STRUCTURE, EXPRESSION, AND GENETIC LINKAGE OF THE MOUSE BCM1 (OX45 OR Blast-1) ANTIGEN

Evidence for Genetic Duplication Giving Rise to the BCM1 Region on Mouse Chromosome 1 and the CD2/LFA3 Region on Mouse Chromosome 3

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An antigen named MRC OX45 in the rat, or Blast-1 in the human, is of particular interest because it forms a subset within the Ig superfamily (IgSF)[1] along with the CD2 and LFA3 antigens that are known to form a recognition pair (1, 2). Since the loci for OX45/Blast-1 were recently designated *Bcm-1* in mouse and *BCM1* in human by the genetic nomenclature committee, for simplicity, we will call the antigen BCM1 in this report. CD2 and LFA3 are involved in adhesion reactions between T cells and accessory cells (3), and CD2 can also act as a target for mAb-induced mitogenesis on human (4) and rat (5) T lymphocytes. In the rat, the BCM1 antigen is found on most leukocytes and endothelial cells, with high levels of expression on macrophages and activated T lymphocytes (6, 7). The tissue distribution of BCM1 resembles LFA3 (8) more than CD2 antigen, which is restricted to human T lymphocytes (4), rat T cells plus some macrophages (5), and mouse T plus B cells (9).

Both rat and human BCM1 antigens are attached to the cell surface via a glycosyl phosphatidylinositol (GPI) anchor, a property shared with LFA3 antigen, which is found in GPI- or protein-anchored forms (1, 2, 10, 11). While the mouse GPI-anchored molecules Thy-1 and Ly-6 can function as targets for mAb-induced mitogenesis of T lymphocytes in the presence of phorbol esters (12, 13), mAbs against rat BCM1 have not been found to be mitogenic but, instead, inhibit T lymphocyte responses in cultures that contain macrophages (7). The basis of this effect is obscure, but it appears to occur via potentiation of suppression by macrophages or other bystander cells.

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[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; GPI, glycosyl phosphatidylinositol; IgSF, Ig superfamily; PFGE, pulsed field gel electrophoresis; RFLV, restriction fragment length variant.

The genetic loci for CD2 and LFA3 are closely linked (14–16), presumably reflecting evolution from a common precursor. It is of particular interest that two molecules apparently derived from a common precursor have evolved into a recognition pair for cell interactions. The locus for the human BCM1 antigen is also linked to the CD2 and LFA3 loci, but the linkage interval is much greater than between CD2 and LFA3 (2).

In this paper, we report the cloning and sequencing of mouse BCM1 antigen, and partially determine its tissue distribution with a rabbit anti-rat BCM1 serum that crossreacts with the mouse antigen. Mouse BCM1 has been expressed in Chinese hamster ovary (CHO) cells as a preliminary step to raising mAbs for functional studies. The locus for the mouse antigen has been genetically mapped, and long-range restriction site analyses have been compared with those for the human homologue. The results show conservation of a chromosomal segment in human and mouse that has similarities to the region containing the CD2 and LFA3 loci.

Materials and Methods

Mice. C3H/HeJ-gld/gld and *Mus spretus* (Spain) mice and [(C3H/HeJ-gld/gld × *M. spretus*)F_1 × C3H/HeJ-gld/gld] interspecific backcross mice were bred and maintained as previously described (17). C57BL/6J, DBA/2J, and BXD recombinant inbred strains were obtained from The Jackson Laboratory, Bar Harbor, ME; Balb/c mice were from the Sir William Dunn School of Pathology.

Cloning of cDNA, Sequencing, and Sequence Comparisons. Standard cloning and sequencing methods were used as outlined previously (18). The ALIGN program of Dayhoff et al. (19) was used to evaluate the significance of sequence similarities with parameters as done previously (1). The ALIGN program compares similarities between test sequences in terms of the number of SD that a score obtained with the best alignment of the sequences is away from the random mean best score obtained by scrambling the sequences a number of times (e.g., 100–150). Scores of >3 and >5 SD U can be expected by chance in 2 and 0%, respectively, of comparisons (20).

Southern Hybridization and Pulsed Field Gel Electrophoresis (PFGE). DNA was isolated from mouse organs by standard techniques and digested by restriction endonucleases, and 10-μg samples were subjected to electrophoresis in 0.9% agarose gels. DNA was transferred to Nytran (Schleicher & Schuell, Inc., Keene, NH) or Hybond-N (Amersham International, Amersham, UK) membranes and hybridized with washing under stringent conditions, as previously described (17). PFGE was performed as described previously (21).

Molecular Probes. All probes were labeled by the hexanucleotide technique with α-[^{32}P] dCTP or dATP as previously described (17). Probes from mouse BCM1 cDNA are indicated in Fig. 1, and the *Bcm 1* RFLV were detected with the 1,150-bp Xho I insert (Fig. 1 *C*). The human BCM1 probe was a 1,200-bp Eco RI insert from clone p06154 (2). The mouse α-spectrin (*Spna-1*), *Ly-17* (*CD32*), and α 3 subunit of Na^+K^+-ATPase (*Atpa-3*) RFLV were detected as previously described (17, 22). The ATP1A2 probe was a 2,700-bp Eco RI insert from a human cDNA clone (kindly provided by Dr. Dackowski, Integrated Genetics, Inc., Framingham, MA) (16).

Gene Linkage Analyses. Maximum likelihood estimates of recombination probabilities and their standard errors among backcross progeny were calculated according to Green (23). The best gene order was determined according to Bishop (24).

Expression of Soluble Mouse BCM1 Antigen. A stop codon was inserted in the mouse BCM1 cDNA clone to replace nucleotides 693–695 by use of oligonucleotide-directed polymerase chain reaction. The mutated cDNA was ligated into the pEE6HCMV.GS plasmid (kindly provided by Celltech, UK, Ltd.) and was transfected into CHO cells (25, 26). Positive cells were selected with 15–25 μM of methionine sulphoximine. To enhance the expression of soluble BCM1, 2 mM of butyrate was added to the culture medium. The use of this system has been fully described for expression of soluble CD4 (27).

