Differential Role for p38 Mitogen-Activated Protein Kinase in Regulating CD40-Induced Gene Expression in Dendritic Cells and B Cells

Alexandra Aicher, Geraldine L. Shu, Dario Magaletti, Thera Mulvania, Antonio Pezzutto, Andrew Craxton and Edward A. Clark

*J Immunol* 1999; 163:5786-5795; ;
http://www.jimmunol.org/content/163/11/5786

**References**
This article cites 60 articles, 39 of which you can access for free at:
http://www.jimmunol.org/content/163/11/5786.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Differential Role for p38 Mitogen-Activated Protein Kinase in Regulating CD40-Induced Gene Expression in Dendritic Cells and B Cells

Alexandra Aicher,* Geraldine L. Shu,* Dario Magaletti,* Thera Mulvania,* Antonio Pezzutto,† Andrew Craxton,* and Edward A. Clark*

We investigated whether human monocyte-derived dendritic cells (DCs) differed from tonsillar B cells in the set of cell fate genes they express constitutively and in the way these genes are affected after CD40 ligation. In particular, Bel-2, TNF receptor-associated factor-2 (TRAF2), and TRAF4 were clearly inducible via CD40 in B cells but not in DCs. DCs, unlike B cells, were induced to increase expression of IL-1β, IL-1Ra, IL-8, IL-12 p40, RANTES, macrophage inflammatory protein-1α, and monocyte chemoattractant protein-1 after CD40 ligation. We next tested whether CD40-induced signaling pathways were different in DCs vs B cells. In DCs, as in B cells, CD40 ligation activated p38 mitogen-activated protein kinase (MAPK), its downstream target, MAPKAPK-2, and the c-Jun N-terminal kinase. The p38 MAPK-specific inhibitor, SB203580, blocked CD40-induced MAPKAPK-2 activation, but did not affect activation of c-Jun N-terminal kinase. Furthermore, unlike in B cells, extracellular signal-regulated kinase-1 and -2 were activated after CD40 ligation in DCs. SB203580 strongly blocked CD40-induced IL-12 p40 production in DCs at both mRNA and protein levels, while having minimal effect on CD40-induced expression of the chemokine RANTES. In contrast, no detectable IL-12 p40 protein was secreted in CD40-stimulated B cells. Furthermore, CD40-induced mRNA expression of cellular inhibitor of apoptosis protein-2 was also dependent on the p38 MAPK pathway in DCs and differed compared with that in B cells. In conclusion, CD40 induces distinct programs in DCs and B cells, and the set of p38 MAPK-dependent genes in DCs (IL-12 p40 and cellular inhibitor of apoptosis protein-2) is different from that in B cells (IL-10 and IL-1β). The Journal of Immunology, 1999, 163: 5786–5795.

Dendritic cells (DCs) are APCs that play a dominant role in the initiation of primary immune responses (1). Ag presenting via DCs activates T cells to express CD40 ligand (CD154) (2), which, in turn, activates both CD40+ DCs and B cells. CD40 ligation of DCs induces the expression of IL-12 (3), which can promote the differentiation of naive B cells into plasma cells (4). DC-secreted IL-12 also plays a pivotal role in shifting CD4+ T cell differentiation to a Th1-cytokine pattern, producing IFN-γ, but not Th2 cytokines such as IL-4 (5). Therefore, CD40-induced secretion of cytokines and chemokines in DCs and B cells and the signaling pathways mediating these CD40-induced cellular responses are of special interest. CD40 is also involved in the regulation of cell survival/apoptosis (6). Therefore, CD40-induced death-inhibiting or death-promoting molecules may be relevant to regulate the duration of an Ag-induced immune response.

MAPK family members are essential for the signal transduction of a variety of cellular functions in response to CD40 or other stimuli (7–9). Three major MAPK subfamilies have been characterized extensively: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs; or stress-activated protein kinases), and the p38 MAPKs. Bacterial constituents such as LPS, proinflammatory cytokines, and ligation through TNF receptor (TNFR) family members, including CD40, can activate p38 MAPK (7–10). A well-characterized substrate for p38 MAPK is the MAPK-activated protein kinase-2 (MAPKAPK-2), which, in turn, phosphorylates the 27-kDa heat shock protein (11). Several transcription factors, such as ATF-1, ATF-2, and cAMP response element binding protein, are also activated via a p38 MAPK-dependent pathway in response to certain stimuli (12, 13). A p38 MAPK-specific inhibitor, the pyridinyl imidazole compound SB203580, has facilitated extensive investigation of the role of the p38 MAPK pathway (14, 15). Using this inhibitor, p38 MAPK has been implicated in the regulation of cytokines such as IL-1, IL-6, and TNF-α (9, 16). In some cell types, the ERK pathway is also involved in CD40-induced production of several cytokines (17, 18).

