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Biochimica et Biophysica Acta 1510 (2001) 354–366

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Glycans are involved in RANTES binding to CCR5 positive as well as to CCR5 negative cells

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Received 13 September 2000; received in revised form 7 November 2000; accepted 8 November 2000

Abstract

We show that cell surface glycans, sialic acid and mannose-containing species, are involved beside glycosaminoglycans (GAGs), heparan sulfate and chondroitin sulfate in the binding of full length (1–68) RANTES not only to CCR5 positive human primary lymphocytes or macrophages but also to CCR5 negative monocytic U937 cells. Pretreating the cells with neuraminidase, heparitinase, chondroitinase or adding soluble glycans such as mannan or GAGs (heparin or chondroitin sulfate), significantly inhibited RANTES binding. Such effects were not observed with truncated (10–68) RANTES. Heat-denaturation of (1–68) RANTES strongly decreased its binding to the cells, demonstrating involvement of the three-dimensional structure. Accordingly, full length, but not truncated (10–68) RANTES, specifically bound to soluble mannan as well as to mannose-divinylsulfone-agarose affinity matrix and to soluble heparin or chondroitin sulfate as well as to heparin-agarose. Soluble heparin exerts, depending on its concentration, inhibitory or enhancing effects on RANTES binding to mannose-divinylsulfone-agarose, which indicates that RANTES interaction with glycans is modulated by GAGs. These data demonstrate that full length RANTES, but not its (10–68) truncated counterpart, interacts with glycans and GAGs, in soluble forms or presented either by affinity matrices or CCR5 positive as well as CCR5 negative cells.

Keywords: Regulated on activation normal T cell expressed and secreted; CCR5 positive cell; CCR5 negative cell; Glycan; Glycosaminoglycan; Heparin; Chondroitin sulfate; Mannan

1. Introduction

Infection of target cells by human immunodeficiency virus type 1 (HIV-1) is mediated by interactions of the HIV envelope glycoproteins with CD4 and a coreceptor [1–3]. The preferential tropism of different HIV strains for either macrophages or transformed $CD4^+$ cell lines is related to their use of different coreceptors [1–3]. The major coreceptor of X4 HIV strains is CXCR4 [1,4–6], the physiological ligand of which is α chemokine stromal-cell-derived factor (SDF)-1α [4,5]. CXCR4, also present on macrophages [7], is not used for entry by R5 strains that use CCR5, the receptor of β chemokines RANTES (regulated on activation normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α and MIP-1β [8,9]. The ligands of

Abbreviations: RANTES, regulated on activation normal T cell expressed and secreted; GAG, glycosaminoglycan; PL, peripheral lymphocytes; MDM, monocyte-derived macrophages; SDF, stromal-cell-derived factor; HIV, human immunodeficiency virus; MIP, macrophage inflammatory protein; C_{50}, 50% inhibiting concentration

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0005-2736/01/$ – see front matter
PII: S0005-2736(00)00368-0

these receptors can modify, in vitro, HIV-1 infection in several ways, the most commonly observed being inhibition of virus entry because of competition between the virus and the chemokine for binding sites on the same receptor [9–11]. While some chemokines have been shown to bind to glycosaminoglycans (GAGs) [12], the role of GAGs binding properties on receptor binding or signaling has not been clearly established [12–14]. However, after enzyme digestion of their membrane GAGs, some cell lines or human primary macrophages are resistant to the antiviral effects of RANTES and MIP-1β for R5 HIV strains [10,15]. There is some controversy concerning the quaternary structure of biologically active chemokines when they bind to receptors, with evidence to suggest either monomers, dimers or higher molecular weight associations [16,17]. Not all chemokines self-associate, leading to the question of the biological significance of such an association. RANTES has been shown to self-associate [16,17] and to inhibit [15,18] or increase [19] in vitro HIV infection. It has been reported that one mechanism of infectivity enhancement may be caused by cross-linking of HIV virions to cell surfaces by oligomers of the RANTES form after binding to GAGs [19] and that a second mechanism of viral infectivity enhancement arises from the prolonged interaction of RANTES oligomers with cell surface GAGs, which activates a herbimycin A-sensitive, tyrosine-kinase-dependent signal transduction [19]. In addition, it has been demonstrated that aggregation of μM concentrations of RANTES is responsible for its inflammatory properties [16]. However, RANTES serum levels are respectively 2.2 nM in healthy HIV seronegative patients and 3.5 nM in HIV-infected patients [20].

The aim of this study was to test whether RANTES, at nM concentrations, close to those observed in human sera, interacts, like SDF-1 [21] and gp120 of X4 strain HIV-1lai [22–26], not only with soluble or cellular GAGs, as previously described [10,14,15], but also with soluble or cellular glycans presented by CCR5 negative (CCR5−) or CCR5 positive (CCR5+) cells. The behavior of synthetic full length (1–68) RANTES was compared with that of truncated (10–68) RANTES in order to determine the role of the chemokine N-terminus domain on its carbohydrate-binding properties.

2. Materials and methods

2.1. (1–68) and (10–68) RANTES radiolabeling

Synthetic (1–68) and (10–68) RANTES (gifts from C. Vita, CEA, Saclay, France) were synthesized on a fully automatic peptide synthesizer (Applied Biosystems Model 433, Foster City, CA, USA) on a mmol scale according to the FastMoc protocol [27]. Purity was tested by analytical HPLC and capillary electrophoresis. Identity of the purified products was verified by amino acid analysis and mass spectrometry. It was previously reported that the binding affinities of synthetic RANTES for CD4+ CCR5+ cells were similar to those observed for recombinant RANTES [28]. In the present study, synthetic (1–68) and (10–68) RANTES were radiolabeled with iodobeads (Pierce Europe, BA, Oud-Beijerland, The Netherlands) according to the manufacturer's instructions. Specific activities were about 0.2–0.7 MBq/μg. Iodide-labeled (1–68) RANTES was analyzed by electrophoresis on polyacrylamide gels (16%), in native (PAGE) and in denaturing and reducing conditions (SDS-PAGE).

2.2. Cells

CCR5− CD4+ CXCR4+ cells of the monocytic U937 line were cultured at 37°C in RPMI 1640, 10% heat-inactivated fetal calf serum (FCS, Bio-Whittaker, Emarainville, France), 1% penicillin/streptomycin, 1% L-glutamine (Gibco BRL, Paisley, UK).

To prepare peripheral lymphocytes (PL) and MDM, Ficoll–Hypaque-separated blood mononuclear cells (PBMC) of healthy volunteers (Seine-Saint-Denis or Pitié-Salpêtrière Blood Banks) were cultured at $2–5\times10^6$ cells/ml for 5 days, in RPMI 1640, 1% penicillin/streptomycin, 1% L-glutamine (Gibco BRL), 20% FCS (Boehringer Mannheim, Germany), 10% heat-inactivated normal human pooled AB serum (Seine Saint-Denis blood bank) [15,18,29]. Nonadherent cells which represent more than 90% T or B CD3+ or CD19+ lymphocytes, as assessed by immunofluorescent labeling with FITC-conjugated mAb anti-CD3 and anti-CD19 mAb (Becton Dickinson Immunocytometry, Mountain View, CA, USA), followed by flow cytometry analy-

sis with a FACScan (Becton Dickinson Immunocytometry), were then removed by several washes in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS).

Adherent cells were cultured in the same medium without AB serum, but with 20% FCS for another 24–48 h, before they were subjected to binding experiments as adherent cells or scraped off with a rubber policeman. This procedure yielded >90% CD14+ viable MDM that expressed CCR5 and low membrane CD4 as reported [15,18,29]. No contaminating T cells were noted under these conditions (data not shown).

