*Immunology* 1993 **79** 616–620

# B7/BB-1 is a leucocyte differentiation antigen on human dendritic cells induced by activation

D. N. J. HART, G. C. STARLING, V. L. CALDER & N. S. FERNANDO *Haematology Department, Christchurch Hospital, Christchurch, New Zealand*

*Accepted for publication 4 March 1993*

## SUMMARY

Activation of a primary T-lymphocyte response requires additional signals apart from interaction of the T-cell receptor (TcR)/CD3 complex with major histocompatibility complex (MHC) antigens on the antigen-presenting cell. The CD28 antigen on T lymphocytes provides an important co-stimulatory signal to T lymphocytes and we therefore searched for the presence of its ligand, the B7/BB-1 antigen, on blood and tonsil dendritic cells (DC). Blood DC, prepared from peripheral blood mononuclear cells with a minimal period of *in vitro* culture, did not stain with the monoclonal antibody BB-1 using flow cytometry analysis. In contrast, tonsil DC stained weakly for B7/BB-1 compared to positive control cell lines. Polymerase chain reaction (PCR) was used to amplify a 605 base pair (bp) fragment from human B7/BB-1 mRNA and demonstrated significant amounts of B7/BB-1 mRNA in tonsil DC but no specific product was obtained from blood DC, confirming the surface-staining results. Weak expression of B7/BB-1 antigen was detected by immunofluorescence analysis following culture of blood DC with either interferon-$\gamma$ (IFN-$\gamma$) or granulocyte–macrophage colony-stimulating factor (GM-CSF). These data support the concept that blood DC give rise to tissue and/or lymphoid DC, which acquire co-stimulatory ligands as a result of activation and/or differentiation.

## INTRODUCTION

Successful initiation of a primary T-lymphocyte response to foreign antigen requires at least two signals to be delivered to the T lymphocyte by the antigen-presenting cell (APC). The first signal is delivered by the T-cell receptor (TcR) interacting with the major histocompatibility complex (MHC) class II–peptide complex on the APC.[1] A potential candidate for the second (co-stimulatory) signal is one delivered by CD28, a T-cell-restricted 44,000 MW homodimer.[2] Signals delivered through CD28 are co-stimulatory in the presence of a TcR/CD3-mediated signal.[3–11] CD28 signalling is thought to be mediated by stabilization of cytokine mRNA[12] and increased cytokine transcription,[13] via a cyclosporin-A independent pathway.[14] CD28 and the closely related T-cell molecule, CTLA-4, both bind to the B7/BB-1 molecule,[15,16] which was first described as an activation antigen of B lymphocytes.[17,18]

The most potent APC for primary T-lymphocyte responses is the dendritic cell (DC), a leucocyte with a complex and only partially characterized differentiation pathway. DC appear to differentiate from a relatively immature blood DC to become the Langerhans' cells (LC) of the skin, the interstitial DC (found in solid organs) and the interdigitating cell of lymph nodes (reviewed in ref. 19). Expression of the B7/BB-1 antigen on human DC would have important implications for DC-mediated T-cell activation. As DC are susceptible to activation and differentiation *in vitro*,[20] and B7/BB-1 is an activation antigen of B cells[17,18] and monocytes,[21] we isolated blood DC using a technique which employed a minimal period of *in vitro* culture and activation. We show that B7/BB-1 is not expressed on blood DC but that it is expressed on isolated tonsil DC (interdigitating cells) and that B7/BB-1 can be induced on blood DC by culture in interferon-$\gamma$ (IFN-$\gamma$) or granulocyte–macrophage colony-stimulating factor (GM-CSF).

Abbreviations: DC, dendritic cell; LC, Langerhans' cell.

Correspondence: Dr G. C. Starling, Haematology Dept., Christchurch Hospital, Private Bag 4710, Christchurch, New Zealand.

