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

By Mark L. Watson,* Stephen F. Kingsmore,* Geoffrey I. Johnston,‡ Mark H. Siegelman,§ Michelle M. Le Beau,‖ Richard S. Lemons,¶ Nalini S. Bora,** Thad A. Howard,* Irving L. Weissman,§ Rodger P. McEver,‡ and Michael F. Seldin*

*From the Department of Medicine and Department of Microbiology, Duke University, Durham, North Carolina 27710; ‡St. Francis Medical Research Institute and Department of Medicine, University of Oklahoma Health Science Center and Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104; §Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305; ‖Section of Hematology/Oncology, The University of Chicago, Chicago, Illinois 60637; ¶Department of Pediatrics, The University of Utah School of Medicine, Salt Lake City, Utah 84132; **Howard Hughes Medical Institute Laboratories, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110*

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 antigen, a murine lymph node homing receptor (LHR)[1, 2] also 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 lymphocyte 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 endothelium (16–18). When these cells are activated by agonists such as thrombin, GMP-140, also known as PADGEM protein is rapidly redistributed to the plasma membrane where it mediates adhesion of neutrophils and monocytes (19–21).

[1] *Abbreviations used in this paper:* ELAM-1, endothelial leukocyte adhesion 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 designations for the same genes indicated above: LHR, *Lnhr*; ELAM-1, *Elam*; GMP-140, *Grmp*; coagulation factor V, *F-5*.

In addition to their functional relationship, the selectins share extensive amino acid similarity and overall domain organization. Each molecule contains an NH_2-terminal domain homologous to Ca^{2+}-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 studies was selected from a series of hybrids that contained the entire rodent genome but that have selectively lost different combinations of human chromosomes. A [^{32}P]GMP-140 probe labeled by the random oligonucleotide priming method to a specific activity of 1–3 × 10^9 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/SalI genomic fragment from the 5′ end of the gene) were prepared by nick translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0 × 10^8 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*)F_1 × 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× SSC/0.1% SDS wash followed the initial 2× 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× 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-[^{32}P]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 SalI 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 asynteny
	+/+	+/−	−/+	−/−	
1	20	0	0	18	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	1	12	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	65
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.

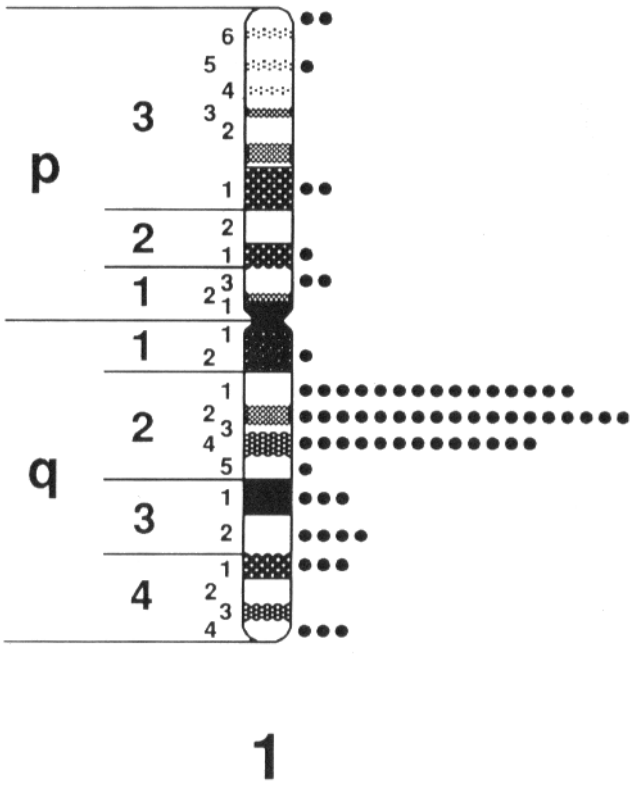

Figure 1. Distribution of labeled sites on human chromosome 1 in 100 metaphase cells that were hybridized with the GMP-140 5′ genomic probe. The labeled sites observed in this hybridization were clustered at bands 1q21–24.

