

Regulation of the IgE System: Experimental and Clinical Aspects

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The IgE antibody system could, in my view, be considered as the "master hypocrite" of our body's defense mechanisms. On the one hand, it serves as a paradigm example of how a finely tuned network of interacting cells and soluble products from such cells carefully regulate the quality and quantity of a given humoral immune response (reviewed in 1-10). Indeed, I have become convinced that the IgE antibody system is perhaps among the most important and sophisticated of the existing antibody defense systems. On the other hand, this system is also responsible for IgE-mediated allergic diseases, diseases constituting a major health problem with consequences that not only affect the physical well-being of affected individuals but also impose a substantial economic impact on both individual and society alike. The theme of this review article will be centered around understanding the basis for this hypocrisy in the IgE system, because only by understanding it will we be able to grasp the steering wheel that will enable us to guide the system in an effective therapeutic fashion.

This review was not written for the purpose of providing a compendium of experimental or clinical observations derived from numerous sources around the world. It is, instead, an attempt to present a perspective of our present understanding of the IgE system and, in particular, some thoughts on what lies ahead regard-

ing the treatment and/or amelioration of IgE-mediated allergic disorders. In many respects, therefore, this is a personal statement in which hard experimental data have been mixed with anecdotal information (some of which was personally communicated to me during lecture trips, etc.) that tend to go along with the general theme that I will develop here.

Components of the allergic response

The major cellular and molecular components of the IgE antibody system, and the manner in which they interact together, is summarized in Fig. 1. The IgE antibodies themselves, depicted at the lower middle of the figure, are the products of IgE precursor B lymphocytes (upper left), whose ultimate maturation and function is regulated by a network of cells and molecules, including the B cells themselves, and T cells that play helper and suppressor functions, respectively, and whose activities are mediated by soluble factors that serve as helper and suppressor substances, respectively.

As stated above, I believe that the IgE antibody system is one of the most, if not the most, important antibody defense system that exists. It is clear that this system plays an important defense role against certain offending exogenous antigens, particularly those which gain access through mucoepithelial and epithelial linings,

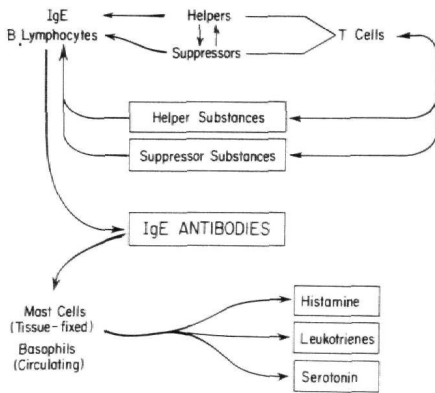


Fig. 1. Components of the allergic response. See text for explanation.

such as the respiratory and gastrointestinal tracts and skin. What is particularly unique about the IgE system is the inherent amplification of its physiological capacity. Thus, as indicated in Fig. 1, since IgE molecules bind specifically and avidly to receptors displayed on the surface membranes of tissue-fixed mast cells and circulating basophils (among other cell types), and since such cells are actually small factories of potent pharmacological mediators – including histamine, leukotrienes, and serotonin – only a small number of IgE molecules are required to exert the biological effects for which the system was designed. It makes sense, therefore, for regulatory mechanisms concerned with IgE antibody synthesis to evolve with particular emphasis on minimizing the quantities of IgE antibody molecules produced following sensitization. In this way, IgE antibody responses could be sufficient to provide protection for the individual without resulting in undesirable and/or deleterious reactions.

Indeed, as will become clear throughout this review, this is precisely how the system has been designed to operate under normal circumstances. It will also become clear, however, that the regulatory control of IgE synthesis is so delicate that it becomes susceptible to certain perturbations that can upset the balance and thereby result in production of higher than necessary quantities of IgE which, in turn, becomes translated into symptomatic manifesta-

tions. Hence lies the basis for the hypocrisy of the system: the manner in which its regulatory mechanisms have evolved is *so* sophisticated and intelligent that it has become more susceptible to external perturbations, some of which are perhaps of a man-made nature and therefore not anticipated by evolution, and allergic diseases have been the consequence. This may be analogous, for example, to common problems with the most sophisticated computers – namely, the more sophisticated they are, the more susceptible they appear to be to common environmental influences such as temperature fluctuations and even specks of dust.

"Allergic breakthrough": Pathogenesis of the allergic phenotype

The putting together of numerous pieces of information that were obtained in studies in our laboratory – as well as those of others – concerning regulation of IgE antibody production in experimental animals over several years, formed the genesis of a new concept concerning the possible pathogenesis of the allergic phenotype (5, 6, 11). This concept, termed "allergic breakthrough", embodies the essence of the hypocrisy of the IgE antibody system and, more importantly, has been subsequently validated in both experimental and clinical terms.

The essence of this concept of "allergic breakthrough" is schematically illustrated in Fig. 2. The dotted lines in the middle demarcate two zones, the allergic and non-allergic zones determined by the magnitude of IgE antibody production made by a given individual in response to a given sensitization. Quite simply, the concept states that, normally, IgE antibody production is maintained at a low, albeit effective, magnitude following sensitization because of the existence of a normal suppressive, or "damping", mechanism that exists precisely to limit the quantity of IgE antibodies produced in any given response. The normal damping mechanism would, of course, reflect the net balance of suppressive versus enhancing regulatory activities concerned with this antibody response. If any one of a number of

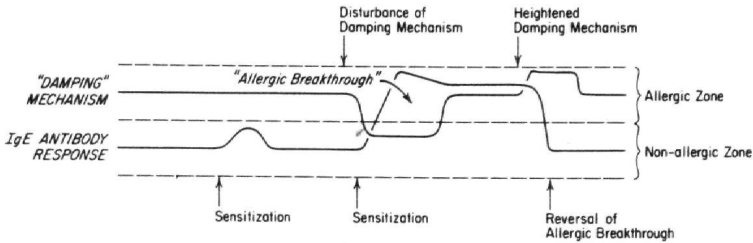


Fig. 2. Possible pathogenesis of "allergic breakthrough". See text for explanation.

possible perturbations disturbs this damping mechanism, in such a way as to diminish the overall damping capabilities to a sufficiently low level, and if then, when the damping threshold is lowered, the individual becomes sensitized to one or more allergenic substances, this unfortunate juxtaposition in time can result in allergic breakthrough. This simply means that the height of IgE antibody production to that given allergen rises into the allergic zone, in terms of quantities produced, thereby leading to the development of allergic symptomatology.

This "allergic breakthrough" concept makes several important predictions, most of which have now been either experimentally or clinically verified:

1. The damping mechanism should be perturbable by certain definable manipulations, and probably is subject to similar perturbations by subtle events to which we are commonly exposed in our natural environments. Experimentally, we know that the exposure of inbred mice that are genetically low IgE-responders, to such manipulations as low dose whole-body irradiation or suitable doses of immunosuppressive drugs, disturbs the normally dominant suppressive mechanism (12-17). If while this "window" is open, albeit transiently, such animals are sensitized with antigen, they display allergic breakthrough as manifested by conversion into high IgE responders (to that particular antigen).

Clinically, the relatively high frequency of allergic disorders in man implies that something more subtle and probably rather commonplace in our environment can result in similar disturbances. I believe that common upper respira-

tory viruses are the most likely candidates for this role; indeed, this suggestion (18) was experimentally verified in mice and dogs which, following intranasal exposure to respiratory viruses, manifested allergic breakthrough responses in the IgE class indistinguishable from that induced by exposure to low dose irradiation (19). An important prospective clinical study performed with high-risk allergy-prone children (both parents of whom were allergic), demonstrated the onset of allergic disorders within weeks of the occurrence of upper respiratory infections with parainfluenza, respiratory syncytial virus or cytomegalovirus (20).

It seems obvious that also certain endogenous events can contribute to perturbations of the regulatory mechanisms controlling the IgE system, both in a positive and a negative manner. Marked shifts in endogenous hormone production are prime candidates in this regard, and previous studies in our laboratory demonstrated that normal diurnal variations in endogenous steroid production exerted significant effects on the magnitude of IgE responses developed by sensitized inbred mice (21). Clinically, the wide variations in hormone production during pregnancy probably account for the often seen fluctuations in symptomatology in allergic women during pregnancy. The abrupt onset of sex hormone production during puberty probably explains the sudden disappearance of allergic diseases in many patients who manifested one or more allergic disorders during childhood. This may represent a natural process for heightening the damping mechanism, a process which, in turn will depress IgE antibody production back into the non-allergic

zone, as graphically depicted at the far right of Fig. 2.

2. A second prediction of this concept is that once "breakthrough" has occurred, the height of IgE production, specific for the antigen(s) to which sensitization has occurred, should remain elevated in the allergic zone even though the damping mechanism has returned to its normal threshold. When low responder mice are exposed to low dose irradiation this opens a transient window for approximately two days, during which sensitization with antigen results in conversion to high IgE responses for that specific antigen (allergic breakthrough); after the two-day window has closed, the damping mechanism returns to normal and subsequent sensitization with an unrelated antigen results in the normal low-level IgE response pattern (11). However, IgE production to the original sensitizing antigen administered when the window was open remains high in such mice, essentially for the remainder of their lives (22).

3. A third prediction is that any manipulation which either effectively re-establishes the damping mechanism, such as administration of soluble suppressive molecules (see below), or counteracts it, such as administration of soluble enhancing molecules, should persist for long periods (11).

4. A fourth prediction is that allergic breakthrough should be highly specific for the antigen(s) to which the individual became sensitized coincident with disturbance in normal damping. As stated above, this is precisely what occurs experimentally, in that once the window closes and normal damping recovers, subsequent sensitizations for high IgE responses can *only* be accomplished with the sensitizing antigen administered during the transient disturbance. Unrelated antigens will only result in low-level IgE responses (11) unless the individual is exposed once again to a manipulation which will effectively disturb normal damping and this is accompanied by sensitization to a second antigen.

