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T-Cell-Induced Expression of Human Immunodeficiency Virus in Macrophages

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Macrophages are major reservoirs of human immunodeficiency virus (HIV) in the tissues of infected humans. As monocytes in the peripheral blood do not show high levels of infection, we have investigated the expression of HIV in T-cell-activated, differentiated macrophages. Peripheral blood mononuclear cells were isolated from HIV-seropositive individuals and stimulated with antigens or mitogens, and the nonadherent fraction was removed. Macrophages were cultured alone for 2 weeks, and HIV expression was assessed. Results from p24 antigen capture assays demonstrated that the presence of autologous T cells and concanavalin A or autologous T cells and allogeneic cells for the initial 24 h of culture induced HIV expression in 35 of 47 (74%) HIV-seropositive patients tested. The macrophage monolayers could be immunostained with anti-HIV antibodies to reveal discrete infectious centers, indicating that complete virus replication was occurring in the macrophages and that infection of adjacent cells was mediated by cell-cell contact. Time course studies of the interval of coculture of the adherent and nonadherent cells indicated that 24 h (but not 2 h) was sufficient for induction of HIV in the macrophages. Direct contact between the adherent cells and activated T cells was required as well. Since the presence of autologous T cells also appeared to be necessary, induction of HIV expression in macrophages may be genetically restricted. HIV-seronegative nonadherent cells were able to induce HIV expression in macrophages from HIV-seropositive donors, demonstrating that the virus originated in the monocytes and was reactivated in the context of a classic T-cell-mediated immune reaction. The high percentage of monocytes from HIV-seropositive donors which can be induced to replicate HIV by activated T cells suggests that infection of monocytes may be critical to the pathogenesis of this lentivirus infection.

A hallmark of advanced human immunodeficiency virus (HIV) infection is the depletion of helper T cells in the peripheral blood correlating with increased susceptibility to opportunistic infections. Peripheral blood T cells contain viral nucleic acids as detected by polymerase chain reaction (27) and can be easily induced to replicate virus upon activation. Investigation of the brain (11, 25, 34), lymph nodes (14, 30), and skin (1, 26, 31) has revealed that monocyte/macrophages are the most commonly identified infected cells in the tissues of HIV-seropositive individuals. The source of the macrophage virus may be other cells in the tissues, or more likely, the monocyte/macrophages are infected in the bone marrow or peripheral blood prior to migration into the tissues. Several laboratories have reported that HIV can be cultured from blood monocytes from a majority of infected individuals (4, 8, 10). However, very little virus can be directly detected in monocytes in the blood (27), indicating that the infection in monocytes may be limited to a small percentage of cells which are latently infected. A variety of different culture conditions have been employed to reactivate HIV, and some viral strains obtained in this manner have been used to study HIV infection of macrophages in vitro (5, 7, 8, 10).

It has been reported that function is impaired in macrophages from HIV-seropositive donors and also in cells infected in vitro (17, 24, 29, 32, 33). Such data suggest that macrophage dysfunction could enhance the susceptibility to infection in a host already compromised by T-cell depletion. Since T-helper function is dependent on macrophage processing and presentation of antigen and since T-cell-mediated inflammation involves macrophage recruitment and activation, it has been difficult to determine whether the macrophage impairment is due to direct infection or is a secondary result of T-cell depletion and reduced ability to produce gamma interferon (17).

While HIV infection may affect macrophage function, the metabolic state in turn strongly influences the ability of the virus to replicate. Monocyte/macrophages are remarkably versatile in this respect. The blood monocyte has the ability to exit from the blood and differentiate into a resident cell in numerous tissues with metabolic characteristics specific to that tissue (e.g., Langerhans cells of the skin, microglia of the brain, and Kupfer cells of the liver). Macrophages may act as nonspecific mediators of inflammation or can be directed in the course of an antigen-specific reaction by activated T cells.