## MATERIALS AND METHODS

*Monoclonal antibody and labelling techniques*

Monoclonal antibodies used in this study were BB-1 (anti-B7/BB-1, IgM, a gift of Dr J. A. Ledbetter, Seattle, WA), (anti-HLA-DP, IgG1, a gift from Professor F. Brodsky, San Francisco, CA), HuNK-2 (CD16, IgG2a, Professor I. F. C. McKenzie, Melbourne, Australia). Antibodies CMRF-15 (anti-sialogycophorin, IgM control), CMRF-20 (anti-HLA class II, IgM), L227 (anti-HLA-DQ, biotin conjugated, IgG1), CMRF-31 (CD14, IgG1), OKT3 (CD3, IgG2), FMC-63 (CD19, IgG2a, hybridoma from Dr H. Zola, Adelaide, Australia), HNK-1 (CD57, IgM) were produced from hybridomas made in this

laboratory (CMRF series) or from hybridomas gifted by colleagues or purchased from the American Type Culture Collection [(ATCC) Rockville, MD]. Phycoerythrin (PE)-conjugated IgG1 control and PE–Leu-16 (CD20, IgG1) were purchased from Becton Dickinson (Mountain View, CA). Cells were labelled with primary antibodies for 30 min prior to addition of fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse (Tago, Burlingame, CA). Mouse serum was added to block non-specific binding in double-labelling experiments using PE-conjugated antibodies or biotin–PE–streptavidin. Immunofluorescence analysis was performed on a Coulter Epics Profile analyser (Coulter, Hialeah, FL).

*Isolation of human peripheral blood DC*

Leucocytes were obtained from normal volunteers by leucopheresis. Mononuclear cells (MNC) were separated on Ficoll–Hypaque (F/H) density gradients. MNC were T-cell depleted by rosetting with neuraminidase (Behring, Marburg, Germany) treated sheep red blood cells (SRBC), and then cultured at 37° in 5% $CO_2$ overnight in medium [10% foetal calf serum (FCS) in RPMI-1640 (Gibco, Auckland, New Zealand) supplemented with 2 mM glutamine and penicillin and streptomycin]. Low-density cells were obtained on bovine serum albumin (BSA) (Intergen, Purchase, NY) gradients, depleted of Fc receptor positive cells by panning on human immunoglobulin (this laboratory)-coated Falcon 1001 plates (Becton Dickinson, Lincoln Park, NJ) for 30 min at 37°. The non-adherent cells were then depleted of B cells by panning on Petri dishes coated with affinity-purified rabbit anti-human immunoglobulin (this laboratory) at 4° for 60 min. The non-adherent cells were harvested and separated on a Nycoprep (Nycomed, Oslo, Norway) gradient. DC were further purified using a cocktail of monoclonal antibodies against CD3, CD14, CD19, CD56, CD57 and FITC-conjugated sheep anti-mouse (Tago) to label the cells and then sort the negative cell fraction on a FACS IV (Becton Dickinson, Mountain View, CA) cell sorter. The resulting cell population was 50–90% DC enriched as judged by morphological analysis and strong expression of the MHC class II products HLA-DQ or HLA-DP. Further gating for immunofluorescence analysis was possible on the basis of high longitudinal and forward scatter to yield preparations of cells > 85% positive for HLA-DP. The highest purity sorted preparations were used for mRNA extraction and analysis by PCR.

*Tonsil DC preparation*

Tonsil DC were obtained as described previously.[22] Briefly, single-cell suspensions were prepared from tonsils obtained at routine tonsillectomy and MNC isolated by F/H gradients followed by T-cell depletion with SRBC. Cells were then cultured overnight at 37° in medium. Following culture, a BSA gradient was used to separate out the low-density DC-enriched fraction. Tonsil DC were then purified by labelling with FMC63, OKT3, and CMRF-31 antibodies followed by sorting as described above, leaving 80–90% pure DC as judged by morphology and staining with anti-HLA-DQ monoclonal antibody.

