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Activated human T lymphocytes display new surface glycoproteins

(T-cell subsets/differentiation markers/blast transformation/killer cell antigen)

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ABSTRACT **We have analyzed the surface glycoproteins of resting and *in vitro* activated human T lymphocytes by the galactose oxidase/NaB^3H_4 and the periodate/NaB^3H_4 labeling techniques. The labeled glycoproteins were separated by polyacrylamide slab gel electrophoresis and visualized by fluorography. A "new" glycoprotein with an apparent molecular weight of 130,000 (GP130) was strongly labeled on alloantigen-activated T blasts but only weakly or not at all on mitogen-stimulated T blasts and resting T lymphocytes. These results demonstrate that human T cells, as earlier found in the mouse system, express different surface molecules in relation to the particular mode of activation and stage of differentiation.**

The functionally differentiated cells in multicellular organisms frequently carry specific surface structures which are involved in intercellular communication. A detailed characterization of such membrane molecules may allow a deeper insight to the mechanisms operating during cell-to-cell cooperation.

A number of different cells interact in the immune system to create optimal responses to various immunogenic substances. The mode and magnitude of the immune response are largely controlled by the T lymphocytes (1). Endowed with the ability to display functions like help, suppression, or killing, T lymphocytes apparently are comprised of functionally discrete subsets (2). Some of these subsets express specific differentiation antigens which, in the mouse, can be detected by appropriate alloantisera (3). Corresponding antisera specific for the subsets of human T lymphocytes are not yet available, but the surface molecules found in the murine system will most likely have their counterparts on human lymphocytes.

The surface molecules on different lymphoid cells have recently been studied by selective radiolabeling of the membrane glycoproteins (4). These investigations have disclosed that both murine and human resting T and B lymphocytes have their own, distinct, surface glycoprotein patterns (5, 6). Blast transformation and differentiation of the murine T cells *in vitro* induced changes in their surface glycoprotein patterns which were different depending on the mode of activation. T blasts from cultures stimulated with allogeneic cells in mixed lymphocyte culture (MLC) had a surface glycoprotein pattern that was different from that of mitogen-activated T cells (5). Detailed analysis revealed the expression of a unique surface glycoprotein, called T145 (molecular weight, 145,000), that appeared concomitantly with the expression of cytotoxic ability by activated T cells. The cellular distribution of this particular membrane protein indicated it to constitute a specific differentiation marker for a subset of T cells in the mouse—namely, killer cells (7).

In the present communication we report on the extension of this work to include surface glycoproteins of human T cells at various stages of differentiation. Mitogen- and alloantigen-activated (in MLC) human T blasts displayed different cell surface glycoprotein patterns compared to normal resting T lymphocytes. The MLC blasts carried a membrane glycoprotein, GP130 (molecular weight, 130,000) that appears to be a human counterpart of the murine killer T cell glycoprotein T145.

MATERIALS AND METHODS

Isolation of Blood Lymphocytes. Buffy coats from fresh human blood were obtained from the Finnish Red Cross Blood Transfusion Service. Mononuclear cells were isolated by Ficoll/Isopaque gradient centrifugation (8). Phagocytic cells were removed by treatment with iron powder and a magnet. T lymphocytes were purified by passage of the cells over a human Ig/rabbit anti-human-Ig column (9). The contamination of the T cells by surface-immunoglobulin-bearing cells was <1% as judged by immunofluorescence staining. Blood B lymphocytes and null cells (with lymphocytic morphology but lacking the typical T and B cell surface markers) were purified as reported in detail elsewhere (10).

Cultivation and Purification of T Lymphoblasts. Lymphocytes enriched for T cells were cultivated at a density of 10^6 cells per ml in RPMI-1640 culture medium supplemented with 10% normal human AB plasma for the indicated times with optimal concentrations of phytohemagglutinin (PHA) or concanavalin A (Con A). T lymphocytes were also cultivated with mitomycin C-treated allogeneic mononuclear blood cells in MLC. Blast cells were purified from the mitogen cultures and the MLC by 1-*g* velocity sedimentation (11). The blast cell populations consisted of >95% lymphoblasts as judged from May–Gruenwald–Giemsa-stained cytocentrifuged smears. More than 85% of the blasts formed rosettes with sheep erythrocytes. Viability of all cells exceeded 95% as judged by the trypan blue exclusion test.

