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Increased Expression of CD80 and CD86 in *in Vitro*-Infected $CD3^+$ Cells Producing Cytoplasmic HIV Type 1 p24

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ABSTRACT

Determining the effects of HIV infection on the expression of cell surface molecules has been limited by an inability to differentiate between productively infected cells and those without productive infection. We inoculated human peripheral blood mononuclear cells from healthy, human immunodeficiency virus type 1 (HIV) antibody-negative donors with HIV; noninoculated cells were also examined. Using multiparameter flow cytometry, we differentiated cells actively producing HIV cytoplasmic p24 antigen during acute, *in vitro* HIV infection from those not producing detectable cytoplasmic p24. For both resting and PHA-stimulated cells inoculated with HIV (R/H and P/H), a higher proportion of $p24^+$ cells expressed CD25, compared with $p24^-$ cells ($p = 0.031$ and $p = 0.008$, respectively), consistent with either increased viral replication in stimulated cells or increased stimulation secondary to productive HIV infection. Findings were similar for the expression of CD38, HLADR, and CD28. A striking proportion of $p24^+$ cells expressed CD80 or CD86, antigens not usually expressed by $CD3^+$ lymphocytes. The increased expression appeared to be independent of stimulation status in that it occurred in both the R/H and P/H treatment groups but not in resting or PHA-stimulated uninfected cells. CD28 expression was generally comparable between $CD3^+$ cells that did and did not express CD80 or CD86. Multiparameter flow cytometry, in association with improved techniques for cell permeabilization and cytoplasmic fluorescent staining, should prove useful in examining the effects of productive HIV infection on surface and cytoplasmic cellular molecules. Using this approach, we found an association between productive infection and increased expression of CD80 and CD86. This association has implications for HIV disease pathogenesis and, potentially, HIV therapy.

INTRODUCTION

DETERMINING THE EFFECTS of human immunodeficiency virus type 1 (HIV) infection on the expression of cell surface molecules has been limited by an inability to differentiate between productively infected cells and those without productive infection. Early attempts to do so showed promise[1–4] but proved to be inadequately sensitive in detecting cytoplasmic antigen positivity to elicit widespread application. Since these attempts, a number of polymerase chain reaction (PCR)-based approaches have been developed to quantitate plasma HIV viral RNA levels. This assessment may assist in predicting clinical disease progression and determining the efficacy of a growing number of HIV therapeutic agents.[5] However, it is less useful in experimental settings, since it does not permit an examination of the HIV-infected cells themselves.

One laboratory has described a system permitting detection of HIV proviral DNA and surface molecules on the cells harboring it; this approach does not differentiate productive and quiescent HIV infection.[6] To examine the cell-specific effects of productive HIV infection, we used recently improved reagents for and approaches to intracellular/cytoplasmic flow cytometric fluorescent staining.[7] Using multiparameter flow cytometry, we differentiated human peripheral blood lymphocytes actively producing HIV cytoplasmic p24 antigen during acute, *in vitro* HIV infection from those not producing detectable cytoplasmic p24. This system also permitted an efficient, simultaneous examination of $CD8^+$ cells.

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Cell activation is associated with increased production of HIV.[8–12] Thus, determining the effects of HIV infection on cell surface molecules is confounded by the effects of cell activation on both HIV infection and a wide variety of T cell surface molecules. We inoculated resting and phytohemagglutin (PHA)-stimulated human peripheral blood mononuclear cells (PBMCs) with HIV, to examine the interactive effects of cell activation status and HIV infection itself on cytoplasmic HIV p24 production and the expression of various surface antigens previously implicated in HIV infection and/or cell activation. For $CD3^+$ cells, these included CD25, CD28, CD38, CD49d, CD80, CD86, CD45RO, and HLA-DR. For the $CD8^+$ cells in these cultures, we also examined CD11a, CD27, CD30, CD71, and CD95.

Our findings, described herein, are consistent with activation being associated with productive HIV infection and validate the usefulness of this system in examining the effects of acute HIV infection at a single-cell level. Most interesting, we found that a relatively higher proportion of $p24^+$ $CD3^+$ lymphocytes expressed CD80 and CD86 than did $p24^-$ $CD3^+$ lymphocytes. These findings may have important implications for HIV transmission, pathogenesis, and therapy.

