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Regulation of IgE synthesis in humans: A tale of two signals

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It is an unambiguous sign of vitality for a field when reviews become rapidly obsolete. After a decade of relatively slow progress, this is happening in the field of IgE regulation. After years of cell and cytokine cocktails, molecular biology is now providing an insight into the IgE system at a deeper, more meaningful level. The study of IgE regulation becomes important now not only because of the role of IgE in allergic disease but also, more generally, as a model to study the signals involved in isotype-specific regulation of immunoglobulin synthesis.

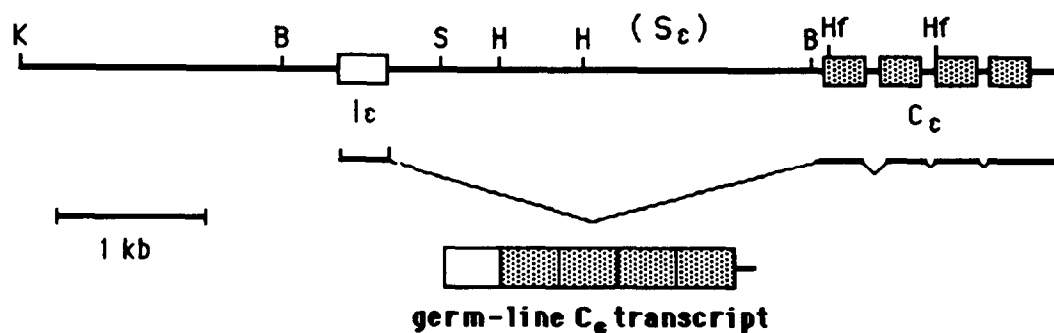
In the past 2 to 3 years, evidence from different laboratories has indicated that induction of IgE synthesis requires two signals: One is delivered by a cytokine, IL-4, and is IgE isotype specific. The other signal is a B cell-activating signal.¹ The challenge in IgE studies now is to determine what role the two signals play in the induction of IgE synthesis at the level of gene regulation.

Abbreviations used

Con A:	Concanavalin A
HIE:	Hyper-IgE
IFN- γ :	Interferon- γ
IL:	Interleukin
LPS:	Lipopolysaccharide
MAb:	Monoclonal antibody
PMA:	Phorbol myristate acetate
S region:	Switch region
TCR:	T cell receptor
Ab:	Antibody
MHC:	Major histocompatibility complex
Ag:	Antigen
EBV:	Epstein-Barr virus
sIgE:	Surface IgE
rIL:	Recombinant IL
cIgE:	Cytoplasmic IgE
TNF:	Tumor necrosis factor
FcR:	Fc receptor

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In this article, we will first briefly discuss the molecular mechanisms underlying immunoglobulin synthesis; then we will focus on the synthesis of IgE in humans and on IgE-inducing signals.



K = KpnI, B = BamHI, S = SmaI, H = HindIII, Hf = HinfI

FIG. 1. Structure of ϵ germ-line transcripts.

ISOTYPE SWITCHING AND IMMUNOGLOBULIN SYNTHESIS

The antigen-binding specificity of an immunoglobulin molecule is determined by the NH_2 -terminus of the immunoglobulin heavy and light chain, which is highly variable. By contrast, the COOH terminus of the heavy chain has a constant amino acid sequence that determines the effector functions of the immunoglobulin molecule, such as binding to FcRs on different cell types and activation of secondary pathways. The variable region of immunoglobulin is encoded by multiple germ-line elements that are assembled into complete V(D)J variable regions during B cell differentiation by a common enzymatic activity (recombinase), which is likely to be encoded by the RAG-1 and RAG-2 genes.^{2,3}

During an immune response, a B-lymphocyte can express different heavy chain isotypes sharing the same VDJ region. This phenomenon (heavy chain class switching) allows a single B cell clone to produce Abs that retain variable region specificity in association with a different C_H region gene, that is, with a different effector function. Class switching is believed to result from a recombination event that juxtaposes a downstream C_H gene to the expressed V(D)J gene. Intervening sequences, including the previously expressed C_H gene, are deleted. Recombination involves characteristic repetitive sequences (S regions) located 5' of the C_μ gene and corresponding S regions located immediately 5' of each C_H gene, except $\text{C}\delta$. S regions are 2 to 10 kb in length, and their sequence consists of short tandem repeats (e.g., GAGCT and GGGCT). S region recombination sites are not flanked by obvious consensus elements, and joining has been observed at various locations within the S regions.^{4,5} Switch recombination is generally believed to occur within one chromosome. However, recent studies in transgenic mice carrying an immunoglobulin μ heavy

chain transgene demonstrate that switch recombination can occur between two different chromosomes.⁶

Several studies have suggested that isotype switching may be a directed (nonrandom), rather than a stochastic, process. In particular, the observation that switch recombination in hybridomas and normal murine B cells is directed to the same S region on both the productive and the nonproductive C_H alleles⁷⁻⁹ favors directed switching. C_H rearrangements in human B cells appear to be more random; that is, switching to the same C_H gene on both alleles is not uniformly observed.¹⁰ However, the limited sample size may have influenced the pattern observed in these studies.

