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T Lymphocyte activation results in an increased expression of β-1,4-Galactosyltransferase: Phorbol ester induces a similar enhancement in the absence of mitosis

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We previously showed that *in vitro* activated human T lymphocytes expressed increased amounts of β-1,6-branched N-linked oligosaccharides (Lemaire S *et al.* (1994) *J Biol Chem* 269: 8069-74), which have been proposed to participate in the regulation of the immune process. In the present paper, we compared the activity and expression of β-1,4-galactosyltransferase (GalT), one of the glycosyltransferases involved in the biosynthesis of these β-1,6-branched N-linked oligosaccharides, before and after *in vitro* activation of T lymphocytes after a 40 h treatment with a mixture of phorbol 12-myristate 13-acetate and *Phaseolus vulgaris* lectin. After treatment, the enzymatic activity of the GalT was significantly increased and immunoblot experiments performed with a monoclonal antibody to human GalT showed an increased intensity of the GalT band at 49 kDa, attributable to an enhancement of GalT mRNA level, as shown by Northern blots. However, treatment of the same T-lymphocytes by phorbol ester alone, which is unable to induce mitosis, resulted in a comparable increase of the expression of GalT. Moreover, these phorbol ester-treated T lymphocytes, analysed by flow cytometry exhibited a two-fold increase in the expression of GalT. Finally, confocal fluorescence microscopy performed on all T lymphocytes (treated or not) showed that the flow cytometric signal of GalT originates from intracellular, Golgi-associated antigen only since no surface GalT was detected.

***Keywords*: human T lymphocytes, activation, glycosylation, galactosyltransferase**

Introduction

We recently showed [1] that the *in vitro* activation of human T-lymphocytes was associated with important changes in the carbohydrate structures of cellular glycoproteins, the most characteristic change being a dramatic increase in the expression of β-1,6-branched N-linked carbohydrate structure. A similar β-1,6-branched structure has previously been found in several tumour cells, such as human colon carcinoma cells [2] and appears involved in the metastatic process in some human and murine tumour cells [2, 3]. Thus, expression of the same carbohydrate structure by both metastatic tumour cell lines and by activated human T lymphocytes suggests that both cell types have some similarities in their cell behaviour, probably with respect to the cell adhesion, as it was observed for epithelial cells [4]. Two glycosyltransferases were directly involved in the biosynthesis of this β-1-6-branched structure: *N*-acetylglucosaminyltransferase V (GnTV) and β-1,4-galactosyltransferase (GalT). We have observed, for GnTV, a significant enhancement of its activity after T-cell activation [1] and for GalT, the aim of the present work is to investigate its possible changes after T lymphocyte activation.

GalT (EC 2.4.1.38) is a *trans*-Golgi resident type II membrane-bound glycoprotein, responsible for the biosynthesis of the N- and O-linked carbohydrate moieties on glycoproteins and glycolipids, by transferring galactose to the acceptor sugar *N*-acetylglucosamine (for review, see [5]) and according to species and cell types, various galactosyltransferase transcripts and proteins have been described (for review, see [6]). In the mouse, the *GalT* gene specifies two size sets of mRNA of 3.9 kb and 4.1 kb [7] which are a consequence of initiation at two different sets of start sites, separated by about 200 bp. Harduin-Lepers *et al.* [8] showed that the region upstream of the 4.1 kb

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transcriptional start site functions as a housekeeping promoter, when the 3.9 kb transcriptional start site functions as a mammary cell-specific promoter. In human, the genomic organization appears to be similar to the corresponding mouse gene [9], but several cell types express only one transcript of 4.2 kb, corresponding to the short form observed in the mouse [9]. Two GalT proteins could result from the translation of the transcripts, the longer protein having a unique N-terminal 13-amino acid extension, which does not appear to be involved in Golgi retention [10, 11].

In the present paper, the human T lymphocytes were *in vitro* activated by a mixture of phorbol 12-myristate 13-acetate ester (PMA) and *Phaseolus vulgaris* leukoagglutinin (L-PHA) and we observed a significant increase in activity and expression of GalT after T-cell activation, attributable to an increase in the transcription of the *GalT* gene. Moreover, stimulation of the same lymphocytes by PMA alone, which is unable to induce mitosis, resulted in a similar increase in GalT.

