Interaction of B cells with activated T cells reduces the threshold for CD40-mediated B cell activation

Gerry G. B. Klaus, Mary Holman, Caroline Johnson-Léger, Jillian R. Christenson and Marilyn R. Kehry

Division of Cellular Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK
Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT 06877, USA

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Abstract

CD154–CD40 interactions are of central importance for the induction of antibody responses to T-dependent antigens. Since most anti-CD40 mAb are only weak B cell mitogens, it is believed that under physiological conditions, signals through CD40 synergize with those from other receptors on B cells to induce B cell activation. We show here that the interaction of either normal B cells, or those from CBA/N (xid) mice, with CD3-activated primary T cells in whole spleen cell cultures markedly reduces the threshold for B cell activation via CD40. Hence, these pre-activated cells undergo vigorous proliferation when stimulated with either optimal or suboptimal concentrations of weakly mitogenic anti-CD40 mAb, or with soluble CD40 ligand. Blocking experiments indicate that the establishment of this priming effect requires stimulation via CD40 itself, plus T cell-derived IL-2. In support of this concept, only CD3/CD28-pre-activated, but not CD3-pre-activated T cells induce this effect, unless the co-cultures of B cells with the latter T cells are supplemented with IL-2. Although B cells activated in this fashion do express higher levels of CD40 than naive cells, we believe that this is insufficient to explain the observed dramatic effects on their proliferative capacity. Rather we propose that T cell-dependent B cell activation induces fundamental changes in the signalling machinery invoked by ligation of CD40. It is likely that this amplification loop could play an important role during the initiation of antibody responses to T-dependent antigens, when activated CD4 T cells only express low levels of CD154.

Introduction

The interaction of CD40 on B cells with the CD40 ligand (CD40L or CD154) on activated CD4 T cells represents the key event in the initiation of antibody responses to T-dependent antigens (reviewed in 1). Signals via CD40, in conjunction with appropriate cytokines, such as IL-4 and IL-5 in the mouse, then initiate the program of B cell activation, Ig secretion, isotype switching and B cell memory formation. Many of the pioneering studies on the role of CD40 in B cell activation have entailed the use of anti-CD40 mAb: most of these are only weak B cell mitogens, in the absence of co-stimuli, such as anti-Ig, or cytokines such as IL-4 (2, 3). These findings have led to the generally accepted view that, under physiological conditions, CD40-derived signals act to induce cell cycle progression of B cells that had been pre-activated, say by contact with antigen (reviewed in 2). This concept was supported by findings with CD3-activated primary T cells, which only express low levels of CD154 (unlike pre-activated T cell clones) and are ineffective in inducing the activation of resting B cells (4,5). We have recently shown, however, that the major reason that naive T cells activated via CD3 alone are ineffective helper effector cells, is because they secrete insufficient IL-2 (6). In contrast, T cells stimulated via CD3 and CD28 [as would occur on CD80/CD86+ antigen-presenting cells, such as dendritic cells (7)] induce B cells to become IL-2 responsive, via a combination of CD40-mediated signals and IL-2 itself. In this study we also showed that B cells cultured with CD3/CD28-primed T cells need continuous stimulation via CD40 for some 36 h before becoming committed to DNA synthesis. The capacity of such T cells to induce B cell activation was absolutely dependent on their re-stimulation via CD3 during the co-culture period. Since CD154 is rapidly down-regulated following contact with CD40...
(8,9), these findings therefore suggested that T cells must re-express CD154 in a cyclical fashion during the period of T cell–B cell interaction. We therefore concluded that a critical level of IL-2 is required to enable primary T cells, expressing low levels of CD154, to induce B cell activation.

During the course of these experiments we also noted that B cells which had been exposed to activated T cells became markedly responsive to stimulation with normally poorly mitogenic anti-CD40 mAb. This suggested that T cell–B cell interactions set in train an amplification loop which reduces the threshold of signalling via the CD40 receptor. Here we present the results of experiments which analyze the mechanisms involved in this phenomenon.

