

Jun N-terminal kinase is essential for CD40-mediated IgE class switching in B cells

Haifa H. Jabara, BS, and Raif S. Geha, MD *Boston, Mass*

Background: CD40 ligation activates nuclear factor κ B (NF- κ B) and the mitogen-activated protein kinases p38 and C-Jun N-terminal kinase (JNK) and causes immunoglobulin class-switch recombination (CSR) in B cells. Both NF- κ B and p38 are important for CD40-mediated CSR. The role of JNK activation in CD40-mediated isotype switching is unknown. **Objective:** We sought to determine the role of JNK activation in CD40-mediated isotype switching.

Methods: Splenic B cells from BALB/c mice were stimulated with anti-CD40 mAb and IL-4 or with soluble CD40 ligand in the presence or absence of SP600125, an anthrapyrazolone inhibitor of JNK. The following events were examined: IgE production by means of ELISA; S μ -Se deletional switch recombination by means of digestion circularization PCR; C ϵ germline, mature ϵ , and activation-induced deaminase (AID) transcription by means of RT-PCR; and proliferation by tritiated thymidine incorporation and surface expression of CD23, CD54, and CD86 by means of FACS analysis.

Results: SP600125 at 10 μ M drastically inhibited JNK phosphorylation but had little effect on CD40-mediated p38 phosphorylation and expression of the NF- κ B dependent genes c-Myc and bcl-xL. SP600125 inhibited IgE synthesis by approximately 88% but had no effect on B-cell proliferation and survival in response to anti-CD40 + IL-4 or on upregulation of CD23, CD54, and CD86 in response to CD40 ligation. Analysis of molecular events involved in IgE class switching revealed that SP600125 had no effect on the expression of C ϵ germline and AID transcripts. In contrast, SP600125 severely reduced S μ -Se switch recombination and expression of mature ϵ transcripts.

Conclusion: These results demonstrate that JNK activation is essential for CD40-mediated CSR to IgE and suggest that JNK is important for AID activity in B cells. (*J Allergy Clin Immunol* 2005;115:856-63.)

Key words: CD40, B cells, isotype switching, Jun terminal kinase

Immunoglobulin class switching in the course of the response to T-dependent antigens involves deletional switch recombination and requires 2 signals.¹ One signal is delivered by cytokines that target the specific C heavy

Abbreviations used

AID:	Activation-induced cytidine deaminase
AP-1:	Activation protein 1
CD40L:	CD40 ligand
CSR:	Class switch recombination
ERK:	Extracellular signal-regulated kinase
GLT:	Germline transcript
JNK:	C-Jun N-terminal kinase
MAPK:	Mitogen-activated protein kinase
NF- κ B:	Nuclear factor κ B
TRAF:	TNF receptor-associated factor
UNG:	Uracil-N-glycosylase

chain for switch recombination by causing their transcription. The other signal is delivered by means of ligation of the B-cell surface antigen CD40. Both signals synergize to induce optimal transcription of immunoglobulin heavy chain germline genes and of the gene for activation-induced deaminase (AID), which plays a critical role in deletional switch recombination.²

CD40 is a member of the TNF receptor family of surface molecules and is expressed on all B cells.³ CD40 ligand (CD40L) is expressed transiently on activated T cells. The critical role of CD40-CD40L interactions in isotype switching is illustrated by several observations: anti-CD40 mAb bypasses the requirement for T cells in IL-4-driven IgE isotype switching *in vitro*,⁴ mutations in the CD40L underlie the isotype switch defect in patients with X-linked hyper-IgM syndrome,⁵ and mice deficient in CD40 or CD40L fail to undergo isotype switching in response to T-dependent antigens.⁶⁻⁸

The intracellular domain of human and mouse CD40 has a TNF receptor-associated factor (TRAF) 6 binding K/RxxPx motif⁹ and a PxQxT motif that binds TRAF2 and TRAF3.⁹⁻¹² TRAF2 and TRAF3 bind TRAF1 and TRAF5, respectively. CD40 ligation in B cells causes activation of the transcription factor nuclear factor κ B (NF- κ B) and of the mitogen-activated protein kinases (MAPKs) p38 and C-Jun N-terminal kinase (JNK), whereas activation of extracellular signal-regulated kinase (ERK) has been controversial.¹³⁻¹⁸ Studies in CD40^{-/-} mice reconstituted with mutant CD40 molecules suggest that TRAF2/TRAF3 binding is essential for CD40 activation of NF- κ B, p38, and JNK.¹⁹

