Interleukin 2 is a lectin that associates its receptor with the T-cell receptor complex

Jean-Pierre ZANETTA*‡, Catherine ALONSO† and Jean-Claude MICHALSKI†

*Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67000 Strasbourg, France, and †Laboratoire de Biochimie, CNRS UMR 111, Université des Sciences et Technologies de Lille, 59655 Villeneuve D'Ascq Cédex, France

To determine the nature of the mechanism by which the binding of interleukin-2 (IL-2) to its receptor (IL-2R β) induces IL-2R β phosphorylation by the tyrosine kinase p56^{lck} associated with the T-cell receptor (TCR) complex, we investigated the possibility that this mechanism was due to the putative lectin activity of IL-2 ([Sherblom, Sathyamoorthy, Decker and Muchmore (1989) J. Immunol. 143, 939–944]. Here we demonstrate that IL-2 is a

calcium-independent lectin specific for oligomannosidic N-glycans with five and six mannose residues. This lectin activity is preserved after binding of IL-2 to IL-2R β . IL-2 behaves as a bifunctional molecule that associates IL-2R β with specific glycoprotein ligands of the TCR complex including a glycosylated form of CD3.

INTRODUCTION

The proliferative response induced by the binding of interleukin 2 (IL-2) to its receptor IL-2R β is a key determinant affecting the magnitude of the immune response [1–3]. IL-2R β constitutively expressed on T-cells plays a critical role in the IL-2-induced intracellular pathways [2]. IL-2R β lacks tyrosine kinase domains but IL-2 binding induces its rapid tyrosine-phosphorylation by the intracellular p56^{lck} [4,5], a member of the Src family of tyrosine kinases, in the immediate vicinity of the T-cell receptor (TCR) complex [6]. To explain these observations it was suggested that the IL-2-dependent association between IL-2R β and the TCR complex resulted from conformational changes in the intracytoplasmic domain of IL-2R β . However, domain-specific anti-IL-2 antibodies and site-directed mutagenesis identified two different domains in IL-2, one necessary for the binding to its receptor and another, of which the function was uncertain, necessary for its activity [7,8]. Because it was reported that IL-2 has a lectin activity specific for oligomannosidic N-glycans with five and six mannose residues [9], we formed the hypothesis that this carbohydrate-binding property could be responsible for the association between the two receptor complexes. This hypothesis was reinforced by the finding that the calcium-independent cerebellar soluble lectin (CSL) [10], endowed with carbohydrate-binding properties close to that of IL-2 (CSL specifically binds Man₆GlcNAc₂Asn oligomannosidic N-glycans [11]), recognized N-glycosylated forms of CD3 [12], a constituent of the CD3–TCR complex.

The present study demonstrates that (1) IL-2 is a calcium-independent lectin specific for oligomannosidic N-glycans with five and six mannose residues, and (2) IL-2 associates IL-2R β with glycoprotein constituents of the TCR complex through a specific carbohydrate-dependent mechanism.

MATERIALS AND METHODS

Materials and chemicals

Microtitration plates were from Nunc (Naperville, IL, U.S.A.). Ribonuclease B (RNAse B; type III-B from bovine pancreas),

agarose–Protein A beads, BSA, RPMI 1640 culture medium, cysteine/methionine-free RPMI 1640 culture medium, alkaline phosphatase (AKP)-labelled extravidin, *p*-nitrophenyl phosphate platelets, Brij97 detergent, PMSF, *p*-tosyl-arginine methyl ester, Nitro Blue Tetrazolium (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), α 2-microglobulin and leupeptin were from Sigma (St. Louis, MO, U.S.A.). [³⁵S]Met/Cys mixture and Hyperfilm MP were from Amersham (Amersham, Bucks., U.K.). Normal goat serum was from Biosys (Compiègne, France). Fetal calf serum (FCS) was from Gibco BRL (Paisley, Scotland,

Figure 1 Structures determined by NMR spectroscopy of the oligosaccharides used in this study

(a) M5 oligosaccharide. (b) M6 glycoasparagine; according to Wyss et al. [37], the Man α 1-2 residue interacts specifically with the GlcNAc(β 1-4)GlcNAc(β 1-4) disaccharide. The recognition by CSL of the specific conformation of this glycoasparagine (this conformation is not observed for glycoasparagine with higher mannose content or for any oligomannosidic oligosaccharide with a single GlcNAc residue) might explain previous results on the carbohydrate-binding properties of CSL [11]. (c) M9 oligosaccharide.