Isotype switching is regulated by mitogens and lymphokines. These agents are believed to control class switching by modulating the accessibility of specific S regions to a putative common switch recombinase.¹¹⁻¹⁵ The accessibility model is supported by several observations:

1. Before switching, the chromatin structure of the relevant S regions undergoes changes, such as increased sensitivity to DNase I digestion and hypomethylation.^{12,16,17} Inhibition of methylation by 5-azacytidine during B cell activation profoundly alters the direction and frequency of class switching.¹⁷
2. In murine B cells that are predisposed to undergo class switching to a particular isotype ($\gamma 1$, $\gamma 2\text{b}$, or ϵ), the C_H locus to which switching is directed is transcribed, before switching, into a germ-line transcript.^{11-15,18,19} The transcripts initiate ≈ 2 kb upstream of the S region involved in switch recombination and are processed to an mRNA in which a 100 to 500 bp upstream exon (designated I exon) is spliced to the C_H coding exons (Fig. 1). The resulting germ-line transcripts do not appear capable of initiating the synthesis of a mature protein, because the I exon contains stop codons in

all three reading frames. Therefore, germ-line transcripts are also referred to as "sterile" transcripts. Because the I exon is usually 200 to 300 bp shorter than the VDJ exon present in mature transcripts, germ-line transcripts are usually 0.2 to 0.3 kb smaller than mature transcripts.

The precise role of germ-line transcripts in isotype switching remains to be defined. All germ-line transcripts initiate 5' of the Switch region, consistent with the possibility that transcription through a targeted region may be directly involved in promoting accessibility. However, other possibilities, not necessarily mutually exclusive, should be considered. Transcription may be a byproduct of other events (e.g., binding of specific factors) involved in promoting recombinational accessibility. In addition, germ-line transcripts may themselves play a role in recombination events. The germ-line transcripts characterized so far share a similar general structure. Each consists of 5' exon derived from germ-line sequences upstream of the corresponding S region that is spliced to the C_H1 gene immediately downstream. These structural similarities suggest an analogous function, although the different transcripts contain no significant sequence homologies. Germ-line RNA transcripts themselves may promote specific recombination events through interaction with DNA in the S regions or could conceivably be used in putative interchromosomal RNA transsplicing for heavy chain class switching.²⁰

IL-4, THE FIRST SIGNAL FOR ISOTYPE SWITCHING TO IgE

Recent studies from various laboratories, including our own, have identified, in the cytokine IL-4, a crucial factor for isotype switching to IgE. The role played by IL-4 in the induction of IgE synthesis first became evident in 1986, when it was demonstrated that rIL-4 (at the time, still termed B cell stimulatory factor-1) could induce IgE production by LPS stimulated murine B cells.^{21, 22} More recently, IL-4 was demonstrated to be sufficient by itself to activate transcription through the ϵ locus. In murine B cells, stimulation with IL-4 induces the appearance of 1.7 to 1.9 kb germ-line C_ε transcripts^{15, 18} that contain an I_ε exon, located $\approx$ 2 kb upstream of S_ε, spliced to the C_ε1-C_ε4 exons. The I_ε exon has been reported to span $\approx$ 245 bp in I29 B-lymphoma cells²³ and 60 to 100 bp in the Abelson murine leukemia virus-transformed pre-B cell line A20.²⁴

The ϵ germ-line transcripts expressed by human B cells after stimulation with IL-4 consist of an $\approx$ 140 bp I_ε exon that is spliced to C_ε1 by removal of the intervening sequences from the primary transcript.²⁵ The germ-line transcript contains all four C_ε exons,

as well as the 3' untranslated region. Interestingly, ϵ germ-line transcripts were also induced in resting B cells isolated by Percoll-density gradients, indicating that no previous *in vivo* activation is required for expression of ϵ germ-line transcripts after IL-4 stimulation (Vercelli D, Shapira SK, Geha RS, et al., *In preparation*).

Thus, IL-4 is by itself sufficient for the initiation of germ-line transcription through the ϵ locus. However, additional stimuli are required for the expression of mature ϵ mRNA and IgE synthesis.

A SECOND B CELL-ACTIVATING SIGNAL IS REQUIRED FOR IgE SYNTHESIS

IL-4 is necessary, but not sufficient, for the induction of IgE synthesis by B cells. Highly purified normal human B cells could not be induced to produce IgE by IL-4 and mixtures of T cell-derived lymphokines.²⁶ The ability to produce IgE was restored on addition of autologous²⁷ or allogeneic²⁸ T cells. This observation led us first to propose a two-signal model for the induction of IgE synthesis.²⁷ LPS typically acts as a second signal for murine B cells.²¹⁻²⁴ In humans, a variety of second IgE-inducing signals have been described, which synergize with IL-4 in the induction of IgE synthesis (Fig. 2).

