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Downregulation of HLA Class I Antigens in HIV-1–Infected Cells

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ABSTRACT

By means of indirect immunofluorescence analysis we investigated the effect of HIV-1 infection on HLA class I surface antigens. We report here that in CD4$^+$ HeLa cells, in H9 cells, and in peripheral T lymphocytes HLA class I antigens are downregulated following infection with HIV-1. The downregulation is effected at a posttranscriptional level since the amounts of HLA class I specific mRNA are similar in infected and uninfected cells. This phenomenon is not only correlated with the state of infection, that is, the presence of P24 of HIV-1 in the cells, but also depends on the time of infection. Upon HLA class I downregulation by HIV infection, the specific lysis of peripheral blood cells by allogeneic CTL is reduced.

INTRODUCTION

As with most virus infections, the infection with the human immunodeficiency virus (HIV) also results in the generation of immunologic effector cells. Thus HIV-specific antibodies are maintained for years after infection and CD8$^+$ major histocompatibility complex (MHC) class I as well as CD4$^+$ MHC class II restricted cytolytic T cells have been demonstrated. Yet, in contrast to most other virus infections, infection with HIV is not contained or terminated immunologically. The failure of virus neutralization by HIV-specific antibodies is at least partially because of the constantly occurring variations of the critical viral gp120 antigen and because even antibody-complexed HIV can still infect macrophages via Fc receptor uptake. It is, however, not clear why demonstrably present cytotoxic T lymphocytes (CTL) and in particular class I restricted CTL fail to eliminate HIV infections.

In this context it is interesting to note that in all reports on HIV-specific MHC class I restricted CTL from patients so far published, target cells other than HIV-infected CD4$^+$ T helper cells were used.[1,2] Since these CTL can usually recognize and lyse a target cell only if the processed antigen is presented in association with MHC class I structures,[3,4] we investigated the effects of HIV infections on the expression of HLA

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class I antigens by means of indirect immunofluorescence. In this study we report a HLA class I downregulation occurring a few days postinfection in all HIV-infected cells so far investigated. We also show that peripheral $CD4^+$ T cells displaying MHC class I downregulation as a consequence of HIV infection are worse targets for class I restricted allogeneic CTL than uninfected cells.

MATERIALS AND METHODS

HLA class I and HIV-1 p24-specific indirect double-immunofluorescence analysis of peripheral $CD4^+$ lymphocytes after various times in culture

From buffy coat cells of a HIV-negative donor, $CD8^+$ cells were removed by a magnet after complexing them to Dynabeads coupled with a monoclonal antibody (MAb) to human CD8. The remaining $CD8^-$ cells were stimulated for 2 days with phytohemagglutinin (PHA, 2 μg/ml) in the presence of rIL-2 (interleukin-2, 40 U/ml). Half the cells were infected with MOI 0.1 of HIV-1 (HTLV-IIIB), and the other remained uninfected as controls. All cells were further cultivated in the presence of rIL-2. At the times indicated, aliquots from controls and infected cultures were removed for staining. Sedimented cells were suspended in a 1:50 dilution of mouse MAb W6/32-HLK I ($IgG2\alpha$, Serotec) in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.02% sodium azide and incubated for 30 minutes on ice. This antibody recognizes HLA A, B, and C. After three cycles of washing by centrifugation in PBS, the cells were stained on ice for 30 minutes with FITC-conjugated $F(ab')_2$ GaMIgC (Dianova). After another three washing cycles cells were fixed with 3.5% formaldehyde for 30 minutes at room temperature, washed once with PBS, and treated for 20 minutes at room temperature with 0.25% Triton X-100 in PBS. Cells were washed twice with PBS, incubated for 30 minutes at room temperature in 10% heat-inactivated sheep serum, washed twice, and incubated with a 1:25 solution of a purified biotinylated polyclonal sheep p24 antibody (Biochrom) for 1 hour at room temperature. After another three washing cycles cells were incubated with a 1:25 dilution of streptavidin-phycoerythrin (Becton-Dickinson) for 1 hour at room temperature, washed three times more in PBS, and analyzed in a FAC-SCAN (Becton-Dickinson).

