Genomic Organization of the Selectin Family
of Leukocyte Adhesion Molecules on Human
and Mouse Chromosome 1

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Summary

A structurally and functionally related group of genes, lymph node homing receptor (LHR),
granule membrane protein 140 (GMP-140), and endothelial leukocyte adhesion molecule 1 (ELAM-1)
are shown to constitute a gene cluster on mouse and human chromosome 1. In situ hybridization
mapped GMP-140 to human chromosome 1 bands 21–24 consistent with chromosomal localization
of LHR. Gene linkage analysis in the mouse indicated that these genes and serum coagulation
factor V (FV) all map to a region of distal mouse chromosome 1 that is syntenic with human
chromosome 1, with no crossovers identified between these four genes in 428 meiotic events.
Moreover, long range restriction site mapping demonstrated that these genes map to within
300 kb in both the human and mouse genomes. These data suggest that LHR, ELAM-1, and
GMP-140 comprise an adhesion protein family, the selectins, that arose by multiple gene duplication
events before divergence of mouse and human. Furthermore, the location of these genes on mouse
and human chromosome 1 is consistent with a close evolutionary relationship to the complement
receptor-related genes, which also are positioned on the same chromosomes in both species and
with which these genes share a region of sequence homology. These data characterize the
organization of a genomic region that may be critical for intercellular communication within
the immune system.

The primary structures of three glycoproteins defining a
novel class of adhesion receptors, termed selectins, have
recently been determined (1–5). The first is the Mel-14 an-
tigen, a murine lymph node homing receptor (LHR).1, 2 also

1 Abbreviations used in this paper: ELAM-1, endothelial leukocyte adhe-
sion molecule; FV, coagulation factor V; GMP-140, granule membrane
protein 140; LHR, lymph node homing receptor.
2 The Human Gene Mapping Nomenclature Committee has assigned
the following designations for the genes utilized in the current
report: LHR, LYAM; ELAM-1, ELAM; GMP-140, GRMP; FV, F5.
The Mouse Nomenclature Committee has assigned the following des-
ignations for the same genes indicated above: LHR, Lnhr; ELAM-1,
Elam; GMP-140, Grmp; coagulation factor V, F5.

found on neutrophils and monocytes (3, 4). The gene for
the human counterpart, encoding the Leu-8/TQ1 antigen
or LAM-1, has also been cloned (6–9). In addition to lym-
phocyte homing, this molecule may play a role in neutrophil
adhesion to endothelium at sites of inflammation (10). The
second selectin is endothelial leukocyte adhesion molecule 1
(ELAM-1), a cytokine-inducible receptor for neutrophils on
human endothelium (1, 11). The third is granule membrane
protein 140 (GMP-140) (3), a membrane glycoprotein found
in secretory granules of human platelets (12–15) and end-
thelium (16–18). When these cells are activated by agonists
such as thrombin, GMP-140, also known as PADGEM pro-
tein is rapidly redistributed to the plasma membrane where
it mediates adhesion of neutrophils and monocytes (19–21).
In addition to their functional relationship, the selectins share extensive amino acid similarity and overall domain organization. Each molecule contains an NH₂-terminal domain homologous to Ca²⁺-dependent lectins (22), followed by an epidermal growth factor-like domain, a variable number of tandem consensus repeats related to those in complement-binding proteins (23–25), a transmembrane segment, and a short cytoplasmic tail.

In the current study we examine the genomic organization of LHR, ELAM-1, and GMP-140. Using a combination of cytogenetic, interspecific backcross mouse linkage data, and long range restriction site mapping, we demonstrate that LHR, ELAM-1, and GMP-140 are physically linked both on human chromosome 1 and on distal mouse chromosome 1. These studies define an adhesion receptor gene complex and raise the possibility that these genes may have common modes of regulation similar to other clusters of related genes such as the major histocompatibility locus.

Materials and Methods

Somatic Cell Hybrids. Somatic cell hybrids were generated by PEG 1000-mediated cell fusion of human VA2, A549, or IMR90 fibroblast or peripheral human lymphocyte cells to either Chinese E36 or Syrian BHK-B1 hamster cells as previously described (26). A panel for mapping purposes was selected from a series of hybrids that contained the entire rodent genome but that have selectively lost different combinations of human chromosomes. A [32P]GMP-140 probe labeled by the random oligonucleotide priming method to a specific activity of 1–3 x 10⁹ dpm/μg was hybridized to Southern blots of high molecular weight DNA from these hybrid clones that had been digested to completion with EcoRI, electrophoresed, and blotted as previously described (26).

