MHC-Antigens: Constituents of the Envelopes of Human and Simian Immunodeficiency Viruses

Hans Gelderblom, Hilmar Reupke, Thorsten Winkel, Rudolf Kunze, and Georg Pauli

Robert Koch-Institut des Bundesgesundheitsamtes, Nordufer 20, D-1000 Berlin 65

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Immunoelectron microscopy was applied to study the antigenic make-up of human and simian immunodeficiency viruses (HIV, SIV) grown in cells expressing either MHC class I (Molt-3) or MHC class I and II (H9) antigens. A variety of antibodies directed against the surface glycoprotein gp120 of HIV and against MHC class I and II antigens were employed. Consistent with earlier observations on the loss of HIV envelope components, gp120 was only weakly demonstrable on the mature virion.

MHC class I determinants were present regularly in small amounts on HIV and SIV. Class II antigens, *e.g.* HLA-DR were found in high density on HIV and SIV grown in H9 cells, but were absent, as expected, on virus grown in Molt-3 cells. These cellular surface antigens are constituents of the virion.

The presence of MHC class II antigens in virus preparations used for diagnostic purposes might explain some of the false positive results in HIV serology. Possible biological implications of these virus associated cellular antigens for the pathogenicity of HIV are discussed.

Introduction

The acquired immunodeficiency syndrome (AIDS) is caused by the infection with a human retrovirus [1–3] recently named human immunodeficiency virus (HIV, [4]); HIV infects CD4+ antigen expressing cells (for review see [5] and [6]). This antigen is expressed on different cell types, *i.e.* macrophages, Langerhans cells and T-helper/inducer lymphocytes. The latter are directly involved in the regulation of the immune response. The progressive depletion and/or loss of function of these cells leads ultimately to severe defects of the immune system, *i.e.* to opportunistic infections, malignancies and to primary neurological syndromes. MHC class I antigens are expressed on virtually all cells of the body (exept neurons, mature trophoblasts and erythrocytes). The class II antigens are more restricted in their tissue distribution. They are detectable on antigen presenting cells (APC), *i.e.* on B cells, macrophages, dendritic cells and Langerhans cells as well as on T cells [7, 8]. Virus production is only found in activated T helper cells [9], which at the same time express MHC class II antigens. In resting CD4+ lymphocytes HIV is in latency. We demonstrate, that HIV and SIV incorporate in their envelopes MHC antigens. The role of these glycoproteins involved in the recognition of foreign antigens and in the immune regulation for the development of AIDS will be discussed.

Reprint requests to Dr. Hans Gelderblom.

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Materials and Methods

Viruses and cells

The two T-cell lines, H9 and Molt-3, and the HIV-1 strain HTLV-IIIB were kindly supplied by Dr. R. C. Gallo and Dr. M. Popovic, National Cancer Institute, Bethesda, MD, USA. HIV-2 strain SBL 6669 [10] and SIV strain STLV-IIImac [11] were kindly supplied by Dr. Eva Maria Fenyö, Stockholm, Sweden, and Dr. M. D. Daniel, New England Regional Primate Research Center, Southborough, MA, USA, respectively. Vesicular stomatitis virus (VSV, strain Indiana) was grown in H9 and Molt-3.

HIV can be propagated to high titers in both cell lines [12]. Freshly infected cultures were prepared for immunoelectron microscopy by mixing 1 part of cells chronically infected with HIV in H9 and Molt-3 or SIV (grown in H9) with 4 parts of uninfected cells. After propagation for three days in RPMI 1640 medium supplemented with 10% fetal calf serum, cells were washed with PBS and 2×10^3 cells per well seeded into Microtiter plates (Falcon Plastics, Oxnard, CA) (for details see [13] and [14]). The plates were pretreated with 1% Alcian blue 8 GS (Serva Feinbiochemica, Heidelberg) in 1% acetic acid in or-

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der to fix the cells by cationic interaction to the bottom of the wells.

