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Abstract

To search for lymphocyte marker antigens on the surface of human T-cell leukemia virus (HTLV), an immunoelectron microscopic study was performed on a HTLV-producing human T-cell line, MT-2, using monoclonal antibodies, such as anti-Leu-1, -Leu-2b, -Leu-3a, -Leu-5, -Leu-10 and -HLA-DR and OKIa1. The reactivity of each antibody with MT-2 cells was tested by the immunoperoxidase method at the light microscopic level. OKIa1, anti-HLA-DR and -Leu-10 gave positive results. At the ultrastructural level, the surface of HTLV as well as the plasma membranes of MT-2 cells were labeled with ferritin by the monoclonal antibodies OKIa1, anti-HLA-DR and -Leu-10, but not by anti-Leu-1 and -Leu-3a. These findings suggest that HLA-D region -associated antigens are common antigenic determinants shared by the surface of HTLV and the plasma membranes of MT-2 cells. These antigens on the virus surface are probably picked up selectively from the plasma membranes and may play an important role in the interaction of HTLV and target T-cells.

KEYWORDS: adult T-cell leukemia, monoclonal antibodies, HLA-D antigens, immunoelectron microscopy, human T-cell leukemia virus

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DETECTION OF HLA-D-REGION-ASSOCIATED ANTIGENS ON THE SURFACE OF ADULT T-CELL LEUKEMIA VIRUS PARTICLES BY IMMUNOELECTRON MICROSCOPY

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Abstract. To search for lymphocyte marker antigens on the surface of human T-cell leukemia virus (HTLV), an immunoelectron microscopic study was performed on a HTLV-producing human T-cell line, MT-2, using monoclonal antibodies, such as anti-Leu-1, -Leu-2b, -Leu-3a, -Leu-5, -Leu-10 and -HLA-DR and OKIa1. The reactivity of each antibody with MT-2 cells was tested by the immunoperoxidase method at the light microscopic level. OKIa1, anti-HLA-DR and -Leu-10 gave positive results. At the ultrastructural level, the surface of HTLV as well as the plasma membranes of MT-2 cells were labeled with ferritin by the monoclonal antibodies OKIa1, anti-HLA-DR and -Leu-10, but not by anti-Leu-1 and -Leu-3a. These findings suggest that HLA-D region-associated antigens are common antigenic determinants shared by the surface of HTLV and the plasma membranes of MT-2 cells. These antigens on the virus surface are probably picked up selectively from the plasma membranes and may play an important role in the interaction of HTLV and target T-cells.

Key words : adult T-cell leukemia, monoclonal antibodies, HLA-D antigens, immunoelectron microscopy, human T-cell leukemia virus.

Adult T-cell leukemia (ATL) virus (ATLV)-producing ATL-related cell lines have been shown to express human T-cell antigens and Ia antigens (1-3). Human T-cell leukemia/lymphoma virus (HTLV)-producing leukemia/lymphoma cell lines (4) and HTLV-transformed human cord blood T-cells (5) express HLA-DR antigens. ATLIV isolated in Japan (1, 6) and HTLV isolated in the USA (7) are considered to be closely related or even the same (8). In the present report, the term HTLV is used.

The presence of ATL-related antigens on the envelope of HTLV has been demonstrated by electron microscopy (9-11), and homology between the envelope gene region of HTLV and the region of the HLA-B locus gene has also been established (12). However, the expression of cell marker antigens on viruses has not been reported.

In the present report, we describe the relationship between cell marker antigens of the plasma membrane and those expressed on the viral envelope in a HTLV-producing human T-cell line, MT-2 (1), in which HTLV have been well

characterized biochemically (13) and ultrastructurally (14).

MATERIALS AND METHODS

MT-2 established from human cord T-cells by co-cultivation with ATL cells (1) was used as a HTLV producer. MT-2 cells were cultured in RPMI 1640 supplemented with 20 % fetal calf serum. The monoclonal antibodies defining human pan-T cell antigen (anti-Leu-1), human cytotoxic/suppressor T cell antigen (anti-Leu-2b), human helper/inducer T cell antigen (anti-Leu-3a), human lymphocyte antigen associated with the receptor for sheep erythrocytes (anti-Leu-5), human D-region associated antigen equivalent to murine I-A subregion products and distinct from HLA-DR products (anti-Leu-10), and human HLA-DR, non-polymorphic antigen (anti-HLA-DR), all from Becton Dickinson Monoclonal Center Inc., USA, and OKIa1, equivalent to anti-HLA-DR, from Ortho Diagnostic Systems, USA, were used at 1 : 20 dilution. The IgG fraction of peroxidase- or ferritin- conjugated goat anti-mouse IgG serum (Cappel Lab. Inc., USA) was used as the second reagent.

For indirect immunoperoxidase reaction at the light microscopic level, the cultured cells were placed on glass slides after washing three times with PBS, and fixed in ethanol or acetone for 10-15 min at 4 °C. After blocking of intrinsic peroxidase with 0.3 % H₂O₂ containing methanol and pretreatment with normal goat serum, the fixed cells were incubated with each monoclonal antibody and then with peroxidase-conjugate at 1 : 20 dilution. Then, each slide was examined under a light microscope following DAB reaction and hematoxylin stain for the nucleus.