In Situ Chromosomal Hybridization. Human metaphase cells were prepared from PHA-stimulated PBL. Radiolabeled GMP-140 genomic probes (a 3-kb HindIII genomic fragment from the 3' end of the gene and a 2.1-kb HindIII/Sall genomic fragment from the 5' end of the gene) were prepared by nick translation of the entire plasmid with all four [3H]labeled deoxynucleoside triphosphates to a specific activity of 1.0 x 10⁶ dpm/μg. In situ hybridization was performed as described previously (27). Metaphase cells were hybridized at 1.0 and 3.0 ng of probe per mL of hybridization mixture. Autoradiographs were exposed for 11 days.

Mice. C3H/HeJ-gld/gld and Mus spretus (Spain) parental mice and [(C3H/HeJ-gld/gld × M. spretus)F₁ × C3H/HeJ-gld/gld] interspecific backcross mice were bred and maintained as previously described (28). Mus spretus was chosen as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants (RFLV) in comparison with crosses using conventional inbred strains.

Southern Hybridization. DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases, and 10 μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schull Inc., Keene, NH), hybridized at 65°C, and washed as previously described (28) except for low stringency conditions utilized for the human cDNA probes. Low stringency conditions were identical to high stringency conditions except that a 0.5 x SSC/0.1% SDS wash followed the initial 2 x SSC/0.1% wash, and the first and second wash temperatures were lowered from 65 to 58°C. Wash time was decreased from 30 to 15 min for the first blot wash, and from 30 to 10 min for the second blot wash.

Mouse Gene Linkage Analyses. Maximum likelihood estimates of recombination probabilities and their standard errors among backcross progeny were calculated according to Green (29). The best gene order was determined according to Bishop (30).

Pulsed Field Electrophoresis. Single donor human PBL and C3H/HeJ-gld/gld mouse peripheral lymph node cells were prepared and suspended in agarose blocks as previously described (31). Digests of nuclei suspended in agarose blocks were carried out using 0.5–20 U restriction endonuclease/μg DNA (Boehringer-Mannheim Biochemicals, Indianapolis, IN) in 1 x appropriate restriction buffer for 4 h at the manufacturer’s recommended temperature. Reactions were terminated by the addition of 0.5 M EDTA.

Pulsed field gel electrophoresis (PFGE) was performed as previously described (31). Large DNA fragments of size 1,000–6,000 kb were separated using pulse times of 15–90 min, linearly ramped, using an LKB-Pharmacia apparatus (Piscataway, NJ), and a running time of 144 h at 54 V. DNA fragments of size 200–1,200 kb were separated using pulse times of 70–145 s at 145 V for 46 h. DNA fragments of size 20–800 kb were separated using pulse times of 30–80 s at 170 V for 20 h. High molecular weight size standards included chromosomes of yeast Saccharomyces cerevisiae, strain YNN295 (Bio-Rad Laboratories, Rockville Center, NY), yeast Schizosaccharomyces pombe, strain 972, and concatamers of intact bacteriophage λ DNA (FMC Bioproducts, Rockland, ME). After electrophoresis, gels were stained with ethidium bromide to visualize size standards, which were marked with India ink before alkali transfer onto nylon membranes as previously described (31). Hybridizations of radioactive labeled probe were performed as described elsewhere (28). Hybridization of mouse DNA with human probes was performed under low stringency conditions as indicated above for standard genomic blots.

Molecular Probes. All probes were labeled by the hexanucleotide technique with α-[32P]dCTP as previously described (28). Gene probes used for Southern blot hybridizations were: a 2.7-kb XbaI fragment from human ELAM-1 cDNA (kind gift of Brian Seed and Michael Bevilacqua) (1); a 3.2-kb Sall insert containing a full-length human GMP-140 cDNA (2); a 1.5-kb HindIII/EcoRI fragment from a human factor V (FV) cDNA (32); a 2.3-kb EcoRI fragment from human LHR cDNA clone hLHRc (7); a 1.5-kb Not I fragment from mouse LHR cDNA (3).

Results

Localization of GMP-140 to Human Chromosome 1, Bands q21–24. To obtain a chromosomal assignment a cDNA probe for GMP-140 was hybridized to genomic DNA blots containing samples from a series of somatic cell hybrids. The results showed perfect concordance between the human GMP-140 restriction endonuclease bands and human chromosome 1 (Table 1). All of the other human chromosomes showed a significant discordance (32–65%).

