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Changes in the expression of lectins in human T lymphocyte membrane upon mitogenic stimulation*

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ABSTRACT

Surface lectins, specific for given sugar structures, are expressed on human T cells, as shown by flow cytofluorometry using F-neoglycoproteins bearing either β- and α-D-galactosyl, β-D-galactosyl 6-phosphate, or α-L-rhamnosyl groups, but not by F-neoglycoproteins bearing other sugar groups (such as α-D-mannosyl groups). After stimulation with *Phaseolus vulgaris* mitogen, the number of cells that bind β-D-galactosyl 6-phosphate groups (6-*P*-β-D-Gal*p* lec$^+$ cells) increased fourfold during the first five days; these cells are helper (CD4$^+$) T cells. Conversely, cells that bind α-L-Rha groups belong to the T suppressor (CD8$^+$) family and their number moderately increased. Upon stimulation by concanavalin A, the number of cells expressing the lectin recognizing α-L-Rha groups increased during the first two days and then decreased within the next two days. These results are discussed with regard to the implication of lymphocyte membrane lectins in the suppressor mechanism and in the homing process.

INTRODUCTION

Lectins[1] in mammalian cells have raised many questions regarding their biological implications, since the initial discovery of the hepatic β-D-galactose-binding lectin by Ashwell and Morell (see ref. 2 for a review).

Lectins of mouse lymphocytes were first identified as components of membrane extracts[3] and then visualized at the surface by use of glycosylated cytochemical markers[4] and, later on, neoglycoproteins[5]. Since, cell surface lectins have been identified in many other normal cells and tissues, as well as in transformed cells (see refs. 6–8 for reviews). It was shown that the number and the immunological type of F-neoglycoproteins-labeled cells are a function of the carbohydrate structure carried by the synthetic glycoprotein[9,10]. Neoglycoproteins proved themselves to be valuable tools to detect and study cell sugar receptors because they carry enough sugar molecules to provide an avidity factor which allows neoglycoproteins to bind specifically membrane lectins[7]. It has subsequently been shown that membrane lectins are involved in the interactions between lymphocytes and endothelial cells in microveinules of lymphoid organs[11,12]. Recently, the involvement of lectins in the homing process received more support by use of two

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approaches, (a) inhibition of adhesion by sugar or glycoconjugates[13–15], and (b) inhibition of adhesion by monoclonal antibodies[16,17] specific for lymphocyte antigens involved in the homing process. So far, two of these antigens have been sequenced[18,19] and have been named LEC-CAM (cell adhesion molecule) with Lectin, EGF, Complement domain homology. They present some homology with carbohydrate domains of known lectins[20]. So far, however, there is no direct evidence that these two cloned LEC-CAM antigens recognize carbohydrate components of their natural glycoconjugate receptors.

We report herein, that the expression of cell surface lectins on human lymphocytes is modulated as a function of lymphocyte activation and maturation after *in vitro* polyclonal mitogenic stimulation induced by the plant lectins, PHA (*Phaseolus vulgaris*[21,22] agglutinin) and Con A (concanavalin A, from *Canavalia ensiformis*[23,24]).

EXPERIMENTAL

Cells. — Mononuclear, cell-enriched buffy coats from the blood of healthy donors were obtained from the Centre de Transfusion Sanguine (Centre Hospitalier Régional, Orléans, France). Human peripheral blood leukocytes (HPBL) were isolated according to the Ficoll-hypaque gradient technique[25]. Adherent cells were removed by plastic adhesion[26] on Petri dishes (Falcon, Becton and Dickinson, Grenoble, France). Lymphocytes were washed with phosphate-buffered saline (PBS), pH 7.3, supplemented with mM $CaCl_2$ and 0.5mM $MgCl_2$ (c-PBS). The T cell-enriched population was obtained by two successive rosettings with sheep red blood cells[27] (Institut Mérieux, Lyon, France).

Cell variability was assessed during cytochemical studies by the propidium iodide-labeling technique[28] adapted by Sasaki *et al.*[29].

