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Structural differences between the putative carbohydrate-recognition domains of human IL-1 alpha, IL-1 beta and IL-1 receptor antagonist obtained by *in silico modeling*

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Abstract In a previous report (Cebo *et al.* J Biol Chem 276 (2001) 5685–5691), it was established that biologically active recombinant human IL-1α and IL-1β had different carbohydrate-binding properties. IL-1α recognized a di-antennary N-glycan with two α2-3-linked sialic acid residues, whereas IL-1β recognized the GM_4, a α2-3-linked sialylated glycosphingolipid. These different carbohydrate-binding properties of two interleukins binding to the same receptor (IL-1R) could explain why these molecules had different biological effects and cell specificities. Molecular modeling of the ligands and *in silico* docking experiments defined putative carbohydrate-recognition domains localized in the same area of the two molecules, a domain different from that defined as the type I IL-1R binding domain. The calculated pattern of hydrogen bonding and of van der Waals interactions fulfilled the essential features observed for calcium-independent lectins (mammalian, viral or bacterial). The analysis of the same domain of the third members of this family of molecules, the IL-1R-antagonist, indicated it did not fulfill the criteria for carbohydrate-recognition domains. It is proposed that its role as a pure antagonist is due to the absence of lectin activity and consequently explained its inability to associate IL-1R with other surface molecular complexes necessary for signaling.

Keywords Cytokine · Lectin · Oligosaccharide · Ganglioside · Modeling · Signaling

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Abbreviations

IL	interleukin
IL-1R	IL-1 receptor
IL-1Ra	IL-1 receptor antagonist
CRD	carbohydrate-recognition domain
RBD	receptor-binding domain
GRAMM	Global Range Molecular Matching
GOLD	Genetic Optimization for Ligand Docking

Introduction

The heterogeneous interleukin-1(IL-1) family consists of two agonists, IL-1α and IL-1β that are important mediators of inflammatory diseases able to transduce specific signals, and one family of antagonists, known as IL-1 receptor antagonist (IL-1Ra) unable to generate a cell signal, but blocking the IL-1α and IL-1β signaling (for review see [2, 4, 12]) which are the subject of a large variety of studies and of therapeutical trials [11, 14]. Each of these molecules binds to the type I IL-1 receptor (IL-1R). The binding of IL-1α or IL-1β to IL1R is an early step in IL-1 signal transduction and blocking this interaction may therefore be a useful target for the development of new drugs. As observed for almost all interleukins, the binding to their receptor provokes the association of this receptor with other molecular complexes having signal transduction molecules (kinases, phosphatases, etc.) provoking very early changes in phosphorylations of intracellular domain of one of the components of the super-complex initiating the signaling, the nature of the association between the interleukin receptor and the signaling complex remaining in many cases still speculative. For example, it was proposed that the fixation of an interleukin to its receptor changed the conformation

of its intracellular domain some sequences being accessible to kinases or phosphatases. This could not explain the specificity of the changes and the observation that throughout the multiple kinases /phosphatases present close to the plasma membrane only a few could participate to this signal. Once the signal is transmitted and the cell committed, the signaling super-complex is eliminated from the surface and degraded [12], a situation often encountered when surface molecules are clustered by plant lectins and/or polyvalent antibodies [1, 5, 35].

As documented previously, several interleukins had carbohydrate-binding properties (lectin activities). These molecules behave as bi-functional molecules endowed with a receptor-binding domain (RBD) and a carbohydrate-recognition domain (CRD) [7, 39]. In two cases, IL-2 [40] and IL-6 [8], it was demonstrated that the formation of the signaling super-complexes was due to the lectin activities of interleukins and that the interleukin-dependant signal could be eliminated using small concentrations of their high affinity oligosaccharide ligands. Because of their bi-functionality they were able to specifically and extracellularly associate the receptor to the signal-transducing complex containing the glycan ligands, putting specifically intracellular domains of the super-complex into contact [8, 40].

We reported previously that IL-1α and IL-1β had lectin activities different from each other [7], a point that could explain why these two interleukins could have different target cells and different signaling systems. Indeed, IL-1α recognized with a very high specificity a bi-antennary complex-type N-glycan with two α2-3-linked sialic acid residues whereas IL-1β recognized the α2-3-sialylated β-galactosyl-ceramide, GM_4. Therefore, it was of interest to try to localize the carbohydrate-recognition domains of these molecules, performing computational docking of their specific ligands in the three-dimensional structure of IL-1α and IL-1β reported in the literature. This manuscript proposes the localization of the putative CRD for IL-1α and IL-1β and discusses these data in relation with site-directed mutagenesis experiments and co-crystallization of these molecules with type I IL-1R. It proposes a structural basis for the pure antagonist activity of IL-1Ra.

Experimental section

Glycoproteins, glycolipids and oligosaccharides

Fetuin was obtained from Sigma. Bovine lactotransferrin (bLTF) was purified as previously described [9]. Oligosaccharides from urine of patients with sialidoses [33] were kindly provided by Dr. G. Strecker (CNRS UMR 8576, Villeneuve d'Ascq)). GalNAc-O-benzyl derivatives produced by cultured HT-29 cells supplemented with GalNAcα-*O*-benzyl (GalNAc-*O*-Bn) were obtained and characterized as previously described [42]. Gangliosides were purified from developing rat cerebellum [43]. Neutral glycolipids from human meconium [16] were kindly given by Dr. J.C. Michalski (CNRS UMR 8576, Villeneuve d'Ascq). Recombinant human cytokines IL-1α and IL-1β (produced in bacteria) and their respective polyclonal rabbit antibodies were from Chemicon International Inc. (Temecula, CA, USA). Alkaline-phosphatase-labelled anti-rabbit IgG and normal goat serum were from Sigma (St. Louis, MO, USA).