Abbreviations used: AKP, alkaline phosphatase; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CSL, cerebellar soluble lectin; FCS, fetal calf serum; FGF, fibroblast growth factor; IL-2, interleukin 2; IL-2R, interleukin 2 receptor; M5, Man₅GlcNAc₂; M6, Man₆GlcNAc₂Asn; M9, Man₉GlcNAc; NBT, Nitro Blue Tetrazolium; pBSA, periodate-treated BSA; PE-pBSA: PBS buffer containing 5 mM EDTA and 3% (v/v) pBSA; TCR, T-cell receptor.

‡ To whom correspondence should be addressed.

U.K.). Ficoll-Paque was from Pharmacia (Uppsala, Sweden) and nitrocellulose filters (0.45 μm pore size) from Schleicher & Schuell (Dassel, Germany). Human recombinant IL-2 (at least 97% purity) was from UBI (Lake Placid, NY, U.S.A.). In all experiments involving protein-carbohydrate interactions, BSA was previously treated with periodate (to eliminate oligosaccharides present in commercial BSA preparations, which could interfere with lectin-carbohydrate interactions) by the method of Glass et al. [13].

Antibodies

Mouse anti-(human TCR β) monoclonal antibody was from Ancell (Laüfelfingen, Switzerland). This antibody was chosen because it did not inhibit TCR function and was directed against the common part of the TCR β chain. Anti-(human IL-2R β) monoclonal antibody (clone M1K β 1) was from CLB (Amsterdam, The Netherlands); unlabelled goat anti-(mouse IgG) and AKP-labelled goat anti-(rabbit Ig) were from Biosys (Compiègne, France) and AKP-labelled goat anti-(mouse Ig) was from Sigma. The rabbit polyclonal antibody directed against the N-terminal portion (residues 22–51) of human p56^{lck} was from UBI. The rabbit anti-CD3 antibody was initially described as an antibody directed against the Purkinje cell-specific glycoprotein. This antibody, staining only Purkinje neurons within the central nervous system [14,15], specifically immunoprecipitates CD3 from human lymphocyte lysates (J.-P. Zanetta, unpublished work).

Oligosaccharides and glycoasparagines

Man₆GlcNAc₂Asn (M6) was prepared by exhaustive Pronase digestion of ovalbumin followed by cation-exchange chromatography on Dowex 50 $\times$ 2, 200–400 mesh, H⁺ form (1.5 cm $\times$ 150 cm) by the method of Tai et al. [16]. Man₅GlcNAc₂Asn was isolated from the urine of a patient with Gaucher's disease as previously described [17]. Man₅GlcNAc₂ (M5) was obtained by hydrazinolysis followed by re-N-acetylation [18]. Man₆GlcNAc (M9) was obtained from the urine of a patient with α -mannosidosis [19]. The structures of the different glycoasparagines and oligosaccharides (Figure 1) were established by NMR on a Bruker AM-400 WB spectrometer. Resolution enhancement of ¹H-NMR spectra was achieved by Lorentzian to Gaussian transformation by the method of Ernst [20]. ¹H-NMR double-relayed spectra at 400 MHz from correlation spectroscopy were compared with literature data obtained from reference compounds [21].

Determination of the lectin activity of IL-2

The determination of the lectin activity of IL-2 was performed in two steps. The first consisted of the competition of IL-2 (or oligomannosidic N-glycans) for the binding of biotinylated CSL to plates coated with RNase B (a glycoprotein containing Man₅₋₉GlcNAc₂ oligomannosidic N-glycans). The second consisted of demonstrating that the inhibition of the binding of biotinylated CSL by IL-2 was due to the binding of IL-2 to the RNase B N-glycans. CSL was isolated from the cerebella of young rats by immunoaffinity chromatography [10]. The active lectin was biotinylated and its activity was verified as described previously [22].

Microtitration plates were coated (5 h at 37 °C in a ventilated oven) with 1.2 μg of RNase B dissolved in 50 μl of PBS (25 mM sodium phosphate, pH 7.2, containing 150 mM NaCl). After washing with PBS, wells were saturated with periodate-treated BSA (pBSA) for 2 h at room temperature.

