

Role of protein tyrosine kinases in CD40/interleukin-4-mediated isotype switching to IgE

Richard K. S. Loh, MD,* Haifa H. Jabara, BSc,* Clement L. Ren, MD, PhD,*
Shu Man Fu, MD,^b and Raif S. Geha, MD* Boston, Mass., and Charlottesville, Va.

The B-cell antigen CD40 transduces signals, which synergize with interleukin (IL)-4 to induce IgE synthesis in human B cells. IL-4 induces ϵ germline transcription but not mature ϵ transcripts or IgE protein synthesis in B cells. Addition of anti-CD40 monoclonal antibody to IL-4-treated B cells results in deletional $S_{\mu} \rightarrow S_{\epsilon}$ switch recombination, expression of mature ϵ transcripts, and IgE synthesis and secretion. Because both IL-4 and anti-CD40 induce protein tyrosine phosphorylation in B cells, we investigated the role of protein tyrosine kinase in IL-4/CD40-mediated IgE synthesis. The protein tyrosine kinase inhibitors genestein and herbimycin A, but not the protein kinase C inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7) or the protein kinase A inhibitor N-2-guanidinoethyl-5-isoquinolinesulfonamide, inhibited IgE synthesis in B cells stimulated with IL-4 and CD40. Genestein and herbimycin, but not H7, inhibited IL-4-driven ϵ germline transcription in B cells. Both genestein and herbimycin, but not H7, inhibited CD40-mediated IgE synthesis in B cells pretreated for 4 days with IL-4 to allow optimal expression of ϵ germline transcripts. Inhibition of IgE synthesis in these cultures was accompanied by inhibition of $S_{\mu} \rightarrow S_{\epsilon}$ deletional switch recombination as assayed by nested polymerase chain reactions. These results suggest that activation of protein tyrosine kinase plays an important role in both the IL-4 and the CD40 signalling pathways that lead to IgE isotype switching in B cells. (J ALLERGY CLIN IMMUNOL 1994;94:784-92.)

Key words: Protein tyrosine kinase, IgE, deletional switch recombination, interleukin-4, anti-CD40

We have previously demonstrated that the B-cell antigen CD40 transduces signals, which synergize with interleukin (IL)-4 to induce IgE synthesis in highly purified resting human B cells.¹ IL-4 induces ϵ germline transcription but not mature ϵ transcripts or IgE protein synthesis in B cells.^{2, 3} Addition of anti-CD40 monoclonal antibody (mAb) to IL-4-treated B cells results in deletional $S_{\mu} \rightarrow S_{\epsilon}$ switch recombination, expres-

Abbreviations used

H-7:	1-(5-Isoquinolinesulfonyl)-2-methylpiperazine
HA-1004:	N-2-guanidinoethyl-5-isoquinolinesulfonamide
IL:	Interleukin
PCR:	Polymerase chain reaction
PKC:	Protein kinase C
PMA:	Phorbol myristyl acetate
PTK:	Protein tyrosine kinase

From *the Division of Immunology, The Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston; and ^bthe Division of Rheumatology, Department of Medicine and Department of Microbiology, University of Virginia, Charlottesville.

Supported by National Institutes of Health grants 1U01AI31541-01 and 1R01AI31136-01A1.

Received for publication July 7, 1993; revised Apr. 5, 1994; accepted for publication Apr. 29, 1994.

Reprint requests: Raif S. Geha, MD, Division of Immunology, Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/157077

sion of mature ϵ transcripts, and IgE synthesis and secretion.^{4, 5} A ligand for CD40 is expressed on T cells on activation.⁶ Interaction between CD40 and its ligand appears to play an important role in T-cell-dependent isotype switching to IgE because a soluble form of the CD40 ligand inhibits T-cell-driven isotype switching to IgE in IL-4-treated B cells.⁷ Furthermore, Epstein-Barr virus-transformed B cells transfected with the CD40 ligand replace T cells in synergizing with IL-4 to induce IgE synthesis in B cells.⁸

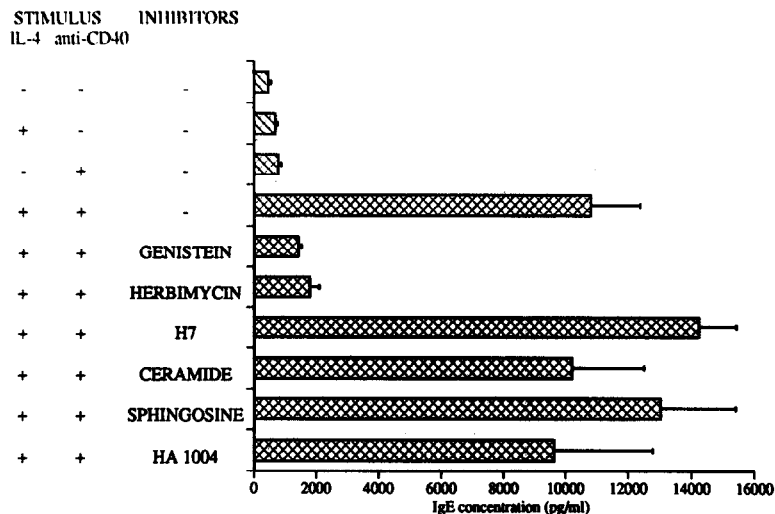


FIG. 1. Effect of protein kinase inhibitors on IgE production by B cells in the presence of IL-4 (50 IU/ml) and CD40 mAb (5 μ g/ml). B cells at a concentration of 1.0×10^6 cells/ml were cultured for 10 days with various inhibitors—genistein (5 μ g/ml), herbimycin (1 μ mol/L), H7 (100 μ mol/L), ceramide (2.5 μ mol/L), sphingosine (2.5 μ mol/L), and HA1004 (5 μ mol/L). Cells were preincubated at 37° C for 1 hour with the indicated concentrations of inhibitors or an equivalent concentration of diluent. For herbimycin A, the preincubation time was 16 hours. Supernatants were harvested after 10 days, and IgE levels were measured by radioimmunoassay. Values represent mean $\pm$ SEM net synthesis IgE (picograms per milliliter) of three or more experiments.

