The EMBO Journal vol.6 no.12 pp.3695–3701, 1987

Molecular cloning of the lymphocyte activation marker Blast-1

Donald E.Staunton and David A.Thorley-Lawson

Department of Pathology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA

Communicated by R.A.Flavell

Blast-1 is an early activation-associated glycoprotein expressed on the surface of human lymphocytes. Here we report the isolation and analysis of a cDNA encoding Blast-1. The translated sequence of the Blast-1 cDNA contains a hydrophobic putative signal peptide and a hydrophobic carboxyl terminus devoid of charged residues. The sequence also contains five N-linked glycosylation sites, the utilization of which was confirmed by the shift in relative mol. wt of Blast-1 upon digestion with N-glycosidase F. The translated sequence reveals that Blast-1 is related to members of the immunoglobulin superfamily, especially to CD4 and MHC class II molecules. The homology to these proteins is greatest in their amino termini where they demonstrate 30–32% identity. This region of Blast-1 also demonstrated 25% identity to a V_κ sequence. Considering conservative amino acid substitutions this homology to CD4, MHC class II and V_κ becomes 60, 49 and 48%, repectively.

Key words: activation/B cell/Blast-1/cDNA/EBV-regulation

Introduction

The molecular mechanisms involved in the transition of resting lymphocytes into an activated state and then into differentiation are currently under intensive study. Small resting B lymphocytes in G_0 respond to a variety of stimuli such as antigens, lymphokines and mitogens. Subsequently, numerous physiological, phenotypic and functional changes occur which are collectively referred to as lymphocyte activation. They include increases in: free intracellular Ca^{2+} (Monroe and Cambier, 1983; Ransom and Cambier, 1986a), phosphatidylinositol hydrolysis (Coggeshall and Cambier, 1984, Ransom *et al.*, 1986b), protein kinase C activation (Altman and Iskav, 1985; Chen *et al.*, 1985), cell size (Muraguchi *et al.*, 1984), RNA and DNA synthesis, and the number of cells traversing the cell cycle (Kehrl *et al.*, 1984). During this period, B cells become susceptible to growth and differentiation factors such as IL-2 (Nakagawa *et al.*, 1985), B cell growth factor (BCGF) (Muraguchi *et al.*, 1984), and B cell differentiation factor (BCDF-1) (Butler *et al.*, 1984). Concomitantly, the expression of a number of cell surface molecules (activation markers) is altered.

Analysis at the molecular level has begun to yield insights into the roles of a variety of lymphocyte-associated markers. Among the activation markers, two broad functional groups may be described. The first, as indicated above, includes receptors for growth or differentiation factors such as IL-2, BCGF and BCDF, which are involved in the progress of the resting cell through proliferation to a terminally differentiated state. The second group would involve molecules associated with the function of the activated/differentiated cell, such as MHC class II molecules in antigen presentation.

The infection of human B cells by Epstein–Barr virus (EBV) *in vitro* provides a model for studying both the normal pathway of activation and the mechanisms of neoplastic transformation of B cells (Thorley-Lawson and Mann, 1985). Using this system we have been able to define two markers that are characteristic of activated B cells, termed Blast-1 (Thorley-Lawson *et al.*, 1982, 1986) and Blast-2 (EBV CS/CD23) (Kintner and Sugden, 1981; Thorley-Lawson *et al.*, 1985, 1986). Blast-1 is a 40–45 kd glycoprotein which is noncovalently associated with another protein of 55 kd (Thorley-Lawson *et al.*, 1986). It was originally described as a B cell restricted activation marker due to its appearance on the plasma membrane of B cell blasts activated *in vitro* by pokeweed mitogen or EBV infection, and on neoplastic B cells from chronic lymphocytic leukemia and poorly differentiated lymphoma. It is not detected on resting peripheral blood B cells and T cells. Blast-1 is also expressed on germinal center cells but not on mantle cells of lymphoid tissue, suggesting that it plays an *in vivo* role in lymphocyte activation (Thorley-Lawson *et al.*, 1982). Recent analysis, however, has demonstrated that Blast-1 is also an activation marker for T cells (Staunton *et al.*, in preparation).

In this paper we describe the isolation and characterization of a Blast-1 cDNA and its application to studying the structure of Blast-1 and its function in cellular activation.

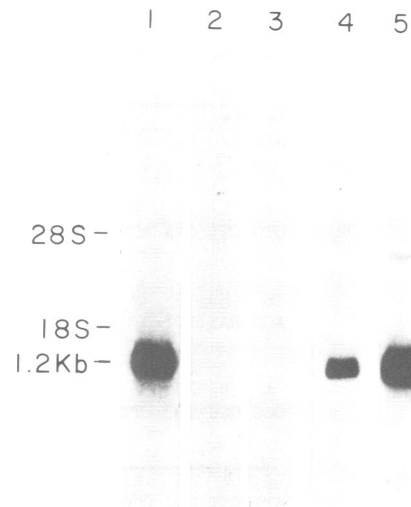

Fig. 1. Hybridization of 17D6 selected 1.2 kb cDNA to cellular RNAs. The ^{32}P labeled 1.2 kb cDNA was hybridized to RNA from: **lane 1**, ER-LCL, a Blast-1 positive B lymphoblastoid cell line; **lane 2**, 350Q, Blast-1 negative primary fibroblasts; **lane 3**, K562, a Blast-1 negative erythroleukemia cell line; **lane 4**, BL-2, a Blast-1 positive Burkitt's lymphoma cell line; **lane 5**, DT-LCL, a Blast-1 positive B lymphoblastoid cell line. Lanes 1, 2 and 5 are poly(A)$^+$ RNAs, and lanes 3 and 4 are total RNAs.

