Recombinant Human Interleukins IL-1α, IL-1β, IL-4, IL-6, and IL-7 Show Different and Specific Calcium-independent Carbohydrate-binding Properties*

A method was developed for the determination of putative lectin activities of cytokines. It involved the immunoblotting measurement of the quantity of these cytokines unbound to a series of different immobilized glycoconjugates and displacement of the bound cytokines with oligosaccharides of known structures. This method allows demonstrating that the following interleukins specifically recognize different oligosaccharide structures in a calcium-independent mechanism: interleukin-1α binds to the biantennary disialylated N-glycan completed with two Neu5Acα2–3 residues; interleukin-1β to a GM₃ sialylated glycolipid Neu5Acα2–3Galβ1-Cer having very long and unusual long-chain bases; interleukin-4 to the 1,7 intramolecular lactone of N-acetyl-neuraminic acid; interleukin-6 to compounds having N-linked and O-linked HNK-1-like epitopes; and interleukin-7 to the sialyl-Tn antigen. Because the glycan ligands are rare structures in human circulating cells, it is suggested that such activities could be essential for providing specific signaling systems to cells having both the receptors and the oligosaccharide ligands of the interleukin at their cell surface.

Cytokines are modulators of the activity of the immune system, their mechanism of action remaining, for the most part, not decrypted. As a general rule, the action of a cytokine results from its binding to membrane receptors, a series of molecules coupled to signaling systems involving kinases and/or phosphatases (1–5). The binding of a cytokine to its receptor(s) generally results in the phosphorylation/dephosphorylation of the intracytoplasmic domain of the receptor(s), the first step of the signaling. In general, the phosphorylation/dephosphorylation mechanism is cell type-specific, the kinases/phosphatases involved in these processes being quite specifically associated with surface molecular complexes different from the cytokine receptor complex (3, 6). Even when two different cytokines use the same receptor, the signal transduction pathways may be specific of the cytokines (7).

We made the hypothesis that the specific association of interleukin receptors with other surface complexes could be due to carbohydrate-binding properties of these cytokines, a property already suggested in the literature (8–14). The lectin activity of interleukin-2 (IL-2)² for specific oligosaccharides (15) appeared to be essential, because IL-2 behaves as a bifunctional molecule able to extracellularly associate its β receptor (IL-2Rβ) to other surface receptor complexes bearing N-glycans recognized by IL-2. This is the case for the CD3/TCR complex in which a N-glycosylated form of CD3 is an IL-2 ligand. This specific extracellular association is responsible for the specific phosphorylation of the IL-2Rβ by the CD3/TCR-associated kinase p56lck (15), considered as a first step in the antigen-specific activation process of CD4⁺ T cells. As a consequence of this carbohydrate-binding property, it was suggested that oligomannosides accumulated in specific diseases or bound to specific microorganisms could alter this essential role of IL-2 (16, 17). Because several groups reported lectin-like activities for cytokines (8, 10–13), we undertook a study of such properties for recombinant human interleukins. This work demonstrates that IL-1α, IL-1β, IL-4, IL-6, and IL-7 recognize very specific oligosaccharide ligands in a calcium-independent mechanism. The implications of these findings are discussed.

EXPERIMENTAL PROCEDURES

Biochemicals—Recombinant human cytokines (produced in bacteria) and their respective polyclonal rabbit antibodies were from Chemicon International Inc. (Temecula, CA). Alkaline-phosphatase-labeled anti-rabbit IgG and normal goat serum were from Sigma. Ovalbumin, ribonuclease B (from bovine pancreas), fetuin, and glycosaminoglycans (chondroitin sulfate A, B, and C, heparan sulfate, heparin, dermatan sulfate, and hyaluronic acid) were from Sigma. Bovine lactotransferrin was from Euromedex (Souffelweyersheim, France). Equine and ovine submaxillary mucins were prepared according to Tettamanti and Pigman (18), and Bufo bufo egg mucins were prepared according to Morelle and Streeker (19). Gangliosides were extracted from young rat cerebella as previously described (20). Neutral glycolipids were extracted from human meconium by Folch extraction and Folch partition followed by separation by silica gel chromatography (21). Asialo-GM₁ was prepared by limited acid hydrolysis (aqueous formic acid, pH 2.0, during 30 min at 50 °C) followed by a Folch partition. Cerebrosides and sulfatides were isolated from the rat cerebellum (phospholipids were eliminated using mild alkaline methanolysis). Sciatic nerve extracts were obtained by homogenization of freshly dissected sciatic nerves from adult Wistar albino rats in 1% SDS at the concentration of 10 mg of protein/ml. Myelin-associated glycoprotein (MAG) was purified from adult rat brain myelin (22) according to Quares et al. (23). The Pr peptide was prepared from rat sciatic nerve myelin according to Kitamura et al. (24). Oligosaccharides were isolated from different sources, and their structures were determined by NMR as pre-