T cells

The important role played by T cells in the development of IgE Ab responses was first reported in the 1970s. These experiments were extensively reviewed.^{1, 29} In the mid-1980s, the identification of the T cell-derived lymphokine, IL-4, provided a rationale for the observation that selected alloreactive T cell clones induced IgE synthesis by B cells bearing the relevant alloantigen.³⁰ Subsequently, we demonstrated that T cells are crucial for IgE induction not only as a source of IL-4 but also because of their ability to deliver a second signal to B cells after cognate interaction between the TCR/CD3 complex and MHC class II Ags. MAbs directed to these molecules completely blocked IgE synthesis. The observation that anti-MHC class II, but not anti-MHC class I, MAb inhibited IL-4-dependent IgE synthesis was consistent with the finding that anti-CD4 MAb, but not anti-CD8 MAb, blocked IgE production and indicated that a CD4⁺ helper T cell subset is involved in B cell recognition.²⁷

To rule out the possibility that inhibition of IgE synthesis by MAbs to the CD3/TCR complex and MHC class II molecules resulted from direct negative signaling to the B and/or T cells, rather than from interference with T-B cell recognition, we used an experimental system in which normal B cells are stim-

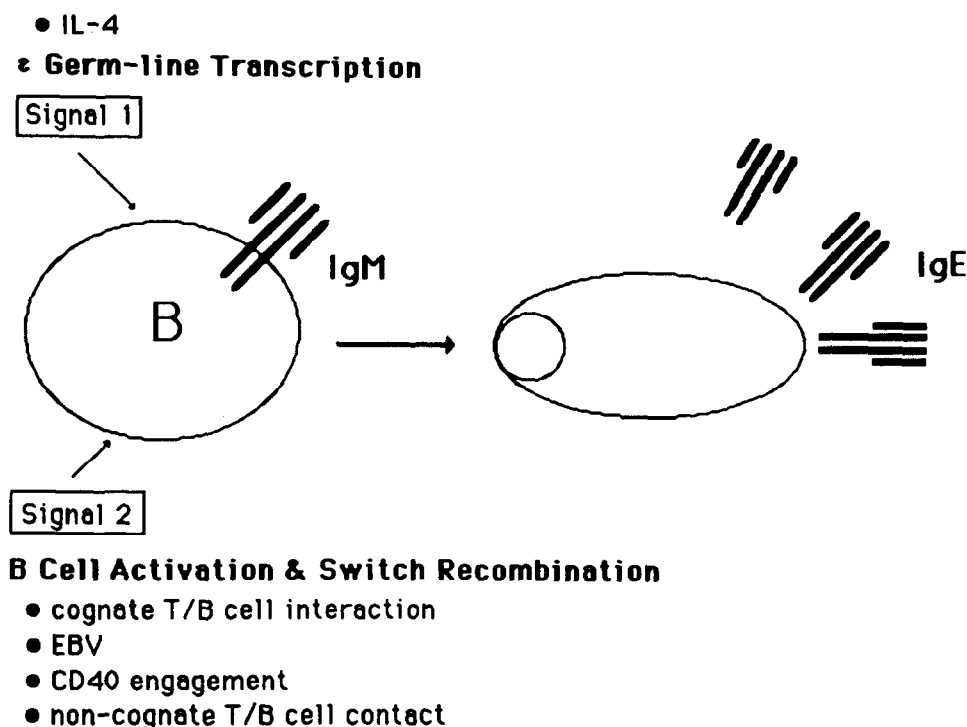


FIG. 2. A two-signal model for the induction of IgE synthesis in humans.

ulated to produce IgE by IL-4-producing alloreactive T cell clones. Such a system, in which no external stimuli can deliver direct negative signals to T and/or B cells, is only constrained by T-B cell recognition. Clone F6, which recognizes MHC class II Ags expressed on both B cells and monocytes,³¹ induced IL-4-dependent IgE synthesis in B cells bearing the relevant alloantigen, but not in HLA-mismatched B cells, even after addition of optimal amounts of exogenous IL-4. In contrast, clone A1, which recognizes MHC class II Ags only on monocytes,³¹ did not induce IgE synthesis when it was incubated with HLA-matched B cells and monocytes, in spite of a strong proliferative and lymphokine response. The addition of exogenous IL-4 did not restore IgE production. A comparison of the results obtained with clone A1 and F6 suggests that the failure of clone A1 to induce IgE synthesis is likely to result from the lack of direct T-B cell recognition.²⁷

The role of T cells in the induction of IgE synthesis has been further emphasized by recent studies with T cell clones obtained from patients with atopic disease and parasitic infections. CD4⁺ T cell clones established from atopic patients, and specific for *Dermaphagoides pteronyssinus*, produced very high levels of IL-4, as compared to CD4⁺ T cell clones from the same patients not specific for *D. pteronyssinus* or CD4⁺ clones from healthy individuals. These high-

level IL-4-producer T cell clones strongly supported the induction of IgE synthesis^{32, 33} (de Vries, JE. Personal communication). Furthermore, most helper T cell clones isolated from patients with filariasis, toxocariasis, and ascariasis produced high levels of IL-4.³⁴ Because IL-4 production is known to require repeated Ag stimulation,³⁵ these data appear to suggest that chronic stimulation by selected Ags (allergens) may select for allergen-specific T cells in individuals whose T cells are intrinsically prone to secrete large amounts of IL-4 on activation.