Among MAPKs, the ERK pathway has been implicated in cell proliferation and survival (19, 20). In a murine DC cell line, ERK was required as a survival factor (20). The role of p38 MAPK in delivering death or survival signals is less clear (19, 21, 22). Previously, we investigated the role of the p38 MAPK pathway in response to CD40 engagement in B cells; we found that p38 MAPK is required for optimal CD40-induced B cell proliferation, CD54 gene expression, and NF-κB activation in B cell lines and

† Robert-Roessle-Klinik, Charite, Humboldt University of Berlin, Berlin, Germany

Address correspondence and reprint requests to Dr. Edward A. Clark, Department of Microbiology, Box 357242, University of Washington, Seattle, WA 98195. E-mail address: eclark@bart.prp.washington.edu

*Department of Microbiology, University of Washington, Seattle, WA 98195; and Robert-Roessle-Klinik, Charite, Humboldt University of Berlin, Berlin, Germany
tonsillar B cells (7). In the study described in this paper we examined the role of p38 MAPK in CD40-dependent regulation of cytokine and chemokine production as well as apoptotic regulators in human DCs compared with B cells.

Materials and Methods

Preparation of monocyte-derived human DCs and tonsillar B cells

PBMC were isolated from peripheral blood samples by centrifugation over Ficoll-Hypaque (Robbins Scientific Corp., Sunnyvale, CA). After sheep erythrocyte rosetting to deplete T cells, CD14+ cells were obtained by positive selection with magnetic anti-CD14 microbeads according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA). CD14+ cells, 93–99% pure as assessed by flow cytometry, were cultured in RPMI 1640 medium plus 100 ng/ml human GM-CSF (RDI, Flanders, NJ) and 30 ng/ml human IL-4 (RDI) (23). Every other day, 50% of the medium was removed, and the same volume of fresh medium containing twice the amount of cytokines was added. After 5–7 days, cells exhibited an immature DC phenotype, i.e., CD14+, CD86+/−, HLA-DR+/−, CD1a+/−, and CD40+/− (23). Lymphocytes from tonsillar cell suspensions were isolated, then subjected to T cell depletion by sheep erythrocyte rosetting; dense B cells were obtained after CD19. These resting B cells contained >70% naive IgD+ CD38− B cells.

Kinase assays

Immune complex assays for p38 MAPK were performed as previously described (7), using 3–5 × 10^6 DCs/sample and stimulated with either 10 μg/ml anti-CD40 (G28-5) or 10 μg/ml anti-CD22 IgG1-iso type control (G28-7) for the indicated times. Assays measuring the inhibition of MAPKAPK-2 following pretreatment with the p38 MAPK inhibitor SB203580 (Calbiochem, San Diego, CA) have been described previously (7). After 30-min preincubation of DCs with 0–20 μM SB203580, cells were stimulated with either 10 μg/ml anti-CD40 or anti-CD22 iso type control for 15 min, since maximal MAPKAPK-2 activation occurred at this time (data not shown). As SB203580 was dissolved in DMSO, the control Ab and CD40-ligated DCs were also pretreated with a 0.1% (v/v) concentration of DMSO shown). As SB203580 was dissolved in DMSO, the control Ab and CD40-ligated DCs were also pretreated with a 0.1% (v/v) concentration of DMSO (G28-7) for the indicated times. Assays measuring the inhibition of MAPKAPK-2 following pretreatment with the p38 MAPK inhibitor SB203580 (Calbiochem, San Diego, CA) have been described previously (7).

To gain insight into the role of CD40 in DCs vs B cells, we tested whether genes that affect cell fate were regulated differentially in these cell types. Apoptotic regulators showed differences in inducible gene expression after CD40 ligation in DCs and B cells. We compared the induction of members of the Bcl-2 family after CD40 ligation of DCs and B cells, including the antiapoptotic members Bcl-2, Bcl-x, and Mcl-1, and the proapoptotic members Bax, Bak, and Bik (25). Bcl-2 was strongly induced in B cells in contrast to DCs. CD40 cross-linking up-regulated Bcl-2, TRAF2, and TRAF4 in B cells. Bik and cIAP1 were constitutively expressed in B cells but not in DCs. NAIP was down-regulated in DCs after CD40 cross-linking and not expressed in B cells.

MultiProbe ribonuclease protection assay (RPA)

Total RNA from SB203580-pretreated and 1 μg/ml anti-CD40 or isotype control mAb-stimulated DCs or tonsillar B cells was extracted using RNasy kits (Qiagen, Santa Clarita, CA) or Trizol (Life Technologies, Grand Island, NY). RPAs were performed according to the supplier’s instructions (PharMingen, San Diego, CA), using human CK-2, CK-5, Apo-2, and Apo-2 template sets with 2–5 μg of total RNA.

Enzyme-linked immunosorbent assays

Supernatants from 1 × 10^6 DCs/ml or 2 × 10^6 tonsillar B cells/ml pre-treated with graded concentrations of SB203580 or 0.5–1 μM U0126 were obtained after CD40 engagement for 6 or 24 h. The cell viability, as determined by trypan blue exclusion, was 78–95%. Samples were immediately frozen and stored at −70°C. The ELISAs for both human p40 IL-12 (OptEIA Set, PharMingen) and the human chemokine RANTES (Quantikine, R&D Systems) were performed according to the manufacturer’s instructions.