The abbreviations used are: IL, interleukin; GPI, glycosyl-phosphatidylinositol; PBS, phosphate-buffered saline; BSA, bovine serum albumin; pBSA, periodate-treated BSA; bLTF, bovine lactotransferrin; OSM, ovine submaxillary mucin; MAG, myelin-associated glycoprotein; GC/MS, gas chromatography-mass spectrometry.

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Lectin Activities of Cytokines

Glycopeptides were obtained from different glycoproteins by extensive Pronase digestion followed by Biogel P2 chromatography (29). Nonreductive glycopeptides (0.45-µm pore size) was from Schleicher & Schuell. GCMS analyses of monosaccharides, long-chain bases, and fatty acid derivatives liberated by acid methanalysis were performed as described (30). Melting derivatives were then obtained by diazomethane flow injection of the derivatives.2 Volatile compounds were analyzed using a Carlo Erba GC 5000 apparatus coupled to a Finnigan MAT 80 mass spectrometry. Analyses were performed routinely in the electron impact mode or, when necessary, in the chemical ionization mode in the presence of NH3 and detection of positive or negative ions as previously described (30–32).

Immobilization of Glycoconjugates on Plastic Microwells—In routine screening experiments, the following classes of glycoconjugates were immobilized to plastic microwells: 1) fetuin, a glycoprotein containing N- and O-glycans but presenting a large microheterogeneity concerned with the degree of sialylation of its N-glycans and its different O-glycans, differently bound sialic acid residues, and minor glycans with unknown structures (33); 2) a mixture of bovine RNase B and of bovine lactotransferrin (bLTF) containing oligomannosidic N-glycans (with 5 and 6 mannoside residues as major glycans for RNase B (34) and with 8 and 9 mannoside residues for bovine lactotransferrin, but for the latter, minor biantennary complex-type N-glycans; 3) ovine mucins containing mostly oligomannosidic N-glycans and hybrid-type N-glycans with a very large structural microheterogeneity (36); 4) a mixture of mucins, the ovine mucins (containing especially globosides and blood group substance glycolipids series (20); and 8) a mixture of neutral lipids from the human meconium

RESULTS

The rationale of the method used for studying the lectin activities of interleukins was to detect the cytokines unbonded to different immobilized glycoconjugates with known glycan structures using an immunoblotting technique as schematized in Fig. 1. The reasons for choosing such a method were as follows: 1) because cytokines could loose their carbohydrate-binding properties upon chemical or radiolabeling (49), we used unlabeled recombinant human cytokines having a preserved biological activity; 2) because no precise carbohydrate-binding properties of most cytokines were found in the literature, we studied the binding of cytokines to a large variety of glycoconjugates or mixtures of glycoconjugates, the structure of their glycans being in large part determined; and 3) because the presence of calcium (above 1 mM in Tris-buffered saline) induced a nonspecific fixation of all interleukins to all immobilized glycoconjugates, the experiments were performed in the presence of 5 mM EDTA. Consequently, the data reported in this manuscript were concerned only with Ca2+-independent carbohydrate-binding properties.

