BLAST-1 POSSESSES A GLYCOSYL-PHOSPHATIDYLINOSITOL (GPI) MEMBRANE ANCHOR, IS RELATED TO LFA-3 AND OX-45, AND MAPS TO CHROMOSOME 1q21–23

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Integral membrane proteins possess a hydrophobic sequence bordered by hydrophilic residues, a configuration that allows for anchorage in a lipid bilayer. Recently, an alternative protein anchoring structure has been characterized which consists of a complex glycosyl-phosphatidylinositol (GPI)[1] containing lipid (reviewed in references 1 and 2). The purification of a phospholipase C (PLC) specific for phosphatidyl inositol (PI-PLC) from various bacterial strains has greatly facilitated the identification and characterization of these proteins.

PI-PLC was demonstrated to specifically release the murine lymphocyte antigens Thy-1, Qa, and T cell-activating protein (TAP). PI-PLC also mediates the release of decay-accelerating factor, LFA-3, and one form of the neural cell adhesion molecule (NCAM). Thus, GPI-anchored proteins are functionally diverse and encompass molecules reported to be involved in T cell activation (Thy-1 and TAP) (3, 4) and adhesion (LFA-3, NCAM) (5, 6). It has been suggested that the GPI anchor may confer increased lateral mobility to a protein, provide a mechanism for its selective release from the cell surface, and/or provide a mechanism for cell activation (7–9).

Blast-1 is a cell surface 40–45-kD glycoprotein expressed on activated B cells, activated T cells, and monocytes (reference 10, and Staunton, D. E., and D. A. Thorley-Lawson, manuscript in preparation). Analysis of a cDNA encoding Blast-1 reveals amino acid sequence similarly to members of the Ig superfamily (11). The sequence also revealed characteristics of GPI anchored proteins.

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[1] *Abbreviations used in this paper:* ER-LCL, EBV immortalized B-lymphoblastic cell line; GPI, glycosyl-phosphatidylinositol; NCAM, neural cell adhesion molecule; PI, phosphatidyl inositol; PLC, phospholipase C; TAP, T cell-activating factor.

Here we show that Blast-1 is anchored through GPI and extend the analysis of homologous proteins to show that Blast-1 is the human homologue of OX45 and most closely related to LFA-3. Last, we show that the gene for BLAST-1 is localized to chromosome 1q21–q23.

Materials and Methods

Cells and Antibodies. The EBV immortalized B-lymphoblastic cell line (LCL) ER-LCL was established by in vitro EBV infection of peripheral blood B lymphocytes as previously described (11). Peripheral blood T cells were isolated by Ficoll-Hypaque density centrifugation followed by 1-h adherence to plastic at 37°C in RPMI with 10% FCS. Nonadherent cells were then rosetted with SRBC and the rosetting cells (T cells) were then isolated and the sheep cells were lysed. This preparation was determined, by indirect immunofluorescence with OKT3, to contain 96% T cells. T cells were then cultured at 10^6 cells/ml RPMI with 10% FCS and 1 μg/ml PHA (HA-16, Bourroughs-Wellcome Co., Greenville, NY) for 3 d before PI-PLC treatment.

The generation of the anti-Blast-1 mAb 17D6 was described previously (10). MOPC-11 is a negative control isotyped-matched mAb. W6/32 is an anti-MHC class I mAb. EBVCS-5 is an anti-Blast-2 (CD23) mAb (12). Anti-CD2 (TS2/18) was a kind gift of Dr. T. Springer (Center for Blood Research, Boston, MA). Indirect immunofluorescence and immunoprecipitations were performed as previously described (10).

Phospholipase C Treatment of Cells. For analysis by indirect immunofluorescence, cultured cells (10^6) were washed once with PLC buffer (RPMI with 2 mg/ml BSA, 10 mM Hepes pH 7.4, and 5×10^{-5}M 2-ME). Cells were then resuspended in 1 ml of PLC buffer and incubated at 37°C for 60 min with 1 μM/min/ml (final activity) of PI-specific PLC (1) (a kind gift from Dr. Martin Low), or no enyme. The cells were then washed once with PLC buffer followed by two washes with PBS containing 0.5% FCS and immediately incubated at 4°C with antibodies for indirect immunofluorescence. For immunoprecipitation analysis, 2×10^7 ^{125}I surface-labeled cells were washed with PLC buffer and incubated in 1 ml of PLC buffer at 37°C for 60 min with 1 μM/min/ml (final activity) of PI-specific PLC or no enzyme. The cells were pelleted and the supernatant was centrifuged for 30 min at 13,000 *g*. The cells were washed once in PLC buffer, twice in PBS, and then solubilized by the addition of 0.5% NP-40. Supernatant and solubilized cells were precleared with formalin fixed Staphylococcus A before immunoprecipitation and SDS-PAGE analysis, which were carried out as described previously (11).

