Immunology 1995 **86** 325–330

Alloantigen presentation by B cells: analysis of the requirement for B-cell activation

J. L. WILSON, A. C. CUNNINGHAM & J. A. KIRBY *Department of Surgery, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

SUMMARY

This paper describes a model for investigation of the functional implications of B-cell activation for antigen presentation. Mixed lymphocyte cultures were used to assess the ability of freshly isolated B cells, mitogen-activated B cells and Epstein–Barr virus (EBV)-transformed B-cell lines to stimulate the activation and proliferation of allogeneic T cells under a variety of experimental conditions. It was found that resting B cells presented antigen poorly, while activated cells were highly immunogenic. Paraformaldehyde fixation completely eliminated antigen presentation by resting B cells, despite constitutive expression of class II MHC antigens. However, fixation had little effect on antigen presentation by activated B cells that expressed B7-1 and B7-2 in addition to class II major histocompatibility complex (MHC) molecules. Arrest of B-cell activation by serial fixation after treatment with $F(ab')_2$ fragments of goat anti-human IgM produced cells with variable antigen-presenting capacity. Optimal antigen presentation was observed for cells fixed 72 hr after the initiation of B-cell activation. Although both B7-1 and B7-2 antigen expression increased after B-cell activation, it was found that the rate of T-cell proliferation correlated most closely with B7-2 expression. Stimulation of T cells by fixed activated B lymphocytes could be blocked by antibodies directed at class II MHC molecules, indicating involvement of the T-cell antigen receptor. In addition, T-cell proliferation was inhibited by antibodies specific for B7-1 and B7-2 and by the fusion protein CTLA4–Ig, demonstrating a requirement for CD28 signal transduction. The sole requirement of B7 family expression for antigen presentation by B lymphocytes was shown by demonstration of T-cell stimulation by fixed resting B cells in the presence of CD28 antibody as a source of artificial costimulation.

INTRODUCTION

In 1975 Lafferty & Cunningham[1] proposed that T-cell proliferation is dependent on simultaneous delivery of two activation signals. Engagement between the T-cell receptor (TCR) and an immunogenic peptide–major histocompatibility complex (MHC) molecule complex produces the first signal, while the second, or costimulatory, signal is generated by interaction between non-polymorphic cell-surface molecules on the T cell and corresponding ligands on the antigen-presenting cell (APC).[2]

The well-characterized T-cell surface molecule CD28 can transduce costimulatory signals. Antibody stimulation of CD28 on resting T cells produces no observable effects but lymphocyte proliferation occurs when this is coupled with TCR stimulation.[3] Signal transduction by CD28 results in increased interleukin-2 (IL-2) production by antigen-stimulated T cells.[4] This is caused by the binding of specific factors to regions of the IL-2 gene promoter that enhance transcription,[5] and by inhibition of the degradation of mRNA encoding this cytokine.[6]

A second T-cell surface molecule, CTLA4, has been cloned and has significant homology to CD28.[7,8] CTLA4 mRNA transcripts are present at lower levels than CD28 transcripts in human T cells. Monoclonal antibodies to CTLA4 do not costimulate T-cell growth but have a synergistic effect when added together with CD28-specific antibodies.[9] Using a chimeric fusion protein CTLA4–immunoglobulin (Ig), it is possible to inhibit T-cell responses. CTLA4–Ig binds to B7-1 with 20-fold higher affinity than a similar CD28 construct.[8]

It was shown initially that the B-cell antigen B7 was a natural ligand for both CD28 and CTLA4. Transfected cells expressing high levels of B7 could provide costimulatory signals to antigen-activated T cells.[10] Furthermore, antibodies to the B7 antigen could inhibit an allogeneic mixed lymphocyte reaction (MLR).[11] Evidence for the additional ligand B7-2 came from analysis of B7-deficient mice[12] that only had a 70% loss of costimulation in MLR. B7-1 (CD80) and B7-2 (CD86) are both members of the immunoglobulin superfamily. The expression of these ligands on B cells is increased on activation

Received 5 June 1995; revised 10 July 1995; accepted 12 July 1995.

