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Immunology

B29: A member of the immunoglobulin gene superfamily exclusively expressed on B-lineage cells

(subtractive library screening/B-cell gene isolation/membrane glycoprotein)

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ABSTRACT **A number of the glycoproteins identified on the surfaces of cells of the immune response belong to the immunoglobulin superfamily. We have isolated and characterized cDNA clones and the complete genomic gene encoding a B-cell-specific member of the immunoglobulin superfamily called "B29." This isolate is expressed at all stages in B-cell development beginning with the earliest precursor B cells undergoing immunoglobulin heavy chain gene diversity region → joining region gene ($D_H \rightarrow J_H$) rearrangements. The protein sequence predicted by the *B29* coding region contains a leader sequence and a single extracellular immunoglobulin-like domain, followed by a hydrophobic transmembrane segment and a charged intracytoplasmic domain. The immunoglobulin-like domain contains cysteines and other conserved amino acids characteristic of light chain variable and joining regions, but overall the sequence is only distantly related to immunoglobulins. Each of these domains is encoded in separate exons in the *B29* gene, in analogy to other members of the immunoglobulin superfamily. The conserved structural features of the immunoglobulin-like domain in the *B29* gene product resemble those of other members of the immunoglobulin superfamily involved in cell recognition and adhesion.**

Many glycoproteins involved in the immune response exhibit conserved structural features like those first reported for immunoglobulin chains. These molecules are encoded by genes of the immunoglobulin superfamily and are presumed to have a common evolutionary origin (1, 2). Immunoglobulin genes are exclusively expressed in B-lineage cells, and their products are the most extensively studied superfamily members (reviewed in refs. 3 and 4). A considerable number of immunoglobulin superfamily members on T cells, such as the T-cell antigen receptor, have now been defined by molecular cloning (reviewed in refs. 1, 5, and 6).

However, in contrast to the genes encoding the collection of surface molecules now defined on T cells, little is known about genes that are specifically expressed in B cells other than the immunoglobulin genes. An immunoglobulin light (L) chain gene called "λ5" and two genes resembling immunoglobulin L chain variable (V) region genes have been reported (7–10). These genes do not undergo rearrangement and are selectively expressed in pre-B-cells. We have isolated a set of B-cell-specific clones from a B-cell-enriched library prepared by subtractive hybridization of B-cell cDNA and T-cell mRNA (11). Here we describe the characterization of cDNA clones and the sequence¶ for a previously unreported B-cell-specific gene, designated "*B29*," which is expressed throughout B-cell development. This gene encodes an integral membrane protein with a single extracellular immunoglobulin-like domain containing three potential N-linked glycosylation sites. The predicted coding sequence of this domain exhibits a high degree of identity with the conserved residues in immunoglobulin κ L chain V and joining (J) regions. When analyzed by the PROFILE method (12), the immunoglobulin-like domain of B29 also was related to other superfamily members including the V regions of T-cell antigen receptor (TCR) α, β, and γ chains, immunoglobulin λ L chain V regions, and the T-cell accessory molecules, CD4 and CD8. These features and the organization of the *B29* gene establish B29 as a new member of the immunoglobulin superfamily. The widespread expression of B29 throughout B-cell development and its structural relatedness to molecules on T cells involved in cellular recognition and adhesion (e.g., CD4; ref. 13) suggests that this molecule may be important in the cellular interactions involved in B lymphopoiesis.