Interleukin 1a Is a Lectin Specific for Disialylated Biantennary N-Glycans with 2,3-Linked Sialic Acid Residues—As shown in Fig. 2a, IL-1α binds significantly to fetuin, ovalin, OSM, gangliosides, neutral glycolipids, and RNase B, 1.2 µg for equine submaxillary mucin and the mucin of B. bufo, and 1.5 µg for the mixture of glycosaminoglycans. These quantities of bound glycanes remained the same after incubations with the cytokines as determined in the wells after recovery of the supernatant.

2 J.-P. Zanetta, A. Pons, M. Iwersen, C. Mariller, Y. Leroy, P. Timmerman, and R. Schauer, submitted for publication.

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biantennary cation-independent lectin endowed with a higher affinity for the ligand of the cytokine). For example in 5-bromo-4-chloro-3-indolyl phosphate reagent (a). Note the large amounts of pBSA used for eliminating nonspecific interactions with glycoconjugates that could in some cases perturb electrophoresis. Blots are then revealed using the proper anti-cytokine primary antibody followed by alkaline phosphatase-labeled anti-rabbit secondary antibody and then staining with the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate reagent (b). For example in b, compare lane 1 (control) and lane 4 (coincubation with a high affinity ligand of the cytokine). Note an increased amount of the cytokine in the supernatant, thus indicating an inhibition of the fixation of the cytokine to the glycoconjugate.

bLTF, the binding of IL-1α was observed only to the latter. From the quantity of IL-1α bound to bLTF, it could be calculated that IL-1α binding sites were present on about 7% of the bLTF molecules, a result that indicated that the ligands of IL-1α corresponded to minor glycans of this molecule, as they were minor glycans of fetuin. In fact, the O-glycan fraction obtained from fetuin after reductive β-elimination and Biogel P2 chromatography, as well as the two major O-glycans of fetuin, were devoid of inhibitory activity, in contrast with the N-glycan-enriched fraction obtained by extensive Pronase digestion of fetuin. However, the purified major triantennary N-glycan of fetuin and the products obtained after its partial or extensive Pronase digestion of fetuin. However, the purified major triantennary N-glycan of fetuin and the products obtained after its partial or extensive Pronase digestion of fetuin. However, the purified major triantennary N-glycan of fetuin and the products obtained after its partial or complete desialylation did not show any inhibitory activity. Because bLTF possessed minor biantennary N-glycans with the Neu5Acα2,6GalNAcβ1,4GlcNAc sequence (35), we tested the inhibitory activity of this compound. As for the previous ones, no significant inhibitory activity was obtained at the 10⁻⁴ M concentration range. We therefore tested a variety of biantennary oligosaccharides isolated from the urine of patients with sialidoses (25, 26). The one containing two α2,6-linked Neu5Acα residues was ineffective at 10⁻⁴ M, whereas the binding of IL-1α was completely inhibited using 10⁻⁶ M of the biantennary N-glycan containing two α2,3-linked Neu5Acα residues (Fig. 2, b and c). A quasi-equimolar mixture of the two isomers of biantennary N-glycans containing α2,3- and α2,6-linked Neu5Acα residues showed a weak inhibition only at the 10⁻⁶ M concentration range. Therefore, it was concluded that IL-1α is a calcium-independent lectin endowed with a higher affinity for the biantennary N-glycan having the Neu5Acα2,3Galβ1,4GlcNAcβ1,2 sequence on its two branches (Fig. 2d).

Previous authors (8) reported that IL-1α was able to bind to uromodulin, a urine glycoprotein rich in sialylated N-glycans, the binding being inhibited by the bulk of glycopeptides isolated by Pronase digestion of fetuin. These authors suggested that IL-1α was a lectin specific for the major glycan of fetuin, the triantennary triantennary N-glycan. Our experiments demonstrate that the inhibitor of the interaction was not the previous glycan but the biantennary N-glycan with two α2,3-linked Neu5Acα residues. 50% inhibition of the binding to fetuin was obtained using 0.5–1 μM of this compound, i.e. 100–200-fold higher efficiency than the total N-glycans of fetuin. The reason why the biantennary N-glycan was a high affinity ligand, whereas α2,3-sialyl-lactose or the mixture of the linear monoantennary α2,3-sialylated glycan isolated from patients with sialidosis (25, 26) was ineffective, remained speculative. This might be related to the tendency of IL-1α to associate into dimers, with each subunit being able to bind one of the branches of the biantennary N-glycan. Such a specifically increased affinity upon formation of oligomers of lectins was previously demonstrated for the asialo-fetuin receptor (50).