The mechanisms by which IL-4 induces ϵ germline transcription and engagement of CD40 leads to switch recombination are not understood. It has been reported that IL-4 results in the activation of tyrosine kinases.^{9, 10} Monoclonal antibody to CD40 induces the phosphorylation of proteins on tyrosine, as well as on serine and threonine residues in B cells.¹¹ In this report we show that activation of protein tyrosine kinases (PTKs) plays an important role in IL-4-mediated induction of ϵ germline transcripts, as well as in CD40-mediated deletional switch recombination in B cells treated with IL-4.

METHODS

Cell preparation

Highly purified B cells were prepared as previously described.^{12, 13} In summary, peripheral blood mononuclear cells from healthy subjects were isolated by Ficoll (Pharmacia, Uppsala, Sweden) density gradient centrifugation, rosetted twice with 2-aminoethylisothiuronium bromide-treated sheep red blood cells, and treated twice with anti-CD3 mAb (OKT3; Ortho Diagnostics, Raritan, N.J.) plus rabbit complement (Pel-Freez Biologicals, Inc., Rogers, Ark.). Monocytes were depleted by adherence to plastic Petri dishes. The B-cell populations thus obtained contained less than 1% CD3+ cells and did not proliferate in response to phytohemagglutinin (10 μ g/ml) but strongly proliferated in response to phorbol myristyl acetate (PMA) (25

ng/ml) + ionomycin (0.5 μ mol/L). B cells were suspended in RPMI 1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Hyclone Sterile Systems, Inc., Logan, Utah), 2 μ mol/L L-glutamine, 100 U/ml penicillin, and 50 μ g/ml streptomycin (complete medium) in the presence or absence of IL-4 (50 IU/ml) and anti-CD40 mAb (5 μ g/ml). Control cultures for the evaluation of preformed immunoglobulin were set up in the presence of cycloheximide (100 μ g/ml; Sigma Chemical Co., St. Louis, Mo.). On day 10 of culture, supernatants were assessed for their IgE level by a radioimmunoassay with a sensitivity limit of 150 pg/ml.¹⁴ Net IgE synthesis was calculated by subtracting the values of preformed IgE. For inhibition experiments, cells were preincubated at 37° C for 1 hour with the indicated concentrations of inhibitor or an equivalent concentration of diluent. For experiments involving herbimycin A, the preincubation time was 16 hours. For all the inhibitors, there was no difference between the viability of treated and untreated cells.

Reagents and antibodies

The anti-CD40 mAb 626.1 has previously been described.¹⁵ Sphingosine and PMA were purchased from Sigma Chemical Co.; herbimycin A and genistein were from Gibco BRL, Gaithersburg, Md.; and N-2-guanidinoethyl-5-isoquinolinesulfonamide (HA-1004) and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) were from Seikagaku America, Inc., Rockville, Md. Ceramide was a kind gift of Dr. Y. Hannun (Duke University, Durham, N.C.).

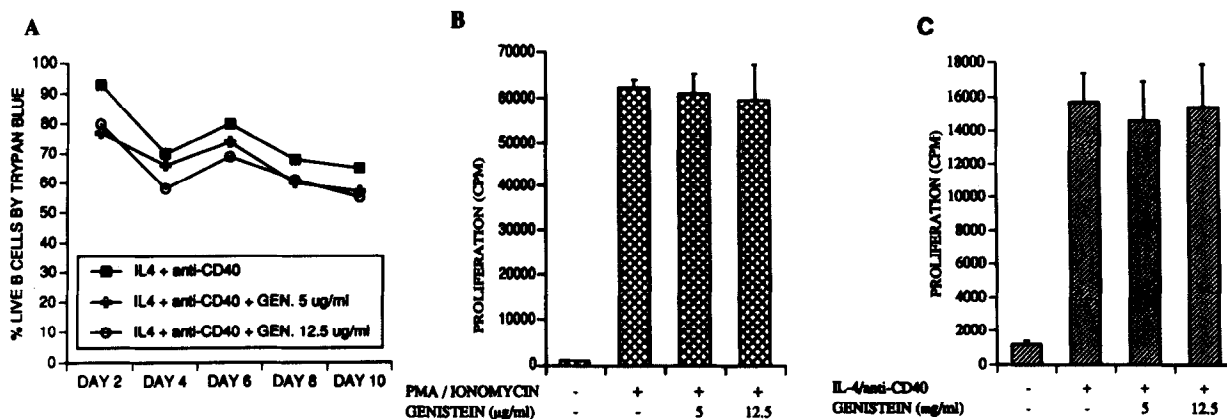


FIG. 2. A, Effect of increasing concentrations of genistein on B-cell viability in the presence of IL-4 and CD40 mAb. B cells at a concentration of 1.0×10^6 cells/ml were cultured for 10 days with IL-4 (50 IU/ml) and genistein (5 µg/ml and 12.5 µg/ml). Every 48 hours a sample was taken, and cell viability was assessed by staining with trypan blue. B, B cells were induced to proliferate by PMA (20 ng/ml) and ionomycin (0.5 µmol/L). The B cells were cultured for 72 hours at 1×10^6 cells/ml in the presence and absence of genistein. During the last 16 hours of culture, tritiated thymidine was added to the culture. Results are mean counts per minute of three experiments $\pm$ SEM. C, B cells were induced to proliferate by IL-4 (50 IU/ml) and anti-CD40 (5 µg/ml). The B cells were cultured for 72 hours at 1×10^6 cells/ml in the presence and absence of genistein. During the last 16 hours of culture tritiated thymidine was added to the culture.

