Histol Histopathol (1996) 11: 1101-1108

Histology and Histopathology

Invited Review

Lectin activities of cytokines and growth factors: function and implications for pathology

J.-P. Zanetta[1], T. Scior[2], J. Wantyghem[3], C. Wermuth[2], M. Aubery[3], G. Strecker[4] and J.-C. Michalski[4]

[1]Centre de Neurochimie du CNRS, Strasbourg, [2]Laboratoire de Pharmacologie Moléculaire, Strasbourg, [3]INSERM U 180, Paris, [4]Laboratoire de Chimie Biologique USTL et UMR 111 du CNRS, Villeneuve d'Ascq Cédex, France

Summary. The discovery that certain cytokines have carbohydrate-binding (lectin) properties opens new concepts in the understanding of their mechanism of action. The carbohydrate-recognition domain, which is localized opposite to the receptor-binding domain, makes these molecules bi-functional. The expression of the biological activity of the cytokine relies on its carbohydrate-binding activity which allows the association of the cytokine receptor with molecular complexes comprising the specific kinase involved in receptor phosphorylation and in specific signal transduction. It is expected that blood accumulation of free or membrane-bound glycan ligands of cytokines may dramatically perturb their endogenous function inducing specific immunodeficiencies.

Key words: Lectin, Cytokine, Clustering, Signal transduction, Tyrosine kinase, Immunodeficiency, Aids, α-mannosidosis, Mannose, Glycan, Il-2, CSL

Introduction

There is increasing evidence that carbohydrate-binding proteins (lectins) are widespread in mammals, and especially in cells of the immune system. More than ten calcium-dependent lectins (C-type lectins; Drickamer, 1988) have been identified each endowed with a different carbohydrate-binding specificity. For example, L-selectin (Bevilacqua et al., 1989; Springer, 1991; Foxall et al., 1992; Lasky, 1992; Bevilacqua and Nelson, 1993) recognizes the 6'-sulphated sialyl-Lewis^x epitope. CD69, which represents the first member of a family of related lectins of the surface of natural killer cells (Lanier et al., 1994; Bezouska et al., 1995) is specific for galactose. Likewise, the calcium-independent lectins comprise the lactose-binding galectins (Barondes et al., 1994), the NeuNAcα2-6 binding lectin CD22 (Powell and Varki, 1994; Powell et al., 1995), the NeuNAcα2-3 binding sialoadhesin (Kelm et al., 1994; Nath et al., 1995) and CSL, which is specific for oligomannosidic N-glycans with 6 mannose residues (Zanetta et al., 1987). The MR60-ERGIC-53 mannose-binding lectin (Fiedler and Simons, 1994; Arar et al., 1995) is related to plant lectins. Besides these molecules involved either in homotypic or heterotypic cell adhesion, or in intracellular traffic of glyco-conjugates, those which are polyvalent (having two carbohydrate-recognition domains (CRD) or organized as oligomers) may have clustering effects on their ligands. This may represent the endogenous mechanism mimicked by specific polyvalent antibodies or plant lectins (Feizi and Childs, 1987). For example, the lectin CSL, which is rapidly over-expressed and externalized after stimulation of human cells with phorbol esters and which binds to its surface ligands (including the glycosylated forms of CD3 on T cells and CD24 on B cells) is responsible for the major tyrosine-phosphorylation changes occurring in the early stages of cell activation (Zanetta et al., 1995).

Such a role of soluble extracellular lectins in signal transduction raises the question of how small soluble proteins, through their binding to a specific receptor, may generate specific intracellular signals. It has been frequently observed that when a cytokine binds to its receptor, the intracytoplasmic receptor domain becomes phosphorylated. This phosphorylation could not be explained simply by the putative oligo-merization of the cytokine (often dimeric in crystals) since these intracytoplasmic domains are frequently devoid of kinase activities. Indeed, the intracellular phosphorylation is due to a kinase which is associated with another surface receptor complex. When phosphorylation studies are performed on an intact cell, the cytokine-specific receptor is always phosphorylated by a unique kinase which suggests that, rather than unspecific conformational changes of the intracyto-plasmic receptor domain upon cytokine binding, the cytokine itself would be responsible for the specific extracellular association of its receptor to another surface complex. This review will document the hypothesis that such a cytokine-dependent association of surface receptor complexes may be linked to the

Offprint requests to: Dr. J.-P. Zanetta, CNRS UMR 111, Laboratoire de Chimie Biologique USTL, 59655 Villeneuve d'Ascq Cédex, France

presence of a carbohydrate-recognition domain in cytokines. Pathological implications are also discussed focused on the lectin activity of IL-2.

Putative lectin activities of cytokines and growth factors

Site-directed mutagenesis experiments and the action of domain-specific antibodies sustain the evidence that cytokines and growth factors contain two different domains. The first is defined as the receptor-binding domain, a complex site which often includes the N- and C-terminal sequences. A specific loop which interacts with the receptor is generally stabilized by disulphide bridges or ß-strand interactions. However, these molecules possess another undefined domain, not implied in receptor binding, but necessary for the expression of the biological activity of the cytokine. Subtle changes in this second domain do not modify the conformation of the receptor-binding domain, but suppress the biological activity. In the three-dimensional structure of cytokines, this second domain is localized at opposite to the receptor-binding site (Wells et al., 1994).

The nature of this second domain involved in cytokine activity, but not in its binding to the receptor, remained unknown until recently, although lectin-like activities have been previously described for several cytokines like IL-1α and IL-1ß, TNFα and TNFß (Sherblom et al., 1988), a series of molecules presenting three-dimensional homologies with heparin-binding growth factors. Likewise, interleukin-2 (IL-2) was described as a lectin specific for oligomannosidic N-glycans with 5 and 6 mannose residues, suggesting that IL-2 recognized an unsubstituted Manα1-6(Manα1-3)Man structure (Sherblom et al., 1989). Unfortunately, this work was not further developed, probably because the homology of IL-2 with C-type lectins suggested by the authors was not convincing. Although the trypanolytic activity of TNFß has been determined as dependent on its lectin activity (Lucas et al., 1994), the roles in signalling of the lectin activities of cytokines have not been analyzed.