T cell-independent systems of IgE induction

Systems in which T cells deliver the B cell-activation signal(s) that synergize with IL-4 are highly complex and do not allow for dissection of second IgE-inducing signals in molecular terms. Several laboratories have therefore sought to identify T cell-independent systems for the induction of IgE synthesis. The classic polyclonal B cell activators (e.g., anti-IgM Abs, *Staphylococcus aureus* Cowan I, pokeweed mitogen, and phorbol esters) failed to synergize with IL-4 in inducing IgE synthesis in B cells²⁷ (authors' unpublished observation).

EBV. Recently, it has been demonstrated that stimulation with IL-4 and EBV induces T cell-independent IgE synthesis in human B cells.^{36, 37} IgE production is likely to result from de novo induction of isotype

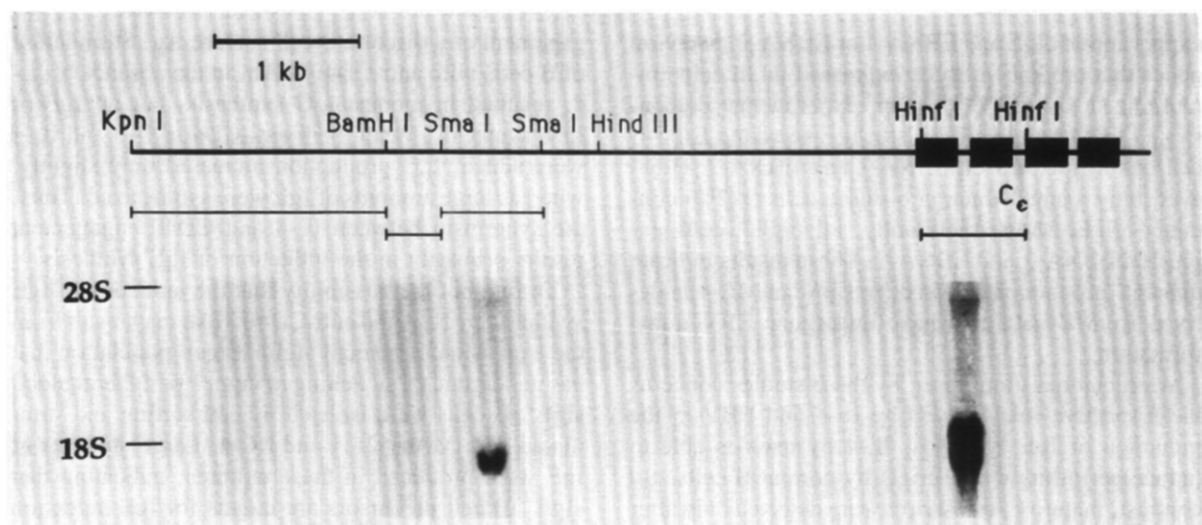


FIG. 3. Human B cells stimulated with EBV and IL-4 express both germ-line and mature C_ϵ transcripts.

switching rather than from expansion of a precommitted $slgE^+$ B cell population, which has undergone C_ϵ switching in vivo, because $slgE^-$ B cell precursors, isolated by cell sorting, were stimulated by EBV and IL-4 to produce IgE.³⁷

To study the molecular events that accompany IgE induction by EBV and IL-4, we cloned a series of contiguous human genomic DNA fragments: a 1.8 kb Kpn I-BamH I, a 0.48 kb BamH I-Sma I and a 0.7 kb Sma I fragment (Fig. 3). These DNA fragments span an ≈ 3 kb region that contains the I_ϵ exon and were used as probes to analyze RNA from IgE-secreting B cell lines obtained by EBV transformation in the presence of IL-4. Furthermore, we used a genomic 0.88 kb Hinf I fragment that encompasses most $C_\epsilon 1$ exon and the whole $C_\epsilon 2$ exon.³⁸ The Hinf I fragment would hybridize with mature C_ϵ transcripts (in which mRNA for the rearranged VDJ has been spliced to C_ϵ), as well as with germ-line transcripts containing the C_ϵ region. By contrast, the other probes would only hybridize to transcripts containing sequences upstream of S_ϵ , that is, to germ-line C_ϵ transcripts. Our results demonstrated that the Hinf I fragment hybridized to two mRNA species in IgE-secreting EBV-B cells, a 2 kb band and a more prominent 1.8 kb band. The 1.8 kb band, but not the 2 kb band, also hybridized with the Sma I fragment and therefore corresponded to germ-line C_ϵ transcripts (Fig. 3). No signal was detected when the Hinf I and the Sma I fragments were used to probe RNA from EBV-B cells that were not treated with IL-4 and secreted immunoglobulin isotypes other than IgE. These results indicate that IgE-secreting B cells obtained by activation with EBV