Hybrid Cell Lines and Chromosome Localization. Chromosomal location was analyzed as described previously (13). All cell hybrids except series XIX were derived from fusions between human cells and Chinese hamster V79/380-6 cells. The construction and characterization of these hybrids have been summarized recently (13). Series XIX hybrids were derived from fusions between human skin fibroblasts and rat Morris hepatoma 7777 cells. In some hybrids of this series spontaneous translocation events have separated the short arm and long arm of human chromosome 1. The hybrid containing region 1qter→q32 was derived from a human donor with a t(1;2)(q32;q13) translocation (13a).

Genomic DNA was extracted from cultured cells as described. 10 mg of hybrid and parental control cell DNA were digested with restriction enzymes according to the manufacturers' instructions. A three- to fourfold excess of enzyme was used and spermidine HCl (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 4 mM. Hind III digestions were carried out in TA buffer. DNA fragments were separated by electrophoresis in 0.8% agarose and transferred to Hybond-N (Amersham Corp., Arlington Heights, IL) as described (13). Filters were pretreated and hybridized with gel-purified, ^{32}P-labeled probe as previously described, except that SDS (Bio-Rad Laboratories) was added to the pre- and hybridization solutions to a final concentration of 1%.

In Situ Chromosomal Hybridization. Human metaphase cells were prepared from PHA-stimulated peripheral blood lymphocytes. For autoradiographic localization, a radiolabeled Blast-1 cDNA probe was prepared by nick translation of the entire plasmid with all four

^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0×10^{8} dpm/μg. In situ hybridization was performed as described previously (14). Metaphase cells were hybridized at 2.0 and 4.0 ng of probe per μl of hybridization mixture. Autoradiographs were exposed for 11 d. For chromosomal localization performed with biotinylated probes, a modification of a previously described procedure (15) was used, details of which will be published elsewhere (Lawrence, J. B., et al., manuscript in preparation). Normal human lymphocytes were hybridized with 2.5 μg/ml of two genomic clones (designated 2c and 5c, both of 16 kb) that span the Blast-1 gene. The probes were labeled by nick translation with biotin-dUTP and were detected with fluorescein-avidin. The nature of the analysis with the fluorescence technique differs from that of autoradiography; a statistical analysis is unnecessary because the identical labeling of sister chromatids verifies the position of bona fide hybridization in a single metaphase figure (15) and because the scatter of signal observed with autoradiography is eliminated. Hence, careful analysis of a few well-banded metaphase chromosomes after hybridization provides a comparatively precise placement of the gene. The position of hybridization signals was aligned with G bands on normal human metaphase chromosomes which had been Giemsa/trypsin banded and photographed before hybridization.

Results

Linkage of Blast-1 to PI. The translated sequence of a Blast-1 cDNA predicts a 19 amino acid hydrophobic COOH terminus devoid of charged residues that might serve as a cytoplasmic anchor. This is a characteristic of proteins that have been found anchored to the plasma membrane by processing which links the COOH terminus to GPI. Therefore, the possible linkage of Blast-1 to GPI was examined. Treatment of ER-LCL with PI-PLC resulted in a marked decrease in binding of an anti-Blast-1 mAb (17D6) as determined by indirect immunofluorescence (Fig. 1 *A*). This decrease was specific for Blast-1 and was not observed with CD23 (Fig. 1 *B*), which is known to possess a cytoplasmic tail. Interestingly, ~60% of Blast-1 on ER LCL was resistant to PI-PLC digestion (Fig. 1 *A*), even in the presence of increasingly higher concentrations of enzyme (data not presented). By contrast all of the Blast-1 detected by immunofluorescence on PHA-activated T cells could be removed by digestion with PI-PLC (Fig. 1 *C*). This decrease was again shown to be specific since in this case the non-PI-linked CD2 marker was unaffected (Fig. 1 *D*). Digestion with PLC lacking PI specificity did not effect Blast-1 expression (not shown).