Single-immunofluorescence analysis of $CD4^+$ HeLa cells, H9 cells, and peripheral $CD4^+$ lymphocytes

For single-fluorescence analysis, $CD4^+$ HeLa cells,[5] H9 cells,[6] and peripheral $CD4^+$ lymphocytes infected with MOI 0.1 of HIV-1 (HTLV-IIIB) and uninfected controls were stained with mouse MAb W6/32-HLK I and FITC-conjugated $F(ab')_2$ GaMIgC as described until the formaldehyde fixation step.

Northern blot analysis of HLA class I-specific mRNA of H9 cells uninfected or infected with HIV-1 for various times

Cells were harvested at the times indicated and sedimented, and total RNA was extracted following the guanidinium isothiocyanate-cesium chloride method.[7] RNA samples were denatured, electrophoresed on 1.5% agarose gels in 2.2 M formaldehyde, and transferred onto Hybond N filter (Amersham). They were hybridized with a HLA-B–specific 1.5 kb PstI cDNA fragment in MF 28 (a kind gift of Dr. E. Weiss, Munich). For control purposes, the blots were rehybridized with an Eco fragment of a 45S rRNA in PUC 12 coding for 28S rRNA (a kind gift of Dr. I. Grummt, Würzburg).

Comparison of HIV-infected and noninfected $CD4^+$ PBL as allogeneic targets for class I restricted CTL

From buffy coat cells of a HIV-negative donor A, $CD8^+$ cells were removed by a magnet after complexing them to Dynabeads coupled with a MAb to human CD8. Cells (2×10^7) of the remaining $CD8^-$ cells were irradiated with a dose of 3000 rad and cocultivated as stimulator cells with 2×10^7 buffy coat

cells of a HIV-negative donor B. The remaining $CD8^-$ cells of donor A were stimulated for 2 days with PHA (2 μg/ml) in the presence of rIl-2 (40 U/ml). Half the cells were infected with MOI 0.1 of HIV-1 (HTLV-IIIB); the other remained uninfected as controls. All cells were further cultivated in the presence of rIL-2. The quality of these HIV-infected and noninfected $CD4^+$ PBL of donor A as allogeneic targets for class I restricted CTL of donor B was tested in a 4 hour ^{51}Cr release assay at the effector to target (E/T) ratios shown in the figures.

RESULTS AND DISCUSSION

To correlate the effect of HIV infection on HLA class I antigens we investigated $CD4^+$ HeLa cells, H9, and $CD4^+$ PBL by means of indirect immunofluorescence FACS analysis after staining the cells with labeled monoclonal antibodies against cellular class I antigens. Figure 1 shows the results obtained with $CD4^+$-positive HeLa cells. In this case, a gradual downregulation of HLA class I fluorescence intensities