Expression of mouse BCM1 was assayed by inhibition of an indirect radioactive binding assay using mouse thymocyte targets, rabbit anti-rat BCM1 in the first step, and ^{125}I horse anti-rabbit IgG antibody in the second step (28).

Preparation of Rabbit Anti-rat BCM1 Antisera and FACS Analysis. Rat BCM1, purified as in reference 1, was injected at 100 μg per dose i.m. with CFA in two doses at an interval of 10 d, and bleeds were taken from day 22. IgG was purified, and $F(ab')_2$ fragments were prepared using standard methods (28). Cells were obtained from various lymphoid organs of BALB/c mice, and 5×10^6 cells were incubated at 4°C with 3.75 μg of $F(ab')_2$ anti-rat BCM1 antibody with FITC-labeled pure horse anti-rabbit IgG in the second step (28). Analysis of labeling cells was carried out on a FACScan instrument (Becton Dickinson & Co., Mountain View, CA) with gating to exclude dead cells and erythrocytes.

Results

Cloning and Sequencing of Mouse BCM1. A murine thymocyte cDNA library in λgt11 (kindly provided by Dr. Franco Calabi, MRC Laboratory of Molecular Biology) was screened with an 894-bp Bam HI/Nsi I fragment of rat BCM1 cDNA as a probe (Fig. 1 *A*). A positive clone (clone 1) containing a 1.4-kb insert was obtained and sequenced to reveal a coding sequence for the C2 domain of BCM1 with flanking noncoding sequence (Fig. 1 *B*). It is assumed that this cDNA clone was derived from mRNA containing unspliced introns, which seems to be often found in mRNA from thymocytes (29). The positions of introns suggested by this clone are shown in Fig. 2.

Another cDNA library constructed with the mouse C5 cell line in a pCD vector (30, 31) was screened using the $\alpha[^{32}P]$-dATP-labeled Dra I/Eco RV fragment of clone 1 as a probe (Fig. 1 *B*). Six independent positive clones all had the same sized inserts. One of them was sequenced and contained a full-length sequence, as shown schematically in Fig. 1 *C*. On Northern blots, one band only of ~1.2 kb was seen with RNA from thymus, spleen, and peritoneal exudate cells (data not shown).

The sequence for the full-length clone is shown in Fig. 2, with the coding sequence within the open reading frame defined by homology with the rat molecule (1). The features of the sequence are indicated in Fig. 2, and the similarity to rat and human sequences can be seen from Fig. 3. In all cases, the molecules contain two IgSF domains with the first being V like without a disulphide bond, and the second being a C2SET domain. The sequences at the COOH terminus have the features of signal

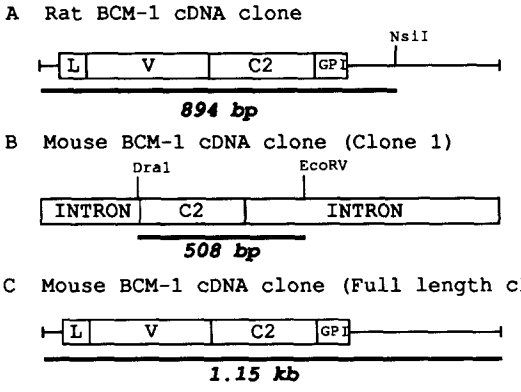

FIGURE 1. Probes for cloning of mouse BCM1 cDNA. (*A*) Rat BCM1 cDNA was digested with Bam HI (cloning site) and Nsi I. A 894-bp fragment containing the whole coding sequence was used as a probe in the first screening of a mouse thymus cDNA library. (*B*) A partial mouse BCM1 cDNA clone was isolated from a mouse thymus cDNA library in λgt11. A Dra I/Eco RV-digested fragment of 508 bp was isolated and used to screen the mouse C5 library, and also for Southern blot analysis. (*C*) A full-length cDNA clone of mouse BCM1 antigen was isolated from the mouse C5 library in the pCD vector. The whole mouse BCM1 cDNA clone was used for RFLV and pulsed field analysis. All probes were labeled with either α-$[^{32}P]$dATP or α-$[^{32}P]$dCTP. Filters were hybridized at 42°C and washed with 0.2–1× SSC at 65°C.

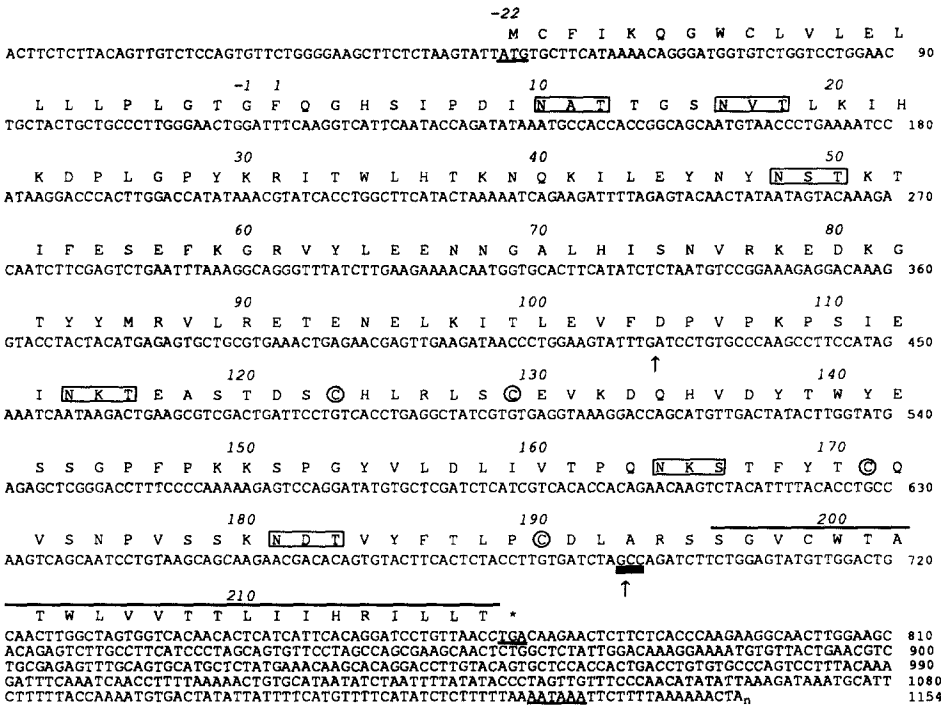

FIGURE 2. Sequence of mouse BCM1. The mouse BCM1 cDNA was sequenced from both directions, and its full length is 1.1 kb. The leader peptide is designated as residues −22 to −1, on the basis of a comparison with the rat sequence for which the NH_2 terminus is known (1). The initiation, termination, and poly(A) signals are underlined. The GPI-anchoring signal (residues 196–219) is overlined. The six possible N-linked glycosylation sites and four cysteine residues are boxed and circled, respectively. The presumed intron positions in clone 1 are marked (↑). The bold bar indicates the position where a stop codon (TGA) was introduced to mutate the cDNA for expression of a soluble form of the antigen. These sequence data have been submitted to the EMBL/GenBank/DD B J Nucleotide Sequence Database, under the accession number X17501.

