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HLA class II glycoprotein expression by monocytes from HIV-infected individuals: differences in response to interferon-gamma

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SUMMARY

The expression of HLA class II glycoproteins by monocytes from HIV-infected individuals was evaluated. We found no differences in HLA-DR, HLA-DQ, or HLA-DP expression by freshly isolated monocytes from patients with clinical AIDS, HIV-infected patients without AIDS or from uninfected individuals. However, monocytes from HIV-infected individuals without AIDS responded better to stimulation with recombinant human interferon-gamma (IFN-γ) than did monocytes from patients with clinical AIDS or from uninfected individuals. The response to IFN-γ stimulation by monocytes from patients with clinical AIDS was different. Thus, the largest increases in the percentage of cells expressing HLA-DR was associated with the smallest changes in the fluorescence intensity of the population that is observed following stimulation of monocytes from other patient populations.

Keywords monocytes HLA class II HIV

INTRODUCTION

Infection of individuals with HIV results in a progressive suppression of immunological responsiveness. Initial reports indicated that $CD4^+$ lymphocytes served as the sole site of HIV replication. Thus antigen-induced activation of infected $CD4^+$ lymphocytes resulted in HIV replication and depletion of the $CD4^+$ population. The progressive depletion of the $CD4^+$ population rendered the host susceptible to opportunistic infections (Fauci, 1988). More recently, studies have shown that the macrophage can also serve as an important site for HIV replication (Koenig *et al.*, 1986; Gendelman *et al.*, 1988).

In addition to their role as phagocytic cells that can remove foreign material and invading microorganisms, macrophages process antigenic material (Unanue, 1984). Processed antigen becomes associated with MHC class II glycoproteins which are then recognized by class II-restricted $CD4^+$ lymphocytes. Interaction of macrophages with T cells during antigen presentation may result in the transmission of HIV. Infection of mononuclear phagocytes by HIV may result in a defect in antigen processing or presentation that can also result in immunological unresponsiveness of HIV-infected individuals. The importance of HLA class II expression by mononuclear phagocytes in the recognition of antigen by $CD4^+$ lymphocytes led us to compare the expression of MHC glycoproteins by monocytes from individuals infected with HIV. We found that in response to interferon-gamma (IFN-γ) there was an increase in the amount of HLA-DR and HLA-DP in HIV-seropositive subjects without AIDS when compared with HIV-negative individuals or patients with clinical AIDS. Interestingly, the largest increases in the percentage of monocytes from patients with AIDS expressing HLA-DR after treatment with recombinant human IFN-γ (rhIFN-γ) was associated with the smallest changes in the amount of HLA-DR expressed by the population. This correlation was not observed for seronegative patients nor for patients infected with HIV without clinical AIDS and was not observed with changes in HLA-DQ nor HLA-DP.

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MATERIALS AND METHODS

Reagents

Hybridomas, producing monoclonal antibodies to HLA-DR, clone L243 (ATCC HB55), or to HLA-DQ, clone Genox 3.53 (ATCC HB103) were obtained from the American Type Culture Collection (Rockville, MD) and grown in our laboratory. These antibodies were purified by Protein A affinity chromatography and adjusted for reactivity to conform with the commercial reagents obtained from Becton Dickinson (Mountain View, CA). The monoclonal antibody reacting with HLA-DP was purchased from Becton Dickinson. The anti-HLA class II reagents were conjugated to FITC by standard methods as described by Mishell & Sheigii (1980). Anti-Leu M5, which

reacts with CD11c, was used as a monocyte marker and was purchased from Becton Dickinson. The antibody was biotinylated according to the method of Bayer & Wilchek (1980). Strepavidin–phycoerythrin was purchased from Tago (Burlingame, CA) and adjusted to 10 μg/ml in phosphate-buffered saline (PBS) just prior to use. The recombinant human interferon was kindly provided by Genentech (South San Francisco, CA).

