The Mel 14 Antibody Binds to the Lectin Domain of the Murine Peripheral Lymph Node Homing Receptor

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Abstract. Murine and human leukocytes express surface glycoproteins, termed homing receptors (HRs), containing lectin-like, EGF-like (egf), and complement binding–like domains, that apparently endow these cells with the ability to home to peripheral lymph nodes (pln's) by virtue of an adhesive interaction with the pln postcapillary venule endothelium. The murine pln HR was initially characterized with a rat monoclonal antibody, Mel 14, that was specific for the murine form of the receptor. This work demonstrated that Mel 14 blocked the binding of murine lymphocytes to pln endothelium both in vitro and in vivo, a result consistent with the possibility that this monoclonal antibody recognizes a region of the HR that is involved with endothelium recognition and adhesion. In addition, this antibody also blocked the binding to the HR of PPME, a polyphosphomannan carbohydrate known to inhibit lymphocyte-pln endothelium interactions, suggesting that Mel 14 may recognize the lectin domain

of the pln HR. Here we show that, while Mel 14 recognized truncated HR containing both the lectin and egf domains, antibody recognition was lost when the lectin domain alone was expressed. Chimeric molecules, in which regions of the lectin domain of the non-Mel 14-reactive human pln HR were replaced with homologous regions of the murine pln HR, demonstrated that the Mel 14 recognition site is within the NH_2-terminal 53 amino acids of the lectin domain. These results suggest that the Mel 14 monoclonal antibody recognizes a determinant within the lectin domain of the pln HR whose conformation may be dependent upon the presence of the egf domain. Since Mel 14 efficiently blocks lymphocyte–endothelial interactions, these results support the hypothesis that the pln HR lectin domain may be directly involved with binding of lymphocytes to a carbohydrate ligand on the pln postcapillary venule endothelium.

T HE trafficking of lymphocytes between the blood circulation and various lymphoid organs is a critical aspect of the immune system that allows for cells with various antigenic specificities to be readily exposed to their cognate antigenic stimuli. This trafficking is due, in large part, to adhesive interactions between homing receptors (HRs)[1] on the lymphocyte cell surface and ligands on the high endothelium of the postcapillary venules of the various lymphoid organs (2, 8, 11, 29). Several lines of evidence suggest that peripheral lymph nodes (pln's), gut-associated lymphoid tissues, synovial lymphoid tissues, and bronchial lymphoid tissues depend upon different adhesive interactions to accomplish tissue-specific lymphocyte trafficking and homing (4, 6, 7, 12, 13). It has been hypothesized that a diversity of lymphocyte HRs and endothelial cell ligands might be the means by which this lymphoid organ-specific lymphocyte homing is attained (3, 14, 15).

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1. *Abbreviations used in this paper:* cbd, complement binding–like domain; egf domain, epidermal growth factor–like domain; HR, homing receptor; mHR-L, mHR-LE, and mHR-LEC, the murine mHR lectin, lectin-egf, and lectin-egf-cbd domains, respectively; PCR, polymerase chain reaction; PPME, polyphosphomannan ester.

The most well-characterized lymphocyte HR is the murine pln HR (mHR) (10). This 90-kD surface glycoprotein was initially characterized with a rat monoclonal antibody, Mel 14, and is here referred to as gp90[mel 14]. The Mel 14 antibody was able to specifically block pln postcapillary venule binding of lymphocytes in both the in vitro frozen section (10) assay as well as in vivo in MRL-*lpr/lpr* mice (18), suggesting that it recognized an adhesion molecule involved in pln endothelium binding. In addition, this antibody was able to block the binding of a polyphosphomannan ester, PPME, that was previously shown to inhibit lymphocyte-pln endothelium interactions (31). This latter result suggested that the Mel 14 monoclonal antibody might recognize a lectin-like molecule that was involved with pln recognition, a notion that was confirmed when a murine spleen cDNA encoding the antigen recognized by Mel 14 was cloned (17, 24). This cDNA encoded a Mel 14–reactive surface glycoprotein, as determined by transient transfection analysis, that contained an NH_2-terminal lectin-like domain followed by an EGF-like domain (herein referred to as egf domain), two repeats of a complement binding–like domain (cbd), a transmembrane anchor, and a short cytoplasmic domain. The overall structure of the cDNA, together with previous data suggest-

ing that the binding of murine lymphocytes to pln endothelium was a sugar-inhibitable phenomenon (26, 27, 30), suggested that the cDNA corresponding to gp90$^{mel\ 14}$ encoded a new class of molecules that may use lectin–sugar interactions to accomplish cell–cell adhesion. Subsequently, a human lymphocyte cDNA showing a high degree of sequence homology with the murine pln HR cDNA was cloned (1) suggesting, in agreement with data regarding the sugar blocking of human lymphocyte–pln interactions as well as the lack of species specificity for lymphocyte–pln endothelium interactions, that human immune cells home to pln using adhesive interactions similar to those used in the murine immune system (28).

