Brief Definitive Report

The CD28 Ligand B7/BB1 Provides Costimulatory Signal for Alloactivation of CD4+ T Cells

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Summary

Activation via the T lymphocyte cell surface molecule CD28 provides a potent amplification signal for interleukin 2 (IL-2) production in several in vitro systems. The B lymphocyte activation antigen, B7/BB1, is a natural ligand for CD28. Here we investigate the role of CD28 and B7/BB1 in primary activation of CD4+ T lymphocytes stimulated with allogeneic B lymphoblastoid cell lines. A subset of peripheral CD4+ T cells that is unresponsive to crosslinking of CD3/T cell receptor (TCR) with CD3 monoclonal antibody (mAb) does proliferate in response to allogeneic B lymphoblasts. TCR binding to allogeneic major histocompatibility complex antigens was an absolute requirement for activation of these cells because mAbs to either CD3 or human histocompatibility leukocyte antigen (HLA) class II completely inhibited activation. CD28 and B7/BB1 antibodies inhibited T cell proliferation 90% and 84%, respectively. Similar results were obtained with the total CD4+ T lymphocyte population. Crosslinking of HLA-DR antigens on small, resting B cells induced rapid expression of B7/BB1, which peaked at 6 h and returned to baseline levels within 18 h. These data demonstrate that CD28-B7/BB1 binding provides an important early second signal for alloactivation of CD4+ T lymphocyte by B lymphoblasts. The results also suggest that T cells interacting with allogeneic resting B cells may induce B7/BB1 expression in the alloantigen-presenting cell as a consequence of interaction between the TCR and class II molecules.

Primary antigen-specific immune responses require TCR-Ag/MHC occupancy (first signal) and additional stimuli from the APC (second signal) (reviewed in reference 1). Similarly, alloantigen activation of T cells also requires costimulatory signals in addition to TCR occupancy by the allogeneic MHC antigen. Peripheral blood dendritic cells and anti-Ig-activated B lymphoblasts are potent stimulators, in contrast to monocytes and small resting B cells (2). Several cell surface molecules on T lymphocytes are potential candidates for providing the "second signal" necessary for alloactivation as a result of binding to their natural ligand: CD2/LFA-3, LFA-1/ICAM-1 and -2, CD4/HLA class II, CD8/HLA class I (reviewed in reference 3), and CD28/B7-BB1 (4, 5). We have recently described a subset of peripheral CD4+, CD45RA+ T cells that is unresponsive to CD3/TCR crosslinking with anti-CD3 in presence of autologous APC (6). We report here that this CD4+ T cell subset proliferates in response to allogeneic B lymphoblastoid cell lines (B-LCL). The responses are potently inhibited by mAbs to two sets of cell surface–expressed receptor ligands: TCR-CD3/HLA class II and CD28/B7-BB1. Similar results were obtained with the total CD4+ T cell population. Crosslinking of HLA-DR molecules on resting B lymphocytes induces B7/BB1 expression which peaks within 6 h, suggesting that binding of B7/BB1 to CD28 provides an important second signal for primary alloactivation of CD4+ T cells by B lymphoblasts.

Materials and Methods

mAbs Used. Anti-HLA class I (HLA-A,B,C): W6/32 (IgG2a) and 4E (HLA-B,C and Aw19) (IgG2a); anti-HLA-DR: L243 (IgG2a) and HB10a (IgG2b); anti-HLA-DQ: TU 22; anti-CD2: 9.6 (IgG2a) and 9-1 (IgG3); anti-CD3: OKT3 (IgG2a) and SP34 (IgG2a); anti-CD28: 9.3 (IgG2a); anti-CD8: OKT8 (IgG2a) and SA-19-11 (IgG1); anti-CD25: 6G (IgG2a); anti-CD5: H65 (IgG1) and 6-2 (IgG2a); anti-CD37: G28-1 (IgG1); anti-CD22: G28-7 (IgG1); anti-B7/BB1: BB1 (IgM); anti-CD54 (ICAM-1): LB-2 (IgG2b); anti-CD45: 9.4 (IgG2a), reactive with all chains of CD45; and anti-CD1c: M241 (IgG1).