MATERIALS AND METHODS

Cells and virus

PBMCs were obtained from healthy human donors seronegative for HIV and hepatitis viruses. PBMCs were gradient-separated and cultured in RPMI 1640 containing antibiotics, at 37°C, in 5% CO_2, in a humidified incubator, at a concentration of 2×10^6 cells/ml. Cells were either stimulated for 1.5 days with PHA (5 μg/ml; Advanced Biotechnologies, Columbia, MD)* and 10% heat-inactivated (56°C for 45 min) fetal calf serum (FCS) or retained without stimulation in the preceding medium and 10% heat-inactivated (56°C for 45 min) donor serum. [Others have shown that under the latter conditions cellular DNA synthesis does not occur for up to 2 weeks.[12] Cells were then (at day 0 of infection) either infected or not infected with the HIV-1 T cell-tropic isolate LAI.[10] Medium was changed at that time, with resting cells again placed in medium plus donor serum. PHA-stimulated cells were placed into medium with 10% FCS and 10% interleukin 2 (volume percent; 640 half-maximal units/ml; Advanced Biotechnologies). Resting cells had extremely poor survival (results for resting cells infected with HIV will be provided only when the number of $p24^+$ $CD3^+$ cells collected per tube was >800 [>500 for one experiment]). The number of viable resting cells not inoculated with LAI was adequate for flow cytometric analysis in only two experiments; those results will be described herein but will not be included in aggregated data. Thus, comparison arms for each donor's cells included some or all of the following: day 0 resting (D0-R), day 0 PHA-stimulated (D0-PHA), resting/HIV-inoculated (R/H), PHA-stimulated/HIV-inoculated (P/H), and PHA-stimulated (PHA) cells. P/H and PHA cells were harvested on the same day, at day 4–7 postinoculation, with timing based on the decline in CD4 expression; for some experiments, a second harvest was done at 12–14 days. The R/H cells were harvested on the same or following day.

Reagents

Fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to HIV-1 p24 antigen was purchased from Chemicon International (Temecula, CA). Other FITC-conjugated reagents included murine monoclonal antibodies to: CD11a (clone G-30; Becton Dickinson Immunochemistry Systems [BD], San Jose, CA), CD25 (clone 2A3; BD), CD27 (clone M-T271; PharMingen [PMG], San Diego, CA), CD30 (clone Ki-1; Dako, Carpinteria, CA), CD71 (clone L01.1; BD), CD80 (clone BB1; PMG), CD86 [clone 3551(FUN-1); PMG], and CD95 (clone UB2; Immunotech, Westbrook, ME). Phycoerythrin (PE)-conjugated monoclonal reagents were to CD3 (clone SK7; BD), CD14 (clone MϕP9; BD), CD25 (clone 2A3; BD), CD28 (clone L293; BD), CD38 (clone HB-7; BD), CD49d (clone L25; BD), CD80 (clone L307.4; BD), CD86 [clone 3551(FUN-1); PMG], CD95 (clone DX2; PMG), CD45RO (clone UCHL-1; BD), and HLA-DR (clone L243; BD). PE–indodicarbocyanine (Cy5) tandem-conjugated reagents included monoclonal antibodies to CD3 (clone UCHT1; PMG), and CD8 (clone RPA-T8; PMG). Peridinin chlorophyll protein reagents (BD) were to CD3 (clone SK7; BD), CD4 (clone SK3; BD), and CD8 (clone SK1; BD); allophycocyanin reagents were to CD3 (clone SK7; BD), CD14 (clone MϕP9; BD), and CD19 (clone 4G7; BD). Unconjugated reagents included monoclonal antibody to CD80 ($2\ \mu g/5 \times 10^5$ cells, clone BB1; PMG) and CD86 [$2\ \mu g/5 \times 10^5$ cells, clone 3551(FUN-1)]. CTLA4Ig (10 μg/ml) was kindly provided by P. Linsley (Bristol-Myers-Squibb Pharmaceutical Research Institute, Seattle, WA). Permeabilization was done with Ortho PermeaFix (Ortho Diagnostic Systems, Raritan, NJ). Blocking with unconjugated antibodies was done for 15 min at room temperature; with CTLA4Ig, it was done for 2 hr at 4°C, in the dark, prior to fluorescent staining.

Flow cytofluorometry

Experiments were initially done using three-color cytofluorometry and Lysis II software on a FACSort flow cytometer (BD); when the FACSort system was upgraded, four-color staining and Cellquest software were used. As isotype controls, cell aliquots were stained with FITC–mouse IgG_1 (BD), PE–mouse IgG_{2a} (BD), and PECy5–mouse IgG_1 (PMG). Staining for surface antigens was routinely done following a 15-min incubation of cells with heat-inactivated normal mouse serum ($20\ \mu l/10^6$ cells) and prior to permeabilization and internal(/surface) staining. Prior permeabilization did not appreciably affect the proportion of cells reacting with antibodies to CD3, CD4, and CD8; however, reactivity to several other surface antigens markedly decreased if staining was done after permeabilization (data not shown). For both HIV-inoculated and control cultures, <1% of lymphocytes reacted with antibody to HIV-1 p24 when staining was done prior to permeabilization. Cytoplasmic p24 positivity was positively associated with supernatant HIV p24 antigen levels (data not shown). For all experiments, monocyte contamination within the lymphocyte scatter gate, based on pos-

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itive staining with anti-CD14–PE, was ≤4.0% on day −1.5 (immediately after cell separation), ≤1.3% on day 0 (day of HIV inoculation), and ≤0.7% thereafter.