Radiolabeling of Cell Surface Proteins. Cells were surface labeled by reduction with NaB^3H_4 after oxidation with either neuraminidase plus galactose oxidase (4) or periodate (12). About 50×10^6 cells suspended in 1 ml of Dulbecco's phosphate-buffered saline (P_i/NaCl) were incubated with 25 units of *Vibrio cholera* neuraminidase (NE) (Behringwerke, Mahrburg, Lahn) and 5 units of galactose oxidase (Kabi, Stockholm). The enzyme preparations did not contain proteolytic activity. Alternatively, the same amount of cells was suspended in 1 ml of ice-cold P_i/NaCl, 10 μl of 0.1 M sodium metaperiodate was added, and the cells were kept on ice for 10 min. After treatment with enzymes or periodate, the cells were washed with P_i/NaCl and then suspended in 0.5 ml of P_i/NaCl, and 0.5 mCi of NaB^3H_4 was added. After 30 min at room temperature, the cells were washed with cold P_i/NaCl, and 0.2

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Abbreviations: MLC, mixed lymphocyte culture; Con A, concanavalin A; GO, galactose oxidase; NE, *Vibrio cholera* neuraminidase; PHA, phytohemagglutinin; P_i/NaCl, phosphate-buffered saline.

ml of cold P_i/NaCl containing 1% Triton X-100 and 2 mM phenylmethylsulfonyl fluoride was added. After 5 min on ice, the nuclei were pelleted and the supernatants were used for electrophoresis.

Polyacrylamide Slab Gel Electrophoresis. Electrophoresis was performed on 8% polyacrylamide gels with ^{14}C-labeled marker proteins in peripheral slots (5). The gels were fixed and treated for fluorography as described (13). Quantitative measurements of the individual fluorographic profiles were performed on a Joyce–Loebl recording densitometer.

RESULTS

Surface Glycoprotein Patterns of Resting Blood Lymphocytes. Fluorographic patterns of slab gels of surface-labeled T and B and null lymphocytes purified from normal human blood are shown in Fig. 1. The patterns of the main lymphocyte populations are clearly different. In the fluorography pattern of resting T lymphocytes, four closely spaced bands of equal labeling intensity are seen in the high molecular weight region: GP200, GP180, GP165, and GP160. B cells and null cells have one major band in this region with an apparent molecular weight of 210,000 (GP210). Both T and B lymphocytes express a major band, GP120, which is weakly labeled in null cells. The basic patterns of these lymphocyte populations were remarkably constant and did not differ between corresponding cells obtained from different donors.

Surface Glycoprotein Patterns of *In Vitro*-Generated T Blasts. T lymphoblasts recovered after activation *in vitro* with mitogens or with allogeneic cells still showed the basic glycoprotein patterns of resting T lymphocytes. On comparison of the glycoprotein patterns of T blasts isolated from mitogen-stimulated cultures with those of T blasts isolated from MLC, some apparent differences were observed. The MLC blasts expressed a major band, GP130, that was seen after 3 days in culture and was strongly labeled after 6 days in culture (Figs. 2 and 3). This was only weakly expressed on PHA- and Con A-stimulated blast cells and on T lymphocytes kept in unstimulated cultures. GP120, on the other hand, was virtually absent from MLC blasts at 6 days but was the major protein in this molecular weight range on mitogen-activated T blasts.

Another major difference between mitogen- and alloantigen-stimulated T blasts was in the labeling of the four closely spaced bands in the high molecular weight region. As shown, in Figs. 1, 2, and 3, GP200, GP180, GP165, and GP160 showed approximately identical labeling intensities on resting T lymphocytes and on T lymphocytes kept in unstimulated cultures for 6 days. GP200 and GP180 were weakly labeled on MLC-activated blasts whereas on mitogen-activated T blasts, GP180 and GP165 were relatively strongly labeled. Stimulation of lymphocytes from different individuals consistently yielded these characteristic differences between the fluorography patterns of their MLC T blasts and mitogen-induced T blasts (Fig. 4).