While comparative viral tropisms (2, 5, 7) and drug sensitivities (23) of HIV-infected macrophages and T cells have received considerable attention, relatively little is known concerning the consequences of an immune response on HIV replication in the infected monocyte/macrophage. Given the interdependency of T cells and macrophages and the evidence that both can be productively infected with HIV, we initiated experiments to examine macrophage expression of HIV as regulated by T-cell activation. We found that blood monocytes from 35 of 47 (74%) HIV-seropositive individuals could be induced to differentiate and produce HIV in the context of T-cell activation.

MATERIALS AND METHODS

Isolation and growth of macrophages. Peripheral blood mononuclear cells (PBMC) were isolated from HIV-seropos-

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itive and -seronegative donors. Patients and controls were enrolled in studies described elsewhere (28) and included all stages of HIV disease progression. Blood was collected in lithium heparin-coated tubes (VACUTAINER; Baxter, McGaw Park, Ill.), underlayered with Histopaque (Sigma Chemical Co., St. Louis, Mo.), and spun at 200 × g for 25 min at room temperature. The mononuclear band was collected and washed twice in sterile saline. Plasma samples were also frozen and stored. Mononuclear cells were suspended to 3×10^6 to 4×10^6 cells per ml in Iscoves (GIBCO Laboratories, Grand Island, N.Y.) medium with pen/strep and 10% human AB serum (seronegative for HIV, cytomegalovirus, and herpes simplex virus). Cell suspensions (2 ml) were cultured either alone or pooled or were stimulated as described for each experiment in wells of a six-well tissue culture plates for 24 h at 37°C with 5% CO_2. Following incubation, the nonadherent cells were removed and 2 ml of fresh medium (60% AIM V, 30% Iscoves [both from GIBCO], 10% pooled human serum) was added. Serum stocks were obtained from laboratory personnel. In certain experiments, the initial incubation with the nonadherent cells was reduced from 24 to 2 h, as noted in the figure legends. Cultures were fed additional medium every 4 to 5 days, with only small amounts of old medium removed at each point. At 24 h, the adherent cells were greater than 95% esterase positive. As these cells differentiated during the culture period, the intensity of the esterase reaction in all cells diminished but remained detectable. Adherent cells were harvested between 10 and 20 days after the initiation of culture. After removal, the nonadherent population was also cultured and assayed for HIV expression in some experiments. Nonadherent cells remained in the initial medium and were given interleukin-2 (5%) (Cellular Products Inc., Buffalo, N.Y.) at day 7.

Wells of adherent cells to be tested in the HIV p24 antigen capture assay were rinsed with saline, solubilized in 0.5 ml of sample buffer, and stored frozen at −70°C until use. For immunocytochemistry or plaquing, the monolayers were rinsed twice with saline and fixed (methanol or 3% paraformaldehyde) for 15 min and stained directly; or placed on ice for 20 min, removed with a scraper, and dried on polylysine- or organosilane-coated slides; or transferred to HeLa cell monolayers for a 1-week incubation.

To assess DNA synthesis in the monocyte/macrophages during differentiation, some cultures were labeled with ^{3}H-thymidine (30 μCi per well) during the final week of culture. Cells were then removed from the wells as described, dried on slides, and either stained for HIV p24 or directly dipped in NTB2 emulsion (Eastman Kodak Co., Rochester, N.Y.) and developed 6 days later.

HIV assay methods. Cultures were tested for the expression of HIV by p24 antigen capture, immunocytochemistry, and infectious-center assays. p24 antigen capture kits were purchased from Dupont, NEN Research Products, Boston, Mass., and Coulter Electronics, Inc., Hialeah, Fla., and used as directed. All values shown are means of duplicate wells (which varied less than 10%) and represent 0.1 ml (out of 0.5 ml from a confluent 35-mm well) of solubilized cells. Lysed cells were found to contain more p24 protein than did cell supernatants. Standard curves relating optical density readings to protein concentration were derived for each assay to allow comparison across experiments. The lower limit of detection of the p24 antigen capture assay was 10 to 15 pg/ml (at this concentration, the negative and positive controls overlapped), and therefore, the lowest significant level was considered to be 30 pg/ml. The values in the table and figures marked with an asterisk registered at the maximum optical density (2.00) for the enzyme-linked immunosorbent assay reader and were not diluted out for more exact determination.

