CD28 co-stimulation stabilizes the expression of the CD40 ligand on T cells

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Abstract
The ligand for CD40 (CD40L) is a protein which is expressed on CD4 T cells following their activation: CD40–CD40L interactions are absolutely required for the induction of T cell-dependent antibody responses, yet little is known about the mechanisms whereby CD40L+ primary T cells activate naive B cells, since the protein is only transiently expressed and is rapidly down-regulated following T cell–B cell contact. We show here, using a variety of assays, that co-stimulation of primary murine T cells via CD3 and CD28 stabilizes the expression of the CD40L protein. Firstly, T cells stimulated in this manner express higher levels of CD40L when activated in the presence of B cells, compared to CD3-activated T cells. Secondly, the CD40L expressed on CD28-co-stimulated T cells is more resistant to B cell-induced down-regulation. Finally, CD3/CD28-preactivated, rested T cells re-express higher levels of CD40L more rapidly following re-stimulation via CD3 than T cells preactivated via CD3 alone. CD3/CD28-preactivated T cells, but not CD3-activated cells, are competent to induce DNA synthesis in naive B cells, and this requires re-stimulation via CD3 and prolonged ligation of CD40. These data therefore reinforce the concept that naive T cells need to be activated initially by cognate interaction with B7-bearing antigen-presenting cells (such as dendritic cells), before becoming competent helper effector cells capable of driving B cells into proliferation via a CD40-dependent pathway.

Introduction
The induction of antibody responses to T cell-dependent (TD) antigens requires direct physical contact between Tc cells with B cells, as well as the participation of a variety of cytokines. The key receptor on B cells for the reception of contact-mediated helper signals is CD40, which transduces activating signals following its interaction with the CD40 ligand (CD40L or gp39), a protein expressed on activated CD4 T cells (reviewed in 1). The evidence for this concept has emerged from many sources. Thus, patients with the immunodeficiency X-linked hyper IgM syndrome have mutations in the gene encoding CD40L. These lead to a gross deficiency in switched Ig isotypes and an incapacity to form germinal centres (reviewed in 2). Mice with targeted mutations in the genes for CD40 or CD40L have a similar phenotype (3–5). Finally, administration of anti-CD40L antibodies to mice abrogates TD antibody responses (6). It is therefore now clear that signals generated via CD40 on B cells, in conjunction with a variety of cytokines (most notably IL-4 and IL-5 in the mouse), drive the program of B cell proliferation, differentiation to Ig-secreting cells, isotype class switching, and the formation of germinal centres and B memory cells.

The expression of the CD40L on T cells is tightly regulated, presumably to minimize bystander B cell activation. The protein is rapidly induced following the stimulation of either primary T cells, or T cell clones via the TCR–CD3 complex, and then wanes within some 16–24 h (7–9). The interaction of CD40L-bearing T cells with CD40+ cells, such as B cells, induces very rapid down-regulation of the protein. This is initially due to receptor-mediated endocytosis (10), but may eventually involve down-regulation of mRNA for the CD40L as well (11). In addition, CD40L expression seems to be controlled by similar mechanisms to those which regulate the production of many T cell-derived cytokines. Hence, the expression of the CD40L is abrogated by the immunosuppressive drugs cyclosporine and FK506 (12,13). These agents inhibit cytokine (e.g. IL-2) gene transcription via their action on calcineurin, with consequent inhibition of the assembly of the heterotrimeric transcription factor NF-AT (reviewed in (14). Recent studies have shown that the promoter of the CD40L gene does indeed contains four functional NF-AT sites (15, 16). Optimal production of a number of T cell-derived cytokines requires co-stimulation via CD28 [which interacts with B7-1...
and/or B7-2 counter-receptors on antigen-presenting cells (APC), reviewed in (17). It is clear that the induction of the CD40L during T cell interactions with normal APC does not require signalling via CD28, although the presence of B7 is required when primary T cells are stimulated by antigen presented on class II-transfected fibroblasts (18-21). However, co-stimulation via CD28 does accelerate the induction of CD40L mRNA and protein in T cells, and increases their capacity to activate B cells (13,18,20,22). The findings that CD40L is only transiently expressed on activated CD4 T cells and is rapidly down-regulated following T cell–B cell interactions raise fundamental questions about how signals are generated for sufficient periods via CD40 to induce B cell activation, during the initiation of TD antibody responses. We undertook the studies described here following two observations. The first was that B cells require ~36 h exposure to CD40L-transfected fibroblasts before becoming committed to DNA synthesis. The second was that, in agreement with published data (13,20,22), primary T cells stimulated via CD3 alone are incapable of inducing B cell proliferation, despite the fact that most of them express CD40L (8). One reason for the failure of such T cells to activate B cells could therefore be the rapid down-regulation of the CD40L following T cell–B cell interaction (10). These observations therefore suggested that there might be mechanisms to stabilize the expression of the CD40L under physiological conditions. A logical candidate for providing such a mechanism is CD28.