Because the Mel 14 antibody blocks lymphocyte–pln endothelium binding (10), determination of the gp90$^{mel\ 14}$ epitope recognized by this monoclonal antibody may have important implications for characterizing the relative roles of the various pln HR protein domains in lymphocyte–endothelial interactions. Previous work indicated that this monoclonal antibody appeared to recognize a unique, branched form of ubiquitin in association with a gp90$^{mel\ 14}$ core sequence. These data included amino acid sequence analyses of immunoaffinity-purified gp90$^{mel\ 14}$ which revealed a strong amino acid sequence signal that corresponded to the NH$_2$ terminus of the murine pln HR and a very weak signal corresponding to ubiquitin (23). Perhaps more convincing was the isolation of a subset (~1%) of ubiquitin-containing clones from a lambda gt 11 cDNA library that were reactive with the Mel 14 antibody, a result suggesting that a relatively unique form of ubiquitin was recognized by Mel 14 (25). In addition, antibodies directed against the bacterially produced material generated from these Mel 14–positive lambda gt 11 ubiquitin clones reacted with cell surface components of various cell lines, although, surprisingly, the reactivity was not dependent upon the presence of gp90$^{mel\ 14}$ (25). In contrast to these results, subsequent analysis by our group did not reveal a clear ubiquitin sequence when a gp90$^{mel\ 14}$ antigen preparation isolated from murine spleen by Mel 14 immunoaffinity chromatography was analyzed by gas phase–amino acid sequencing (17). In addition, we also found that a human homologue of the murine pln HR was not reactive with Mel 14 when transfected into mammalian cells (1), suggesting, if the antibody was indeed recognizing a unique form of ubiquitin, that this form could be reproduced in a bacterial system (lambda gt 11), but could not be produced with a highly homologous human glycoprotein in a mammalian cell expression system.

The work reported in this article describes the mapping of the Mel 14 recognition region on the murine pln HR. A combination of deletion mutants and murine–human chimeras demonstrates that this antibody recognizes an epitope contained within the first 53 amino acids of the lectin domain of gp90$^{mel\ 14}$ whose recognition by Mel 14 is dependent upon the presence of the adjacent egf domain. These results are consistent with the notion that the lectin domain of the HR is critical for the recognition and binding of lymphocytes to the postcapillary endothelium of pln.

Materials and Methods

Construction of Recombinant Molecules

Deletion mutants containing various domains of the murine pln HR were produced by in vitro mutagenesis of the pln HR cDNA (32). Briefly, a double-stranded plasmid (pRK; reference 9) containing an m13 origin of replication was converted to a single-stranded form by rescue with the m13 helper phage K07. The resultant single-stranded template was mutagenized by insertion of two adjacent stop condons (TAA and TAG) after amino acid positions 158 (the mHR lectin-like domain [mHR-L]), 193 (the mHR lectin-egf domain [mHR-LE]), and 332 (the mHR lectin-egf-cbd domain [mHR-LEC]) using in vitro mutagenesis with 48-mer oligonucleotides (32). The resultant constructs were transformed into bacteria, mutants were selected by hybridization with ^{32}P 21-mer oligonucleotides, and the DNA sequence of the mutants was determined by supercoil sequencing. mHR lectin-IgG and mHR lectin-egf-human IgG constant region chimeras were constructed by insertion of a human gamma 1 IgG constant region cassette (5) 3' to the mHR gene, and loop-out in vitro mutagenesis of the resultant construct to give chimeras containing amino acids 1-158 of the murine pln HR (corresponding to the lectin domain) or amino acids 1-193 (corresponding to the lectin and egf domains) in translational phase with the hinge, CH2, and CH3 regions of the human IgG heavy chain. The Bgl II chimera (corresponding to amino acid 131) between the murine and human pln HR was produced by ligating an Eco RI-Bgl II fragment from the murine homing receptor to the homologous region of the human HR cDNA clone. An Mro I chimera (corresponding to amino acid 91) was produced by ligating an NH$_2$-terminal Eco RI-Mro I fragment derived from the mHR cDNA by polymerase chain reaction (PCR) (22) using a 3' synthetic primer containing an Mro I site to the homologous region of the human HR cDNA. Finally, an Mro I-Bgl II chimera that replaced amino acids 91-131 of the human receptor with the homologous region of the murine receptor was constructed using a similar PCR mutagenesis approach.

