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Cloning, Expression, and Function of BLAME, a Novel Member of the CD2 Family

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The CD2 family is a growing family of Ig domain-containing cell surface proteins involved in lymphocyte activation. Here we describe the cloning and expression analysis of a novel member of this family, B lymphocyte activator macrophage expressed (BLAME). BLAME shares the structural features of the CD2 family containing an IgV and IgC2 domain and clusters with the other family members on chromosome 1q21. Quantitative PCR and Northern blot analysis show BLAME to be expressed in lymphoid tissue and, more specifically, in some populations of professional APCs, activated monocytes, and DCs. Retroviral forced expression of BLAME in hematopoietic cells of transplanted mice showed an increase in B1 cells in the peripheral blood, spleen, lymph nodes, and, most strikingly, in the peritoneal cavity. These cells do not express CD5 and are CD23+"Macl-", characteristics of the B1b subset. BLAME may therefore play a role in B lineage commitment and/or modulation of signal through the B cell receptor. The Journal of Immunology, 2001, 166: 5675–5680.

Members of the CD2 family, a subset of the Ig superfamily (1), function as coreceptors for lymphocyte activation and/or adhesion. The family currently consists of CD2 (LFA-2) (2), CD48 (Blast-1, BCM-1, OX-45) (3), CD58 (LFA-3), CD84 (4), CD150 (signaling lymphocytic activation molecule (SLAM)) (5, 6), Ly-9 (7), and CD244 (2B4) (8, 9). Members have been defined primarily by homology and structural features, each consisting of an Ig variable-like domain and a membrane proximal Ig constant-2 domain (5). The exception is Ly-9, which contains two pairs of IgV-C domains. The cytoplasmic domains of CD150, CD84, CD244, and Ly-9 also contain some homology, most notably, multiple copies of the tyrosine-based motif TyxYxxV/I/A. Disregulated signaling through SAP/SH2DIA (SLAM-associated protein/SH domain-containing gene 1A), which binds to this tyrosine-based motif, is thought to contribute to the phenotype of a sometimes fatal immunodeficiency X-linked lymphoproliferative syndrome (10, 11).

The genes encoding these proteins are found in two pericentric loci in humans, 1p13 (CD2 and CD58) (11) and 1q21–24 (CD48, CD84, CD150, CD244, and Ly-9) (12, 13). In the mouse, CD244, Ly-9, and CD48 are clustered on chromosome 1 (14), and CD2 is found on chromosome 3.

The ligands for CD2 family members that have been identified are within the CD2 family, CD2 to CD58 in humans (15) or CD48 in rodents (16), CD48 to CD244 (17), and the homotypic interaction of CD150 with itself (18). CD84 and Ly-9 are currently orphans.

CD2 is expressed on the surface of T cells and on NK cells (19). The extracellular domain of CD2 is an important adhesion molecule for the initial transient interaction of the T cell with an APC (20). Activation of the TCR by a MHC Ag complex leads to recruitment of CD2AP, an SH3 domain-containing adaptor protein, to the cytoplasmic tail of CD2. This leads to CD2 clustering and helps in the formation of the “immunological synapse” (21). Conversely, CD2 cross-linking has been shown to recruit the adaptor protein CD2BP1, which binds to the same region of the cytoplasmic domain of CD2 and is thought to down-regulate CD2-mediated adhesion (22). The ligand for CD2, CD58, is widely expressed on both hematopoietic and nonhematopoietic tissue.

Signaling via CD244 and CD150 involves competition between SAP and SHP-2 (SH2-domain containing protein tyrosine phosphatase (SAP) 2) for binding to the tyrosine-based motifs (11, 23). CD244 is expressed on NK cells, CD8 T cells, and γδ T cells. Ligation of CD244 on NK cells by CD48 on target leukocytes results in an increase in target cell lysis. Although ligation of CD244 on CD8 cells does not increase their cytolytic activity, CD8 "CD244-" cells have been shown to be responsible for non-MHC-restricted “natural” cytotoxicity (24). CD150 is expressed on activated T and B cells. Ligation on T cells results in costimulation of activation and an increase in IFN-γ secretion (25), but not the Th2-type cytokines IL-4 and IL-5, and on B cells induces proliferation and Ig secretion (26).