Isolation of peripheral blood monocytes

Monocytes were isolated using a non-adherent, two-step density gradient technique (Böyum, 1968). Sixty millilitres of blood were drawn by venepuncture from consenting donors with 1 ml of 5% EDTA in PBS as anti-coagulant. The blood was diluted with an equal volume of Sepracell (Sepratech, Oklahoma City, OK), distributed equally into three 50-ml conical centrifuge tubes (Falcon, Oxnard, CA) and centrifuged at 1500 **g** for 20 min at room temperature. The mononuclear cells were removed from the top 7 ml of each tube and transferred to separate 15-ml polypropylene centrifuge tubes containing 3·5 ml of PBS/1% bovine serum albumin (BSA) and centrifuged at 300 **g** for 10 min. To isolate the monocytes, the cell pellet was resuspended in 5 ml PBS/BSA, mixed with 7 ml of Sepracell and centrifuged at 1500 **g** for 20 min at room temperature. The monocytes were removed, washed and spun through a layer of 25 ml of bovine calf serum at 300 **g** for 10 min in order to remove platelets. This procedure routinely yielded $10–30 \times 10^6$ monocytes/60 cc of peripheral blood. The purity of the population, as assessed by differential staining and by staining for nonspecific esterase was greater than 95%. Leu M5, which recognizes about 90% of peripheral blood monocytes, reacted with 78% of the Sepracell-purified monocytes.

Culture of monocytes

Monocytes were suspended in Iscove's modified Dulbecco's medium (Whittaker Bioproducts) supplemented with 10 mM glutamine, 100 U/ml penicillin, 100 μg streptomycin, 10^{-4} M 2-mercaptoethanol and 20% fresh pooled human serum to 5×10^6/ml. The serum, which was not heat-inactivated prior to use, can be stored at 4°C for not more than 30 days. One millilitre of the cell suspension was added to Teflon vials (Cole Palmer, Chicago, IL) and cultured for 4 days in the absence or presence of 500 U rhIFN-γ.

Quantification of MHC class II expression

Monocytes were assayed for surface HLA class II expression immediately or after 96 h of *in vitro* culture in the presence or absence of rhIFN-γ. Briefly, 5×10^5 cells were aliquoted into 250-μl microfuge tubes and fixed by treatment with 1% paraformaldehyde for 30 min at 4°C. The cells were then washed and treated for 30 min with 100 μl of a mixture of IgG2a and IgG2b monoclonal antibody, containing 4 mg/ml, in order to block Fc receptors. After washing, the cells were incubated with a cocktail containing 0·4 μg of biotinylated anti-Leu M5 monoclonal antibody and either 0·4 μg of FITC-conjugated monoclonal anti-HLA-DR, HLA-DQ or HLA-DP. The cells were incubated for 45 min, washed with PBS and further incubated with 1 μg of strepavidin–phycoerythrin for 45 min. After washing, the cells were resuspended in 0·5 ml of PBS and analysed for MHC class II expression using an Ortho System 50H flow cytometer. The monocytes were identified by forward

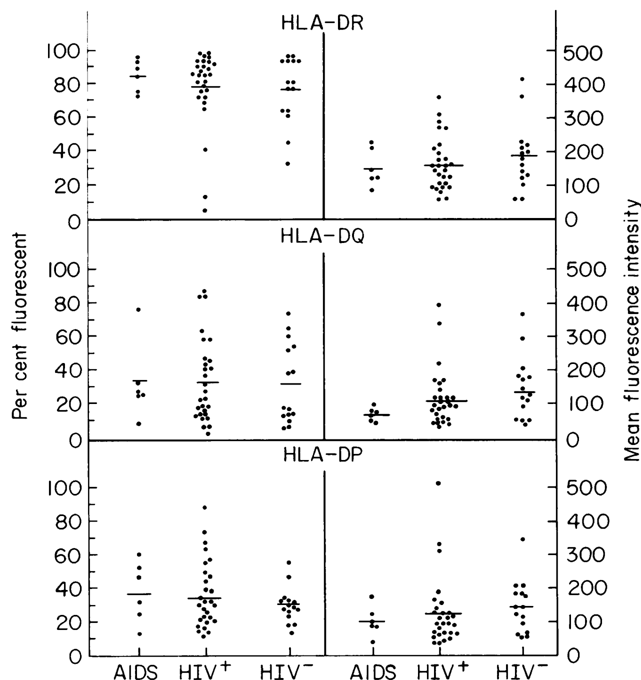

Fig. 1. HLA Class II expression by monocytes from HIV-infected individuals. Peripheral blood monocytes were isolated by differential centrifugation on Sepracell gradients. The monocytes were identified by Leu M5 phycoerythrin and MHC class II by FITC-conjugated monoclonal anti- HLA-DR, HLA-DQ or HLA-DP antibody. The per cent of fluorescent cells and the mean fluorescence were determined by dual-parameter flow cytometry using an Ortho System 50.