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* × *M. spretus*) F_1 × 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 RLFV 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* × *M. spretus*) F_1 × C3H/HeJ-*gld/gld*] were digested with the appropriate

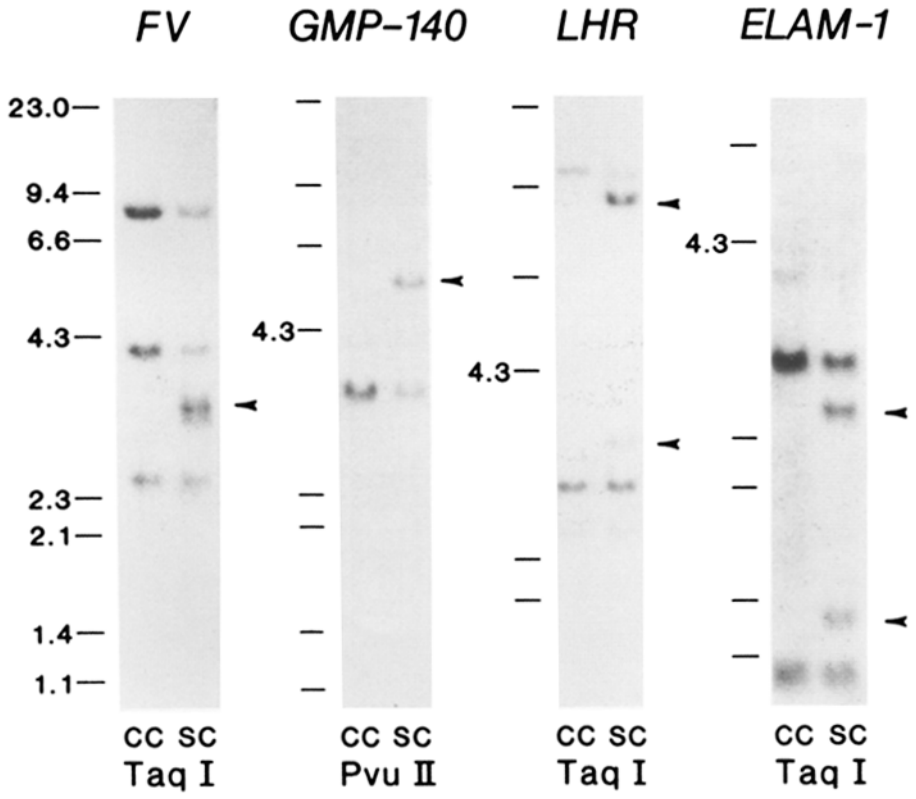

Figure 2. Identification of RFLV unique for *M. spretus* detected with FV, GMP-140, LHR, and ELAM-1 gene probes. Restriction endonucleases are indicated at the bottom, and molecular size standards (in kilobases) are shown at the left of each panel. "CC" represents the parental (C3H-*gld/gld*) pattern and "SC" represents the parental (C3H-*gld/gld* × *M. spretus*)F_1 pattern. The arrows to the right of each panel signify bands present in DNA from interspecific F_1 mice that are not present in DNA from the C3H-*gld/gld* parental mice.

Table 2. *Gene Mapping Using (C3H/HeJ-gld/gld x M. spretus)F_1 x C3H/HeJ-gld/gld Backcross Mice*

	Number of recombination events*							
Mouse genes	None		One					
At-3	SC	CC	SC x CC	CC x SC	CC x SC	SC x CC	CC x SC	SC x CC
ELAM-1, LHR, GMP-140, FV	SC	CC	CC	SC	CC	SC	CC	SC
Atp-b	SC	CC	CC	SC	SC	CC	CC	SC
T3z	SC	CC	CC	SC	SC	CC	SC	CC
Number of mice	51	365‡	2	7	1	0	2	0
	416		9		1		2	
Linkage interval§	r		(cM)		r		r̄	
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, F_1 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).