To directly demonstrate the PI-PLC sensitivity of Blast-1, immunoprecipitations were performed from ^{125}I surface-labeled ER-LCL and PHA-activated T cells and their cell supernatants after PI-PLC digestion or mock digestion. Whereas Blast-1 was only detected on the membranes of mock-digested ER-LCL, it was found in

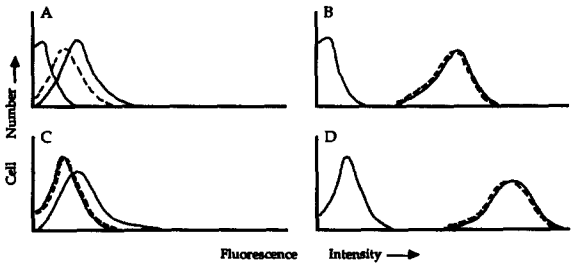

FIGURE 1. Release of Blast-1 from B and T lymphocytes with PI-PLC. The B-lymphoblastoid cell line ER-LCL (*A* and *B*) or PHA-activated T cells (*C* and *D*) were treated (--) or not treated (---) with PI-PLC before indirect immunofluorescence using anti-Blast-1 (*A* and *C*). Anti-CD23 (*B*) and CD-2 (*D*) mAbs were used as specificity controls for the digestion. The weakest fluorescent profile represents staining with fluorescein-labeled goat anti-mouse IgG alone.

both the membrane and supernatants of PI-PLC-digested cells (Fig. 2, *left*). Similarly, Blast-1 was precipitated from the supernatant of PI-PLC-digested PHA-activated T lymphocytes (Fig. 2, *right*). Unlike the B cells, and consistent with the fluorescence analysis, residual Blast-1 was not detected on membranes of activated T cells after PI-PLC digestion. Thus, digestion with PI-PLC results in the specific release of Blast-1 rather than the nonspecific destruction of the epitope recognized by 17D6. As a control for the specificity of the enzyme, HLA-class I that possesses a cytoplasmic tail was shown not to be released from ER LCL cells by PI-PLC (Fig. 2). These experiments indicate that there exists at least one form of Blast-1 anchored to the surface of T and B cells by linkage to GPI and that, in contrast to B lymphoblasts, the majority of Blast-1 on PHA-activated T cells appeared to be sensitive to PI-PLC digestion.

Sequence and Structural Similarities Between Blast-1, OX45 and LFA-3. Blast-1 was previously demonstrated to possess amino acid sequence homology with members of the Ig superfamily (11). A direct comparison was therefore made with proteins that recently have been demonstrated to possess a PI anchor and Ig-like domains. Using protein alignment programs, no significant homology was observed between Blast-1 and NCAM, decay-accelerating factor, Qa, TAP, and Thy-1 (data not presented).

The alignment, however, over the entire length of Blast-1 (217 residues) and OX45 (218 residues) yields 50% identity (Fig. 3). Considering conservative amino acid substitutions (16), the overall homology becomes 81%. There is 45 and 54% identity within the first and second Ig-like domains (D1 and D2), respectively. Their hydrophobic COOH-terminal sequences are 58% identical. This degree of homology is consistent for human and rodent protein homologues.

The alignment over the entire length of Blast-1 and LFA-3 (210 residues) yields 25% identity (Fig. 3). There is 31% identity between the D1 of Blast-1 and LFA-3. Considering conservative amino acid substitutions, the similarly between the two D1 sequences becomes 63%. The percentage of identical residues that aligned in D2 was considerably less (18%). The most NH_2-terminal *N*-linked glycosylation sites in Blast-1, LFA-3, and OX45 were also aligned. It can also be observed that the same conserved regions of strong homology in D1 between Blast-1 and OX-45 also occur in LFA-3 (Fig. 3 *A*).

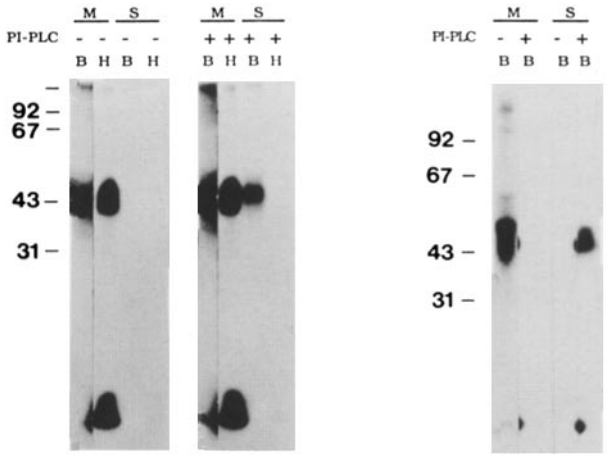

FIGURE 2. Blast-1 (*B*) and HLA class I (*H*) were immunoprecipitated from ^{125}I-labeled ER-LCL (*left panel*) and day 3 PHA-stimulated T cell blasts (*right panel*). Proteins were immunoprecipitated from both membranes (*M*) and culture supernatants (*S*) after PI-PLC treatment (+) or no PI-PLC treatment (–).

