PRIMARY STRUCTURE OF LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN 3 (LFA-3)
The Ligand of the T Lymphocyte CD2 Glycoprotein

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The organization of specialized cells within tissues during embryogenesis and development, as well as in inflammation, is thought to be due to a collection of cell surface receptors that interact with molecules in the extracellular matrix and on the surface of other cells, thereby imparting positional information. Recently, we found (1) that lymphocyte function-associated antigen 3 (LFA-3), a surface glycoprotein expressed on many cell types, binds to the T lymphocyte surface glycoprotein CD2 with a high affinity (10^{-10} M). This interaction is important in mediating thymocyte interactions with thymic epithelial cells, antigen-independent and -dependent interactions of T lymphocytes with target cells and antigen-presenting cells, and the T lymphocyte rosetting with erythrocytes (1-7). In addition to mediating cell adhesion, it has been suggested that the LFA-3/CD2 interaction may prime responses by both the CD2^{+} and LFA-3^{+} cells (8-10). Although receptors for extracellular matrix components (11), and receptors that appear to engage in like-like interactions (N-CAM) (12), have been defined, to our knowledge CD2 and LFA-3 are the first example in which an integral membrane protein on one cell binds to a distinct integral membrane protein on another cell. These molecules thus represent an ideal model system for studying cell-cell adhesion. To understand the structural basis for cognate interactions between cell adhesion molecules, we have cloned an LFA-3 cDNA and determined the primary structure of the LFA-3 protein.

Materials and Methods

LFA-3 Purification and Sequencing. LFA-3 was purified by immunoaffinity chromatography from human erythrocytes as described (6). LFA-3 purified by one cycle of affinity chromatography was deglycosylated using peptide:N-glycosidase F (N-glycanase) according to the supplier's instructions (Genzyme, Boston, MA). The glycosylated and deglycosylated forms were further purified by preparative SDS-PAGE and electroeluted exactly

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Abbreviations used in this paper: LFA-3, lymphocyte function-associated antigen 3; PIPLC, phosphatidylinositol-specific phospholipase C.
as described (13). Deglycosylated LFA-3 was digested with trypsin and the tryptic peptides were separated by HPLC (14). Intact LFA-3 (500 pmol), N-glycanase-treated LFA-3 (50 pmol), and tryptic fragments (5–50 pmol) were subjected to automated Edman degradation on a gas-phase sequencer (model 470A; Applied Biosystems, Inc., Foster City, CA) in the presence of polybrene (15). PTH-amino acids were identified on-line using a PTH analyzer (model 120A; Applied Biosystems, Inc.).

Construction of the PBL cDNA Library. PBL from leukapheresis were stimulated with 100 U/ml IFN-γ and 10 µg/ml PHA for 24 h. RNA was prepared by phenol extraction (16), and double-stranded cDNA was synthesized from poly(A)-selected RNA by the methods of Okayama and Berg (17) and Gubler and Hoffman (18). The cDNA was ligated to an Eco RI/blunt-ended linker, designed to allow the cDNA insert to be excised by Not I. Size-selected (800 bp and longer) cDNA was ligated to Eco RI–digested Agt10 arms, and aliquots of the ligation mixture were packaged in Gigapak (Stratagene, San Diego, CA). The packaged phage was used to infect Escherichia coli. The resulting library contained 1.1 × 10⁶ independent recombinant phages.

Isolation of the cDNA Clones. 10⁶ recombinants of either the human PBL Agt10 cDNA library or a human tonsil Agt11 cDNA library (a generous gift of Lloyd Klickstein, Harvard Medical School, Boston, MA) (19) were screened with 3²P-labeled oligonucleotide probes as described (20). Antisense oligonucleotide probes were synthesized on an Applied Biosystems, Inc., 30A DNA Synthesizer. Oligonucleotide probe pool LF1 was a 20 mer, 32-fold degenerate, corresponding to amino acids 28–34 of the NH₂-terminal sequence of LFA-3 (Table I). Probe pools LF2 to LF5 were synthesized to residues 3–7 of the NH₂-terminus and were a set of four subpools, each 20 bp long and 96-fold degenerate. The nucleotide sequence of each subpool was identical except for the nucleotide in the third position of the codon for glycine. Probes were 5’ endlabeled using γ-[³²P]ATP (7,000 Ci/mmol; New England Nuclear, Boston, MA or ICN Pharmaceuticals, Inc., Irvine, CA) and polynucleotide kinase according to the protocol of Maxam and Gilbert (21).