Cell sorting

Purified B cells were labeled with fluorescein isothiocyanate-conjugated anti-CD19 mAb and sorted on a FACStar Plus flow cytometer (Becton Dickinson, Mountain View, Calif.). Setting of the markers and analysis of the sorted cells were performed as previously described.³

Northern blot analysis

Total cellular RNA was prepared by cell lysis with guanidium isothiocyanate followed by centrifugation of the lysate through a cesium chloride step gradient. Northern blot analysis was carried out with 10 µg of total cellular RNA by using phosphorus 32-labeled gel-purified DNA probes. A 0.88 kb *Hinf* I fragment, which spans the first 2 exons of *C ϵ* , detects both germline and mature *C ϵ* transcripts. The human cDNA β -actin probe was kindly provided by Dr. C. Terhorst (Beth Israel Hospital, Boston, Mass.). Prehybridization, hybridization, washing, and autoradiography conditions were as previously described.³ Densitometric analysis was performed with an LKB Ultrascan XL densitometer (LKB, Bromma, Sweden).

PCR primers/amplification, cloning, and sequencing of S μ /S ϵ "switch fragments"

High molecular weight DNA was prepared from 5 to 10×10^6 cultured B cells with the A.S.A.P. Genomic DNA Isolation kit (Boehringer Mannheim Biologicals, Indianapolis, Ind.). Nested primer PCR analysis for S μ /S ϵ "switch fragments" was performed on high mo-

lecular weight DNA isolated from cultured B cells with the use of the following four primers as previously described⁴:

1. S6/5'-CTGCAGACTCAGAAGGGAGGGGAT-GCTCCG-3'
2. S4/3'-ACTGATCCAAGACAGGAGTGTG-GCGGATGT-5'
3. S7/5'-GAGGGTGGTAATGATTGGTAAT-GCTTTGGA-3'
4. S9/3'-GAACCCTGAGGTCCGFTCCCCGCT-TCCCGG-5'

The first round of PCR was performed with primers S6/S4. The second round of PCR was performed on a 5 ml aliquot of the first round PCR mixture with primers S7/S9. PCR amplification and purification of the amplified fragments were carried out as previously described.⁴

RESULTS

IL-4/CD40-induced IgE synthesis is inhibited by PTK inhibitors

We measured IgE synthesis by highly pure human peripheral blood B cells stimulated with human IL-4 and anti-CD40 mAb 626.1 in the presence or absence of various protein kinase inhibitors. Fig. 1 shows that anti-CD40 mAb plus IL-4 induced vigorous IgE synthesis in B cells. IgE synthesis was strongly inhibited by both PTK inhibitors genistein (5 µg/ml) and herbimycin A

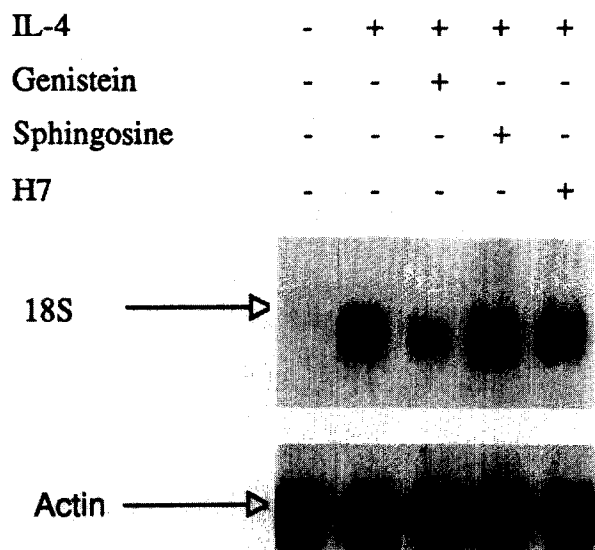


FIG. 3. Effect of genistein on the induction of germline $C\epsilon$ transcripts in B cells stimulated with IL-4. Total RNA (10 μ g) was prepared from normal peripheral blood B cells incubated with medium or IL-4 (50 IU/ml) or IL-4 (50 IU/ml) and various inhibitors for 5 days, then electrophoresed on a 1% formaldehyde-agarose gel, transferred to a nitrocellulose membrane, and hybridized to a ^{32} P-labeled 0.88 kb *Hinf* I fragment. The autoradiograph was exposed for 6 days. Concentrations of inhibitors used were genistein (5 μ g/ml), sphingosine (2.5 μ mol/L), and H7 (100 μ mol/L).

(1 μ mol/L). In contrast, the protein kinase C (PKC) inhibitors sphingosine and H7 and the protein kinase A inhibitor HA1004 failed to inhibit IgE synthesis.

