EXPRESSION OF Le^y ANTIGEN IN HUMAN IMMUNODEFICIENCY VIRUS–INFECTED HUMAN T CELL LINES AND IN PERIPHERAL LYMPHOCYTES OF PATIENTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) AND AIDS-RELATED COMPLEX (ARC)

By MASAKAZU ADACHI,*‡ MASANORI HAYAMI,§ NOBUHITO KASHIWAGI,* TOSHINOBU MIZUTA,* YOSHIHIRO OHTA,§ MICHAEL JOHN GILL,‖ DAVID S. MATHESON,¶ TAIKI TAMAOKI,** CHIAKI SHIOZAWA,*·** and SEN-ITIROH HAKOMORI‡‡

*From the *Japan Immunoresearch Laboratories, Takasaki 370; the ‡Department of Immunological Research, Otsuka Pharmaceutical, Tokushima 771; the §Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan; the ‖Departments of Medical Microbiology and Infectious Disease and Medicine, ¶Department of Paediatrics and Internal Medicine, and the **Department of Medical Biochemistry, University of Calgary, Calgary, Alberta T2N 4N1, Canada; and ‡‡The Biomembrane Institute, Departments of Pathobiology, Microbiology, and Immunology, University of Washington, Seattle, Washington 98119*

Acquired immune deficiency syndrome (AIDS) is recognized as a distinct new disease whose etiology has been identified as being associated with infection of a new class of lymphotrophic retrovirus termed human immunodeficiency virus (HIV) (1, 2).[1] The disease is characterized by a disorder associated with an impaired cell-mediated immunity and absolute lymphopenia, particularly reduced helper T lymphocytes ($T4^+$ or CD4). This is due to the fact that HIV preferentially infects the CD4 lymphocyte population. AIDS may be preceded by a presyndrome that is usually manifested by a complex of designated clinical features and helper T lymphopenia (3–5). The presyndrome is called AIDS-related complex (ARC).[2] Diagnosis of infection with HIV is usually made on the basis of detecting antibodies directed against HIV. The exact antibody profile may vary with the stage of the disease (1). Despite significant progress in understanding the pathogenesis of HIV, no studies have been performed regarding carbohydrate antigens in T lymphoid cells infected with HIV, although carbohydrate changes associated with functional change of cellular phenotypes have been well documented (6). In this note, we present evidence that Le^y

[1] Previously called, in various terms, human T cell lymphotropic virus III (HTLV-III), human lymphadenopathy-associated virus (LAV), or AIDS-related virus (ARV) (reviewed in references 1, 2).

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[2] *Abbreviations used in this paper:* ARC, AIDS-related complex; HTLV-III, human T cell lymphotropic virus III.

TABLE I

mAbs Used in this Study and the Structures Defined by Them*

mAb	Structure defined	Reference
FH2 (IgM)	Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer 3 ↑ Fucα1	11
ACFH18 (IgM)	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 3 3 ↑ ↑ ↑ Fucα1 Fucα1 Fucα1	11
FH6 (IgM)	NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 3 ↑ ↑ Fucα1 Fucα1	12
KH-1 (IgM)	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 2 3 3 ↑ ↑ ↑ Fucα1 Fucα1 Fucα1	13
BM-1‡ (IgM)	Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 ↑ Fucα1	14
CU-1 (IgG3) anti-Tn	GalNAcα1→0-Ser/or Thr	Takahashi, H., and Hakomori, S., unpublished data
HH8 (IgG$_3$) Anti-T	Galβ1→3GalNAcα1→3Galβ1→R 3 ↑ (Fucα1)	Clausen, H., and Hakomori, S., unpublished data

* All hydridomas secreting these antibodies were originally selected and established as being directed to tumor-associated antigens strongly expressed in various human cancer cell lines or human cancer tissues.

‡ A subclone of a hybridoma originally described as AH6 (14).

determinant is highly expressed at the surface of human T cell lines only after infection with HIV, as well as in T lymphocytes of peripheral blood of patients with AIDS and ARC but not in normal lymphocytes of healthy individuals.