For the first series of experiments, biotinylated CSL (0.1–4.5 μg) was added with or without different concentrations of oligomannosidic inhibitors (from 10⁻⁷ to 10⁻⁴ M) or of IL-2 (from 0.1 to 4 μg) in PBS containing 5 mM EDTA and 3% (v/v) pBSA (PE-pBSA). After ten washings for an overall 2 h period in PE-pBSA, detection of the binding of biotinylated CSL was performed with AKP-labelled extravidin as follows. A 3% (v/v) solution of pBSA in PBS was added for 1 h followed by AKP-labelled extravidin (1:1000 dilution). After incubation for 2 h, wells were washed ten times with PBS, once with PBS containing 0.1% Triton X-100, ten more times with PBS and twice with water. Bound extravidin was revealed by using *p*-nitrophenyl phosphate reagent and measuring the absorbance at 405 nm.

For the second series of experiments, RNase B-coated plates were incubated for 2 h at room temperature with 2 μg of IL-2 in PE-pBSA supplemented or not with 10 μM oligomannosidic N-glycans. After one washing with PE-pBSA containing the same oligomannosidic N-glycans in the absence of IL-2 and additional washings with PE-pBSA, biotinylated CSL (1 μg , corresponding to the maximal binding) was added for 2 h followed by washings and detection of biotinylated CSL as described above.

Study of the function of the lectin activity of IL-2

Peripheral blood lymphocytes of normal individuals were isolated by Ficoll-Paque centrifugation. After elimination of adherent cells on plastic for 30 min in RPMI 1640 containing 10% (v/v) FCS, cells were washed three times in cysteine/methionine-free RPMI 1640 containing 10% FCS. Cells (2 $\times$ 10⁷) were cultured in humidified air containing 5% CO₂ for 24 h in the same medium supplemented with 1 mCi of [³⁵S]Met/Cys mixture. After being washed in RPMI 1640 containing 10% FCS, cells were supplemented or not with 20 ng/ml human recombinant IL-2 and incubated for 15 min at 37 °C and then cooled. After being washed with cold PBS, cells were lysed at 4 °C in 750 μl of 10 mM Tris/HCl buffer, pH 7.4, containing 150 mM NaCl, 5 mM EDTA, 50 $\mu\text{g}/\text{ml}$ pBSA and 1% (v/v) Brij97, supplemented with protease inhibitors (10 mM PMSF, 10 mM *p*-tosyl-arginine methyl ester, 20 $\mu\text{g}/\text{ml}$ microglobulin and 10 μM leupeptin). After centrifugation to eliminate nuclei (12 500 *g* for 15 min at 4 °C), the supernatants were incubated for 2 h at room temperature with rotation with 100 μl of agarose-Protein A beads previously coated with 100 $\mu\text{g}/\text{ml}$ swollen gel of rabbit anti-mouse IgG, followed by the mouse anti-(human TCR β) monoclonal antibody (the binding of the antibodies to agarose-Protein A beads was performed for 2 h at 4 °C and the beads were exhaustively washed in the lysis buffer before use).

After three washings at room temperature in 750 μl of the lysis buffer, beads were incubated for 5 min at room temperature in the same buffer containing 50 $\mu\text{g}/\text{ml}$ M9. After centrifugation, the beads were incubated for 5 min at room temperature in the same buffer containing 10 $\mu\text{g}/\text{ml}$ M6 (or M5). The last washing of the anti-TCR β beads and the M9 and M6 (or M5) eluates were immunoprecipitated overnight at 4 °C with 25 μg of mouse anti-(human IL-2R β) monoclonal antibody followed by addition of 50 μg of goat anti-(mouse IgG) (2 h at 4 °C). Immunoprecipitates were recovered by centrifugation and washed in the buffer, followed by boiling in Laemmli [23] dissociating buffer. For control of anti-IL-2R β precipitations, all supernatants were precipitated with 10% (w/v) trichloroacetic acid and the precipitates were washed in methanol and boiled in Laemmli [23] dissociation buffer before electrophoresis. Beads containing the anti-(human TCR β) antibodies after M6 (or M5) elutions were also boiled in this buffer.