MATERIALS AND METHODS

Cells. The pattern of *B29* gene expression was analyzed in established cell lines and in tissues. The earliest B-lineage cell examined was an Abelson murine leukemia virus (Ab-MuLV)-transformed bone marrow line, KD87-12 (μ^-, κ^-), which shows no rearrangement of either H or L chain immunoglobulin genes (K. Denis, personal communication; G.G.H., unpublished results) but does contain λ5 mRNA, which is specifically expressed in pre-B-cells (7). The Ab-MuLV-transformed fetal liver line JS61-11 (μ^-, κ^-; ref. 14) and Ab-MuLV-transformed bone marrow line KD87-10 (K. Denis, personal communication) represent pre-B-cell lines exhibiting only the first stage of μ chain gene segment rearrangement [i.e., heavy (H) chain diversity region (D_H) → J_H; ref. 6]. Both κ L chain alleles in these two cell lines are in the germ-line configuration. Pre-B-cell lines (μ^+, κ^-) are represented by the Ab-MuLV-transformed cell line 18-81 (15) and the Ab-MuLV-transformed fetal liver cell line JS61-10 (14). B-cell lines expressing IgM (but little IgD) include W279 (16), L10A, and Bal 17 (17). M2-5 corresponds to a mature IgM^+ IgD^+ B cell (μ^+, δ^+, κ^+; R.W. and O. Witte, unpublished results). IgM-secreting hybridoma cell lines MxW231 and MxW279 (18) and myeloma cell line S107A represent terminally differentiated immunoglobulin-secreting plasma cells (19). T-cell lines screened include T-cell lymphomas BW5147 and R1.1 (17), a helper T cell, EL4 (20); a helper T-cell hybridoma, AODK10.4 (21); suppressor T-cell hybridomas TS6, 7C, and VL3 (22); and a cytotoxic T-cell line, MD 90 (23). Other cell lines screened include 2813 (macrophage), Friend cells (hematopoietic cell precursor),

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Abbreviations: V, variable; C, constant; D, diversity; J, joining; Ab-MuLV, Abelson murine leukemia virus; TCR, T-cell antigen receptor; H, heavy; L, light.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03857).

3T3 (fibroblasts), and two stromal cell lines, SS1 and SSN1 (P.W.K., unpublished results).

cDNA Library Screening. Bacterial colonies from the B cell − T cell subtracted library (11) were picked and grown to confluency in eight 96-well microtiter plates containing L broth, tetracycline, and 3% (vol/vol) glycerol. Partially purified plasmid DNA from each colony was obtained by alkaline lysis of bacteria in each well. The plasmid DNA was applied to nitrocellulose filters by using a Bethesda Research Laboratories dot-blot apparatus, and duplicate filters were probed with radiolabeled cDNA from Bal 17 (B-cell line) or BW5147 (T-cell line). The resulting clone, 1A94-1, was used to isolate 1A94-2 from a Bal 17 library constructed in pTZ18. Clone 1A94-3 was obtained from a 70Z/3 library constructed in λgt10 (provided by A. Bothwell, Yale University, New Haven, CT).

Genomic Library Screening. The B29 genomic clone was isolated from a BALB/c sperm library in λ Charon 4A. The library was plated on *Escherichia coli* LE392, transferred to nylon filters, denatured, and UV-crosslinked. The filters were probed with 1A94-3 by standard methods (24).

DNA Sequencing. Restriction fragments and exonuclease III (25)-generated deletion fragments were cloned into phage M13 vectors and sequenced by using the dideoxy chain-termination technique (26).

RESULTS

Isolation of B-Cell-Specific cDNA Clones. The source for our B-cell-specific clone isolation was a cDNA library prepared from Bal 17 B lymphoma mRNA and enriched approximately 20-fold for B-cell sequences by subtractive hybridization with RNA from Bal 4 thymoma (11). Initially, 27 clones were identified that preferentially reacted with the B-cell probe. These included 18 clones of immunoglobulin L and H chain mRNAs identified by hybridization with complete *V* and constant (*C*) region genomic DNA clones encoding μ, κ, and λ chains. The remaining presumptive B-cell-specific clones were hybridized with RNA from different tissues and cell lines. Presumptive B-cell-specific clone B29 (initially isolated as p1A94-1) hybridized with RNA from all B-lineage cells tested but not with RNA from any non-B-lineage tissue or cell line examined (Fig. 1). To the effective limit of these screening methods for detecting mRNA (approximately one or two copies per cell), this clone was not detectably expressed in any T-cell line or in thymus. Since B and T cells express an estimated 98% of their genes in common (29), this finding is an especially compelling indication of the B-cell specificity of this clone. The expression of this newly isolated gene is more stringently restricted to B-lineage cells than the majority of antibody-defined surface markers now used to isolate and characterize murine B lymphocytes and their precursors (30).