Results

CD40-induced apoptosis-related genes in DCs and B cells

Expression of apoptosis-related genes after CD40 ligation in DCs and B cells

Table 1. Expression of apoptosis-related genes after CD40 ligation in DCs and B cells

<table>
<thead>
<tr>
<th>Apoptosis-Related Genes</th>
<th>DCs</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutive level</td>
<td>Change after anti-CD40</td>
</tr>
<tr>
<td>Caspase-1</td>
<td>Intermediateb</td>
<td>None</td>
</tr>
<tr>
<td>Caspase-2</td>
<td>Intermediate</td>
<td>None</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>Intermediate</td>
<td>None</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>Intermediate</td>
<td>None</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>Intermediate</td>
<td>None</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Not detectable or low</td>
<td>None</td>
</tr>
<tr>
<td>Bcl-x</td>
<td>Intermediate</td>
<td>Higher</td>
</tr>
<tr>
<td>Mcl-1</td>
<td>High</td>
<td>None</td>
</tr>
<tr>
<td>Bik</td>
<td>Not detectable</td>
<td>None</td>
</tr>
<tr>
<td>Bak</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td>Bax</td>
<td>Intermediate</td>
<td>None</td>
</tr>
<tr>
<td>TRAF1</td>
<td>High</td>
<td>Much higher</td>
</tr>
<tr>
<td>TRAF2</td>
<td>Not detectable or low</td>
<td>None</td>
</tr>
<tr>
<td>TRAF3</td>
<td>Low</td>
<td>None or slightly higher</td>
</tr>
<tr>
<td>TRAF4</td>
<td>Not detectable or low</td>
<td>None</td>
</tr>
<tr>
<td>XIAP</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td>cIAP1</td>
<td>Not detectable</td>
<td>None</td>
</tr>
<tr>
<td>cIAP2</td>
<td>Intermediate</td>
<td>Higher or much higher</td>
</tr>
<tr>
<td>NAIP</td>
<td>Not detectable or low</td>
<td>None or lower</td>
</tr>
</tbody>
</table>

b In contrast to DCs, CD40 cross-linking up-regulated Bcl-2, TRAF2, and TRAF4 in B cells. Bik and cIAP1 were constitutively expressed in B cells but not in DCs. NAIP was down-regulated in DCs after CD40 cross-linking and not expressed in B cells.

given are absolute expression levels and their changes after anti-CD40 ligation. Based on kinetic studies 0–24 h after CD40 cross-linking.
response to CD40 ligation, but poorly expressed in DCs (Table I and Fig. 1, A and B). Bcl-x, however, was strongly induced after CD40 engagement in both cell types, whereas Mcl-1, Bak, and Bax were constitutively expressed in both cell types; Bik was constitutively expressed in B cells, but was not detectable in DCs even after CD40 ligation.

The TNFR-associated factor (TRAF) family members represent intracellular adapter molecules for coupling TNFRs to effector molecules (26). Some TRAFs have been shown to interact with the cytoplasmic tail of CD40 (27–29). TRAF1 and TRAF2 molecules also associate with inhibitor of apoptosis protein (IAP) molecules such as cIAP2 and cIAP1 (30). TRAF2 and TRAF4 mRNA were increased in B cells after CD40 cross-linking, but were not detectable or were only constitutively expressed in DCs (Table I; see also Fig. 5, A and B). TRAF1 and TRAF3 expression were up-regulated in both DCs and B cells upon CD40 ligation. In some experiments, however, DCs only showed a constitutive expression of TRAF3. In both DCs and B cells the survival molecule cIAP2 was up-regulated upon CD40 ligation, whereas cIAP1 was constitutively expressed in B cells and was not detectable in DCs (Table I; see Fig. 5, A and B). The neuronal inhibitor of apoptosis protein (31) however, was constitutively expressed in DCs and was down-regulated in response to CD40 ligation, but was never detected in B cells (Table I). We also examined expression of members of the caspase family, which are death-promoting cysteine proteases (32). The only caspase markedly induced in DCs by CD40 cross-linking was caspase-7. Caspase-7 was also weakly up-regulated in B cells (Table I).

Thus, DCs and B cells differ both in the set of cell fate genes they express constitutively and in how these genes are affected after CD40 ligation. In particular, Bcl-2, Bik, TRAF2, TRAF4, and cIAP1 were clearly expressed and/or inducible in B cells but not in DCs.

CD40-induced cytokine/chemokine genes in DCs and B cells

In response to CD40 engagement, APCs such as DCs and B cells secrete cytokines and chemokines that act as mediators for the induction of cellular and humoral immune responses. Thus, we compared whether the cytokine/chemokine profiles of CD40-stimulated DCs and B cells were different. IL-12 is known as a pivotal Th1-skewing factor and consists of p40 and p35 subunits (33). Following CD40 cross-linking, the p40 subunit of IL-12 was induced in DCs, while IL-12 p35 mRNA was undetectable (Table II and Fig. 2, A–C). In contrast, in B cells, IL-12 p40 mRNA and/or IL-12 p35 mRNA were constitutively expressed at very low levels, but were not up-regulated by CD40 cross-linking (Table II).