Biological function of the lectin domain of IL-2

A recent report concerns the biological function of the lectin activity of IL-2 (Zanetta et al., 1996). IL-2 is a 15 kDa molecule, produced essentially by activated $CD4^+$ T cells, which can stimulate the proliferation of T cells, induce the NK activity of $CD8^+$ cells and the activation of B cells (and other leukocytes having IL-2 receptors at their surface). Three IL-2 receptors (IL-2R) have been identified (α, ß, gamma), only the IL-2Rß is constitutively expressed on resting cells (Zola et al., 1991), receiving first the IL-2 signal. Studies on the signal transduction pathway resulting from IL-2 binding (see for review Waldmann, 1991; Taniguchi and Minami, 1993) showed that, although IL-2Rß has no kinase activities, its intracytoplasmic domain is tyrosine-phosphorylated upon IL-2 binding (Farrar et al., 1990) by the $p56^{lck}$ kinase (Hatakeyama et al., 1991; Shibuya et al., 1994). As this kinase is considered to be associated with the CD3/TCR complex, it is suggested that when IL-2 binds to its receptor, IL-2Rß and the CD3/TCR complex become associated. After tyrosine phosphorylation, $p56^{lck}$ binds through an SH2 domain specific of the src family kinases to a short sequence of the IL-2Rß receptor containing the phosphotyrosine residue and remains firmly attached to the IL-2Rß.

The common hypothesis to account for the interaction between $p56^{lck}$ and the IL-2Rß receptor is that IL-2 binding initiates a conformational change of the intracytoplasmic domain of IL-2Rß, providing a site for tyrosine phosphorylation. However, this could neither explain why only $p56^{lck}$ recognizes the intracytoplasmic domain of IL-2Rß when experiments are performed on an intact cell nor the data acquired by site-directed mutagenesis of IL-2. Indeed, it was demonstrated (Cohen et al., 1986; Ju et al., 1987) that IL-2 needs two domains for expressing its full biological activity: one is involved in the binding to its receptors, and the other, opposite to the receptor-binding site on crystallized IL-2, is necessary for the expression of the biological activity. Furthermore, using domain-specific antibodies reinforced the concept of two domains, suggesting that in vivo, IL-2 behaves as a bi-functional molecule. However, the nature of the second site, indispensable for the biological activity remained uncertain.

Several years ago, IL-2 was reported to be a lectin binding to oligomannosidic N-glycans with 5 and 6 mannose residues but not to olgomannosidic N-glycans with 9 mannose residues (Fig. 1) in the absence of calcium at neutral pH (Sherblom et al., 1989). Since the loss of lectin activity at acidic pH was partially restored in the presence of calcium ions, the authors suggested that IL-2 was a calcium-dependent lectin. This view was not supported by sequence comparisons with the well-defined C-type mammalian lectins identified by Drickamer (1988). We recently suggested (Zanetta et al., 1995) that in the early stages of lymphocyte activation, CD3/TCR complexes could be clustered by a polyvalent endogenous lectin, CSL (Zanetta et al., 1987), because the CD3/TCR complex bears N-glycans recognized by this lectin. In fact, CSL, which recognizes the $Man_6GlcNAc_2Asn$ structure (Fig. 1), is able to bind the N-glycosylated forms of CD3. As the described carbohydrate-binding properties of IL-2 were less tight than those described for CSL, it was assumed that the lectin IL-2 could also recognize N-glycosylated forms of CD3. Following its binding to IL-2Rß, IL-2 could associate this latter with the CD3/TCR complex. Hence, the data of Sherblom et al. (1989) were reexamined, using a different methodology. It occurs that IL-2 is a specific for oligomannosidic N-glycans with 5 and 6 mannose residues, but not for those with 9 mannose residues (Fig. 1) as previously described (Sherblom et al., 1989) and is active even in the presence of 5mM

EDTA, indicating that IL-2 is a calcium-independent lectin (Zanetta et al., 1996).

Using a complex experimental design, we showed that in the presence of IL-2, and under these conditions, an anti-TCR antibody co-immunoprecipitated IL-2Rß from which IL-2Rß is released in a mechanism independent of oligomannoside with 9 mannose residues, but dependent of oligomannosides with 5 and 6 mannose residues. Moreover, using an anti-IL-2Rß antibody, this specifically released IL-2Rß co-immunoprecipitated with the $p56^{lck}$ kinase, verifying the strong association between IL-2Rß and $p56^{lck}$ (Zanetta et al., 1996). This confirms that the lectin activity of IL-2 provokes an extracellular association between IL-2Rß and glycoprotein constituents of the TCR complex. Detection of the putative IL-2 ligands in the TCR complex by CSL identifies two glycoprotein subunits (Zanetta et al., 1996), one corresponding to a glycosylated form of CD3 and the other to an unidentified glycoprotein of 55 kDa. This suggests that when IL-2 interacts with its receptor, two molecules of IL-2Rß become associated with the CD3/TCR complex. Furthermore, from the similarities of the carbohydrate-binding properties of CSL and IL-2, it might be suggested that once bound to its receptor, IL-2 could associate IL-2Rß to other complexes, different from the CD3/TCR complex, containing other ligands of IL-2. This possibility was not tested, since only the association with the CD3/TCR complex was examined. Nevertheless, these experiments indicated that in resting cells, the super-complex CD3/TCR:IL-2Rß/$p56^{lck}$ is not associated with other surface complexes in an IL-2-dependent mechanism.