Isolation of CD4+ T Lymphocyte Populations. Peripheral blood CD4+ T cells were isolated as previously described (6). Anti-CD3-unresponsive CD4+ cells were isolated from CD4+ T lymphocytes that had been cultured for 40 h at 37°C with the CD3

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mAb SP34 (4 μg/ml) in the presence of 15–20% APC and rIL-2 (Amgen Biol., Thousand Oaks, CA). The cells were negatively selected with mAbs L243 and 6G (6).

B Lymphocyte Preparations. Tonsillar B cells were isolated as previously reported (7) on discontinuous Percoll gradients (Pharmacia Fine Chemicals, Piscataway, NJ) of 55%, 45%, and 35%. Cells that pelleted below 55% Percoll were used.

Primary Mixed Lymphocyte Culture. T cells from different preparations were incubated at 5×10^4 cells/well in 96-well plates in RPMI 1640 supplemented with 15% human serum (Gibco Laboratories, Grand Island, NY) with equal number of irradiated (9,000 rad) EBV-transformed B lymphocytes (B-LCL) as stimulators. The proliferation was measured by the [^{3}H]TdR uptake (6). Inhibition of MLR with mAbs was performed by preincubating T cells for 30 min at 4°C with anti-CD3 (OKT3) and anti-CD28 (9.3). Stimulator B-LCL were preincubated in the same manner with HLA class II mAbs (anti-DR, L243, and anti-DQ, Tu22), and anti-B7/BB1 (BB1). Anti-HLA class I (W6/32 and 4E) were added to both responding and stimulator cells. The percentage of inhibition was calculated relative to the maximum proliferative response in unblocked control cultures.

Induction of BB1. Small, dense, tonsillar B cells were treated with 5 μg/ml or either biotinylated HB10a, 9.4, or G28-1 for 30 min on ice, washed, and then incubated at 37°C with 20 μg/ml avidin (Sigma Chemical Co., St. Louis, MO) in biotin-free RPMI 1640 with 10% dialyzed FCS for the indicated times. In some experiments B cells were treated with 10 ng/ml of 12-0-tetradecanoylphorbol 13-acetate (PMA) (Sigma Chemical Co.). After incubation, cells were stained with either BB1-FITC or LB-2-FITC mAb.

Results

Anti–CD3-unresponsive CD4$^+$ T cells proliferated to anti-CD3 and anti-CD2 stimulation only when exogenous rIL-2 was supplied, indicating that anti-CD3 or anti-CD2 mAbs did not provide sufficient activation signals for endogenous IL-2 production. The addition of anti-CD28 (mAb 9.3) to the anti-CD2 combination resulted in a strong mitogenic response (6). Thus, crosslinking of CD3/TCR with anti-CD3 in the presence of APC was not sufficient to induce proliferation of the anti–CD3-unresponsive CD4$^+$ T cell subset. Allogeneic B-LCL provided, however, all the signals necessary to induce proliferation in the anti–CD3-unresponsive subset of CD4$^+$ cells (Fig. 1 and Table 1).

The effects of mAbs on the allogeneic response by the anti–CD3-unresponsive CD4$^+$ T cells are shown in Fig. 1. Cell proliferation was completely abrogated by CD3 mAb and by HLA class II mAbs. CD28 mAb 9.3 and mAb BB1 inhibited cell proliferation by 87% and 93%, respectively. This inhibition of MLR by CD28 mAb was similar to that previously obtained with total peripheral T cells (8). Anti-HLA class I mAbs W6/32 plus 4E also inhibited the MLR by 76%. In contrast, mAbs binding either CD1c, CD5, or CD22 did not inhibit MLR (data not shown).