CD84 and Ly-9 contain two tyrosine-based domains; however, they are currently orphans and their function is unclear. CD84 is expressed on B cells, thymocytes, memory T cells, monocytes, and platelets, and Ly-9 is found on thymocytes, T and B cells, and bone marrow lymphoid cells (27, 28). The remaining two family members, CD58 and CD48, are GPI-linked membrane proteins and so contain no cytoplasmic domains (13).

Several members of the family exist in different forms. Splice variants of CD150, CD244, and CD84 can be found with different cytoplasmic domains containing three or one, four or one, and two or zero tyrosine-based motifs respectively (6, 29, 30). In addition,
there is a predicted secreted form of CD150 (26) and a transmembrane form of CD58 with a cytoplasmic domain of 12 amino acids containing no obvious signaling motifs.

In this paper, we describe the cloning, expression analysis, and possible function of a novel member of this family, B lymphocyte activator macrophage expressed (BLAME).

Materials and Methods

Cloning BLAME

Mixed lymphocyte response library. Approximately 100 ml of blood was collected from 24 healthy donors with informed consent, and PBMCs were isolated by Ficoll gradient. Total lymphocytes were cultured at 1 × 10⁷ cells/ml in RPMI 1640 with 10% FCS. Equal numbers of starting cells were harvested at 4, 8, and 24 h, and RNA was purified using standard techniques. The cDNA library was prepared as previously described (31).

Identification of human BLAME and the mouse ortholog and isolation of full-length clones. The MLR library was studied by high throughput single-pass sequencing and computer analysis. BLAME was originally identified by basic local alignment search tool analysis (32) as a homologue of SLAM (6), and a full-length clone was identified. The mouse ortholog was identified in the Millennium Pharmaceutical database as a full-length clone in a lung library from a mouse asthma model 3 h after Ag challenge (33).

Mapping. The chromosomal location of BLAME was mapped using the GeneBridge 4 Human Radiation Hybrid mapping panel (Research Genetics, Huntsville, AL). PCR amplified to introduce unique 5′- and 3′-EcoRI restriction sites produced a full-length clone, GGTTGAGAACTTGGTAACCC as the forward primer and GGGTGAGAGAAACTGTGAACC as the reverse primer (34). PCR products were run on a 2% agarose gel and scored for the presence or absence of the band in each of the 93 cell line DNAs. The results were analyzed using Map Manager software program.

Expression of BLAME in human leukocytes

TagMan analysis. PBMC and granulocytes were isolated from whole blood using Ficoll-Hypaque density gradient centrifugation. Whole blood was centrifuged in a step gradient of Ficoll at 1.077 g/ml. Granulocytes were isolated from the lower interface. Both cell layers were depleted of erythrocytes and washed before activation or subsequent isolation of cell populations. Individual cell populations (CD3, CD4, CD8, CD14, and CD19) were isolated using Abs to respective cell surface markers conjugated to magnetic beads and passed through separation columns (Miltenyi Biotec, Auburn, CA). PBMC and T cells were activated with PHA (5 µg/ml), and CD14+ cells were activated with LPS (100 ng/ml). Cells were cultured in RPMI 1640 medium with 10% FCS (Sigma, MO) supplemented with 2 mM l-glutamine, 0.1 M nonessential amino acids, and 1 M sodium pyruvate (Life Technologies, Rockville, MD).

Dendritic cells (DC) were differentiated from two sources, bone marrow-derived CD34+ cells to give DC1 or CD14+ monocytes to give DC2, as shown in Fig. 3b. Bone marrow-derived DC 1 were propagated from CD34+ cells cultured with GM-CSF (100 ng/ml), stromal cell factor (SCF; 120 ng/ml), and TNF-α (10 ng/ml) for 7 days (35–37). CD14+ cells were sorted, and fresh cytokones (GM-CSF and TNF-α) were added to replenish the medium. Cells were grown for 15–17 more days, stained for FACS analysis, and lysed for RNA isolation. CD14+–derived DC2 were generated according to the method of Sullasto and Lanzavecchia (38) and Pickl et al. (39). Monocytes were cultured in medium containing GM-CSF (50 ng/ml; R&D Systems, Minneapolis, MN) and IL-4 (50 ng/ml; PeproTech, Rocky Hill, NJ) for 12–14 days, replacing half of the medium every 3 days. Cells were stimulated with TNF-α (100 ng/ml) for 4 days to promote maturation; they were then activated with LPS (1 µg/ml) for 24 h. These cells were CD83+ after LPS stimulation.

