Dendritic cells (DCs) are potent antigen-presenting cells (APCs) that have the unique property of being able to generate primary T-cell responses (3, 4, 60). The roles that DCs play in the immunopathogenesis of human immunodeficiency virus (HIV) disease have been extensively studied, but some questions still remain unanswered. It was not clear whether DCs can be directly infected with HIV or if the virus is only present on the cell surface or if both processes occur simultaneously (37, 46, 47). However, recent work has demonstrated that DCs can take up HIV into endosome-like compartments, where it can be transferred to T cells (35). Turnville et al. have demonstrated that DCs transfer HIV type 1 (HIV-1) to CD4+ T cells in two distinct phases (63). In the first phase DCs divert the virus from the endosomesomal pathway to the DC–T-cell synapse, while in the second phase the transmission occurs by de novo infection of the DCs. Study of the immunologic function of HIV-infected DCs, as well as DCs isolated from HIV-infected individuals, has yielded ambiguous results (7, 8, 10, 11, 12, 13, 19, 24, 25, 44, 45, 53). Some reports have identified APC defects with DCs, whereas others have not. This could be partially explained by different effects of HIV infection on DC subpopulations, the purity of the DC population studied, and different percentages of infected DCs. A cloned stable cell line that can be infected with HIV may be useful for studying the effect of HIV infection on DC APC function, although it may not be reflective of the different types of DCs, including Langherhans cells, monocyte-derived, immature, mature, and myeloid and plasmacytoid DCs. There are currently no available cloned and stable human DC lines. However, it is possible to fuse DCs with tumor cells by using either either polyethylene glycol or electrofusion to create a tumor-DC hybrid (54).

We have previously demonstrated that both human peripheral blood monocytes and macrophages are able to fuse with the U937 promonocytic cell line to form stable hybrids (56). Using these cell lines, we demonstrated different monocyte defects after HIV infection (57, 58, 65). These studies have been extended here; we have made human DC hybridomas by fusing immature DCs obtained from peripheral blood monocytes with HGPRT-deficient U937 cells, followed by aminopterin selection. The hybridomas express DC markers and do not express class I and II antigens and have functional properties not found in the U937 fusion partner, including interleukin-12 (IL-12) production and the ability to stimulate allogeneic T-cell responses. In addition, these hybridomas can be uniformly infected with HIV. In the present study we have used one of these cell lines (HB-2) to investigate DC function after HIV infection and have found that infection leads to defective accessory cell function manifested by an inability to stimulate allogeneic T-cell proliferative responses. This is due to multiple factors including gp120, IL-10, and the induction of apoptosis.

**MATERIALS AND METHODS**

**DC generation.** Peripheral blood mononuclear cells were isolated from buffy coats obtained from healthy blood donors by using Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, N.J.) density gradient centrifugation (65). Cells were washed three times with sterile phosphate-buffered saline (PBS) and re-suspended in RPMI 1640 (Life Technologies, Grand Island, N.Y.) supplemented with 10% male AB human serum (Life Technologies) and 1% penicillin-strep-
tomycin-glutamine (Life Technologies). The freshly isolated peripheral blood mononuclear cells were incubated at 37°C in 5% CO2 in culture flasks and allowed to adhere for 45 min. The nonadherent cells were removed by several washes with sterile PBS. The adherent monocytes were then cultured in RPMI 1640 (Life Technologies) supplemented with 1% male AB human serum (Life Technologies) and 1% penicillin-streptomycin-glutamine (Life Technologies) and 100 U of granulocyte-macrophage colony-stimulating factor (BD Pharmingen, San Diego, Calif.)/ml and 1,000 U of IL-4 (BD Pharmingen)/ml. The medium was replaced at day 3 and day 7, yielding immature DCs. On day 7, the medium was replaced and supplemented with tumor necrosis factor alpha (TNF-α; BD Pharmingen) at a final concentration of 5 ng/ml. Cells were cultured in TNF-α-supplemented medium for 3 days to yield mature DCs at day 10.