B-cell-specific clone B29 hybridized with an mRNA of 1.4 kb expressed in all B-lineage cells tested, including myeloma and hybridoma cells secreting different classes of immunoglobulin (Fig. 1). A selected panel of established pre-B-cell lines representing the earliest stages in B-cell development were analyzed to determine when expression of *B29* is initiated in B-cell development (Fig. 1). B29 mRNA was detected in the μ^+ pre-B-cell lines (JS61-10 and 18-81) and in the μ^- pre-B-cell lines (JS61-11 and KD87-10), which contain rearranged $D_H \rightarrow J_H$ gene segments (6). B29 RNA also was found in a cell line (KD87-12) whose immunoglobulin gene segments are in the germ-line configuration and which is presumed to represent an early B-cell precursor (Fig. 1). RNA hybridizing with the B29 probe also was detected in total RNA isolated from murine fetal livers at 16–19 days of gestation (results not shown). This survey strongly suggests that the expression of *B29* is activated concurrent with the earliest events in immunoglobulin H chain gene rearrangement (3, 4). Further experiments will be necessary to confirm that *B29* expression is activated in precursor B-lineage cells prior to $D_H \rightarrow J_H$ rearrangements. The present results indicate that *B29* is a useful molecular probe for identifying early precursor cells in the B lineage and for analyzing normal as well as aberrant pathways of B-cell development.

Sequences of the B29 cDNA and Gene Clones. The insert sequences in B29 cDNA clones (p1A94-1 and p1A94-2, Fig. 2), were short due to the stringent hybridization procedures used in the generation of the B cell − T cell subtracted library (11). A larger cDNA clone (p1A94-3) was subsequently isolated from a different cDNA library prepared from the pre-B-cell line 70Z/3 (Fig. 2). This cDNA clone was shown to contain an almost full-length copy of the B29 mRNA. The complete sequences of both strands of all three cDNA clones were determined. The sequences comprising the 5′ end of the mRNA and the complete coding sequence were determined from the *B29* genomic gene clone (Fig. 2). A computer search of the GenBank nucleic acid sequence data base and of the NBRF protein sequence data base established that *B29* is a previously unreported sequence.

Genomic Southern blotting experiments performed with the B29 cDNA clone p1A94-3 on DNA from liver, spleen, and the Bal 17 lymphoma cell line established that the *B29* gene is present in one copy per haploid genome and that it is not rearranged (results not shown). The complete genomic *B29* gene was isolated from a library of BALB/c sperm DNA cloned in λ Charon 4A by screening with P1A94-3 DNA (Fig. 2). The intron–exon structure of this gene was determined by sequencing along both strands, except for intron 2 which was sequenced in only one direction. The combined maps of the three B29 cDNA clones, the genomic gene clone, and the

