Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11814–11818, December 1995
Immunology

CD38 ligation induces tyrosine phosphorylation of Bruton tyrosine kinase and enhanced expression of interleukin 5-receptor α chain: Synergistic effects with interleukin 5

(X-chromosome-linked deficiency/Blimp1)

YUJI KIKUCHI*, TOKUTARO YASUE*, KENSUKE MIYAKE†, MASAO KIMOTO†, AND KIYOSHI TAKATSU*‡

*Department of Immunology, Institute of Medical Science, the University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan; and †Department of Immunology, Saga Medical School, Nabeshima-cho, Saga 970, Japan

Communicated by Alfred Nisonoff, Brandeis University, Waltham, MA, September 5, 1995 (received for review July 14, 1995)

ABSTRACT **Mouse CD38 has been implicated in the regulation of both B-cell proliferation and protection of B cells from irradiation-induced apoptosis. CD38 ligation on B cells by CS/2, an anti-mouse CD38 monoclonal antibody, induced proliferation, IgM secretion, and tyrosine phosphorylation of Bruton tyrosine kinase in B cells from wild-type mice. B cells from X chromosome-linked immunodeficient mice did not respond at all to anti-CD38 antibody, although CD38 expression on these B cells was comparable to that on wild-type B cells. We infer from these results that Bruton tyrosine kinase activation is involved in B-cell triggering after cross-linkage of CD38. Analysis of the synergistic effects of various cytokines with CD38 ligation on B-cell activation revealed that interleukin 5 (IL-5) showed the most potent effect on B-cell proliferation, *Blimp1* gene expression, and IgM production. These synergistic effects were not seen with B cells from X chromosome-linked immunodeficient mice. Flow cytometry analysis revealed that CD38 ligation increased surface expression of the IL-5-receptor α chain on B cells. These data indicate that CD38 ligation increases IL-5 receptor α expression and synergizes with IL-5 to enhance *Blimp1* expression and IgM synthesis.**

CD38 is a type II transmembrane glycoprotein and is an ectoenzyme that possesses both ADP-ribosyl cyclase and cADP-ribosyl hydrolase activities, which generate cADP-ribose and ADP-ribose from NAD^+ (1–4). CD38 has been implicated in the regulation of both B-cell proliferation and rescue of B cells from apoptosis (5, 6). A role for CD38 in regulation of B-cell activation has been suggested by analysis using agonistic monoclonal antibody (mAb) to mouse CD38, which mitogenically stimulates resting B cells (5, 6) and induces tyrosine phosphorylation of cellular proteins (7). We have reported that ligation of CD38 by CS/2, an agonistic mAb against mouse CD38, also demonstrated mitogenic activity on resting B cells (8). At present, the molecular mechanisms underlying CD38-mediated activation of B cells are poorly understood.

Interleukin 5 (IL-5) is a cytokine that stimulates proliferation and differentiation of B cells, eosinophils, and basophils (9, 10). IL-5 signals through the IL-5 receptor (IL-5R) complex, which is composed of an α chain (IL-5Rα) and a β chain (11–13). IL-5Rα specifically binds IL-5 and forms with the β chain, which cannot bind IL-5 by itself, a high-affinity receptor complex indispensable for IL-5 signal transduction (12–17). The β chain is shared among receptors for IL-5, IL-3, and granulocyte/macrophage colony-stimulating factor (GM-CSF) (14, 18, 19). IL-5 induces rapid tyrosine phosphorylation of IL-5R β chain, phosphatidylinositol 3-kinase, Shc, Vav, and HS1 and activates B-cell-specific nonreceptor-type Bruton tyrosine (Btk) and JAK2 kinases (20, 21).

X chromosome-linked immunodeficient (Xid) mice, which carry the gene (*xid*) responsible for X chromosome-linked immunodeficiency, are known to have defects manifested by low levels of circulating IgM and IgG3, a high surface IgM-to-IgD ratio, and a failure to respond to type II thymus-independent antigen (22, 23) and anti-IgM stimulation. Low responsiveness of Xid B cells to CD40 (24), RP14 (25), and cytokines (26–28) also has been reported. Recently, it has been demonstrated that Xid mice have a point mutation in the pleckstrine homology domain of Btk (29–31). We also have reported that Xid B cells show an impaired proliferative response to CD38 ligation, despite expressing normal levels of CD38 on their surface (8). Xid B cells also demonstrate reduced IgM synthesis in response to IL-5 (26). It is not clear whether aberrant expression of Btk in Xid mice is involved in this impaired B-cell responsiveness. In this study, we have examined the role of Btk activation in signaling through CD38 ligation and the synergistic effect of CD38 and IL-5 on IgM secretion. We show that Btk is tyrosine phosphorylated after CD38 cross-linking. Moreover, CD38 ligation with IL-5 costimulation enhances B-cell proliferation, *Blimp1* (B-lymphocyte-induced maturation protein) gene expression, and IgM production.