Neoglycoproteins and their fluorescent derivatives. — Neoglycoproteins were synthesized by a method previously described[30,31], adapted from McBroom *et al.*[32], allowing phenyl isothiocyanate glycosides to react with bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 0.1M Na_2CO_3 buffer at pH 9.3. Such a reaction is approximately quantitative; the sugar content, determined by the resorcinol–H_2SO_4 method[33], indicated that the average number of sugar groups per molecule of protein was 23 ± 3. After purification by gel filtration on Ultrogel GF 05 (IBF Biotechnics, Villeneuve la Garenne, France) in pure distilled water, the freeze-dried neoglycoproteins were fluoresceinated for cytochemical applications.

Fluorescein isothiocyanate (Molecular Probes, Junction City, OR, U.S.A.) was coupled to neoglycoproteins in a threefold molar excess, at pH 9.5, in 0.1M Na_2CO_3 buffer, by stirring for 5 h at 25°. Fluorescent neoglycoproteins were purified by gel filtration on Ultrogel GF 05 (IBF Biotechnics) in 1:19 (v/v) butanol–water[31]. Neoglycoproteins were freeze-dried and kept at −20°.

Stimulation of human T cells by plant lectins. — PHA stimulation of human T cells. T cells were resuspended in the culture medium RPMI 1640 (Eurobio, Paris, France) supplemented with 10% heat-inactivated fetal bovine serum (Flow Laboratories, Asnières, France), 2mM glutamine, 100 units/mL of penicillin, 100 μg/mL of streptomycin

(Eurobio). The final cell concentration was $5 \cdot 10^6$ cells/mL. Aliquots of this cell suspension (3 mL) were kept in 14-mL culture tubes (Falcon, Becton and Dickinson) in the presence of PHA (10 μg/mL) (IBF Biotechnics). The cells were cultured in an incubator at 37°, under a 19:1 air–CO_2, humidified atmosphere for 12–24 h. After extensive washings with RPMI, the cells were further cultured for various lengths of time, up to 7 days.

Concanavalin A stimulation. T cells were stimulated by Con A (5.0 μg/mL) (IBF Biotechnics) for 18 h under the conditions described above. The cells were then washed with RPMI containing 20mM methyl α-D-mannopyranoside, and then with RPMI alone, and resuspended in the complete culture medium, under the conditions described above, for various time lengths.

Cytochemical studies of T cells. — Fluorescent neoglycoprotein labeling. For most of the labeling experiments, neoglycoproteins were used at a final concentration of 100 μg/mL, in c-PBS containing BSA (1 mg/mL) (Armour, IBF Biotechnics) (c-PBS-BSA). After 1 h of preincubation at 37°, the cell concentration was adjusted to $5 \cdot 10^6$ cells/mL and F-neoglycoprotein binding was allowed during a 1-h incubation at 4°.

Double labeling for identification of T cells by neoglycoproteins and monoclonal antibodies. In these experiments, fluorescein-free neoglycoproteins were used as described above (100 μg/mL, 4°, 1 h). Cell-bound neoglycoprotein molecules were revealed by a second-step reagent, rabbit anti-BSA, fluorescein-labeled antibodies (Cappel, Flobio, Courbevoie, France). The monoclonal antibodies derivatized with phycoerythrin (PE) were anti-T4-PE from Coultronics (Margency, France) and Leu 2a-PE from Becton and Dickinson. The cells were double-labeled by incubation with both reagents, either separately and successively or concommitantly. The last step was always the detection of the bound neoglycoprotein by rabbit anti-BSA, fluorescein-labeled antibodies.

Flow cytometry studies. — These were performed with a FACS 440 cell sorter (Becton and Dickinson, Sunnyvale, CA, U.S.A.), equipped with an Ar laser. With an excitation wavelength of 488 nm, both green (530 ± 15 nm) and red (>580 nm) fluorescent-emitted lights were collected by photomultipliers, as well as forward (FSC) and side (SSC)-scattered light.