To independently localize GMP-140, genomic probes were hybridized to normal metaphase chromosomes. This resulted in specific labeling only of chromosome 1. Of 100 metaphase cells examined from the hybridization of the 5' genomic probe, 32 (32%) were labeled on the region q2 of one or both chromosome 1 homologues. The distribution of labeled sites on this chromosome is illustrated in Fig. 1; of 194 labeled sites
Table 1. Synteny Test of GMP-140 Gene and Human Chromosomes in Rodent × Human Hybrid Clones

| Human chromosome | GMP-140 gene* / human chromosome† | Percent syteny |
|------------------|-----------------------------------|---------------|
|                  | +/+                               | +/−           |
| 1                | 20                                | 0             | 0             |
| 2                | 8                                 | 10            | 2             | 13            | 36            |
| 3                | 14                                | 6             | 3             | 5             | 32            |
| 4                | 4                                 | 5             | 7             | 5             | 57            |
| 5                | 10                                | 6             | 10            | 6             | 50            |
| 6                | 12                                | 7             | 11            | 4             | 53            |
| 7                | 2                                 | 7             | 1             | 4             | 57            |
| 8                | 6                                 | 8             | 8             | 7             | 52            |
| 9                | 12                                | 6             | 9             | 6             | 45            |
| 10               | 8                                 | 4             | 4             | 7             | 35            |
| 11               | 14                                | 6             | 11            | 3             | 51            |
| 12               | 12                                | 7             | 8             | 8             | 43            |
| 13               | 13                                | 3             | 10            | 3             | 45            |
| 14               | 14                                | 6             | 8             | 7             | 40            |
| 15               | 10                                | 7             | 9             | 5             | 52            |
| 16               | 8                                 | 3             | 8             | 5             | 46            |
| 17               | 17                                | 12            | 1             | 1             | 42            |
| 18               | 4                                 | 10            | 2             | 7             | 52            |
| 19               | 12                                | 8             | 10            | 5             | 51            |
| 20               | 9                                 | 7             | 12            | 6             | 56            |
| 21               | 2                                 | 11            | 0             | 4             | 63            |
| 22               | 0                                 | 6             | 0             | 5             | 55            |
| x                | 9                                 | 11            | 10            | 5             | 60            |

* Numerator corresponds to the presence (+) or absence (−) of GMP-140 coding sequences determined by Southern hybridization.
† Denominator represents the presence (+) or absence (−) of specific human chromosomes in somatic cell hybrid clones as assayed by gene-enzyme systems.

Figure 1. Distribution of labeled sites on human chromosome 1 in 100 metaphase cells that were hybridized with the GMP-140 5' genomic probe. The labeled sites observed in this hybridization were clustered at bands 1q21-24.

observed, 69 (35.6%) were located on this chromosome. These sites were clustered at bands 1q21-24, and this cluster represented 23.7% (46/194) of all labeled sites (cumulative probability for the Poisson distribution is <0.0005). A small, yet significant, cluster of grains was observed at bands 1q31-41 (10/194, p < 0.0005), suggesting that this probe cross-hybridizes to DNA sequences that are located in this region. Hybridizations with both 5' and 3' probes were repeated twice and gave similar results. Since the two GMP-140 genomic probes used for in situ analysis did not contain sequence encoding the complement receptor consensus repeat units, the cross-hybridization at bands 1q31-41 could not be attributed to complement receptor genes that are tightly clustered at 1q32 (23-25).

Localization of GMP-140, LHR, and ELAM-1 to Mouse Chromosome 1. The cytogenetic localization of GMP-140 to human chromosome 1, bands q21-24, suggested that GMP-140 could map near LHR, previously localized to human chromosome 1, bands q23-24 (6). However, cytogenetically localized genes have indeterminate physical relationships, since chromosomal band segments may include up to several million base pairs of DNA. Previous studies from our laboratory have defined a 30 cM span of distal mouse chromosome 1 in which over 20 genes are syntenic with human chromosome 1, bands q21-32 (28, 33, 34). We therefore reasoned that this region of the mouse genome for which we have characterized a large panel of interspecific backcross mice [(C3H/HeJ-gld/gld x M. spretus) F1 x C3H/HeJ-gld/gld] would enable precise linkage studies.