IL-1β Is Likely a Lectin Specific for the GM4 Glycolipid—IL-1β binds only significantly to the fraction containing the mixture of gangliosides isolated from the rat cerebellum (Fig. 3a). Because these gangliosides were previously fractionated according to their charges by ion-exchange chromatography (20), we examined the binding of IL-1β to the different classes of mono-, di-, tri-, and tetrasialo-gangliosides, respectively. Only the monosialo-ganglioside fraction showed a binding of IL-1β (Fig. 3b). The different constituents of the fraction of monosialo-gangliosides isolated by preparative TLC (20) were immobilized individually. As shown in Fig. 3c, IL-1β binds to a single fraction, previously identified as the sialylated galactosylceramide GM4 (20).

This binding was surprising, because GM4 is a very simple sialylated glycolipid, Neu5Acα2,3Galβ1-Cer. It was therefore expected that the binding could be inhibited by small oligosaccharides with similar structures. In fact, sialyl-lactoses (with α2,3- or α2,6-linked Neu5Acα) were without inhibitory effects at the concentration of 10⁻⁷ M. Because it was described that IL-1β binds to glycolipids of GPI anchors (13), we tested the possibility that the GM4 sample used in this study was contaminated by GPs. This possibility was ruled out using GC/MS analysis of this GM4 sample (30, 31). Using a new procedure allowing the complete liberation of all GPI constituents, the analysis showed the total absence of D-mannose, inositol, and GlcN, which are essential constituents of GPs. To further document this point, the binding of IL-1β was tested to an immobilized glycosylphosphatidylinositol-steramide isolated from Tulamen (kindly provided by Drs. J. and L. Previato). No binding was observed. Furthermore, the binding of IL-1β to GM4 was not inhibited by Man-6-P at the concentration of 10⁻³ M, indicating that the binding to GM4 observed here was unrelated to the binding to GPI oligosaccharides (13).

The question remained to know how a compound with a simple carbohydrate composition could be a specific ligand of a cytokine. Based on the observations of Karlsson (51) on the influence of the lipid moiety on the binding of microbe lectins, we made the hypothesis that part of the specificity could be due to the lipid portion of the rat cerebellum GM4. This question was answered using GC/MS analysis (30). Although the fatty acid composition was classical (C16:0 and C18:0 in equivalent amounts representing more than 98% of total fatty acids), the long-chain base composition was unusual. Indeed, the long-chain base composition was classical (C16:0 and C18:0 in equivalent amounts representing more than 98% of total fatty acids), the long-chain base composition was unusual. Indeed, the linear C22:1 phytosphingosine (60%) and the linear C22:1 sphingosine (30%) were the major constituents. A minor constituent (10%) was identified as 18-O-ethyl-C18:1 sphingosine. Although it was not demonstrated that the long-chain base composition of the GM4 was important for the binding of IL-1β, this remained a stimulating possibility. Because of the lack of an inhibitor, the lectin activity of IL-1β could not be ascertained as such. Nevertheless, the completely different behavior as lectins of IL-1α and IL-1β could explain why these interleukins, endowed with a common receptor, have different biological functions (see “Discussion”).

IL-4 Is a Lectin Specific for the 1,7 Intramolecular Lactone of

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3. A. Pons, J. Previato, L. Previato-Mendoza, P. Timmerman, Y. Leroy, and J.-P. Zanetta, manuscript in preparation.
**FIG. 2.** Illustration of the data obtained for identifying the carbohydrate-binding property of IL-1α. *a,* immunoblotting screening of the binding of 0.1 μg of the cytokine to the different classes of immobilized glycoconjugates. Note the binding of IL-1α to fetuin and overall on the mixture of RNase B and bovine lactotransferrin bLTF (arrow heads). *b,* inhibition of the binding of IL-1α to bLTF using the following different biantennary oligosaccharides: IbI (lane 2), IbII (lane 3), and IbIII (lane 1); the structures are shown in *c.* Concentrations of inhibitors used in this experiment were 10−4 M for compounds IbIII and IbII, and 10−6 M for compound IbI. Note the increased amount of supernatant IL-1α obtained by coincubation of 0.1 μg of IL-1α with 10−6 M of compound IbI, having two α2,3-linked Neu5Ac residues. Because ligands of IL-1α were only minor glycans of bLTF, the control lane without inhibitor was not significantly different from *lanes* 1 and 3. In *a* and *b,* the direction of the electrophoresis was to the left. Quantitation of the experiment reported in *b.*