Correspondence: Dr J. A. Kirby, Department of Surgery, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK.

but neither B7-1 nor B7-2 binds CD28 or CTLA4 preferentially. Differential antibody-labelling provides limited evidence of the existence of a third ligand for CD28 and CTLA4.[13]

It is reasonable to suppose that B cells should be able to present antigen to $CD4^+$ T cells by virtue of their constitutive expression of class II MHC antigens and their ability to capture antigen with membrane immunoglobulins. Early studies showed that the state of B-cell differentiation plays a critical role in determining this antigen-presenting capacity.[14] It is known that activated B cells and dendritic cells share many antigen-presenting properties[15] but that B cells generally provide inferior costimulation of T-cell activation.[16]

It has been proposed recently that antigen presentation by resting B cells can anergize T cells but that T-cell activation occurs when antigen is presented by activated B cells.[17] Further studies have suggested that presentation of antigen by activated or resting B cells always induces naive T cells to become tolerant.[18]

The current study examined the expression of B7-1 and B7-2 by resting B cells and during the process of B-cell activation induced by stimulation of the cell-surface immunoglobulin receptors. The effect on the activation of allogeneic T cells of differing expression of these antigens was determined by fixation of B cells at various times during the activation process and by blockade of specific costimulatory and MHC molecules.

MATERIALS AND METHODS

Isolation of tonsil lymphocytes

Tonsillar lymphocytes were prepared by a modification of the method of Watanabe *et al.*[19] Briefly, human tonsil tissue was obtained at tonsillectomy and was stored in RPMI-1640 medium (Gibco BRL, Paisley, UK) at 4° for a maximum of 2 hr before processing.

Tonsil tissue was chopped into small pieces and the cells were dispersed by teasing and filtration through a wire mesh. The cell suspension was layered over Ficoll–Hypaque at 1·077 g/ml (Lymphoprep; Nycomed, Birmingham, UK). The gradients were centrifuged at 400 ***g*** for 20 min at room temperature, and the interfacial cells were collected and washed twice in complete medium consisting of RPMI-1640 containing 10% (v/v) fetal calf serum (FCS; Gibco), HEPES buffer (pH 7·3, 10^{-2} M), 2-mercaptoethanol (5×10^{-5} M), penicillin and streptomycin (both at 100 μg/ml).

Peripheral blood mononuclear cells (PBMC)

PBMC were isolated from heparinized blood samples taken from healthy volunteers by 1 : 1 dilution with RPMI-1640 and centrifugation for 25 min at 400 ***g*** over a Ficoll–Hypaque gradient. Interfacial cells were recovered as described above, washed and resuspended in complete RPMI-1640 medium at a concentration of 1×10^6 cells/ml.

$CD4^+$ T-cell purification

$CD4^+$ T cells were isolated by positive selection using the Dynabead–Detachabead system (Dynal UK Ltd, Merseyside, UK). Briefly, 10^7 PBMC were resuspended in 1 ml of phosphate-buffered saline (PBS; Gibco), supplemented with 1% FCS (PBS/FCS). Sufficient mouse anti-human CD4 antibody-coated magnetic immunobeads were added to achieve a final ratio of three beads per target cell. The suspension was rotated on ice for 60 min. The rosetted cells were removed from the lymphocyte suspension using a magnet and were washed in PBS/FCS to remove contaminating cells.

The $CD4^+$ cells were resuspended in 100 μl of RPMI-1640 medium supplemented with 1% FCS (RPMI/FCS) and were released from the beads by adding one unit of Detachabead solution (sheep anti-mouse antibody) and rotating gently at room temperature for 45 min. The magnetic beads were removed from the cell population with a magnet. The remaining cell suspension was washed three times in RPMI/FCS to remove any remaining Detachabead solution and was resuspended in complete media at a concentration of 1×10^6 cells/ml. The purity and pheotype of the positively selected cells was determined using flow cytometry and found to be > 99% $CD4^+$; the cells were refractory to stimulation by phytohaemagglutinin (PHA).

$CD19^+$ B-cell purification

$CD19^+$ B cells were also isolated by positive selection using the Dynabead–Detachabead system. B cells were purified from tonsillar lymphocytes, using a final bead to cell ratio of 5 : 1, and were released from the beads as described above.[20] More than 99% of the purified cells expressed CD19; contamination by CD14-expressing mononuclear phagocytes was routinely less than 0·5%.

Pre-activation of B cells

For some experiments it was necessary to pre-activate B cells before use as APC. Postivively selected $CD19^+$ B cells were incubated at a concentration of 1×10^6/ml with 20 μg/ml of goat anti-human IgM $F(ab')_2$ fragment (Sera-Lab, Crawley Down, UK).[21] The cells were harvested at daily intervals and washed twice in complete media. Some of these cells were labelled with optimal concentrations of antibodies specific for class II MHC (CR3/43; Dako, High Wycombe, UK), B7-1 (CD80; Becton Dickinson, Oxford, UK) or B7-2 (CD86; Pharmingen, San Diego, CA) and were incubated at 4° for 30 min. The cells were then washed, counterstained with a fluorescein isothiocyanate (FITC)-conjugated polyclonal secondary antibody preparation (Becton Dickinson; fluorochrome : protein ratio of 4·3) and incubated for a further 30 min at 4°. The cells were then washed and analysed by flow cytometry (FACScan; Becton Dickinson); in each case a minimum of 10 000 events was accumulated.