Th1 and Th2 cells were differentiated according to Somsasse et al. (40).

Total RNA was prepared from purified cells by a single-step extraction method using RNA STAT-60 according to the manufacturer’s instructions (Tel-Test, Friendswood, TX). Each RNA preparation was treated with DNase I (Ambion, Austin, TX) at 37°C for 1 h. DNase I treatment was determined to be complete if the sample required at least 38 PCR amplification cycles to reach a threshold level of fluorescence using β2-microglobulin as an internal amplon reference. After phenol extraction, cDNA was prepared from the sample using the SuperScript Choice System following the manufacturer’s instructions (Life Technologies).

BLAME expression was measured by TaqMan quantitative PCR (Applied Biosystems, Foster City, CA). PCR probes designed by PrimerExpress software (Applied Biosystems) were as follows: β2-microglobulin forward primer, ATGCTCGGCGTGTGAAACAGTG; β2-microglobulin reverse primer, CTGAGACTCTTGCTGGTACCAAAG; BLAME reverse primer, GCTACAGACTTGAGTAACCG; BLAME probe, CATTGGCGCCTAGAAAGTTACG; and BLAME forward primer, TGTCACACACTGCGTTCCTA.

BLAME probe was labeled using 6-carboxyfluorescein, and the β2-microglobulin probe was labeled with VIC. Each reaction contained 200 nM of forward and reverse primers plus 100 nM probe for β2-microglobulin and 600 nM forward and reverse primers plus 200 nM probe for BLAME, and reactions were conducted in TaqMan Universal PCR Master Mix (Applied Biosystems) using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Conditions were as follows: hold for 2 min at 50°C and 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 s followed by 60°C for 1 min. ΔCt value (expression of BLAME relative to β2-microglobulin) was calculated using the following formula: 

\[ \Delta Ct = Ct_{BLAME} - Ct_{\beta 2-microglobulin} \]

Relative expression was then calculated using the arithmetic formula given by 2^-ΔΔCt.

Northern blot analysis. Human poly(A)+ immune blot (Clontech Laboratories, Palo Alto, CA) was probed using a 32P-labeled probe corresponding to aa 1–233 of human BLAME according to the manufacturer’s instructions.

Total PBMCs were stimulated for 4 h in RPMI 1640 with 10% FCS supplemented with IL-2, IL-6, IL-9, IL-12, IFN-γ (10 ng/ml), IL-10, TGF-β, IL-4, IL-5 (20 ng/ml), or TNF-α (100 U/ml). Resting monocytes were isolated from PBMCs by Percoll gradient centrifugation and were >90% CD14+. CD4+, CD8+, and CD19+ cells were isolated from PBMCs by positive selection using MACS magnetic beads according to manufacturer’s protocols (Miltenyi Biotec). Monocytes were stimulated for 4 h in RPMI 1640 with 10% FCS with or without LPS or IFN-γ. RNA was prepared using RNeasy Mini Kit (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions, and Northern blots were probed for commercial blots.

Overexpression of BLAME in bone marrow-reconstituted irradiated mice

Construction and production of retroviruses. Full-length BLAME was PCR amplified to introduce unique 5′ KhoI and a 3′ EcoRI restriction sites and a Kozak sequence (ACCGCC) in the original cDNAs (Advantage-HF kit; Clontech Laboratories). The PCR products were ligated into the murine stem cell virus Neo retroviral vector (41), and clones were sequenced and selected for base-perfect match with the original cDNA. Viral supernatants were generated into the 293-EBV nuclear Ag cells (Invitrogen, Carlsbad, CA) by cotransfecting three constructs: the BLAME retroviral construct or control (empty murine stem cell Neo virus), pNSe vector containing the gagpol genes from the murine Moloney murine leukemia virus virus and a pNSe vector containing the vesicular stomatitis virus envelope glycoprotein G gene. Concentrated viral supernatants were prepared by centrifugation for 2 h at 50,000 × g (SW28 rotor, 25,000 rpm) at 4°C. Pellets were resuspended in DMEM with 10% FCS (Cell Technology, Vancouver, Canada), shaken at 4°C for 24 h, filtered, and frozen at −80°C.