Methods

Experimental animals

Specific pathogen-free (CBA×C57BL/10)F1, mice bred at the National Institute for Medical Research were used at the age of 3–4 months.

Reagents

The following mAbs were used: hamster anti-CD3 (145-2C11), hamster anti-CD28 (37-51.1, a gift from J. Allison), hamster anti-CD40L (MR-1, 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) (both obtained from H. Waldmann), and rat anti-μ (b7.6). These 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 and biotinylated anti-CD69 were from PharMingen (San Diego, CA). AffiniPure goat anti-mouse IgG and IgM (H + L) and phycoerythrin-conjugated streptavidin (PE–SA) were from Southern Biotechnology Associates (Birmingham, AL). CD40L-transfected L cells (clone K47) and transfectants of 3–4 months.

Preparation of T cells and B cells

Spleenic CD4 T cells were prepared as follows. Single-cell suspensions were loaded onto a discontinuous (50–75–85%) Percoll (Pharmacia) gradient. After centrifugation, cells at the 75–85% layer were harvested and resuspended in medium with a saturating concentration of anti-CD8. After 40 min at 4°C, the cells were washed, resuspended in PBS/3% FCS at 3–5×10^6/ml and plated onto washed bacteriological Petri dishes previously coated overnight at 4°C with 10 µg/ml affinity purified goat anti-mouse Ig in 0.05 M Tris–HCl, pH 9.5. Plates were swirled after 40 min to redistribute unattached cells and left for a further 30 min. Non-adherent cells were recovered and subjected to a second round of ‘panning’, as before. The non-adherent cells were typically >90% CD4+, with <5% B cell contamination.

Small, dense B cells were prepared from mouse spleen by a modification of a method previously described (23). Briefly, T cells were killed by incubation with a cocktail of anti-Thy-1, anti-CD4 and anti-CD8 mAbs, and guinea pig complement for 40 min at 37°C. The remaining cells were washed and layered onto a 50–75–85% Percoll gradient. After centrifugation, the cells banding at the 75–85% interface were recovered. These were typically >90% B220+, with <1% T cell contamination.

Induction and re-expression of CD40L

T cells were cultured (at 10^6/ml) in supplemented RPMI 1640 medium plus 5% FCS, in flasks coated with anti-CD3 (in PBS, 10 µg/ml, for 24 h at 4°C). Some cultures received in addition soluble anti-CD28 (or normal hamster IgG) at 1–5 µg/ml. CD40L expression was first detectable by 4 h, reached maximal levels by 12–16 h and subsequently waned. Hence in most experiments T cells were stimulated for 12–14 h, as indicated. In some instances, T cells stimulated (primed) with anti-CD3, in the presence or absence of anti-CD28, were harvested, washed and re-cultured for a further 24 h in media, to allow time for spontaneous decay of CD40L. These rested cells were then replated in anti-CD3-coated flasks, which were spun briefly to accelerate contact between cells and the plastic.

Down-regulation of CD40L by B cells

In most experiments B cells were added directly to the flasks in which T cells had been stimulated to induce CD40L expression, at the ratios indicated and in the presence or absence of various mAbs. These mixtures of cells were incubated (generally for 1 h at 37°C), prior to flow cytometry (FCM).

FCM analyses of CD40L expression

T cells (or T and B mixtures) were suspended in PBS/0.2% BSA/0.1% NaN₃ and stained with appropriate combinations of mAbs by conventional methods. Appearance of CD40L was assessed by two-color FCM analyses, using a combination of FITC–anti-CD4 and biotinylated anti-CD40L, revealed by PE–SA. Flow cytometric analyses were performed on either a FACStar Plus or a FACSc Vantage (Becton Dickinson, Mountain View, CA).

