JOURNAL OF VIROLOGY, Sept. 1989, p. 3748–3754
0022-538X/89/093748-07$02.00/0

Vol. 63, No. 9

Depletion of the Surface CD4 Molecule by the Envelope Protein of Human Immunodeficiency Virus Expressed in a Human CD4$^+$ Monocytoid Cell Line

IKUO KAWAMURA,[1] YASUHIRO KOGA,[2]* NOBUHIRA OH-HORI,[1] KAZUKIYO ONODERA,[3] GENKI KIMURA,[2] AND KIKUO NOMOTO[1]

Department of Immunology[1] and Department of Virology,[2] Medical Institute of Bioregulation, Kyushu University 69, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, and Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Yayoi, Tokyo 113,[3] Japan

Received 7 February 1989/Accepted 30 May 1989

A CD4$^+$ human monocytoid cell line, U937, was transfected with a constructed plasmid which has the envelope gene of human immunodeficiency virus under the transcriptional control of the human metallothionein IIA promoter and was cloned thereafter. These cloned cell lines (EH and EL cells) expressed the viral gp160 in the cytoplasm. The expression of surface CD4 antigen examined by Leu3a and OKT4 monoclonal antibodies, however, disappeared completely in EH cells, which produce a larger amount of gp160, while diminishing only partly in EL cells, which produce a smaller amount of gp160. These results indicate that the level of expression of surface CD4 antigen correlates inversely with the amount of intracellular gp160. Moreover, immunoprecipitation studies using lysate from EH cells showed that OKT4 monoclonal antibody precipitated a significant number of CD4 molecules even after surface CD4 disappeared. However, Leu3a monoclonal antibody, which recognizes the binding site for envelope protein, could not precipitate any CD4 molecules in the same cell lysate. Taken together, these results suggested that CD4 molecules are still synthesized normally after the augmented production of gp160 in the cells but form a complex with the envelope protein in the cytoplasm and become unable to be transported to the cell surface, resulting in the observed depletion of surface CD4 antigen. This mechanism may explain the decrease or absence of surface CD4 antigens in human lymphocytes infected with human immunodeficiency virus.

Infection of CD4$^+$ T cells by human immunodeficiency virus (HIV) causes an extensive depletion of CD4$^+$ helper-inducer cells, resulting in devastation of the host defense mechanism involved in cellular and humoral immune responses (4, 6, 8). A physical interaction between the HIV envelope glycoprotein and CD4, i.e., a complexing of these two molecules, was demonstrated by immunoprecipitation (14, 19). Moreover, a gene transfer study confirmed that CD4-negative cells become susceptible to infection by HIV after the transfer of the CD4 gene and the expression of human CD4 molecules on their surface (15). These studies indicated that the CD4 molecule is a high-affinity receptor for HIV.

It has been demonstrated that CD4 functions as an adhesion molecule that binds to the nonpolymorphic region of major histocompatibility complex class II antigens and stabilizes the major histocompatibility complex class II–T-cell receptor complex (3, 7). Thus, the disturbance of CD4 expression by HIV infection will lead to a significant functional defect in these T cells. Actually, in HIV infection, functional abnormalities of CD4$^+$ cells are also reported, in addition to the quantitative depletion of CD4$^+$ T cells (13, 27). Hoxie et al. (9) have already reported that there are at least three factors concerning the decreased expression of CD4 antigen on the surface of HIV-infected cells: reduced levels of CD4-specific mRNA, reduced amounts of immunoprecipitable CD4 antigen, and the complexing of surface CD4 antigen with viral envelope protein (gp120). However, it still remains obscure as to what viral component or which stage of viral infection of cells is responsible for each of the pathophysiological events involving CD4 in the cells infected with HIV.

In this study, we have constructed a plasmid containing the *env* gene from the human T-cell leukemia virus III B strain of HIV under the transcriptional control of the human metallothionein IIA (hMTIIA) promoter, whose activity can be augmented 10 times or more by heavy metal ions (25). By transferring this plasmid into a CD4$^+$ human monocytoid cell line, we examined the effects of an envelope protein produced in cells on the expression of the CD4 molecule and found the depletion of surface CD4 antigen by the increased production of envelope protein, gp160.