Materials and Methods

Cell culture

Normal human lymphocytes, obtained by cytapheresis from the peripheral blood of volunteer blood-bank platelet donors, were isolated by density-gradient centrifugation (mononuclear cells) [12]. To eliminate the majority of the monocytes, the recovered cells were incubated in culture flasks for 1 h at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The non-adherent cells were further treated to isolate the T-cells from other lymphocytes, using a human T-cell recovery column kit (Cedarlane, Hornby, Ontario, Canada) coated with polyclonal goat anti-human IgG (H + L) , which binds B cells. Cell purity was more than 90%, as assessed by specific immunofluorescent antibody staining.

T-cells were cultured at 5×10^6 cells per ml in RPMI 1640 medium supplemented with 15% heat-inactivated FCS, 1% glutamine and 0.2% penicillin/streptomycin, at 37 °C in a 5% CO_2 atmosphere. They were stimulated by adding to the culture medium either 50 ng ml^{-1} phorbol 12-myristate 13-acetate (PMA) (Sigma, St Louis, MO, USA) and/or 1 μg ml^{-1} of leukoagglutinin from *Phaseolus vulgaris* lectin (L-PHA) (E.Y. Laboratories, San Mateo, CA, USA).

[^{3}H]Thymidine, [^{3}H]Mannose and [^{3}H]galactose incorporation

Cells were cultured in 96-well (flat-bottomed) polystyrene plates (Nunc, Kamstruip, Denmark) for 40 h. Each well contained 5×10^5 cells pulsed for 20 h with 40 kBq of [^{3}H] thymidine or 300 kBq of [^{3}H] mannose or 300 kBq of [^{3}H] galactose (Dupont NEN, Les Ulis, France). Cells were collected using a semiautomatic harvester (Skatron, Lierbyen, Norway) [13], treated with a 10% trichloroacetic solution and washed. The radioactivity incorporated into the acid-insoluble cell fractions was counted in a β-scintillation counter (Packard Instruments, Rungis, France).

β-1,4-galactosyltransferase assays

Five $\times 10^7$ cells were washed three times with 0.15 M NaCl and lysed for 45 min at 0 °C in 200 μl 0.15 M NaCl containing 1% TritonX100. Cellular lysates were centrifuged for 20 min at 30 000 × **g** at 4 °C and the protein concentration of the supernatant was determined with the bicinchoninic acid reagent (Pierce Chemical Co, Rockford, IL, USA), using bovine serum albumin as a standard. Kinetic assays were performed with samples containing between 50 ng and 330 ng of proteins in 25 μl of cellular extract, according to the method previously described by Yousefi *et al.* [14], using UDP-[^{14}C]-galactose (11.2 GBq mmol^{-1}, Dupont NEN) at the specific activity of 40 Bq nmol^{-1} and different concentrations of *N*-acetylglucosamine as acceptor (8 mM, 5 mM, 3 mM, 1 mM or 0.8 mM). Radioactivity obtained from controls, without cellular extract or without exogenous acceptor, was subtracted from the radioactivity of respective sample assays.

Immuno blot analysis

Cells (2×10^8) were lysed in 0.5 mM Hepes buffer, pH 7.0, at 0 °C, in a 1 ml potter, kept on ice for 30 min and centrifuged at 4 °C, for 30 min at 30 000 × **g**. Cell pellets were treated overnight, under mild agitation, at 4 °C by the same buffer containing 1% Triton-X100 and centrifuged as above. Protein concentration of the supernatants was determined, using the bicinchoninic acid reagent and 150 μg protein was subjected to SDS gel electrophoresis in a 10% polyacrylamide gel according to Laemmli [15] and transferred onto a nitrocellulose membrane. The membrane was washed, under mild agitation, for 1 h in buffer 1 (composed of 50 mM TrisHCl, pH 8.0, containing 80 mM NaCl, 2 mM $CaCl_2$, 5% milk powder and 0.2% NP40), three times for 15 min in buffer 2 (composed of buffer 1 without NP40) and for 90 min in monoclonal anti-human galactosyltransferase antibody (GT2/36/118) [16], diluted 1 : 10 in buffer 2. The membrane was then washed three times for 15 min with buffer 1, twice times for 15 min with buffer 2 and treated with a 1 : 300 diluted antimouse Ig-horseradish peroxidase-linked F(ab′)$_2$ fragment from sheep (Amersham, Les Ulis, France), for 1 h at room temperature. The membrane was washed again three times for 15 min with buffer 1, twice for 15 min with buffer 2 and finally once with PBS. Antibody-labelled proteins were stained with the 'Renaissance chemiluminescence reagent' (Dupont–NEN).