The cells (5×10^5 in 100 μl PBS, 0.05% BSA, 0.05% sodium azide) (PBS-BSA-A) were incubated for 30 min at 4°C with 2.5 μg mAb anti-CCR5, specific for the second extracellular loop (2D7) or with 2.5 μg anti-CXCR4 mAb12G5 (both from Pharmingen, San Diego, CA, USA) or with 2.5 μg of the isotype control (IgG2a; Sigma). Cells were then washed and incubated for 30 min at 4°C in 100 μl of FITC-labeled anti-mouse immunoglobulin goat antibodies (Coulter Coultronics, France) diluted 1/20 in buffer. In parallel, cells were incubated for 30 min at 4°C in PBS-BSA-A with 1 μg FITC-CD4 (clone 13B.8.2) or its isotype control (1 μg; IgG1-FITC) both from Immunotech, Marseille, France or with FITC-labeled specific lectins (0.75–1 μg/ml; RCA120 specific for β-galactosyl, erythrina cristagalli specific for Galβ1–4GlcNAc, lathyrus odoratus specific for α-mannose and GlcNAc and concanavalin A specific for α-mannose and α-glucose (all from Sigma or Boehringer, Mannheim, Germany). After three washes at 4°C, cells were fixed in 1% paraformaldehyde (Sigma) in PBS (500 μl) and analyzed by flow cytometry. Data were analyzed using CELL Lysis (Becton Dickinson Immunocytometry).

2.3. Enzyme treatment

An amount of 5×10^5 cells was incubated for 1 h at 37°C in 50 μl PBS, 0.05% BSA, and 1 mU heparitinase (heparitin sulfate eliminase, EC 4.2.2.8) or chondroitinase (chondroitinase ABC eliminase, EC 4.2.2.4), both from Sigma (St Louis, MO, USA) or 2 mU neuraminidase (Boehringer, Mannheim, Germany) supplemented or not with protease inhibitors (10 mM PMSF, 25 mM phenanthroline, 5 mM iodoacetamide, 100 μg/ml aprotinin, all from Sigma). The enzymes were not toxic for the cells, as assessed by trypan blue exclusion.

2.4. (1–68) and (10–68) RANTES binding to the cells

Cells (5×10^5) in 100 μl RPMI 1640, 0.05% BSA (RPMI-BSA) were incubated for 2 h at 4°C with 50 μl of native or heat-treated (10 min at 100°C, in the presence of 0.3% β-mercaptoethanol) [^{125}I](1–68) or [^{125}I] (10–68) RANTES (0.5×10^5 cpm; 3×10^{-9} M). After two washes ($700\times g$, 10 min, 4°C), cell-bound radioactivity was measured with a γ counter (LKB, Paris, France). The effect of heparitinase, chondroitinase or neuraminidase on native [^{125}I](1–68) RANTES binding to the cells was determined. In parallel, the effect of heparin (sodium salt, from porcine intestinal mucosa), mannan (from *Saccharomyces cerevisiae*), chondroitin sulfate (70% chondroitin sulfate A; sodium salt from bovine trachea) or dextran (up to 330 μg/ml; all from Sigma), or a mix of these compounds, on native [^{125}I](1–68) or [^{125}I] (10–68) RANTES binding to the cells was examined, at different concentrations that did not alter cell viability, as follows: [^{125}I] RANTES in RPMI-BSA was incubated for 60 min at 37°C with different concentrations of heparin, chondroitin sulfate, mannan or dextran, and the mixture was then added to the cells and incubated as described above. In some experiments, mixes of these compounds, were incubated with native [^{125}I](1–68) RANTES. In some experiments, cells were preincubated for 45 min at 4°C with anti-CCR5 mAb 2D7 or its isotype control (IgG2a) (both at 25 μg/ml), and the mixture was incubated with native [^{125}I](1–68) RANTES as described above.

2.5. (1–68) or (10–68) RANTES binding to heparin or mannose either in solution or presented by heparin-agarose or mannose-divinylsulfone-agarose

Binding of native or heat-treated (10 min at 100°C, in the presence of 1% SDS, 5% β-mercaptoethanol) [^{125}I](1–68) or [^{125}I] (10–68) RANTES (0–12 nM) to heparin-agarose (Sigma) or to mannose-divinylsulfone-agarose (E.Y Laboratories, San Mateo, CA,

USA) was investigated as follows: 20 μl of affinity matrix were suspended in an equal volume of buffer 0.02 M Tris, 0.15 M NaCl, 0.01 M $CaCl_2$, 0.05% BSA, pH 7.4 (Tris–Ca–BSA). After incubation for 1 h at 37°C, unbound RANTES was washed out twice in 500 μl of buffer. Solid phase-bound radioactivity was counted, and the results were expressed as means of duplicates of at least three independent experiments. The physicochemical characteristics of the interaction were analyzed by performing the assays under different conditions: [^{125}I] RANTES diluted in 50 μl of buffer was incubated with 20 μl affinity matrix in 0.02 M Tris, 0.15 M NaCl, 0.05% BSA buffer (Tris–BSA), pH 7.4, supplemented by 0–0.01 M $CaCl_2$ or by 0.01 M disodium salt EDTA (Sigma); or the experiments were conducted in Tris–Ca–BSA at 4, 20 or 37°C, or at different pH ranging from 4 to 8.2. In parallel, native [^{125}I](1–68) or [^{125}I] (10–68) RANTES was preincubated for 45 min at 37°C with 0–330 μg/ml heparin, chondroitin sulfate, mannan or dextran (all from Sigma), before adding the affinity matrix (20 μl). Coincubation was then performed as above. Unbound [^{125}I] RANTES was washed out and bound radioactivity was counted.

Reversal of RANTES binding to heparin-agarose or mannose-divinylsulfone-agarose was investigated as follows: 250 μl of [^{125}I](1–68) RANTES (3–9 nM) were incubated for 1 h at 37°C with 250 μl of affinity matrices in Tris–Ca–BSA. Unbound [^{125}I]-(1–68) RANTES was washed out thrice in 1 ml of buffer, and the matrix was incubated at 20°C for 5 h with gentle shaking in 250 μl buffer supplemented or not with heparin, mannan dextran or methyl-α-mannose (5 mg/ml). Unbound and specifically reversed molecules were then analyzed by SDS-PAGE (16%) in native or denaturing conditions. In some experiments, native [^{125}I](1–68) RANTES (3–9 nM) was preincubated for 1 h at 37°C in Tris–BSA, Tris–EDTA, Tris–BSA supplemented with 2.5 or 10 mM $CaCl_2$, in PBS, or in Tris–Ca–BSA supplemented with mannan, heparin or dextran (all at 330 μg/ml) and analyzed by PAGE (16%) in native conditions.

2.6. (1–68) RANTES binding to dotted mannan

Mannan or dextran (5 μg) were dotted onto nitrocellulose strips; strips were then incubated with 10^6 cpm of [^{125}I](1–68) RANTES for 2 h at 37°C. After six washes, strips were exposed at −20°C for 48 h. Alternatively, [^{125}I](1–68) RANTES was preincubated for 45 min at 37°C with 5 mg/ml of mannan, heparin, or dextran before incubation with the strips, during which the compounds were maintained at the initial concentration.

3. Results

3.1. Cell surface glycans and GAGs mediate (1–68) RANTES binding to CCR5+ primary lymphocytes, macrophages and CCR5− monocytic U937 cells

In the present study, no exogenous cytokine was added, but the monocyte-derived macrophages (MDM), were first cultured in the presence of autologous lymphocytes, which may release endogenous cytokines, before lymphocytes were carefully washed away. During cell coculture, the MDM may also release endogenous cytokines. In these conditions, 45–60% of the primary lymphocytes (PL) clearly expressed CD4, while a low amount of CD4 was detected on all the MDM (Fig. 1); all the PL and MDM expressed CCR5 and CXCR4, while the U937 cells expressed CD4 and CXCR4, but not CCR5 (Fig. 1).