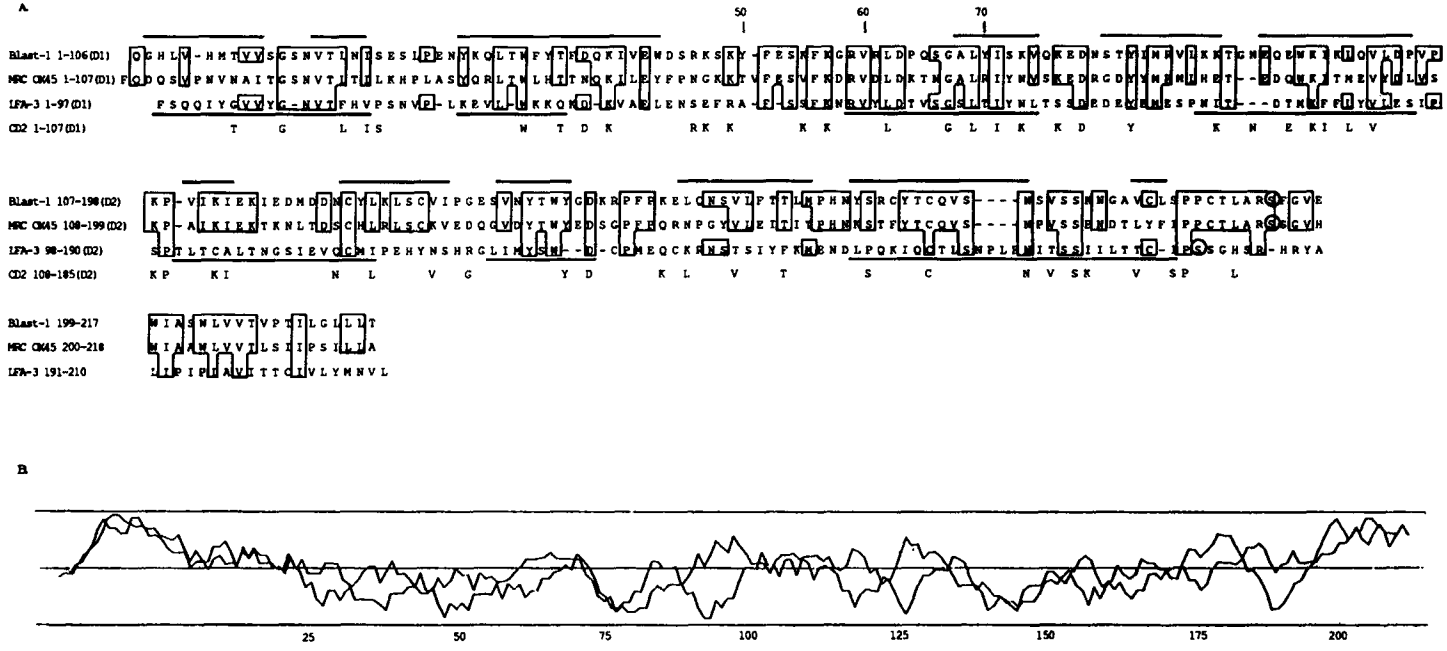

FIGURE 3. (*A*) Homology between Blast-1, MRC OX-45 and LFA-3. Putative Ig domains (D1, D2) are defined approximately by the first and second set of sequences. Horizontal bars indicate regions of predicted β strands (17) for Blast-1 (above sequences) and LFA-3 (below sequences). Circles indicate sites of predicted GPI attachment. (*B*) Hydrophobicity profiles of Blast-1 and LFA-3. Numbers indicate amino acid residue. Sequence above and below the central axis are hydrophobic or hydrophilic, respectively.

Secondary structures were predicted using the criteria of Chou and Fasman (17). The majority of β strand predictions for Blast-1 and LFA-3 are similarly located (Fig. 3 *A*). Alignment of the hydrophobicity profiles (18) of Blast-1 and LFA-3 also suggests an overall similar organization (Fig. 3 *B*).