The farthest reaching implications of this concept are that *any* individual can, under ap-

propriate circumstances, develop IgE-mediated allergies. This is perhaps best exemplified by the reported development of IgE-mediated allergies to antibiotics in bone marrow transplant patients who were iatrogenically immunosuppressed and concomitantly being treated prophylactically with antibiotics to avoid infectious disease complications (23, 24). It is noteworthy that such patients had no prior history of IgE-mediated allergies prior to such therapy. Why, then, are IgE-mediated allergies of one sort or another not observed in everyone, and what is the explanation for the apparent genetically inherited tendencies to the development of allergic disorders? Perhaps this is explained by the differences in susceptibility of the normal damping mechanism to fluctuations in response to various perturbations. In other words, perhaps the relative threshold of disturbance of regulatory control of the IgE system is the major variable, and heritable, trait.

I often like to exemplify the concept of allergic breakthrough by using the analogy of the development of an IgE-mediated allergy to shellfish protein(s). In this case, the story might go as follows: An individual develops an upper respiratory viral infection during the week preceding a scheduled Saturday evening dinner engagement. Not realizing that he/she has undergone, as a result of the viral infection, a transient disturbance in the damping mechanism normally controlling the IgE system, and feeling sufficiently recovered from the symptoms of the upper respiratory viral infection, he/she goes out and enjoys a marvelous seafood dinner. Unbeknownst to him/her, sensitization to the ingested shellfish proteins results in allergic breakthrough, the consequences of which will only become apparent at some later point when the individual again indulges in a seafood dinner.

Now that we have considered the concept of allergic breakthrough, and its implications for the pathogenesis of IgE-mediated allergic diseases, let us explore the experimental basis for our current understanding of regulatory mechanisms controlling IgE antibody synthesis.

Control of IgE antibody synthesis is under the dominant influence of a suppression, or "damping", mechanism which can be disturbed by exposure to low-dose irradiation

The fact that inbred strains of mice can be divided into categories of low and high IgE-responder phenotypes, makes the use of such animals for experimental analysis of IgE regulation particularly convenient. Thus, certain strains of mice respond to antigen sensitization with production of relatively high quantities of specific IgE antibodies, whereas other strains produce considerably lower, or undetectable, antibody responses in this class following comparable modes of immunization (25). These strain differences are restricted to the IgE class, since comparable quantities of antibodies to the same antigen in other immunoglobulin (Ig) classes are made by both types of strains.

An important advance in our understanding of the regulation of IgE synthesis and the development of the allergic breakthrough concept came with the realization that the IgE response phenotype of a given experimental animal could be willfully manipulated in various ways, including exposure to low-dose whole-body irradiation

(12–17). Collectively, such observations indicated that regulation of IgE synthesis is dominated by a suppressive, or "damping", mechanism which normally serves to limit the magnitude of IgE antibody production following sensitization. Under appropriate circumstances, certain perturbations can effectively disturb this damping mechanism, resulting in heightened production of IgE.

One such experiment illustrating this phenomenon is presented in Fig. 3 (22). The inbred SJL mice used here are the prototype low IgE-responder mouse strain. Thus, as shown by the control group (indicated by the dashed line and open circles), these mice respond to routine sensitization regimens by producing no, or barely detectable, IgE antibodies; note that these unirradiated control mice failed to develop appreciable IgE antibody responses at any point during the course of this extended experiment, even despite secondary restimulation on day 60.

In marked contrast, SJL mice exposed to whole-body irradiation at the time of initial sensitization manifest very high IgE responses – i.e., a clear allergic breakthrough pattern. Note that the height of IgE production tends to remain at elevated levels in such mice, and restimulation

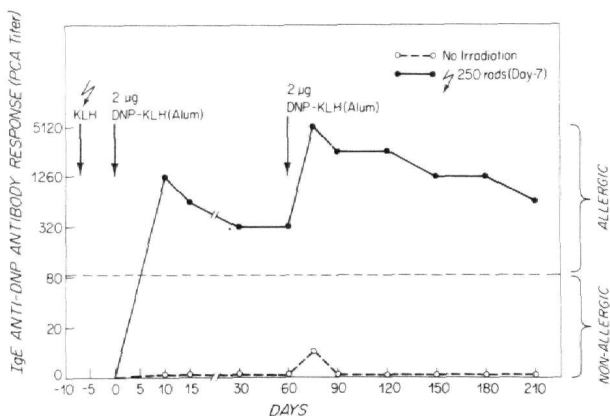


Fig. 3. The height of IgE antibody production persists at elevated levels for long periods following irradiation-induced allergic breakthrough in SJL mice. SJL mice were either not pretreated (open circles) or pretreated with 250 rads irradiation (closed circles) shortly before preimmunization with 2 μ g of KLH in alum on day -7. All mice were primarily immunized on day 0 with 2 μ g of DNP-KLH in alum and given a secondary challenge with the same antigen and dose on day 60. The IgE anti-DNP antibody responses are illustrated as PCA titers at the indicated intervals over a 7-month period. (From Ref. 22 with permission of the publisher).

on day 60 elicits a clear secondary elevation of IgE production. It is particularly noteworthy how the IgE levels of such mice tend to persist at such relatively high magnitudes, even without further antigen restimulation, well into seven months after initial sensitization and irradiation. Thus, although the deliberate disturbance of the damping mechanism to which they were exposed occurred only once many months earlier, the irradiated SJL mice continued to display high IgE response patterns for extended periods.

Two other points should be emphasized about this, and comparable, experiments. First, responses in the IgG class were indistinguishable between the control and experimental groups – in other words, the breakthrough phenomenon provoked by irradiation is selective for the IgE response. Second, antigen specificity of this breakthrough phenomenon is quite strict. Thus, if the two groups of mice shown in Fig. 3 were subjected to sensitization with an unrelated antigen, such as ragweed extract, on day 60, *both* groups would make low level IgE responses of the character displayed in Fig. 3 for the unirradiated control mice (11).

Irradiation-enhanced IgE responses can be suppressed by a circulating "suppressive factor of allergy" (SFA)

Our success in obtaining a molecular handle on IgE antibody regulation began to emerge with the discovery of soluble factors that manifested selective regulatory effects on IgE antibody responses. One such factor, termed suppressive factor of allergy (SFA), was discovered in our laboratory nearly eight years ago almost by accident (26, 27). The remarkable biological effects of SFA on *in vivo* production of IgE antibodies is illustrated in Fig. 4. Again, mice of the low responder SJL strain are employed in this study and, as shown by control group I, which were immunized but not irradiated, such mice develop low-level IgE antibody responses following antigen sensitization. Exposure to irradiation, on the other hand, results in conversion to very high IgE antibody production while having no detectable effect on responses in the IgG class (group II). Administration of small quantities of serum obtained from normal (i.e., unmanipulated) SJL donor mice has little or no

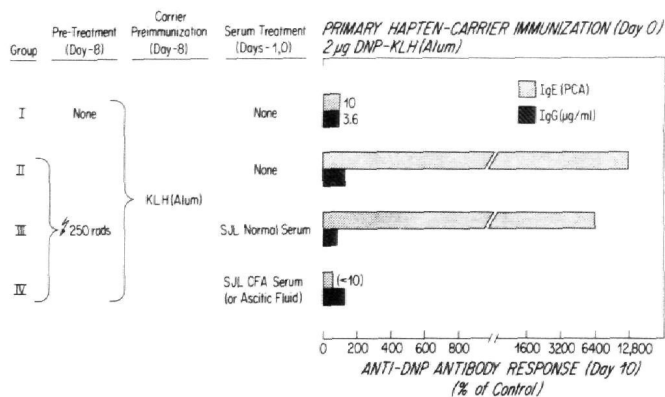


Fig. 4. Selective suppression of irradiation-enhanced primary IgE antibody responses of low-responder SJL mice by passive administration of CFA-induced suppressive factor of allergy (SFA). SJL mice were either not irradiated (group I) or irradiated with 250 rads (groups II-IV) shortly prior to carrier preimmunization with 2 μ g of KLH in alum on day -8. On days -1 and 0, mice in groups III and IV were injected with serum from normal SJL mice or from CFA-immune SJL donors, respectively; such injections consisted of 0.1 ml of the respective sera per injection given four times spaced at 12-h intervals over a 48-h period. On day 0, all mice were primarily immunized with 2 μ g of DNP-KLH in alum. The IgE and IgG anti-DNP antibody responses on day 10 after primary sensitization are presented as percentages of the respective untreated control responses (group I) with the actual titers listed beside the control bars. (From Ref. 22 with permission of the publisher).

effect on the irradiation-enhanced IgE responses of mice in group III. In striking contrast, this converted high IgE response pattern can be completely reversed back to the normal low-level response when such irradiated mice are injected intravenously with preparations of serum (or ascites fluid) from SJL donor mice inoculated 7 days prior to bleeding with mycobacteria-containing complete Freund's adjuvant (CFA). (Early observations demonstrated that inoculation of donor mice with CFA provoked the production of large quantities of SFA.)

Several points are noteworthy about the results presented in Fig. 4. First, the effects of an active serum or ascites fluid preparation are highly selective for antibody responses in the IgE class (26, 27); no appreciable effects are exerted on responses of the IgG class or on other immunological responses as far as we can tell. It is because of this selective effect on IgE production that we have termed the active molecules in such preparations suppressive factor of allergy, or SFA (26, 27). Second, although not obvious from the preceding data, variable quantities of SFA can be found in the serum of normal donor mice, particularly those of the

low IgE responder types; the effects of CFA (or certain other manipulations) are related to the abilities of such materials to stimulate production of exaggerated quantities of SFA in such donor mice. Third, the biological effect of SFA is completely *nonspecific* in the sense that IgE responses elicited by any number of antigens can be effectively suppressed by appropriate doses of SFA. Fourth, SFA displays very high activity even when administered in minute quantities. Finally, it cannot be overemphasized that SFA is produced by living animals and is biologically active when passively transferred to living recipients, thereby underscoring the physiological relevance of this potent factor; moreover, in many thousands of mice that we have injected with these and similar materials over the years, we have never observed any manifestations of acute chronic toxicity.