Immunoelectron microscopy

Five different murine monoclonal antibodies (MAB) specific for MHC antigens were used: One antibody was directed against the class I antigen beta-2-microglobulin (a gift from Dr. W. Ax, Behring-Werke AG, Marburg) and four against MHC class II determinants: anti-HLA-DR clone L243 purchased from Becton Dickinson, Heidelberg; anti-alpha-chain and anti-beta-chain kindly provided by Dr. L. Akerblom and Dr. B. Morein, Biomedical Center, Uppsala, and anti-HLA-DR (clone Tü35), a generous gift from Dr. A. Ziegler, Tübingen. Two antibodies specific for HIV gp120 were applied: A goat hyperimmune serum obtained through courtesy of Dr. D. Bolognesi, Durham, NC, USA, and affinity purified anti-peptide antibodies kindly provided by Drs. B. Frenzel and W. Röhnspeck, Biochrom KG, Berlin. High titered human patient sera were obtained from the AIDS-Arbeitsgruppe of the Robert Koch-Institut.

Immunolabeling followed published techniques [13–15]. Briefly, the Microtest plates were washed with PBS free from floating cells and debris and treated for 15 min with a fixative consisting of 0.05% glutaraldehyde and 4% paraformaldehyde in PBS. This prefixation inactivates HIV, thus avoiding any biohazard, and circumvents problems due to the redistribution of antigens during immunolabeling. The MAB (10 µl/well, 0.5 to 1 mg/ml) were reacted for 30 min at 37 °C with the cells. After three washes bound MAB was detected by an anti-mouse-IgG ferritin conjugate (30 min, 37 °C; Cappel Laboratories, Cochranville, PA, USA). Sera of HIV infected individuals as well as the other polyclonal antibodies were used at a dilution of 1:10 and detected by the appropriate ferritin-conjugate. After thorough washing the cells were fixed with 2.5% glutaraldehyde for 15 min, postfixed for 1 h with 1% osmium tetroxide and treated before dehydration for 1 h with 1% uranyl acetate [16]. The cells together with the plates were Epon-embedded and processed in situ. Ultrathin sections were poststained with lead citrate and evaluated using a Zeiss EM 10 A operated at 60 kV.

Results and Discussion

In order to control the experimental conditions HLA phenotyping of uninfected H9 and Molt-3 cells was performed by flow cytometry (Epics C, Coulter) using standard procedures.

Consistent with observations by Weiss *et al.* [17], it was shown that MHC class I antigens were expressed on both cell lines, while class II HLA-DR was present only on H9 cells.

Infected H9 and Molt-3 cells were investigated with MAB or polyspecific antisera in the sensitive pre-embedding mode [13]. Antibodies directed against HIV-1 gp120 and human patient sera with high neutralizing titers [14, 18] react preferentially on early HIV-1 budding stages, appearing in clusters on metabolically impaired cells [18], whereas mature virus particles are hardly labeled (Fig. 1) independent from the cell used for virus propagation.

Different reaction patterns on H9 compared to Molt-3 cells were observed with MHC specific antibodies. The MAB directed against the beta-2-microglobulin reacted with epitopes on the cell surface, on budding and on mature HIV and SIV particles (Fig. 2 and 3) independent of the host cell line. All four class II specific antibodies, directed against alpha- and beta-chains, recognized corresponding antigens on H9 cells and showed a strong signal – higher than on the cell surface – on budding structures and on the surface of mature HIV. In contrast, no class II specific labeling of Molt-3 cells and HIV grown in these cells was detectable (Fig. 2). These findings indicate that the labeling of HIV grown in H9 cells is specific for HLA-DR and that no cross-reaction of these determinants with the gp120 of HIV is detectable. Thus, our results do not support the hypothesis that MHC class II antigens and gp120 share common epitopes, which might be involved in immunopathogenesis [19, 20].

The same antibodies were applied on HIV-2 and SIV infected cells. Results comparable to HIV-1 infected cells were obtained for HIV-2 (Fig. 3). Attempts to grow SIV in Molt-3 cells were unsuccessful and virus was propagated therefore only in H9 cells, which express both class I and II MHC antigens. Our finding of MHC class II determinants on the surface of SIV corroborates recent results by Kannagi *et al.* [21].