Characterization of adherent cells and immunocytochemistry. Cells were characterized by immunocytochemistry with anti-Leu M1, M3, 3a, and 3b (Becton Dickinson and Co., Mountain View, Calif.); RCA-1 (Vector Laboratories, Inc., Burlingame, Calif.); anti-HIV p24 (1:10) (Dupont NEN); anti-HIV gp41 (Genetic Systems, Seattle, Wash.); and a protein G-purified human anti-HIV serum (S-Hum 89-3). The details of our immunochemical procedures have been published previously (34). Briefly, biotinylated or anti-species secondary antibodies (Tago, Burlingame, Calif., at 1:100) were used, followed either by avidin-biotin-peroxidase (Vector) or by avidin-fluorescein isothiocyanate (1:40) (Becton Dickinson). Nonspecific esterase activity was determined by using a kit from Sigma. The CD4-transfected HeLa cells were a gift from Bruce Chesbro, Rocky Mountain Laboratory, Mont., as was the protocol for immunochemically identifying the infectious foci.

RESULTS

Macrophages from HIV-seropositive individuals can be induced to replicate HIV by activated T cells. In our initial studies on the induction of HIV type 1 expression in macrophages, we noted that cultured macrophages from separate HIV-positive individuals did not express HIV protein. However, when PBMC from several HIV-infected donors were pooled, given an adherence interval of 24 h (in contact with the pooled nonadherent lymphocytes), significant amounts of HIV p24 were produced within 2 weeks. As macrophages cannot independently initiate an allogeneic reaction, this observation suggested that T cells present for the first 24 h of culture were activated (by the foreign cells) and directed the monocytes to differentiate and express HIV. The induction of HIV p24 expression in seven seropositive individuals is shown in Fig. 1. The adherent cells of each donor were cultured with (i) autologous nonadherent cells, (ii) autologous nonadherent cells and concanavalin A (ConA), and (iii) autologous nonadherent cells and allogeneic (HIV-seronegative) nonadherent cells. In all of these cultures, ConA and all nonadherent cells were removed at 24 h and the adherent cells were lysed at 2 weeks for assay by HIV p24 antigen capture. Adherent cells from six of the seven HIV-seropositive donors tested in this experiment produced appreciable levels of p24 in response to either ConA or allogeneic stimulation. ConA in the absence of nonadherent cells did not induce HIV expression (data not shown). It is unlikely that the virus detected in the macrophages derived from T cells in vitro since (i) the T-cell fraction did not contain detectable virus during the first 24 h of culture, (ii) the adherent cells were greater than 98% esterase positive, (iii) no CD4-positive cells were detected at 2 weeks of culture, and (iv) the culture conditions which were used for macrophages did not contain interleukin-2 and were not favorable for T-cell survival.

Out of a total of 47 populations of monocytes from HIV seropositives tested to date, 35 (74%) have expressed HIV p24 in response to T cells activated by either allogeneic cells or ConA. In the experiment shown in Fig. 1, two donors (841 and 205) expressed p24 in response to either activating agent, while two others (459 and 615) produced p24 with ConA only and two more (1181 and 970) generated detectable p24 exclusively following 24 h of coculture with alloge-