A summary of the proliferative in vitro responses of total CD4$^+$ and anti–CD3-unresponsive CD4$^+$ T lymphocytes is presented in Table 1. The results indicate that the anti–CD3-unresponsive CD4$^+$ subset proliferated less in response to alloantigens compared with total CD4$^+$ cells. In spite of this difference, the pattern as well as the degree of blocking of primary MLR caused by the mAbs were similar for both T cell preparations. This would imply that the activation process is initiated in the same manner in both total CD4$^+$ T cells and anti–CD3-unresponsive T cells and is further dependent on the same costimulatory secondary signals. Anti-CD28 inhibited MLR when added during the first 24 h of culture, while this antibody had no significant inhibitory effect when added at 72 h (data not shown).

Efficient crosslinking of HLA class II antigens on B cells transmits an activation signal(s) to human B cells (9). As shown in Fig. 2 *A*, crosslinking of HLA-DR antigens on B cells stimulated an increased expression of B7/BB1. The effect was specific since (*a*) PMA did not induce increases in B7/BB1 although it did increase CD54 in some cells, and (*b*) crosslinking CD37, which like class II is expressed at high levels on B cells, did not induce significant increases in B7/BB1. Crosslinking CD45 on B cells also increased B7/BB1 levels, but unlike stimulation via HLA class II, signaling via CD45 also rendered a dramatic increase in CD54 in some cells. The

Table 1. *Inhibition of Allogeneic Responses by Peripheral CD4$^+$ T Lymphocytes and Anti–CD3-unresponsive CD4$^+$ T lymphocytes*

Responder cells	CD4$^+$ T cells	Anti–CD3-unresponsive CD4$^+$ T Cells
Mean (cpm)	82,036	45,200
Range (cpm)	17,717–187,555	10,415–74,640
No. of experiments	13	12
Anti-CD3		
Mean (percent inhibition)	99	99
Range (percent inhibition)	99–100	98–99
No. of experiments	3	2
Anti-HLA class II		
Mean (percent inhibition)	86	97
Range (percent inhibition)	66–96	96–97
No. of experiments	5	2
Anti-CD28		
Mean (percent inhibition)	82	90
Range (percent inhibition)	62–96	87–91
No. of experiments	4	4
Anti-B7/BB1		
Mean (percent inhibition)	75	84
Range (percent inhibition)	62–89	75–93
No. of experiments	3	2

Peripheral CD4 T lymphocytes and anti–CD3-unresponsive CD4 T cells were isolated as described in Materials and Methods. Allogeneic B-LCL (irradiated 9,000 rad) were used as stimulator cells. mAbs were used in dilutions ranging from 6.25 to 300 μg/ml, as described in Materials and Methods. Maximum percent inhibition is shown. The allogeneic responses of CD4$^+$ T cells differed significantly from the response by anti–CD3-unresponsive CD4$^+$ cells ($p = 0.0025$, Wilcoxon Rank Test).