RESULTS

Detection of cell surface lectins on unstimulated human peripheral blood T lymphocytes. — A T cell-enriched population was isolated by double E rosetting procedure, kept in culture for 12 h, and then labeled by fluorescein-substituted neoglycoproteins. Defined proportions of cells were able to bind fluorescent neoglycoproteins bearing either α- and β-D-galactopyranosyl, β-D-galactopyranosyl 6-phosphate, α-L-rhamnopyranosyl, or 2-acetamido-2-deoxy-β-D-glucopyranosyl groups in the proportions indicated in Fig. 1. Conversely, almost no T lymphocytes were labeled by fluorescent bovine serum albumin (BSA) or neoglycoproteins containing α-D-mannopyranosyl 6-phosphate or α-D-mannopyranosyl groups. These data obtained with nonstimulated

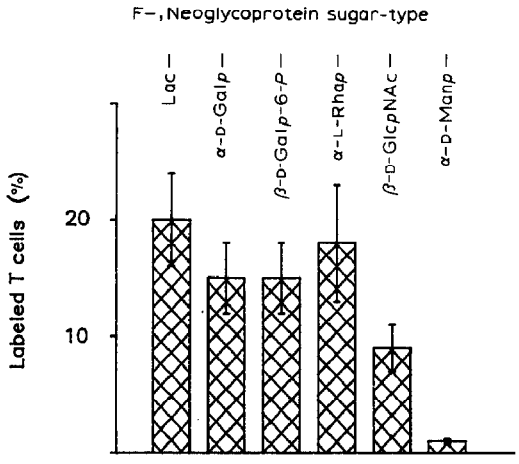

Fig. 1. Number (%) of human peripheral blood T lymphocytes labeled with fluorescein-substituted neoglycoproteins bearing 23 ± 3 sugar residues. Fluorescent neoglycoproteins were used at a 100 μg/mL concentration, to label unstimulated T cells for 1 h at 4°; cells were analyzed by flow cytometry on a FACS 440 cell sorter. Data are mean values obtained from eight separate donors, assays were made in triplicate.

human T cells, isolated from normal, healthy donor blood, represent a variable sampling and, indeed, the proportions of lymphocytes labeled with a given fluorescent neoglycoprotein was quite dependent on the blood sample. In some cases, for instance, the proportion of T lymphocytes labeled with neoglycoproteins bearing either α-L-Rha*p* or β-D-Gal*p*-6-*P* groups was quite low. We studied the variability of the labeling in 34 separate experiments. Such a variability was noticeable mainly for α-L-Rha*p*-BSA and β-D-Gal*p*-6-*P*-BSA. For this reason, we undertook to investigate whether the variability reflects a biological state of the donors in terms of immunologically defined subpopulations of T cells. Fig. 1 gives results as mean values obtained from eight different donors (each assay was made in triplicate) responding positively to α-L-Rha*p*-BSA and β-D-Gal*p*-6-*P*-BSA.

Modulation of the expression of T cells membrane lectins specific for β-D-*Gal*p-6-P *or for* α-L-*Rha*p *groups upon mitogenic stimulation by* Phaseolus vulgaris *mitogen.* — By use of a mitogen concentration inducing blast transformation in such an amount that, on day 3 during routine dose response experiments, $5 \cdot 10^4$ T lymphocytes/well incorporated [^{3}H]thymidine to provide an activity ranging from $2 \cdot 10^4$ to $3 \cdot 10^4$ c.p.m., the number of cells expressing on their surface a lectin was estimated with the various fluorescent neoglycoproteins mentioned above. It appeared that the number of cells recognizing β-D-Gal*p*-6-*P*- and α-L-Rha*p*-substituted neoglycoproteins changed in significant proportions as a function of time in culture. Because these two neoglycoproteins repeatedly displayed a change in the number of cells that they recognize, we investigated the cell populations to which these two neoglycoproteins bound. Indeed, the cell population recognizing the neoglycoprotein bearing β-D-Gal*p*-6-*P* groups increased up to fourfold after the five first days, and then decreased (Fig. 2). Meanwhile, the number of cells recognizing neoglycoproteins bearing α-L-Rha*p* groups was only