MATERIALS AND METHODS

Antibodies. Anti-CD4 monoclonal antibodies Leu3a (Becton Dickinson and Co., Mountain View, Calif.), NU-$T_{H/I}$ (Nichirei Corp., Tokyo, Japan), OKT4 (Ortho Diagnostics, Inc., Raritan, N.J.), and C4.3138 and anti-CD45 monoclonal antibody NU-Lpan (Nichirei) were used. Leu3a and NU-$T_{H/I}$ recognize HIV-binding sites on the CD4 molecule. OKT4 and C4.3138 recognize CD4 epitopes other than the HIV-binding site. The C4.3138 monoclonal antibody was supplied by H. Wigzell, Karolinska Institute, Stockholm, Sweden. The specificity of C4.3138 was examined by assaying inhibition of HIV-induced syncytium formation by monoclonal antibodies to CD4, by the method of Dalgleish et al. (2). The inhibitory activity of C4.3138 was as ineffective as that of OKT4 (unpublished data). Anti-gp120 monoclonal antibody 0.5β was supplied by S. Matsushita et al. (18). Antisera containing high titers of antienvelope antibody was obtained from patients with acquired immunodeficiency syndrome (AIDS) (a gift from H. Wigzell, Karolinska Institute).

* Corresponding author.

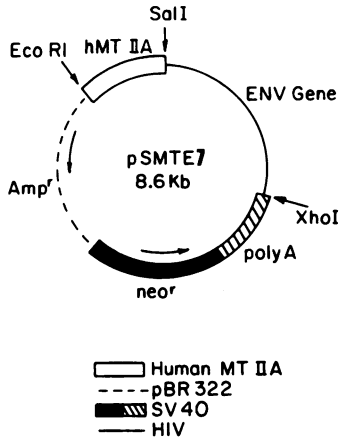

FIG. 1. Structure of the plasmid for transfer of the *env* gene. Plasmid pSMTE7 was constructed with insertions of the promoter portion of the hMTIIA gene (nucleotides −770 to +70) (10) and HIV sequence downstream from the promoter into pSV2neo, which has the gene encoding resistance to G418. The HIV sequence includes the entire envelope gene (nucleotides 5496 to 8474) (21).

Cells. A human $CD4^+$ monocytoid cell line, U937 subclone 2, was derived from a histiocytic lymphoma (1) and maintained in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (GIBCO), 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Dojin, Tokyo, Japan), 5×10^{-5} M 2-mercaptoethanol (Wako Pure Chemical Industries Ltd., Japan), and 10 μg of Gentacin (Schering Corp., Kenilworth, N.J.) per ml.

Establishment of cell lines which express HIV *env*. Plasmid pSMTE7 (Fig. 1) was constructed by inserting the promoter portion of the hMTIIA gene (nucleotides −770 to +70) (10) and the sequence of the whole HIV *env* gene (BH10 clone, nucleotides 5496 to 8474) (21) into plasmid pSV2neo, which possesses a gene encoding resistance to the antibiotic G418. The plasmid was transferred by the electroporation method (20) into U937 clone 2 cells, which express CD4 antigen on their surfaces. Cells were selected for resistance to the antibiotic G418 in the absence of heavy metal ions. About 30 of the isolated clones were then tested for their level of expression of *env* message by Northern (RNA) blot analysis after 10 μM cadmium chloride was added as an inducer. Hybridization was done by using the 3.0-kilobase *SalI-XhoI* *env* fragments from the plasmid vector pSMTE7 as a probe. Finally, around 20 cell clones which express *env* message were selected for the experiments in this study. The control cell clone, transfected with the plasmid that has no *env* gene insert, was also constructed and designated EC.

Western immunoblot analysis for expression of envelope protein. Cells (2×10^7) were washed three times with phosphate-buffered saline. One milliliter of lysing buffer (1% Nonidet P-40 [Sigma Chemical Co., St. Louis, Mo.] and 2 mM phenylmethylsulfonyl fluoride [Sigma] in phosphate-buffered saline) was added to the cell pellet and vigorously stirred with a Vortex mixer for 1 min. After being left at room temperature for 15 min, the preparations were centrifuged ($1,500 \times g$ for 5 min), and the supernatants were collected. Volumes of the supernatants containing about 10 μg of proteins were then diluted twice with a sample buffer (0.5 M Tris hydrochloride [pH 6.8] containing 2% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, 0.001% bromphenol blue, and 10% glycerol) and heated at 95°C for 5 min. The equilibrated samples were run on a 7.5% SDS-polyacrylamide gel and electroblotted to a nitrocellulose membrane at 30 V overnight. The filter was incubated with sera from AIDS patients or antibody diluted with 10 mM phosphate buffer (pH 7.2) containing 1% Tween 20 (Wako)–3% bovine serum albumin (Sigma) at 4°C overnight. Any antibody bound to the filter was detected by using ^{125}I-labeled protein G (Pharmacia LKB, Uppsala, Sweden), and an autoradiograph was taken with X-ray film, using intensifying screens.