Detection of the lectin activities of recombinant human IL-1α and IL-1β

The rationale and the experimental details of the method used for determining the lectin activities of IL-1α and IL-1β were previously reported [7]. Briefly, in an initial screening, glycoconjugates (purified or mixtures of glycoconjugates were immobilized to plastic microwells (fetuin; a mixture of bovine ribonuclease B (RNAse B) and of bovine lactotransferrin (bLTF); ovalbumin; a mixture of mucins (ovine and the equine submaxillary mucins and mucins from the eggs of *Bufo bufo*; a glycosaminoglycan mixture containing chondroitin sulphate A and C, dermatan sulphate, keratan sulphate, heparan sulphate, heparin and hyaluronic acid; a sciatic nerve SDS extract; a total mixture of gangliosides isolated from young rat cerebella in the absence of alkaline treatment; a reconstituted mixture of neutral lipids from the human meconium supplemented with galactosylceramides, sulfatides and with asialo-GM1). After saturation of the wells using 3% periodate-treated BSA (pBSA) in phosphate-buffered saline (PBS), the interleukins (100 ng in 5µl) were added to wells containing 45 µl of PBS containing 0.3% pBSA and 5 mM EDTA and incubated for 2 h. The supernatants were recovered, submitted to SDS-PAGE, blotted onto nitrocellulose and revealed using the specific anti-interleukin antibodies. A binding of the interleukin was revealed by the decreased amount of interleukin in the incubation supernatants, quantified by scanning the blots [7]. Dilution curves obtained varying the amount of interleukins indicated that the binding was saturable and proportional to the quantity of immobilized ligands.

Once a binding was observed to a mixture of glycoconjugates, the experiments were repeated on individual immobilized glycoconjugates and attempts to inhibit the binding were performed in the same conditions adding the interleukins as above to the same buffer containing 2 mM of the tested glycans. An inhibitory activity was revealed by increased quantities of the interleukins in the supernatants analyzed by immuno-detection. Dilutions of the quantity of glycans showing an inhibitory activity allowed determining the molarity providing a 50% inhibition of the binding.

Molecular modeling of the ligand of IL-1α and IL-1β

The structures of some lower energy conformers of the IL-1α and IL-1β ligands were obtained using the minimization and random searching tools within the SYBYL software [SYBYL, Tripos Inc., St Louis, Mo, USA [http://www.tripos.com]. The Tripos force field was mainly used except for the internal rotation and electrostatic terms for carbohydrates specific parameters as previously determined [23]. In particular, partial atomic charges were calculated using quantum chemical methods based on the Density Functional Theory (B3LYP hybrid functional and 6-31G* basis set). For that purpose, the Jaguar software was used [http://www.schroedinger.com]. To derive atomic charges, the molecular electrostatic potential was fitted to a set of point charges located at the atomic centers also reproducing the dipole moment. The electrostatic potential itself was computed on a spherical grid.

In silico docking of the IL-1α and β ligands in their respective partner molecules

Contrary to what is mainly observed in pharmaceutical chemistry (where the docking of a relatively small molecule occurs within a cleft inside a protein), usually the carbohydrate-protein interactions take place at the protein surface. The location of this interaction region has to be at first carefully determined. Due to the intrinsic charge flexibility of the carbohydrate ligand, a two step procedure involving successively rigid and flexible docking technique was employed. For the chosen low energy conformers, a surface docking with IL-1α [15] (pdb file "2ILA") and [36] (pdb file "1ITA"); and IL-1β [37] (pdb file "1ITB") was investigated using the rigid body docking GRAMM program (Global Range Molecular Matching [19]). This technique allows locating the area of the global minimum of intermolecular energy for the structure of different accuracy. The generic mode and a grid step of 1.7 were used. One thousand matches were stored among which 100, 10 and 5 were respectively displayed. The retained interaction site was compared with the possible clusters proposed by the flexible ligand docking program DOCK 4.0 ([http://www.compbio.ucsf.edu/dock4] [19, 20, 25]. It was also used in the flexible ligand program GOLD [Genetic Optimization for Ligand Docking; [http://www.ccdc.cam.ac.uk] [17]. Within DOCK, the program SPHGEN identified several sites of interest (clusters) among which at least one was selected according the results given by the GRAMM program. Sphere centers, which filled the site were generated. The program DOCK matched spheres with ligand atoms and used scoring grids from the program GRID to evaluate the ligand orientation. The GOLD program was used for performing a conformational search on the binding site with the aim of improving the hydrogen bonding network between the protein and its ligand. Energy functions were partly based on conformation and non-bonded contact information contained in the Crystallographic Structural Database ([http://www.ccdc.cam.ac.uk] [17]. The putative carbohydrate-recognition domain (CRD) was again defined according to the results of the program GRAMM. Structures were analyzed as ".pdb" and/or ".mol2" files using the Weblab ViewerLite 4.2 graphic program [http://www.accelerys.com].