Northern blot hybridization and stability assay for β-1,4-galactosyltransferase mRNA

Total RNA was extracted as described previously [17] and subjected to electrophoresis in a 1.1% agarose gel as

described by Thomas [18]. Two controls, λ-DNA *Hind*III-digested and an RNA ladder (0.25–9.5 kb) were routinely included as a size standard. One tenth of the treated RNA was run on a separate 1.1% agarose gel, which was then stained with ethidium bromide to control RNA integrity.

Gels were transferred onto hybond N^+ filters (Amersham) in 20 mM NaOH for 5 h at room temperature. The blots were then washed at room temperature with Sodium Salt Citrate (SSC) buffer 6 × (900 mM NaCl + 90 mM sodium citrate), prehybridized for 30 min at 65 °C, in the CHURCH buffer (composed of 0.5 mM sodium phosphate, pH 7.2, 1 mM EDTA, 7% SDS) containing 100 μg ml^{-1} salmon sperm DNA, followed by hybridization, for 16 h at 65 °C, in CHURCH buffer containing 100 μg ml^{-1} salmon sperm DNA and a GalT HeLa cDNA probe [19]. The blots were then washed successively with SSC buffer 2 × (300 mM NaCl + 30 mM sodium citrate) and SSC buffer 0.1 × (15 mM NaCl + 1.5 mM sodium citrate), both SSC buffers containing 0.1% SDS. Probes were amplified in ampicillin-resistant *E. coli* and, after isolation, labelled with [α-^{32}P] dCTP at 111 TBq $mmol^{-1}$ (Dupont - NEN), using the 'Ready To Go DNA labelling Kit (-dCTP)' from Pharmacia. The blots were also hybridized with a [^{32}P] labelled human β-actin DNA probe (Clontech Lab., Palo Alto, CA, USA), as an internal standard.

To determine the relative stability of the GalTmRNA, T lymphocytes were cultured for 24 h in absence or in presence of stimulated agents (PMA alone or PMA/L-PHA), then resting and both stimulated T lymphocytes were incubated for an additional 3, 6, 12, 18 and 40 h, in the presence of 100 μM 5, 6-dichlorobenzimidazole riboside (DBR) which inhibits the transcription. Total RNA was extracted, analysed for GalT and actin mRNA by Northern blot hybridization and quantified using a Beta-Imager 1200 operating with a beta-Vision 3.2 software (Biospace Instruments, Paris, France).

Flow cytometry

For detection of β-1,4,-galactosyltransferase, we used the murine monoclonal antibody to human GalT 2/36/118 [16] as primary antibody and a goat anti-mouse Ig-FITC from Southern Biotechnology Associates, as a secondary antibody.

All of the following procedures were carried out at room temperature, in a volume of 100 μl for a pellet of 10^6 cells. Cells were fixed (10 min) with 3% paraformaldehyde/PBS, centrifuged and treated (10 min) with 20 mM glycine/PBS to quench free aldehyde groups. The cells were either labelled with antibodies directly for further surface staining or permeabilized for intracellular staining.

For surface staining, the cell pellets were washed with 1% bovine serum albumin (w/v)/PBS, incubated in 100 μl 1 : 1 working solution of the primary antibody, washed twice with PBS, incubated with 1 : 40 diluted secondary antibody for 30 min and washed twice with PBS.

For intracellular staining, cells were permeabilized in 0.1% (w/v) saponin/PBS for 30 min, suspended in 100 μl of 1 : 1 diluted primary antibody/0.1% (w/v) saponin (Sigma)/PBS and incubated for 30 min. The cells were washed twice with 0.1% (w/v) saponin/PBS, incubated with 1 : 40 diluted secondary antibody for 20 min and washed twice with 0.1% (w/v) saponin/PBS.

Cells were analysed for immunofluorescence on an EPICS Profile flow cytometer by collecting data for 1×10^4 cells for each histogram. Corresponding negative controls were performed using a secondary antibody alone.

Immunofluorescence confocal microscopy

Approximately 1×10^4 cells in 20 ml of PBS without Ca^{2+} and Mg^{2+} (PBS^-) were plated on a circular glass slide (diam. 12 mm), previously treated with 3-aminopropyltriethoxysilane (Sigma) and placed in the well of an eight well chamber slide. Cells were fixed with 3.7% (w/v) paraformaldehyde for 15 min at room temperature and washed once with PBS^-. Cells were treated with 50 mM ammonium chloride and further permeabilized with 'buffer SP', composed of 0.075% saponin and 0.2% bovine serum albumin (w/v) in PBS^-. Cells were incubated for 45 min in mAb GT2/36/118 [16] 1 : 10 diluted in 'buffer SP', then washed three times with 'buffer SP', followed by incubation for 45 min with 1 : 10 diluted sheep anti-mouse Ig-FITC (Amersham). Cells were washed five times with 'buffer SP'. Treated cells were sedimented onto glass slides by a cytospin centrifuge and mounted in a medium containing 5% (w/v) n-propyl-gallate in 30 mM Tris-HCl pH 9.5 supplemented with 70% (v/v) glycerol. The slides were then examined with a Leica confocal laser scanning fluorescence microscope (Heidelberg) using the Planapo 100 × objective numerical aperture 1.4 and a pinhole diameter of 0.25 μM. Images were processed by image processing software Imaris (Bitplane, Zürich).

