

Workshop on measurement of in vitro IgE synthesis and regulation of IgE synthesis

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Twenty years have passed since the isolation of IgE, the antibody responsible for immediate-type hypersensitivity. This discovery led investigators to search for an understanding of the mechanisms regulating the synthesis of IgE with the ultimate goal being the treatment, and perhaps even prevention, of allergic diseases via the regulation of IgE synthesis.

Many distinguished and diligent scientists have been and currently are engaged in studies in this field. Significant strides have been made, but the research is hampered by some technical problems, one of the most significant being the accurate measurement of low levels of IgE synthesis in vitro. On May 17, 1985, the Workshop on Measurement of in vitro IgE Synthesis and Regulation of IgE Synthesis was held at the National Institutes of Health in Bethesda, Md. The goal of this workshop was to provide a forum where those pursuing work in the field of IgE regulation could (1) present their work, (2) discuss common problems, (3) talk about possible solutions to these technical difficulties, and (4) come to an agreement on future goals for these studies. Dr. Ishizaka gave an overview of the field, and 11 other investigators gave short presentations. A condensed version of each presentation is included in this article. Approximately 20 other interested persons, from all over the world, attended and joined in the discussions.

T CELL FACTORS INVOLVED IN THE ISOTYPE-SPECIFIC REGULATION OF THE IgE RESPONSE AND APPROACHES TO CONTROL IgE FORMATION

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IgE synthesis is regulated, like other antibody classes, via antigen-specific T cells, both helper and

Abbreviations used

PF:	Potentiating factor
SF:	Suppressive factor
BF:	Binding factor
GEF:	Glycosylation-enhancing factor
GIF:	Glycosylation-inhibiting factor
PBMNC:	Peripheral blood mononuclear cell
PWM:	Pokeweed mitogen
id:	Idiotypic
RIA:	Radioimmunoassay
TCS:	T cell supernatants
AthCC:	Alloreactive helper T cell clone
EBV:	Epstein-Barr virus
Con A:	Concanavalin A
R:	Receptor

suppressor. However, there also functions a unique mechanism that is selective for the IgE isotype. Dr. Ishizaka and his coworkers during the last 6 years have examined this phenomenon at the cellular and molecular levels in the rodent systems and most recently have evidence that a similar mechanism is at work in man. His keynote lecture summarized what is known regarding T cell factors involved in isotype-specific regulation of IgE synthesis and possible clinical applications.

Table I lists selected influences on IgE production. These indicated the possibility of an isotype-specific mechanism. Using *Nippostrongylus*-infected rats, they found soluble factors with affinity for IgE that were derived from W 3/25⁺ (Lyt 1⁺) T cells. Depending on when the cells were taken, the factors either enhanced or suppressed IgE synthesis. Both IgE-PF and IgE-SF have similar molecular weights (13,000 to 15,000 daltons). Both are glycoproteins but contain different oligosaccharides.

These IgE-BFs could also be obtained after injections of complete Freund's adjuvant or *Bordetella pertussis* vaccine. Genetic factors as well as low-dose X rays play a role in determining the nature of the IgE-BF released. IgE-PF is detected whenever IgE synthesis is enhanced, and IgE-SF is found when the IgE response is selectively suppressed. This suggested that the IgE-BFs were involved in the regulation of the IgE antibody response in vivo.¹

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The same T cells have the capacities to produce both IgE-PF and IgE-SF. The environment of the cells determines the nature of the IgE-BF. The major difference between the factors is in their carbohydrate moieties. IgE-PF has both N-linked, mannose-rich oligosaccharide and O-linked oligosaccharide with both oligosaccharides having sialic acid as the terminal sugar residues. IgE-SF contains O-linked oligosaccharide with galactose → *N*-acetyl galactosamine as the terminal sugar.

T cells also produced 60K and 30K IgE-BF, some of which either suppressed or enhanced the IgE response. Recently, **Drs. Kevin Moore and Chris Martens** (in DNAX Institute of Molecular Biology) in collaboration with **Dr. Ishizaka's** group, cloned the gene for rat IgE-BFs, and four cDNA clones were obtained.² Transfection of COS7 monkey kidney cells by each cDNA clone resulted in formation of IgE-BFs. None of them suppressed IgE synthesis; however, IgE-BF derived from two clones selectively potentiated it. The IgE-BFs derived from clone 8.3 consisted of two species, one 60,000 and another 11,000 to 12,000 daltons. Both potentiated the IgE response. Since the cDNA were constructed from messenger RNA of cells that form IgE-SF, these results suggested that IgE-PF and IgE-SF share a common structural gene and that posttranslation glycosylation events decide the difference. Nucleotide sequencing of cDNA clone 8.3 revealed a coding region for 556 amino acids, corresponding in size to the 60K IgE-BF. Thus, the 11K factor must be a cleavage product. It is hoped that gene cloning will elucidate the structural basis of the function of IgE-BFs.

The effective substance to induce the factor formation is an interferon-like substance. However, this inducer substance does not determine the nature of the IgE-BFs formed. The activities of the factors are controlled by two T cell factors that either enhance or inhibit the *N*-glycosylation of IgE-BFs. GEF is derived from a subset of Lyt 1⁺ T cells. Thus, when Fc R⁺ T cells are stimulated in the presence of GEF, these cells selectively form glycosylated IgE-BF that potentiates the IgE response. The same Fc R⁺ T cells selectively form IgE-SF when the cells were stimulated by inducers in the presence of GIF. In the mouse the major source of GIF is Lyt 2⁺, I-J⁺ antigen-specific suppressor T cells.