DNA Sequencing. DNA sequences were determined by the method of Maxam and Gilbert (21) and in some cases by the related procedure of Church and Gilbert (22).

Northern Blot Analysis. A431, CEM, THP1, U937, HL60, Jurkat, HT1080 cell lines were obtained from the American Type Culture Collection, Rockville, MD. L428 and U266 cell lines were a gift of Steve Shaw, NIH, Bethesda, MD (23, 24), and the ROHA-9 cell line was obtained from A. Muchmore, NIH (25). RNA from human tissues and from 10⁷ cells of all cell lines was prepared by the guanidinium thiocyanate–GCl method (26). Poly(A) RNA was selected by passage over an oligo(dT)–cellulose column. Aliquots of 10 µg of total RNA or 3 µg of poly(A) RNA were electrophoresed on a 1% agarose-formaldehyde gel, transferred to a GeneScreen membrane (New England Nuclear) and hybridized to the respective ²P-labeled probe, essentially as described (27).

Genomic Southern Blot Analysis. DNA was extracted from the human lymphoblastoid cell line GM1416,48,XXX, obtained from the Human Mutant Cell Repository, Camden, NJ, as previously described (28). Digestion of 20-µg aliquots of DNA with restriction endonucleases Eco RI, Bam HI, Pvu II, or Pst I, separation of the digested DNA on an agarose gel, transfer to nylon membrane, and hybridization to ³²P-labeled cDNA, were performed essentially as described (29).

Results and Discussion

LFA-3 was purified from human erythrocytes by mAb affinity chromatography (6). In SDS-PAGE it migrated as a diffuse band of 45–66,000 Mₐ (lane i in Fig. 1, A–C), as previously reported (6). One batch of LFA-3 was subjected to a second affinity chromatography step to remove minor contaminants. No contaminants were detectable by SDS-PAGE, in 10% (Fig. 1 A) or 15% gels (not shown). This batch of LFA-3 was directly subjected to gas-phase microsequencing. The NH₂-terminal 38 amino acid residues, except for one unidentified residue at position 12, were determined (Table I). A second batch of LFA-3 purified by one cycle of affinity chromatography was divided into two aliquots, and N-linked
Intact, deglycosylated LFA-3 or tryptic fragments were subjected to NH$_2$-terminal protein sequence analysis. Tryptic peptide designations correspond to peaks off the HPLC. Amino acid sequences corresponding to oligonucleotide probes are underlined. N-glycosylated residues are indicated by asterisks.

carbohydrate was removed from one aliquot with N-glycanase. The glycosylated and deglycosylated forms (Fig. 1B, lanes 1 and 2) were purified by preparative SDS-PAGE and electroelution (Fig. 1C, lanes 1 and 2). Deglycosylated LFA-3 ran as a relatively sharp band at 25,600 M$_r$, but still retained some heterogeneity in M$_r$ (Fig. 1B, lane 2; Fig. 1C, lane 2). The NH$_2$-terminal sequence of the glycosylated, SDS-PAGE-purified protein was in complete agreement with that for the LFA-3 purified by two cycles of affinity chromatography. The only difference in the sequence of deglycosylated LFA-3 (Table 1) was an aspartic acid.
FIGURE 2. DNA sequence and deduced amino acid sequence of human LFA-3 cDNA. The signal sequence is indicated by the numbers -1 to -28. The amino acids of the mature LFA-3 are numbered starting with +1 and continued at the end of each line. Nucleotide numbering is given at the end of each line. Amino acid sequences that correspond to the sequences of the tryptic peptides are underlined with solid lines. Potential N-glycosylation sites are indicated by an arrow. The potential transmembrane domain is underlined with a broken line, and the poly(A) addition signal is indicated by a line above the sequence. These sequence data have been submitted to the EMBL/GenBank Data Libraries under accession number Y006536.