We also examined the expression of mRNA encoding proinflammatory cytokines, including IL-1, as well as anti-inflammatory cytokines, such as IL-10 and IL-1R antagonist (IL-1Ra). In DCs, IL-1, IL-1Ra, IL-8, IL-12 p40, RANTES, and MIP-1α in DCs.

### Table II. Expression of cytokine/chemokine genes after CD40 ligation in DCs and B cells

<table>
<thead>
<tr>
<th>Cytokine/Chemokine Genes</th>
<th>DCs</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutive level</td>
<td>Change after anti-CD40</td>
</tr>
<tr>
<td>IL-1α</td>
<td>Not detectable or low&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Higher</td>
</tr>
<tr>
<td>IL-1β</td>
<td>High</td>
<td>Much higher</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>High</td>
<td>None or much higher</td>
</tr>
<tr>
<td>IL-8</td>
<td>Not detectable</td>
<td>None</td>
</tr>
<tr>
<td>IL-10</td>
<td>Intermediate or high</td>
<td>Higher</td>
</tr>
<tr>
<td>IL-12 p35</td>
<td>Not detectable</td>
<td>Slightly higher or higher</td>
</tr>
<tr>
<td>IL-12 p40</td>
<td>Not detectable</td>
<td>Slightly higher or higher</td>
</tr>
<tr>
<td>IL-15</td>
<td>Intermediate</td>
<td>Higher</td>
</tr>
<tr>
<td>RANTES</td>
<td>Low</td>
<td>Slightly higher</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>Low</td>
<td>Higher</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>Intermediate or high</td>
<td>Higher</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>I-309</td>
<td>Intermediate</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>a</sup> In contrast to B cells, CD40 cross-linking up-regulated IL-1β, IL-1Ra, IL-8, IL-12 p40, RANTES, and MIP-1α in DCs.

<sup>b</sup> Given are absolute expression levels and their changes after anti-CD40 ligation. Based on kinetic studies 0–24 h after CD40 cross-linking.
IL-1β mRNA was strongly up-regulated and IL-1Ra mRNA was weakly induced by CD40 ligation; in contrast, in B cells, both IL-1β and IL-1Ra mRNA were down-regulated. The Th2-skewing cytokine IL-4 was not detected in either cell type, whereas IL-15 mRNA, a T/B cell growth and differentiation factor with chemotactic properties (34), was induced in both DCs and B cells via CD40.

Chemokines are a family of mediators with chemotactic activity. Of the chemokines, RANTES and IL-8 mRNA were up-regulated in DCs by CD40 cross-linking but were not induced after CD40 ligation in B cells (Table II and Fig. 2D). In the experiment shown, there is already a substantial IL-8 mRNA expression in unstimulated DCs. In five independent experiments we found an increase in IL-8 mRNA after CD40 engagement ranging from 1.7- to 6-fold. Although macrophage inflammatory protein-1β (MIP-1β) was induced in both cell types, MIP-1β was induced only in DCs and was constitutively expressed in B cells. Macrophage chemoattractant protein-1 (MCP-1) mRNA was up-regulated by CD40 in DCs, but was apparently not expressed in B cells. Another chemokine, I-309, was constitutively expressed in DCs, but not in B cells.

In summary, constitutive cytokine and chemokine gene expression and the set of cytokine/chemokine genes induced after cross-linking CD40 are very different in DCs and B cells. Untreated DCs, unlike dense B cells, expressed the chemokine I-309, and, after CD40 ligation, were induced to increase expression of IL-1β, IL-1Ra, IL-8, IL-12 p40, RANTES, MIP-1-α, and MCP-1.

**FIGURE 2.** CD40 ligation induces cytokines and chemokines via both SB203580-sensitive and -insensitive pathways in DCs and B cells. A. After CD40 cross-linking for 3 h, mRNAs for cytokine genes were induced as determined by RPA in DCs with or without SB203580. B. After CD40 ligation for 1, 3, and 6 h, induction of IL-12 p40 mRNA transcripts in DCs in the presence of a control solvent vehicle (DMSO; black bars) was strongly inhibited in the presence of 20 μM SB203580 (white bars) as shown by scanning densitometry. C. After CD40 cross-linking for 0–6 h, mRNAs for cytokine genes were induced as determined by RPA in B cells in either the presence or the absence of SB203580. D. CD40 engagement for 3 h induces mRNAs for chemokine genes in DCs with or without SB203580. This figure is representative of three similar experiments performed with cells from different donors.