The inhibition of IL-4/CD40-triggered IgE synthesis by PTK inhibitors was not due to a toxic effect of these compounds on B cells. Fig. 2, A shows that addition of genistein for 10 days in concentrations of up to 12.5 μ g/ml did not affect the viability of the B cells stimulated with anti-CD40 and IL-4 as determined by staining with trypan blue. Furthermore, genistein (up to 12.5 μ g/ml) did not inhibit the capacity of the B cells to proliferate in response to PMA and ionomycin (Fig. 2, B) or to anti-CD40 + IL-4 (Fig. 2, C). Similar results were obtained with herbimycin A used in concentrations of up to 2 μ mol/L (data not shown).

Inhibition of PTK inhibits IL-4 induction of ϵ germline transcription

The effect of PTK inhibitors on IgE synthesis could be exerted at the level of induction of ϵ germline transcription by IL-4 or at the level of CD40-mediated switch recombination or at both

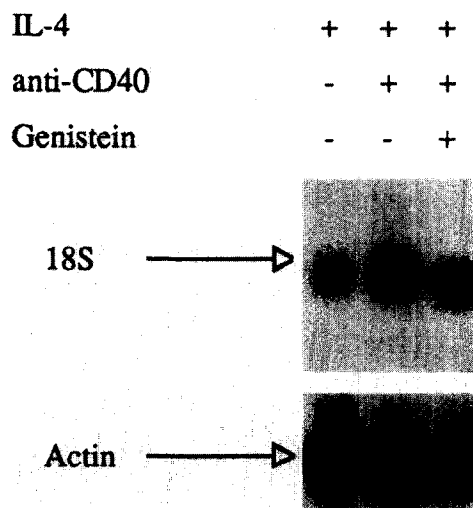


FIG. 4. Effect of genistein on induction of germline $C\epsilon$ transcripts in B cells stimulated with IL-4 and anti-CD40. Total RNA (10 μ g) was prepared from normal peripheral B cells incubated with medium or IL-4 (50 IU/ml) plus anti-CD40 mAb (5 μ g/ml) or IL-4 (50 IU/ml) plus CD40 mAb (5 μ g/ml) and genistein (5 μ g/ml) for 5 days, then proceeded as described for Fig. 3, except that the autoradiograph was exposed for only 3 days. Similar results were obtained in two other experiments.

levels. Fig. 3 shows that genistein (5 μ g/ml) had a strong inhibitory effect on the accumulation of ϵ germline transcripts in B cells. In two experiments the mean ratio of ϵ germline transcript to actin of B cells in the presence of genistein was 0.26 ± 0.07 times that of the B cells that were stimulated in the absence of genistein (74% inhibition). In contrast, the PKC inhibitors sphingosine and H7 had minimal effect on ϵ germline transcription.

We have previously shown that anti-CD40 mAb upregulates ϵ germline transcription induced by IL-4. To mimic the conditions required for IgE synthesis, we examined the effect of genistein on the level of ϵ germline transcript mRNA in B cells cultured with IL-4 and CD40. Fig. 4 shows that anti-CD40 enhanced the level of ϵ germline transcripts induced by IL-4 by fourfold. Genistein inhibited the upregulatory effect of CD40 ligation on ϵ germline transcripts in IL-4-treated B cells. In three experiments the mean ratio of ϵ germline transcript to actin of B cells stimulated with IL-4 and anti-CD40 in the presence of genistein was 0.34 ± 0.07 times that of B cells treated with IL-4 and anti-CD40 alone (66% inhibition).

We next attempted to determine whether the levels of ϵ germline transcripts in B cells treated

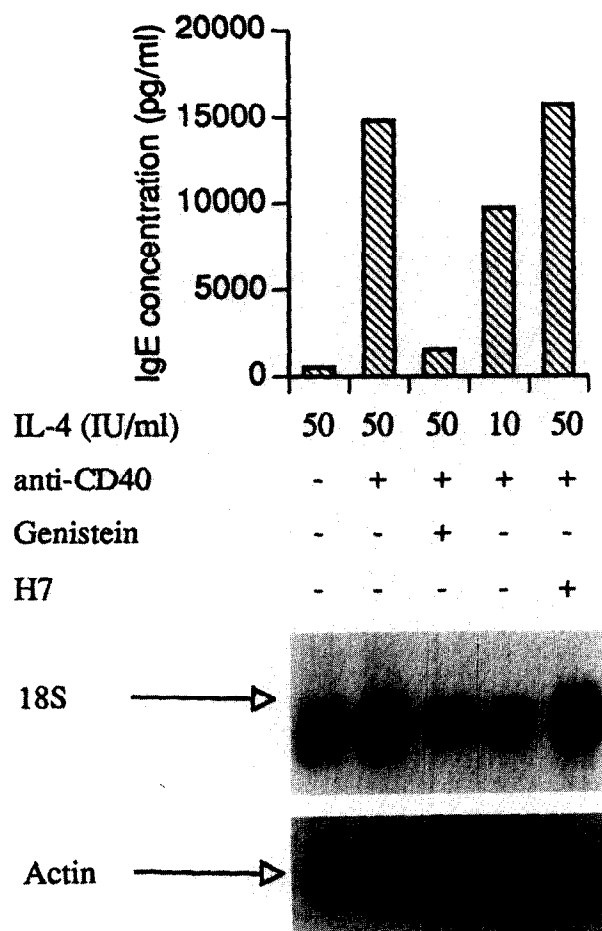


FIG. 5. Effect of genistein on induction of germline ϵ transcripts in B cells stimulated with IL-4 anti-CD40. Total RNA (10 μ g) was prepared from normal peripheral B cells incubated for 5 days with lane 1: IL-4 (50 IU/ml), lane 2: IL-4 (50 IU/ml) plus anti-CD40 mAb (5 μ g/ml), lane 3: IL-4 (50 IU/ml) plus anti-CD40 mAb (5 μ g/ml) and genistein (5 μ g/ml), lane 4: IL-4 (10 IU/ml) plus CD40 mAb (5 μ g/ml), lane 5: IL-4 (50 IU/ml) plus anti-CD40 mAb (5 μ g/ml) and H7 (100 μ mol/L). The RNA was processed as described for Fig. 3, except that the autoradiograph was exposed for only 3 days. IgE synthesis was measured by radioimmunoassay. Similar results were obtained in a second experiment.