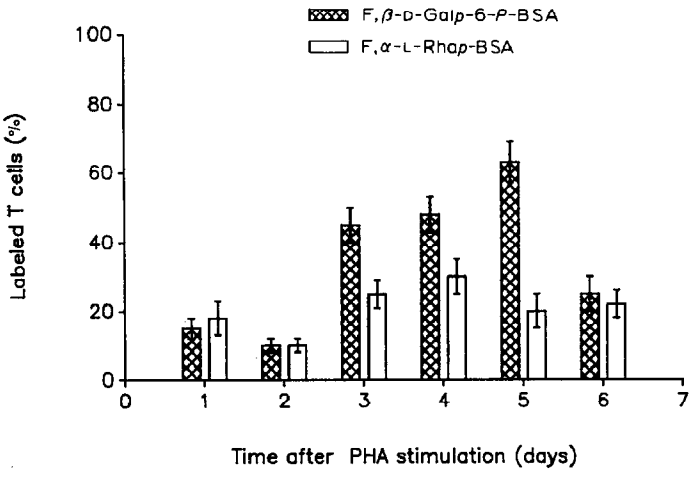

Fig. 2. Time course study of the expression of cell surface lectins on T lymphocytes upon stimulation with *Phaseolus vulgaris* mitogen. Data are expressed as the percentages of fluorescent neoglycoprotein-labeled cells.

slightly dependent on the elapsed time of culture. Therefore, the expression of these two types of lectins is independently modulated during the blastic transformation of purified human T lymphocytes. Furthermore, upon culture for two days after a stimulation step by *Phaseolus vulgaris* mitogen, the T cells that bound neoglycoprotein bearing β-D-Gal*p*-6-*P* groups behaved as a separate population from the Leu $2a^+$ T cells (Fig. 3a; no doubly labeled cells were detected with these markers), but the majority of β-D-Gal*p*-6-*P* group-binding population (80%) was also labeled by anti-T4 monoclonal antibody, as shown on Fig. 3b (mean fluorescence values were Fl1 = 60 and Fl2 = 105); the cells that were not labeled by the neoglycoprotein were partly labeled by the antibody (Fig. 3b, marker 7).

An opposite pattern was obtained with cells labeled by a neoglycoprotein bearing α-L-Rha*p* groups; a large proportion of cells labeled with this neoglycoprotein clearly bound anti-Leu 2a monoclonal antibodies (75%, Fig. 3c; mean fluorescence intensity values were Fl1 = 210 and Fl2 = 100, marker 8), *i.e.*, most cells labeled by anti-Leu 2a antibody are binding α-L-rhamnopyranosyl groups. Anti-T4 monoclonal antibody did not label cells that bind the neoglycoprotein bearing α-L-Rha*p* groups (Fig. 3d; Fl1 = 20 and Fl2 = 80).

Neoglycoprotein-binding experiments were performed with a 1 mg/mL solution of cold BSA, thus providing an internal control for positive binding of neoglycoproteins.

Furthermore, other neoglycoproteins bearing such groups as α-D-Man*p* did not label T cells under these conditions and showed the background level for the green fluorescence (FL1) (ranging between channels 5 and 40) – as indicated by the control experiments. These used fluorescent anti-BSA antibodies, in the absence of neoglycoprotein, doubly labeled by anti-T4 monoclonal antibodies (mean value for Fl1 = 10 and Fl2 = 100), as shown in Fig. 3e (marker 5).