Methods

**Experimental animals**

In most experiments unfractionated whole spleen cell (WSC) suspensions or B cells were prepared from spleens of male (CBA/Ca×C57BL10)F1, mice aged 3–4 months. Some experiments used WSC or B cells from male (xid) or female (normal) (CBA/N×C57BL10)F1, mice. All mice were bred under specific pathogen-free conditions.

**Reagents**

The following mAb were used: hamster anti-CD3 (145-2C11), hamster anti-CD28 (37.5.1.1, from J. Allison), hamster anti-CD40L (MR1, a gift from R. Noelle), rat anti-Thy-1 (NIMR-1), rat anti-CD8 (YTS 169.4.2.1) and anti-CD4 (YTS 191.1.1.2) (the latter two both obtained from H. Waldmann), rat anti-μ (b.7.6), rat anti-IgD (1.19), rat anti IL-2 (1A12 or S4B6) and rat anti-ovine placental lactogen (Mac-193, from G. Butcher). The following rat anti-CD40 mAb were used: 3/23 (10), DOM185 (from D. Gray), FGK45.5 (from A. Rolink) and 1C10 (from M. Howard) (11). These mAb were purified on Protein G-Sepharose (Pharmacia, Uppsala, Sweden) and coupled with biotin or FITC by standard methods. Biotinylated or FITC-coupled rat anti-B220 were from PharMingen (San Diego, CA). Mouse IL-2 was from supernatants of a stably transfected cell line kindly provided by F. Melchers (12). IL-2 bioactivity was titrated using the CTLL indicator cell line, by standard procedures. The production of the soluble CD40L–CD8 fusion protein in Sf9 insect cells has been described elsewhere (13). The protein was affinity purified from an anti-CD154 column and eluted with Gentle Elution Buffer (Pierce & Warriner, Chester, UK). Lipopolysaccharide (LPS) and normal hamster IgG were from Sigma (Poole, UK).

**Preparation of T cells and B cells**

Small, dense B cells were prepared from mouse spleen as described previously (6). In brief, this involved killing T cells by a cocktail of mAb against Thy-1, CD4 and CD8, plus guinea pig complement, followed by Percoll fractionation. These preparations were typically >90% B220⁺, with <1% T cell contamination. Splenic CD4⁺ T cells were also prepared as described previously. These were typically >90% CD4⁺, with <5% B cell contamination.

**WSC**

WSC suspensions were freed of erythrocytes by Percoll fractionation and were cultured at 10⁶/ml in flasks coated with 10 µg/ml of anti-CD3 as above (unless otherwise indicated), generally for 24–48 h. The cultures were harvested, depleted of T cells and B cells were recovered by centrifugation through a 50/85% Percoll gradient. The resulting preparations (92–95% B220⁺) were re-cultured (10⁵/200 µl) with the indicated mitogens and cultures were labelled with [³H]thymidine as above at the times indicated under individual experiments.

**Flow cytometric analyses**

Freshly prepared B cells or those recovered from WSC cultures were stained with the indicated mAb and analysed on either a FACStar Plus or FACS Vantage flow cytometer (Becton Dickinson, Mountain View, CA).

