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Regulation of IgE synthesis in humans

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In the past 2 to 3 years, evidence from different laboratories has indicated that induction of IgE synthesis requires two signals. One signal is delivered by a cytokine, IL-4, and is IgE isotype specific. The other signal is a B-cell-activating signal.¹ The challenge is to determine what role the two signals play in the induction of IgE synthesis at the level of gene regulation. We will first briefly discuss the molecular mechanisms underlying immunoglobulin synthesis, and then we will focus on the synthesis on IgE in humans and on IgE-inducing signals. Finally, we will discuss, in the light of our recent understanding of IgE synthesis, future strategies aimed at suppressing the IgE response.

ISOTYPE SWITCHING AND IMMUNOGLOBULIN SYNTHESIS

The antigen-binding specificity of an immunoglobulin molecule is determined by the NH₂ 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 Fc receptors

Abbreviations used

IFN- γ :	Interferon- γ
IL:	Interleukin
rIL:	Recombinant IL
LPS:	Lipopolysaccharide
bp:	Base pair
MAb:	Monoclonal antibody
S region:	Switch region
EBV:	Epstein-Barr virus
mRNA:	Messenger ribonucleic acid
sIgE:	Secreting IgE
PCR:	Plasma clearance rate
s-IL-4 R:	Soluble IL-4 receptor
GLT:	Germ-line transcript

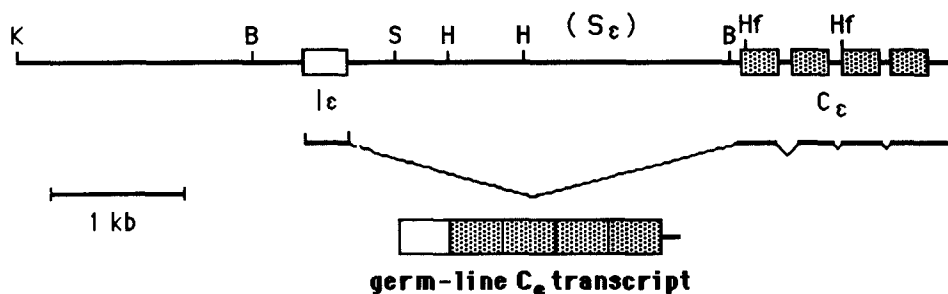
on different cell types and activation of secondary pathways. The variable region of immunoglobulin is encoded by multiple germ-line elements that are assembled into complete V(D)J variable regions during B-cell differentiation by a common enzymatic activity (recombinase), which is likely to be encoded 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 antibodies that retain variable region specificity in association with a different CH region gene, that is,

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

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

Sterile
 ϵ germ-line transcript



Productive
mature ϵ transcript



FIG. 2. Comparison of sterile versus productive ϵ mRNA. The I exon region is ≈ 200 bp shorter than the VDJ exon region. Thus, the sterile ϵ germ-line transcript is ≈ 200 bp shorter than the productive mature ϵ transcript.

with a different effector function. Class switching is believed to result from a recombination event that juxtaposes a downstream CH gene to the expressed V(D)J gene. Intervening sequences, including the previously expressed CH 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 CH gene, except C δ . 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

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.⁵ 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.^{6, 12}
2. In murine B cells that are predisposed to undergo class switching to a particular isotype ($\gamma 1$, $\gamma 2b$, or ϵ), the CH locus to which switching is directed is transcribed before switching into a germ-line

transcript.^{5, 7} 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 CH 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 (Fig. 2).

The precise role of germ-line transcripts in isotype switching remains to be defined. The germ-line transcripts characterized thus far share a similar general structure. Each consists of a 5' exon derived from germ-line sequences upstream of the corresponding S region that is spliced to the CH1 gene immediately downstream. These structural similarities suggest an analogous function.