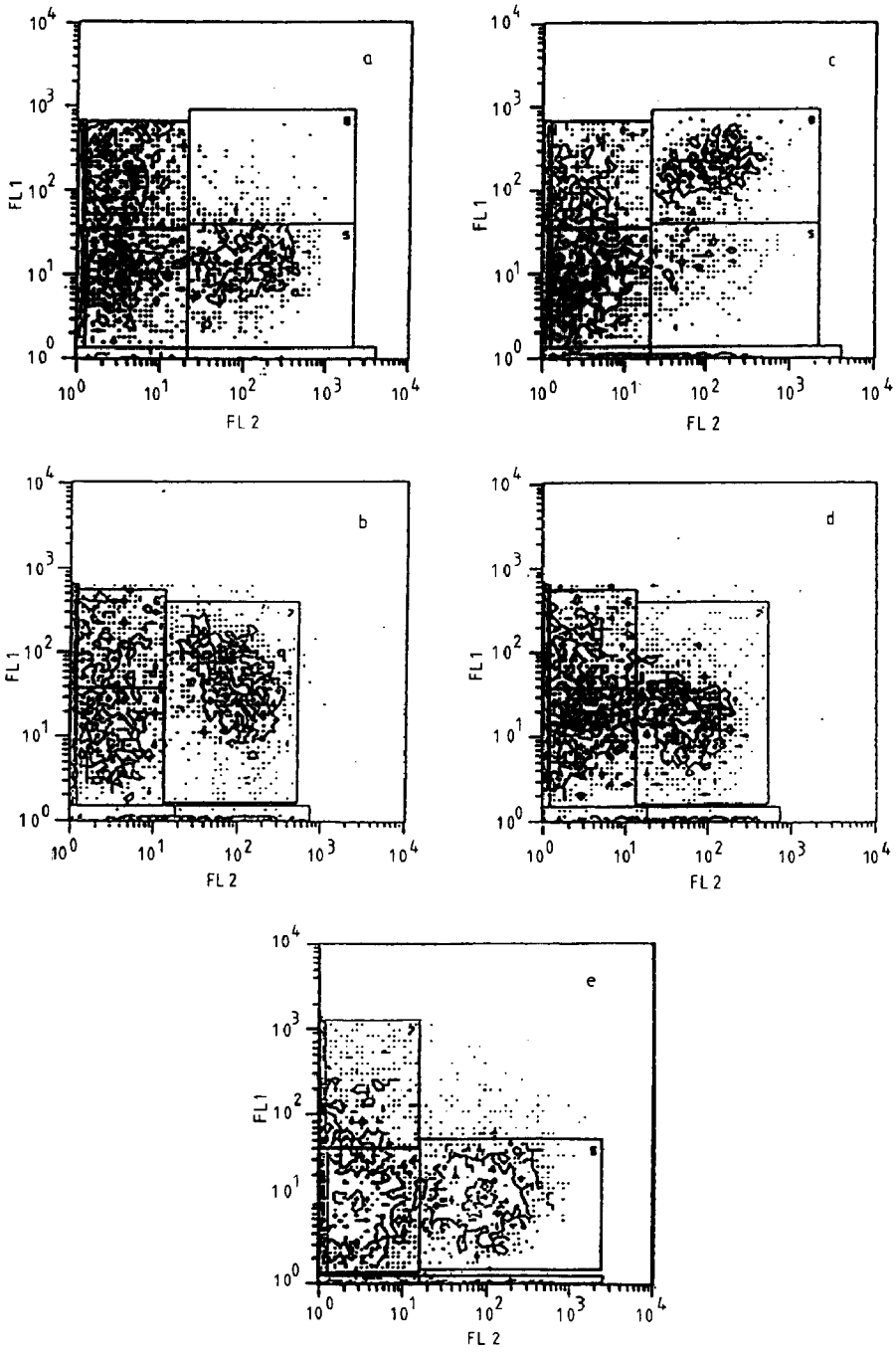

Fig. 3. Characterization of human T lymphocytes cultured for two days after the activation step by *Phaseolus vulgaris* mitogen: (a, b) Labeling with neoglycoproteins bearing *β*-D-Gal*p*-6-*P* groups and phycoerythrin-labeled anti-Leu 2a monoclonal antibodies (a) or phycoerythrin-labeled anti-T4 monoclonal antibodies (b); neoglycoprotein binding was detected by fluorescein-labeled rabbit anti-BSA antibody. (c, d) Labeling with neoglycoproteins bearing *α*-L-Rha*p* groups (detected by fluorescein-labeled rabbit anti-BSA antibody) and phycoerythrin-labeled anti-Leu 2a (c), or phycoerythrin-labeled anti-T4 antibodies (d). (e) Control for nonspecific binding of the second-step reagent for the detection of neoglycoprotein binding, fluorescein-labeled rabbit anti-BSA antibodies; the first-step labeling was with nonglycosylated BSA and phycoerythrin-labeled anti-T4 monoclonal antibody. FL1, green fluorescence (520 nm); FL2, red fluorescence (580 nm).

TABLE I

Binding of fluorescein-labeled bovine serum albumin substituted with α-L-rhamnopyranosyl groups to human peripheral T lymphocytes activated by concanavalin A, for 18 h[a]

Blood sample	*Culture day (%)[b] α-L-Rhap lec⁺ T cells*		
	1[c]	*2[c]*	*3[c]*
A	20 (6)	17 (9)	15 (10)
B	20 (5)	12 (6)	6 (8)
C	39 (16)	17 (18)	15 (20)

[a] Time course after removal of the mitogen. [b] Data are from triplicate experiments. Data are expressed as percentages of cells binding F-(α-L-Rha*p*)-BSA minus the control obtained with F-BSA. Numbers in parentheses are data obtained with unstimulated T cells. [c] Number of days in culture.

Modulation of cell surface lectins specific for neoglycoproteins bearing α-L-Rhap groups upon stimulation with concanavalin A. — By use of the same approach, the number of T lymphocytes expressing on their surface a lectin recognizing neoglycoprotein bearing α-L-Rha*p* groups was shown to increase rapidly upon culture, after incubation in the presence of a mitogenic concentration of concanavalin A and subsequent removal of the