sequences for GPI anchors, except for the unusual presence of His and Arg residues at position 213 and 214 in the mouse sequence. The point of attachment of the GPI group has been established for rat BCM1 to be at the Ser, marked with an asterisk in Fig. 3. In all the clones, including the six mouse clones mentioned above, there has been no evidence for an additional form of this molecule with a transmembrane protein sequence, as is the case for LFA3. The sequences are conserved between species in a manner that is similar to that seen for CD2 or CD4.

The similarities in sequence between BCM1, CD2, and LFA3 were evaluated by use of the ALIGN program in comparisons of sequences that included both IgSF domains of each antigen. The definition of the domains and the parameters used in the ALIGN program were as previously described (1) (see also, Materials and Methods). The comparisons between human, rat, and mouse BCM1, and human, rat, and mouse CD2 gave scores in SD units: 6.51, 6.68, and 6.13 (BCM1 sequences vs. human CD2); 5.63, 5.43, and 5.93 (vs. rat CD2), and 5.53, 6.05, and 6.24 (vs. mouse CD2). In comparisons between human, rat, and mouse BCM1 sequences

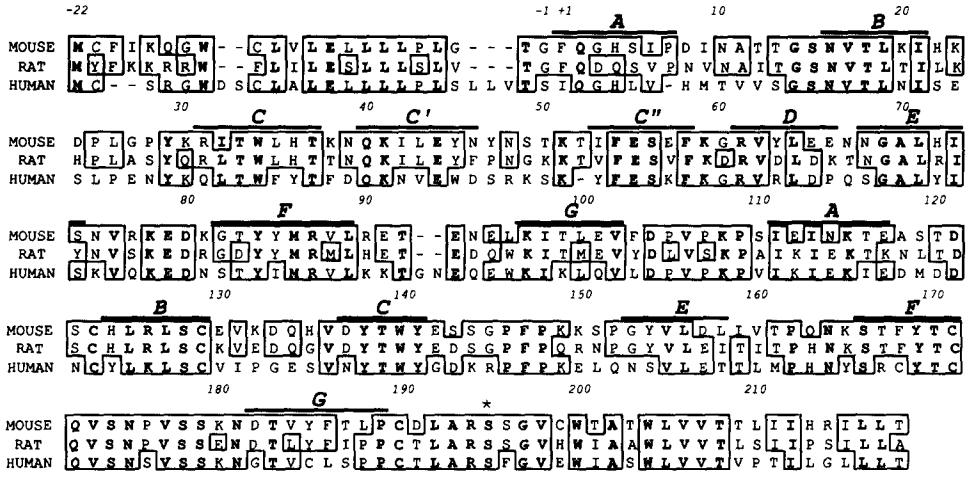

FIGURE 3. Comparison of mouse, rat, and human BCM1 sequences. The predicted protein sequences of mouse, rat, and human BCM1 are aligned. Identities between at least two species are boxed, while residues identical in all three species are in bold letters. The bars and letters above the sequences indicate the positions of postulated β strands in IgSF domains. The asterisk above residue 195 indicates the proposed site for the attachment of GPI anchor.

and human LFA3 sequence, the scores were: 7.26, 7.5, and 10.0. For human, rat, and mouse CD2 vs. LFA3, the scores were: 6.52, 6.0, and 4.94. Thus, the BCM1 sequence seems more similar to LFA3 than CD2 on the basis of these comparisons, and LFA3 also scores better with BCM1 than CD2.

Expression of Mouse BCM1. To express soluble mouse BCM1, a stop codon was inserted by site-directed mutagenesis in the mouse sequence replacing nucleotide residues 693–695. This yields a coding sequence ending at the Leu-192 residue that comes two amino acids after the last Cys of the second disulphide bond. The mutated cDNA was then cloned into the pEE6HCMV.GS expression vector (25, 26), which has been shown to give high level expression of soluble CD4 in CHO cells (27). The expression system works on the basis of a cytomegalovirus promoter with selection via glutamine synthetase in the presence of the glutamine synthetase inhibitor methionine sulphoximine.

Secretion of mouse BCM1 into the medium of selected CHO cells was assayed using a rabbit anti-rat BCM1 serum that crossreacts with the mouse BCM1. The results are shown in Fig. 4 *A*, which shows inhibition of antibody binding to mouse thymocytes by pure rat BCM1 and extracts from the CHO cells. These extracts were from the first round of selection, and the best culture gave antigen at the level of ~3 mg/l.

