Immunobiol., vol. 184, pp. 402–409 (1992)

Rapid Communication

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Mechanism of MHC Class I Downregulation in HIV Infected Cells

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Received December 23, 1991 · Accepted December 30, 1991

Abstract

HIV infection of $CD4^+$ peripheral blood lymphocytes leads to a loss of MHC class I molecules on the surface of the infected cells as detectable by monoclonal antibody staining and flow cytometry. Incubation of the infected cells at 26 °C or treatment at 37 °C with peptides leads to upregulation of MHC class I to levels equal to those found on uninfected cells cultured under the same conditions. The data suggest that, after HIV infection, the mechanisms responsible for peptide generation, peptide transport and thus stable association between peptides and MHC class I molecules are severely affected.

Introduction

Following virus infection a variety of host defense mechanisms are being mobilized by the organism, including the activation of $CD8^+$ cytolytic T lymphocytes which eventually help to eliminate virus infected cells. These $CD8^+$ T cells recognize antigenic fragments in the context of major histocompatibility class I (MHC class I) molecules which bind and transport peptides derived from intracellularly degraded proteins to the cell surface, where the class I/peptide complexes are recognized by the T cells (1–6). A failure to express such complexes on the surface of infected cells would thus be expected to severely compromise the host and favor virus spread. To escape the hosts' defensive mechanisms, several viruses have developed strategies that lead to downregulation of MHC class I expression on the surface of the cells in which they replicate (reviewed in 7). Thus, adenovirus 12 inhibits class I production at the transcriptional level, while other viruses either bind β2 microglobulin or the heavy chain of class I and thus prevent their export, together with viral peptides, to the cell surface. We and others (8, 9) have previously shown that HIV infection of peripheral blood lymphocytes or susceptible cell lines also leads to a reduction of class I expression on the surface of those cells heavily infected. Since we found that the amounts of class I specific mRNA were similar in

infected and uninfected cells, it seemed likely that class I downregulation was due to posttranscriptional or posttranslational mechanisms. The present study was undertaken to more closely analyze the basis for the escape mechanism from the immune system operative in HIV infection.

Material and Methods

Cell culture and HIV infection

From buffy coat cells of HIV negative donors $CD8^+$ cells were removed by a magnet after complexing them to Dynabeads (Dianova, Hamburg, Germany) coupled with a monoclonal antibody to human CD8. The resulting $CD8^-$ cells were cultured in RPMI/10 % FCS at a cell concentration of 1×10^6/ml for 2 days with phytohemagglutinin (PHA-P; Difco, Detroit, MI, USA, 2 µg/ml) in the presence of human rIL-2 (40 U/ml, a kind gift of PROF. SEBALD, Würzburg). Half of the cells were then infected with an MOI of 0.1 of HIV-1 (HTLV III B), the other half remained uninfected as a control.

Upregulation of MHC class I at low temperature or by peptides

Aliquots of the infected or uninfected cells were placed in a 26 °C water bath for the last 6 h of a 4 day culture period, after which time all cells were processed for surface fluorescence staining. Alternatively, 5×10^5 cell aliquots of the infected cultures were exposed for the last 5 h of a 4 day culture period at 37 °C to HIV gag peptide p 17-8 or p17-3 (Cambridge Research Biochemicals, England) at a concentration of 1×10^{-4} M in a total volume of 1.4 ml.

Immunofluorescence staining and FACS analysis

On day 4 after infection, aliquots from controls and infected cultures – cultured at 37 °C/ 26 °C or treated with peptides as described above – were incubated with the following antibodies for 30 min on ice: mAb W6/32 (Serotec, Indianapolis, IN, USA), antihuman β2-microglobulin polyclonal antibody (DAKO, Hamburg, Germany), anti HLA A2 mAb and anti HLA Bw6 mAb (Biotest, Dreieich, Germany). The cells were washed twice in PBS and then incubated with 0.1 ml fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Dianova, Hamburg) or FITC-conjugated swine anti-rabbit-immunoglobulin (DAKOPATTS, Denmark) for 30 min on ice, washed twice in PBS, fixed in 3.5 % formaldehyde and analyzed on a FACScan (Becton Dickinson, Heidelberg, Germany).

