Subtractive cDNA Cloning of a Novel Member of the Ig Gene Superfamily Expressed at High Levels in Activated B Lymphocytes

By Eric J. Kozlow, Gaye Lynn Wilson, Cecil H. Fox, and John H. Kehrl

Using subtractive cDNA cloning we have isolated a series of cDNA clones that are exclusively or selectively expressed in B lymphocytes. mRNA transcripts from such cDNA clone, referred to as BL11, were found to be expressed at low levels in RNA from normal B lymphocytes, but at very high levels in RNA from in vitro activated B lymphocytes. One major 2.5-kb BL11 mRNA transcript was detected, while low levels of 4.8-, 1.8-, and 1.6-kb transcripts were also found. BL11 mRNA transcripts were absent or present at low levels in RNA prepared from resting or mitogen activated T cells, a variety of lymphoid cell lines including several B-cell lines, and several different tissues. Low levels of BL11 transcripts were found in poly(A) RNA purified from brain and lung. A study of the kinetics of BL11 mRNA accumulation in B lymphocytes stimulated in vitro with Staphylococcus aureus Cowan strain I showed a rapid induction of BL11 mRNA within 2 hours of stimulation with peak expression by 16 hours and a mild decrease with time following the peak levels. Consistent with the in vitro data, in situ hybridization using antisense BL11 RNA probes and human tonsil tissue localized BL11 transcripts in B-cell-enriched areas. Multiple BL11 cDNA and genomic clones were isolated and sequenced to complete and verify the BL11 cDNA sequence (2,404 bp). A 615-nucleotide open reading frame predicted to encode for a 205-amino acid protein with a molecular weight of 23 Kd was identified. Search of protein data bases with the predicted BL11 protein showed homologies to several members of the Ig superfamily. Analysis of the predicted protein showed a likely signal peptide, a single membrane spanning region, and one V-like Ig domain with three predicted N-glycosylation sites. Southern blot analysis of human genomic DNA suggested that BL11 is a single copy gene without evidence of rearrangement. Primer extension and S1 nuclease mapping identified four tightly clustered transcriptional start sites approximately 40 bp upstream of the predicted translation start site. The first 270 bp of the promoter region were sequenced and found to contain a CATATAA box rather than a TATAA box and several DNA motifs found in activation genes. BL11 should prove to be an interesting gene that likely encodes for a protein involved in B-cell activation. This is a US government work. There are no restrictions on its use.

The unique phenotype of a cell results in large part from the present or past selective expression of a specific set of regulatory and structural genes. The protein products of these genes are essential not only for specifying the identity of a cell type, but also for the performance of the unique functions of that cell. B lymphocytes are known to express cell-type specific genes that distinguish them, both phenotypically and functionally, from T lymphocytes. It has been estimated that B- and T-lymphocyte tumor cell lines express approximately 200 to 300 genes that are unique.1 Comparison of two-dimensional gels of membrane proteins extracted from normal B or T lymphocytes identified approximately 0.5% of the spots as being unique to one cell type versus the other (J.H.K., unpublished observation, September 1988). The production of monoclonal antibodies (MoAbs) against cell-surface determinants present on B lymphocytes has identified a number of unique proteins. Besides Ig, CD19, CD20, CD22, and CD72 are uniquely expressed on B cells and not other types.2 Differential screening and subtractive cloning techniques have been used to isolate genes specifically expressed in one cell type versus another, and they have been applied to lymphocytes with considerable success to isolate cDNAs for genes specifically expressed in T cells, T-cell subsets, activated T cells, and B cells.3-10 For example, cDNA clones for several B-cell-specific cell surface proteins have been isolated including CD19, CD20, CD22, and B29.7-10 We have used subtractive cloning techniques to isolate a series of cDNAs from a Staphylococcus aureus Cowan (SAC)-activated B lymphocyte cDNA library.11-12 These subtracted cDNAs represent genes expressed in B lymphocytes, but not expressed in T lymphocytes, and thus are potentially important genes that determine the phenotype and function of B lymphocytes. One of these subtracted cDNAs, BL11, is an activation gene in B lymphocytes, a member of the Ig gene superfamily, and is the subject of this report.