The alignment between Blast-1 and LFA-3 also includes three cysteines in their second domains (Fig. 3). The existence of a disulfide-bonded second domain in Blast-1 and LFA-3 is supported here by their decreased mobility under reducing conditions relative to nonreducing conditions. ^{125}I-labeled Blast-1 immunoprecipitated from ER-LCL and LFA-3 purified from the B-lymphoblastoid cell line JY were first digested with *N*-glycosidase F to increase the resolution on SDS-PAGE. In Fig. 4 *A* the products of the *N*-glycosidase digestion of Blast-1 corresponding to zero and one *N*-linked oligosaccharide forms migrated more slowly under reducing conditions, demonstrating the presence of a disulfide-bonded domain in Blast-1. A similar reduction in mobility was observed in deglycosylated LFA-3 when subjected to SDS-PAGE under reducing conditions (Fig. 4 *B*).

Thus, Blast-1 is a homologue of OX45 and possesses several similarities to LFA-3, including polypeptide length, amino acid sequence, predicted secondary structure, hydrophobicity, and a disulfide-bonded second domain.

Chromosome Localization of the Gene for Blast-1. It has been recently been shown (19) that the genes for two members of the Ig superfamily, LFA-3 and CD2, map to the same band of chromosome 1. To demonstrate if the gene for Blast-1 was linked to any member of the superfamily, its chromosomal localization was determined. This was initially done by Southern blot analysis of DNA from 15 Chinese hamster × human hybrid cell lines (Materials and Methods) with the Blast-1 cDNA probe. Fragments of 3.1, 2.5, 1.3, and 0.95 kb were detected by this probe on Southern blots of Bgl II–digested human DNA (Fig. 5 *A*, lane *1*) and in all hybrids which had retained human chromosome 1 (Fig. 5 *A*, lanes *5*, *8*, *10*, and *12*). All other human chromosomes were ruled out as possible sites of any one of these hybridizing fragments by three or more discordant hybrids (Table I). No significant crosshybridization was seen with Chinese hamster DNA (Fig. 5 *A*).

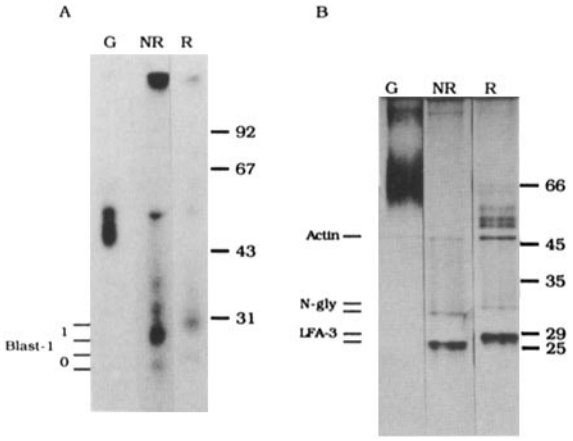

FIGURE 4. (*A*) Immunoprecipitation of Blast-1 and LFA-3 from ER-LCL under reducing (*R*) and nonreducing (*NR*) conditions. Blast-1 was immunoprecipitated and digested with *N*-glycosidase (*NR* and *R*) or not treated (*G*-glycosylated) before SDS-PAGE on a 10% polyacrylamide gel. *1* and *0* indicate one and no *N*-linked oligosaccharide forms of Blast-1. (*B*) LFA-3 purified from the lymphoblastoid cell line JY was subjected to SDS-PAGE as described above for Blast-1. An expected reduction in *N*-glycosidase (*N-gly*) mobility under reducing conditions is evident. Molecular mass (kilodaltons) shown to right of each panel.