**Results**

**B cells exposed to CD3-activated T cells become hyper-responsive to CD40 stimulation**

In our earlier study we showed that B cells harvested from WSC cultures stimulated with anti-CD3 for 24 h, or following contact with CD3/CD28-co-stimulated CD4 T cells, became responsive to IL-2 (6). We subsequently investigated the effects of these priming cultures on the responses of B cells to the anti-CD40 mAb 3/23, which by itself is only a weak mitogen for resting B cells. Figure 1 illustrates the effects of exposing B cells to CD3-stimulated T cells in WSC cultures (priming cultures) for 24 or 48 h on their subsequent proliferative responses to anti-CD40 or anti-μ. Fresh B cells gave only a very weak response to anti-CD40 and a good response to anti-μ, which peaked on day 3 of culture, as expected. Some B cells harvested from WSC cultures after 24 or 48 h were already committed to DNA synthesis, as manifested by the elevated levels of [³H]thymidine uptake at the time they were re-cultured (designated time 0 in Fig. 1). These could be naturally pre-activated cells. However, after both 24 and 48 h priming periods, clearly the majority of B cells still required re-stimulation for commitment to DNA synthesis. Re-stimulation with (both optimal and suboptimal concentrations of) anti-CD40 induced a striking proliferative response (some 15- to 25-fold higher than that of fresh, resting B cells), which peaked
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Fig. 1. Effects of priming B cells in anti-CD3-stimulated WSC cultures on their subsequent responsiveness to stimulation via CD40. WSC cultures were primed in anti-CD3-coated plates for 24 or 48 h, as indicated. Then T cells were depleted and the recovered B cells or fresh B cells (A) were cultured (at time 0, in each case) with the mitogens shown after the arrows and cultures were harvested on the days indicated. In each case aliquots of B cells were labelled with [3H]thymidine at the initiation of the (re)culture period and harvested 4 h later. Fresh B cells received 3/23 anti-CD40 (10 µg/ml) or anti-µ (10 µg/ml). Primed B cells were re-stimulated in media alone (designated 0), or with 0.1, 1.0 or 10 µg/ml anti-CD40 as indicated by the figures in brackets, or 10 µg/ml anti-µ. In additional groups (not shown) B cells primed for 24 h and re-cultured with 100 U/ml IL-2 incorporated 28,000 ± 4100 c.p.m. on day 2, whilst those primed for 48 h and re-cultured for a further 24 h with IL-2 incorporated 75,800 ± 4200 c.p.m. Fresh B cells cultured with 100 U/ml IL-2 gave background levels of proliferation (not shown). Fresh B cells cultured with 10 µg/ml LPS for 3 days incorporated 76,800 ± 3100 c.p.m., whilst 24 or 48 h primed B cells re-cultured with LPS for 48 or 24 h incorporated 72,600 ± 3500 and 96,600 ± 1900 c.p.m. respectively. Data are means ± SEM, n = 3 from one of two experiments which gave comparable results.

at 48 h in groups primed for 24 h and after 24 h with 48 h primed cells. Re-stimulation with IL-2 also induced dose-dependent proliferation of primed B cells, but not fresh B cells, as expected (see figure legend). It is noteworthy that the priming of B cells in this fashion had much less striking effects on their responses to anti-µ or LPS (see figure legend) which, although they reached a peak earlier, were only enhanced by ~2-fold or barely at all in other experiments (see below). In addition, it should be mentioned that T cells in WSC cultures (unlike purified T cells, see below) do not
require co-stimulation via CD28 to induce B cell responsiveness to IL-2, or hyper-responsiveness to anti-CD40, thereby suggesting that there are sufficient B7+ cells in these cultures.

In order to explore the mechanisms involved in this effect we repeated this experiment, including a variety of blocking mAb to molecules which might play a role. Figure 2(A) shows the results of a representative experiment, where WSC were activated on immobilized anti-CD3 in the presence of anti-CD154 or anti-IL-2. B cells from these cultures were then re-cultured with anti-CD40 (either 3/23 or FGK45.5, which is also weakly mitogenic for resting B cells), IL-2 or anti-µ (see figure legend). It is clear that blocking the CD154–CD40 interaction substantially (but not completely) abrogated the priming effect. A neutralizing anti-IL-2 mAb [but not anti-IL-4, or an irrelevant isotype-matched control (Mac-193); data not shown] also suppressed the heightened responsiveness to both anti-CD40 mAb tested, but to a lesser degree. Anti-IL-2 had a much more dramatic effect on the induction of IL-2 responsiveness in B cells, in agreement with our earlier findings (6). In additional experiments (not shown) we also found that the addition of anti-LFA-1 substantially abrogated both the induction of IL-2 responsiveness and the heightened responsiveness to anti-CD40. These results therefore suggest that both CD154–CD40 interactions and IL-2 contribute to this priming effect, a conclusion which is strengthened by further experiments presented below.