GIF, with a molecular weight of about 15 K, is a fragment of phosphorylated lipomodulin. It inhibits *N*-glycosylation of IgE-BFs via inactivation of phospholipase. Meanwhile, GEF has serine protease activity and is a kallikrein-like enzyme. Under physiologic conditions, the balance between GEF and GIF appears to determine the nature of IgE-BFs formed. The production of IgE-SF is always accompanied by

TABLE I. Correlation between the IgE response and selective formation of IgE-potentiating factor and IgE-suppressive factor

Influence on IgE production	IgE response	IgE-BF
Infection with nematodes		
14 days	↑	PF
8 days	(-)	SF
Adjuvant treatment		
<i>Bordetella pertussis</i> vaccine	↑	PF
Complete Freund's adjuvant	↓	SF
Antigen priming		
Aluminum hydroxide	(+)	PF
Complete Freund's adjuvant	(-)	SF
Genetic factor		
BDF, mice	(+)	PF
SJL mice	(-)	SF
Irradiation with x-ray or cyclohexamide treatment	↑	PF

↑ Indicates enhancement of IgE response; ↓ indicates suppression of IgE response; (+) indicates IgE antibody formation; and (-) indicates no IgE response.

the formation of GIF, whereas GEF is detected along with IgE-PF. This same principle may even explain genetic differences. Those mice that are poor IgE producers have cells that constitutively form GIF, whereas those strains that are high IgE producers have cells constitutively forming GEF.¹

IgE-BFs are produced by peripheral blood lymphocytes of ragweed-sensitive patients when the cells are incubated with specific antigen and homologous IgE. With the use of peripheral blood T cells of patients with hyper-IgE syndrome or with atopic dermatitis, Saryan et al.³ were able to demonstrate the release of an IgE-specific potentiating factor. Alternatively, they found, in the serum of normal individuals with very low IgE levels, an IgE-SF. Thus, human T cells can form IgE-PF and IgE-SF essentially the same as those found in rodent systems.

Dr. Ishizaka has constructed human T cell hybridomas that produce IgE-BFs on incubation with human IgE.⁴ Since human IgE-BFs have affinity not only for human IgE but also for rat IgE, their effect on IgE synthesis can be determined by use of rat MLN cells. With T cells from a subject with no known allergies and a very low serum IgE level, the IgE-BFs produced by the hybridomas suppressed IgE synthesis.⁵

With the use of another human T cell hybridoma, 166A2, the nature of the IgE-BFs formed could be switched from PF to SF, depending on whether incubated with bradykinin (which enhances glycosylation) or with GIF. Thus, a common mechan-

ism appears to regulate the IgE-BFs in man and rodents.

The genes encoding human IgE-BFs are now being cloned by **Drs. Moore and Martens**. Mixtures of some cDNA clones have been used to transfect COS7 cells and produce human IgE-BFs.

If the nature of IgE-BFs is determined by the balance between GEF and GIF, administration of GIF might be effective to suppress the IgE response. GIF has been purified from culture filtrates of a hybridoma cell and injected into mice. It completely suppressed both the IgE and IgG primary antibody response to DNP-OA. GIF is also effective in suppressing ongoing IgE antibody synthesis.⁵

Gene cloning of GIF is now in progress. Many hurdles remain to be cleared before clinical application. However, it is hoped that basic mechanisms discovered in animal systems will lead to new approaches to regulating the IgE antibody response in allergic patients in the not too distant future.

MULTICENTER PILOT STUDY OF METHODS TO MEASURE IgE PROTEIN IN CELL-CULTURE SUPERNATANTS⁶⁻⁸

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Numerous immunoassays have been used to measure low levels of IgE in supernatants from cultured PBMNCs. Published articles are at variance as to whether IgE is produced by such cells either spontaneously or following mitogen stimulation. To evaluate the sensitivity, specificity, and precision of IgE immunoassays being used by various investigators, a multicenter collaborative study was organized in which lyophilized test samples were distributed to 22 laboratories.

Sensitivity was tested by including samples containing tissue-culture medium alone or medium spiked with 0.05 ng/ml, 0.25 ng/ml, or 0.50 ng/ml of polyclonal IgE (United States reference standard). Only 13 of 22 laboratories were able to quantitate IgE in the 0.5 ng/ml sample, and only five were able in the 0.25 ng/ml and 0.05 ng/ml samples.

Specificity was assayed by including samples containing 5 ng/ml of polyclonal IgE (unheated or heated at 56° C for 4 hours), IgE (YU), and IgE (ND). All but one or two laboratories were able to quantitate the IgE; however, the values for the polyclonal IgE were in the 1 to 3 ng/ml range. Half of the laboratories were unable to measure the heated polyclonal IgE sample, indicating their antisera were recognizing heat-labile determinants on the ϵ chain.

The ability to quantitate IgE in cell-culture supernatants was examined by including a sample from a

12-day, pokeweed mitogen-stimulated PBMNC culture. Control samples included culture medium with pokeweed mitogen alone, culture medium from unstimulated cells, and culture medium from stimulated cells that had been freeze-thawed five times on day 0. To test the ability of the IgE assays to quantitate IgE in the presence of large quantities of IgG, a stimulated cell-culture supernatant was spiked with 10 ng/ml of polyclonal IgE. Eighteen laboratories measured IgE in the stimulated cultures (values ranging from 0.25 ng/ml to 6.5 ng/ml) with >70% in the lower part of the range. Only those measuring the higher values found a net increase in IgE over control samples. Most were able to quantitate the IgE in the spiked sample.

