GENOMICS **40,** 217–221 (1997)
ARTICLE NO. GE964590

The Genomic Structure of the Human Charcot–Leyden Crystal Protein Gene Is Analogous to Those of the Galectin Genes

KIMBERLY D. DYER,*,† JEFFREY S. HANDEN,* AND HELENE F. ROSENBERG*,[1]

*Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and †Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, DC 20057

Received September 16, 1996; accepted December 11, 1996

The Charcot–Leyden crystal (CLC) protein, or eosinophil lysophospholipase, is a characteristic protein of human eosinophils and basophils; recent work has demonstrated that the CLC protein is both structurally and functionally related to the galectin family of β-galactoside binding proteins. The galectins as a group share a number of features in common, including a linear ligand binding site encoded on a single exon. In this work, we demonstrate that the intron–exon structure of the gene encoding CLC is analogous to those encoding the galectins. The coding sequence of the CLC gene is divided into four exons, with the entire β-galactoside binding site encoded by exon III. We have isolated CLC β-galactoside binding sites from both orangutan (*Pongo pygmaeus*) and murine (*Mus musculus*) genomic DNAs, both encoded on single exons, and noted conservation of the amino acids shown to interact directly with the β-galactoside ligand. The most likely interpretation of these results suggests the occurrence of one or more exon duplication and insertion events, resulting in the distribution of this lectin domain to CLC as well as to the multiple galectin genes.

INTRODUCTION

The Charcot–Leyden crystal protein (CLC) is an intracellular protein characteristic of human eosinophilic and basophilic leukocytes. It has been localized to the eosinophil cell membrane and cytoplasm and is found extracellularly as distinct hexagonal crystals at sites of eosinophil infiltration and inflammation (Ackerman, 1993). The complete amino acid sequence of the CLC protein as encoded by its cDNA (Ackerman *et al.*, 1993) revealed a moderate degree of sequence homology to the class of proteins known as galectins (Barondes *et al.*, 1994a,b). Galectins are expressed in a wide variety of mammalian cell types (Barondes *et al.*, 1994b; Hynes *et al.*, 1990; Regan *et al.*, 1986), and galectins 1 and 3 have been shown to be secreted via a nonclassical pathway into the extracellular space (Barondes *et al.*, 1994b; Sato and Hughes, 1994; Lindstedt *et al.*, 1993; Cooper and Barondes, 1990). The physiologic ligands for galectins include laminin (Sato and Hughes, 1992; Cooper *et al.*, 1991; Woo *et al.*, 1990), lysosome-associated membrane proteins (Do *et al.*, 1990; Skrincosky *et al.*, 1993), and the α-7 β-1 integrins (Gu *et al.*, 1994), suggesting a role for galectins in mediating cell–cell and cell–extracellular matrix interactions. Additionally, galectin-3 interacts with both IgE and the IgE receptor (Cherayil *et al.*, 1989, 1990; Frigeri *et al.*, 1993) and can activate and induce superoxide production in peripheral blood neutrophils (Yamaoka *et al.*, 1995), suggesting a role for this protein in mediating cellular immune responses.

In previous work, we evaluated the amino acid sequences shared by the CLC protein and human galectins and determined that CLC shares 9 of the 13 residues characterized as invariant in this family; of these invariant residues, CLC shares 6 of the 8 residues shown to be directly involved in binding to the β-galactoside sugar (Dyer and Rosenberg, 1996). We determined that recombinant CLC binds to a lactose-conjugated agarose resin and that binding was inhibited in a dose-dependent fashion by unconjugated lactose, indicating that CLC possessed functional as well as sequence homology to the galectin family of proteins (Dyer and Rosenberg, 1996). The crystal structure determined by Leonidas and colleagues (1995) demonstrated that the three-dimensional structure of CLC was topologically identical to those determined for human galectins 1 and 2.

In the work presented here, we have elucidated the structure of the gene encoding the CLC protein. The coding sequence of the CLC gene is divided into four exons, with the entire β-galactoside binding site encoded by a single exon, as is the case for all galectins characterized to date (Barondes *et al.*, 1994a; Gitt and

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. U68393–U68398.