IgE synthesis can be enhanced by an "enhancing factor of allergy" (EFA) present in serum of both high and low IgE-responder mice

After the discovery of SFA, it eventually became clear that molecules exist which exert precisely

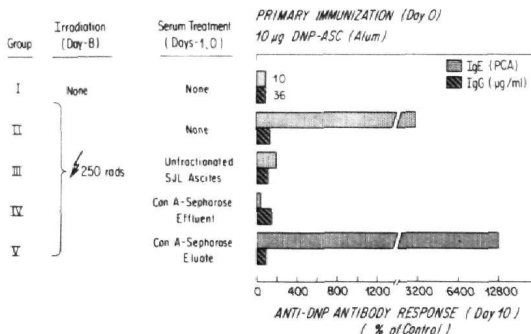


Fig. 5. Separation of suppressive factor of allergy (SFA) and enhancing factor of allergy (EFA), which selectively modulate IgE responses of low responder SJL mice, by affinity chromatography on Con A-Sepharose. Groups of SJL mice were employed in the protocol summarized on the left. CFA-induced ascites fluids from SJL donors were precipitated by ammonium sulfate and then administered to SJL recipients either without further fractionation (unfractionated ascites, group III) or after fractionation on Con A-Sepharose, the pass-thru effluent fraction administered to group IV, and the fraction eluted from the column by α -methyl-D-mannoside administered to group V. The immunization schedules and the schedule of injections of the various ascites fluid samples were identical to those described in Fig. 4; the only difference was the nature of the antigen used for sensitization which, in this experiment, was unconjugated *Ascaris* (on day -8) and DNP-ASC on day 0. The IgE and IgG anti-DNP antibody responses on day 10 after primary sensitization with DNP-ASC in alum are illustrated as percentages of the control response with the group I control values listed beside the corresponding bars. (Modified from data presented in Ref. 28).

the opposite regulatory effects, also in a selective manner, on IgE responses of both low and high IgE responder individuals (28). Moreover, such "enhancing factors of allergy", or EFA, can be conveniently separated from SFA by affinity chromatography on lectin-Sepharose columns (28, 29) as illustrated in Fig. 5.

In this experiment, the irradiation-enhanced IgE responses of SJL mice (group II) are again completely suppressed by administration of an ammonium sulfate precipitate of otherwise unfractionated ascites fluid (group III). When the ascited fluid ammonium sulfate precipitate is fractionated by affinity chromatography on concanavalin A-Sepharose, the material recovered in the effluent – i.e., molecules of low glycosylation which therefore bind poorly to lectin columns – is highly enriched for SFA activity (group IV). In contrast, the fraction that initially binds to the column, and then is eluted by competitive sugar inhibition, – i.e., those molecules of glycoprotein nature that have affinity for lectins – is substantially enriched in EFA activity as revealed by the fact that mice receiving this material (group V) develop 4-fold higher responses than the already enhanced responses obtained in group II. Note that again all effects observed, whether enhancing or suppressive, are selective for the IgE system, since IgG responses are essentially indistinguishable among the various groups in this study.

The demonstration of the existence of two distinct factors with opposing activities, SFA and EFA, which could be chromatographically separated from one another on lectin affinity columns, as illustrated in the preceding experiment, represented a significant breakthrough for our subsequent analysis of these biologically active entities.

For one thing, recognition of the fact that both molecular entities could exist in varying quantities in a given preparation of body fluid, or culture fluid, emphasized to us the importance of using appropriate separatory techniques before using such preparations in various bioassay systems. For example, the presence of EFA in somewhat higher quantities in a given

tissue culture fluid can completely mask the activity, and hence the presence, of SFA in that same culture fluid. Moreover, quantitation of these respective entities, SFA and EFA, made it possible to experimentally document that the IgE response phenotype of an individual reflects the relative balance of these two opposing regulatory factors that exists at the time of antigen sensitization. Hence, one of the very early events following irradiation of mice (either low or high IgE-responders) is a precipitous drop in the amounts of circulating SFA, and a corresponding elevation in the relative amounts of EFA. This preponderance of EFA lasts only a few days, after which SFA production resumes and restores the balance of these two respective factors to its original state. It is during the time of the altered ratio of EFA to SFA that the transient window for eliciting irradiation-enhanced IgE responses remains open – i.e. the period during which allergic breakthrough can occur.

Normal lymphoid cells exposed to elevated quantities of IgE, either in vivo or in vitro are induced to synthesize and express membrane-bound Fc receptors for IgE (FcRe)

Receptors for determinants present on the Fc portion of immunoglobulin molecules have been known for some time to exist on the cell membranes in macrophages and lymphocytes, as well as neutrophils (reviewed in 30). During recent years, Fc receptor (FcR) heterogeneity on T lymphocytes both in experimental animals and man has received increasing attention. Pertinent to the subject of this review are the demonstrations in recent years of FcR specific for IgE antibody molecules (FcRe). These have been discovered on the membranes of macrophages (31–33) and on subpopulations of lymphocytes. Other cell types express FcRe, including mast cells, basophils and eosinophils, although these cells express FcRe constitutively. Conversely, normal lymphocyte populations express only very low levels of FcRe (34–36). However, when elevated IgE levels develop, a significant induction of FcRe expression occurs in certain lymphocytes (36–40a). Thus, relatively

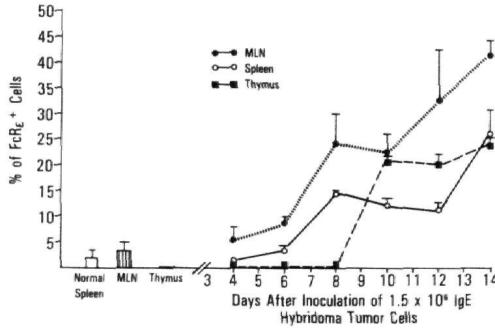


Fig. 6. Induction of FcεR⁺ cells in IgE-secreting hybridoma-bearing CAF₁ mice. CAF₁ mice were inoculated intraperitoneally with 1.5×10^6 hybridoma cells secreting DNP-specific IgE. At varying times thereafter mesenteric lymph nodes, spleen and thymus were removed from each mouse (3 mice per time-point) and examined for frequencies of FcεR⁺ cells. FcεR⁺ cells were enumerated by the development of rosettes when lymphoid cells were incubated with IgE-coated sheep erythrocytes. Frequencies of FcεR⁺ cells in the same organs obtained from normal mice are shown at the left. The data are presented as geometric means and standard errors of the frequencies of FcεR⁺ cells detected at each time-point following tumor inoculation in 3 individual mice per point.

high expression of FcεR has been shown among lymphocytes derived from animals stimulated to produce high levels of IgE, or from animals bearing IgE-secreting hybridoma cells; likewise, in atopic individuals, or patients with hyper-immunoglobulin E syndrome, increased circulating levels of FcεR⁺ cells have been documented (34, 37, 41). In addition, exposure of normal lymphocytes to appropriate concentrations of IgE *in vitro* induces the expression of FcεR in these cells.

The data presented in Fig. 6 (42) demonstrate the progressive rise and frequency of FcεR⁺ lymphoid cells in three different lymphoid organs of mice inoculated with progressively growing hybridoma cells secreting high levels of monoclonal DNP-specific IgE (43). As indicated on the left portion, only low numbers of FcεR⁺ cells are normally detected in spleen, mesenteric lymph nodes or thymus of normal donor mice. However, these same organs obtained from mice inoculated with hybridoma cells manifest a progressive rise in the frequency of FcεR⁺ cells that corresponds in time with the progressive elevation of circulating IgE secreted by the hybridoma cells. This effect is indeed startling when one notes that by day 14 over 40% of the total lymphocyte population in mesenteric lymph nodes are FcεR⁺.

The ability to induce *de novo* expression of FcεR by *in vitro* exposure of normal murine lymphoid cells to suitable concentrations of monoclonal IgE (43) is demonstrated in Fig. 7 (36). Thus, as shown by the left panel, normal splenic lymphocytes cultured in the presence of IgE for 24 hours display 5-fold higher numbers of FcεR⁺ cells than the corresponding control cultures lacking IgE. This IgE-mediated induction process requires intact energy metabolism and functional cytoskeletal structures, as demonstrated by the ability to inhibit this process by treatment of such cultures with the inhibitory drugs shown in the right panel of Fig. 7 (42). The ability of monoclonal IgE to induce increased expression of FcεR, either *in vivo* or *in vitro*, is truly isotype-specific since such manipulations have no influence on the frequencies of FcR⁺ cells specific for other immunoglobulin molecules (36).

Studies such as these, which demonstrated that FcεR expression by lymphoid cells is a sensitive indicator of total IgE concentrations in the environment, suggested an important role(s) for such cells in the isotype-restricted regulation of the IgE system (reviewed in 6, 7). More importantly, Ishizaka and colleagues made the important discovery that FcεR⁺ lymphoid cells were the source of IgE-selective regulatory fac-

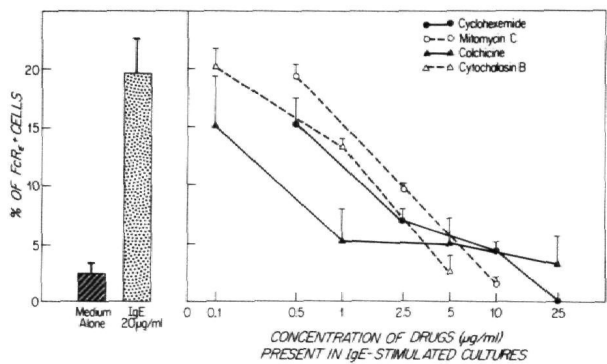


Fig. 7. Requirement of intact energy metabolism and cytoskeletal structures for the induction of FcRe⁺ cells by monoclonal IgE. Spleen cells from normal CAF₁ mice were incubated in 24-well macroplates (10 × 10⁶ cells in 0.5 ml) either in the absence or in the presence of DNP-specific monoclonal IgE (20 µg/ml). In addition, IgE-stimulated cultures were carried out either in the absence (left panel) or in the presence of varying concentrations of inhibitor drugs, as indicated in the right panel. At the end of 24 h incubation, all cells were harvested, washed, and then assayed for rosette-forming capacity against IgE-coated sheep erythrocytes as indicator cells. The values presented represent the arithmetic mean percent and standard errors of rosette-forming cells of each type determined on 3–4 counts of approximately 400 living small lymphocytes examined in each sample pool.