We next examined whether full length [^{125}I](1–68) RANTES bound to CCR5− U937 cells as well as to CCR5+ PL and MDM.

[^{125}I](1–68) RANTES bound in a dose-dependent manner to each cell type; the binding was significantly inhibited by heat-treatment of the chemokine, demonstrating the involvement of RANTES three-dimensional structure (Table 1 and data not shown). We have already reported [18] that in agreement with Ben Baruch et al., [30], a 10-fold molar excess of cold (1–68) RANTES only partially competed with the binding of [^{125}I](1–68) RANTES to MDM and that a 100–250-fold excess of cold (1–68) RANTES led to increased binding of [^{125}I](1–68) RANTES, which was related to aggregates of cold and [^{125}I](1–68) RANTES. Therefore, in the present study we considered that non-specific [^{125}I](1–68) RANTES binding to the cells was that of the heat-denatured [^{125}I]-(1–68) chemokine, which was a mean of 21, 47 and

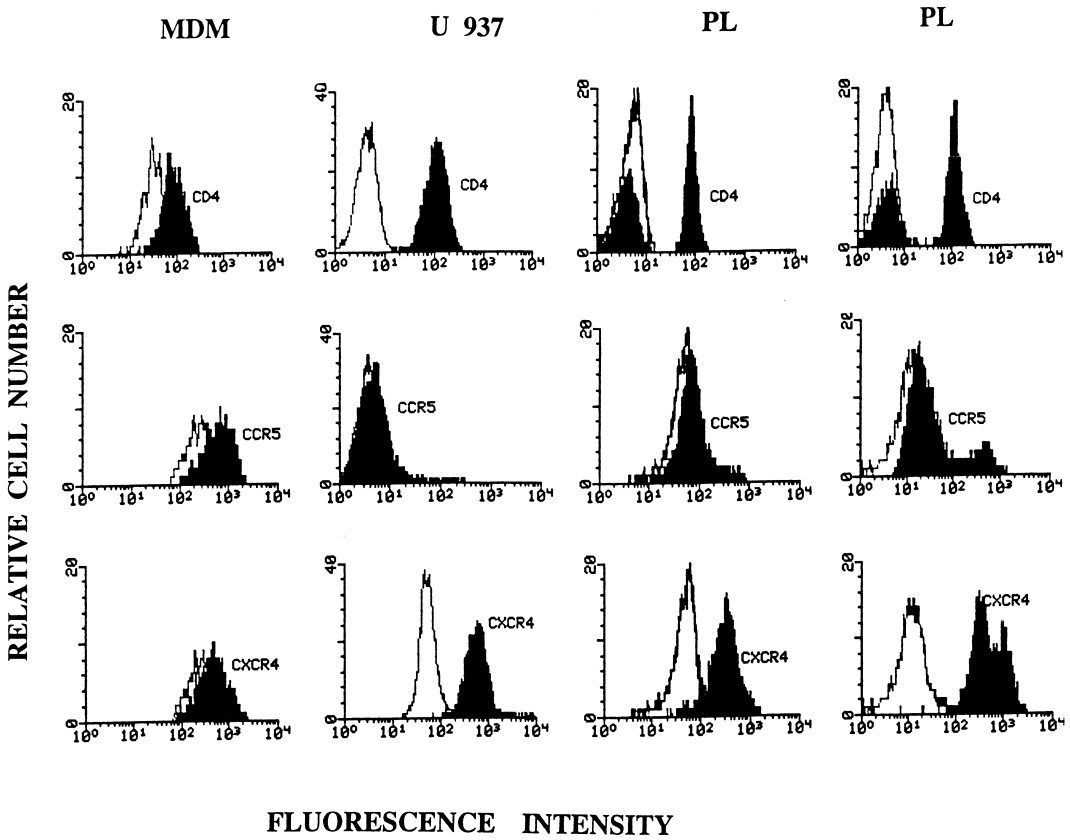

Fig. 1. MDM, U937 cells and PL and membrane expression of CD4 and chemokine receptors. MDM, PL and U937 cells were labeled with CD4 (by direct immunofluorescence assay), CCR5, and CXCR4 mAb (by indirect immunofluorescence assay) (black peaks) or with isotype control antibodies (white peaks). Data are representative of two to four different experiments.

5% of the total bound radioactivity by respectively PL, MDM and U937 cells (Table 1).

[^{125}I](1–68) RANTES binding to primary CCR5+ PL and CCR5+ MDM, but not as expected to CCR5− U937 cells, was significantly, inhibited by anti-CCR5 mAb, 2D7, specific for the second extracellular loop (ECL2) (Table 1); this indicates, as expected, CCR5 involvement in RANTES attachment to the CCR5+ cells.

We next analyzed the role of cellular glycans and GAGs in (1–68) RANTES binding to the cells. For this purpose, the cells were pretreated by neuraminidase, heparitinase or chondroitinase. Neuraminidase treatment increased their binding to FITC-labeled RCA and erythrina cristagalli, specific for β-galactose residues, demonstrating efficiency of this enzyme treatment (data not shown). The efficiency of heparitinase or chondroitinase treatment was previously tested by a metabolic labeling during a 20 h culture in a medium supplemented with [^{35}S]0_4 [21].

In the present study, we observed that not only heparitinase but also chondroitinase or neuraminidase pretreatment of the cells, partially, but significantly inhibited [^{125}I](1–68) RANTES binding (Table 1). These data indicate that (1–68) RANTES attachment to CCR5+ PL, CCR5+ MDM and also to CCR5− U937 cells occurs not only through heparan sulfate but also through chondroitin sulfate and sialic acid-containing glycans.

Alternatively, we examined whether soluble glycans as well as soluble GAGs (heparin or chondroitin sulfate) competed with (1–68) RANTES binding not only to CCR5+ cells but also to CCR5− cells. We first observed that [^{125}I](1–68) RANTES binding to the cells was partially but significantly inhibited not only by heparin but also by chondroitin sulfate; in addition, soluble mannan had also a significant inhibitory effect, with indicates that mannose-containing species were also involved in this interaction (Tables 2 and 3). These inhibitions were dose-depen-

Table 1
Effect of anti-CCR5 2D7 mAb, heparitinase, chondroitinase and neuraminidase treatment on [^{125}I](1–68)RANTES binding to primary lymphocytes, monocyte-derived macrophages and U937 cells

	Lymphocytes		Macrophages		U937 cells	
	Total bound radioactivity (cpm)	Inhibition of binding (%)	Total radio-activity (cpm)	Inhibition of binding (%)	Total bound radioactivity	Inhibition of binding (%)
Native [^{125}I]RANTES	9620 ± 1320		7570 ± 2600		11500 ± 3000	
Anti-CCR5 2D7 mAb	4320 ± 1000*	55 ± 10	4010 ± 1000*	47 ± 12	11500 ± 2000	0
IgG2a	9600 ± 1200	0	7500 ± 1500	0	11500 ± 2000	0
Heparitinase	5715 ± 1320***	41 ± 8	5730 ± 1350*	22 ± 6	6740 ± 3000***	45 ± 17
Chondroitinase	6470 ± 880**	31 ± 15	5600 ± 1600****	25 ± 5	7200 ± 2700**	35 ± 5
Neuraminidase	6010 ± 1000*	43 ± 6	4580 ± 2500***	48 ± 17	6020 ± 2000*	42 ± 25
Heat-treated [^{125}I]RANTES	2020 ± 800*	79 ± 8	3558 ± 1000*	53 ± 10	600 ± 150***	95 ± 2