[1] To whom correspondence should be addressed at LHD/NIAID/NIH, Building 10, Room 11N104, 10 Center Drive MSC 1886, Bethesda, MD 20892-1886. Telephone: (301) 402-9131. Fax: (301) 402-4369. E-mail: hr2k@nih.gov.

0888-7543/97 $25.00

Barondes, 1991; Oda *et al.*, 1991, 1993; Gitt *et al.*, 1992). Sequences encoding the CLC β-galactoside binding site were isolated from two additional species, and the rate of evolutionary divergence calculated for the human/mouse pair was compared to those calculated for the analogous domains of galectins 1 and 3.

MATERIALS AND METHODS

Isolation, cloning, and sequencing of human CLC genomic fragments. Genomic fragments of CLC were isolated by extension from both the 5′ and the 3′ directions using a unidirectional PCR method (Promoter Finder kit; Clontech, Palo Alto, CA). The following gene-specific primers were used to isolate each of the genomic fragments in conjunction with linker primers provided with the kit: E451 was generated using primers corresponding to the 5′ end of the CLC promoter (L01665, GSP 1, ATGCTCTCCCAGAACCACTGGAGAA; and GSP 2, TTGCCCCCAGGATCTCCAGCTTTTT); E461 was generated using primers designed to the 3′ end of the E451 fragment and shown in italics in Fig. 2B (E451); E465 was generated using primers corresponding to the 3′ end of the E461 fragment and shown in italics in Fig. 2B (E461); E462 was generated using primers corresponding to the 5′ end of fragment E458; and E458 was generated using primers designed to the 3′ CLC cDNA (L01664: GSP 3, ATGAGCAGGAGTAAGGATTGAAGTGAG; and GSP 4, TCATGCTGTTAGCTGGCTTTGGAATCC). The PCR products were gel purified (BIO 101, Vista, CA) and subcloned into the pCR 2.1 vector (Invitrogen, San Diego, CA) for dideoxy sequencing (USB, Cleveland, OH). Sequences were submitted to GenBank, and the accession numbers are listed in Fig. 2B.

Isolation, cloning, and sequencing of orangutan and mouse CLC orthologs. Primers corresponding to nucleotides 129–158 and 304–333 of the human CLC cDNA (Ackerman *et al.*, 1993b) were used to PCR-amplify the corresponding region from orangutan (*Pongo pygmaeus*) and mouse (*Mus musculus*) genomic DNA (Bios, New Haven, CT) as previously described (Rosenberg *et al.*, 1995). The 204-bp products were subcloned into the pCR 2.1 vector for dideoxy sequencing. Sequence analysis and amino acid translation were performed with the assistance of algorithms within the Wisconsin Genetics Computer Group Program online at the National Institutes of Health. The GenBank accession numbers for the orangutan and mouse CLC β-galactoside binding site orthologs are U67984 and U67985, respectively.

Genomic Southern blots. Human genomic DNA from leukocytes was prepared as described previously, and Southern blots were generated (Rosenberg and Dyer, 1995). Membranes were hybridized overnight at 37°C in a formamide-based buffer (Rosenberg and Dyer, 1995) with either 2×10^6 cpm/ml ^{32}P-radiolabeled CLC ORF or CLC promoter ((see Fig. 1A) Random prime labeling kit; Boehringer Mannheim) and washed at 65°C in 5× SSPE containing 0.1% SDS for 1 h; autoradiographs were developed after exposure at −80°C.

Calculation and analysis of evolutionary rates. Calculations shown in Figs. 3B and 3C were derived from sequences reported to GenBank (Galectin-1: X14829, bp 104–268 and X51903, bp 150–314; Galectin-3: M64303, bp 457–615 and X16834, bp 524–682). Nonsynonymous substitutions per site (K_a = Nd/N) and synonymous substitutions per site (K_s = Sd/S) were calculated from the β-galactoside binding site analogous to the portion of CLC examined (Dyer and Rosenberg, 1996; Oda *et al.*, 1991, 1993; Gitt *et al.*, 1992). The value T was determined from consensus values for species divergence reported by Sibley and Ahlquist (1984); $2T$ represents the total years of divergent evolution, which includes 26 million years (my) for human/orangutan and 160 my for human/mouse pairs.