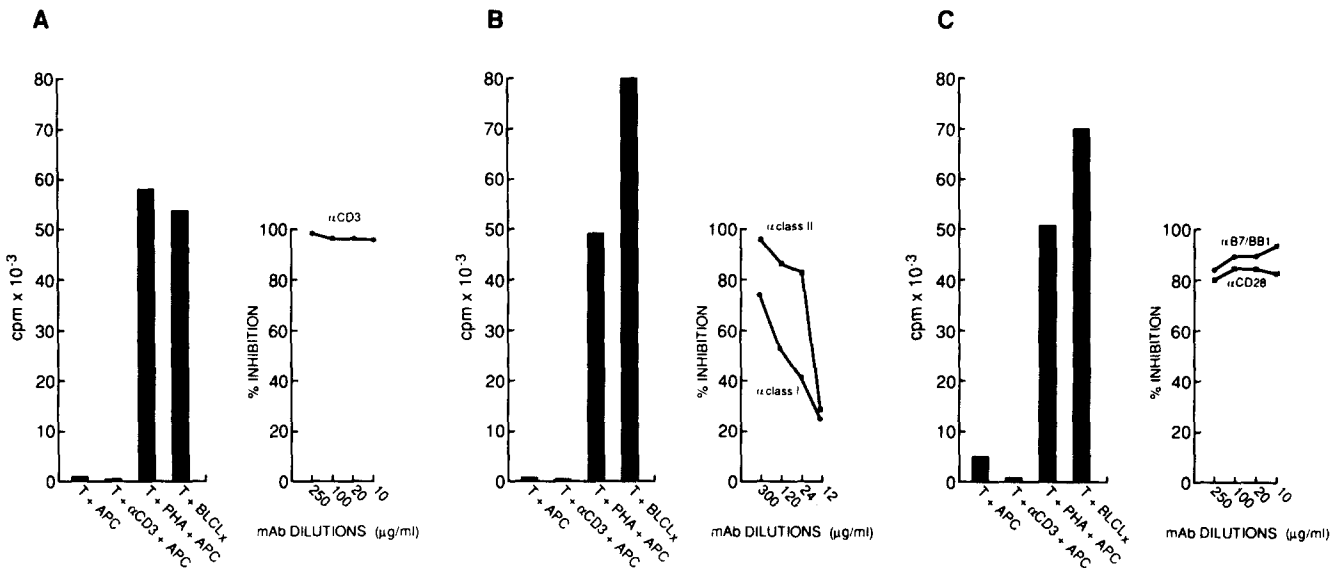

Figure 1. Inhibition of the allogeneic proliferation of anti-CD3-unresponsive CD4+ T cells by mAbs. Freshly isolated anti-CD3-unresponsive CD4+ lymphocytes (see Materials and Methods) were cultured with irradiated EBV-transformed B cells ($BLCL_x$) for 6 d. At the onset of the cultures the following mAbs were added: anti-CD3 (OKT3), anti-HLA class II (L243 and Tu22), anti-HLA class I (W6/32 and 4E), anti-CD28 (9.3), and anti-B7/BB1 (BB1), in concentrations as indicated. The maximum proliferative response in the absence of antibodies was determined in the control unblocked cultures ($T+BLCL_x$). The purity of the responder population was assessed by the lack of proliferation when stimulated with anti-CD3 in the presence of 20% APC (T + anti-CD3 + APC).