Materials and Methods

Cell culture and cell lines. Human tonsils were obtained from healthy patients undergoing routine tonsillectomies. The tonsil mononuclear cells were teased from the tonsillar tissue and fractionated into T and B cells by rosetting with aminothiolosmethionine bromide-treated sheep red blood cells.12 The B cells, rosette-negative cells, were further purified by repeating the rosetting step. The T cells were further purified by nylon wool columns. The B-cell preparations were routinely greater than 96% CD20 positive, whereas the T-cell preparations were greater than 98% CD3 positive as assessed by flow cytometry. The HS-Sultan, Jurkat, IM9, T24, 70Z, RAMOS, and RPMI 8226 lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). The BJA-B cells were a kind gift of Dr Edward Oates (Miami, FL). The M.J. EBV-3, M.T2, and SupT1 cell lines were a gift from Dr Scott Koenig (MedImmune, Rockville, MD).

cDNA libraries and isolation of BL11 cDNA clones. The isolation of the original BL11 cDNA clone was performed by screening an SAC-activated B-lymphocyte cDNA library in lambda ZAP phage with a 32P-labeled B-lymphocyte-specific subtracted cDNA probe as previously described.13 The isolation of additional BL11 cDNA clones

From www.bloodjournal.org by guest on November 11, 2017. For personal use only.
was performed by screening the B-lymphocyte cDNA library with the original 32P-labeled cDNA probe or with various 32P-labeled polymerase chain reaction (PCR)-amplified cDNA probes constructed from different regions of the original BL1 cDNA. Further 5′ cDNA sequence information was obtained by PCR using purified DNA from approximately 1 × 10⁶ phage of the SAC-activated B-cell cDNA library with two primers whose DNA sequences were derived from the lambda phage vector adjacent to the cloning site (sense) and the 5′ portion of the known BL1 cDNA (antisense). The resulting PCR product was directly subcloned into the pCRII plasmid (Invitrogen, San Diego, CA) and DNA sequenced.

Genomic libraries and isolation of BL11 genomic clones. BL11 genomic clones were obtained by screening 1 × 10⁶ phage from a human lymphocyte genomic library in lambda DASH (Stratagene, La Jolla, CA) phage with a full-length 32P-labeled BL1 cDNA probe and subsequently with a PCR-generated probe corresponding to positions +44 to +209 in Fig 1. The PCR probe was prepared using standard methodologies with a Perkin Elmer Cetus DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) and 5′ and 3′ primers whose sequences were based on the cDNA sequence. Nicktocrucile filters were hybridized with the probes as previously described.13 Positive plaques were identified and three positive clones were isolated from each screening. The initial BL11 genomic clones were partially sequenced via thermal cycle DNA sequencing, but were found to lack the coding region for the amino terminus of the predicted BL11 protein on the basis of a failure to sequence or hybridize to specific oligonucleotides whose sequence was based on the 5′ end of the BL11 cDNA. The subsequent BL11 genomic clones isolated with the above PCR fragment were partially mapped by restriction enzyme analysis and a 6-kb EcoRI restriction fragment that hybridized to a 5′ BL11 oligonucleotide was subcloned into pBluescript (Stratagene) for DNA sequencing.

DNA sequencing of BL11 and analysis of predicted protein. DNA sequencing was performed on double-stranded plasmid DNA templates or purified phage DNA using the dyeodeoxy chain termination technique with Sequenase (US Biochemicals, Cleveland, OH) or with T7 polymerase following the manufacturer’s protocols (Life Technologies, GIBCO/BRL, Gaithersburg, MD) using a Perkin Elmer Cetus DNA thermal cycler. Oligonucleotides were synthesized using an Applied Biosystems 392 DNA synthesizer (Foster City, CA). The sequencing project was performed with the aid of the Assembl program in PCGENE (IntelliGenetics, Mountain View, CA). The DNA and protein data base searches were performed with the FASTA program (Advanced Scientific Computing Center, Frederick, MD). The protein alignments were performed with the PCOMPARE program (IntelliGenetics).