The incorporation of MHC antigens into enveloped viruses is not unique for retroviruses [22, 23]. Using cytotoxicity inhibition assays Hecht and Summers described the presence of such antigens in purified VSV [24]. In an attempt to study their findings on the ultrastructural level we infected H9 and

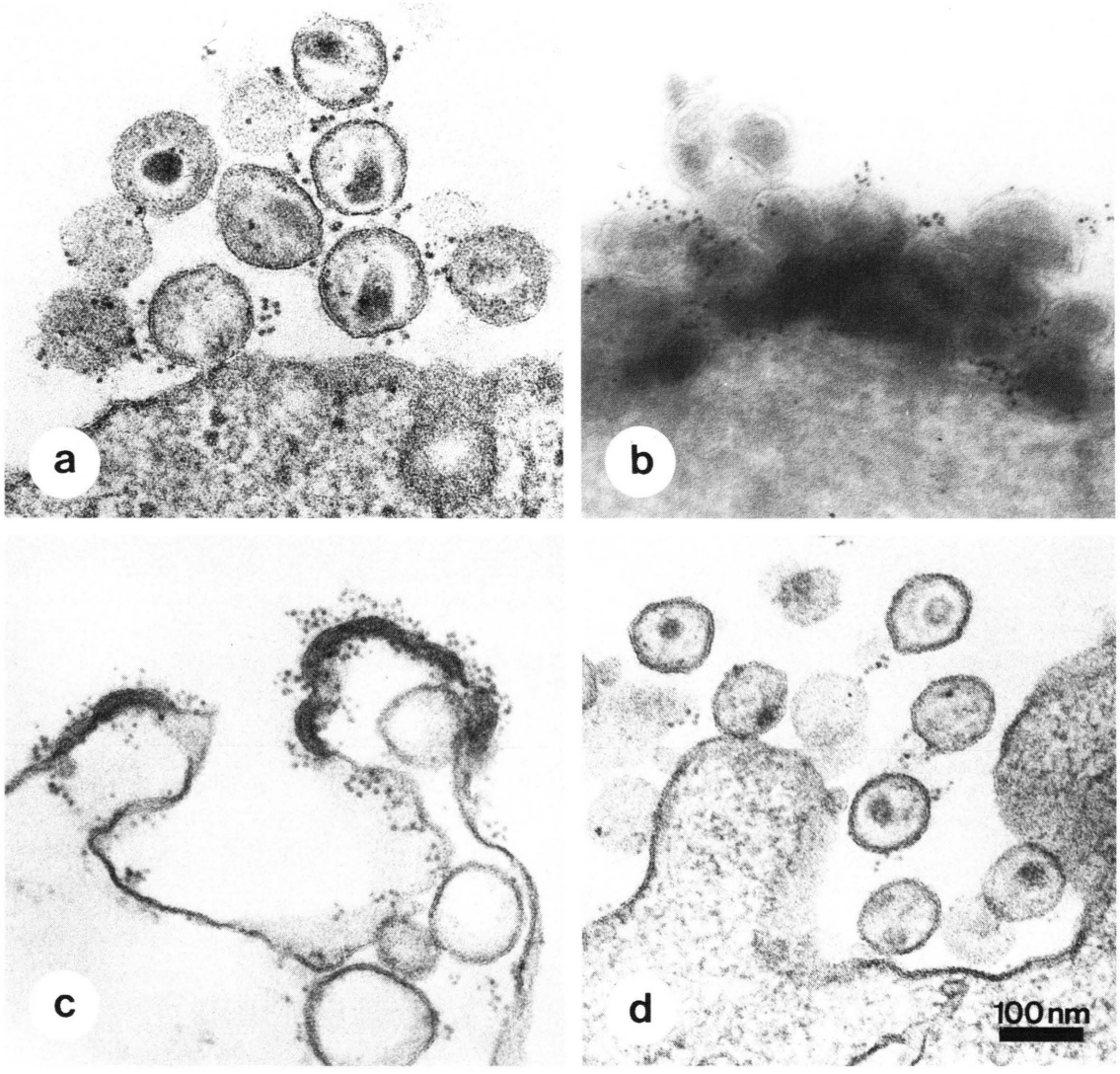

Fig. 1. Immunoelectron microscopy of HIV-1 strain HTLV–IIIB infected H9 cells. Monospecific antisera directed against gp120 were investigated in pre-embedding IEM (a, sheep-anti-peptide serum) and in immunocryoultramicrotomy (b, goat-anti-gp120 hyperimmune serum). In both techniques the surface of mature HIV is weakly labeled. In pre-embedding IEM early budding stages are heavily tagged (c, HIV positive human serum), whereas mature virus particles (d) in the same specimen show a low label intensity indicating a loss of antigen from the virion's surface.

