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Binding of RANTES, MCP-1, MCP-3, and MIP-1α to Cells in Human Skin

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Based on their ability to induce leukocyte chemotaxis and adhesion to endothelial cells (ECs), chemokines have been implicated in driving inflammatory leukocyte emigration. Recently, it was suggested that chemokines can accomplish their pro-emigratory role more effectively while being bound to the luminal surface of the ECs. Previously, such binding was demonstrated *in situ* in human skin for the prototype α-chemokine interleukin (IL)-8. Here we used an *in situ* binding assay to investigate the binding characteristics of several β-chemokines in intact human skin. RANTES, MCP-1, and MCP-3 bound, similar to IL-8, in a specific saturable manner to the ECs of venules and small veins but not arteries or capillaries. RANTES inhibited MCP-1 and MCP-3 binding and *vice versa*, indicating that the EC binding sites are shared among these β-chemokines; moreover, IL-8 and RANTES cross-competed for each other's binding, suggesting that the same chemokine binding sites are used by members of α- and β-chemokine subfamilies. Conversely, MIP-1α did not bind to the ECs and did not compete for binding of RANTES. Analogous to IL-8, all of the tested β-chemokines bound to the resident dermal cells. As a novel aspect of chemokine interaction with cells in normal skin, we observed specific, saturable binding of RANTES, MCP-1, and MCP-3 but not MIP-1α or IL-8 to the ECs of dermal afferent lymphatic vessels. RANTES, MCP-1, and MCP-3 also cross-competed for each other's binding to lymphatics, suggesting a common binding site with a novel chemokine binding profile. We suggest that the chemokine binding to the ECs of lymphatics may be involved in the process of leukocyte entry into the afferent lymphatic vessels. *(Am J Pathol 1998, 152:749–757)*

The family of chemokines comprises structurally homologous peptides with potent *in vitro* chemotactic activity for different leukocyte types.[1,2] Due to their ability to induce firm leukocyte-endothelial cell (EC) adhesion and transendothelial migration these molecules have been suggested to drive inflammatory leukocyte recruitment from blood into the tissues in diseases of different pathologies.[1–4] By analogy with *in vitro* leukocyte chemotaxis, ie, migration along soluble attractant gradients, it was thought initially that during leukocyte emigration *in vivo* chemokines also act as soluble mediators. However, specific saturable binding sites for the α-chemokine interleukin (IL)-8 were described on the surface of the ECs of postcapillary venules (PCVs) and small veins,[5] and an alternative mechanism of *in vivo* pro-emigratory IL-8 action was suggested.[6] It was argued that soluble gradients could not persist at the blood-endothelium interface as they would immediately be diluted by the blood flow.[6] Therefore, to efficiently trigger firm leukocyte-EC adhesion and due to the requirement of sequential involvement of leukocyte adhesion molecules, first selectins and then activated integrins,[7] IL-8 has to be immobilized by the ECs and presented in solid phase to the adherent leukocytes. The IL-8 binding sites on the PCVs and small veins[5] provided the morphological substrate for such immobilization and presentation, which recently had been visualized using immunoelectron microscopy.[8] A similar mechanism of chemokine binding to the EC surface had been suggested also for β-chemokines.[9,10] Although MIP-1β and RANTES immunoreactivity had been previously detected associated with the ECs, it had not been elucidated whether these chemokines, similar to IL-8, bound to specific, saturable surface receptors on the ECs or were produced by the ECs and retained within their cytoplasm.[8,9] We used the *in situ* binding assay[5] to investigate the EC binding of β-chemokines in human skin. We observed that RANTES, MCP-1, and MCP-3, similar to IL-8,[5] bound to the venular ECs, whereas MIP-1α did not. In addition, saturable binding sites for RANTES, MCP-1, and MCP-3 but not for MIP-1α or IL-8 were detected on the ECs of the afferent lymphatic vessels. Similar to IL-8,[5] all of the studied β-chemokines bound to perivascular resident cells in the dermis. Cross-competition studies were performed to characterize the specificity of the chemokine binding to the different cell types.

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Materials and Methods

In Situ *Binding Assay*

A modification of the described procedure was used.[5] Pieces of normal human skin were obtained after informed consent from patients undergoing reductive surgery and immediately cut into 1- to 2-mm^3 cubes with a scalpel blade. Four or five tissue pieces were placed in tubes containing 0.5 to 5 ng of either ^{125}I-labeled recombinant human (rh^{125}I)-RANTES, rh^{125}I-MIP-1α, rh^{125}I-MCP-1, rh^{125}I-MCP-3, or rh^{125}I-IL-8 (Du Pont de Nemours, Brussels, Belgium; specific radioactivity, 250 μCi/μg) in 500 μl of Hanks' balanced salt solution supplemented with 0.1% bovine serum albumin (Calbiochem, La Jolla, CA) and 20 mmol/L HEPES and incubated for 1 hour at room temperature on a platform shaker (~300 rpm). Control tubes contained 1000X excess of the corresponding unlabeled chemokine (PeproTech EC, London, UK). To assess the chemokine specificity of the binding, tissue pieces were incubated with either ^{125}I-RANTES and 1000X excess of unlabeled MCP-1, MCP-3, MIP-1α, and IL-8 or with ^{125}I-MCP-1, ^{125}I-MCP-3, ^{125}I-MIP-1α, ^{125}I-IL-8, and 1000X excess of unlabeled RANTES. After the incubation, the pieces were washed four times for 10 minutes each in 2 ml of Hanks' balanced salt solution (containing 0.1% bovine serum albumin and 20 mmol/L HEPES) on a platform shaker and fixed overnight in buffered 4% paraformaldehyde. The samples were washed, dehydrated in increasing concentrations of ethanol, and embedded in paraplast (Monoject Scientific, Athy, Ireland). The 5-μm thin sections were cut on an automatic microtome (Ultracut 2050, Reichert-Jung, Vienna, Austria), deparaffinized, rehydrated, air dried, and coated with K.2 nuclear track emulsion (Ilford, Mobberley, UK). The slides were sealed in black boxes, exposed at 4°C for 2 to 8 weeks and afterwards developed with D-19 developer (Kodak-Pathe, Paris, France), fixed (Unifix, Kodak-Pathe), and counterstained with hemalaun. All sections were studied and photographed using a BX 60 Olympus microscope (Olympus Austria, Vienna, Austria), and the numbers of grains over different cells and microanatomical structures were scored by two observers. As we have used equimolar amounts of different chemokines, which were labeled with the same radioisotope with the same specific radioactivity, the number of chemokine molecules that bound to defined microanatomical structures should be proportionate to the number of radioactive grains detected over these structures. All of the observations were made in at least three different experiments on samples from different donors. Arteries, capillaries, venules, and lymphatic vessels were identified based on their characteristic histological appearance.