The nature of the secreted product was determined by SDS-PAGE and Western blotting, and, under reducing conditions, a band of similar size to pure rat BCM1 was seen (Fig. 4 *B*). However, in nonreducing gels, the mouse molecule ran at the size of a dimer compared with the monomer form seen for rat BCM1. It seems possible that the second disulphide bond of domain 2 may not have formed leading to the presence of free sulphydryls and the formation of disulphide-linked multimers. Presumably, the GPI signal sequence is present when the protein fold is formed,

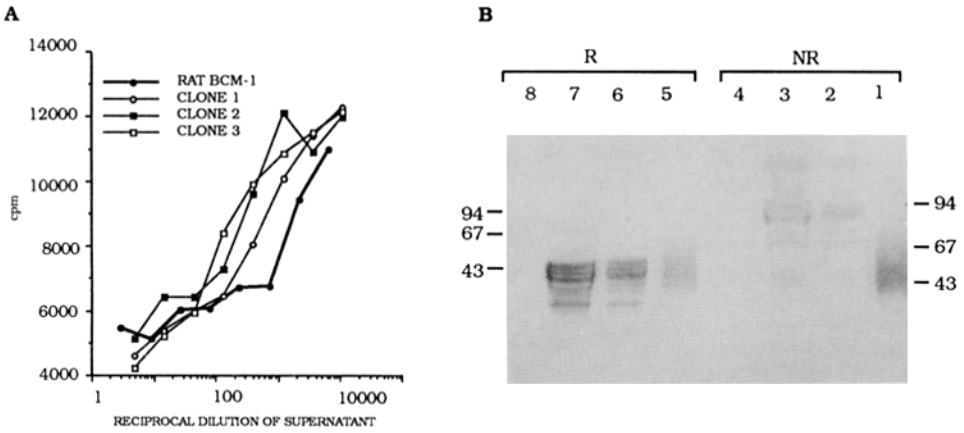

FIGURE 4. Expression of soluble mouse BCM1 antigen (sBCM1). (*A*) Inhibition of crossreacting rabbit anti-rat BCM1 antibodies with soluble mouse BCM1. Rabbit antiserum against rat BCM1 was titrated on mouse thymocyte targets (5×10^6), and a dilution of 1:800 was chosen for the inhibition assays. Aliquots of the antibody were incubated with dilutions of pure rat BCM1 antigen or of supernatants from the CHO cells, and then binding was assayed on the thymocyte targets (28). The concentration of undiluted rat BCM1 antigen was 8.6 μg/ml. Three different clones were assayed and shown to produce 1-3 μg/ml of sBCM1. (*B*) Western blot analysis of sBCM1. Culture supernatant from clone 1 (*A*) was concentrated, and ~10 μg total protein was subjected to electrophoresis on polyacrylamide gels in SDS and transferred to nitrocellulose membrane. The filter was incubated with 200× diluted rabbit anti-rat BCM1 for 1 h at 4°C and then incubated with peroxidase-conjugated swine anti-rabbit Ig as second antibodies for 1 h at 4°C. Positive bands were developed by incubating with 0.5 mg/ml chloronapthol in buffer with H_2O_2. Lanes *1* and *5*, rat BCM1 antigen; lanes *2* and *6*, culture supernatant containing sBCM1; lanes *3* and *7*, culture supernatant (with butyrate) containing sBCM1; lanes *4* and *8*, culture supernatant containing soluble rat CD8 antigen (kindly provided by Dr. B. Classon) as a control. Lanes *1-4* and *5-8* were under nonreducing and reducing conditions, respectively.

and this may be important for correct alignment of Cys residues for the second disulphide bond. This requires further investigation, but the point for now is that the size of the expressed product is as expected for mouse BCM1.

Cell Distribution of Mouse BCM1. Labeling of leukocytes was assessed with an $F(ab')_2$ form of the rabbit anti-rat BCM1 antibody with control labeling being assessed by blocking with the expressed form of the mouse molecule. As shown in Fig. 5, most leukocytes were labeled with anti-BCM1 antibody, as is the case in the rat. Labeling of erythrocytes was not clearly seen, and this may be a difference in comparison with the rat, where erythrocytes are $BCM1^+$. Alternatively, there may be a sensitivity problem with the crossreacting antibodies and a final assessment will require the production of mAbs. Immunoperoxidase studies were attempted to look for labeling of endothelium, but the results were unsatisfactory and this point will also be later addressed with mAbs.

Genomic Analysis by Southern Blotting. To determine the number of mouse BCM1 genes, Southern blotting was undertaken using the clone that contains coding sequence for only the C2 domain of BCM1 (Fig. 1 *B*). With four restriction enzymes that do not cut in the C2 domain sequence, single bands were seen leading to the conclusion that only one BCM1 gene is present (Fig. 6 *A*). These data also supported the possibility that the C2 domain is contained in only one exon, as was sug-

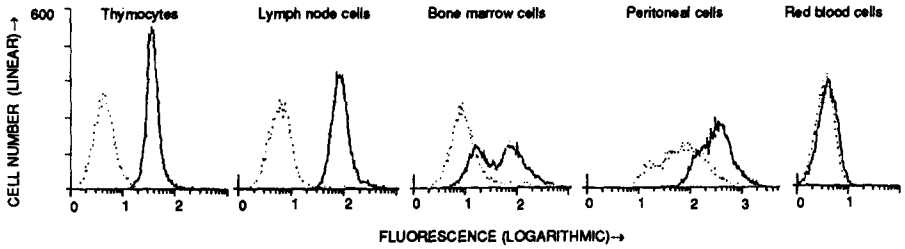

FIGURE 5. Distribution of mouse BCM1 antigen. Cells were prepared from BALB/c mice, and 5×10^6 cells were incubated with 3.75 μg purified F(ab')$_2$ of rabbit anti-rat BCM1, which was absorbed with 1 mg/ml crude mouse liver homogenate (————) or with liver homogenate and 75 ng/ml soluble mouse BCM1 antigen (----) as negative control. FITC-conjugated purified horse anti-rabbit IgG antibody was used to detect binding of the first antibody, and analysis was carried out on a FACScan instrument.

gested by the thymus BCM1 cDNA clone that is thought to contain unspliced introns (Figs. 1 *B* and 2).