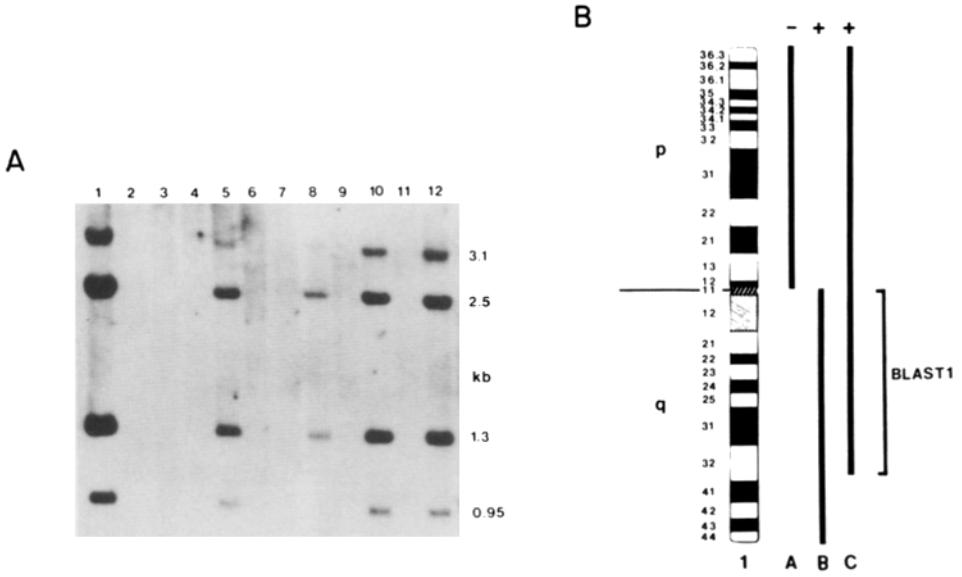

FIGURE 5. Chromosomal localization of the Blast-1 gene. (*A*) Hybridization of ^{32}P-labeled Blast-1 cDNA probe to a Southern blot of *Bgl VII*-digested DNA from human × Chinese hamster hybrid cell lines and controls. (Lane *1*) Human DNA; (lanes *2–12*) human × Chinese hamster hybrid cell DNAs. The four human bands were always present together, as was evident in lane *8* on a longer exposure. (*B*) Analysis of three additional hybrids containing portions of chromosome 1. The heavy vertical bars indicate the regions present in the three hybrids. + and − sign at the top indicate absence and presence of human Blast-1 restriction fragments in these hybrids. The bracket indicates the localization of the Blast-1 sequence.

To define the regional location of Blast-1 sequences on chromosome 1, we analyzed three additional hybrids containing portions of human chromosome 1 in the absence of the intact chromosome. The results of these analyses are presented graphically in Fig. 5 *B*. The heavy vertical bars indicate the portions of chromosome 1 present in three independently derived hybrids. No Blast-1 sequences were detected when only the short arm of chromosome 1 (region A) was present, while all four restriction fragments were detected in hybrids that had retained either the entire long arm (region B) or the short arm and a portion of the long arm (region C). The shortest region of overlap in the two positive hybrids was the proximal long arm of chromosome 1 (region cen→q32), and we assigned Blast-1 to this region.

To further sublocalize the Blast-1 gene on chromosome 1, we hybridized the Blast-1 cDNA probe to normal metaphase chromosomes. This resulted in specific labeling only of chromosome 1. Of 100 metaphase cells examined, 30 (30%) were labeled on region q2 of one or both chromosome 1 homologues. The distribution of labeled sites on this chromosome is illustrated in Fig. 6; of 204 labeled sites observed, 64 (31.4%) were located on this chromosome. These sites were clustered at bands q21–q24, and this cluster represented 19.1% (39/204) of all labeled sites (cumulative probability for the Poisson distribution is <0.0005). The largest number of grains was observed at 1q21–q23. All hybridizations were repeated three times and gave similar results. Thus, the Blast-1 gene was localized to chromosome 1, at bands q21–q23.

TABLE I

Correlation of Human Blast-1 Sequence with Human Chromosomes in Rodent × Human Somatic Cell Hybrids

Hybridization chromosome	Human chromosomes																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
+ / +	5	2	3	2	0	4	0	4	1	1	1	3	1	4	3	4	1	4	5	1	4	5	1
− / −	10	8	3	5	6	5	6	6	7	8	5	7	4	3	4	6	8	4	7	6	6	4	2
+ / −	0	3	2	2	5	1	4	1	3	4	2	2	4	1	2	1	4	1	0	4	0	0	0
− / +	0	2	6	3	3	5	3	4	3	2	5	3	6	5	6	3	2	5	3	4	4	5	3
Discordant hybrids	0	5	8	5	8	6	7	5	6	6	7	5	10	6	8	4	6	6	3	8	4	5	3
Informative hybrids	15	15	14	12	14	15	13	15	14	15	13	15	15	13	15	14	15	14	15	15	14	14	6

The numbers of hybrids showing concordant & + / + or − / −) and discordant (+ / − or − / +) segregation with Blast-1 are given for each chromosome. Data on rearranged chromosome or chromosomes present in <10% of cells were excluded.