Identification of Dermal Cells

For immunohistochemical staining of tissue macrophages, selected sections were incubated with a mouse monoclonal anti-HLA-DR antibody (LN3, Clonab, Biotest, Vienna, Austria). The bound primary antibody was detected using a rabbit secondary antibody and an alkaline phosphatase-anti-alkaline phosphatase detection kit (Dako, Glostrup, Denmark). Mast cells were identified by staining the selected sections with toluidine blue after additional methanol fixation of the developed autoradiographs.

Results

In Situ *Chemokine Binding*

In direct analogy with the previous observation with IL-8,[5] RANTES, MCP-1, and MCP-3 selectively bound to the ECs of venules and small veins but failed to bind to either arterial or capillary endothelium (Figure 1, A–C). Conversely, we observed no binding of MIP-1α to the ECs of venules or any other segment of the circulatory tree (Figure 1D). Venular ECs bound lower amounts of MCP-1 and MCP-3 than of IL-8 and RANTES (Table 1). All of the chemokines tested bound to the dermal resident cells (Figure 2, A–D). However, the estimated relative numbers of cells that bound a particular chemokine and the number of radioactive grains over a positive cell differed greatly among the chemokines tested (Table 1).

Immunohistochemical techniques identified most of the resident dermal cells that expressed binding sites for MIP-1α, RANTES, or IL-8 as HLA-DR-positive macrophages (Figure 2, A and B), although not all HLA-DR-positive cells bound these chemokines. Conversely, among the much less numerous resident cells that bound MCP-1 and MCP-3, macrophages were rare (Figure 2, C and D). Also, melanophages, which are present in the upper dermis and characterized by yellow-brown granules in the cytoplasm, have been shown to bind MIP-1α (Figure 3B), RANTES, IL-8, and only rarely MCP-1 and MCP-3 (not shown). Additionally, as confirmed by toluidine blue staining, few mast cells bound MIP-1α, RANTES (Figure 3A), and IL-8. Most of the dermal resident cells that bound MCP-1 and MCP-3 and some that bound other chemokines remained unaccounted for by the described staining procedures.

A low amount of IL-8 binding was observed over the basal and suprabasal keratinocyte layers (Figure 4A). In contrast, all of the β-chemokines tested failed to bind to keratinocytes, although, occasionally, scattered cells present in the basal and suprabasal layers of the epidermis expressed binding sites for RANTES (Figure 4B), MIP-1α, and MCP-1 (not shown).

Finally, the β-chemokines RANTES, MCP-1, and MCP-3 bound to the ECs of afferent lymphatic vessels of various sizes throughout the dermis (Figure 5, A–C). The number of silver grains over these structures was comparable among the three chemokines (Table 1). In contrast, neither MIP-1α (Figure 5D) nor IL-8 (not shown) bound to lymphatic ECs.

Excess of unlabeled chemokines removed the specific binding of the corresponding radiolabeled chemokines to venular and lymphatic ECs and to dermal resident cells, indicating the saturable nature of the binding sites (Figure 1E). However, the addition of unlabeled chemokines