Molt-3 cells with VSV and investigated the antigenic make-up of the virion in parallel to HIV and SIV. A high VSV production was observed in both cell lines. However, in contrast to HIV and SIV, the MHC specific labeling of the cell surface and the VSV envelope was relatively weak (Fig. 3f). This low amount of MHC determinants is probably due to the VSV induced shut-off of the cellular protein synthesis and the high amount of virions produced.

Enveloped viruses obviously tolerate some degree of error in respect to their envelope constituents. The incorporation of cellular antigens, however, seems not to be due to a random event. Bubbers and Lilly [23] have shown for murine oncoviruses that a

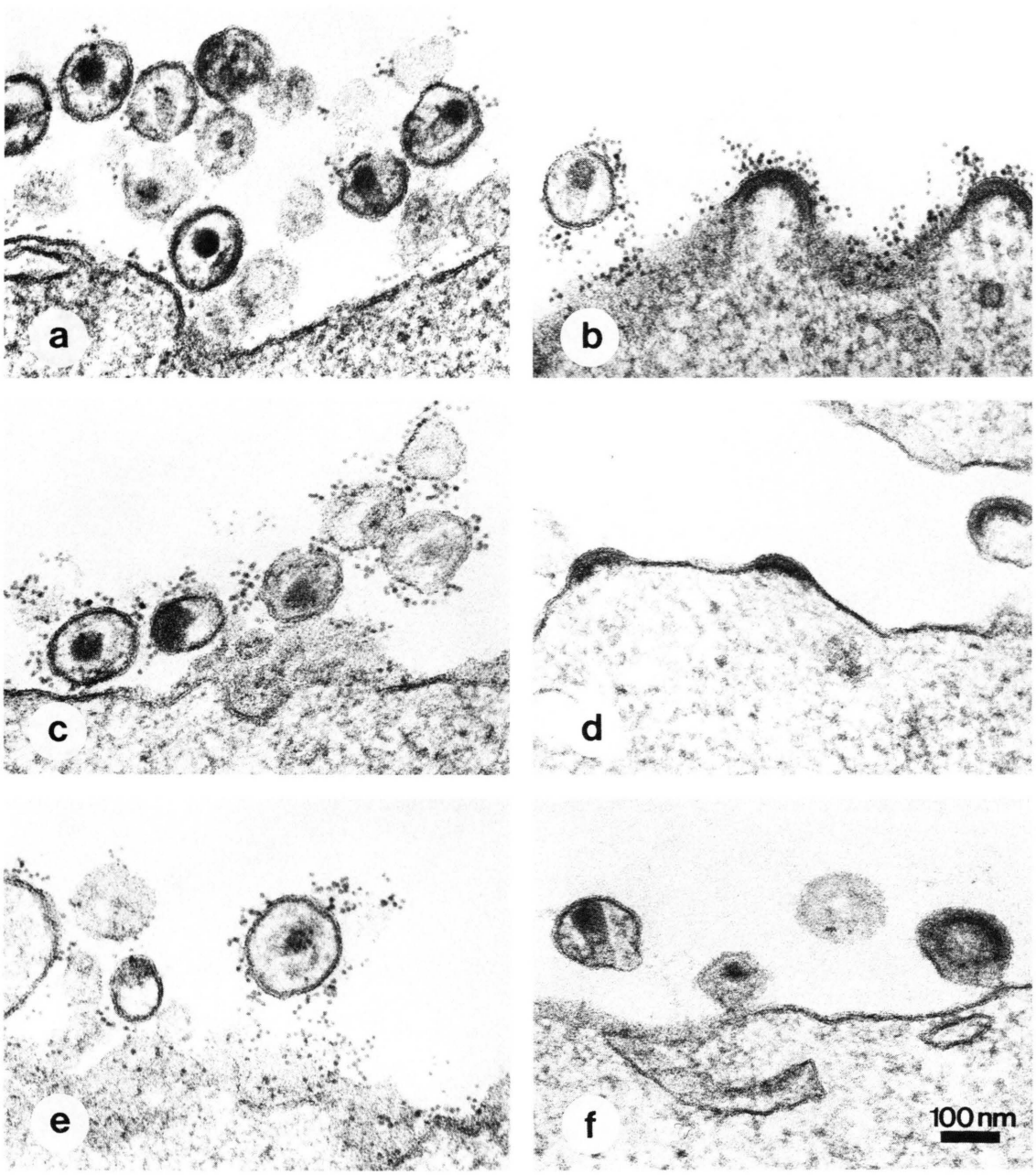

Fig. 2. Immunoelectron microscopy of HIV-1 strain HTLV–IIIB grown in H9 (a, c, e) and Molt-3 cells (b, d, f), respectively. Class I specific determinants are detected on HIV grown in both cell lines (a, b; revealed after incubation with anti-beta-2-microglobulin MAB). Incubation with class II specific MAB (c, d = anti-HLA-DR; e, f = anti-beta-chain) demonstrates that corresponding epitopes are present on HIV grown in H9 cells (c, e), but not on Molt-3 cells and the respective virus (d, f).