Analysis of Constructs by Radioimmunoprecipitation

Various constructs were analyzed by immunoprecipitation of transfected 293 cell supernatants labeled with ^{35}S cysteine and methionine using Mel 14 monoclonal antibody (1 μg/ml) or a polyclonal antibody directed against purified gp90$^{mel\ 14}$ (the gift of Dr. Steven Rosen, University of California, San Francisco) as previously described (17). Since the Mel 14 monoclonal antibody is a rat antibody, Mel 14 immunoprecipitations were accomplished in the absence of protein A-Sepharose with rabbit anti-rat IgG polyclonal antibodies conjugated directly to Sepharose beads. Precipitation of the mHR lectin-IgG and mHR lectin-egf-IgG chimeras was accomplished with protein A-Sepharose beads in the absence of added antibodies. Immunoprecipitated glycoproteins were analyzed on 7.5 or 10.0% polyacrylamide-SDS gels after reduction at 100°C with beta mercaptoethanol. Immunoprecipitation of murine-human chimeric homing receptors was accomplished using transfected cell supernatants, since previous work demonstrated that the full-length murine and human HR cDNAs generated both membrane-bound and secreted forms of the receptor (17). Pulse-chase analysis was performed by labeling 293 cells that had been transfected with an expression plasmid encoding the cell-associated form of the pln HR for 10 min with 500 microcuries each of ^{35}S methionine and cysteine in methionine-cysteine-free medium. The cells were washed and incubated for various times in methionine-cysteine-containing medium, after which the cells were lysed with 1% NP-40 and immunoprecipitated with either the Mel 14 monoclonal antibody (1 μg/ml) or with the rabbit polyclonal antibody directed against purified HR antigen.

Results

Pulse-Chase Analysis of the Mel 14 Epitope

To examine the possibility of posttranslational modification of the pln HR and its role in Mel 14 antibody reactivity (23, 25), pulse-chase experiments were performed. Cells were transfected with an expression plasmid encoding the full-length murine pln HR, pulse labeled with ^{35}S methionine and cysteine for 10 min, and chased in the presence of unlabeled amino acids for various periods of time. Cells were lysed, and the reactivity of labeled proteins with the polyclonal anti-pln HR antibody and with Mel 14 was analyzed by immunoprecipitation. Fig. 1 shows that the pln HR is initially produced as a lower molecular mass precursor of ~80 kD which is partially converted with time to a higher molecular mass glycoprotein of ~90 kD. Glycosidase experiments
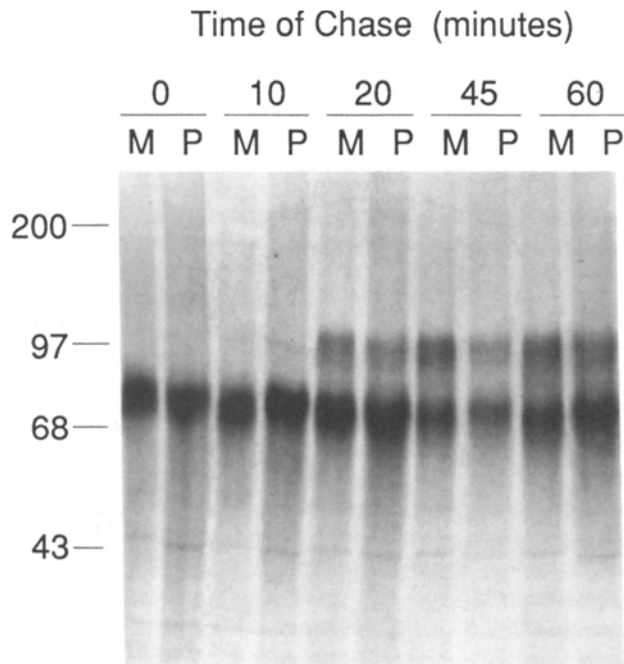
Time of Chase (minutes)

Figure 1. Pulse–chase analysis of murine pln HR reactivity with Mel 14 monoclonal antibody and anti-gp90[mel 14] polyclonal antibodies. Cells were labeled for 10 min with ^{35}S methionine and cysteine and chased for the indicated times in cold methionine-cysteine-containing media. The cells were lysed and the labeled glycoproteins were analyzed for reactivity with Mel 14 and polyclonal anti-gp90[mel 14] antibodies. Molecular mass markers in kD are shown. *M*, Mel 14 antibody reactivity; *P*, polyclonal anti-gp90[mel 14] reactivity.