Primer extension and S1 nuclease mapping. Primer extension was performed according to previously published methods.14 Briefly, 15 μg of total RNA from tonsillar B cells activated with SAC for 72 hours or from phorbol-12-myristate-13-acetate (PMA)-activated Jurkat cells was mixed with a 32P-end-labeled 96-base oligonucleotide complementary to a 5′ portion of BL11 mRNA (bases +56 to +85 in Fig 1) in an 80% formamide based hybridization buffer, heated to 85°C for 10 minutes, and incubated overnight at 35°C. After purification of the RNA-DNA hybrid the extension reaction was performed with 40 U AMV reverse transcriptase (RT) in RT buffer, at 42°C for 90 minutes. The extension products were treated with RNase, size fractionated by electrophoresis on an 8% denaturing polyacrylamide gel, and visualized by autoradiography. The S1 nuclease assay was performed according to previously published methods.13 Briefly, a 32P-end-labeled synthetic 96-base oligonucleotide (antisense to bases -48 through +58), which spanned the promoter-exon 1 junction based on the primer extension assay, was mixed with 10 μg of total RNA from SAC-activated tonsillar B cells or PMA-activated Jurkat cells in an 80% formamide-based hybridization buffer, heated to 85°C for 10 minutes, and incubated at 35°C overnight. Digestion with various amounts of S1 nuclease was performed at 37°C for 60 minutes. Products from the S1 nuclease treatment were visualized and sized as above for primer extension.

Northern and Southern blot analysis. Total RNA was prepared by a guanidine thiocyanate method.14 The RNA was size fractionated on a 1% agarose/formaldehyde gel, transferred to nitrocellulose, UV cross-linked, and hybridized to a 32P-labeled BL11 random primed cDNA probe using a nonformamide-based hybridization method.13 The blots were washed at high stringency and hybridization signals were detected by autoradiography. The multiple tissue RNA blot was purchased from Clontech (Palo Alto, CA). The blot was hybridized and washed according to the manufacturer’s suggestions.

In situ hybridization. A protocol similar to that described by Pardue17 was used for RNA hybridization with previously published modifications.10 Paraffin-fixed human tonsil sections were mounted on silanized slides, deparaffinized, and digested with proteinase K. The slides were then acetylated and prehybridized at 45°C for 2 hours in a 50% formamide-buffered saline solution previously published.21 Hybridization was performed in the same solution with the addition of an equal volume of 20% dextran sulfate in 50% formamide and probe at 8 × 10⁶ cpm per 25 mm² of specimen. Ordinary coverslips were sealed at the edges and the slides hybridized at 42°C. 32P dCTP-labeled sense and antisense

---

**Fig 1.** Nucleotide and predicted protein sequence of BL11. The complete DNA sequence of BL11 attained from sequence information derived from SAC-activated B-lymphocyte cDNA library clones (nucleotides 18 through 2404), nested primer PCR of the B-lymphocyte library (nucleotides 44 through 209), and genomic sequence (nucleotides 1 through 17). The transcriptional start sites, as determined by primer extension and S1 nuclease mapping, are indicated with dots. The amino acid sequence is numbered on the left and the nucleic acid sequence is numbered on the right. The N-terminal signal peptide and the hydrophobic transmembrane region are underlined.
RNA probes were made from the pBluescript BL11 plasmid using the T3 and T7 promoters. The probes were hydrolyzed in a carbonate buffer to a length of approximately 300 bp. They were purified by repeated precipitation with ethanol and had specific activities of \(2 \times 10^6 \text{cpm/\mu g}\). After overnight hybridization, the coverslips were removed and the slides were washed once in 50% formamide \(1 \times \text{SSC}\) with 1 mmol L \(-\text{EDTA}\) and 5 mmol L dithiothreitol (DTT), and five times at 60°C with \(2 \times \text{SSC}\) with EDTA and DTT followed by digestion of single-stranded RNA with RNase A and RNase T1. Slides were then washed in 0.3 mol L ammonium acetate in alcohol and dried overnight. Slides were dipped in NTB2 emulsion, exposed for 3 days, and developed in Kodak X-OMAT film.