Immunoelectron microscopy employing the indirect immunoferritin method was performed as described previously (10). In the present experiment, following prefixation in phosphate-buffered 2 % paraformaldehyde solution containing 0.25 % glutaraldehyde for 10 min at 4 °C, cells were first incubated with each monoclonal antibody, anti-Leu-1, -Leu-3a, -Leu-10 and -HLA-DR, and OKIa1, and then with ferritin conjugate as the second reagent at 1 : 5 dilution for 90 min at room temperature. Control specimens were reacted only with the second reagent.

RESULTS

Immunoperoxidase reaction at the light microscopic level revealed that OKIa1 gave the strongest reaction in all MT-2 cells (Fig. 1a) which showed a positive reaction with both anti-HLA-DR and -Leu-10. However, other monoclonal antibodies tested were non-reactive (Fig. 1b). Immunoelectron microscopic studies disclosed that OKIa1, anti-HLA-DR and anti-Leu-10 gave positive ferritin labeling of both virus particles and plasma membranes (Figs. 2-5). Heavy or patchy ferritin labeling of single (Figs. 4a, b and 5) or clustered (Figs. 2, 3) virus particles was observed. Labeled immature particles were also found (Fig. 3 arrow head). Virus particles were often labeled in the same manner as the plasma membranes (Figs. 4a and 5). Distribution of ferritin particles on the virus particles and plasma membranes usually appeared to be patchy as shown in Fig. 4a, and only some times diffuse as in Fig. 5. The degree of ferritin labeling was observed in the decreasing order of OKIa1, anti-Leu-10 and anti-HLA-DR. No qualitative difference in the labeling was noticed among these three antigens. On the other hand, monoclonal antibodies, such as anti-Leu-1 and -Leu-3a which were non-reactive by light