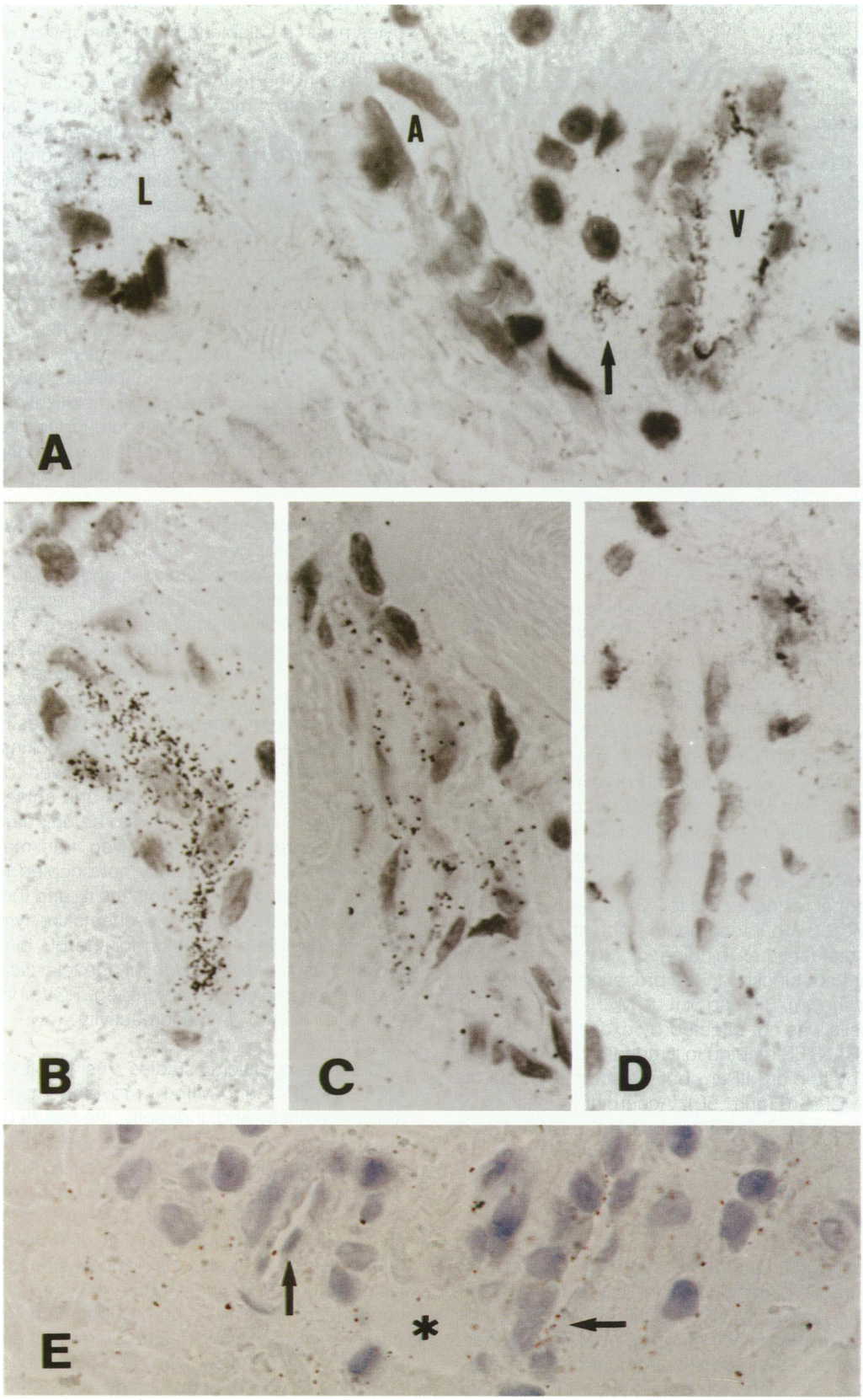

Figure 1. *In situ* binding of RANTES, MCP-1, MCP-3, and MIP-1α to venular ECs in human dermis. **A:** RANTES, visualized as silver autoradiographic grains, binds to the ECs of the venule (V) and the lymphatic vessel (L) but not to the ECs of the artery (A). Additionally, RANTES binds to a single cell in the perivascular tissue (**arrow**). **B** and **C:** MCP-1 (**B**) and MCP-3 (**C**) bind to the ECs of the venule. **D:** MIP-1α does not bind to venular ECs but is seen over resident cells in the dermis. **E:** Binding of RANTES in the presence of 1000X excess of unlabeled ligand; the lumen of an afferent lymphatic, a small artery, and few residual grains on venular ECs are marked by an **asterisk**, a **vertical arrow** , and a **horizontal arrow**, respectively. All counterstained with hemalaun; magnification, ×1125.

Table 1. Scores of *in Situ* Binding of the Chemokines IL-8, RANTES, MCP-1, MCP-3, and MIP-1α to Cells in Human Skin

	IL-8	RANTES	MCP-1	MCP-3	MIP-1α
ECs of venules and small veins	+++	+++	++(+)	++(+)	−(+)
Resident cells in the dermis	++	++	+	+	+++
ECs of lymphatic vessels	−	+++	+++	+++	−
Basal and suprabasal keratinocytes	++(+)	−	−	−	−

Scores as judged by two observers reflect the number of radioactive grains seen over the cells. The estimated relative number of chemokine-binding resident cells in the dermis was approximately 1/5, 1/3, <1/10, <1/10, and 1/2 of the whole dermal cell population for IL-8, RANTES, MCP-1, MCP-3, and MIP-1α, respectively. Each score was derived from three observations on skin from three different donors. Scores in parentheses show variant binding seen in one experiment.

increased the nonselective background binding of radiolabeled chemokines, primarily to the extracellular matrix.

Cross-Competition Binding Studies

In these studies, the ability of unlabeled RANTES to compete for binding of radiolabeled IL-8, MCP-1, MCP-3, and MIP-1α and *vice versa* was assessed. IL-8 binding to the ECs was strongly reduced but not blocked completely in the presence of unlabeled RANTES (Figure 6A); in contrast, unlabeled IL-8 entirely removed RANTES from its venular EC binding sites (Figure 6B). Venular EC binding of MCP-1 and MCP-3 was abolished by excess of RANTES (Figure 6C), and MCP-1 and MCP-3 competed efficiently with RANTES for its endothelial binding sites (Figure 6D). MIP-1α failed to inhibit the endothelial cell binding of RANTES but was able to interfere with its binding to dermal resident cells for which neither IL-8 nor MCP-1 or MCP-3 could compete (not shown). Conversely, the resident cell binding of MIP-1α was only decreased but not blocked in the presence of unlabeled RANTES (not shown). Binding of IL-8, MCP-1, and MCP-3 to resident cells was not affected by excess of RANTES (not shown). RANTES binding to lymphatic ECs could not be removed by IL-8 or MIP-1α but was competed off by MCP-1 and MCP-3 (Figure 5E). Accordingly, excess of RANTES competed efficiently for binding of MCP-1 and MCP-3 to the lymphatic endothelium (not shown).

Discussion

Our *in situ* binding studies demonstrate the presence of specific, saturable binding sites for the β-chemokines RANTES, MCP-1, and MCP-3 on ECs of PCVs and small veins in human skin and their absence from ECs of arteries and capillaries. PCVs are the segment of the circulatory tree where leukocytes have been shown to emigrate in skin and in other tissues. The selective binding of several β-chemokines and IL-8[5] to the venular ECs supports the concept that in the process of leukocyte emigration chemokines are transported to the luminal EC surface where they are presented to emigrating leukocytes not in a soluble form but immobilized by presentation molecules.[5,6,8] In contrast, we could not observe MIP-1α binding to the ECs *in situ*. This is confirmed by immunoelectron microscopic studies that also show the lack of MIP-1α localization to the ECs after its *ex vivo* injection into human skin.[8] The failure to observe MIP-1α binding to the ECs in the skin vasculature does not necessarily exclude a possible role of MIP-1α in leukocyte emigration. On one hand, in tissues other than skin the EC binding sites for MIP-1α could be expressed. On the other hand, it is possible that MIP-1α can participate in leukocyte emigration in a soluble rather than in an EC-bound form. In several anatomical sites, eg, lung alveoli, kidney glomeruli, and heart muscle, leukocytes may leave the circulation via the capillaries where there is no apparent need for the EC immobilization of chemokines.[11] In addition, MIP-1α could induce leukocyte emigration by stimulating the release of secondary chemotactic mediators from the resident macrophages or other cells.