Co-culture of T cells and B cells

CD4 T cells which had been preactivated with anti-CD3, with or without anti-CD28, were harvested, irradiated (3000 rad) and then plated into 96-well microtiter wells (10^4/well), which were uncoated, or had been coated with anti-CD3 as above, together with an equal number of resting B cells and appropriate mAbs, as indicated. These cultures were labelled with [3H]thymidine (0.5 µCi/well) generally after 68 h of culture, harvested 4 h later and incorporation of radiolabel into DNA.
Commitment to DNA synthesis in B cells requires prolonged interaction with CD40L

The present study was prompted by experiments which investigated how long B cells need to be stimulated via CD40 before becoming committed to DNA synthesis. For this purpose, B cells were cultured with CD40L-transfected fibroblasts: in this system B cell proliferation was completely abrogated by adding the anti-CD40L mAb MR-1 at the initiation of the culture and even after 24 h (Fig. 1). Most significantly, however, the antibody still suppressed the proliferative response by ~50% when added after 48 h of the 3-day culture period. These results therefore indicate that in vitro B cells need to be stimulated via CD40 for at least 36 h before becoming committed to DNA synthesis. Similar findings have been reported with other lymphocyte mitogens (e.g. 24) since contact of T cells with CD40L-bearing T cells induces dose-dependent down-regulation of CD40L (10), it was possible that the B:T cell ratio was too high in WSC cultures to permit the visualization of CD40L+ cells. We therefore stimulated a 1:1 mixture of CD4 T cells and B cells with anti-CD3, in the absence of anti-CD28, for 13 h (Fig. 2). Only 26% of T cells stimulated via CD3 in the presence of B cells were positive when incubated with B cells. Of T cells stimulated via CD3 and CD28, in the absence of B cells, 74% (MFI = 489) were positive, whilst 57% (MFI = 415) expressed CD40L in the presence of B cells.

Taken together, these results suggested that mechanisms might exist which stabilize the expression of the CD40L on activated T cells.

Co-stimulation with via CD3 and CD28 leads to the induction of detectable CD40L in mixtures of T and B cells

Stimulation of WSC cultures via CD3 and CD28 did not lead to the appearance of CD40L+ T cells (not shown). However, since contact of T cells with CD40+ cells induces dose-dependent down-regulation of CD40L (10), it was possible that the B:T cell ratio was too high in WSC cultures to permit the visualization of CD40L+ cells. We therefore stimulated a 1:1 mixture of CD4 T cells and B cells with anti-CD3, in the presence or absence of anti-CD28, for 13 h (Fig. 2). Only 26% of T cells stimulated via CD3 in the presence of B cells were very weakly positive for CD40L. In contrast, 57% of T cells stimulated with via CD3 and CD28, in the absence of B cells, 74% (MFI = 489) were positive, whilst 57% (MFI = 415) expressed CD40L in the presence of B cells.
Stabilization of CD40L by CD28 co-stimulation

Fig. 3. Effects of CD28 co-stimulation on spontaneous decay or CD40-induced down-regulation of CD40L. In Experiment 1 (A) purified CD4 T cells were activated on immobilized anti-CD3, in the presence or absence of anti-CD28 for 12 h. At this time (t = 0) the cells were replated in uncoated tissue culture wells, which were subsequently harvested and subjected to FCM analyses at the times indicated. The curves show the loss of CD40L-bearing T cells with time: data are expressed as percentages of values at t = 0, whilst figures in brackets are MFI values (on a logarithmic scale). In Experiment 2 (B and C) CD4 T cells were activated as above and then resting B cells were added directly to the flasks at various ratios. After a further 1 h incubation, cultures were analysed as above: (B) shows the profiles of CD3-primed (shaded histogram) or CD3/CD28-primed (open histogram) in the absence of B cells, whereas (C) shows comparable profiles of cells incubated with B cells at a 1:1 ratio. Comparable results were obtained with a 3:1 B cell:T cell mixture. Each experiment is representative of two or three that gave comparable results.