Materials and Methods

Cells and Viruses. Two human T cell leukemia lines, H-9 (7) and TALL-1 (8), used in this experiment were originally isolated from T cell leukemia patients as previously described. These cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS containing 50 μg/ml of gentamycin. The cell population density of the culture was maintained within 10^5–10^6/ml and subcultured every 3 d. Human T cell lymphotropic virus III (HTLV-III) of HIV was isolated and characterized as previously described (1, 7) and was maintained in TALL-1 cells in the laboratory of M. Hayami.

Method of HIV Infection. A cell suspension of H-9 and TALL-1 cells at a cell population density of 3×10^5/ml was mixed with 2 ml of culture supernatant containing HIV having reverse transcriptase activity of $6–30 \times 10^4$ cpm/ml in the presence of 1 μg/ml Polybrene (Sigma Chemical Co., St. Louis, MO) and subcultured every 3 d. Infection of HIV was observed by syncytium formation and by the detection of immunofluorescence antigens using patients' antisera (9) and by mAb VAK-4 directed to p24 gag protein (10). Furthermore, a release of HIV virus in culture media after established infection was confirmed by the activity of reverse transcriptase.

mAbs and the Structures Defined by These Antibodies. Seven types of anticarbohydrate mAbs were used for immunofluorescence and cytofluorometric analysis. The structures defined by these antibodies and their origin are described in Table I. The antibody FH-2 is directed to Le^x, ACFH-18 is directed to a long type 2 chain fucosylated structure showing strong reactivity with trifucosyl Le^x (trimeric Le^x) (11), the antibody FH-6 is

directed to sialyl Le^x carried by a long type 2 chain with internal fucosylation (12), and KH-1 reacts specifically with trifucosyl Le^y (13). The antibody BM-1 is a subclone of AH-6 directed to Le^y structure as previously described (14). The antibody CU-1 is directed to Tn antigen (Takahashi, H., and S. Hakomori, unpublished observations), and HH-8 is directed to Galβ1→3GalNAcα residue of galactosyl A and is similar to T antigen associated with glycoprotein (Clausen, H., and S. Hakomori, unpublished data).

Cytofluorometric Analysis of Membrane Antigen. A cell suspension of 5×10^5 H-9 or TALL-1 cells was mixed with 100 μl of purified mAbs, reacted at 4°C for 60 min, washed twice with RPMI 1640 medium, and subsequently added with 100 μl of 35 times–diluted FITC-conjugated goat $F(ab)_2$ fragment directed to anti–mouse IgM (No. 4352; Tago Inc., Burlingame, CA) or IgG (No. 4350; Tago Inc.). After reaction at 4°C for 30 min, cells were washed twice with RPMI medium and fixed with 1.5% formalin-PBS. A purified mouse IgM (Coulter Electronics, Hialeah, FL) was used instead of primary antibody for a control. Cytofluorometry was performed using an EPICS-C (Coulter Electronics). The details of the conditions are described in the legend for Fig. 1.

Immunofluorescence and Two-Color Cytofluorometric Analysis of Lymphocytes from HIV-infected Patients. 10 ml of heparinized peripheral blood drawn from a vein of HIV-infected patients was mixed with 1 ml of KAC-2™ (5% iron silicate sol; Japan Immunoresearch Laboratories, Takasaki, Japan) and maintained at 37°C for 1 h to eliminate phagocytotic cells. The KAC-2-treated blood was then subjected to Ficol-Hypaque gradient centrifugation to obtain mononuclear cell fraction free from granulocytes and monocytes. Aliquots of the lymphocyte suspension were stained by immunofluorescence with various mAbs as described above, and examined by fluorescence microscopy. Aliquots of cells were washed with PBS containing 0.1% gelatin and 0.1% sodium azide. BM-1 antibody was added and incubated for 60 min at 4°C, followed by washing with PBS-gelatin. FITC-labeled anti–mouse IgM $(F[ab]_2$ fragment, purchased from Tago Inc.) was then added and incubated for 30 min at 4°C followed by washing with PBS-gelatin. Subsequently, phycoerythrin coupled to CD3, CD4, or CD8 antibody (Becton Dickinson & Co., Oxnard, CA) was added and incubated for 30 min at 4°C, washed with PBS, fixed with 1.5% formalin for 15 min at 4°C, washed and resuspended in PBS, and analyzed by cytofluorometry (FACS-3). The ratio of CD4/CD8 was determined by analysis with phycoerythrin-labeled CD4 and CD8 by the procedure described above.