© IRL Press Limited, Oxford, England

EcoRI AluI SacI SacI SphI EcoRI

5' 3'

0.1Kb

```
                                          -26
                                          M   C   S   R   G   W   D   S
AACTGTCTCCTGCGTTGCTGGGAAGTTCTGGAAAGC ATG TGC TCC AGA GGT TGG GAT TCG  60

                                   -10                                     +1
 C   L   A   L   E   L   L   L   L   P   L   S   L   L   V   T   S   I   Q
TGT CTG GCT CTG GAA TTG CTA CTG CTG CCT CTG TCA CTC CTG GTG ACC AGC ATT CAA 117

                                +10                                     +20
 G   H   L   V   H   M   T   V   V   S   G   S  [N   V   T]  L  [N   I   S]
GGT CAC TTG GTA CAT ATG ACC GTG GTC TCC GGC AGC AAC GTG ACT CTG AAC ATC TCT 174

                                    +30
 E   S   L   P   E   N   Y   K   Q   L   T   W   F   Y   T   F   D   Q   K
GAG AGC CTG CCT GAG AAC TAC AAA CAA CTA ACC TGG TTT TAT ACT TTC GAC CAG AAG 231

+40                                     +50
 I   V   E   W   D   S   R   K   S   K   Y   F   E   S   K   F   K   G   R
AAT GTA GAA TGG GAT TCC AGA AAA TCT AAG TAC TTT GAA TCC AAA TTT AAA GGC AGG 288

    +60                                     +70
 V   R   L   D   P   Q   S   G   A   L   Y   I   S   K   V   Q   K   E   D
GTC AGA CTT GAT CCT CAG AGT GGC GCA CTG TAC ATC TCT AAG GTC CAG AAA GAG GAC 345

        +80                                     +90
[N   S   T]  Y   I   M   R   V   L   K   K   T   G   N   E   Q   E   W   K
AAC AGC ACC TAC ATC ATG AGG GTG TTG AAA AAG ACT GGG AAT GAG CAA GAA TGG AAG 402

            +100                                    +110
 I   K   L   Q   V   L   D   P   V   P   K   P   V   I   K   I   E   K   I
ATC AAG CTG CAA GTG CTT GAC CCT GTA CCC AAG CCT GTC ATC AAA ATT GAG AAG ATA 459

                +120                                    +130
 E   D   M   D   D   N   C   Y   L   K   L   S   C   V   I   P   G   E   S
GAA GAC ATG GAT GAC AAC TGT TAT CTG AAA CTG TCA TGT GTG ATA CCT GGC GAG TCT 516

                    +140                                    +150
 V  [N   Y   T]  W   Y   G   D   K   R   P   F   P   K   E   L   Q   N   S
GTA AAC TAC ACC TGG TAT GGG GAC AAA AGG CCC TTC CCA AAG GAG CTC CAG AAC AGT 573

                        +160                                    +170
 V   L   E   T   T   L   M   P   H  [N   Y   S]  R   C   Y   T   C   Q   V
GTG CTT GAA ACC ACC CTT ATG CCA CAT AAT TAC TCC AGG TGT TAT ACT TGC CAA GTC 630

                            +180                                    +190
 S   N   S   V   S   S   K   N   G   A   V   C   L   S   P   P   C   T   L
AGC AAT TCT GTG AGC AGC AAG AAT GGC ACG GTC TGC CTC AGT CCA CCC TGT ACC CTG 687

                                +200                                    +210
 A   R   S   F   G   V   E   W   I   A   S   W   L   V   V   T   V   P   T
GCC CGG TCC TTT GGA GTA GAA TGG ATT GCA AGT TGG CTA GTG GTC ACG GTG CCC ACC 744

                        +217
 I   L   G   L   L   L   T
ATT CTT GGC CTG TTA CTT ACC TGA GATGAGCTCTTTTAACTCAAGCGAAACTTCAAGGCCAGAAGAT 811

CTTGCCTGTTGGTGATCATGCTCCTCAGCAGGACAGAGACTGTATAGGCTGACCAGAAGCATGCTGCTGAATTAT 886

CAACGAGGATTTTCAAGTTAACTTTTAAATACTGGTTATTATTTAAT                             933
```

Fig. 2. Sequencing strategy, nucleotide and protein sequence of the Blast-1 cDNA. **Top:** Partial restriction endonuclease map and sequencing strategy of the 1.2 kb Blast-1 cDNA. The largest open reading frame is indicated by the bold line. **Bottom:** Nucleotide sequence and translated sequence of the largest open reading frame. The long hydrophobic sequences derived from Kyte and Doolittle (1982) analysis are overlined. The predicted signal peptide runs from residue -26 to -1. The five N-linked glycosylation sites are indicated by boxes.