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

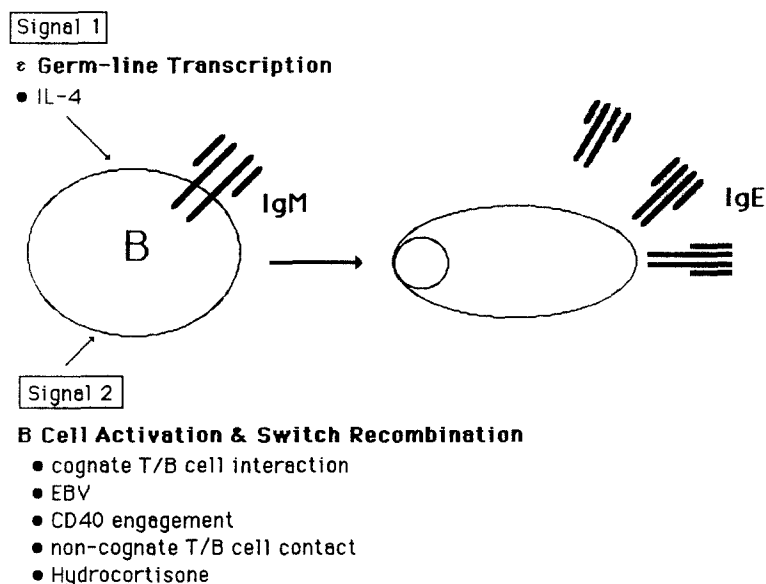


FIG. 3. A two-signal model for the induction of IgE synthesis in humans.

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.⁸ More recently, IL-4, by itself, was demonstrated to be sufficient 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 that contain a I ϵ exon, located $\approx$ 2 kb upstream of S ϵ , spliced to the C ϵ 1 to C ϵ 4 exons.

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 ϵ 1 by removal of the intervening sequences from the primary transcript.⁸ The germ-line transcript contains all four C ϵ exons, as well as the 3' untranslated region.

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. LPS typically acts as a second signal for murine B cells.⁹ In humans, a variety of second IgE-inducing signals have been described, which synergize with IL-4 in the induction of IgE synthesis (Fig. 3).

T cells

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 T cell receptor/CD3 complex and major histocompatibility complex class II antigens.¹⁰ 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 *Der-matophagoides pteronyssinus*, produced very high levels of IL-4 compared with that of CD4⁺ T cell clones from the same patients not specific for *D. pteronyssinus* or with that of CD4⁺ clones from healthy individuals. These high IL-4-producer T-cell clones strongly supported the induction of IgE synthesis¹¹ (de Vries, J. E. Personal communication). Furthermore, most helper T-cell clones isolated from patients with filariasis, toxocariasis, and ascariasis produced high levels of IL-4.¹² These data appear to suggest that chronic stimulation by selected antigens (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

EBV virus. Recently, stimulation with IL-4 and EBV has been demonstrated to induce T-cell-independent IgE synthesis in human B cells.^{13, 14} To study the molecular events that accompany IgE induction by EBV and IL-4, we cloned a genomic 0.88 kb *Hinf*I fragment that encompasses most C ϵ 1 exon and the whole C ϵ 2 exon (Fig. 3). The *Hinf*I fragment hybridized with mature 2 kb C ϵ transcripts present in U266 plasmacytoid cells and in EBV⁺ IL-4-stimulated B cells in which mRNA for the rearranged VDJ has been spliced to C ϵ , as well as with 1.8 kb germ-line transcripts containing the C ϵ region. These results indicate that sIgE B cells obtained by activation with EBV and IL-4 contain both mature (2 kb) and germ-