mitogen (Table I). [^{3}H]Thymidine incorporation ranged from $3 \cdot 10^4$ to $4 \cdot 10^4$ c.p.m. for $5 \cdot 10^5$ T cells seeded per well. The binding of neoglycoprotein containing α-L-Rha*p* groups was optimal at the beginning of the culture, as early as one day after removal of the lectin, by a cell population that represented up to 39% of the total cell population. This proportion decreased after a longer duration of the culture as shown in Table I. In these experiments, the extent of differences in the expression of lectins was also dependent on the blood sample. The doses of concanavalin A used in these experiments were determined as being optimal, but they were such that this mitogen was tenfold less efficient than PHA to induce blast transformation; these conditions are used to generate Con A-induced T suppressor cells[23,34–36]. In our hands, concanavalin A stimulation indeed resulted in an increase in the proportion of CD8$^+$ T cells, as evaluated by flow-cytometry measurements of concanavalin A-stimulated, cells-enriched population. As an example, the ratio of the percentages of Leu1$^+$ cells to Leu3a$^+$ cells was 2.0 $\pm$ 0.1 for control cells, PHA-stimulated, and concanavalin A-stimulated cells, but the ratio of the percentages of Leu2a$^+$ cells to Leu3a$^+$ cells was 0.65 $\pm$ 0.05 for control cells and PHA stimulated cells, and it reached 1.0 $\pm$ 0.05 for concanavalin A-stimulated cells after a two-day post-stimulation culture; meanwhile the ratio of the percentages of Leu1$^+$ cells to Leu2a$^+$ cells decreased from 3.0 $\pm$ 0.1 to 2.0 $\pm$ 0.1 upon concanavalin A stimulation.

DISCUSSION

Human T lymphocytes have many properties in common with murine T lymphocytes. In this paper, we show that, in addition, human T lymphocytes do express cell

surface lectins analogous to those found in murine T lymphocytes[4]. It is interesting to note that human T lymphocytes do not express a lectin able to recognize neoglycoproteins bearing α-D-mannopyranosyl groups in contrast to human B lymphocytes[37].