RESULTS

Figure 1A shows a schematic of the human CLC cDNA (Ackerman *et al.*, 1993b) and promoter (Gomolin *et al.*, 1993). The regions used as probes for Southern blot analysis are as indicated (CLC open reading frame (ORF), bp 34–462; CLC promoter, bp 1–510).

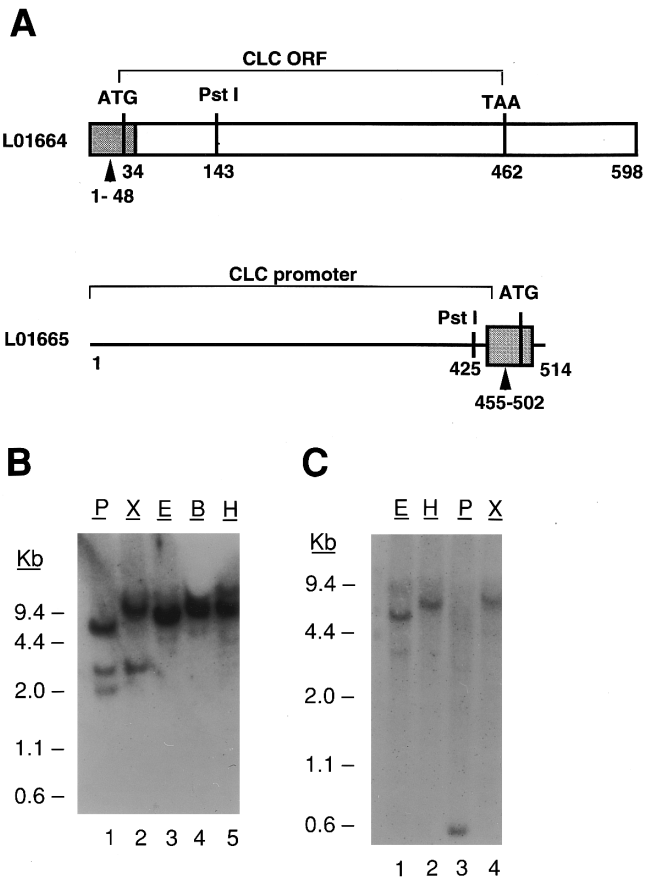
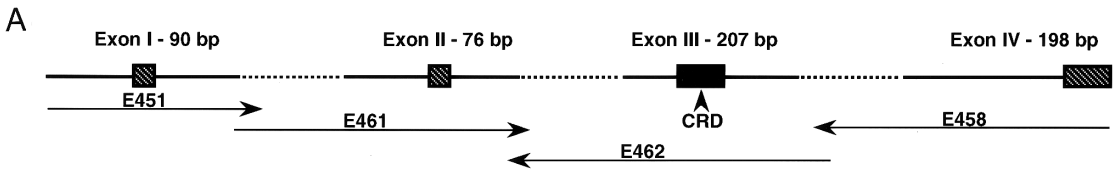


FIG. 1. (**A**) Schematic of the cDNA and promoter regions of the human Charcot–Leyden crystal protein (CLC) gene (GenBank Accession nos. L01664 and L01665, respectively). The CLC open reading frame (ORF) and promoter probes are as indicated, and the shaded portions indicate sequence found in both the promoter and the cDNA clone. (**B**) Human genomic DNA digested with the following restriction enzymes and probed with the CLC ORF shown in (**A**): *Pst*I (**P,** lane **1**), *Xba*I (**X,** lane **2**), *Eco*RI (**E,** lane **3**), *Bam*HI (**B,** lane **4**), and *Hin*dIII (**H,** lane **5**). (**C**) Human genomic DNA digested with *Eco*RI (**E,** lane **1**), *Hin*dIII (**H,** lane **2**), *Pst*I (**P,** lane **3**), and *Xba*I (**X,** lane **4**) probed with the CLC promoter shown in (**A**).