Native or heat-treated (10 min at 100°C) [^{125}I](1–68) RANTES (3 nM; 50000 cpm), was incubated for 2 h at 4°C with the cells. In parallel, the cells were preincubated for 1 h at 4°C in the presence or the absence of anti-CCR5 2D7 mAb or the isotype control, IgG2a (2.5 μg, each), mixes were then incubated, as described above, with native [^{125}I](1–68) RANTES. Enzyme-treated or untreated cells were incubated with native [^{125}](1–68) RANTES for 2 h at 4°C. Results are means ± S.D. of three to eight independent experiments performed in duplicate. The statistical significance of differences relative to controls was determined by the paired Student's *t*-test $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.02$.

dent (data not shown). Concentrations for maximum inhibitions were respectively 0.3–3 μg/ml for the CCR5+ PL, 33 μg/ml for the CCR5+ MDM and 330 μg/ml for the CCR5− U937 cells, which demonstrates that higher concentrations of soluble GAGs or glycans are required to inhibit RANTES binding to the CCR5− U937 cells as compared to the CCR5+ MDM or the CCR5+ PL. Dextran had no significant effect, except for binding to PL (Tables 3 and 4). Most of these effects were additive (Tables 2 and 3).

These data strongly indicate that beside heparin, another GAG-chondroitin sulfate as well as glycans, have a role in (1–68) RANTES attachment not only to CCR5+ cells but also to CCR5− cells.

However, the fact that soluble heparin, chondroi-

Table 2
Effect of soluble mannan, heparin, chondroitin sulfate or mixes of these compounds on [^{125}I](1–68) RANTES binding to primary lymphocytes and monocyte-derived macrophages

	Lymphocytes		Macrophages	
	Total bound radioactivity (cpm)	Inhibition of binding (%)	Total bound radioactivity (cpm)	Inhibition of binding (%)
Control	7500 ± 1600		5810 ± 1550	
Heparin	4100 ± 940***	45 ± 5	3230 ± 740**	42 ± 13
Dextran	5100 ± 1380*	32 ± 7	7340 ± 3000	8 ± 8
Mannan	4800 ± 1100***	35 ± 11	4240 ± 1800*	32 ± 20
Chondroitin sulfate	5780 ± 1010**	27 ± 13	4096 ± 1088***	30 ± 3
Heparin+chondroitin sulfate	3000 ± 900*	60 ± 7	1900 ± 900*	67 ± 16
Heparin+mannan	2400 ± 800*	68 ± 14	1700 ± 400*	71 ± 4
Mannan+chondroitin sulfate	3500 ± 200*	54 ± 3	2320 ± 50**	60 ± 1
Heparin+chondroitin sulfate+mannan	2200 ± 200*	71 ± 4	1370 ± 400*	77 ± 12

Native [^{125}I](1–68) RANTES (3 nM; 50000 cpm) (control) was incubated for 1 h at 37°C with 0–330 μg/ml of heparin, dextran, mannan or chondroitin sulfate or mixtures of these compounds. The mixture was added to the cells, and incubated for 2 h at 4°C. Concentrations for maximum inhibitions were 0.3–3 μg/ml for lymphocytes and 33 μg/ml for macrophages. Results are presented as means ± S.D. of the maximum inhibition observed in four to nine independent experiments performed in duplicate. The statistical significance of differences relative to controls was determined by the paired Student's *t*-test: $*P < 0.02$; $**P < 0.01$; $***P < 0.001$.

Table 3
Effect of mannan, heparin, chondroitin sulfate or mixtures of these compounds on [^{125}I](1–68) and (10–68) RANTES binding to U937 cells

	[^{125}I](1–68) RANTES		[^{125}I](10–68) RANTES	
	Total bound radioactivity (cpm)	Inhibition of binding (%)	Total bound radioactivity (cpm)	Inhibition of binding (%)
Control	5230 ± 1500		2190 ± 440	
Heparin	2290 ± 780**	56 ± 12	1825 ± 410	17 ± 10
Dextran	4960 ± 2350	11 ± 4	2250 ± 605	0
Mannan	3460 ± 350*	24 ± 9	3110 ± 1290	0
Chondroitin sulfate	3880 ± 2150*	28 ± 20	3480 ± 605	0
Heparin+mannan	1950 ± 600**	63 ± 14		
Chondroitin sulfate+mannan	2300 ± 700**	57 ± 14		
Heparin+chondroitin sulfate	2300 ± 650**	55 ± 14		
Heparin+chondroitin sulfate+mannan	1800 ± 600**	67 ± 15		

[^{125}I](1–68) or (10–68) RANTES (3 nM; 30 000–50 000 cpm) was incubated for 1 h at 37°C with 0–330 μg/ml of heparin, dextran, mannan or chondroitin sulfate or mixtures of these compounds. The mixture was added to the cells, and incubated for 2 h at 4°C. Results are presented as means ± S.D. of the maximum inhibition observed in three to five independent experiments performed in duplicate. The C_{50} of heparin was 160 μg/ml. The statistical significance of differences relative to controls was determined by the paired Student's *t*-test: $*P < 0.05$; $**P < 0.01$.

tin sulfate or mannan did not significantly compete with the binding of radiolabeled truncated (10–68) RANTES to the CCR5− U937 cells or the CCR5+ PL strongly indicates the involvement of the N-terminus domain of RANTES in its interactions with soluble or cellular GAGs and glycans (Table 3 and data not shown).

Table 4
[^{125}I](1–68) RANTES or [^{125}I](10–68) RANTES binding to heparin-agarose or mannose-divinylsulfone-agarose

	(1–68) RANTES		(10–68) RANTES	
	Total bound radioactivity (cpm).	Inhibition of binding (%)	Total bound radioactivity (cpm)	Inhibition of binding (%)
Heparin-agarose				
Control	8130 ± 3500		8010 ± 1945	
Heparin	4075 ± 2700**	53 ± 16	6225 ± 1210**	21 ± 8
Chondroitin sulfate	10325 ± 4500	0		
Dextran	8560 ± 4000	0	7600 ± 1940	2 ± 2
Mannose-divinylsulfone-agarose				
Control	27750 ± 5675		7810 ± 1905	
Mannan	7415 ± 1450****	73 ± 3[a]	6100 ± 1280*	21 ± 5
Heparin	8750 ± 730***	70 ± 4[b]	5140 ± 1280*	29 ± 8
Chondroitin sulfate	9160 ± 1640***	68 ± 11	8930 ± 3855	0
Dextran	25935 ± 4050	0	7280 ± 2270	3 ± 3

[^{125}I](1–68) RANTES or [^{125}I](10–68) RANTES (3–6 nM; 50 000–100 000 cpm) was incubated for 1 h at 37°C with heparin, mannan, chondroitin sulfate or dextran, and the mixture was then added to the matrix. Results are presented as means ± S.D. of three to nine experiments performed in duplicate. Concentrations for maximum inhibition are 330 μg/ml. The statistical significance of differences relative to controls was determined by the paired Student's *t*-test: $*P < 0.05$; $**P < 0,02$; $***P < 0.01$; $****P < 0.001$.
[a] $C_{50} = 18$ μg/ml.
[b] $C_{50} = 160$ μg/ml.