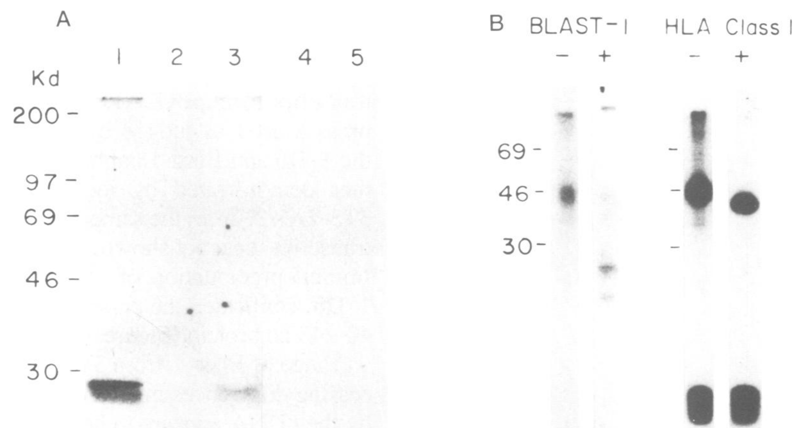

Fig. 3. Comparison of the 1.2 kb cDNA *in vitro* transcription–translation product with the Blast-1 core protein. **(A)** [^{35}S]methionine labeled *in vitro* translation product using RNA transcribed from the 1.2 kb cDNA (**lane 1**) or no RNA (**lane 2**). Product of translation as in lane 1, immunoprecipitated with the 17D6 anti-Blast-1 antibody (**lane 3**), or negative control antibodies (**lanes 4** and **5**). **(B)** Blast-1 and HLA-class I immunoprecipitated from ^{125}I surface labeled ER-LCL, digested wth (+) or without (-) N-glycosidase F. Samples were run on 10% polyacrylamide gels under reducing conditions.

Results

Isolation of cDNAs from λgtll cDNA library

A λgtll cDNA library was constructed using poly(A) selected RNA from an EBV$^+$ lymphoblastoid cell line (ER-LCL). 2 × 10^5 plaques were screened with the Blast-1 specific monoclonal antibody 17D6, resulting in the isolation of three positive phage clones. Specificity was demonstrated by the failure of their fusion proteins to react with an isotype matched negative control antibody or a panel of eight monoclonal antibodies with different specificities. The cDNA inserts were of distinct sizes — 1.2 kb, 780 bp and 760 bp. They cross-hybridized under stringent conditions (data not shown) suggesting good homology or identity. The largest cDNA was subcloned into the vector Bluescribe (Materials and methods) for further characterization. To determine if this cDNA represented an approximately full length copy of its corresponding RNA, the purified 1.2 kb cDNA fragment was nick-translated and hybridized to poly(A)$^+$ RNA from ER-LCL. The cDNA probe hybridized to a 1.2 kb poly(A)$^+$ transcript from the Blast-1-positive ER-LCL, DT-LCL and Burkitt lymphoma cell line BL-2 (Figure 1), as well as from all other Blast-1 positive cells examined (6 out of 6, data not presented). A 4 kb transcript was also detected in RNA from some LCLs (Figure 1, lane 5). No hybridization was observed to RNA from Blast-1 negative fibroblasts or the erythroleukemia line, K562. Subsequently, using a 21 bp oligonucleotide probe corresponding to a 5′ sequence, eight additional cDNAs were isolated, none of which were greater than 1.2 kb in size. Thus, the cDNA is, or is close to, full length.

Nucleotide and protein sequences

The 1.2 kb cDNA nucleotide sequence was determined and used to predict the amino acid sequence and structure of the encoded protein. The sequencing strategy is depicted in Figure 2. The sequence reveals the longest open reading frame beginning with the first ATG at position 47 and ending with a TGA terminating triplet at position 766 (Figure 2). The presence of a cytosine 1 bp and an adenine 3 bp, 5′ of the AUG, is consistent with this being close to an optimal higher eukaryotic translational start site (Kozak, 1986). A protein initiating at this first methionine has a deduced mol. wt of 28 kd and five N-linked glycosylation sites which, if used, could result in a glycoprotein with a mol. wt consistent with that of Blast-1 (43 kd). A hydrophobicity analysis of the translated sequence (Kyte and Doolittle, 1982), revealed a 19-amino acid hydrophobic stretch at the amino terminus (Figure 2) which is within a predicted 26-residue signal sequence. The assignment of this putative signal sequence and its cleavage site is supported by analysis of homologous signal sequences in related proteins (discussed below). There is a 19-amino acid hydrophobic sequence at the carboxyl terminus which might serve as a membrane anchor, although a hydrophilic cytoplasmic tail is absent. The presence of 22 acidic and 29 basic amino acids in the predicted processed protein sequence, is in agreement with the previously determined slightly basic pI (7.2) of Blast-1 (Thorley-Lawson *et al.*, 1986). Thus, the predicted biochemical properties of the protein encoded by this cDNA are consistent with those of Blast-1 from LCLs.

Proof of the cDNA identity by in vitro and in vivo transcription and translation

To establish the identity of the cDNA, it was transcribed and translated *in vitro*, and the protein product was compared to the core protein of Blast-1. The sense strand of the cDNA was transcribed and 5′ capped from linearized plasmid, using T7 RNA polymerase. The transcript was translated in a reticulocyte lysate system to yield a non-glycosylated 28 kd polypeptide. This is in agreement with the size expected from the cDNA sequence (Figure 3A, lane 1). This polypeptide was specifically precipitated with 17D6 and not by two monoclonal antibodies with unrelated specificities. Thus, the translated protein itself, rather than a product of its fusion with β-galactosidase, is antigenically related to Blast-1. The 28 kd *in vitro* translation product includes the uncleaved predicted signal sequence of about 3 kd (discussed below). Thus, the mol. wt of the core protein encoded by the cDNA would be approximately 15–20 kd less than mature glycosylated cellular Blast-1. In order to determine the core protein size of Blast-1, it was precipitated from ER-LCL and treated with N-glycosidase F (Materials and methods). N-glycosidase F digested Blast-1 yielded a core protein of 25 kd which is in good agreement with the size of the cDNA *in vitro* transcription–translation product minus the putative signal pep-