CD40 cross-linking activates the p38 MAPK pathway in DCs

Because the sets of genes induced by CD40 in DCs and B cells were quite different, we next tested whether upstream CD40-induced signaling pathways were different in DCs compared with B cells. As with human B cells (7), p38 MAPK activity was rapidly increased in DCs upon cross-linking with CD40 mAb (Fig. 3A).
Maximal activation of p38 MAPK peaked at 15 min and then declined to unstimulated levels at 45 min. A secondary increase, observed between 60 and 90 min, suggested biphasic kinetics of p38 MAPK activation not previously seen in B cells. The availability of the p38 MAPK inhibitor, SB203580, enabled us to examine the role of p38 MAPK in CD40 signaling in DCs as in B cells (7). SB203580 also blocked CD40-induced MAPKAPK-2 activation in a dose-dependent manner. DCs were preincubated for 30 min with graded doses of SB203580 and then ligated with CD40 mAb or treated with CD22 mAb for 15 min. Maximal activation of MAPKAPK-2 in this representative experiment of three was 102,832 cpm after CD40 ligation vs 57,447 cpm in the isotype control. The background counts per minute (isotype control) were subtracted.

CD40 ligation phosphorylates ERK in DCs but not in B cells

Previously, we found that cross-linking CD40 on B cells preferentially induced activation of JNKs rather than ERKs (35). This is not the case in DCs. Within minutes after CD40 stimulation, the phosphorylated (active) forms of ERK1 (p44 MAPK) and ERK2 (p42 MAPK) were strongly (>7-fold) up-regulated in DCs (Fig. 3C) but not in B cells (Fig. 3D). PMA strongly increased ERK2 phosphorylation in B cells showing that these cells were responsive to other stimuli. The inhibitor U0126 specifically blocks MEK1/2, the kinases upstream of ERK (36, 37). We could not detect any influence of U0126 on the activation of p38 MAPK (data not shown), thereby confirming the selectivity of U0126. The MEK1/2 inhibitor U0126 reduced the CD40-induced increase in ERK2 phosphorylation in DCs (C). For B cells, PMA (50 ng/ml) was used as a positive control (D). Total ERK2 served as a loading control.

CD40 ligation induces c-Jun phosphorylation in DCs

Using in vitro JNK kinase assays, we found that JNK was also activated in DCs after CD40 ligation; maximal activity occurred at 15 min (data not shown), similar to that in B cells (35). CD40 cross-linking also increased phosphorylation of endogenous c-Jun, a substrate for JNK/stress-activated protein kinase (Fig. 3E). In these experiments phosphorylated c-Jun migrates slower than nonphosphorylated c-Jun, as evident in control lanes. Although SB203580 is specific for p38 MAPK at lower doses, it may also affect the JNK pathway when used at higher doses in some cell types.
types (38, 39). However, following preincubation of DCs with graded doses of SB203580 (0 to 20 μM) and subsequent CD40 stimulation, there was no change in the ratio of the upper phosphorylated c-Jun band to the lower nonphosphorylated c-Jun band (Fig. 3E). Based on these observations, we conclude that SB203580 at the doses used in this study does not appear to affect CD40-induced activation of the JNK pathway.

A role for p38 MAPK in cytokine/chemokine expression in DCs and B cells after CD40 ligation

Having defined which MAPK family kinases were activated in DCs vs B cells, we examined the roles of these kinases in CD40-induced gene and protein expression. Because p38 MAPK is involved in TNF- and LPS-induced cytokine expression in other cell types (9, 15, 16), and SB203580 is a specific p38 MAPK inhibitor in DCs and B cells, we decided to identify SB203580-sensitive cytokines/chemokines after CD40 cross-linking. The induction of IL-12 p40 mRNA after CD40 ligation was significantly reduced by up to 80% in DCs in the presence of graded doses of SB203580 (0–20 μM; Fig. 2, A and B). As with MAPKAPK-2 activation (Fig. 3B), as little as 1 μM SB203580 was sufficient to inhibit mRNA increases by ~60%. CD40 ligation also induced a strong increase in IL-12 p40 protein secretion by DCs as detected by ELISA, and blockade of p38 MAPK with graded doses of SB203580 dramatically reduced IL-12 protein expression up to 85% (Fig. 4A). Even at low SB203580 doses, e.g., 1 μM, which minimally affect JNK2 β splice variants (38, 39), CD40-induced IL-12 production in DCs was strongly blocked. Consistent with the inability of CD40 ligation to up-regulate IL-12 p40 mRNA in dense B cells (Fig. 2C), we were also unable to detect any CD40-induced IL-12 p40 protein secretion by ELISA after 24 h of CD40 ligation in three independent experiments (Fig. 4C). As a negative control for SB203580 sensitivity of CD40-induced IL-12 mRNA and protein expression, CD40-induced RANTES mRNA and protein expression at doses of 1–5 μM were only minimally affected (Fig. 4B).