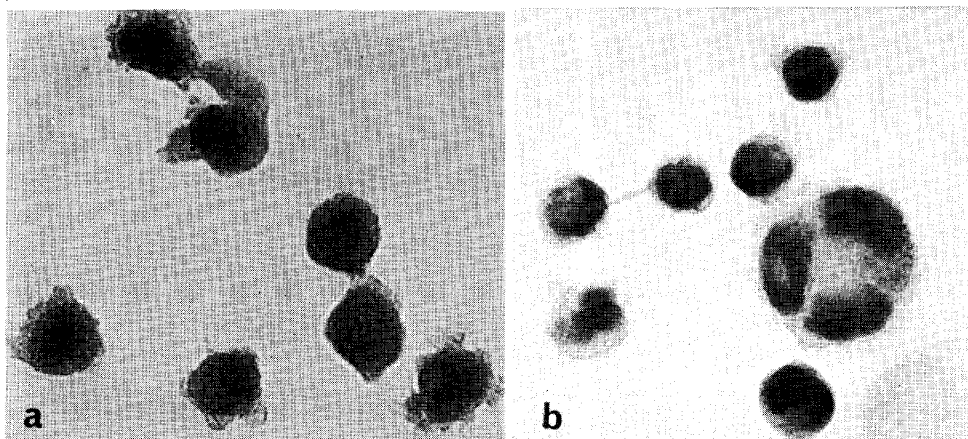


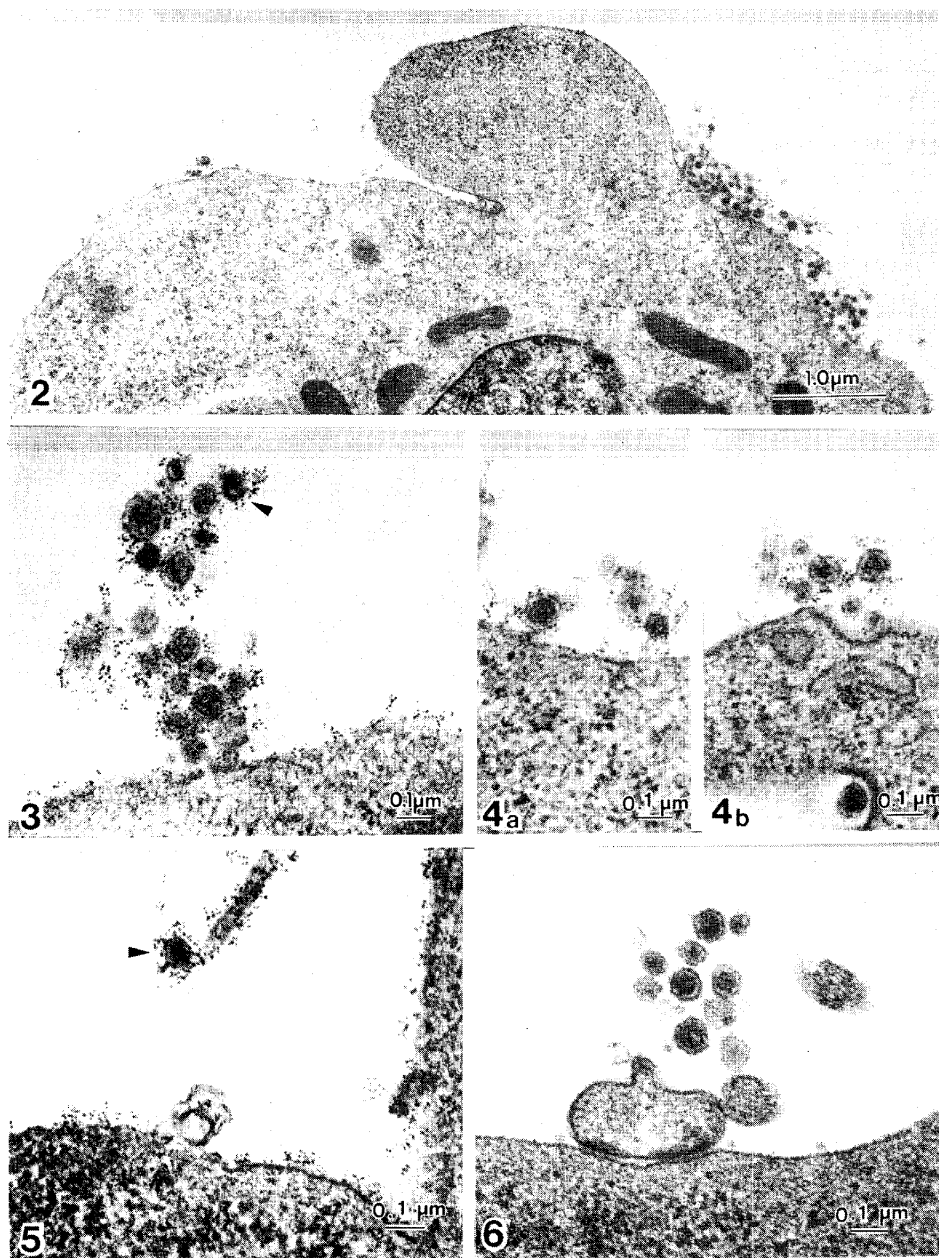
Fig. 1. Strongly positive cytoplasmic reaction of MT-2 cells with OKIa1 by indirect immunoperoxidase method (a), but not with anti-Leu-1 (b) a, b: $\times 660$.

microscopy, did not specifically label virus particles or plasma membranes of MT-2 cells (Fig. 6). MT-2 cells treated with only the ferritin conjugate were not labeled at all.

DISCUSSION

HLA-DR antigens are generally thought to be expressed on B-cells and monocytes/macrophages. Normal resting T-cells express little or no HLA-DR, but they synthesize HLA-DR antigens after activation (15, 16) or in continuous tissue culture (17). Cultured fresh ATL cells (3) and ATL/HTLV-transformed T-cells (1, 2, 4, 5, 18, 19) also express HLA-DR determinants. Anti-Leu-1, which defines the human T-cell antigen, initially reacted with MT-2 cells (1), but its reactivity seems to have been lost during continuous cultivation as shown by immunoelectron microscopy.

As for the origin of these antigens expressed on HTLV, most probably they are selectively picked up from the plasma membranes during the detachment of the virus particles. Interestingly, it has been reported that the region of the HLA-B locus gene of class I HLA is homologous to that of the HTLV envelope gene, but not to the class II HLA gene region in which the HLA-D region is included (12). Therefore, the presence of HLA-D region-associated antigens detected on the surface of HTLV appear to be expressed not as a genomic manifestation but as incorporated antigenic determinants associated with the plasma membrane. This view is supported by our observation that both virus particles and plasma membranes were labeled in the same patchy or diffuse pattern. In addition, the T-cell growth factor receptor recognized by the tac-1 monoclonal antibody considered to have been derived from the plasma membrane during virus was found to be associated with the HTLV envelope (20), and it is budding (21) as in the present case.



Concerning the HLA alloantigen expression after HTLV infection, it has been reported that HTLV-producing cutaneous T-cell lymphoma line and HTLV-infected cord blood lymphocytes expressed additional HLA-A and -B locus antigens, not observed in B cells from the same host and non-infected cord blood lymphocytes, respectively (4, 22). Further studies are needed to determine the pres-

ence of HLA alloantigen expression on HTLV. HLA-D region-associated antigens detected on HTLV may play an important role in viral infection of the target T-cells, especially during T-cell transformation by co-cultivation.

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Fig. 2. Ferritin labeling of isolated or clustered virus particles and plasma membrane of MT-2 cells with OKIa1. $\times 15,000$.

Fig. 3. Heavy ferritin labeling of mature and immature (arrow head) virus particles as well as patchy labeling of plasma membrane with OKIa1. $\times 46,000$.

Fig. 4. Virus particles individually labeled with anti-HLA-DR. Note the similarity in the Patchy labeling of virus particles and plasma membrane with ferritin. a, b: $\times 46,000$.

Fig. 5. A virus particle (arrow head) and plasma membrane heavily labeled with anti-Leu-10. $\times 51,000$.

Fig. 6. Virus particles and plasma membrane not labeled with anti-Leu-1. $\times 46,000$.

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