Gene probes were initially hybridized to nylon membranes containing genomic DNAs from M. spretus and C3H/HeJ-gld/gld mice that were digested with various restriction endonucleases in order to detect informative RFLV between the two species. Unique informative RFLV for probes which detect FV, GMP-140, ELAM-1, and LHR are shown in Fig. 2. FV was included in this study since previous studies had indicated that this gene mapped to human chromosome 1 bands q21-25 (32). A panel of genomic DNAs from 428 interspecific backcross mice [(C3H/HeJ-gld/gld x M. spretus) F1 x C3H/HeJ-gld/gld] were digested with the appropriate
Table 2. Gene Mapping Using (C3H/HeJ-gld/gld x M. spretus)F1 x C3H/HeJ-gld/gld Backcross Mice

| Mouse genes | Number of recombination events* |
|-------------|---------------------------------|
|             | None | One |
| At-3        | SC   | CC  | SC  | CC  | x   | CC  | SC  | CC  |
| ELAM-1, LHR, GMP-140, FV | SC   | CC  | CC  | SC  | x   | x   | x   | x   |
| Atp-b       | SC   | CC  | CC  | SC  | SC  | CC  | SC  | CC  |
| T3z         | SC   | CC  | CC  | SC  | SC  | CC  | SC  | CC  |

| Number of mice | 51 | 365# | 2 | 7 | 1 | 0 | 2 | 0 |
|----------------|----|------|---|---|---|---|---|---|
|                | 416| 9    | 1 | 1 | 2 | 0 |

| Linkage interval$^5$ | r   | (cM) | r  | f  |
|----------------------|-----|------|----|----|
| At-3 – Selectins, FV | 9/428 | (2.10) | 0.96 | 3.99 |
| GMP-140, LHR, ELAM-1, FV | 0/428 | (0.00) | 0.00 | 0.86 |
| Selectins, FV – Atp-b | 1/428 | (0.23) | 0.01 | 1.28 |
| Atp-b – T3z         | 2/428 | (0.47) | 0.06 | 1.66 |

* Columns indicate the haplotype of individual backcross mice as determined by loci typed by RFLV illustrated in Fig. 2 and those previously reported (28, 34, 35). CC, C3H/HeJ-gld/gld genotype; SC, F1 genotype; x, crossover.
# The larger number of chromosome markers typing as C3H/HeJ-gld/gld reflects selection of many of the backcross mice (~75%) for the gld/gld phenotype, consistent with the previous study localizing gld to this segment of mouse chromosome 1 (28).
$^5$ r, recombination frequency; r and f represent 95% confidence intervals based on binomial distribution.

Note: Multiple crossovers were observed between genes listed above and the gld locus, excluding above loci as candidate genes (Watson, M.L., and M.F. Seldin, unpublished observations).
restriction enzyme, transferred to nylon membranes, and hybridized with probe. Each probe yielded either the homozygous pattern (C3H/HeJ-gld/gld = CC) or the heterozygous pattern ((C3H/HeJ-gld/gld x M. spreus)F1 = SC). The haplotype data for all 428 interspecific backcross mice at these loci and loci defined by previous studies (28, 34, 35) are shown (Table 2). The best gene order (30) ± standard deviation (29) was: Centromere A3- 2.10 ± 0.69 cM-(ELAM-1, LHR, GMP-140, FV)-0.23 ± 0.23 cM-Atpb-0.47 ± 0.33 cM-T3z-telomere. There were no crossovers between ELAM-1, LHR, GMP-140, and FV in 428 meiotic events (95% upper confidence limit r = 0.86).

Long Range Restriction Mapping of the Selectins on Human Chromosome 1. Since the genetic data in the mouse indicated that FV, GMP-140, LHR, and ELAM-1 loci were tightly linked with each other on distal mouse chromosome 1, long range restriction site mapping studies using PFGE were done to determine the physical relationship of these genes on human chromosome 1. These studies employ restriction endonucleases that recognize genomic sequence containing the relatively rare hypomethylated CpG dinucleotide (36). Unique regions known as CpG islands contain stretches of hypomethylated CpGs, and these sequences may be cleaved by methylation sensitive rare cutting endonucleases, resulting in DNA fragments averaging up to one million base pairs in size (36). Although some rare restriction sites are methylated in a tissue-specific manner, estimates of intergenic distances and the identification of hypomethylated CpG islands can be made using these restriction endonucleases. The results are summarized in Table 3, indicating all restriction fragment sizes detected.

Initial PFGE studies were performed to determine the presence of physical linkage of the selectins and FV using several restriction endonucleases and digestion conditions that produce DNA fragments in the megabase pair size range. Partial digests in which preferential restriction sites are cleaved were