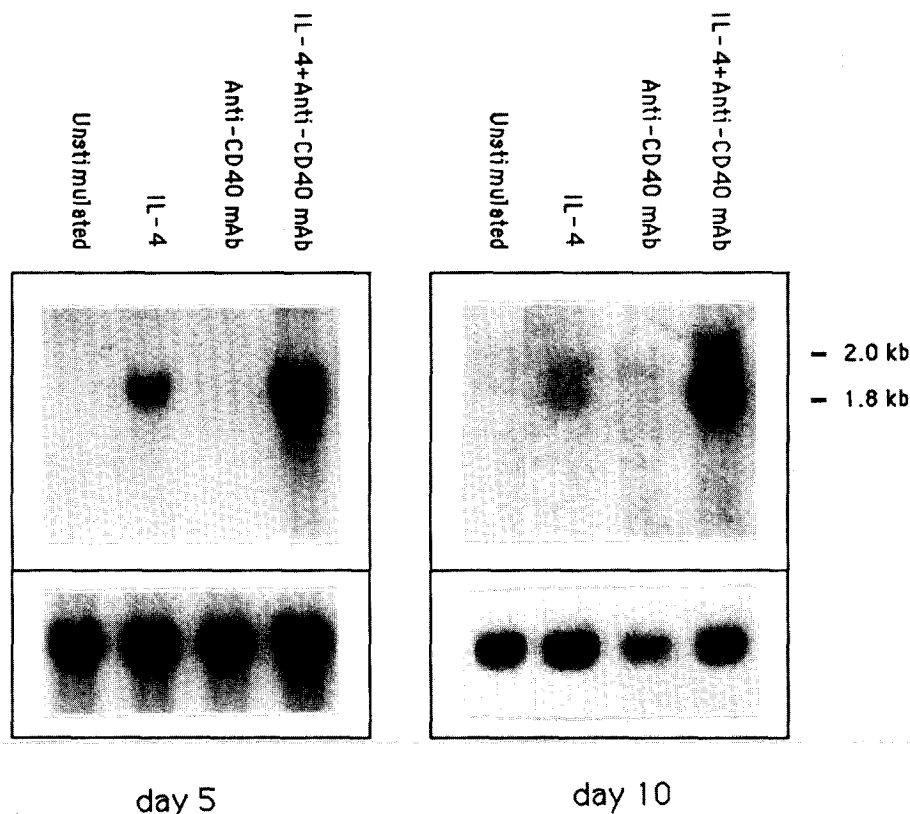


FIG. 4. Induction of germ-line and mature $C\epsilon$ transcripts in normal human B cells stimulated with rIL-4 and anti-CD40 MAb. Total RNA (10 μ g) from normal peripheral blood B cells stimulated with rIL-4 and/or anti-CD40 MAb for 5 and 10 days and from U266 cells was subjected to electrophoresis on a 1% formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized to [^{32} P]-labeled 0.88 kb *Hin*I (top panels) or actin (bottom panels) probes. Top panels were exposed for 3 days with the exception of the U266 lane (4 hours). Bottom panels were exposed for 1 day. Similar results were obtained in two additional experiments.

line (1.8 kb) $C\epsilon$ transcripts. In contrast, only sterile $C\epsilon$ transcripts were induced by IL-4 alone (see below).

CD40. 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. We therefore sought to identify B-cell-surface antigens that are important in IgE synthesis. Antibodies to surface IgM, CD20, and major histocompatibility 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.¹⁵ CD40 is a 50 kd surface glycoprotein expressed on all human B lymphocytes. Molecular cloning has revealed that the CD40 gene is closely related to the receptors for nerve-growth factor and tumor necrosis factor- α . The natural ligand of CD40 is not known.

RNA of B cells incubated with anti-CD40 MAb 626.1, rIL-4, or both, for 5 and 10 days, was analyzed

by Northern blotting with the 0.88 kb *Hin*I probe that hybridizes to both 1.8 kb ϵ germ-line transcript and 2 kb mature $C\epsilon$ transcripts. That 1.8 kb ϵ GLT, but not mature 2 kb $C\epsilon$ mRNA, were detected in B cells stimulated with IL-4 for 5 or 10 days is illustrated in Fig. 4. Anti-CD40 MAb by itself did not induce either GLT or mature $C\epsilon$ transcription but synergized with IL-4 to enhance ϵ GLT accumulation. Densitometric analysis of ϵ GLT mRNA bands versus actin mRNA demonstrated a sevenfold increase in ϵ GLT mRNA after addition of anti-CD40 MAb at both time points. A 2 kb band, corresponding to mature ϵ mRNA, as indicated by comigration with the major band in RNA from the sIgE plasmacytoma U266, was detected only in B cells stimulated with both IL-4 and anti-CD40 MAb for 10 days. These results indicate that the CD40 signal induced isotype switching and the production of mature ϵ mRNA. The effects of CD40 engagement were totally dependent on the presence of IL-4. These findings are compatible with the hypothesis that the