Mapping of the Mouse BCM1 Locus (Bcm-1). Previous studies have shown that the human BCM1 locus (*BCM1*) and the CD2 and LFA3 loci (*CD2* and *LFA3*) are located in genetic regions on either side of the centromere of human chromosome

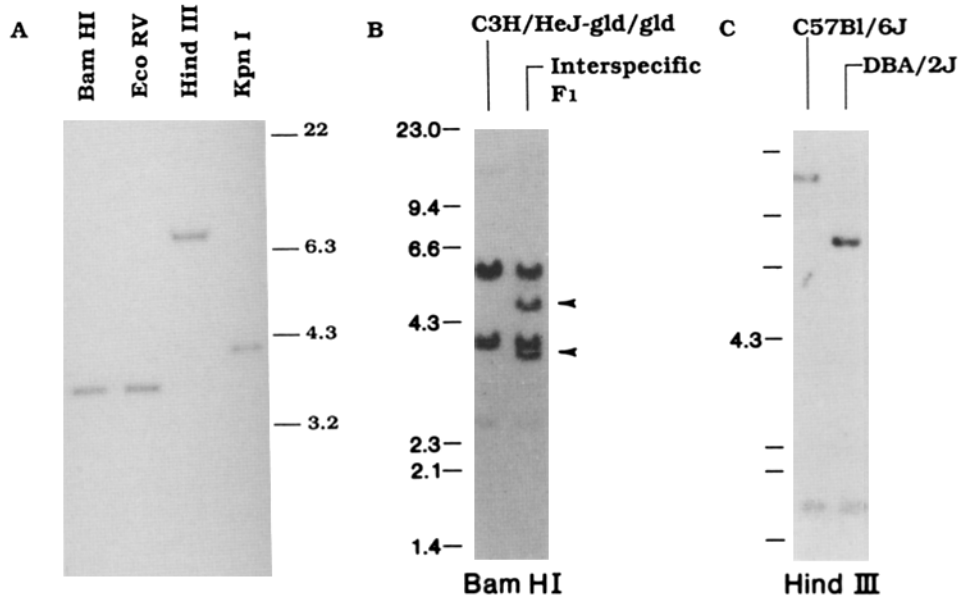

FIGURE 6. Southern blots with mouse BCM1 probes. (*A*) Genomic DNA was isolated from BALB/c male mice, aged 3–5 wk. 10 μg of genomic DNA was digested with Bam HI, Eco RV, Hind III, and Kpn I. Digests were run on a 0.9% agarose gel and transferred to nitrocellulose membrane. The filters were hybridized with $\alpha[^{32}P]$-labeled cDNA probe, as shown in Fig. 1 *B*. (*B* and *C*) Southern blot identification of RFLV detected with the *Bcm-1* gene probe. Restriction endonucleases are indicated at the bottom, and molecular size standards (in kilobases) are shown at the left of each panel. (*B*) The arrows signify bands present in DNA from (C3H/HeJ-gld/gld × *M. spretus*) interspecific F_1 mice that are not present in DNA from the C3H/HeJ-gld/gld parental mice. (*C*) Variant bands that distinguish DBA/2J from C57BL/6J mice are shown.

1 in the 1q21-23 and 1p12 regions, respectively (2, 14, 15). In the mouse, there is a large region on chromosome 1 that is syntenic with human chromosome 1q21-32, while the human 1p12 region is syntenic with a region on mouse chromosome 3 (discussed later) (16, 21, 22). We therefore examined the linkage relationship of *Bcm-1* restriction fragment length variants (RFLV) among other mouse chromosome 1 markers previously localized in a panel of genomic samples generated from [(C3H-gld/gld × *M. spretus*)F_1 × C3H-gld/gld] interspecific backcross mice. RFLVs are readily detectable between the DNA of *M. spretus* and laboratory mice. *Bcm-1*-related RFLVs that differentiate (C3H-gld/gld × *M. spretus*)F_1 mice and the homozygous C3H/HeJ-gld/gld mice were detected with the use of the full-length mouse BCM1 probe (Fig. 1 *C*), and are shown in Fig. 6 *B*. A panel of DNA from 428 interspecific backcross mice was then analyzed for segregation of the *Bcm-1* RFLV and previously described RFLV for *Ly-17* (*CD32*), *Atpa-3*, and *Spna-1* (17, 22). At each locus, mice displayed either the homozygous C3H (CC) or the heterozygous F_1 pattern (SC). The best gene order (24), illustrated in Table I, resulted in elimination of double crossover events. These data indicated that *Bcm-1* was located (± SD) 0.7 ± 0.4 cM telomeric of Ly-17 and 1.2 ± 0.5 cM centromeric of *Atpa-3*.

TABLE I

Gene Mapping Using (C3H/HeJ-gld/gld × M. spretus) F_1 × C3H/HeJ-gld/gld Backcross Mice

A. Genotypes* and numbers of mice

Marker	No recombination event		One recombination event				Human homologue‡
Ly-17 (CD32)	CC§	SC	CC x SC	CC	SC	CC	*CD32* 1q23-24
Bcm-1	CC	SC	SC	CC x SC	SC x CC	CC	*BCM1* 1q21-23
Atpa-3	CC	SC	SC	SC	CC	CC x SC	*ATP1A2* 1q
Spna-1	CC	SC	SC	SC	CC	SC	*SPTA1* 1q22-25
	346‖	71	3	2	3	3	

B. Summary of gene linkage data

Linkage interval	r¶	cM	r	r̄
Ly-17 - Bcm-1	3/428	0.7	0.15	2.0
Bcm-1 - Atpa-3	5/428	1.2	0.36	2.6
Atpa-3 - Spna-1	3/428	0.7	0.15	2.0

* Columns indicate the genotype of individual backcross mice as determined in this study. Genotypes for mouse gene probes were determined by RFLV, illustrated in Fig. 6, and as previously reported (17, 22). With the gene order given, no multiple crossovers were seen.

‡ Nomenclature of human homologues and previous chromosomal assignments (2, 39, 40, 41).

§ CC, C3H/HeJ homozygous genotype; SC, F_1 genotype; x, crossover.

‖ Number of mice. The larger number of mice typing as C3H homozygous reflects selection of many of the backcross mice for the gld/gld phenotype, consistent with a previous study mapping the *gld* gene on distal mouse chromosome 1 (17).

¶ r, recombination frequency; r and r̄ represent 95% confidence intervals based on binomial distribution.

Since *Bcm-1* was localized to a region of distal mouse chromosome 1 that contains *Mlsa* (32, 33), and previous studies suggested that the BCM1 antigen might have functional properties consistent with those of Mlsa (7), we examined the distribution of *Bcm-1* RFLV in a series of BXD recombinant inbred strains that had previously been typed for Mlsa (33). *Bcm-1* RFLVs which distinguish the two parental strains C57BL/6J and DBA/2J are shown in Fig. 6 *C*. A definitive crossover between *Bcm-1* and *Mlsa* was demonstrated in the BXD 29 strain (Table II), indicating that it is extremely unlikely that *Bcm-1* encodes Mlsa.