Fusion and selection of DC hybridomas. Our previously generated HGPRT-deficient U937 promonocytic cell line was fused with DCs in the presence of 35% polyethylene glycol (pH 8.2) (aldehyde free, MW1500; Sigma Chemicals, St. Louis, Mo.) for 8 min (55). A ratio of 10:1 DCs to U937 cells was used to maximize fusion efficiency and to increase chances of DC-U937 fusions. After fusion, the cells were cultured in complete medium (CM), i.e., RPMI 1640 (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies) and 1% penicillin-streptomycin-glutamine (Life Technologies) supplemented with tumor necrosis factor alpha (TNF-α; BD Pharmingen) for 12 weeks in 96-well plates to select products. Cells were fed three times a week with HAT medium. After 10 to 12 weeks, the viable hybridomas were removed from HAT medium and cultured in CM.

HLA typing. The tumor-DC hybridomas (HB), along with the parent U937 cell line, were typed for class I and class II antigens at the Mount Sinai Blood Bank Tissue Typing Laboratory using factors binding CAPS sequences of the class I and class II primers supplied by One Lambda, Inc. (Los Angeles, Calif.). HLA allele designations follow the guidelines of the WHO Nomenclature Committee.

Flow cytometry analysis. The HB-2 cell line was characterized by immunofluorescence staining (41, 56). Immature DCs, the U937 cell line, and the DC hybridomas were stained with fluorescein isothiocyanate (FITC)-conjugated antibodies directed against different cell surface markers, including HLA class I (WB865, a gift from M. G. Mackay, Canadian National Collection, Man- nington, Iowa)/ml and CD40 (626; a gift from Shu Man Fu, University of Virginia), and HLA-DR, CD80, CD83, CD14, CD4, CD54, CD85, CD80, CD86, and CD11c (Pharmingen). Cells were analyzed by flow cytometry (FACSCalibur and Cellquest software), with gating on live cells (56). For intracytoplasmic staining, cells were fixed and permeabilized with 70% ethanol for 30 min at 4°C. The cells were then washed three times with PBS, and the 5145 polyclonal human anti-HIV-1 antibodies (17) or control human antibodies were added for 30 min at 4°C, followed by affinity-purified FITC-conjugated goat anti-human immunoglobulin antibodies (Tago, Burlingame, Calif.), and then analyzed as described above. Our laboratory has routinely used 70% ethanol to permeabilize cells for intracytoplasmic staining (17, 39, 54).

Isolation of purified CD3+ and CD3−CD45RA+ T cells. We isolated purified CD3+ T cells and CD3−CD45RA+ T-cell populations by RosetteSep (Stem Cell Technologies, Vancouver, British Columbia, Canada) (59). RosetteSep is a rapid, easy cell separation kit for the isolation of highly purified CD3 CD45RA+ T cells from whole blood. The cells were obtained by negative selection. Whole blood was added to a RosetteSep cocktail, and cells are cross-linked with tetramer complexes and then incubated at room temperature, layered over Ficoll-Hypaque, and centrifuged for 20 min. The enriched CD3+ CD45RA+ T cells were then isolated.

HIV-1 infection of HB-2 cells. The HB-2 cells were infected with the HIV-1MN (50) T-cell-tropic virus and the HIV-1NL (29) monocytotropic virus. These HIV-1 strains were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS Research, National Institutes of Allergy and Infectious Diseases (Germantown, Md.). A multiplicity of infection of 1 was used to infect the HB-2 cells with both HIV-1MN and HIV-1NL, for 90 min at 37°C, followed by three PBS washes and culture in CM (57). The cells were stained intracytoplasmically to determine the presence of HIV-1 proteins as described above (17). The p24 antigen levels in the culture supernatants were measured at day 5 according to manufacturers’ protocol by using a commercial antigen capture enzyme-linked immunosorbent assay (ELISA; Dupont, Wilmington, Del.) (58).

MLR. The HIV-infected and uninfected HB-2 cells were used as stimulators in unidirectional mixed lymphocyte reactions (MLRs). The stimulator population was irradiated at a dose of 6,000 rads (cesium source). One hundred thousand CD3+ T cells or CD3−CD45RA+ T cells were cocultured with various concentrations of irradiated human DC hybridomas (104 to 107) in 0.2 ml of CM in triplicate in round-bottom plates (Linbro, Oxnard, Calif.) at 37°C in 5% CO2 incubator for 5 days. One hundred thousand T cells in 100 μl were used as the responder cells in all of the experiments. The total cells in the coculture experiments varied from 101,000 (107 HB-2 or 107 HB-2MN) in 1 μl to 110,000 (104 HB-2 or 104 HB-2MN) in 110 μl and 200,000 (107 HB-2 or 107 HB-2MN) in 200 μl. At 16 h before harvesting, 1 μCi of [3H]thymidine (ICN Pharmaceuticals, Aurora, Ohio) was added to each well. The cells were harvested onto glass fiber filters, and incorporated radiolabel was measured by scintillation counting (39).