§ r, recombination frequency; r̲ and r̄ 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).

Table 3. *Sizes of Human Restriction Endonuclease Fragments*

Enzyme	FV	GMP-140	LHR	ELAM-1
		kb		
NotI	1,800	1,800	1,800	1,800
MluI	260	260	260	1,050
			1,050	
SalI	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
SalI/MluI	260	260	260	510
			510	460
			460	
ClaI	245	300	300	300
	380	380	380	380
	610	490	490	490
	670	610	610	610
		670	670	670
ClaI/MluI	260	260	260	410
			410	350
			350	
NaeI	390	390	390	390
	470	470	470	470
	510	510	510	510
	590	590	590	590
	690	690	690	690
	770	770	770	770
NaeI/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	
XhoI	300	300	300	300
	380	380	380	380
	420	420	420	420
SmaI	200	40	220	220
	360	200	260	260
		260	360	360
		360		
SmaI/MluI	170	40	170	170
	330	170	260	260
		260	330	330
		330		

continued

Table 3. *(continued)*

* Underscore indicates autoradiograph prominent fragments.

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* × *M. spretus*) F_1 = 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 *At3*-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

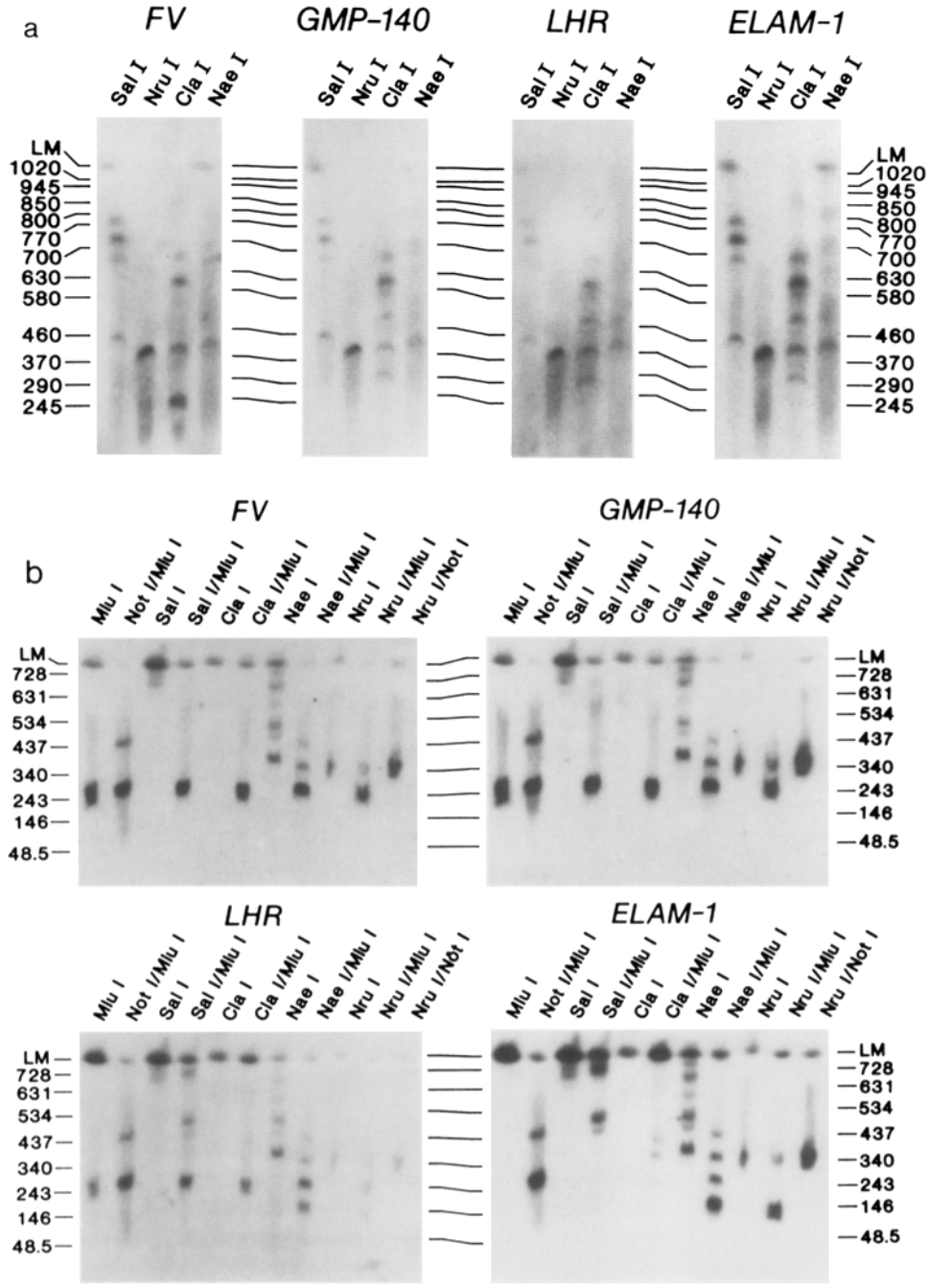