Results

Both H-9 and TALL-1 cells were essentially negative with immunofluorescence staining and cytofluorometric assay with seven anticarbohydrate antibodies, as listed in Table I. In striking contrast, both H-9 and TALL-1 cells after infection with HIV became strongly positive with antibody BM-1 after infection; the expression of the BM-1-defined antigen was found subsequent to the appearance of the HIV-specific gag p24 protein antigen defined by mAb VAK-4, as well as by antibodies in the patients' sera (Fig. 1). The cytofluorometric pattern with BM-1 antibody is shown in Fig. 2, and the percent of positive cells, defined by various antibodies, before and after infection with HIV virus, is shown in Fig. 3. All these infected cells were characterized by the presence of positive reaction with mAbs directed to HIV gag protein p24 and by syncytium formation.

The lymphocyte fractions of 17 HIV-infected patients were prepared, and their reactivity with BM-1 mAb was observed under immunofluorescence. Lymphocytes from AIDS patients showed a strong immunofluorescence with BM-1, and those from ARC patients showed positive staining also, but with less frequency as compared with lymphocytes from the AIDS patients (Fig. 4). With hematoxylin and eosin staining of these BM-1^+ cells, their morphology was distinctively different from monocytes but was characteristic of lymphoid cells.

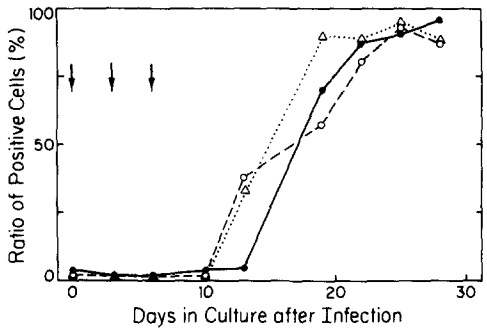

FIGURE 1. Time-dependent changes in Le^y antigen and HIV-associated antigens expressed in human T cell leukemia cell line TALL-1. TALL-1 cells in culture were infected by the addition of supernatant from HIV-infected TALL-1 culture, which contained reverse transcriptase activity with 58,000 cpm/ml at the 1st, 3rd, and 6th days (*arrow*), and subsequently, expression of HIV-associated antigen in cells was determined by indirect immunofluorometry using anti-p24 mAb and by serum of an AIDS patient. Simultaneously, the expression of Le^y antigen was monitored by cytofluorometry with mAb BM-1. Expression of antigens is shown by the ratio of positive cells in percent. (●) Reactivity with BM-1; (○) reactivity with patient's serum; (△) reactivity with anti-p24.

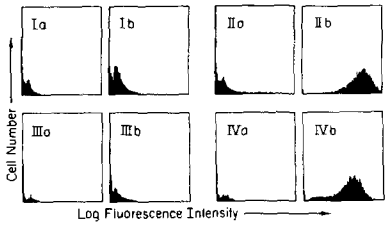

FIGURE 2. Cytofluorometric pattern of expression of Le^y antigen in human T cell leukemia cells H-9 and TALL-1 before and after infection with HIV. (*Ia, Ib, IIa,* and *IIb*) H-9 cells; (*IIIa, IIIb, IVa,* and *IVb*) TALL-1 cells. (*Ia*) control H-9 cells (uninfected) treated with mouse IgM; (*Ib*) H-9 cells infected with HIV treated with mouse IgM; (*IIa*) H-9 cells uninfected and treated with mAb BM-1; (*IIb*) H-9 cells infected with HIV treated with mAb BM-1. (*IIIa*) control uninfected TALL-1 cells treated with mouse IgM; (*IIIb*) TALL-1 cells infected with HIV treated with mouse IgM; (*IVa*) uninfected TALL-1 cells treated with mAb BM-1; (*IVb*) TALL-1 cells infected with HIV treated with mAb BM-1.