We next wished to determine if this priming effect could also be observed in response to a soluble construct of CD40L, which is known to be poorly mitogenic for resting B cells, unless secondarily cross-linked (14,15). The results in Fig. 2(B) show that primed B cells respond much more vigorously to stimulation with CD40L–CD8 fusion protein than fresh B cells, in the absence of secondary cross-linking: the proliferative response of the latter is, as expected, markedly enhanced by cross-linking the fusion protein with anti-CD8 (see figure legend).

Flow cytometric analyses of B cells harvested from these CD3-stimulated WSC cultures revealed that they have the typical phenotype of activated cells, in that they are enlarged and express uniformly high levels of MHC Class II, and are virtually all CD25 and CD86+. In addition, exposure of B cells to activated T cells for 24 h leads to a ~5-fold increase in their levels of CD40, which is not simply due to cellular enlargement, since the levels of B220 on these cells only increased some 2-fold (data not shown). The up-regulation of CD40 was substantially blocked by including anti-CD154 in the priming cultures and is therefore dependent on CD40/CD154 interactions, and highly potentiated by anti-CD40 mAb tested, but to a lesser degree. Anti-IL-2 had a much more dramatic effect on the induction of IL-2 responsiveness in B cells, in agreement with our earlier findings (6). In additional experiments (not shown) we also found that the addition of anti-LFA-1 substantially abrogated both the induction of IL-2 responsiveness and the heightened responsiveness to anti-CD40. These results therefore suggest that both CD154–CD40 interactions and IL-2 contribute to this priming effect, a conclusion which is strengthened by further experiments presented below.

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Fig. 3. Effects of stimulating WSC cultures with differing concentrations of anti-CD3 on the responses of B cells to anti-CD40 or IL-2. WSC were cultured on flasks which had been coated with 10, 1.0 or 0.1 µg/ml anti-CD3 for 24 h. T cells were depleted and the resulting B cells, and fresh B cells were re-cultured with the indicated concentrations of 3/23 anti-CD40 (A) or IL-2 (B). Cultures were harvested after a further 48 h. Responses to anti-µ were as follows: fresh B cells 27,000 ± 300 c.p.m.; B cells ex 10 µg/ml anti-CD3 58,000 ± 1300 c.p.m.; B cells ex 0.1 µg/ml anti-CD3 54,000 ± 1700 c.p.m.; B cells ex 0.1 µg/ml anti-CD3 35,700 ± 1500 c.p.m.

anti-Ig to the WSC cultures stimulated with anti-CD3. Figure 4 shows that anti-IgD induced a 20–30% increase in the levels of proliferation induced by anti-CD40, but had no effects on the responses of B cells to IL-2. Interestingly, the addition of exogenous IL-2 during the priming culture caused a similar level of enhancement of responses induced by suboptimal concentrations of anti-CD40 and also significantly enhanced proliferation induced by IL-2. This suggests that the concentrations of IL-2 may be limiting in these cultures, at least in some experiments (see below).

B cells from xid mice become responsive to anti-CD40 stimulation following exposure to activated T cells