The molecular nature of the chemokine binding sites on the ECs still needs to be clarified. As chemokines, similar to many other cytokines and growth factors, bind heparin and related molecules,[12,13] heparan sulfate proteoglycans, which are abundantly expressed on the ECs, have been suggested as possible chemokine anchoring molecules.[6,13] Another possible candidate molecule that could function as a receptor for chemokines on the ECs is the Duffy antigen/receptor for chemokines (DARC).[14–16] First described as a promiscuous chemokine receptor on erythrocytes,[14] this molecule has been demonstrated also on the ECs of brain, kidney, and spleen blood vessels.[15] DARC has been found to be unique among other chemokine receptors with seven transmembrane domains in that it is apparently not coupled to G-proteins and binds chemokines of both the α- and the β-family but not MIP-1α, MIP-1β, or the C-chemokine lymphotactin.[17] A third possible explanation for the EC binding of chemokines is the expression of the classical G-protein-coupled chemokine receptors; eg, PCVs display CXCR-2[18] and CCR-5 immunoreactivity (own unpublished observation).

Cross-competition studies using IL-8, RANTES, MCP-1, MCP-3, and MIP-1α provided important hints as to the specificity and, indirectly, the nature of the chemokine binding sites on the tissue cells. RANTES could compete with MCP-1 and MCP-3 for EC binding and *vice versa*, whereas MIP-1α failed to bind and to compete with the EC-bound RANTES. IL-8, a member of the α-chemokine family, inhibited the binding of RANTES to venular ECs, whereas *vice versa* inhibition was not complete. The lack of MIP-1α binding and the ability of chemokines to compete for EC binding across the chemokine subfamily barrier mirrors the chemokine binding characteristics of DARC.[17] Alternatively, the EC heparan sulfates and DARC could cooperate in binding chemokines. As RANTES, MCP-1, and MCP-3 can cross-compete efficiently for each others' venular endothelial binding sites, the involvement of one of the known β-chemokine receptors, eg, CCR-2[19] or CCR-5,[20] is not plausible, whereas

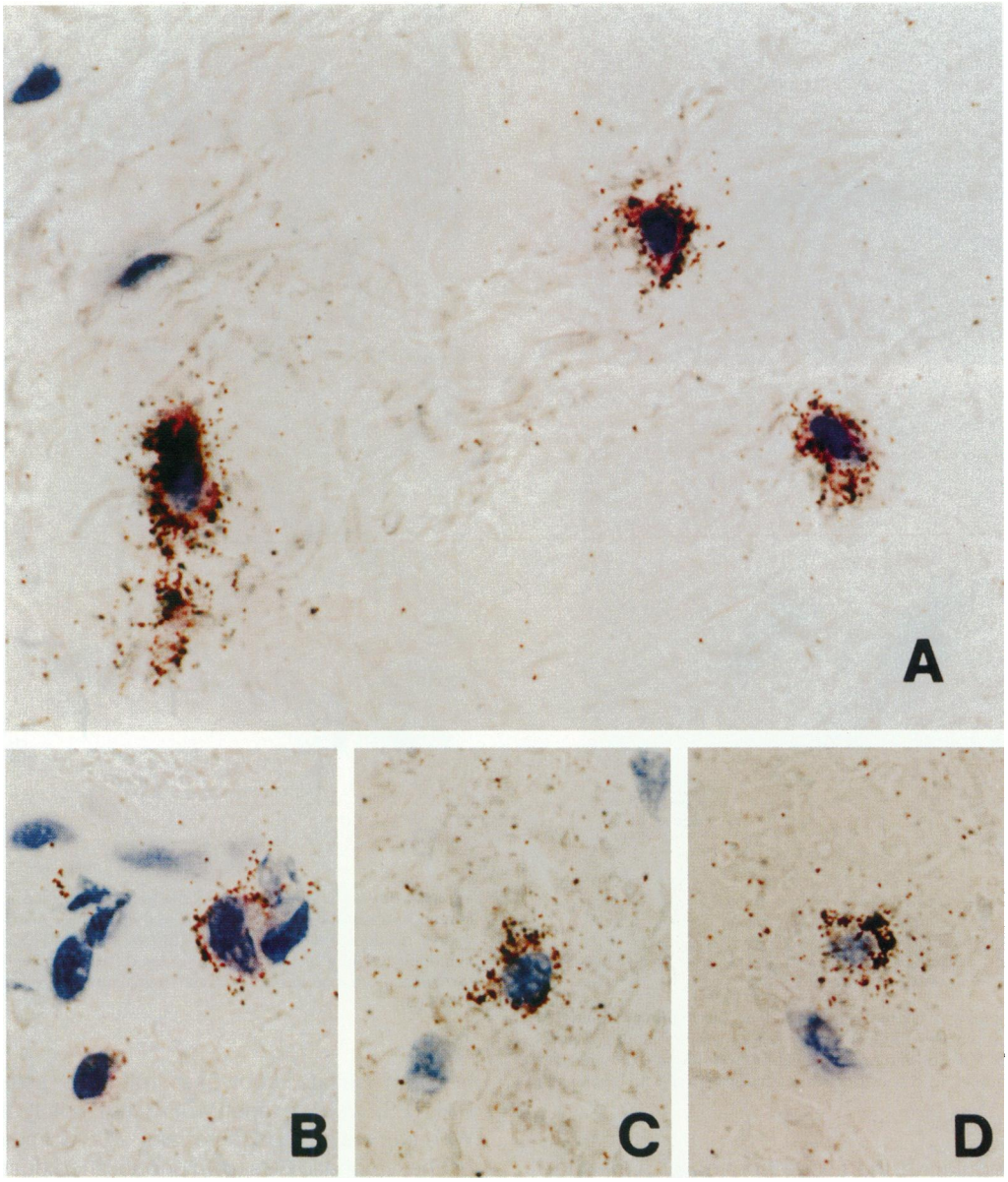

Figure 2. *In situ* binding of MIP-1α, RANTES, MCP-1, and MCP-3 to resident cells in human dermis. **A:** MIP-1α binds to resident macrophages in the dermis identified by immunostaining with an anti-HLA-DR antibody. **B** and **C:** RANTES (**B**) and MCP-1 (**C**) bind to dermal macrophages. **D:** *In situ* binding of MCP-3 to an HLA-DR-negative cell in the dermis. All counterstained with hemalaun; magnification, ×1125.