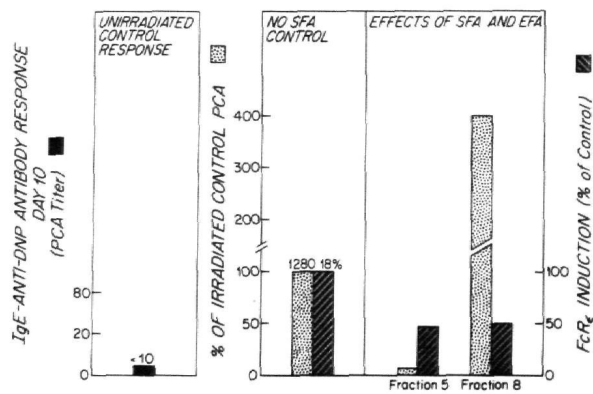


Fig. 8. Contrasting effects of SFA-enriched fraction 5 and EFA-enriched fraction 8 on *in vivo* IgE responses and *in vitro* FcRe induction. Ammonium sulfate-precipitated SJL ascites fluid was subjected to ion-exchange chromatography on DEAE cellulose followed by gel filtration chromatography on Sephadex G-200. Fraction 5 was found to be enriched for SFA activity and consisted of molecules in the 30,000–50,000 dalton range, while EFA-enriched fraction 8 consisted of molecules in the 10,000–15,000 dalton range. These fractions were tested for their biological activities on *in vivo* irradiation-enhanced IgE responses of SJL mice and on *in vitro* IgE-mediated induction of FcRe⁺ cells in cultures of CAF₁ spleen cells. The unirradiated low-level *in vivo* response is shown in the left panel. The respective positive *in vivo* IgE responses and *in vitro* FcRe induction responses, obtained in the absence of exposure to SFA or EFA, are depicted in the middle panel as 100% positive control values, with the actual experimental values listed atop the corresponding bars. The *in vivo* IgE responses and *in vitro* FcRe induction responses obtained in experimental groups either treated or exposed to SFA (fraction 5) or EFA (fraction 8), respectively, are depicted in the right panel as relative percent of the 100% control responses in each bioassay. Results presented represent the mean responses obtained *in vivo* in groups of 4 mice each and the mean frequencies of FcRe⁺ cells enumerated in each group determined from 3–4 counts of approximately 400 live small lymphocytes examined in each sample pool. (Taken from Ref. 48 with permission of the publisher).

tors that have the property of binding specifically to the Fc domain of IgE (44–47). The associated production of such IgE-binding factors and FcRe⁺ lymphoid cells strongly suggested that the regulation of IgE synthesis is mediated by a series of cellular interactions revolving around the expression of FcRe by lymphocytes. Recent studies in our own laboratory directed at unravelling the physiologic functions of FcRe⁺ lymphocytes, and the manner in which they themselves may be regulated, have provided some new insights that will be summarized in the following sections.

FcRe induction by in vitro exposure to IgE can be modulated by both SFA and EFA

Recently, studies in our laboratory by Chen et al. (36) demonstrated that SFA-containing ascites fluid from SJL mice selectively inhibited the IgE-mediated *in vitro* induction of FcRe⁺ lymphocytes. In a more detailed analysis designed to further characterize SFA and EFA, and to directly compare their biochemical and biological properties, we documented that it was possible to distinguish SFA and EFA by certain chromatographic techniques (48). The unanticipated finding was that although SFA and EFA exert dramatically distinct biological effects in regulating IgE antibody synthesis *in vivo*, the two respective molecular entities exert precisely identical modulating effects on the induction of FcRe⁺ lymphocytes by IgE *in vitro*.

The results summarized in Fig. 8 illustrate these points. Ammonium sulfate-precipitated ascites fluid was subjected to ion-exchange chromatography on DEAE-cellulose followed by gel filtration chromatography on Sephadex G-200. Two active fractions were identified on the basis of exerting IgE-selective regulatory effects on *in vivo* IgE antibody responses in a manner similar to those illustrated in Figs. 4 and 5. One fraction (denoted fraction 5), containing molecules in the 30,000–50,000 dalton range, exerted marked suppressive activity characteristic of SFA, while a second fraction (fraction 8) with molecules eluting in the 10,000–15,000 dalton range was clearly enriched for EFA activity. These respective frac-

tions were then compared for their biological effects on *in vivo* IgE production and *in vitro* FcRe expression.

As shown in Fig. 8, control responses of unirradiated mice (left panel) are typical, barely detectable low IgE responses. In contrast, irradiated SJL mice (serving as 100% controls) yielded good IgE responses (middle panel); likewise, the positive control response, shown by the adjacent bar, in the FcRe induction assay yielded a frequency of 18%. As shown in the far right panel, SFA-enriched fraction 5 completely suppresses the *in vivo* IgE antibody response, whereas EFA-enriched fraction 8 enhances these same IgE responses by 400%. In striking contrast, both fractions inhibit the induction of FcRe⁺ lymphocytes by approximately 50% of the control value. Thus, while SFA and EFA have contrasting suppressive and enhancing effects, respectively, on *in vivo* IgE responses, the two molecular entities behave identically in inhibiting, by around 50%, the *in vitro* induction of FcRe⁺ lymphocytes. Since, in each case, 50% inhibition of FcRe induction is usually observed, such results suggest that SFA and EFA may be acting on different populations of FcRe⁺ lymphocytes; some data demonstrating this to be the case will be presented below.

It should be pointed out here that this modulation of receptor expression by SFA and EFA is highly selective for IgE-specific Fc receptors, since these same factors do not affect the expression of Fc receptors with specificities for other Ig classes.

It must also be emphasized that the fact that SFA and EFA, which can be distinguished on the basis of molecular size and the dramatically opposite functional effects they exert on *in vivo* IgE responses, display identical effects in both quality (inhibitory) and magnitude on IgE-mediated FcRe induction on lymphocytes, strongly suggests that FcRe⁺ lymphocytes are important constituents of the IgE class-selective regulatory mechanisms, and probably belong to at least two distinct subpopulations of lymphocytes. It is difficult to imagine that molecules such as SFA and EFA, which serve as such potent regulators of the physiologic *in vivo* IgE

response, and which do so in a highly isotype-selective manner, would exert effects on cells bearing receptors for IgE, also in a highly selective fashion, only by coincidence.

Evidence for a physiologic role of FcRε⁺ lymphocytes in in vivo regulation of IgE antibody production

In order to further define meaningful physiologic participation of lymphoid cells capable of binding IgE through the expression of FcRε in isotype-restricted regulation, we reasoned that the IgE antibody system might be susceptible to manipulation with molecules that interact specifically with the relevant cellular components in immature animals. We thus administered soluble IgE or cell-bound IgE – i.e., IgE covalently coupled to cell membranes of syngeneic spleen

cells – to mice beginning at the neonatal period of life. When these animals were tested for their ability to develop antibody responses after they had matured to adulthood, we observed a novel type of immunological tolerance induced selectively and polyclonally in the class of IgE antibody responses (49).

Fig. 9 demonstrates a representative experiment of this type. All mice in the various groups indicated were treated on three occasions during the neonatal period of life with either control materials or soluble or cell-bound IgE. At 2.5 months of age, all mice were immunized according to a regimen designed to elicit both IgE and IgG antibody responses. As shown in Fig. 9, the two control groups, consisting of mice treated either with saline (group I) or with unconjugated syngeneic spleen cells from adult donors (group III) during the neonatal period of life, develop good antibody responses in both the IgE and IgG antibody classes. In striking contrast, however, mice treated neonatally with either soluble IgE (group II) or with two different forms of cell-bound IgE (groups IV and V) are completely unable to mount antibody responses in the IgE class. This unresponsiveness is, however, restricted to the IgE class since responses in the IgG class, as shown here (49), or in the IgA class, presented elsewhere (50), are perfectly intact.

Detailed analysis of this IgE class-restricted tolerance following neonatal exposure to soluble or cell-bound IgE has revealed that this state persists for long periods, essentially throughout the lifetime of the treated mouse, and that it is mediated by IgE-restricted suppressor T cells (50). The process can be induced with IgE, irrespective of antigen specificity employed for the induction events, and the effects are polyclonal in the sense that IgE responses of any and all specificities are blunted by this process (49, 50).

Since the hypothesis leading to the design of the immediately preceding experiments was that FcRε⁺ lymphocytes played important regulatory roles in the IgE antibody system, it was naturally of pertinence to examine whether mice treated neonatally with IgE, and thus rendered tolerant in the IgE class-restricted

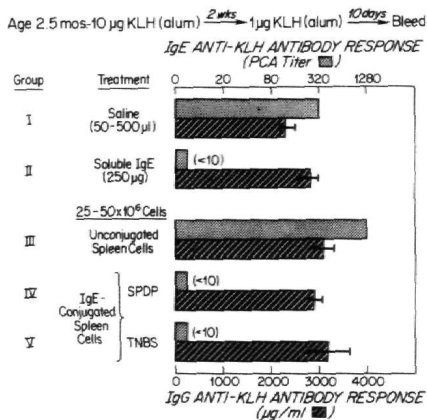


Fig. 9. Induction of IgE class-restricted tolerance by neonatal exposure of (BALB/c × SJL)_{F1} mice to soluble IgE or IgE-conjugated syngeneic spleen cells. (BALB/c × SJL)_{F1} hybrid mice were injected i.p. with 250 μg of soluble IgE, IgE bound to 25-50 × 10⁶ splenocytes or control material on day 0 and 7 after birth in 50 μl saline and in 100 μl saline on day 17. Mice in each group were later given the same material in 500 μl saline i.p. on day 24, 34 and 59. At approximately 2.5 months of age, the mice were immunized i.p. with 10 μg of KLH in 2 mg alum, and 14 days later were boosted with 1 μg of KLH in 2 mg alum. Sera were collected 10 days later. The IgE anti-KLH antibody responses were measured by PCA reactions and the IgG anti-KLH antibody responses were measured by RIA. Concentration of IgG anti-KLH antibody response is expressed as geometric means ± standard errors of the means of at least 4 individual values from each group. (Taken from Ref. 49 with permission of the publishers).

fashion, displayed abnormal cellular behavior with regard to *in vitro* induction of FcRe⁺ lymphocytes by exposure to monoclonal IgE. To make this analysis, the ability of spleen cells from mice treated neonatally with monoclonal IgE to respond to IgE by expression of FcRe was compared to parallel control groups of mice (50).