Accordingly, when bound to their receptors, cytokines endowed with monovalent lectin activities may induce the carbohydrate-dependent surface association of different receptor complexes. Because they are actually bi-functional, they are able to recognize specific glycans at the cell surface leading to specific intracytoplasmic associations. The specific phosphorylation of the IL-2Rß by the $p56^{lck}$ can be explained because of the specific carbohydrate-dependent association of IL-2Rß with the CD3/TCR. Thus, these experiments demonstrate that an extracellular glycobiological interaction, which occurs in vivo, can modify specifically the intracellular organization of molecules.

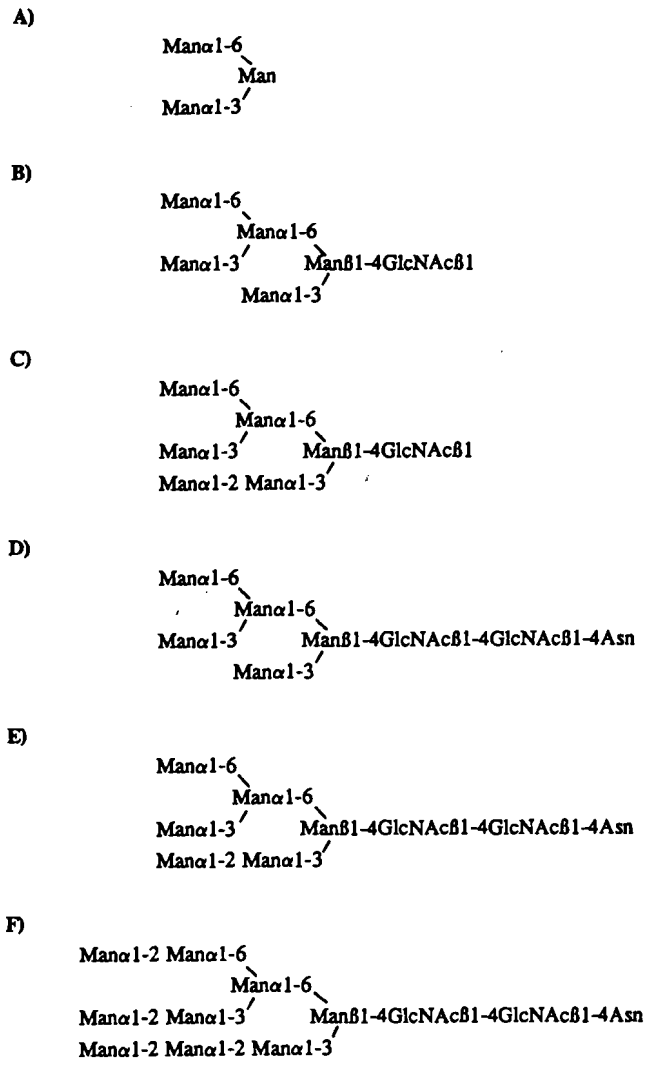

Fig. 1. Structure of oligomannosidic N-glycans: **A)** Manα1-6(Manα1-3)Man; **B)** Man5GlcNAc; **C)** Man6GlcNAc; **D)** Man5GlcNAc2Asn; **E)** Man6GlcNAc2Asn; **F)** Man9GlcNAc2Asn. Compounds A)-E) are ligands of IL-2. Compound E) is the ligand of CSL present two fold in the gp120 envelope of the HIV-1 virus. Compound F) is not a ligand of IL-2 and CSL. Compounds A)-C), but not D)-F), accumulate in α-mannosidosis. Compounds D) and E) are ligands of IL-2 present four times in the gp120 HIV-1 envelope glycoprotein.

Other cytokines

IL-1α and IL-1ß are cytokines produced by macrophages, epithelial cells and T and B lymphocytes which display multiple activities in the immune system. Evidence that IL-1 is a lectin was inferred from the observation that it interacts with the N-glycans of uromodulin (Hession et al., 1987; Muchmore and Decker, 1987; Brody and Durum, 1989), in particular with polyantennary complex-type N-glycans. Interestingly, Martin et al. (1994) demonstrated that IL-1 induces the co-precipitation of a protein kinase with the type I interleukin-1 receptor in T cells, in a mechanism similar to that produced by IL-2. Similarly, IL-7 induces either the association of $p56^{lck}$ and $p59^{fyn}$ with the p90 IL-7 receptor (Page et al., 1995) or the association of the phosphatidylinositol 3-kinase with the α chain of the IL-7 receptor (Venkitaraman and Cowling, 1994). This cell type-dependent association of the IL-7 receptors with different kinases could be explained by the fact that glycan ligands of IL-7 are cell type-specific. Although it is not clear if IL-7 has a lectin activity, recent data showed that this cytokine could interact with

glycosaminoglycans (Clarke et al., 1995). Moreover, sequence comparisons suggest that IL-7 could be a calcium-independent lectin (Zanetta et al., unpublished data). A lectin activity has been found in the tumor necrosis factors (TNF; Lucas et al., 1994). The first evidence that TNFα could have a lectin-like activity was obtained from its interaction with uromodulin (Sherblom et al., 1988). TNFα and TNFß apparently differ in their carbohydrate-binding properties. The TNFß trypanolytic activity can be specifically inhibited by di-N-acetyl-chitobiose (GlcNAcß1-4GlcNAc) whereas the TNFα cytotoxicity appears to be inhibited by polysialylated oligosaccharides. In fact, TNF are members of a superfamily of molecules which includes CD70/CD27 ligand and the CD40 ligand (Hintzen et al., 1994; Gruss and Dower, 1995). These molecules bind to receptors similar to other cytokine receptors which have a short intracytoplasmic domain without kinase activity but which can be phosphorylated by specific kinases. A TNF-dependent association of the TNF receptor with a Ser/Thr kinase has been demonstrated in lymphoma cells (Darnay et al., 1994).