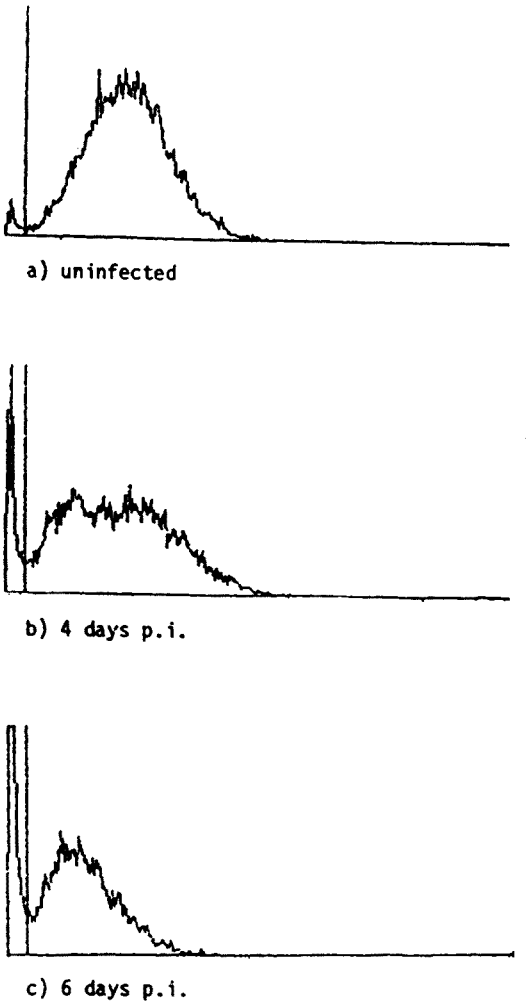

FIG. 1. HLA class I downregulation in HIV-infected $CD4^+$ HeLa cells. HLA class I-specific immunofluorescence (3 log scale) was analyzed by epics V of uninfected and HIV-infected $CD4^+$ HeLa cells at various days postinfection. The cells were stained as described in Materials and Methods.

was observed beginning on day 4 postinfection compared with noninfected cells. At 6 days postinfection the phenomenon became even more pronounced.

Figure 2 depicts results obtained with H9 cells. Here an HLA class I downregulation with infected cells first became apparent on day 7 postinfection, increasing further on days 11 and 14 postinfection.

As shown in Figure 3, HLA class I downregulation in HIV-infected peripheral $CD4^+$ T cells was rather rapid and very pronounced on day 5 postinfection.

To find out whether the observed phenomenon was directly due to HIV infection, peripheral $CD4^+$ T cells were investigated by double-fluorescence FACS analysis. After staining with an HLA A, B, and C

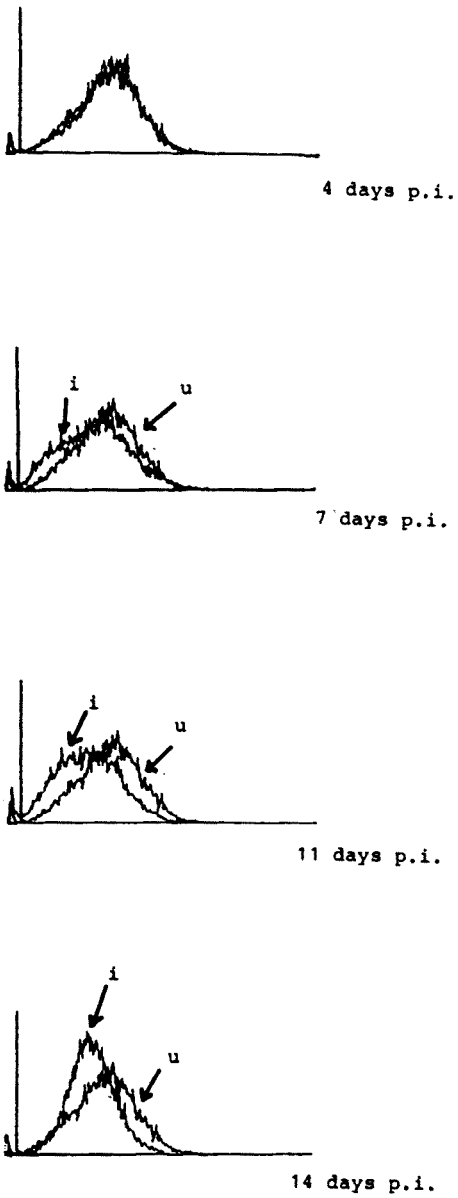

FIG. 2. HLA class I downregulation in HIV-infected H9 cells. HLA class I-specific immunofluorescence (3 log scale) of H9 cells on various days after infection with HIV-1 (HTLV-IIIB) at MOI 0.1. FACS analysis was performed as described in Materials and Methods. u = uninfected, i = HIV infected.