NF- κ B is known to play an important role in class switch recombination (CSR). It plays essential roles in CD40 induction of germline transcripts (GLTs), including C ϵ GLT²⁰ and in transcription of the gene encoding AID.²¹ More importantly, mice deficient in p50 units, p65

From the Division of Immunology, Children's Hospital, and the Department of Pediatrics, Harvard Medical School.

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Reprint requests: Haifa Jabara, KARP Bldg #10126, 1 Blackfan Circle, Division of Immunology, Children's Hospital, Boston, MA 02115. E-mail: Haifa.jabara@childrens.harvard.edu.

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units, or both of NF- κ B and patients with mutations in I κ B kinase (IKK γ)/NF- κ B essential modulator (NEMO) and defective activation of NF- κ B exhibit defects in CSR.²²⁻²⁴

The MAPK p38 has been shown to be required for CD40-mediated class switching to IgE. CD40/IL-4-dependent IgE induction in human B cells was inhibited by the specific p38 inhibitor SB203580 but not by the ERK-specific inhibitor PD98059.²⁵ The inhibitory effects of SB203580 on IgE production were directly related to its ability to suppress production of I ϵ GLTs, with subsequent inhibition of S μ -S ϵ recombination. SB203580 and a dominant negative form of p38 were shown to decrease CD40 activation of the C ϵ promoter by reducing the ability of CD40 to increase the transactivation potential of NF- κ B.²⁶ These results suggest that p38 is important in mediating CD40 activation of NF- κ B, which acts to induce C ϵ GLTs, ultimately facilitating IgE switching.

The role of JNK in CD40-mediated isotype switching is not defined. We took advantage of the availability of SP600125, an anthracycline inhibitor of JNK,²⁷ to examine the role of JNK in CD40-driven isotype switching. The results suggest that JNK activation is essential for CD40-mediated CSR to IgE. JNK inhibition had no effect on the expression of either C ϵ germline or AID transcripts but strongly inhibited S μ -S ϵ switch recombination, suggesting that JNK is essential for AID-dependant deletional switch recombination.

METHODS

Mice

BALB/c mice were purchased from Charles River Laboratories (Wilmington, Mass). Animal studies were performed according to the Children's Hospital Institutional Animal Care and Use Committee guidelines.

Cell preparation

B cells were purified from spleens of 8- to 10-week-old BALB/c mice by using Dynabeads M-280 Streptavidin (Dyna) after incubation with biotinylated antibody cocktail of Thy1.2 (53-2.1), Mac1 (M1/70), CD43 (Ly-48), and Gr-1 (RB6-8C5) (PharMingen, San Diego, Calif). Purified cells were greater than 95% B220⁺ by means of FACS analysis and were suspended in RPMI containing 10% FCS, L-glutamine, and 50 μ M β -mercaptoethanol (complete medium).

Flow cytometric analysis

Single-cell suspensions were stained with FITC- or phycoerythrin-conjugated antibodies in PBS containing 5% rat serum (Sigma), 0.05% sodium azide, and Fc-block (PharMingen); washed; and analyzed on a FACSCalibur cytometer (Becton Dickinson, Franklin Lakes, NJ). FITC- or phycoerythrin-conjugated mAbs used in these studies were as follows: CD3 (145-2C11), B220 (RA3-6B2), rat IgG2a (R35-95), and hamster IgG (A19-3) (PharMingen). Annexin V-FITC (Biovision Inc) staining was performed as per the manufacturer's instructions.