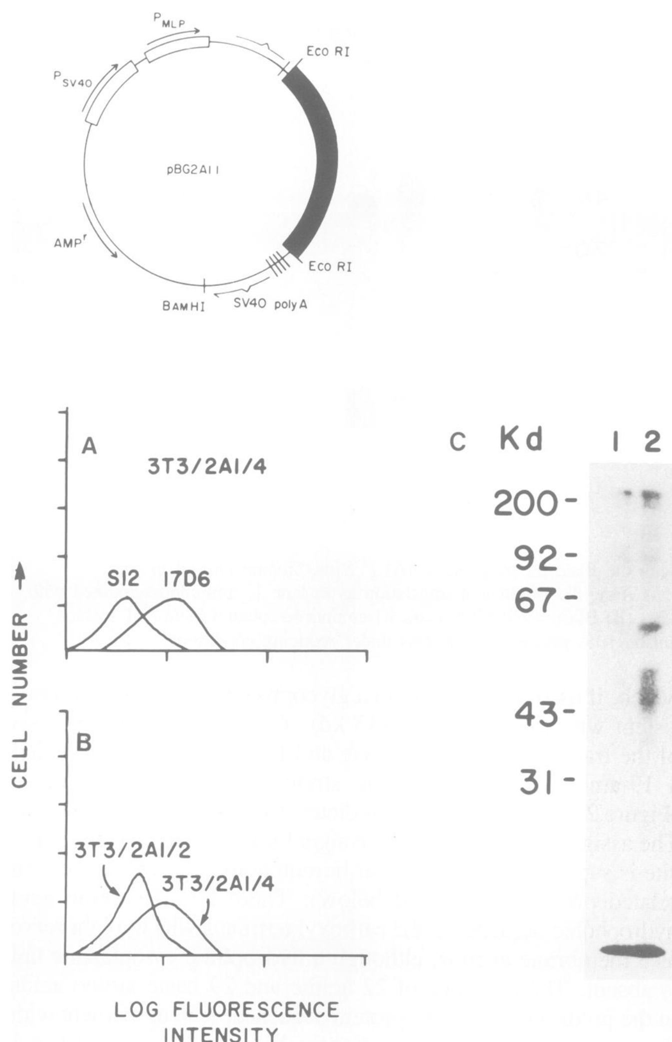

Fig. 4. Expression of the 1.2 kb cDNA encoded product in transfected NIH 3T3 cells. **Top:** The eukaryotic expression construct containing the 1.2 kb cDNA (pBG2A1.1). (**A**) Cytofluorographic analysis of the pBG2A1.1 transfectant 3T3/2A1/4 following indirect immunofluorescent staining with anti-Blast-1 (17D6) or a negative control monoclonal antibody (S12). (**B**) Comparison of 17D6 binding to the positive 3T3/2A1/4 and a negative control transfectant, 3T3/2A1/2, by indirect immunofluorescence. (**C**) Immunoprecipitation of ^{125}I surface labeled Blast-1 from the positive transfectant 3T3/2A1/4 using anti-Blast-1 (17D6) (**lane 2**) or control antibody (S12) (**lane 1**).

tide (Figure 3B). The decrease in size is also consistent with N-linked glycosylation occurring at five sites, as predicted from the cDNA sequence.

A minor coprecipitated polypeptide of 20 kd was also observed following N-glycosidase F digestion in some experiments. The origin of this polypeptide is unknown. As a control for N-glycosidase F digestion, HLA class I heavy chain was fully digested in the same experiment (Figure 3B) yielding a 3 kd shift in mobility, which was expected from published data (Krangel *et al.*, 1979).

The protein encoded by the cDNA possesses the correct size and antibody reactivity to be the Blast-1 core protein. The question was then asked if the processed (glycosylated) product encoded by the cDNA was also of correct size and antibody reactivity to be Blast-1. To determine this, the cDNA was inserted into the eukaryotic expression vector pBG367 to yield the pBG2A1.1 construct (Figure 4). This was cotransfected with the selectable marker, pSV2neo, into NIH 3T3 cells. A stable transfectant, 3T3/2A1/4 was found to transcribe a 2 kb RNA which hybridizes to the cDNA and is the expected size of the transcript from pBG2A1.1 (data not presented). These cells express Blast-1 as judged by indirect immunofluorescence using the 17D6 anti-Blast-1 antibody (Figure 4A). Specificity was further demonstrated by the isolation of another transfectant, 3T3/2A1/2, from the same cotransfection which lacked the 2 kb transcript (data not shown) and failed to bind 17D6 (Figure 4B). Immunoprecipitation of ^{125}I surface labeled 3T3/2A1/4, with 17D6, confirmed the presence of Blast-1 as it yielded a specific 40−48 kd protein (Figure 4C). The slightly broader relative mol. wt range of Blast-1 from 3T3/2A1/4 is probably a result of processing differences in a foreign cell. Thus, the product encoded by the cDNA appears to be processed by glycosylation to yield a mature protein which reacts with anti-Blast-1 and had an apparent mol. wt on SDS−PAGE in good agreement with that of mature Blast-1 from LCLs.

In conclusion, the unprocessed and glycosylated forms of the protein derived from the cDNA specifically react with 17D6 and have the mol. wt expected for Blast-1. Therefore, we conclude that the cDNA encodes Blast-1.