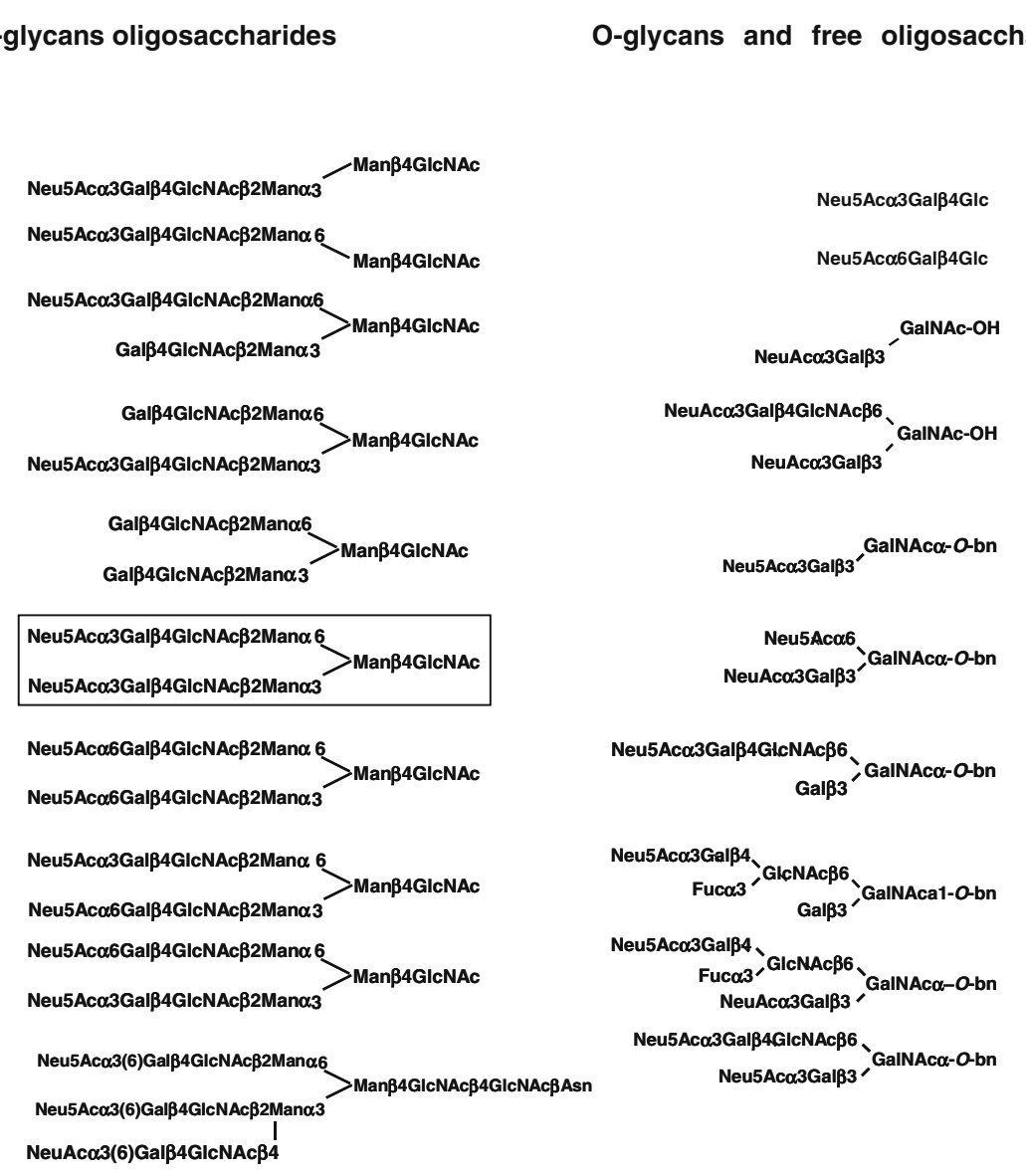
Results and discussion

Identification of the ligands of IL-1α and IL-1β

In a previous manuscript [7], it was reported that IL-1α and IL-1β were endowed with very specific carbohydrate-binding properties. Indeed, IL-1α recognized only the di-antennary N-glycan terminated by two α2-3-linked sialic acids (Table 1) whereas IL-1β recognized only the GM_4 ganglioside. The conclusion for IL-1α was based on the results of inhibition experiments of the binding of Il-1α to fetuin using 20 different pure oligosaccharides or GalNAc-*O*-bn derivatives. None of these glycans could inhibit the interaction except the di-antennary oligosaccharide reported above, even at their limit of solubility, indicating that this compound was at least 10^4 to 10^5 more efficient than the other compounds in inhibiting this interaction. A 50% inhibition of the binding was observed using 0.1 μM, suggesting a very high affinity of this minor ligand apparently (present in bLTF and in fetuin, but never identified). Because the affinity (Kd) was low and since the previous glycan was rare in normal human tissues and cells, based on its level in the urine of patients with sialidosis [33], it was suggested that this lectin activity could be compatible with an important biological function. The absence of efficiency of the isomers di-antennary N-glycans with one α2-3 and one α2-6 sialic acids suggested that the ligand could interact with a dimeric form of IL-1α. This hypothesis was *a priori* rejected because IL-1α was crystallized as a monomer [15] and because all subsequent docking experiments based on this hypothesis (*in silico* production of a putative IL-1α dimer) were unsuccessful (not shown). This strongly suggested that the interaction of this peculiar ligand with IL-1α was due to a specific conformation of this glycan not found in mono-antennary compounds. Based on inhibition experiments it was suggested that the two α2-3-linked sialic acid residues should be close from each other and localized in a same site of the monomeric IL-1α.

The initial screening of the putative lectin activity of IL-1β showed that this molecule bound only to gangliosides isolated from rat synaptosomal plasma membranes, and after

Table 1 Structures of the oligosaccharides used for inhibition of the binding of IL-1α and IL-1β to bovine lactotransferrine and GM_4, respectively



Only a single compound (in the box) was an inhibitor of the binding of IL-1α (50% inhibition at 10^{-7} M), indicating that the presence of two α2-3-linked sialic acids was a determining parameter for the binding. None of these glycans were inhibitors of the binding of IL-1β to GM_4, indicating that the lipid portion of this glycosphingolipid was fundamental for the binding.

differential binding studies only to monosialo-gangliosides. Throughout these compounds, only GM_4 isolated to purity by preparative HPTLC was a ligand [7]. Attempts to inhibit this binding using the compounds shown in Table 1 were unsuccessful when used at their limit of solubility. It should be emphasized that the long-chain base (LCB) composition of this glycolipid was very peculiar, since the major compounds were the C22 sphingenine and the C22 phytosphingosine. This LCB composition was specific for GM_4 since it was found in the series of compounds of the true ganglio series having a predominance of C18 and C20 LCBs. Since the fatty acid composition was normal for gangliosides (excess of saturates linear fatty acids with C16 and C18 carbon atoms), it was concluded that the specificity of the interaction not only involved the sugar chain, but also the non-polar head of the GM_4, the hydroxyl and amido group of the long LCBs being presumably also accessible for interaction with IL-1β. Such a role of the glycolipid nature

in interaction with lectins was largely documented previously for microbe lectins [18]. Recently, the evidence for such a role of the lipid portion was obtained for the mammalian lectin galectin-4 recognizing very strictly sulfatides (3-sulfated-β-galactosyl-ceramide) having long-chain fatty acids hydroxylated in position 2 [10]. This suggested that the interaction of IL-1β with its ligand should take into consideration not only the glycan part of this glycolipid but also the lipid portion and, especially the possible interactions with the accessibility of the polar head due to the presence of unusual LCBs. Thus, the absence of inhibitory activity of all oligosaccharides and benzyl-derivatives shown here could find an explanation considering the role of the lipid portion of GM_4.