Heparin-binding growth factors

Several growth factors have been isolated using their affinity to heparin. Their biological function depends on the presence or not of the addition of exogenous heparin. The present consensus is that heparin is important for cells lacking specific endogenous cell surface heparan sulphate proteoglycans, but that a second site of the molecule is necessary for its binding to a high affinity receptor (Heath et al., 1991; Zhu et al., 1995). The fibroblast growth factors, FGFα and FGFß, are the most common heparin-binding growth factors. It was initially assumed that these factors bound to the highly negatively-charged heparin through cationic domains. However, site-directed mutagenesis of these putative cationic sites to hydrophilic or acidic amino acids (Presta et al., 1992) produce FGF molecules with similar affinities for heparin and induce only subtle differences in their biological properties (i.e. receptor binding and induction of cell proliferation). The minimal carbohydrate moieties of heparin interacting with FGF were defined as [L-IduUraß1-3(or D-GlcUraß1-3)GlcN(2-SO_4)ß1-4]2 (Maccarana et al., 1993; Tyrell et al., 1993; Rusnati et al., 1994). This favours a role of a CRD rather than of unspecific ionic interactions between heparin and FGF, a view which is reinforced by the similarities of the three dimensional structure of FGFß, TNFα and interleukin 1 suggested to behave as calcium-independent lectins.

Heparin needs repetitive carbohydrate sequences to increase the biological activity of FGF, since shorter oligomers are inhibitory (Maccarana et al., 1993). This suggests that the binding of heparin does not primarily induce a conformational change from a poorly active to an active form of FGF receptor. Recent data (Pantoliano et al., 1994; Spivak-Kroizman et al., 1994) have resolved this critical problem. The binding of FGF to specific repetitive domains of heparin initiates a clustering of FGF molecules which, consequently, induces an oligomerization of FGF receptor molecules. This results in the trans-phosphorylations of the FGF receptor complexes and therefore the generation of an intracellular signal. The possibility that heparin also binds to the FGF receptor is still being discussed. But convincing evidence has been provided that the binding of FGF to its receptors is not primarily dependent on the presence of heparin (Roghani et al., 1994).

The question why FGF needs heparin to induce the proliferation of some cells and does not need heparin for others may find an answer considering the previous mechanism. When cells do not have an endogenous surface proteoglycan recognized by FGF, the repetitive oligosaccharide sequences of heparin would allow the clustering of FGF receptor molecules. When endogenous proteoglycan ligands of FGFß are present at the cell surface, the binding of FGFß to its high affinity receptor leads to its association with the proteoglycan-containing complex. Therefore, the resulting signal may be interpreted in terms of surface molecule clustering, as in other models concerned with lectins. Sequence homologies with FGFß suggest that other heparin-binding molecules, including other FGF molecules, INT-2 (Goldfarb et al., 1991) and members of the midkine family of growth factors, also have a lectin activity. Unfortunately, it is not known if these molecules, defined as heparin-binding, recognize the same oligosaccharide moiety of heparin. Site-directed mutagenesis of individual cationic aminoacids may help to understand if heparin binding is due to strong ionic interactions or to specific glycobiological interactions.

Mechanisms of signalling

The proposed mechanism of cytokine receptor association with other cell surface complexes due to the bi-functionality of cytokines could explain the initial step of signalling, which consists in the phosphorylation of the intracytoplasmic domain of the cytokine receptor. The association is specific to the oligosaccharide ligands of the cytokine or growth factor present at the surface of a cell. The association does not depend initially on intracytoplasmic changes but primarily on cell surface interaction of the lectin cytokine with its ligands. If several glycan ligands of the cytokine are present at the cell surface, the association of the cytokine receptor could take place with different surface complexes. However, the possibility remains that the phosphorylation of a single cytokine receptor could be performed by different kinases; those which are associated with the complex having the cytokine glycan ligand. For example, in the case of IL-2, several IL-2 glycoprotein ligands are present on T cells, not all of them being associated with the CD3/TCR complex. Thus, it is expected that IL-2Rß could be immunoprecipitated with antibodies other than the anti-TCR

antibodies and, consequently, IL-2Rß could be phosphorylated (and associated) with a kinase different from $p56^{lck}$. Furthermore, IL-2Rß is expressed on cells devoid of the CD3/TCR complex at their surface, as in B cells, where one major ligand of IL-2 is CD24. Thus, a cell-specific IL-2-dependent association of IL-2Rß with the surface complex including CD24 is expected.

The outcome of the initial receptor phosphorylation results in the commitment of specific pathways and in the expression of new molecules involved in cell proliferation or differentiation. However, due to the cell-specific association of the cytokine receptor with a peculiar molecular surface complex, the cascade of signal transduction would be cell-specific and would explain the divergence between the cascades which are engaged. The actual relevance to an in vivo situation of experiments of gene transfection of cytokine receptors in malignant or immortalized cells which can express cytokine ligands entirely different from the endogenous ones is questioned.