*PCR analysis of B7 mRNA*

Total mRNA was isolated from approximately $5 \times 10^4$ DC using the Fast Track mRNA isolation kit (Invitrogen, San Diego, CA), and cDNA prepared using oligo-dT priming and AMV reverse transcriptase (Promega, Madison, WI).

For polymerase chain reaction (PCR), a 50 $\mu$l reaction mix containing 1/100 of the cDNA, 0·5 $\mu$M of each B7/BB-1 primer 5′(5′AGTACAAGAACCGGACCATC3′) (nt 573–593) and 3′(5′GGCGTACACTTTCCCTTCTC 3′) (nt 1157–1177) from the published B7 cDNA sequence,[23] 0·2 $\mu$M each dNTP, 1·5 mM $MgCl_2$, 10 mM Tris–HCl (pH 9·0), 50 mM KCl, 1% Triton X-100 and 2·0 U Taq polymerase (Promega) was amplified for 35 cycles in a DNA thermal cycler (Perkin Elmer Cetus, Emeryville, CA). Each cycle comprised 96° (1 min), 50° (1 min) and 72° (1 min) with a 10-min extension at 72° for the final cycle. The PCR amplified products (605 bp for B7/BB-1) were run on a 1% agarose gel, ethidium stained and visualized under ultraviolet (UV).

The amplified DNA was transferred to Hybond-N plus membranes (Amersham International, Amersham, U.K.) by vacuum, dried, and UV fixed for 45 seconds. The membrane was prehybridized for 30 min at 37° and then hybridized overnight at 37° with an internal $\gamma$ ATP-$^{32}$P (Amersham) end-labelled oligonucleotide (5′ TTGATGAGACACATGAAG 3′) (nt 956–973). The blots were washed with 6 × SSC for 10 min at 37°, 2 × SSC + 0·1% SDS at 50° for 10 min and 1 × SSC + 0·1% SDS at 50° (Tm for internal oligonucleotide = 50°) for 10 min. The blots were exposed to Kodak X-Omat film for 1–23 hr before developing.

Amplification of the dihydrofolate reductase gene was used as a positive control for PCR amplification and produced a 499 bp product.

*Monocyte preparation*

Enriched preparations were obtained by removing Fc receptor positive cells from the human Ig panning plates of the blood DC preparation described above. Monocytes were gated on the basis of size and forward scatter on flow cytometric analysis and were > 90% CD14$^+$.

*Activation of DC and monocytes*

Activation experiments were carried out in medium using cells at $1 \times 10^6$/ml. IFN-$\gamma$ (a gift from Hoffman-La Roche, Nutley, NJ) was used at 500 U/ml. GM-CSF (Leucomax, a gift from Sandoz Pharma, Auckland, New Zealand) was used at 100 U/ml or 500 U/ml.

## RESULTS

### Resting blood dendritic cells do not express B7/BB-1 antigen

Immunofluorescence analysis failed to reveal the presence of B7/BB-1 antigen on highly purified DC ($n = 5$, Fig. 1a), despite characteristic immunofluoresence staining of B-cell lines Raji (Fig. 1b), Mann and JY (not shown). The DC were strongly HLA class II positive, had typical DC morphology, and were strongly stimulatory in a mixed lymphocyte reaction (MLR).[24] These results were in direct contrast to a previous report[25] showing B7/BB-1 antigen expressed at detectable levels on a population of lineage marker negative cells (blood DC) identified within a PBMC preparation of highly MLR stimulatory cells. Given this result we decided to look at an alternative DC source, to investigate whether another population of DC did in fact express the B7/BB-1 antigen.