MAPK phosphorylation

Splenic B cells were suspended in 1% FCS/RPMI-1640 for 1 hour at 37°C, followed by preincubation with either 0.1% dimethyl

sulfoxide (vehicle) or with different concentrations of SP600125 (a kind gift of Celgene, San Diego, Calif) for 30 minutes before stimulation with hamster IgM anti-mouse CD40 (anti-CD40, 10 μ g/mL, HM40-3; PharMingen). Activated MAPKs were detected by using immunoblotting lysates from 1×10^6 cells with antibodies to phospho-p38 (Cell Signaling) and phospho-SAPK/JNK (Biosource). Membranes were reprobed with kinase-specific antibodies to p38 and SAPK/JNK (Cell Signaling) as loading controls. Immunoreactive bands were detected by means of enhanced chemiluminescence (Amersham Pharmacia Biotech, Little Chalfont, Bucks, United Kingdom).

Expression of the NF- κ B-dependent genes c-Myc and bcl-xL

Purified B cells were pretreated with either vehicle or SP600125 (10 μ M) for 30 minutes, followed by stimulation with medium or anti-CD40 (1 μ g/mL) for 2 hours at 37°C. RNA was extracted from the cultured cells, and RT-PCR was performed as described previously.¹⁹ The primers used for bcl-xL were previously described,¹⁹ and the primers for c-Myc were as follows: c-MycF, 5'-GGGCACAGCGTCTGCTCC AC-3'; c-MycR, 5'-CACCAGAGTTTCGAAGC-TGTTCGAG-3'.

Proliferation and IgE synthesis of B cells

Purified B cells (0.8×10^6 /mL) were pretreated with either vehicle or various concentrations of SP600125 for 30 minutes, followed by stimulation with medium alone or with anti-CD40 (1 μ g/mL) plus IL-4 (50 ng/mL; R&D systems, Minneapolis, Minn). Supernatants were collected after 6 days and assayed for IgE by means of ELISA. For proliferation, after 72 hours, cultures were pulsed with 1 μ Ci tritiated thymidine for an additional 16 hours and then harvested, and scintillation was counted.

Surface expression of CD23, CD54, and CD86

Purified splenic B cells were preincubated with either vehicle or SP600125 (10 μ M) for 30 minutes and then were cultured overnight with medium, soluble CD40L (1:20 dilution of supernatants from muCD40L:muCD8 transfected J558L cells), or control supernatants (1:20 dilution of J558L cells transfected with empty plasmid). Cells were then washed and double stained as described above with B220-phycoerythrin and FITC-conjugated mAbs to either CD23 (B3B4), CD54 (3E2), or CD86 (GL1) or with the appropriate isotype controls, all from PharMingen. Analysis was performed by gating on B220⁺ B cells.

RT-PCR for C ϵ GLT, AID, and I μ -C ϵ transcripts

RNA was extracted from cultured splenic B cells on day 4 by using TRIzol (Invitrogen) and was reverse transcribed with Superscript II RT (Invitrogen) according to the manufacturer's instructions. PCR primers used for C ϵ GLT, AID, I μ -C ϵ and β_2 -microglobulin were as described previously.¹⁹

Digestion circularization PCR

Genomic DNA was isolated from cultured splenic B cells on day 6. DNA was digested with *Eco*R1, circularized, and used as a template for PCR by using primers for nicotinic acetylcholine receptor β unit²⁸ and for S μ -S ϵ .²⁹

RT-PCR and digestion circularization PCR reactions were performed on various dilutions to ensure that the products measured were in the linear range.