CD40-induced increases in IL-1α mRNA in both DCs and B cells were also partially reduced in the presence of the p38 MAPK inhibitor (Fig. 2, A and C). IL-10 mRNA increases after CD40 cross-linking, however, were reduced by about 50% in B cells, but were not affected in DCs (Fig. 2, A and C). CD40-induced increases in mRNA levels of IL-1β in DCs did not depend on the p38 MAPK pathway, thereby providing a negative control for p38 MAPK specificity in DCs, while in B cells decreases in IL-1β mRNA appeared to be SB203580 sensitive. Furthermore, IL-1Ra levels were not significantly affected by SB203580 and served as a negative control for SB203580 specificity in B cells. We also pretreated CD40-stimulated DCs with the MEK1/2 inhibitor U0126 and measured IL-12 p40 secretion by ELISA (Fig. 4C). Blockade of the ERK pathway with 1 μM U0126 reduced CD40-induced IL-12 p40 production in DCs by about 40%, while under the same conditions the p38 MAPK inhibitor blocked IL-12 p40 production by >80%.

Role of p38 MAPK in regulating expression of cIAP2 mRNA in DCs and B cells after CD40 ligation

CD40-induced expression of cIAP2 mRNA in DCs was also strongly reduced by SB203580, but induction of the same gene in B cells was not sensitive to the p38 MAPK inhibitor (Fig. 5, A and B). Similarly, blocking p38 MAPK did not affect CD40-induced cIAP2 up-regulation in Daudi cells at the transcriptional level (7).

In summary, CD40-induced increases in IL-1α mRNA (Fig. 2A) and cIAP2 (Fig. 5A) in DCs, like changes in IL-12 expression, were reduced in the presence of the p38 MAPK inhibitor, while CD40-induced increases in IL-10, IL-1β, and IL-1Ra mRNAs (Fig. 2A); RANTES, MIP-1β, MIP-1α, MCP-1, and IL-8 mRNAs (Fig. 2D); and TRAF1 mRNA (Fig. 5A) were not. Thus, changes in expression of IL-12, IL-1α, and cIAP2 mRNAs via CD40 require p38 MAPK in DCs. The requirement for p38 MAPK in CD40-induced gene expression was different in B cells (Figs. 2C and 5B). Although a small increase in IL-1α mRNA was blocked by SB203580 in B cells as in DCs (Fig. 2C), unlike in DCs, the induction of cIAP2 mRNA increases via CD40 was not sensitive to the p38 MAPK inhibitor (Fig. 5B). In contrast, IL-10 mRNA increases stimulated via CD40 were sensitive to the p38 MAPK inhibitor in B cells, with a reduction ranging from 50–65% in three independent experiments (Fig. 2C), but not in DCs (Fig. 2A).
Role of p38 MAPK in regulating cIAP2 protein expression in DCs and B cells after CD40 ligation

We also tested the effect of the p38 MAPK inhibitor on cIAP2 protein levels in DCs and B cells (Fig. 5, C–E). As expected, CD40-induced cIAP2 protein expression in DCs could be blocked by SB203580 (Fig. 5, C and D). Although SB203580 had no effect on CD40-induced cIAP2 mRNA in B cells, it did inhibit CD40-induced cIAP2 protein increases in B cells (Fig. 5E), but significantly higher doses of SB203580 were required. In DCs, CD40-induced cIAP2 also exhibited a weak sensitivity to U0126 (D). For the B cells (E), PMA (2 ng/ml) and ionomycin (10 ng/ml) were used as a positive control.

Discussion

During a primary immune response, activated T cells expressing CD40 ligand trigger both CD40+ DCs and B cells. The consequences of CD40 ligation on expression of cytokine, chemokine, and apoptotic regulator genes, however, appear to be quite different for each cell type. A program is initiated in B cells to express a number of cell fate genes not induced in DCs. We found that the apoptotic regulator genes Bcl-2, TRAF2, and TRAF4 were clearly inducible in B cells but not in DCs. Therefore, these survival-related genes are specifically regulated by CD40 in B cells but not in DCs. In another study, Bcl-2 protein expression was reported to be up-regulated after CD40 ligation in DCs (40). These DCs, however, were derived from CD34+ precursors. In both DCs and B cells, CD40-induced mRNA increases were observed for Bcl-x, cIAP2, and TRAF1 after CD40 ligation, suggesting that these apoptotic regulators are common to the survival programs of both APCs. CD40-induced up-regulation of Bcl-x was consistent with previous reports in monocyte-derived DCs after ligation with CD40 and TRANCE (41). Here, we describe the CD40-induced expression of cIAP2 and TRAF1 in DCs and normal B cells after CD40 ligation, as shown previously for B cell lines (7). In conclusion, CD40 ligation induced survival program genes in both cell types, but in B cells the survival gene profile involved additional genes. Differences were also observed in the pattern of CD40-induced cytokine and chemokine gene expression in these two APC cell types. Unlike B cells, DCs were induced to increase expression of IL-1β, IL-1Ra, IL-8, IL-12 p40, RANTES, MIP-1α, and MCP-1 after CD40 ligation, consistent with previous reports (42–45), whereas MIP-1β, IL-10, and IL-15 were induced in both DCs and B cells. Thus, while CD40-induced cytokines and chemokines strongly contributed to the inflammatory and chemotactic properties of DCs, CD40 ligation was only a poor inflammatory
and chemotactic stimulus in B cells. Interestingly, the cytokines IL-10 and IL-15 induced in both cell types are B cell growth/differentiation factors (34, 46). Thus, CD40 ligation may promote B cell growth/differentiation via endogenous and DC-produced factors.