Possible interferences of the IL-2-dependent mechanisms with other glycobiological interactions

Experiments on the role of the lectin activity of IL-2 (Zanetta et al., 1996) were performed on resting human lymphocytes because of the possible interferences with another lymphocyte lectin endowed with very similar carbohydrate-binding properties (Zanetta et al., 1995). The lectin CSL recognizes the specific conformation of Man6GlcNAc2Asn. In this glycan, the interaction of Manα1-2 residue with the GlcNAcß1-4GlcNAcß1-disaccharide ensures a different conformation from the lower or higher mannose N-glycans or other Man6 isomers (Wyss et al., 1995). According to Sherblom et al. (1989), IL-2 probably recognizes a portion of this structure (Manα1-6(Manα1-3)Man), also present and unaffected in oligomannosides with 5 and 6 mannose residues (Fig. 1), but does not interact with oligomannosidic N-glycans with additional Manα1-2 residues on the different branches. Upon lymphocyte stimulation (Zanetta et al., 1995), CSL, which is immunologically related to CDw70, is rapidly expressed and externalized. Because CSL recognizes N-glycosylated forms of CD3, CSL and IL-2 may compete for the same CD3 ligand, the two lectins having almost identical affinities for the Man6GlcNAc2Asn N-glycans. But, as other ligands of CSL are present in the early stages of activation, the surface association of molecular complexes would be of higher order than single TCR/CD3 clustering. This situation occurs early, long before the increased IL-2R (including other forms than the ß form) and IL-2 expression in cells stimulated by phorbol esters (Aggarwal et al., 1994). Thus, uncertain mechanisms remain during early stages of activation for the possible competition between CSL and IL-2. Nevertheless, the divergence between the functions of CSL and IL-2 is evident. Although CSL over-expression persists during three days after activation with phorbol esters or plant lectins and only the 31.5 kDa form of CSL is expressed, on the fourth day, this form completely disappears (Zanetta et al., unpublished data) and is replaced by the 43 kDa form of CSL (which has the same carbohydrate-binding properties as the 31.5 kDa form). This suggests that between days 3 and 4, the early CSL, and probably its ligands, have been internalized and degraded. This occurs at a time when IL-2 and IL-2Rs are expressed at a maximal level. This complete shift in the molecular expression of CSL corresponds to a period where proliferation is maximal; a process when may well be IL-2 dependent.

Possible interferences of the IL-2-dependent mechanisms under pathological conditions

The lectin activity of cytokines raises important fundamental questions in particular pathologies, since interferences with the cytokine function could be specifically achieved using the cytokine glycan ligand. As far as IL-2 is concerned, the carbohydrate-binding properties for oligomannosidic N-glycans with 5 and 6 mannose residues permit the proposed of new understanding in the field of immunodeficiency. Based on knock-out experiments of the gene of IL-2 (Horak, 1995), the pattern of an IL-2-dependent immunodeficiency has been characterized. These symptoms resemble those found in human or animal diseases which include α-mannosidosis, mycobacterial infections and AIDS. These homologies suggest a decreased IL-2 response as a primary defect in these diseases which may find explanation considering the lectin activity of IL-2.

α-Mannosidosis

This lysosomal storage disease is characterized (Öckerman, 1967, 1969), as all other lysosomal storage diseases, by the lysosomal accumulation of undegraded material. This induces cell vacuolization associated with metabolic defects. As in all lysosomal storage diseases, α-mannosidosis is characterized by a severe mental retardation. However, in contrast with all of them, α-mannosidosis is accompanied with a severe immunodeficiency concerned with a severe leukopenia responsible for permanent bacterial and viral infections (Kjellman et al., 1969). This immunodeficiency pattern suggests an inhibition of the IL-2-dependent mechanisms. Patients with α-mannosidosis accumulate very high concentrations of oligomannosides with 3 to 9 mannose residues attached to a single GlcNAc residue in their lysosomes and biological fluids (Yamashita et al., 1980; Daniel et al., 1981). In fact, the blood levels of oligomannosidic N-glycans with 5 and 6 mannose residues, which are ligands of IL-2, are expected to be by far higher than those (in the micromolar range) which are necessary for detaching IL-2 from its ligands at the T cells surface (Zanetta et al., unpublished data). These glycans (Fig. 1) will produce a complete inhibition of the IL-2-dependent mechanisms of cell proliferation and differentiation, inhibiting the association of IL-2Rß with

other cell surface complexes. Hence, $CD4^+$ T cell survival, differentiation of $CD8^+$ T cells into NK cells and antigen-specific differentiation of B cells will be blocked. In contrast, such an effect cannot take place in ß-mannosidosis since the accumulated glycans are shorter than in α-mannosidosis. Furthermore, the glycans accumulated in α-mannosidosis do not bind CSL, since they do not possess the first GlcNAc of the N-glycan core necessary for the recognition by CSL (Marschal et al., 1989). This absence of interaction with CSL of the accumulated glycans could also explain why the CSL-dependent ontogenetic processes in the brain (i.e. neuron migration and myelination (Kuchler et al., 1988; Lehmann et al., 1990; Zanetta et al., 1994) are not significantly affected in this disease. Thus, from the immunological point of view, α-mannosidosis could be considered as an immunodeficiency disease concerned essentially with the IL-2-dependent mechanisms.

The acquired immuno-deficiency syndrome (AIDS)

The immunodeficiency observed in AIDS presents the characteristic features of the immunodeficiency observed in animals in which the IL-2 gene has been knocked-out. Nevertheless, the $CD4^+$ T cells are able to produce normal levels of IL-2 and $CD4^+$ and $CD8^+$ and B cells express normal levels of IL-2R. Thus, apparently, the IL-2-dependent system is present but immunodeficiency pattern suggests that it is not functional. Therapeutical trials showed that the administration of human recombinant IL-2 to patients stabilizes the disease and particularly the number of $CD4^+$ T cells. This suggested that the IL-2 produced by the patients was not accessible. The lectin activity of IL-2 of fers a new understanding of the immunodeficiency observed in AIDS. Indeed, the major envelope glycoprotein of the HIV-1 virus gp120/gp160 is N-glycosylated (23 potential N-glycosylation sites on gp120). Based on the N-glycan composition of the gp120 produced by CHO cells or immortalized cell lines (Geyer et al., 1988; Mizuochi et al., 1988; Yeh et al., 1993), about 50% of the N-glycans are of the oligomannosidic type. In fact, gp120 possesses four IL-2-binding N-glycans (Fig. 1). An interaction of IL-2 with the gp120/gp160 is expected and could take place in three different ways (Zanetta et al., unpublished data):

- i) the gp120/gp160 (either circulating, or virus-associated or membrane-associated on infected cells) can trap IL-2, thus reducing the levels of free IL-2 in the blood.
- ii) the IL-2 bound to circulating gp120 could be eliminated in the liver by specific clearance system.
- iii) when bound to IL-2R, the IL-2/gp120 complex interferes with the normal IL-2-dependent association of IL-2R with surface molecular complexes (this point has been recently demonstrated (Hubert et al. 1995), inhibiting the production of IL-2.