Isolation of BL11 cDNAs and DNA sequence analysis. The original 2.0-kb BL11 cDNA clone was isolated via screening a human B-cell cDNA library with a B-T subtracted cDNA probe. Initial Northern blots with the BL11 clone verified that the BL11 transcripts were differentially expressed as a major 2.5-kb mRNA transcript and three lesser transcripts of 1.6, 1.8, and 4.8 kb present in RNA derived from B lymphocytes, but not from T lymphocytes (see below). Partial DNA sequence analysis and search of Genbank (Advanced Scientific Computing Center) with the derived sequence information did not show any significant homologies to known DNA sequences. The initial BL11 clone was fully sequenced but was found to lack an open reading frame that was predicted to encode a protein. The original BL11 cDNA was used to re-screen the SAC-activated B-cell cDNA library. Ten cDNA clones were isolated and the longest cDNA (2.2 kb) was sequenced. This cDNA diverged from the original BL11 cDNA clone, and on closer observation the original BL11 cDNA was found to have an unspliced intron at the 5′ end that accounted for the failure to find an open reading frame. This was based on a subsequent comparison with a BL11 genomic clone. The 2.2-kb cDNA was sequenced and found to contain an open reading frame, but no initiating methionine was identified. The 5′ 165 nucleotides of the 2.2 kb clone were amplified by PCR and used as a probe to isolate an additional 50 clones from the SAC-activated B-lymphocyte cDNA library. Eight of these clones appeared to be full length (approximately 2.3 kb) and the 5′ ends were sequenced. Although the derived sequence information verified the presence of the open reading frame, a translational start codon could not be identified. In an attempt to screen the entire SAC-activated B-lymphocyte cDNA library for additional BL11 5′ sequence information, nested primer PCR was performed on purified DNA from \(1 \times 10^6\) phage particles using primers corresponding to the 5′ sequence of BL11 cDNA and lambda phage vector sequence. This amplification resulted in the generation of DNA fragments with an additional 25 nucleotides of 5′ information that contained a start codon. To confirm the sequence information derived from PCR amplification, genomic sequence information corresponding to this 5′ region was attained. Three genomic clones were isolated from the screening of \(1 \times 10^6\) genomic phage particles with the 5′ 165 nucleotide BL cDNA probe. Sequencing of these clones showed that the PCR-generated sequence information was accurate. The PCR-generated 5′ fragment was again used as a probe to rescreen the SAC-activated cDNA library and a 2.3-kb cDNA clone was isolated that contained the initiation ATG. Analysis of the 2.3-kb BL11 cDNA showed an ATG-initiated open reading frame of 615 bases, which encoded for a predicted protein of 205 amino acids with a molecular mass of 23.5 Kd (Fig 1). The ATG was in fair context for initiation of translation. Hydrophobicity plots showed the presence of the signal peptide and a single membrane spanning domain (amino acids 144 through 164) with a predicted intracyto-
plasmic region of 42 amino acids. The predicted extracellular portion of the molecule has three potential N-linked glycosylation sites. Near the completion of this study, Zhou et al. reported the isolation and characterization of an essentially identical cDNA sequence.

**Homologies to other proteins and domain structure.** The BL11 predicted protein was used to search the National Biomedical Research Foundation (NBRF; Advanced Scientific Computing Center) protein data base and the translated Genbank database. Significant homologies with members of the Ig gene superfamily were found. The best scores on search of the protein data bases were with proteins that possessed Ig V-like domains. The best match was with the chicken B-G antigens, which are part of the chicken major histocompatibility complex and contain a single IgV-like domain. A quantitative measurement of BL11 amino acid sequence homology with several members of the Ig family was performed using the PCOMPARE program (Table 1). PCOMPARE measures the similarities between two sequences before and after 100 random shufflings of each of the sequences. The degree of similarity between the two sequences is indicated by the number of standard deviations that this sequence deviates from what is expected when two random sequences are compared. This analysis confirmed that BL11 has the highest degree of sequence homology with other members of the Ig superfamily that contain an Ig V domain. This contrasts with the lower scores found with Ig C domains (Table 1).