Fig. 10 illustrates a dose-response plus kinetics of such spleen cells to respond to IgE *in vitro*. As shown in panel A, spleen cells from mice treated neonatally with saline give rise to 26% FcRe⁺ cells upon exposure to IgE *in vitro*. Likewise, spleen cells from mice treated neonatally with soluble IgA manifest about 12–15% FcRe⁺ cells in response to the same exposure. In contrast, spleen cells from mice treated neonatally with soluble IgE of either one of two different specificities (DNP or ragweed), or with IgE-conjugated spleen cells, are significantly impaired in their ability to express FcRe. As shown in panel B, similar results were obtained in the same cultures allowed to incubate in the presence of IgE for an additional 24 hours (50).

These results, demonstrating that mice that are unable to develop normal IgE antibody re-

sponses following the induction of IgE class-restricted tolerance by neonatal treatment with IgE, have an associated defect in the ability of their lymphoid cells to respond to IgE-mediated induction of FcRe expression, strongly implicate a true physiologic role for FcRe-positive lymphocytes in regulation of the IgE antibody system.

B lymphocytes can exert isotype-restricted regulatory effects on the IgE antibody system

As stated earlier, the IgE antibody system serves as a paradigm example of how a finely tuned network of interacting cells and soluble products from such cells carefully regulate the quality and quantity of a given humoral immune response. In terms of the cellular regulatory mechanisms governing such responses, and other types of immune responses as well, most of the emphasis in past years has focused on the complex array of regulatory T cells which participate in the generation and limitation of such responses. Other than scattered reports of regulatory effects of B lymphocytes on some cell-mediated, and even fewer humoral, immune responses, comparatively little is

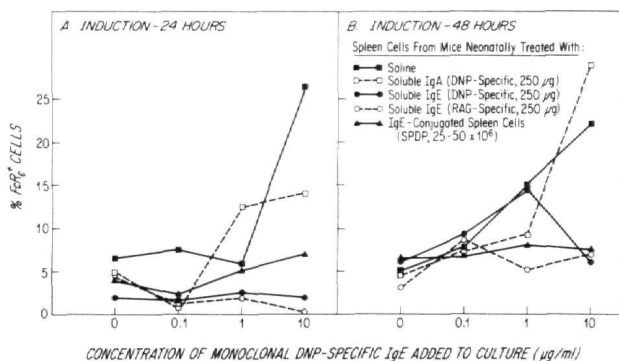


Fig. 10. Induction of FcRe⁺ cells in culture of (BALB/c × SJL)_{F1} spleen cells exposed to different doses of monoclonal DNP-specific IgE. (BALB/c × SJL)_{F1} hybrid mice were injected neonatally with IgE or control material in the experiment summarized in Fig. 9. These mice were pooled from groups of mice receiving the same materials 3 to 4 times from the aforementioned three separate experiments. All mice had been immunized with 1 µg KLH in alum 3 times at 10–14 day intervals, and were rested for approximately 3 months. Mice were about 6 months old at the time of assay. Single cell suspensions were made from spleens of 2–3 mice from each group. 10⁷ splenic lymphocytes were cultured in RPMI with 5% FCS in the presence of different concentrations of monoclonal DNP-specific IgE for 24 h and 48 h. The appearance of FcRe⁺ cells was enumerated by their capacity to form rosettes with IgE-coated TNP-SRBC as indicator cells. The values presented represent the arithmetic means of rosette-forming cells determined from 3–4 counts of approximately 400 live small lymphocytes examined in each sample pool. (Taken from Ref. 50 with permission of the publishers).

known about the extent to which B lymphocytes can perform regulatory functions in the immune system.

During the course of experiments designed to continue earlier work from this laboratory analyzing biological differences between B lymphocytes involved in IgE antibody responses and antibody responses of other immunoglobulin isotypes (51, 52), we made the unexpected observation that mature splenic B lymphocytes exert very definite regulatory effects on the IgE antibody responses of mice undergoing spontaneous hematopoietic reconstitution following sublethal irradiation (53). Moreover, the type of regulatory influence observed appears to be determined by the IgE responder type of both the donors and recipients of such cells.

Fig. 11 depicts the results obtained in low responder SJL, low $\times$ intermediate responder (SJL $\times$ B6) F_1 and low $\times$ high responder (SJL $\times$ BALB/c) F_1 recipients of corresponding parental donor lymphoid cells (53). Such cells were obtained from mature donors treated *in vivo*

with anti-lymphocyte serum to deplete ALS-sensitive peripheral T cells, and then the recovered spleens from such donors were further T cell-depleted by *in vitro* treatment, twice consecutively, with anti-Thy 1.2 + complement. All recipients were sublethally irradiated prior to cell transfer and then were immunized for generation of IgE and IgG antibody responses at varying times. In each case, control groups consisted of parental or F_1 mice subjected to the same dose (700 rads) or irradiation, but *not* reconstituted with T cell-depleted donor lymphoid cells. Such control groups manifested clear evidence of self-reconstitution of their immune systems as verified by their ability to mount antigen-specific IgE responses by day 14 after irradiation and immunization, and such responses rose progressively thereafter.

As shown in the left panel of Fig. 11, the magnitude of such self-reconstituting responses, as manifested by control mice, was substantially decreased in SJL recipients of SJL donor cells at all time points during the course

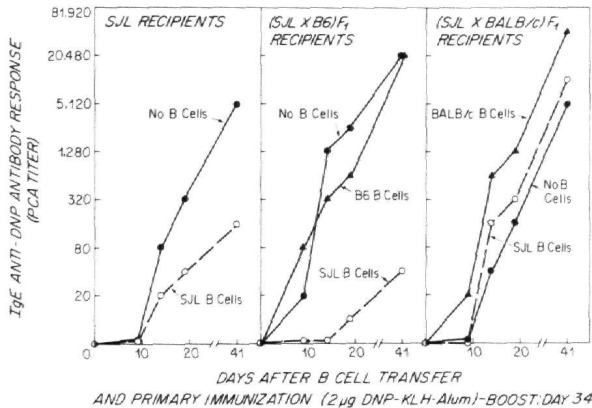


Fig. 11. Low IgE responder SJL B cells exert suppressive effects on IgE responses of low responder and (low $\times$ intermediate responder) F_1 hybrid mice, but not (low $\times$ high responder) F_1 mice, exposed to sublethal irradiation (700 rads). Recipients of the types indicated were primed with 2 μ g of KLH adsorbed on 2 mg of alum, i.p. 7 days prior to irradiation (day -7). On day 0, all recipients were exposed to 700 rads of irradiation, and then injected intravenously with 25×10^6 T cell-depleted donor spleen cells, of the donor type indicated. Control groups were KLH-primed and exposed to irradiation, but did not receive donor spleen cells. The donor spleen cells transferred were obtained from normal donor mice, of the strain indicated, which had been treated *in vivo* with 150 μ l of ALS 3 days prior to sacrifice in order to eliminate the ALS-sensitive T cell subset. The spleen cells harvested from such mice were then subjected to two successive *in vitro* treatments with anti-Thy 1.2 + C. Soon after donor cell transfer, all recipients, including controls, were immunized i.p. with 2 μ g of DNP-KLH adsorbed on 2 mg of alum. All mice were bled on days 10, 14 and 19, boosted a second time on day 34 with the same dose of DNP-KLH followed by a final bleeding on day 41. The data are presented as mean IgE levels, as determined by PCA reactions, of groups of five mice each bled on the days indicated. (Taken from Ref. 53 with permission of the publishers).

of the experiment. Transferred low responder SJL B cells likewise significantly suppressed self-reconstituting responses of (SJL $\times$ B6) F_1 (middle panel), whereas intermediate parental responder B6 B cells did not exert a comparable suppressive effect in such recipients. In contrast, when (SJL $\times$ BALB/c) F_1 mice were employed as recipients in this system (right panel), neither low parental responder SJL, nor high parental responder BALB/c B cells exerted suppressive influences on the self-reconstituting IgE responses; indeed, such responses in these recipients were enhanced 4-fold or more by the transfer of high responder parental BALB/c cells. Although not shown in order to maximize clarity of the figure, both (SJL $\times$ B6) F_1 and (SJL $\times$ BALB/c) F_1 recipient mice were injected with their corresponding syngeneic F_1 -type donor cells; in both cases the responses obtained were essentially identical to those obtained in the self-reconstituting F_1 mice which did not receive transferred B cells.

It should be noted that IgG responses in all groups depicted in the figure were comparable, therefore documenting the IgE-selective nature of the regulatory effects exerted by the transferred B cells (53).

These findings are novel in that they present evidence for isotype-restricted regulation by B cells – in this case the responses of the IgE antibody class – as contrasted to the more classical examples of such regulation mediated by T cells. Such findings prompted us to determine whether thymus-deficient mice could be induced to produce SFA and/or whether SFA would be effective in selectively suppressing IgE antibody production in such thymus-deficient environments.

The experiment summarized in Fig. 12 demonstrates that thymectomized low responder C57BL/6 mice produce SFA in response to inoculations with complete Freund's adjuvant, and are susceptible to suppression by SFA obtained from both intact and athymic B6 donors (54). All of the mice employed in groups I–IV were thymectomized at 8–10 weeks of age and then treated with anti-lymphocyte serum to remove any residual peripheral T cells. Several weeks later, these mice were used either as recipients in groups I–IV or as donors of SFA-containing serum obtained 7 days after inoculation with CFA. For comparison, SFA-containing serum from normal (euthymic) B6 mice of the same age was also tested.

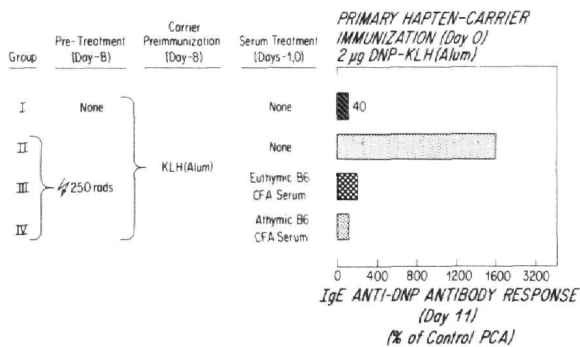


Fig. 12. Thymectomized low responder C57BL/6 mice produce SFA following CFA-priming and are susceptible to suppression by SFA obtained from both intact and athymic B6 donors. B6 mice were thymectomized at 8–10 weeks of age and then treated with anti-lymphocyte serum intravenously to remove any residual peripheral T cells. Several weeks later, some of these mice were inoculated with CFA and bled 7 days thereafter as a possible source of SFA-enriched serum. Normal, non-thymectomized (euthymic) B6 mice of corresponding ages were similarly primed with CFA and bled 7 days thereafter. The remaining thymectomized mice were divided into groups I–IV and subjected to the protocol of this experiment as indicated on the left side of the figure. The immunization schedule and the schedule of injections of serum samples were identical to those described in Fig. 4. The IgE anti-DNP antibody responses on day 11 after primary sensitization are presented as percentages of the respective untreated control response with the actual group I titers listed beside the corresponding bar.