the residual IL-8 binding to the ECs, which could be seen in the presence of unlabeled RANTES, may be mediated by a low number of specific IL-8 receptors possibly expressed by the ECs, eg, CXCR-2.[18]

Additionally, RANTES, MIP-1α, and to a lesser extent also MCP-1 and MCP-3 bound to perivascular resident dermal cells, as previously described for IL-8.[5] Immunohistochemical techniques identified a part of the dermal chemokine-binding cells as macrophages. Only a low percentage of mast cells, identified by toluidine blue staining, expressed binding sites for RANTES, MIP-1α, and IL-8. Resident cells bound high amounts of MIP-1α, and the relative number of resident cells binding MIP-1α exceeded the number of cells binding IL-8, RANTES, MCP-1, or MCP-3. In the presence of an excess of unlabeled RANTES, binding of MIP-1α was reduced but not abolished, whereas MIP-1α was able to displace RANTES completely from its binding sites on dermal resident cells. This suggests that MIP-1α may bind to two distinct receptors; one is shared with RANTES, eg, CCR-1[21] or CCR-5,[20] whereas the other appears to be specific for MIP-1α. Also, we cannot exclude the possibility that MIP-1α, which is known to form aggregates,[22] is phagocytosed by macrophages and thus cannot be displaced by RANTES. However, as unlabeled MIP-1α competes almost completely for the binding of ^{125}I-MIP-1α, the latter possibility is unlikely. The function of receptors for MIP-1α and RANTES on resident dermal cells is not known. It is possible that MIP-1α and RANTES are

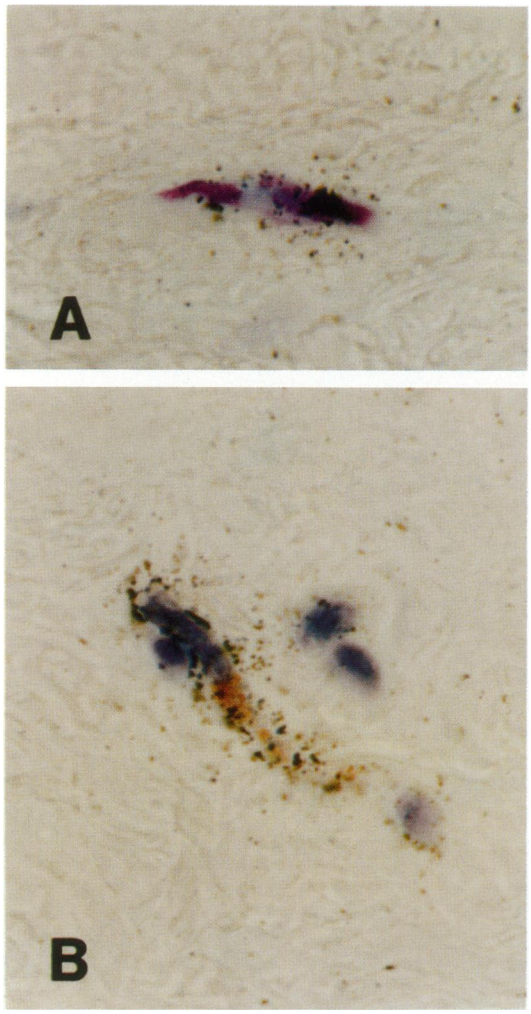

Figure 3. *In situ* binding of chemokines to resident cells in human dermis. **A:** RANTES binding to a mast cell identified by toluidine blue staining. Magnification, ×1125. **B:** MIP-1α binding to a melanophage identified by characteristic brown granules in the cytoplasm. Counterstained with hemalaun; magnification, ×1125.

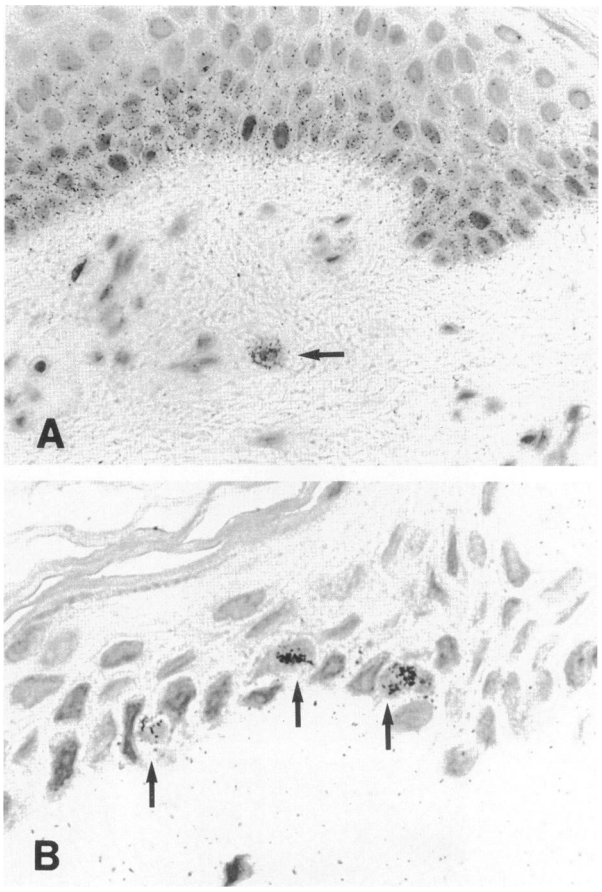

Figure 4. *In situ* binding of chemokines to human epidermis. **A:** IL-8 binds to keratinocytes in the basal and suprabasal epidermis. Also, IL-8 can be seen over a resident cell in the dermis (**arrow**). **B:** RANTES does not bind to keratinocytes but is localized to isolated cells in the lower epidermis (**arrows**). Both counterstained with hemalaun; magnification, ×310 **(A)**, ×775 **(B)**.

involved in regulating the tissue homing of bone-marrow-derived cell populations, ie, macrophages, mast cells, dendritic cells, etc.

Whereas it has already been known that keratinocytes express IL-8 receptors,[23] our *in situ* binding studies show that only the basal and suprabasal keratinocytes bind IL-8. This binding is most likely mediated via specific IL-8 receptors[18,23] because none of the β-chemokines is able to bind to keratinocytes and RANTES fails to compete with IL-8 for its keratinocyte binding sites.