Results

Lymphocyte stimulation

Lymphocyte stimulation was evaluated by measurement of [^{3}H]thymidine uptake. Table 1 indicates a dramatic increase (13-fold) of [^{3}H]thymidine uptake into (PMA/L-PHA)-treated lymphocytes, in comparison with resting and PMA-treated lymphocytes where no significant changes were observed. Thus, only the combined treatment is able to induce an increase in DNA synthesis. Results obtained with [^{3}H]mannose and [^{3}H]galactose incorporation into glycoproteins were quite different: treatment with PMA alone led respectively to a five-fold and a nine-fold increase in [^{3}H]mannose and [^{3}H]galactose incorporation, while the combined treatment (PMA/L-PHA) induced respectively

Table 1. [^{3}H]thymidine uptake, [^{3}H]mannose and [^{3}H]galactose incorporations of human T-lymphocytes. Values are mean counts per min $\times 10^{-3}$ per 10^6 cells $\pm$ SD of five different experiments.

Lymphocytes	*[^{3}H]thymidine*	*[^{3}H]mannose*	*[^{3}H]galactose*
Resting	12.5 $\pm$ 1.8	8.9 $\pm$ 2.2	4.7 $\pm$ 0.4
PMA	13.7 $\pm$ 2.0	45.8 $\pm$ 11.4	44.3 $\pm$ 5.2
PMA + L-PHA	162.3 $\pm$ 16.1	131.3 $\pm$ 35.0	96.2 $\pm$ 7.1

a 15-fold and a 20-fold increase in [^{3}H]mannose and [^{3}H]galactose incorporation. This suggests that the PMA-treatment which is unable to promote proliferation is sufficient to induce, like the combined PMA/L-PHA-treatment, important changes in the glycosylation of the cell glycoproteins.

Activity and expression of the β-1,4-galactosyltransferase

As shown in Figure 1, stimulation of T lymphocytes by combined PMA/L-PHA-treatment resulted in a time-dependent increase in the activity of GalT and, after a 40 h treatment, the activity was three-fold higher than in resting T lymphocytes. Table 2 shows that both PMA- and PMA/L-PHA-treatments produced comparable increases in GalT activity: as early as 6 h, a significant increase in the transferase activity was observed, but no significant changes were detected before. The delay observed before changes in GalT activities became apparent, favours the assumption of *de novo* synthesis of GalT rather than its activation by post-translational modification. To test this hypothesis, immunoblot, Northern blot and flow cytometric analyses have been performed.

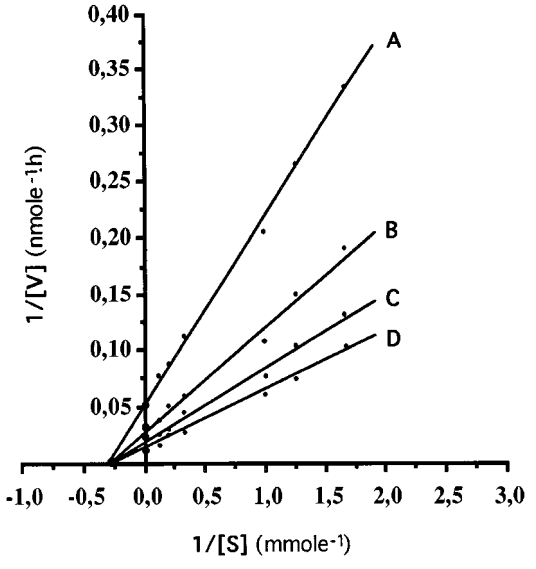

Figure 1. β1,4-galactosyltransferase activity in lysates of resting T lymphocytes (A) and in 6 h (B), 24 h (C) and 40 h (D) PMA/L-PHA-activated T lymphocytes as a function of GlcNAc acceptor concentration, plotted according to Lineweaver-Burk. The incubation was carried out, as described in Materials and methods, for 1 h at 37 °C. The product was eluted from a AG 1X8 anion-exchange column and radioactivity measured in a β-counter. PMA-activated T lymphocytes produced similar results (not shown).