Immunoprecipitation against CD4. Lysates were derived from 2×10^7 cells induced for the expression of *env* gene with 10 μM $CdCl_2$ and radioiodinated with Iodogen (Pierce Chemical Co., Rockford, Ill.) as previously described (12). Briefly, lysates were mixed with 500 μCi of $Na^{125}I$ (Amersham International Inc., Buckinghamshire, England) in tubes which were precoated with 50 μg of Iodogen (Pierce), and the mixture was incubated at room temperature for 10 min by shaking gently. The reaction was stopped by adding 0.1 ml of 0.1 M potassium iodide solution. Then the ^{125}I-labeled lysates were absorbed with anti-CD4 antibodies at 4°C overnight and then immunoprecipitated with 50 μl of protein A-Sepharose CL-4B (Pharmacia) for 1 h with rotation. Sepharose absorbents were washed five times with phosphate-buffered saline and heated at 95°C with 100 μl of sample buffer to elute absorbed materials. Electrophoresis was performed in a 7.5% SDS-polyacrylamide gel, and autoradiographs were taken with X-ray film.

Quantitative analysis of CD4 and envelope protein on the cell surface and in the cytoplasm. The levels of expression of CD4 and envelope protein on the cell surface were evaluated by flow cytometry, using a fluorescence-activated cell sorter (FACS 440; Becton Dickinson). Both the location and distribution of envelope protein in the cytoplasm were determined by indirect immunofluorescence analysis. Cells incubated with or without $CdCl_2$ were fixed with ethanol and added with 0.5β anti-gp120 monoclonal antibody. After being washed twice with phosphate-buffered saline, the cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin G (IgG; Tago) and mounted for viewing with a fluorescence microscope.

Northern blot analysis. RNA was extracted from cells in the presence of guanidine hydrochloride, and 10 μg of total RNA was glyoxylated, electrophoresed though a 0.8% agarose gel in 10 mM sodium phosphate buffer (pH 7.0), and transferred to GeneScreen Plus (New England Nuclear Corp., Boston, Mass.) according to the directions of the manufacturer. Filters were hybridized overnight at 65°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–5× Denhardt solution (1× = 0.02% polyvinylpyrrolidone–0.02% bovine serum albumin–0.02% Ficoll)–10% dextran sulfate–1% SDS–100 μg of salmon sperm DNA per ml, washed in 2× SSC–0.1% SDS at 65°C for 60 min with two changes of washing buffer, and then exposed on X-ray film. Molecular hybridization was done by using ^{32}P-nick-translated probes.

RESULTS

Expression of HIV envelope protein in *env* gene-transfected cells. We transferred the plasmid containing the HIV *env* gene downstream from the promoter of the hMTIIA gene to a $CD4^+$ human monocytoid cell line, U937 clone 2, and selected the clones which expressed *env* gene by Northern blot analysis (data not shown). Twenty cell clones were obtained by the screening and tested for their level of expression of *env* protein by Western blot analysis, using

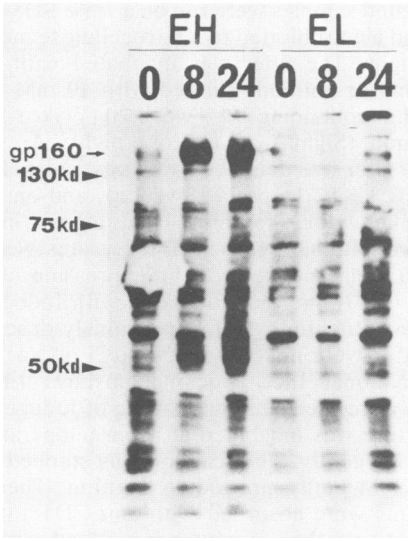

FIG. 2. Western blot analysis for expression of envelope glycoprotein in *env* gene-transfected cells. EH and EL cells were incubated with 10 μM Cd for 0, 8, and 24 h. Cell lysates were prepared from the treated cells and loaded on an SDS-polyacrylamide gel. The gel was electroblotted to a nitrocellulose membrane, and the filter was incubated with sera from AIDS patients. Envelope glycoprotein was determined by binding of ^{125}I-labeled protein G. Size markers (in kilodaltons) are shown at the left.