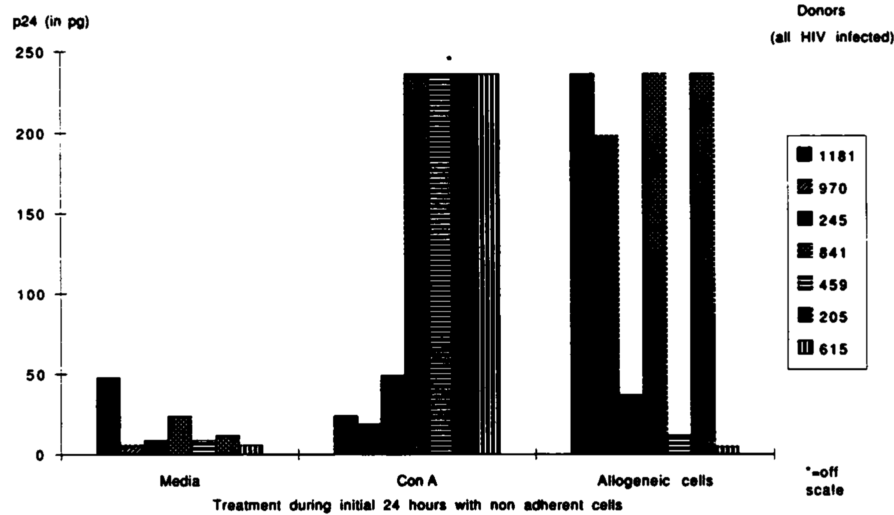

FIG. 1. Expression of p24 in adherent cells of HIV-positive individuals. PBMC were isolated from HIV-seropositive individuals and cultured for 24 h with either medium alone, ConA, or PBMC from HIV-seronegative (allogeneic) donors. All mitogens and nonadherent cells were removed at 24 h, and the adherent cells were cultured alone for 2 weeks. At this time, the adherent cell monolayers were solubilized in sample buffer and tested for HIV p24 expression by an antigen capture assay.

neic cells. Therefore, adherent cells from many HIV-positive donors express HIV p24 following an initial 24-h incubation with autologous nonadherent cells and either foreign lymphocytes or mitogen.

HIV expression by macrophages varies with the interval of coculture of adherent and nonadherent cell populations. To begin investigating the time course and magnitude of increase in HIV p24 expression in macrophages, experiments were designed to test the levels of p24 during the initial phase of culture. The interval of coculture of adherent and nonadherent cells required to induce HIV p24 expression in the adherent population was also examined. Figure 2 illustrates data collected according to two different protocols. In panel 1, unseparated PBMC were solubilized immediately (T0). Separated adherent and nonadherent cell populations were solubilized at 2 h (2 non ad, 2 ad). No p24 was detected at these times. In an experiment not shown in Fig. 2, the p24 value for pooled nonadherent HIV-positive lymphocytes at 24 h was below the lower limit of detection (25 pg/ml) at 24 h but rose to over 400 pg/ml (off the scale) by 2 weeks. The absence of significant levels of HIV p24 at the initial time points suggests, but does not prove, that later production of p24 by the adherent cells was not due to an early transfer of virus from the nonadherent cells.