and 90° light scatter and by reactivity with Leu M5. All cells staining positive for Leu M5 were analyzed for reactivity with the anti-HLA class II reagents. The per cent of Leu M5 positive cells expressing each of the HLA class II glycoproteins as well as the mean fluorescence intensity of the population were recorded.

Statistical analysis

Differences between patient groups in their response to interferon-gamma were examined using a one-way analysis of variance (ANOVA) model with the differences between values obtained after culture in the presence of rhIFN-γ minus the value obtained after culture without rhIFN-γ serving as the dependent variable. The effect of the 4 days of *in vitro* culture was similarly examined using the differences between values from cultured cells and from freshly isolated cells. The intercept term in these models was used to gauge the overall effects of interferon or of culture. Group differences in the correlations between effects were examined using Fisher's transformation and a weighted by $(1/n-3)$ ANOVA. In each case, probit plots of model residuals and checks on their homoscedasticity supported the validity of the statistical methods used.

RESULTS

Multi-parameter flow cytometric analysis was used to evaluate the expression of HLA-DR, HLA-DQ and HLA-DP by peripheral blood monocytes from 35 individuals infected with HIV and 16 HIV-negative subjects. The data obtained from

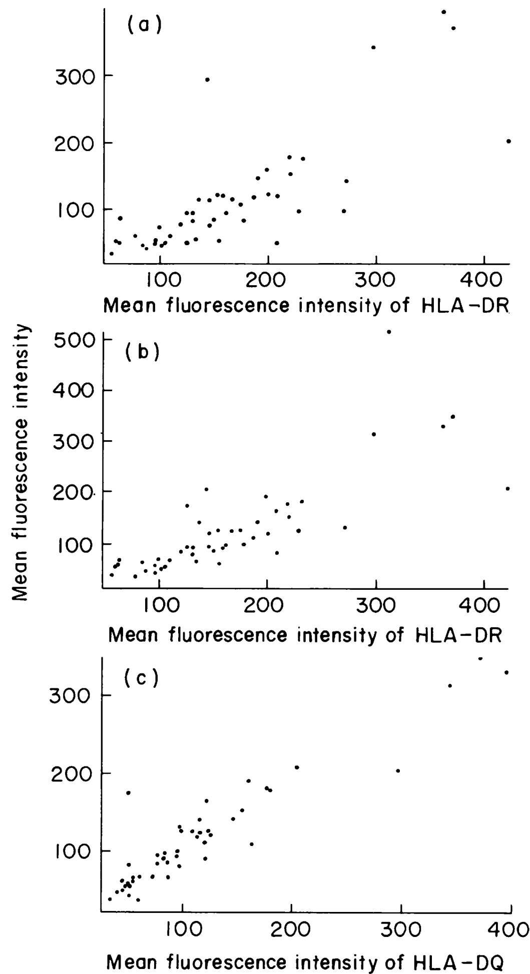

Fig. 2. Relation of expression of HLA class II glycoproteins by peripheral blood monocytes. Monocytes were isolated as described and analyzed immediately for expression of HLA- DR (a); HLA-DQ (b); and HLA-DP (c); by dual-parameter flow cytometry. The relation of the expression of the MHC class II glycoproteins was analysed by plotting the fluorescence intensity of HLA-DR *versus* HLA-DP or HLA-DQ and by plotting the intensity of HLA-DP *versus* HLA-DQ.

