Structure, Expression, and T Cell Costimulatory Activity of the Murine Homologue of the Human B Lymphocyte Activation Antigen B7

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Summary

Following occupancy of the T cell receptor by antigen, T cell proliferation and lymphokine production are determined by a second costimulatory signal delivered by a ligand expressed on antigen presenting cells. The human B cell activation antigen B7, which is expressed on antigen presenting cells including activated B cells and γ interferon treated monocytes, has been shown to deliver such a costimulatory signal upon attachment to its ligand on T cells, CD28. We have cloned and sequenced the murine homologue of the human B7 gene. The predicted murine protein has 44% amino acid identity with human B7. The greatest similarity is in the Ig-V and Ig-C like domains. Murine B7 mRNA was detected in murine hematopoietic cells of B cell but not T cell origin. Cells transfected with murine B7 provided a costimulatory signal to human $CD28^+$ T lymphocytes. These results demonstrate the costimulatory activity of murine B7 and provide evidence that the ligand attachment site is conserved between the two species.

Although occupancy of the TCR complex by antigen in association with the MHC is necessary for the initiation of T cell activation, several lines of evidence suggest that a second costimulatory signal is essential for the induction of proliferation and lymphokine secretion (1–4). In murine and human systems, this costimulatory signal is delivered by APC and requires cell to cell contact (2, 4). Cells which can deliver this costimulatory signal include activated, but not resting B lymphocytes (5), INF-γ activated monocytes, and dendritic cells (2, 6).

Several recent studies in human systems have provided compelling evidence that the B cell activation antigen B7 can provide one such costimulatory signal (7–9). B7, a member of the Ig supergene family, has been shown to be a ligand for another member of this family, the T cell surface protein CD28, (10–13). CD28 is constitutively expressed on 95% of human $CD4^+$ T cells, 50% of $CD8^+$ T cells, and on thymocytes which coexpress CD4 and CD8 (14–16). Following suboptimal activation of T cells with anti-CD3 mAb (16), anti-CD2 mAb, or phorbol ester (17), crosslinking of CD28 by anti-CD28 mAb results in enhanced T cell proliferation and greatly augments synthesis of multiple lymphokines (18). B7 is likely to be an important regulator of T cell proliferation and lymphokine production as evidenced by its pattern of expression and functional activity. B7 is not expressed on resting B cells (19) but appears following crosslinking of surface Ig (10, 19) or class II MHC (9). Moreover, B7 is not expressed on unstimulated monocytes and is specifically induced by INF-γ but not other stimuli which activate monocytes (20). Human B7 (hB7)[1] transfected cells or recombinant B7-Ig fusion protein augment proliferation and induce IL-2, but not IL-4, synthesis in T cells which have been treated with phorbol ester or anti-CD3 mAb (7–9).

In murine systems, a homologue for CD28 has recently been cloned (21); however, a conserved functional activity has not yet been demonstrated. In the present study, we have cloned, determined the nucleotide sequence, and structurally analyzed the murine homologue of B7. We demonstrate that murine B7 (mB7) is costimulatory for human $CD28^+$ T cells, suggesting the existence of a highly conserved binding domain.

Materials and Methods

Isolation of Murine cDNA Clones. In preliminary experiments, low stringency hybridization of the human B7 cDNA insert (10) to blots of poly(A)$^+$ RNA from the murine B cell lines 70Z, A20,

[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary cells; hB7, human B7; mB7, murine B7.

TA3, and NS-1 suggested the presence of crosshybridizing mRNAs in 70Z and A20. The ^{32}P-labeled, 1.5-kb human B7 cDNA insert was used to screen a λgt11 cDNA library generated from the mouse pre-B cell line, 70Z/3 (22). Hybridization at reduced stringency was performed in 5× SSPE, 5× Denhardt's solution, 0.2% SDS, 500 μg/ml salmon sperm DNA at 50°C. Final washes were in 2× SSC, 0.1% SDS at 52.5°C for 20 min. A single cDNA clone was isolated. DNA sequence analysis revealed that it contained a region homologous to the human B7 Ig-C, transmembrane, cytoplasmic, and 3′ untranslated domains. This cDNA insert was used to screen a cDNA library prepared from the mouse B cell line, A20, in the pCDM8 vector. Four additional cDNA clones were isolated. One of these was sequenced and found to contain regions homologous to human B7 5′ untranslated, signal peptide, Ig-V, and Ig-C domains. The Ig-C region was identical between the two cDNA clones and contained a BamHI restriction enzyme site. A complete murine B7 cDNA clone was constructed by ligation of the 70Z cDNA clone into the EcoRI site of the eukaryotic expression vector, pCDNAI (Invitrogen, San Diego, CA), followed by digestion with BamHI, and ligation of the BamHI fragment from the A20 cDNA clone.