Figure 3. Sequential hybridization with FV, GMP-140, LHR, and ELAM-1 gene probes on PFGE blots separating DNA fragments. Human single donor PBL DNA was separated by PFGE using ramped pulses from 70 to 145 s (*a*) or from 30 to 80 s (*b*). Restriction enzymes are indicated at the top and molecular size standards (in kilobases) are to the right and left of each panel. LM indicates limiting mobility. All gene probes recognize common SalI (440, 660, 720, and 770 kb), NruI (380 kb), ClaI (380, 610, and 670 kb), and NaeI (390, 510, and 680 kb) restriction fragments (*a*). Informative double digests indicate FV and GMP-140 probes recognize an internal fragment of 260 kb not recognized by the ELAM-1 probe for digests using MluI and either SalI, ClaI, NaeI, or NruI (*b*). Instead the ELAM-1 probe hybridizes to 510- and 460-kb SalI/MluI fragments, 410- and 350-kb ClaI/MluI fragments, a 170-kb NaeI/MluI fragment, and a 120-kb NruI/MluI fragment, placing *ELAM-1* at most 120 kb from *LHR* (*b*). All four probes recognize common NaeI (390 kb) and NruI (380 kb) fragments, confirming a maximum distance of 380 kb separating all three selectin family genes and *FV* (*b*).

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 SalI partial fragments, strongly suggesting physical linkage (data not shown). Further studies demonstrated common SalI fragments 440, 660, 720, and 770 kb in size (Fig. 3 *a*), and a common NruI 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