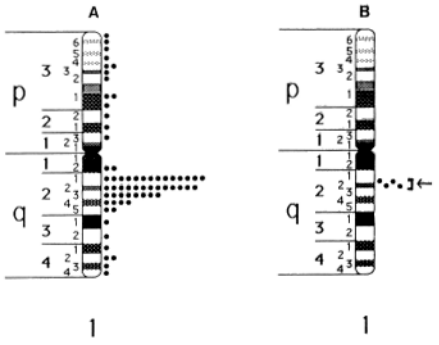

FIGURE 6. (*A*) Autoradiographic distribution of labeled sites on chromosome 1 in 100 normal human metaphase cells from PHA-stimulated peripheral blood lymphocytes that were hybridized with the Blast-1 cDNA probe. The labeled sites observed in this hybridization were clustered at 1q21–q24; the largest cluster of grains was located at 1q21–q23. (*B*) Fluorescence localization of the Blast-1 gene after hybridization of biotinylated genomic Blast-1 probes to G-banded cytogenetic preparations of PHA-stimulated normal human lymphocytes. Because of the high resolution and signal-to-noise ratios of this technique, label was observed at essentially the same localization in four independent measures. Within the limits of resolution of this technique the placement of the gene is narrowed to a region corresponding to 1q21.3–1q22 as indicated.

Recently, a higher resolution method of gene localization has been developed (15) using biotinylated DNA probes detected with fluoresceinated avidin. Use of this technique provides more precise localization, since the resolution of fluorescence (0.1 μm) is 10–20-fold greater than that of tritium autoradiography, due to the scatter of radioactive disintegrations. Using two biotinylated genomic clones for Blast-1 DNA, collectively representing ~32 kb of DNA, in situ hybridization was performed and Blast-1 gene localization was assessed after fluorescein-avidin detection. As indicated in Fig. 6 *B*, the signals fell on or near the border of 1q21 and 1q22. No signal was detected on any other region of chromosome 1 or on any other chromosome. Signals on each chromosome were independently assessed by two investigators with the same results. This result agrees well with the above results from autoradiographic analysis, which were derived entirely independently. Further, using this nonisotopic approach, label was not observed over 1q23 or the upper two-thirds of 1q21, narrowing the most likely placement to within 1q21 and 1q22.

Discussion

The recent isolation and sequence analysis of a cDNA encoding Blast-1 revealed that, except for a single hydrophobic COOH-terminal sequence, it is a predominantly hydrophilic polypeptide (11). The hydrophobic COOH terminus could act as a membrane anchor, although it lacks charged residues that might function as a cytoplasmic tail and contains a proline residue that would disrupt an α-helix structure that could traverse the plasma membrane. These characteristics are common to a group of proteins that are anchored to the membrane by linkage to a lipid containing glycosyl-phosphatidylinositol (1, 2). The prediction that Blast-1 may be anchored to the membrane in a similar fashion was confirmed by the PI-PLC-mediated release of Blast-1 from the cell surface.

The PI-PLC-mediated release of Blast-1 from B cells was incomplete. This suggests that a fraction of Blast-1 on B cells either lacks a GPI anchor, possesses a modified GPI anchor that is not a substrate of the specific PI-PLC used, or is associated with another surface molecule that inhibits PI-PLC digestion. The reason for a PI-PLC-resistant form of Blast-1 on B-LCL remains to be determined. In contrast, the PI-

PLC-mediated release of Blast-1 from PHA-stimulated T cells is relatively complete, suggesting that one GPI-anchored form of Blast-1 may exist on these cells.

The predicted site of GPI attachment to serine at position 195 (Fig. 3) is based on that determined for MRC OX45 (20). Attachment to serine has been observed and predicted for a number of proteins. In these proteins the attachment site does not appear to include residues with large side chains and occurs approximately 5-10 residues from the carboxyl terminal hydrophobic sequence (2). The predicted site of GPI attachment in Blast-1 is within six residues of that predicted for LFA-3 (2) in the alignment depicted in Fig. 3 *A*.

The nature of Blast-1's membrane linkage might be expected to play a role in its function. It has been suggested that a GPI anchor may confer greater mobility, provide a specific mechanism of membrane release, and/or result in protein kinase C activation via release of diacylglycerol. Further investigation is required to determine such a function(s) for Blast-1.

