

The IgE response in atopy and infections

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The IgE antibody system represents an important defense mechanism against offending agents coming from the respiratory and gastrointestinal tracts. The human pathological condition most commonly associated with hyperproduction of IgE is atopy, the familial allergic disorder of immediate-type hypersensitivity to environmental allergens. Dysregulation of IgE synthesis has also been observed in clinical disorders often associated with immunodeficiency and/or infections, such as the Hyper-IgE syndromes (1), parasitic infestations, some primary and secondary immunodeficiencies (2, 3), AIDS (4), the acute phase of GVHD (5). Therefore, the understanding of mechanisms involved in IgE regulation is of primary importance in order to define the alterations responsible for allergic diseases, as well as other pathological conditions characterized by hyperproduction of IgE.

In the first part of this paper we have summarized the mechanisms that regulate the synthesis of IgE in humans. In the second part, we discuss the pathophysiological aspects of IgE dysregulation in disorders characterized by hyperproduction of IgE.

A. MECHANISMS REGULATING IgE SYNTHESIS IN HUMANS.

A.1. The *in vitro* human IgE synthesis is reciprocally regulated by Interleukin 4 (IL-4) and interferon-gamma (IFN- γ) and requires physical interaction between B cells and activated T cells.

Some years ago we showed that a noticeable proportion of phytohemagglutinin (PHA)-induced T cell clones (TCC), obtained from the peripheral blood (PB) of either atopic or nonatopic individuals, were able to support the IgE synthesis on normal B cells (6). Supernatants (SN) of PHA- or anti-CD3 antibody-stimulated TCC, selected for their helper activity on IgE synthesis, also induced IgE production (7). When PHA-induced TCC or their SN were assayed, a significant positive correlation between helper function for IgE synthesis and production of IL-4 was found (8, 9). In contrast, there was a significant inverse correlation between the IgE helper activity of TCC (or SN) and their ability to produce IFN- γ (8). The opposite regulatory role of IL-4 and IFN- γ on the synthesis of human IgE was definitely proved by the observation that human recombinant IL-4 was able to induce the synthesis of IgE in PB mononuclear cells (MNC) and this effect was inhibited by addition of recombinant IFN- γ (9). More importantly, an anti-IL-4 antibody consistently abolished the IgE synthesis induced by an active TCC or their SN tested, whereas it had poor or no inhibitory effect on the synthesis of IgM and IgG. IL-4 alone, however, was not sufficient for the

induction of human IgE synthesis *in vitro*; in fact, it was consistently ineffective in inducing IgE synthesis in highly purified tonsillar B cell suspensions (containing less than 1% T lymphocytes and monocytes) (10). T cells and monocytes did not support the IL-4-dependent IgE synthesis merely via the production of other cytokines, since we and others were unable to reconstitute the synthesis of IgE in highly purified B cells by using IL-2, IL-6, IL-1, TNF- α or mixtures of these lymphokines. IgE synthesis, in the presence of IL-4, occurred only when T and B cells were cultured in the same well of a double chamber system, but not when they are cultured in different chambers separated by a microporous membrane. This data suggest that a physical interaction between T and B cells is required for the IL-4-dependent IgE synthesis. Kinetics studies also demonstrated that the physical interaction with T cells was required by B cells prior to the signal provided by IL-4. Thus, our data provide evidence that IgE synthesis is dependent on two main signals: the first signal consists of physical interaction between T and B cells and the second one is provided by T cell-derived (or exogenously added) IL-4 (11).

A 2. Both cognate and noncognate T-B cell interaction can support the IL-4-dependent IgE synthesis.

Several data obtained in our laboratory suggest that not only cognate, but also noncognate, interaction with T cells may render B cells susceptible to the activity of IL-4. First evidence of it was provided by an alloreactive TCC (TR46), which was able to produce IL-4, but not IFN- γ , and to induce the synthesis of IgE when cultured with B cells possessing the appropriate alloantigen (HLA-DR4). When DR4-positive B cells were irradiated, IgE synthesis did not occur any more, but it could be restored by the addition of DR4-negative unirradiated B cells. Furthermore, in the presence of exogenously added IL-4, TR46 enabled B cells from all donors tested to produce IgE, irrespective of whether they possessed the DR4 alloantigen (11). Moreover, several TCC specific for soluble antigens (*Bordetella pertussis* exotoxin, tetanus toxoid - TT - and *Dermatophagoides pteronyssinus* - DP -), producing IL-4 and no or limited amounts of IFN- γ provided helper function for IgE synthesis in autologous B cells upon stimulation with the specific antigen (cognate interaction). However, many TCC, including those producing IFN- γ but not IL-4 in response to the specific antigen, showed the ability to induce IgE synthesis when cultured without antigen, but in the presence of exogenously added IL-4 (11). One may suggest that under the above experimental conditions IgE synthesis is the result of cognate interaction between B cells and a few autoreactive T cells contaminating the B cell population. More recently, however, it has been shown that cells from a subclone of the mouse EL-4 thymoma are able to support the synthesis of IgM, IgG and IgA by human B cells in the presence of PMA. When the B cells were added to EL-4 cultures one cell per well the pattern of Ig secreted by the B clones was found to be similar, thus excluding requirements for human T cells for the EL-4/PMA-dependent B response (12). The addition of very low amounts of IL-4 to such a system resulted in the production of high amounts of IgE, as well (13). Recently, we have shown that treatment of PMA-prestimulated EL-4 thymocytes with