**FIG. 3.** Evidence for the specific binding of IL-1β to GM4. *a,* immunoblotting screening of the binding of 0.1 μg of IL-1β to the different classes of immobilized glycoconjugates. Note the binding of IL-1β to the ganglioside mixture. *b,* study of the binding of IL-1β to the families of mono-, di-, tri-, and tetrasialo-gangliosides. Note the specific binding to the monosialo-ganglioside fraction (arrow head). *c,* comparative binding of IL-1β to the different isolated monosialylated glycosphingolipids. Note the decreased amount of the cytokine in the supernatant issued from the microwell in which GM4 was immobilized (arrow head). The amount of immobilized gangliosides were proportional to that present in the initial mixture of monosialo-gangliosides in which GM4 represented only 5% of the total compounds. *d,* structure of the three long-chain bases identified in the immobilized GM4. In *a,* *b,* and *c,* the direction of the electrophoresis was to the left.

**FIG. 4.** Evidence for a lectin activity of IL-4. *a,* binding of 0.1 μg of IL-4 to the different mixtures of immobilized glycoconjugates. Note the specific binding IL-4 to the mucin mixture fraction. *b,* fixation of IL-4 to the B. bufo mucins (BbM). Wells were coated by 50 or 100 μg of the different mucins, except in control wells. Note the strong fixation of the cytokine to the B. bufo mucins, and to a lesser extend, to OSM. ESM, equine submaxillary mucin. *c,* inhibition of the fixation of IL-4 by sialic acids isolated from the mucins of the eggs of B. bufo by mild acid hydrolysis. Wells were coated by the B. bufo mucins, and different fractions prepared from the mucins of the eggs of B. bufo were used to inhibit the fixation of IL-4. Only the sialic acids were able to inhibit the fixation of IL-4 to the mucins, suggesting that the high affinity ligand for IL-4 was a particular sialic acid present in this fraction. Results are representative of at least three independent experiments. *d,* structure of the ligand of IL-4, the 1,7 lactone of Neu5Ac.
β-elimination were Neu5Ac and Neu5Gc in equivalent amounts. Because the commercial compounds were not inhibitory, it was concluded that the ligand of IL-4 was an O-acetylated derivative of one of these two compounds.

When GC/MS analyses of the sialic acids present in this mucin were performed, four major peaks were detected (Fig. 5). Two of them corresponded to Neu5Ac and Neu5Gc, respectively, but Neu5Ac was a very minor constituent of the mucin. In contrast, the sialic acids derived from the oligosaccharide-alditols obtained by reductive β-elimination showed an equivalent ratio of Neu5Ac and Neu5Gc (27). Classical O-acetylated and O-lactylated sialic acids were absent from this mucin. In contrast, the major peak corresponded to a compound derived from Neu5Ac. Based on the fine fragmentation pattern of the compound in the electron impact mode of ionization, it was concluded that this compound was the 1,7 lactone of Neu5Ac (Fig. 4d). The fourth peak corresponded to the 1,7 lactone of Neu5Gc. The existence of such intramolecular lactones of sialic acids was previously suggested in the literature but never obtained in sufficient amounts to be identified with security.

The problem remained to know whether the ligand of IL-4 in the B. bufo mucins was the 1,7 lactone of Neu5Ac or that of Neu5Gc or both. Because OSM, showing only a weak binding for IL-4, contains a significant amount of the 1,7 lactone of Neu5Ac but no trace of the corresponding lactone of Neu5Gc, it was concluded that the ligand of IL-4 in the B. bufo mucins could not be the 1,7 lactone of Neu5Gc but was the 1,7 lactone of Neu5Ac.