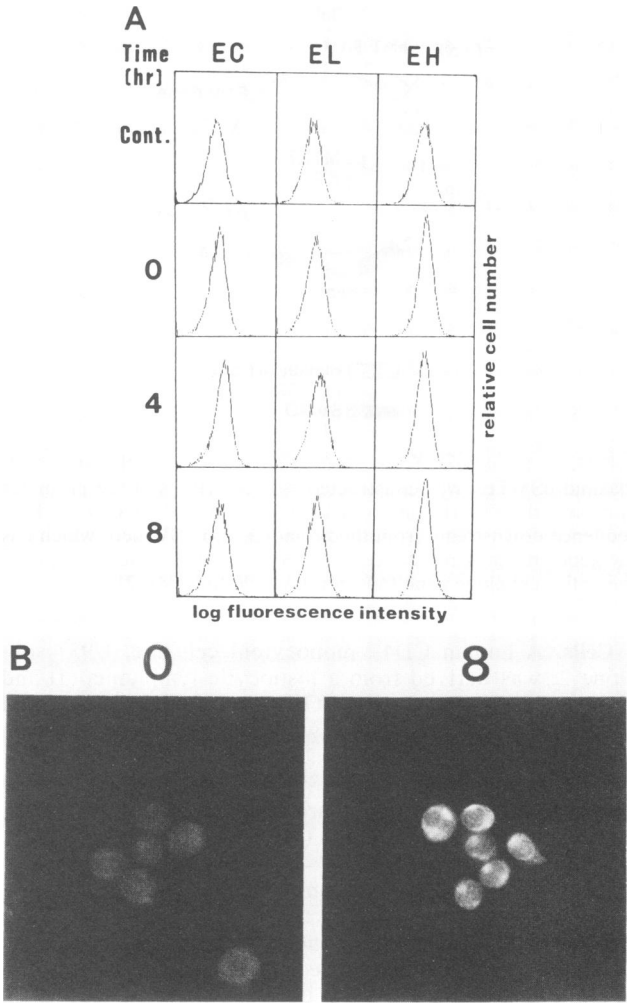

FIG. 3. Localization of envelope glycoprotein (gp160) in *env* gene-transfected cells. (A) Levels of expression of cell surface envelope glycoprotein after the induction of *env* gene expression by Cd. EC, EL, and EH cells incubated with Cd for O, 4, and 8 h were treated with AIDS patient sera preabsorbed by EC cells and then with FITC-conjugated anti-human IgG. Fluorescence intensities were analyzed by FACS. The control group (Cont.; treated with FITC-conjugated anti-human IgG only) represents control fluorescence intensity. (B) Immunofluorescence microscopy of cytoplasmic gp160. EH cells were fixed and incubated with 0.5β monoclonal antibody 0 or 8 h after induction by Cd. The fluorescence patterns were observed with a fluorescence microscope.

sera from AIDS patients or 0.5β monoclonal antibody, and two clones were selected for further analysis. Figure 2 shows the results of immunoblotting analysis for the two clones. One was designated EH cells, which expressed a higher level of envelope protein, and the increased expression was observed 8 h after the induction by cadmium (Cd). The other was designated EL cells, which expressed a lower level of the protein, and its expression level after the induction was almost the same as that of EH cells 0 h after stimulation. Although gp160 was detectable, the protein band corresponding to gp120 could not be detected in either EH or EL cells by using sera from AIDS patients (Fig. 2) or 0.5β monoclonal antibody (data not shown).

Distribution of envelope protein in *env* gene-transfected cells. In order to determine the distribution in cell compartments of envelope protein in EH cells, an immunofluorescence assay was performed, using 0.5β antibody or AIDS patient sera preabsorbed with EC cells, which have no *env* gene. When membrane fluorescence was examined with viable cells by FACS, little or no fluorescence intensity was detected on either EH or EL cells both before and after the induction of gene expression by Cd (Fig. 3A). Cell samples of parallel cultures were fixed with ethanol to determine the intracellular localization of the envelope protein by fluorescence microscopy (Fig. 3B). EH cells showed practically no detectable fluorescence without induction (0 h), whereas almost all the cells showed intracellular fluorescence 8 h after induction. Taken together, these results indicated that the Cd-induced HIV envelope protein gp160 was located mostly in the cytoplasm but not on the outer-membrane portion of cells.

Expression levels of surface CD4 antigens on *env* gene-transfected cells after induction by Cd. We determined the levels of expression of surface CD4 antigen on parental U937 cells and EC, EL, and EH cells after induction with Cd, using flow cytometry (Fig. 4a, b, c, and d, respectively). Two types of anti-CD4 antibodies were used to evaluate the level of expression on the cell surface. One type consisted of Leu3a and NU-$T_{H/I}$, which both recognize the HIV-binding site on the CD4 molecule, in contrast to the other type, OKT4 and C4.3138, which bind to epitopes other than the HIV-binding site. CD4 antigens recognized in both types of antibodies did not change their levels of expression on U937 or EC cells 4 or 8 h after induction (Fig. 4a, A and B; Fig. 4b, A, B, and C). On the other hand, in EH cells (Fig. 4d), 30 to 40% of the cells were already negative for the expression of all the CD4 antigens detected by a series of antibodies, including Leu3a (A), NU-$T_{H/I}$ (B), and C4.3138 (C), at 0 h. Moreover, when EH cells were stimulated with Cd, the ratio of the cells positive for these antigens decreased significantly