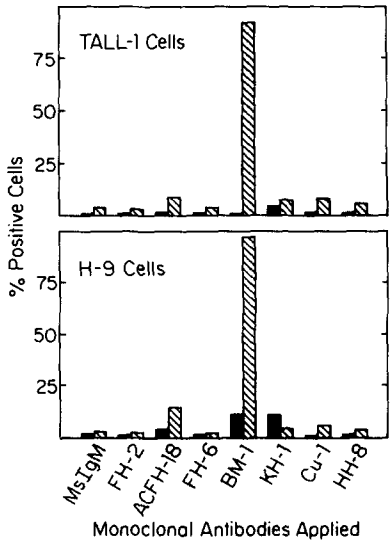

FIGURE 3. Comparative reactivities of human T leukemia cells with various anticarbohydrate antibodies. (*Solid bar*) before infection; (*hatched bar*) after infection with HIV. The top panel shows reactivity of TALL-1 cells; the bottom panel shows reactivity of H-9 cells. Antibodies tested for reactivity are identified at the abscissa. The structures defined by these antibodies are shown in Table I. The results from TALL-1 cell line are determined at the 20th day after infection.

Furthermore, monocytes were eliminated by KAC-2 treatment. These BM-1$^+$ cells were characterized as being CD3$^+$, as indicated by dual staining (see below), and therefore are considered to be T cells. In addition, those cells showing strongly positive reactivity with BM-1 as well as CD3 antibody were characterized