3.2. Binding of (1–68) RANTES to heparin-agarose or mannose-divinylsulfone agarose

According to these findings, we next examined whether full length (1–68) RANTES interacts with heparin or mannose either in solution or presented by affinity matrices, heparin-agarose or mannose-divinylsulfone-agarose. [^{125}I](1–68) RANTES bound in a dose-dependent manner not only to heparin-agarose, as expected, but also to mannose-divinylsulfone-agarose (Figs. 2a and 3a). The chemokine heat-denaturation strongly and significantly decreased these bindings, which indicates involvement of its three-dimensional structure (Figs. 2e and 3e). The percent of inhibition induced by the chemokine heat-treatment in the presence of SDS and β-mercaptoethanol were respectively 90 ± 2 and 87 ± 3 for heparin-agarose and mannose-divinylsulfone-agarose ($P < 0.001$ and < 0.01; $n = 4$). As aggregation of RANTES has been reported [30], non-specific [^{125}I](1–68) RANTES binding to the matrices was therefore considered here as the binding of heat-treated [^{125}I](1–68) RANTES in the presence of SDS and β-mercaptoethanol which was respectively 6–12% for heparin-agarose and 6–16% for mannose-divinylsulfone-agarose of the total bound radioactivity (Figs. 2e and 3e). Binding to mannose-divinylsulfone-agarose was strongly dependent on temperature, while binding to heparin-agarose was only slightly increased at 37°C as compared to 4 and 20°C (Figs. 2b and 3b). Both bindings were strongly pH-dependent, increasing from pH 7 up to 7.8, with a maximum at pH 7.8, for mannose-divinylsulfone-agarose and from pH 5 to 8.2 for heparin-agarose (Figs. 2c and 3c). Binding to heparin-agarose was Ca^{2+}-independent (Fig. 2d) and was not prevented when EDTA was added before initiation of the reaction (data not shown); on the contrary, binding to mannose-divinylsulfone-agarose was strongly Ca^{2+}-dependent, prevented by EDTA added before initiation of the reaction (Fig. 3d). This calcium depen-

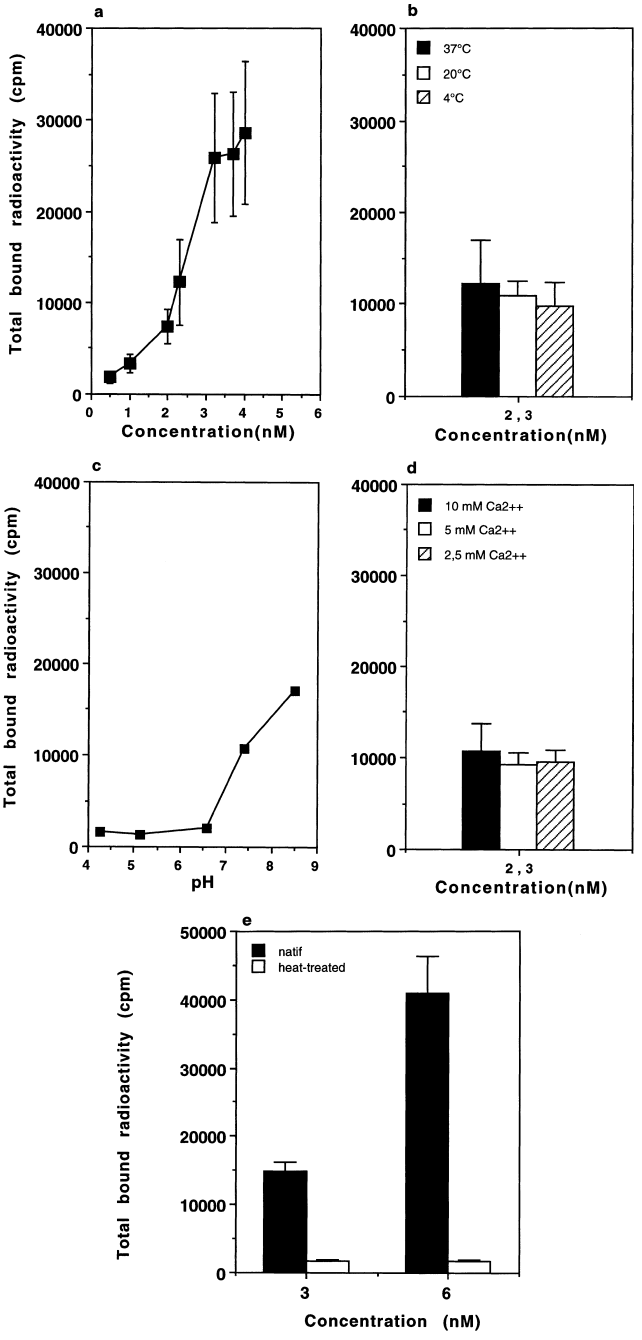

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Fig. 2. Influence of physicochemical conditions on the binding of full length (1–68) RANTES to heparin-agarose. (a) RANTES concentration: [^{125}I](1–68) RANTES was incubated for 1 h at 37°C with 20 μl of matrix in Tris–Ca–BSA (pH 7.4). (b) Temperature: [^{125}I](1–68) RANTES was incubated with 20 μl of heparin-agarose at 4°C, 20°C and 37°C. (c) Influence of pH: 3 nM [^{125}I](1–68) RANTES were incubated with 20 μl of heparin-agarose at pH ranging from 4 to 8.2. (d) Influence of Ca^{2+} concentration: 2.3 nM [^{125}I](1–68) RANTES was incubated with 20 μl of heparin-agarose in Tris–BSA in the presence of 2.5, 5 or 10 mM Cl_2Ca. (e) Influence of heat-treatment of [^{125}I](1–68) RANTES. Results are representative as means ± S.D. of three to four independent experiments, each performed in duplicate.