Generally, 20,000 ungated events were collected, followed by an additional collection of up to 3000 cells with the following characteristics: (1) forward scatter/side scatter (FSC/SSC) consistent with resting/blastic lymphocytes,[13] (2) cytoplasmic p24 antigen positivity, and (3) CD3 positivity, if anti-CD3–PECy5 was used in fluorescence channel 3 (Fl3) or CD3 positivity/CD8 negativity, if both anti-CD3–PECy5 and anti-CD8–PECy5 were used in Fl3. The latter was done in two experiments, after titration experiments showed clear separation between $CD3^+CD8^-$ and $CD3^+CD8^+$ cells to be feasible under these conditions (data not shown). Data for $p24^+$ cells were analyzed only if ≥500 cells could be collected using the latter gating (>800 cells for all but one experiment).

Statistical techniques

Because most of these parameters are not normally distributed, a Wilcoxon Signed rank test was used to compare results on day 0 (prior to HIV-1 inoculation) and days 4/5. This provides a nonparametric comparison of results, matched by blood donor. The number of experiments extended to days 12–14 was too small for statistical testing. Since activation is known to be associated with productive HIV infection, findings for antigens known to be associated with activation (CD25, CD38, CD71, HLA-DR) were assessed using a one-tailed test. For those without a clearly defined relationship to activation (CD11a, CD27, CD28, CD30, CD49d, CD80, CD86, and CD95), a two-sided test was used. A statistical result is not given if the p value was ≥0.05.

RESULTS

Associations with both activation and p24 positivity

As expected, CD25, CD38, and HLA-DR expression increased with PHA activation. The percentage of $CD3^+$ cells expressing CD25 after PHA stimulation alone was significantly greater at the time of virus inoculation (D0-PHA) than after no stimulation (D0-R), as was the percentage at the first harvest date (day 4/5 PHA) compared with $p24^-$ resting cells inoculated with HIV (R/H) (one-sided $p = 0.031$ for both comparisons). For both R/H and PHA-stimulated cells inoculated with HIV (P/H), a higher proportion of $p24^+$ cells expressed CD25, compared with $p24^-$ cells ($p = 0.031$ and $p = 0.008$, respec-