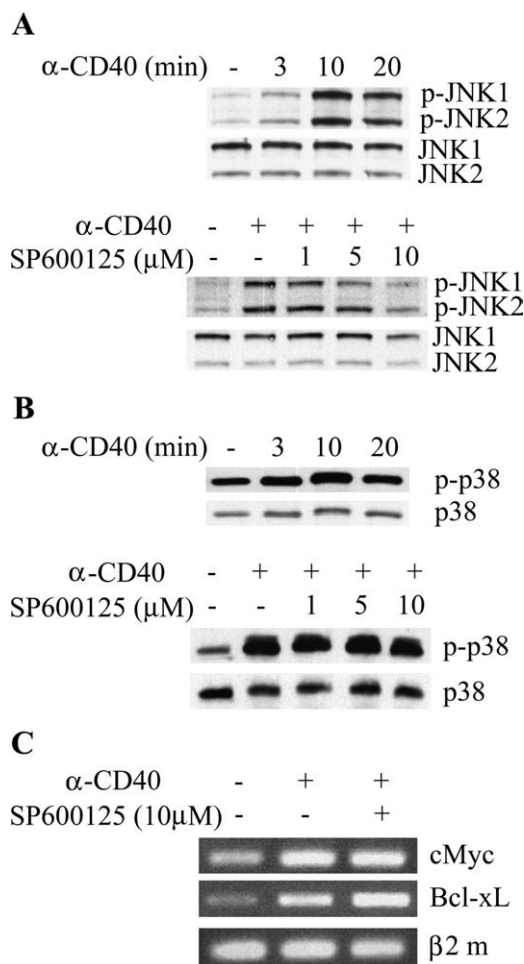


FIG 1. Effect of SP600125 on phosphorylation of JNK and p38 and expression of NF-κB-dependent genes. **A** and **B**, Phosphorylation of JNK (**Fig 1, A**) and p38 (**Fig 1, B**) in B cells stimulated with anti-CD40 mAb for 0 to 20 minutes (*upper panels*) or for 10 minutes after treatment with dimethyl sulfoxide (vehicle) or SP600125 (*lower panels*). **C**, RT-PCR of c-Myc and bcl-xL mRNA expression in B cells. β₂-Microglobulin was used as a control. Similar results were obtained in 3 experiments.

RESULTS

SP600125 inhibits JNK phosphorylation but not p38 phosphorylation or expression of NF-κB-dependent genes after CD40 activation of B cells

CD40 ligation in resting mouse B cells results in the phosphorylation and activation of the MAPKs p38 and JNK but not ERK.³⁰ **Fig 1, A**, shows that CD40 ligation in purified B cells caused phosphorylation of JNK that peaked at 10 minutes after stimulation. SP600125 inhibited JNK phosphorylation measured 10 minutes after stimulation in a dose-dependent manner. Almost complete inhibition was achieved with 10 μM SP600125.

The MAPK p38 had been implicated in CD40/IL-4 induction of IgE isotype switching. We therefore exam-

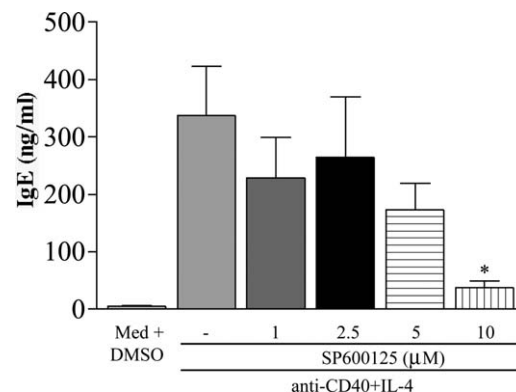


FIG 2. Effect of SP600125 on CD40-mediated IgE synthesis by purified B cells stimulated with anti-CD40 + IL-4 alone or in the presence of various concentrations of SP600125. Results shown are the mean of 4 experiments. **P* < .02, Student *t* test. DMSO, Dimethyl sulfoxide.

ined the effect of SP600125 on CD40-mediated phosphorylation of p38. **Fig 1, B**, shows that CD40 ligation caused phosphorylation of p38 in purified B cells that peaked at 10 minutes after stimulation. However, SP600125 had little effect on p38 phosphorylation in B cells stimulated with anti-CD40.

CD40 ligation in B cells causes activation of NF-κB, which is important for isotype switching^{12,20,23,24,31} and upregulation of NF-κB-dependent genes that include c-Myc and bcl-xL.^{3,32} **Fig 1, C**, shows that SP600125 (10 μM) had no effect on c-Myc and slightly enhanced bcl-xL mRNA expression in B cells stimulated with anti-CD40.

These results suggest that SP600125 selectively inhibits JNK activation induced by CD40 ligation in B cells, has little effect on the activation of p38, and has no detectable effect on the activation of NF-κB.