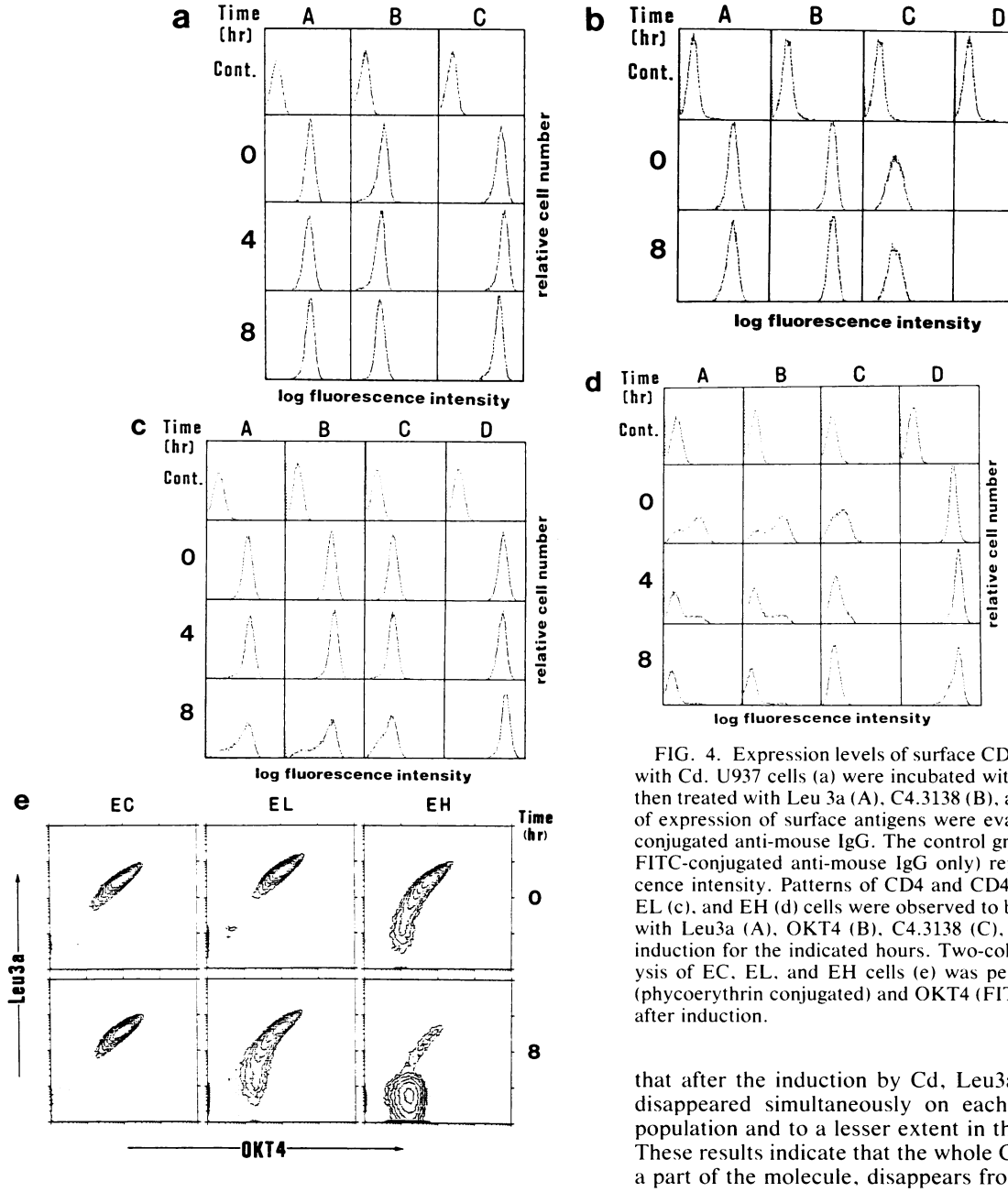

FIG. 4. Expression levels of surface CD4 on cells after induction with Cd. U937 cells (a) were incubated with Cd for 0,4 and 8 h and then treated with Leu 3a (A), C4.3138 (B), and Nu-Lpan (C). Levels of expression of surface antigens were evaluated by adding FITC-conjugated anti-mouse IgG. The control group (Cont.; treated with FITC-conjugated anti-mouse IgG only) represents control fluorescence intensity. Patterns of CD4 and CD45 expression on EC (b), EL (c), and EH (d) cells were observed to be similar after treatment with Leu3a (A), OKT4 (B), C4.3138 (C), and NU-Lpan (D) after induction for the indicated hours. Two-color flow cytometric analysis of EC, EL, and EH cells (e) was performed by using Leu3a (phycoerythrin conjugated) and OKT4 (FITC conjugated) 0 and 8 h after induction.

after 4 h and became undetectable 8 h after induction. The level of expression of CD45 antigen determined by NU-Lpan antibody did not change during induction of this cell line. These results show that the depletion of surface antigens on EH cells accompanied by the increased production of intracellular gp160 was restricted to the antigens of the CD4 series. In EL cells, which express a smaller amount of gp160 (Fig. 4c), all the cells were positive for CD4 antigens up to 4 h, and 30 to 40% of them became negative 8 h after induction. The amount of intracellular gp160 was almost the same for EL cells at 24 h and EH cells at 0 h (Fig. 2). These data support the idea that the degree of surface CD4 depletion correlates with the amount of intracellular gp160.