MATERIALS AND METHODS

Reagents. Mouse recombinant IL-5 was prepared and purified using anti-mIL-5 mAb coupled beads as described (11). Rat anti-mouse CD38 mAb, CS/2, and anti-mouse IL-5Rα mAb, H7, were prepared as described (8, 32). Rat anti-mouse Btk polyclonal antibodies was prepared by immunizing rats with glutathione *S*-transferase–Btk fusion protein (33). Anti-phosphotyrosine mAb, 4G10, was obtained from Upstate Biotechnology (Lake Placid, NY).

Flow Cytometry. One million cells were stained with 50 μl of biotinylated H7 or CS/2 mAb (25 μg/ml) and 50 μl of fluorescein isothiocyanate-labeled RA3-6B2 mAb (10 μg/ml) for 30 min at 4°C, with the addition of 2.4G2 mAb (anti-mouse Fcγ fragment receptor, 10 μg/ml) to avoid nonspecific binding of the labeled mAb. After being washed, cells were incubated with 50 μl of phycoerythrin-conjugated streptavidin at 4°C for 20 min. After being stained, cells were suspended in phosphate-buffered saline buffer/0.1% 7-amino-actinomycin D (Sigma) to exclude dead cells from the analysis. Fluorescence intensity was measured with a FACScan instrument (Becton Dickinson) equipped with an argon laser (480 nm), operating

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Btk, Bruton tyrosine kinase; Xid, X chromosome-linked immunodeficient; IL-5, interleukin 5; IL-5R, IL-5 receptor; IL-5Rα, IL-5R α chain; GM-CSF, granulocyte/macrophage colony-stimulating factor; LPS, lipopolysaccharide.

‡To whom reprint requests should be addressed.

at 200 mW. Only cells within the lymphocyte gate were counted.

Assays for B-Cell Proliferation and IgM Secretion. C57BL/6 (B6) and C57BL/6.xid (Xid) mice were anesthetized with ether and then sacrificed. An enriched population of B cells was obtained from splenocytes by eliminating T cells, using anti-Thy-1.2 mAb (Serotec) and guinea pig complement as described (26). These T-cell-depleted cells were cultured at a concentration of 1×10^5 cells per 200 μl per well with or without stimulants in RPMI 1640 medium/8% fetal calf serum/2 mM L-glutamine/50 μM 2-mercaptoethanol/penicillin at 50 μg/ml/streptomycin at 50 μg/ml in 96-well flat-bottom microtiter plates. Cultures were performed in triplicate. The cells were pulse-labeled with [^{3}H]thymidine (0.2 μCi per well; 1 Ci = 37 GBq) during the last 6 hr of a 72-hr culture period, and incorporation of radioactivity was measured by tritium-sensitive avalanche gas-ionization detection on a Matrix 96 direct β counter (Packard) (34). Results were expressed as the mean cpm ± SEMs of triplicate cultures.

For determining IgM secretion, cells were cultured for 7 days, and IgM levels in cultured supernatants were determined by ELISA (34). With myeloma proteins (Miles Scientific), a standard curve was generated, and the IgM concentration was determined with the DELTA-SOFT computer program (Biometallics, Princeton).

Immunoprecipitation and Immunoblot Analysis. Cells (6×10^7 cells per 300 μl) were stimulated with CS/2 (50 μg/ml) or anti-IgM mAb (50 μg/ml) for 10 min at 37°C. Cell lysates were prepared and subjected to immunoprecipitation as described (21). Briefly, cell lysates were precleared with protein G-Sepharose 4B and incubated at 4°C for 60 min with 2–10 μg of anti-Btk antibody. Immune complexes were collected on protein G–Sepharose during a 60-min incubation at 4°C, washed five times with lysis buffer, and boiled for 5 min with 2× Laemmli's sample buffer. Samples were electrophoresed on SDS/8% polyacrylamide gels and transferred to an Immobilon-P membrane (Nihon Millipore, Tokyo). After blocking with Tris/borate/saline (TBS) containing 5% bovine serum albumin, membranes were incubated with the appropriate primary antibody and washed in TBS/0.1% Tween 20 (TBS-T). After incubation with goat anti-rat or anti-rabbit secondary antibodies coupled to horseradish peroxidase, membranes were washed four times with TBS-T and subjected to an ECL detection system (Amersham).

Preparation of Poly(A)$^+$ RNA and Northern Blot Analysis. Splenic B cells were cultured for 3 days with mAb CS/2 or mAb CS/2 plus IL-5. Total RNA was prepared from cultured cells by the acid-guanidinium-phenol-chloroform (AGPC) methods (35). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose column chromatography. Poly(A)$^+$ RNA (5 μg) was subjected to electrophoresis through 1% agarose gel containing 2.2 M formaldehyde as described (12) and transferred to GeneScreen (DuPont). Hybridization was done according to vendor specifications. A 535-bp fragment (nt 562–1095) of Blimp1 cDNA (36) was prepared by PCR, labeled with ^{32}P by the random primer-labeling method, and used as a probe.