Electrophoretic and blotting techniques

Samples were subjected to SDS/PAGE [13 % (w/v) acrylamide] in the buffer system of Laemmli [23] and transferred to nitrocellulose by the method of Towbin et al. [24]. The blots corresponding to the anti-IL-2R β immunoprecipitates of M9 and M6 (or M5) elutions were submitted to autoradiography and subsequently revealed for the presence of p56^{lck} or the presence of ligands of CSL (see below).

For the detection of p56^{lck} by immunoblotting, blots were saturated for 1 h at room temperature with PBS containing 3 % (v/v) BSA and a 1:20 dilution of normal goat serum. The polyclonal antibody was added (1:500 dilution) and incubation was performed for 4 h at room temperature. Blots were rinsed ten times over a 2 h period with PBS containing 0.3 % BSA and saturated again as above. After addition of AKP-labelled goat anti-(rabbit Igs) (1:1000 dilution), the blots were incubated overnight at 4 °C, then rinsed as above, followed by 2 h of washing with PBS and two washings with distilled water. The blots were revealed with the NBT/BCIP substrates [25]. Blanks were made by using a preimmune rabbit serum instead of the anti-p56^{lck} specific antibody.

The detection of CSL ligands on blots was performed by incubations with biotinylated CSL followed by AKP-labelled extravidin and staining with NBT/BCIP substrates as previously described [12,20]. Controls of specificity were made by using boiled biotinylated CSL (30 min at 100 °C).

RESULTS AND DISCUSSION

Determination of the lectin activity of IL-2

To verify the lectin activity of IL-2, reported by Sherblom et al. [9] to be specific for M5 and M6 but not for M9 N-glycans, we

Figure 2 Binding and inhibition of binding of biotinylated CSL to RNase B

(a) The binding of biotinylated CSL to RNase B (▲) reached a plateau above 1 µg, indicating that it was saturable. This binding was inhibited with 10 µM M6 but not with the same concentrations of M5 and M9. The binding of biotinylated CSL was significantly decreased when 0.4 µg of IL-2 was co-incubated with CSL. (b) Concentration dependence of the inhibition of the binding of 1 µg of biotinylated CSL to RNase B by different concentrations of M5, M6 and M9 and different amounts (expressed as grams) of IL-2. Results are means of three experiments (standard deviations were less than 5%).

Table 1 Inhibition of the binding of 1 µg biotinylated CSL by 2 µg IL-2 and double inhibition of the binding using oligomannosidic N-glycans

The simultaneous incubation of IL-2 with 10 µM M5 or M6 did not inhibit the binding of biotinylated CSL to RNase B. The inhibition of the binding of biotinylated CSL when IL-2 was incubated in the presence of 10 µM M9 indicated that M9 was unable to inhibit the IL-2 binding to RNase B N-glycans. Results are the means of three experiments (standard deviations were less than 5%).

Pre-incubation	Biotinylated CSL binding (%)
None	100
IL-2	26
IL-2 + M9	24
IL-2 + M5	97
IL-2 + M6	102

used a competition of binding between recombinant human IL-2 and biotinylated CSL to common N-glycans found on RNase B. We chose this method because of the possibility of activity loss of the putative lectin site of IL-2 on radioactive or chemical labelling. As shown in Figure 2(a), biotinylated CSL bound to RNase B-coated microwells in a concentration-dependent and saturable process. The binding was inhibited by the M6 isomer shown in Figure 1 (IC₅₀ 0.75 µM) but not by M5 and M9 up to 100 µM (Figures 2a and 2b). The binding of biotinylated CSL was also inhibited (in an approximately molar ratio) by IL-2, suggesting very similar affinities of the two proteins for RNase B (Figure 2b).

Because there was 3 % pBSA in the incubation medium, it was suggested that the inhibition of the binding of biotinylated CSL to RNase B by IL-2 could be due to a competitive binding to the same N-glycans of the RNase B. To determine whether this inhibition was actually carbohydrate-dependent we used a double-inhibition system. IL-2 was first added to RNase B-coated wells in the presence or absence of 10 µM M5, M6 or M9. After washings, biotinylated CSL was added. As shown in Table 1, the binding of CSL was inhibited only when IL-2 was incubated in the absence of oligomannosidic N-glycans or in the presence of 10 µM M9 but not when IL-2 was added in the presence of 10 µM M5 or M6. This indicated that M5 and M6 (but not M9) inhibited the binding of IL-2 to RNase B. Therefore these experiments verified that IL-2 was a lectin specific for M5 and M6 at neutral pH. However, because these experiments were performed in the presence of 5 mM EDTA, it became evident that IL-2 was not a C-type lectin as suggested previously [9], as calcium is directly involved in the binding of carbohydrates in C-type lectins [26].