have shown that the lower molecular mass glycoprotein is a high mannose endoglycosidase H–sensitive precursor form, while the higher molecular mass antibody-reactive glycoprotein is neuraminadase sensitive and localized on the cell surface (data not shown). This figure also clearly illustrates that the pln HR is reactive with both the polyclonal anti–pln HR antibody as well as with the Mel 14 monoclonal antibody at the initial time of the chase, suggesting that if the epitope recognized by Mel 14 is a result of posttranslational modification of the pln HR core sequence then this modification must occur very rapidly after or cotranslationally with polypeptide synthesis.

Reactivity of Mel 14 with pln HR Domain Deletion Mutants

Previous work demonstrated the ability of the Mel 14 monoclonal antibody to block both lymphocyte–pln high endothelium (10) interactions as well as the binding of PPME (31), a yeast cell wall polyphosphomannan carbohydrate known to inhibit this same cell–cell interaction. These data, in conjunction with the interesting domain-like structure of the gp90[mel 14] cDNA clone (17, 24), suggested that an investigation of the interaction of this antibody with various gp90[mel 14] domain deletion mutants might provide some insights into the relative importance of these domains to pln endothelial cell recognition. Fig. 2 shows the various domain deletion mutants that were produced by in vitro mutagenesis. The deletion of the putative transmembrane domain in each of these

mutants was expected to allow for efficient secretion of the truncated proteins from transfected mammalian cells, an expectation that was fulfilled (see below). The truncated proteins that were analyzed contained either the lectin-like, egf and cbd domains (mHR-LEC), or the lectin and egf domains (mHR-LE), or the lectin domain alone (mHR-L).

Mammalian cells were transfected with each type of deletion mutant, and the secreted materials were analyzed with the Mel 14 monoclonal antibody as well as with a polyclonal antibody directed against the purified gp90[mel 14] antigen. As can be seen in Fig. 3, the nontruncated, cell-associated mHR migrating at about 80–90 kD, and the secreted mHR-LEC (~80 kD) and mHR-LE (multiple glycosylated forms at ~30–40 kD) deletion mutants appeared to all be recognized by both the Mel 14 monoclonal antibody as well as the anti-gp90[mel 14] polyclonal antibody. However, neither antibody was able to recognize the deletion mutant containing the lectin-like domain alone (mHR-L). This result was consistent with one of three possibilities: the Mel 14 monoclonal antibody recognized an epitope at least in part contained within the egf domain; the Mel 14 antibody recognized a conformational determinant in the lectin domain that required the presence of the egf domain; the monoclonal antibody recognized an epitope solely within the lectin-like domain, but the expressed protein was unstable and was, therefore, not detectable by the antibody.

The third of these possibilities was examined by producing lectin- and lectin-egf–containing deletion mutants that could be detected by other means. Chimeric molecules containing the lectin-like and lectin-egf domains of the gp90[mel 14] cDNA clone and the hinge, CH2, and CH3 regions of the human gamma 1 IgG were constructed (Fig. 2, *mHR-L+ IgG, mHR-LE+IgG*) (5). Because of the efficient reactivity of the IgG constant region with Staphylococcal protein A antigen, these chimeric molecules could be easily detected by precipitation of transfected cell supernatants with protein A–Sepharose beads in the absence of added antibodies (5). As can be seen
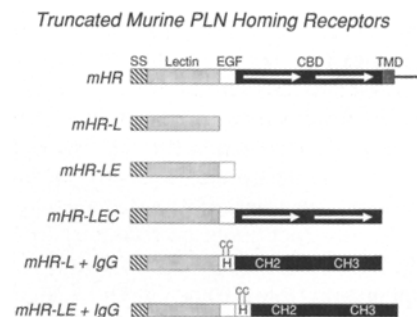