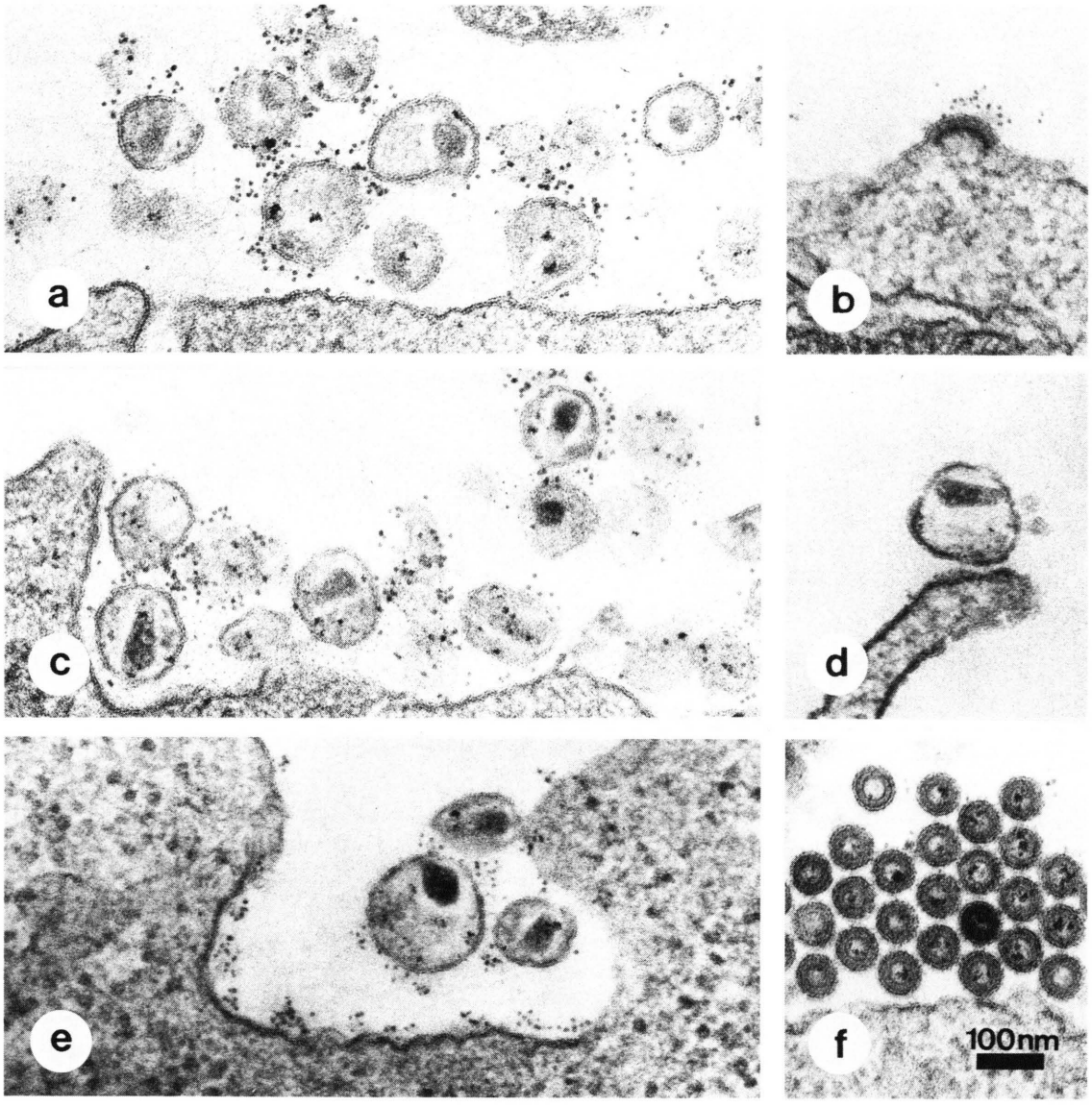

Fig. 3. Immunoelectron microscopy of HIV-2, strain SBL 6669 (a–d), SIVmac (e) and VSV (f). Class I specific determinants are detected on HIV-2 grown in H9 (a) and Molt-3 (b). Class II specific MAB react with HIV grown in H9 cells (shown as the reaction with anti-beta-chain MAB, c) and not with Molt-3 grown HIV-2 (shown as the reaction with HLA-DR, d). The presence of class II antigens on SIVmac grown in H9 cells is demonstrated in (e). VSV propagated in H9 cells is weakly labeled after incubation with class I specific (f), and class II specific MAB (not shown).

close physical association of Friend leukemia virus glycoproteins with MHC antigens exists on the surface of Friend leukemia virus and on infected cells. The presence of cellular proteins therefore seems to be a consequence of the association with virus glycoproteins at the site of virus budding. From our results we cannot conclude that MHC class I and II specific proteins are incorporated in a selective way as was observed by Bubbers and Lilly in the Friend leukemia virus system [23].

Specific incorporation of MHC class I and II antigens in HIV grown in H9 cells was reported by Hoxie and coworkers [40] using ELISA tests. In agreement with our results they could show that beta-2-microglobulin and MHC class II determinants were associated with virions. Class I antigens, however, are obviously incorporated in a selective way. Only one of the class I determinants present on the H9 cell was detected in virus preparations indicating that only a specific haplotype associates with viral envelope proteins.

However, an immediate practical implication is obvious. Cell grown HIV preparations, in contrast to genetically engineered probes, contain host cell specific proteins. When virus was propagated in MHC class II positive cells, these normal host components can lead to false positive ELISAs, especially when sera of multitransfused probands are tested [25, 26, 39]. Virus grown in cells devoid of MHC class II antigens (KE 37, Molt-3, CEM) represent a more specific probe and should therefore be used for diagnostic purposes.