To assess precision, coded duplicate test samples containing 0.5 ng/ml of polyclonal IgE were included. Only 50% of the participants could measure IgE in both samples, and frequently the values were neither precise nor accurate.

In this study a number of different assays were used, including RIA, enzyme immunoassay, and particle-counting immunoassay. Each used their own anti-IgE preparations and their own IgE standards. The accuracy, precision, sensitivity, and specificity of the assays were not dependent on the type of assay. It was concluded that there was a wide variation in the sensitivity and precision of the assays used to quantitate low levels of IgE. Only three laboratories obtained results consistent with de novo IgE production by the mitogen-stimulated PBMNCs that were used in this study.

ANTIBODIES TO IDIOTYPIC DETERMINANTS OF HUMAN IgE MYELOMA PROTEINS REACT WITH POLYCLONAL IMMUNOGLOBULIN IN SUPERNATANTS OF POKEWEE MITOGEN-STIMULATED CELLS AND CAUSE AN OVERESTIMATION OF IgE SYNTHESIS IN VITRO^{7, 9, 10}

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Several years ago **Dr. Spiegelberg** discovered a subpopulation of lymphocytes carrying Fc receptors for IgE. The number of such cells is increased in patients with allergic diseases. He speculated that these cells may be involved in the regulation of IgE synthesis and attempted to study the effect of Fc_γ receptor-positive B and T cells on PWM-induced IgE synthesis in vitro.

The ability of human PBMNC to synthesize IgE in vitro in response to PWM is controversial. To determine whether the conflicting results obtained by different laboratories could be, in part, the result of

qualitative differences in the anti-IgE antibodies used to measure low concentrations of IgE in culture supernatants, they compared the specificities of anti-IgE reagents prepared by various methods. Immunosorbent-purified antibodies were isolated from a goat antiserum to the λ IgE myeloma protein PS and a rabbit antiserum to the κ IgE protein Bed to yield (1) anti-PS (anti- ϵ and anti-id PS), (2) anti- ϵ , and (3) anti-id PS and anti-id Bed. These three types of antibodies were then used in a solid-phase radioimmunosorbent test to quantitate the amount of IgE synthesized by PBMNC from atopic and nonatopic donors in the presence and absence of PWM. Anti-PS antibodies detected a PWM-induced IgE formation in all culture supernatants of both nonatopic and atopic donors, whereas anti- ϵ antibodies did not. However, anti-id PS detected an increase in the concentration of idiotypic antigen in nine of 10 culture supernatants, whereas anti-id Bed detected such an increase in five of 10 culture supernatants. The anti-id PS and anti-id Bed antibodies also detected epitopes on F(ab')₂ (but not Fc) fragments of polyclonal IgG. Thus, anti-IgE antibodies isolated by affinity chromatography with the same myeloma protein that was used for immunization cannot be used for measuring IgE concentrations on cell-culture supernatants or sera from nonatopic donors because anti-idiotypic antibodies in these preparations react with Fab determinants of polyclonal immunoglobulin.

To assure that polyclonal anti-IgE is measuring IgE in supernatants of cultured cells, three control experiments should be performed. (1) Different IgE myeloma proteins including that used for immunization have to be demonstrated to react equally. (2) No reaction should be obtained with polyclonal IgG and/or Fab fragments of polyclonal IgG measured at concentrations up to 1 mg/ml. (3) The culture supernatants should be tested at three serial dilutions in order to establish that the IgE in the culture supernatant has the same slope as the IgE standard and elicits the same IgE concentration at all three dilutions. (4) The assay should yield 900 IU of IgE per milliliter in the standard serum of National Institutes of Health. The slope of the polyclonal IgE in the serum of National Institutes of Health should be identical to that of the myeloma proteins.

With the use of these criteria, **Dr. Spiegelberg** was unable to detect PWM-induced IgE synthesis in vitro in either cells from normal or atopic patients. In contrast, spontaneous IgE formation in vitro was observed with cells from atopic donors, particularly those with severe disease. However, the IgE level seldom exceeded background released from cells cultured in the presence of cycloheximide. Therefore, it was at-

tempted to enrich the mononuclear cells for B cells by depleting them of monocytes and T cells. B cell preparations from four of six patients with severe atopic dermatitis produced 4 to 25 ng of IgE. This was four to 10 times more than the IgE released from cells incubated with cycloheximide. Whether such B cell preparations may be suited for studying the regulation of IgE synthesis by Fc ϵ receptor-positive lymphocytes and cell-free factors is currently being investigated.

DETECTION OF V REGION DETERMINANTS IN IgE: ASSESSMENT BY MONOCLONAL ANTIBODIES¹¹⁻¹³

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Drs. Saxon, Chapman, and Kanowith-Klein have developed a series of monoclonal antibodies against the IgE myeloma protein PS. These were used to develop IgE-specific immunoassays and to assess the occurrence of a PS-related V region determinants (cross-reacting idiotypic) in sera and peripheral blood lymphocyte cultures. Such an id is present on immunoglobulin classes other than IgE, and its detection may confound assays in which polyclonal antisera are used that were raised against IgE protein PS. Murine hybridoma B cell lines were raised to intact IgE PS, and the antibodies were screened for binding to intact PS protein. Three were found to bind to four IgE myelomas (PS and ND, IgE λ ; and Bedora and Forshey, IgE κ) and to elicit similar binding curves for the standard sera of United Kingdom and World Health Organization. In contrast, antibody 5.12 only bound to myeloma IgE PS, suggesting that it was an anti-id antibody. This was investigated by use of different fragments of PS IgE as well as other immunoglobulins to inhibit the binding of radiolabeled PS to the various monoclonal antibodies. The results indicated that antibody 5.12 binds to a V region-determinant id (5.12 id) displayed on the λ light chain of PS protein, whereas the other three monoclonal antibodies are specific for the Fc portion of IgE.