Fluorescence standard curves were routinely generated using FITC-conjugated beads (FCSC; Becton Dickinson) to quantify the logarithmic photomultiplier signals. All fluorescence results were expressed as linear median equivalents of soluble FITC (MESF) values, which may be compared directly across a time–course experiment.

Generation of Epstein–Barr virus (EBV)-transformed cell line

An immortalized B-cell line was obtained by transformation of tonsillar lymphocytes. Tonsil lymphocytes (10^7) were added to culture superntant from the EBV-secreting B95-8 cell line (ECACC 8501419; ECACC, Porton Down, UK). Cyclosporin A (Sandoz, Basle, Switzerland) was added at 1 μg/ml to the cell culture to abolish T-cell growth; the outgrowing B cells were cultured in complete media.

Culture of CD19$^+$ B lymphocytes with allogeneic lymphocytes

Freshly isolated or pre-activated B cells were either irradiated (25 Gy; ^{137}Cs source) or fixed using paraformaldehyde as described by Jenkins *et al.*[11] In brief, cells were incubated for 10 min at room temperature in 0·15% (w/v) paraformaldehyde (Sigma, Poole, UK) in PBS and were then incubated for 20 min at 37° in complete media. Fixed cells were washed twice in PBS and resuspended in complete media before use. A series of experiments was performed in which B cells were activated by treatment with anti-human IgM F(ab')$_2$ antibody fragment and were harvested daily for fixation and use as stimulators in allogeneic mixed cell cultures.

B lymphocytes were titrated into the wells of round profile 96-well plates (Falcon, Oxnard, CA) in 50-μl aliquots containing between 1×10^5 cells and 12 500 cells. Fifty microlitres of PBMC or CD4$^+$ T cells were then added to each well to give stimulator : responder cell ratios ranging from 1 : 1 to 0·125 : 1. Wells were made up to a total volume of 200 μl by adding a range of concentrations of either the fusion protein CTLA4–Ig (kindly provided by Dr P. S. Linsley, Bristol Meyers Squibb, Seattle, WA) and antibodies specific for B7-1, B7-2 and CD28 (provided by Dr P. S. Linsley) or anti-class II MHC antigen. Each microculture was established in triplicate on at least three separate occasions.

Fifty microlitres of medium was removed from some assays for IL-2 bioassay after culture for 3 days. Lymphoproliferation was assessed after 5 days by addition of 1 μCi of [^{3}H]thymidine (TRA61; Amersham International, Amersham, UK) to each microculture 6 hr before harvesting (Tomtec; Wallac, Milton Keynes, UK) and β-scintillation counting (Microbeta plus; Wallac).

IL-2 bioassay

Culture supernatants were assayed for IL-2 using the CTLL-2 cell line (ECACC) maintained in complete culture medium supplemented with recombinant IL-2 at a concentration of 2 U/ml (Boehringer Mannheim, Lewes, UK). Fifty thousand CTLL-2 cells were added to each culture supernatant in round-profile 96-well plates. After incubation for 24 hr cell proliferation was assessed by measurement of [^{3}H]thymidine incorporation as described above. The IL-2 concentration in the supernatants was determined by interpolation using the equation (FigP Software; Biosoft, Cambridge, UK) describing a curve generated using known concentrations of a standard IL-2 preparation (NIBSC, South Mimms, UK).

Statistical analysis

Assay results were analysed by Student's *t*-test using Minitab statistical software (Clecom, Birmingham, UK).

RESULTS

The antigen-presenting capacity of B cells was examined at different stages of activation. Freshly isolated and irradiated B cells were found to stimulate weak proliferation of allogeneic T cells; this response was maximal at a stimulator : responder cell ratio of 1 : 1 ($P < 0·002$; Fig. 1). However, the same B cells were unable to stimulate allogeneic T-cell proliferation following chemical fixation. Transformation of B cells from the same donor by infection with EBV produced a cell line that retained the ability to stimulate efficient T-cell proliferation after fixation (Fig. 1).