As discussed before[5,7], the use of neoglycoproteins helps to elucidate the sugar specificity because the structure of these molecules is well defined and allows comparable experiments. Screening for the definition of membrane lectins specificity is made possible by cross-inhibition studies of a fluorescent-neoglycoprotein binding by non-labeled neoglycoproteins. Because of the avidity phenomenon that allows the binding of a synthetic neoglycoprotein to a natural endogeneous lectin, it is very difficult to get clear inhibition by free oligosaccharides.

Phaseolus vulgaris mitogen[21,22] is one of the most efficient inducers of blast transformation of T lymphocytes. This mitogen induces an increase in the number of cells bearing lectins that recognize β-D-Gal*p*-6-*P*-neoglycoprotein; these cells also express T4 antigen, which belongs to the cluster of differentiation characteristic for helper–inducer T cells ($CD4^+$). They do not express T8 antigen (detected by anti Leu 2a monoclonal antibodies) which is characteristic of suppressor–cytotoxic T cells ($CD8^+$). *Phaseolus vulgaris* mitogen does not increase so much the number of cells which express lectins specific for neoglycoproteins bearing α-L-Rha*p* groups. The subpopulation of cells that bind α-L-Rha*p* groups were shown to be also labeled by anti-Leu 2a antibodies, confirming that they belong to the T suppressor cell family, as previously shown[9,10]. Furthermore, concanavalin A, which is not an efficient inducer of human T cell transformation, is known to enhance suppressor activity[23,24] and especially enhances the release of soluble suppressor factors into the culture supernatant[38]. α-L-Rhamnose-binding molecules having a M_r 14 500 Da could be isolated from human lymphocytes; they were water soluble and were studied for their suppressive properties[9,39]. The data presented herein showed that the number of α-L-Rha*p* binding cells that are known to participate in the suppression of the immunoglobulin production by B cells[9,10,38] increases. These results confirm and extend the observation that activated human lymphoid cells express cell surface lectins[40], on the basis of membrane glycoproteins micelles binding to concanavalin A-activated lymphoid cells and lymphoblastoid cell lines, and subsequent inhibitions by glycoproteins containing complex-type oligosaccharides, such as fetuin or thyroglobulin, as opposed to high mannose-type oligosaccharides. Additional precisions to the previous observation[40] result from the present work: expression of α-L-rhamnose-specific molecules is related to suppressive-cell induction, whereas β-D-galactose 6-phosphate-specific molecules are expressed preferentially on $CD4^+$ T cells.

The rapid expression of various receptors upon adequate activation with interleukin 1 (IL1) or tumor necrosis factor (TNFα) has been reported for other cells, especially endothelial cells. Such a modulation has been documented for various cell surface adhesion molecules, namely the "endothelial leukocyte adhesion molecule 1" (ELAM)[41,42] upon specific action of cytokines and by the initial binding of neutrophils to endothelial cells (see ref. 43 for a review). The extensively studied MEL14/LAM1 (lymphocyte adhesion molecule 1) molecules present on lymphocyte and neutrophils

are also highly "activation dependent" in their expression. These molecules, along with GMP 140 from platelets and Weibel–Palade bodies of endothelial cells, are members of a family of molecules called selectins (see review by Springer[44]). They possess a lectin-homology domain in their sequence[16,18,45] and are fundamental early adhesion molecules[44].

The expression of many lectin molecules has been shown to be developmentally regulated[46]; these molecules are stimulation-dependent for their activity in leukocytes[40,47]. The knowledge of the mechanism of their expression might be very important for the modulation of the immune response, which may be accomplished by increasing or lowering the activity of a population, as compared to another one, by chemoattraction or modification of the homing properties of lymphocytes[17], thus suggesting immunomodulation potencies for endogeneous lectins.

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