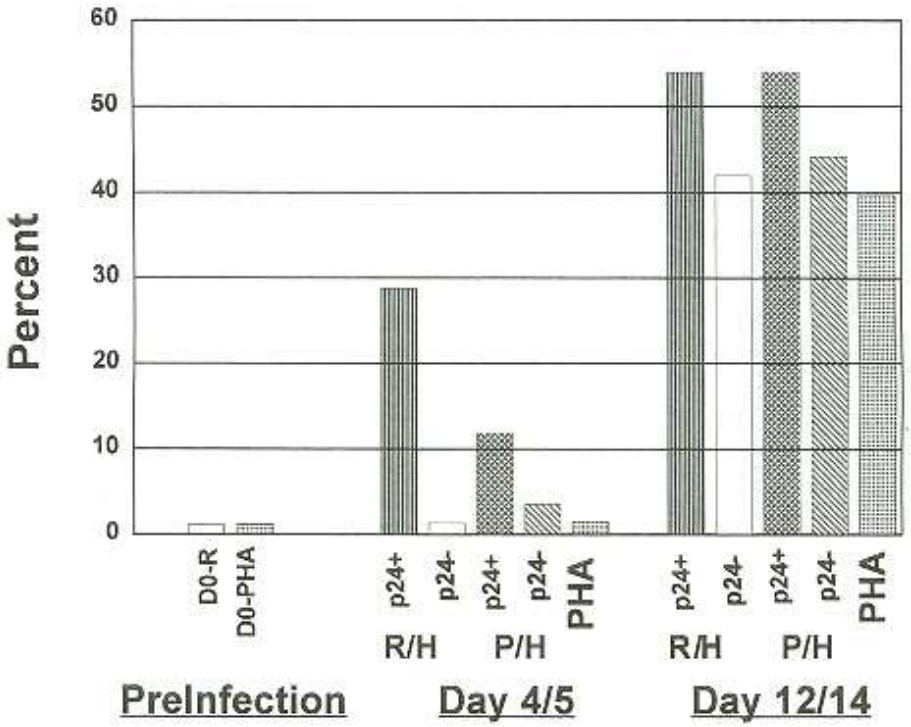

FIG. 1. Median percentage of $CD3^+$ lymphocytes expressing surface CD80 at time of HIV inoculation and on days 4/5 or 12/14 postinoculation, in relation to stimulation status and detection of cytoplasmic HIV-1 p24 by multiparameter flow cytometry, as described in Materials and Methods. Resting cells on day 0 (D0-R) (unstimulated cells in medium and autologous serum) were evaluated cytometrically and the remaining cells were inoculated with HIV-1 (R/H). Cells stimulated with PHA for 1.5 days were examined cytometrically on day 0 (D0-PHA) and the remaining cells were inoculated with HIV-1 (P/H) or were not inoculated (PHA), as described in Materials and Methods. Median values are given for six experiments (P/H and PHA on days 4/5), four experiments (R/H on days 4/5), three experiments (D0-R, D0-PHA), and two experiments (days 12/14). For one of these experiments, values were determined for $CD4^+$ cells and for another, $CD3^+CD8^-$ cells, rather than $CD3^+$ cells. Statistically significant results of Wilcoxon signed rank testing were as follows: $p24^+$ P/H compared with $p24^-$ P/H (two-sided $p = 0.031$) and $p24^+$ P/H compared with PHA (two-sided $p = 0.031$).

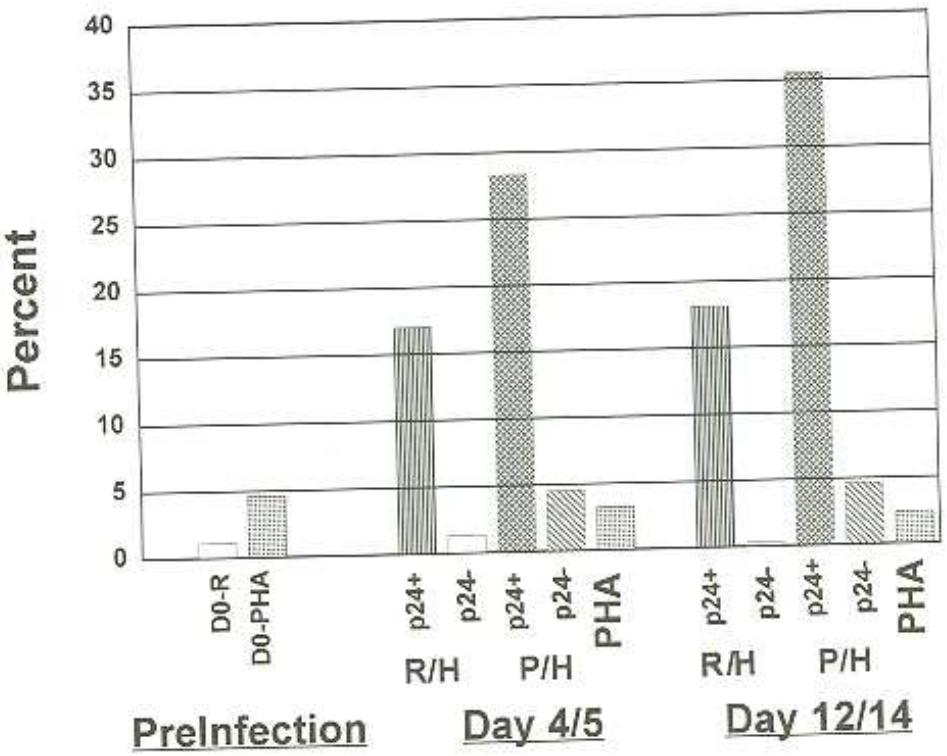

FIG. 2. Median percentage of $CD3^+$ lymphocytes expressing surface CD86 at time of HIV inoculation and on days 4/5 or 12/14 postinoculation, in relation to stimulation status and detection of cytoplasmic HIV-1 p24 by multiparameter flow cytometry, as described in Materials and Methods. Resting cells on day 0 (D0-R) (unstimulated cells in medium and autologous serum) were evaluated cytometrically and the remaining cells were inoculated with HIV-1 (R/H). Cells stimulated with PHA for 1.5 days were examined cytometrically on day 0 (D0-PHA) and the remaining cells were inoculated with HIV-1 (P/H) or were not inoculated (PHA), as described in Materials and Methods. Median values are given for seven experiments (P/H and PHA on days 4/5), five experiments (R/H on days 4/5), four experiments (D0-R, D0-PHA), and three experiments (days 12/14). For one of these experiments, values were determined for $CD4^+$ cells and for another, $CD3^+CD8^-$ cells, rather than $CD3^+$ cells. Statistically significant results of Wilcoxon signed rank testing were as follows: $p24^+$ P/H compared with p24 P/H (two-sided $p = 0.016$) and $p24^-$ P/H compared with PHA (two-sided $p = 0.016$).

tively), consistent with either increased viral replication in stimulated cells or increased stimulation secondary to productive HIV infection. Findings were similar for the expression of CD38 and HLA-DR. However, of these three activation-associated antigens, HLA-DR expression appeared to be more strikingly related to p24 positivity than activation per se, that is, the difference in HLA-DR appeared greater for $p24^+$ compared with $p24^-$ cells than for PHA-stimulated cells compared with resting cells. Findings were similar for CD28. Over 70% of $CD3^+$ lymphocytes in all treatment groups expressed surface CD28. However, a significantly higher proportion of $p24^+$ cells expressed surface CD28 than did $p24^-$ cells; the proportion was highest for $p24^+$ R/H. Inoculated, $p24^-$ cells expressed less CD28 than did PHA cells, especially those in the P/H group.