Computational determination of the conformations of the IL-1α and docking in the IL-1α molecule

When performing a conformational search of the IL-1α ligand, all low energy conformers within a range of 50 Kcal. mole^{-1} adopted a double wing shape with the two sialic acids residues far from each other. Rigid docking experiments for these conformers displayed a quasi random distribution of the ligand with only one of the two sialic acids interacting with IL-1α. Most of these interactions did not show hydrogen bonding and van der Waals interactions able to correspond to a putative CRD. Furthermore, the fact that only one sialic acid residue was in contact with IL-1α indicated that docking experiments were not in agreement with the biochemical binding experiments. Nevertheless, it still remained a possible dimerization of IL-1α. Computer modeling of the IL-1α dimer was performed together with docking of the wing-shaped low energy conformers of the ligand. Again in this case, docking occurred with only one sialic acid residue. This suggested that there was no need to consider IL-1α dimer and that, may be, the ligand conformation was wrong. In fact, due to the number of rotable bonds (59 possibilities) in the α2-3-di-sialylated di-antennary N-glycans, the configurational space generated in the Monte Carlo procedure was not correctly sampled. All the proposed low energy structures exhibited a wing shape, a situation contrasting with our biochemical data.

Therefore, the conformation of the IL-1α ligand was reconsidered on the basis of free rotation for the torsional angles related to the Manα1-3 and Manα1-6 branches of the glycan in order to fix the two sialic acid residues in vicinity. Conformers thus computed showed an energy lower than those of the wing-shaped structures (by about -15 Kcal.mole^{-1}). These "new" conformers exhibited a compact structure stabilized by a large number of intramolecular hydrogen bonds (14 for the lowest energy conformer) and, especially, hydrogen bonds between the two branches of the di-antennary N-glycan (Fig. 1a–c).

These conformers were used for docking experiments into the IL-1α molecule. All of them found their place in the same part of the IL-1α molecule, *i.e.* in the vicinity of Trp-139. After energy minimization in the dynamic mode, the conformers showed a very strong association with the IL-1α molecule (Fig. 1d–g). Indeed, the ligand presented nine hydrogen bonds with the IL-1α. Three hydrogen bonds were observed with Gln-88, three with Pro-89 (CO of the peptide chain with the carboxyl group of Neu5Ac of the Manα1-6 branch and between the NH of the peptide chain with the same). One hydrogen bond was observed between the NH group of the ring of Trp-139 and the oxygen atom of the pyranose ring of Neu5Ac of the Manα1-3 branch). The ligand was forming strong van der Waals interactions with cyclic amino acids: 6.17–8.02 A (av. 7.09 A) between Tyr-138 and the pyranose ring of Neu5Ac of the Manα1-3 branch, 6.40–7.92 A (av. 7.16 A) between Tyr-138 and the pyranose ring of Gal of the Manα1-3 branch, 3.01–7.63 A (av. 5.32) between Trp-139 and the pyranic ring of Gal of the Manα1-3 branch, 5.27–9.97 A (av. 7.62) between Trp-139 and the pyranose ring of Neu5Ac of the same branch, 4.02–7.39 A (av. 5.70) between Tyr-39 and the pyranose ring of Gal of the Manα1-3 branch and 7.54–8.23 av. 7.89) between Tyr-39 and the pyranic ring of Neu5Ac of the Manα1-3 branch, 5.45–7.39 A (av. 6.84 A) between Pro-89 and the pyranose ring of Neu5Ac of the Manα1-6 branch and 5.57–10.04 A (av. 7.80 A) between Pro-89 and the Gal residue of the Manα1-3 branch. Furthermore, van der Waals interactions were found between sugar residues of the ligands (4.64–8.28 (av. 6.46 A) between the two sialic acid residues, 7.26–981 A (av. 8.53 A) between the two Gal residues and 8.48–13.70 (av. 11.1 A) between the two GlcNAc residues).

Computational data defined a putative carbohydrate-recognition domain in which the ligand made nine hydrogen bonds with Gln-38 (1; CO of the acetamido group with the hydroxyl of the C4 carbon atom of Gal of the Manα1-3 branch), Tyr-39 (1; phenol with hydroxyl of the C6 carbon atom of Gal of the Manα1-6 branch), Asp-85 (2; Carboxylic group with the NH of the acetamido of Neu5Ac of the Manα1-6 branch and with the hydroxyl of the C4 carbon atom of the same Neu5Ac), Asp-87 (3; OH of the C4 carbon atom of Neu5Ac of the Manα1-3 branch with the carboxyl group of Asp-87 and the CO group of the peptide chain of Asp-87 and the NH of the acetamido group of Neu5Ac of the Manα1-3 branch with the carboxyl group of Asp-87), Gln-88 (1; NH of the acetamido of Neu5Ac of the Manα1-6 branch with the CO of the peptide bond) and Trp-139 (1; NH of the indol ring with the carboxyl group of Neu5Ac of the Manα1-6 branch). Strong van der Waals interactions were observed with Tyr-39, Pro-89, Tyr-138 and Trp-139: between Tyr-39 and Gal residues of the two branches (4.48 and 6.40 A, respectively), between Tyr-39 and Neu5Ac of the Manα1-6 branch (8.43 A); between Pro-89 and the Gal