The occurrence of 5.12 id on IgG in normal human sera was assessed by use of a solid-phase RIA. Sera containing no IgE demonstrated binding to 5.12 but not to the monoclonal anti-IgE antibodies. It was calculated that 5.12 id was present on approximately 1 out of every 10,000 IgG molecules. Antibody 5.12 bound strongly to six of 22 λ myeloma proteins (including IgG 1, 2, 3, and IgA1 proteins); however, it bound no κ myeloma proteins or free κ or λ Bence-Jones proteins. Additionally, three of 30 sera from patients with high IgE levels had strong IgE binding to 5.12.

The occurrence of 5.12 id in culture supernatants with and without PWM was assessed by measuring the Ig produced by use of two RIA for IgE, one with monoclonal antibodies and one with polyclonal antisera, and by directly measuring 5.12 id on IgG in the same cultures. The PWM-stimulated cultures demonstrated an increase in IgE of 306 pg per culture by monoclonal assay and 1256 pg per culture by polyclonal assay; 40% of this latter increase could be accounted for by 5.12 id in the culture. Additionally, the monoclonal and polyclonal anti-IgE RIAs were done in the presence of unlabeled 5.12 anti-PS id antibody to block any id binding. With this there was a 36% decrease in the IgE detected by the polyclonal RIA but no change in the monoclonal RIA.

Human fetal tissue was examined for the presence of IgE because it had been reported that human IgE was synthesized *in vitro* by fetal liver or lung cells from as early as 11 weeks of gestation, much earlier than would have been predicted from rodent data. By use of immunohistochemical staining of human fetal lung, liver, and spleen from up to 28 weeks of gestation, there was no binding of ϵ -specific monoclonal antibody. In contrast, antibody 5.12 demonstrated clear reactivity on these tissues as did control staining with anti- μ antiserum. Thus, the earlier observations may have been due to detection of a common id.

The determinant called 5.12 id is present in the λ light chain of PS IgE protein. It appears to be a common or cross-reacting light chain id or possibly a V λ family framework determinant. It is found in normal human immunoglobulin from serum and from lymphocyte-culture supernatants. This may result directly from limited heterogeneity in the λ V gene family or from cross-reactivity within the family. The detection of this determinant or related ones by antisera against PS protein may lead to the inaccurate determination of IgE. This may not be overcome by absorption of the antisera by non-IgE myeloma proteins since they are likely to be 5.12 id negative. However, this reactivity could be removed by absorption with polyclonal IgG. The use of Fc ϵ -specific monoclonal antibodies or polyclonal anti-IgE antisera that have been affinity purified on an IgE myeloma other than the one used for immunization are ways to circumvent the difficulty of detecting this type of cross-reacting determinant.

TECHNICAL ASPECTS OF MEASURING IN VITRO SYNTHESIS OF IgE:

CONTROLLING FOR PREFORMED IgE^{6, 14}

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Studies of IgE immunoregulation with human PBMNC have been and continue to be very difficult due to the extremely small quantities of IgE syn-

thesized by cultured circulating B-lymphocytes. Two crucial points must be addressed in all such studies: (1) IgE measurements should be made only with reagents known to be solely ϵ -specific and (2) of preformed IgE present in or on the cells at the initiation of the experiment must be completely accounted for in order that this can be subtracted from the total IgE found at its termination.

In 1978 **Dr. Buckley** first reported the detection of IgE in supernatants of mononuclear cells from patients with atopic eczema. From the beginning, however, it was demonstrated that PWM either had no effect or actually inhibited spontaneous IgE production by cells from patients with elevated serum IgE in contrast to its effect on the synthesis of other classes of immunoglobulins. Another peculiarity was that the time of detection of IgE after culture initiation was earlier, i.e., at 2 to 3 days.

Because of these differences and because of discrepancies in the literature regarding the effect of PWM, **Dr. Buckley** and coworkers reexamined their method of evaluating the quantity of preformed IgE present in the cells at initiation of culture. Parallel cultures were frozen and thawed, and the supernatants were either analyzed immediately or incubated with the live-cell culture and harvested at specified times. A crucial finding was that, in contrast to freshly cultured cell supernatants that contained very little IgE, supernatants from cells frozen on day 0 and analyzed immediately contained considerably more IgE, and those frozen and thawed on day 0 and held in the incubator for 7 days before analysis contained much more. In the atopic patients' live-cell cultures, held for 10 days, there was considerably more IgE than in the frozen and thawed cultures. Treating the cultures with 2000 rad at initiation did not result in IgE concentrations statistically different from nonirradiated cultures. The IgE in the PWM-stimulated, nonirradiated cultures was less and approximately the same as the irradiated cultures. Normal control subjects did not have more IgE in their live-cell cultures than in their frozen and thawed counterparts, whether PWM stimulated or not.