The differential pattern of gene expression observed upon ligation of CD40 in DCs and B cells may reflect heterogeneity in the signal transduction machinery. We first examined whether p38 MAPK can also be stimulated in DCs in response to CD40 ligation. In a previous study we showed that the p38 MAPK pathway is activated in normal and transformed human B cells after CD40 ligation (7). Here, we demonstrate that CD40 ligation also activates the p38 MAPK pathway in human monocyte-derived DCs. To test which genes are dependent on the p38 MAPK pathway, we used the p38 MAPK-specific inhibitor SB203580 that effectively abrogated activation of the p38 MAPK target MAPKAPK-2 in DCs. Because certain JNK isoforms, particularly the β splice variants of JNK2, are also sensitive to higher doses of SB203580 (38, 39), we attempted to exclude this possibility by examining the influence of SB203580 on the JNK downstream target c-Jun (7). In this assay we did not see any inhibitory effect of SB203580 on the JNK pathway. In addition to the p38 MAPK pathway, we also demonstrated that CD40 ligation activates the ERK pathway in DCs, as detected by the phosphorylation of ERK1 and ERK2, but not in B cells. Previously, we showed that CD40 ligation did not induce ERK in B cell lines (35), consistent with other results (47, 48). However, CD40 ligation resulted in activation of ERK in murine splenic B cells and monocytes (17, 48–50). Activation of ERK in murine splenic B cells, in contrast to human tonsillar B cells, may be due to B cell stage or species differences in MAPK required for CD40 signaling. To inhibit the ERK pathway, we used the U0126 inhibitor of the ERK upstream kinase MEK1/2 (36). U0126 suppressed the CD40-induced ERK phosphorylation by >70%.

Because CD40 ligation plays an important role in the regulation of cell death (6), we tested the effect of SB203580 on the expression of apoptosis-related genes. Up-regulation of the Bcl-2 family members Bcl-2 and Bcl-x remained unaltered in both DCs and B cells in the presence of SB203580 (data not shown). Likewise, the CD40-induced TRAF molecules were not affected by the p38 MAPK inhibitor. Thus, the p38 MAPK pathway did not seem to be involved in CD40 induction of these apoptotic regulators in either DCs or B cells. The exception was the survival molecule cIAP2, which promotes antideath activity by blocking distinct caspases (51). CD40-induced cIAP2 mRNA induction and protein expression in DCs were clearly dependent on the p38 MAPK pathway. The fact that cIAP2 mRNA expression in B cells was not affected by SB203580 is consistent with our previous work in Daudi cells (7). Furthermore, because significantly higher doses of SB203580 were necessary to inhibit CD40-induced cIAP2 protein induction in B cells, it is possible that this is due to the effects of higher SB203580 doses (>10 μM) on JNK isoforms (39). Blockade of the ERK pathway also showed a weak inhibition of the survival protein cIAP2 protein expression. In a recent paper ERK was implicated in cell survival of a murine LPS-stimulated DC cell line (20). In this DC cell line, LPS activated the ERK pathway that was required for cell survival. This effect might also be due to an induction of apoptotic regulators such as cIAP2.

We investigated the p38 MAPK requirement for CD40-induced cytokines and chemokines in DCs and B cells. Because CD40-induced IL-12 p40 was one of the most SB203580-sensitive cytokines we found in DCs, we studied IL-12 p40 extensively and compared its qualities in DCs and B cells. In DCs, the p40 subunit of IL-12, a strong Th1 differentiation factor (5), was induced, whereas IL-12 p35 mRNA could not be detected. In dense B cells, however, only low constitutive expression of either IL-12 subunit was observed. To confirm the lack of IL-12 production in B cells after CD40 ligation, we also examined IL-12 p40 protein expression in B cells by ELISA. We were unable to detect any IL-12 p40 production in B cells after CD40 engagement, although we doubled the cell numbers typically used to detect IL-12 p40 production and collected supernatants after 24 h, consistent with similar negative results measuring IL-12 p70 in other studies (4, 52). In contrast, Schultze et al. (53) reported CD40-induced IL-12 production in nongerminal center human tonsillar B cells. However, in our studies, dense B cells, which were about 90% CD38-IgD−/IgD+, showed no detectable IL-12 production after CD40 ligation. Schultze et al. stimulated B cells using CD40 ligand-transfected fibroblasts that may have provided more cross-linking or may have added an additional signal. In our experiments we observed a clear difference in IL-12 mRNA and protein expression between DCs and B cells. IL-12 gene expression in B cells was not CD40 inducible, possibly because of a lack of ERK activation, which appears to contribute to IL-12 production in DCs. Alternatively, the expression of constitutive normal B cell-specific repressors might block IL-12 induction via CD40. As a consequence of the lack of IL-12 production in B cells, DCs might be expected to be superior to B cells in their ability to generate a Th1 response.

