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

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Background: CD40 ligation activates nuclear factor κB (NF-κB) 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-κB 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; Sμ-Sε deletional switch recombination by means of digestion circularization PCR; Cε 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 μM drastically inhibited JNK phosphorylation but had little effect on CD40-mediated p38 phosphorylation and expression of the NF-κB 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 Cε germline and AID transcripts. In contrast, SP600125 severely reduced Sμ-Sε 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*

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-κB: Nuclear factor κB
TRAF: TNF receptor–associated factor
UNG: Uracil-N-glycosylase

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 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/RxxPxE motif[9] 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 κB (NF-κB) 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-κB, p38, and JNK.[19]

NF-κB is known to play an important role in class switch recombination (CSR). It plays essential roles in CD40 induction of germline transcripts (GLTs), including Cε GLT[20] and in transcription of the gene encoding AID.[21] More importantly, mice deficient in p50 units, p65

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units, or both of NF-κB and patients with mutations in IkappaB kinase (IKKγ)/NF-κB essential modulator (NEMO) and defective activation of NF-κB 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 Iε GLTs, with subsequent inhibition of Sμ-Sε recombination. SB203580 and a dominant negative form of p38 were shown to decrease CD40 activation of the Cε promoter by reducing the ability of CD40 to increase the transactivation potential of NF-κB.[26] These results suggest that p38 is important in mediating CD40 activation of NF-κB, which acts to induce Cε 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 anthrapyrazolone 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 Cε germline or AID transcripts but strongly inhibited Sμ-Sε 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 (Dynal) 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 μM 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 μg/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-κB–dependent genes c-Myc and bcl-xL

Purified B cells were pretreated with either vehicle or SP600125 (10 μM) for 30 minutes, followed by stimulation with medium or anti-CD40 (1 μg/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′-CACCAGAGTTTCGAAGCTGTTCGAG-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 μg/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 μCi 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 μM) 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 Cε GLT, AID, and Iμ-Cε transcripts

RNA was extracted from cultured splenic B cells on day 4 by using TRIzol (Invitrogen) and was reverse transcribed with Supercript II RT (Invitrogen) according to the manufacturer's instructions. PCR primers used for Cε GLT, AID, Iμ-Cε 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 unit[28] and for Sμ-Sε.[29]

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