RESULTS

Impaired Proliferative Response of B Cells from Xid Mice to IL-5 and CD38 Ligation. We have described (9, 10) that stimulation of activated B cells with IL-5 and CS/2, an anti-CD38 mAb, can induce a proliferative response. We then asked whether CD38 ligation by mAb CS/2 and IL-5 stimulation act differently on B cells. To address this question, T-cell-depleted splenic B cells prepared from B6 or Xid mice were stimulated with mAb CS/2, IL-5, or mAb CS/2 plus IL-5 for 3 days, and uptake of [^{3}H]thymidine was determined. As a control, B cells were also stimulated with lipopolysaccharide (LPS). mAb CS/2 alone (>40 ng/ml) could induce proliferation of B cells from B6 mice. This response reached a plateau at a mAb CS/2 concentration of 0.2 μg/ml (data not shown). Stimulation of splenic B cells with mAb CS/2 (0.5 μg/ml) induced a more profound proliferative response than that with IL-5 alone (Table 1). These results agree with our previous results (8) and those of others (6) and indicate that mAb CS/2 induces B-cell proliferation. A remarkable proliferative response was observed when B cells from B6 mice were stimulated with IL-5 plus mAb CS/2. When we cultured B cells with a fixed IL-5 concentration (5 units/ml) plus dilutions of mAb CS/2, a significant proliferative response was demonstrated down to a mAb CS/2 concentration of 8 ng/ml (data not shown). Intriguingly, splenic B cells from Xid mice did not show a significant proliferative response to mAb CS/2 plus IL-5 (Table 1).

To compare the synergistic effect of IL-5 on mAb CS/2-induced proliferation with that of IL-2, IL-3, IL-4, or GM-CSF, we cultured splenic B cells with mAb CS/2 and each cytokine. Except for IL-2, each cytokine did not induce a significant proliferative response alone. Each cytokine marginally enhanced the mAb CS/2-induced proliferative response (Table 1). These responses were weaker than that induced by IL-5 plus mAb CS/2. Stimulation of B cells from Xid mice with IL-2, IL-4, or GM-CSF slightly induced proliferation, but these cytokines did not cooperate with mAb CS/2 to enhance proliferation. These results indicate that mAb CS/2 can act synergistically with IL-5 to enhance proliferation of B cells from B6 mice but not proliferation of B cells from Xid mice. We compared CD38 expression on splenic B cells and bone marrow cells from Xid mice with that from B6 mice by flow cytometry. The results revealed no significant difference of CD38 expression on B220$^+$ B cells between B6 and Xid mice (data not shown) (8, 37). We infer from these results that the impaired responsiveness of B cells from Xid mice is not due to a reduced level of CD38 expression but rather to an impaired signaling pathway through CD38.

Induction of Blimp1 mRNA Expression in mAb CS/2- and IL-5-Stimulated B Cells. To evaluate the role of CD38 ligation in B-cell differentiation, we examined Blimp1 mRNA expression in B cells because the *Blimp1* gene has been identified as one of the early genes induced during B-cell differentiation (36). We cultured splenic B cells with mAb CS/2 or mAb CS/2 plus IL-5 and monitored Blimp1 expression by Northern blot

Table 1. Effect of cytokine and CD38 ligation on B-cell proliferation

Stimulation†	mAb CS/2, 0.5 μg/ml	[^{3}H]Thymidine incorporation* C57BL/6	Xid
None	−	253 ± 21	220 ± 7
	+	16,210 ± 1,649	252 ± 125
IL-2	−	10,737 ± 898	7,309 ± 1,239
	+	22,716 ± 1,162	8,802 ± 1,135
IL-3	−	601 ± 116	1,145 ± 251
	+	18,686 ± 740	851 ± 211
IL-4	−	2,030 ± 125	3,210 ± 732
	+	8,002 ± 718	3,976 ± 654
IL-5	−	519 ± 47	107 ± 10
	+	52,756 ± 2,298	132 ± 5
GM-CSF	−	1,501 ± 175	1,420 ± 399
	+	19,301 ± 2,046	1,612 ± 109
LPS	−	27,965 ± 4,146	13,484 ± 1,113

*T-cell-depleted splenic B cells (1×10^5 per well) were cultured for 3 days with various stimuli. Cells were pulse-labeled with [^{3}H]thymidine (0.2 μCi per well) during the last 6 hr of a 72-hr culture period, and incorporation of radioactivity was measured. Results are expressed as mean cpm ± SEM of triplicate cultures.