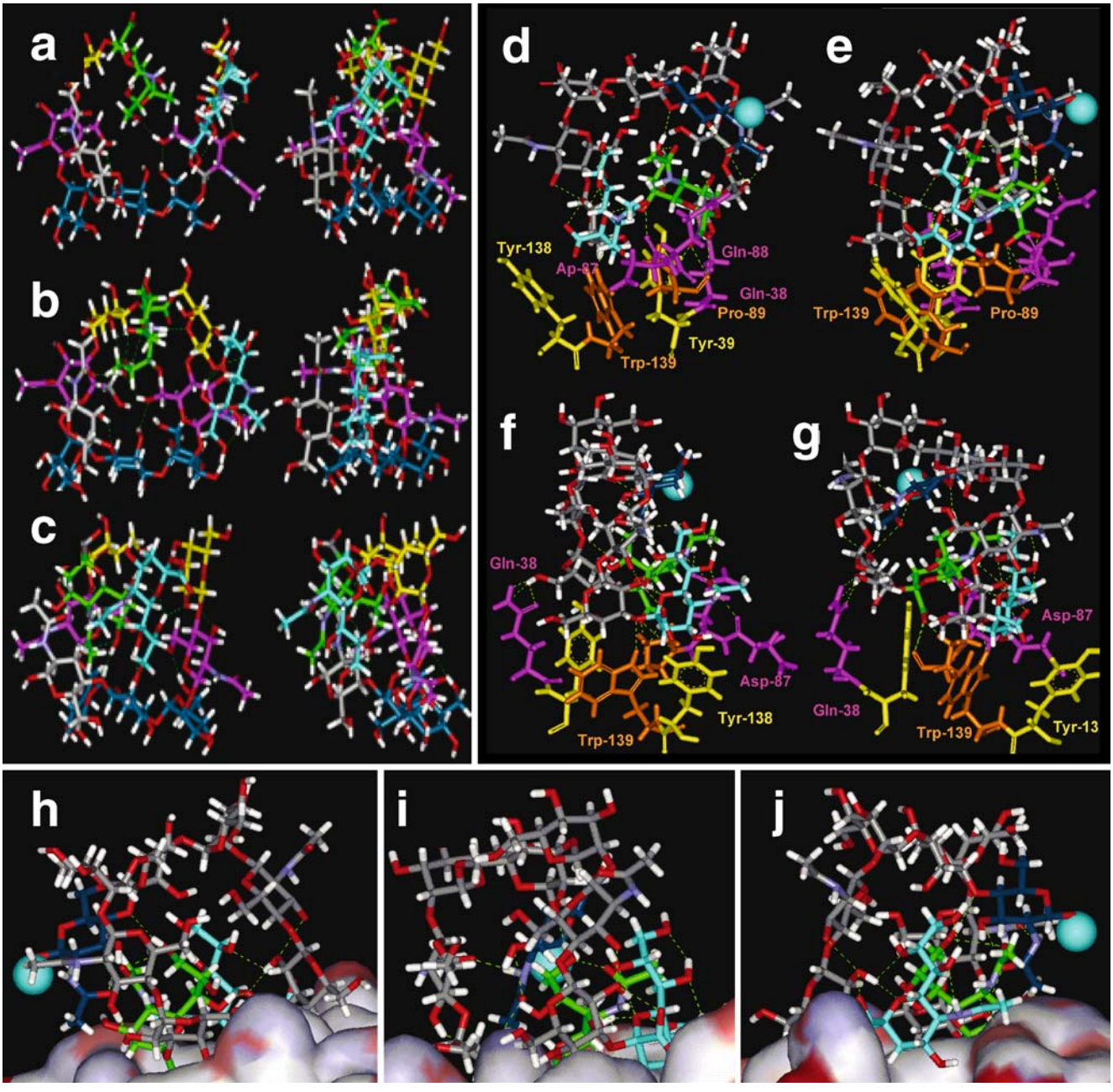


Fig. 1 Representation of the conformations of the three low energy conformers of the IL-1 ligand (*Neu5Acα3Galβ4GlcNAcβ2Manα6 [Neu5Acα3Galβ4GlcNAcβ2Manα3]Manβ4GlcNAc*) (**a**, **b**, **c**) and of the docking experiments of conformer **A** into the IL-1α molecule (**d**–**j**) In **a**–**c**, the left and right parts correspond to two views of the same compound with a 90° rotation. In order to clarify the representation, the carbon atoms of the GlcNAc residues at the reductive end are represented in grey color, those of the three Man residues in dark-blue color, the two internal GlcNAc residues in pink color, the Gal residues in yellow color, the sialic acid of the α1-6 branch in green color and that of the α1-3 branch in pale-blue color. Note the compacted structure of the ligands, these structures being stabilized by numerous internal hydrogen bonds (nine for conformer **a**, 14 for conformer **b** and 13 for conformer **c**. For conformer **b**, two hydrogen bonds are found between the two branches: NH of Neu5Ac of the α1-6 branch and the ring oxygen of the Gal residue of the α1-3 branch and the OH of the $C_{(9)}$ with the OH of the $C_{(6)}$ of the GlcNAc residue of the α1-3 branch. **d**–**g** represented four sequential views (each with a 72° rotation) of the putative calculated CRD of IL-1α. The same color code for constitutive sialic acids was used as in **a**–**c**. The reducing end of the ligand is represented by a pale blue sphere. The amino acids involved in van der Waals interactions are in yellow, those involved in hydrogen bond in pink and those involved in the two types of bonds in orange color. Not only sialic acids were involved in hydrogen bonding but also the two penultimate Gal residues (with Gln-38 for the Gal residue of the α1-6 branch (*green sialic acid*) and with Trp-139 for the Gal residue of the α1-3 branch (*pale-blue sialic acid*). **h**–**j** represented three sequential views (each after a rotation of 90°) of the position of the ligand on the surface of the molecule. Only Neu5Ac residues are colored (*green* for that of the α1-6 branch and *pale-blue* for that of the α1-3 branch)