**Expression of BL11 mRNA and in situ hybridization.** Northern blot hybridization of total cellular RNA samples with the BL11 cDNA full-length probe showed the presence of a major transcript of approximately 2.5 kb in SAC-activated B lymphocytes (Fig 2). In addition, there are three minor transcripts of approximately 1.6, 1.8, and 4.8 kb seen in SAC-activated B lymphocytes. There is only a small amount of BL11 RNA expression in unstimulated tonsil B-lymphocytes. BL11 mRNA transcripts were increased in RNA derived from tonsil B cells stimulated with SAC and PMA for 2 hours, peaked at 24 hours, and gradually decreased over a 120-hour culture period (Fig 3). Only very low or negligible expression was seen in phytohemagglutinin (PHA)/PMA-activated T cells and various cell lines of T-lymphocytic origin including MJ and MT-4, which are transformed with the human T-cell leukemia virus (Fig 3A). Two-day exposures of the autoradiographs did identify a BL11 signal in mitogen activated T cells (data not shown). Northern blot analysis of total RNA derived from various B-cell lines including RAMOS, RPMI 8226, IM-9, or BJAB showed only low levels of BL11 mRNA transcripts relative to stimulated tonsil B cells (Fig 3B). The 2.5-, 1.8-, and 1.6-kb BL11 mRNA transcripts were also detected in poly(A) RNA prepared from human brain and lung. In contrast, there was minimal to nonexistent expression in other human tissues including heart, placenta, liver, skeletal muscle, kidney, and pancreas (Fig 4). Thus, based on the Northern blot data, BL11 mRNA is expressed at high levels in activated B cells, but expressed at lower levels in several other cell types as well. The nature of the cells in the brain and lung that express BL11 mRNA remains to be determined.
transcriptional start sites of the BL11 gene. Primer extension was performed with 15 μg of total RNA from 72-hour SAC-activated B lymphocytes and a BL11 reverse primer. The results show a collection of extension products ranging from 38 to 41 bp 5′ of the presumed initiator ATG (Fig 6A). RNA from Jurkat cells, a human T-lymphocyte–derived leukemic cell line that expressed very low amounts of BL11 mRNA had no detectable levels of BL11 extension products in the region corresponding to the B-cell transcripts. However, a single extension product 28 nucleotides 5′ of the B-cell transcripts was detected with the Jurkat RNA. An S1 nuclease assay was performed to confirm the results obtained with primer extension. An oligonucleotide that extended 78 bp 5′

Fig 4. Northern blot analysis of BL11 transcripts in different human tissues. A human multiple tissue Northern blot containing 2 μg of poly A RNA from the indicated tissues was probed with the full-length BL11 cDNA insert. The positions of RNA size markers are shown at the right of the figure.

To characterize the expression of BL11 mRNA in vivo, we used in situ hybridization to localize BL11 mRNA in human tonsillar tissue. 35S-labeled sense and antisense RNA probes were made and sheared to 200 to 300 bases. The antisense probe is complementary to BL11 mRNA and should detect BL11 transcripts. In situ hybridization with paraffin-fixed tonsillar tissue identified a clear hybridization signal with the antisense probe, but not with the sense probe (Fig 5). The strongest signal was in the mantle zone of the lymphoid follicle, and a less intense although clearly present signal was also seen in the germinal center; both sites markedly enriched with B lymphocytes.

Southern blot analysis of human genomic DNA with the BL11 cDNA. Human genomic DNA was prepared from various lymphoid cell lines, tonsil B cells, and placenta. The DNA was digested with EcoRI or HindIII and the Southern blot was hybridized with a full-length BL11 cDNA. An identical restriction pattern was found with each of the DNAs (data not shown). Thus, BL11 appears to be a unique gene without evidence of rearrangement. A 6-kb EcoRI band identified on the Southern blot likely corresponds to the 6-kb band that was subcloned from the human genomic library and found to contain the promoter region and the 5′ portion of the BL11 cDNA (see below).

Mapping the BL11 transcriptional start sites. Primer extension and S1 nuclease assays were used to determine the

Fig 5. In situ hybridization with BL11 RNA probe. 35S dCTP-labeled sense (A) and antisense (B and C) BL11 RNA were hybridized to human tonsil tissue and then stained with hematoxylin and eosin. Panels A and B were photographed using dark field illumination, whereas panel C was photographed with visible light. When viewed with dark field illumination, a positive signal is light while a negative signal is dark.
Fig 6. Primer extension and S1 nuclease mapping of the BL11 transcription start sites. (A) The primer extension reactions were performed by annealing a 32P-end-labeled 30-bp oligonucleotide (antisense to bp +56 through +85) to 15 μg of 72-hour SAC-activated B-lymphocyte total RNA (lane 1) or Jurkat total RNA (lane 2) and extended with AMV reverse transcriptase. The size in nucleotides of the major products 5' to the primer is indicated on the left. (B) S1 nuclease analysis was performed by annealing a 32P-end-labeled 96-bp oligonucleotide probe (antisense to bp -48 to +58) to 15 μg of 72-hour SAC-activated B-lymphocyte total RNA (lanes 1 and 2) or Jurkat total RNA (lane 3), followed by digestion with S1 nuclease. The amount of S1 used is indicated below each lane. An unrelated DNA sequence was used to determine the length of the primer extension and size of the protected fragments after S1 digestion.