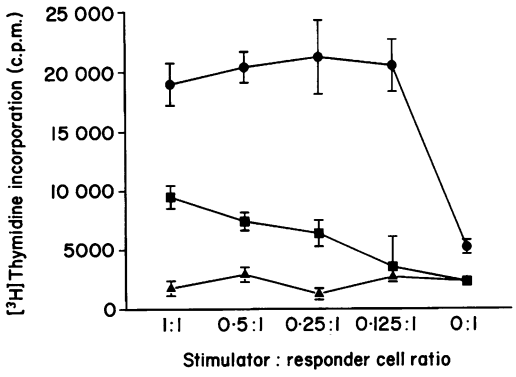

Figure 1. Lymphocyte proliferation after a 5-day mixed lymphocyte culture at a range of stimulator : responder cell ratios. Stimulator cells were all derived from the same donor: (●) fixed EBV-transformed B-cell line; (■) irradiated resting B lymphocytes; (▲) fixed resting B lymphocytes. The points show mean values of five replicate microcultures; the error bars show the standard deviation.

The expression of B7-1 and B7-2 by this EBV-transformed B-cell line was then examined. Both antigens were expressed at high levels by these cells (Fig. 2). The B7-1 antibody bound a mean of $5·9 \times 10^4$ FITC molecules, while the B7-2 antibody bound $3·9 \times 10^4$ FITC molecules; fixation did not alter the labelling of these cell-surface molecules.

Expression of these costimulatory ligands was then measured on B cells immediately after isolation and at increasing times after addition of the anti-human IgM F(ab')$_2$ preparation. These B cells constitutively expressed class II MHC antigens at a high level but had minimal expression of either B7-1 or B7-2 when freshly isolated (Fig. 3). However, during activation the expression of B7-1 was induced and increased gradually to a peak after 96 hr, whereas B7-2 expression increased rapidly on activation and reached peak expression after 72 hr. Analysis of irradiated B cells showed that these cells were also able to increase their expression of B7-2.

Assays were established to determine the activation period required by B cells in order to overcome the effect of fixation on antigen presentation. Purified B cells were treated with anti-IgM F(ab')$_2$ antibody fragments and samples were fixed with paraformaldehyde at 24-hr intervals. These cells were used to stimulate mixed lymphocyte cultures at a stimulator : responder

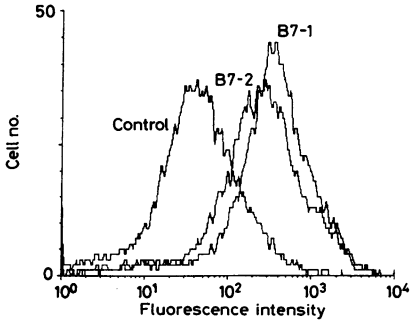

Figure 2. Flow cytometer fluorescence histograms showing B7-1 and B7-2 expression by an immunofluorescence-labelled, EBV-transformed B-cell line.

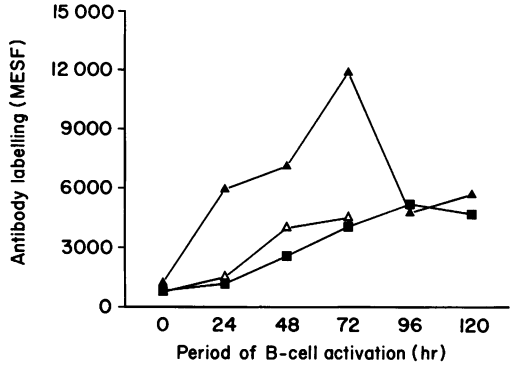

Figure 3. Flow cytometric antigen quantification showing variation of the expression of B7-1 (■) and B7-2 (▲) antigens with time after the initiation of mitogen-stimulation of B lymphocytes. The open symbol (△) shows changes in the expression of B7-2 by irradiated B cells. Data in this figure are from a single experiment but are representative of results from three separate investigations.

cell ratio of 1 : 1. Lymphoproliferation was assessed after 5 days. It was found that B cells that had been activated for a period of 72 hr before fixation produced the greatest T-cell proliferation in mixed culture (Fig. 4); by 96 hr this capacity for allostimulation had decreased. There was a significant correlation between T-cell proliferation and B7-2 expression ($P < 0{\cdot}01$). Assays were duplicated using PBMC and $CD4^+$ T cells to control for bystander or 'trans-costimulation' by B7-expressing cells in the responder PBMC population; similar results were obtained using either responder cell preparation.

The concentration of IL-2 in culture supernatants collected from the above experiments was assessed by bioassay. It was found that IL-2 production was significantly elevated ($P < 0{\cdot}01$) when activated but fixed B cells were used as stimulator cells.