and IL-4 contain both mature (2 kb) and germ-line (1.8 kb) C_ϵ transcripts. No signal was detectable when RNA was probed with the BamH I-Sma I fragment or with the Kpn I-BamH I fragment, which are located immediately 5' of the Sma I fragment. These results suggest that the initiation site for the germ-line C_ϵ transcripts induced by IL-4 and EBV is probably contained within the Sma I fragment. These results are consistent with results obtained with IL-4-stimulated peripheral blood mononuclear cells.²⁵

We next investigated whether rIL-4 is able to induce IgE synthesis by established EBV-B cell lines secreting immunoglobulin isotypes other than IgE. To this purpose, rIL-4 (100 U/ml) was added to different EBV-B cell lines that had been maintained in culture for more than 3 months and secreted IgM and IgG. IL-4 failed to induce mature C_ϵ transcripts and IgE synthesis but induced germ-line C_ϵ transcription. Thus, in established EBV-B cell lines stimulated with IL-4, there is a dissociation between germ-line C_ϵ transcription and isotype switching to IgE expression.

The failure of IL-4 to induce mature C_ϵ transcripts in established EBV-B cell lines raised the question whether IgE production in cultures treated with IL-4 and EBV was derived from B cells that were infected with EBV or from bystander noninfected B cells activated by growth factors secreted by the EBV-infected cells. In this regard, only few circulating B cells (<2%) are susceptible to EBV transformation and immortalization.³⁹ To address this issue, IgE-secreting B cells obtained by activation of peripheral blood B cells with IL-4 and EBV were analyzed by double immunofluorescence for the simultaneous

expression of cIgE and EBNA-1 or EBNA-2, two viral genes that are known to be expressed in all cells immortalized by EBV.⁴⁰⁻⁴² Our experiments demonstrated that virtually all cells in IgE-secreting EBV-B cell cultures expressed EBNA-1 and EBNA-2. However, only a small fraction of these cells ($\leq 2\%$) contained cIgE. Most importantly, all cIgE⁺ cells expressed EBNA-1 and EBNA-2. These results indicate that IgE production induced by EBV and IL-4 is derived from B cells that have undergone EBV transformation.

A crucial question raised by these studies concerns the respective roles played by IL-4 and EBV in the induction of IgE synthesis. IL-4 by itself is able to induce germ-line ϵ transcripts by normal B cells. By contrast, mature C ϵ transcripts were detected only after stimulation with both IL-4 and EBV. These data are consistent with a two-signal model for isotype switching to IgE. EBV delivers an activating signal functionally equivalent to the signal provided in mice by LPS. The nature of the EBV-derived signal is presently unknown. Activation by EBV does not simply result from engagement of the EBV receptor/CD21/CR2. MAbs to different CD21 epitopes, including MAb OKB7, which recognizes an epitope in the EBV-binding site⁴³ and induces activation of human B-lymphocytes,⁴⁴ could not synergize with IL-4 in IgE induction. EBV infection results in the expression of viral proteins, at least some of which (e.g., EBNA-2, EBNA-3C, and latent membrane protein) are important effectors of B-lymphocyte gene expression and cell-growth transformation and proliferation.⁴⁵⁻⁴⁸ These EBV genes could facilitate recombination indirectly through effects on transcription or cell proliferation.

Because EBV-infected cells express virally encoded as well as endogenous proteins, characterization of the second signal required for IgE induction is difficult in the EBV system. Furthermore, although established infection with EBV does not appear to interfere with IL-4-dependent transcriptional activation of the ϵ locus, stable expression of the EBV genome dissociates germ-line ϵ transcription from isotype switching. Thus, the EBV system is still too complex to be amenable to dissection.

CD40. We therefore sought to identify B cell surface Ags that are important in IgE synthesis. Abs to IgM, CD20, and MHC class II molecules, used in combination with IL-4, were found to be ineffective. However, anti-CD40 MAb 626.1 potently induced IgE synthesis by highly purified human B cells isolated by positive sorting for CD20 expression.⁴⁹ This is the first system in which the B cell-activating signal required for human IgE synthesis is delivered by en-

gagement of a discrete B cell surface Ag. Stimulation of B cells with anti-CD40 MAb, in the absence of IL-4, resulted in modest IgG synthesis, but no IgE or IgM synthesis. When both anti-CD40 MAb and IL-4 were added, IgG production increased only slightly, whereas large amounts of IgE were synthesized. Thus, the synergism between IL-4 and CD40 engagement results primarily in the induction of IgE synthesis.