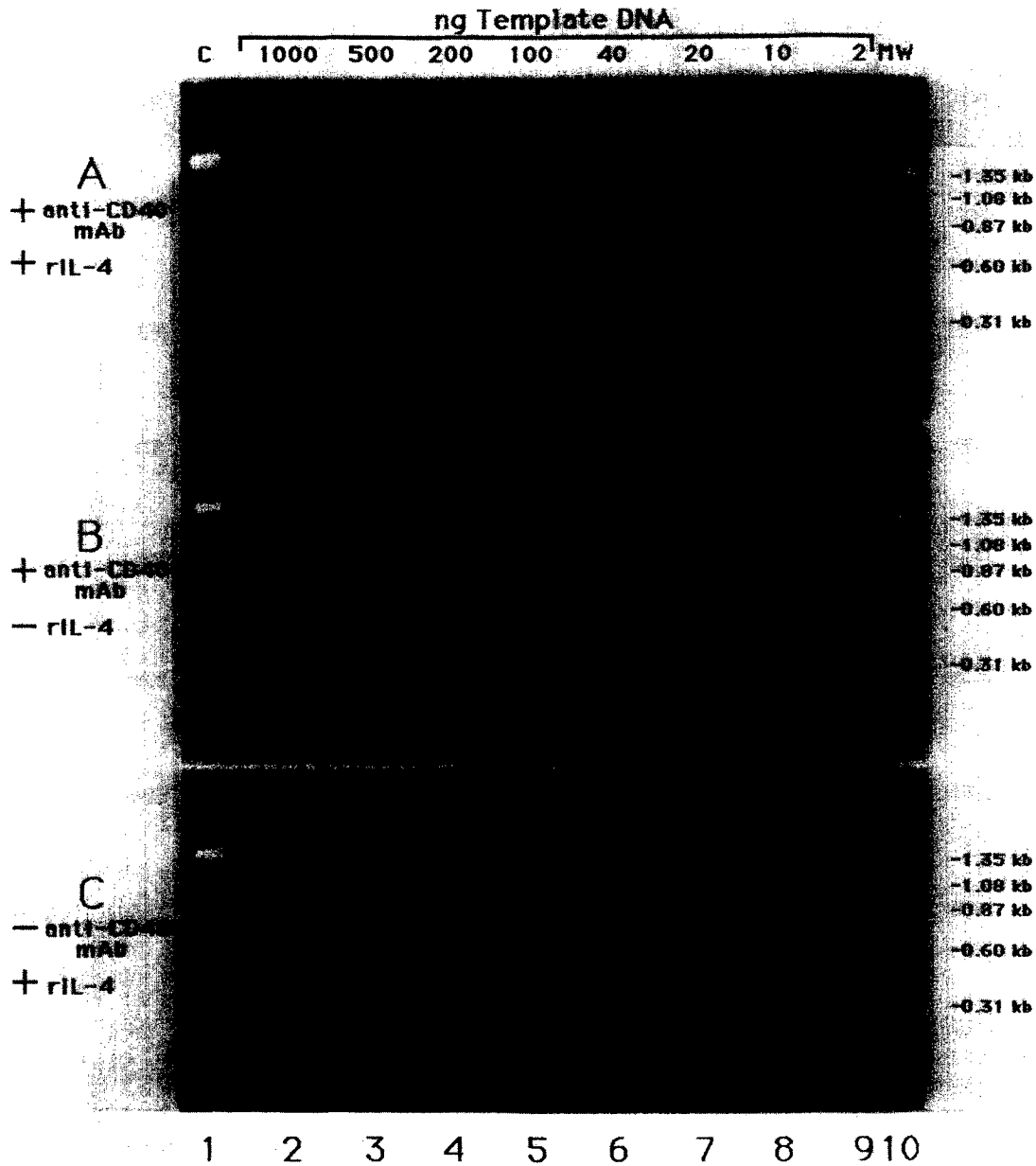


FIG. 5. Nested primer PCR amplification of "switch fragments." **A**, Serially diluted aliquots of total cellular DNA from B cells stimulated with anti-CD40 MAb and IL-4. **B**, Anti-CD40 MAb alone. **C**, IL-4 alone. Amplified by nested primer PCR. **A**, Serially diluted template DNA sample amounts used in the first round of PCR are noted above the gel. Second or nested round of PCR used 10% of the original PCR reaction mixture as DNA template. Final PCR products were subjected to agarose gel electrophoresis. PCR-amplified products were generated only from DNA of B cells stimulated with both anti-CD40 MAb and IL-4 (**A**, lanes 2 to 8). PCR-amplifiable DNA was initially present in all three samples, as evident by the "control" band (lane 1), for which primers specific for the human IL-1 β promoter (-1323/+72) yield a 1.4 kb fragment by PCR. **C**, Control molecular weight markers.

induction of GLT may result in increased accessibility of the downstream S region for deletional switch recombination with S μ and that the CD40-derived signal may function as an activator of a DNA recombination system.

Noncognate T-B cell interactions. 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,¹⁶ and mouse EL-