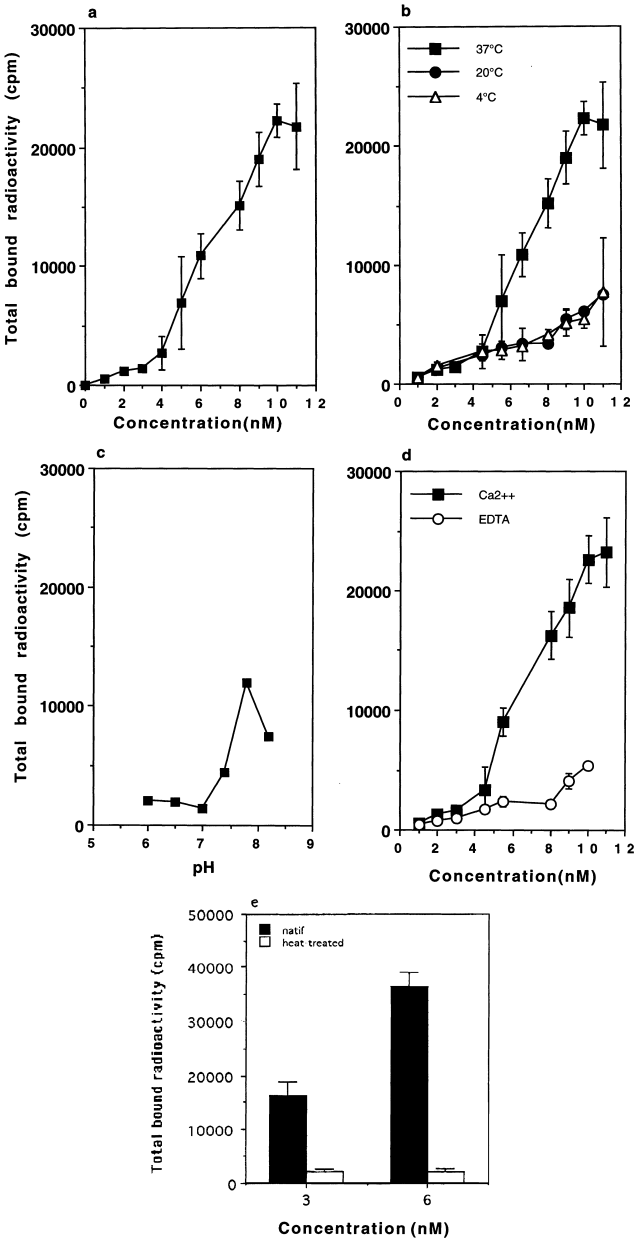

Fig. 3. Influence of physicochemical conditions on the binding of full length (1–68) RANTES to mannose-divinylsulfone-agarose. (a) RANTES concentration: [^{125}I](1–68) RANTES was incubated for 1 h at 37°C with 20 μl of matrix in Tris–Ca–BSA (pH 7.4). (b) Temperature: [^{125}I](1–68) RANTES was incubated with 20 μl of matrix at 4, 20 and 37°C. (c) Influence of pH: 3 nM [^{125}I](1–68) RANTES were incubated with 20 μl of mannose-divinylsulfone-agarose at pH ranging from 6 to 8.2. (d) Influence of Ca^{2+} concentration: [^{125}I] RANTES was incubated with 20 μl of mannose-divinylsulfone-agarose in Tris–BSA in the presence of 10 mM Cl_2Ca or 10 mM EDTA. (e) Influence of heat-treatment of [^{125}I](1–68) RANTES. Results are representative as means ± S.D. of three to four independent experiments, each performed in duplicate.

dency is in accordance with the occurrence of lectin–carbohydrate interaction.

Soluble heparin significantly competed with [^{125}I](1–68) RANTES binding to heparin-agarose, while chondroitin sulfate or dextran had no effect, which demonstrates the specificity of such interaction (Table 4).

Soluble mannan significantly competed with [^{125}I](1–68) RANTES binding to mannose-divinylsulfone-agarose: a 18 μg/ml concentration was required to achieve 50% reduction of the binding; on the contrary, dextran had no significant effect, which demonstrates specificity (Table 4). However, sugars or sugar derivatives such as mannose, methyl-α-mannose, glucose, methyl-α-glucose, galactose, methyl-α-galactose up to 20 mM, had no effect (data not shown). Taken together, our data indicate that RANTES specifically interacts with mannan in solution and with mannose presented by an affinity matrix, and that these interactions strongly depend on the carbohydrate moiety presentation.

Nevertheless, high concentrations of heparin or chondroitin sulfate, 330 μg/ml, also significantly competed with [^{125}I](1–68) RANTES binding to mannose-divinylsulfone-agarose (Table 4), while lower concentrations of heparin, 40–80 μg/ml, induced a two-fold enhancing effect on [^{125}I](1–68) RANTES binding to mannose-divinylsulfone-agarose ($P < 0.05$; $n = 3$). These data demonstrate that RANTES specifically interacts not only with heparin but also with soluble chondroitin sulfate, and indicate that some GAGs modulate RANTES binding properties to mannose.

We next determined whether [^{125}I](1–68) RANTES binding to the matrices was reversed by soluble mannan or soluble heparin, respectively. [^{125}I](1–68) RANTES original preparation (9 nM) was characterized as a major band by SDS-PAGE (16%) with an apparent 8 kDa MW and as a minor one with a 16 kDa MW (Fig. 4a, lane 3); this indicates the presence of monomers and dimers resistant to SDS and β-mercaptoethanol treatment. In these denaturing and reducing conditions, the chemokine molecules eluted from heparin-agarose by soluble heparin or from mannose-divinylsulfone-agarose by soluble mannan, had the same migration patterns as the original preparation (Fig. 4a, lanes 1 and 2 versus 3). These data indicate the reversibility of RANTES

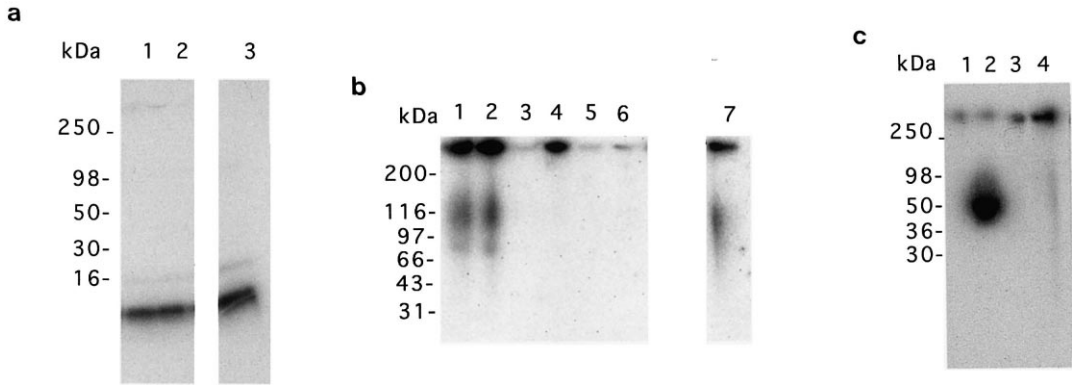

Fig. 4. Reversion of [^{125}I](1–68) RANTES binding to heparin-agarose and to mannose-divinylsulfone-agarose. (a) SDS-PAGE (16%) of [^{125}I](1–68) RANTES after binding to heparin-agarose, and elution by heparin (lane 1) or after binding to mannose-divinylsulfone-agarose and elution by mannan (lane 2) or before binding to the matrices (lane 3). (b) PAGE (16%) in native conditions of [^{125}I]-(1–68) RANTES unbound by mannose-divinylsulfone-agarose (lanes 1 and 2) and after binding to mannose-divinylsulfone-agarose, and elution by buffer (lane 3), mannan (lane 4), methyl-α-mannose (lane 5) or dextran (lane 6). [^{125}I](1–68) RANTES before incubation with mannose-divinylsulfone-agarose (lane 7). (c) PAGE (16%) in native conditions of [^{125}I](1–68) RANTES after binding to heparin-agarose, and elution by buffer (lane 1), heparin (lane 2), dextran (lane 3) or before binding to heparin-agarose (lane 4). Data are representative of two to three independent experiments.

binding to the matrices by respectively heparin or mannan.

We next further analyzed, by electrophoresis in native conditions, the reversibility of RANTES binding to the matrices. The original [^{125}I](1–68) RANTES preparation (9 nM) was characterized by PAGE (16%), as a major band of MW >250 kDa and as a minor diffuse one, of apparent MW between 64 and 168 kDa which indicates the presence of multimers and aggregates (Fig. 4b,c, lanes 7 and 4). Similar patterns were observed for the chemokine molecules which were not bound by mannose-divinylsulfone-agarose (Fig. 4b, lanes 1 and 2 versus 7), while the chemokine molecules bound by mannose-divinylsulfone-agarose and then eluted by soluble mannan, were exclusively characterized as aggregates of apparent MW >250 kDa (Fig. 4b, lane 4). No elution or slight elution from mannose-divinylsulfone-agarose by buffer, methyl-α-mannose or dextran, were observed which demonstrates that elution by soluble mannan is specific (Fig. 4b, lanes 3, 5, 6).

Moreover, the chemokine molecules bound by heparin-agarose and eluted by soluble heparin, were characterized in native conditions as a major band with an apparent MW between 40 and 80 kDa and as a minor one of apparent MW >250 kDa (Fig. 4c, lane 2), which indicates that these molecules are mainly multimers, and not aggregates, as described above for the original preparation (Fig. 4c, lane 4 versus 2). The fact that, in the same conditions, buffer supplemented or not with dextran, only eluted from the matrix some chemokine molecules which migrated as a minor band of apparent MW >250 kDa, demonstrates the specificity of the 40–80 kDa complex formed by RANTES with soluble heparin (Fig. 4c, lanes 1 and 3). Furthermore, while soluble RANTES (6 nM) preincubated for 1 h at 37°C with soluble mannan migrated, in native conditions, as a major band of MW >250 kDa, soluble RANTES (6 nM) preincubated for 1 h at 37°C with soluble heparin migrated as two bands of apparent MW of about 40 and 80 kDa (data not shown).