The 1,040-bp-long sequence of LFA-3 cDNA contains a 5' untranslated region of 9 bp, followed by an open reading frame of 750 bp (Fig. 2). The 281-bp 3'
untranslated region contains a consensus polyadenylation signal (Fig. 2, overlined). The first ATG located at 84 bp upstream of the NH$_2$-terminus of the mature LFA-3 glycoprotein is the probable translational initiation site. This indicates that LFA-3 is synthesized as a 250-amino-acid preprotein containing a hydrophobic signal sequence of 28 residues. The mature protein of 222 amino acids is 24,400 $M_r$, which is in close agreement with the $M_r$ determined by SDS-PAGE of the deglycosylated polypeptide chain of 25,500 $M_r$ (Fig. 1) or 29,000 $M_r$ (see below).

The cDNA sequence defines a typical membrane bound protein with an NH$_2$-terminal domain of average hydrophobicity, followed by a highly hydrophobic transmembrane region and a COOH-terminal hydrophilic domain. The NH$_2$-terminal domain of 188 amino acids contains all six potential N-glycosylation sites (Asn-Xaa-Ser/Thr) (Fig. 2). Sequencing of glycosylated and deglycosylated LFA-3 showed that this domain is glycosylated and hence is extracellular. Cleavage by N-glycanase of N-linked carbohydrates occurs within the amide bond of the asparaginyl moiety, converting the N-glycosylated asparagine residue to an aspartic acid residue. NH$_2$-terminal sequencing showed N-glycanase converted position 12 of LFA-3 from a blank (glycosylated asparagine are not identified) to an aspartic acid and the cDNA sequence shows asparagine at this residue. Thus, the LFA-3 protein is N-glycosylated at this position. Similarly, the cDNA predicts an asparagine in a potential N-glycosylation site at position 66, while the deglycosylated tryptic peptide T91 shows aspartic acid at this position (Table I, asterisk), indicating that this potential N-glycosylation site is also used.

Pulse-chase labeling of diverse LFA-3** cell types (Dustin, M. L., and T. A. Springer, unpublished observations) with $[^{35}S] $-methionine has revealed, in all cell lines thus far tested, two LFA-3 precursors of $M_r$ 43,000 and 41,000 $M_r$. Digestion of these precursors, in which the carbohydrate moieties are in the high mannose form, with N-glycanase or Endo H reduces their apparent $M_r$ to 29,000 and 25,500, respectively. This indicates a contribution of 14–15,000 $M_r$ by high mannose carbohydrates. An estimate of 2,500 $M_r$ per high mannose carbohydrate (30) predicts six N-linked carbohydrates, suggesting that all of the other potential N-glycosylation sites are used. The mature LFA-3 glycoprotein is heterogeneous in $M_r$ on individual cell types and varies from 43,000 to 76,000 $M_r$, depending on cell type (Dustin and Springer, unpublished observations). The polypeptide chain of $M_r$ 24,400 defined by the cDNA sequence shows that the mature glycoprotein is 44–68% carbohydrate.

The predicted transmembrane domain consists of 23 uncharged amino acids, most of which are hydrophobic in character. The putative cytoplasmic domain is short, consisting of 12 hydrophilic residues. While this work was in progress we found, corresponding to the two LFA-3 precursors, two forms of mature LFA-3 that differ in membrane attachment as distinguished by their sensitivity to phosphatidylinositol-specific phospholipase C (PIPLC) (31; Dustin, M. L., et al., manuscript in preparation). The high molecular weight precursor remains membrane bound, while the lower molecular weight form is released upon PIPLC treatment, indicating membrane attachment through a PI-glycolipid membrane anchor (32). This suggests that the structural difference between these two proteins resides in the COOH-termini.

Phosphatidylinositol-glycan moiety is attached to the extracellular domain of
membrane proteins by a transamidation reaction in which a COOH-terminal hydrophobic sequence in the protein is displaced (32). We have determined that the human genome contains a single LFA-3 gene (see below), suggesting generation of two forms at the protein or mRNA levels. Although most PI-anchored proteins have hydrophobic transmembrane precursor sequences with no or insignificant cytoplasmic domains, the "signal" for PI attachment remains undefined (32). It is thus possible that this LFA-3 sequence we have defined is expressed as the membrane-integrated form with the transmembrane domain intact and that it may also act as a precursor for PI attachment. This would generate both forms from a single protein and mRNA precursor. Since PI is added extremely rapidly (32), this is consistent with the detection of two protein precursors at the earliest time points. Alternatively, differential mRNA splicing could generate two distinct transmembrane domains, only one of which is exchanged for the PI anchor, as has been demonstrated recently for N-CAM (reviewed in reference 32).