CBA/N mice carrying the xid mutation in Bruton’s tyrosine kinase mount poor primary responses to T-dependent antigens in vivo (16,17). In vitro, B cells from these animals do not proliferate in response to a variety of mitogens, including anti-Ig and three out of four anti-CD40 mAb (18–20). It was therefore of interest to determine the effects of T-dependent activation of xid B cells on their responses to CD40 stimulation. Figure 5 shows that B cells harvested from CD3-stimulated WSC cultures from xid mice responded as vigorously as those from normal littermates when re-stimulated with anti-CD40, although their responses to IL-2 were lower. Interestingly, pre-activated xid B cells did not become responsive to anti-µ, but gave markedly enhanced responses to LPS, whereas pre-activation had little effect on the responses of normal B cells to these mitogens. B cells from CBA/N mice survive poorly in culture, because they have lower levels of the key anti-apoptotic protein Bcl-2 (21,22). We therefore examined the effects of T-dependent activation of xid and normal B cells on their survival, by flow cytometric analyses of propidium iodide-stained cells. The results (not shown) revealed that B cells harvested from WSC cultures of both wild-type and xid mice survived significantly longer than fresh B cells, especially if re-stimulated with either IL-2 or anti-CD40.

CD3/CD28-co-stimulated T cells are more effective than CD3-stimulated T cells at inducing hyper-responsiveness to CD40 stimulation

The results obtained thus far suggested that the observed priming effect was induced by a combination of CD40-mediated signals and IL-2. Since it is well-established that optimal IL-2 production by T cells requires co-stimulation via CD28 (reviewed in 23), we next studied the capacity of purified CD4 T cells to activate B cells. These T cells were pre-stimulated via CD3 alone, or via CD3 and CD28 for 16 h. They were then re-cultured on immobilized anti-CD3 with B cells for 20 h, in the presence or absence of mAb to CD40L, or to IL-2. Subsequently, the T cells were depleted and the B cells were re-cultured with various mitogens (Fig. 6). As we described previously (6), only CD3/CD28-co-stimulated
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T cells effectively induce IL-2 responsiveness in B cells and this effect is significantly abrogated by blocking CD40L–CD40 interactions or by neutralizing IL-2. Figure 7(A) shows that, in addition, T cells pre-activated via CD3 alone were significantly less effective than co-stimulated T cells in priming B cells to respond to anti-CD40 stimulation. The capacity of co-stimulated T cells to prime B cells to respond to anti-CD40 was completely blocked by the inclusion of anti-CD40L and significantly inhibited by neutralizing IL-2.

In a second similar experiment B cells were cultured with CD3- or CD3/CD28-pre-activated CD4 T cells on immobilized anti-CD3 for 20 h, in the presence or absence of exogenous IL-2. The T cells were then depleted and the recovered B cells, or fresh B cells, were re-cultured with various mitogens (Fig. 7). In this experiment, fresh B cells gave a higher response to anti-CD40 than in previous ones (possibly due to the use of a different batch of FCS); however, exposure of B cells to CD3-primed T cells had minimal effects on their response to anti-CD40 and did not render them IL-2 responsive, as we described previously (6). In contrast, B cells harvested from co-cultures with CD3/CD28-pre-activated T cells again gave a significantly greater response to anti-CD40 and responded well to IL-2, as expected. Co-cultures of CD3-primed T cells and B cells which were supplemented with IL-2 yielded B cells which responded as well to either anti-CD40, or IL-2, as those harvested from co-cultures with CD3/CD28-pre-activated T cells. The addition of IL-2 to the latter co-cultures did not affect B cell responses to anti-CD40, but further enhanced their responsiveness to IL-2 (an effect not seen in most experiments). These results therefore reinforce the conclusion that CD40L–CD40 interactions, in conjunction with IL-2, lead both to IL-2 responsiveness and hyperresponsiveness to CD40 stimulation in B cells.

Discussion

The induction of proliferation in quiescent B cells via ligation of CD40 clearly depends critically on the level of cross-linking
Secondly, B cells activated in this T-dependent fashion respond much more vigorously than naive cells to re-stimulation via CD40, even when stimulated with suboptimal amounts of anti-CD40 or with a soluble CD40L-CD8 fusion protein (Figs 1 and 2). It is noteworthy that this priming effect is seemingly specific for responses elicited by engaging CD40, since activation of B cells in this fashion has much less dramatic effects on their responses to anti-Ig or LPS (Figs 1 and 5). The powerful nature of the effect is underscored by the fact that B cells from xid mice, which do not proliferate at all when stimulated with most anti-CD40 mAb, respond vigorously after being exposed to activated T cells (Fig. 5).