Further experiments demonstrated that the source of the IgE was not IgE being shed from the Fc receptors on the cells. Preformed IgE continued to be released from cultures frozen and thawed on day 0 through the tenth day of incubation. Mitomycin C had no significant effect on the amount of IgE produced; however, puromycin and cycloheximide clearly inhibited IgE production. The fact that neither mitomycin C nor irradiation had an effect on IgE synthesis is very similar to findings in mice and rats that the circulating cell producing IgE is a long-lived, radio-resistant plasma cell. This suggests that the human

blood cell spontaneously producing IgE is terminally differentiated and not likely to be easily modulated by other cells or regulatory factors.

Additional findings during the previous year make it likely that most if not all of the IgE measured is actually preformed in vivo and merely released from the cells during culture (rather than being synthesized in vitro). Dr. Buckley confirmed Dr. Turner's report that brief treatment of PBMNC with 1 N HCl (to bring the pH to 2.0 to 2.5) is more efficient in releasing preformed IgE than multiple freeze-thaws plus prolonged incubation. Moreover, the use of protein-synthesis inhibitors in parallel tubes is not an adequate way to account for preformed IgE because it does not measure the IgE remaining in the cell pellets. Table II illustrates that the amount of IgE detected in the live cells is less than twofold more than the amount of preformed IgE in the day 0 HCl-treated cultures. Further experiments have demonstrated that the 1 N HCl treatment is not merely exposing more antigenic sites for the anti-IgE reagent.

These, and results from similar studies, bring into serious question whether any true in vitro IgE synthesis is occurring by cultured PBMNC, even by those from atopic donors.

ASSESSMENT OF SOME MODELS OF HUMAN IgE PRODUCTION IN VITRO¹⁵⁻¹⁷

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In studies of models of in vitro IgE production, two different types of reagents have been used. First, the Pharmacia anti-D 2 reagent (Pharmacia Fine Chemicals, Piscataway, N. J.) was tried, then an anti- ϵ monoclonal antibody as the solid-phase antibody, and a polyclonal antibody served as the labeled reagent. The polyclonal antibody was raised against the PS myeloma protein and purified by affinity chromatography with the ADZ myeloma protein. By use of B cells from atopic patients, no significant differences in the amount of IgE released into the supernatants were observed regardless of whether freeze-thawing, acid extraction, or cycloheximide was employed. For this reason the last was routinely used as the simplest way to discriminate *de novo*-synthesized IgE from cell-associated (preformed) IgE. Spontaneous IgE synthesis was studied in both atopic and nonatopic individuals. De novo synthesis of IgE in vitro was found in B cell cultures from many atopic patients with multiple sensitivities with or without atopic dermatitis and in a lower proportion of patients with pollenosis, but only during or soon after the pollination season. Most spontaneously IgE-producing cells bore surface-membrane IgE but not surface-membrane IgM and could be influenced, but only in

TABLE II. Evaluation of methods to determine preformed IgE

Treatment	No. of days incubation	IgE (pg/ml)	
		Donor No. 1	Donor No. 2
Freeze-thaw four times day 0	0	380	<200
	2	490	<200
	4	620	<200
	6	1030	<200
	8	1190	<200
	10	1320	<200
	12	1240	<200
Freeze-thaw four times day 0 plus 1 N HCl	0	2250	410
1 N HCl only	0	2480	380
Culture cells 12 days and then freeze-thaw four times	12	2930	<200
Culture cells 12 days and then freeze-thaw four times plus 1 N HCl	12	2250	320
Culture cells 12 days and then 1 N HCl only	12	2080	—

a negative way, by lectins, T cells, specific antigen, and low-dose irradiation.

In attempts to induce IgE production by signals delivered in vitro, classic T cell-dependent and T cell-independent polyclonal B cell activators did not lead to the production of detectable amounts of IgE. Soluble factors capable of potentiating IgE production in vitro were detected in TCS from some patients with severe atopy and elevated serum IgE levels. The molecular weight of this factor was in the 10 K to 45 K range. Thirty TCS were tested with B cells from atopic patients. Eighteen TCS had no activity, eight consistently potentiated IgE synthesis, and four inhibited IgE synthesis. The mean serum IgE levels of TCS donors were higher in those subjects whose TCS demonstrated potentiating activity than in either of the other two groups of donors. However, the increase in IgE synthesis induced by these soluble factors was usually small and could vary in relation to the target B cell donor.

Recently, an AThCC was established that was able to induce significant IgE production by B cells from atopic and nonatopic donors possessing the appropriate alloantigen. The AThCC-induced IgE synthesis started after the fourth day of culture and peaked between days 7 and 12. Virtually no IgE was detected

in supernatants of cultures treated with cycloheximide. Depletion experiments demonstrated that at least a proportion of B cells activated to high-rate IgE production by the AThCC bore both surface-membrane IgE and surface-membrane IgM, suggesting that circulating IgE-forming cell precursors are demonstrable in both atopic and nonatopic people provided that suitable stimulation is achieved.

HUMAN IgE SYNTHESIS IN VITRO BY POKEWEED MITOGEN-STIMULATED MONONUCLEAR CELLS¹⁸⁻²⁰

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This study was performed to address the controversy concerning human IgE biosynthesis in vitro induced by stimulation with PWM. The controversy has focused on the specificity of reagents used for quantitative assay of human IgE in culture-supernatant fluids. At times the same myeloma protein has been used both as an agent for immunization and for purification of the IgE class-specific antibody reagents. This raises the question of whether certain antihuman IgE antibody reagents possess anti-idiotypic reactivities, thereby resulting in reactions with Fab determinants of polyclonal immunoglobulins that would yield false positive readings of IgE protein levels.