Then a two-dimensional flow cytometry analysis was done, using Leu3a (phycoerythrin-conjugated) and OKT4 (FITC-conjugated) antibodies (Fig. 4e). The results show that after the induction by Cd, Leu3a and OKT4 epitopes disappeared simultaneously on each cell in the EH cell population and to a lesser extent in the EL cell population. These results indicate that the whole CD4 molecule, not just a part of the molecule, disappears from the cell surface.

Interaction of CD4 and gp160 protein in the cytoplasm. To determine why CD4 molecules diminished from the cell surface after the induction of gp160 protein production, the possible relation of CD4 and gp160 protein in the cytoplasm was evaluated by immunoprecipitation analysis, using OKT4 and Leu3a. When OKT4 antibody was used (Fig. 5a), the number of CD4 molecules precipitated from these cell lysates did not decrease even 8 h after induction, either in EL or EH cells. When immunoprecipitation assay was performed with Leu3a (Fig. 5b), although a significant amount of CD4 was precipitated in EL cells after induction, few and no CD4 molecules were precipitated at 4 and 8 h, respectively, after induction in EH cells.

Northern blot analysis, performed to examine the level of expression of CD4 gene message, showed no significant reduction in the amount of CD4 transcripts in EH cells (Fig. 6) or EL cells (data not shown) after induction. These results

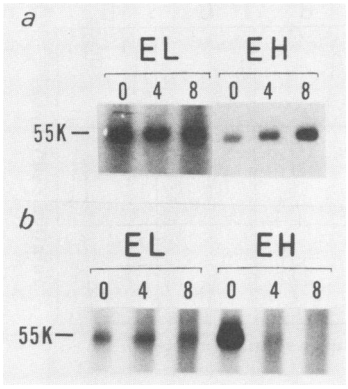

FIG. 5. Immunoprecipitation analysis of CD4 in EL and EH cells. EL and EH cells were incubated with Cd for 0, 4, and 8 h. Cell lysates were prepared and then radioiodinated with $Na^{125}I$. The lysates were incubated with OKT4 (a) and Leu3a (b) and immunoprecipitated with protein A-Sepharose. The absorbents were eluted and run on a 7.5% SDS-polyacrylamide gel. Molecular weight (in thousands) is indicated at the left.

supported the idea that the CD4 molecule is still produced at the same level even after the increased production of gp160; however, it is bound intracellularly to some molecule, possibly gp160, with its Leu3a epitope.

DISCUSSION

The precise mechanism which causes a dysfunction or depletion of $CD4^+$ T cells in HIV infection remains unclear. Recent studies have indicated that the interaction between CD4 and HIV envelope protein is a process important in abnormalities of functions in the cells infected with HIV as well as for viral entry (17, 26). However, a live HIV virion has various viral structures and regulatory genes which participate in the complicated life cycle of virus replication (5). Thus, in studies using live HIV, it is often difficult to elucidate the role of envelope protein itself on the pathophysiological events of the infected cells. In this study, we used the cloned $CD4^+$ U937 cell lines which express HIV envelope protein under the transcriptional control of the hMTIIA promoter in order to find out the effects of the envelope protein on the expression of surface CD4 antigens.

The cell lines EH and EL expressed the gp160 envelope protein but not gp120; the gp160 was located in the cytoplasmic portion but not on the surface membrane (Fig. 3). The HIV envelope protein is produced as a 160-kilodalton glycoprotein at first, followed by a posttranslational cleavage into the gp120 protein, which is located on the cell surface to bind

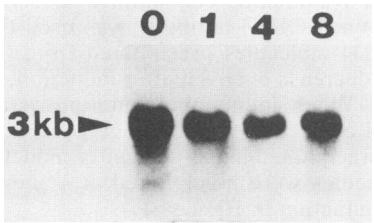

FIG 6. Northern blot analysis of CD4 transcripts in EH cells. Total RNA was extracted from EH cells treated with 10 μM Cd for 0, 1, 4, or 8 h. A probe for CD4 gene message was a 1.7-kilobase cDNA fragment from pT4B (16). kb, Kilobase.

CD4, and the gp41 protein, which is anchored in the cell membrane (22, 29). Such posttranslational proteolysis may have not occurred in EH or EL cell lines. Also, the absence of detectable production of gp120 in these cells might explain the loss of expression of the *env* protein on the cell surface (Fig. 3). Asjo et al. (1) previously reported that U937 subclone 2, the same cell line used for transfection with the *env* gene in our study, was able to be infected with human T-cell leukemia virus IIIB and expressed *env* protein on its cell surface, resulting in syncytium formation. Thus, neither the production of gp120 nor the expression of *env* protein on the cell surface in our system should be ascribed to the property of our *env* gene construct.