DNA Sequence Analysis. B7 cDNA inserts were subcloned into the pKSII$^-$ plasmid (Stratagene, La Jolla, CA). Nested deletions were constructed using the Erase-A-Base kit according to the manufacturer's directions (Promega, Madison, WI). The cDNA insert was sequenced using dye labeled primers and Taq polymerase (Applied Biosystems, Foster City, CA) and the sequencing reactions were analyzed on an Applied Biosystems (model 373) automated fluorescent sequencer. Sequence data obtained from overlapping deletion clones on both strands were assembled to yield the final murine B7 sequence. These sequence data are available from EMBL/GenBank/DDBJ under accession number X60958.

B7 Hybridization Probe. A DNA fragment corresponding to the protein coding region of the murine B7 cDNA was used as a probe for RNA and DNA blot hybridizations because of a repetitive element in the 3′ untranslated region of the B7 mRNA. The complete murine B7 cDNA was used as a template for PCR amplification of the coding region using a sense primer (ATGGCTTGCAATTGTCAG) and anti-sense primer (CTAAAGGAAGACGGTCT) corresponding to nucleotides 249-266 and 1169-1153 of the cDNA sequence. The 921 bp coding region PCR product was gel purified and used for all blot hybridizations.

RNA and DNA Blot Hybridizations. RNA was prepared from various organs isolated from a 4-wk-old Balb/c mouse. RNA was also prepared from the murine pre-B cell lines 38B9 and 300.19, the B cell lymphomas AJ9, CH1 and A20, the plasmacytoma lines, Ag8.653 (P3 × 63-Ag8.653) and NS-1 (P3/NS1/1-Ag4-1), the T cell lymphoma lines, EL4, BW5147, and YAC, and the thymoma line RADA. RNA preparation, detailed characterizations, and sources of these cells are as described (23). 2 μg of poly(A)$^+$ RNA were denatured with glyoxyl, electrophoresed on an agarose gel, and blotted onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Isolation of genomic DNA and DNA blot hybridizations were performed as described (24). The 921 bp murine B7 coding region PCR product and the actin cDNA insert were labeled by random oligonucleotide priming using α-^{32}P-labeled dCTP and the Klenow fragment of DNA polymerase. Hybridization, washing, and autoradiography were performed as previously described (10, 24).

Cells. Human CD28$^+$ T cells were isolated from peripheral blood mononuclear cells as described (7).

Monoclonal Antibodies. 4B10 (IgG1) is an anti-CD28 mAb (7). Optimal stimulation of T cells with anti-CD28 mAb was obtained at a concentration of 1 μg/ml and this dose was used throughout the experiments. 4B10 was purified using a protein A Sepharose column (Bio-Rad) as described (7). The anti-B7 mAb, 133, (IgM) was characterized in our laboratory (10, 19) and was used at a final concentration of 10 μg/ml.

B7-Transfection. Transient expression of B7 cDNA clones in COS cells was performed as previously described (11). COS cells transfected with the pCDNAI vector alone were also prepared. Transfected COS cells were used 72 h after the addition of DNA. A stabley transfected Chinese hamster ovary (CHO) cell line expressing hB7 was constructed as previously described and is referred to as CHO-hB7 (7).

Cell Fixation. COS and CHO cells were detached from tissue culture plates and fixed with paraformaldehyde as described (7).

Proliferation Assay. The capacity of B7 to costimulate T cell proliferation was measured as described (7). Briefly, human CD28$^+$ T lymphocytes were stimulated with phorbol myristate 13-acetate (PMA) (Calbiochem, La Jolla, CA) at 1 ng/ml final concentration (17). The fixed CHO-hB7 and COS cell transfectants were added at a concentration of 2 × 10^4 cells/well. The specificity of the stimulation with COS-hB7 cells was assayed by the addition of anti-B7 mAb to the cultures at a final concentration of 10 μg/ml. The cells were pulsed with 1 μCi of ^{3}H-thymidine (ICN Flow, Costa Mesa, CA) during the last 8 h of a 72-h culture, harvested onto filters, and counted.