Protein homologies to Blast-1

In an attempt to gain further insights into the function of Blast-1, databases, maintained by the National Biomedical Research Foundation, were searched for homologous protein sequences using the FASTP program (Lipman and Pearson, 1985). Homologous sequences were further analyzed using the Beckman Microgenie system (Queen and Koru, 1984). Blast-1 demonstrated homology to several members of the immunoglobulin superfamily, though the strongest was to the T lymphocyte accessory molecule CD4 (Maddon *et al.*, 1985). Over its entire 243 residues, Blast-1 possesses 27% identity to CD4 (data not presented), however, in the amino terminal sequence −26 through +85, the homology rises to 32% (Figure 5A). From the FASTP program analysis, a significance score of Z=7.5 was calculated, which suggests that the sequences in this region are related. In one stretch of 33 amino acids within this region, from 53 to 85, the identity increases to 40%. Interestingly, a 10 amino acid stretch within the homologous CD4 sequence (69−79) is also very similar (70% identity) to another Blast-1 sequence outside this region (110−119) (Figure 5B). When conservative amino acid substitutions are considered, the 32% homology in the amino terminus increases to 60% resulting in a predicted β sheet structure that is very similar to CD4 (Figure 5A). When the Blast-1 sequence was compared with CD4 on the basis of homology, a highly hydrophobic sequence (−18 to −1) was found to align with the signal sequence of CD4 suggesting that this is the Blast-1 signal sequence and that Blast-1 has an N-terminal +1 glutamine.

The amino terminal 85 amino acids of Blast-1 also demonstrated homology to other members of the immunoglobulin superfamily (Figure 5B), including MHC class II (30% identity with a DQ α chain) (Auffray *et al.*, 1984) and a V_κ (25% identity) (Kabat *et al.*, 1983). The putative Blast-1 signal sequence aligns with that of several MHC class II molecules (data not shown) and the predicted +1 glutamine also aligns with the +1 glutamine of V_κ. The relatedness of Blast-1 to the immunoglobulin superfamily is further supported by the alignment of seven V_κ invariant residues (Kabat *et al.*, 1983) with Blast-1. Overall, Blast-1 is related by sequence to several members of the immunoglobulin superfamily.

Fig. 5. Protein sequences homologous to the Blast-1 cDNA translated sequence. Amino acid sequences homologous to Blast-1 were aligned by protein analysis programs (Lipman and Pearson, 1985; Queen and Koru, 1984). Residues identical in type and position with those in the Blast-1 sequence are boxed. (**a**) Conservative amino acid changes between Blast-1 and CD4 (Maddon *et al.*, 1985) are indicated by vertical bars. Predicted beta-sheets (Chou and Fasman, 1978) for Blast-1 and CD4 are indicated by the brackets. The signal peptide sequences for Blast-1 and CD4 are between -26 and -1. (**b**) Class II alpha is an HLA-class II DQ alpha chain (Auffray *et al.*, 1984). Class II beta[1] is an H-2 class II beta chain (Malissen *et al.*, 1983), and class II beta[2] is an HLA class II beta chain (Gorski *et al.*, 1984). INV = invariant residues of the V-kappa light chain variable region (Kabat *et al.*, 1983). TM = transmembrane region of the MHC class II proteins.

Discussion

In this study the cDNA encoding the lymphocyte activation marker Blast-1 was isolated, sequenced and used to characterize its translated protein with respect to structure and relatedness to other proteins. The identity of the Blast-1 cDNA was demonstrated by the fact that the non-glycosylated *in vitro* translation product and the glycosylated *in vivo* product are: (i) consistent with the respective relative mol. wt of Blast-1 from LCLs; and (ii) bind the anti-Blast-1 monoclonal antibody.

Our understanding of the structure of Blast-1 was greatly elucidated as a result of sequence and biochemical information. A diagrammatic representation summarizing the structural features of Blast-1 is presented in Figure 6. The cDNA translated sequence contains a hydrophobic carboxyl terminus which could act as a membrane anchor although it lacks a cytoplasmic tail and contains a proline residue which would disrupt an α helix structure that could traverse the plasma membrane. These characteristics are common to a group of proteins which are anchored to the membrane by linkage to a complex lipid containing phosphatidylinositol (Low and Finean, 1977; Low *et al.*, 1986; Cross, 1987). This prediction was recently confirmed by demonstrating that Blast-1 is released from the membrane by digestion with a phosphatidylinositol specific phospholipase C (Staunton and Thorley-Lawson, in preparation).

The translated sequence from the Blast-1 cDNA also predicts five N-linked glycosylation sites. Previous analysis had failed to detect N-linked carbohydrate chains on Blast-1 (Thorley-Lawson *et al.*, 1986). The use of these sites however, was unequivocally confirmed in this study. The shift in the relative mol. wt of Blast-1 from LCLs, following N-glycosidase F treatment was ~18 kd, which is in good agreement with the relative mol.

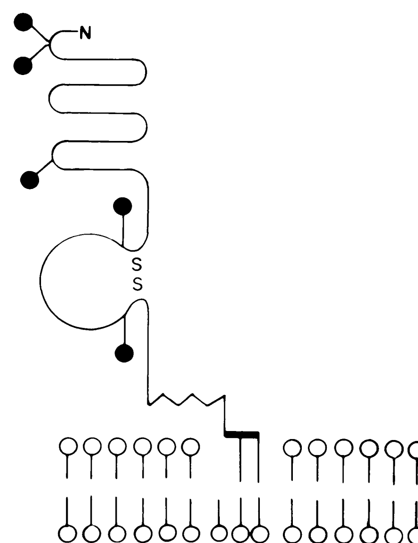

Fig. 6. The major structural features of Blast-1 based on cDNA sequence and protein analyses. The amino terminus is indicated by N. N-linked oligosaccharides are indicated by ⚫ and the predicted disulfide bond by SS. The PI containing membrane anchor is indicated by the zig-zag line attached to the membrane lipid layer.

wt of Blast-1 on transfected NIH 3T3 cells compared to the unglycosylated *in vitro* translation product. A single N-linked carbohydrate side chain is approximately 3–4 kd, therefore, the observed change in size of Blast-1 is exactly consistent with the use of all five N-linked sites.