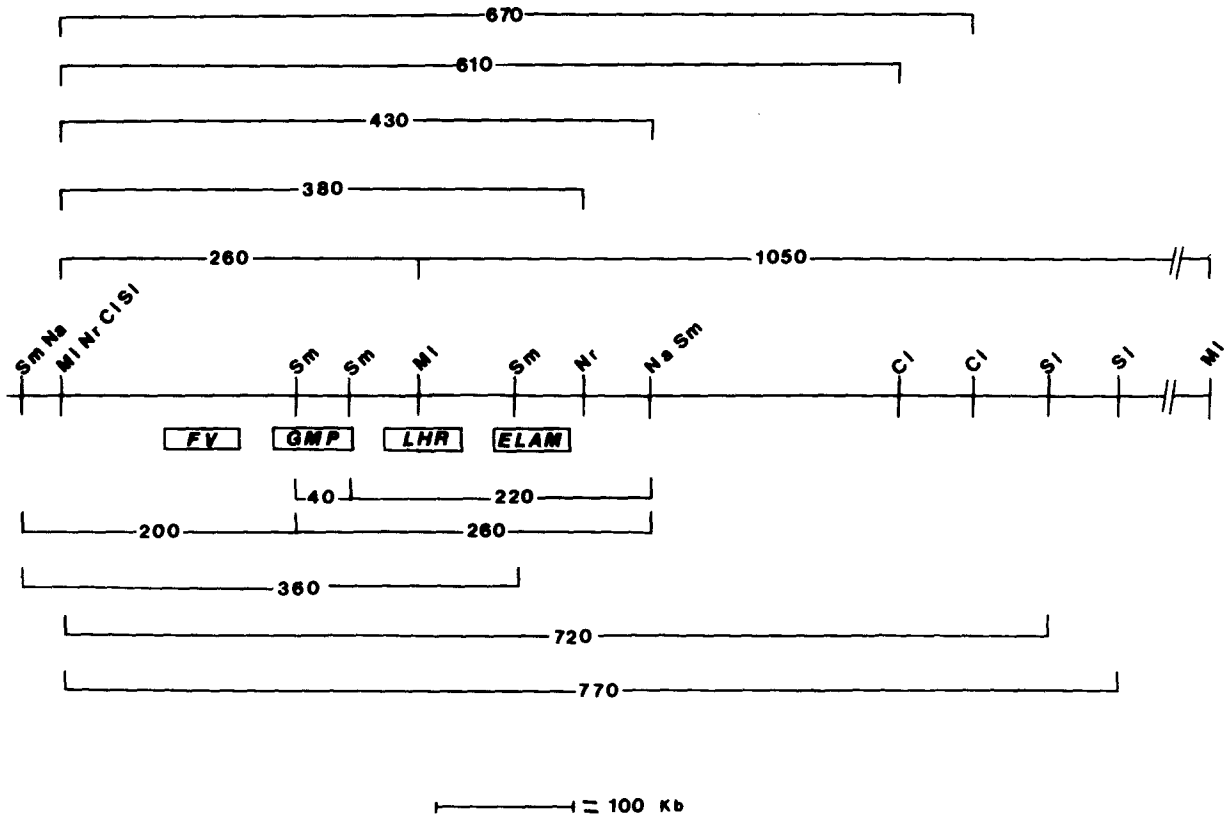

Figure 4. Long-range restriction map of a segment of human chromosome 1 containing the selectin gene complex. Restriction endonuclease sites for endonucleases MluI (Ml), NruI (Nr), SalI (Sl), ClaI (Cl), NaeI (Na), SmaI (Sm) are shown. *FV*, *GMP-140*, *LHR*, and *ELAM-1* genomic regions are indicated by boxes and positioned in agreement with data above. Gene sizes have not been determined and are not drawn to scale. Restriction fragments corresponding to Table 3 are given in kilobases and are shown to scale.

defined the relative location of different restriction sites in this region. For example, ELAM-1 hybridized to a prominent 510-kb SalI/MluI band and a 460-kb minor band, indicating the presence of a SalI 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 SmaI 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 SmaI fragment, physically separating FV from the other three genes. While the ELAM-1 and LHR probes hybridized to a common 220-kb SmaI fragment, GMP-140 and FV recognized a common 200-kb SmaI fragment. Only the GMP-140 probe hybridized to a prominent 40-kb SmaI fragment. An order of *FV-GMP-140-LHR-ELAM-1*, is 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 SalI 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.

Table 4. *Sizes of Murine Restriction Endonuclease Fragments*

Enzyme	FV	GMP-140	LHR	ELAM-1
			kb	
NotI	570	570	570	570
MluI	340	340	340	340
ClaI	310	ND	310	ND
	470		470	
	560		560	
SalI	370	ND	370	ND
	520		520	
	650		650	
NaeI	350	ND	350	ND
NruI	290	ND	290	ND
BssHII	300	ND	300	ND
SacII	340	ND	340	ND

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 these 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 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.

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Address correspondence to Dr. Michael F. Seldin, Department of Medicine, Box 3380, DUMC, Durham, NC 27710.

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