Long-range Restriction Site Analysis of Bcm-1 and BCM1. Recent studies examining long-range restriction maps indicated that the genomic organization of five genes, α-spectrin (*Spna-1/SPTA1*), serum amyloid P component (*Sap/APCS*), C-reactive protein (*Crp/CRP*), the α chain of the high affinity Fc ϵ receptor (*Fcε1a/FCE1A*), and the α 3 subunit of Na^+K^+-ATPase (*Apta-3/ATP1A2*) had been conserved during evolution of mouse and human genomes (21). In addition, studies in mouse and man indicated close physical linkage of *Ly-37/CD2* and α-1 subunit of Na^+K^+-ATPase (*Atpa-1/ATP1A1* (16). It was therefore of interest to determine whether *Bcm-1/BCM1* were closely physically linked with *Atpa-3/ATP1A2* in both species.

Analysis of long-range restriction fragments on PFGE blots containing mouse lymphoid DNA indicated that *Bcm-1* was localized to several fragments in common with *Atpa-3*. Probes to both genes detected prominent 1,800-kb Mlu I, 2,400-kb Not I, and 2,800-kb Nru I bands (Fig. 7). BCM1 and Atpa-3 probes also hybridized to a faint 1,600-kb Nru I band and a common 1,800-kb band with Nru I/Mlu I double digestion (Fig. 7), confirming the linkage of these two genes within the 1,600–1,800 kb range. Combined with previous studies of this region of the genome (21), these data indicated physical linkage in the order *Bcm-1 - Atpa-3 - Fcε1a - Sap - Spna-1* (where *Crp* is adjacent to *Sap* and *Fcε1a* (Fig. 8 *A*).

TABLE II

Mapping of Bcm-1 in BXD Recombinant Inbred Strains

	Recombinant inbred strain number					
Locus	1 12 14 16 18 19 21	5 9 11 22 24 25 27 28 30 32	2 8	13 23	29	15
At-3	B	D	B	D	D	B
			x	x	x	
Apo-A2/Ly-17	B	D	D	B	B	B
Bcm-1	B	D	D	B	B	B
Sap	B	D	D	B	B	B
					x	
Spna-1	B	D	D	B	D	B
Mlsa	B	D	D	B	D	B

BXD strain distribution pattern. B and D are generic symbols for alleles inherited from C57BL/6J and DBA/2J, respectively. RFLV assignments determined for *Bcm-1* were as shown in Fig. 6; those for other markers were as previously determined (33). The BXD-29 crossover between *Mlsa* and *Bcm-1* was confirmed by determining *Bcm-1* RFLV using DNA from two individual mice of this strain that had previously been typed for *Mlsa* (33).

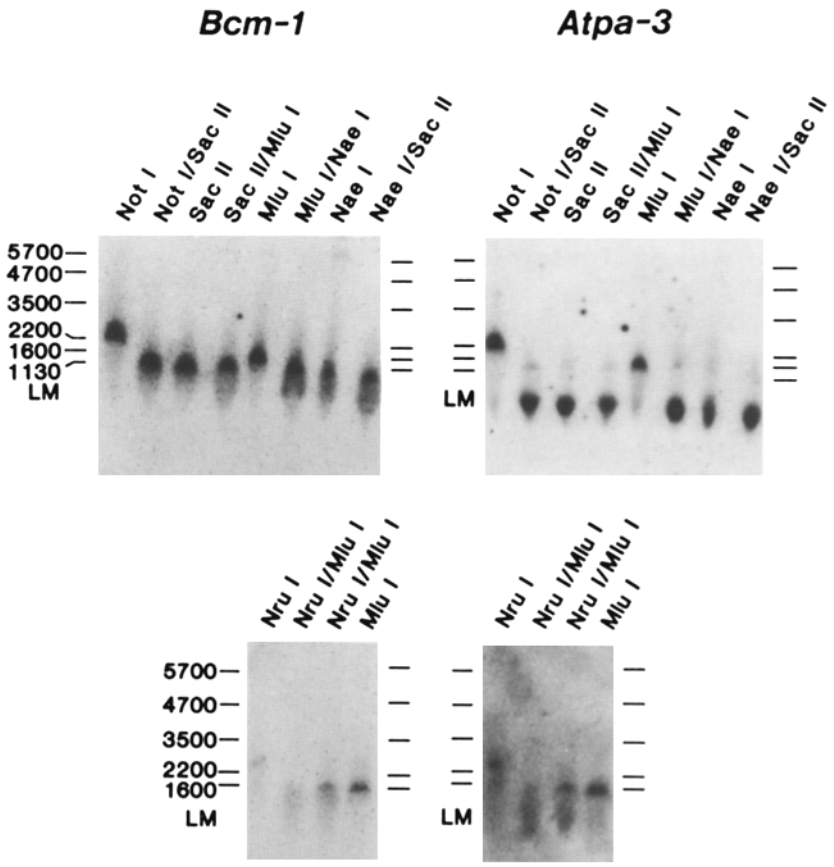

FIGURE 7. Autoradiographs of a mouse PFGE Southern blot sequentially hybridized with *Bcm-1* and *Atpa-3* gene probes. C3H/HeJ-gld/gld DNA was separated by PFGE using ramped pulses from 15–90 min. Restriction endonucleases are indicated at the top, and molecular size standards in kilobases are shown to the right of each panel. LM indicates limiting mobility. The top panels indicate that the two probes hybridized to common 2,400-kb Not I and 1,800-kb Mlu I bands. The bottom panel shows a common 2,800-kb Nru I band, the common 1,800-kb Mlu I band, and a common 1,800-kb band when an Nru I/Mlu I double digestion was performed.