Taken together, these data indicate that RANTES binding to heparin-agarose or to mannose-divinylsulfone-agarose is reversed in a carbohydrate-specific manner.

To further confirm RANTES-specific interaction with mannosylated derivatives, [^{125}I](1–68) RANTES was incubated in parallel with dotted mannan or dextran. In these conditions, [^{125}I](1–68) RANTES interacted with dotted mannan but not with dextran (Fig. 5, lane a, 1 versus 2). This interaction with dotted mannan was abolished by mannan or heparin in solution (Fig. 5, lanes b,c), as well as by RANTES heat-denaturation (Fig. 5, lane e), but not by dextran (Fig. 5, lane d), which demonstrates the specificity

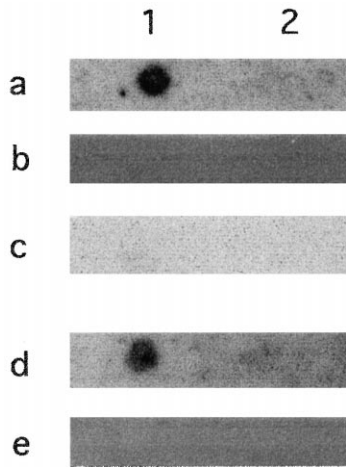

Fig. 5. Carbohydrate-specific binding of [^{125}I](1–68) RANTES to dotted mannan. Binding of native (lane a) or heat-denatured (lane e) [^{125}I](1–68) RANTES to dotted mannan (1) or dextran (2). In lanes b, c and d, RANTES was incubated with mannan, heparin or dextran in solution.

and involvement of RANTES three-dimensional structure.

In order to determine whether the RANTES N-terminus domain is involved in its GAG or mannose-binding properties, we have analyzed (10–68) RANTES interactions with heparin-agarose or mannose-divinylsulfone-agarose. Soluble heparin or mannan only slightly competed with [^{125}I] (10–68) RANTES binding to both matrices, while chondroitin sulfate or dextran had no effect (Table 4).

Taken together, these data indicate specific interactions between full length (1–68) RANTES and GAGs or mannose presented by mannan or an affinity matrix.

4. Discussion

Since the chemokine receptors used for entry of HIV-1 R5 and X4 strains are respectively CCR5 and CXCR4 [1,4,5,6,8,9], we have analyzed the effects of soluble or cellular glycans or GAGs on RANTES binding to primary CXCR4+ CCR5+ lymphocytes and macrophages as well as to CCR5− CXCR4+ monocytic U937 cells. Nevertheless, it has been reported that human peripheral blood monocytes differentiated in vitro into macrophages and cultured in vitro for a few days express, in addition to CCR5, other specific RANTES receptors such as CCR1 and CCR3 [31]. In addition, a small proportion (about 5%) of unstimulated U937 cells responses minimally to RANTES and MIP-1α (ligands to CCR1, CCR3, CCR4 and CCR5) whereas no response is observed with MIP-1β, the most specific CCR5 ligand, as assessed by changes in calcium flux [31]. However, in the present study we did not observe by cytofluorometric analysis any labeling of the U937 cells either by anti-CCR1 or anti-CCR3 antibodies (data not shown). These data indicate that the CCR5− U937 cells used here do not express RANTES receptors different from CCR5.

In the present study, we observed that (1–68) RANTES specifically bound not only to CCR5+ primary cells but also to CCR5− monocytic cells. These binding strongly depended on the chemokine three-dimensional structure. However, (1–68) RANTES attachment to the CCR5+ cells depended as expected, at least in part, on CCR5. In addition, we observed that cellular GAGs, not only heparan sulfate but also chondroitin sulfate, mediated (1–68) RANTES-specific attachment not only to the CCR5−, but also to the CCR5+ cells inasmuch as these attachments were significantly, but partially, decreased by heparitinase or chondroitinase pretreatment of the cells. GAGs in solution, heparin or chondroitin sulfate, also competed with (1–68) RANTES binding. However, higher concentrations of soluble GAGs were required to achieve maximum competition of RANTES binding to the CCR5− cells as compared to the CCR5+ cells which indicates differences in RANTES affinity for the cellular GAGs according to their presentation by the different cells. The fact that additivity was observed for these competitions suggests the involvement of more than one type of GAG in this binding. These data indicate that if the chemokine is complexed with soluble GAGs, it will be unable to bind cellular GAGs. They suggest that soluble GAGs modulate RANTES binding to the cells. Modulation of macrophage and B cell function by GAGs has indeed been recently reported [32].

Otherwise, we observed that (1–68) RANTES binding to the CCR5− as well as to the CCR5+ cells was inhibited either by soluble mannan or by neuraminidase treatment of these cells. This suggests that glycans, such as mannose- and sialic acid-containing species, may be used by the chemokine at some at-

tachment step not only to to CCR5+ primary cells (lymphocytes or macrophages) but also to CCR5− cell lines, in addition to cell surface GAGs.

Accordingly, we observed dose-dependent and specific interactions between full length (1–68) RANTES and either heparin- or mannose-immobilized on an affinity matrix. Heat-denaturation of the chemokine significantly decreased its binding to both matrices, which indicates involvement of the three-dimensional structure.

(1–68) RANTES interaction with heparin-agarose was pH-dependent which indicates, as expected, involvement of electrostatic interactions. It was inhibited by soluble heparin, but not by dextran nor by chondroitin sulfate, which demonstrates in accordance with [14], specificity.

(1–68) RANTES binding to mannose-divinylsulfone-agarose was also pH-dependent, which may be related to an indirect effect mediated by changes of RANTES three-dimensional structure according to pH. This binding was strongly calcium-dependent, which is in accordance with lectin–carbohydrate interactions. Indeed, it was inhibited by soluble mannan, with a 18 μg/ml 50% inhibiting concentration (C_{50}), which is indicative of affinity interactions in the μM range. Dextran had no effect, which confirms specificity. Mannose or Me-α-Man had no effect, which indicates that RANTES interaction with glycans strongly depends on their presentation. However, chondroitin sulfate also significantly inhibited (1–68) RANTES binding to mannose-divinylsulfone-agarose, which strongly indicates specific interaction between the chemokine and soluble chondroitin sulfate. However, soluble heparin, depending on its concentrations, had inhibitory or enhancing effects on (1–68) RANTES binding to mannose-divinylsulfone-agarose. Taken together, these data indicate that (1–68) RANTES has specific binding properties to glycans, which can be modulated by GAGs.

The fact that mannan, heparin or chondroitin sulfate only slightly modify truncated (10–68) RANTES binding to both matrices and to the CCR5− and the CCR5+ cells strongly suggests that RANTES N-terminus domain is involved in its carbohydrate binding properties. However, the role of this domain has not been clearly established. It has been reported that truncation did or did not modify RANTES affinity for CCR5 [28,33]. While equivalent and/or enhanced inhibition of SIVmac251 infection/replication in vivo by N-terminal truncated RANTES has been reported [34], 10-fold higher concentrations of truncated RANTES were necessary to achieve in vitro the same inhibitory effect as full length RANTES on HIV-1 infection of primary macrophages [28]. Nevertheless, truncated RANTES is not chemotactic [28].

The role of GAGs association in the function of chemokines is not clearly elucidated. However, the fact that specific interactions of chemokines with GAGs modulate receptor binding and cellular responses has been strongly suggested [14] and a specific association between a chemokine, SDF-1, and glycans such as mannan was recently suggested [21,35]. The present study demonstrates that besides GAGs, glycans are involved in RANTES conformation and attachment not only to CCR5+ primary cells but also to CCR5− cells.