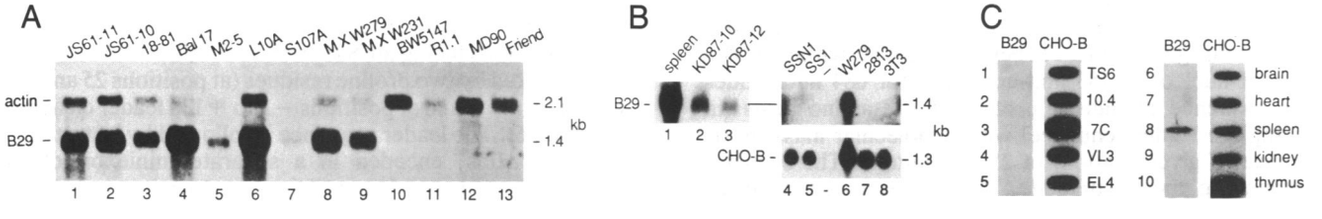

FIG. 1. Expression of B29 mRNA is restricted to B-lineage cells. (*A*) Blot-hybridization analysis of 1–2 μg of poly(A)-containing mRNA from pre-B-cell lines (lanes 1–3), B-cell lines (lanes 4–6), immunoglobulin-secreting hybridoma or myeloma cell lines (lanes 7–9), T-cell lines (lanes 10–12), and a Friend hematopoietic precursor cell line (lane 13) hybridized with ^{32}P-labeled B29 and α-actin probes (27). (*B*) Blot-hybridization analysis of 1–2 μg of poly(A)-containing mRNA from BALB/c splenic B cells (lane 1) and early pre-B-cell lines (lanes 2 and 3) and of 20 μg of total RNA from two stromal cell lines (lanes 4 and 5), a B-cell lymphoma (lane 6), a macrophage line (lane 7), and 3T3 fibroblasts (lane 8). All samples were hybridized with ^{32}P-labeled *B29* plasmid DNA. Lanes 4–8 were also hybridized with ^{32}P-labeled *CHO-B* plasmid DNA (a ubiquitously expressed housekeeping gene; ref. 28). (*C*) Slot blots of duplicate 1-μg samples of poly(A)-containing mRNA from a panel of T-cell lines (slots 1–5) and different tissues (slots 6–10). Samples were hybridized with either ^{32}P-labeled B29 or a ubiquitously expressed gene, *CHO-B* (28). Cell lines are described in *Materials and Methods*. kb, Kilobases.