Infection procedure. Bone marrow cells were collected from C57BL/6SJL mice (Tacfonics Farms, Germantown, NY) 4 days after 5-fluouracil treatment of 150 mg/kg administrated i.v. Lin− cells were selected using a MACS depletion column (type BS; Miltenyi Biotec). Briefly, cells were labeled with a mixture of four FITC-conjugated Abs against CD3ε, CD11b, CD45R, and Ly-6G (BD Pharmingen, San Diego, CA). Cells were washed and incubated with anti-FITC microbeads (Miltenyi Biotec). Labeled cells were removed using depletion columns according to manufacturer’s instructions. After separation, Lin− cells were washed and resuspended in DMEM with 10% FCS.

Before infection, Lin− cells (10⁶ cells/ml) were prestimulated with recombinant mouse (rm)IL-3 (10 ng/ml; Endogen, Woburn, MA), rmIL-6 (10 ng/ml; Endogen), rmSCF (100 ng/ml; R&D Systems), rm fm-slike tyrosine kinase-3 ligand (100 ng/ml; R&D Systems) and mouse thrombopoietin (10 U/ml, conditioned medium) for 2 days. Cells were centrifuged, resuspended in DMEM with 10% FCS and viral supernatant (1/1 v/v) in the presence of rmIL-3, rmIL-6, rmSCF, rm fm-slike tyrosine kinase-3 ligand, and thrombopoietin, and incubated at 37°C, 10% CO₂. This infection procedure was repeated 24 h later and 4 h after this second infection, the cells were collected, washed twice, and injected into lethally irradiated C57BL/6J mice (9.5 Gy; γ rays generated by cobalt source).
Analysis of mice. Major organs were harvested and tissue fixed in 10% buffered formalin stained with hematoxylin and eosin and subject to histologic analysis. Tissue examined included skin, kidneys, stomach, spleen, thymus, brain, heart (weighed), ovaries, muscles, skeletal muscle, thyroid/parathyroid, femur, brain (weighed), brown and white fat, pituitary, head, eyes, testes, aorta, spleen (weighed), stomach, intestines, liver (weighed), and adrenals.

RNA expression of BLAME was confirmed in the spleens of the transduced mice using a slot blot analysis (Slot Blot Manifold, Amersham Pharmacia Biotech, Piscataway, NJ). Total RNA (5 μg/sample) was loaded onto duplicated membranes using the slot blot apparatus. The membranes were washed with 10× SSC and irradiated with an UV transilluminator. Hybridization was conducted using 75 ng of randomly 32 P-labeled (Rediprime; Amersham Pharmacia Biotech) BLAME (0.7 kb corresponding to the extracellular domain) or GAPDH (1.2 kb) cDNA probes displaying similar specific activities. After washing, signals were analyzed using a phosphorimager (FujiFilm BAS-2500; Fuji Medical Systems, Stamford, CT). Using the same virus containing a green fluorescent protein (GFP) gene in place of the BLAME gene, we found that 74 ± 6% of the peripheral blood cells were GFP positive when mice were studied 15 wk after transplantation. Among these cells, similar percentages of GFP-positive cells were found in the Mac1 + population (87 ± 6%), the CD3 + population (67 ± 8%) and the B220 + cell populations (74 ± 8%). This result strongly suggests that the ectopic expression of BLAME is observed in all hematopoietic cell populations, including macrophages, T cells, and B cells.

Blood was collected from the tail vein or at necropsy by heart puncture. RBC were lysed, and FACS was conducted using FITC, PE, and Cy-5 (as predicted by MEMSAT (43)), and a short 31 amino acid cytoplasmic tail. Using the HMMER software (44) and the PFAM database of models (45), the extracellular domain was shown to contain the two Ig-like domains typical of the CD2 family, an N-terminal IgV-like fold that does not contain the conserved disulfide bonds, and a membrane proximal C2-like fold. There are no SAP/SH2D1A binding sites in BLAME (predicted by MEMSAT (43)) of CD2 family members. Peritoneal lavage was conducted at necropsy according to the manufacturer’s instructions. Peritoneal lavage was conducted at necropsy by washing the peritoneum twice with 2 ml PBS. All FACS was gated for viable leukocytes on the basis of forward and side scatter.