cyclosporin A or paraformaldehyde (in order to block the release of cytokines) had no inhibitory effect on the IgE synthesis. This finding suggests that cognate interaction is required for inducing T cells to produce IL-4, but is not essential for providing the first (physical) signal to the B cells. Noncognate, contact dependent, B cell activation can also function as an efficient signal for IL-4-dependent IgE synthesis to occur. The nature of molecule(s) responsible for nonspecific physical interaction occurring between T and B cells is still unclear.

The pathophysiological implications of these findings are noteworthy. When a B cell acts as antigen-presenting cell for the T helper cell, cognate interaction provides the first signal to the B cell. If the T cell is able to release IL-4, but not inhibitory amounts of IFN- γ , production of antigen-specific IgE does occur. However, B cells specific for other antigens eventually present in the microenvironment, can also undergo both a physical noncognate signal by the activated T cell and the influence of IL-4, thus being induced to produce IgE. This might explain why in some pathological conditions, such as parasitic infestations or the acute phase of GVHD, strong polyclonal IgE production occurs (14).

B. PATHOPHYSIOLOGICAL ASPECTS OF IgE PRODUCTION IN ATOPY AND INFECTIONS.

We tried to investigate whether alterations of the above mentioned IgE-regulatory mechanisms are involved in the genesis of human diseases characterized by hyperproduction of IgE.

Patients with common atopic diseases show increased production of IgE, that is usually limited to antibodies specific for environmental allergens and several significant associations have been observed between particular HLA haplotypes and responsiveness toward different purified allergens (15). An imbalance in PB of T cell subsets responsible for IL-4 and IFN- γ secretion seems to be one of the main alterations responsible for the hyperproduction of IgE in common atopy. This imbalance is quite well defined in tissue allergic inflammatory infiltrates. It has been shown at the clonal level that the majority of T cells isolated from conjunctival infiltrates of patients with vernal conjunctivitis (a disease suggested to have an allergic etiology) exhibited the Th2-like pattern of cytokine production and helper function for IgE, whereas the number of Th1-type cells producing IFN- γ was very low (Maggi et al., unpublished data). Recently, eye alterations histologically similar to those of VC have been observed in a cohort of IL-4 transgenic mice expressing IL-4 at T cell level (16). These findings suggest that dysregulation of IL-4 production plays a role in the genesis of such a type of lesions. The reason why in individuals genetically determined to recognize allergenic epitopes, these latter induce B cells to switch not only to IgG, but also to IgE, synthesis is unclear. One possibility is that allergens differ from other antigens for their ability to "select" helper T cells with peculiar profiles of cytokine production. To explore such a possibility, we have recently established DP- and TT-specific TCC from PBMNC of one atopic donor showing both immediate-type hypersensitivity to DP and delayed cutaneous hypersensitivity to TT. Virtually

all TT-specific TCC produced both IL-4 and IFN- γ in response to PMA plus anti-CD3 antibody, whereas 4 of 12 DP-specific TCC failed to produce IFN- γ upon the same stimulus. When assayed with the specific antigen, the proportion of DP-specific TCC able to produce IL-4, but not IFN- γ , was even higher (50%), whereas only 18% of TT-specific TCC displayed a similar pattern of cytokine production. In addition, the mean concentrations of IL-4 produced by DP-specific TCC upon both stimulations were significantly higher than those produced by TT-specific TCC, whereas the mean concentrations of IFN- γ were significantly lower (Parronchi P et al, submitted for publication). Taken together these data suggest that in atopic individuals allergen-specific TCC have higher probability than TCC specific for microbial antigens to belong to the IL-4-, but not IFN- γ , producing (IgE-helper) T cell subset.