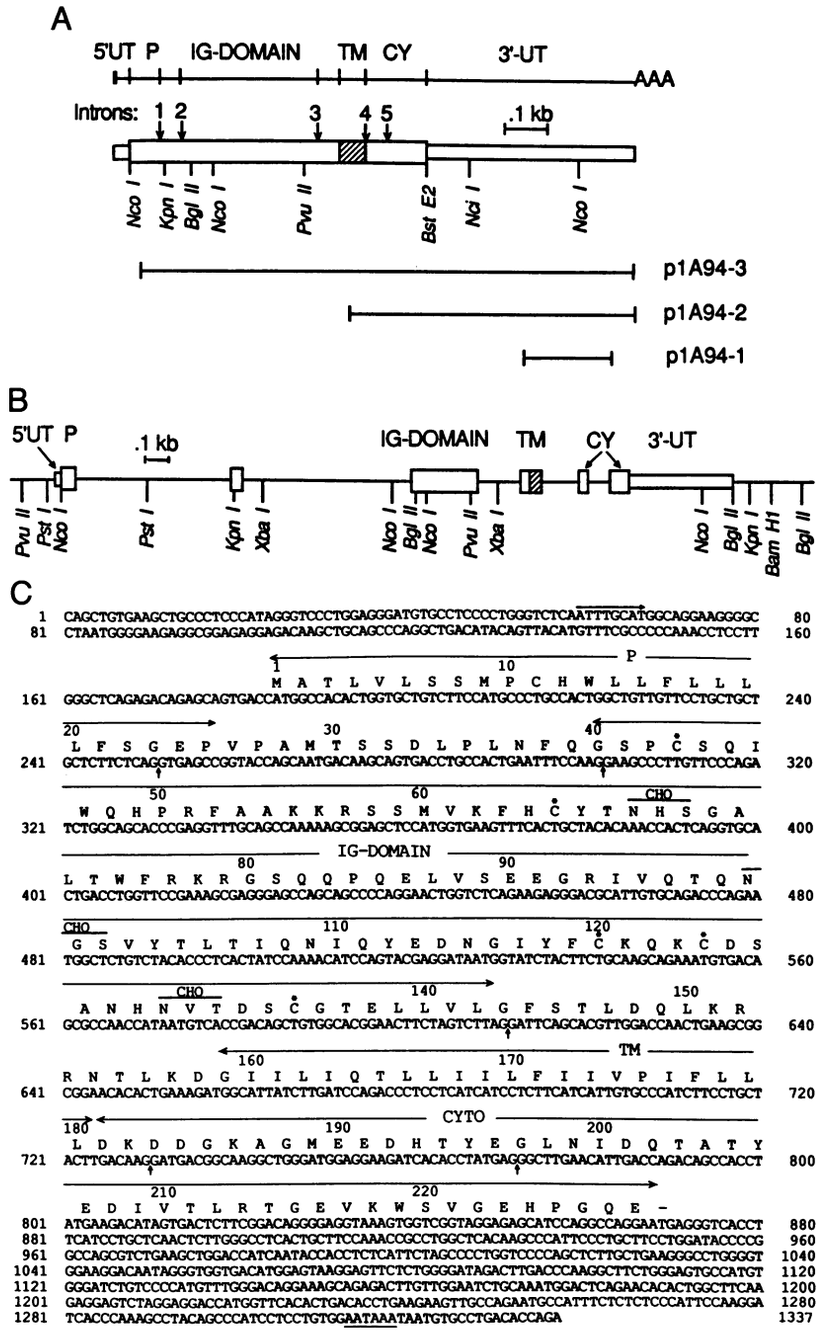

FIG. 2. The structure and sequence of B29 cDNA and genomic gene clones. (*A*) B29 cDNA clones (p1A94-1, -2, -3) are aligned along a schematic diagram of B29 mRNA. (*B*) Structure of the *B29* genomic gene. The exon/intron structure of the isolated *B29* genomic gene was established by nucleotide sequencing as described in the text. (*C*) The sequence of the B29 mRNA and the deduced amino acid sequence of the longest B29 open reading frame. The 5′ sequence of the mRNA was derived from the combined nucleotide sequences of the p1A94-3 cDNA clone and the genomic *B29* gene. The 5′ region of the *B29* contains an octamer motif, 5′-ATTTGCAT-3′, in the same orientation as in the promoters of the κ *V* regions (31–33). Positions of introns in the mRNA sequence are noted by (↑) below the nucleotide sequence. The precursor (P), immunoglobulin-like domain (IG-DOMAIN), transmembrane segment (TM), and cytoplasmic (CYTO) segment are denoted by bars over the deduced amino acid sequence. Cysteine residues in the amino acid sequence are denoted by asterisks. The AATAAA in the 3′-untranslated region of the B29 mRNA is underlined.

coding sequence deduced from the complete cDNA and genomic gene sequences are summarized in Fig. 2.

The longest open reading frame from the combined cDNA and genomic gene sequences begins with ATG within the *Nco* I site at nucleotide 183 and continues for 684 nucleotides. This sequence predicts a protein of 228 amino acids (including the leader sequence) with a molecular mass (in the absence of glycosylation) of 25,697 daltons. The sequence surrounding the postulated ATG initiation codon closely approximates the consensus translation initiation sequence RNNATGR (34), where R is an unspecified purine nucleoside. The predicted B29 amino acid sequence includes a hydrophobic leader segment, an immunoglobulin L chain V region-like domain of 103 residues, a positively charged extracellular segment followed by a hydrophobic transmembrane sequence of 22 residues, and an intracytoplasmic tail of 48 residues. The leader sequence is predicted to be 25 amino acids long based on the hydrophobicity and charge characteristics of other leader peptides (35, 36). Like most proteins of the immunoglobulin superfamily, the leader is encoded in a single exon. It is uncertain if the predicted leader cleavage site is used (producing an NH_2 terminal valine at residue 26) as it is flanked by two proline residues (at positions 25 and 27) reportedly not found at positions -3 to $+1$ in leader cleavage sites (35, 36). The leader sequence is followed by a stretch of 16 amino acids, encoded in a separate miniexon of 51 nucleotides, with no clear relationship to known proteins. This small segment is followed by an exon-encoded 103 amino acids containing multiple cysteine residues. This domain was subjected to computer analysis by the PROFILE method (12) to determine its relatedness to other known protein sequences. In PROFILE analysis, a position-dependent scoring table, called a profile, is generated from one or more aligned sequences. (In the present case, only the 103 residue immunoglobulin-like B29 domain was used). Then the profile is compared to other sequences, and a score

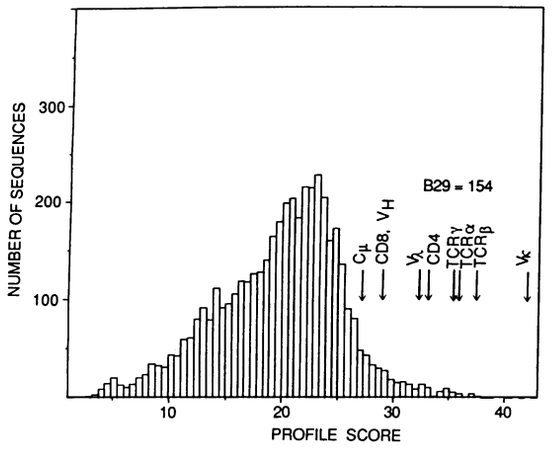

FIG. 3. B29 is structurally related to κ L chain V and J regions and to V-region-like immunoglobulin superfamily members. PROFILE analysis (12) of the deduced B29 amino acid sequence of the Ig domain (residues 40–142 of Fig. 2*C*) was carried out on 4525 protein sequences.‖ This profile yielded a maximum score of 42.1 for the sequence most related to B29 (i.e., the V_κ region from S107A) to 1.9 for the least similar sequence. The mean value for profile scores was 19.8, and the standard deviation was 5.6. The arrows denote the highest profile scores for different members of the immunoglobulin superfamily.