Results

Isolation of Murine B7 cDNA, Sequencing, and Analysis. A murine cDNA clone was isolated by low stringency hybridization with the hB7 cDNA employing a cDNA library prepared from the murine pre-B cell line, 70Z. The murine cDNA isolated from 70Z was composed of 1180 bases of intron followed by a splice acceptor and sequences homologous to the hB7 Ig-C, transmembrane, cytoplasmic, and 3′ untranslated domains. This cDNA was used to isolate additional clones under stringent conditions from a cDNA library prepared from the murine B cell line, A20. Comparison of the A20 cDNA clone with hB7 showed that the A20 cDNA contained sequences homologous to the hB7 5′ untranslated, signal peptide, Ig-V, and Ig-C domains followed by a splice donor and 1900 bases of intron sequence. This intron DNA contained murine B1 repetitive Alu-like elements. The two murine cDNA clones share 320 bases of identical sequence corresponding to the Ig-C domain. The Ig-C domain contained a unique BamHI site and a complete murine B7 cDNA clone was constructed by ligating the two cDNA clones at this BamHI site.

A search of the GenBank and EMBL databases with the mB7 nucleotide sequence revealed that only the hB7 sequence exhibited significant homology with the murine sequence (sigma = 24 SDs above the mean). Comparison of the mB7 cDNA sequence with that of hB7 showed that the two were 60% identical. Homologous domains include the 5′ (50%) and 3′ (40%) untranslated regions in addition to the protein coding sequence (63%). A poly(A) tract following a consensus polyadenylation signal (bases 1678 - 1683) was identified.

Analysis of the mB7 cDNA reveals a single, long open reading frame of 918 bases initiated by one of three closely spaced ATG codons beginning at nucleotides 225, 249, and

270. We have chosen the second of these ATG codons (nt 249) as the initiating methionine because the DNA sequence GCTATGG around this ATG is consistent with the consensus translation initiation sequence RCCATGG defined by Kozak (25). In addition, the region 5′ of this ATG is highly similar (15 of 17 nucleotides identical) to the human start site. Initiation at this methionine predicts an open reading frame of 918 bases encoding a protein of 306 amino acids.

Fig. 1 shows the alignment of the murine and human B7 protein sequences and the structural features associated with these molecules. The structural domains shown for mB7 are based on a comparison with hB7 and with other members of the Ig supergene family. The initiatory methionine codon is followed by a 37 amino acid signal peptide. The length of the signal peptide was chosen to correspond to the signal cleavage site experimentally determined for hB7 expressed in CHO cells. Amino terminal sequencing of a soluble hB7 purified from the culture media of transfected CHO cells revealed that the mature hB7 began with the amino acid sequence valine, isoleucine, histidine, and valine (our unpublished results). Hydrophobicity analysis reveals that the putative signal sequence agrees with the profile for a consensus signal

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          |--------------Signal---------------|
Mouse   1 MACNCQLMQDTPLLKFPCPRLILLFVLLIRLSQVSSDVDEQLSKSVKDKV 50
Human   1   MGHTRRQGTSPSKCPYLNFFQLLV.LAGLSHFCSGV.IHVTKEVKEVA 46

         51 LLPCRYNSPHEDESEDRIYWQKHDKVVLSVIAGKLKVWPEYKNRTLYDNT 100
         47 TLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGDMNIWPEYKNRTIFDIT 96

        101 .TYSLIILGLVLSDRGTYSCVVQKKERGTYEVKHLALVKLSIKADFSTPN 149
         97 NNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPS 146

        150 ITESGNPSADTKRITCFASGGFPKPRFSWLENGRELPGINTTISQDPESE 199
        147 ISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETE 196

        200 LYTISSQLDFNTTRNHTIKCLIKYGDAHVSEDFTWEKPPEDPPDSKNTLV 249
        197 LYAVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQEHF.PDNLLP 245

                 _____Transmembrane_____|-------Cytoplasmic--------
        250 LFGAGFGAVITVVVIVVIIKCFCKHRSCFRRNE.ASRETNNSLTFGPEEA 298
        246 SWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV*..... 289