To rule out the possibility that the induction of IgE synthesis by IL-4 and anti-CD40 MAb may result from the expansion of a small sIgE⁺ B cell population that has undergone C ϵ switching in vivo,⁵⁰ peripheral blood sIgE⁻ B cells were isolated by cell sorting and stimulated with MAb 626.1 and rIL-4. Large amounts of IgE were produced in these cultures, indicating that sIgE⁻ B cell precursors are targets for IgE induction by IL-4 and anti-CD40 MAb.

CD40 is a 50 kd surface glycoprotein expressed on all human B-lymphocytes and by certain carcinomas and melanomas, but not by T cells and monocytes.^{51, 52} Anti-CD40 MAb (such as MAb G28.5 and MAb S2C6) deliver a strong progression signal to resting B cells in the presence of primary activators, such as phorbol esters, anti-CD20 MAb, and anti-IgM Abs.⁵³⁻⁵⁵ By contrast, MAb 626.1 (used in our studies) delivers a competence signal that results in significant proliferation of highly purified resting B cells in the absence of other costimuli.⁵⁶ In addition, MAb to CD40 induce homotypic B cell adhesion.⁵⁷

Molecular cloning has revealed that the CD40 gene is closely related to the receptors for nerve growth factor^{58, 59} and TNF- α .⁶⁰ This suggests that CD40 may be involved in the regulation of B cell activation and growth. Interestingly, selected anti-CD40 MAb cross-linked on Fc γ R synergize with IL-4 in inducing long-term, factor-dependent growth of B cell lines in the absence of EBV transformation.⁶¹

The natural ligand of CD40 is not known. Because of its homology with the nerve growth factor receptor and the TNF- α receptor, CD40 has been suggested to be a receptor for a soluble growth factor. However, none of the known soluble B cell growth factors uses CD40 as its receptor.⁶² We are examining the possibility that T cells express a ligand for CD40 on activation. This would implicate CD40 in noncognate, contact-dependent, T-B cell interactions (see below).

It is not clear at which step(s) the IL-4 and CD40-dependent signaling pathways actually synergize to induce IgE production. IL-4 has been demonstrated to costimulate human B cells only when it is combined with competence signals that activate protein kinase C and induce increase in intracellular calcium.⁶³ Anti-CD40 MAb 626.1, however, does not induce calcium mobilization, even after cross-linking.⁵⁶ Interestingly,

IL-4 has been demonstrated to up regulate CD40 expression on B cells.⁶³ This up regulation may amplify the signals generated by the reaction between CD40 and the MAb 626.1 and may be important in IgE induction.

Noncognate T-B cell interactions. It has been recently suggested that, in addition to cognate T-B cell interactions involving the TCR/CD3 complex and MHC class II molecules, MHC-unrestricted, noncognate T-B interactions might provide a second signal for induction of IgE synthesis in humans. Evidence for the role of noncognate T-B interactions comes from two distinct experimental systems: (1) phytohemagglutinin-induced T cell clones, both CD4⁺ and CD8⁺, have been reported to induce IgE synthesis by B cells from randomly selected donors if large amounts of exogenous IL-4 are added to the cultures,⁶⁴ and (2) mouse EL-4 thymoma cells potently induce IgE synthesis by human B cells if both PMA and exogenous human rIL-4 are added. In both cases, requirement for cell-cell contact has been demonstrated by culturing T and B cells in chambers partitioned by a semipermeable membrane.⁶⁴⁻⁶⁶ The significance of these models will only become clear once the molecules that deliver the contact-dependent signal(s) are characterized. In particular, it will be interesting to determine whether any of the B cell surface Ags already known to be involved in IgE induction play a role in noncognate T-B cell interactions.

Hydrocortisone. IgE synthesis has been recently induced in human CD5⁺ leukemic B cells by a combination of IL-4 and hydrocortisone in the absence of T cells. Furthermore, hydrocortisone up regulates IL-4-dependent IgE synthesis by normal unfractionated mononuclear cells.⁶⁷ Normal polyclonal sIgE⁻ B cells isolated by cell sorting are also inducible by hydrocortisone and IL-4 (Jabara H, Ahern D, Vercelli D, Geha R. In press). The mechanisms by which hydrocortisone synergizes with IL-4 are unknown. However, the observation that hormone-receptor interactions can provide a second signal for IgE synthesis is of great interest while it warrants some caution in prescribing steroids for allergic disease.