Results and Discussion

During our investigations of the mechanisms responsible for the downregulation of MHC class I molecules in HIV-1 infected $CD4^+$T-cells, we were puzzled by the observation that the extent of downregulation varied between individual blood donors and, occasionally, was more pronounced for β2 microglobulin than for the heavy chain of class I (Fig. 1). In view of the comparable amounts of class I heavy chain specific mRNA in infected and uninfected cells (8) and the recent finding that β2-microglobulin loss from «empty» MHC class I molecules may precede loss of total class I complexes from the surface (10) we suspected that a posttranslational mechanism, possibly affecting the stability of class I/β2 complexes, was

operative. The phenomenon was thus reminiscent of the reduced MHC class I expression recently described for the mutant mouse cell line RMA-S (11, 12) which appears to have a defect in the transport of peptide to the MHC class I molecules resulting in drastically reduced class I surface expression. The empty class I molecules thus formed are unstable at 37 °C and those molecules still exported to the surface rapidly lose β2-microglobulin. At reduced temperature (26 °C) the complexes can be stabilized. They can also be stabilized by peptides binding to the class I molecule in question, or by β2 microglobulin at body temperature (5, 6, 10, 13–15).

To see whether an instability of MHC class I might also be responsible for the loss of surface MHC class I in HIV-infected cells, we infected PBL's of an HIV negative donor with HIV *in vitro*. At day 4 after infection we observed in a proportion of cells previously shown to be those that are heavily infected (8), a downregulation on the cell surface of, in this case,