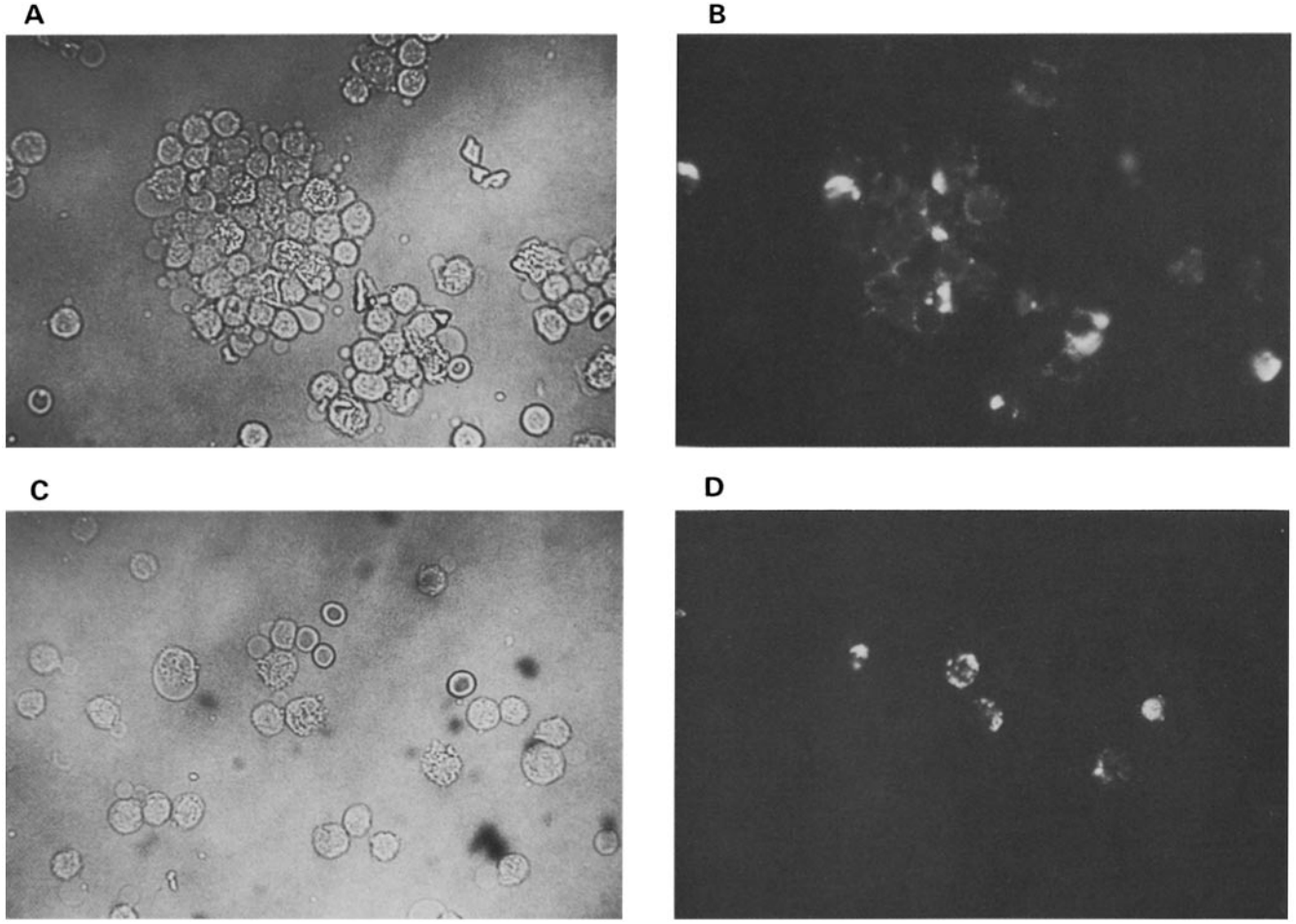

FIGURE 4. Immunofluorescence micrograph of lymphocytes separated from blood of patients with AIDS and ARC. (*A* and *C*) Phase-contrast microscopy; (*B* and *D*) indirect immunofluorescence microscopy with mAb BM-1. (*A* and *B*) From blood of a patient with AIDS, (*C* and *D*) from blood of a patient with ARC.

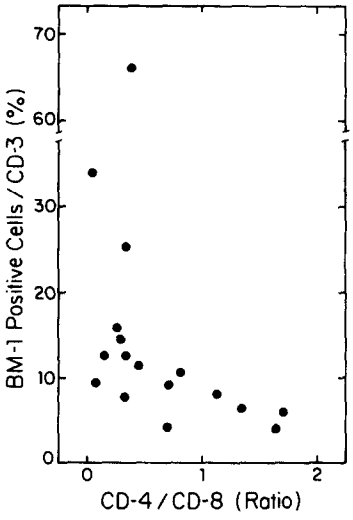

FIGURE 5. Incidence of BM-1$^+$ cells in CD3$^+$ population in AIDS patients and its correlation with the size of the CD4$^+$ population (CD4$^+$/CD8$^+$ ratio). Incidence of BM-1$^+$ cells in CD3$^+$ population and the ratio of CD4$^+$/CD8$^+$ populations were analyzed by two-color cytofluorometry, as described in the text. Note that BM-1 expression tends to increase when the CD4$^+$ population decreases on progression of AIDS. Normal subjects have a CD4$^+$/CD8$^+$ ratio of ~1.8, and BM-1 expression is <1%.

by granular structures in the cytoplasm, and therefore, are considered to be granular lymphocytes, particularly at blast stage. None of the carbohydrate antigens defined by these seven mAbs were found to be expressed in normal lymphocytes, except the antigen defined by FH-6, which reacts with an unknown population of human lymphocytes (data not shown). Two-color cytofluorometry of peripheral blood lymphocytes of HIV-infected patients with a combination of BM-1 and CD3 antibodies clearly indicates that BM-1$^+$ cells are a T lymphocyte population. Incidence of the BM-1$^+$ population in the CD3$^+$ population increased greatly when the CD4$^+$ population decreased greatly in AIDS patients, as characteristically indicated by the lower ratio of CD4$^+$/CD8$^+$ in advanced stages of the disease (Fig. 5). The clinical stage of symptoms in AIDS patients correlates well with the CD4$^+$/CD8$^+$ ratio. In normal subjects, in which the CD4$^+$/CD8$^+$ ratio is ~1.8, the incidence of BM-1 reactivity among CD3$^+$ lymphocytes (mature T cells) is very low (see legend for Fig. 5).