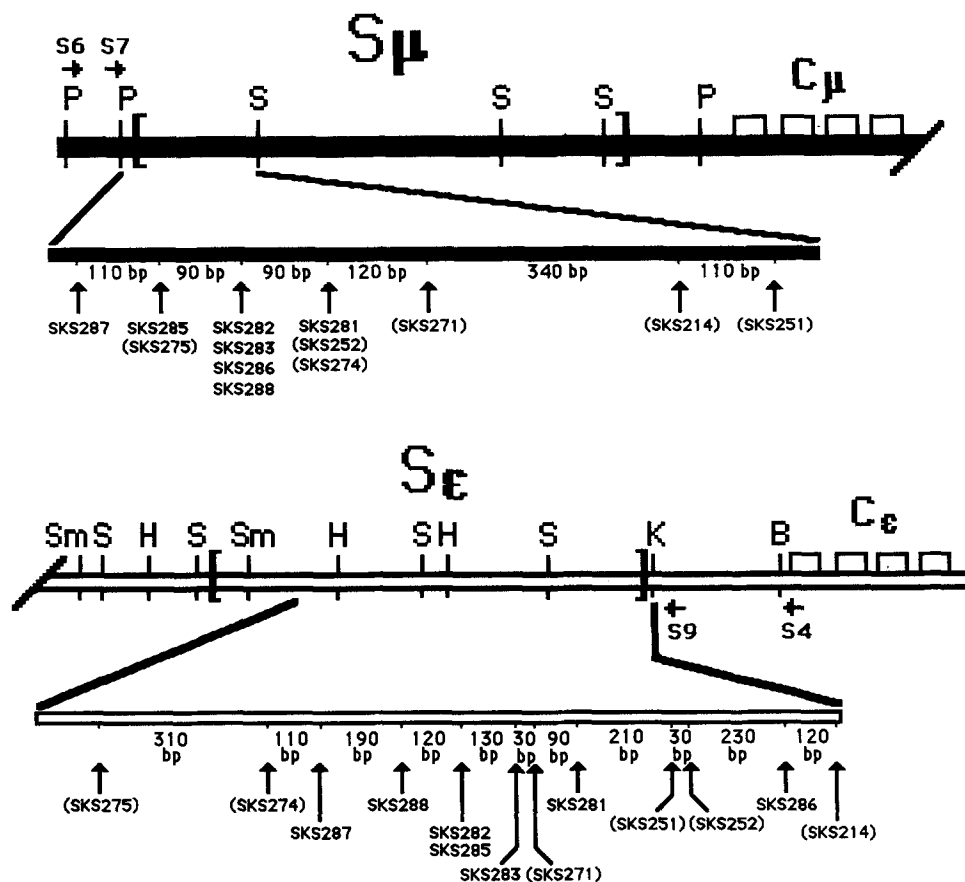


FIG. 6. Switch recombination sites for the sequenced S_{μ}/S_{ϵ} "switch fragments." Map positions of PCR primers upstream for S_{μ} (S6, S7) and downstream from S_{ϵ} (S4, S9) are illustrated. Only in "switch fragments" in which there has been recombination between S_{μ} and S_{ϵ} , forming a hybrid switch region, will a primer pair amplify a product by PCR. First round of PCR used primers S6/S4, whereas second or nested round of PCR used primers S7/S9. Schematic representations of each switch region and recombination sites for sequenced "switch fragments" are illustrated. Each "switch fragment" (designated as SKS2NN) is comprised of DNA segment upstream of recombination site in S_{μ} joined to DNA segment downstream of recombination site with same designation of S_{ϵ} . Sites of recombination for "switch fragments" described in Shapira et al.¹⁹ isolated from sIgE-EBV-transformed B cells and from U266 are illustrated in parentheses. Approximate map distances between recombination sites are noted (rounded off to nearest 10 bp): B, *Bam*HI; H, *Hind*III; K, *Kpn*I; P, *Pst*I; S, *Sac*I; Sm, *Sma*I.

4 thymoma cells potentially induce IgE synthesis by human B cells if both phorbol myristate acetate 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. The significance of these models will only become clear once the molecules that deliver the contact-dependent signal(s) are characterized.

Hydrocortisone. Hydrocortisone induces IL-4-dependent IgE synthesis by normal unfractionated mononuclear cells, and sIgE-B cells isolated by cell sorting are also inducible by hydrocortisone and IL-4.^{17, 18} The mechanisms by which hydrocortisone syn-

ergizes with IL-4 are unknown. The observation that steroid hormone-receptor interactions can provide a second signal for IgE synthesis dictates some caution in prescribing steroids for allergic diseases.

Switch recombination or alternative splicing?

We have recently demonstrated deletional switch rearrangement from IgM to IgE by using PCR amplification of ϵ switch fragments, followed by cloning and DNA sequencing. These data obtained indicate that switch recombination occurs after human B-cell stimulation with EBV and IL-4¹⁹ or anti-CD40 and IL-4 (Fig. 5). The length of the switch fragments

isolated varies, consistent with different switch junctions (Fig. 6). Our results 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