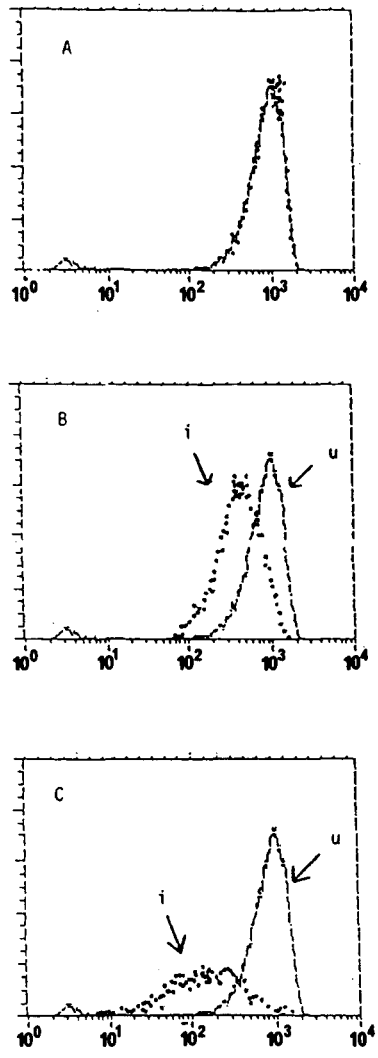

FIG. 3. HLA class I downregulation in peripheral CD4$^+$ T cells. The cells were isolated and cultured as described in Materials and Methods. Uninfected and HIV-infected (MOI 0.1) were stained only for HLA class I as described in Materials and Methods. (A) uninfected; (B) 4 days postinfection, (C) 5 days postinfection.

specific monoclonal antibody, cells were subsequently stained with a differently labeled monoclonal antibody specific for p24 of HIV. This antigen is only demonstrable in truly HIV-infected cells when viral gene expression occurs. The results are shown in Figure 4. Clearly, the decrease in HLA class I immunofluorescence (green) is observed only with the cells that also display immunofluorescence specific for the HIV p24 antigen (red). This indicates that actual infection is required for the downregulation to occur. At least partially due to the relatively low MOI, the demonstrable loss of MHC class I antigens was gradual: in peripheral CD4$^+$ cells it took 5 days following infection to fully develop. Interestingly, 7 days postinfection the cultures appeared to return to a rather noninfected state with respect to HLA class I expression. However, as shown by the simultaneous disappearance of p24-positive cells, this was not due to a reversible downregulation of class I antigens in fully infected cells. Such a mechanism was suggested by Scheppler et al.[8] Rather, the reappearance of a majority of cells normally expressing MHC class I antigens reflects a selection process in favor of noninfected or only recently infected cells that develops spontaneously in the culture.