with IL-4 + anti-CD40 in the presence of genistein could account for the severe inhibition of IgE synthesis in these cultures. B cells were treated with decreasing concentrations of IL-4 and a constant concentration of anti-CD40 and examined for ϵ germline transcript expression and IgE synthesis. Fig. 5 shows that B cells stimulated with anti-CD-40 and IL-4 at 10 IU/ml expressed levels of ϵ germline transcripts comparable to those of B cells treated with IL-4 (50 IU/ml) + anti-CD40 in the presence of genistein. However, IgE synthesis was

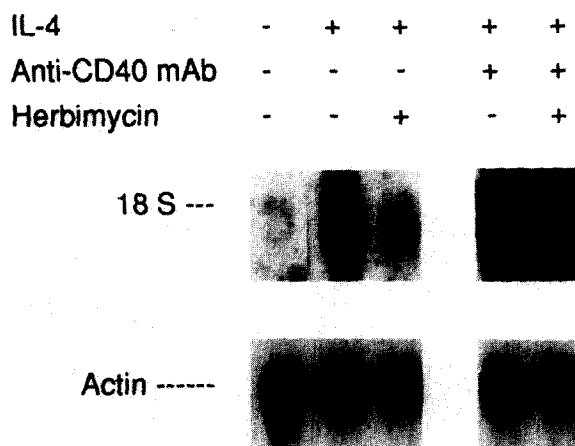


FIG. 6. Effect of herbimycin (1 μ mol/L) on the induction of germline ϵ transcripts in B cells stimulated with IL-4 $\pm$ anti-CD40. Northern blot analyses were performed as described for Fig. 3, and the autoradiographs were exposed for 6 days.

vigorous in the former cultures and absent in the latter cultures. Similar results were obtained in a second experiment. These findings suggested that PTK inhibitors block the CD40 signal that leads to IgE synthesis.

The effect of the PTK inhibitor genistein on ϵ germline transcripts was not peculiar to genistein because another PTK inhibitor, herbimycin, exerted similar effects. Fig. 6 shows that herbimycin (1 mmol/L) strongly inhibited IL-4-induced accumulation of ϵ germline transcripts in B cells (Fig. 6, lanes 1 to 3). In two experiments the mean ratio of ϵ germline transcript to actin of B cells in the presence of herbimycin was 0.22 ± 0.06 times that of B cells stimulated in the absence of herbimycin (78% inhibition). Herbimycin also inhibited the upregulatory effect of CD40 ligation on ϵ germline transcripts in IL-4-treated B cells (Fig. 6, lanes 4 to 5). In two experiments the mean ratio of ϵ germline transcript to actin of B cells stimulated with IL-4 and anti-CD40 in the presence of genistein was 0.51 ± 0.09 times that of B cells treated with IL-4 and anti-CD40 alone (49% inhibition).

PTK inhibitors inhibit CD40-mediated induction of IgE and $S\mu\text{--} \rightarrow S\epsilon$ switch recombination

Accumulation of ϵ germline transcripts in B cells treated with IL-4 is optimal after 4 days in culture (data not shown). To confirm that PTK inhibitors interfere with CD40-mediated IgE syn-