To investigate the importance of costimulation by B cells that had been activated for 72 hr prior to fixation, either the CTLA4–Ig fusion protein or blocking antibodies was added to the assay microcultures (Fig. 5). It was found that addition of the CTLA4–Ig fusion protein at 5 μg/ml reduced lymphocyte proliferation by 76% ($P < 0{\cdot}01$). Addition of anti-B7-1 at 5 μg/ml caused a reduction of 69% ($P < 0{\cdot}05$), while anti-B7-2 caused a 56% reduction ($P < 0{\cdot}02$). Addition of both B7-1 and

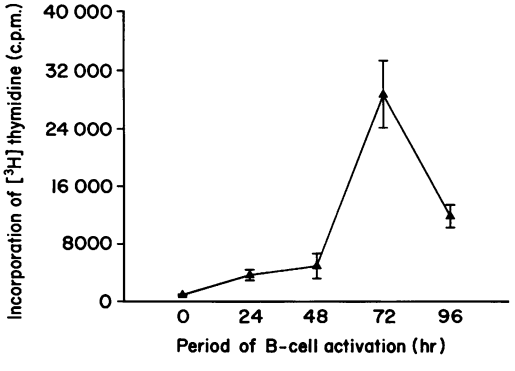

Figure 4. Lymphocyte proliferation after a 5-day mixed lymphocyte culture stimulated with B cells that were activated for increasing periods before chemical fixation. The points represent the mean of five replicate microcultures; the error bars show the standard deviation.

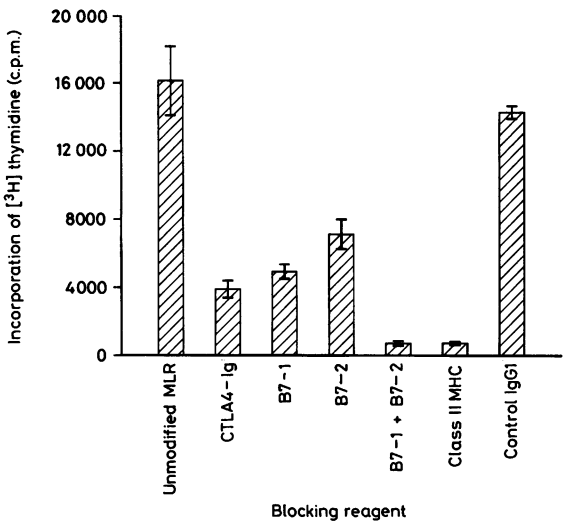

Figure 5. Lymphocyte proliferation after a 5-day mixed lymphocyte culture stimulated with B cells that were activated for 72 hr prior to fixation. Blocking reagents were added at the initiation of the microcultures; CTLA4–Ig, anti-B7-1, anti-B7-2 and the control antibody were added at 5 μg/ml; antibody specific for class II MHC antigens was added at 1·6 μg/ml. The bars represent the mean of three replicate microcultures; the error bars show the standard deviation.

B7-2 abrogated almost all lymphocyte proliferation ($P < 0{\cdot}006$). An anti-class II MHC antibody added to these cultures at a final concentration of 1·6 μg/ml inhibited lymphoproliferation by 96% ($P < 0{\cdot}006$).

The production of IL-2 in these cultures was also reduced by addition of these reagents (Table 1). By day 3 there was 3·4 ± 0·2 U/ml of IL-2 in medium from the basic MLR system. This was reduced by 79% by the addition of CTLA4–Ig. Addition of B7-1 and B7-2 reduced the concentration of IL-2 by 44% and 35%, respectively, while a combination of both of these antibodies reduced IL-2 production by 68%. Antibody specific for class II MHC antigens reduced the concentration of IL-2 by 97%.

Addition of bivalent anti-CD28 at 15 μg/ml increased lymphoproliferation in cultures containing fixed resting B lymphocytes and allogeneic $CD4^+$ T cells. Lymphocyte proliferation in the absence of this antibody was at a background

Table 1. Inhibition of IL-2 production by blockade of mixed lymphocyte culture stimulated by B cells that were activated for 72 hr prior to fixation

Blocking reagent	IL-2 conc. (U/ml)*	Significance of inhibition
Unmodified MLR	3·4 ± 0·2	
CTLA4–Ig	0·7 ± 0·1	$P < 0{\cdot}01$
B7-1	1·9 ± 0·5	$P < 0{\cdot}05$
B7-2	2·2 ± 0·5	$P < 0{\cdot}05$
B7-1 + B7-2	1·1 ± 0·02	$P < 0{\cdot}01$
Class II MHC antigens	0·09 ± 0·11	$P < 0{\cdot}01$

* Mean ± SD; $n = 5$.

level of 2669 ± 267 c.p.m.; this was significantly ($P < 0.01$) increased to 40 450 ± 6590 c.p.m. in the presence of the anti-CD28 antibody. The antibody produced no mitogenesis in control lymphocyte cultures.