Due to the presence of two Man6GlcNAc2Asn N-glycans in the gp120 (Fig. 1), an interference with the CSL-dependent activation mechanisms is also expected, reducing the general activation of the immune system. In contrast with α-mannosidosis a double action of the glycoprotein on two essential oligomannoside-dependent mechanisms could occur in AIDS.

The possible glycobiological interaction between gp120/gp160 and the IL-2- and CSL-dependent mechanisms of activation/differentiation of human lymphocytes may have further consequences. AIDS-Related Complexes (ARC) are infectious diseases playing a fundamental role in the evolution of the disease. They are generally considered as "opportunist" diseases. Most of them are due to infections with mycobacteria which are characterized by the presence of cell wall mannans. Although the most abundant structures of microorganism cell wall are not potential IL-2 or CSL ligands, recent data (Zanetta et al., unpublished data) indicate that *Candida albicans* cell wall glycans bind (specifically compared to other yeast strains) huge amounts of IL-2 in a mechanism dependent on oligomannosides with 5 and 6 mannose residues but independent of the oligomannoside with 9 mannose residues. Thus, the higher prevalence of candidiasis in AIDS may be suggestive that ARC are not "opportunist" but "complementary" or "synergistic" pathologies also inhibiting the IL-2-dependent mechanisms. The stabilizing effect of the IL-2 and anti-mycobacterial agent therapies in AIDS could be explained because they are reducing the immunodeficiency they provoke using the same mechanism as the HIV-1 virus.

Acknowledgments. This work was supported by a grant from the National Multiple Sclerosis Society and the Association Française Contre le Cancer. The authors thank C. Alonso, A. Meyer and J.-G. Lorentz for technical assistance.

References

Aggarwal S., Lee S., Mathur A., Gollapudi S. and Gupta S. (1994). 12-Deoxyphorbol-13-O-phenylacetate 20 acetate [an agonist of protein kinase C ß1 (PKCß1) induces DNA synthesis, interleukin-2 (IL-2) production, IL-2 receptor α-chain (CD25) and ß-chain (CD122) expression, and translocation of PKCß isozyme in human peripheral blood lymphocytes: Evidence for a role of PKCß1 in human T cell activation. J. Clin. Immunol. 14, 248-256.

Arar C., Carpentier V., Le Caer J.-P., Monsigny M., Legrand A. and Roche A.-C. (1995). Ergic-53, a membrane protein of the endoplasmic reticulum-Golgi intermediate compartment is identical to MR60, an intracellular mannose-specific lectin of myelomonocytic cells. J. Biol. Chem. 270, 3551-3553.

Barondes S.H., Cooper D.N.W., Gitt M.A. and Leffler H. (1994). Galectins. Structure and function of a large family of animal lectins. J. Biol. Chem. 269, 20807-20810.

Bevilacqua M.P. and Nelson R.M. (1993). Selectins. J. Clin. Invest. 91, 379-387.

Bevilacqua M.P., Stengeli S., Gimbrone M.A. and Seed B. (1989). Endothelial leukocyte adhesion molecule I: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 243, 1160-1164.

Bezouska K., Nepovim A., Horvath O., Pospisil M., Hamann J. and Feizi

T. (1995). CD69 antigen of human lymphocytes is a calcium-dependent carbohydrate-binding protein. Biochem. Biophys. Res. Commun. 208, 68-74.

Brody D.T. and Durum S.K. (1989). Membrane IL-1. IL-1α precursor binds to the plasma membrane via a lectin-like interaction. J. Immunol. 143, 1183-1187.

Clarke D., Katoh O., Gibbs R.V., Griffiths S.D. and Gordon M.Y. (1995). Interaction of interleukin 7 (IL-7) with glycosaminoglycans and its biological relevance. Cytokine 7, 325-330.

Cohen F.E., Kosen P.A., Kuntz I.D., Epstein L.B., Ciardelli T.L. and Smith K.A. (1986). Structure-activity studies of interleukin-2. Science 234, 349-352.

Daniel P.F., Defeudis D.F. and Lott I.T. (1981). Mannosidosis: Isolation and comparison of mannose-containing oligosaccharides from gingiva and urine. Eur. J. Biochem. 114, 235-237.

Darnay B.G., Reddy S.A.G. and Aggarwal B.B. (1994). Physical and functional association of a serine-threonine protein kinase to the cytoplasmic domain of the p80 form of the human tumor necrosis factor receptor in human histiocytic lymphoma U-937 cells. J. Biol. Chem. 269, 19687-19690.

Drickamer K. (1988). Two distinct classes of carbohydrate-recognition domains in animal lectins. J. Biol. Chem. 263, 9557-9560.

Farrar W.L., Linnekin D., Evans G., Garcia G.G. and Michiel D. (1990). Interleukin-2 regulation of a tyrosine kinase associated with the P70-75 ß-chain of the receptor complex. Mol. Cell. Biol. Cytokines 10, 265-269.

Feizi T. and Childs R.A. (1987). Carbohydrates as antigenic determinants of glycoproteins. Biochem. J. 245, 1-11.

Fiedler K. and Simons K. (1994). A putative novel class of animal lectins in the secretory pathway homologous to leguminous lectins. Cell 77, 625-626.