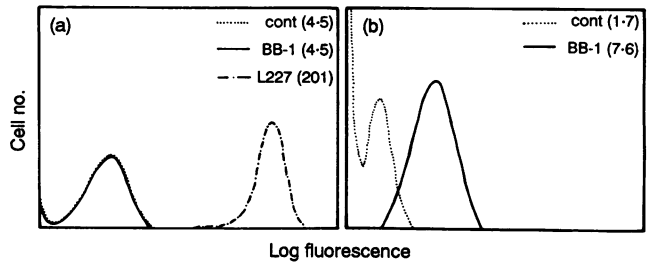


**Figure 1.** Blood dendritic cells do not express B7/BB-1 antigen. Immunofluorescence profiles of (a) blood DC or (b) the Raji cell line. Blood DC were labelled with IgM control (cont) or double labelled with BB-1 and L227-biotin/streptavidin-PE, and histograms redrawn to the same scale. Figures in brackets are mean channel fluorescence.

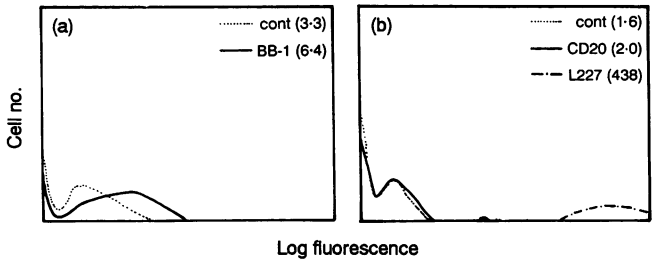


**Figure 2.** Tonsil DC express B7/BB-1 antigen. Superimposed immunofluorescence profiles of (a) IgM control (cont) and BB-1 staining, and (b) a double-labelling analysis confirming strong HLA-DQ and lack of CD20 labelling on purified tonsil DC. Figures in brackets are mean channel fluorescence.

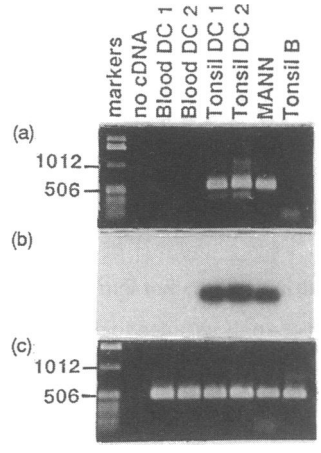


**Figure 3.** FACS-purified tonsil but not blood DC express B7 mRNA. mRNA was prepared from 50,000 lineage marker negative DC, and cDNA synthesized using oligo-dT primer and AMV reverse transcriptase. (a) Ethidium bromide stained gel showing a 605 bp band amplified from tonsil DC and the Epstein-Barr (EBV)-transformed B-cell line MANN. (b) Specificity of the reaction was determined using Southern blotting with a $^{32}P$ end-labelled primer. (c) Dihydrofolate reductase PCR as a cDNA control.

### Isolated tonsil DC express B7/BB-1 without prior stimulation

DC were enriched from human tonsil mononuclear cells as already described. These preparations were stained with monoclonal antibody BB-1 and double labelled with CD20 or anti-HLA-DQ antibodies. The BB-1 antigen was expressed on a population of cells which lacked CD20, but were strongly positive for HLA-DQ (Fig. 2). As the only contaminating cells in our tonsil DC preparations are B lymphocytes,[22] the $CD20^-$ $DQ^+$ cells were DC.

### PCR analysis of B7/BB-1 mRNA in DC

Given our findings that blood DC lacked B7/BB-1, although the antigen was weakly expressed on tonsil DC, and a conflicting report describing the presence of the B7/BB-1 antigen on blood DC (also obtained using BB-1 mAb),[25] we performed additional PCR studies. PCR analysis for B7 gene expression, using cDNA prepared from highly purified blood ($n=2$) and tonsil ($n=2$) DC confirmed our immunofluorescence data indicating that blood DC, prepared by our method, did not express either B7/BB-1 mRNA or surface protein. In contrast PCR analysis of tonsil DC resulted in a B7-specific amplifed band derived from mRNA comparable to the control Mann B-cell line (Fig. 3). It is possible that B7/BB-1 is expressed on DC according to their state of differentiation and that tissue (tonsil) DC express this ligand upon interaction with antigen, tissue epithelium, or T lymphocytes. A non-exclusive alternative explanation is that B7/BB-1 is expressed as an activation antigen on DC.