†Concentrations of cytokines and LPS were 50 units/ml and 1 μg/ml, respectively.

analysis. BCL_1 cells were also stimulated with IL-5, and their Blimp1 mRNA expression was examined. Stimulation of BCL_1 cells served as a positive control because in response to IL-5 these cells differentiated into IgM-secreting cells with an accompanying increase in Blimp1 expression (36). As expected, stimulation of BCL_1 with IL-5 enhanced expression of the ≈6-kb Blimp1 mRNA (Fig. 1 lane 4 vs. lane 5). Two additional bands that migrated faster than 6 kb might be alternatively spliced mRNA for Blimp1 or a degradation product of Blimp1 mRNA. Significant enhancement of Blimp1 mRNA expression was observed in splenic B cells stimulated with mAb CS/2 plus IL-5 (lane 1 vs. lane 3), whereas expression was only marginally increased upon stimulation with mAb CS/2 alone (lane 2). Stimulation of B cells from Xid mice with mAb CS/2 plus IL-5 did not induce any significant Blimp1 expression (data not shown).

Synergistic Effect of mAb CS/2 with IL-5 on B-Cell Differentiation. To examine the effect of mAb CS/2 on B-cell differentiation, splenic B cells from B6 mice were cultured for 7 days with either mAb CS/2 or mAb CS/2 plus various cytokines, and the amounts of secreted IgM were measured by ELISA. Fig. 2 shows that stimulation of B cells with mAb CS/2 alone induced a basal level of IgM production. Stimulation of B cells with mAb CS/2 plus IL-5 dramatically enhanced IgM production, whereas stimulation with mAb CS/2 plus IL-2, IL-4, or GM-CSF showed only marginal enhancement of IgM production. Conversely, stimulation of splenic B cells from Xid mice again did not induce detectable IgM production even when stimulated with IL-5 plus mAb CS/2 (data not shown). We carried out frequency analysis of IgM-producing cells using the ELISPOT assay. The frequency of IgM-producing cells in unstimulated, IL-5 stimulated, and mAb CS/2-stimulated B cells was 1/10,500, 1/350, and 1/2,200, respectively. The frequency of IgM-producing B cells stimulated with IL-5 plus mAb CS/2 was 1/5.4, which was nearly equivalent to cells stimulated with LPS (1/4.2).

Tyrosine Phosphorylation of Btk by CD38 Ligation. It has been shown that the activation of B cells through antigen receptor or growth factor receptor is accompanied by tyrosine phosphorylation of cellular proteins (38, 39). To examine tyrosine phosphorylation of cellular proteins by mAb CS/2 stimulation, splenic B cells were cultured for 10 min with mAb CS/2 or IL-5. Cell lysates were prepared and analyzed by SDS/PAGE followed by immunoblot analysis using anti-phosphotyrosine mAb. Results revealed that tyrosine phosphorylation of cellular proteins was significantly enhanced in response to mAb CS/2 but not to IL-5 (data not shown).

To gain further insight into the impaired responsiveness of Xid B cells to CD38 ligation, we focused on tyrosine phosphorylation of Btk because Btk has been shown to be mutated in Xid mice (30, 31), whose B cells show impaired responsiveness to CD38 ligation. Splenic B cells from either B6 or Xid mice were unstimulated or stimulated with CS/2 or anti-IgM mAb for 5 min. Cell lysates from each group were prepared and immunoprecipitated with rat anti-Btk antibody followed by immunoblotting with anti-phosphotyrosine mAb or rabbit anti-Btk antibody. Results revealed that significant tyrosine phosphorylation of Btk was induced in splenic B cells of B6 mice stimulated with mAb CS/2 or anti-IgM mAb (Fig. 3*A*) but was not induced in unstimulated cells. We also carried out a similar set of experiments using splenic B cells from Xid mice and found that splenic B cells from Xid mice did not show any significant tyrosine phosphorylation of Btk with either mAb CS/2 or anti-IgM stimulation (Fig. 3*B*), even though immunoblot analysis revealed equal amounts of Btk were immunoprecipitated with anti-Btk antibody. These results clearly indicate that CD38 ligation or cross-linking of antigen receptor

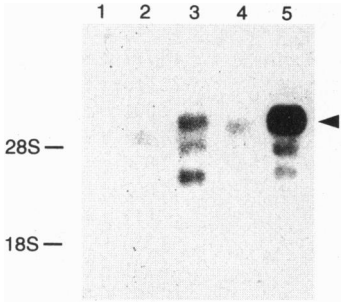

FIG. 1. Induction of Blimp1 mRNA expression. T-cell-depleted splenic B cells (lanes 1–3) and BCL_1 cells (lanes 4 and 5) were cultured for 3 days and 1 day, respectively, with or without stimuli. After culturing, cells were harvested and poly(A)$^+$ RNA was prepared. A Northern blot analysis containing 5 μg per lane of poly(A)$^+$ RNA from splenic B cells or BCL_1 cells treated with mAb CS/2 (lane 2), mAb CS/2 plus IL-5 (lane 3), or IL-5 (lane 5) was carried out and hybridized to a Blimp1 probe. Arrowhead, Blimp1 RNA.