These data discussed above clearly indicate that the second signal(s) required for IgE production can be delivered to B cells through different pathways. At the present time, no unifying explanatory mechanism can reconcile all different observations. Not much is known about signaling via MHC class II molecules or CD40; signaling via CD21 is not sufficient for IgE induction, and EBV-induced proteins essential for IgE induction have not been identified. The role played by hydrocortisone indeed is not clear, and the molecules involved in noncognate T-B cell interactions are

as yet unknown. It appears, however, plausible to speculate that the pathways mentioned above may share the ability to activate the switch recombination machinery in B cells in which the ϵ locus has been transcriptionally activated and/or rendered accessible by IL-4. Alternatively, the IL-4 signal may be sufficient for switch recombination, and the activating signal(s) may simply allow B cells to survive in culture long enough for switching to become detectable. A strategy based on transfection of "switch plasmids" containing S _{μ} and S _{ϵ} into IL-4-responsive B cell lines should allow distinguishing between these two possibilities.

SWITCH RECOMBINATION OR ALTERNATIVE SPLICING?

In addition to the second signals required for switching to IgE, the molecular mechanisms that underlie the production of mature ϵ mRNA are also a matter of debate. No formal evidence has been provided to date to demonstrate that IgE synthesis results from switch recombination rather than from alternative mechanisms (e.g., alternative splicing of a long mRNA transcript, transsplicing). The human IgE myeloma cell line, U266, has undergone switch recombination to S _{ϵ} , at least on the productive chromosome.^{68, 69} In contrast, a cloned EBV-B cell line obtained by limiting dilution, in the absence of IL-4, has been found to coexpress IgE and IgM.⁵⁰ IgE production was not accompanied by DNA rearrangement because the ϵ locus was maintained in its germ-line configuration on both alleles with no deletional switch rearrangement.⁷⁰ Expression of mature VDJCE mRNA was believed to occur via alternative processing of a long transcript, as previously proposed for the simultaneous expression of multiple isotypes, including IgE, in neoplastic B cell lines as well as in normal B cells.⁷¹⁻⁷³ However, the existence of a long primary transcript has never been demonstrated. In contrast, analysis of immunoglobulin circular DNA from murine IL-4-stimulated B cells has recently provided evidence for successive class switching from μ to ϵ via $\gamma 1$.⁷⁴ Thus, the mechanisms underlying IgE production by B cells remain unclear. We have recently addressed this issue by use of plasmidase chain reaction amplification of ϵ switch fragments, followed by cloning and DNA sequencing. This approach was validated by demonstrating S _{μ} /S _{ϵ} rearrangement with deletion of intervening sequences in the IgE myeloma cell line U266.⁷⁵ Preliminary data obtained with the same approach indicate that switch recombination does also occur after human B cell stimulation with EBV and IL-4.⁷⁶ The length of the switch fragments thus isolated varies, consistent with different switch

junctions. Our results therefore provide formal evidence for the occurrence of switch recombination in B cells that have been stimulated with IL-4 and an appropriate second activating signal.

CYTOKINE-DEPENDENT MODULATION OF IL-4-DRIVEN IgE SYNTHESIS

Evidence coming from several laboratories indicates that multiple cytokines, derived from T as well as non-T cells, are able to modulate IL-4-dependent IgE synthesis. We have demonstrated that IL-5 and IL-6 up regulate the IgE response induced by IL-4 in unfractionated mononuclear cells. IgE up regulation by rIL-5 was more evident when suboptimal concentrations of rIL-4 were used, whereas rIL-6 could up regulate IgE synthesis even in the presence of high concentrations of rIL-4. The effects of the combination of rIL-4, rIL-5, and rIL-6 were additive in some experiments and clearly synergistic in others. By contrast, IL-2, IL-1, and TNF- α had no amplifying effect. Most interestingly, we found that endogenous IL-6 is crucially involved in IL-4-dependent IgE induction, since an anti-IL-6 Ab strongly inhibited the production of IgE induced by IL-4.²⁶ Furthermore, endogenous IL-6 has an important amplification role in IgE synthesis induced by IL-4 and CD40 engagement.⁴⁹ Interestingly, both IL-4 and anti-CD40 MAb induced IL-6 production in B cells.^{62, 77} Furthermore, IL-6 increases the phosphorylation of CD40 in human B cells, thus leading to a CD40-IL-6 loop.⁶² IL-6 is known to act at a late stage in B cell differentiation with no isotype preference.⁷⁸ IL-6 induces the secretion of IgG1 by coordinated transcriptional activation, selective accumulation of mRNA for the secreted form of IgG, and possibly differential mRNA stabilization.⁷⁹ IL-6 may amplify IgE secretion by similar mechanisms.

At least one more lymphokine appears to be involved in IgE regulation. IFN- γ has been reported to inhibit IL-4-dependent IgE synthesis in both mice^{21, 80, 81} and humans.^{82, 83} IFN- γ also antagonizes the effects of IL-4 in other systems; for example, it blocks the IL-4-dependent induction of Fc ϵ R II/CD23 on B cells,⁸⁴ although it does not block on monocytes.⁸⁵ However, alloreactive T cell clones secreting both IL-4 and IFN- γ in large amounts can induce IgE synthesis by normal B-lymphocytes.²⁷ Of particular interest is the finding that the expression of IFN- γ is down regulated by IL-4, both at the protein and at the mRNA level.^{28, 86} Induction of IgE synthesis by IL-4 may thus reflect not only the ability of this lymphokine to target the S ϵ region for switch recombination but also the simultaneous suppression of a major functional IL-4 antagonist, IFN- γ .