is assigned for comparison. The score is higher for sequences more closely related to the profile sequence. The results in Fig. 3 indicated that this B29 exon-encoded segment is most closely related to immunoglobulin κ L chain V regions. In fact, 90 of the top 100 profile scores (i.e., indicative of the sequences most closely related to B29) were different κ chain V regions. The profile analyses also revealed significant structural relatedness of B29 to other V region-related superfamily members including V regions of TCR α, β, γ chains, λ L chains, CD4, and CD8 (Fig. 3).

Overall, the sequence of this B29 exon-encoded protein is only 28% identical to the most similar immunoglobulin κ L chain V region (Fig. 4). However, the B29 sequence contains 13 of the 18 invariant residues in L chain V and J regions (37). The cysteine residues at 65 and 120 in B29 define a potential disulfide-bridged immunoglobulin-like domain of 55 amino acids (Fig. 2). This loop includes invariant aspartic acid and arginine residues capable of forming a salt bridge within the immunoglobulin-like domain (2). The immunoglobulin-like domain of B29 is shorter than that commonly found in immunoglobulin and TCR V regions and lacks the internal C′ and C″ β strands in typical V regions (2). In this regard, the structure of B29 more closely resembles immunoglobulin C regions. In addition to the two cysteine residues postulated to form the disulfide-bridged immunoglobulin-like domain, two additional cysteine residues found at positions 124 and 135 (Fig. 2) could be linked in interchain disulfide bridges to form homo- or heterodimers as is almost universally the case for immunoglobulin superfamily proteins (1). Finally, the amino acid sequence of the immunoglobulin-like domain contains three potential N-linked glycosylation sites at asparagine residues 68, 99, and 130 (Fig. 2).

The immunoglobulin-like domain is followed by a 16 amino acid long charged sequence preceding a hybrophobic transmembrane segment of 22 amino acids related to γ2b, γ2a, and μH chain transmembrane segments (38). Like H chains, the charged extracellular and transmembrane sequences are encoded in a single exon (Fig. 2). The transmembrane segment is followed by a highly charged intracytoplasmic domain of 48 amino acids encoded in two separate exons in an arrangement also analogous to that in immunoglobulin H chains (38). This intracellular domain has the unusual feature for such structures of a relatively high net negative (−7) charge. This intracellular sequence also contains a serine residue (position 220) and two threonine residues (positions 194 and 214) directly preceded by basic residues which could comprise potential sites for phosphorylation by protein kinase C (39).

‖Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 13.0.

DISCUSSION

To date, the immunoglobulin gene superfamily includes nine multigene families, and 22 single gene members (see ref. 2). All of the products of the immunoglobulin gene superfamily are organized on a common protein structure termed the immunoglobulin homology unit. This basic structure is composed of a domain approximately 100 amino acids long containing a centrally placed intrastrand disulfide loop that stabilizes a series of antiparallel β chain strands into the characteristic immunoglobulin fold (1, 2). The overall amino acid sequence identity between homology units ranges from 15% to 40% in different superfamily proteins (1, 2). Despite this low level of overall sequence identity, all homology units are thought to form the immunoglobulin fold. Therefore, it appears that sequence divergence within the superfamily has occurred within constraints imposed by the need to conserve the basic structure of the immunoglobulin fold. This conserved structure has only been confirmed by x-ray crystallography for immunoglobulin V and C domains, major histocompatibility complex class I antigens, and β_2-microglobulin.