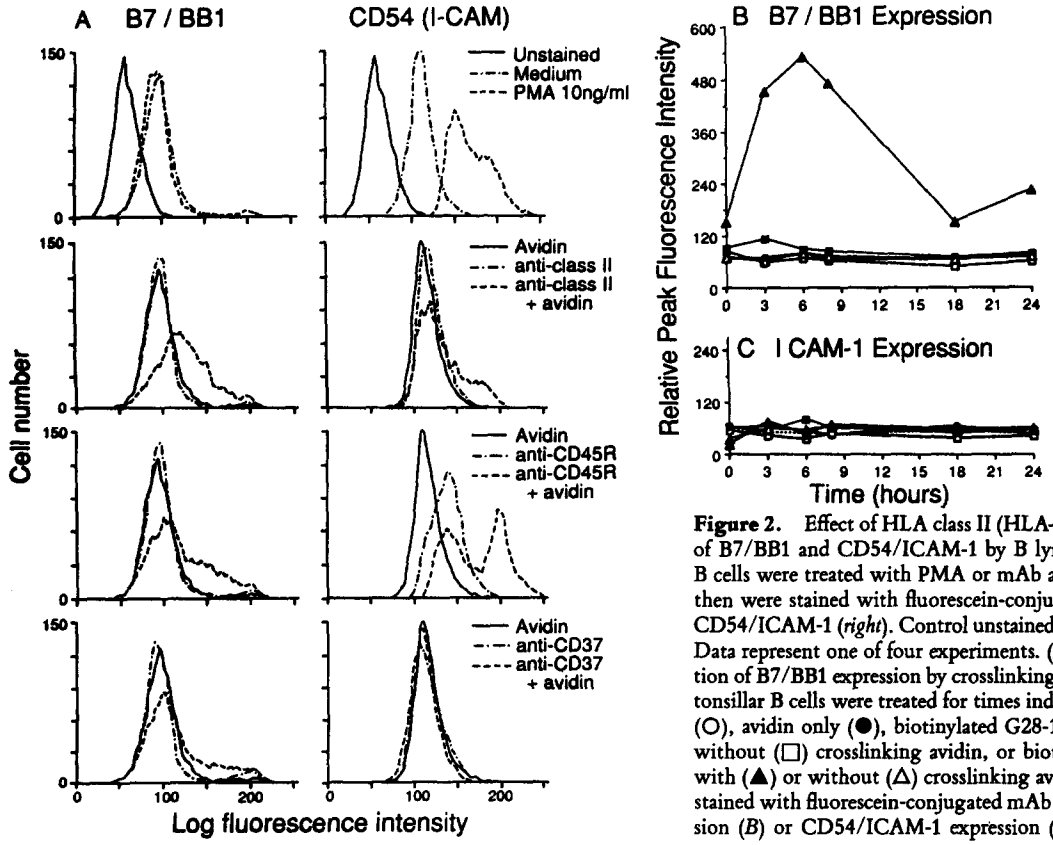

Figure 2. Effect of HLA class II (HLA-DR) crosslinking on expression of B7/BB1 and CD54/ICAM-1 by B lymphocytes. (*A*) Dense tonsillar B cells were treated with PMA or mAb as indicated for 6 h at 37°C and then were stained with fluorescein-conjugated mAb to B7/BB1 (*left*) or CD54/ICAM-1 (*right*). Control unstained cells are shown in the top panel. Data represent one of four experiments. (*B* and *C*) Kinetics of the induction of B7/BB1 expression by crosslinking MHC class II molecules. Dense tonsillar B cells were treated for times indicated with either medium only (○), avidin only (●), biotinylated G28-1 anti-CD37 mAb with (■) or without (□) crosslinking avidin, or biotinylated HB10a anti-DR mAb with (▲) or without (△) crosslinking avidin. Stimulated cells were then stained with fluorescein-conjugated mAb to detect either B7/BB1 expression (*B*) or CD54/ICAM-1 expression (*C*).

kinetics of class II induction of B7/BB1 was relatively rapid peaking about 6–8 h after stimulation and then waning (Fig. 2 *B*), while ICAM-1 was not upregulated (Fig. 2 *C*).

Discussion

Recent data suggest that only activated APC are able to mediate antigen presentation to T cells and that a key second signal provided by APC is not a cytokine but a cell surface molecule (reference 10 and reviewed in reference 1). Crosslinking of MHC class II antigens on B cells by mAb leads to an increase in the second messengers such as intracellular free Ca^{2+} and cAMP, and activation of phospholipase C and new protein tyrosine phosphorylation (9). The importance of the signals delivered to the APC through MHC class II molecules is indicated by the observation that B lymphoma cells expressing truncated class II antigens display profound defects in antigen presentation (11). We here demonstrate that crosslinking of HLA-DR antigens on resting B lymphocytes induces maximal expression of B7/BB1 antigen after only 6 h. Taken together these data indicate that presentation of HLA class II alloantigen by B cells to the TCR on $CD4^+$ T cells produce bidirectional activation signals: one towards the T lymphocyte (first signal) and another towards the B lymphocyte, resulting in the induction of B7/BB1. Increased expression of B7/BB1 in turn may facilitate its binding to CD28 to provide a second signal for induction of endogenous IL-2 production.

The interactions between the molecules on the APC and their natural ligands on the T lymphocytes in most instances involve constitutively expressed receptor ligands, some of which become upregulated after TCR-Ag/MHC engagement (3). Activation of CD28 will depend on the capacity of the APC to induce the natural ligand B7/BB1. The present study demonstrates that HLA class II crosslinking on resting B cells will mediate this effect and imply that TCR-Ag/MHC interactions represent the corresponding physiologic activation signal. The finding that soluble B7/BB1 can directly activate T cells via CD28 (12) further supports this model. The B7/BB1 may constitute a unique second signal during primary T cell activation, since B7/BB1 is absent from resting B cells.

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