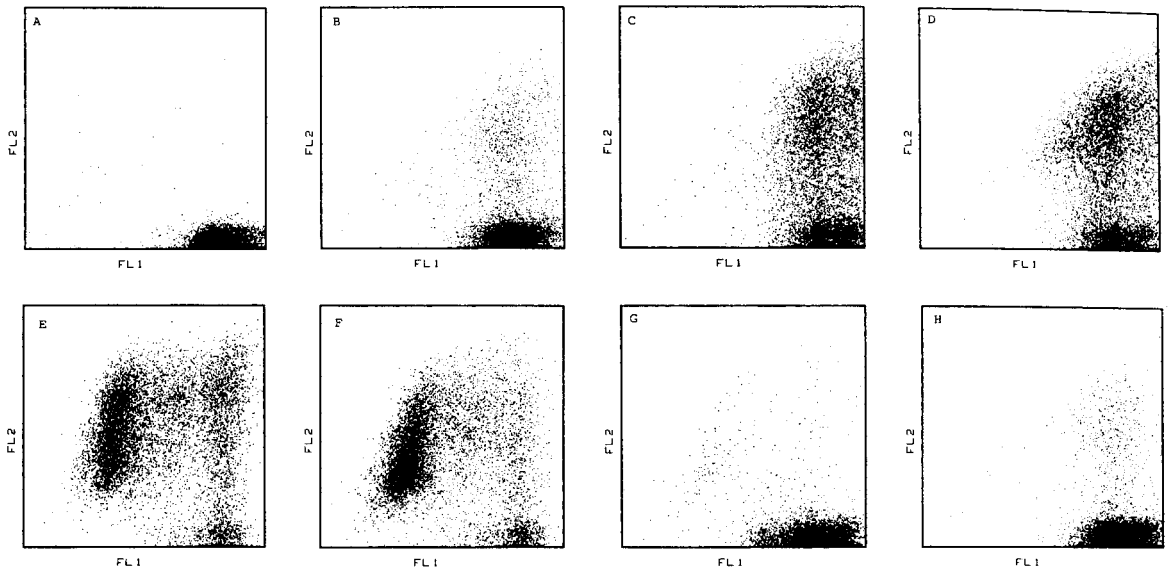

FIG. 4. HLA class I (abscissa) and HIV-1 p24 (ordinate) specific indirect double-immunofluorescence analyses (4 log scale) of peripheral CD4$^+$ T lymphocytes after various times in culture. (A) Uninfected control (day 2). (B–H) Cultures infected with HIV-1 for 2 days (B), 3 days (C), 4 days (D), 5 days (E), 6 days (F), 7 days (G), and 8 days (H).

The seeming loss of p24-positive cells in peripheral CD4$^+$ cells at day 7 postinfection may be due to mechanical stress in addition to the direct cytopathic effects of virus infection. At the various times indicated, aliquots of infected cells were removed from the culture flask and washed several times by centrifugation during the staining procedure. It is possible that cells infected for several days become sufficiently labile and disintegrate with this treatment. Dye exclusion analysis with nonmanipulated cells revealed only about 10% of dead cells even at these times. A second cycle of infection seems to begin on day 8 of culture of CD4$^+$ peripheral cells, as indicated by the cloud of p24-positive cells resembling the situation on day 3 after primary infection (Fig. 4).

The gradual loss of HLA class I antigen demonstrable by immunofluorescence could have several explanations. First we investigated the steady state of class I-specific mRNA, as shown in Figure 5. Two independent Northern blot analyses of H9 cells did not suggest that a downregulation of HLA class I antigen expressed in HIV-infected cells occurs at the level of transcription or posttranscription up to mature mRNA. These cells were infected for the same periods of time as those shown in Figure 2. This confirms a previous report[9] showing downregulation of several surface molecules (CD3, 4, 8, and 11) of HIV-1–infected cells without impairment of corresponding mRNAs.

Another possible mechanism could be the loss of MHC class I by budding viruses as described by Gelderblom et al.[10] This implies that downregulation of MHC class I is not a consequence of HIV infection per se but depends on the number of virus particles released from a cell. Consequently, the phenomenon should occur, not immediately after infection, but only after virus production has become sufficiently great. This is in keeping with our observation that MHC class I downregulation is observed only in cultures infected for at least 5 days, although many p24-positive cells are already clearly demonstrable on day 4 postinfection (Fig. 4). However, other mechanism, such as lack of mRNA translation and retention of class I antigen inside the cell, cannot as yet be excluded.

Of course, the downregulation of HLA class I antigens following HIV infection described here does not yet preclude that these cells could still be recognized by HIV-specific class I restriced CTL. However, it is intriguing that in all reports on HIV-specific class I restricted CTL from patients so far published, target cells other than freshly HIV-infected normal CD4$^+$ T helper cells were used[1,2] and that unusually high incidences of class II rather than class I restricted CTL have been reported.[11,12]