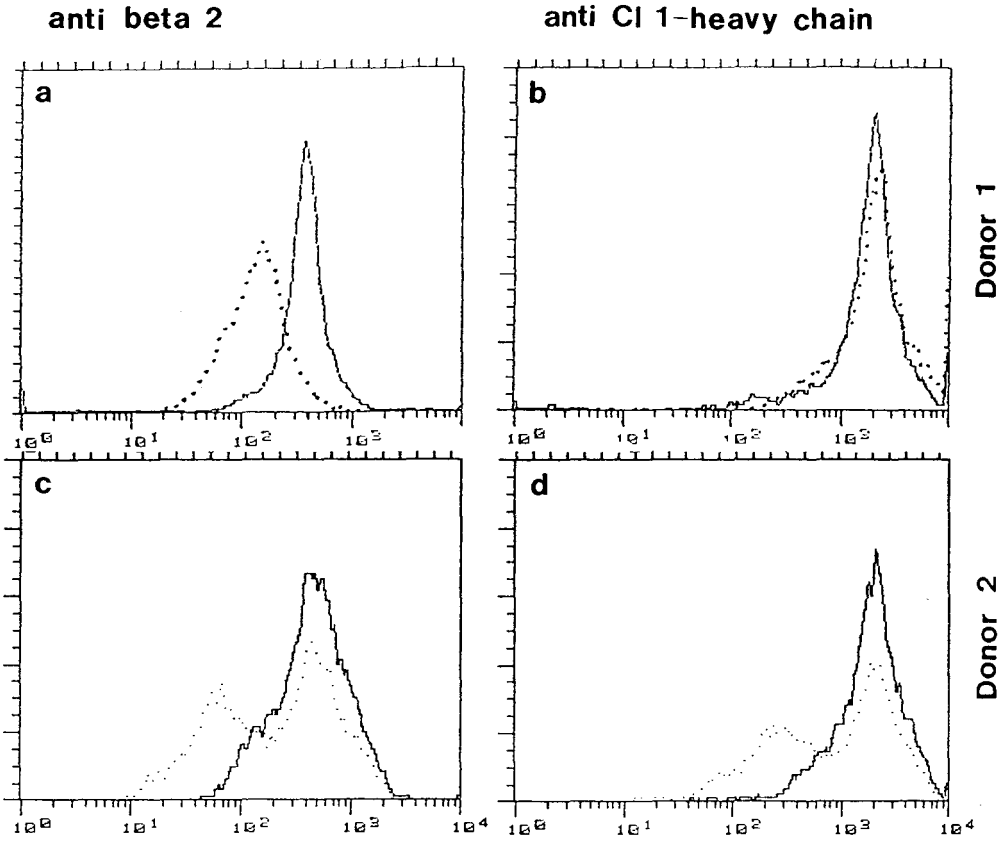

Figure 1. Indirect immunofluorescence staining of uninfected (——) and HIV-infected (·····) PBL's of two different blood donors with anti-human β2-microglobulin (a+c) or with monoclonal antibody W6/32 directed against class 1 heavy chain (b+d).

both the heavy and light chain of class I (Fig. 2). When we cultured the infected cells at 26 °C for 6 h and subsequently measured the expression of class I molecules on the surface by fluorescence analysis we observed a marked increase of class I molecules to values equal to those found in uninfected cells also cultured at 26 °C. The surface fluorescence of both populations surpassed that observed in uninfected cells cultured at 37 °C (Fig. 2), in keeping with previous reports showing that some empty MHC class I molecules are always present even in normal cells and can be stabilized at low temperatures (10). Since in spite of equal numbers of p24 positive cells (data not shown) increased surface class I on HIV infected cells could be due to inefficient virus replication at the reduced temperature, we attempted to upregulate surface MHC class I expression by the addition of peptides at 37 °C. In this experiment PBL's were infected that derived from an A2 donor which also reacted with the broad specificity anti HLA-B monoclonal antibody Bw6. Figure 3 shows reduced staining with both