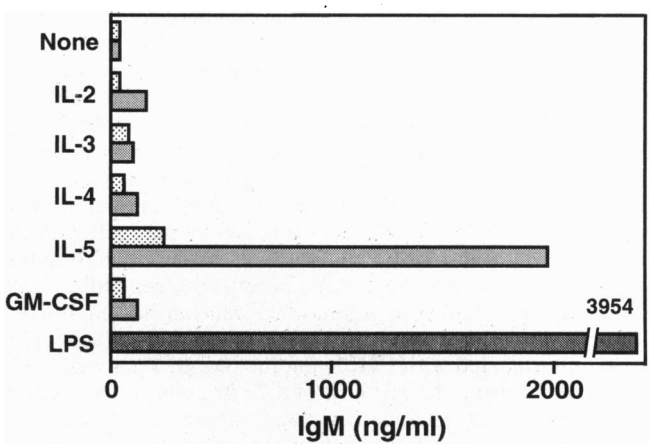

FIG. 2. Synergistic effect of mAb CS/2 and various cytokines on IgM secretion by splenic B cells. T-cell-depleted splenic B cells (1×10^5 per well) were cultured for 7 days. Cells were unstimulated or stimulated with mAb CS/2 (0.5 μg/ml) or cytokines (50 units/ml) (▨), or mAb CS/2 plus cytokines (■). After culturing, the amount of IgM in the culture supernatant was determined by ELISA.

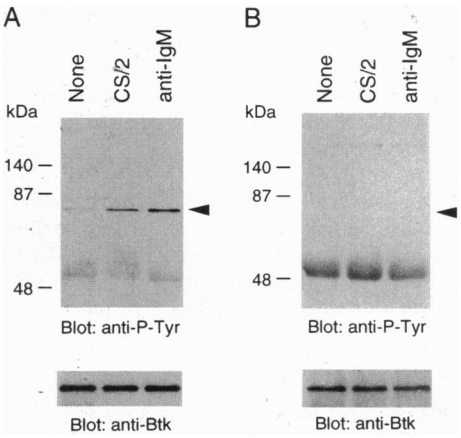

FIG. 3. Induction of tyrosine phosphorylation of Btk by cross-linking CD38 or surface IgM. Splenic B cells (6×10^7/300 μl) from wild-type (*A*) or Xid (*B*) mice were treated with mAb CS/2 at 50 μg/ml or anti-IgM mAb at 50 μg/ml for 10 min at 37°C. Cells were lysed, and lysates were immunoprecipitated with anti-Btk antibodies. Immunoprecipitates were subjected to SDS/8% PAGE analysis and immunoblotted with anti-phosphotyrosine mAb. The membrane was reprobed with anti-Btk antibody (*Lower*). Positions to which Btk kinase migrated are indicated by arrowheads, and marker proteins (in kDa) are shown at left.

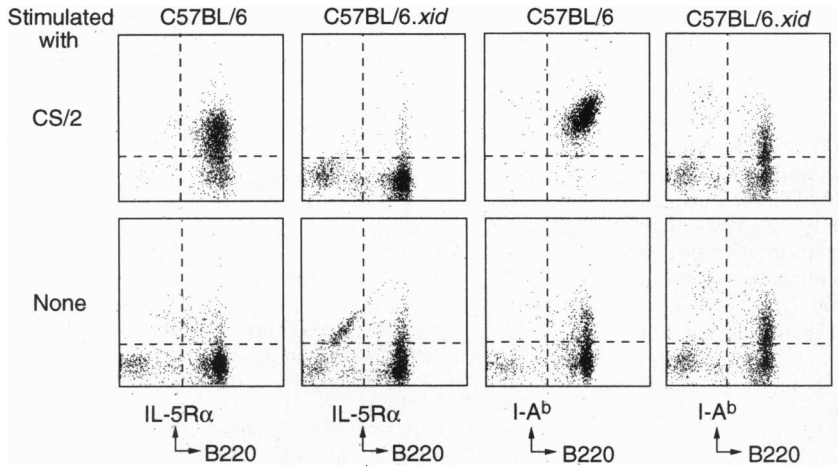

FIG. 4. Flow cytometric analysis. Splenic B cells were cultured with mAb CS/2 (1 μg/ml) for 48 hr and stained with biotinylated mAb H7 (anti-IL-5Rα mAb), or anti-major histocompatibility complex class II mAb followed by avidin–phycoerythrin and fluorescein isothiocyanate-labeled RA3-6B2 (anti-B220 mAb). Cells in the lymphoid gate were analyzed by flow cytometry.

(IgM) can induce the tyrosine phosphorylation of Btk in B cells from wild-type mice but not from Xid mice.