### Induction of B7/BB-1 following activation of blood DC

As *in vitro* culture may alter the phenotype and function of DC,[26] we tested whether the B7/BB-1 antigen could be induced on blood DC. To activate blood DC we first used IFN-$\gamma$, a stimulus known to up-regulate the expression of B7/BB-1 on peripheral blood monocytes.[21] In our hands, monocytes ($>90\%$ $CD14^+$) up-regulated B7/BB-1 following incubation in 500 U of IFN-$\gamma$ for 24–32 h. To our surprise, B7/BB-1 was expressed on the DC surface following culture for this period in medium alone as well as with IFN-$\gamma$, at levels similar to those on tonsil DC ($n=2$). In the blood DC preparation shown (Fig. 4d–f), small lymphocytes (negative for lineage markers and HLA-DP) were contaminants of the preparation (gated to 85% positive for HLA-DP) and it is possible that these cells produced cytokines that up-regulated B7/BB-1 expression during the period of tissue culture.

We then tested the effect of GM-CSF on the expression of BB-1 antigen on blood DC since this cytokine alters DC surface antigen expression and DC function.[27] We found that GM-CSF was also able to induce B7/BB-1 antigen in a dose-dependent manner ($n=2$). In contrast to experiments using impure DC preparations, culture in medium alone did not induce B7/BB-1 in these experiments (Fig. 5), which used DC preparations of greater purity. This provides evidence that incubating DC with other cell types for prolonged periods in culture can activate DC.

## DISCUSSION

Resting blood DC do not express the B7/BB-1 antigen but can be induced to do so by culturing them. Tonsil DC (interdigitat-

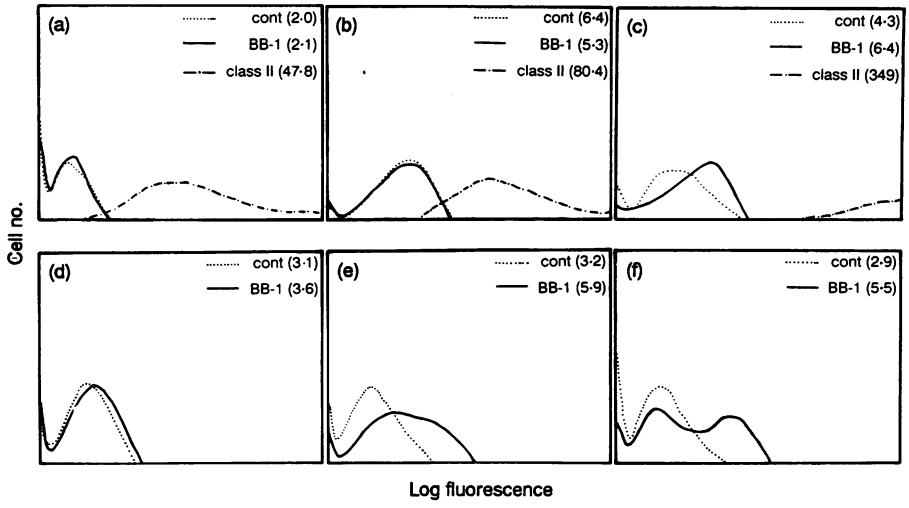


**Figure 4.** Expression of BB-1 antigen on gated monocytes and DC following *in vitro* culture. Monocytes [gated Fc receptor positive cells (a–c)] and DC [FACS sorted and gated to >85% HLA-DP positive (d–f) to exclude contaminating small mononuclear cells], were incubated in medium alone for 0 hr (a, d) or for 32 hr in medium in the absence (b, e) or presence (c, f) of 500 U/ml IFN-γ. Figures in brackets are mean channel fluorescence.