The physiopathological role of the glycan- and GAG-binding properties of RANTES, which may thus be compared to that of gp120lai and of SDF-1, is presently under investigation. We and others have previously reported that the suppressive effect of RANTES on HIV-1 infection by HIV R5 strains is inhibited by enzymatic removal of GAGs from CD4+ CCR5+ cells [15,36]. In addition, it has been recently reported that complexes formed between RANTES and soluble GAGs failed to induce intracellular Ca^{2+} mobilization on either glycosaminoglycanase-treated or untreated PMBC and to stimulate chemotaxis [36]. However, such complexes bound in a saturable manner to glycosaminoglycanase-treated PMBC and their binding was partially reversed by anti-CCR5 antibody, which indicated that they represent seven-transmembrane ligands that do not activate receptors [36]. Whether the RANTES ability to bind to soluble or cellular glycans, described here, also modulates cellular responses and HIV-1 infection deserves further investigation.

Acknowledgements

This work was supported by the Agence Nationale de Recherche sur le SIDA, the Direction de la Recherche et des Enseignements Doctoraux (Ministère de l'Enseignement Supérieur et de la Recherche),

Sidaction Ensemble contre le SIDA, Université Paris XIII (France).

References

[1] Y. Feng, C.C. Broder, P.E. Kennedy, E.A. Berger, Science 272 (1996) 872–876.
[2] B.A. Premack, T. Schall, Nature Med. 2 (1996) 1174–1178.
[3] M. Baggiolini, B. Dewald, B. Moser, Annu. Rev. Immunol. 15 (1997) 675–705.
[4] C.C. Bleul, M. Farzan, H. Chloe, C. Parolin, I. Clark-Lewis, J. Sodroski, T.A. Springer, Nature 282 (1996) 829.
[5] E. Oberlin, A. Amara, F. Bachelirie, C. Bessia, J.L. Virelizier, F. Arenzana-Seisdedos, O. Schwartz, J.M. Heard, I. Clark-Lewis, D.F. Legler, M. Loetscher, M. Baggiolini, B. Moser, Nature 382 (1996) 833–837.
[6] E.A. Berger, R.W. Doms, E.M. Fenyô, B.T.M. Korber, D.R. Littman, J.P. Moore, Q.J. Sattentau, H. Schuitemaker, J. Sodrosky, R.A. Weiss, Nature 391 (1998) 240.
[7] Y. Yi, S. Rana, J. Turner, N. Gaddis, R.G. Collman, J. Virol. 72 (1998) 772–777.
[8] G. Alkhatib, C. Combadiere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy, E.A. Berger, Science 272 (1996) 1955–1958.
[9] T. Dragic, V. Litwin, G.P. Allaway, S.R. Martin, U. Huang, K.A. Nagashima, C. Cayanan, P.J. Maddon, R.A. Koup, J.P. Moore, A. Paxton, Nature 381 (1996) 667–673.
[10] T. Oravecz, M. Patel, J. Wang, G. Roderiguez, M. Ditto, M.A. Norcross, J. Immunol. 159 (1997) 4587–4591.
[11] F. Arenzana-Seisedos, J.L. Virelizier, D. Rousset, L. Clark-Lewis, P. Loetscher, B. Moser, M. Baggiolini, Nature 383 (1996) 400–404.
[12] W. Koopmann, S. Krangel, J. Biol. Chem. 272 (1997) 10103–10119.
[13] A. Amara, S. Le Gall, O. Schwartz, J. Salamero, M. Montes, P. Loetscher, M. Baggiolini, J.L. Virelizier, F. Arenzada-seisdedos, J. Exp. Med. 186 (1997) 139–146.
[14] G.S.V. Kuscher, F. Coulin, C.A. Power, A.E.I. Proudfoot, R.E. Hubbard, A.J. Hoogewerf, N.C. Wells, Biochemistry 381 (1999) 2959–12968.
[15] S. Amzazi, L. Ylisastigui, Y. Bakri, L. Rabehi, L. Gattegno, M. Parmentier, J.C. Gluckman, A. Benjouad, Virology 252 (1998) 96–105.
[16] V. Appay, A. Brown, S. Cribbes, E. Randle, L.G. Czaplewski, J. Biol. Chem. 274 (1999) 27505–27512.
[17] L. Czaplewski et al., J. Biol. Chem. 274 (1999) 16077–16084.
[18] L. Rabehi, N. Seddiki, A. Benjouad, J.C. Gluckman, L. Gattegno, AIDS Res. Hum. Retroviruses 14 (1998) 1605–1615.
[19] A. Trkola, C. Gordon, J. Matthews, E. Maxwell, T. Ketas, L. Czaplewski, A.E.I. Proudfoot, J.P. Moore, J. Virol. 73 (1999) 6370–6379.
[20] L. Weiss, A. SI-Mohamad, P. Giral, P. Castiel, A. Ledur, C. Blondin, M.D. Kazatchkine, N. Haeffner-Cavaillon, J. Infect. Dis. 176 (1997) 1621–1624.
[21] E. Mbemba, J.C. Gluckman, L. Gattegno, Glycobiology 1 (2000) 21–29.
[22] L. Gattegno, H. Sadeghi, L. Saffar, D. Bladier, B. Clerget-Raslain, J.C. Gluckman, E. Bahraoui, Carbohydr. Res. 213 (1991) 79–93.
[23] M. Haidar, N. Seddiki, J.C. Gluckman, L. Gattegno, Glycoconj. J. 9 (1992) 315–323.
[24] E. Mbemba, J. Czersky, L. Gattegno, Biochim. Biophys. Acta 1180 (1992) 123–129.
[25] E. Mbemba, J.C. Gluckman, L. Gattegno, Glycobiology 4 (1994) 13–21.
[26] E. Mbemba, V. Carre, A. Atemezem, L. Saffar, J.C. Gluckman, L. Gattegno, AIDS Res. Hum. Retroviruses 12 (1996) 47–53.
[27] C.G. Fields, D.H. Lloyd, R.L. McDonald, KM. Otteson, R.L. Noble, Pept. Res. 4 (1991) 91–101.
[28] L. Ylisastigui, J. Vizzavona, E. Drakopoulou, P. Paindavoine, C.F. Calvo, M. Parmentier, J.C. Gluckman, C. Vita, A. Benjouad, AIDS 12 (1998) 977–984.
[29] A. Valentin, S. Von Gegerfelt, K. Matsuda, K. Nilsson, B. Asjö, J. Acquired Immune Defic. Syndr. 4 (1991) 751–759.
[30] A. Ben-Baruch, L. Xu, P.R. Young, K. Bengali, J.J. Oppenheim, J.M. Wang, J. Biol. Chem. 270 (1995) 22123–22128.
[31] D. Zella, O. Barabitskaajaa, L. Casareto, F. Romerio, P. Secchiero, M.V. Reitz, R.C. Gallo, F.F. Weichold, J. Immunol. 163 (1999) 3169–3178.
[32] L.E. Wrenshall, R.B. Stevens, F.B. Cerra, J.L. Platt, J. Leukoc. Biol. 6 (1999) 391–400.
[33] J.H. Gong, M. Ugoccioni, B. Dewad, M. Baggiolini, I. Clark-Lewis, J. Biol. Chem. 271 (1996) 10521–10527.
[34] P. Bostik, F. Villinger, A.A. Ansari, T.M. Folks, Immunol. Today 18 (1997) 555–556.
[35] E. Mbemba, A. Benjouad, L. Saffar, L. Gattegno, Virology 10 (1999) 354–364.
[36] J.M. Burns, G.K. Lewis, A.L. Devico, Proc. Natl. Acad. Sci. USA. 96 (1999) 14499–15208.