Results

BLAME is a novel cell surface protein of the CD2 family

BLAME was originally cloned from a human MLR library. The open reading frame encodes a 28 amino acid protein (Fig. 1) with a 22 amino acid leader sequence predicted by signal P (42). The mature protein is a type I transmembrane protein with a 212 amino acid extracellular domain, 21 amino acid transmembrane domain (predicted by MEMSAT (43)), and a short 31 amino acid cytoplasmic domain. The human BLAME mapping indicated that the gene was located on chromosome 3. This region of chromosome 1 (1q21–24) also contains multiple other family members: CD48, CD84, CD150, CD244, and Ly-9 (12) (13).

Homology between BLAME and other CD2 family members

Interestingly, CD48 and CD58 are currently the only two members of the superfamily member with structural similarity to CD2, 19A (GenBank accession number CAB81950) was included in this analysis. The human BLAME mapping indicated that the gene was located at chromosomal location 1q21. (with synteny to mouse chromosome 3). This region of chromosome 1 (1q21–24) also contains multiple other family members: CD48, CD84, CD150, CD244, and Ly-9 (12) (13).

Expression

Human immune Northern blot analysis revealed two transcripts of ~2 and 3.5 kb (Fig. 3a). In the lymph node, spleen, thymus, and bone marrow, the smaller transcript was more abundant, and highest BLAME expression was seen in lymph node. To identify the specific cell types expressing BLAME, we used real-time quantitative PCR and primers/probes within the 3’ untranslated region of BLAME. Significant expression was seen in PBMCs, monocytes, and certain DCs (Fig. 3b). However, Northern blot analysis showed no detectable expression in resting PBMCs. Using 4 h of stimulation with a variety of cytokines, we found that BLAME was induced by IFN-γ (Fig. 3c). In fact, purification of monocytes by adhesion to plastic provided sufficient stimulation to induce relatively high expression (data not shown), and all additional experiments were conducted using resting monocytes purified by Percoll gradient. Northern blot analysis of isolated monocytes stimulated with IFN-γ (but not LPS) confirmed the data generated using the sensitive TaqMan technique suggesting that BLAME is expressed in activated monocytes (Fig. 3d).

Therefore, BLAME is expressed on at least two populations of professional APCs, DCs, and activated monocytes.

Retroviral forced expression of BLAME in vivo using bone marrow reconstitution of lethally irradiated mice

To investigate the in vivo function of BLAME, we used reconstitution of lethally irradiated mice with retrovirally infected bone marrow to give expression of mouse BLAME in all hematopoietically derived cells. As a control, empty vector was used. Mice predicted by signal P or the transmembrane and intracellular domains) shows BLAME to be most closely related to CD85 (Fig. 2).

Interestingly, CD48 and CD58 are currently the only the two members of the family that, like BLAME, have no cytoplasmic signaling motifs. Annotated as an early response gene that encodes an Ig superfamily member with structural similarity to CD2, 19A (GenBank accession number CAB81950) was included in this analysis.

FIGURE 1. Sequence of mouse and human BLAME. Amino acid sequence of mouse and human BLAME. Putative signal peptides are italicics and transmembrane domains are in bold.

FIGURE 2. Homology between BLAME and other CD2 family members. Results as predicted by clustal analysis. Dendrograms showing relationship of the (A) full-length or (B) extracellular domains (as predicted by MEMSAT (43)) of CD2 family members.
revealed that these were in fact the same population of mice ($3 \times$ GAPDH RNA level for BLAME mice compared with control mice). The spleen of transduced mice was infected with virus as evaluated by resistance to G418 in methyl cellulose cultures (data not shown). The level of BLAME RNA in infected cells is expected. Fifty-four percent of progenitor cells were infected when a full hematopoietic reconstitution from these in-... were examined between 8 and 16 wk after bone marrow transplantation, when a full hematopoietic reconstitution from these infected cells is expected. Fifty-four percent of progenitor cells were infected with virus as evaluated by resistance to G418 in methyl cellulose cultures (data not shown). The level of BLAME RNA in the spleen of transduced mice was ~10 times that found in control mice (~3 times GAPDH RNA level for BLAME mice compared with 0.3 times GAPDH RNA for endogenous BLAME level in control mice).