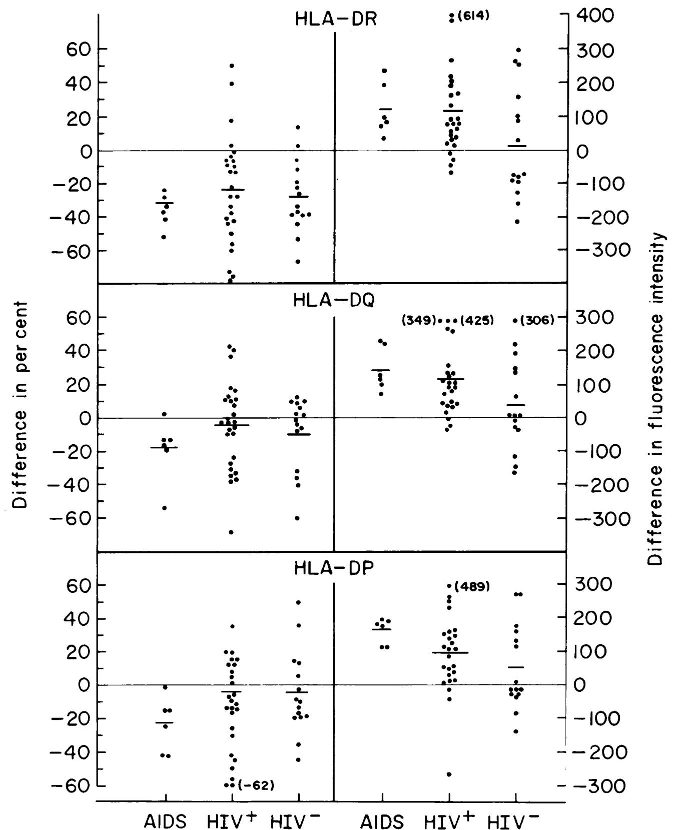

Fig. 3. The effect of *in vitro* culture on the expression of HLA class II glycoprotein by peripheral blood monocytes from HIV-infected individuals. Monocytes were incubated for 4 days in teflon vessels. Following *in vitro* culture, the cells were analysed for MHC class II expression by dual-parameter analysis using an Ortho System 50 flow cytometer. The data are plotted as the difference in the per cent of cells or mean fluorescence intensity of the population from each individual patient obtained after 4 days of culture minus that obtained immediately after isolation of the monocytes.

each of the individual patients are summarized in Fig. 1 as dot plots of the percentage of cells expressing HLA class II or the mean peak fluorescence intensity of the population. Seventy-seven per cent to 84% of the monocytes expressed HLA-DR, 33% expressed HLA-DQ and 30–37% expressed HLA-DP. No statistically significant differences were observed between monocytes from the different patient groups.

The lack of any disease-related differences in the percentage of cells expressing HLA class II or in the amount of HLA class II expressed by the monocytes from the different populations allowed us to compare the relation between the levels of expression of each of the class II glycoproteins. The scatter plots in Fig. 2 show that there was a linear relationship between the mean fluorescence intensity of HLA-DR versus that of HLA-DQ ($r=0{\cdot}762$) or HLA-DP ($r=0{\cdot}789$) and a stronger relation between the intensity of HLA-DQ and HLA-DP ($r=0{\cdot}936$). That is, if the level of expression of HLA-DQ was high, then the level of HLA-DP was also high.

The expression of HLA class II by monocytes declines during 4 days of *in vitro* culture (Gonwa & Stobo, 1984). The data in Fig. 3 represent the differences in the percentage of cells expressing each of the HLA class II glycoproteins when evaluated initially after isolation or after 4 days of *in vitro* culture. The dot plots represent the data calculated for each of the individual patients. Thus, dots appearing below the 0 line indicate that the percentage of cells expressing HLA class II from a given individual declined during *in vitro* incubation, while those above the line indicate that the percentage of cells expressing HLA class II increased during the 4 days of *in vitro* culture. As can be seen, the percentage of cells expressing HLA class II from the majority of patients, declined after 4 days of culture. The decline was most pronounced for HLA-DR (27% average drop, $P\leq0{\cdot}0001$) but still significant for HLA-DQ and HLA-DP (8%, $P\leq0{\cdot}05$). Interestingly, despite the decline in the percentage of cells expressing HLA class II, the mean fluorescence intensity for each increased significantly ($P\leq0{\cdot}0001$). Again, no disease-related differences were observed. It is possible that the increase in fluorescence intensity may have been due to the presence of IFN-γ synthesized by a small number of contaminating lymphocytes or natural killer (NK) cells. To