The novel and most striking result of our current *in situ* binding study is the binding of the β-chemokines RANTES, MCP-1, and MCP-3 to the ECs of the afferent lymphatic vessels of the dermis. The failure of IL-8 to bind to lymphatic endothelium suggests that these binding sites are clearly distinct from those on the ECs of venules and are not identical to DARC. Also, heparan sulfate proteoglycans are not likely candidates. However, the binding affinity of chemokines to different heparan sulfate molecules may vary,[24] and therefore we cannot exclude completely the possibility that lymphatic ECs express heparan sulfate subtypes that can selectively bind RANTES, MCP-1, and MCP-3 but not IL-8 or MIP-1α. Whereas we can observe faint CCR-5 immunoreactivity on the lymphatic ECs (own unpublished observation), the *in situ* chemokine binding and cross-competition pattern excludes the significant involvement of this receptor or any other hitherto described β-chemokine receptor. However, it is possible that a yet unidentified G-protein-coupled receptor is responsible for the observed pattern of *in situ* chemokine binding.

So far we can only speculate on a possible function for chemokine binding sites on the ECs of lymphatic vessels in the dermis of the skin. It is well documented that T cells[25] and Langerhans' cells[26] migrate from skin to the regional draining lymph nodes via the afferent lymphatics, but not much is known about the mechanisms that regulate this migratory pathway. It is possible that RANTES, MCP-1, and MCP-3 immobilized on the lymphatic endothelium of the dermis direct the migration of cutaneous T lymphocytes and Langerhans' cells from the extravascular tissue across the lymphatic endothelial cell barrier. Recently, it has been reported that the chemokines MIP-1β and IL-8, derived from the afferent lymphatic, can be detected in the reticular fiber network of

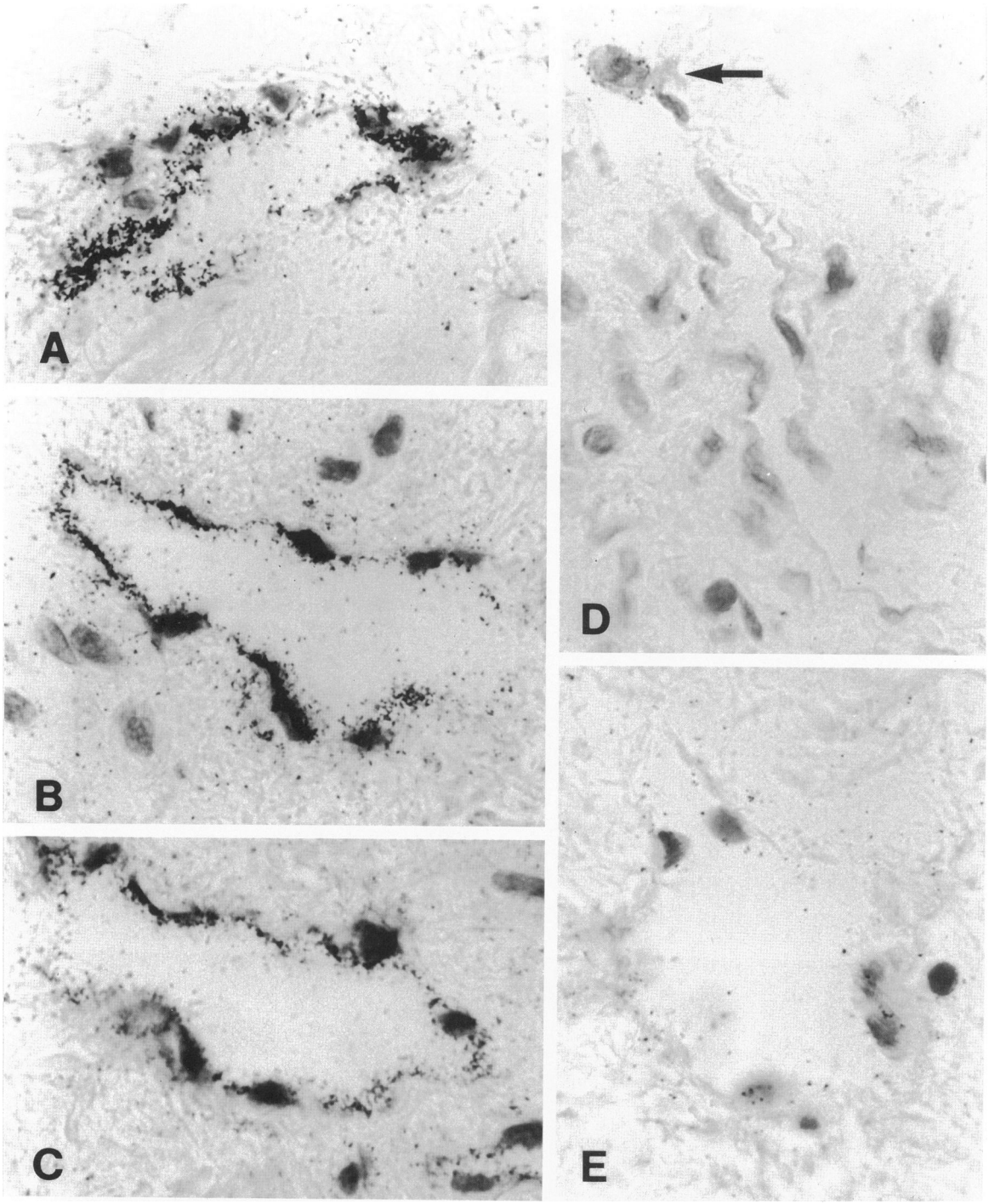

Figure 5. *In situ* binding of RANTES, MCP-1, MCP-3, and MIP-1α to lymphatic ECs in human dermis. **A** to **C**: RANTES (**A**), MCP-1 (**B**), and MCP-3 (**C**) bind to the ECs of lymphatic vessels. **D**: MIP-1α does not bind to the ECs of the lymphatic vessel but is seen bound to a resident cell in the extravascular space (**arrow**). **E**: *In situ* binding of RANTES is removed by 1000X excess of MCP-3. All counterstained with hemalaun; magnification, ×1125.

the lymph node.[27] It is possible that binding sites for the chemokines RANTES, MCP-1, and MCP-3 on the ECs of afferent lymphatic vessels serve to immobilize these chemokines and thus prevent them from being transported into the draining lymph node.