Enhancement of IL-5Rα Expression by CD38 Ligation. As mentioned above, CD38 ligation of B cells from B6 mice together with IL-5 stimulation synergistically enhances cell proliferation and IgM secretion. One interpretation of this synergistic effect of mAb CS/2 and IL-5 on B-cell growth and differentiation is that CD38 ligation may enhance IL-5R expression. To test this hypothesis, we stimulated splenic B cells from B6 or Xid mice with mAb CS/2 for 48 hr. IL-5Rα expression was then monitored by flow cytometry using an anti-IL-5Rα mAb. As a control, expressions of major histocompatibility complex class II antigen was also examined. An increased expression of IL-5Rα was seen in B cells from B6 mice stimulated with mAb CS/2 but not in B cells from Xid mice. More than 60% of B cells expressed IL-5Rα after 48-hr culture (Fig. 4). Expression of major histocompatibility complex class II was also enhanced by mAb CS/2 (Fig. 4) as described (6). mAb CS/2 stimulation did not affect the expression of major histocompatibility complex class II antigens on Xid B cells.

DISCUSSION

We demonstrated four major observations in this study. (*i*) We showed an agonistic effect of mAb CS/2 on B-cell proliferation and, to a lesser extent, on IgM secretion. (*ii*) IL-5 and mAb CS/2 synergistically act on B cells to induce not only proliferation but also expression of Blimp1 mRNA and IgM secretion. These synergistic effects were seen in B cells from B6 mice but not from Xid mice. Among the cytokines tested, IL-5 was the most potent cytokine to enhance B-cell proliferation and differentiation together with mAb CS/2. (*iii*) Stimulation of B cells with CS/2 or anti-IgM mAb induced tyrosine phosphorylation of Btk. (*iv*) Stimulation of B cells with mAb CS/2 enhanced IL-5Rα expression.

Blimp1 is a gene whose transcripts are rapidly induced during the differentiation of B cells into immunoglobulin-secreting cells and whose expression is characteristic of late B cells and plasma cell lines (36). Turner *et al.* (36) reported that Blimp1 is induced by IL-5 and IL-2 treatment of BCL_1 cells. We confirmed their original observation using BCL_1 cells and additionally found that stimulation of resting B cells with mAb CS/2 modestly induced Blimp1 mRNA expression (Fig. 1). Intriguingly, mAb CS/2 plus IL-5 further enhanced expression of Blimp1 mRNA. Blimp1 mRNA expression was detected on day 3 when IgM-secreting cells were hardly detectable. Thus, an increase in the level of Blimp1 mRNA expression appears to precede terminal differentiation of B cells.

CD38 ligation by mAb CS/2 induced cell proliferation, IgM production, and tyrosine phosphorylation of Btk in B cells from B6 mice. Stimulation of B cells with mAb CS/2 plus IL-5 further enhanced cell proliferation, Blimp1 expression, and IgM secretion. By contrast, neither CD38 ligation nor CD38 plus IL-5 stimulation showed any of these effects on B cells from Xid mice (Fig. 3). When we consider that Xid mice carry a mutation that alters the primary sequence of Btk (30, 31), we can infer from these results that Btk must be required, directly or indirectly, for CD38-mediated signal transduction. Because IL-5 stimulation of an IL-5-dependent early B-cell line, Y16, enhances Btk activity (21), Btk activation appears involved in IL-5 signal transduction. There may be a functional relationship between the signals through IL-5R and CD38 on B cells. The synergistic effect of mAb CS/2 with IL-5 on B-cell activation would partly be accounted for by enhanced expression of IL-5Rα chain by mAb CS/2, as will be discussed.

Unresponsiveness of Xid B cells to anti-CD38 and IL-5 for proliferation and differentiation suggests that subpopulations of B cells respond to anti-CD38 because Xid mice lack $CD5^+$ B (B-1) cells. Although we do not show data, peritoneal B-1 cells from normal mice, which express both CD38 molecules and IL-5R, did not respond to anti-CD38 for proliferation but did respond to IL-5. Our observations agree with those reported by Lund *et al.* (40), who found that peritoneal $CD5^+$ B cells and neonatal "immature" B cells do not respond to anti-CD38 stimulation by proliferation. Furthermore, stimulation of normal splenic B cells with mAb CS/2 did not induce CD5 expression (data not shown). Taking these results together, we propose that conventional ($CD5^-$) B cells with mature phenotype rather than B-1 cells respond to anti-CD38 stimulation. We also point out that the signaling pathway used by IL-5R may differ in part from that used by CD38.

One specific defect of Xid mice is an impaired responsiveness to IL-5; this has been interpreted as reflecting a B-cell-specific defect of IL-5 signaling (26, 34). It has been reported that there is no significant difference in Btk expression between Xid B cells and non-Xid B cells and that the *xid* mutation does not affect the kinase activity of Btk (29–31). Therefore, the *xid* mutation may interfere with the ability of Btk to interact with regulatory or substrate molecules important to the Btk signaling cascade. To date, physical association between the PH domain of Btk and protein kinase C (41) or G protein βγ subunits (42, 43) have been reported. Binding of the proline-rich sequence of Btk to Fyn, Lyn, or Hck through a Src homology 3 domain also has been reported (44, 45). Analysis of the physical association between CD38, IL-5Rα, Btk, and adaptor proteins may allow us to understand the mechanisms of the functional relationship between signals through IL-5R and CD38.