DISCUSSION

Previous studies have shown that chemical fixation reduces the capacity of resting splenic APC to support a T-cell response, by blocking their ability to provide costimulation.[22] The observation that EBV-transformed B cells can present alloantigen after chemical fixation suggests that, despite metabolic inactivity, these cells possess and retain all the cell-surface molecules necessary to initiate lymphocyte activation.

It has been demonstrated that EBV-transformed cells express high levels of B7-1.[23] Detailed analysis of these cells revealed that both the B7-1 and B7-2 ligands and class II MHC antigens were expressed at high levels. Specific monoclonal antibodies still bound these antigens after fixation of the cells. This indicates that mild paraformaldehyde modification produces only minor changes in antigen structure and is consistent with the observed interaction between ligands on fixed cells and receptors on T lymphocytes.

Previous studies have demonstrated that B7-1 is not present on freshly isolated B cells[24,25] but that cross-linking surface antigen receptors[24,25] or stimulation with lipopolysaccharide (LPS)[26] induces expression of this molecule. Reports have suggested that B7-2 expression is detectable at extremely low levels on freshly isolated B cells[27] but that this expression is increased by treatment with LPS, anti-IgD or IL-5.[27,28] The freshly isolated B cells used in this study expressed no B7-1 or B7-2 molecules that could be detected by immunofluorescence staining and sensitive flow cytometry. It has been demonstrated that co-ligation of CD19 and the B-cell antigen receptor can augment B-cell activation.[29] However, extensive studies have shown that B cells remain quiescent following positive selection using anti-CD19-conjugated immunomagnetic beads.[30]

Fixation of B cells immediately after isolation rendered the cells unable to stimulate the proliferation of allogeneic lymphocytes. This may be due to the inability of fixed cells to up-regulate the expression of costimulatory molecules. It must be noted that the same B cells could stimulate weak lymphoproliferation in a 5-day MLR if irradiated but not fixed after isolation. Irradiated resting B cells could be induced to express low levels of B7-2 following activation *in vitro*. Increased expression of this molecule by irradiated B cells may be a reflection of the constitutive expression of mRNA encoding B7-2.[31] It is known that irradiated lymphocytes can also produce cytokines such as IL-2 in response to mitogen stimulation.[32]

Serial examination of B7-1 and B7-2 expression during the activation of freshly isolated B cells showed that B7-1 increased slowly and peaked after 96 hr, while B7-2 increased rapidly, peaked after 72 hr at a level higher than B7-1, and then decreased. Similar experiments have been performed previously using murine B lymphocytes.[27] It was found that B7-2 peaked at a level higher than B7-1 but that the kinetics for murine cells were more rapid than those observed for human B cells. Murine cells showed peak B7 antigen expression after activation for 42 hr but expression was decreased after 60 hr.

It is known that resting B cells are poor APC.[33] If activated, however, the ability of B cells to present antigen improves.[14,34,35] It has been shown previously that short periods of B-cell activation prior to mitotic arrest by irradiation or treatment with mitomycin c are sufficient to allow the cells to stimulate maximal proliferative responses during a 5-day mixed lymphocyte culture.[36] This is explained by the observation that briefly activated B cells can continue to increase their ability to costimulate T-cell proliferation after mitomycin c treatment and during mixed lymphocyte culture.[33] In order to study the relationship between B7 antigen expression and alloantigen presentation it was necessary to fix B cells serially at a range of time-points during the activation process, to prevent changes in costimulatory potential during subsequent mixed lymphocyte culture.

It was found that B cells that had been pre-activated for 72 hr before fixation had the ability to present antigen to allogeneic T lymphocytes and to costimulate the proliferation of these cells. This observation is consistent with the elevated B7-1 expression and maximal B7-2 expression 72 hr after initiation of B-cell activation. Cells activated for less than this critical time were unable to generate a maximal proliferative response as their expression of B7-1 and B7-2 had not yet peaked. After reaching maximal expression, the level of B7-2 on activated B cells decreased rapidly; this was reflected by the observation that B cells fixed 96 hr after activation stimulated a reduced lymphoproliferative response.