RESULTS

In this study we generated stable clonal human DC hybridoma cell lines that we used to study DC function after HIV infection. The human DC hybridomas were obtained by allowing peripheral blood monocytes to become immature DCs by treatment with IL-4 and granulocyte-macrophage colony-stimulating factor for 7 days, followed by TNF-α for 3 days to obtain mature DCs. Both immature and mature DCs were then fused with a mutagenized HAT-sensitive U937 promonocyto-cyte cell line. Only the immature DCs were capable of forming stable hybrids. Interestingly, the phenotype of the DC hybridomas (CD80+ and CD86+) represents a mature phenotype. However, this does not necessarily mean that the mature DCs were the fusion partner for the human DC hybridomas. Immature DCs without maturation stimuli (TNF-α, Cpg, etc.) undergo apoptosis, making it unlikely that any of the immature DCs could have matured before fusion with the U937 cell line (4). What more likely happened was that the fusion process itself may have caused some maturation in the immature DCs. Mature DCs were capable of fusion, but such hybrids were unstable in culture. It is possible that the terminally differentiated state of the mature DCs may have precluded the generation of stable cell lines.

Fusion and fusion efficiency. Although the fusion efficiency to make DC-tumor cell hybridomas has been reported to be between 15 and 20% (54), the fusion of the immature DCs with U937 to form stable hybridomas cell lines was an inefficient process. Although conditions were optimized to enhance the possibility of a DC-U937 fusion (10/1 ratio of DCs to 937), only a total of four cell lines were generated. Of the five fusions performed, only one was successful, yielding an overall efficiency of 0.0015%. Even in the successful fusion, the efficiency was only 0.08%. Growth positive wells were noted 10 to 12 weeks after the fusion and were expanded for further analysis. The immature DC hybridomas were relatively stable in con-
Continuous culture for more than 6 months, and the doubling time of the DCs hybridomas was ca. 24 h.

Presence of donor class I antigens on human DC hybridomas, morphological appearance, and adherent growth pattern. In order to document that the growth positive wells represent true hybridomas, we analyzed the expression of class I and class II antigens on these cells. All of the human DC hybridomas came from one fusion. As illustrated in Table 1, a representative human hybridoma cell line, HB-2, from the successful fusion has the A*031012 locus, the B*51011 locus, the C*0701 locus, the DRB3*01011 locus, and the DRB5*01011 locus that were not present on the U937 parent line. Although HLA typing of the donor DCs was not available, the presence of three donor-derived class I antigens and two class II antigens on the HB-2 cells is consistent with fusion and provides proof that the cell lines are true hybridomas. In conjunction with the expression of donor class I antigens, other changes were noted as well. U937 is an oval nondescript cell without any distinguishing features (Fig. 1). In contrast, the four hybridomas have a distinct stellate morphology with dendritic processes similar to primary DCs (Fig. 1). All of the hybridomas grew in an adherent pattern, unlike U937 that grows unattached to plastic surfaces.

Expression of normal dendritic cell antigens and IL-12 production. The human DC hybridomas are representative of

| Class I | HLA type              | Class II | HLA type         |
|---------|-----------------------|----------|-----------------|
| HB-2    | A*0301, A*031012      | DRB1     | DRB1*1401, DRB1*1605 |
| U937    | A*0301                | DRB3     | DRB3*01011      |
|         | B*1801, B*51011       | DRB5     | DRB5*01011      |
|         | Cw*0102, Cw*0701      |          |                 |

Table 1. HLA typing

a HLA typing was performed on the HB-2 human DC hybridoma line HB-2 by using polymerase chain-specific primers for class I and class II.