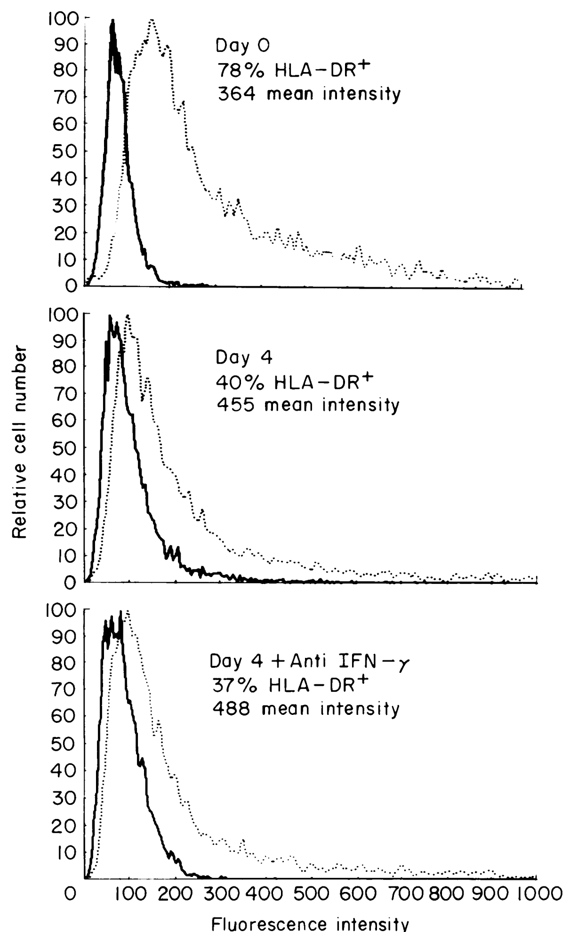

Fig. 4. The effect of anti-IFN-γ on the expression of HLA-DR by peripheral blood monocytes. Monocytes were isolated as described and incubated for 4 days in the presence or absence of monoclonal anti-IFN-γ (ATCC HB8291) in teflon vessels. Following *in vitro* culture, the cells were analysed for HLA-DR expression by dual-parameter analysis. The per cent of cells expressing HLA-DR as well as the mean fluorescence intensity of the population is provided for each treatment group.

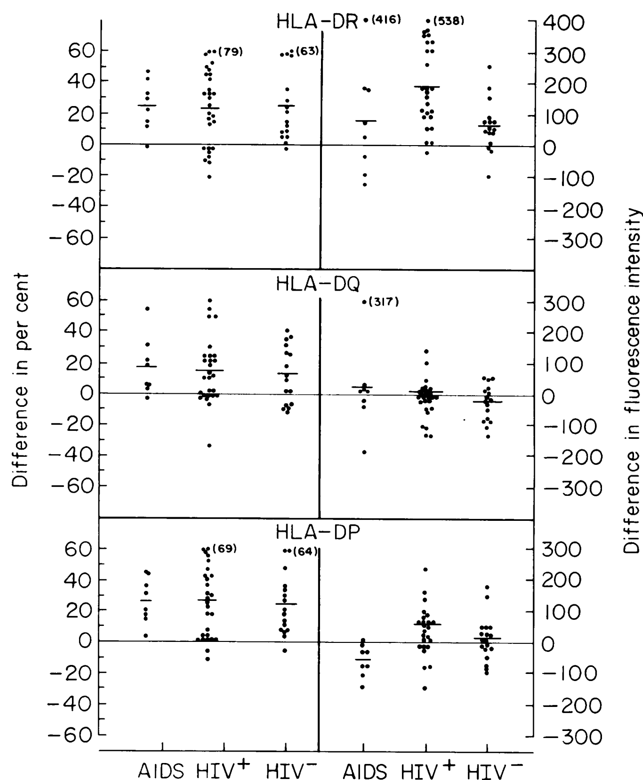

Fig. 5. The effect of rhIFN-γ on HLA class II expression by monocytes from HIV-infected patients. Monocytes were cultured in the presence or absence of 500 U of rhIFN-γ for 4 days. Following culture, the percent of cells and fluorescence intensity of the population was determined. The data are plotted as the difference between the values obtained after culture in the presence of rhIFN-γ minus the values obtained after culture in the absence of rhIFN-γ.

test this possibility, monoclonal anti-human IFN-γ was added to the cultures at the initiation of the experiment. A representative histogram (Fig. 4) shows that the additional anti-hIFN-γ, at concentrations that inactivated 500 U of rhIFN-γ, did not affect the reduction in the percentage of cells expressing HLA-DR nor the increase in fluorescence intensity. Similar observations have been made for HLA-DQ and HLA-DP (data not shown).