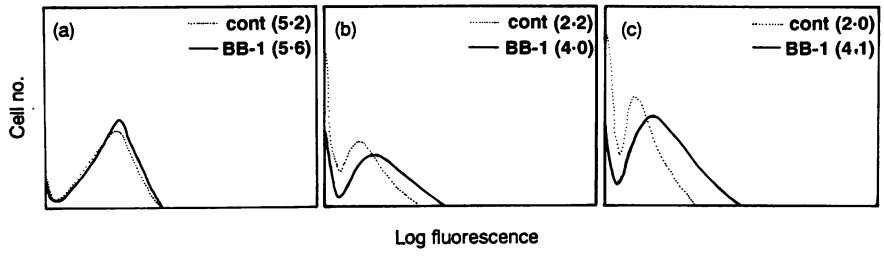


**Figure 5.** Highly purified blood DC weakly express B7/BB-1 antigen following activation with GM-CSF. Blood DC were activated for (a) 0 hr, (b) 24 hr in 100 U/ml GM-CSF, (c) 24 hr in 500 U/ml GM-CSF. Figures in brackets are mean channel fluorescence.

ing cells) do, however, express both B7/BB-1 mRNA and cell-surface antigen. We conclude, therefore, that B7/BB-1 is an activation and/or differentiation antigen of human DC. The fact that others have found B7/BB-1 on blood DC[25] probably reflects differences between the methods of DC preparation, some of which[25] activate the DC. In support of our conclusions, LC in the mouse do not express a CTLA-4 ligand,[28] whereas mouse splenic DC do express a ligand, which is most probably B7/BB-1.[11,28] Activation of the murine LC however induces expression of the CTLA-4 ligand.[28] Given that our tonsil DC preparation is not dissimilar to the blood DC preparation it seems probable that B7/BB-1 expression on these cells represents the situation *in vivo*. This may also be true for mouse spleen DC prepared by a method which involves minimal activating stimuli.[11]

Although freshly isolated LC lacking B7/BB-1 are less stimulatory than cultured LC, our blood DC, which also lacked expression of B7/BB-1 are highly allostimulatory.[24] B7/BB-1 is therefore either not important in human blood DC–T lymphocyte responses (and perhaps a second ligand might exist for CD28), or interactions between the DC and T lymphocytes, during the course of the MLR, may induce expression of B7/BB-1 on DC. Current data support the hypothesis that interaction of the DC with the T lymphocyte results in a T-lymphocyte feedback loop activating the DC. Certainly, HLA class II antigens have the capability to transmit intracellular signals, and one outcome of HLA class II signalling is the induction of B7/BB-1 antigen expression.[5,25,29] Blood DC may therefore up-regulate B7/BB-1 on receiving reciprocal signal(s) from T lymphocytes interacting with DC surface antigen, or by exposure to cytokines released locally from natural killer (NK) or myeloid cells within the tissues. Cytokine stimulation of blood DC in our experiments produced only low B7/BB-1 expression as compared to its level of expression on activated B cells[17,18] or on DC following interactions with allogeneic T lymphocytes.[25] GM-CSF and IFN-γ are probably not optimal stimuli for B7/BB-1 expression. However, given the low level of the expression of the high-affinity B7/BB-1 ligand CTLA-4 on resting T cells,[30] low expression of B7/BB-1 may be sufficient to saturate CTLA-4 on resting T cells. Current views on the differentiation and migration patterns of DC would support lymphoid and spleen DC as having interacted with antigen and T lymphocytes in the tissues.[19] DC in these compartments would therefore be expected to express the B7/BB-1 antigen. It is also possible that B7/BB-1 is not an essential co-stimulatory molecule on human blood DC and that they express alternative co-stimulatory molecules. A potential candidate would be a human homologue of the murine heat-stable antigen, which provides a significant co-stimulatory signal from murine DC.[11]