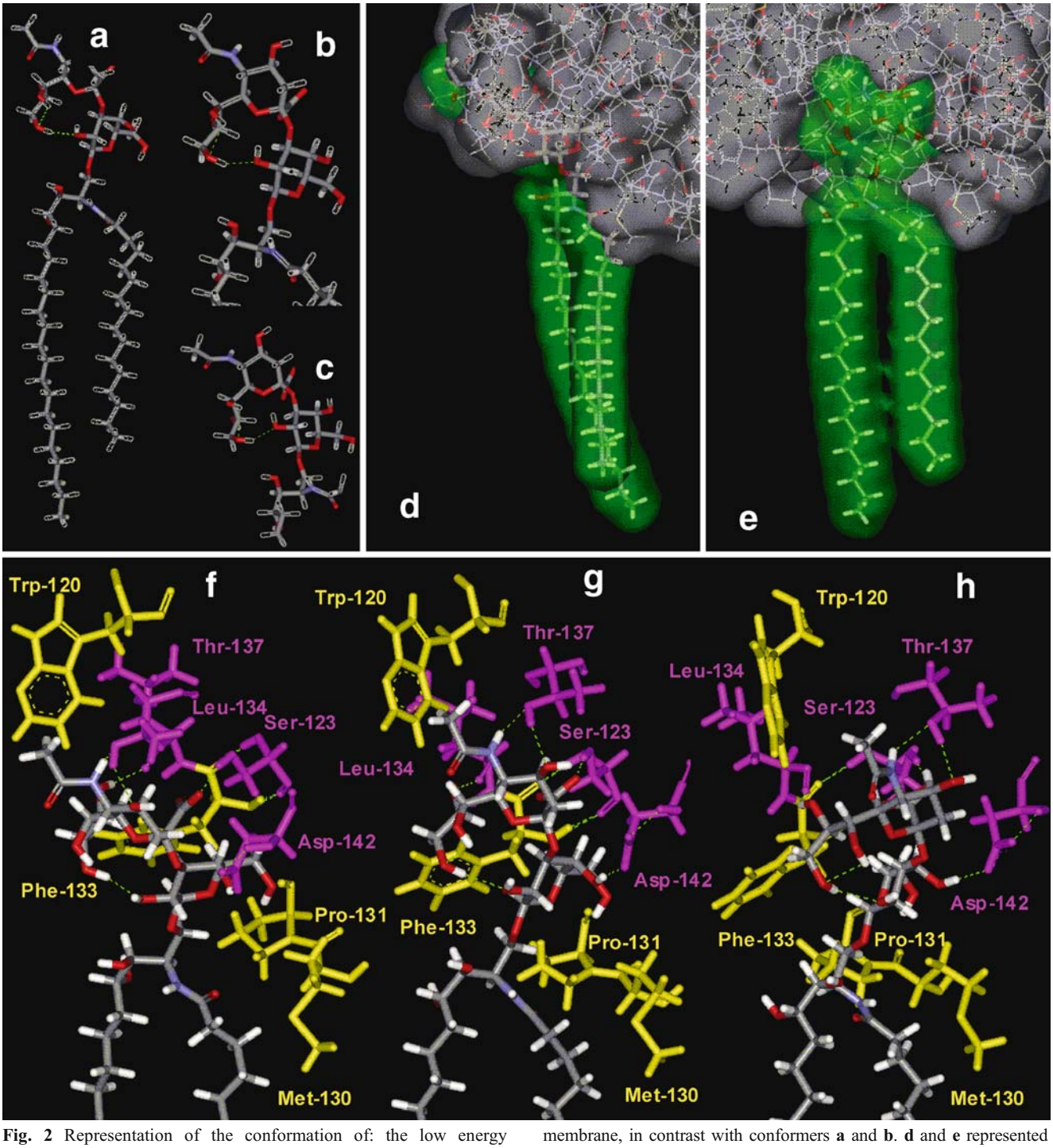


Fig. 2 Representation of the conformation of: the low energy conformers (**a–c**) and of the docking of GM_4 (*Neu5Acα3Galβ1Cer (C16:0 fatty acid;C22:0 phytospingosine)*) into the IL-1β molecule (**d–e** and **f–h**). In **b** and **c**, the ceramide portion was truncated in order to show the fine disposition of the atoms of the carbohydrate part of GM_4. Conformer **c**, the lowest energy conformer was rejected because of the vicinity of the glycan to the lipid bilayer of the plasma membrane, in contrast with conformers **a** and **b**. **d** and **e** represented two views of the surface integration of GM_4 into the IL-1β molecule. Note the perfect adaptation of the glycan part of GM_4 (*green color*) into a cavity of IL-1β. **f**, **g**, and **h** represented three views (each after a 90° rotation of the interaction of GM_4 (conventional atom colors) with the surrounding amino acids through van der Waals interactions (*yellow color*) and hydrogen bonds (*pink color*)

and the Neu5Ac of the Manα1-3 and of the Manα1-6 branch (6.00, 5.41, 8.07 and 6.74 A, respectively); between Tyr-138 and Neu5Ac of the Manα1-3 branch (6.53 A); between Trp-139 and Gal and Neu5Ac of the Manα1-3 branch (4.80 and 5.22 A, respectively) (Fig. 1h–j).

Computational conformation of the GM_4 ligand of IL-1β and docking into the IL-1β molecule

As discussed above, IL-1β recognized an α2-3-sialylated β-galactosyl-ceramide (GM_4 isolated from young rat cerebella) characterized by a ceramide with normal fatty acid composition (linear 16:0 and C18:0), but with an unusual long-chain base (LCB) composition. Indeed, the major LCBs were the C22 sphingenine (30%) and the C22 phytosphingosine (60%; Fig. 1). IL-1β did not recognize all other gangliosides including GM_3, GM_2, GM_1, GD_{1a}, GD_{1b}, GD_3, GD_2, GT_{1b}, GT_3 or tetra-sialogangliosides. Using the same methodology [7], no binding was observed for a large variety of non-sialylated glycolipids and of glycoproteins, proteoglycans and GPI anchors in identical conditions. Unfortunately, the binding of IL-1β to GM_4 could not be inhibited using oligosaccharides such as sialyl-lactose (both α2-3- and α2-6-linked) or using the oligosaccharides tested for inhibiting the lectin activity of IL-1α. These data suggested that the specific binding of IL-1β to this GM_4 was, at least in part due to the nature of the ceramide LCBs, a mechanism frequently observed for bacterial lectins [18] and mammalian lectins [10], the polar head of the ceramide being part of the binding epitope. Therefore, the major GM_4 molecular entity was used for conformational studies and docking experiments.