Role of the lectin activity of IL-2 in the association between IL-2R β and the TCR complex

The hypothesis that the association between IL-2R β and the TCR complex through IL-2 was carbohydrate-dependent was tested on resting human lymphocytes. Although the quantity of IL-2 receptors is minimal, only the IL-2R β is expressed significantly. Lymphocytes from control individuals were labelled with ³⁵S and supplemented or not with 20 ng/ml IL-2 for 15 min at 37 °C [this short time was chosen to minimize IL-2R β internalization after binding of IL-2, whereas a relatively high IL-2 concentration (2–10 times that generally used) was applied to saturate IL-2R β molecules]. Cells were lysed in a mild detergent that had been shown to preserve the associations of cell surface complexes [27]. The supernatants were adsorbed on agarose

Figure 3 Evidence for an IL-2-dependent association of IL-2R β with the TCR complex

(a) Autoradiographs of the blots of the anti-IL-2R β immunoprecipitates of the material eluted from anti-TCR-coated beads with M9 then M6 oligomannosides. Lanes 1 and 3, control cells; lanes 2 and 4, cells treated with IL-2. Lanes 1 and 2 correspond to M9 eluates and lanes 3 and 4 to M6 eluates. Note that radioactive bands were found only in the M6 eluate of the IL-2-treated cells (arrows at the left indicate the positions of the 75, 59, 56 and 45 kDa constituents). (b) Immunoblotting identification of the 56 and 59 kDa bands with p56^{lck} on the same blot as in (a). Note that the immunostained doublet bands were not present in the M9 eluates or in the M6 eluate of material from control cells, but are found specifically in the M6 eluate of cells previously incubated in the presence of IL-2. (c) Analysis of the CSL ligands (lanes 1 and 2) found in the material remaining adsorbed on the anti-(human TCR β) beads after M9 and M6 elutions. Note the presence of a band with the same molecular mass as CD3 (lower arrow at the right). Lanes 3 and 4, controls for the specificity of CSL binding to the same fractions obtained with boiled biotinylated CSL.

beads previously coated with the mouse anti-(human TCR β) monoclonal antibody. After being washed, the beads were sequentially eluted in buffer containing M9 then M6 (or M5). The supernatants of the washings and M9 and M6 (or M5) eluates were immunoprecipitated with a mouse anti-(human IL-2R β) monoclonal antibody and the immunoprecipitates were subjected to SDS/PAGE, blotted on to nitrocellulose and blots subjected to autoradiography.

As shown in Figure 3a, autoradiographs of the anti-IL-2R β immunoprecipitated material indicated that radioactive bands were detected only in the M6 (or M5; result not shown) eluate of the material adsorbed on anti-TCR beads from cells initially incubated in the presence of IL-2. These bands had molecular masses of 75, 59, 56 and 45 kDa. As expected for a material immunoprecipitated with specific anti-IL-2R β antibody, one of these bands (75 kDa) corresponded to IL-2R β itself. The 59 and 56 kDa bands had similar molecular masses to those characteristic of the p56^{lck} shown by others [4] to be co-immunoprecipitated with IL-2R β when human T-cells are treated with IL-2. As shown in Figure 3b, immunoblotting experiments with the specific polyclonal antibody to p56^{lck} revealed that the bands at 56 and 59 kDa were immunologically related to p56^{lck}. The 59 kDa band has been attributed to extensive serine-phosphorylation of the intracytoplasmic domain of p56^{lck} on association with IL-2R β [4].