FIG. 1. Morphological appearance of the human DC hybridomas. Photograph (magnification, ×360) of immature DCs, U937, HB-1, HB-2, HB-3, and HB-9. The human DC hybridomas are larger and have a stellate appearance with dendritic processes similar to the immature DCs but are unlike U937, which is a nondescript cell line that has no remarkable features.
myeloid DCs since they were derived from CD14+ monocytes (40, 51). We will present the results from one representative human DC hybridoma line, HB-2, chosen for its stability and ability to grow in long-term culture. We first determined whether the HB-2 cell line expressed common DCs markers not found on the U937 cells. We stained U937, primary DC and HB-2 cells with antibodies directed against major histocompatibility complex (MHC) class I, MHC class II, CD80, CD86, and CD83 and demonstrated that HB-2 expresses all of these cell surface proteins like primary DCs but unlike U937, which only expresses MHC class I (Fig. 2A). DCs also express CD4, CD11c, and CD40 and have a high surface density of adhesion molecules, including ICAM-1 (CD54) and LFA-3 (CD58), that facilitates interactions with lymphocytes (3, 4,

FIG. 2. Surface expression of DC markers on HB-2 cells. (A) HB-2 cells, U937 cells, and the primary DCs were stained by direct immunofluorescence with FITC-labeled anti-HLA-class I, HLA-DR, CD83, CD80, and CD86 antibodies, along with isotype-matched controls, and then analyzed by flow cytometry, with gating on live cells. These results are representative of an experiment repeated three times. (B) The HB-2 cells, U937 cells, and DCs were directly stained with FITC-labeled anti-CD11c, CD4, CD58, CD54, and CD40 antibodies, along with isotype control antibodies and then analyzed by flow cytometry, gating on live cells. These findings are representative of an experiment repeated three times. (C) Induction of IL-12 production. The HB-2 cells, U937 cells, and DCs were stimulated with anti-CD40 MAb (10 μg/ml) for 48 h. IL-12 production was determined by using an IL-12 p70 antigen capture ELISA. These findings are representative of an experiment repeated three times. *P values (comparing IL-12 production in unstimulated cells and in antibody-stimulated DCs and HB-2 cells) of ≤0.05 are considered statistically significant.
HB-2 cells also expressed CD4, CD11c, CD40, CD54, and CD58, again unlike U937 cells, which did not (Fig. 2B). Analysis of IL-12 secretion by the U937 cells, primary DCs, and HB2 cells revealed no IL-12 production above background in the unstimulated cells, in contrast to CD40-stimulated HB2 cells and primary DCs that secreted significantly large amounts of IL-12 (P = 0.01 and P = 0.02, respectively) (14), unlike the U937 cells, in which IL-12 was not detectable (Fig. 2C). TNF-α and CpG activation of the HB-2 cells produced similar results (data not shown).

**Stimulation of naive and memory T cells.** DCs have the capacity for efficient stimulation of autologous and allogeneic T cells (3, 15). In fact, mature DCs are the most potent APCs and are >100-fold more efficient at stimulating an MLR than are monocytes and are the only cells that can induce primary immune responses (10, 21). We studied the ability of the human DC hybridoma cell line, HB-2, to induce T-cell proliferation in both bulk CD3⁺ and naive T cells. Consistent with the presence of the class II antigens and costimulatory molecules, irradiated HB-2 cells induced allogeneic T-cell proliferation in a concentration-dependent manner like the primary DCs but unlike U937 (Fig. 3). As further evidence that the HB-2 cells were true human DC hybridomas, we investigated whether they could stimulate CD45RA⁻ T-cell proliferation. A dose-dependent increase in T-cell proliferation similar to that seen with the unsorted T-cell populations was observed in both HB-2 and primary DCs but not in U937 (Fig. 3).

**HIV-1 infection of DCs.** DCs are commonly the first immune cells to encounter foreign organisms such as HIV (5, 7, 28, 47). DCs play an important role in the generation of protective immunity but may also be subverted as part of the life cycle of
different pathogens (22, 23). HIV-1 uses receptors expressed by DCs, including DC-SIGN, CD4, CCR5, and CXCR4 for infection (30, 44, 43). HIV-1 can remain dormant in DCs and exploits DC trafficking to lymphoid tissue to infect permissive CD4^+ T cells (31, 33, 46). To investigate whether HIV has any effect on DC accessory cell function, we first infected HB-2 cells with two different strains of HIV: HIV-1 IIIB, a T-cell-tropic strain, and HIV-1BaL, a monocytotropic strain. As noted in the introduction, one problem in studying APC function in HIV-infected DCs is that the percentage of infected cells is small and uninfected cells may give false-negative results in functional assays. A false-negative result means that there were no defects in antigen presentation under experimental conditions since there were sufficient numbers of uninfected DCs to restore normal APC function. As illustrated in Fig. 4, HB-2 cells could be infected with HIV-1IIIB and HIVBaL, as demonstrated by peak shifts corresponding to intracellular virus after staining with the 5145 anti-HIV-1 antibodies.