Physical mapping studies of the human homologues of *Bcm-1* (*BCM1*) and *Atpa-3* (*ATP1A2*) were also undertaken by PFGE of high molecular weight DNA samples from human peripheral blood mononuclear lymphocytes. Probes for these two genes hybridized to a series of common bands with Nru I digestion (Fig. 9). A prominent common 1,050-kb Nru I band was easily visualized using PFGE conditions that resolved DNA between 200 and 1200 kb (*Bottom*), and a common partial Nru I band of 3,400 kb was visualized using PFGE conditions that resolved larger fragments (*Top*). In addition, a faint common 4,300-kb Nru I band was seen on longer exposure of the same autoradiographs (data not shown). The linkage of these two genes was confirmed with an Nru I/Mlu I double digest that resulted in dividing the 1,050-kb common Nru I band into a 700-kb fragment and a 350-kb fragment that hybridized to BCM1 and ATP1A2 probes, respectively. Thus, these studies indicated that *BCM1*

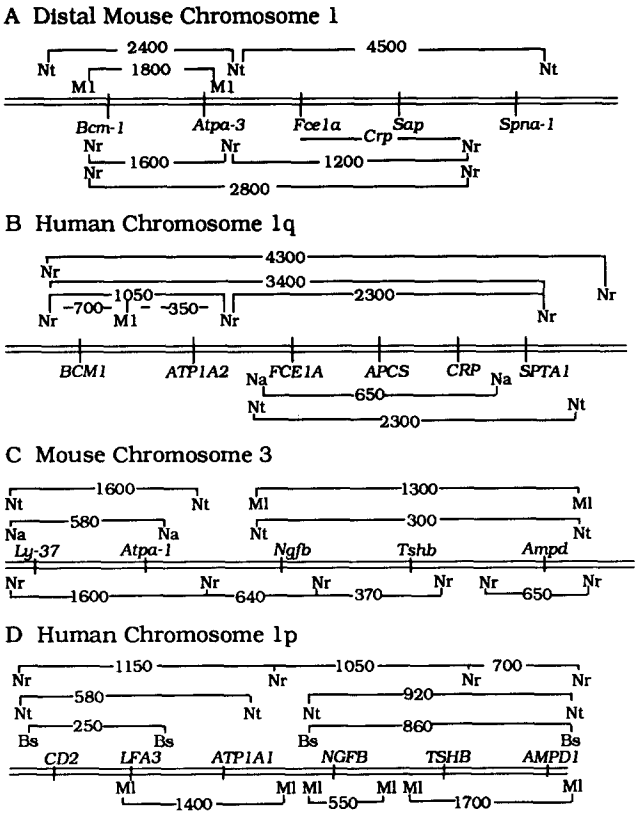

FIGURE 8. Long-range restriction maps of segments of distal mouse chromosomes 1 and 3 and human chromosomes 1p and 1q. *A* and *B* show maps for segments from distal mouse chromosome 1 and human chromosome 1q, as determined using PFGE (current study and reference 22). *C* and *D* show similar maps of segments of mouse chromosome 3 and human chromosome 1p as derived from a previous study (16). Not I (Nt), Mlu I (Ml), Nru I (Nr), Nae I (Na), BssH II (Bs) restriction endonuclease fragment sizes in kilobases are indicated.

and *ATP1A2* were located within 1,050 kb. Together with previous studies (2), the gene order was determined to be *BCM1-ATP1A2-FCE1A-APCS-CRP-SPTA1* (Fig. 8 *B*).

Discussion

In this study, we have cloned and sequenced the mouse BCM1 antigen and demonstrated that it has structural features that are similar to the rat and human homologues. The similarity of the mouse BCM1 sequence to CD2 and LFA3 antigen sequences supports the argument that this group of antigens constitutes a set within the IgSF that is closely related in evolution (1). Among the three sets of sequences, BCM1 and LFA3 scored as being more similar to each other than either was to CD2. The mouse BCM1 sequence was of the type expected for a GPI-anchored molecule and, thus far, there is no evidence for a protein-anchored form of BCM1 in any species.

One cDNA clone for mouse BCM1 showed the characteristics of a sequence with unspliced introns, and this sequence plus Southern blot analysis strongly argued for coding in one exon of the sequence for the second of the proposed IgSF domains. IgSF domains are usually encoded in one exon (34), and this situation has been found for the proposed IgSF domains of CD2 (35, 36). These observations support an IgSF relationship for the BCM1 and CD2 domains, although this relationship has been disputed (37).