The properties of the *B29* gene clearly establish it as a new member of the immunoglobulin gene superfamily. As established by the technique of PROFILE analysis (12), the product of *B29* is most similar structurally to immunoglobulin κ L chain V regions (≤28% identical residues) and related members of the superfamily including TCR V regions, λ L chain V regions, CD4, and CD8 (see Fig. 3). In contrast to many members of the superfamily which contain multiple immunoglobulin-like domains (2), the B29 molecule is relatively simple with a single, disulfide-bridged external domain. In fact, the basic B29 structure with a leader sequence, a single immunoglobulin-like domain (with conserved residues characteristic of immunoglobulin L chain V regions and structural features like C regions) followed by a transmembrane segment and cytoplasmic tail strikingly resembles the structure proposed for the progenitor of the immunoglobulin superfamily (1).

The organization of the genomic *B29* gene further validates it as a member of the immunoglobulin gene superfamily (Fig. 2). The exon structure exactly duplicates that seen in immunoglobulin genes. The leader sequence, the single immunoglobulin-like domain, the transmembrane segment, and intracellular domains are all encoded in separate exons. The splicing signals at the exon/intron junctions also reinforce this similarity to immunoglobulin genes. RNA splicing occurs between the first and second bases of codons in all exons through the 5′ end of the transmembrane segment exactly as in immunoglobulin H chain genes (1). At the 3′ juncture of the transmembrane exon and on both sides of the exons encoding the intracellular segment, RNA splicing occurs between codons as is also the case in immunoglobulin H chain membrane exons (38). Thus, *B29* conclusively fulfills all the criteria short of experimental secondary structure determination for inclusion in the immunoglobulin gene superfamily.

It will be interesting to determine the function of the *B29* gene. Its product is structurally related to V–J-region domains of immunoglobulin superfamily members involved in antigen recognition as well as to CD4 and CD8 molecules.

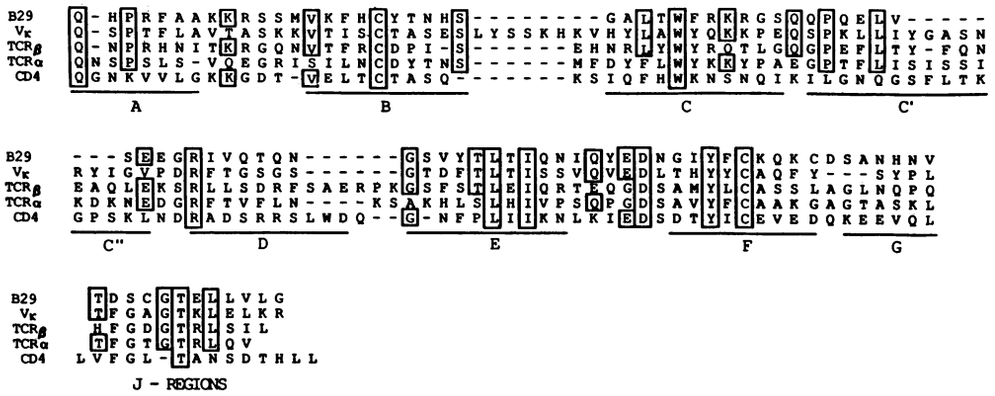

FIG. 4. Alignment of the V and J regions of B29 with related proteins of the immunoglobulin superfamily. The alignment was performed to maximize identity (shown as boxed residues) of related members of the immunoglobulin superfamily with the V–J immunoglobulin-like domain of B29. The lines below the sequences labeled with letters A, B, C, C′, C″, D, E, F, G denote residues that form β strands (2).

These latter superfamily members are postulated to function as cell adhesion molecules in T-cell antigen recognition and binding (reviewed in ref. 40). In support of this idea, Doyle and Strominger (13) have recently demonstrated that CD4 mediates cell adhesion by binding class II major histocompatibility complex molecules. The structural relatedness of B29 to these T-cell accessory molecules may imply a role for this B-cell-specific protein in either homotypic or heterotypic interactions of B cells.

Finally, the DNA sequence in the *B29* gene 5′ of the translation initiation site contains a perfect match to the octamer motif present in all immunoglobulin L and H chain gene promoters (refs. 31 and 32, reviewed in ref. 33). Wirth, Staudt, and Baltimore (33) recently reported that a synthetic oligonucleotide containing the octamer motif alone was sufficient to promote B-cell-specific transcription. Accordingly, the octamer motif functioning as a promoter element could account for the B-cell-specific transcription of the *B29* gene.

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