Foxall C., Watson S.R., Dowbenko D., Fennie C., Lasky L.A., Kiso M., Hasegawa A., Asa D. and Brandley B.K. (1992). The 3 members of the selectin receptor family recognize a common carbohydrate epitope the sialyl Lewis oligosaccharide. J. Cell Biol. 117, 895-902.

Geyer H, Holschbach C., Hunsmann G. and Schneider J. (1988). Carbohydrates of human immunodeficiency virus. Structures of the oligosaccharides linked to the envelope glycoprotein 120. J. Biol. Chem. 263, 11760-11767.

Goldfarb M., Deed R., Macallan D., Walther W., Dickson C. and Peters G. (1991). Cell transformation by Int-2. A member of the fibroblast growth factor family. Oncogene 6, 65-71.

Gruss H.J. and Dower S.K. (1995). Tumor necrosis factor ligand superfamily: Involvement in the pathology of malignant lymphomas. Blood 85, 3378-3404.

Hatakeyama M., Kono T., Kobayashi N., Kawahara A., Levin S.D., Perlmutter R.M. and Taniguchi T. (1991). Interaction of the IL-2 receptor with the src-family kinase p56lck: Identification of novel intermolecular association. Science 252, 1523-1528.

Heath W.F., Cantrell A.S., Mayne N.G. and Jaskunas S.R. (1991). Mutations in the heparin-binding domains of human basic fibroblast growth factor alter its biological activity. Biochemistry 30, 5608-5615.

Hession C., Decker J.-M., Sherblom A.-P., Kumar S., Yue C.C., Mattallano R.J., Tizard R., Kawashima E., Schmeissner U., Heletky S., Chow E.P., Burne C.A., Shaw A. and Muchmore A.V. (1987). Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. Science 237, 1479-1484.

Hintzen R.Q., Lens M.S., Beckmann M.P., Lynch D., Goodwin R.G. and vanLier R.A.W. (1994). Characterization of the human CD27 ligand a novel member of the TNF gene family. J. Immunol. 152, 1762-1773.

Horak I. (1995). Immunodeficiency in IL-2-knockout mice. Clin. Immunol. Immunopathol. 76, 172-173.

Hubert P., Bismuth G., Korner M. and Debre P. (1995). HIV-1 glycoprotein gp120 disrupts CD4-p56(lck)/CD3-T cell receptor interactions and inhibits CD3 signaling. Eur. J. Immunol. 25, 1417-1425.

Ju G., Collins L., Kaffka K.L., Tsien W.H., Chizzonite R., Crowl R., Bhatt R. and Kilian P.L. (1987). Structure-function analysis of human interleukin-2. J. Biol. Chem. 262, 5723-5731.

Kelm S., Schauer R., Manuguerra J.C., Gross H.J. and Crocker P.R. (1994). Modifications of cell surface sialic acids modulate cell adhesion mediated by sialoadhesin and CD22. Glycoconjugate J. 11, 576-585.

Kjellman B., Gamstorp I., Brun A., Öckerman P.A. and Palmgren B. (1969). Mannosidosis: A clinical and histopathologic study. J. Pediat. 75, 366-373.

Kuchler S., Fressinaud C., Sarliäve L.L., Vincendon G. and Zanetta J.-P. (1988). Cerebellar soluble lectin is responsible for cell adhesion and participates in myelin compaction in cultured rat oligodendrocytes. Dev. Neurosci. 10, 199-212.

Lanier L.L., Chang C.W. and Phillips J.H. (1994). Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. J. Immunol. 153, 2417-2428.

Lasky L.A. (1992). Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 258, 964-969.

Lehmann S., Kuchler S., Théveniau M., Vincendon G. and Zanetta J.-P. (1990). An endogenous lectin and one of its neuronal glycoprotein ligands are involved in contact guidance of neuron migration. Proc. Natl. Acad. Sci. USA 87, 6455-6459.

Lucas R., Magez S., Deleys R., Fransen L., Scheerlinck J.P., Rampelberg M., Sablon E. and Debaetselier P. (1994). Mapping the lectin-like activity of tumor necrosis factor. Science 263, 814-817.

Maccarana M., Casu B. and Lindahl U. (1993). Minimal sequence in heparin/heparan sulfate required for binding of basic fibroblast growth factor. J. Biol. Chem. 268, 23898-23905.

Marschal P., Reeber A., Neeser J.-R., Vincendon G. and Zanetta J.-P. (1989). Carbohydrate and glycoprotein specificity of two endogenous cerebellar lectins. Biochimie 71, 645-653.

Martin M., Bol G.F., Eriksson A., Resch K. and Brigeliusflohe R. (1994). Interleukin-1-induced activation of a protein kinase co-precipitating with the type I interleukin-1 receptor in T cells. Eur. J. Immunol. 24, 1566-1571.

Mizuochi T., Spellman M.W., Larkin M., Soloman J., Basa L.J. and Feizi T. (1988). Carbohydrate structures of the human immunodeficiency virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese hamster ovary cells. Biochem. J. 254, 599-603.

Muchmore A.U. and Decker J.-M. (1987). Evidence that recombinant IL1 exhibits lectin-like specificity and binds to homogeneous uromodulin via N-linked oligosaccharides. J. Immunol., 138, 2541-2552.

Nath D., Vandermerwe P.A., Kelm S., Bradfield P. and Crocker P.R. (1995). The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22. J. Biol. Chem. 270, 26184-26191.

Öckerman P.A. (1967). A generalized storage disorder resembling Hurler's syndrome. Lancet 2, 239-241.

Öckerman P.A. (1969). Mannosidosis: Isolation of oligosaccharide storage material from brain. J. Pediat. 75, 360-365.

Page T.H., Lali F.V. and Foxwell B.M.J. (1995). Interleukin-7 activates

p56(lck) and p59(fyn), two tyrosine kinases associated with the p90 interleukin-7 receptor in primary human T cells. Eur. J. Immunol. 25, 2956-2960.