Southern analysis of human genomic DNA probed with the CLC ORF is shown in Fig. 1B. The CLC ORF probe detected three fragments in *Pst*I-digested DNA (lane 1), two fragments in the *Xba*I and *Hin*dIII-digested DNA (lanes 2 and 5, respectively), and one fragment in both the *Eco*RI and the *Bam*HI digests (lanes 3 and 4, respectively). Ackerman and colleagues (1993) detected single hybridizing fragments in *Pst*I, *Xba*I, *Eco*RI, and *Bam*HI-digested DNA using a CLC cDNA probe; the discrepancy between these results is most likely due to differences in hybridization and washing conditions. Southern analysis of human genomic DNA probed with the CLC promoter sequence demonstrated a single hybridizing fragment in the *Pst*I-digested DNA (lane 3), two fragments in the *Eco*RI-digested DNA (lane 1), and a single fragment in both the *Hin*dIII



B

E451 - Promoter/Exon I/5′Intron 1 (Genbank acc. U68393)

*ttgcccccag gatctccagc ttttt*accat tgcacaggtt aggagaacgc acaccttcct ggtagattct gcacagtgga gaaaaggctt
catgagaagc atggaggctg gaactcacac cactcatcct cactccatcg tctttatctg tgcatttccc tggtgacccc ttcctcctcc
agtacctccc tcactgtcgt ttctgctgca tcacaggcac aggcctccaa acaggtgtgg ttgtgactga gtctgtttct gacaccccac
ccctttctcac tgtgtgagga aaccttcctg ggaggggctt ggaatcagga ataaagccag aggcagggct gataggggcc ATTTAAATTC
TGCAGCTCAG AGATTCACAC AGAAGTCTGG ACACAATTCA GAAGAGCCAC CCAGAAGGAG ACAACAATGT CCCTGCTACC Cgtgagttaa
aaaggcacag cttcaataat ctcaggtcac acagaaaagg gaagagggag attttatggg atgaaaatat gaaaatttct actgtgaatg
ctttactgag a.....

E461 - 3′INTRON 1/EXON II/5′INTRON 2 (Genbank acc. U68395)

..... cctgccctgc ctctctcacc cactctcaat ccctgcagG TGCCATACAC *AGAGGCTGCC TCTTTGTCTA CTGGTTCTAC*
*TGTGACAATC AAAGGGCGAC CACTTGTCTG TTTCTT*gtga gtactccatg gtgcaaagat gggaggtgga ggagaggaag aatattttct
aaggggtga cctcatgtgt gaatcatgtg gaaagtctag ttggcaggat ggaagtgcca tgaatgtgca ggtcctggag a.....

E462 - 3′INTRON 2/EXON III/5′INTRON 3 (Genbank acc. U68396)

..... caaggcctag aataccaaca agtcttctct gaccacggaa ggggcaaaga gacttagtgt gatgtgtctt gtgtgtgccc
cacaagaatg gacctgctca acactgtgtg ctctgccctc agGAATGAAC CATATCTGCA GGTGGATTTC CACACTGAGA TGAAGGAGGA
ATCAGACATT GTCTTCCATT TCCAAGTGTG CTTTGGTCGT CGTGTGGTCA TGAACAGCCG TGAGTATGGG GCCTGGAAGC AGCAGGTGGA
ATCCAAGAAC ATGCCCTTTC AGGATGGCCA AGAATTTGAA CTGAGCATCT CAGTGCTGCC AGATAAGTAC CAGgtgagca ccccaggagc
tccctgcacc cagcttccgt gggatctcag agcaggtggc agctcttcat gccctaactc cagataactc ccactgtccc ggcttttcat
cacccacaac tccccttatc ccaggctttt catc.....