Although the nature of the 45 kDa band remains unknown, these experiments verify the IL-2-dependent association between p56^{lck} and IL-2R β described previously [4]. However, a new concept is suggested by the observation that the IL-2R β -p56^{lck} complex is bound to anti-TCR β beads and specifically eluted from the latter with M6 and M5, two glycan ligands of IL-2. In contrast, the IL-2R β -p56^{lck} complex bound to the anti-TCR beads is not detached by using M9, which is not a ligand of IL-2. Because these carbohydrate-binding properties are those of

IL-2, these experiments demonstrate that IL-2 bound to its receptor is able to bring about the association of the IL-2R β with the TCR complex through its carbohydrate-recognition domain.

The putative IL-2 glycoprotein ligands in the TCR complex were analysed on the material attached to the anti-TCR beads after M9 and M6 elution, by using biotinylated CSL followed by AKP-labelled extravidin. Such experiments were justified because IL-2 has common M6 ligands with CSL, although it could have additional ligands of the M5 type. As shown in Figure 3c, only two glycoprotein subunits (55 and 28 kDa) were detected in the material bound to the anti-TCR β beads, independently of whether or not cells were supplemented with IL-2. The 55 kDa glycoprotein was not identified, whereas the second had the same molecular mass as a glycosylated form of CD3 identified by immunoprecipitation [12]. The identification of this ligand of CSL as CD3 was confirmed by its immunoreactivity with the antibody against the Purkinje cell-specific glycoprotein, also specific for human CD3 (J.-P. Zanetta, unpublished work). Therefore, owing to the common lectin properties of IL-2 and CSL, it is suggested that IL-2 can associate IL-2R β with at least two different glycoprotein components of the CD3-TCR complex, one of which corresponds to a glycosylated form of CD3.

The similar lectin properties of IL-2 and CSL raised a specific point because CSL is also a lymphocyte antigen, which could have provoked a similar association. Although CSL is expressed in resting lymphocytes, no evidence was obtained that it is externalized in resting cells [12], in contrast with activated cells where, after externalization, it binds to CD3 glycosylated forms. The presence of the polyvalent CSL could have associated the two types of receptor through its lectin activity. This point was examined by looking for the presence of CSL ligands in the IL-2R β immunoprecipitates. In fact, these experiments gave completely negative results (not shown), indicating that none of the constituents of the IL-2R β -p56^{lck} complex was a ligand of CSL. Furthermore immunodetection of CSL in the material adsorbed on anti-TCR β , in the IL-2R β immunoprecipitate and in the supernatant of this immunoprecipitate gave negative results whether or not the cells were treated with IL-2.

Conclusion and perspectives

Our data suggest a new explanation of how the binding of IL-2 to its receptor β induces the phosphorylation of IL-2R β by the p56^{lck} kinase, which seems to be physically associated with the CD3-TCR complex [6]. As shown in Figure 4, the bifunctional IL-2, having a receptor-binding domain (RBD) and a carbohydrate-recognition domain (CRD), cross-links IL-2R β to the CD3-TCR complex. The lectin activity allows IL-2 to bind extracellularly to glycoproteins of the CD3-TCR complex. This glycan-specific surface association provokes intracellular rearrangements and, consequently, the contact of p56^{lck}, initially bound to the CD3-TCR complex, with IL-2R β , which becomes tyrosine-phosphorylated. The p56^{lck} binds through its SH2 domain to the specific intracytoplasmic domain of the IL-2R β containing the phosphotyrosine residue. Because of the presence of at least two different potential glycoprotein ligands of IL-2 in the CD3-TCR complex, it is suggested that at least two different IL-2R β molecules could be associated with it. Furthermore because other ligands of CSL (consequently potential ligands of IL-2), which are not part of the TCR complex, are present in T-cells [12], it is suggested that IL-2R β could be associated with other surface complexes containing putative glycoprotein ligands of IL-2.

This study opens new insights into understanding the mechanisms of action of cytokines. Carbohydrate-binding