Absence of associations with either activation or p24 positivity

A higher proportion of $p24^+$ cells tended to express CD45RO, consistent with HIV infection of memory T cells; however, this trend did not reach statistical significance (NS). No clear or statistically significant relationships were found with CD49d (six experiments).

$CD3^+CD8^+$ lymphocytes

The expression of the following antigens on $CD3^+CD8^+$ cells was not obviously influenced by, or statistically associated with, inoculation with HIV, or influenced by stimulation with PHA: CD5 (two experiments), CD11a (four experiments), CD27 (two experiments), CD28 (seven experiments), CD30 (five experiments), CD49d (five experiments), CD80 (seven experiments), CD86 (six experiments), CD95 (six experiments), and CD45RO (seven experiments). Expression of the following antigens tended to increase with PHA activation, on day 0 and/or days 4/5: CD38 (one-sided $p = 0.013$ on days 0 and 4/5) (seven experiments) and CD71 (one-sided $p = 0.031$ on day 0 and $p = 0.063$ on days 4/5) (six experiments). CD25 expression was significantly affected by PHA stimulation but may have been further increased by HIV inoculation of stimulated cultures; a similar but more marked effect of HIV inoculation was seen with HLA-DR. For both CD25 and HLA-DR expression, the one-sided p value was 0.031 for D0-R compared with D0-PHA cells (on the day of HIV inoculation); on day 4/5, for R/H compared with P/H cells, and for R/H compared with PHA cells. For P/H compared with PHA cells, for CD25, NS; for HLA-DR, the one-sided $p = 0.023$.

TABLE 1. PERCENTAGE OF $CD3^+$ LYMPHOCYTES EXPRESSING CD28, BY COEXPRESSION OF CD80 OR CD86 AND TREATMENT GROUP[a]

	Treatment group[b]			
	R	*R/H*	*P/H*	*PHA*
$CD80^+$	88.2	80.9	85.0	86.2
$CD86^+$	87.0	65.7	79.5	76.3
All $CD3^+$	77.4	80.7	66.4	78.5

[a]One experiment, duplicate tubes. Mean values on day 6 (P/H and PHA) or day 7 (R and R/H) postinoculation with HIV-1, as described in Materials and Methods.

[b]Resting cells (unstimulated cells in medium and autologous serum) not inoculated (R) or inoculated with HIV-1 (R/H); PHA-stimulated cells inoculated with HIV-1 (P/H); or PHA-stimulated cells not inoculated with HIV-1 (PHA), as described in Materials and Methods.

Associations between HIV p24 positivity and expression of CD80 and CD86

A striking proportion of $p24^+$ inoculated cells expressed CD80 or CD86, antigens not usually expressed by $CD3^+$ lymphocytes (Figs. 1 and 2). This increased expression appeared to be independent of stimulation status in that it occurred in both the R/H and P/H treatment groups and not in PHA-stimulated, noninoculated cells. It also did not occur in resting, noninoculated cells on day 5 of culture (two experiments). CD80 expression may have been slightly greater in the R/H group and CD86, in the P/H group; however, these differences did not reach statistical significance. We next examined the coexpression of CD28 and CD80 or CD86 or $CD3^+$ lymphocytes (Table 1). CD28 expression appeared to be generally comparable between cells expressing CD80 or CD86 and all $CD3^+$ cells within a given treatment group. To assess the specificities of the reactivity with the fluoresceinated anti-CD80 or anti-CD86 reagents, we preincubated cells with unconjugated antibodies to CD80 or CD86 or with CTLA4Ig. Blocking of CD80 reactivity was 64–86% complete with unconjugated anti-CD80 antibody and 11–75% complete with CTLA4Ig but did not occur with anti-CD86 antibody (Table 2A). Blocking of CD86 reactivity was 56–95% complete with unconjugated anti-CD86 antibody and 0–50% complete with CTLA4Ig; some blocking may have occurred with the anti-CD80 antibody (Table 2B). Blocking occurred for both $p24^-$ and $p24^+$ $CD3^+$ lymphocytes (Figs. 3A–D).