Fig 7. BL11 promoter region. Nucleotides upstream of the transcriptional start site and a portion of the first exon until the ATG are numbered relative to the transcription start sites. The major transcription start sites are indicated by dots. Potentially important cis-elements are underlined and labeled.

DISCUSSION

Using a subtractive hybridization technique we have isolated a new member, BL11, of the Ig gene superfamily from an SAC-activated B-lymphocyte cDNA library. Among the subtracted cDNA clones we have isolated, BL11 was the most common following exclusion of the class II gene cDNAs. Furthermore, based on multiple screenings of an activated B-cell cDNA library, BL11 composes approximately 0.1% of the library and, thus, represents a relatively common gene in activated B cells. Despite the frequency of this gene within the cDNA library, numerous difficulties were encountered in obtaining a full-length cDNA clone that contained the initiating ATG. Some of the problems were undoubtedly related to the high GC content and short length of the 5' untranslated region of BL11. In addition, attempts to confirm the cDNA sequence via sequencing genomic clones required two screenings of a human genomic library because of the presence of a relatively large intron at the 5' end of the gene. Eventually, a 2.3-kb cDNA, which includes the likely initiating ATG, was isolated.

The RNA expression pattern seen with various Northern blots probed with BL11 cDNA probe suggests that there is a restricted expression of this gene. No or very low levels of expression were found in total RNA derived from various T-lymphocytic cell lines or nonlymphocytic cell lines. Also,
there was either negligible or only low level expression in total RNA isolated from cell lines of B-lymphocytic origin including Epstein-Barr virus (EBV) immortalized B cells. BL11 transcripts were most striking in RNA derived from tonsillar B lymphocytes stimulated with SAC. RNA isolated from B lymphocytes activated with SAC exhibits expression of four different BL11 RNA transcripts. The major transcript identified on Northern blot analysis with the BL11 cDNA was 2.5 kb, which was representative of the BL11 cDNAs isolated from the cDNA library. No cDNA clones were isolated from the cDNA library that conformed to the larger RNA transcript of 4.8 kb. One BL11 cDNA clone of 4.0 kb was isolated that contained unspliced introns, suggesting that the 4.8-kb BL11 mRNAs observed on Northern blot analysis represents partially spliced RNAs. The 1.8- and 1.6-kb transcripts likely represent the usage of alternative more 3’ polyadenylation sites. In situ hybridization analysis of BL11 RNA expression in human tonsillar lymphoid tissue showed expression in both the germinal center and the surrounding mantle zone, both B-cell–rich regions. There appears to be more prominent expression in the mantle zone, although it is difficult to accurately quantify given the differing density of cells within the germinal center versus the mantle zone.

Hybridization of the BL11 cDNA probe to the multiple tissue blot identified BL11 mRNA transcripts in poly(A) RNA derived from both brain and lung. This tissue expression was somewhat surprising given the restricted pattern of expression seen in various lymphoid and nonlymphoid cell lines. It is unlikely that the levels of expression seen in brain and lung results from B-lymphocyte infiltration of these tissues. There are now a number of cell surface determinants which, while originally thought to be lymphocyte specific, have been identified in brain. Thy-1 is a good example of such a protein that is expressed on T lymphocytes as well as neuronal cells. It will be of interest to identify which cells in the brain express BL11.

The BL11 mRNA is predicted to encode for 23-Kd protein containing 205 amino acids. The initial assessment of the signal peptide cleavage site based on the method of von Heijne predicted the cleavage site between amino acids 19 and 20. However, this site does not conform to the (-3, -1) rule by virtue of a proline at position -2 relative to the cleavage site. The (-3, -1) rule is a feature of the majority of signal peptide cleavage sites previously identified, although not a universal requirement, as there are documented exceptions to this rule. Assuming the cleavage site between amino acids 19 and 20, the core BL11 protein would have a molecular mass of 21 Kd. In the extracellular domain of the BL11 are three potential N-linked glycosylation sites (Asn-X-Ser/Thr). On the basis of hydrophobicity analysis a transmembrane domain is predicted to exist between amino acids 143 and 163, resulting in a relatively small intracytoplasmic tail of 42 amino acids. Search of the protein data bases with the intracytoplasmic tail amino acid sequence failed to identify any significant homologies. The intracytoplasmic region is relatively charged with six positively and three negatively charged amino acids.