Recent data suggest that blocking the B7/BB-1–CD28 interaction by CTLA4 immunoglobulin facilitates acceptance of human islet xenografts.[31] An anti-human B7 antibody (non-reactive with mouse) was also immunosuppressive and this would be consistent with the concept that the xenogeneic

reaction results in induction of B7/BB-1 on pancreatic DC, which are then blocked or destroyed by interaction with the CTLA4 immunoglobulin. This mimics the immunosuppressive effect seen when donor DC are depleted from transplanted tissue.[32] The difference between B7/BB-1 expression on human blood and tonsil DC not only emphasizes the potential influence of activation signals and T-lymphocyte interactions on DC differentiation, but highlights the need to develop methods for obtaining human blood DC in a minimally activated state for functional studies.

## ACKNOWLEDGMENTS

We would like to thank Dr G. Clark for comments and Mrs S. Banks for typing the manuscript. This work was supported by the McClelland Trust, The Cancer Society of New Zealand (Canterbury/Westland Division) and the Health Research Council of New Zealand.

## REFERENCES

1. Jenkins M.K. (1992) The role of cell division in the induction of clonal anergy. *Immunol. Today*, **13,** 69.
2. June C.H., Ledbetter J.A., Linsley P.S. & Thompson C.B. (1990) Role of the CD28 receptor in T-cell activation. *Immunol. Today*, **11,** 212.
3. Damle N.K., Doyle L.V., Grosmaire L.S. & Ledbetter J.A. (1988) Differential regulatory signals delivered by antibody binding to the CD28 (Tp44) molecule during the activation of human T lymphocytes. *J. Immunol.* **140,** 1753.
4. Jenkins M.K., Taylor P.S., Norton S.D. & Urdahl K.B. (1991) CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J. Immunol.* **147,** 2461.
5. Koulova L., Clark E.A., Shu G. & Dupont B. (1991) The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of $CD4^+$ T cells. *J. exp. Med.* **173,** 759.
6. Linsley P.S., Brady W., Grosmaire L., Aruffo A., Damle N.K. & Ledbetter J.A. (1991) Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. exp. Med.* **173,** 721.
7. Freeman G.J., Gray G.S., Gimmi C.D., Lombard D.B., Zhou L-J., White M., Fingeroth J.D., Gribben J.G. & Nadler L.M. (1991) Structure, expression, and T cell costimulatory activity of the murine homologue of the human B lymphocyte activation antigen B7. *J. exp. Med.* **174,** 625.
8. Azuma M., Cayabyab M., Buck D., Phillips J.H. & Lanier L.L. (1992) CD28 interaction with B7 costimulates primary allogeneic proliferative responses and cytotoxicity mediated by small, resting T lymphocytes. *J. exp. Med.* **175,** 353.
9. Harding F.A., McArthur J.G., Gross J.A., Raulet D.H. & Allison J.P. (1992) CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature*, **356,** 607.
10. Lui Y. & Janeway C.A., Jr. (1992) Cells that present both specific ligand and costimulatory activity are the most efficient inducers of clonal expansion of normal CD4 T cells. *Proc. natl. Acad. Sci. U.S.A.* **89,** 3845.
11. Liu Y., Jones B., Brady W., Janeway C.A. Jr & Linley P.S. (1992) Co-stimulation of murine CD4 T cell growth: cooperation between B7 and heat-stable antigen. *Eur. J. Immunol.* **22,** 2855.
12. Lindsten T., June C.H., Ledbetter J.A., Stella G. & Thompson C.B. (1989) Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science*, **244,** 339.
13. Fraser J.D., Irving B.A., Crabtree G.R. & Weiss A. (1991) Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. *Science*, **249,** 313.