Effect of HIV infection on stimulation of an MLR. HIV causes many deleterious effects on APCs resulting in defective function. Since HB-2 cells can stimulate both naive and memory T cells, we infected the HB-2 cells with HIVBaL for 10 days to determine whether its accessory cell function was impaired. We then cocultured the HIV-infected HB-2 cells with unsorted alloreactive T cells and with sorted CD45RA^-/HI1001 T cells from two different donors (T-1 and T-2) and assessed proliferation by measuring thymidine incorporation. Unsorted alloreactive (P<0.04 and P<0.05) and CD45RA^-/HI1001 T cells (P<0.01 and P<0.02) cocultured with HB-2 HIV cells infected with HIV BaL failed to proliferate (Fig. 5). There are several possibilities that could explain the lack of T-cell proliferation, including loss of MHC class II and costimulatory and adhesion molecules, altered cytokine production in the HB-2 cells, transmission of HIV to the cocultured T cells, the effect of an HIV protein, or the induction of apoptosis.

Expression of cell surface markers and IL-10 and IL-12 production in HB-2HIV cells. Loss of MHC class II antigens (HLA-DR) and costimulatory molecules (CD80 and CD86) has been reported after HIV infection in monocytes, which could explain the inability of the HB-2HIV cells to induce T-cell stimulation in our system (38, 49, 52). Furthermore, decreased expression of both CD54 and CD58 has been reported in monocytes of HIV-infected patients (61). This would
also contribute to defective HB-2\textsubscript{HIV}–T-cell interactions. To
address the effect of HIV infection on different regulatory
surface proteins, staining with specific MAbs was performed.
Uninfected HB-2 cells express MHC class I, MHC class II,
CD83, CD86, CD80, CD54 and CD58, CD11c, and CD4 (Fig.
2A and B). As illustrated in Table 2, we compared the MFIs
for HLA class I, HLA class II, CD11c, CD40, CD4, CD80,
CD86, CD83, CD58, and CD54 on uninfected HB-2 cells to
the expression antigens on HB-2 cells infected with either HIVIIIB
or HIVBaL. This was determined by dividing the MFI staining
for HLA class I, HLA class II, CD11c, CD40, CD4, CD80,
CD86, CD83, CD58, and CD54 on uninfected HB-2 cells by
the MFIs for the same antigens on the HB-2 cells infected
with either HIV\textsubscript{HIV} or HIV\textsubscript{BaL}. This was determined by dividing the MFI staining
for HLA class I, HLA class II, CD11c, CD40, CD4, CD80,
CD86, CD83, CD58, and CD54 on uninfected HB-2 cells by
the MFIs for the same antigens on the HB-2 cells infected
with HIV\textsubscript{HIV} and HIV\textsubscript{BaL} and multiplying that value by 100. There
was no significant change in the MFIs of class I, class II,
CD11c, CD40 CD4, CD80, CD86, CD83, CD58, and CD54 in
HB-2 cells infected with HIV\textsubscript{HIV} and HIV\textsubscript{BaL} compared to the
uninfected controls. It has been demonstrated in HIV-infected
monocytes that there is loss of IL-12 production and induction
of IL-10 that has been associated with impaired immune re-
sponses (65). It has also been demonstrated that IL-10 can
cause DC apoptosis (42). The lack of myeloid DC activation by
HIV is also consistent with the findings of Fonteneau et al.,
who reported that HIV activates plasmacytoid DCs and in-
duces bystander maturation of myeloid DCs (27). We stimu-
lated the HB-2 cells through CD40 for 48 h with anti-CD40
antibodies to investigate whether there were any changes in
IL-12 or IL-10 production. There was no significant difference
in IL-10 and IL-12p70 production between the uninfected
HB-2 cells and HB-2 cells infected with HIV\textsubscript{BaL} (Fig. 6).