Expressed protein is down-regulated even more rapidly, following contact of T cells with CD40+ cells. We therefore investigated the effects of CD28 co-stimulation on these phenomena. In the first experiment CD4 T cells were activated for 12 h via CD3 or CD3/CD28. These cells were then recultured in wells containing media alone and the percentages of CD40L+ T cells were determined at varying times thereafter (Fig. 3A). Again, CD28 co-stimulation did not affect the percentage of T cells that initially expressed CD40L (~ 60%) nor the levels of the protein (see figure legend). However, cells stimulated with anti-CD3 alone rapidly lost CD40L following their removal from anti-CD3, so that by 9 h very few of them expressed the protein. In contrast, co-stimulation of T cells via CD3/CD28 prolonged the expression of the protein by ~3 h, so that after 11 h 20% of the cells still expressed significant levels of the protein. In the second experiment T cells activated in the presence or absence of anti-CD28 were mixed with resting B cells. After 60 min incubation, the percentages of T cells which remained CD40L+ were determined (Fig. 3B and C). The addition of B cells to CD3-activated T cells caused dose-dependent down-regulation of CD40L, as expected. In contrast, addition of B cells at a 1:1 ratio (and at a 3:1 ratio, not shown) only induced minimal down-regulation of CD40L expressed by CD3/CD28-activated T cells. This effect could not be reproduced by including anti-CD28 during the T cell–B cell incuba-
Stabilization of CD40L by CD28 co-stimulation

Co-stimulation via CD28 leads to more rapid reappearance of CD40L and reduces B cell-induced down-regulation of the protein

In the next experiments, T cells were activated via CD3, or CD3/CD28, rested for 24 h and subsequently re-stimulated via CD3 in the presence or absence of anti-CD28 and B cells. Very few rested T cells expressed CD40L and both populations re-expressed CD40L following re-stimulation via CD3 for 3 h (Fig. 4). However, at this time only 55% of CD3-primed T cells were CD40L+ (MFI = 379), whilst 76% (MFI = 464) of CD3/CD28-primed cells were brightly positive. The inclusion of anti-CD28 during re-stimulation did not affect these values. Only 23% (MFI = 327) of CD3-primed T cells re-expressed CD40L in the presence of B cells, while 68% (MFI = 408) of CD3/CD28-primed T cells did so. These results therefore further reinforce the concept that primary activation of T cells via CD3 and CD28 stabilizes the expression of CD40L, and hence renders the protein less susceptible to down-regulation upon its contact with CD40.

Reappearance of CD40L on preactivated T cells requires de novo protein synthesis

There is evidence that a subset of preactivated human T cells contains a store of preformed CD40L, which is rapidly expressed on the cell surface following re-stimulation (25). We therefore investigated if the rapid reappearance of CD40L in CD3/CD28-primed cells requires de novo protein synthesis (Fig. 5). T cells were primed as before, rested for 24 h and then re-stimulated for 2 or 4 h in the presence or absence of cycloheximide. Again, CD3/CD28-primed T cells re-expressed CD40L more rapidly than CD3-primed cells (Fig. 5A and B), but this was completely abrogated by cycloheximide, in both populations. Blocking protein synthesis completely inhibited de novo induction of CD40L (and CD69) on fresh T cells (Fig. 5G and H). It is noteworthy that a substantially
greater percentage of CD3/CD28-primed T cells expressed CD69 than the control cells (Fig. 5E and F), presumably reflecting the enhancing effects of CD28 co-stimulation on T cell activation. The levels of CD69 on these two groups of cells were unaffected by cycloheximide. These results therefore indicate that, under these experimental conditions, CD3/CD28-stimulated T cells do not contain intracellular stores of CD40L, so that the rapid re-expression of the protein following re-stimulation of these cells is likely to be due to residual mRNA.

T cells activated via CD3 and CD28 can activate naive B cells

Primary T cells activated via CD3 alone are incapable of inducing B cell activation (unlike preactivated, Th1 or Th2 T cell clones), possibly because they express lower levels of CD40L and/or produce insufficient levels of relevant cytokines (8, 13, 20). However, little is known about how long B cells need to be stimulated via CD40 when they encounter CD40L-bearing T cells to become committed to DNA synthesis. To test this, T cells were first activated via CD3 or CD3/CD28, rested for 24 h and irradiated. They were then recultured on anti-CD3-coated or uncoated wells (not shown), together with B cells and a blocking concentration of anti-CD40L was added to these cultures at varying times. Figure 6 shows that, as expected, only CD3/CD28-primed T cells induced DNA synthesis in B cells, and this was absolutely dependent on the re-stimulation of the T cells via CD3 in the co-cultures (see figure legend). Proliferation was almost completely abrogated by adding anti-CD40L at t = 0 and was still significantly blocked when the mAb was added at 36 h, indicating that B cell proliferation is not driven by cytokines alone, but requires CD40–CD40L interaction. These results indicate that primary T require initial activation via CD3/CD28, followed by re-stimulation via CD3, in order to drive B cells into DNA synthesis, via a CD40L-dependent mechanism. Furthermore, the induction of DNA synthesis by preactivated T cells in B cells requires prolonged stimulation via CD40 (although it may obviously involve cytokines as well).