We also evaluated the response of the monocytes from the different patient groups to rhIFN-γ. The data are presented in Fig. 5. The left-hand panel represents the differences in the percentages of cells expressing HLA class II for each of the individual patients following incubation of the peripheral blood monocytes in the presence or absence of rhIFN-γ for 4 days. As expected, rhIFN-γ stimulation resulted in a significant increase in the percentage of cells expressing HLA-DR, HLA-DQ or HLA-DP ($P \leq 0{\cdot}0001$). The change in the percentage of cells expressing MHC class II in response to rhIFN-γ did not vary with disease status.

The mean fluorescence intensity of the cells from the different patient populations in response to rhIFN-γ was also determined (Fig. 5, right). HIV-seropositive patients without clinical AIDS exhibited a significantly greater response to rhIFN-γ. The increase in the fluorescence of HLA-DR ($P \leq 0{\cdot}006$) and HLA-DP ($P \leq 0{\cdot}02$) was greater on monocytes from the HIV-seropositive individuals than either monocytes from uninfected subjects or from patients with clinical AIDS. This effect was not observed for HLA-DQ (Fig. 5). An analysis of the relation between the change in the mean fluorescence intensity of the population to the change in the percentage of cells expressing HLA-DR showed a negative correlation when the monocytes were obtained from patients with AIDS (Fig. 6). Thus, in response to rhIFN-γ, a large increase in the per cent of cells expressing HLA-DR did not result in a change in the fluorescence intensity of that population. In contrast, for the other patient populations (HIV-infected, non-AIDS and uninfected) as well as for the expression of HLA-DQ and HLA-DP, changes in the per cent of cells expressing HLA class II following treatment with rhIFN-γ showed a modest positive correlation with changes in the amount of glycoprotein expressed by the population (Fig. 6) (data not shown on HLA-DQ and HLA-DP). This change in correlation of HLA-DR with disease status was significant ($P < 0{\cdot}01$). However, as an unplanned significance test, this result should be confirmed by independent experimentation.

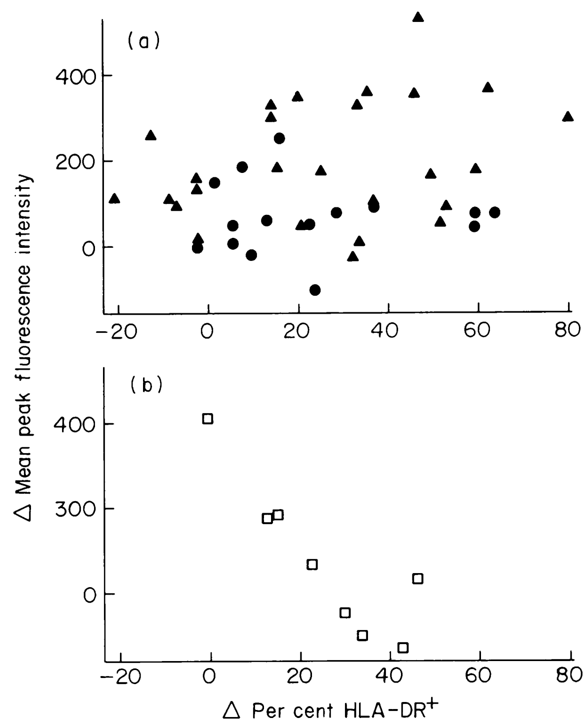

Fig. 6. Relation of the change in percent of monocytes expressing HLA-DR to the change in fluorescence intensity of the population. Peripheral blood monocytes were incubated in the presence or absence of rhIFN-γ for 4 days. The percent of cells expressing HLA-DR and the mean fluorescence intensity of the population was determined. The data represent the difference in the percent or intensity of cells expressing HLA-DR in the presence of rhIFN-γ minus the percent of cells expressing HLA-DR in the absence of the stimulus for each individual patient. The data are plotted as the change in per cent *versus* the change in mean fluorescence intensity for each individual patient. (a) Monocytes obtained from uninfected individuals (●) or from HIV-infected (▲) without AIDS; (b) monocytes obtained from patients with AIDS (□).