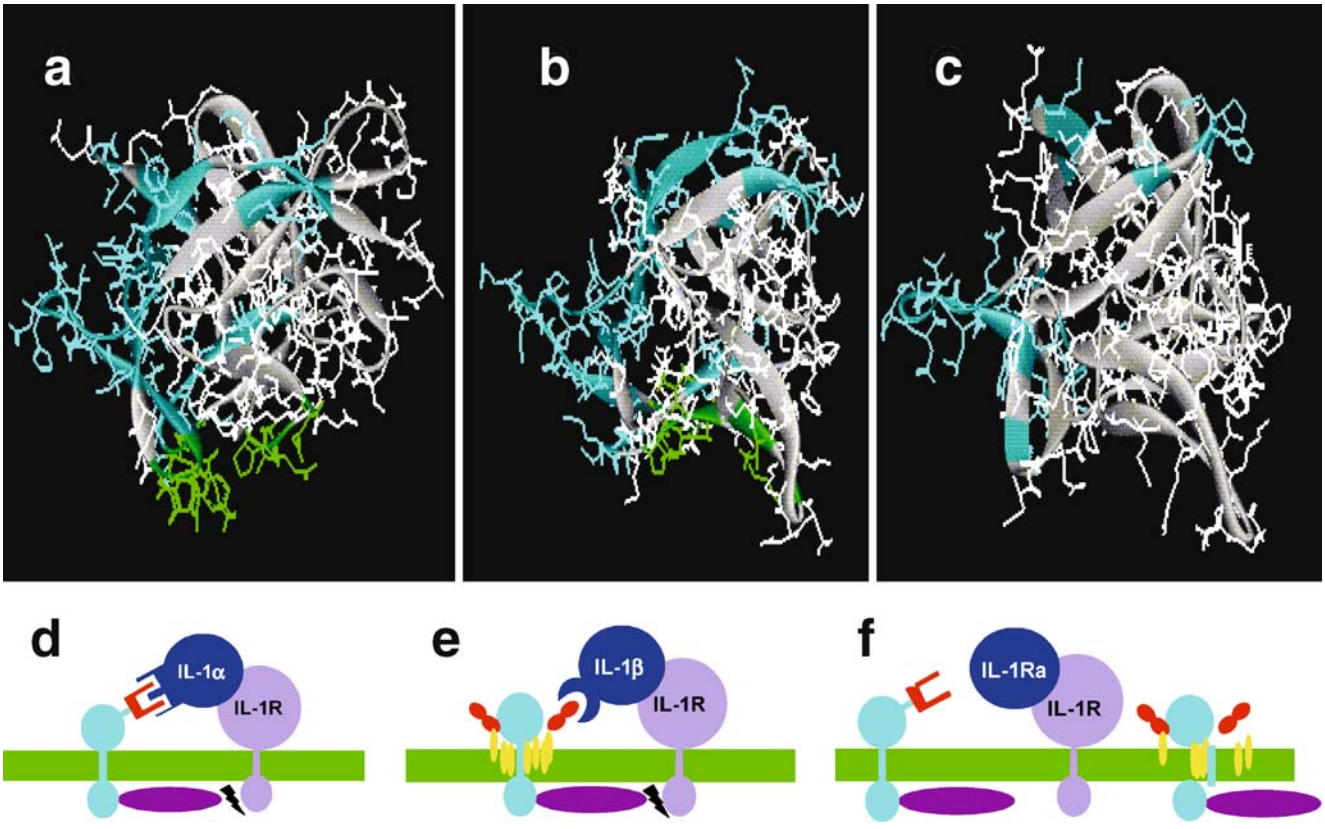


Fig. 3 Comparative representations of the three-dimensional structures of IL-1α, IL-1β and IL-1Ra (**a–c**) and comprehensive scheme of the action of IL-1α and IL-1β on different cells and of the pure antagonist activity of IL-1Ra (**d–f**). The portions of the molecules involved in the binding to type I receptor are represented in *pale blue color* and the putative calculated CRD of IL-1α and IL-1β in *green color*. Although a cavity was also observed in IL-1Ra in the same area, it does not fulfill the criteria expected for calcium-independent lectins because of the absence of possible van der Waals interactions. **d** Scheme proposed for human IL-1α. IL-1α bound to the type I IL-1R recognizes a specific di-antennary N-glycan with two α2-3-linked sialic acids. The bi-functional IL-1α associates IL-1R with the signal-transducing complex by its very specific lectin activity for a N-glycan almost absent from human serum glycoproteins, but present as a minor compound at the surface of specific cells. After signaling these super complexes are internalized and degraded, while the cells are committed. **e** Scheme proposed for human IL-1β. IL-1α bound to the type I IL-1R recognizes a specific glycolipid (GM_4). The bi-functional IL-1α associates IL-1R with the signal-transducing complex by its very specific lectin activity for this sialylated glycolipid also absent from human serum, not yet identified in circulating cells, but present, quite specifically in type-II astrocytes. **f** IL-1Ra binds to type I IL-1R but is not able to associate it with other molecular complexes and then unable to produce a signal and to be internalized (at least during a short period of time)

As shown in Fig. 2, the low energy conformers within a 10 Kcal.mole^{-1} range presented different conformations. The lowest energy conformers (conformer C in Fig. 2) exhibited an organization of the glycan perpendicular to the sphingolipid portion. These conformers were rejected because of their flat shape and the expected proximity of the glycan to the plasma membrane. The same situation was also found for the higher energy conformers within the same range of energy because the disaccharide portion was bent to the sphingolipid portion, a situation impossible because it was located inside the lipid bilayer. In contrast, the intermediate energy conformers (conformers a and b in Fig. 2) showed all a shape escaping the plasma membrane and susceptible to enter in a cavity of the IL-1β molecule.