Using the p38 MAPK inhibitor SB203580, we found that IL-12 p40 mRNA and protein secretion were dependent on the p38 MAPK pathway in DCs, whereas CD40-induced up-regulation of RANTES mRNA and protein secretion were only slightly affected by high concentrations of SB203580. IL-12 is a critical mediator of IFN-γ production by Th1 T lymphocytes (5). IL-12 also directly induces IFN-γ secretion from both T and NK cells (54). Experiments with IL-12−/− mice have demonstrated that IL-12 is essential for the generation of polarized Th1 cytokine profiles after infection with intracellular bacteria and parasites, but not following viral infections (55). Likewise, several animal models suggest a central role for IL-12 in the immunopathology of Th1-dependent diseases, such as multiple sclerosis or Crohn’s disease (56). Recent experiments in human DCs suggest that anti-IL-12 strongly decreased the number of IFN-γ-producing T cells, whereas no shift toward a Th2 pattern occurred (57). Because the results obtained with p38 MAPK inhibitors may be expected to be similar to those obtained with anti-IL-12, the potential therapeutic range of p38 MAPK inhibitors might be extended to disorders with pathologic Th1 cytokine profiles. Furthermore, DC-derived IL-12 is mandatory in inducing naive, but not memory, B cell differentiation (4). CD40-induced IL-12 p40 transcripts require NF-κB activation in B cell lines (58, 59). Because CD40-induced NF-κB activation requires p38 MAPK (7), it is likely that NF-κB activation is also an important component for CD40-mediated IL-12 production in DCs, although the involvement of other signaling pathways, such as ERK, cannot be excluded. Recently, Hacker et al. found that both JNK and p38 MAPK can be induced by synthetic oligonucleotides via a CD40-independent pathway (60). In murine APCs, including DCs, these authors reported that TNF-α and IL-12 protein expression, induced by oligonucleotides, required p38 MAPK; whether there are oligonucleotide-induced cytokines and chemokines that were unaffected by the p38 MAPK was not examined. Transgenic mice that express dominant negative p38 MAPK on the distal lck promoter also showed selectively impaired Th1 responses with blocked production of IFN-γ, whereas IL-4 production by Th2 cells was not affected (8). The possibility that reduced IL-12 production by APCs such as DCs in these mice contributed to a decrease in Th1 T cell-produced IFN-γ was not
addressed, particularly because it is not entirely clear whether the distal lck promoter is active in some APCs.

In addition to the p38 MAPK pathway, we found that in DCs the ERK pathway also contributed to CD40-induced IL-12 p40 protein secretion, albeit to a lesser extent than the p38 MAPK pathway. Thus, the ERK pathway appears to participate in CD40-mediated IL-12 production in DCs. The ERK pathway previously has been shown to be required for the production of IL-1, IL-3, IL-6, IL-8, and IL-10 in LPS-stimulated monocytes and CD3/CD28-stimulated T cells (18, 37). Expression of other CD40-induced cytokines was also differentially dependent on the p38 MAPK pathway in DCs and B cells. Although the Th2-skewing cytokine IL-10 was up-regulated in both DCs and B cells after CD40 ligation, only IL-10 mRNA increases in B cells were sensitive to SB203580. Thus, B cells seem to regulate CD40-induced IL-10 mRNA by a p38 MAPK-dependent pathway, whereas DCs do not. IL-10 expression was also dependent on the p38 MAPK pathway in monocytes and T cells (9, 60). Interestingly, IL-10 is able to suppress IL-12 production (61, 62); thus, the regulation of two opposing cytokines appears to be differentially regulated in DCs and B cells. Furthermore, we detected a differential SB203580 sensitivity of IL-1 in DCs and B cells. IL-1 was one of the first cytokines shown to be dependent on the p38 MAPK pathway (15). CD40-induced IL-1α mRNA expression seemed to be SB203580 sensitive in both cell types, while CD40-induced IL-1β was SB203580 sensitive only in B cells. Differences in DCs vs B cells might be due to a differential SB203580 sensitivities of transcriptional and post-transcriptional mechanisms in the two types of cells.

In conclusion, p38 MAPK has a differential role in the regulation of the IL-12 p40, cIAP2, IL-10, and IL-1β in DCs and B cells. The results presented here show that a Th1 cytokine (IL-12) is regulated by p38 MAPK in DCs and suggest that a Th2 cytokine (IL-10) is regulated by p38 MAPK in B cells. Further studies to investigate the influence of the ERK pathway on the CD40-induced gene program in DCs and B cells are in progress.

Acknowledgments
We thank Dr. Jonathan Graves for critically reading the manuscript, Marj Domenowke for the preparation of figures, Kate Elias for editorial assistance, and members of the Clark laboratory for helpful discussions.

References