We therefore examined a number of LFA-3 cDNA clones for differences in their 3' sequences by Southern blot analysis, and determined the complete DNA sequence of four independent isolates. All clones examined so far had identical 3' regions. More extensive investigation is required to determine whether the two forms of LFA-3 are coded for by a single or two distinct mRNAs.

LFA-3 is a cell surface molecule with a broad tissue distribution. It has been found in most tissues examined, including most blood cells, epithelial, and endothelial cells. It was of interest to determine the presence and size of LFA-3 transcripts in various tissues and cell lines. The LFA-3 cDNA hybridizes to an ~1.3-kb transcript in RNA extracted from human tonsil, kidney, placenta, and skin. LFA-3 transcripts are also present in HT1080 cells (a human fibrosarcoma cell line), A431 (a human carcinoma cell line), CEM (human acute lymphoblastic leukemia), THP1 (human monocyte leukemia), U937 (human histiocytic lymphoma, U266 (plasmacytoma, B lineage), JY (B lymphoblastoid), L428 (Reid-Sternberg, Hodgkin's), ROHA9 (human Burkitt lymphoma), K562 (erythroleukemia), and Jurkat (T lymphoma). No LFA-3 mRNA could be detected in this subline of HL60 cells (a promyelocytic leukemia cell line) (Fig. 3).

A Southern blot analysis on human genomic DNA indicated that LFA-3 is coded for by a single gene in the human genome. The 220-bp fragment from the 5' end of the LFA-3 cDNA insert was used as a hybridization probe. This probe contains an internal Pvu II site, and hybridizes to only two fragments of the Pvu II-digested human DNA (Fig. 4), and to only one fragment of the Pst I digest, which indicates a single gene for human LFA-3. The two hybridizing bands of equal intensity in the Bam HI digest and the two bands of unequal intensity in the Eco RI digest predict that the 220-bp probe sequence is interrupted by an intron in the LFA-3 gene.

No significant homologies with LFA-3 were detected by a FASTP search of the National Biomedical Research Foundation Protein (release 12.0) and NEW (release 27.0) databases (33). CD2, the receptor for LFA-3, has recently been cloned in the human and rat (34–37). Homology of short CD2 segments with the Ig superfamily has been reported by some groups (34, 35, 37). It has been proposed that cell–cell adhesion may be mediated by interactions between Ig-
FIGURE 3. Northern blot analysis. RNA from indicated cell sources was separated on 1% formaldehyde-agarose gel and probed with 32P-labeled LFA-3 cDNA. (A) and (C) 5 μg poly(A) RNA from HT1080 and ROHA-9 cells and 10 μg total RNA from all other cell lines was applied. (B) Human tonsil, kidney, and placenta: 3 μg poly(A) RNA; human skin: 10 μg total RNA. To assure equal loading of RNA in each sample, each Northern blot was also hybridized to 32P-labeled bovine pyruvate kinase cDNA (shown for panel A only).

FIGURE 4. Southern blot analysis. Aliquots of 20 μg human genomic DNA was digested with the indicated restriction endonucleases, separated on 1% agarose gel, transferred to a nylon membrane and hybridized to 32P-labeled 220-bp 5′ fragment of LFA-3 cDNA.
like domains (38). Secondary structure predictions for LFA-3 show $\beta$ strands containing cysteines that might assume an Ig-like structure.

As mentioned above, CD2 and LFA-3 are the first reported example of two distinct integral membrane glycoproteins that interact with one another to mediate cell adhesion. The isolated cDNA clones will facilitate study of the structural features of LFA-3 and CD2 that are important in this interaction, as well as in signal transduction.

Summary

We have isolated the cDNA for human lymphocyte function-associated antigen 3 (LFA-3), the ligand of the T lymphocyte CD2 molecule. The identity of the clones was established by comparison of the deduced amino acid sequence to the LFA-3 NH$_2$-terminal and tryptic peptide sequences. The cDNA defines a mature protein of 222 amino acids that structurally resembles typical membrane-anchored proteins. An extracellular domain with six $N$-linked glycosylation sites is followed by a hydrophobic putative transmembrane region and a short cytoplasmic domain. The mature glycoprotein is estimated to be 44–68% carbohydrate.

Southern blots of human genomic DNA indicate that only one gene codes for human LFA-3. Northern blot analysis demonstrates that the LFA-3 mRNA of 1.3 kb is widely distributed in human tissues and cell lines.