Table 2. β1,4-galactosyltransferase activity measured at different PMA and PMA/L-PHA activation times.

Lymphocytes	V_{max} *(nmol/mg h^{-1})*	K_m *(mM)*
Resting	22.1 $\pm$ 2.3	2.8 $\pm$ 0.3
PMA		
3 h	24.0 $\pm$ 2.1	
6 h	35.7 $\pm$ 1.3	3.1 $\pm$ 0.3
24 h	46.7 $\pm$ 2.3	
40 h	60.0 $\pm$ 6.5	
PMA + L-PHA		
3 h	22.0 $\pm$ 2.1	
6 h	37.1 $\pm$ 1.3	3.1 $\pm$ 0.3
24 h	50.2 $\pm$ 2.3	
40 h	60.1 $\pm$ 4.3	

The expression of GalT was evidenced by immunoblot analysis, using the mAb GT2/36/118 made against GalT from human milk (Figure 2). PMA- or PMA/L-PHA-treatment induced an increased expression of the transferase without any apparent changes in the molecular weight: the intensity of the band at 49 kDa increased significantly after 40 h of both treatments.

Transcription of the β-1,4-galactosyltransferase

To further investigate the modifications in expression of GalT associated with both lymphocyte treatments, Northern blot analyses were performed on total RNA (Figure 3). The GalT mRNA was detected as a band corresponding to 4.5 kb. Comparison with transcripts obtained from HeLa cells, which gave a band at 4.2 kb [9], confirms this result (data not shown). The increase in the level of GalT mRNA after (PMA/L-PHA)- or PMA-treatment was found to be significant after a 24 h treatment.

To determine whether or not T lymphocyte stimulation increased the stability of GalT mRNA, we blocked transcription of the gene by the addition of 5, 6-dichlorobenzimidazole riboside (DBR) and then measured the decay of the mRNA with time (Figure 4). Results show that GalT mRNA from the three species of T lymphocytes could be characterized by their stability, in comparison to β-actin mRNA. Moreover, we observed that GalT mRNA from resting T lymphocytes are not less stable than those from both stimulated T lymphocytes and thus the increase of

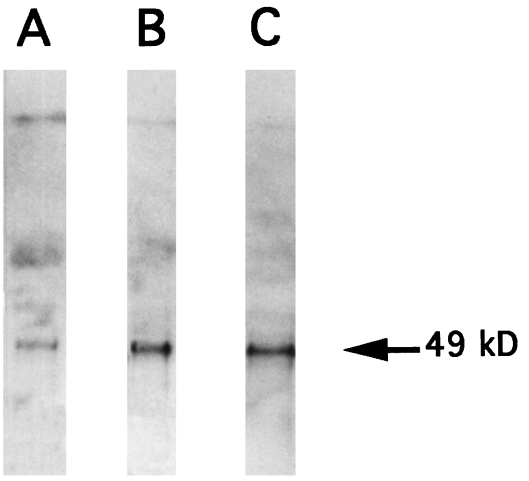

Figure 2. Immuno-blot analysis of the β1,4-GalT. Cell lysates from resting T lymphocytes (A) and from 40 h PMA/L-PHA-activated (B) and 40 h PMA-activated (C) T lymphocytes were subjected to SDS gel electrophoresis in a 10% polyacrylamide gel, transferred onto a nitrocellulose membrane, incubated with a monoclonal antibody against human galactosyltransferase [16], followed by an anti-mouse Ig-horseradish peroxidase-linked $F(ab')_2$ fragment from sheep and stained with the 'Renaissance' chemiluminescence reagent, as indicated in Materials and methods.

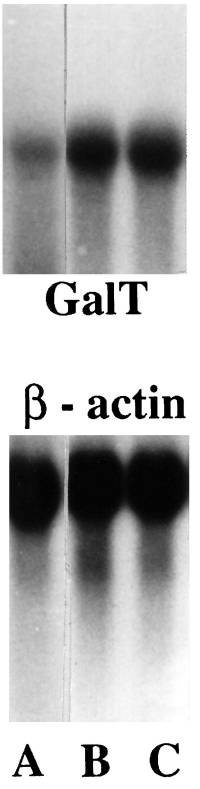

Figure 3. Northern blot hybridization. Total RNA (27 μg) were separated on a 1.1% agarose gel and transferred onto hybond N^+ filters. The blots were hybridized with a [^{32}P]HeLa GalT cDNA probe and a [^{32}P]human β-actin DNA probe as an internal standard. For details, see Materials and methods. A: resting T lymphocytes, B: 24-h PMA/L-PHA-activated T lymphocytes; C: 24-h PMA-activated T lymphocytes.