        --------|
        299 LAEQTVFL*
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Figure 1. Comparison of murine and human B7 amino acid sequences. Amino acid identities are indicated with a vertical bar (|). Possible N-linked glycosylation sites are marked with an (*). The signal peptide, transmembrane, and cytoplasmic domains are indicated. Ig-like domains are defined by the cysteines at position 54 and 121 (Ig-V) and 166 and 220 (Ig-C). The cDNA sequence data are available from EMBL/GenBank/DDBJ under accession number X60958.

peptide and that a highly hydrophobic membrane spanning domain is located at amino acids 248-272. Ig-V (amino acids 38 - 142) and Ig-C (amino acids 143 - 236) domains retain many of the conserved amino acids important for the structure of the Ig supergene family (13). The complete human and murine B7 protein sequences were 44% identical with 47% identity in the Ig-V domain and 57% in the Ig-C domain. The mB7 transmembrane domain contains two cysteine residues, as opposed to three in hB7, and these could be involved in lipid derivatization or covalent interaction with other membrane proteins. The mB7 cytoplasmic domain is not closely related to its human counterpart but retains its highly charged nature. Both murine and human B7 contain eight potential N-linked glycosylation sites of which four are conserved between the two sequences. Three of the common glycosylation sites were found in the Ig-C domain and one in the Ig-V domain. The murine and human B7 proteins differ in that the mB7 exhibits an Ig hinge-like region between the Ig-C and transmembrane domains. This should confer greater flexibility to the mB7. The predicted mature mB7 protein would contain 269 amino acids with a mol wt of 30386 kD as opposed to 254 amino acids and 29311 for hB7. Glycosylation of the hB7 protein leads to an apparent mol wt of 44–54 kD and a similar increase would be expected for mB7.

A search of the PIR (Protein Identification Resource) and the Swiss-Prot (Intelligenetics) protein databases with the mB7 protein sequence revealed similarities with several immunoglobulin variable and constant domains of human and murine origins. Human B7 was not found in the protein homology searches because this sequence was not present in the databases searched. However, searching all three reading frame translations of the Genbank and EMBL databases with the mB7 protein sequence showed that homology with hB7 is much greater than all other sequences.

Expression of B7 mRNA. A DNA fragment corresponding to the protein coding region of the mB7 cDNA (basepairs 249–1169) was synthesized using PCR because of the presence of a repetitive element in the 3′ untranslated region of the B7 mRNA. Using this B7 coding region probe, RNA blot hybridization analysis of B7 mRNA expression in murine lymphoid cells revealed that B7 was expressed in the mature B cell lines AJ9 and CH1, in the plasmacytoma line Ag8.653 and at low levels in the mature B cell line A20 (Fig. 2 *A*). Two mRNA transcripts of 2.2 and 3.9 kb were detected in poly(A)$^+$ RNA. This is a more simple transcript pattern than seen in human B cell lines where transcripts of 1.7, 2.9, 4.2, and 10 kb were detected. A large transcript (approximately 10 kb) was observed in the pre-B cell line 38B9. B7 mRNA expression was not detected in the pre-B cell line 300.19, the plasmacytoma line NS-1, or the T cell lines EL-4, BW5147, RADA, and YAC.

RNA blot hybridization analysis of poly(A)$^+$ RNA isolated from murine organs demonstrated that B7 expression was restricted to murine splenocytes (Fig. 2 *B*). No expression was observed in liver, brain, heart, lung, kidney, muscle, or thymus. In both murine splenocytes and B cell lines, 2.2 and 3.9 kb mRNA transcripts were identified, with the 2.2