The presence of host cell specific proteins on the surface of immunodeficiency viruses might have biological consequences. MHC antigens play a major role in cell-cell interaction and in the regulation of the immune response. It is conceivable that these antigens in combination with the viral envelope glycoprotein can serve as recognition signals. While the complex of viral antigen with class I MHC antigen might activate CD8+ (suppressor) T cells, the other possible configuration, envelope proteins with class II antigens, might stimulate CD4+ (helper/inducer) T cells [27]. This antigen specific regulation with opposite effects could explain some of the disturbances observed in AIDS patients.

It is well established that CD4 serves as the receptor for HIV and that the CD4 gp120 interaction is the first step in HIV replication [28–30]. On the other hand, the association of the MHC antigens with the virion and the spontaneous loss of the virus specific gp120 from the surface of the virion [15, 18] implies speculations on the infectivity of HIV. Virions, which have lost most of their gp120, might be hidden *in vivo* from the elimination by neutralizing antibodies ("wolf in a sheep's skin"). Whether such virions are infectious for certain cells and MHC antigens alone or in connection with the transmembrane glycoprotein gp41 might serve as receptors has to be elucidated. Such a function of MHC antigens appears not unreasonable. Recently it was reported that the MHC class I antigen beta-2-microglobulin associates with the human cytomegalovirus and helps the virus to escape neutralizing antibodies [31].

Finally, assuming that *in vivo* antigen presenting cells (APC) of the monocyte-macrophage lineage are the first to become infected, one can speculate about the role of the MHC antigens closely associated with virus specific envelope proteins. Virus production in these cells persist for considerably longer periods of time and the cytopathic effect is less pronounced than in HIV infected T cell cultures [32–34]. HIV infected APC may react efficiently with specific cells of the immune system and transfer the infection *via* direct contact to T helper/inducer cells [35].

The Langerhans cells of the human epidermis are of special interest among the APC, since in the natural situation, *e.g.* in sexual transmission, they might represent anatomically the first cell to become infected. Indeed, Langerhans cells can be productively infected *in vitro,* and in HIV seropositive persons such cells harbour the virus [36, 37]. Exhibiting 50-fold more MHC HLA-DR antigens than blood-derived monocytes and macrophages [38] Langerhans cells might transmit the virus very efficiently to T helper lymphocytes while exerting part of their natural immune function.

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