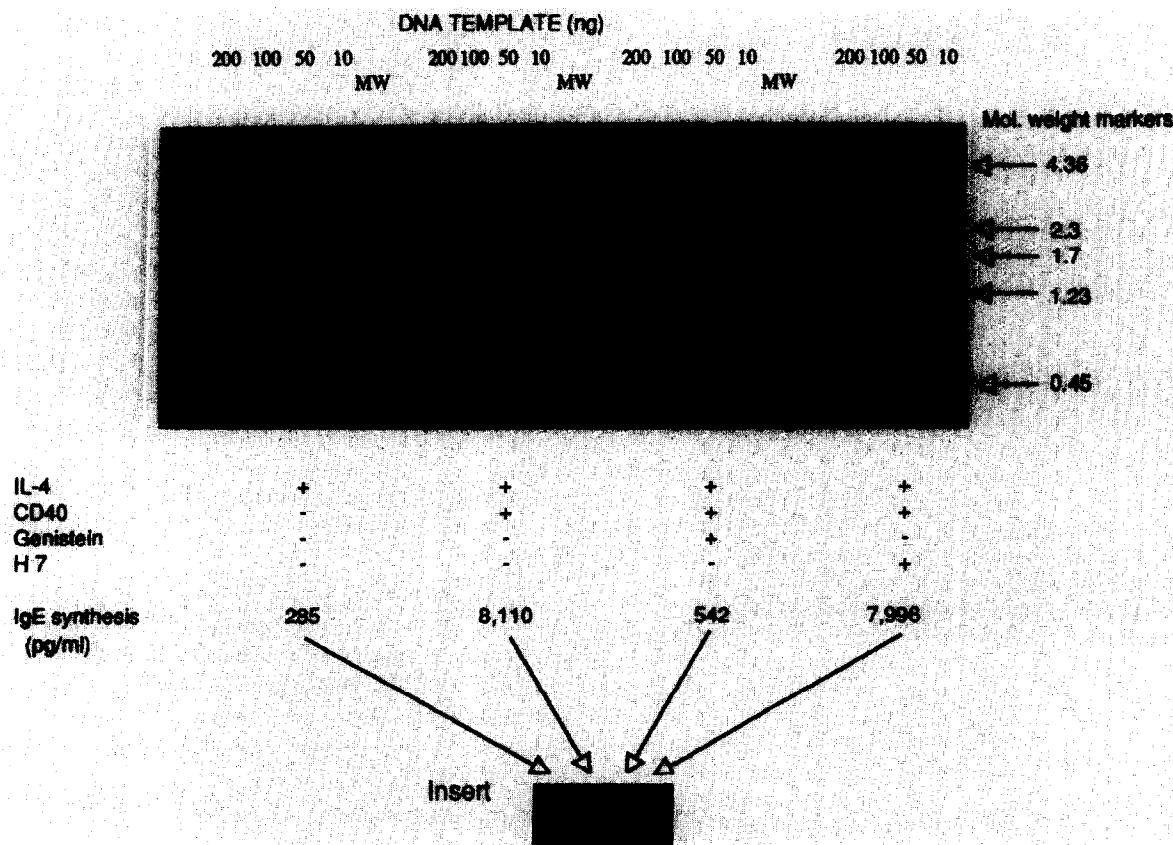


FIG. 7. Effect of genistein on the generation of $S_{\mu} \rightarrow S_{\epsilon}$ switch fragments. B cells were stimulated with IL-4 (50 IU/ml) for 4 days then cultured for 10 days with anti-CD40 $\pm$ genistein (5 μ g/ml) or H-7 (100 μ mol/L). After 10 days, IgE level (picograms per milliliter) in the supernatants was measured by radioimmunoassay. Serially diluted aliquots of total cellular DNA from the B cells were amplified by nested PCR. The serially diluted template DNA sample amounts used in the first round of PCR are noted above the gel. The second round 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-amplifiable DNA was present in all four samples, as evident in the insert in which primers specific for the human IL-1 β promoter (-1323/+72) yield a 1.4 kb fragment by PCR. Similar results were obtained in a second experiment. MW, Molecular weight markers.

thesis independently of their effects on IL-4-induced ϵ germ transcription, we first incubated B cells for 4 days with IL-4, then stimulated the cultures for an additional 10 days with anti-CD40 in the presence or absence of genistein. Fig. 7 shows that addition of anti-CD40 mAb to B cells pretreated with IL-4 for 4 days resulted in vigorous IgE synthesis equivalent to that seen in B cells simultaneously stimulated with IL-4 and anti-CD40 for 10 days. Addition of genistein, but not of the PKC inhibitor H7, together with anti-CD40 to B cells pretreated with IL-4 for 4 days strongly inhibited IgE synthesis.

We have previously shown that addition of anti-CD40 mAb to B cells treated with IL-4 re-

sults in deletional switch recombination as demonstrated by nested primer PCR amplification of recombined S_{μ}/S_{ϵ} regions. To determine whether genistein interfered with switch recombination, we performed nested primer PCR amplification of recombined S_{μ}/S_{ϵ} regions on DNA isolated from B cells pretreated with IL-4 for 4 days then stimulated with anti-CD40 in the presence or absence of genistein or H7. Fig. 7 shows that the generation of S_{μ}/S_{ϵ} switch fragments was strongly inhibited by genistein. This effect was specific because H7 did not inhibit the generation of S_{μ}/S_{ϵ} switch fragments.

The effects of the PTK inhibitor herbimycin on IgE synthesis and $S_{\mu} \rightarrow S_{\epsilon}$ switch recombination