Blast-1 homology to class II α and β carboxyl termini includes an identical 5 amino acid sequence around the +170 cysteine which is used by class II chains to form a disulfide bridge (Kaufman *et al.*, 1984). Another 5 amino acid sequence, around the +128 cysteine of Blast-1, is also identical to the V-region framework 1 cysteine of V_H, which also forms a disulfide bridge (Kabat *et al.*, 1983). It is thus conceivable that Blast-1 may form a 43 amino acid disulfide bridged domain from +128 to 170. Although this disulfide bridged domain would be smaller than that found in the HLA class II alpha 2-domain, domains of similar size have been suggested for the IgG Fc receptor which also belongs to the immunoglobulin superfamily (Ravetch *et al.*, 1986).

The degree of identity between the amino terminal region of Blast-1, CD4, HLA class II α chain and V_x is similar to that found between Ig-like domains of members of different gene families in the immunoglobulin superfamily (Hood *et al.*, 1985; Lee *et al.*, 1982; Williams, 1984). The amino terminus of Blast-1 has the highest degree of homology to CD4. In this region the degree of identity is 32% which, considering conservative changes, results in a 60% homology and predicted secondary structures that are very similar. In approximately half of this region, >40% of the amino acids are identical. Unlike CD4, however, this region does not possess a characteristic Ig-like disulfide bonded domain and has three N-linked carbohydrate sites, whereas CD4 has none. Thus there are considerable structural differences between these proteins. Overall, Blast-1 appears to be structurally more similar to MHC class II α chains, which have a similar polypeptide backbone length. The amino terminal regions of Blast-1 and α chains do not possess disulfide bonded domains, whereas the carboxyl terminal domain of the α chain does, and one is also predicted for Blast-1. Although Blast-1 possesses a greater number of N-linked oligosaccharides, one of these (position 78) is at a similar position to one in the α chains (Krangel *et al.*, 1979). Interestingly, both Blast-1 and α chains are covalently attached to lipid (Kaufman *et al.*, 1984). The limits of the amino terminal region of Blast-1 (1-85) also correspond to the limits of the MHC class II α-1 domain (3-85), which is encoded for by the second exon of the α chain gene (Lee *et al.*, 1982). Blast-1 could therefore have a similar gene organization to MHC class II genes. This is consistent with preliminary analysis of genomic DNA suggesting a maximum size for the Blast-1 gene of ~10 kb. The sequence and structural similarities between Blast-1, CD4 and MHC class II molecules may also indicate possible functions for Blast-1. Both CD4 and MHC class II are involved in cellular interaction during antigen presentation and can also transduce signals when ligands are bound (Swain, 1983; Bank and Chess 1985). It is conceivable, therefore, that Blast-1 could also be involved in cellular interaction.

Blast-1 mRNA is detected within 30 min and surface protein within 4 h post-activation (Staunton *et al.*, in preparation). Furthermore, recent analysis indicates that Blast-1 is also expressed on activated T cells, monocytes and granulocytes. Blast-1 could therefore be of great importance in immunological regulation as it might facilitate cellular interaction only between activated cells and not between resting cells.

In conclusion, Blast-1 is a unique member of the immunoglobulin superfamily. By analogy with homologous proteins, it may act as an accessory molecule in the immune response, providing adhesion or signal transduction functions.

Materials and methods

Cells and monoclonal antibodies

The EBV immortalized B-lymphoblastoid cell lines (LCL) ER-LCL and DT-LCL, were established by *in vitro* EBV infection of B lymphocytes from two individuals, ER and DT. The B lymphocytes were isolated by Ficoll–Hypaque density centrifugation of peripheral blood followed by passage over an immunoaffinity column of rabbit anti-human Ig, as described previously (Chess and Schlossman, 1976).

The erythroleukemia cell line K562, was obtained from the American Type Culture Collection (ATCC; Rockville, MD). The EBV-negative Burkitt lymphoma cell line BL2 was a gift from Dr G.Lenoir. 350 Q primary fibroblasts were a gift from Dr David Waters. The NIH 3T3 murine fibroblasts were a gift from Dr Ted Krontiris.

Immunofluorescence and immunoprecipitation were performed as previously described (Thorley-Lawson and Edson, 1979; Thorley-Lawson *et al.*, 1982). Generation and characterization of the monoclonal antibody with specificity for Blast-1 (17D6) has been reported (Thorley-Lawson *et al.*, 1982). Generation of the negative control murine monoclonal antibodies 1A2, CS5 and S12 has been reported (Edson *et al.*, 1985; Kintner and Sugden, 1981; Mann *et al.*, 1985). The anti-HLA class I (W6/32) monoclonal antibody was obtained from ATCC.