E458 - 3′INTRON 3/EXON IV (Genbank acc. U68398)

..... gggttcagat atatatgagg cattttctgg ttggatgttt caggtactag tacaccatag agagcaggct gaaagcttgt
ttggtgaaat actgtctttc tgatgtattt ttttccttct gtagGTAATG GTCAATGGCC AATCCTCTTA CACCTTTGAC CATAGAATCA
AGCCTGAGGC TGTGAAGATG GTGCAAGTGT GGAGAGATAT CTCCCTGACC AAATTTAATG TCAGCTATTT AAAGAGATAA CCAGACTTCA
TGTTGCCAAG GAATCCCTGT CTCTACGTGA ACTT*GGGATT CCAAAGCCAG CTAACAGCAT GA*

FIG. 2. (**A**) The gene structure determined for the human CLC gene: boxes indicate exons I–IV; solid lines indicate sequenced intronic regions; dots represent intronic regions for which the sequence was not determined. The arrows beneath the diagram indicate the isolated PCR fragments assembled to determine the gene structure. (**B**) Intron–exon boundaries of the human CLC gene. The exons are indicated by uppercase letters, with the translated residues underlined; intronic sequences are indicated by lowercase letters, with the splice sites indicated by open boxes and the consensus branch sites highlighted by shaded boxes. The dots represent unknown intronic sequence, and the sequence corresponding to the PCR extension primers are in italics. The GenBank accession numbers are provided for each gene fragment and additional intronic sequence has been submitted and assigned the following accession numbers: intron 1, U68394; intron 3, U68397.

and the *Xba*I digests (lanes 2 and 4, respectively). The results of the Southern analysis with the CLC ORF probe suggest either multiple gene copies or the existence of multiple exons constituting the CLC gene; the results using the CLC promoter probe are consistent with the latter interpretation.

We determined the intron–exon boundaries within the gene encoding human CLC using a PCR-based gene isolation technique that permitted simultaneous extension in both the 5′ and the 3′ directions using nested primers hybridizing to the known CLC cDNA (see Materials and Methods). Figure 2A is the map of the CLC gene, indicating the division of the open reading frame by three introns; the PCR fragments isolated that encode each portion of this gene are also shown. Figure 2B shows a portion of the sequence of each of these genomic fragments, indicating the presence of a 5′ GT and 3′ AG splice site and a branch site matching the consensus sequence $Py_{80}NPy_{80}Py_{87}Pu_{75}APy_{95}$ (Lewin, 1994) for each intron/exon boundary. Exon III of the CLC gene encodes the entire β-galactoside binding site, a region shown to be both structurally (Leonidas *et al.*, 1995) and functionally (Dyer and Rosenberg, 1996; Leonidas *et al.*, 1995) homologous to the analogous domains of members of the galectin family of carbohydrate binding proteins.

Southern blot analysis of other mammalian DNAs probed with the CLC ORF suggests the existence of sequence orthologs of CLC in several additional species (data not shown). We PCR-amplified regions corresponding to the human CLC exon III from both orangutan (*P. pygmaeus*, U67984) and mouse (*M. musculus*, U67985) genomic DNAs with primers corresponding to bp 129–158 and 304–333 of the human cDNA sequence (Ackerman *et al.*, 1993). Figure 3A shows the predicted amino acid sequences of the CLC β-galactoside binding site orthologs without the primer-encoded residues. The CLC β-galactoside binding site orthologs have 17 residues (identical) in common with at least two of the four galectins examined in previous studies (Dyer and Rosenberg, 1996) and have 6 of the 8 residues in the carbohydrate binding domain either identical or conserved (Barondes *et al.*, 1994b; Dyer and Rosenberg, 1996). Analysis of the percentage identities

A

```
              42                                                                                90
Human        - T E M K E E S D I V  F H F Q V C F G R R  V V M N S R E Y G A  W K Q Q V E S K N M  P F Q D G Q E F E -
Orangutan    - T E M K E E S G I A  F H F Q V H F G C Y  V V M N S R E Y G A  W K K P V E S K N M  P F Q D G Q E F D -
Mouse        - T E M K E D S D I A  F H S R V Y F G H W  V V M N S R V N G A  W Q Y E V T C H N M  P F Q D G K P F N -
```

B

	Human	Orangutan	Mouse
Human	100%	84%	63%
Orangutan	91%	100%	63%
Mouse	79%	76%	100%