As shown in Fig. 12, thymectomized B6 mice respond to low dose irradiation by developing enhanced IgE responses as compared to unirradiated controls (group II versus I). This irradiation-enhanced IgE response (IgG responses were not affected) could be reversed by the administration of SFA-containing serum not only from normal mice (group III) but also from athymic B6 donors (group IV). These results suggest, therefore, that production of SFA is either not a T cell-dependent process or can occur under conditions of very limited T cell availability. Moreover, SFA can mediate IgE-selective suppression either directly on B cells or in conditions of limited T cell participation (54). More recent data obtained in our laboratory suggest that B cells expressing ϵ -specific Fc receptors (FcR ϵ), as a result of exposure to IgE *in vitro* or *in vivo*, can exert very definite and very selective regulatory effects on the IgE antibody system, as discussed in more detail below.

A new class of molecules (IgE-induced regulants (EIR)) can modulate FcR ϵ expression by lymphocytes

Our growing appreciation of the physiologic involvement of FcR ϵ^+ lymphocytes in the regulation of the IgE system, coupled with our curiosity pertaining to B cell-dependent IgE class-specific regulation, prompted us to dissect the sequence of regulatory steps in this system more stringently. Since only a very small proportion of normal lymphocytes express FcR ϵ , we addressed the question of whether the amplification of FcR ϵ expression is caused by the interaction of lymphocytes with IgE directly or is mediated through the production of soluble factors. The results of such studies are summarized below (55, 56).

Lymphoid cells induced to express FcR ϵ by exposure to monoclonal IgE secrete IgE-induced regulants (EIR) that regulate FcR ϵ expression by other lymphoid cells

Normal mouse spleen cells were incubated in the presence or absence of monoclonal IgE.

After 24 hours, induction of FcR ϵ^+ cells in such cultures was confirmed and the supernatants harvested and assayed for the presence of IgE-induced regulants (EIR). In order to eliminate the possibility of carry-over of some of the original IgE placed in the first culture, all such culture supernatants were first passed over affinity columns prepared with anti-IgE antibodies.

The ability of varying concentrations of EIR to induce FcR ϵ was compared to FcR ϵ induction by different doses of monoclonal IgE. As shown in Fig. 13, cells cultured in the presence of increasing doses of IgE display a dose-related induction of FcR ϵ^+ lymphocytes, the optimal IgE concentration being 10 μ g/ml. In addition, 24-hour supernatants from spleen cell cultures exposed to IgE induced high levels of FcR ϵ expression even at final concentrations as low as 1% of the total culture volume, thereby documenting that cells exposed to IgE produce soluble factors, termed EIR, that can, in turn, induce FcR ϵ in fresh spleen cell cultures (55).

Some of the pertinent findings about the EIR generated in the fashion described above can be summarized as follows:

1) As stated above, the ability of EIR to induce FcR ϵ expression is totally unrelated to carry-over of IgE or fragments therefrom.

2) EIR obtained by exposing whole spleen cell cultures to IgE induces FcR ϵ expression preferentially by T cells – such EIR is therefore denoted EIR $_T$. In contrast, IgE alone induces FcR ϵ expression by B cells. In other words, B cell-depleted lymphoid cell cultures fail to respond to monoclonal IgE by FcR ϵ expression, whereas the same cultures will develop high levels of FcR ϵ^+ cells following exposure to EIR.

3) On the other hand, exposure of an intact spleen cell culture (i.e., mixtures of T and B cells) to monoclonal IgE results in the induction of FcR ϵ^+ spleen cells that are equally distributed between T and B lymphocytes; in other words, when B cells are present, exposure to IgE results in the induction of FcR ϵ^+ T cells. On the other hand, even when mixtures of B and T cells are exposed to EIR $_T$, the resulting FcR ϵ^+ cells are restricted to the T lymphocyte class.

Collectively, the aforementioned findings in-

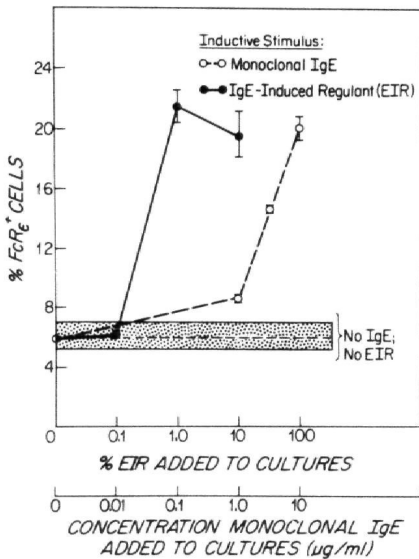


Fig. 13. A 24-h supernatant from IgE-induced $\text{Fc}\epsilon\text{R}^+$ spleen cells – termed IgE-induced regulator (EIR) – can induce $\text{Fc}\epsilon\text{R}$ expression in fresh spleen cell populations. Normal BALB/c spleen cells were incubated for 24 h in either medium alone, in medium containing the indicated concentration of IgE, or in medium containing the indicated concentration of EIR. EIR was obtained as the culture supernatant from another spleen cell culture stimulated for 24 h with IgE; the resulting supernatant was adsorbed on a goat anti-mouse IgE affinity column before being used in the experiment depicted here. After 24 h of exposure to either nothing, IgE or EIR, the cells were harvested and assayed for $\text{Fc}\epsilon\text{R}$ expression by rosette formation with IgE-coated sheep erythrocytes. The geometric mean value for the negative control is indicated by the dashed line with the standard error of the control mean depicted by the surrounding shaded horizontal bar. For the experimental groups, each point represents the geometric mean (vertical lines equal ranges of standard errors) of rosette-forming cells for each culture condition, as determined from 3 counts of 250–300 viable lymphocytes. (Taken from Ref. 55 with permission of the publisher).

indicate that production of EIR_T , and subsequent expression of $\text{Fc}\epsilon\text{R}$ in T cells, is dependent on the initial interaction of IgE with B cells which, in turn, express $\text{Fc}\epsilon\text{R}$ on their surfaces. In view of the association between $\text{Fc}\epsilon\text{R}^+$ T cells and elaboration of IgE class-specific regulatory molecules, these findings further strengthen a crucial role for B lymphocytes in IgE class-specific regulation (55).

$\text{Fc}\epsilon\text{R}^+$ B lymphocytes initiate a cascade of cellular and molecular interactions controlling $\text{Fc}\epsilon\text{R}$ expression and IgE production

As summarized in the preceding section, the production of EIR_T is dependent upon the initial interaction of IgE with B cells. These, and the data described in earlier sections, imply that B cells function as important sensors and triggers for normal homeostatic regulation of IgE levels. Further examination of the cellular and molecular interactions pertinent to regulation of $\text{Fc}\epsilon\text{R}$ expression in B and T lymphocytes has recently substantiated the suggestion that the regulation of $\text{Fc}\epsilon\text{R}$ expression is accomplished through a complex cellular and molecular network which, itself, is an integral component in a larger network that controls IgE synthesis (56).

The results of these analyses have revealed that:

1) Induction of $\text{Fc}\epsilon\text{R}$ expression by EIR_T requires the presence of Lyt-2^+ , but not Lyt-1^+ , T cells, while IgE induction of $\text{Fc}\epsilon\text{R}$ expression requires Lyt-1^+ , but not Lyt-2^+ , T cells.

2) Moreover, when Lyt-1^+ T cells are selectively depleted, the remaining Lyt-2^+ T cells completely inhibit the induction of $\text{Fc}\epsilon\text{R}^+$ B lymphocytes by monoclonal IgE. Indeed, this Lyt-2^+ cell inhibitory activity is mediated through a second, and distinguishable, type of EIR – denoted EIR_I (i.e., inhibitory).

3) EIR displays restricted target cell specificity inability to induce $\text{Fc}\epsilon\text{R}$ expression – i.e., EIR from B cells works selectively on B cells (EIR_B) while EIR from T cells work selectively on T cell targets (EIR_T).

4) EIR_T is a product of Lyt-2^+ T cells and this in turn stimulates the production, by $\text{Fc}\epsilon\text{R}^+$ Lyt-2^+ T cells, of EIR_I which inhibits $\text{Fc}\epsilon\text{R}$ expression in B cells.

5) The inhibitory effects of EIR_I on induction of $\text{Fc}\epsilon\text{R}^+$ B cells can be blocked by the activity of Lyt-1^+ T cells.

Collectively, these and other data have allowed us to assemble the following links in a chain of cellular and molecular interactions in this system. First, IgE interacts with B cells provoking the production of EIR_B which, in turn, provides the inductive stimulus for these,

or other, B cells to express FcR ϵ on their surface membranes. These FcR ϵ^+ B cells can, by themselves, produce SFA, but the magnitude of SFA production is considerably improved in the presence of Lyt-1 $^+$ T cells.

Second, these FcR ϵ^+ B cells provide signals to Lyt-2 $^+$ T cells to produce EIR $_T$ which acts on these or other Lyt-2 $^+$ T cells to express FcR ϵ on their surface membranes. These FcR ϵ^+ , Lyt-2 $^+$ T cells produce EIR $_I$ which can, depending on the relative functional presence of Lyt-1 $^+$ T cells, inhibit further expression of FcR ϵ by B cells.

Third, EFA produced by as yet an undefined subset of T cells exerts its inhibitory effects on FcR ϵ induction by blocking Lyt-1 $^+$ T cells that otherwise would counterbalance the inhibitory effects of EIR $_I$. In other words, EFA is an indirect inhibitor of FcR ϵ induction by IgE *in vitro*.