| Enzyme | FV | GMP-140 | LHR | ELAM-1 |
|--------|----|---------|-----|--------|
| NcoI   | 1,800 | 1,800 | 1,800 | 1,800 |
| MluI   | 260  | 260    | 260  | 1,050  |
| Sall   | 440  | 440    | 440  | 440    |
|        | 660  | 660    | 660  | 660    |
|        | 720  | 720    | 720  | 720    |
|        | 770  | 770    | 770  | 770    |
|        | 2,200 | 2,200  | 2,200 | 2,200  |
|        | 2,800 | 2,800  | 2,800 | 2,800  |
| Sall/MluI | 260  | 260    | 260  | 510    |
|        |      |        |      | 510    |
|        |      |        |      | 460    |
| Clal   | 245  | 300    | 300  | 300    |
|        | 380  | 380    | 380  | 380    |
|        | 610  | 490    | 490  | 490    |
|        | 670  | 610    | 610  | 610    |
|        |      | 670    | 670  | 670    |
| Clal/MluI | 260  | 260    | 260  | 410    |
|        |      |        |      | 410    |
|        |      |        |      | 350    |
| Nael   | 390  | 390    | 390  | 390    |
|        | 470  | 470    | 470  | 470    |

| Enzyme | FV | GMP-140 | LHR | ELAM-1 |
|--------|----|---------|-----|--------|
| NcoI   | 510 | 510     | 510 | 510    |
| MluI   | 590 | 590     | 590 | 590    |
| Sall   | 690 | 690     | 690 | 690    |
|        | 770 | 770     | 770 | 770    |
| Nael/MluI | 260 | 260    | 170 | 170    |
|        | 360 | 360     | 260 | 260    |
|        | 430 | 430     | 360 | 360    |
|        |     |         | 430 | 430    |
| NruI   | 380 | 380     | 380 | 380    |
| NruI/MluI | 260 | 260    | 120 | 120    |
|        | 380 | 380     | 260 | 380    |
|        | 380 | 380     | 380 | 380    |
|        | 380 | 420     | 420 | 420    |
| Smal   | 200 | 40      | 220 | 220    |
|        | 360 | 200     | 260 | 260    |
|        |     | 260     | 260 | 360    |
| Smal/MluI | 170 | 40      | 170 | 170    |
|        | 330 | 170     | 260 | 260    |
|        |     | 260     | 330 | 330    |
|        |     | 330     | 330 | 330    |

* Underscore indicates autoradiograph prominent fragments.
achieved by using a limiting amount of restriction endonuclease. FV, GMP-140, LHR, and ELAM-1 probes all recognized a predominant 1,800-kb NotI fragment, common 2,200-kb and 2,800-kb Sall partial fragments, strongly suggesting physical linkage (data not shown). Further studies demonstrated common Sall fragments 440, 660, 720, and 770 kb in size (Fig. 3 a), and a common Nrul fragment 380 kb in size (Fig. 3, a and b). Together these data demonstrated the physical linkage of these four genes on human chromosome 1q and suggested that the maximum distance encompassing these genes to be 380 kb.

To further define the genomic organization among these four genes, multiple experiments involving additional restriction endonucleases and double restriction endonuclease digests were performed. FV, GMP-140, and LHR probes hybridized to a 260-kb MluI fragment that was not detected with ELAM-1 (Fig. 3 b). In contrast, ELAM-1 and LHR hybridized to a 1,050-kb MluI fragment that was not detected with FV and GMP-140 (data not shown). Informative double digests including MluI, indicated the presence of an MluI restriction site (often coincident with gene associated “CpG” islands, references 36, 37) located within the LHR genomic sequence, and suggest a gene order of FV/GMP-140-LHR-ELAM-1.

Additional studies allowed determination of gene order and
defined the relative location of different restriction sites in this region. For example, ELAM-1 hybridized to a prominent 510-kb Sall/MluI band and a 460-kb minor band, indicating the presence of a Sall restriction site 510 and 460 kb from LHR in the direction of ELAM-1 (Figs. 3 b and 4). Similar restriction site analyses were performed for the rare-cutting restriction endonucleases ClaI, NaeI, and NruI, resulting in the fragments shown in Table 3 and Fig. 4. The coincidence, within experimental error, of cleavage sites for several rare restriction endonucleases suggests the presence of at least one hypomethylated “CpG island” (36, 37) in the region 260 kb from LHR in the direction of FV/GMP-140 (Fig. 4).

Experiments using restriction endonucleases XhoI and Smal were performed to confirm the details of the physical map. XhoI fragments of 420, 380, and 300 kb were also recognized by all four probes (Table 3 and data not shown). ELAM-1, GMP-140, and LHR, but not FV, additionally hybridized to a common 260-kb Smal fragment, physically separating FV from the other three genes. While the ELAM-1 and LHR probes hybridized to a common 220-kb Smal fragment, GMP-140 and FV recognized a common 200-kb Smal fragment. Only the GMP-140 probe hybridized to a prominent 40-kb Smal fragment. An order of FV-GMP-140-LHR-ELAM-1, consistent with the above data and further substantiates a maximum distance of ~300 kb which physically links all four genes (Fig. 4).