C

	Ka/2T X 10^9 (substitutions/site/year)	Ks/2T X 10^9 (substitutions/site/year)
CLC	1.12	1.81
Galectin-1	0.44	2.88
Galectin-3	0.32	3.19

FIG. 3. (**A**) The predicted amino acid alignment of the CLC exon III orthologs. Open boxes highlight the residues (identical) shared between the CLC orthologs and at least two of the human galectins examined previously (Dyer and Rosenberg, 1996). Shaded boxes highlight the amino acids (either identical or conserved) that are involved in β-galactoside recognition and binding (Dyer and Rosenberg, 1996; Leonidas *et al.*, 1995). Numbering is with respect to the CLC cDNA; primer encoded residues have been omitted. (**B**) Nucleotide (open boxes) and amino acid (shaded boxes) sequence comparisons between pairs of CLC orthologs. (**C**) Rates of synonymous and nonsynonymous substitution between human and mouse sequences encoding the β-galactoside binding sites of CLC, galectin-1, and galectin-3.

between the CLC β-galactoside binding site orthologous pairs is presented in Fig. 3B. The human CLC β-galactoside binding site is 91/84% (nucleotide/amino acid) identical to the orangutan ortholog and 79/63% identical to that of the mouse. The rates of nonsynonymous and synonymous substitution for the CLC β-galactoside binding site and for the analogous regions of galectin 1 and 3 human/mouse pairs were calculated as described under Materials and Methods (Fig. 3C). The rate of nonsynonymous substitution calculated for the CLC β-galactoside binding site (1.12×10^{-9} substitutions/site/year (s/s/year)) was higher than that calculated for galectin 1 (0.44×10^{-9} s/s/year) or galectin 3 (0.32×10^{-9} s/s/year). The nonsynonymous and synonymous rates for the human/orangutan pair were calculated to be 3.15 and 6.08×10^{-9} s/s/year, respectively.

DISCUSSION

In this work, we have demonstrated that CLC is genetically related to the members of the galectin gene family. The CLC protein is encoded on four exons with the third exon encoding the β-galactoside binding site. This arrangement is identical to that found in galectins 1 and 2; the β-galactoside binding site of galectin 3 is also encoded on a single exon (exon V) (Barondes *et al.*, 1994a; Gitt and Barondes, 1991; Oda *et al.*, 1991, 1993; Gitt *et al.*, 1992). It is likely that this exon has undergone one or more duplication and insertion events, resulting in its distribution to at least two distinct genes.

As both a lysophospholipase and a lectin, CLC has an unusual combination of activities. Interestingly, CLC is structurally unrelated to the other known mammalian lysophospholipases (Leonidas *et al.*, 1995; Ackerman *et al.*, 1994; Han *et al.*, 1987; Garsetti *et al.*, 1992, 1993). The amino acids within CLC necessary for the lysophospholipase activity have not been fully identified but the cysteines at positions 29 and 57 appear to be involved (Weller *et al.*, 1984; Leonidas *et al.*, 1995). In light of the X-ray crystallography findings (Leonidas *et al.*, 1995), it would be interesting to determine whether galectins 1 and 2 might also have lysophospholipase activity. It remains to be determined whether the lysophospholipase and/or the lectin activity of CLC is physiological. The structural and functional relationship between CLC and the galectins suggests a role for CLC in cell–cell and cell–matrix interactions mediated via the β-galactoside binding properties of CLC.

ACKNOWLEDGMENTS

We thank Dr. Arne Egesten and Dr. Harry Malech for helpful discussions and Dr. John Gallin for his continuing support of our work.

REFERENCES

Ackerman, S. J. (1993). *In* "Eosinophils: Biological and Clinical Aspects" (S. Makino and T. Fukuda, Eds.), pp. 33–74, CRC, Ann Arbor, MI.

Ackerman, S. J., Corrette, S. E., Rosenberg, H. F., Bennett, J. C., Mastrianni, D. M., Nicholson-Weller, A., Weller, P. F., Chin, D. T., and Tenen, D. G. (1993). Molecular cloning and characterization of human eosinophil Charcot–Leyden crystal protein (lysophospholipase). Similarities to IgE binding proteins and the S-type animal lectin superfamily. *J. Immunol.* **150:** 456–468.

