SUPs of the addition to B cell cultures of specific antibodies to different interleukins, such as IL-2 and IL-6. We found that the addition in culture of anti-IL-2 antibody consistently exerted a strong inhibitory effect on the IL-4-induced T cell-dependent IgE synthesis, whereas it had no effect on the B cell proliferation induced by IL-4 in the co-stim-

Table 3

IL-4-dependent IgE synthesis is restored by randomly selected T cell clones regardless of the specificity

T cell clones added to the cultures	Induced IgE synthesis (ng/ml)*	
	Medium	IL-4
Nil	< 0.3	< 0.3
CO 3	< 0.3	11.9
CO 4	< 0.3	21.8
CO 12	< 0.3	8.9
CO 15	< 0.3	13.8
CO 17	0.9	15.9
CO 21	1.2	22.4
CO 22	< 0.3	14.9
CO 26	< 0.3	21.5
CO 28	< 0.3	11.7
Heterogeneous allogeneic T cells	< 0.3	2.4

* B cell (4×10^5) were cultured in the presence of allogeneic randomly selected TCC or heterogeneous T cells (derived from the same subject) and IL-4 (200 U/ml).

ulatory assay with anti-IgM antibody (Fig. 2). These data suggest that some IL-2 is produced in culture even by mitomycin C-treated T cells and, in concert with IL-4, it plays an important role in the differentiation of B cells into IgE-secreting cells. In addition, even though less consistently, anti-IL-6 antibody was also inhibitory in IL-4-induced IgE synthesis (data not shown). Interestingly, purified human IL-6 was able to potentiate the spontaneous IgE synthesis by B cells from a number of atopic subjects (Table 4). The latter observations suggest that at least some B cells in advanced stage of differentiation, which have probably already switched to IgE synthesis *in vivo*, may be directly induced by IL-6 to secrete detectable amounts of IgE *in vitro*. Obviously, two such possibilities (T-B cell contact and additional signals mediated by lymphokines such as IL-2 and IL-6) are not mutually exclusive.

Another main difference between the mouse and the human IgE systems is that in humans

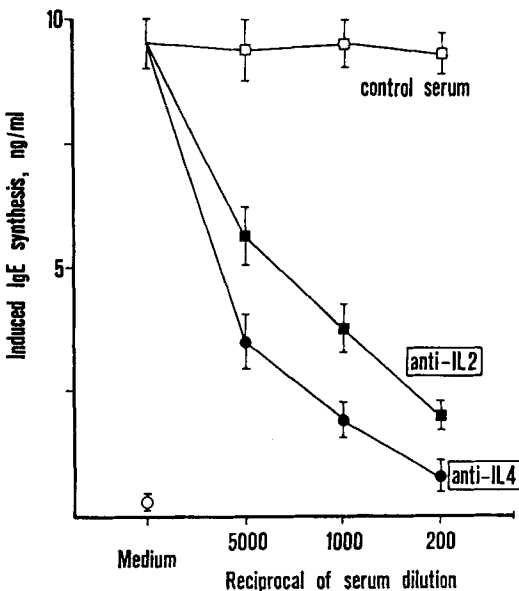


Fig. 2. Inhibition by anti-IL-2 antibody of the IgE synthesis induced by rIL-4 in culture containing appropriate concentrations of B and T cells. Highly purified tonsillar B cells (4×10^5 /ml) were cultured in the presence of autologous T cells (1×10^5 /ml) and rIL-4 (200 U/ml) and different concentrations of anti-IL-2 and anti-IL-4 antibodies. After 10 days of culture supernatants were assayed for their IgE content.

Table 4

IL-6 increases *in vitro* spontaneous IgE synthesis by atopic B cells

Diagnosis	Induced IgE synthesis (ng/ml)*		
	Medium	Cycloheximide	rIL-6
Nonatopic	< 0.3	< 0.3	0.4
Nonatopic	< 0.3	< 0.3	< 0.3
Atopic	1.4	0.4	4.2
Atopic	1.9	0.5	4.9
Atopic	2.6	0.9	3.4

* Peripheral blood B cells (4×10^5) from 3 atopic and 2 nonatopic subjects were cultured for 48 h in medium alone, washed to remove preformed IgE, and cultured for 8 days in the presence of medium alone or recombinant IL-6 (100 U/ml).

we could not find as well-defined a dichotomy between type 1 (Th1) and type 2 (Th2) helper T cells as that reported in mice (3). Mouse Th1 cells are able to produce IL-2, IFN- γ , lymphotoxin (LT), but not IL-4, whereas Th2 synthesize IL-4 and IL-5 but not IL-2, IFN- γ and LT. Only Th2 cells were found to be able to induce IgE production by LPS-stimulated murine B cells (13). A detailed analysis of a series of more than 500 CD4-positive clones derived from 14 different donors and from 4 different sources (PB, tonsils, lymph node and spleen) clearly indicated that human T lymphocyte populations not only consists of Th1- and Th2-like clones, but also of T helper clones able to simultaneously produce IL-4 and IFN- γ , IL-4 and IL-2, or all three lymphokines (Table 5) (6).