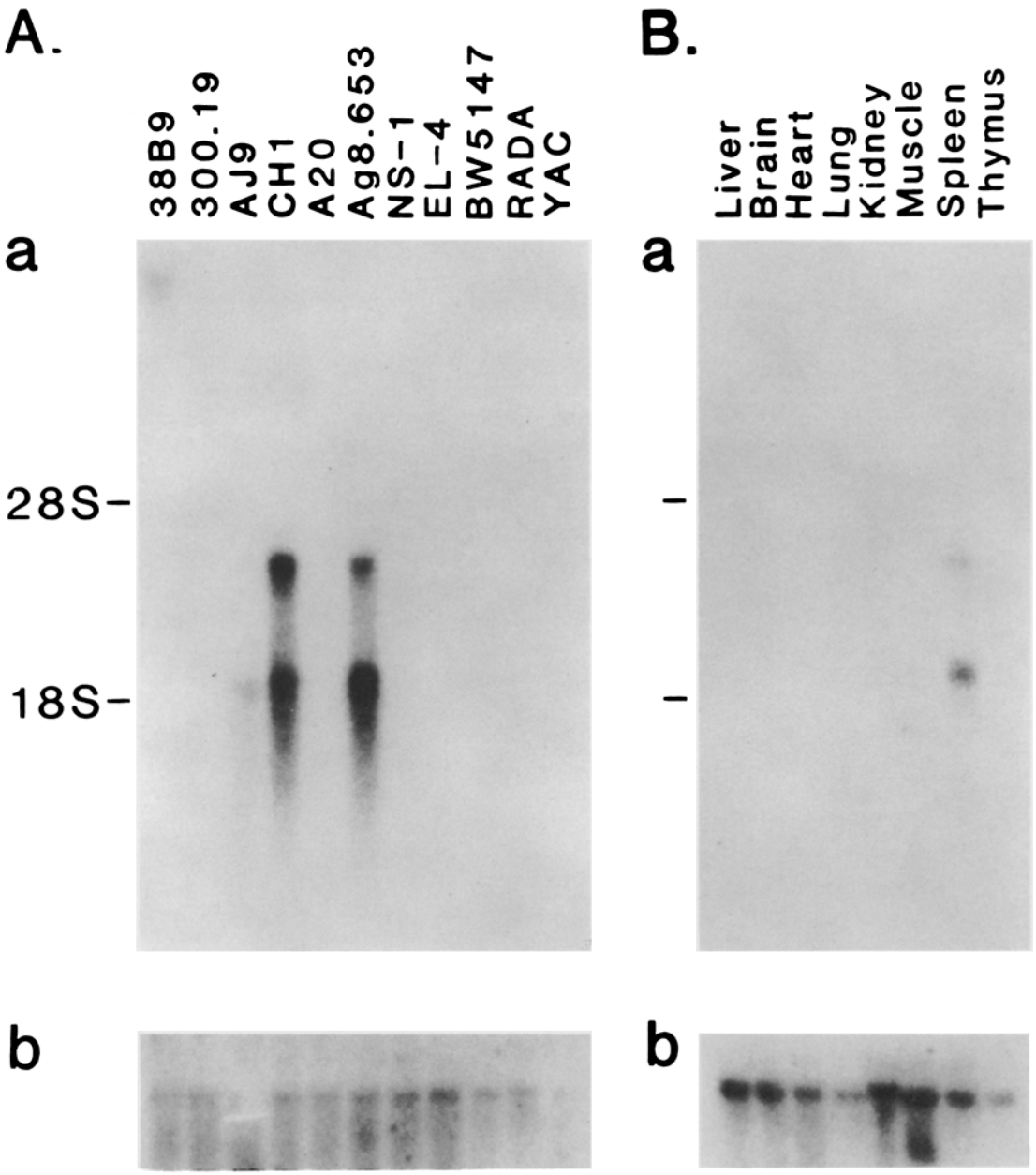

Figure 2. B7 mRNA expression is B cell restricted. RNA blot analysis of (*A*) lymphoid cell lines and (*B*) Balb/c mouse organs. 2 μg of poly(A)$^+$ RNA were glyoxylated, electrophoresed on agarose gels, and transferred to nitrocellulose. The blot was hybridized with (*a*) ^{32}P-labeled mB7 coding region cDNA and reprobed with (*b*) ^{32}P-labeled rat actin cDNA. The lanes contain RNA from the murine pre-B cell lines, 38B9 and 300.19, the B cell lines, AJ9, CH1, and A20, the plasmacytoma lines, Ag8.653 and NS-1, and the T cell lines, EL-4, BW5147, RADA, and YAC. The mobility of rRNAs are indicated on the left.

kb transcript predominating. Thus, B7 expression is restricted in both murine and human lymphoid cells to mature B cells, some pre-B and plasmacytoma cell lines, but is not found in T cell lines.