Comparison of the BL11 protein sequence with the NBRF data base and the translated Genbank data base showed substantial homology with human, murine, and rabbit Ig variable regions and with other members of the V-set of the Ig super family. The assignment of BL11 to the V-set is supported by the presence of the additional C and C’ subsegments possessed by BL11 as well as the higher align scores with the V-set members versus the C1 and C2 set members. Based on the homology with Ig V-regions we would predict that a disulfide bond links amino acids 35 and 107.

The overall structure of the BL11 protein with a single V-set Ig domain, a transmembrane domain, and a short intracytoplasmic domain is similar to several other proteins including CD7, the myelin Po protein,23 the CMRF35 protein,2 and both chains of the rat CD8 heterodimer.34 CD7 and CD8, in contrast to BL11, are expressed predominantly on T cells rather than B cells. The CMRF35 protein is broadly expressed on myeloid cells and subpopulations of peripheral blood B and T cells. Besides BL11 another member of the Ig superfamily has been shown to be induced after B-cell activation; however, instead of a single Ig domain B7 has two Ig domains, one V-like and one C-like. In addition, B7 has been shown to be a ligand for the T-cell surface protein CD28.35 It will be of interest to determine if, similar to B7, BL11 interacts with another cell surface molecule present on T cells.

In addition to isolating and characterizing the BL11 cDNAs, several BL11 genomic clones were isolated and used to verify the BL11 cDNA as well as to localize the BL11 promoter region. Transcriptional initiation was clustered around a 4-bp span located approximately 40 bp 5’ of the translational start ATG as determined by primer extension and S1 nuclease mapping. A CATAA box rather than a TA-TAA box is likely important in transcription initiation. An NF-kB site was found at −73 bp relative to the transcriptional start site and four potential SP-1 sites were identified. Otherwise there were not any other DNA motifs commonly found in B-cell–specific promoters. The promoter region of the BL11 gene is GC rich, a characteristic of “housekeeping” genes rather than tissue specific or activation genes. This was somewhat surprising because BL11 fits into these latter categories. Further studies of the BL11 promoter are in progress to identify genetic elements that are responsible for the restricted expression of this gene, which may lead to the discovery of transactivating factors that participate in the regulation of BL11 expression.

In the course of our study of the BL11 gene, Zhou al identified a cDNA for the same gene. The predicted amino acid sequence was identical, although the BL11 cDNA extends both 5’ and 3’ from their reported DNA sequence. These investigators have also prepared a MoAb which shows that the protein is expressed in lymphoid tissues with a distribution similar to the distribution of the BL11 mRNA. The MoAb also reacted with concanavalin A- or PMA-stimulated peripheral blood lymphocytes, suggesting expression on activated T cells. While we did detect some BL11 mRNA in stimulated T cells, the levels were much less than those found in activated B cells. In conclusion, BL11 is a member of the Ig superfamily that is rapidly induced on at least a portion of B lymphocytes after B-cell activation. Future studies will
concentrate on identifying a ligand for BL11 and characterizing the promoter region.

ACKNOWLEDGMENT

The authors thank Dr Anthony S. Fauci for his advice and support and Mary Rust for her editorial assistance.

REFERENCES

23. Dynan WS, Tjian R: Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerases II. Cell 32:669, 1983
27. Williams AF, Gagnon J: Neuronal cell thy-1 glycoprotein homology with Ig. Science 216:697, 1982
30. Aruffo A, Seed B: Molecular cloning of two CD7 (T-cell leukemia antigen) cDNAs by a COS cell expression system. EMBO J 6:3313, 1987
Subtractive cDNA cloning of a novel member of the Ig gene superfamily expressed at high levels in activated B lymphocytes

EJ Kozlow, GL Wilson, CH Fox and JH Kehrl