**Effect of antiretroviral therapy and anti-gp120 antibodies**
on T cells cocultured with HB-2\textsubscript{HIV} cells. DC cells can transmit
HIV to T cells that could result in the diminished capacity of
T cells to be stimulated by the HB-2\textsubscript{HIV} cells (33, 48). As
shown in Fig. 5, HIV infection of HB-2 cells significantly re-
duced T-cell proliferation in the MLR ($P = 0.02$). We next
determined the effect of HIV infection of the HB-2 cells on
allogeneic T-cell proliferation by adding the antiretroviral
drugs ddI and IDV to our cocultures. IDV ($P = 0.01$) was
more efficient in suppressing the inhibition of the proliferative

![Figure 5: Stimulation of an MLR.](image-url)
TABLE 2. Expression of cell surface antigens on HB-2 cells infected with HIV$_{HIV}$ and HIV$_{HIV}$.

| Cell type | HIV$_{HIV}$ % Modulation of MFI ± SD in cells | HIV$_{HIV}$ % Modulation of MFI ± SD in cells |
|-----------|---------------------------------------------|---------------------------------------------|
| MHC class I | 91 ± 25                                     | 89 ± 13                                     |
| MHC class II | 87 ± 19                                     | 93 ± 20                                     |
| CD11c      | 84 ± 27                                     | 91 ± 18                                     |
| CD40       | 118 ± 36                                    | 102 ± 25                                    |
| CD4        | 99 ± 34                                     | 116 ± 30                                    |
| CD80       | 90 ± 13                                     | 95 ± 27                                     |
| CD86       | 86 ± 25                                     | 96 ± 28                                     |
| CD83       | 93 ± 17                                     | 87 ± 24                                     |
| CD58       | 94 ± 21                                     | 100 ± 25                                    |
| CD54       | 107 ± 31                                    | 110 ± 25                                    |

Values are expressed as the percent modulation of the control MFI on HB-2 cells infected with either HIV$_{HIV}$ or HIV$_{HIV}$. In these experiments, the MFI for HLA class I, HLA class II, CD11c, CD40, CD4, CD1a, CD80, CD86, CD83, CD58, or CD54 antigens on uninfected HB-2 cells was divided by the MFI staining of the same antigens on HB-2 cells infected with either HIV$_{HIV}$ or HIV$_{HIV}$. This number was multiplied by 100 to determine the percent modulation of the control antigens after HIV$_{HIV}$ and HIV$_{HIV}$ infection. These results represent the findings from three different experiments.

Responses than ddI as expected (Fig. 7A). The combination of ddI and IDV (P = 0.01) was no more effective than either drug alone and significantly blocked p24 production in the cocultures as noted in Fig. 7A but did not completely restore reactivity in the MLR (26). Another possibility is that HIV viral proteins, especially gp120, produced by HIV are well known to cause T-cell dysfunction (18, 62). IDV and other protease inhibitors block HIV proteases that prevent replication of mature virions but do not affect the release of HIV gp120 secreted by immature defective virions (20). In addition, it has been reported that gp120 produced by HIV-infected primary DCs can suppress T-cell proliferation in cocultures without inducing apoptosis (37). To determine whether this was happening in our system, we treated HIV-infected HB-2 cells with the poly-

![FIG. 6. IL-12 and IL-10 production after HIV infection. Supernatants from HB-2 and HB-2HIV cells stimulated with anti-CD40 antibodies (10 μg/ml) for 48 h were analyzed for IL-12 and IL-10 production by using a direct binding ELISA (R&D). NS, not statistically significant. These findings are representative of an experiment repeated three times. Columns 1 to 3 show IL-12 production; columns 4 to 6 show IL-10 production. Columns: 1, HB-2; 2, HB-2 + anti-CD40; 3, HB-2HIV + anti-CD40; 4, HB-2; 5, HB-2 + anti-CD40; 6, HB-2HIV + anti-CD40.](image)