DISCUSSION

HIV and HIV antigens have long been known to interact directly with the CD4 molecule, at both a surface and cytoplasmic level. More recently, it has been shown that other surface molecules act as ancillary receptors for HIV.[14] However, the direct and indirect effects of productive HIV infection on other cellular antigens have been far less examined. Previous attempts to use intracellular HIV-1 p24 antigen as a marker for active HIV infection[1–3] were limited by the relatively low signal-to-noise ratio associated with these indirect fluorescence techniques, primitive permeabilization technology, nonspecific staining, and the low proportion of PBMCs that are actively infected at any point in time. Characterization of *in vitro*-infected cells would have been further limited by the small number of parameters that could be concurrently assessed using flow cytometry. We have adapted recently refined techniques for cell permeabilization and cytoplasmic fluorescent staining, and taken advantage of improvements in multiparameter flow cytometry, to examine the effects of acute, productive, *in vitro* HIV infection on a single-cell level and determine the expression of various surface antigens in relation to the presence or absence of detectable cytoplasmic HIV-1 p24 antigen.

In our experimental system, PHA stimulation led to an increased proportion of both $CD3^+$ and $CD8^-$ cells expressing certain known activation markers. Further, a significantly higher proportion of $CD3^+$ cells with detectable cytoplasmic HIV-1 p24 expressed CD25, CD38, and HLA-DR than did $p24^-$ cells within the same culture. This is consistent with either increased viral production in activated cells or additional activation, on the single-cell level, occurring secondary to HIV infection itself. The expression of these antigens on unstimulated, infected cells supports but does not confirm the latter possibility. Alternatively, some of these antigens may have a more direct relationship with HIV or HIV antigens, rather than being related to activation per se. CD25 and HLA-DR have both been reported to be increased in persons with HIV infection[15]

TABLE 2. EFFECTS OF PREINCUBATION WITH MONOCLONAL ANTIBODIES TO CD80, CD86, OR CTLA4Ig ON $CD3^+$ LYMPHOCYTE SURFACE REACTIVITY TO MONOCLONAL ANTI-CD80 AND ANTI-CD86 ANTIBODY[a]

Preincubation with[c]:	*Treatment group*[b]				
	R	*R/H*[d]	*$p24^-$ P/H*	*$p24^+$ P/H*	*PHA*
A. Anti-CD80					
Buffer alone	0.9	4.0	4.8	26.9	7.9
Anti-CD80	0.6	1.3	1.0	9.7	1.1
Anti-CD86	1.2	5.5	3.6	22.3	6.5
CTLA4Ig	0.8	1.5	1.4	16.1	2.0
B. Anti-CD86					
Buffer alone	3.0	8.0	12.8	56.1	10.3
Anti-CD80	2.5	7.4	11.4	51.5	5.9
Anti-CD86	1.2	3.5	0.8	2.6	0.5
CTLA4Ig	3.1	4.0	9.4	48.4	9.1

[a]One experiment, duplicate tubes. Mean values on day 6 (P/H and PHA) or day 7 (R and R/H) postinoculation with HIV-1, as described in Materials and Methods.

[b]Resting cells (unstimulated cells in medium and autologous serum) either not inoculated (R) or inoculated with HIV-1 (R/H); PHA-stimulated cells inoculated with HIV-1 (P/H); or PHA-stimulated cells not inoculated with HIV-1 (PHA), as described in Materials and Methods.

[c]Blocking with unconjugated antibodies was done for 15 min at room temperature; blocking with CTLA4Ig was done for 2 hr at 4°C, in the dark, prior to fluorescent staining.

[d]Number of $p24^+$ R/H cells was inadequate for assessment.