Truncated Murine PLN Homing Receptors

Figure 2. Construction of various truncated mHR and mHR+IgG chimeras. The original mHR cDNA was mutagenized by in vitro mutagenesis with oligonucleotides containing two adjacent stop codons (TAA and TAG) after amino acid positions 158 (the mHR-lectin-like domain [*mHR-L*]), 193 (the mHR lectin-egf domain [*mHR-LE*]), and 332 (the mHR-lectin-egf-cbd domain [*mHR-LEC*]). mHR + IgG chimeras were produced by in vitro loop-out mutagenesis of plasmids containing the original mHR cDNA and a human gamma 1 IgG-containing cassette (5) located 3' of the mHR cDNA. Oligonucleotides were designed to loop out the region between the mHR lectin domain and IgG hinge, CH2, and CH3 regions (*mHR-L + IgG*), or the mHR egf domain and IgG hinge, CH2, and CH3 regions (*mHR-LE + IgG*). *TMD*, transmembrane anchor domain.
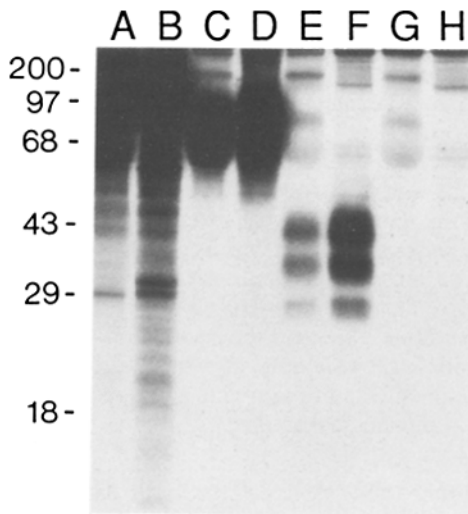
Figure 3. Reactivity of various HR domain deletion mutants with the Mel 14 monoclonal and anti-gp90$^{mel~14}$ polyclonal antibodies. Plasmids containing the various deletion mutants were transfected onto the 293 kidney cell line and the cells were labeled with ^{35}S methionine and cysteine. Since deletion of the transmembrane anchor sequence from the mHR constructs should result in efficient secretion of the glycoproteins, cell supernatants were immunoprecipitated with Mel 14 monoclonal antibody, or with a polyclonal antibody directed against purified gp90$^{mel~14}$ as described previously. The resultant immunoprecipitates were analyzed on 10% polyacrylamide-SDS Gels. Molecular mass markers in kD are shown. (Lane *A*) Cell lysates expressing native (i.e., nontruncated) mHR, gp90$^{mel~14}$ polyclonal antibody; (lane *B*) cell lysates expressing native mHR, Mel 14 antibody; (lane *C*) mHR-LEC, gp90$^{mel~14}$ polyclonal antibody; (lane *D*) mHR-LEC, Mel 14 antibody; (lane *E*) mHR-LE, gp90$^{mel~14}$ polyclonal antibody; (lane *F*) mHR-LE, Mel 14 antibody; (lane *G*) mHR-L, gp90$^{mel~14}$ polyclonal antibody; (lane *H*) mHR-L, Mel 14 antibody.

tage of this fact to further map the Mel 14 recognition region by producing chimeric molecules where portions of the murine lectin domain replaced homologous regions from the human lectin domain. As can be seen in Fig. 5, the first such chimera took advantage of a Bgl II restriction site at amino acid 131 (amino acid 93 of the mature molecule) that was shared by both the human and murine forms of the HR (mhHR Bgl II). A second chimera (mhHR Mro I) was produced by insertion of an Mro I site in the murine receptor cDNA at amino acid 91 (amino acid 53 of the mature molecule) by PCR mutagenesis (22) such that this site could be joined to a homologous site in the human form of the receptor. Finally, a third chimera produced by PCR mutagenesis replaced the region corresponding to amino acids 91-131 of the human receptor with the homologous region from the murine receptor mhHR (Bgl II-Mro I).