GalT mRNA levels observed in T lymphocytes after their stimulation (Figure 3) could be attributable rather to an enhanced transcription, than to an increase in the mRNA stability.

Cellular localization of the β-1,4-galactosyltransferase

Cellular localization of GalT in resting, PMA- and PMA/L-PHA-stimulated T lymphocytes was examined by a confocal laser scanning fluorescence microscope. No fluorescence was detected in non-permeabilized cells (data not shown), suggesting that neither treatment resulted in a cell surface expression of the GalT. On the contrary, GalT has been localized, after permeabilization, within the cell, probably in the Golgi apparatus, in both resting cells and PMA- and PMA/L-PHA-stimulated cells. Figure 5 shows the results obtained with the PMA-treated cells, with or without (negative control) the GalT monoclonal antibody. Results obtained from resting and PMA/L-PHA-treated cells gave similar pictures (data not shown).

Flow cytometry

Cells treated with a mixture of PMA and L-PHA cannot be examined by flow cytometry, since L-PHA-treatment results in cell agglutination. Consequently, to quantify the relative increase of intracellular GalT in stimulated T-lymphocytes compared to resting T-lymphocytes, flow cytometric measurements were carried out on PMA-stimulated and resting cells, respectively. These cells were permeabilized and incubated with the mAb GT2/36/118 [16]. The Mean Fluorescence Intensity (MFI) measurements were carried out after 16, 24 and 40 h in the presence of the stimulant. The shift of the MFI observed in PMA-stimulated cells corresponded to an approximately two-fold increase of GalT antigen per cell, as compared with the corresponding resting cells. A representative example is shown in Figure 6. We noted some batch-to-batch variations in the latency period of induction of GalT and also in the MFI of resting cells which specifies the normal amount of enzyme. Nevertheless, an increase in intracellular GalT in PMA-stimulated cells over resting cells was detected in all experiments, starting either at 16 or 24 h. Cell surface staining, performed on non-permeabilized resting and PMA-stimulated cells at 16, 24 and 40 h, produced no signal in all cases studied (data not shown).

Discussion

In this study, we found that *in vitro* activation of peripheral blood T lymphocytes resulted in an increase in the activity