The molecular basis of how CD38 ligation and IL-5 synergistically trigger B-cell differentiation into immunoglobulin-secreting cells is yet to be elucidated. We may speculate that

mAb CS/2 first enhances IL-5Rα expression followed by binding of IL-5 to induce progression to proliferation and antibody synthesis. Activation of Btk in response to mAb CS/2 may be involved in IL-5Rα expression. Induction of IL-5Rα on B cells by mAb CS/2 stimulation is one of the unique phenomena of CD38-mediated B-cell activation because neither LPS nor anti-IgM stimulation alone induces significant IL-5Rα expression. At present, the physiological role of CD38 in B-cell activation is unclear. CD38, like CD40, may be involved in T-dependent B-cell activation and controlling apoptosis through its interaction with its respective ligand, which might be expressed on helper T cells. Identification of the natural ligand for CD38 will be important to elucidate the role of CD38 in T-dependent or T-independent B-cell activation.

In conclusion, B-cell triggering by CD38 ligation that is synergistically enhanced by IL-5 requires phosphorylation and/or activation of Btk. Further study of Btk-associated molecules, the natural ligand for CD38, and molecular mechanisms of enhanced IL-5Rα expression by CD38 ligation should provide an important opportunity for understanding the role of CD38 and IL-5 in B-cell differentiation and prevention of B cells from undergoing apoptosis.

We are grateful to Richard Dickason for critical review of the manuscript. This study was supported in part by a special grant for Advanced Research on Cancer and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan; and by a Research Grant from the Uehara Memorial Foundation.