The different fluorography patterns of MLC and mitogen-induced T blasts obtained after labeling with the neuraminidase/galactose oxidase technique is apparently not due to differences in accessibilities of galactosyl- or *N*-acetylgalactosyl-aminyl residues, because the same principal differences were also observed after labeling of the sialic acid residues by periodate/NaB^3H_4 treatment (Fig. 5).

DISCUSSION

Analysis of exposed membrane glycoproteins by selective surface labeling (4, 12) allows the identification of certain cells and cell lineages with high accuracy (14, 15). In the present study we have shown that blast cells derived from human peripheral T lymphocytes express different surface glycoprotein patterns as a result of various cellular activations.

The most striking finding was a prominent "new" band, GP130, in the fluorography patterns of MLC blasts. GP130 was undetected in small T lymphocytes of blood and was weakly labeled in mitogen-stimulated T blasts and T lymphocytes kept in nonstimulated cultures. This difference in the expression of glycoproteins on human MLC and resting T cells parallels that reported in the mouse system, where a certain glycoprotein, called T145, was selectively found on $Ly1^{-}2^{+}$ killer T blasts (7).

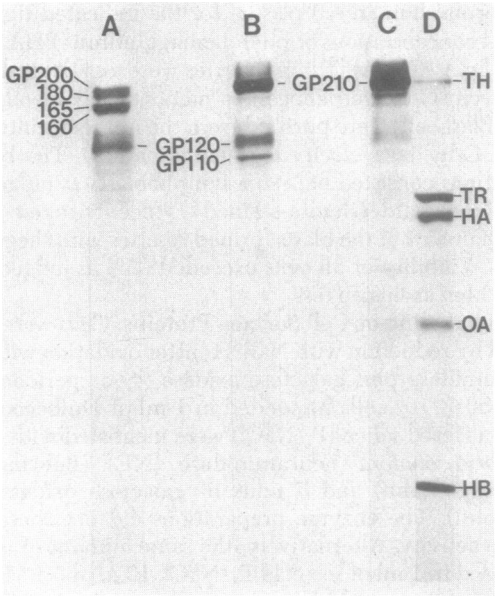

FIG. 1. Fluorography patterns of the surface glycoproteins labeled after neuraminidase/galactose oxidase treatment of human blood lymphocytes. Lanes: A, T lymphocytes; B, B lymphocytes; C, null lymphocytes; D, ^{14}C-labeled marker proteins. TH, thyroglobulin; TR, transferrin; HA, human serum albumin; OA, ovalbumin; HB, hemoglobin. GP210 indicates a glycoprotein with an apparent molecular weight of 210,000, etc. [The smaller glycoproteins (i.e., the HLA-D molecules on B lymphocytes) require longer exposure times to become apparent.]

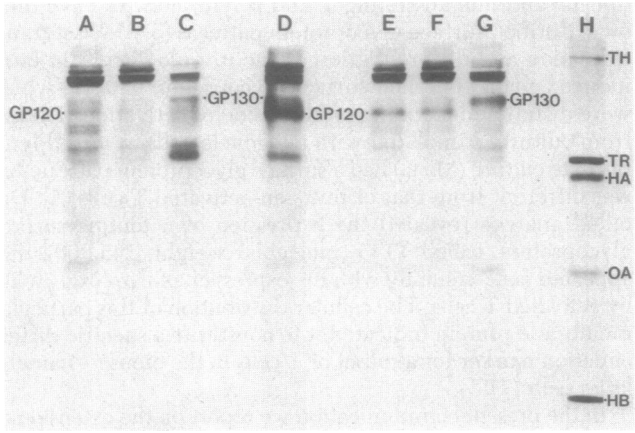

FIG. 2. Fluorography patterns of the surface glycoproteins labeled after neuraminidase/galactose oxidase treatment of T blasts and T lymphocytes cultured for different times. Lanes: A, PHA blasts, 3 days; B, Con A blasts, 3 days; C, MLC blasts, 3 days; D, T lymphocytes, kept in unstimulated cultures for 6 days; E, PHA blasts, 6 days; F, Con A blasts, 6 days; G, MLC blasts, 6 days; H, marker proteins as in Fig. 1.