Ackerman, S. J., Zhou, Z.-Q., Tenen, D. G., Clark, M. A., Tu, Y.-P., and Irvin, C. G. (1994). *In* "Eosinophils in Allergy and Inflammation," pp. 21–54, Dekker, New York.

Barondes, S. H., Castronovo, V., Cooper, D. N., Cummings, R. D., Drickamer, K., Feizi, T., Gitt, M. A., Hirabayashi, J., Hughes, C., Kasai, K., Leffler, H., Liu, F., Lotan, R., Mercurio, A. M., Monsigny, M., Pillai, S. F., Poirer, F., Raz, A., Rigby, P. W., Rini, J. M., and Wang, J. L. (1994a). Galectins: A family of animal B-galactoside-binding lectins. *Cell* **76:** 597.

Barondes, S. H., Cooper, D. N. W., Gitt, M. A., and Leffler, H. J. (1994b). Galectins: Structure and function of a large family of animal lectins. *J. Biol. Chem.* **269:** 20807–20810.

Cherayil, B. J., Weiner, S. J., and Pillai, S. (1989). The Mac-2 antigen

is a galactose- specific lectin that binds IgE. *J. Exp. Med.* **170:** 1959–1972.

Cherayil, B. J., Chaitovitz, S., Wong, C., and Pillai, S. (1990). Molecular cloning of a human macrophage lectin specific for galactose. *Proc. Natl. Acad. Sci. USA* **87:** 7324–7328.

Cooper, D. N. W., and Barondes, S. H. (1990). Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. *J. Cell Biol.* **110**(5): 1681–1691.

Cooper, D. N. W., Massa, S. M., and Barondes, S. H. (1991). Endogenous muscle lectin inhibits myoblast adhesion to laminin. *J. Cell Biol.* **115**(5): 1437–1448.

Do, K. Y., Smith, D. F., and Cummings, R. D. (1990). LAMP-1 in CHO cells is a primary carrier of poly-*N*-acetyllactosamine chains and is bound preferentially by a mammalian S-type lectin. *Biochem. Biophys. Res. Commun.* **173:** 1123–1128.

Dyer, K. D., and Rosenberg, H. F. (1996). Eosinophil Charcot–Leyden crystal protein binds to beta-galactoside sugars. *Life Sci.* **58:** 2073–2082.

Frigeri, L. G., Zuberi, R. I., and Liu, F. T. (1993). Epsilon BP, a beta-galactoside-binding animal lectin, recognizes IgE receptor (Fc epsilon RI) and activates mast cells. *Biochemistry* **32:** 7644.

Garsetti, D. E., Ozgur, L. E., Steiner, M. R., Egan, R. W., and Clark, M. A. (1992). Isolation and characterization of three lysophospholipases from the murine macrophage cell line WEHI 265.1. *Biochim. Biophys. Acta* **1165:** 229–232.

Garsetti, D. E., Steiner, M. R., Holtsberg, F., Ozgur, L. E., Egan, R. W., and Clark, M. A. (1993). Comparison of six mammalian lysophospholipases. *J. Lipid Mediat.* **6:** 223–232.

Gitt, M. A., and Barondes, S. H. (1991). Genomic sequence and organization of two members of a human lectin gene family. *Biochemistry* **30:** 82–89.

Gitt, M. A., Massa, S., Leffler, H., and Barondes, S. H. (1992). Isolation and expression of a gene encoding L-14-II, a new human soluble lactose-binding lectin. *J. Biol. Chem.* **267:** 10601–10608.

Gomolin, H. I., Yamaguchi, Y., Paulpillai, A. V., Dvorak, L. A., Ackerman, S. J., and Tenen, D. G. (1993). Human eosinophil Charcot–Leyden crystal protein: Cloning and characterization of a lysophospholipase gene promoter. *Blood* **82:** 1868–1874.

Gu, M. J., Wang, W. W., Song, W. K., Cooper, D. N. W., and Kaufman, S. J. (1994). Selective modulation of the interaction of alpha 7 beta 1 integrin with fibronectin and laminin by L-14 lectin during skeletal muscle differentiation. *J. Cell Sci.* **107:** 175–181.