Figure 4 Schematic drawing of the consequence of the bifunctionality of IL-2

IL-2 bound to its receptor β (IL-2R) through the receptor-binding domain (RBD) is able to associate (through its carbohydrate-binding domain; CRD) IL-2R β with at least two different ligands in the CD3-TCR complex, one of them being a glycosylated form of CD3. These extracellular interactions put the p56^{lck} in contact with the intracytoplasmic domain of IL-2R β , and induce its phosphorylation and the association of p56^{lck} with IL-2R β . In our experimental design the anti-TCR β antibody on the beads fixes the CD3-TCR-associated material. The complex IL-2R β /p56^{lck} is not detached using M9 (an oligomannoside not binding to IL-2), but is released from the anti-TCR β column using Man₆GlcNAc₂Asn (M6) or M5 (the ligands of IL-2). The complex IL-2R β /p56^{lck} is immunoprecipitated from the M5 or M6 eluates by using anti-IL-2R β antibody. The IL-2-binding glycans are represented by black circles. In this experimental design the analysed material is that related to the CD3-TCR complex (material bound to the anti-TCR β antibody). Because other ligands of CSL (and consequently potential ligands of IL-2) are found in T-cells [12], it is suggested that IL-2 bound to IL-2R β can associate IL-2R β with other surface complexes containing IL-2 ligands. Such a mechanism could be extended to B-cells possessing a major CSL ligand (and consequently IL-2 ligand), CD24.

properties (different from that of IL-2) have been demonstrated for tumour necrosis factors and for interleukin 1 [28–30]. Because binding-dependent associations of cytokine receptors with specific kinases is frequently observed [31–33], it can be speculated that similar glycobiological associations between cytokine receptors and other molecular complexes might occur, owing to bifunctional cytokines having a receptor-binding domain and a carbohydrate-recognition domain. Such bifunctional behaviour was recently demonstrated for fibroblast growth factor β (FGF β) [34,35], which behaves as a lectin specific for a minimal oligosaccharide structure [36]. The binding of FGF β to specific repetitive domains of heparin induces a clustering of FGF β molecules, which consequently induces the oligomerization of FGF receptor molecules [34,35]. This generates an intracellular signal through specific phosphorylations. The similarity of the three-dimensional structures of FGF β and of several cytokines (including tumour necrosis factors and interleukin 1, which have calcium-independent lectin activities [28–30]) suggests that a similar mechanism could take place for a variety of cytokines and growth factors.

The concept that cytokines might have a carbohydrate-binding site different from the receptor-binding site could have important consequences in the field of pathologies of the immune system. Specifically, the dependence of the IL-2-related signal transduction system on oligomannosidic glycans could be of importance in the field of immunodeficiencies observed in several pathologies [α -mannosidosis, candidiasis, AIDS and cancer (J.-P. Zanetta, R. Bonaly, S. Maschke, G. Strecker and J.-C. Michalski, unpublished work)].

We thank J.-C. Barthe, J.-G. Lorentz, A. Meyer and C. Zanetta for technical assistance, and Dr. J.-B. Dietrich for lymphocyte cultures. This work was supported by grants of the National Multiple Sclerosis Society (U.S.A.) and the Association pour la Recherche contre le Cancer (France).