In order to evaluate the pathophysiological role of IgE production in infections, we have examined three pathological conditions characterized by recurrent or chronic infections and high IgE serum levels. PHA-stimulated PBMC from 4 children exhibiting Hyper-IgE syndrome produced lower concentrations of IFN- γ than did PBMC from 8 age-matched healthy controls, particularly at low cell concentrations. The analysis at clonal level revealed that patients with the Hyper-IgE syndrome had significantly lower proportions of circulating T cells able to produce IFN- γ and TNF- α (but not of T cells producing IL-2 or IL-4) in comparison with controls (17). Since IFN- γ inhibits the synthesis of IgE and both IFN- γ and TNF- α play an important role in cell-mediated immune reactions and in the defense against infectious agents, it is reasonable to suggest that the defective (genetically determined?) production of IFN- γ accounts for hyperproduction of IgE and the combined defect of IFN- γ and TNF- α may contribute at least in part to the undue susceptibility to infections of patients with the Hyper-IgE syndrome (17).

It is well known that parasites induce in infested hosts marked production of IgE antibodies, most of which are not specific for parasite antigens. However, the mechanisms by which parasites stimulate IgE production are still unknown. Filarial parasite-specific T cell lines released in their SN soluble factors capable of inducing IgE synthesis in normal B cells (18), but the nature of such factors was not specified. Recently, we have studied a high number of PHA-induced TCC established from the PB of 5 patients with parasitic infestations (2 with filariasis, 2 with toxocariasis and 1 with toxocariasis and ascaridiasis) for their profile of IL-4 and IFN- γ production. Patients infested with parasites showed significantly higher proportions of IL-4-producing, and significantly lower proportions of IFN- γ -producing, CD4+ TCC in comparison with controls. Even if the imbalance between IL-4- and IFN- γ -producing T cells probably accounts for the increased synthesis of IgE in such diseases, we do not know at present the precise relationship between parasitic infestation and IgE production, since we have no information on the specificity of TCC able to produce IL-4, but not IFN- γ that circulate in the blood of these patients.

In AIDS, concomitant to a depression of primary antibody responses, an overreactivity of the B cell compartment has been reported (19). Recently, we have found increased serum IgE levels in a proportion of patients with

constitutional symptoms or full-blown disease. The enhanced IgE response was polyclonal and, at least in part, related to the production of IgE specific for fungal antigens (4). This finding is consistent with previous studies of frequent occurrence of allergic reactions during full-blown AIDS (20) and with our previous observations showing that TCC derived from residual CD4+ PB T cells from stage IV patients, as well as their SN, exhibited increased helper activity for IgG and IgE synthesis in comparison with control CD4+ TCC (21). This finding might account for the imbalance of IL-4- and IFN- γ -producing T cells prevalently found in PB from patients with full-blown disease (22). At present no data are available to explain the selection of CD4+ T cells with such a peculiar functional pattern in PB from AIDS patients. One possibility is that HIV infection can modify CD4+ cells in their cytokine potential. Very recent data indicate that in vitro-infected TCC derived from healthy subjects show a reduced viability, a decreased production of IL-2, IL-4 and IFN- γ , but an increased ability to synthesize TNF- α and to induce Ig synthesis in vitro by co-cultured autologous B cells (23). Thus, HIV-induced changes on the membrane of helper T cells and/or their pattern of cytokine production may be responsible for polyclonal B cell activation seen during the early phases of infection. Subsequently, a selective loss of CD4+ T cell subsets exhibiting Th1-like pattern may further contribute to the hyperproduction of different immunoglobulin classes, including IgE.

CONCLUDING REMARKS

In this paper we have summarized the mechanisms involved in the regulation of human IgE synthesis. IL-4 and IFN- γ are the main regulatory cytokines of IgE production with opposite effects on the synthesis of this immunoglobulin class. However, a prior physical interaction with activated T cells is required by the B cells to synthesize IgE in response to IL-4. Cognate interaction with T cells producing IL-4, but not IFN- γ , leads to specific IgE antibody production. When high numbers of activated helper T cells with such a profile of cytokine production are generated, noncognate interaction with bystander B cells can result in polyclonal IgE production, as well.

An imbalance between IL-4- and IFN- γ -producing helper T cells seems to be the alteration responsible for IgE hyperproduction in patients with atopy and in infectious conditions characterized by high IgE serum levels. Preliminary observations suggest that atopic patients have the ability to "select" helper T cells inducible to high production of IL-4 and low or undetectable production of IFN- γ . In the Hyper-IgE syndrome, a primary defect in the number of circulating T cells able to produce IFN- γ (and TNF- α), may account for both the high IgE serum levels and the susceptibility to infections. In helminth infestations, the imbalance of circulating IL-4- and IFN- γ -producing T cells may be the result of a selective stimulation of Th2-like cells by parasitic antigens. Finally, in AIDS the high IgE serum levels in full-blown disease probably reflect polyclonal B cell activation due to HIV-induced changes on helper T cells as well as the preferential depletion of HIV-infected Th1-like cells.

Acknowledgements: This work was supported by grants from Ministry of Education (MPI 40%), from CNR (Finalized Project FATMA), from ISS (Research Project on AIDS) and from AIRC.

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