DNA Blot Hybridization Analysis of B7. DNA blot analysis to determine the genomic organization of B7 was performed, using the B7 coding region probe described above (Fig. 3). When genomic DNA was digested with eleven different restriction endonucleases, the B7 coding region probe hybridized to between one and five restriction enzyme fragments. Digestion with ApaI or EcoRV produced a single DNA fragment, consistent with a single copy of the B7 gene per haploid genome. Digestion with SacI or BclI, which are not present in the B7 coding region, each produced 5 DNA fragments. These results suggest that the mB7 protein coding region encompasses approximately 20 kb and is divided into at least 5 exons. If these correspond to the hB7 genomic organization, these will encode the signal peptide, Ig-V, Ig-C,

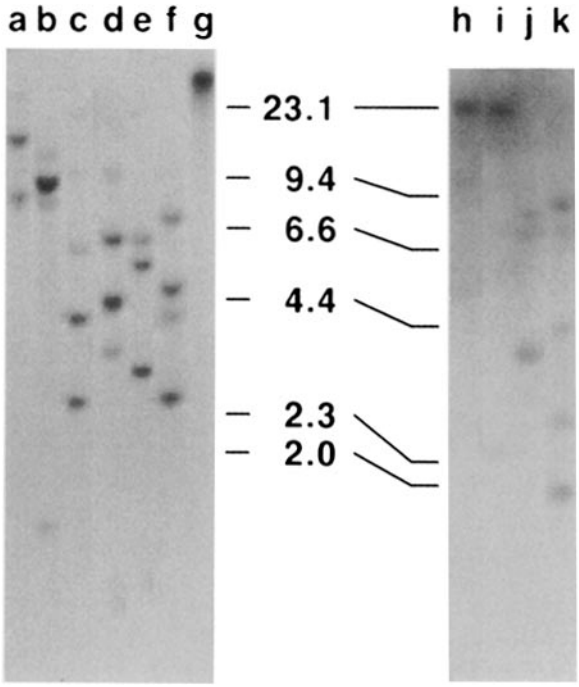

Figure 3. Genomic DNA blot analysis of B7. 5 μg of C57BL/6 splenic DNA were digested with (*a*) BamHI, (*b*) EcoRI, (*c*) BclI, (*d*) KpnI, (*e*) BglII, (*f*) XbaI, (*g*) EcoRV, (*h*) ApaI, (*i*) BglI, (*j*) BstXI, and (*k*) SacI. DNAs were electrophoresed in 0.7% agarose, blotted, and hybridized with ^{32}P-labeled mB7 coding region cDNA. The sizes, in kb, of mol wt markers are indicated.

transmembrane, and cytoplasmic domains (G. Freeman and G. Gray, manuscript in preparation).

Murine and Human B7 Transfected Cells Stimulate the Proliferation of Phorbol Ester Activated Human CD28$^+$ T Cells. We examined whether mB7 might provide a costimulatory signal to human CD28$^+$ T cells. Table 1 summarizes one of three representative experiments. Coincubation of paraformaldehyde fixed COS-mB7 or COS-hB7 cells with PMA treated CD28$^+$ human T cells resulted in 29-fold and 30-fold enhancement of proliferation, respectively, compared to T cells treated with PMA alone. Addition of anti-hB7 mAb could completely inhibit the costimulatory activity of COS-hB7 cells but not of COS-mB7 cells. Addition of paraformaldehyde fixed CHO-hB7 transfected cells resulted in a 51-fold increase in proliferation. In contrast, coincubation of PMA treated T cells with paraformaldehyde fixed COS-Vector resulted in no increase in proliferation. Paraformaldehyde fixed CHO-hB7, COS-hB7, COS-mB7, and COS-Vector transfected cells did not induce untreated human CD28$^+$ cells to proliferate above media controls.

Table 1. *Effect of Murine and Human B7 Expressing Cells on the Proliferation of Phorbol Ester Treated Human CD28$^+$ T Cells*

Human CD28$^+$ T cells cocultured with:	*cpm ± SEM*
Media	148 ± 18
PMA	689 ± 48
Anti-CD28	109 ± 17
CHO-hB7	70 ± 2
COS-hB7	47 ± 7
COS-mB7	40 ± 4
COS-Vector	89 ± 28
PMA + anti-CD28	58,646 ± 3,093
PMA + CHO-hB7	34,910 ± 982
PMA + COS-hB7	20,676 ± 897
PMA + COS-mB7	20,081 ± 1,516
PMA + COS-Vector	392 ± 34
PMA + COS-hB7 + anti-hB7 mAb	356 ± 52
PMA + COS-mB7 + anti-hB7 mAb	17,395 ± 1,367