The mechanisms by which IFN- γ inhibits IgE syn-

thesis are not well defined. IFN- γ profoundly suppressed the expression of ϵ germ-line transcripts in murine B cells stimulated with IL-4 and LPS.⁸⁷ By contrast, no inhibition was observed when IFN- γ was added to highly purified human B cells stimulated with IL-4.²⁵ These results indirectly suggest that activation of germ-line transcription and switch recombination may not be necessarily coupled. IFN- γ may thus prevent recombination events without affecting the expression of ϵ germ-line transcripts.

THE HIE SYNDROME

The HIE syndrome is a complex disorder characterized by high levels of serum IgE, chronic dermatitis, and recurrent serious infections.⁸⁸ The etiology of the HIE syndrome is unknown. There is evidence to suggest that patients with HIE have T cell defects, which include impaired *in vivo* and *in vitro* T cell-mediated responses to specific Ags and defective Ab responses, particularly to polysaccharides.^{89, 90} The mechanisms responsible for increased IgE production in the HIE syndrome are unknown. We have investigated the role played by cellular interactions and lymphokines in the regulation of spontaneous IgE synthesis by peripheral blood mononuclear cells from patients with the HIE syndrome. Our data indicated that spontaneous *in vitro* IgE synthesis in the HIE syndrome is largely independent of endogenous lymphokines (IL-4 and IL-6) and T-B cell cognate interactions and is likely to be due to a terminally differentiated B cell population, no longer sensitive to regulatory signals known to modulate IgE induction in normal subjects.⁹¹

The lymphokine production pattern in patients with HIE was studied by assessing IL-4 and IFN- γ concentrations in supernatants from unfractionated mononuclear cells from patients with HIE stimulated with Con A or PMA and ionophore. Con A activates T cells through the TCR/CD3 complex.⁹² Patients with HIE, however, have defective proliferative responses to specific Ags that may, in turn, reflect alterations in TCR/CD3-mediated signaling. In addition to Con A, we therefore used PMA plus ionophore because signaling of T cells by the latter agents bypasses surface receptors and does not require accessory cells.⁹³ No abnormality in lymphokine secretion was apparent with either modality of stimulation.⁹¹ However, no definitive conclusion can be drawn at the present time because IL-4 secretion *in vitro* could only be detected after activation with PMA and ionophore, a rather nonphysiologic type of stimulation. Thus, although no simple causal relation is to be established between increased IgE production and lymphokine secretion in patients with HIE, our data do not rule out the possibility that hyperproduction of IL-4 (absolute or rel-

ative to IFN- γ) may occur in vivo at a local and/or peripheral level, escaping our detection. In this respect, it has been observed that most circulating B cells from patients with HIE states bear CD23/Fc ϵ R IIb on their membrane (Kishimoto T. Unpublished observation). This indirectly suggests that IL-4 may be abnormally secreted in patients with HIE because the expression of Fc ϵ R IIb on B cells is IL-4 dependent.⁹⁴ The frequent finding of eosinophilia in patients with the HIE syndrome⁹⁰ also supports this hypothesis because eosinophil differentiation is induced by IL-5,⁹⁵ which is produced by T cell clones concomitantly with IL-4.^{1, 96} Furthermore, analysis at the clonal level recently indicated that patients with HIE syndrome have a significantly decreased proportion of circulating T cells that produce IFN- γ and TNF- α , as compared to normal control subjects.⁹⁷ A decreased synthesis of IFN- γ may be responsible for IgE hyperproduction, whereas a defect in TNF- α may underlie the increased susceptibility to infections observed in patients with HIE.⁹⁷

Finally, an additional intriguing possibility should be mentioned. IL-4 may derive, at least in part, from a non-T cell source. It has recently been demonstrated that murine mast cells secrete IL-4 in response to cross-linkage of the high-affinity receptor for IgE or to calcium ionophores.⁹⁸ Furthermore, Fc ϵ R-mediated IL-4 synthesis has been detected in a population of murine spleen cells that lack B cell, T cell, and natural killer cell markers.⁹⁹ This cell population, which is markedly expanded after infection with *Nippostrongylus brasiliensis* or injection of anti-IgD Ab,¹⁰⁰ might represent a local source of IL-4, which may provide one of the two signals necessary to activate B cells to IgE secretion. The second signal may be provided by polyclonal activators (such as EBV or other viruses or CD40 ligands yet to be identified) acting directly on B cells.^{36, 37, 49} It is tempting to speculate that hyperproduction of IgE in the HIE syndrome may derive, at least in part, from T cell-independent mechanisms.

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