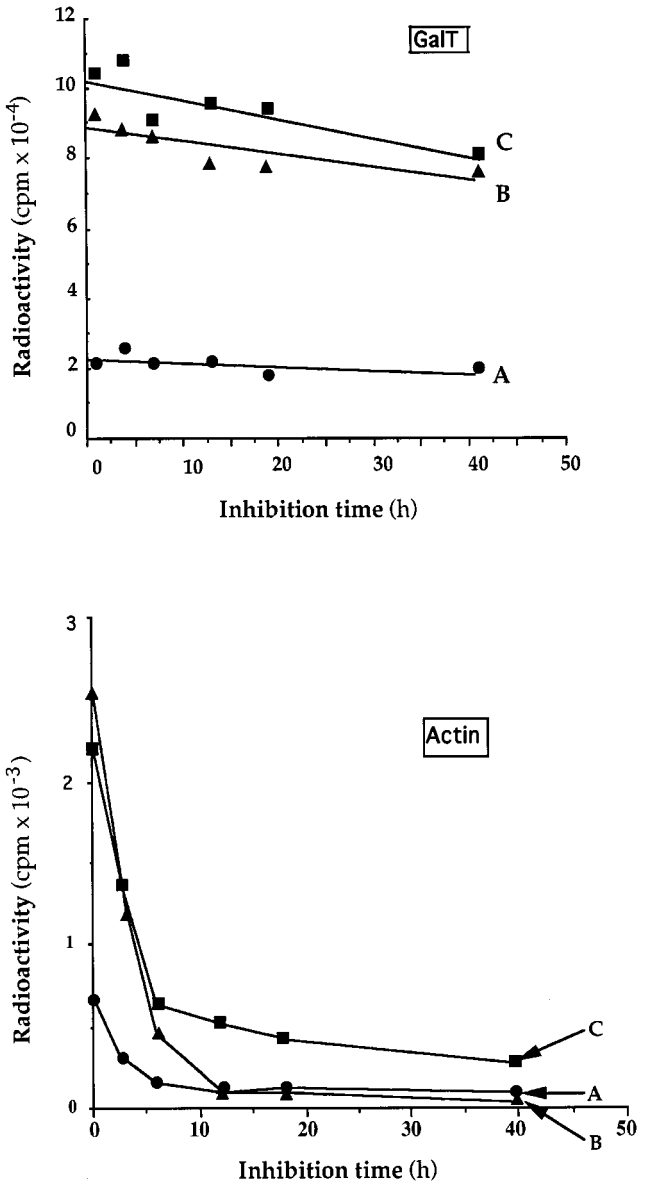

Figure 4. Effect of PMA or PMA/L-PHA on the stability of GalT mRNA in T lymphocytes. T lymphocytes were cultured for 24 h in the absence or in the presence of stimulating agents (PMA or PMA/L-PHA) and then incubated in the presence of 100 μM 5,6-dichlorobenzimidazone riboside for an additional 3, 6, 12, 18 and 40 h. Total RNA was isolated, analysed for GalT mRNA and actin mRNA by Northern blot hybridization (27 μg per lane) and quantified using a Beta-Imager 1200 operating with a beta-Vision 3.2 software. Values on the curves were calculated relatively to a constant ratio of the total mRNA isolated from 10^8 cells. (A) resting T lymphocytes; (B) PMA-activated T lymphocytes; and (C) PMA/L-PHA-activated T lymphocytes.

and expression of β-1,4-galactosyltransferase and consequently, carbohydrate structures with a high galactose residue content, like the β-1,6-branched *N*-acetyllactosamine antennae, could be more specifically affected. Nevertheless, it is unclear if an increase in GalT alone is sufficient to allow the extension of these β-1,6-branched antennae and the formation of polylactosaminoglycans. The limiting factor could be the branching enzyme (GnT V) or iGnT [20].

After activation, increased enzyme activity was observed as early as 6 h and reached a maximum at 40 h with a three-fold increase in the activity. These results are different from those of Piller *et al.* [21] who did not find significant changes in GalT activity after T lymphocyte activation; nevertheless, in this work the incubation times were not specified, and it is possible that in the case of GalT measurements, initial velocity conditions were not met. This could have led to an underestimation of GalT activity in stimulated cells.

Regarding the GalT protein, increased levels of expression were observed using two complementary techniques, *eg* immuno-blotting and flow cytometry. Moreover, confocal fluorescence microscopy shows that the GalT protein is exclusively localized inside the cells, probably in the Golgi apparatus. Thus the possibility of an involvement of GalT as cell surface recognition molecule, as demonstrated for spermatozoa [22] or embryonal cells [23], appears unlikely in the case of activated T lymphocytes.

Northern blot analysis showed that both PMA- and PMA/L-PHA-treatment of human T lymphocytes resulted in an increased expression of GalT mRNA. This transcript has 4.5 kb, contrary to the transcripts of 4.2 kb expressed by most human cells [9]. mRNA stability assays (Figure 4) were in favour of the hypothesis of a control of the GalT expression by transcriptional activation rather than by changes in mRNA stability, since no significant differences in RNA stability were found between resting and both PMA- and L-PHA/PMA-stimulated T lymphocytes. These results are different from those observed by Miyoshi *et al.* [24], in the case of *N*-acetylglucosaminyltransferase V (GnT V) where the up-regulation of the *GnT V* gene activity observed after treatment by the Transforming Growth Factor β is due to an increased stability of the mRNA. This regulation seems to be in conflict with the presence, in the promoter regions of GalT transcript, of features typical of housekeeping genes. Nevertheless, several authors have previously reported changes in GalT associated with different biological events, as in cell growth [25], cell differentiation [26] or human blood cancer [27] and also in NIH 3T3 cells after their transfection by the *N-ras* proto-oncogene [28]. Thus, the regulation of galT transcription at the proximal site is probably more complex than expected and involves a combination of both positive and negative elements [8], The increased transcriptional activity of the *GalT* gene, observed after treatment by phorbol ester suggests an involvement of the PKC transduction pathway [29] but the exact mechanisms and the nuclear factors involved in these processes remains to be clarified.