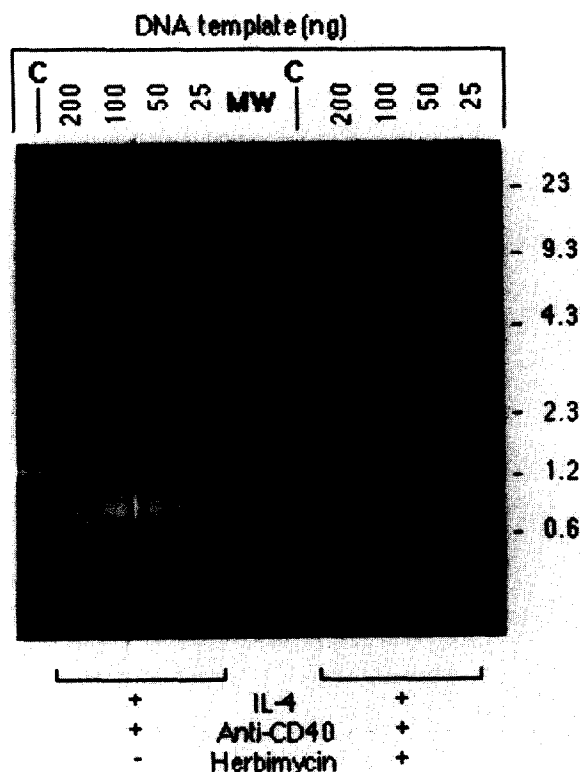


FIG. 8. Effect of herbimycin (1 μ mol/L) on the generation of $S_{\mu} \rightarrow S_{\epsilon}$ switch fragments. B cells were stimulated with IL-4 (50 IU/ml) for 4 days then cultured for 10 days with anti-CD40 $\pm$ herbimycin (1 μ mol/L). After 10 days, IgE levels (picograms per milliliter) in the supernatants measured by radioimmunoassay were 22,725 in anti-CD40 + IL-4-stimulated cells versus 2160 in the presence of added herbimycin. Nested PCR was performed on serially diluted aliquots of total cellular DNA from the B cells, and PCR products were analyzed as described in the legend to Fig. 7. DNA was present in both samples, as evident in the insert in which primers specific for the human IL-1 β promoter (-1323/+72) yield a 1.4 kb fragment by PCR. Similar results were obtained in a second experiment. MW, Molecular weight markers.

paralleled those of genestein. Fig. 8 shows that addition of herbimycin with anti-CD40 to B cells pretreated with IL-4 for 4 days strongly inhibited the generation of S_{μ}/S_{ϵ} switch fragments, as well as IgE synthesis (Fig. 8). These results suggest that activation of PTK plays an important role in CD40-mediated isotype switch recombination.

DISCUSSION

In this article we show that inhibitors of PTK inhibit IgE synthesis in B cells stimulated with IL-4 and anti-CD40. PTK inhibitors inhibited both IL-4-driven ϵ germline transcription and CD40-mediated isotype switch recombination.

These results suggest that PTKs play an important role in IgE isotype switching.

Both PTK inhibitors, genestein and herbimycin A, inhibited IgE synthesis by B cells stimulated with anti-CD40 mAb and IL-4 (Fig. 1). This inhibition occurred at concentrations for which these two agents are relatively specific inhibitors of PTKs: 5 μ g/ml for genestein and 1 μ mol/L for herbimycin A. The observed inhibition could not be explained by a toxic effect of these drugs. Neither B-cell viability nor B-cell proliferation to PMA + ionomycin or anti-CD40 + IL-4 was affected by concentrations of inhibitors in excess of those that effectively blocked IgE synthesis (Fig. 2). The effect of PTK inhibitors on IgE synthesis was specific because IgE synthesis was not affected by inhibitors for PKC and protein kinase A.

Both genestein and herbimycin strongly inhibited the induction of ϵ germline transcripts by IL-4 (Figs. 3 and 6). This inhibition was specific because the PKC inhibitors H7 and sphingosine did not affect ϵ germline transcript levels. These findings strongly suggest that PTK plays an important role in IL-4-mediated induction of ϵ germline transcription. Activation of PTK by IL-4 is suggested by the observation that IL-4 causes tyrosine phosphorylation of a $\sim$ 75 kd membrane protein in murine B cells⁹ and of a $\sim$ 170 kd protein homologous to insulin receptor substrate 1 that associates with P13 kinase in hemopoietic cells.¹⁰

Engagement of CD40 synergizes with IL-4 to increase the level of ϵ germline transcripts in B cells.⁴ Addition of the PTK inhibitor genestein to B cells treated with anti-CD40 + IL-4 reduced the levels of ϵ germline transcripts in these cultures to that observed in B cells treated with IL-4 alone. IgE synthesis was completely inhibited in these cultures, whereas there was vigorous IgE synthesis in B-cell cultures stimulated with anti-CD40 and IL-4 at concentrations that resulted in equivalent expression of ϵ germline transcripts (Fig. 5). These findings suggested that in addition to its effect on the IL-4 signal, genestein exerted an effect on the signal provided by anti-CD40. Herbimycin, a PTK inhibitor that differs from genestein in structure and mode of action, exerted inhibitory effects on IL-4-induced ϵ germline transcription similar to those observed with genestein (Fig. 6).

We directly demonstrated inhibition of the CD40 signal by the PTK inhibitor genestein. B cells were pretreated with IL-4 for 4 days to allow

optimal expression of ϵ germline transcripts (data not shown). Subsequent addition of anti-CD40 for 10 days resulted in vigorous IgE synthesis. IgE synthesis was almost completely inhibited by incorporation of genistein or herbimycin in the second phase of the culture (Fig. 7). These results strongly suggest that the CD40 signal that leads to IgE isotype switching in IL-4-treated B cells involves activation of tyrosine kinases.

Anti-CD40 induces deletional switch recombination in IL-4-treated B cells.⁴ We found that genistein, as well as herbimycin, abrogated $S_{\mu} \rightarrow S_{\epsilon}$ deletional switch recombination in these cultures (Figs. 7 and 8). This observation strongly suggests that activation of PTK plays an important role in CD40-driven isotype switch recombination. This conclusion is further supported by our recent observation that cross-linking the transmembrane phosphatase CD45 to CD40 inhibits IgE synthesis by IL-4-treated B cells (Loh et al. Manuscript submitted).

Anti-CD40 mAb induces the release of IL-6, a cytokine essential for optimal synthesis of IgE by B cells.^{16, 17} Inhibition of IgE synthesis by genistein in cultured B cells pretreated with IL-4 then stimulated with anti-CD40 could not be reversed by addition of IL-6 (data not shown). Thus the effect of PTK inhibitors on IgE synthesis could not be simply due to inhibition of IL-6 release.

