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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.

Abbreviations used

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

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 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^+, $I\text{-}J^+$ antigen-specific suppressor T cells.

GIF, with a molecular weight of about 15 K, is a fragment of phosphorylated lipocortin. 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 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]

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		
Bordetella pertussis 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.

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 SUPERNATANTS[6-8]

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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 POKEWEED 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, 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