The studies reported here not only reexamined **Dr. Katz's** original antihuman IgE reagent, goat antihuman IgE (GAHE) (PS) for which the myeloma protein IgE (PS, ϵ , λ) was used to generate and purify GAHE, but also document results obtained with new GAHE antibody reagents purified on completely unrelated IgE myeloma proteins IgE (DZA, ϵ , κ) and IgE (ND, ϵ , λ). Although his initial GAHE (PS) was purified by affinity chromatography on the same myeloma protein used for immunization, IgE (PS), the resulting antibody did not contain antibody activities reactive with either heavy chain or light chain idiotype determinants specific for the myeloma protein. GAHE (DZA) and GAHE (ND) should be devoid of any reactivities of either heavy chain or idiotype determinants specified by the Fab fragment of the IgE (PS) myeloma protein. Also, IgE (DZA) has κ chains, whereas IgE (PS) has λ chains. Furthermore, the GAHE reagents described here have been demonstrated to specifically react with the human IgE Fc fragment encoded and transcribed by cDNA for a human IgE (ND) providing overwhelming proof of the ϵ -specific properties of these GAHE antibodies.

Data from ϵ -specific RIA with GAHE (PS) and GAHE (DZA) indicate that there is in vitro IgE biosynthesis by PWM-stimulated human PBMNC, and this is de novo synthesis because the addition of cy-

cloheximide to such cultures prevented the production of IgE in stimulated cultures. IgE was also measured with GAHE (ND). By use of PBMNC from one donor and GAHE (PS), GAHE (DZA), GAHE (ND), and a commercially available GAHE, the studies presented here confirm that PWM-stimulated human PBMNCs synthesize increased quantities of IgE, which can be detected in comparable amounts by all of four GAHE antibody reagents in supernatant fluids from such cultures.

There are several important factors involved in the stimulation of IgE protein synthesis from human PBMNCs by PWM:

1. The fetal calf serum must be screened for IgE production. What works in another assay system may not always work well for IgE synthesis.
2. The stimulating activity of PWM differs from lot to lot within a company and between companies. Also the optimal dose for IgE biosynthesis differs from donor to donor.
3. PBMNCs need to be separated out of blood samples as soon as the blood is drawn. Whenever cell recovery from a normal donor exceeds 2×10^6 cells per milliliter of blood, such cells respond very poorly to PWM caused by an abnormally high frequency of monocytes in these samples.
4. The physical health condition of the donor must be checked very carefully.

Collectively, these points suggest that the ability to obtain reproducible PWM-stimulated IgE synthesis in vitro requires optimizing the culture system with healthy cells to avoid any combination of several potential inhibitory influences. **Dr. Katz** finds 25% of donors do not respond to PWM to produce IgE, whereas there is always an increase in IgG synthesis. In conclusion, **Dr. Katz** confirms that human PBMNCs from normal donors can be stimulated with PWM to synthesize IgE under appropriate conditions in vitro.

REQUIREMENTS OF B CELLS FOR INDUCTION OF IgE SYNTHESIS AND IgE POTENTIATING FACTORS^{3, 21, 22}

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Requirements for the induction of IgE synthesis were studied. In vitro PBMNCs from allergic patients spontaneously synthesize IgE, whereas those PBMNCs from normal patients do not. Even after the removal of suppressor cell types, neither PWM nor EBV could induce normal cells to produce IgE. However, these B cells could be triggered to produce IgE after exposure to the supernatant of T cells from patients with elevated serum IgE (hyper-IgE syndrome,

atopic dermatitis, and acute graft-versus-host disease). Cycloheximide and tunicamycin inhibited the release of this IgE isotype-specific helper factor. These results indicate that, whereas classic polyclonal B cell activators (PWM and EBV) fail to induce IgE synthesis by normal B cells, IgE synthesis is readily induced by an IgE-specific helper factor released by T cells from patients with hyper-IgE states.

T cells were isolated from the peripheral blood of patients with hyper-IgE syndrome on the basis of their expression of Fc ϵ receptors for human IgE (Fc ϵ R). After separation, Fc ϵ R+ T cells and Fc ϵ R- T cells were propagated in culture. Only the supernatants of the Fc ϵ R+ T cell lines selectively enhanced IgE synthesis in cultures of B cells and only when these cells were from allergic patients. The surface phenotype of the Fc ϵ R+ T cell line was predominantly T3 $^{+}$, T4 $^{+}$, and Ia $^{+}$. Two T cell clones were grown from the Fc ϵ R+ T cell line, and these had the T4+ helper/inducer phenotype and secreted IgE-enhancing factor(s). The factor(s) had affinity for human IgE, was sensitive to treatment with trypsin and neuraminidase, and had as its target an IgE-bearing B cell. These results suggest that a subset of human T cells bearing an Fc ϵ R secretes an IgE-binding glycoprotein that selectively enhances IgE synthesis by IgE-bearing B cells from atopic patients.

An AThCC (HLA-DR) stimulated PBMNCs from both allergic and nonallergic donors to synthesize IgE. Induction of IgE synthesis in nonallergic B cells occurred only under conditions of cognate interaction. In contrast, IgE synthesis in B cells from allergic donors occurred under these conditions as well as bystander conditions in which the T cells were stimulated by third-party monocytes bearing the relevant alloantigens. Furthermore, such bystander stimulation occurred in the presence of tetanus toxoid antigen-specific T cell clones activated by the appropriate tetanus toxoid-pulsed monocytes. However, the B cells did not differ in their requirements for the induction of IgG synthesis. IgG synthesis was induced in all B cells under conditions of cognate interaction with the T cells as well as under bystander conditions. These results suggest that cognate T-B cell interactions may be important in the development of IgE immune responses in the normal host.