SP600125 inhibits CD40-mediated IgE isotype switching

CD40 ligation in the presence of IL-4 causes isotype switching to IgE.^{4,33} **Fig 2** shows that SP600125 significantly inhibited IgE synthesis in B cells stimulated with anti-CD40 + IL-4. This inhibition was dose dependent (**Fig 2**). The mean inhibition of IgE synthesis at 10 μM was 88% (37.2 ± 11.3 ng/mL for SP600125-treated cultures vs 337 ± 85.8 ng/mL for untreated control cultures, *n* = 4 experiments).

SP600125 has no effect on CD40-mediated B-cell proliferation, B-cell survival, and upregulation of CD23, CD54, and CD86 expression

CD40 ligation causes B-cell proliferation.³ Because isotype switching might be linked to proliferation, we examined whether the inhibition of IgE synthesis by SP600125 is accompanied by inhibition of proliferation. **Fig 3, A**, shows that SP600125 at concentrations of up to 10 μM had no effect on B-cell proliferation in response to anti-CD40 + IL-4.

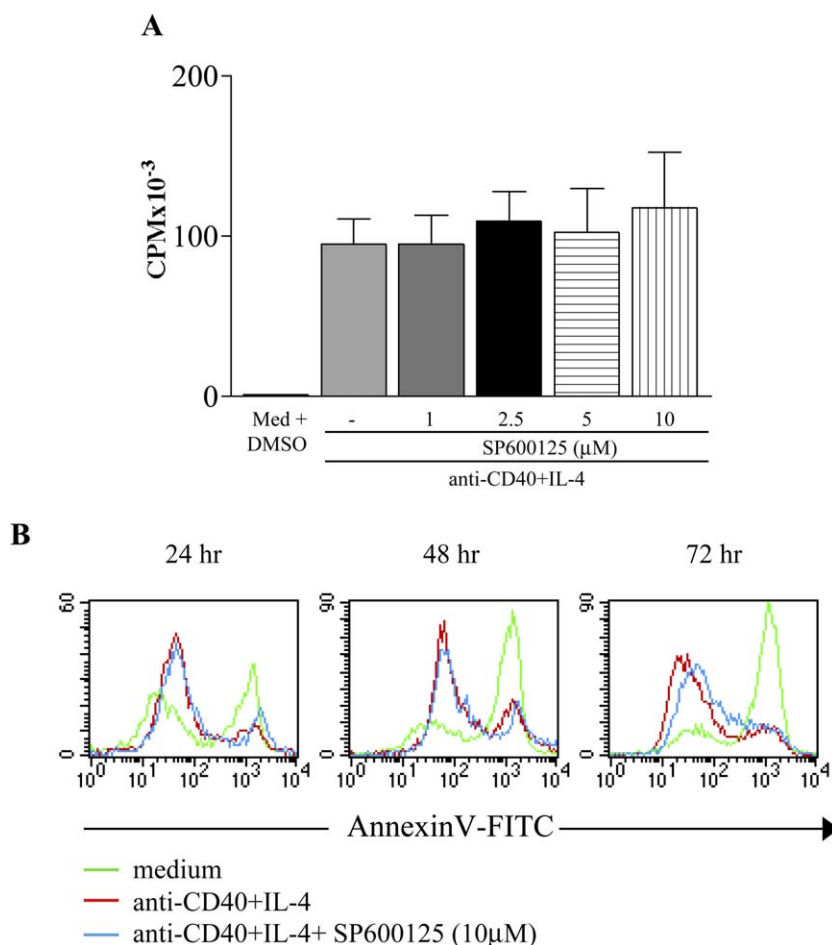


FIG 3. Effect of SP600125 on CD40-mediated B-cell proliferation and survival. **A**, Effect of SP600125 on anti-CD40⁺ IL-4-induced B-cell proliferation. Results are derived from 3 experiments. **B**, Annexin V staining of B cells stimulated for 24, 48, and 72 hours with medium (green), anti-CD40 + IL-4 alone (red), or 10 μM SP600125 (blue). Similar results were obtained in 2 experiments. DMSO, Dimethyl sulfoxide.