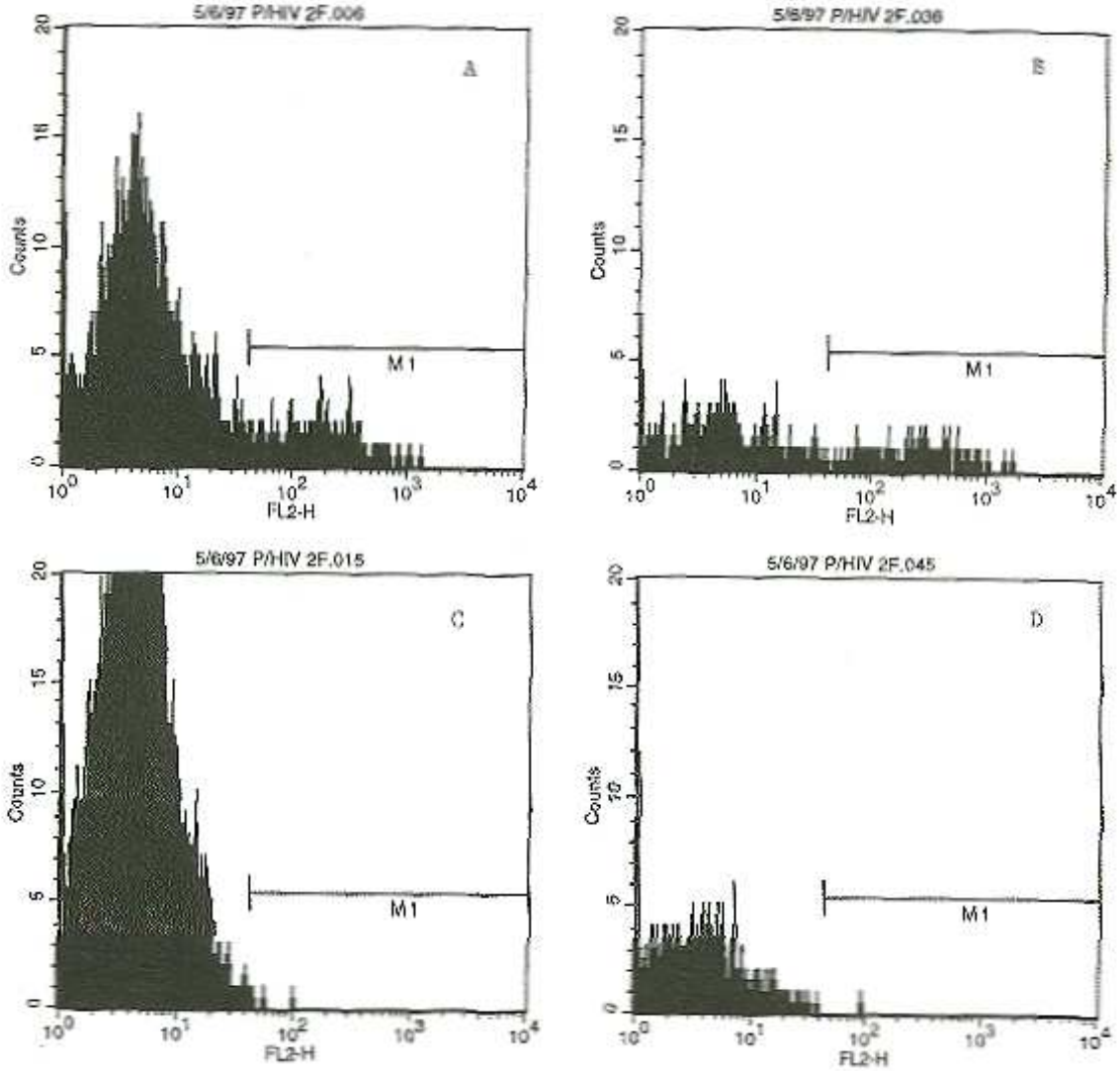

FIG. 3. Histograms of reactivities with murine monoclonal anti-CD86–PE on days 4/5 postinoculation of PHA-stimulated cells with HIV-1, by $CD3^+CD8^-$ HIV-1 $p24^-$ lymphocytes (**A** and **C**) and by $CD3^+CD19^-$ $p24^+$ lymphocytes (**B** and **D**) without (**A** and **B**) and with preincubation with unconjugated anti-CD86 antibody (**C** and **D**), as described in Materials and Methods. Blocking with unconjugated antibodies was done for 15 min at room temperature, in the dark.

and to be incorporated into HIV.[16] CD38 expression on $CD4^+$ cells increases with advancing HIV infection.[17]

This experimental system can also be used to assess the effects of acute, *in vitro* HIV infection on the $CD8^+$ cells within the culture. For $CD3^+CD8^+$ cells, we found that acute stimulation with PHA affected a number of surface antigens. However, only two antigens appear to be additionally affected by infection with HIV: CD25 and HLA-DR. The expression of these antigens on $CD8^+$ cells is increased in clinical HIV infection.[15,18] *$CD8^+HLA\text{-}DR^+$ cells may be associated with less* rapid clinical disease progression.[19] Thus, within our culture system, these cells may represent an attempt to control the acute HIV infection. A decreased expression of CD28 has also been reported for $CD8^+$ T cells of patients with progressive HIV disease.[20–22] We found no stimulation-independent effect of HIV infection on CD28 expression by $CD8^+$ cells within our system.