It has been previously reported that CD40 engagement on B-cell lines induces protein tyrosine phosphorylation and activation of serine/threonine kinases, which include PKC.¹¹ However, PKC inhibitors did not inhibit CD40/IL-4-driven IgE synthesis in B cells. These observations strongly suggest that PKC does not play an important role in CD40-induced IgE synthesis by IL-4-treated B cells.

The nature of the biochemical pathway activated by PTK that leads to isotype switch recombination in B cells is not known. Our recent results indicate that CD40 engagement causes tyrosine phosphorylation and increased enzymatic activity of the *src* type kinase *lyn*, of phospholipase C- γ and of phosphoinositol-3' kinase.¹⁸ The role of these enzymes in CD40-mediated isotype switching needs to be determined.

The signal delivered through CD40 plays an important role in T-cell-driven switching to all isotypes. We and others have recently shown that patients with X-linked agammaglobulinemia and normal or elevated levels of IgM who are unable to switch from IgM/IgD to other immunoglobulin

isotypes fail to express the CD40 ligand on their T cells.¹⁹⁻²³ Our results, which suggest an important role for PTKs in CD40-mediated switching to IgE, may be applicable to switching to other isotypes.

REFERENCES

1. Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 mAb and IL-4 in the induction of IgE synthesis by highly purified human B cells. *J Exp Med* 1990;172:1861-4.
2. Gauchat J-F, Lebman DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germline ϵ transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172:463-73.
3. Jabara HH, Schneider LC, Shapira SK, et al. Induction of germ-line and mature C ϵ transcripts in human B cells stimulated with rIL-4 and EBV. *J Immunol* 1990;145:3468-73.
4. Shapira S, Vercelli D, Jabara H, Fu SM, Geha R. Molecular analysis of the induction of IgE synthesis in human B cells by IL-4 and engagement of CD40 antigen. *J Exp Med* 1992;175:289-92.
5. Zhang K, Clark E, Saxon A. CD40 stimulation provides an IFN- γ -independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991;146:1836-42.
6. Noelle RJ, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA, Aruffo A. A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. *Proc Natl Acad Sci U S A* 1992;89: 6550-4.
7. Fanslow WC, Anderson DM, Grabstein KH, Clark EA, Cosman D, Armitage RJ. Soluble forms of CD40 inhibit biologic responses of human B cells. *J Immunol* 1992;149: 655-60.
8. Armitage R, Fanslow WC, Strockbine L, et al. Molecular and biological characterization of a murine ligand for CD40. *Nature* 1992;357:80-3.
9. Mire-Sluis AR, Thorpe A. Interleukin-4 proliferative transduction involves the activation and the dephosphorylation of an 80-kDa protein. *J Biol Chem* 1991;266:18113-8.
10. Wang L, Keegan AD, Paul WE, Heideran MA, Gutkind JV, Pierce JA. IL-4 activates a distinct signal transduction cascade from IL-3 in factor-dependent myeloid cells [Abstract]. *J Cell Biochem* 1993;17B(suppl):119.
11. Uckun FM, Schieven GL, Dibirdik I, Chandan-Langlie M, Tuel-Ahlgren L, Ledbetter JA. Stimulation of protein tyrosine phosphorylation, phosphoinositide turnover, and multiple previously unidentified serine/threonine-specific protein kinases by the pan-B-cell receptor CD40/Bp50 at discrete developmental stages of human B-cell ontogeny. *J Biol Chem* 1991;266:17478-85.
12. Mourad W, Scholl P, Diez A, Geha RS, Chatila T. The staphylococcal toxin TSST-1 triggers B cell proliferation and differentiation via MHC unrestricted cognate T/B cell interaction. *J Exp Med* 1989;170:2011-22.
13. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. *J Exp Med* 1989;169:1295-1307.
14. Umetsu DT, Leung DYM, Siraganian R, Jabara HH, Geha RS. Differential requirements of B cells from nor-

- mal and allergic subjects for the induction of IgE synthesis by an alloreactive T cell clone. *J Exp Med* 1985;162:202-14.
15. Gruber MF, Bjorndahl JM, Nakamura S, Fu SM. Anti-CD45 inhibition of human B cell proliferation depends on the nature of activation signals and the state of B cell activation. *J Immunol* 1989;142:4144-52.
 16. Clark EA, Shu G. Association between IL-6 and CD40 signaling. IL-6 induces phosphorylation of CD40 receptors. *J Immunol* 1990;145:1400-6.
 17. Vercelli D, Jabara HH, Arai K, Yokota T, Geha RS. Endogenous IL-6 plays an obligatory role in IL-4 induced human IgE synthesis. *Eur J Immunol* 1989;19:1419-24.
 18. Ren CL, Morio T, Fu SM, Geha RS. Signal transduction via CD40 involves activation of *lyn* kinase and phosphatidylinositol-3-kinase, and phosphorylation of phospholipase C γ 2. *J Exp Med* 1994;179:673-80.
 19. Fuleihan R, Ramesh N, Loh R, et al. Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. *Proc Natl Acad Sci U S A* 1993;90:2170-3.
 20. Allen RC, Armitage RJ, Conley ME, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993;259:990-3.
 21. Aruffo A, Farrington M, Hollenbaugh D, et al. The CD40 Ligand, gp39, is defective in activated T cells from patients with X-linked hyper IgM syndrome. *Cell* 1993;72:291-300.
 22. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. *Nature* 1993;361:541-3.
 23. Korthauer U, Graf D, Mages HW, et al. Defective Expression of T-cell CD40 Ligand Causes X-linked Immunodeficiency with Hyper-IgM. *Nature* 1993;361:539-41.