Discussion

The initiation of TD antibody responses involves complex reciprocal cross-talk between T cells and B cells, once the

Fig. 5. Requirements for de novo protein synthesis for the re-expression of CD40L on preactivated T cells. Purified CD4 T cells were primed for 12 h with immobilized anti-CD3 in the presence or absence of anti-CD28. These, and fresh unstimulated T cells, were then rested in uncoated flasks for 24 h, when some were incubated with cycloheximide (10 µg/ml) for 2 h, when they were again replated onto anti-CD3-coated flasks. The cultures were harvested after a further 2 or 4 h for FCM analyses. The data show the levels of CD40L (left panels) or CD69 (right panels) on CD4 T cells, which were originally primed with anti-CD3 alone (A and E), anti-CD3/CD28 (B and F), unprimed T cells stimulated with anti-CD3 alone (C and G) or unprimed T cells stimulated with anti-CD3/CD28 (D and H). In each panel the thick histogram represents cells 2 h after (re)stimulation, the thin histogram is the profile given after 4 h of (re)stimulation and the dashed lines the profile given by cells cultured for 4 h in the presence of cycloheximide.
cells have formed antigen-specific conjugates (reviewed in 26). The principal avenues of communication utilized by these cells are the receptor–counter-receptor pairs CD40–CD40L and CD28–B7-1 (CD80) and/or B7-2 (CD86). Hence, a major consequence of B cell stimulation via CD40 is up-regulation of CD80 and CD86 (27), which are essential for these cells to become competent APC. Signals via CD28 are in turn required for T cells to become competent effector cells and to prevent the induction of T cell anergy (reviewed in 17). The results presented here provide additional evidence for this concept. Although co-stimulation of CD3-activated naive CD4 T cells via CD28 does not affect the levels of CD40L they express (13, 18, 19, 21), it significantly prolongs the expression of the protein on the cell surface. This stabilizing effect was manifest under a variety of experimental conditions. Firstly, significantly higher levels of CD40L+ T cells were observed in cultures of B cells plus CD3/CD28-co-stimulated T cells than in mixtures of CD3-activated T cells and B cells (Fig. 2). Secondly, CD28 co-stimulation retarded the spontaneous decay of CD40L on T cells removed from the inducing stimuli (Fig. 3A) and markedly reduced the down-regulation of the CD40L induced by the addition of B cells (Fig. 3C). In addition, CD3/CD28-primed T cells re-expressed CD40L more rapidly, and at higher levels following re-stimulation via CD3, than CD3-primed T cells (Fig. 4). Finally, and most importantly, T cells preactivated via CD3/CD28 (unlike CD3-activated T cells) became competent to induce DNA synthesis in B cells (20–22), provided they were re-stimulated via CD3 and this involved prolonged stimulation of B cells via CD40 (Fig. 6).