1. Malavasi, F., Funaro, A., Roggero, S., Hornstein, A., Calosso, L. & Mehta, K. (1994) *Immunol. Today* **15,** 95–97.
2. Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Argumedo, L., Parkhouse, R. M. E., Walseth, T. F. & Lee, H. C. (1993) *Science* **262,** 1056–1059.
3. Zocchi, E., Franco, L., Guida, L., Benatti, U., Bargellesi, A., Malavasi, F., Lee, H. C. & Flora, A. D. (1993) *Biochem. Biophys. Res. Commun.* **196,** 1459–1465.
4. Gelman, L., Deterre, P., Gouy, H., Boumsell, L., Debre, P. & Bismuth, G. (1993) *Eur. J. Immunol.* **23,** 3361–3364.
5. Harada, N., Santos-Argumedo, L., Chang, R., Grimaldi, J. C., Lund, F. E., Brannan, C. I., Copeland, N. G., Jenkins, N. A., Heath, A. W., Parkhouse, R. M. E. & Howard, M. (1993) *J. Immunol.* **151,** 3111–3118.
6. Santos-Argumedo, L., Teixeira, C., Preece, G., Kirkham, P. A. & Parkhouse, R. M. E. (1993) *J. Immunol.* **151,** 3119–3130.
7. Kirkham, P. A., Santos-Argumedo, L., Harnett, M. M. & Parkhouse, R. E. M. (1994) *Immunology* **83,** 513–516.
8. Yamashita, Y., Miyake, K., Kikuchi, Y., Takatsu, K., Noda, T., Kosugi, A. & Kimoto, M. (1995) *Immunology* **85,** 248–255.
9. Takatsu, K. (1992) *Curr. Opin. Immunol.* **4,** 299–306.
10. Takatsu, K., Takaki, S. & Hitoshi, Y. (1994) *Adv. Immunol.* **57,** 145–190.
11. Mita, S., Tominaga, A., Hitoshi, Y., Sakamoto, K., Honjo, T., Akagi, M., Kikuchi, Y., Yamaguchi, N. & Takatsu, K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2311–2315.
12. Takaki, S., Tominaga, A., Hitoshi, Y., Mita, S., Sonoda, E., Yamaguchi, N. & Takatsu, K. (1990) *EMBO J.* **9,** 4367–4374.
13. Murata, Y., Takaki, S., Migita, M., Kikuchi, Y., Tominaga, A. & Takatsu, K. (1992) *J. Exp. Med.* **175,** 341–351.
14. Takaki, S., Mita, S., Kitamura, T., Yonehara, S., Yamaguchi, N., Tominaga, A., Miyajima, A. & Takatsu, K. (1991) *EMBO J.* **10,** 2833–2838.
15. Devos, R., Plaetinck, G., Heyden, J. V. D., Cornelis, S., Vandekerckhove, J., Fiers, W. & Tavernier, J. (1991) *EMBO J.* **10,** 2133–2137.
16. Takaki, S., Murata, Y., Kitamura, T., Miyajima, A., Tominaga, A. & Takatsu, K. (1993) *J. Exp. Med.* **177,** 1523–1529.
17. Tavernier, J., Devos, R., Cornelis, S., Tuypens, T., Heyden, J. V. D., Fiers, W. & Plaetinck, G. (1991) *Cell* **66,** 1175–1184.
18. Hayashida, K., Kitamura, T., Gorman, D. M., Arai, K., Yokota, T. & Miyajima, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9655–9659.
19. Kitamura, T., Sato, N., Arai, K. & Miyajima, A. (1991) *Cell* **66,** 1165–1174.
20. Takaki, S., Kanazawa, H., Shiiba, M. & Takatsu, K. (1994) *Mol. Cell. Biol.* **14,** 7404–7413.
21. Satoh, S., Katagiri, T., Takaki, S., Kikuchi, Y., Hitoshi, Y., Yonehara, S., Tsukada, S., Kitamura, D., Watanabe, T., Witte, O. N. & Takatsu, K. (1994) *J. Exp. Med.* **180,** 2101–2111.
22. Scher, I., Ahmed, A., Strong, D. M., Steinberg, A. D. & Paul, W. E. (1975) *J. Exp. Med.* **141,** 788–803.
23. Scher, I. (1982) *Immunol. Rev.* **64,** 117–136.
24. Hasbold, J. & Klaus, G. G. B. (1994) *Eur. J. Immunol.* **24,** 152–157.
25. Miyake, K., Yamashita, Y., Hitoshi, Y., Takatsu, K. & Kimoto, M. (1994) *J. Exp. Med.* **180,** 1217–1224.
26. Hitoshi, Y., Sonoda, E., Kikuchi, Y., Yonehara, S., Nakauchi, H. & Takatsu, K. (1993) *Int. Immunol.* **9,** 1183–1190.
27. Go, N. F., Castle, B. E., Barret, R., Kastelein, R., Dang, W., Mosmann, T. R., Moore, K. W. & Howard, M. (1990) *J. Exp. Med.* **172,** 1625–1631.
28. Howard, M., Pesavento, P. & Stein, P. (1986) *J. Immunol.* **136,** 4531–4537.
29. Tsukada, S., Rawlings, D. J. & Witte, O. N. (1994) *Curr. Opin. Immunol.* **6,** 623–630.
30. Rawlings, D. J., Saffran, D. C., Tsukada, S., Largaespada, D. A., Grimaldi, J. C., Cohen, L., Mohr, R. N., Bazan, J. F., Howard, M., Copeland, N. G., Jenkins, N. A. & Witte, O. N. (1993) *Science* **261,** 358–361.
31. Thomas, J. D., Sideras, P., Smith, C. I. E., Vorechovsky, I., Chapman, V. & Paul, W. E. (1993) *Science* **261,** 355–361.
32. Yamaguchi, N., Hitoshi, Y., Mita, S., Hosoya, Y., Murata, Y., Kikuchi, Y., Tominaga, A. & Takatsu, K. (1990) *Int. Immunol.* **2,** 181–187.
33. Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., Sparkes, R. S., Kubagawa, H., Mohandas, T., Quan, S., Belmont, J. W., Cooper, M. D., Conley, M. E. & Witte, O. N. (1993) *Cell* **72,** 279–287.
34. Koike, M., Kikuchi, Y., Tominaga, A., Takaki, S., Akagi, K., Miyazaki, J.-I., Yamamura, K.-I. & Takatsu, K. (1995) *Int. Immunol.* **7,** 21–30.
35. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
36. Turner, C. A., Mack, D. H. & Davis, M. M. (1994) *Cell* **77,** 297–306.
37. Santos-Argumedo, L., Lund, F. E., Heath, A. W., Solvason, N., Wu, W. W., Grimaldi, J. C., Parkhouse, R. M. E. & Howard, M. (1995) *Int. Immunol.* **7,** 163–170.
38. Kock, C. A., Anderson, D., Moran, F. M., Ellis, C. & Pawson, T. (1991) *Science* **252,** 668–674.
39. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B. & Silvennoinen, O. (1994) *Trends Biochem. Sci.* **19,** 222–227.
40. Lund, F. E., Solvason, N. W., Cooke, M. P., Heath, A. W., Grimaldi, J. C., Parkhouse, R. M. E., Goodnow, C. C. & Howard, M. C. (1995) *Eur. J. Immunol.* **25,** 1338–1345.
41. Yao, L., Kawakami, Y. & Kawakami, T. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9175–9179.
42. Tsukada, S., Simon, S. I., Witte, O. N. & Katz, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11256–11260.
43. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G. & Lefkowitz, R. J. (1994) *J. Biol. Chem.* **269,** 10217–10220.
44. Alexandropoulos, K., Cheng, G. & Baltimore, D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3110–3114.
45. Cheng, G., Ye, Z. S. & Baltimore, D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8152–8155.