Fourth, in addition to B cell production of SFA, SFA can also be produced by T cells of the Lyt-1 $^+$ subset, and its inhibitory effect on FcR ϵ expression also appears to result from a blocking effect on the Lyt-1 $^+$ T cells that normally counterbalance these inhibitory effects of EIR $_I$.

Uede et al. have reported that Lyt-1 $^+$ T cells are required for IgE-induced production of IgE-binding factors – those soluble mediators that also selectively modulate IgE synthesis (47). The results summarized above demonstrate that Lyt-1 $^+$ T cells function to potentiate FcR ϵ expression in other lymphoid cells. Moreover, Lyt-1 $^+$ cells appear also to be necessary for optimal levels of SFA production. However, Uede et al. also observed that B cell depletion did not abrogate IgE-binding factor production in response to IgE. In our hands, B cell depletion resulted in the loss of FcR ϵ induction by IgE, but the resulting culture supernatants have not been tested for IgE-binding factors. We do know, however, that neither EIR $_T$ nor EIR $_B$ possesses such IgE-binding properties. In ongoing investigations, we are trying to sort out the interrelationships between SFA, EFA, IgE-binding factors, and FcR ϵ expression in T and B lymphocytes.

Recent studies indicate the existence of IgE-selective regulatory mechanisms in man similar to those discovered in mice

The clinical relevance of the aforementioned findings in experimental animals, and those related to them, will only be demonstrated after such information has been translated to the human IgE antibody system in an appropriate fashion so as to verify the extent to which parallel mechanisms operate in man. Some studies in other laboratories demonstrated the biosynthesis of IgE antibody molecules by human lymphocytes *in vitro*, both spontaneously and following stimulation by suitable polyclonal mitogenic substances (57–60). We set out to develop a system in our own laboratory whereby human lymphocytes could be stimulated by polyclonal mitogens and/or by specific antigens to develop primary IgE responses *in vitro*. This type of system could then be used to assay for the existence of, and to investigate the properties of, IgE-selective regulatory cellular and molecular interactions in man.

1) *Mechanisms of the in vitro human IgE antibody system.* Heparinized venous blood was obtained from healthy volunteer donors with no history of allergy; mononuclear cells (MNC) were isolated from such samples and used either as unfractionated MNC or MNC fractionated into T cell and non-T cell-enriched fractions. The samples of human MNC thus obtained were then cultured in a special system as described in detail elsewhere (61–63). An important feature of this system is the separation of the culture period into two stages, a first-stage stimulation culture during which cells are exposed to mitogen (such as pokeweed mitogen, PWM), to antigen, or both, for 3 days following which they are washed thoroughly and then resuspended in fresh medium. The cells are then re-plated in either micro- or macrowell culture plates for an additional 6-day second-stage incubation during which time the cultures are devoid of any mitogen, antigen or

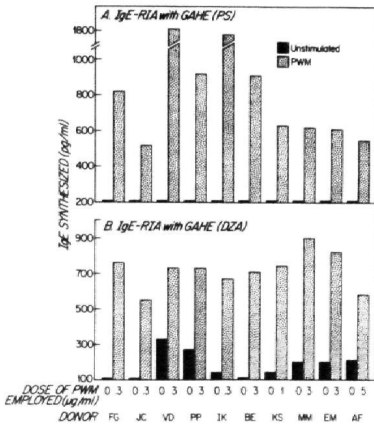


Fig. 14. IgE synthesis *in vitro* by cells from 10 different human donors can be detected equally well by GAHE (PS) and GAHE (DZA). Peripheral MNC from 10 different donors were cultured according to the system described in the text in the absence or presence of varying concentrations of pokeweed mitogen (PWM). The two-stage culture system described in the text was employed. At the end of the culture period, the level of IgE secreted into culture supernatants was determined by RIA using two different goat anti-human IgE reagents. The top panel illustrates the values obtained with GAHE adsorbed on the myeloma protein PS, while the bottom panel depicts the values obtained from the very same culture supernatants measured with GAHE that had been affinity-purified on the unrelated myeloma protein, DZA. The values shown represent geometric mean levels of IgE production ascertained on triplicate assays on supernatants obtained from cultures set up in replicates of 10 per group.

other substances. At the conclusion of the second-stage incubation, the culture supernatants are recovered and analyzed by radioimmunoassay (RIA) for the levels of IgE and IgG antibodies. The RIA for human IgE employed in this system has been described in detail elsewhere (62) and has been shown to be 1) highly specific for the ϵ heavy chain, and 2) sufficiently sensitive to measure human IgE down to levels as low as 10 μ g/ml in culture fluids.

2) *Stimulation of IgE synthesis by pokeweed mitogen.* Using the two-stage culture system described above, isolated MNC from a variety of different normal donors have been cultured with PWM at varying doses, and culture supernatants were assayed for IgE protein. Although PWM at a concentration of 1 μ g/ml most often proved to

be optimal, the optimal PWM dose may vary downward or upward for different donors and/or the same donor at different times. In general, at the optimal dose employed, PWM stimulated increases, substantial in most cases, in IgE levels ranging from 4- to 75-fold, with an average 18-fold increase in a selected group of 14 donor individuals (62). These data are representative of more than 300 determinations of this type performed over a several-year period. It is true that, on occasion, we fail to observe stimulation of IgE biosynthesis after stimulation by PWM, but this failure seldom occurs in disease-free, nonatopic donors.

Fig. 14 summarizes the results obtained with MNC from 10 different donors stimulated with varying doses of PWM. The distinction between the upper panel A and lower panel B lies in the goat anti-human IgE (GAHE) reagent employed for detecting IgE responses by RIA of the culture supernatants. The values shown in the upper panel were obtained with GAHE that had been affinity-purified on the same IgE myeloma protein (PS) as that used for hyperimmunization to raise the goat antiserum; the values depicted in the lower panel were those obtained on the same culture supernatants using GAHE that had been affinity-purified on a completely unrelated IgE myeloma protein (DZA) than that used for immunization. The point being that irrespective of which reagent was used for the RIA, cultures stimulated with PWM clearly displayed levels of detectable IgE that were significantly above the baseline values of IgE detected in the parallel unstimulated control cultures (64). Such responses represent *de novo* biosynthesis of IgE as a result of PWM stimulation, since incorporation of the protein synthesis inhibitor, cycloheximide, totally abolishes the capacity of human MNC to synthesize IgE in response to PWM (62).

3) *Pokeweed mitogen stimulation of human IgE synthesis in vitro is T cell-dependent.* MNC from normal donors were separated into T cell and non-T cell populations and then cultured either as isolated fractions or varying mixtures of the respective fractions. As shown in Fig. 15, while unfractionated MNC respond to PWM by

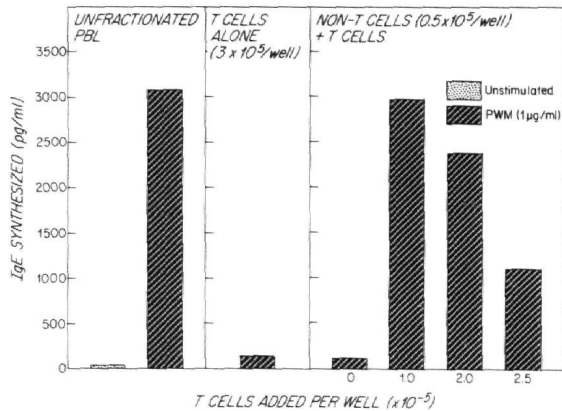


Fig. 15. T cell dependence of pokeweed mitogen-stimulated human IgE synthesis *in vitro*. Human MNC were separated into T cell and non-T cell fractions by standard B-rosette fractionation procedures. Unfractionated or fractionated MNC were cultured in the indicated numbers in tubes with or without stimulation with pokeweed mitogen. At the end of the stimulation stage of the culture, cells were washed and re-plated in 10 identical aliquots for 6 days in V-bottom microtiter plates. (Modified from data presented in Ref. 62).

the synthesis of substantial quantities of IgE (left panel), cultures of the isolated T cell-enriched (middle panel), or non-T cell-enriched fractions alone (1st bar, right panel) fail to synthesize IgE in response to PWM. In contrast, addition of appropriate quantities of the two respective fractions to one another (right panel, last 3 bars) completely reconstituted the IgE response to PWM (62). Additional experiments also demonstrated that this system of *in vitro* synthesis of human IgE is dependent on the presence of surface IgE-bearing cells in the cultured MNC population in order to obtain such responses (62).

4) *Selective inhibition of IgE synthesis by human suppressive factor of allergy (SFA)*. Previous studies have demonstrated that SFA-like biological activity could be recovered from culture supernatants of two-way human mixed lymphocyte reactions (MLR) and could exert its selective inhibitory effects on IgE antibody responses of experimental mice (65). Having a system available for stimulating and measuring human IgE synthesis *in vitro*, we next addressed the question of whether human SFA could effectively and selectively suppress IgE synthesis by human MNC. We now have numerous experiments

which confirm that human SFA exists and is capable of selectively inhibiting IgE antibody synthesis.

Fig. 16 illustrates the capacity of SFA to selectively inhibit IgE synthesis, even when exposure of cells to the inhibitory material is delayed until after stimulation by PWM (62). Thus, MNC were placed in culture tubes on day -4; one culture group was exposed at this time to an MLR supernatant at a final concentration of 15%, while the other two culture groups were not exposed to SFA-containing supernatant. On day -3, PWM was added to all three culture groups, and the stimulation phase of the culture was allowed to proceed in the usual manner for 3 additional days. On day 0, the MNC were thoroughly washed and then plated in microwell cultures for 6 additional days; in one case, cells were cultured in this second stage in the presence of the same MLR supernatant, while the other two culture groups were not exposed to SFA during this period.