Discussion

The results of this study clearly indicate a dramatic change in expression of Le^y antigen in human T cell lines infected with HIV, as well as in peripheral T lymphocytes of HIV-infected patients. Six other carbohydrate antigens defined by respective mAbs did not show significant differences between infected and noninfected cells. Two-color cytofluorometric analysis of the lymphocyte fraction of 17 HIV-infected patients indicates that Le^y expressors are within the CD3$^+$ population and that there is a large degree of variation in Le^y expression in CD3$^+$ population among HIV-infected patients. The morphology of the BM-1$^+$ cells was distinct from monocytes but characteristic of lymphocytes. Some populations showing strong expression of Le^y and CD3 have been characterized by granular structures in the cytoplasm and are considered to be granular lymphocytes. The incidence of Le^y expression in the T cell population in AIDS and ARC patients was much higher than the incidence of expression of the viral antigen. This situation is different from HIV-infected T cell lines in vitro, in

which Le^y expression paralleled viral antigen expression. The reason for this discrepancy is unknown at this time. It is possible that Le^y is expressed at the very early stage of viral infection and that the expression can be induced by other in vivo factors, such as cellular interactions. Nevertheless, the incidence of Le^y expression increased progressively in patients with advanced stages of AIDS, in which the $CD4^+$ population greatly decreased. A progressive increase in the incidence of Le^y expression in the $CD3^+$ population associated with clinical stages of AIDS was of particular importance, e.g., 8.6% in Center for Disease Control (CDC) classification II, 10.1% in CDC classification III, and 19.3% in CDC classification IV. Human T cells infected with HIV were characterized by syncytium formation and the presence of HIV gag protein antigen (p24).

The change of glycosylation pattern in Herpes virus–infected cells has been described (15, 16) although the extent of changes is less pronounced than in glycosylation associated with oncogenic transformation (17). A striking appearance of Le^y antigen in HIV-infected cells and in lymphocytes of patients with AIDS and ARC is therefore a peculiar phenomenon indicating the possibility that HIV infection alters the membrane phenotype of T cells, remarkably similar to the alteration observed in cells oncogenically transformed by tumor viruses. Since no changes of Le^x determinant or sialyl Le^x determinant defined by FH-2 or FH-6 were observed in HIV-infected cells, the appearance of Le^y determinant defined by BM-1 may not be associated with induced fucosylation of preexisting Le^x determinant. The entire process of glycosylation, including the extension of type 2 chain coupled with terminal and subterminal fucosylation, is induced in HIV-infected T cells as well as T lymphocytes of AIDS and ARC. Interestingly, cell growth of HIV-infected cells was strongly inhibited or cytolysis was induced in the presence of BM-1 antibody and human serum complement, and noninfected cells were not affected by BM-1 antibody and complement (Adachi, M., N. Kashiwagi, and S. Hakamori, unpublished data). Le^y antigen defined by BM-1 antibody was not detectable in virions of HIV as determined by competitive RIA and Western blotting (data not shown). Therefore, the expression of Le^y antigen in HIV-infected cells is obviously a virus-dependent induction of glycosylation changes in host cells. In previous studies it was demonstrated that T cell leukemia cells showed significant changes in glycosylation pattern (18, 19) in particular, the appearance of GD_3 in adult T cell leukemia was noticeable (20), while T lymphocytes infected with HIV have now been characterized by a remarkable expression of Le^y, although the phenotype changes induced by HIV are strikingly different; i.e., clear reduction of cell growth, anisocytosis, and syncytium formation. It is of great interest to determine whether Le^y determinant, highly expressed in HIV-infected cells, may have some role in the altered phenotype of the $CD3^+$ cell subpopulation.

Summary

Le^y determinant (Fucα1→2Galβ1→4[Fucα1→3]GlcNAcβ1→R) defined by mAb BM-1 is highly expressed in human immunodeficiency virus (HIV)-infected T cell lines and in $CD3^+$ peripheral mature T cells of patients with acquired immune deficiency syndrome (AIDS) or with AIDS-related complex (ARC). Le^y expression increased greatly in the $CD3^+$ population in the advanced stage of

AIDS when the CD4$^+$ population decreased greatly. Six other carbohydrate antigens tested by their respective mAbs were not detected in these same cells. None of the carbohydrate antigens tested by the seven mAbs used in this study were found in noninfected T cell lines and in normal peripheral blood lymphocytes.

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