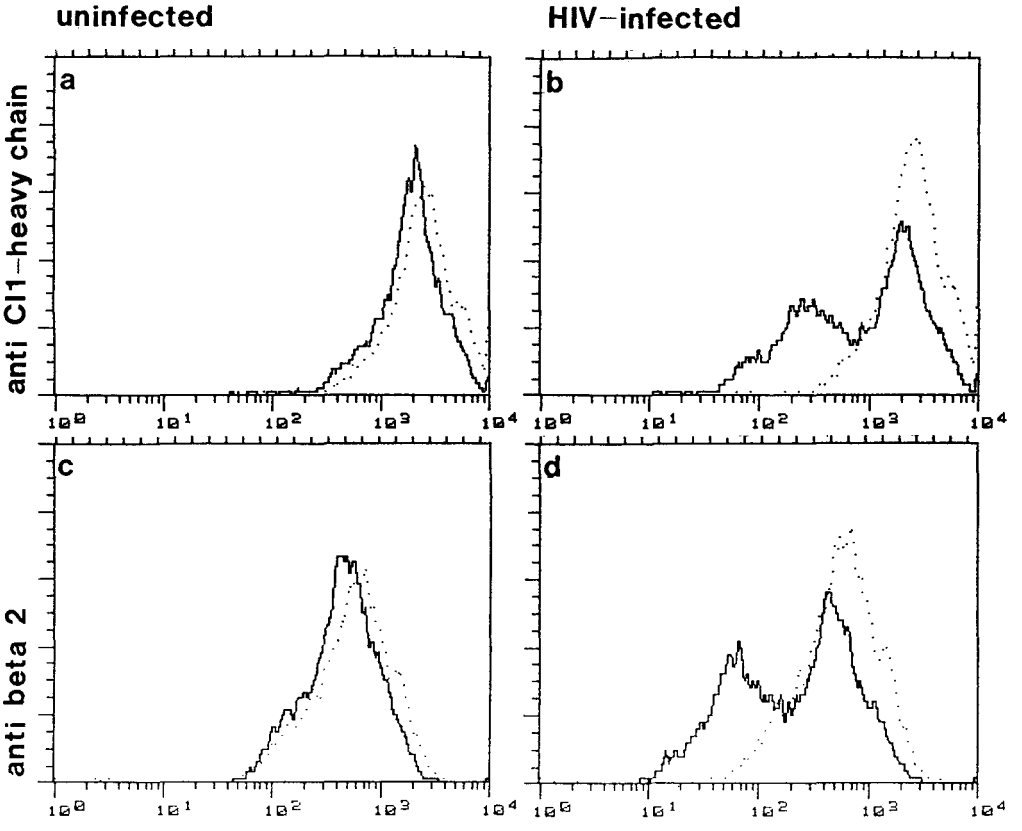

Figure 2. Expression of class 1-heavy chain (a+b) and β2-microglobulin (c+d) on uninfected and HIV-infected cells cultured at 37 °C (——) or at 26 °C (·····) for the last 6 h of a 4 day culture period.

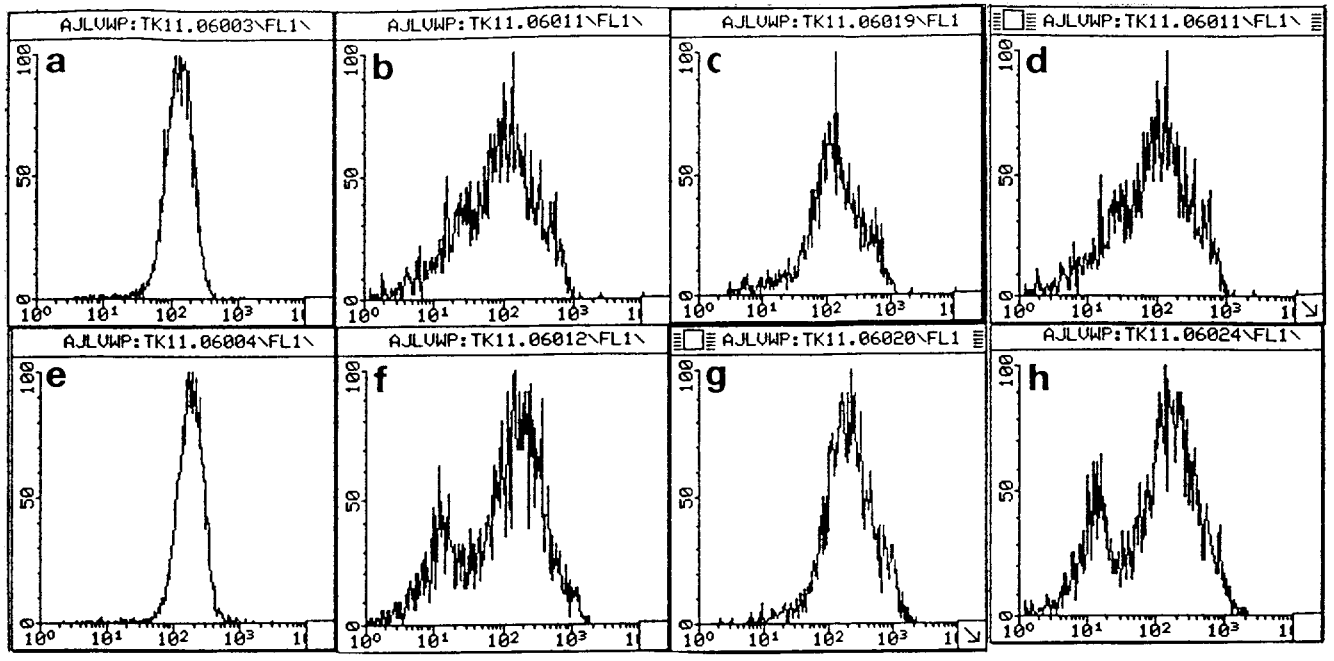

Figure 3. Expression of HLA A2 (a-d) or HLA Bw6 (e-h) on HIV-infected PBL's after exposure to various peptides for the last 5 h of a 4 day culture period. a+e, uninfected control culture; b+f, PBL's infected with HIV-1 for 4 days; c+g, PBL's infected with HIV-1 for 4 days and exposed to peptide p 17-8 for 5 h; d+h, PBL's infected with HIV-1 for 4 days and exposed to peptide p 17-3 for 5 h.