Fig. 5 shows radioimmunoprecipitation analysis of the various murine–human chimeric HRs. Transfected cell supernatants were analyzed for reactivity with either the murine-specific Mel 14 antibody or the murine- and human-reactive polyclonal anti-gp90$^{mel~14}$ antibody, since we previously demonstrated that both transfected cells as well as cells normally synthesizing both the murine and human HR produce secreted forms, albeit relatively inefficiently, of the proteins that apparently correspond to extracellular domains cleaved at or near the transmembrane anchor region (17). As can be seen in this figure, the ∼80-kD murine receptor reacts with both the Mel 14 monoclonal antibody as well as the gp90$^{mel~14}$ polyclonal antibody, while the ∼70-kD human receptor reacts only with the gp90$^{mel~14}$ polyclonal antibody, in agreement with previous fluorescence-activated cell sorter analysis (1). The difference in molecular mass between the two shed receptors was most likely due to the fewer potential NH₂-linked glycosylation sites found in the human vs. the murine receptors (1, 16). In addition, the relatively weak reactivity of the
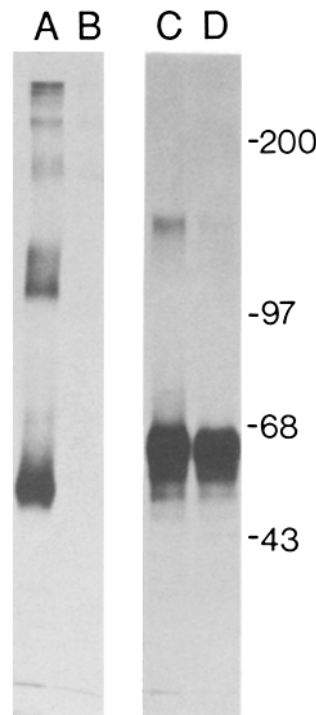
in Fig. 5, chimeric proteins of ∼50 kD (*mHR-L+IgG*) and ∼60 kD (*mHR-LE+IgG*) molecular mass were efficiently produced in transfected mammalian cell lines as protein A-reactive, secreted molecules (the fainter, higher molecular mass bands are reduction-resistant multimers (Fennie, C., and L. Lasky, unpublished observations). The fact that these molecules were efficiently secreted demonstrates that the gp90$^{mel~14}$ signal sequence was functioning normally in the context of these constructs. However, Fig. 4 shows that, while the mHR-LE+IgG construct could be recognized by the Mel 14 antibody, the mHR-L+IgG was unable to interact with Mel 14, in agreement with results shown in Fig. 3, suggesting that the apparent lack of reactivity by the lectin construct was not due to instability of this deletion mutant.

Reactivity of Mel 14 with Murine–Human pln HR Chimeras

Previously, we described a human homologue of the murine pln HR that was isolated from a peripheral blood lymphocyte library (1). This homologue contained an identical domain structure to that discovered for the murine pln HR. In addition, while the overall sequence homologies, especially in the lectin-like and egf domains, were quite high (∼85%), cells transfected with the human homologue showed no reactivity with the Mel 14 antibody (at 1 μg/ml). We took advan-

Figure 4. Reactivity of mHR + IgG chimeras with protein A-Sepharose and with the Mel 14 monoclonal antibody. 293 cells were transfected with plasmids encoding mHR + IgG chimeras and labeled with ^{35}S methionine and cysteine. Cell supernatants were analyzed with protein A-Sepharose in the absence of added antibody or with Mel 14 monoclonal antibody and rabbit anti-rat IgG polyclonal antibody conjugated directly to Sepharose beads. Molecular mass markers in kD are shown. (Lane *A*) mHR-LE + IgG, protein A; (lane *B*) mHR-L + IgG, Mel 14; (lane *C*) mHR-LE + IgG, protein A; (lane *D*) mHR-LE + IgG, Mel 14.
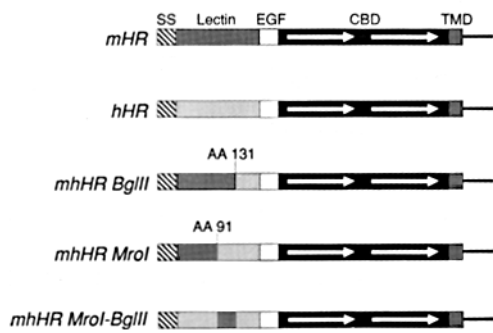
Figure 5. Construction of murine–human HR chimeric molecules. Previous work demonstrated that a human cDNA (*hHR*) encodes a lymphocyte glycoprotein that is highly similar to the murine pln HR (*mHR*), but that does not react with the Mel 14 monoclonal antibody (1). mHR Bgl II was constructed by replacing the hHR region corresponding to amino acids 1–131 with the homologous region from the murine receptor. mHR Mro I was constructed by replacing the hHR region corresponding to amino acids 1–91 with the corresponding region of the mHR produced by PCR mutagenesis Mro I–Bgl II was produced by replacing the region corresponding to amino acids 91–131 of the hHR with the corresponding region of the murine receptor using PCR mutagenesis.

human HR was probably due to poor cross-reactivity between the anti–murine gp90^{mel} [14] polyclonal antibody and the human HR homologue (1). Fig. 6, lanes *I* and *J*, shows that the construct containing the first 131 amino acids (including the signal sequence) of the murine receptor (mhHR Bgl II) reacts with both monoclonal and polyclonal antibodies, suggesting that the Mel 14 epitope is contained within the first 93 amino

acids of the mature (i.e., signal sequence–cleaved) receptor. A chimeric protein containing the first 53 amino acids of the mature receptor (mhHR Mro I; Fig. 6, lanes *G* and *H*) also reacts with both antibodies, while a chimera that contains the first 53 amino acids of the human receptor followed by 42 amino acids of the murine receptor (mhHR Bgl II–Mro I; Fig. 6, lanes *E* and *F*) shows reactivity only with the polyclonal antibody. These results in combination unambiguously assign at least part of the Mel 14 epitope to the first 53 amino acids of the murine homing receptor.