The mice showed normal blood cell counts (white blood cells, RBC, platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils), and lymphoid organs (spleen, peripheral lymph nodes, and thymus) were normal size and showed no gross alteration in architecture. Pathologic examination of a panel of major organs showed no differences from control animals. However, FACS analysis of peripheral blood using a panel of Abs (CD3/NK1.1, CD4/CD8, GR1/Mac1, and B220/IgD) in combination with a marker for donor cells (CD45.1) showed an increase in Mac1low cells of blood, spleen, and peritoneal lavage (~3 times GAPDH RNA level for B220low cells) compared with an average of 35.9% in BLAME mice; SD 7.6 and 5.0, respectively ($p = 0.0001$). The spleen showed a less dramatic but still statistically significant increase in B1 cells (an average of 9.2% in control mice compared with an average of 12.6% in BLAME mice; SD 2.3 and 1.0, respectively; $p = 0.04$), whereas thymus and bone marrow were similar to control. Three-color FACS analysis showed the cells to be predominantly B220highMac1lowCD5−CD23lowIgD+ cells (Fig. 5), which is the phenotype of B1b “sister” cells. Both B220 and IgD expression levels are slightly lower than in B cells of control mice, as one would expect for B1b cells. In addition, the total percentage of B cells in the peritoneal lavage, as defined by expression of surface Ig, was increased from 46% (SD 9.7) to 73% (SD 9.7). This phenotype was duplicated in three separate experiments with five mice per group in each experiment.

Serum titers of blood from BLAME and control retroviral mice at 7 and 11 wk after reconstitution showed no differences in total IgM or IgG (data not shown).

Discussion

Two B cell lineages can be readily distinguished on the basis of cell surface markers, Ab repertoire, and anatomic location (46). Conventional B2 cells are found predominantly in the spleen, lymph nodes, and blood, whereas B1 cells are predominantly found in the peritoneal and pleural cavities. B1 cells can be further divided on the basis of CD5 expression. B1a cells express CD5, and B1b sister cells are CD5 negative. These three lineages have previously been shown to develop independently and will replenish their own populations on adoptive transfer (47). B2 cells are bone marrow derived, whereas B1 cells are derived from the fetal liver and then form a self-renewing population of cells (48, 49). B1b cells differ from B1a cells in that they can also be derived from adult bone marrow (50, 51).

In normal mice, the majority of B cells in the peritoneal cavity are B1a CD5+ cells. However, because these cells are not bone marrow derived, they are almost completely absent in the peritoneal cavity of lethally irradiated bone marrow-reconstituted mice. In their place, there is an increase in the percentage of B2 cells (B220−CD5−CD23high) from ~15–20% in wild-type mice to 40–
It is possible that forced BLAME expression is modulating the signal through the B cell receptor complex by binding to a presumed receptor on B cells and acting as a costimulator or an adhesion molecule. This may occur during initial differentiation to the B1 phenotype or by increasing proliferation or survival of B1b cells (there are no B1a cells in bone marrow-reconstituted mice) once they have reached the peritoneal cavity. Although overexpression of a cell surface gene may lead to constitutive signaling, without the necessity of interaction with a ligand/coreceptor, this seems unlikely in this case because BLAME does not contain the signaling motifs usually used by this family. However, we cannot exclude the possibility that BLAME associates with another chain that does contain a signaling domain. In the retroviral system, BLAME is expected to be expressed on all bone marrow-derived cells, whereas expression is normally restricted to activated macrophages and DCs. The possibility of a direct interaction between B cells and other APCs has been well established (58), and it is possible that in normal mice interaction between BLAME on activated macrophages/DCs and its receptor on B cells is responsible for B1 cell differentiation or maintenance.

The phenotype of the retrovirally transduced mice is similar to that described for IL-9-transgenic mice (59). Although IL-9 does not directly induce expression of BLAME, it is possible that it modulates the expression of the ligand for BLAME or that, conversely, BLAME modulates the expression of IL-9. However, unlike the IL-9-transgenic mice, we saw no alteration in the circulating Ig levels in mice overexpressing BLAME.

It was proposed (23), and has since been validated (12, 60), that the ligand/receptor pairs within the CD2 family are genetically linked. It seems likely that the ligands for the orphan receptors CD84 and Ly-9 would map to the same location, chromosome 1q21–23. Both of these receptors contain long cytoplasmic domains that include the TxYxxV/I/A motifs and may be likely to bind to a nonsignaling ligand. Both CD84 and Ly-9 are currently of unknown function and are expressed on B cells. It will be interesting to see whether BLAME is in fact the ligand for either of these two genes or for a novel CD2 family member.

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References

CD48 associates with very low affinity.