anti-A2 and anti-Bw6 mAb in a proportion of cells. Exposure of the infected cells to exogenous A2 binding peptide p17-8 (16) for 5 h clearly increased the expression of HLA A2 (Fig. 3c) whereas the B8 binding peptide p17-3 (16) failed to do so (Fig. 3d). The peptide p 17-8 was also shown to upregulate class I molecules reacting with the anti-Bw6 antibody (Fig. 3g) while p17-3 (Fig. 3h) did not. Unfortunately we do not have any information on the HLA-B binding properties of these two peptides. However, it has been shown that peptide binding may occasionally be promiscuous (16–18).

Our data indicate that HIV-infected cells behave very similarly to RMA-S cells with respect to reversion of class I surface expression, suggesting a defect in peptide generation/transport or loading of class I in the virus infected cells. Peptides derived from cytosolic proteins assemble with MHC class I molecules in the endoplasmatic reticulum or pre Golgi compartment (5, 19). In the absence of recognizable N-terminal signal sequences, such cytosolic peptides must be translocated across the membranes of the endoplasmatic reticulum by a novel mechanism. Recently, several investigators described the cloning-and-sequencing of «novel» genes encoded within the MHC which are candidates for peptide transporters (20–23) and may supply nascent class I MHC molecules with peptides, thereby fuelling the endogenous pathway of antigen presentation. Since downregulation of MHC class I molecules in HIV-1 infected cells can be overcome either by culture at reduced temperature or by the exposure to extracellular peptides we suggest the following hypothesis: In HIV-1 infected cells peptide generation and/or the peptide transporters may be «switched off» – possibly by HIV-1 derived gene products. As a consequence of this, MHC class I molecules would remain empty. Such empty class I molecules would either not associate with β2-microglobulin and not be transported further to the cell surface or, if so, be unstable at body temperature. Differences in the stabilities of different MHC Class I allelic products, as recently suggested for B27 (24) might account for the variations in class I downregulation observed between different blood donors. Also, loss of β2 has been shown to affect the conformation of the heavy chains still residing in the membrane (10). Conformational stability after β2 loss may vary from allele to allele and thus render detection by the monoclonal antibody used more or less efficient.

A number of observations previously reported for AIDS patients could be explained on the basis of our findings. Thus, the increase in circulating β2-microglobulin in sera of patients infected with HIV (25) might may be due to β2-microglobulin chains having dissociated from peptide-less, unstable MHC complexes. This would eventually lead to the progressive decrease of MHC class I-positive lymphocytes and a defective expression of class I molecules recently reported for AIDS patients (26). Since peptide loading would not be possible in infected cells, the data could also explain why the $CD8^+$ cytotoxic T cells detectable *in vitro* on target cells either incubated with peptides or infected with recombinant vaccinia virus

expressing individual HIV proteins (27–30) do not seem to be very efficient in containing or terminating the disease *in vivo*. The unusally high frequency of $CD4^+$, Class II restricted cytolytic T cells observed in HIV positive donors might reflect an attempt of the host to compensate for the loss of an efficient class I restricted cytolytic response.

Acknowledgments

We thank URSULA SAUER for technical assistance and EBERHARD WECKER and THOMAS HÜNIG for many stimulating discussions. This work was funded by the grant BGA II.031-87 of the Bundesgesundheitsministerium and the Fonds der Chemischen Industrie.

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