Discussion

The data reported in this article demonstrate that the Mel 14 monoclonal antibody recognizes an epitope contained, at least in part, within the lectin domain of the murine pln HR. This result is entirely consistent with previous data which demonstrated that this monoclonal antibody blocks the binding of PPME (30), a polyphosphomannan carbohydrate, presumably to the carbohydrate-binding lectin domain of the lymphocyte surface-localized gp90^{mel} [14] HR. When taken in the context of the ability of this same antibody to block the binding of lymphocytes to pln high endothelium both in vitro (10) and in vivo (18), the results reported here imply that the lectin domain is directly involved in the adhesive interaction between lymphocytes and the pln endothelium. This latter notion is consistent with previous work demonstrating that sialidase treatment of lymph node sections in vitro (20) or of lymph nodes in vivo (21) abolishes the adhesive interaction between lymphocytes and pln endothelium, possibly by destroying a uniquely located sialic acid–containing oligosaccharide recognized by the pln HR lectin domain.

The finding that removal of the egf domain abolishes the ability of Mel 14 to recognize the lectin region of the HR sug-
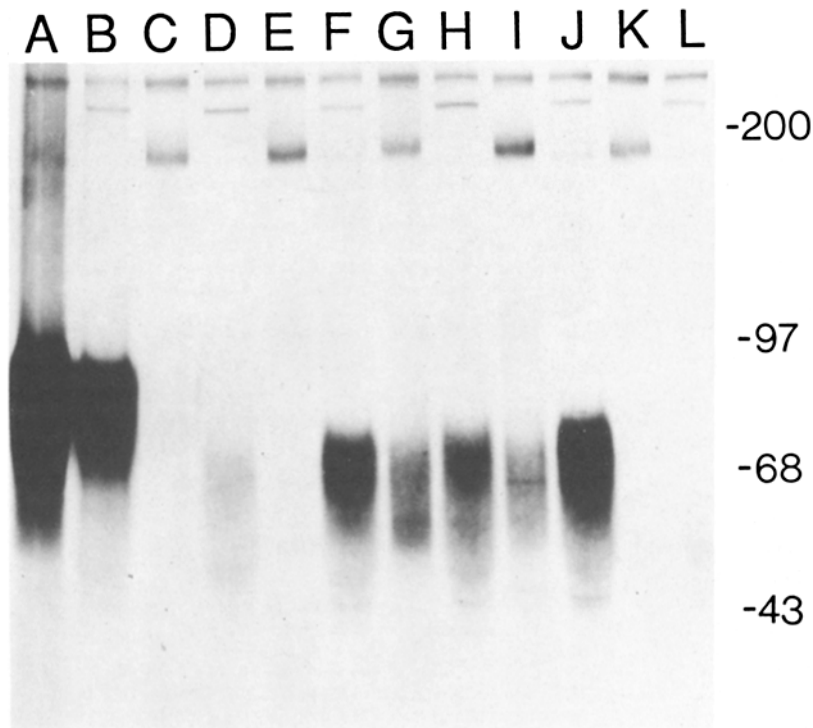

Figure 6. Radioimmunoprecipitation analysis of murine–human chimeric HRs. The constructs shown in Fig. 4 were transfected onto 293 cells, and the cells were labeled with ^{35}S cysteine and methionine. Since previous work had demonstrated that both transfected cells as well as cell lines normally synthesizing both the murine and human HR produce secreted forms of the molecules, labeled cell supernatants were analyzed for reactivity with both the Mel 14 monoclonal antibody (1 μg/ml), specific for the murine form of the receptor, as well as with a polyclonal antibody directed against purified gp90^{mel} [14], which reacted with both murine and human receptors. (Lane *A*) Secreted gp90^{mel} [14], Mel 14 antibody; (lane *B*) secreted go90^{mel} [14], gp90^{mel} [14] polyclonal antibody; (lane *C*) human HR, Mel 14 antibody; (lane *D*) human HR, gp90^{mel} [14] polyclonal antibody; (lane *E*) mhHR Mro 1–Bgl II, Mel 14 antibody (lane *F*) mhHR Mro 1–Bgl II, gp90^{mel} [14] polyclonal antibody; (lane *G*) mhHR Mro 1, Mel 14 antibody; (lane *H*) mhHR Mro 1, gp90^{mel} [14] polyclonal antibody; (lane *I*) mhHR Bgl II, Mel 14 antibody; (lane *J*) mhHR Bgl II, gp90^{mel} [14] polyclonal antibody; (lane *K*) mock transfected, Mel 14 antibody; (lane *L*) mock transfected, gp90^{mel} [14] polyclonal antibody.