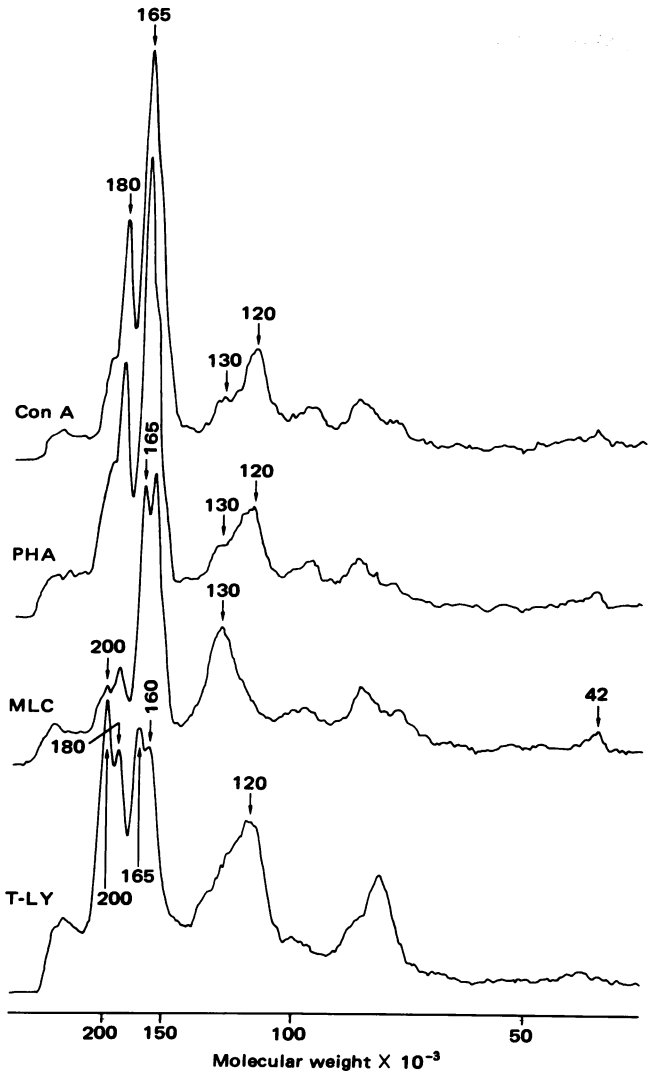

FIG. 3. Densitometric tracings of the fluorography patterns obtained with surface labeled T blasts and T lymphocytes after 6 days in culture. T-LY, T lymphocytes from nonstimulated cultures; MLC, MLC blasts; PHA, PHA blasts; Con A, Con A blasts. The major labeled proteins are indicated. The quantitative differences in the labeling intensities (GP200–GP160) and the different expressions of GP130 and GP120 are obvious. GP42, location of the heavy chain of the HLA antigen.

Although of slightly different apparent molecular weight, the behavior of this protein strongly indicates that it represents the mouse counterpart of the human GP130. The question of whether GP130 represents a marker for a functional differentiation step or is already expressed on a small, undetectable subpopulation of small T lymphocytes being strongly selected for during the MLC is yet to be answered. The kinetics for the appearance of GP130, however, are more compatible with a sequence of differentiation because, although already present on MLC blasts cultivated for 3–4 days, GP130 requires 5–7 days to reach maximal expression. Moreover, we have not found detectable expression of GP130 on thymocytes or on subpopulations of human blood, spleen, and thymocytes or on subpopulations of human blood, spleen, and tonsillar T lymphocytes selected for the presence of receptors for Fcγ, for Fcμ (16), for the ability to form "fast" or "slow" rosettes with sheep erythrocytes, etc.