REFERENCES

- 1 Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. and Sugamura, K. (1992) *Science* **257**, 379–382
- 2 Taniguchi, T. and Minami, Y. (1993) *Cell* **73**, 5–8
- 3 Waldmann, T. A. J. (1991) *J. Biol. Chem.* **266**, 2681–2684
- 4 Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S.D., Perlmutter, R. M. and Taniguchi, T. (1991) *Science* **252**, 1523–1528
- 5 Shibuya, H., Shibuya, H., Kohu, K., Yamada, K., Barsoumian, E. L., Perlmutter, R. M. and Taniguchi, T. (1994) *Mol. Cell. Biol.* **14**, 5812–5819
- 6 Altman, A., Mustelin, T. and Coggeshall, K. M. (1990) *Crit. Rev. Immunol.* **10**, 347–391
- 7 Cohen, F. E., Kosen, P. A., Kuntz, I. D., Epstein, L. B., Ciardelli, T. L. and Smith, K. A. (1986) *Science* **234**, 349–352
- 8 Ju, G., Collins, L., Kaffka, K. L., Tsien, W. H., Chizzonite, R., Crowl, R., Bhatt, R. and Kilian, P. L. (1987) *J. Biol. Chem.* **262**, 5723–5731
- 9 Sherblom, A. P., Sathiyamoorthy, N., Decker, J. M. and Muchmore, A. V. (1989) *J. Immunol.* **143**, 939–944
- 10 Zanetta, J.-P., Meyer, A., Kuchler, S. and Vincendon, G. (1987) *J. Neurochem.* **49**, 1250–1257
- 11 Marschal, P., Reeber, A., Neeser, J. R., Vincendon, G. and Zanetta, J. P. (1989) *Biochimie* **71**, 645–653
- 12 Zanetta, J.-P., Wantyghem, J., Kuchler-Bopp, S., Badache, A. and Aubery, M. (1995) *Biochem. J.* **311**, 629–636
- 13 Glass, W. F., Briggs, R. C. and Hnilica, L. S. (1981) *Anal. Biochem.* **115**, 219–224
- 14 Reeber, A., Vincendon, G. and Zanetta, J.-P. (1981) *Brain Res.* **229**, 53–65
- 15 Langley, O. K., Reeber, A., Vincendon, G. and Zanetta, J.-P. (1982) *J. Comp. Neurol.* **208**, 335–344
- 16 Tai, T., Yamashita, K., Ogata, A. M., Koide, N., Miramatsu, T., Inashita, S., Inoue, Y. R. and Kobata, A. (1975) *J. Biol. Chem.* **250**, 8569–8575
- 17 Michalski, J.-C., Montreuil, J., Strecker, G., Van Halbeek, H., Dorland, L., Vliegthart, J. F. G., Cartigny, B. and Farriaux, J.-P. (1983) *Eur. J. Biochem.* **132**, 375–381
- 18 Michalski, J.-C., Peter-Katalinic, K., Paz-Parente, J., Montreuil, J. and Strecker, G. (1984) *Carbohydr. Res.* **134**, 177–189
- 19 Van Halbeek, H., Dorland, L., Veldink, G. A., Vliegthart, J. F. G., Strecker, G., Michalski, J.-C., Montreuil, J. and Hull, W. E. (1980) *FEBS Lett.* **121**, 71–77
- 20 Ernst, R. R. (1966) in *Advances in Magnetic Resonance* (Wangh, S. S., ed.), vol. 2, Academic Press, London
- 21 Vliegthart, J. F. G., Dorland, L. and Van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem.* **41**, 209–374
- 22 Zanetta, J.-P., Maschke, S. and Badache, A. (1993) in *Lectins and Glycobiology* (Gubius, H. J. and Gubius, S., eds.), pp. 182–187, Springer-Verlag, Heidelberg
- 23 Laemmli, U.K. (1970) *Nature (London)* **227**, 680–685
- 24 Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- 25 Leary, J. J., Brigati, D. J. and Ward, D. C. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4045–4049
- 26 Weis, W. I., Drickamer, K. and Hendrickson, W. A. (1992) *Nature (London)* **360**, 127–134
- 27 Bohuslav, J., Cinek, T. and Horejsi, V. (1993) *Eur. J. Immunol.* **23**, 825–831
- 28 Lucas, R., Magez, S., Deleys, R., Fransen, L., Scheerlinck, J. P., Rampelberg, M., Sablon, E. and Debaetselier, P. (1994) *Science* **263**, 814–817
- 29 Brody, D. T. and Durum, S. K. (1989) *J. Immunol.* **143**, 1183–1187
- 30 Muchmore, A. U. and Decker, J. M. (1987) *J. Immunol.* **138**, 2541–2552
- 31 Farrar, W. L., Linnekin, D., Evans, G., Garcia, G. G. and Michiel, D. (1990) *Molec. Cell. Biol.* **10**, 265–269
- 32 Martin, M., Bol, G. F., Eriksson, A., Resch, K. and Brigelius-Flohe, R. (1994) *Eur. J. Immunol.* **24**, 1566–1571
- 33 Venkataraman, A. R. and Cowling, R. J. (1994) *Eur. J. Immunol.* **24**, 2168–2174
- 34 Spivak-Kroizman, T., Lemmon, M. A., Dikic, I., Ladbury, J. E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J. and Lax, I. (1994) *Cell* **79**, 1015–1024
- 35 O'Brien, C. (1995) *Science* **267**, 1263–1264
- 36 Maccarana, M., Casu, B. and Lindahl, U. (1993) *J. Biol. Chem.* **268**, 23898–23905
- 37 Wyss, D. F., Choi, J. S., Li, J., Knoppers, M. H., Willis, K. J., Arulanandam, A. R. N., Smolyar, A., Reinherz, E. L. and Wagner, G. (1995) *Science* **269**, 1273–1278