All the conformers with shapes similar to those of conformers a and b in Fig. 2, were used for static docking experiments in the IL-1β molecule. A majority of them were found to be localized in the same site of IL-1β close to Phe-133 (Fig. 2). Therefore, dynamic docking computations were performed for the ligands docking in this area, in which a complete freedom of the bonds of IL-1β and of the GM_4 conformers was assign, the selection being made with the criteria of lower energy of the complex.

The magnification of the results showed that the GM_4 ligand was included into a cavity of IL-1β (Fig. 2), the interaction with IL-1β involving seven hydrogen bonds and four van der Waals interactions. The hydrogen bonds were: two with Thr-137, 1 with Ser-123, one with Ser-125, one with Leu-134 and one with Asp-142. The van der Waals interactions involved Pro-131, Phe-133 and Trp-120. The pyranic ring of Neu5Ac was in interaction with Trp-120 (6.74–8.15 A) and Phe-133 (6.44–8.95 A) and the pyranic rings of Gal with Phe-133 (5.56–7.15 A) and Pro-131 (4.10–6.94 A). The surface representation of this putative CRD indicated that both the Neu5Ac and the Gal residues of GM_4 were included into a cavity of the IL-1β molecule (Fig. 2).

The shape of this putative CRD could explain why other members of the family of sialylated glycolipids were recognized by IL-1β. Indeed, studies of the conformations of gangliosides such as GM_3 or GM_1 indicated a bended structure, with a formation of hydrogen bonds between the OH group of the C(7) of Neu5Ac with the OH group of the C(2) of Gal.

This localization of the putative CRD of IL-1β was compatible with the data of the co-crystallization of IL-1β with its type I receptor published in the literature (pdb file 1ITB). Indeed, the receptor-binding domain (RBD) involved residues 1–19, 26–36, 38–40, 46–48, 93–94, 105, 108–109, 121–129, 142–143, 147–150. Although the putative CRD we determined was close to the RBD, dynamic docking of the ligand into the IL-1β/IL-1R complex did not change significantly neither the shape of the ligand nor that of the IL-1β/IL-1R complex relative to X-ray crystallographic data.

Absence of a putative CRD for IL-1Ra

Although many studies were concerned by site-directed mutagenesis of IL-1α and IL-1β [3, 6, 13, 21, 22, 32, 38] the interpretation of the data remained difficult. More interesting were the data of co-crystallization of the two interleukins with the extracellular domain of the type I IL-1R, defining a binding site common to both molecules and to IL-1Ra [28–31].

The question remained to know the differences between IL-1α, IL-1β and IL-1Ra. The latter also bound to type I IL-1R, but in contrast with the two other members of the IL-1 family, it did not produce any signal on all cells so far tested. Considering the calculated localizations of the CRD of IL-1α and IL-1β, it was important to see if the similar site in IL-1Ra could have the classical properties of a CRD. In fact, the careful examination of the corresponding site (Fig. 3) indicated the total absence of aromatic and cyclic amino acids in this domain. Therefore, the corresponding site of IL-1Ra did not fulfill the features of classical CRD of lectins. The antagonist effect of this member of the IL-1 family could be explained by the absence of a CRD and, consequently, to the absence of association of type I IL-1R with a signal transducing complex (Fig. 3). Not only IL-1Ra did not initiate an IL-1 signal but also did not produce after fixation of IL-1R the internalization of the receptor complex. The latter observations were of importance because such internalizations occurred frequently when surface molecules are clustered. Therefore, due to the absence of lectin activity, it could not associate IL-1R with other surface complexes possessing the ligands of the two IL-1: a signal transducing complex containing a glycoprotein with the di-antennary N-glycan ligand for IL-1α and a signal transducing complex containing GM_4 for IL-1β. As a consequence of this hypothesis, only cells having both the receptor and the ligand of the cytokine could respond to the cytokine. This could explain why different cytokines having the same receptor can stimulate specifically certain cell types and not the others. As an example, IL-1α and IL-1β have the same receptors but have different signaling pathways in different cells. IL-1β, but not IL-1α, is able to stimulate human astrocytes, a mechanism responsible for the nervous regulation of fever [24]. It is noteworthy considering that astrocytes are the only cells of the central nervous system producing IL-1β and possessing both IL-1 receptors [34] and the GM_4 glycolipid [26, 27].

Conclusion and perspectives

The question of the function of such lectin activities of cytokines remains largely unanswered. As suggested by the function of the IL-2 lectin activity [40], an essential role in cytokine signaling is expected, consisting in association of

the cytokine receptor complex with specific glycoprotein or glycolipid ligands of another surface complex. Based on previous data [41, 44], it was hypothesized that oligomannosides present on *C. albicans*, cancer cells and HIV-1 virus could monopolize circulating IL-2, inducing severe immuno-deficiencies. More recently (Zanetta, J.P, Malm, D., and Vergoten, G. submitted), it was shown that the steady-state concentration of IL-2 ligands (oligomannosides with 5 and 6 mannose residues) in the blood of a young patient with α-mannosidosis was 20 fold higher than that able to completely block the IL-2 signaling in cultures of human lymphocytes. This constant level of these oligomannosides in the blood could explain the severe immunodeficiency observed in these patients. In contrast, it could be suggested that injections of small amounts of these compounds in the blood of patients with an hyperactivation of the immune system (such as autoimmune diseases) could be beneficial. Such approach could be hypothesized for the di-antennary N-glycan ligand of IL-α, but the glycolipid nature of the IL-1β ligand is yet, *a priori*, difficult to overcome.

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