DISCUSSION

Our results indicate that the expression of HLA-DR in response to IFN-γ is altered in monocytes from HIV-infected individuals. We found that incubation with rhIFN-γ could not produce a large increase in both the percent of cells expressing HLA-DR and in the level of that expression. However, this was quite common for seronegative or other HIV-infected individuals. In contrast, the response of the monocytes from HIV-infected individuals without clinical AIDS was increased. Also, the increase in the intensity of HLA-DR and HLA-DP was greater for HIV-infected individuals without clinical AIDS than for individuals with AIDS or individuals without HIV infection.

We did not observe any disease-related differences in the percentage of freshly isolated monocytes expressing MHC class II glycoproteins. Our observations support those of Haas *et al.* (1987) who also compared the expression of HLA class II by monocytes from HIV-infected individuals with that of monocytes from uninfected individuals. Several other reports, however, have indicated that the expression of MHC class II is suppressed or stimulated as a result of HIV infection (Heagy *et al.*, 1984; Petit, Terpstra & Miedema, 1987). The differences in our observations may be accounted for by the differences in the isolation of the peripheral blood monocytes. We used a density gradient technique that did not require culture of the monocytes. Others, who have observed differences in HLA class II expression, have used adherence and prolonged culture to isolate the monocytes. This can result in an alteration in MHC class II glycoprotein expression (Gonwa & Stobo, 1984). We also used Leu M5 monoclonal antibody to identify macrophages; we found that this antibody reflected more accurately the percentage of monocytes as determined by differential and by stains for non-specific esterase. Others have used monoclonal antibody Leu M3 or Mo2 as a monocyte marker. We found in preliminary studies that this antibody did not detect the percentage of monocytes in our preparations.

The level of expression of MHC class II that we observed are consistent with that reported by others for monocytes from individuals not infected with HIV (Gonwa & Stobo, 1984; Gonwa, Frost & Karr, 1986; Gibbons *et al.*, 1989). Thus, nearly 80% of the monocytes expressed HLA-DR while 30% expressed HLA-DQ or HLA-DP. Incubation of the monocytes without rhIFN-γ resulted in a decrease in the percentage of cells expressing HLA class II but an increase in the level expressed by the population. However, the addition of rhIFN-γ to the culture medium resulted in a stimulation of HLA class II expression by the monocytes which resulted in an increase in the percentage of cells expressing HLA class II as well as in the level of HLA class II expressed by the cells. This observation is consistent with those of others who also reported an increase in the percentage of HIV-infected monocytes expressing HLA class II (Heagy *et al.*, 1984). The only exception to this observation was the expression of HLA-DR by monocytes from patients with AIDS.

The presentation of antigen by antigen-presenting cells is thought to require a certain density of MHC class II glycoproteins on the surface (Matis *et al.*, 1983). The decrease in the density of HLA-DR on the monocytes of patients with clinical AIDS following treatment with rhIFN-γ may be responsible for the decrease in accessory cell function that has been reported by others (Petit *et al.*, 1988). It is not known whether the decrease in HLA-DR expression that we observed is the result of HIV infection. The differences we observed in the expression of HLA-DR did not occur in monocytes from patients with ARC or asymptomatic carriers. Instead, the expression of HLA-DR and HLA-DP by monocytes from these individuals was enhanced following stimulation with rhIFN-γ. This enhanced response to rhIFN-γ may be the result of priming of the monocytes *in vivo* which may have occurred due to responses to HIV or to the opportunistic infections associated with the disease. Our failure to observe an increase in HLA-DQ may be because the antibody we used recognizes epitopes not found on HLA-DR3 or HLA-DR7. Since HIV infects only a small percentage of peripheral blood monocytes, the significance of our observations to the clinical course of HIV infection is not known. Studies evaluating the effect of HIV infection on MHC class II expression by cultured monocytes are currently in progress.

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