Both PBMC and $CD4^+$ T cells responded equally to activated B lymphocytes but failed to respond to fixed, resting allogeneic B cells. The absence of evidence for trans-costimulation by B7-expressing cells within the PBMC responder cell population is consistent with the view that such costimulation is extremely inefficient during a primary immune response.[37]

The functional role of individual B-cell-associated costimulatory molecules was assessed by addition of reagents capable of blocking specific ligand–receptor interactions. Blockade of class II MHC molecules was found to inhibit antigen presentation by activated B cells; this inhibition was caused by the prevention of T-cell receptor engagement. It was also found that the fusion protein CTLA4–Ig, a soluble CD28 antagonist, and monoclonal antibodies specific for either B7-1 or B7-2 significantly reduced T-cell proliferation. This illustrates the importance of the costimulatory interaction between CD28 and CTLA4 on T cells and the B7-1 and B7-2 ligands expressed by activated B cells.

The observation that addition of both B7-1- and B7-2-specific antibodies in the same microculture reduced proliferation to background levels shows that these two ligands share a similar function. The rationale for expression of both these signalling molecules remains unclear. However, kinetic differences in the up-regulation of these molecules support the notion that B7-2 may be involved in the initiation of costimulation while B7-1 may amplify the response.[38] The fact that addition of both antibodies ablates lymphocyte proliferation and inhibits IL-2 production in this system suggests that no further costimulatory ligand is expressed or required. Further evidence of the importance of this costimulation was provided by the observation that T-cell hyporeactivity in cultures where fixed resting B cells were used as stimulator cells could be overcome by antibody stimulation of CD28 on the T lymphocytes.

In conclusion, it has been shown that class II MHC antigen-expressing, resting B cells cannot stimulate the activation of allogeneic $CD4^+$ T cells. However, mitogen-activated B cells are able to initiate the proliferation of T cells in mixed lymphocyte

culture. Activation of B cells for 72 hr optimally induces expression of the B7-1 and B7-2 ligands. These ligands generate all the costimulatory signals required for antigen presentation by B lymphocytes and the proliferation of alloantigen-specific resting $CD4^+$ T cells.

ACKNOWLEDGMENTS

The authors are grateful to the University of Newcastle Research Committee, the Northern Counties Kidney Research Fund and the Harker Bequest for financial support.