gests that the nature of the epitope that interacts with this antibody is potentially quite interesting. If it is assumed that a unique ubiquitin–HR linkage within the lectin domain is the Mel 14 epitope (23), then it would appear that either the structure of the ubiquitinated epitope or the actual ubiquitination of the site may be dependent upon sequences within the egf domain. Since it was previously shown that Mel 14 can apparently recognize bacterially produced ubiquitin in the absence of any egf sequences, the former of these two possibilities is clearly not tenable (25). Thus, if the notion that ubiquitin comprises part of the Mel 14 epitope is accepted, then it seems clear that the egf domain must have some effect on the ubiquitination of the site within the lectin domain.

An alternative possibility is that the epitope recognized by Mel 14 is not actually a unique form of ubiquitin. One argument for this possibility is the lack of Mel 14 reactivity with the human homologue of the pln HR. The high degree of sequence homology between these two lectin domains ($\sim$85%) (1), together with the fact that our transient transfection system can clearly produce a Mel 14–reactive murine HR (17), is difficult to reconcile with a unique ubiquitinated epitope, since it might be assumed that the invariant nature of the ubiquitin primary sequence would be such that this uniquely linked form of ubiquitin should also be found on the human homologue. An additional argument against a ubiquitin-like epitope is the pulse–chase experiment described in Fig. 1 which suggests that ubiquitin would have to be added cotranslationally to the core sequence, a possible, although as yet undescribed, mechanism of ubiquitination (19). In addition, we have failed to demonstrate antiubiquitin polyclonal antibody reactivity with cells that are highly positive for reactivity with both Mel 14 monoclonal and polyclonal anti- gp90^{mel} [14] antibodies (Lasky, L., and S. Stachel, unpublished observations). Finally, although we have been unable to reproduce the Mel 14 epitope by expression of the murine pln HR cDNA sequence in an *Escherichia coli* expression system (Fennie, C., and L. Lasky, unpublished observations), it can be argued that the protein is not glycosylated and/or correctly folded in bacterial expression systems. Alternatively, the relatively minor amino acid differences between the human and murine receptors might be sufficient to disrupt the binding of the ubiquitinated site in the human HR to the Mel 14 antibody, although the recognition of divergent lambda gt 11 ubiquitin clones by Mel 14 (25) seems to argue against this. We thus feel the nature of the Mel 14 recognition sequence remains somewhat controversial.

If one assumes that ubiquitin is not actually part of the Mel 14 recognition site, then the lack of Mel 14 recognition of the truncated HR missing the egf domain may be interpreted in one of two ways. One possibility is that the egf domain induces the lectin domain to assume a conformation that is recognized by Mel 14. Alternatively, the Mel 14 recognition site may contain distantly located sequences derived from both the lectin-like and egf domains. The argument against the latter possibility is the binding of Mel 14 to the murine–human chimera, where the egf domain derived from the human HR contains a number of amino acid substitutions when compared to the murine HR egf domain (6 changes out of 34 total residues). Thus, if the egf domain is actually a component of the Mel 14 epitope, then this antibody must recognize one of the relatively small egf regions found to be con-

served between the human and murine receptors. An additional argument in favor of the role of the egf domain in inducing an appropriate conformation on the lectin domain is the finding that deletion of a similar egf domain from tissue plaminogen activator results in mutant molecules with decreased substrate-binding activities (16). We thus favor the possibility that Mel 14 recognizes a conformational determinant in the murine pln HR and that one of the functions of the egf domain is to induce an appropriate conformation in the lectin domain.

In conclusion, the data reported here suggest that the Mel 14 monoclonal antibody recognizes a determinant within the NH$_2$ terminal region of the murine HR lectin domain whose conformation may be dependent upon the presence of the egf domain. The binding of this cell adhesion–blocking monoclonal antibody to the lectin domain of the pln HR suggests a direct involvement of this domain in endothelial cell recognition by lymphocytes, a possibility that we are currently testing by direct binding of various soluble HR to pln endothelium.

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