EXPRESSION OF IgE-RECEPTORS AND SECRETION OF IgE-BINDING FACTORS BY HUMAN LYMPHOCYTES²³⁻²⁷

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The present knowledge regarding the role of IgE-BFs and of lymphocytes bearing receptors for IgE (Fc ϵ

R) is mainly derived from studies based on the use of IgE-coated erythrocytes (E-IgE) to identify both Fc ϵ R-bearing cells and IgE-BFs. These assays are limited by the availability of pure IgE and the low affinity of IgE for its lymphocyte receptor and probably also for IgE-BFs. **Dr. Delespesse** has recently reported on his production and characterization of mouse monoclonal antibodies specific to human lymphocyte Fc ϵ R (MabER). The affinity of these MabER for Fc ϵ R is more than 100 times greater than that of IgE, and their specificity has been documented. At least some of these MabER react not only with Fc ϵ R on B cells but also with macrophage and T cell Fc ϵ R. In addition, some MabER also react with IgE-BFs. The present article is a summary of investigations, with the use of high-affinity MabER, of the expression of Fc ϵ R and the in vitro secretion of IgE-BFs by normal (untransformed) human lymphocytes.

Fc ϵ R-bearing cells were identified by means of a rosette assay with MabER coupled to bovine erythrocytes by chromium chloride. On the average, 7% of PBMNCs from normal donors expressed Fc ϵ R with values ranging from <1% to 15%. Several normal subjects were tested repeatedly and found to have rather stable proportions of circulating Fc ϵ R-bearing cells. Comparable values were observed in PBMNCs from allergic donors and in umbilical cord blood, whereas about 30% of tonsillar lymphocytes were found to express Fc ϵ R. The difference between these results and earlier studies with IgE-E is attributed to the much higher affinity of MabER than IgE for Fc ϵ R. Also Skatchard plot analysis indicated the density of Fc ϵ R on normal (untransformed) PBMNC Fc ϵ R-bearing cells is much lower than on Fc ϵ R-bearing cells lines such as RPM1 8866 or EBV-transformed cells. The great majority of Fc ϵ R-bearing cells were B cells; Fc ϵ Rs were hardly detected on NK cells, macrophages, or T-lymphocytes. During incubation at 37°, but not at 4° C, Fc ϵ Rs rapidly disappeared from the cell surface and were released into the culture supernatant. After 2 to 3 days, the receptors began to be reexpressed. The disappearance of Fc ϵ Rs was inhibited by IgE.

Activation of B-lymphocytes by EBV, a T-independent B cell activator, resulted in the expression of Fc ϵ R by virtually all the transformed B cells. When PBMNCs were stimulated with PWM, a T-dependent B cell activator, the proportion of Fc ϵ R-bearing cells peaked at 48 hours followed by a rapid drop to values below those of the unstimulated control cells. At the peak of the PWM response, most Fc ϵ R-bearing cells were B cells, and very few T cells also expressed Fc ϵ R. Similar results were obtained with T cell mitogens such as PHA or Con A. In these cases, however, a

TABLE III. Monoclonal antibodies to human IgE

Bound	Type 1	Type 2	Type 3
To myeloma PS	+	—	+
To serum IgE	+	—	+
To myeloma ND	—	—	+
To IgG	—	—	—
To heated IgE (PS or ND)	±	+	±
Histamine release (activation of basophils)	2/3	0/1	5/8

higher proportion of T cells bearing $Fc_\epsilon R$ were detected after 2 to 3 days. Additionally, it was noted that the culture supernatant of T cells stimulated with either PWM, PHA, or Con A could induce further expression of $Fc_\epsilon R$ when these were added to cultures of highly purified B cells. In conclusion, T-dependent mitogens are able to induce $Fc_\epsilon R$ expression in both B and T cells; however, the effect is more pronounced on B cells.

$Fc_\epsilon R$ positive and negative B cells were purified and separated. They were cultured in the presence or absence of T cells, and some were also supplemented with either PWM or EBV. The production of immunoglobulins as well as the proportion of $Fc_\epsilon R$ -bearing cells was then determined after 1 or 3 weeks. Regardless of whether the cultures were initiated with either $Fc_\epsilon R$ (—) or (+) cells the results were the same, indicating these two groups of cells do not correspond to discrete B cell subpopulations.

A solid-phase sandwich RIA for the measurement of IgE-BFs was developed with 2 MabER, and the specificity was documented. This RIA allows IgE-BFs to be detected in human serum and in culture supernatants. Current studies include analysis of the cellular interactions leading to the in vitro production of IgE-BFs and investigation of the serum levels of IgE-BFs in various clinical conditions.

IgE PRODUCTION IN VITRO BY HUMAN BLOOD MONONUCLEAR CELLS: T CELL REGULATION²⁸⁻³⁰

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In vitro IgE synthesis by PBMCs from atopic and nonatopic subjects was examined. A modified Phadebas IgE PRIST was used for the quantitation of supernatant IgE concentration. The maximum concentration was reached at day 7. Cells from 28/39 atopic patients spontaneously synthesized significant amounts of IgE in vitro, whereas none of the cells from 40 nonatopic control subjects produced detect-

able amounts of IgE. Polyclonal activators such as PWM failed to induce or enhance in vitro IgE synthesis in normal or atopic subjects.