Construction and screening of EBV-LCL λgtll cDNA library

Total RNA was isolated from the EBV-immortalized lymphoblastoid cell line ER by homogenizing in 4 M guanidinium isothiocyanate and ultracentrifugation through a CsCl discontinuous gradient (Chirgwin *et al.*, 1979). Poly(A)$^+$ RNA was isolated by oligo-(dT)-cellulose (Type 3, Collaborative Research) chromatography (Cereghini *et al.*, 1979). Double strand cDNA was synthesized using a modification of a procedure described previously (Gubler and Hoffman, 1983). First strand cDNA was synthesized using 8 μg of poly(A)$^+$ RNA, avian myeloblastosis virus reverse transcriptase and an oligo(dT) primer. The hybrid was digested with RNase H and the second strand synthesized with DNA polymerase I. The product was methylated with *Eco*RI methylase, blunt end ligated to *Eco*RI linkers, digested with *Eco*RI and size selected on a low melting point agarose gel. cDNAs greater than 500 bp were ligated to λgtll, which had been cut and dephosphorylated at the *Eco*RI site in LacZ (Promega). *Escherichia coli* strain Y1090 was infected with the λgtll cDNA library and plated for 4 h at 42°C. Nitrocellulose disks which were presoaked in 10 mM IPTG and air-dried, were placed in contact with plaques for 2 h at 37°C to induce synthesis of and bind β-galactosidase fusion protein. The nitrocellulose filters were then processed in an ELISA using 17D6 or control antibodies and goat anti-mouse Ig alkaline phosphatase conjugated antibody (Miles Scientific).

Northern hybridization

RNA was denatured by formaldehyde–formamide and electrophoresed in a formaldehyde, 1% agarose gel (Maniatis *et al.*, 1982). The RNA was then electrotransferred to Zeta Probe as suggested by the manufacturer (BioRad). Hybridization was carried out in 50% formamide—5 × SET (1 × SET = 0.1 M NaCl/0.015 M EDTA/Tris pH 7.5) at 42°C for 16 h with nick-translated DNA probes. Filters were washed successively with 2 × SET, 1 × SET, 0.5 × SET at 42°C and finally with 0.25 × SET at 55°C for 30 min.

DNA sequencing

Restriction fragments of the Blast-1 cDNA (Figure 2) were subcloned into Bluescribe (Stratagene). Double strand sequencing was performed using the dideoxy termination method (Sanger *et al.*, 1977).

In vitro transcription and translation

The 1.2 kb Blast-1 cDNA was subcloned into the *Eco*RI site of the plasmid vector Bluescribe (Stratagene). This plasmid (pBS2A1.12) was then linearized by *Bam*HI digestion, using the conditions recommended by the manufacturer (New England Biolabs). A transcription reaction was performed using the linearized plasmid T7 RNA polymerase and m7G (5′) ppp (5′) G (Pharmacia), according to the manufacturers recommended conditions (Strategene). Approximately 100 ng of transcript was added to a reticulocyte lysate translation system as described (Cereghini *et al.*, 1979).

Transfection of NIH 3T3 cells

Blast-1 cDNA was subcloned into the *Eco*RI site of the eukaryotic expression vector pBG367 (Cate *et al.*, 1986), a gift from Biogen Research Corp. (Cambridge, MA). The construct, pBG2A1.1, was cotransfected with the Genticin selectable plasmid, pSV2neo (Mulligan and Berg, 1981) by standard calcium phosphate precipitation procedure (Graham and Van Der Eb, 1973). Transfectants were then selected for by growth in 500 μg/ml Genticin (Gibco), and isolated with cloning cylinders.

N-Glycosidase F digestion

Blast-1 was immunoprecipitated as previously described (Thorley-Lawson and Edson, 1979). The immune complex precipitated on formalin fixed *Staphylococcus aureus* (Sph A), was washed once with 100 μl of peptide:N-glycosidase F (N-glycosidase F) buffer (50 mM Tris pH 8.5, 50 mM EDTA, 0.1% SDS, and 0.3 M 2-mercaptoethanol). The protein was then eluted from the pelleted Sph A by re-suspending the pellet in 45 μl of N-glycosidase F buffer, incubating in 100°C H_2O bath for 5 min and pelleting the Sph-A again. The supernatant containing the eluted material was transferred to a fresh tube to which 5 μl of 10% NP-40 was added. This material was then incubated with or without 1.5 units of N-glycosidase F (Genzyme) at 37°C for the times indicated, followed by the addition of 45 μl of 2 × SDS−PAGE sample buffer and electrophoresing through a 10% polyacrylamide gel (SDS−PAGE) under reducing conditions.

Acknowledgements

We are grateful to Theodore Krontiris, Andy Leiter, Gerry Waneck and Clark Edson for generously providing material and advice. We gratefully acknowledge Blair Ardman for performing the FACS analysis and Margaret Coutts for performing the *in vitro* translation. Finally we wish to thank Cindy Welch for expert assistance in preparation of the manuscript and John Gotshall for creative photography. This work was supported by US Public Health Grants AI 15310 and CA 28737. David Thorley-Lawson is a recipient of US Public Health Research Career Development Award Grant AI 00549. Donald Staunton was supported in part by a U.S. Public Health Training Grant AI 07077 and by the Ivan Cottrell Endowment.