Although we consider the *in situ* binding assay used by us the best possible method to study the binding sites for chemokines or other small molecules in the intact tissues, it entails several pitfalls. There is no possibility to quantify the results and to standardize the number of radioactive grains, ie, the number of chemokine molecules, accumulated by the various cell types. Also, by using this assay it is not possible to obtain any information on the binding affinity or the number of binding sites involved. Therefore, a large number of radioactive grains, which has been inter-

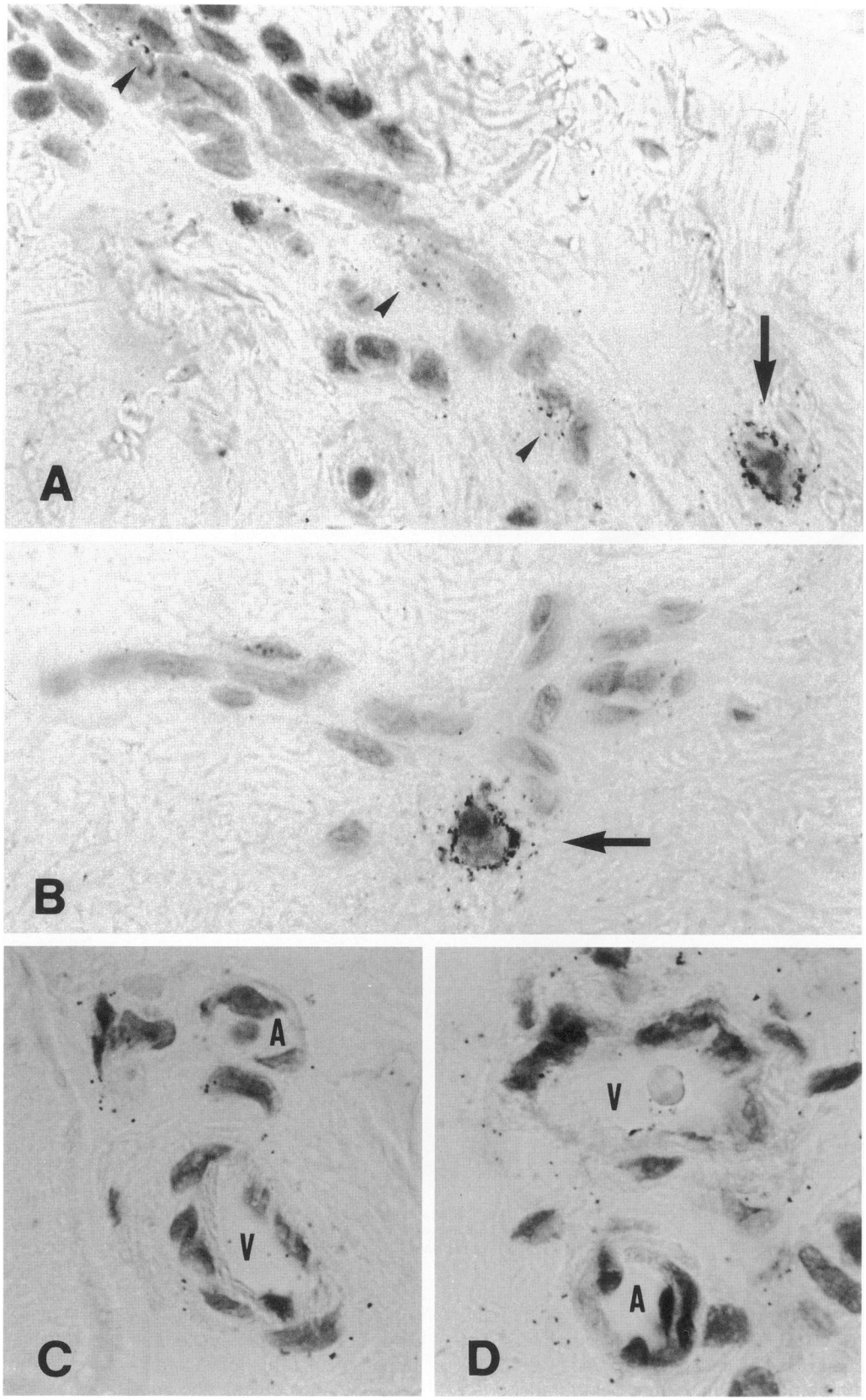

Figure 6. Chemokine cross-competition for binding sites on the venular ECs and resident cells in human dermis. **A:** In the presence of excess of unlabeled RANTES, IL-8 binding to the venular ECs is diminished but not entirely removed (**arrowheads**); conversely, IL-8 binding to the resident dermal cell (**arrow**) is unaffected. **B:** Unlabeled IL-8 completely removes RANTES from its binding sites on the venular ECs but not from its receptors on dermal resident cells (**arrow**). **C:** MCP-1 binding to venular ECs is blocked by excess of unlabeled RANTES. **D:** *Vice versa*, unlabeled MCP-1 competes with RANTES for its binding sites on the ECs. All counterstained with hemalaun, magnification, ×1040.

preted in the text as "high amounts of chemokine bound," could mean either that chemokines bind with high affinity or that the cells express a large number of chemokine binding sites.

In addition to the difficulties in obtaining the parameters of *in situ* binding, the ligand-receptor equilibrium may be biased by the restriction of the Brownian motion of the labeled ligand by the intact tissue studied. This factor imposes limitations on the use of the *in situ* binding assay with ligands larger than chemokines. In addition, the requirement for free diffusion of the molecules to and from the binding sites prohibits the use of high molecular weight blocking reagents, eg, antibodies against putative chemokine receptors. Anomalies in free diffusion of the ligands may have also influenced the results of our current experiments. We have encountered a tendency toward preferential accumulation of the radiolabeled β-chemokines at the edges of the pieces studied; this has not been seen before with IL-8.[5] It is possible that the diffusion of β-chemokines may have been hampered due to their known tendency to oligomerize.[22] Therefore, in the case of larger tissue pieces with signs of uneven distribution of grains, only binding to the peripheral parts was taken into account. For the same reason, the results of cross-competition assays could have been affected by insufficient diffusion of the unlabeled chemokine. Although unlikely, it is still possible that the iodination of the chemokine molecules affected their ability to oligomerize, thus allowing the radiolabeled chemokine molecules to diffuse more readily than the unlabeled ones. When excess of unlabeled chemokine was added to compete with the radiolabeled one, we could observe an increase in background binding, ie, more grains associated with extracellular matrix. This could reflect the oligomerization of labeled and unlabeled chemokines, which is enhanced by the proteoglycans present in the matrix. The resulting chemokine complexes may be resistant to a washing step.