The induction of this heightened responsiveness is largely dependent on the interaction of CD40 with CD154, since it is substantially abrogated by blocking the interaction of these two molecules (Figs 2A and 6). However, it is clear that other T cell-derived stimuli also play a significant role, most notably IL-2, which apparently acts in synergy with CD154. Hence, purified CD4 T cells activated via CD3 alone are much less effective at enhancing CD40 responsiveness in B cells than CD3/CD28-co-stimulated cells (Fig. 6), even though both populations of T cells re-express similar levels of CD154 when re-stimulated via CD3 (6). Addition of IL-2 to co-cultures of CD3-primed T cells with B cells rendered these T cells competent at both inducing IL-2 responses in B cells and in enhancing their responsiveness to CD40 stimulation (Fig. 7). It is possible that under physiological conditions, other T cell-derived cytokines (such as IL-4) may play a role as well, although in our hands neutralizing anti-IL-4 mAb had no effect. In addition, co-stimulation of B cells with anti-Ig during the priming cultures further enhances their subsequent responses to anti-CD40 (Fig. 4), thereby suggesting that signals via the B cell antigen receptor can also contribute to lowering the threshold for CD40 stimulation. We have attempted to mimic the effects of activated T cells by priming populations of T cells re-express similar levels of CD154 when cultured with anti-CD40 (N. van Emmerik and G. G. B. Klaus, unpublished data). Rather we believe that some fundamental biochemical changes occur in the signal transducing machinery associated with CD40 as a result of B cell activation.

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Fig. 7. Effects of exogenous IL-2 on the capacity of CD3-pre-activated T cells to induce heightened responsiveness to CD40 stimulation in B cells. Purified CD4 T cells were pre-activated with anti-CD3 alone (designated T) or with anti-CD3 and anti-CD28 (T*) for 16 h, as in Fig. 6. These cells were then replated with B cells in anti-CD3-coated wells in the presence or absence of 100 U/ml IL-2. These co-cultures were depleted of T cells after a further 20 h and the recovered B cells, or fresh B cells, were re-cultured for 48 h with the indicated mitogens: (A) (anti)-CD40 (3/23 µg/ml in brackets); (B) IL-2 (U/ml in brackets) or anti-µ (10 µg/ml).

of the receptor. Hence, CD154-transfected cell lines or pre-activated T cell clones, which express high levels of CD154, can effectively activate B cells, in the absence of exogenous co-stimuli (24–26). Soluble constructs of CD154 are poorly mitogenic, unless secondarily cross-linked (14,15). Similarly, primary T cells activated via CD3 alone, which express some 10- to 20-fold less CD154 than T cell clones, are poor T cell activators, in the absence of co-stimuli, such as IL-2, or unless the B cells have been pre-activated, by anti-Ig, for example (4–6). Most anti-CD40 mAb also elicit minimal levels of DNA synthesis in resting B cells, in the absence of co-stimuli, such as anti-Ig, or appropriate cytokines, or unless they are presented on a matrix, as provided by CD32-transfected fibroblasts, for example (27). The data we present here indicate that when naive B cells encounter CD3-activated T cells in WSC cultures, or CD3/CD28-co-stimulated T cells, this leads to (at least two) profound changes in their responsiveness. Firstly, the cells become responsiveness to IL-2 (6). Secondly, B cells activated in this T-dependent fashion respond much more vigorously than naive cells to re-stimulation via CD40, even when stimulated with suboptimal amounts of anti-CD40 or with a soluble CD40L-CD8 fusion protein (Figs 1 and 2). It is noteworthy that this priming effect is seemingly specific for responses elicited by engaging CD40, since activation of B cells in this fashion has much less dramatic effects on their responses to anti-Ig or LPS (Figs 1 and 5). The powerful nature of the effect is underscored by the fact that B cells from xid mice, which do not proliferate at all when stimulated with most anti-CD40 mAb, respond vigorously after being exposed to activated T cells (Fig. 5).