B cells undergo apoptosis in culture. This is rescued by CD40 ligation and IL-4 stimulation. To rule out the possibility that inhibition of isotype switching by SP600125 was due to impaired B-cell survival, B cells were cultured for 24, 48, and 72 hours with anti-CD40 + IL-4 in the presence or absence of 10 μM SP600125 and then were counted and stained with annexin-V. There were no significant differences in the numbers of B cells cultured in the presence of SP600125 or dimethyl sulfoxide vehicle control (data not shown). Furthermore, SP600125 caused little change in the percentage of annexin-V⁺ B cells (Fig 3, B).

CD40 ligation upregulates the expression of a number of surface markers on B cells, including CD23, CD54, and CD86.³ As expected, stimulation of B cells with CD40L-containing supernatants from J558L cells transfected with muCD40L:muCD8 upregulated all 3 surface molecules. Fig 4 shows that SP600125 (10 μM) had no effect on CD40-mediated upregulation of these 3 surface molecules on B cells. Stimulation with control supernatants of J558L cells transfected with empty

plasmid caused no upregulation of either marker (data not shown).

These data suggest that JNK inhibition did not impair CD40-mediated B-cell proliferation and survival and did not interfere with CD40-mediated B-cell activation, as assessed by upregulation of surface markers.

SP600125 has no effect on the expression of Cε germline and AID transcripts in B cells stimulated with anti-CD40 + IL-4 but inhibits Sμ-Sε deletional switch recombination and expression of mature ε transcripts

CD40 ligation synergizes with IL-4 in activating a number of molecular events that result in CSR to IgE. These include expression of Cε GLTs and expression of the gene for AID, followed by Sμ->Sε deletional switch recombination and expression of mature Iμ-Cε transcripts.³⁴ The effect of SP600125 on the ability of anti-CD40 + IL-4 to activate these events in B cells was examined. Fig 5, A, shows that SP600125 (10 μM) had no

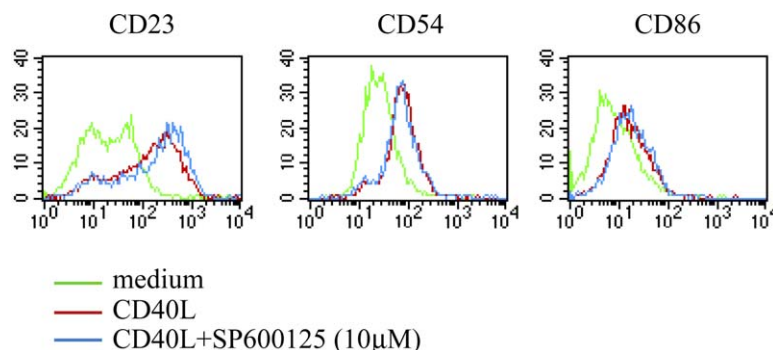


FIG 4. Effect of SP600125 on CD40-mediated upregulation of CD23, CD54, and CD86 expression on B cells. B cells were cultured in medium (green) or were stimulated overnight with sCD40L plus vehicle (red) or 10 μ M SP600125 (blue) and were stained as described in the Methods section. Analysis was performed by gating on B220⁺ cells. Results shown are representative of 3 experiments.

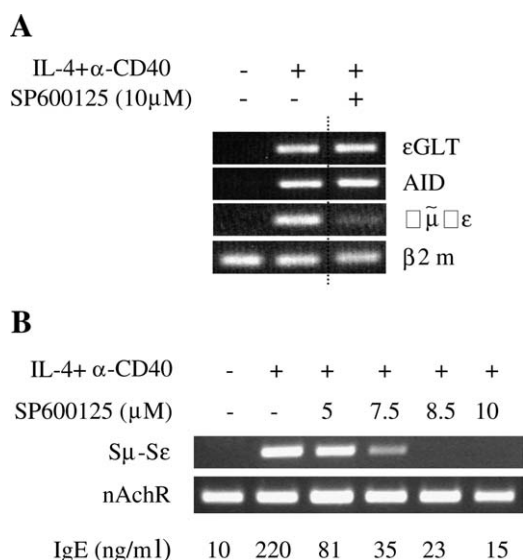


FIG 5. Molecular events in isotype switching. **A**, Effect of SP600125 (10 μ M) on expression of C ϵ germline, AID, and I μ -C ϵ mature transcripts in B cells stimulated with CD40 + IL-4. The dotted lines show where irrelevant lanes were cut out. **B**, Dose-response curve of SP600125 effect on S μ ->S ϵ by means of digestion circularization PCR. IgE values in the corresponding supernatants are shown. Similar results were obtained in 3 independent experiments.