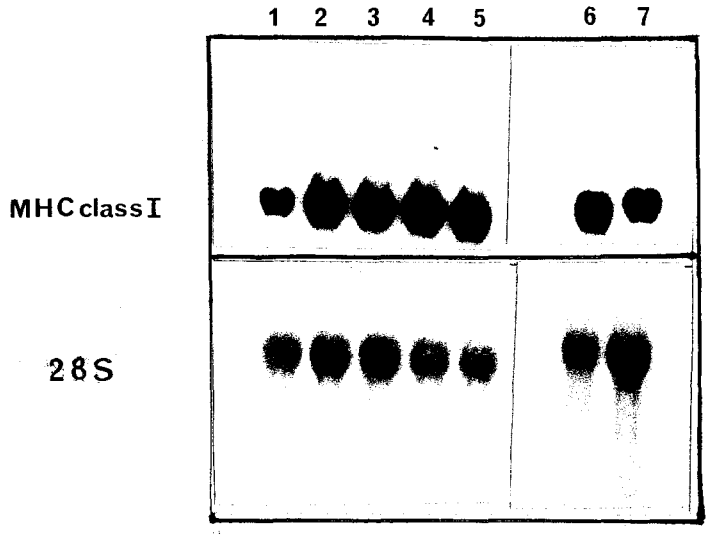

FIG. 5. Northern blot analyses of HLA class I-specific mRNA of H9 cells uninfected or infected with HIV-1 for various times. Two stocks of HTLV-IIIB virus were used. We produced stock I in H9 cells; stock II produced in H9 cells by Behringwerke (Marburg, FRG). Both virus stocks were used at an approximate MOI of 0.1. Lanes: (1) H9-uninfected culture; (2) infected with stock I, 4 days; (3) infected with stock II, 4 days; (4) infected with stock I, 7 days; (5) infected with stock II, 7 days; (6) H9-uninfected control, 16 days; (7) infected with stock I, 16 days.

In three preliminary experiments we addressed this question by investigating the quality of HIV-infected and noninfected $CD4^+$ PBL as allogeneic targets for class I restricted CTL. As shown in Figure 6D, HIV-infected cells not yet displaying class I downregulation were lysed to the same extent as noninfected controls. This indicates that HIV infection per se does not make the cells worse targets for lysis by CTL. However, once HIV infection caused class I downregulation, the extent of lysis by CTL was reduced by a factor of about 3 (Fig. 6A–C). Since in these cultures only $CD8^+$ cells had been removed and thus they still contained in addition to $CD4^+$ cells also B lymphocytes, granulocytes, and macrophages, the effect could not be expected to be more pronounced.

A final answer will only be provided by experiments in which HIV-specific class I restricted CTL are used. However, the phenomenon of class I downregulation described here is likely to explain at least

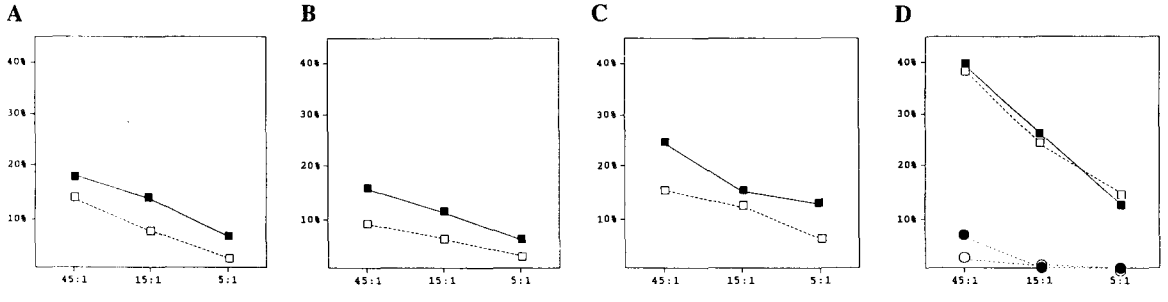

FIG. 6. Effect of HIV infection on peripheral $CD4^+$ cells as allogeneic targets. (A–C) Percentage specific chromium release of uninfected (■) and HIV-infected cells (□) in three independent experiments. In each case the infected target cells were used after HLA class I downregulation occurred. (D) Results obtained when the infected target cells were p24 positive but prior to class I downregulation. After treatment of either the effector cells with a mouse anti-CD8 antibody (●) or the target cells infected with a mouse anti-HLA class I antibody (○) the chromium release was nearly totally abolished. An equal inhibition of chromium release after the relevant antibody treatments was also observed in the experiments shown in A–C.

partially the failure of cellular immune responses to contain HIV infections. Although infected $CD4^+$ helper cells initially remain good targets for class I restricted CTL, they lose this quality just at the time of maximal virus production and release.

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