Previously Blast-1 was shown to possess amino acid sequence homology to members of the Ig superfamily including CD4 (11). In an attempt to gain functional insights into Blast-1, a comparision was made to Ig-related, GPI-anchored proteins for which amino acid sequence has recently become available. No striking sequence similarities were observed except to OX45 (20) and LFA-3 (21, 22). The degree of homology between Blast-1 and OX45 suggests that they are homologues. The similar tissue distribution of OX45 (23) and Blast-1 (Staunton, D. E., and D. A. Thorley-Lawson, manuscript in preparation) is consistant with this notion. The first domain of Blast-1 and LFA-3 are clearly related (Fig. 3). A similar degree of homology between distinct GPI-anchored proteins has not been previously observed. It is also not surprising, given the homology between LFA-3 and CD2 (22), that Blast-1 is also homologous and structurally similar to CD2 (Fig. 3 *B*). The degree of homology between Blast-1 and CD-2 is equivalent to that between CD2 and LFA-3. Blast-1, LFA-3, and OX45 also have similar size polypeptide lengths (217, 207, and 219, respectively), a similar number of *N*-linked glycosylation sites (5, 6, and 5, respectively), similar secondary structure predictions and hydrophobicity profiles, and they all lack a disulfide bond in their NH_2-terminal domain. On this basis, Blast-1 has recently been placed in a subfamily of the Ig superfamily, along with OX45, LFA-3, CD2, carcinoembryonic antigen (CEA), and noncrossreactive antigen (NCA) (24).

We have localized the gene for Blast-1 to chromosome 1 between bands q21–q23 using two completely independent methods: traditional autoradiography and a new fluorescence-based technique using biotinylated probes. The latter technique allows for more precise mapping than the traditional approach due to the high resolution of fluorescence and lack of signal scatter associated with autoradiography. This represents the first report of mapping of an unknown gene by these complementary techniques. Also mapping to chromosome 1 are other members of the Ig superfamily, including LFA-3 and CD2 (19) at 1p13, the CD1 cluster at 1q22–q23 (25), and the IgG Fc receptor at 1q32 (Ravetch, J., personal communication). The gene localization for Blast-1 is indistinguishable from that of the CD1 cluster, and thus they may be linked. However, a more detailed molecular analysis will need to be performed to ascertain the precise distance between these two genes. This raises the possibility that there could be clusters of immunoglobulin superfamily members located in several regions of chromosome 1, since it has recently been observed that

the LFA-3 and CD2 genes are located on a single 130 kb piece of genomic DNA (reference 19, and Crumpton, M. J., personal communication). This is of interest since both CD2 and Blast-1 are closely related to LFA-3 in their extracellular domains. This implies that related genes have arisen from a common precursor, both close to and distant from each other on chromosome 1. Alternatively, these genes may have converged due to having related functions, implying that Blast-1 may have a role in cell adhesion.

Blast-1, OX45, LFA-3, and CD2 are thus evolutionarily and perhaps functionally related. OX45 has been demonstrated to enhance macrophage-mediated suppression of an allogenic MLR and a secondary T cell response to soluble antigen (26). The failure of 17D6 to inhibit MLR (data not presented) may be due to its poor affinity and the fact that its epitope occurs in the COOH half of Blast-1 (11). LFA-3 is a ligand for CD2 and functions in Tc adhesion to target cells and thymocyte adhesion to thymic epithelial cells (5). Blast-1 may function similarly in cell–cell interaction. It could conceivably function as an alternative ligand for CD2. Interaction between CD2 and an additional ligand other than LFA-3 has been proposed (27). We are currently re-examining the function of Blast-1 using Blast-1-expressing transfectants and by generating additional anti-Blast-1 mAbs.

Summary

Blast-1 is a human activation-associated glycoprotein expressed on the surface of leukocytes. Analysis of a translated sequence from a Blast-1 cDNA reveals a single hydrophobic sequence which could traverse the plasma membrane, but is devoid of charged residues that might represent a cytoplasmic tail. Consistent with this characteristic, Blast-1 is demonstrated here to be anchored to the cell surface through a glycosyl-phosphatidylinositol (GPI)-containing lipid. Comparison of Blast-1 to other GPI-anchored membrane proteins revealed a striking primary and secondary structure similarity with MRC OX45 and the lymphocyte function antigen LFA-3. The degree of overall amino acid sequence homology reveals that OX45 is a rat homologue of Blast-1. The greatest homology to LFA-3 occurs between their NH_2-terminal Ig-like domains. Evidence is presented that demonstrates that Blast-1 and LFA-3 possess a disulfide-bonded second domain. These common characteristics demonstrate a structural and evolutionary relationship between Blast-1, OX45, LFA-3, and CD2, which in turn suggests a functional role for Blast-1 in cell–cell interactions in the immune response. The gene for Blast-1 has been localized to chromosome 1 q21–q23, indistinguishable from the CD1 cluster of Ig superfamily genes, raising the possibility that they may be linked.

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