In summary, our *in situ* binding studies show the presence of binding sites for IL-8, RANTES, MCP-1, and MCP-3 but not MIP-1α on venular ECs in the dermis and for RANTES, MCP-1, and MCP-3 on lymphatic ECs. Additional investigations will be necessary to identify the biochemical nature of the molecules expressed by these different EC types that are responsible for the binding. Also, as venular ECs seem to lack binding sites for MIP-1α, it would be interesting to investigate the mechanism by which MIP-1α can guide leukocyte recruitment into inflamed tissues.

Acknowledgment

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References

1. Baggiolini M, Dewald B, Moser M: Interleukin-8 and related chemotactic cytokines: CXC and CC chemokines. Adv Immunol 1994, 55:97–179
2. Schall TJ: The chemokines. The Cytokine Handbook, ed 2. Edited by Thomson AW. New York, Academic Press, 1994, pp 419–460
3. Schall TJ, Bacon KB: Chemokines, leukocyte trafficking, and inflammation. Curr Opin Immunol 1994, 6:865–873
4. Strieter RM, Standiford TJ, Huffnagle GB, Colletti LM, Lukacs NW, Kunkel SL: "The good, the bad, and the ugly": the role of chemokines in models of human disease. J Immunol 1996, 156:3583–3586
5. Rot A: Binding of neutrophil attractant/activating protein-1 (interleukin 8) to resident dermal cells. Cytokine 1992, 4:347–352
6. Rot A: Endothelial cell binding of NAP-1/IL-8: role in neutrophil emigration. Immunol Today 1992, 13:291–294
7. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994, 76:301–314
8. Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, Auer M, Hub E, Rot A: Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell 1997, 91:385–395
9. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U, Shaw S: T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1β. Nature 1993, 361:79–82
10. Wiedermann CJ, Kowald E, Reinisch N, Kaehler CM, von Luettichau I, Pattison JM, Huie P, Sibley RK, Nelson PJ, Krensky AM: Monocyte haptotaxis induced by the RANTES chemokine. Curr Biol 1993, 3:735–739
11. Hub E, Middleton J, Rot A: Mechanism of chemokine-induced leukocyte adhesion and emigration. Chemoattractant Ligands and Their Receptors. Edited by Horuk R. Boca Raton, FL, CRC Press, 1996, pp 301–325
12. Webb LMC, Ehrengruber MU, Clark-Lewis I, Baggiolini M, Rot A: Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. Proc Natl Acad Sci USA 1993, 90:7158–7162
13. Tanaka Y, Adams DH, Shaw S: Proteoglycans on endothelial cells present adhesion-inducing cytokines to leukocytes. Immunol Today 1993, 14:111–115
14. Neote K, Darbonne W, Ogez J, Horuk R, Schall TJ: Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. J Biol Chem 1993, 268:12247–12249
15. Hadley TJ, Lu Z, Wasniowska K, Martin AW, Peiper SC, Hesselgesser J, Horuk R: Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functionally identical to the erythroid isoform, which is the Duffy blood group antigen. J Clin Invest 1994, 94:985–991
16. Peiper SC, Wang Z, Neote K, Martin AW, Showell HJ, Conklyn MJ, Ogborne K, Hadley TJ, Lu Z, Hesselgesser J, Horuk R: The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy negative individuals who lack the erythrocyte receptor. J Exp Med 1995, 181:1311–1317
17. Szabo MC, Soo KS, Zlotnik A, Schall, TJ: Chemokine class differences in binding to the Duffy antigen-erythrocyte chemokine receptor. J Biol Chem 1995, 270:25348–25351
18. Nanney LB, Mueller SG, Bueno R, Peiper SC, Richmond A: Distribution of melanoma growth stimulatory activity of growth regulated gene and the interleukin-8 receptor B in human wound repair. Am J Pathol 1995, 147:1248–1260
19. Charo IF, Myers SJ, Herman A, Frenci C, Connolly AJ, Coughlin SR: Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxy-terminal tails. Proc Natl Acad Sci USA 1994, 91:2752–2756
21. Neote K, DiGregorio D, Mak JY, Horuk R, Schall TJ: Molecular cloning, functional expression and signaling characteristics of a C-C chemokine receptor. Cell 1993, 72:415–425
20. Combadiere C, Ahuja SK, Tiffany HL, Murphy PM: Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1α, MIP-1β, and RANTES. J Leukocyte Biol 1996, 60:147–152
22. Graham GJ, MacKenzie J, Lowe S, Tsang ML, Weatherbee JA, Issacson A, Medicherla J, Fang F, Wilkinson PC, Pragnell IB: Aggregation of the chemokine MIP-1α is a dynamic and reversible phenomenon. J Biol Chem 1994, 269:4974–4978
23. Kemény L, Ruzicka T, Dobozy A, Michel G: Role of interleukin-8 receptor in skin. Int Arch Allergy Immunol 1994, 104:317–322
24. Witt DP, Lander AD: Differential binding of chemokines to glycosaminoglycan subpopulations. Curr Biol 1994, 4:394–400
25. Butcher EC, Picker LJ: Lymphocyte homing and homeostasis. Science 1996, 272:60–66
26. Lukas M, Stössel H, Hefel L, Imamura S, Fritsch P, Sepp NT, Schuler G, Romani N: Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model. J Invest Dermatol 1996, 106:1293–1299
27. Gretz JE, Kaldjian EP, Anderson AO, Shaw S: Sophisticated strategies for information encounter in the lymph node. J Immunol 1996, 157:495–499