When levels of expression of surface CD4 antigens were analyzed by FACS, all the epitopes of the CD4 antigen examined by a series of monoclonal antibodies disappeared completely in EH cells and some of them disappeared in EL cells after the induction of expression of the *env* gene (Fig. 4). A larger amount of gp160 was produced in EH cells; on the other hand, a smaller amount of it was produced in EL cells after induction (Fig. 2). However, the number of CD4 molecules immunoprecipitated with OKT4 antibody was almost the same after induction both in EH and EL cells (Fig. 5a). These results show that the level of expression of surface CD4 antigen in our system will be determined by the amount of gp160 relative to that of CD4. Membrane fluorescence analysis could not detect the envelope protein on the surface membranes of these cells (Fig. 3A). Furthermore, not only Leu3a but also OKT4 epitopes disappeared simultaneously from the surfaces of individual cells (Fig. 4). Therefore, it seems unlikely that envelope protein, especially gp120, is bound to the Leu3a epitope and masks the CD4 antigen on the cell surface.

Our immunoprecipitation study using cell lysates showed that OKT4 monoclonal antibody could precipitate a significant number of CD4 molecules in EH cells even after induction, when surface CD4 had already disappeared. However, Leu3a antibody, which recognizes the envelope-binding site of CD4, could not precipitate anything in these samples (Fig. 5b). From these results taken together, it was thought that CD4 molecules were synthesized normally after induction but formed a complex at the Leu3a epitope with the gp160 protein in the cytoplasm and could not be transported to the cell surface, resulting in the decrease of surface CD4 antigens. Thus, an excessive amount of gp160 relative to that of CD4 will be required to deplete all the surface CD4 antigen, as shown in EH cells after induction. Northern blot analysis also showed no decrease in the level of expression of CD4 gene message after the induction, supporting the notion that the production of CD4 molecules is not arrested in these cells (Fig. 6). Thus, it was concluded in our system that the HIV envelope protein, at least gp160, does not affect the expression of the CD4 gene.

Stevenson et al. (28) noted that the reduction in the cell surface CD4 antigens observed early in the HIV infection period was not directly attributable to the reduction in CD4 mRNA. They also proposed the contribution of a posttranscriptional mechanism to the loss of surface CD4 antigens in their system. On the other hand, Salmon et al. (23) reported that the loss of CD4 mRNA transcripts also affected the disappearance of surface CD4 molecules in peripheral blood mononuclear cells infected with HIV. While the mechanism of disappearance of CD4 mRNA has yet to be elucidated, in our system using a human $CD4^+$ monocytoid cell line, it was shown that at least gp160 did not affect the level of CD4 transcript in the cells.

During the initial attachment of HIV to cells, the binding of Leu3a antibody to the cells is inhibited, whereas the binding of OKT4 is not (24). However, after the production of viral antigens by infected cells, the expression of all epitopes of CD4 is diminished or absent, showing the loss of CD4 antigens from the cell surface (2, 11, 24). This change in CD4 expression after the initiation of the synthesis of viral gene products could result from the modulation of the CD4 molecule at the cell surface, from the direct effects of viral products on the synthesis of CD4, or from an abnormality of transport of CD4 to the cell surface. This study supports the idea that the surface CD4 molecules are deleted by gp160 not because of the inhibition of production of CD4 protein but possibly because of the block of transport of CD4 by formation of a complex in the cytoplasm. No consistent data were obtained to support the argument that the masking of CD4 antigens on the surface is the cause of the disappearance of surface CD4.

ACKNOWLEDGMENTS

We thank H. Wigzell, Ewa Lindstrom, and Shunji Matsuda, Karolinska Institute, Stockholm, Sweden, and T. W. Mak, The Ontario Cancer Institute, Toronto, Canada, for encouragement and advice; Shuzo Matsushita, Kumamoto University, Kumamoto, Japan, for providing 0.5β antibody; and Nichirei Corp., Tokyo, Japan, for providing NU-$T_{H/I}$ and NU-Lpan antibodies. We also thank Brian Quinn, Kyushu University, for helpful comments on the manuscript.