Long Range Restriction Analysis on Mouse Chromosome 1. To further define the conservation of gene organization between the human and mouse, PFGE studies were performed using mouse genomic DNA. These studies were in part limited by utilization of the human probes for FV, ELAM-1, and GMP-140 since the mouse homologues have not yet been cloned. Although human probes FV, GMP-140, and ELAM-1 cross-hybridized with mouse DNA under low stringency, the visualization of bands was considerably reduced. Nevertheless, FV, GMP-140, LHR, and ELAM-1 probes all recognized a common 570-kb NotI fragment and a 340-kb MluI restriction fragment (Table 4). Further analysis of mouse pulsed field blots showed that FV and LHR probes hybridized to common ClaI (310 kb, 470 kb, 560 kb) and Sall fragments (370 kb, 520 kb, 650 kb), and also to common NaeI (350 kb), NruI (290 kb), BssHII (300 kb), and SacII (340 kb) fragments. These results set an upper limit of 290 kb separating FV and LHR, and suggested even closer physical linkage, since no nonlinking fragments were obtained for the two probes. These data are markedly similar to the detailed human physical map of this region presented above.
Discussion

The results indicate that ELAM-1, LHR, and GMP-140 are members of a gene complex on human chromosome 1 and distal mouse chromosome 1. Long range restriction site analysis established that the selectins are included within a genomic segment no larger than 300 kb in human, and 340 kb in mouse. These data, combined with the sequence similarity among these genes (1-9), argue in favor of their generation from a primordial ancestor(s) (38). Since the three genes are similarly clustered within a much larger syntenic segment in both the mouse and human genome, gene duplication probably occurred before the divergence of man and mouse ~80 million years ago. Examination of the organization of the selectin genes in genomes of species more phylogenetically divergent than man and mouse may clarify the evolution of these genes as a family. In addition, it remains to be determined whether further selectin-like glycoproteins are present in this region of the genome.

Human and mouse chromosome 1 include all the characterized genes belonging to the complement receptor (CR)-related gene family including the genes for C4 binding protein, complement factor H, and CR 1 and CR 2 (23-25, 39). These genes share a highly conserved 60 amino acid repeat, similar to the homologous repeat motif present in one of the selectin domains (1-9). The selectin homologous repeats differ from the CR repeats particularly in the 5' half of each repeat, in which there are several amino acid differences (1-9). Notably, the consensus selectin motif contains 6 cysteines instead of the four characteristic of CR genes (1-9). Although other genes, including the IL-2 receptor, contain homologous repeat motifs similar to CR genes, the selectin genes have the primary sequence with the closest similarity to the consensus sequence for the complement receptor motif (5). This sequence similarity and the physical relationship of the selectin gene complex and CR genes on mouse and human chromosome 1, suggest that the selectin genes have evolved by elaborating the basic CR motif through domain fusions with other gene domains to create the present primary structure. Alternatively, the selectins may represent an older locus and the numerous CR proteins are the result of several exonic reduction and duplication processes. Of interest, in situ experiments, GMP-140 genomic probes that did not contain CR-related sequence cross-hybridized to the region of human chromosome 1 that contains the CR genes. This suggests the presence of non-CR encoding sequence related to the selectins in this region of the genome. Together these findings imply a strong evolutionary relationship between the region encoding the selectins (human chromosome 1 bands q21-24) and that encoding the CR genes (human chromosome 1 band q32).

Long range restriction analyses of the selectin region demonstrated conservation of intergenic distances and hypomethylated CpG islands between human and mouse genomes. This type of conservation yields similar restriction fragment sizes for various rare cleaving restriction endonucleases (Tables 3 and 4), and is consistent with observations in another region of human and mouse chromosome 1 (31). The close physical linkage of GMP-140, LHR, and ELAM-1 on conserved chromosomal segments in the mouse and human genomes may reflect a duplication event so recent that stochastic processes have not occurred. Alternatively, these genes may have remained linked due to their need for a common regulatory mechanism. A combination of both mechanisms is possible. The close linkage of FV with the selectin genes in both mouse and human suggests that certain conserved features of genomic organization are independent of gene function. Future experimentation may elucidate the driving forces that shape relationships between genomic organization and gene expression.

We thank Dr. Enriqueta Guinto for her kind gift of the human factor V cDNA probe, Drs. Brian Seed and Michael Bevilacqua for their kind gift of the ELAM-1 probe, and Rafael Espinosa III and Matt Rebentsch for technical assistance.