Han, J. H., Stratowa, C., and Rutter, W. J. (1987). Isolation of full-length putative rat lysophospholipase cDNA using improved methods for mRNA isolation and cDNA cloning. *Biochemistry* **26:** 1617–1625.

Hynes, M. A., Gitt, M. A., Barondes, S. H., Jessell, T. M., and Buck, I. B. (1990). Selective expression of an endogenous lactose-binding lectin gene in subsets of central and peripheral neurons. *J. Neurosci.* **10:** 1004–1013.

Leonidas, D. D., Elbert, B. L., Zhou, Z., Leffler, H., Ackerman, S. J., and Acharya, K. R. (1995). Crystal structure of human Charcot–Leyden crystal protein, an eosinophil lysophospolipase, identifies it as a new member of the carbohydrate-binding family of galectins. *Structure* **3:** 1379–1393.

Lewin, B. (1994). *In* "Genes V," pp. 916–918, Oxford Univ. Press, Oxford, England.

Lindstedt, R., Apodaca, G., Barondes, S. H., Mostov, K., and Leffler, H. (1993). Apical secretion of a cytosolic protein by Madin–Darby canine kidney cells. Evidence for polarized release of an endogenous lectin by a nonclassical secretory pathway. *J. Biol. Chem.* **268:** 11750–11757.

Oda, Y., Leffler, H., Sakakura, Y., Kasai, K.-I., and Barondes, S. H. (1991). Human breast carcinoma cDNA encoding a galactoside-binding lectin homologous to mouse Mac-2 antigen. *Gene (Amsterdam)* **99:** 279–283.

Oda, Y., Herrmann, J., Gitt, M. A., Turck, C. W., Burlingame, A. L., Barondes, S. H., and Leffler, H. (1993). Soluble lactose-binding lectin from rat intestine with two different carbohydrate-binding domains in the same peptide chain. *J. Biol. Chem.* **268:** 5929–5939.

Regan, L. J., Dodds, J., Barondes, S. H., and Jessell, T. M. (1986). Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. *Proc. Natl. Acad. Sci. USA* **83:** 2248–2252.

Rosenberg, H. F., Dyer, K. D., Tiffany, H. L., and Gonzalez, M. (1995). Rapid evolution of a unique family of primate ribonuclease genes. *Nature Genet.* **10:** 219–223.

Rosenberg, H. F., and Dyer, K. D. (1995). Human ribonuclease 4 (RNase 4): Coding sequence, chromosomal localization and identification of two distinct transcripts in human somatic tissues. *Nucleic Acids Res.* **23:** 4290–4295.

Sato, S., and Hughes, R. C. (1992). Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. *J. Biol. Chem.* **269:** 4424–4430.

Sato, S., and Hughes, R. C. (1994). Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. *J. Biol. Chem.* **269:** 4424–4430.

Sibley, C. G., and Ahlquist, J. E. (1984). The phylogeny of the hominoid primates, as indicated by DNA–DNA hybridization. *J. Mol. Evol.* **20:** 2–15.

Skrincosky, D. M., Allen, H. J., and Bernacki, R. J. (1993). Galaptin-mediated adhesion of human ovarian carcinoma A121 cells and detection of cellular galaptin-binding glycoproteins. *Cancer Res.* **53:** 2667–2675.

Weller, P. F., Bach, D. S., and Austens, K. F. (1984). Biochemical characterization of human eosinophil Charcot–Leyden crystal protein (lysophospholipase). *J. Biol. Chem.* **259:** 15100–15105.

Woo, H. J., Shaw, I. M., Messier, K. M., and Mercurio, A. M. (1990). The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). *J. Biol. Chem.* **265:** 7097–7099.

Yamaoka, A., Kuwabara, I., Frigeri, I. G., and Liu, F. T. (1995). A human lectin, galectin-3 (epsilon bp/Mac-2), stimulates superoxide production by neutrophils. *J. Immunol.* **154:** 3479–3487.