![FIG. 7. Effect of antiretroviral therapy and anti-gp120 and anti-IL-10 antibodies on altered APC function of HIV-infected HB-2 cells. (A) Antiretroviral therapy with anti-IL-10 and anti-gp120 antibodies. A 10 μM concentration of ddI and 10 μg of IDV/ml were added to cocultures of HB-2 and HB-2HIV cells and T cells. Thymidine incorporation was measured by scintillation counting, and p24 antigen levels measured by antigen capture ELISA were assessed simultaneously at day 5 in the cocultures of HB-2, HB-2HIV, and T cells. Then, 10 μg of anti-gp120, 5145 and anti-IL-10 antibodies/ml were added alone or in combination with or without ddI and IDV to cocultures of HB-2 cells, HB-2HIV cells, and T cells. P values comparing either thymidine incorporation or p24 levels between uninfected HB-2-T-cell cocultures and T-cell cocultures are as follows: P = 0.02, DD only; P = 0.01, IDV alone; P = 0.03, ddI + IDV; P = 0.05, ddI + IDV + anti-IL-10 + 5145. Significant changes are denoted by P < 0.05. These results represent the findings from three different experiments.](image)
clonal anti-gp120 antibody 5145 (10 µg/ml) and repeated the MLR (17). Compared to the uninfected HB-2 cells, the 5145 antibodies partially restored T-cell proliferation in the HB-2HIV cocultures (Fig. 7A). We also treated uninfected HB-2 cells with ddI and IDV at the same concentrations that we used with the HB-2HIV cells to ensure that the effects on T-cell proliferation that were observed were not due to the toxicity of the drugs (Fig. 7A).

**Effect of anti-IL-10 antibodies on T-cell proliferation and the induction of apoptosis.** Although there was no upregulation of IL-10 after HIV infection, Granelli-Piperno et al. (33) have reported that cocultures of HIV-infected DCs and T cells suppress allogeneic proliferative responses through the generation of a population of IL-10-producing T cells. To test whether this was occurring in our system, we added anti-IL-10 antibody to the MLRs and determined cellular proliferation by measuring thymidine incorporation. Similar to the results obtained with the 5145 antibody, the addition of anti-IL-10 antibody (10 µg/ml) to the HB-2HIV T-cell cocultures partially restored T-cell proliferation (Fig. 7A). The combination of the 5145 and anti-IL-10 antibodies restored the reactivity in the MLR but was still less than the response observed in the uninfected HB-2 cells. Even the combination of ddI, IDV, anti-IL-10, and 5145 antibodies that suppressed HIV replication (P = 0.01) and blocked IL-10 and gp120 still did not restore complete reactivity (Fig. 7A). Similar to the experiments performed with ddI and IDV, we used uninfected HB-2 in MLRs with 5145 and anti-IL-10 antibodies to ensure that the effects on T-cell proliferation were not due to the toxicities of the antibodies. Another possibility to explain the lack of T-cell proliferation in the HB-2HIV cocultures is the induction of apoptosis.

In support of this, Lichtner et al. have reported that HIV-infected DCs induce apoptotic death in CD4 T cells through the induction of TNF-α-related death factors, including FasL, TRAIL, TNF-α, and TWEAK (39). We determined whether the HB-2HIV-infected cells were inducing apoptosis by measuring T-cell apoptosis assay with annexin V and anti-CD3 antibodies. T cells were cocultured with uninfected HB-2, HB-2HIV, and HB-2HIV cells treated with ddI, IDV, and the 5145 anti-gp120 and anti-IL-10 antibodies. Compared to the uninfected HB-2 cells, T cells cocultured with the HB-2HIV cells underwent more apoptosis than T cells cocultured with HB-2 cells, as determined by the presence of a CD3+ Annexin V...
costaining population with four different donors (Fig. 7B). T cells cocultured with HB-2HIV, along with ddI, IDV, 5145, and anti-IL-10 still underwent apoptosis.

**DISCUSSION**

We have previously reported that macrophages can be fused with an appropriate fusion partner for the generation of immortalized cloned human macrophage hybridomas (55). We have extended these studies to DCs to show that, like macrophages, they can also be fused and immortalized to make hybridomas. Our cell lines were proven to be true hybridomas by the acquisition of donor class I antigens present in the donor cells but not on the U937 parent line (Table 1). In addition, the hybridomas displayed distinctive stellate DC morphology, since they were larger than U937 and grew in an adherent pattern (Fig. 1). Further evidence that our cell lines were true hybridomas came from cell surface staining experiments that demonstrated the presence of DC specific markers (Fig. 2A and B), the capacity to produce IL-12 (Fig. 2C) and induce primary T-cell responses unlike the U937 parent line (Fig. 3). As noted in the Introduction, DCs have been previously shown to be capable of cell fusion forming hybrid cell lines with tumor cells to make cancer vaccines, but these were not clonal or stable in long-term culture (20).