This work was supported in part by grant 01653506 to Y. Koga from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Asjo, B., I. Ivhed, M. Gidlund, S. Fuerstenberg, E. M. Fenyo, K. Nilsson, and H. Wigzell.** 1987. Susceptibility to infection by the human immunodeficiency virus (HIV) correlates with T4 expression in a parental monocytoid cell line and its subclones. Virology **157**:359–365.
2. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
3. **Doyle, C., and J. L. Strominger.** 1987. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature (London) **330**:256–259.
4. **Fahey, J. L., H. Prince, M. Weaver, J. Groopman, B. Visscher, K. Schnartz, and R. Petels.** 1984. Quantitative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immune deficiency syndrome from other immune subsets disorders. Am. J. Med. **76**:95–100.
5. **Fauci, A. S.** 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science **239**:617–622.
6. **Friedman-Kein, A. E., L. J. Laubenstein, P. Rubinstein, E. Buimovici-Klein, M. Harmor, R. Stahl, I. Spigland, K. S. Kim, and S. Zolla-Pazner.** 1982. Disseminated Kaposi's sarcoma in homosexual men. Ann. Intern. Med. **96**:693–700.
7. **Gay, D., P. Maddon, R. Sekaly, M. A. Talle, M. Godfrey, E. Long, G. Goldstein, L. Chess, R. Axel, J. Kappler, and P. Marrack.** 1987. Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA-DR antigen. Nature (London) **328**:626–629.
8. **Gottleib, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon.** 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men. Evidence of a new acquired cellular immunodeficiency. N. Engl. J. Med. **305**:1425–1431.
9. **Hoxie, J. H., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Cedarbaum, and J. C. Reed.** 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. Science **234**:1123–1127.
10. **Karin, M., and R. I. Richards.** 1982. Human metallothionein genes—primary structure of the metallothionein-II gene and a related processed gene. Nature (London) **299**:797–802.
11. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J.-C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.
12. **Kubo, R. T., and M. L. Pelanne.** 1982. Tunicamycin inhibits the expression of membrane IgM in the human lymphoblastoid cell line Daudi. Mol. Immunol. **20**:67–76.
13. **Lane, H. C., J. M. Depper, W. C. Greene, G. Whalen, T. A. Waldmann, and A. S. Fauci.** 1985. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. N. Engl. J. Med. **313**:79–84.
14. **Lasky, L. A., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
15. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348
16. **Maddon, P. J., D. R. Littman, M. Godfrey, D. E. Maddon, L. Chess, and R. Axel.** 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. Cell **42**:93–104.
17. **Mann, D. L., F. Lasane, M. Popovic, L. O. Arthur, W. G. Robey, W. A. Blattner, and M. J. Newman.** 1987. HTLV-III large envelope protein (gp120) suppresses PHA-induced lymphocyte blastogenesis. J. Immunol. **138**:2640–2644.
18. **Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takatsuki, and S. Putney.** 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J. Virol. **62**:2107–2114.
19. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson.** 1986. Binding of HTLV-III/LAV to $T4^+$ T cells by a complex of the 110K viral protein and the T4 molecule. Science **231**:382–385.
20. **Potter, H., L. Weir, and P. Leder.** 1984. Enhancer-dependent expression of human immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. Proc. Natl. Acad. Sci. USA **81**:7161–7165.
21. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
22. **Robey, W. G., B. Safai, S. Oroszlan, L. O. Arthur, M. A. Gonda, R. C. Gallo, and P. J. Fischinger.** 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. Science **228**:593–595.
23. **Salmon, P., R. Oliver, Y. Rivier, E. Brisson, J.-C. Gluckman, M.-P. Kieny, L. Montagnier, and D. Klatzmann.** 1988. Loss of CD4 membrane expression and CD4 mRNA during acute human immunodeficiency virus replication. J. Exp. Med. **168**: 1953–1969.
24. **Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverley.** 1986. Epitopes of the CD4 antigen and HIV infection. Science **234**:1120–1123.
25. **Schmidt, C. J., and D. H. Hamer.** 1986. Cell specificity and an effect of *ras* on human metallothionein gene expression. Proc. Natl. Acad. Sci. USA **83**:3346–3350.
26. **Shalaby, M. R., J. F. Krowka, T. J. Gregory, S. E. Hirabayashi, S. M. McCabe, D. S. Kaufman, D. P. Stites, and A. J. Ammann.** 1987. The effects of human immunodeficiency virus recombinant envelope glycoprotein on immune cell functions *in vitro*. Cell. Immunol. **110**:140–148.
27. **Shearer, G. M., D. C. Bernstein, K. S. K. Tung, C. S. Via, R. Redfield, S. Z. Salahuddin, and R. C. Gallo.** 1986. A model for

the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development of acquired immune deficiency syndrome (AIDS). J. Immunol. **137**:2514–2521.

28. **Stevenson, M., X. Zhang, and D. J. Volsky.** 1987. Downregulation of cell surface molecules during noncytopathic infection of T cells with human immunodeficiency virus. J. Virol. **61**: 3741–3748.
29. **Veronese, F. D., A. L. DeVico, T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1985. Characterization of gp41 as the transmembrane protein coded by the HTLV-III/LAV envelope gene. Science **229**:1402–1405.