As shown in the bottom panel of Fig. 16, substantial inhibition of IgE synthesis was observed in both culture groups exposed to SFA, irrespective of whether the SFA was added 1 day prior to PWM stimulation (day -4) or at the termination of the stimulation stage and at the

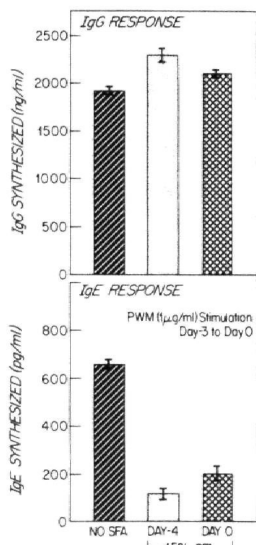


Fig. 16. Selective suppression of IgE synthesis by SFA added to human lymphocytes either before or after stimulation by pokeweed mitogen. 3×10^6 MNC from a single normal donor were cultured in plastic tubes either alone (left and right bars) or in the presence of culture supernatant from an 8-day 2-way human MLR at a final concentration of 15% (center bar). 24 h later, PWM (1 μ g/ml) was added to each of the 3 cultures. 3 days later (day 0) the cells in each culture group were washed and plated in microcultures (10 replicate microwells per group) for 6 additional days either in the absence of MLR supernatant (left and center bars), or in the presence of 15% MLR supernatant (right bar). The mean levels of IgG (upper panel) and IgE (lower panel) synthesized by each culture group are depicted. (Taken from Ref. 62 with permission of the publishers).

onset of the second, synthetic stage of the culture (day 0). Importantly, the inhibitory effects of SFA are selective for the IgE response since, as shown in the top panel, IgG synthesis in the same cultures was not all inhibited. This lack of effect on IgG synthesis in the same cultures furthermore rules out the possibility of SFA exerting its inhibitory effects as a result of some sort of nonspecific toxicity. It is also worth emphasizing that in other experiments we have shown that SFA can successfully inhibit *in vitro* IgE synthesis by MNC derived from patients with hyperimmunoglobulinemia E syndrome (62).

5) *Human SFA inhibits the growth of the human IgE myeloma cell line, U-266.* Studies by Kishimoto et al. (66) have demonstrated that the suppressive activity of certain IgE-selective suppressive factors (IgE-SF) is removed after adsorption with primed B cells, and that treatment of primed B cells with such IgE-SF abolishes their IgE responses, suggesting that certain IgE-SF can recognize and act directly on IgE-precursor and/or IgE-secreting B cells. These results prompted us to test the biological effects of human SFA on the human IgE myeloma cell line, U-266.

The IgE-secreting human myeloma cell line, U-266 (67) was kindly provided by Drs. K. Nilsson and A. Saxon. In order to determine what effects, if any, the addition of SFA-enriched human MLR supernatants might have on the kinetics or amounts of IgE secretion by the U-266 line, human MLR supernatants were added (at a final concentration of 25%) to the cells, and the secreted IgE in the supernatants was measured by RIA on days 2 and 3. As shown in Fig. 17, such experiments demonstrated that human MLR supernatants inhibited IgE secretion by U-266 cells quite substantially, particularly early in the course of cell growth (68). Thus, on day 2, control supernatants of U-266 cells alone contained 94 ng/ml of IgE, while U-266 cells cultured with human SFA secreted only 43 ng/ml IgE, a 54% suppression. However, this highly reproducible inhibitory effect of SFA on IgE synthesis was only transient, as the degree of suppression declined dramatically to only 2% on day 3. Although the precise reason for this transience of suppression is unclear, it nevertheless demonstrates that the suppression by SFA of the kinetics of IgE production by U-266 cells is not due to cytotoxic effects of SFA on the cells themselves.

The fact that human SFA can regulate IgE secretion by a human IgE myeloma cell line indicates that human SFA can act directly on at least certain cells of the B cell lineage. In addition, the selective suppression of IgE production by U-266 cells exposed to human SFA gives us an extremely helpful and, more importantly, a powerful and rapid bioassay for human SFA.

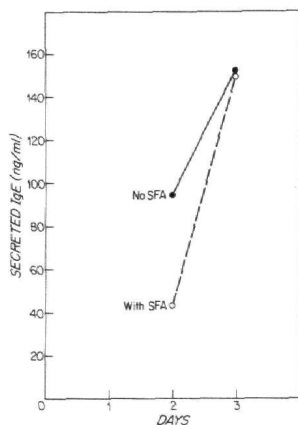


Fig. 17. Suppression of IgE secretion by U-266 cells cultured in the presence of SFA. 8×10^4 U-266 cells were cultured alone or with SFA-containing 2 way-MLR supernatants (final concentration = 25%). Culture supernatants were assayed for IgE on day 2 and 3. (Taken from Ref. 68 with permission of the publishers).

6) *Production of IgE-selective regulatory molecules by stable human T cell lines.* Since one long-range objective of these studies is to obtain substantial amounts of IgE-selective regulatory molecules of human origin, we are planning to utilize genetic engineering technology to achieve this end. Isolation of the relevant mRNA and cDNA corresponding to such molecules would be greatly facilitated by the existence of continuous cell lines producing ample quantities of such molecules. Experiments have, therefore, been undertaken to screen culture supernatants of a number of T cell lymphocytic leukemia lines for immunoregulatory molecules whose activities can be demonstrated to be exerted on the *in vitro* human IgE biosynthesis system described above.

Indeed, culture of various T cell leukemia lines in serum-free medium, in the absence of exogenous stimulation, has revealed the constitutive release of an array of immunoregulatory activities which can influence fresh cultures of PWM-stimulated normal human MNC. Most notably, one T cell acute lymphocytic line constitutively synthesizes and releases into culture

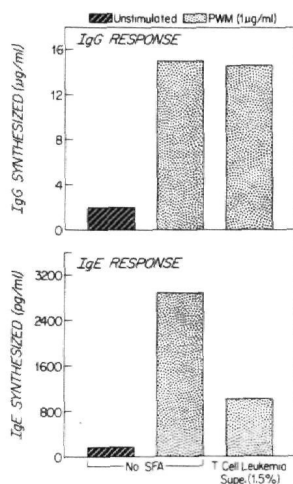


Fig. 18. Selective suppression of human IgE synthesis by SFA-like materials secreted into serum-free medium by a human T cell leukemia line. 3×10^6 MNC from a single normal donor were cultured either alone or in the presence of a serum-free culture supernatant from a T cell leukemia line at a final concentration of 1.5%. 24 h later, PWM was added to two of these cultures (middle and right bars). 3 days later (day 0), the cells in each culture group were washed and plated in microcultures (10 replicate microwells per group) for 6 additional days. The mean levels of IgG (upper panel) and IgE (lower panel) synthesized by each culture group are depicted.

medium regulatory molecules which *selectively* suppress IgE synthesis without affecting synthesis of IgG by the same cultured cells. Thus, as shown in Fig. 18, PWM-stimulated IgE responses of cultured human MNC are inhibited by over 70% when exposed to serum-free culture supernatant of this T cell leukemia line, even at the very low concentration of 1.5% (bottom panel). This inhibitory activity is clearly selective for IgE synthesis since, as shown in the top panel, IgG response in the very same cultures is not detectably affected. Such studies document, therefore, the existence of a stable human cell line which constitutively produces human SFA and which, accordingly, should serve as a valuable source of materials for genetic engineering approaches which are now underway.

Concluding remarks – toward clinical control of the IgE antibody system

In the foregoing discussion, I have summarized a considerable body of experimental data pertaining to regulation of IgE antibody production derived from studies performed in animal and human systems. It is essential to do this in order to emphasize that fundamental research concerned with the regulation of IgE synthesis has produced new insight that promises to lead to more successful therapy for, and even prevention of, IgE-mediated disorders.

This review began by making the point that the IgE antibody system serves as one of our more important defense mechanisms against the hostilities of our natural environment. Consider, for example, the exquisite sophistication of the regulatory network that has evolved to control IgE antibody production, the ubiquitous display of the effector cells (i.e., mast cells, etc.) throughout the lining surfaces of our bodies, and the potent inflammatory mechanisms associated with the IgE system, and it becomes obvious that without IgE we would be in considerable danger in terms of survival. Indeed, the severe paucity or complete absence of IgE may be incompatible with human life; one indication of this presumption is the lack of any reports of living humans that have been stringently documented to be completely devoid of IgE. Other indications of the significant usefulness of IgE include: 1) the fact that the IgE antibody system is one of the first immune mechanisms to become reconstituted after bone marrow transplantation, thereby signaling an ontogenic portrayal of "first things first"; 2) the fact that those patients with immune deficiency disorders that have paradoxical elevations of IgE production tend to fare better in terms of resistance to opportunistic infection than those who do not have high IgE production; and 3) the cleverness of parasites, whose main purpose is to live successfully in symbiosis with their hosts, in stimulating extraordinary amounts of polyclonal IgE, perhaps as a mechanism to enhance survival of the host.

Conversely, the opening statement of this review referred to the hypocritical aspects of the

IgE system because of its pathogenetic involvement in IgE-mediated allergic diseases. It is worth re-emphasizing that this undesirable consequence of IgE antibody production reflects the enormous delicacy of the control mechanisms that normally guide IgE synthesis and, hence, the associated susceptibility of such control mechanisms to various perturbations – the more finely-tuned the instrument, the more likely it will go out of tune.

What is most important here is that now that we have developed a better understanding of how finely tuned the IgE system is, and what types of external perturbations can upset the balance of such tuning, the better position we are in to approach IgE-mediated allergic disorders in a meaningful way. We can realistically look forward, I believe, to the availability of new therapies, based on IgE-selective lymphokines such as suppressive factor of allergy, and others like it, in the very near future. These therapies will be tailored to recapture the fine tuning of the regulatory network controlling IgE synthesis so that ample, but not excessive, quantities of this important protective antibody are produced. The fact that IgE-selective suppressor molecules can be obtained from human cells and be shown to act in a selective fashion on IgE responses of human lymphocytes in culture in a manner similar to those previously observed in animals, as described herein, represents a significant step in the proper direction.

Finally, the concept of allergic breakthrough, and an understanding of its ramifications may perhaps provide new approaches to the *prevention* of IgE-mediated allergies. Thus, for example, removing likely sensitizing agents from the immediate environment of an allergy-prone child during the presence of an upper respiratory viral infection, or other transient febrile illness, could minimize the subsequent development of allergy as a result of an allergic breakthrough process. Moreover, further clinical exploration should be undertaken to identify more precisely other agents, such as commonly used drugs, environmental factors, etc., which can transiently disturb the normal IgE damping mechanism. Ultimately, however, it will be necessary to educate both the public and

the medical profession about the undesirable consequences of allergic breakthrough, and what steps can be taken to avoid them.

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