Eczema B cells were cocultured with either atopic or nonatopic (autologous or allogeneic) T cells to study the regulatory role of the T cell on spontaneous IgE synthesis. Supernatant IgE was measured by a modified PRIST assay, and cultures with cycloheximide were used as controls for preformed IgE levels. No significant suppression of net IgE synthesis occurred when atopic eczema T cells were cocultured with autologous B cells. In allogenic combinations, nonatopic T cells significantly (mean suppression = 59%) suppressed net IgE synthesis by atopic eczema B cells. If the allogeneic control T cells were heat-killed, irradiated, or depleted of suppressor cells, the suppression was not observed. Even greater suppression of net IgE synthesis was observed when histoidentical nonatopic T cells were used, indicating that the autologous eczema T cells have a partial defect in their suppressor function. This apparent defect in immunoregulatory function may be overcome by in vitro activation of atopic eczema T cells by Con A.

Suppressor cells generated during immunotherapy with ragweed were studied for their effect on total and specific IgE synthesis by PBMC. Suppressor cells were removed from unfractionated blood mononuclear cells by treatment with monoclonal antibodies to Leu 2 antigen and complement. IgE was measured before starting immunotherapy and 6, 9, and 13 months into immunotherapy. The removal of suppressor cells did not affect IgE production before immunotherapy but resulted in a statistically significant increase in IgE after 6 and 13 months of immunotherapy. Before immunotherapy, preincubation with ragweed did not change the IgE produced. However, 6, 9, and 13 months into treatment, ragweed suppressed IgE production, but only the last measurement was significant. The suppression of total and specific IgE induced by ragweed could be abolished when suppressor cells were depleted. In conclusion, immunotherapy can induce de novo generation of suppressor cells capable of suppressing specific IgE synthesis (and perhaps the IgE isotype). The suppression is specific and can be induced by preactivation of Leu 2+ cells by ragweed antigen.

PRODUCTION OF IgE BY HUMAN B CELL LINES

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To aid in the study of IgE synthesis and regulation in vitro, Dr. Chiorazzi has generated three sets of cell lines of human B cell lineage secreting mono-

clonal human IgE and IgG4. Two techniques have been used: somatic cell hybridization and EBV transformation.

The first series secretes large quantities of human IgM (10 to 30 $\mu\text{g/ml}$) and smaller quantities of IgE (0.05 to 0.50 $\mu\text{g/ml}$). The cells appear to be the products of hybridization of blast cells from a PWM-stimulated culture of PBMNCs from a patient with severe ragweed allergy and a human HGPRTase-deficient, EBV-transformed cell line. The IgE was purified and functionally assayed. The secreted IgE behaved both serologically and functionally as an intact human IgE molecule. This IgE antibody is presently being sequenced to determine its amino acid composition.

The second series came from the peripheral blood B cells of a patient with a history of hypernephroma, eosinophilia, recurrent herpes simplex infection and elevated IgE titer (7500 IU). These cells were transformed with EBV and 3/32 cell lines secreted moderate amounts of IgE (50 to 100 ng/ml), whereas one cell line secreted $>10 \mu\text{g/ml}$ of IgE. Only negligible amounts of IgM were detected. The cell lines are now being cloned.

The last series are human B cells secreting IgG4. They were obtained from EBV transformation of a bone marrow sample from a patient with documented IgG3 myeloma. These cells may be valuable in mast cell-binding studies and also possibly in IgG subclass switch analysis.

Thus, multiple human B cell lines have been derived that secrete antibodies that may be helpful in the study of allergic disease. Hopefully, such lines will allow a more detailed evaluation of IgE/IgG4 synthesis and regulation, V region assembly, isotype switching, and mast cell binding and triggering.

DIFFERENTIAL BINDING OF MONOCLONAL ANTIBODIES TO HUMAN IgE

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Monoclonal antibodies were used to define sites on the human IgE molecule. The hybridomas were derived from the spleen cells of mice repeatedly immunized with the human IgE myeloma protein PS. Twelve monoclonal antibodies, all of the IgG class, were selected for study. By immunoblotting, all bound predominantly to the E chain.

Table III groups these antibodies according to some of their properties; 5/12 do not release histamine. Hybridomas that bind IgE in solution but are incapable of releasing histamine may be directed toward determinants hidden when IgE is on the basophil surface. Competitive binding assays indicate three related

groups of distinct isotypes. Therefore, these monoclonal antibodies define several different functional domains on the IgE molecule that are either hidden or modified when IgE is bound to the $\text{Fc}_\epsilon\text{R}$ on basophils.

DISCUSSION

A copious amount of data was presented with free and open discussion following. Unfortunately, technical problems still remain regarding the measurement of low levels in vitro IgE synthesis. One recommendation was that the multicenter study be repeated with the same IgE and anti-IgE standards used by each laboratory. Also there is unresolved disagreement regarding (1) the best control to use to measure preformed IgE and (2) whether or not PWM can induce IgE synthesis in vitro. Hopefully, these obstacles and discrepancies will not be insurmountable to future progress and collaboration.

Promising investigations are moving forward with the use of monoclonal antibodies, hybridomas, and recombinant DNA technology to probe the structure and functions of IgE, IgE receptors, and IgE-binding factors. Regulatory cells and regulatory factors are being studied for their role in IgE synthesis.

Therefore, one can remain optimistic that ultimately the mystery of regulation of IgE synthesis will be unraveled and that allergic diseases will be controlled and even prevented.

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