effect on the ability of CD40 ligation to synergize with IL-4 in inducing C ϵ GLT and AID transcripts. Consistent with its inhibition of IgE synthesis, SP600125 inhibited the expression of mature I μ -C ϵ transcripts. Fig 5, B, shows that SP600125 severely inhibited the ability of anti-CD40 + IL-4 to cause S μ ->S ϵ deletional switch recombination in B cells in a dose-dependent manner. These results suggest that JNK activation is essential for S μ ->S ϵ deletional switch recombination but not for C ϵ GLT or AID expression.

DISCUSSION

Our results suggest that JNK activation is important for CD40-mediated CSR to IgE in murine B cells.

We used the JNK inhibitor SP600125 to assess the role of JNK in CD40-mediated isotype switching. *In vitro*, SP600125 selectively inhibits the activity of all 3 JNK isoforms with an inhibitory concentration of 50% (IC₅₀) of approximately 0.10 to 0.15 μ M but has no detectable effect (IC₅₀ >10 μ M) on 20 other enzymes that include ERK2, p38 β , IKK α , KKK β (IC₅₀ >30 μ M each), phosphodiesterases, inducible nitric oxide synthase, 5'-lipooxygenase, and cyclooxygenase-2.²⁷ In fibroblast-like synoviocytes, SP600125 inhibits JNK phosphorylation, JNK activity, c-Jun mRNA expression, activator protein 1 (AP-1) binding activity, reporter gene expression driven by an AP-1 promoter, and AP-1-dependent gene expression.³⁵ *In vivo* administration of SP600125 in a model of adjuvant arthritis in rats inhibits JNK activity in joint lysates, JNK-dependent synovial collagenase gene expression, and radiographic joint damage.³⁵ SP600125 selectively inhibited JNK in primary mouse B cells, as evidenced by almost complete inhibition of CD40-mediated JNK phosphorylation in splenic B cells (Fig 1, A). In contrast, SP600125 had little effect on CD40-mediated p38 phosphorylation (Fig 1, B). SP600125 had no effect on CD40-mediated transcription of c-Myc and slightly increased CD40-driven transcription of bcl-xL (Fig 1, C), 2 genes that are induced in B cells after CD40 ligation in an NF- κ B-dependent manner.³⁶ The increased transcription of bcl-xL is consistent with observations that suggest that JNK might negatively regulate NF- κ B-dependent transcription.³⁷ These results suggest that SP600125 is also a selective inhibitor of JNK in primary mouse B cells. This is in agreement with previous data on the selective inhibition of JNK by SP600125 in T cells. SP600125 had no detectable effect on the phosphorylation of ERK and p38 or on NF- κ B activation in T cells stimulated with phorbol 12-myristate 13-acetate and anti-CD3 + anti-CD28.²⁷

SP600125 at 10 μ M drastically inhibited IgE synthesis in purified B cells stimulated with anti-CD40 + IL-4 (Fig 2, A). At this concentration, SP600125 inhibited CD40-mediated JNK phosphorylation almost completely (Fig 1, A) but had little effect on p38 phosphorylation or on NF- κ B activation. This suggests that the inhibition of

CD40-driven IgE switching by SP600125 is due to its inhibition of JNK. A role for JNK in CD40/IL-4 induction of IgE switching is consistent with our previous observation that both JNK phosphorylation and CSR to IgE were severely impaired in B cells from CD40^{-/-} mice that had been reconstituted with a mutant CD40 transgene that lacks the ability to associate with TRAF2 and TRAF3 (Δ TRAF2/3 mice), although CD40-mediated p38 phosphorylation and NF- κ B activation were also deficient in these B cells.¹⁹