Pantoliano M.W., Horlick R.A., Springer B.A., Vandyk D.E., Tobery T., Wetmore D.R., Lear J.D., Nahapetian A.T., Bradley J.D. and Sisk W.P. (1994). Multivalent ligand-receptor binding interactions in the fibroblast growth factor system produce a cooperative growth factor and heparin mechanism for receptor dimerization. Biochemistry 33, 10229-10248.

Powell L.D. and Varki A. (1994). The oligosaccharide binding specificities of CD22ß, a sialic acid-specific lectin of B cells. J. Biol. Chem. 269, 10628-10636.

Powell L.D., Jain R.K., Matta K.L., Sabesan S. and Varki A. (1995). Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22. Evidence for a minimal structural recognition motif and the potential importance of multisite binding. J. Biol. Chem. 270, 7523-7532.

Presta M., Statuto M., Isacchi A., Caccia P., Pozzi A., Gualandris A., Rusnati M., Bergonzoni L. and Sarmientos P. (1992). Structure-function relationship of basic fibroblast growth+ factor. Site-directed mutagenesis of a putative heparin-binding and receptor-binding region. Biochem. Biophys. Res. Commun. 185, 1098-1107.

Roghani M., Mansukhani A., Dellera P., Bellosta P., Basilico C., Rifkin D.B. and Moscatelli D. (1994). Heparin increases the affinity of basic fibroblast growth factor for its receptor but is not required for binding. J. Biol. Chem. 269, 3976-3984.

Rusnati M., Coltrini D., Caccia P., Dellera P., Zoppetti G., Oreste P., Valsasina B. and Presta M. (1994). Distinct role of 2-O-, N-, and 6-O-sulfate groups of heparin in the formation of the ternary complex with basic fibroblast growth factor and soluble FGF receptor-1. Biochem. Biophys. Res. Commun. 203, 450-458.

Sherblom A.-P., Decker J.-M. and Muchmore A.V. (1988). The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. J. Biol. Chem. 263, 5418-5424.

Sherblom A.P., Sathyamoorthy N., Decker J.M. and Muchmore A.V. (1989). IL-2 a lectin with specificity for high mannose glycopeptides. J. Immunol. 143, 939-944.

Shibuya H., Kohu K., Yamada K., Barsoumian E.L., Perlmutter R.M. and Taniguchi T. (1994). Functional dissection of p56(lck), a protein tyrosine kinase which mediates interleukin-2-induced activation of the c-fos gene. Mol. Cell. Biol. 14, 5812-5819.

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). Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. Cell 79, 1015-1024.

Springer T.A. (1991). Cell adhesion. Sticky sugars for selectins. Nature 349, 196-197.

Taniguchi T. and Minami Y. (1993). The IL-2/IL-2 receptor system: a current overview. Cell 73, 5-8.

Tyrrell D.J., Ishihara M., Rao N., Horne A., Kiefer M.C., Stauber G.B., Lam L.H. and Stack R.J. (1993). Structure and biological activities of a heparin-derived hexasaccharide with high affinity for basic fibroblast growth factor. J. Biol. Chem. 268, 4684-4689.

Venkitaraman A.R. and Cowling R.J. (1994). Interleukin-7 induces the association of phosphatidylinositol 3-kinase with the a chain of the interleukin-7 receptor. Eur. J. Immunol. 24, 2168-2174.

Waldmann T.A. (1991). The interleukin-2 receptor. J. Biol. Chem. 266, 2681-2684.

Wells T.N.C., Graber P., Proudfoot A.E.I., Arod C.Y., Jordan S.R., Lambert M.H., Hassel A.M. and Milburn M.V. (1994). The three-dimensional structure of human interleukin-5 at 2.4-angstroms resolution: Implication for the structures of other cytokines. Cell. Cytok. Lung Inflamm. 25, 118-127.

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). Conformation and function of the N-linked glycan in the adhesion domain of human CD2. Science 269, 1273-1278.

Yamashita K., Tachibana Y., Mihara K., Okada S., Yabuuchi H. and Kobata A. (1980). Urinary oligosaccharides of mannosidosis. J. Biol. Chem. 255, 5126-5133.

Yeh J.C., Seals J.R., Murphy C.I., Vanhalbeek H. and Cummings R.D. (1993). Site-specific N-Glycosylation and oligosaccharide structures of recombinant HIV-1 gp120 derived from a baculovirus expression system. Biochemistry 32, 11087-11099.

Zanetta J.-P., Meyer A., Kuchler S. and Vincendon G. (1987). Isolation and immunochemical study of a soluble cerebellar lectin delineating its structure and function. J. Neurochem. 49, 1250-1257.

Zanetta J.-P., Badache A., Maschke S., Marschal P. and Kuchler S. (1994). Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. Histol. Histopathol. 9, 385-412.

Zanetta J.-P., Wantyghem J., Kuchler-Bopp S., Badache A., Aubery M. (1995). Human lymphocyte activation is associated with the early and high level expression of the endogenous lectin CSL at the cell surface. Biochem. J., 311, 629-636.

Zanetta J.-P., Alonso C. and Michalski J.-C. (1996). Interleukin 2 is a lectin which associates its receptor to the T cell receptor complex. Biochem. J. (in press).

Zhu H.Y., Ramnarayan K., Anchin J., Miao W.Y., Sereno A., Millman L., Zheng J.H., Balaji V.N. and Wolff M.E. (1995). Glu-96 of basic fibroblast growth factor is essential for high affinity receptor binding. Identification by structure-based site-directed mutagenesis. J. Biol. Chem. 270, 21869-21874.

Zola H., Weedon H., Thompson G.R., Fung M.C., Ingley E. and Hapel A.J. (1991). Expression of IL-2 receptor p55 and p75 chains by human lymphocyte. Effects of activation and differentiation. Immunology 72, 167-173.