Our most interesting findings thus far concern the expression of CD80 and CD86 by $p24^+$ $CD3^+$ lymphocytes. CD28 is highly expressed by both $CD4^+$ and $CD8^+$ human peripheral blood lymphocytes; CD28 and the closely related molecule, CTLA-4, have physiological ligands that include CD80 (B7-1) and CD86 (B7-2), expressed on dendritic cells, activated B cells, monocytes, and, rarely, repeatedly activated T cells. CD28/CTLA-4 and CD80/86 have important costimulatory roles in both T lymphocyte proliferation and the generation of cytotoxic T lymphocytes.[23–26] Indeed, evidence suggests that the interaction of these molecules may prevent the induction of anergy following T cell receptor (TCR) stimulation.[27] Further, debatably, the specific molecules (CD80 versus CD86) interacting with CD28/CTLA-4 may influence the cytokine profile expressed by cells following stimulation. B7-1 (CD80) may be associated with precursor commitment to a type 1 cytokine pattern and/or B7-2 (CD86), to a type 0 or 2 cytokine pattern.[28–31]

Long-term human T cell lymphotropic virus type I (HTLV-I) cultures express B7 (CD80/86) on their surface.[32,33] Several HTLV-I-transformed T cell lines were found to express mRNA for B7 (CD80/86).[34] Haffar *et al.*[35] showed similar B7 induction with long-term alloantigen-primed $CD4^+$ T cell lines from HIV-infected patients. B7 expression also increased in cell lines from HIV-negative donors; however, this induction occurred over a much longer time period. We found an upregulation of both CD80 and CD86, specifically on $p24^+$ inoculated cells. These $CD80^+$ or $CD86^+CD3^+$ cells are unlikely to represent misidentification of monocytes as lymphocytes since (1) CD14 staining is minimal within the lymphocyte scatter gate; (2) other antigens present on monocytes did not follow this same pattern; and (3) following HIV infection, monocytes reportedly lose CD80/86 expression.[21]

The described alterations in CD80 and CD86 may affect the course of HIV infection in at least six physiologically meaningful ways, only one of which might benefit the host. First, CD80/86 on the surface of T cells permits direct T–T cell contact through CD28/B7 interaction and perhaps also direct transmission of HIV.[35] Second, B7 cross-linking on activated T cells may produce a protein tyrosine phosphorylation pattern distinct from those seen with signaling through other molecules, leading to clonal expansion of T cells.[36] If these cells are infected with HIV, this could potentiate HIV proliferation. Third, CD28 activation in resting T cells by either CD80 or CD86 reportedly induces the association of phosphatidylinositol 3-kinase (PI3-K) in $CD4^+$ cells far more than in $CD8^+$ cells.[37] When resting T cells were cocultured with CHO cells, if the CHO cells expressed CD80 or CD86, the proliferation of the resting T cells was promoted.[26] In our experiments, CD80/86 induction was seen in resting as well as stimulated cells infected with HIV. Thus, CD80 and CD86 might increase activation of both resting and stimulated HIV-infected T cells, leading to greater viral production. Fourth, if CD28 signal transduction can prevent apoptosis in HIV-infected cell cultures, cross-linking of CD28 with CD80 or 86 on HIV-infected T cells[38,39] might lead to prolonged survival of the latter cells. Fifth, dependent on the pattern and balance between CD80 and CD86 expression on T lymphocytes, the expression of these molecules may contribute to a controversial, theoretical shift from a helper T cell type 1 (Th1) to a Th0/2 pattern with advancing HIV infection.[40,41] It has been suggested that Th2 priming may be more dependent on CD28/B7 interaction than is Th1 priming[31,42] or that CD28 activation promotes Th2 differentiation.[43] Petro *et al.* showed anti-CD80 antibody to be associated with decreased interferon γ production by T cells and anti-CD86 antibody, with decreased production of interferon γ, IL-2, IL-4, and IL-5.[29] This implies that CD28/CD80 engagement favors a Th1 cytokine pattern and CD28/CD86 engagement favors a Th0/Th2 pattern.[29] Results were similar in an experiment using a murine experimental allergic encephalomyelitis model.[28] Thus, the balance of CD80/CD86 upregulation on T cells may influence HIV disease progression. Sixth, and related to the concept of cytokine modulation by CD80/86, interferon γ can lead to an increase in CD80/86 on resting monocytes, while tumor necrosis factor α (TNF-α) can lead to a decrease in CD86 but not CD80 on resting monocytes.[44] Downregulation of CD80 and CD86 on HIV-infected monocytes[21] might be secondary to the cytokine effects of CD80/CD86 induction on HIV-infected lymphocytes. Thus, these T cell alterations might indirectly affect antigen presentation by monocytes, as well as antigen presentation by the T cells themselves.

In summary, we present data from a simple system for examining the effects of experimental, *in vitro* HIV infection. Using advances in cell permeabilization and fluorocytometric techniques, we could differentiate productively infected cells by the presence of cytoplasmic viral p24 antigen. Many findings were consistent with those in the clinical literature or using other experimental systems. Novel data include the association of productive infection with increased expression of CD80 and CD86 by $CD3^+$ lymphocytes. This has implications in terms of HIV disease pathogenesis and, potentially, HIV therapy.

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