REFERENCES

1. Lafferty K.J. & Cunningham A.J. (1975) A new analysis of allogeneic interactions. *Aust J Exp Biol Med Sci* **53,** 27.
2. Kirby J.A. & Wilson J.L. (1994) Allograft rejection: the role played by adhesion molecules. *Transpl Rev* **8,** 114.
3. Linsley P.S. & Ledbetter J.A. (1993) The role of the CD28 receptor during T cell response to alloantigen. *Annu Rev Immunol* **11,** 191.
4. Martin P.J., Ledbetter J.A., Morishita Y., June C.H., Beatty P.G. & Hansen J.A. (1986) A 44 kilodalton cell surface homodimer regulates interleukin 2 production by activated human T lymphocytes. *J Immunol* **136,** 3282.
5. O'Neill E.A. & Tocci M.J. (1993) Transcriptional activation of the IL-2 gene. *Transplant Sci* **3,** 77.
6. 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.
7. Harper C., Balzano C., Rouvier E., Mattei M.G., Luciani M.F. & Goldstein P. (1991) CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *J Immunol* **147,** 1037.
8. 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.
9. Linsley P.S., Greene J.L., Tan P. *et al.* (1992) Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. *J Exp Med* **176,** 1595.
10. Linsley P.S., Brady W., Grosmaire L., Aruffo A., Damle N.K. & Ledbetter J.A. (1991) Binding fo the B cell activation antigen B7 to CD28 costimulates T cell proliferation and IL-2 mRNA accumulation. *J Exp Med* **173,** 721.
11. 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.
12. Freeman G.J., Borriello F., Hodes R.J. *et al.* (1993) Uncovering of functional alternative CTLA-4 counter receptor in B7 deficient mice. *Science* **262,** 907.
13. Boussiotis V.A., Freeman G.J., Gribben J.G., Daley J., Gray G. & Nadler L.M. (1993) Activated human B lymphocytes express three CTLA-4 counter-receptors that costimulate T cell activation. *Proc Natl Acad Sci USA* **90,** 11059.
14. Chesnut R.W., Colon S.M. & Grey H.M. (1982) Antigen presentation by normal B cells, B cell tumours, and macrophages: functional and biochemical comparison. *J Immunol* **128,** 1764.
15. Metlay J.P., Pure E. & Steinman R.M. (1989) Distinct features of dendritic cells and anti-Ig activated B cells as stimulators of the primary mixed leukocyte reaction. *J Exp Med* **169,** 239.
16. Cassell D.J. & Schwartz R.H. (1944) A quantitative analysis of antigen-presenting cell function: activated B cells stimulate naive CD4 T cells but are inferior to dendritic cells in providing costimulation. *J Exp Med* **180,** 1829.
17. Eynon E.E. & Parker D.C. (1992) Small B cells as antigen presenting cells in the induction of tolerance to soluble protein antigens. *J Exp Med* **175,** 131.
18. Fuchs E.J. & Matzinger P. (1992) B cells turn off virgin but not memory T cells. *Science* **258,** 1156.
19. Watanabe T., Yoshizaki K., Yagura T. & Yamamura Y. (1974) *In vitro* antibody formation by human tonsil lymphocytes. *J Immunol* **113,** 608.
20. Rasmussen A.-M., Smeland E.B., Erikson B.K., Caignault L. & Funderud S. (1992) A new method for detachment of Dynabeads from positively selected B lymphocytes. *J Immunol Meth* **146,** 195.
21. Sieckmann D.G., Asofsky R., Mosier D.E., Zitron I.M. & Paul W.E. (1978) Activation of mouse lymphocytes by anti-immunoglobulin. I. Parameters of the proliferative response. *J Exp Med* **147,** 814.
22. Jenkins M.K., Ashwell J.D. & Schwartz R.H. (1988) Allogeneic non T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically modified antigen presenting cells. *J Immunol* **140,** 3324.
23. Yokochi T., Holly R.D. & Clark E.A. (1982) B lymphoblast antigen (BB1) expressed on Epstein–Barr virus activated B cell blasts, B lymphoblastoid cell lines and Burkitt's lymphomas. *J Immunol* **128,** 823.
24. 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.
25. Freedman A.S., Freeman G.J., Horowitz J.C., Daley J. & Nadler L.M. (1987) B7, a B cell restricted antigen that identifies preactivated B cells. *J Immunol* **139,** 3260.
26. Yocuchi T., Holly R.D. & Clark E.A. (1982) B lymphoblast antigen (BB-1) expressed on EBV-activated B cell blasts, B lymphoblastoid lines, and Burkitt's lymphoma. *J Immunol* **128,** 823.
27. Hathcock K.S., Laszlo G., Pucillo C., Linsley P. & Hodes R.J. (1994) Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* **180,** 631.
28. Lenschow D.J., Sperling A.I., Cooke M.P. *et al.* (1994) Differential up regulation of the B7-1 and B7-2 costimulatory molecules after Ig receptor engagement by antigen. *J Immunol* **153,** 1990.
29. Carter R.H. & Fearon D.T. (1992) CD19: lowering the threshold for antigen stimulation of B lymphocytes. *Science* **256,** 105.
30. Funderud S., Erikstein B., Asheim H.C. *et al.* (1990) Functional properties of $CD19^+$ B lymphocytes positively selected from buffy coats by immunomagnetic separation. *Eur J Immunol* **20,** 201.
31. Freeman G.J., Gribben J.G., Boussiotis V.A. *et al.* (1993) Cloning of B7-2: a CTLA-4 counter receptor that costimulates human T cell proliferation. *Science* **262,** 909.
32. Vie H. & Miller R.A. (1986) Estimation by limiting dilution analysis of human IL-2 secreting T-cells; detection of IL-2 produced by single lymphokine secreting T cells. *J Immunol* **136,** 3292.
33. Krieger J.I., Chesnut R.W. & Grey H.M. (1986) Capacity of B cells to function as stimulators of a primary mixed leukocyte reaction. *J Immunol* **137,** 3117.
34. Krieger J.I., Grammer S.F., Grey H.M. & Chesnut R.W. (1985) Antigen presentation by splenic B cells: resting B cells are ineffective, whereas activated B cells are effective accessory cells for T cell responses. *J Immunol* **135,** 2937.
35. Ho W.Y., Cooke M.P., Goodnow C.C. & Davies M.M. (1994) Resting and anergic B cells are defective in CD28-dependent costimulation of naive $CD4^+$ T cells. *J Exp Med* **179,** 1539.
36. Kakiuchi T., Chesnut R.W. & Grey H.M. (1983) B cells as antigen-presenting cells: the requirement for B cell activation. *J Immunol* **131,** 109.
37. Liu Y. & Janeway C.A. (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 USA* **89,** 3845.
38. Freeman G.J., Borriello F., Hodes R.J. *et al.* (1993) Murine B7-2, an alternate CTLA4 counter-receptor that costimulates T cell proliferation and interleukin 2 production. *J Exp Med* **178,** 2185.