On the other hand, we recently found that GP130 is a

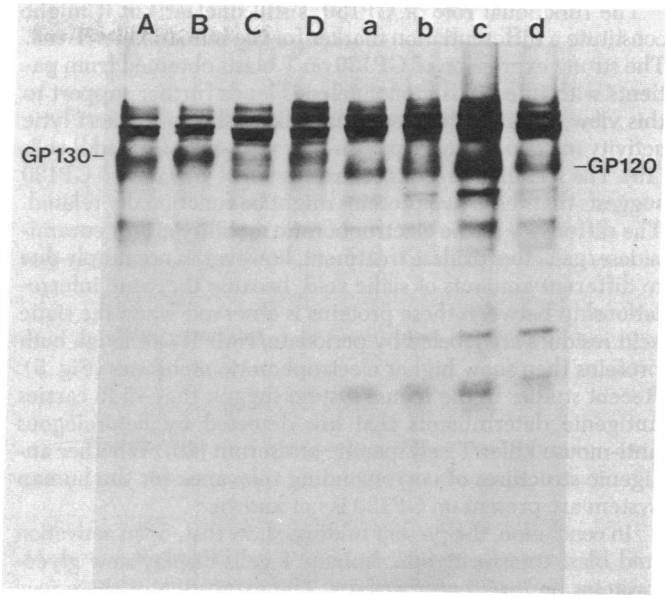

FIG. 4. Fluorography patterns of the surface glycoproteins labeled after neuraminidase/galactose oxidase treatment of MLC and PHA blasts from four different persons. Lanes: A–D, MLC blasts; a–d, PHA blasts; A and a originate from the same donor, etc.

prominent surface glycoprotein on blood T lymphoblasts from patients with acute infectious mononucleosis (17), proving that T blasts may also express this surface protein *in vivo*.

T145, which is the apparent murine counterpart of GP130, has clearly been shown to constitute a differentiation marker for the murine killer T cell. It is absent from resting spleen T lymphocytes, regardless of their Ly phenotype, but the activation of the cytotoxic capacity *in vivo* or *in vitro* coincides with the surface appearance of T145. T145 is only expressed on T killer blasts of relevant Ly phenotype but is retained on "secondary" T cells (18) that have reverted to memory lymphocytes upon prolonged culture of isolated MLC blasts in the absence of stimulator cells.

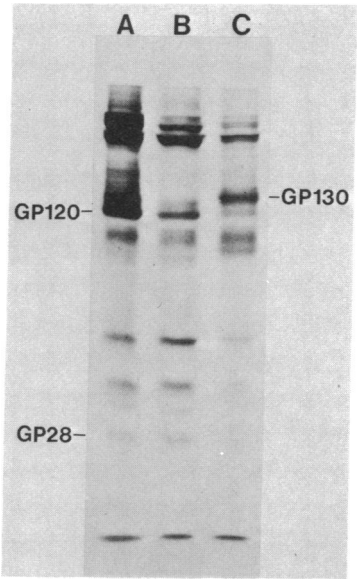

FIG. 5. Fluorography patterns of surface glycoproteins labeled by periodate/NaB^{3}H$_4$ treatment of Con A blasts (lane A), PHA blasts (lane B), and MLC blasts (lane C). The presence of negatively charged sialic acid residues gives the GP120 and GP130 higher electrophoretic mobilities compared to those seen after the enzyme treatment.

The functional role of GP130 is still unclear but it might constitute a differentiation marker for the human killer T cell. The strong expression of GP130 on T blasts obtained from patients with infectious mononucleosis lends further support to this view, because these cells have been shown to exert lytic activity *in vitro* against Epstein–Barr virus-transformed B cells (19). The seemingly reciprocal expression of GP120 and GP130 suggests that these two proteins might be functionally related. The difference in the electrophoretic mobility after neuraminidase/galactose oxidase treatment, however, is not simply due to different amounts of sialic acid, because the same interrelationship between these proteins is observed when the sialic acid residues are labeled by periodate/NaB^3H_4 although both proteins then show higher electrophoretic mobilities (Fig. 5). Recent studies in the mouse system suggest that T145 carries antigenic determinants that are detected by heterologous anti-mouse killer T cell specific antiserum (20). Whether antigenic structures of corresponding relevance for the human system are present on GP130 is not known.

In conclusion, the present findings show that, upon activation and blast transformation, human T cells display new glycoproteins on their outer surface. The expression of these new glycoproteins varies according to mode of activation and requires different time periods to achieve full expression. This suggests that these glycoproteins represent markers for subsets of T cells at different stages of differentiation.

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