IL-6 Is a Lectin Specific for Specific Compounds with a Glucuronic Acid-3-sulfate (HNK-1) Group—As shown in Fig. 6a, IL-6 binds only to the sciatic nerve extract, suggesting the presence of a unique ligand in this heterogeneous fraction. We made the hypothesis that a possible ligand was the HNK-1 epitope (SO₃H (3-)GlcA) present both on glycolipids and glycoproteins found in this tissue (52). IL-6 also binds to the mucins isolated from the eggs of R. temporaria, also rich in glycans bearing this epitope (see Ref. 28 and Fig. 6b). Because the two major glycoproteins of rat peripheral nervous system myelin (MAG and overall P₀) possessed this epitope, we immunoblot these two isolated glycoproteins on plastic and measured the binding of IL-6 relative to the other immobilized glycoconjugates. IL-6 actually binds to these two isolated glycoproteins. The binding of IL-6 was not inhibited by glycopeptides isolated from RNase B or from fetuin. In contrast, the binding was inhibited using 10⁻⁴ M of a glycopeptide fraction obtained from the rat brain synaptosomal fraction (53) in which the HNK-1 epitope was present as minor constituents based on matrix-assisted laser desorption ionization/time of flight analysis. Because a series of reduced oligosaccharides possessing the SO₃H (3-)GlcA motif were isolated from the mucins of the eggs of R. temporaria (28), we tested these compounds for their inhibitory activity of the binding of IL-6 to the P₀ glycoprotein. Interestingly, the best inhibitor was a compound comprising a Fuc residue (see Ref. 28 and Fig. 6, c–e), whereas compounds lacking the Fuc residue were not inhibitory at 10⁻⁴ M. The presence of an additional Gal residue also abolished the inhibitory potency of the oligosaccharides (Fig. 6, c–e). Because none of the other oligosaccharides lacking the SO₃H (3-)GlcA group were inhibitory (data not shown), it was concluded that, although the SO₃H (3-)GlcA motif was necessary for the binding to IL-6, it was not sufficient. Considering the structure already determined of the N-glycans possessing the HNK-1 epitope (41) and the structure of the O-glycans inhibiting the binding of IL-6 to these N-glycans, it was suggested that the recognition domain of IL-6 consisted in the sequence SO₃H (3-)GlcAβ1,3Galβ1,4 followed by a monoosaccharide sequence possessing a methyl group in the vicinity of this structure (issued either from the acetamido group in GlcNAc in N-glycans or from the methyl group in the Fuc residue in R. temporaria oligosaccharide alditols).

IL-7 Binds Specifically to a Glycopeptide of the Ovine Submaxillary Mucin—As shown in Fig. 7a, IL-7 binds to fetuin and the mixture of mucins and not to the glycosaminoglycan mixture, as contrasted from the data of previous authors (12).

When the analysis was subsequently performed on the individual mucins, IL-7 binds only to the ovine submaxillary mucin (Fig. 7b). The binding was not changed when the OSM was submitted to partial or total deacetylation with NH₃ gas, indicating that O-acetylation of sialic acid residues was not important for the binding. In contrast this binding was inhibited when the mucin was desialylated by mild acid hydrolysis, indicating that a sialic acid residue was important for the binding. However, this binding could not be inhibited using 10⁻⁴ M of purified Neu5Ac (the major sialic acid present in OSM) or purified Neu5Gc or by the mixture of sialic acids isolated from OSM by mild acid hydrolysis. Furthermore, O-glycans isolated from OSM by a reductive β-elimination procedure did not show any inhibitory activity. This suggested that the presence of a GalNAc-OH instead of GalNAc residue abolished the binding or that the binding of IL-7 involved a portion of the polypeptide chain. These assumptions were sustained by the observation that complex glycopeptide mixtures obtained by prolonged Pronase digestion of OSM were inhibitory of the interaction of IL-7 with OSM. As shown in Fig. 7c, an active inhibitory compound was found as a low Mᵣ fraction isolated by Biogel P2 chromatography. This fraction contained a single resorcinol-positive spot migrating faster than the major monosialo-glycoserine of fetuin Neu5Acα2,3Galβ1,3GalNAcα1-LSer and containing one Ser, one GalNAc, and one Neu5Ac residue (Fig. 7d). Consequently, these data were compatible with the hypothesis that the IL-7 ligand was the sialyl-Tn antigen, an onco-fetal antigen relatively abundant on the ovine submaxillary mucin and comprising both an oligosaccharide and peptide determinant (54).