Discussion

The mB7 homologue was cloned by probing murine B cell cDNA libraries with the human gene. DNA sequence analysis of the mB7 cDNA reveals that this gene is closely related to the hB7 gene and exhibits 63% identity in the protein coding region. Murine B7 exhibits structural features similar to the human gene and is composed of a signal peptide, Ig-V and Ig-C domains, a transmembrane region, and a cytoplasmic domain. The complete human and murine B7 protein sequences were 44% identical overall with 47% identity in the Ig-V domain and 57% in the Ig-C domain. Both murine and human B7 contain four essential cysteine residues and other conserved amino acids which define the Ig supergene family (13). In the transmembrane domain, mB7 has two and hB7 has three cysteines that may be involved in lipid derivatization or crosslinking to other membrane proteins. Both murine and human B7 contain eight potential N-linked glycosylation sites of which four are located in conserved positions in each molecule. These conserved sites are found in the Ig-V and Ig-C domains and are presumably sites of functional glycosylation. The signal peptide, transmembrane, and cytoplasmic domains are less well conserved. An interesting difference between murine and human B7 is the presence of an Ig hinge-like sequence (26) adjacent to the transmembrane domain of the murine protein.

Expression of mB7 mRNA in tissues was restricted to splenocytes and was not detected in other organs. Transcription of mB7 was detected in mature murine B cell lines (AJ9, A20, and CH1) and a plasmacytoma line (Ag8.653) and yields two polyadenylated transcripts of 2.2 and 3.9 kb. A large 10 kb transcript was found in the pre-B cell line 38B9. This pattern of transcription was reminiscent of the large number of hB7 transcripts which included 1.7, 2.9, 4.2, and 10 Kb mRNAs (10). The high degree of sequence homology and pattern of mRNA expression demonstrate that the hB7 gene and the murine gene reported here are closely related.

In humans, we and others have shown that a recombinant B7-Ig fusion protein or cells expressing B7 can augment proliferation of human T cells that have been stimulated with

phorbol ester or anti-CD3 (7–9). This stimulation is delivered via attachment of B7 to the T cell CD28 glycoprotein and results in T cell proliferation and secretion of IL-2, but not IL-4. The specificity of this pathway has been demonstrated by inhibition of proliferation and IL-2 secretion by mAbs to either B7 or CD28. Murine T cells express a homologue of human CD28 which has 68% amino acid identity to the human gene (21). The expression of murine CD28 is T cell restricted; however, the existence of a pathway for T cell activation has not been demonstrated. COS cells transfected with mB7 can act as costimulators of human $CD28^+$ T cells, enhancing proliferation of $CD28^+$ T cells that have been treated with phorbol ester. The stimulation of human T cells by both mB7 and hB7 shows that the ligand binding site on B7 is conserved. The interspecies costimulatory activity of B7 was somewhat surprising since the protein sequence identity was only 44%.

In humans, engagement of the CD28 pathway greatly augments synthesis of multiple lymphokines including IL-2, INF-γ, tumor necrosis factor α (TNF-α), granulocyte macrophage colony stimulating factor (GM-CSF), but not IL-4 (7, 18, 27). This division of lymphokine synthesis corresponds to that produced by the Th1 subset of T cells in the mouse, whereas the Th2 subset produces IL-4 but not IL-2 (28). In the mouse, it has been shown that Th1 and Th2 clones proliferate optimally in response to different APC populations (29). B cells stimulated optimal proliferation of Th2 clones and splenic adherent cells optimally stimulated Th1 clones (29). Other studies have also demonstrated the existence of different costimulatory factors for Th1 and Th2 cells (29, 30). IL-1 and IL-6 can function to costimulate some murine Th2 clones (4, 30). It will be of great interest to determine if mB7 costimulates only a subset of T cells.

The pattern of expression of B7 on activated but not resting human B cells and IFN-γ activated human monocytes is consistent with the phenotype of the T cell costimulatory signal present on murine APCs. Following occupancy of the T cell receptor by antigen in association with MHC, the outcome of T cell activation in the mouse is determined by a costimulatory signal (1). Schwartz and others have shown that if the costimulatory signal is not delivered, the T cell enters a prolonged period of anergy during which the T cell is unresponsive to signals of T cell activation (1, 3). Thus the failure to synthesize costimulatory factors such as B7 may be related to the generation of tolerance. Conversely, inappropriate expression of B7 might lead to unregulated costimulatory activity potentially resulting in autoimmune phenomena.

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