There were no differences between the four human DC cell lines. When we generated the human macrophage hybridomas (55), different populations of macrophages were identified based on staining with $V_{max}$ antibodies (55) that identify macrophages at different anatomical sites (55). The antibodies that were used in these experiments to characterize the DC lines may not completely identify subpopulations of myeloid DCs, so it is conceivable that HB-1, HB-2, HB-3, and HB-9 may still represent different DC subpopulations. Furthermore, culturing DCs from peripheral blood monocytes either represents a limited subpopulation of DCs or, alternatively, only limited numbers of subpopulations of DCs may be capable of fusion to form stable hybridomas. Thus, it is also possible that selection bias may have occurred during the generation of HB-1, HB-2, HB-3, and HB-9, and the experiments presented here may represent events occurring only in a selected DC subpopulation. We are currently performing experiments with HIV-infected primary DCs to compare to the results obtained with the human DC hybridomas. This must be kept in mind since the data from functional studies with different HIV-infected subtypes of DCs, Langerhans cells, monocyte-derived, immature, mature, and myeloid and plasmacytoid DCs have given variable results (7, 8, 9, 11, 12, 19, 24, 25, 43, 44, 45, 53).

Nevertheless, the HB-2 hybridoma cells have many characteristics of normal DCs and were used to assess DC function after HIV infection (Fig. 4). Using this cell line we demonstrated defects in APC function (Fig. 3). The HB-2HIV cells did not stimulate primary immune responses in an MLR (Fig. 5) despite the fact that there were no alterations in MHC class I, class II, CD11c, CD40, CD80, CD86, CD83, CD58, and CD54 expression (Table 2) or IL-12 and IL-10 production (Fig. 6). These results differ from our previous studies with HIV-infected human macrophage hybridomas, where there was loss of MHC class II expression, downregulation of IL-12, and induction of IL-10 (49, 65). Differences in APC function between DCs and monocytes have been observed in parasitic and mycobacterial infections in studies using murine model systems (34, 64).

HIV infection did not activate the DC hybridomas and reduces their intrinsic allostimulatory capacity. As noted above, the lack of myeloid DC activation by HIV is consistent with recent work by Granelli-Piperno et al. (33) and Fonteneau et al. (27). The impaired stimulatory capacity of the HB-2HIV cell was caused by multiple mechanisms, including gp120, IL-10, and induction of apoptosis, unlike the results of other groups that have identified single pathways for the inhibition of T-cell activity (Fig. 7). Kawamura et al. (36), using HIV-infected primary human DCs, have demonstrated that gp120 blocks HIV proliferation in an allo-MLR and that T-cell proliferation was completely restored with sCD4. Granelli-Piperno et al. have shown that HIV-infected DCs induce a T-cell population that produces IL-10 and that proliferation could be restored by using anti-IL-10 antibodies (32). Lichtner et al. demonstrated that HIV-infected DCs induced apoptosis in CD4+ T cells by TNF-α-related death factors, including FasL, TRAIL, TNF-α, and TWEAK (39). Experiments are currently under way to block induced apoptosis by using anti-FasL, antITRAIL, anti-TNF-α, and anti-Tweak antibodies. It has also been reported that thymic cytotoxic factors are released from HIV-infected thymic DCs after HIV infection (6). An inability to induce naive and memory T-cell responses by HIV-infected DCs early in the course of HIV infection either through gp120 and IL-10 or the induction of apoptosis could delay immune responses, allowing for increased dissemination of the virus. Induction of apoptosis by HIV-infected DCs could also contribute to naive and memory T-cell depletion seen in AIDS patients.

In conclusion, DCs can be fused with the U937 cell line to create stable hybridomas, thereby yielding immortalized cell lines. The representative human DC hybridoma cell, HB-2, expresses DC surface molecules found on monocyte-derived DCs, including CD83, and possesses functional characteristics of monocyte-derived DCs, including stimulation of naive T cells and IL-12 production. The HB-2 cell line can be infected with HIV-1 and used as a model to investigate DC T-cell interactions after HIV infection. Using this model system, we have demonstrated impaired APC function manifested by an inability to stimulate an MLR that was caused by multiple mechanisms, including IL-10 and gp120-induced apoptosis. The HB-2 cell line could be a useful model system to study APC function in HIV-infected DCs.

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