References

Altman,A., and Iskav,N. (1985) *Immunol. Today*, **7**, 155−157.
Auffray,C., Lillie,J., Arnot,D., Grossherger,D., Kappes,D. and Strominger,J., (1984) *Nature*, **308**, 327−333.
Bank,I. and Chess,L. (1985) *J. Exp. Med.*, **162**, 1294−1302.
Butler,J.L., Falkoff,R.J.M. and Fauci,A.S. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2475−2479.
Cate,R.L., Mattaliano,R.J., Hession,C., Tizard,R., Farber,N.M., Cheung,A., Ninfa,E.G., Frey,A.Z., Gash,D.J., Chow,E.P., Fisher,R.A., Bertonis,J.M., Torres,G., Wallner,B.P., Ramachandaran,K.L., Ragin,R.C., Manganaro,T.F., MacLaughlin,D.T. and Donahue,P.K. (1986) *Cell*, **45**, 685−698.
Cereghini,S., Geoghegan,T., Bergmann,I. and Brawerman,G. (1979) *Biochemistry*, **18**, 3153−3158.
Chen,Z.Z., Coggeshall,K.M. and Cambier,J.C. (1985) *J. Immunol.*, **136**, 2300−2304.
Chess,L. and Schlossman,S. (1976) In Rose,R. and Friedman (eds) *Manual of Clinical Immunology, 1st ed.* Am. Soc. Microbiol., Washington, DC, p. 77.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294−5299.
Chou,P.Y. and Fasman,G.D. (1978) *Annu. Rev. Biochem.*, **47**, 251−278.
Coggeshall,K.M. and Cambier,J.C. (1984) *J. Immunol.*, **133**, 3382−3386.
Cross,G.A. (1987) *Cell*, **48**, 179−181.
Edson,C.M., Hosler,B.A., Respees,R.A., Waters,D.J. and Thorley-Lawson,D.A. (1985) *J. Virol.*, **56**, 333−336.
Gorski,J., Rollini,P., Long,E. and Mach,B. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3934.
Graham,F.L. and Van Der Eb,A.J. (1973) *Virology*, **52**, 456−467.
Gubler,U. and Hoffman,B.J. (1983) *Gene*, **25**, 263−269.
Hood,L., Kronenberg,M. and Hunkapiller,T. (1985) *Cell*, **40**, 225−229.
Kabat,E.A., Wu,T.T., Bilosky,H., Reid-Miller,M. and Perry,H. (1983) *Sequences of proteins of immunological interest.* U.S. Department of Health and Human Sciences, Washington, DC, p. 281.
Kaufman,J.F., Auffray,C., Korman,A., Schackelford,D. and Strominger,J. (1984) *Cell*, **36**, 1−13.
Kehrl,J., Muraguchi,A. and Fauci,A.S. (1984) *J. Immunol.*, **132**, 2857−2851.
Kintner,C. and Sugden,B. (1981) *Nature*, **294**, 458−460.
Kozak,M. (1986) *Cell*, **44**, 283−292.
Krangel,M.S., Orr,H.T. and Strominger,J.L. (1979) *Cell*, **18**, 979−991.
Kyte,J. and Doolittle,R.F. (1982) *J. Mol. Biol.*, **157**, 105−132.
Lee,J.S., Trowsdale,J., Travers,P., Carey,J., Grosveld,F., Jenkins,J. and Bodmer,W. (1982) *Nature*, **299**, 750−752.
Lipman,D.J. and Pearson,W.R. (1985) *Science*, **227**, 1435−1440.
Low,M.G. and Finean,J.B. (1977) *Biochem. J.*, **167**, 281−284.
Low,M.G., Ferguson,M.A., Futerman,A. and Silman,I. (1986) *Trends Biochem. Sci.*, **11**, 212−215.
Maddon,P.J., Littleman,D., Godfrey,M., Maddon,D., Chess,L. and Axel,R. (1985) *Cell*, **42**, 93−104.
Malissen,M. Hunkapiller,T. and Hood,L. (1983) *Science*, **221**, 750−753.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Mann,K.P., Staunton,D.E. and Thorley-Lawson,D.A. (1985) *J. Virol.*, **55**, 710−720.
Monroe,J.C. and Cambier,J.C. (1983) *J. Exp. Med.*, **158**, 1589−1599.
Mulligan,R.C. and Berg,P. (1981) *Mol. Cell. Biol.*, **1**, 854−864.
Muraguchi,A., Kehrl,J.H., Butler,J.L. and Fauci,A.S. (1984) *J. Immunol.*, **132**, 176−180.
Nakagawa,T. Hirano,T., Nakagawa,N., Yoshizaki,K. and Kishimoto,T. (1985) *J. Immunol.*, **134**, 959−966.
Queen,C. and Koru,J.L. (1984) *Nucleic Acids Res.*, **12**, 581−599.
Ransom,J.T. and Cambier,J.C. (1986a) *J. Immunol.*, **136**, 66−71.
Ransom,J.T., Harris,L.K. and Cambier,J.C. (1986b) *J. Immunol.*, **137**, 708−714.
Ravetch,J.V., Luster,A.D., Weinshank,R., Kochan,J., Pavlove,A., Portnoy,D.A., Hulmes,J., Pan,Y. and Unkeless,J.C. (1986) *Science*, **234**, 718−725.
Sanger,F., Nicklen,S. and Coulson,A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463−5467.
Swain,S.L. (1983) *Immunol. Rev.*, **74**, 129−142.
Thorley-Lawson,D.A. and Edson,C.M. (1979) *J. Virol.*, **32**, 458−467.
Thorley-Lawson,D.A. and Mann,K.P. (1985) *J. Exp. Med.*, **162** 45−59.
Thorley-Lawson,D.A., Schooley,R.T., Bhan,A.K. and Nadler,L.M. (1982) *Cell*, **30**, 415−425.
Thorley-Lawson,D.A., Nadler,L.M., Bhan,A. and Schooley,R.T. (1985) *J. Immunol.*, **134**, 3007−3012.
Thorley-Lawson,D.A., Swendeman,S. and Edson,C.M. (1986) *J. Immunol.*, **136**, 1745−1751.
Williams,A. (1984) *Nature*, **308**, 12−13.

Received on July 22, 1987; revised on August 31, 1987