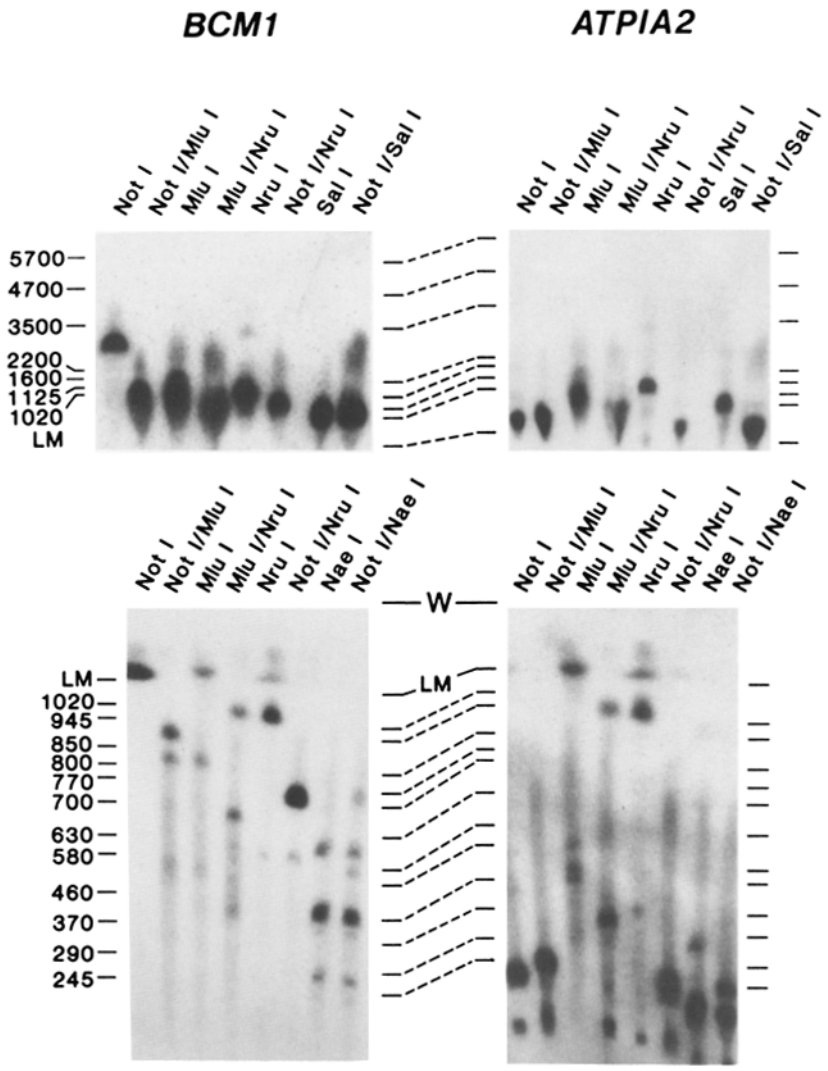

FIGURE 9. Autoradiographs of human PFGE Southern blots sequentially hybridized with *BCM1* and *ATP1A2* gene probes. Human PBL DNA was separated by PFGE using ramped pulses from 15–90 min (*top panels*) or using ramped pulses from 70–145 s (*bottom panels*). Molecular size standards in kilobases are shown on the sides of each panel. LM indicates limiting mobility. The *BCM1* and *ATP1A2* probes hybridized to common Nru I fragments of 1,050 kb (best visualized in the *bottom panels*) and 3,400 kb (*top panels*). Double digestion with Mlu I and Nru I resulted in the detection of a prominent 700-kb fragment with the *BCM1* probe and a 350-kb fragment with the *ATP1A2* probe, further suggesting that the common Nru I fragment size of 1,050 kb represents physical linkage of these two genes.

Gene linkage analysis established the mouse BCM1 locus (*Bcm-1*) to be on distal mouse chromosome 1. While *Bcm-1* is closely linked to *Mlsa*, analysis of recombinant inbred strains of mice indicated that it was extremely unlikely that BCM1 is the *Mlsa* product. *Bcm-1* was mapped within a 30-cM mouse linkage group that contains

>20 genes that have been localized to a large segment of ~70 cM of human chromosome 1q (Table I; references 17 and 22). In addition, long range restriction analysis of *BCM1/Bcm-1* and closely linked genes in both human and mouse demonstrate conservation of genomic organization for a segment of this linkage group (Fig. 8). Thus, a chromosome region has apparently been highly conserved in evolution subsequent to divergence of the mouse and human species.

The loci for the related antigens CD2 and LFA3 map to human chromosome 1p (14) in a position that is adjacent to 1q, with the centromere intervening between these regions. In the mouse, *CD2* (*Ly-37*) is on chromosome 3 (22, 38), and once again, a set of markers defining a region of the chromosome is conserved between mouse and human, as is evident from the gene maps in Fig. 8 (16). Previous studies suggest that a chromosomal rearrangement event occurred in this region during mammalian evolution, resulting in adjacent segments of human chromosome 1 being syntenic with linkage groups on mouse chromosomes 1 and 3 (22). The location of *Bcm-1* and *CD2* (*Ly-37*) on disparate mouse autosomes, in contrast to the location of the human homologues on the pericentric region of chromosome 1, supports the suggestion that the human gene order in this region more closely resembles the putative ancestral chromosomal organization than the mouse.

In the current study, *Bcm-1/BCM1* were physically linked with *Atpa-3/ATP1A2* within 1,600 kb on mouse chromosome 1, and within 1,050 kb on human chromosome 1q (Fig. 8). The related genes *Ly-37/CD2* and *Atpa-1/ATP1A1* were linked within 580 kb on mouse chromosome 3 and human chromosome 1p (5) (Fig. 8). Thus, the related molecules CD2 (and LFA3) and BCM1 are all linked in apparently similar orientation to α subunits of ATPases that are also related in evolution. This suggests that these segments of human chromosomes 1p and 1q resulted from duplication of a chromosome region containing a number of genes, and that this event occurred before the diversion of mouse and human. In the evolution of the *Mus* species, the loci appear to have become dispersed to mouse chromosomes 3 and 1, respectively. If this thinking is correct, the genetic regions adjacent to the BCM1 loci may contain a second gene that codes for the recognizer of BCM1 such that BCM1 and the proposed related structure form a recognition pair analogous to CD2 and LFA3.

Summary

The mouse BCM1 (OX45, Blast-1) antigen has been cDNA cloned and sequenced to provide data supporting the view that BCM1, LFA3, and CD2 constitute a subgroup within the Ig superfamily. Mouse BCM1 is widely expressed on leukocytes and is likely to be anchored to the cell surface by a glycosyl-phosphatidylinositol anchor, as is the case for rat and human BCM1 antigen.

Genetic linkage studies by recombination and pulse field analysis showed the BCM1 locus (*Bcm-1*) to be on distal mouse chromosome 1 and to be linked within 1,600 kb to the locus for an ATPase α chain gene (*Atpa-3*). A similar relationship was established between the human BCM1 locus (*BCM1*) and *ATP1A2*, and other markers on chromosome 1q. Conservation of genomic organization within a segment of human chromosome 1q and mouse chromosome 1 was demonstrated. A similar situation is seen in the region of the CD2 and LFA3 genes between mouse chromosome 3 and human chromosome 1p. Furthermore, the CD2/LFA3 genes are linked within

580 kb to *Atpa-1/ATP1A1* genes to provide a parallel situation to the linkage between *Bcm-1/BCM1* and *Atpa-3/ATP1A2* on chromosomes 1 (mouse) and 1q (human). Taken together, the data suggest duplication of a chromosome region including the precursors of the genes for BCM1, CD2, and LFA3, and the ATPase genes to give rise to the linkage groups now observed. The duplicated regions may have stayed together on chromosome 1 in the human (with the insertion of a centromere), while in the mouse, the genetic regions are proposed to have become dispersed in the formation of chromosomes 1 and 3. CD2 and LFA3 are more dissimilar in sequence than BCM1 and LFA3, and if the precursors of the CD2 and LFA3 loci formed before the proposed chromosome segment duplication, then a gene encoding a recognizer molecule for BCM1 may exist in linkage with *Bcm-1/BCM1* on chromosome 1 (mouse) and 1q (human).

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