**DISCUSSION**

This paper focuses on a new methodology allowing the discovery of lectin activities of cytokines. Because for most of cytokines no data exist on the exact nature of the carbohydrate ligand, we undertook a systematic screening of such carbohydrate-binding properties for several interleukins including IL-

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4 J.-P. Zanetta, unpublished data.
1α, IL-1β, IL-4, IL-6, and IL-7. Similar studies have been undertaken for other cytokines or chemokines. However, lectin activity has not been demonstrated for some of them, possibly because we lack the specific ligand. The case of IL-4 is very relevant to this view, because its lectin activity would have been missed in the absence of the B. bufo mucin as a ligand. Several authors have demonstrated that cytokines could loose their lectin activity upon chemical or radiochemical labeling. Indeed, as demonstrated for IL-2, the labeling of the cytokine by iodine or biotin inhibited its biological function, although the binding to the receptor was not affected (49). Thus, the principal interest of this technique is to allow studies with proteins having a fully preserved biological activity. The discovery of the high affinity ligand will permit the labeling of the cytokine in the presence of the carbohydrate epitope to protect the carbohydrate recognition domain and leads to further investigations, like quantitative studies and identification of the endogenous ligands of cytokines on cells or on blots.

We demonstrate that except for IL-1β (for which a soluble high affinity oligosaccharide ligand was not yet isolated), the other interleukins have high affinity oligosaccharide or glycopeptide ligands inhibiting the binding of the cytokine to the immobilized ligands at a concentration range lower than 10^{-6} M. Although the $K_d$ of the interaction could not be determined using the present methodology, the identified ligands were of high affinity. For example, the ligand of IL-6 from R. temporaria (44). The ligands found in R. temporaria had a significantly different structure, $\text{HSO}_3\beta\text{GlcA}1,3\text{Gal}1,4\text{GlcNAc}1,2\beta\text{Glc}1,3$, whereas the linear oligosaccharide lacking the Fuc residue was inactive at 10^{-4} M. This suggested that, besides a site for the $\text{SO}_3\text{H}1,3\text{GlcA}$ determinant, the carbohydrate recognition domain of IL-6 contains a domain interacting with a methyl group provided either by the 2-acetamido group of $\text{GlcNAc}$ or the methyl group of Fuc, the $\text{HSO}_3\beta\text{GlcA}1,3\text{Gal}1,4\text{Fuc}1,2\text{Gal}1,3$, being not sufficient for a high affinity interaction. For IL-4, the ligand, the 1,7 lactone of Neu5Ac, is a compound actually present in glycoproteins of the human lymphocyte membrane.5

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 (15), 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. 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 profiles in different cells. IL-1β, but not IL-1α, is able to stimulate human astocytes, a mechanism responsible for the nervous regulation of fever (55). It is noteworthy considering that astrocytes are the only cells of the central nervous system producing IL-1β and possessing both IL-1 receptors (56) and the GMα glycolipid (57).

The experimental approaches to answer the question of the

\[ \text{\textsuperscript{5} C. Mariller, A. Pous, and J.-P. Zanetta, manuscript in preparation.} \]
A biological function of the lectin activity of interleukins appears relatively simple, because the binding of a cytokine to its receptor induces specific changes in phosphorylation/dephosphorylation, as observed for IL-2 (15). Indeed, the high-affinity oligosaccharide ligand should inhibit the changes of phosphorylation/dephosphorylation induced by the cytokine. This is actually the case for the IL-6 ligand from B. The binding of a cytokine to its receptor induces specific changes in phosphorylation/dephosphorylation.

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