We have demonstrated that IL-5 and IL-6 upregulate the IgE response induced by IL-4 in unfractionated mononuclear cells. IFN- γ has been reported to inhibit IL-4-dependent IgE synthesis in both mice⁸ and humans.²⁰ 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.²¹ 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 synthesis 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. By contrast, no inhibition was observed when IFN- γ was added to highly purified human B cells stimulated with IL-4.⁹ 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.

Future therapies to suppress IgE synthesis

Based on our present understanding of IgE regulation, several modalities aimed at suppressing IgE synthesis may be available in the future. These include:

1. *IL-4 antagonists.* Recombinant IL-4 conjugated to diphtheria toxin chain B has been genetically engineered. This reagent may serve to eliminate selectively cells that express relatively high-number IL-4 receptors or that express high-affinity IL-4 receptors. The applicability of this approach is contingent on demonstrating selectivity to IgE with minimal interference with other immune responses.
2. *s-IL4R.* There is a natural form of s-IL4R that arises by alternative splicing of the IL-4 R RNA. Because s-IL4R occurs naturally and because mice in which the IL-4 gene is made nonfunctional are essentially healthy, this form of therapy holds good promise.
3. *Modulation by cytokines.* IFN- γ has already undergone clinical trials in atopic dermatitis and in the hyper-IgE syndrome. The results to date demonstrate minimal effect on IgE levels. This finding is perhaps explained by the observation that B cells that have already switched to IgE synthesis (IgE B cells) are no longer subject to inhibition by IFN- γ .

Because IgE B cells no longer require IL-4, the above modalities of therapy will not abolish an existing IgE response, although they will prevent the further differentiation of B cells into the IgE pathway. Therefore, these therapies may need to be combined with therapies aimed toward the elimination of already existing B cells and/or the neutralization of IgE antibody. Recently a MAb that recognizes an amino acid sequence present in IgE expressed on the surface of human B cells, but not present in sIgE, has been described. Properly conjugated, this type of antibody may be used to kill IgE B cells. In addition, peptides and drugs that compete with IgE for binding to its receptors, as well as soluble IgE receptors derived by genetic engineering, may also be used to eliminate the effects of already existing allergen-specific IgE antibodies.

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