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References

1. Selvaraj, P., M. L. Plunkett, M. Dustin, M. E. Sanders, S. Shaw, and T. A. Springer. 1987. The T lymphocyte glycoprotein CD2 (LFA-2/T11/E-rosette receptor) binds the cell surface ligand LFA-3. Nature (Lond.). 326:400.
2. Shaw, S., G. E. G. Luce, R. Quinones, R. E. Gress, T. A. Springer, and M. E. Sanders. 1986. Two antigen-independent adhesion pathways used by human cytotoxic T cell clones. Nature (Lond.). 323:262.
3. Vollger, L. W., D. T. Tuck, T. A. Springer, B. F. Haynes, and K. H. Singer. 1987. Thymocyte binding to human thymic epithelial cells is inhibited by monoclonal antibodies to CD-2 and LFA-3 antigens. J. Immunol. 138:358.
4. Krensky, A. M., F. Sanchez-Madrid, E. Robbins, J. Nagy, T. A. Springer, and S. J. Burakoff. 1983. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J. Immunol. 131:611.
5. Plunkett, M. L., M. E. Sanders, P. Selvaraj, M. L. Dustin, and T. A. Springer. 1987. Rosetting of activated human T lymphocytes with autologous erythrocytes: definition of the receptor and ligand molecules as CD2 and lymphocyte function-associated antigen 3 (LFA-3). J. Exp. Med. 165:664.
6. Dustin, M. L., M. E. Sanders, S. Shaw, and T. A. Springer. 1987. Purified lymphocyte
function-associated antigen-3 (LFA-3) binds to CD2 and mediates T lymphocyte adhesion. J. Exp. Med. 165:677.
7. Krensky, A. M., E. Robbins, T. A. Springer, and S. J. Burakoff. 1984. LFA-1, LFA-2, and LFA-3 antigens are involved in CTL-target conjugation. J. Immunol. 132:2180.
8. Hunig, T., G. Tiefenthaler, K.-H. Meyer zum Buschenfelde, and S. C. Meuer. 1987. Alternative pathway activation of T cells by binding of CD2 to its cell-surface ligand. Nature (Lond.). 326:298.
9. Denning, S. M., D. T. Tuck, L. S. Wolf, T. A. Springer, K. H. Singer, and B. F. Haynes. 1987. Monoclonal antibodies to LFA-1, CD-2, and LFA-3 antigens inhibit human thymic epithelial accessory cell function for mature thymocyte activation. J. Immunol. In press.
10. Le, P., S. Denning, T. Springer, B. Haynes, and K. Singer. 1987. Anti-LFA-3 monoclonal antibody induces IL-1 release by thymic epithelial cells and monocytes. Fed. Proc. 46:447a.
11. Ruoslahti, E., and M. D. Pierschbacher. 1986. Arg-Gly-Asp: A versatile cell recognition signal. Cell. 44:517.
12. Edelman, G. M. 1986. Cell adhesion molecules in the regulation of animal form and tissue pattern. Annu. Rev. Cell Biol. 2:117.
13. Hunkapiller, M. W., E. Lujan, F. Ostrander, and L. E. Hood. 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid analysis. Methods Enzymol. 91:227.
14. Pepinsky, R. B., L. K. Sinclair, J. L. Browning, R. J. Mattaliano, J. E. Smart, E. P. Chow, T. Falbel, A. Riboloni, J. L. Garwin, and B. P. Wallner. 1986. Purification and partial sequence analysis of a 37-kDa protein that inhibits phospholipase A2 activity from rat peritoneal exudates. J. Biol. Chem. 261:4239.
15. Hewick, R. M., M. W. Hunkapiller, L. E. Hood, and W. J. Dreyer. 1981. Agas-liquid solid phase peptide and protein sequenator. J. Biol. Chem. 256:7990.
16. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 121.
17. Okayama, H., and P. Berg. 1982. High efficiency cloning of full-length cDNA. Mol. Cell. Biol. 2:161.
18. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. Gene (Amst.). 25:263.
19. Wong, W. W., L. B. Klickstein, J. A. Smith, J. H. Weis, and D. T. Fearon. 1985. Identification of a partial cDNA clone for the human receptor for complement fragment C3b/C4b. Proc. Natl. Acad. Sci. USA. 82:7711.
20. Benton, W. D., and R. W. Davis. 1977. Screening Agt recombinant clones by hybridization to single plaques in situ. Science (Wash. DC), 196:180.
21. Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labelled DNA with base specific chemical cleavages. Methods Enzymol. 68:499.
22. Church, G. M., and W. Gilbert. 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA. 81:1991.
23. Diehl, V., H. H. Kirchner, M. Schaadt, C. Fonatsch, H. Stein, J. Gevdes, and C. Boie. 1981. Hodgkin's Disease: establishment and characterization of four in vitro cell lines. J. Cancer Res. Clin. Oncol. 101:111.
24. Goldstein, M., J. Hoxie, D. Zembryski, D. Matthews, and A. I. Levinson. 1985. Phenotypic and functional analysis of B cell lines from patients with multiple myeloma. Blood. 66:444.
25. Scala, G., Y. D. Kuang, R. E. Hall, A. V. Muchmore, and J. J. Oppenheim. 1984. Accessory cell function of human B cells. I. Production of both interleukin 1-like activity and an interleukin 1 inhibitory factor by an EBV-transformed human B cell line. J. Exp. Med. 159:1637.
LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN 3 cDNA