Dr. M. F. Seldin is a Charles E. Culpeper Foundation Medical Scholar, the recipient of the Arthritis Foundation Regina S. Loeb Investigator award, and a Basil O'Connor Investigator Award from the March of Dimes; Dr. R. P. McEver is a recipient of Research Career Development award HL-01733 from the National Institutes of Health; Dr. M. M. LeBeau is a Scholar of the Leukemia Society of America; Dr. R. Lemons is a PEW scholar in the biomedical sciences. The current work was supported by these awards and by NIH grants HL-34364 and HG-00101.

Table 4. Sizes of Murine Restriction Endonuclease Fragments

| Enzyme | FV | GMP-140 | LHR | ELAM-1 |
|--------|----|---------|-----|--------|
| NotI   | 570| 570     | 570 | 570    |
| MluI   | 340| 340     | 340 | 340    |
| ClaI   | 310| ND      | 310 | ND     |
| Sall   | 370| ND      | 370 | ND     |
| Nael   | 350| ND      | 350 | ND     |
| Nrul   | 290| ND      | 290 | ND     |
| BssHII | 300| ND      | 300 | ND     |
| SacII  | 340| ND      | 340 | ND     |
References

1. Bevilacqua, M.P., S. Stengelin, M.A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule I: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science (Wash. DC). 243:1160.

2. Johnston, G.I., R.G. Cook, and R.P. McEver. 1989. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. Cell. 56:1033.

3. Siegelman, M.H., M. van de Rijn, and I.L. Weissman. 1989. Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. Science (Wash. DC). 243:1165.

4. Lasky, L.A., M.S. Singer, T.A. Yednock, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel, and S.D. Rosen. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. Cell. 56:1045.

5. Bevilacqua, M.P. 1989. Endothelial leukocyte adhesion molecule I: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Circulation 80(Suppl.II):1.

6. Tedder, T.F., C.M. Isaacs, T.J. Ernst, G.D. Demetri, D.A. Adler, and C.M. Distech. 1989. Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM-1. Homology with the mouse lymphocyte homing receptor and other human adhesion proteins. J. Exp. Med. 170:123.

7. Siegelman, M.H., and I.L. Weissman. 1989. Human homologue of mouse lymph node homing receptor: evolutionary conservation at tandem cell interaction domains. Proc. Natl. Acad. Sci. USA. 86:5562.

8. Camerini, D., S.P. James, I. Stamenkovic, and B. Seed. 1989. Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor. Nature (Lond.). 342:78.

9. Bowen, B.R., T. Nguyen, and L.A. Laskey. 1989. Characterization of a human homologue of the murine peripheral lymph node homing receptor. J. Cell Biol. 109:421.

10. Kishimoto, T.K., M.A. Jutila, E.L. Berg, and E.C. Butcher. 1989. Neutrophil Mac-1 and MEL/14 adhesion proteins inversely regulated by chemotactic factors. Science (Wash. DC). 245:1238.

11. Bevilacqua, M.P., J.S. Poher, D.L. Mendrick, R.S. Cotran, and M.A. Gimbrone, Jr. 1987. Identification of an inducible endothelial-leukocyte adhesion molecule, ELAM-1. Proc. Natl. Acad. Sci. USA. 84:9238.

12. McEver, R.P., and M.N. Martin. 1984. A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. J. Biol. Chem. 259:7979.

13. Hsu-Lin, S.-C., C.L. Berman, B.C. Furie, D. August, and B. Furie. 1984. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J. Biol. Chem. 259:9121.

14. Stenberg, P.E., R.P. McEver, M.A. Shuman, Y.V. Jacques, and D.F. Bainton. 1985. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J. Cell Biol. 101:880.

15. Berman, C.L., E.L. Yeo, J.D. Wencel-Drake, B.C. Furie, M.H. Ginsberg, and B. Furie. 1986. A platelet alpha granule membrane protein that is associated with the plasma membrane after activation. J. Clin. Invest. 78:130.

16. McEver, R.P., J.H. Beckstead, K.L. Moore, L. Marshall-Carlson, and D.F. Bainton. 1989. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J. Clin. Invest. 84:92.

17. Hattori, R., K.K. Hamilton, R.D. Fugate, R.P. McEver, and P.J. Sims. 1989. Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. J. Biol. Chem. 264:7768.

18. Bonfanti, R., B.C. Furie, B. Furie, and D.D. Wagner. 1989. PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. Blood. 73:1109.

19. Larsen, E., A. Celi, G.E. Gilbert, B.C. Furie, J. K. Erban, R. Bonfanti, D.D. Wagner, and B. Furie. 1989. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell. 59:305.