14. June C.H., Ledbetter J.A., Gillespie M.M., Lindsten T. & Thompson C.B. (1987) T cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol. cell. Biol.* **7,** 4472.
15. Linsley P.S., Clark E.A. & Ledbetter J.A. (1990) T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc. natl. Acad. Sci. U.S.A.* **87,** 5031.
16. Linsley P.S., Brady W., Urnes M., Grosmaire L.S., Damle N.K. & Ledbetter J.A. (1991) CTLA-4 is a second receptor for the B cell activation antigen B7. *J. exp. Med.* **174,** 561.
17. Yokochi T., Holly R.D. & Clark E.A. (1982) B lymphoblast antigen (BB-1) expressed on Epstein-Barr virus-activated B cell blasts, B lymphoblastoid cell lines and Burkitt's lymphomas. *J. Immunol.* **128,** 823.
18. Freedman A.S., Freeman G., Horowitz J.C., Daley J. & Nadler L.M. (1987) B7, a B cell-restricted antigen that identifies preactivated B cells. *J. Immunol.* **139,** 3260.
19. Hart D.N.J. & McKenzie J.L. (1990) Interstitial dendritic cells. *Int. Rev. Immunol.* **6,** 127.
20. Romani N., Lenz A., Glassel H., Stossel H., Stanzl U., Majdic D., Fritsch P. & Schuler G. (1989) Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. *J. invest. Dermatol.* **93,** 600.
21. Freedman A.S., Freeman G.J., Rhynhart K. & Nadler L.M. (1991) Selective induction of B7/BB-1 on interferon-$\gamma$ stimulated monocytes: a potential mechanism for amplification of T cell activation through the CD28 pathway. *Cell. Immunol.* **137,** 429.
22. Hart D.N.J. & McKenzie J.L. (1988) Isolation and characterization of human tonsil dendritic cells. *J. exp. Med.* **168,** 157.
23. Freeman G.J., Freedman A.S., Segil J.M., Lee G., Whitman J.F. & Nadler L.M. (1989) B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J. Immunol.* **143,** 2714.
24. Hart D.N.J. & Prickett T.C.R. (1993) Intercellular adhesion molecule-2 (ICAM-2) expression on human dendritic cells. *Cell. Immunol.* **148,** 447.
25. Young J.W., Koulova L., Soergel S.A., Clark E.A., Steinman R.M. & Dupont B. (1992) The B7/BB1 antigen provides one of several costimulatory signals for the activation of $CD4^+$ T lymphocytes by human blood dendritic cells *in vitro*. *J. clin. Invest.* **90,** 229.
26. Girolomoni G., Simon J.C., Bergstresser P.R. & Cruz P.D. Jr. (1990) Freshly isolated spleen dendritic cells and epidermal Langerhans cells undergo similar phenotypic and functional changes during short term culture. *J. Immunol.* **145,** 2820.
27. MacPherson G.G., Fossum S. & Harrison B. (1989) Properties of lymph-borne (veiled) dendritic cells in culture. II Expression of the IL-2 receptor: role of GM-CSF. *Immunology*, **68,** 108.
28. Larsen C.P., Ritchie S.C., Pearson T.C., Linsley P.S. & Lowry R.P. (1992) Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell populations. *J. exp. Med.* **176,** 1215.
29. Nabavi N., Freeman G.J., Gault A., Godfrey D., Nadler L.M. & Glimcher L.H. (1992) Signalling through the MHC class II cytoplasmic domain is required for antigen presentation and induces B7 expression. *Nature*, **360,** 266.
30. Linsley P.S., Greene J.L., Tan P., Bradshaw J., Ledbetter J.A., Anasetti C. & Damle N.K. (1992) Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. *J. exp. Med.* **176,** 1595.
31. Lenschow D.J., Zeng, Y., Thistlewaite J.R., Montag A., Brady W., Gibson M.G., Linsley P.S. & Bluestone J.A. (1992) Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science*, **257,** 789.
32. McKenzie J.L., Beard M.E.J. & Hart D.N.J. (1984) Effective depletion of donor dendritic cells prolongs rat cardiac allograft survival. *Transplantation*, **38,** 371.