T lymphocyte activation involves several signal transduction pathways and is characterized by the expression of specific cell surface antigens, such IL-2 receptor and by

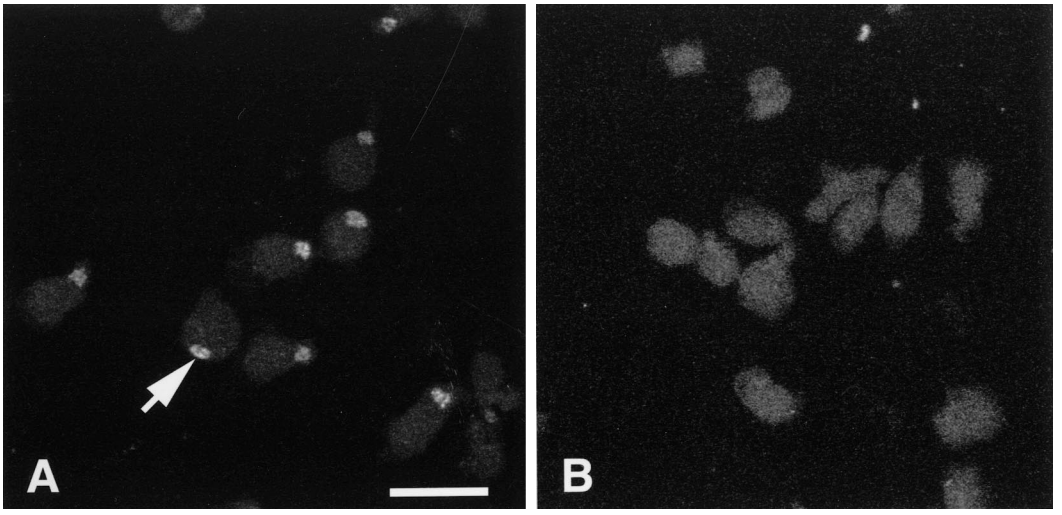

Figure 5. Confocal fluorescence microscopy. The cells were fixed, permeabilized and incubated with mAb GT2/36/118 (against human galactosyltransferase) [16], followed by anti-mouse Ig-FITC-labelled sheep second antibody (A) or with second antibody only (B), as described in Materials and methods. They were examined with a confocal laser scanning fluorescence microscope. The Golgi apparatus of T lymphocytes is shown as the compact juxtanuclear fluorescent structure (arrow). Bar 10 μm.

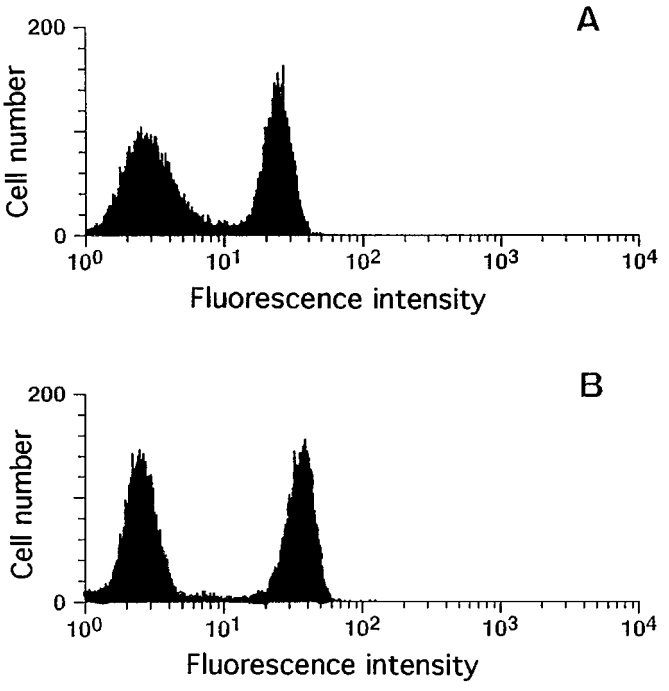

Figure 6. Expression of intracellular β1,4-galactosyltransferase in resting and PMA-stimulated T lymphocytes. Flow cytometric analyses were performed on permeabilized T lymphocytes either without (A) or with stimulation with PMA for 16 h (B). After permeabilization, cells were labelled with GT 2/36/118 mAb. Corresponding negative controls with secondary antibody alone were included (right peak). The increase in MFI in panel B (left peak) over the MFI in panel A (left peak) is two-fold.

a mitogenic response. After activation, T lymphocytes are able to migrate through the organism to promote an immune response. The change in GalT that we observed after T cell treatments, together with the other glycosylation changes in cellular glycoproteins, suggested by the increased amounts in incorporated [^{3}H]mannose and [^{3}H]galactose (Table 2), and also by affinity chromatography on immobilized lectins (Lemaire, unpublished results), could be involved in the modulation of the inflammatory process and immune response [30], probably by changing the adhesion properties of T-cells after their activation, as suggested by Yagel *et al.* [31] for tumour cells.

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