It has been proposed that class switching is linked to cell proliferation.³³ Inhibition of IgE switching by SP600125 was not caused by inhibition of B-cell proliferation because SP600125 at the concentration of 10 μ M, which drastically blocked IgE switching, had no detectable effect on B-cell proliferation (Fig 3, A). JNK activation has been reported to promote or inhibit apoptosis in different cell types.³⁸ JNK is activated in the course of mIgM-induced apoptosis of human B lymphocytes,³⁹ and its activation promotes apoptosis in hairy leukemia B cells and certain B-lymphoma cell lines.⁴⁰⁻⁴² In contrast, JNK activation promotes survival in transformed B lymphoblasts.⁴³ JNK inhibition by SP600125 had little effect on the survival of B cells stimulated with anti-CD40 + IL-4 (Fig 3, B). Thus our results suggest that JNK activation is not necessary for B-cell proliferation and survival in response to CD40 ligation and IL-4. More importantly, they suggest that inhibition of IgE switching by SP600125 is unlikely to be caused by impaired proliferation or survival of these cells.

CD40 ligation upregulates CD23, CD54, and CD86 gene expression in B cells.³ JNK activation has been implicated in the upregulation of CD86 gene expression in THP-1 monocytic cells.⁴⁴ The transcription factor AP-1, the activation of which is dependent on JNK, has been reported to play a role in the upregulation of CD54 gene expression in epithelial and endothelial cells.⁴⁵⁻⁴⁷ SP600125 had no effect on the upregulation of CD23, CD54, and CD86 expression in B cells stimulated with anti-CD40. This suggests that JNK is not important for CD40-mediated upregulation of these activation markers on B cells. The lack of a demonstrable role for JNK in CD40 upregulation of these markers is consistent with our previous observations that upregulation of these markers is largely preserved in B cells of Δ TRAF2/3 mice, which fail to phosphorylate JNK after CD40 ligation.¹⁹ It is also consistent with the observation that CD40-mediated upregulation of CD23 is normal in B cells from JNK1^{-/-} mice.⁴⁸ Furthermore, these results support the notion that SP600125 did not interfere nonspecifically with CD40 activation of the B cells.

CSR to IgE in B cells stimulated with anti-CD40 + IL-4 involves a number of molecular events that include expression of C ϵ GLTs, expression of the gene for AID, S μ ->S ϵ deletional switch recombination, and expression of mature I μ -C ϵ transcripts.³⁴ SP600125 (10 μ M) had no effect on CD40/IL-4 induction of C ϵ GLT and AID transcripts. In contrast, it inhibited CD40/IL-4 induction of S μ ->S ϵ deletional switch recombination and of mature

I μ -C ϵ transcripts. These results suggest that JNK activation is essential for S μ ->S ϵ deletional switch recombination.

AID is a single-strand DNA-specific cytidine deaminase that initiates CSR and somatic hypermutation (SHM) by converting cytidine to uracil in transcribed switch regions.^{49,50} The dU:dG alterations are converted into double strand breaks that initiate CSR through a pathway that involves uracil-N-glycosylase (UNG). Generation of double strand breaks in 2 separate switch regions results in their ligation to effect CSR. The importance of AID and UNG in CSR is demonstrated by defective class switching in patients with hyper-IgM syndrome with mutations in AID and UNG and in mice with disruption of these 2 genes.⁵¹⁻⁵⁴ There are recent data to suggest that phosphorylation of AID might be important for its recruitment to the ssDNA binding replication protein A and hence for its activity on ssDNA.⁵⁵ In addition, AID activity in CSR likely involves several cofactors. Human AID mutant proteins with insertions, replacements, or truncations in the C-terminal region retain strong SHM activity but almost completely lose CSR activity.^{56,57} In addition, a number of patients with autosomal recessive hyper-IgM syndrome and defective CSR have normal AID and UNG genes, implicating other gene products in CSR. In this regard B cells deficient in 53BP1 exhibit no defect in C heavy chain germline transcription or AID expression; however, they have a profound decrease in switch junctions.⁵⁸ These findings raise the possibility that JNK might be important for the activation of AID by regulating its phosphorylation and/or for the expression of necessary cofactors that might be dependent on the transcription factors AP-1 and ATF2 that are regulated by JNK.⁵⁹

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