26. Chirgwin, J. M., A. E. Przybla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 18:5294.

27. Wallner, B. P., R. J. Mattaliano, C. Hession, R. L. Cate, R. Tizard, L. K. Sinclair, C. Foeller, E. P. Chow, J. L. Browning, K. L. Ramachandran, and R. B. Pepinsky. 1986. Cloning and expression of human lipocortin, a phospholipase A2 inhibitor with potential anti-inflammatory activity. Nature (Lond.). 320:77.

28. Cate, R. L., W. Chick, and W. Gilbert. 1983. Comparison of the methylation patterns of the two rat insulin genes. J. Biol. Chem. 258:6645.

29. Cate, R. L., R. J. Mattaliano, C. Hession, R. Tizard, N. M. Farber, A. Cheung, E. G. Ninfa, A. Z. Frey, D. J. Gash, E. P. Chow, R. A. Fisher, J. M. Bertonis, G. Torres, B. P. Wallner, K. L. Ramachandran, R. C. Ragin, T. F. Manganaro, D. T. McLaughlin, and P. K. Donahoe. 1986. Isolation of the bovine and human genes for Mullerian Inhibiting Substance and expression of the human gene in animal cells. Cell. 45:685.

30. Parham, P., B. N. Alpert, H. T. Orr, and J. L. Strominger. 1977. Carbohydrate moiety of HLA antigens. Antigenic properties and amino acid sequences around the site of glycosylation. J. Biol. Chem. 252:7555.

31. Selvaraj, P., M. L. Dustin, R. Silber, M. G. Low, and T. A. Springer. 1987. Deficiency of lymphocyte function-associated antigen 3 (LFA-3) in paroxysmal nocturnal hemoglobinuria: functional correlates and evidence for a phosphatidylinositol membrane anchor. J. Exp. Med. 166:1011.

32. Low, M. G. 1987. Biochemistry of the glycosyl-phosphatidylinositol membrane protein anchors. Biochem. J. 244:1.

33. Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. Science (Wash. DC). 227:1435.

34. Sewell, W. A., M. H. Brown, J. Dunne, M. J. Owen, and M. J. Crumpton. 1986. Molecular cloning of the human T-lymphocyte surface CD2 (T11) antigen. Proc. Natl. Acad. Sci. USA. 83:8718.

35. Sayre, P. H., H.-C. Chang, R. E. Hussey, N. R. Brown, N. E. Richardson, G. Spagnoli, L. K. Clayton, and E. L. Reinherz. 1987. Molecular cloning and expression of T11 cDNAs reveal a receptor-like structure on human T lymphocytes. Proc. Natl. Acad. Sci. USA. 84:2941.

36. Seed, B., and A. Aruffo. 1987. Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. Proc. Natl. Acad. Sci. USA. 84:3365.

37. Williams, A. F., A. N. Barclay, S. J. Clark, D. J. Paterson, and A. C. Willis. 1987. Similarities in sequences and cellular expression between rat CD2 and CD4 antigens. J. Exp. Med. 165:368.

38. Williams, A. F. 1984. The immunoglobulin superfamily takes shape. Nature (Lond.). 308:12.