Mechanisms possibly involved in the dysregulation of IgE synthesis in pathological conditions characterized by hyperproduction of IgE

We asked whether alterations in these new mechanisms were at least in part responsible for the altered regulation of IgE antibody production in patients with atopic diseases or other disorders characterized by high IgE levels. To answer this question, we analyzed the functional activities of a large number of T cell clones derived from normal donors, patients with com-

Table 5

Profile of lymphokine activity of human CD4+ T cell clones derived from different lymphoid organs

Source of T cells	No. of TCC tested	No. of lymphokine-producing clones*		
		IL-2	IFN- γ	IL-4
PB	232	127 (55%)	126 (54%)	58 (25%)
Tonsil	151	107 (71%)	82 (54%)	103 (68%)
Lymph node	61	23 (38%)	27 (44%)	9 (15%)
Spleen	59	45 (76%)	38 (64%)	36 (61%)

* The amount of lymphokines was evaluated in the TCC SUPs after a 24-h pulse with PHA (1%). IL-2 content was evaluated by a proliferative assay of CTLL murine cells; IFN- γ and IL-4 by a calibrated RIA and ELISA, respectively.

mon atopy or patients with Buckley's syndrome.

When we looked at patients with common atopy or with the hyper-IgE syndrome, we found that they possessed in their peripheral blood a significantly higher number of T cells able to provide helper function for IgE synthesis in comparison with nonatopic donors. There was also a significant decrease in the proportion of IFN- γ -producing clones in patients with common atopy in comparison with that of nonatopic donors (Table 6). In addition, interestingly, we found that 4 children with the hyper-IgE syndrome had in their peripheral blood a very low number of T cells able to produce IFN- γ (Table 5). When we looked at the ability of T cell clones of patients with common atopy to produce IL-4, we found a higher proportion of both CD4-positive and CD8-positive T cells potentially inducible to IL-4 production (13), whereas there was no significant difference in the proportion of IL-4-producing clones be-

tween nonatopic donors and patients with hyper-IgE syndrome. These data provide a reasonable explanation of why patients with common atopy possess a significantly higher proportion of T cells in their blood able to provide helper function for IgE than nonatopics, as well as of why this type of helper T cell is even more common in the peripheral blood of patients with hyper-IgE syndrome. Taken together, these data suggest that an enhanced aptitude to produce IL-4 may represent one of the defects allowing the elevated and prolonged IgE production in patients with common atopy, whereas a severe deficiency in T cells able to produce IFN- γ , which negatively regulates the production of IgE, might be the pre-eminent feature of patients with Buckley's syndrome.

CONCLUSIONS

The studies on human IgE synthesis here reported provide further insight into the cellular

Table 6

Functional profile of CD4+ T cell clones derived from nonatopic subjects and from patients with common atopy or hyper-IgE syndrome

Diagnosis	No. of CD4+ clones tested	No. of clones showing:		
		Production of IL-4%*	Production of IFN- γ *	Helper function for IgE
Nonatopic subjects	336	146 (43%) ^a	185 (55%) ^d	56 (17%) ^g
Patients with:				
Common atopy	357	216 (61%) ^b	145 (41%) ^e	104 (29%) ^h
Hyper-IgE syndrome	142	67 (47%) ^c	17 (12%) ^f	79 (55%) ⁱ

* IL-4 was evaluated in the supernatant of PHA-induced T cell clones by a ELISA. IFN- γ production was evaluated by a calibrated RIA.

χ^2 analysis: b vs a = $P < 0.0005$; e vs d = $P < 0.0005$; h vs g = $P < 0.0005$; f vs d = $P < 0.0005$; i vs g = $P < 0.0005$.

and molecular mechanisms involved in IgE regulation, and alterations responsible for IgE dysregulation in some pathological conditions. It is clear that IL-4 is the essential factor for the induction of human IgE synthesis. No synthesis of IgE can be obtained *in vitro* in the absence of this lymphokine. Another T cell-derived lymphokine, IFN- γ , negatively regulates the IgE synthesis induced by IL-4. These two lymphokines can be produced by different T helper cells, as shown in mice, but they can also be the product of the same T cell clones. In such cases, the possibility that a given clone provides helper function for IgE seems to be related to a balance between the amounts of the two lymphokines produced. Other cellular and/or molecular signals such as activated T cells, IL-2 and IL-6 seem to be involved in the induction of IL-4-mediated IgE synthesis as well, but their precise role in this process still needs to be determined. Finally, alterations of one or more of these regulatory mechanisms begin to be detected in patients with pathological conditions characterized by hyperproduction of IgE. In particular, the increased prevalence of T cell clones able to produce IL-4, appears to be a distinctive feature of patients with common atopy, whereas a reduction in the proportion of IFN-producing T cells seems to be peculiar to patients with hyper-IgE syndrome.

Obviously, on the basis of such emerging findings, we do not presume to draw conclusions on the pathogenesis of atopy and of other diseases. Further studies will be required to better define the cellular and molecular alterations responsible for the dysregulation of IgE antibody production.

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