The data provide additional evidence for the concept that the transcription of the CD40L gene is controlled in a similar (but not identical) fashion to that of many other cytokine genes (the prototypical example being that for IL-2, reviewed in 28). The induction of the CD40L is exquisitely sensitive to inhibition by cyclosporine and FK506 (12, 13), presumably a reflection of the presence of four functional NF-AT sites within the CD40L promoter (16). However, CD28/CD3 co-stimulation does not render the induction of the CD40L FK506 insensitive (13). Stimulation of human T cells via CD28 and phorbol esters induces cyclosporine/FK506-resistant cytokine gene transcription (29). We have been unable to induce CD40L expression via this combination of stimuli (data not shown), reinforcing the concept that an elevation of intracellular Ca²⁺ is critical for the induction of this protein (30). The effects of CD28 stimulation on cytokine gene transcription are mediated by CD28-response elements (CD28RE), characteristic nucleotide motifs found in the promoters of many cytokine genes, which bind members of the NF-κB/rel family of transcription factors (reviewed in 17). We therefore considered the possibility that the CD40L promoter might also contain a CD28RE. Homology searches with known CD28RE sequences indeed revealed a potential motif, between positions −1125 and −1114 of the promoter (numbered according to 15), with the sequence GAGAGATTCC. There is good agreement between this sequence and the consensus sequence of the CD28RE from eight cytokine promoters, apart from position 9, where there is a T instead of a C (15, 31–34). CD3/CD28 stimulation of human T cells accelerates the initial appearance of CD40L mRNA, but does not affect the levels of the protein ultimately expressed (13, 21). Our data substantiate the latter observation (e.g. Figs 2 and 3), but show instead that CD28 co-stimulation stabilizes the expression of the CD40L protein. A second (poorly understood) effect of CD28 co-stimulation is to stabilize cytokine mRNAs, apparently by reducing their degradation by RNA endonucleases (35, 36). This applies especially to mRNAs with AUUUA motifs in their 3′ untranslated regions. There are five such motifs in mRNA for CD40L (37) and we believe that stabilization of mRNA is responsible for the effects we describe here. We have attempted to verify this by both RT-PCR and Northern analyses (not shown). The former approach proved too variable and difficult to quantify, whilst to date we have been unable to extract sufficient RNA from purified primary T cells to generate quantitative data from Northern blotting.

The original purpose of this study was to investigate how primary T cells activate B cells via CD40, given the transient expression of the CD40L and its rapid down-regulation following interaction with CD40. We showed that B cells require some 36 h contact with CD40L-transfected fibroblasts before becoming committed to DNA synthesis (Fig. 1). We then demonstrated that only CD3/CD28-co-stimulated T cells induced B cell proliferation, provided that they were re-stimulated via CD3 and this again requires prolonged stimulation via CD40 (Fig. 6). These results therefore suggest that during the interaction of T cells and B cells there may be a continuous cycle of down-regulation and re-expression of CD40L, the latter being dependent on the
presence of antigen. It is indeed possible that the stabilization of the CD40L we describe here may become more pronounced with time, as B cells become activated and hence up-regulate B7. Obviously, in vivo B cells which have been preactivated by antigen may not require such prolonged exposure to CD40L to become committed to DNA synthesis. In addition, since there is evidence that as T cells develop into memory cells they carry an intracellular store of CD40L (25), this could also play an important role in driving B cell activation.

T cells initially activated via CD3 alone do eventually re-express substantial levels of CD40L when re-stimulated via CD3, even in the presence of B cells (not shown), yet still fail to induce B cell proliferation. We show elsewhere that an additional reason for their failure to act as effector cells is because they fail to secrete sufficient levels of cytokines (most notably IL-2), which synergize with CD40L to induce B cell proliferation (Johnson-Léger et al., submitted). It is therefore clear that CD28/B7 interactions play a central role during the initiation of TD antibody responses, in line with the phenotype of CD28−/− mice, which respond poorly to TD antigens and fail to form germinal centres (38, 39).

In conclusion, the available data suggest the following hypothesis. Initial activation of naive T cells occurs on dendritic cells in the T cell areas of lymphoid organs (reviewed in 26). Dendritic cells are CD40+, but since they also express high levels of CD80 and CD86 (40), this encounter should induce maximal levels of stable CD40L mRNA (and mRNA for relevant cytokines) in CD4 T cells. It is likely that this cellular interaction will induce internalization of CD40L (41). Nevertheless, we predict that subsequent cognate interaction of such preactivated T cells with B cells will induce rapid, stable re-expression of CD40L and signalling through CD40. The efficiency of this interaction will be maximized if B cells are themselves preactivated and hence express some CD80/CD86. Activation of B cells via CD40 will further increase expression of B7 molecules, thereby establishing a feed-forward amplification loop to maintain stable expression of CD40L and hence long-term engagement of CD40 on B cells. This, in combination with appropriate cytokines, would then drive the program of B cell clonal expansion, Ig secretion and isotype switching.

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Abbreviations

- APC: antigen-presenting cell
- CD40L: CD40 ligand
- CD28RE: CD28-response element
- FCM: flow cytometry
- MFI: median fluorescence intensity
- PE: phycoerythrin
- SA: streptavidin
- TD: T cell dependent
- WSC: whole spleen cells
- TD: T cell dependent

References

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