20. Hamburger, S.A., and R.P. McEver. 1990. GMP-140 mediates adhesion of stimulated platelets to neutrophils. Blood. In press.

21. Geng, J.-G., M.P. Bevilacqua, K.L. Moore, T.M. Mcintyre, S.M. Prescott, J.M. Kim, G.A. Bliss, G.A. Zimmerman, and R.P. McEver. 1990. GMP-140 mediates rapid neutrophil adhesion to activated endothelium. Nature (in press).

22. Drickamer, K. 1980. Two distinct classes of carbohydrate-recognition domains in animal lectins. J. Biol. Chem. 265:9557.

23. Reid, K.B.M., D.R. Bentley, R.D. Campbell, L.P. Chung, R.B. Sim, T. Kristensen and B.F. Tack. 1986. Complement system proteins which interact with C3b or C4b. Immunol. Today. 7:230.

24. Ahearn, J.M., and D.T. Fearon. 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). In Advances in Immunology. Vol. 46. F.J. Dixon, editor. Academic Press, New York. 183–219.

25. Hourcade, D., V.M. Holes, and J.P. Atkinson. 1989. The regulators of complement activation (RCA) gene cluster. In Advances in Immunology. Vol. 45. F.J. Dixon, editor. Academic Press, New York. 381–416.

26. Le Beau, M.M., M.J. Pettenati, R.S. Lemons, M.O. Diaz, C.A. Westbrook, R.A. Larson, C.J. Sherr, and J.D. Rowley. 1986. Assignment of the GM-CSF, CSF-1, and FMS genes to human chromosome 5 provides evidence for linkage of a family of genes regulating hematopoiesis and for their involvement in the deletion 5q in myeloid disorders. Cold Spr. Harb. Symp. Quant. Biol. 51:899.

27. Le Beau, M.M., C.A. Westbrook, M.O. Diaz, and J.D. Rowley. 1984. Evidence for two distinct c-src loci on human chromosomes 1 and 20. Nature (Lond.). 312:70.

28. Seldin, M.F., H.C. Morse III, J.P. Reeves, C.L. Scribner, R.C. LeBoeuf, and A.D. Steinberg. 1988. Genetic analysis of autoimmune gld mice. I. Identification of a restriction fragment length polymorphism closely linked to the gld mutation within a conserved linkage group. J. Exp. Med. 167:668.
29. Green, E.L. 1981. Linkage, recombination and mapping. In Genetics and Probability in Animal Breeding Experiments. E. Green, editor. MacMillan, New York. 77-113.
30. Bishop, D.T. 1985. The information content of phase-known matings for ordering genetic loci. Gen. Epidemiol. 2:349.
31. Kingsmore, S.F., M.L. Watson, T.A. Howard, and M.F. Seldin. 1989. A 6000 kb segment of chromosome 1 is conserved in human and mouse. EMBO (Eur. Mol. Biol. Organ.) J. 8:4073.
32. Wang, H., D.C. Riddell, E.R. Guinto, R.T.A. MacGillivray, and J.L. Hamerton. 1988. Localization of the gene encoding human factor V to chromosome 1q21-25. Genomics. 2:324.
33. Seldin, M.F., H.C. Morse, R.C. LeBoeuf, and A.F. Steinberg. 1988. Establishment of a molecular genetic map of distal mouse chromosome 1: further definition of a conserved linkage group syntenic with human chromosome 1q. Genomics. 2:48.
34. Moseley, W.S., and M.F. Seldin. 1989. Definition of mouse chromosome 1 and 3 gene linkage groups that are conserved on human chromosome 1: evidence that a conserved linkage group spans the centromere of human chromosome 1. Genomics. 5:899.
35. Baniyash, M., V.W. Hsu, M.F. Seldin, and R.D. Klausner. 1989. The isolation and characterization of the murine T cell antigen receptor zeta chain gene. J. Biol. Chem. 264:13252.
36. Bird, A.P. 1987. CpG islands as gene markers in the vertebrate nucleus. Trends Genet. 3:342.
37. Lindsay, S., and A.P. Bird. 1987. Use of restriction enzymes to detect potential gene sequences in mammalian DNA. Nature (Lond.). 327:336.
38. McLachlan, A.D. 1987. Gene duplication and the origin of repetitive protein structures. Cold Spr. Harb. Symp. Quant. Biol. 52:411.
39. Kingsmore, S.F., D.P. Vik, C.B. Kurtz, P. Leroy, B.F. Tack, J.H. Weis, and M.F. Seldin. 1989. Genetic organization of complement receptor related genes in the mouse. J. Exp. Med. 169:1479.