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It is possible that under physiological conditions, other T cell-derived cytokines (such as IL-4) may play a role as well, although in our hands neutralizing anti-IL-4 mAb had no effect. In addition, co-stimulation of B cells with anti-Ig during the priming cultures further enhances their subsequent responses to anti-CD40 (Fig. 4), thereby suggesting that signals via the B cell antigen receptor can also contribute to lowering the threshold for CD40 stimulation. We have attempted to mimic the effects of activated T cells by priming populations of T cells re-express similar levels of CD154 when cultured with anti-CD40 (N. van Emmerik and G. G. B. Klaus, unpublished data). Rather we believe that some fundamental biochemical changes occur in the signal transducing machinery associated with CD40 as a result of B cell activation.
T-dependent B cell activation reduces the threshold for CD40 signalling

We have consistently failed to detect protein tyrosine kinase activation following the stimulation of small dense B cells with anti-CD40 (data not shown), whilst anti-CD40 induces robust protein tyrosine phosphorylation in human germinal centre B cells (31). The precise nature of the second messenger systems invoked by ligation of CD40 is still somewhat confused and may indeed vary according to the state of maturity and/or activation of the target cells (reviewed in 32). However, it is clear that signalling via CD40 involves several members of the TNF receptor-associated factors (TRAF) protein family, which link the receptor to downstream events, such as the induction of NF-κB (reviewed in 33). The present results suggest that the biochemical mechanisms which lead to the establishment of IL-2 responsiveness and hyper-responsiveness to CD40 stimulation in B cells may well be similar, since both apparently depend on a combination of CD40-mediated signals and IL-2 itself, acting in synergy (Figs 2, 6 and 7). However, there are apparent differences, as illustrated by the requirements for more extensive TCR cross-linking for the induction of IL-2 responses in cells (Fig. 3), a phenomenon which deserves further investigation. The IL-2 receptor is coupled to two members of the JAK family of protein tyrosine kinases JAK1 and JAK3, which act on two of the STAT family of transcription factors (STAT3 and STAT5) (reviewed in 34). It may therefore be relevant that both JAK3 and STAT5 are up-regulated in human B cells stimulated via CD40 (35). In addition, Hanissian and Geha (36) have recently shown that JAK3 is associated with CD40, and that it and STAT3 become activated following ligation of CD40 on a human B cell line, although these results were not confirmed by another group (37).

We therefore postulate that the initial encounter of B cells with activated T cells sets in train an amplification loop, which not only induces B cells to become IL-2 responsive, but also markedly reduces the threshold for their activation via the CD40 receptor. This effect is likely to play an important role during the initiation of antibody responses, when T cells only express low levels of CD154, which are by themselves insufficient to induce full-blown B cell activation. Other amplification mechanisms may well be important at this stage of the response, as well. For example, Jaiswal and Croft (38) have shown that the interaction of pre-activated (i.e. effector) T cells with resting B cells, presenting a cognate peptide, leads to the expression of higher levels of CD154, than the interaction of naive T cells with resting B cells. In addition, CD40 ligation reduces the threshold of B cell activation via their antigen receptors (3). Taken together, it is evident that the immune system has evolved multiple amplification mechanisms to maximize responses to antigen, which probably play their most important roles during the early phases of antibody responses. Our future experiments will address the biochemical mechanisms involved in the priming effects we have described here and also how T cell–B cell interactions affect components of the cell cycle regulatory machinery in B lymphocytes.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CD40L</td>
<td>CD40 ligand</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>WSC</td>
<td>whole spleen cell (cultures)</td>
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References


20. Santos Argumedo, L., Lund, F. E., Heath, A. W., Solvason, N.,
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