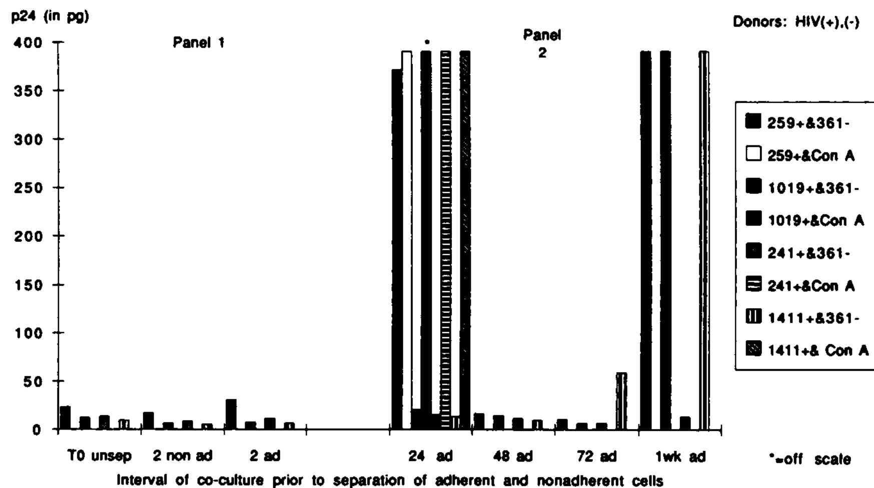

FIG. 2. Expression of p24 in macrophages varies with the interval of coculture of adherent and nonadherent cells. PBMC were isolated from four HIV-infected donors. Samples were taken and assayed for the presence of HIV p24 immediately following isolation (T0), the cells were cultured for 2 h and separated into adherent (ad) and nonadherent (non ad) fractions, and samples were collected for p24 assay as well (panel 1). Panel 2 shows p24 expression of the adherent cells only, from the same infected individuals, after 2 weeks of culture. For this experiment, cells from the donors were cultured with medium alone, ConA, or allogeneic (uninfected) cells. The different time points indicate when the nonadherent cells (and ConA, if included) were removed from the adherent monolayers. Individual PBMC cultured with medium alone produced no p24 (less than 30 pg).

In panel 2 of Fig. 2, the time denotes the separation point of the adherent and nonadherent cell populations. All cultures were harvested at 2 weeks. As shown earlier, separation of the adherent and nonadherent cells at 24 h (where either ConA or allogeneic cells are included) triggers HIV p24 production. Continued coculture for 48 to 92 h abrogated p24 expression. However, coculture for 1 week prior to separation again restored p24 expression by the adherent population. In an additional experiment, we found that coculture of 6 h was insufficient, 18 h was suboptimal, and 36 h was acceptable for induction of HIV expression in macrophages. An assay of nonadherent PBMC from all HIV-seropositive donors tested to date revealed p24 production in excess of 100 pg/ml between 1 and 2 weeks (data not shown).

T-cell-activated macrophages from HIV-seropositive individuals produce replicating virus. In the experiments described above, HIV expression was assayed by p24 antigen levels. Because this *gag* protein appears to be overproduced in macrophages (7) and could, theoretically, be expressed in the absence of viral replication, infectious-center assays were used to detect adherent cell production of infectious HIV. Pooled, individual, and ConA-treated cells from HIV-seropositive and -seronegative donors were cocultured for 24 h as described above, and the nonadherent cells were removed. After 2 weeks, the adherent cells were scraped off the dishes and plated on a confluent monolayer of CD4-transfected HeLa cells. Six days later, these cultures were fixed with methanol and stained with human antibody to HIV or antibody to p24 and infectious centers were detected by immunofluorescence. The results confirmed the pattern observed with p24 antigen expression (Fig. 3). Donors 842 and 843 were HIV seropositive, and when cultured individually, their monocytes yielded no infectious centers (monocytes from 843 are shown in Fig. 3A). However, when their PBMC were pooled prior to the 24-h adherence step, infectious foci were produced (panel B). Pooled macrophages from an HIV-seronegative donor generated no infectious foci, but the addition of macrophages from a seronegative donor to cells from either seropositive donor resulted in infectious centers (panel C). The combination of macrophages from 843 and an HIV-seronegative donor resulted in more infectious foci than the pooling of macrophages from a seronegative donor and 842. Macrophages from 843 activated by ConA also induced virus replication (panel D).

Cultures of pooled macrophages from HIV-seropositive donors (with a 24-h adherence step) were also directly stained with an antibody to HIV p24. Infectious foci (ranging from 104 to 390 in one well) could be easily identified (Fig. 3E). Intensely staining central cells, often with extensive cytoplasm, were surrounded by neighboring cells which produced less p24 as the distance from the central cells increased. In many of these foci, the index cells appeared to be contacting and detaching, creating the beginnings of a plaque. This pattern is a characteristic footprint of viral spread via cell contact. Thus, it appears that the 24-h allogeneic or mitogen stimulation of adherent cells from HIV-seropositive donors results in production of infectious HIV. Quantitation of eight experiments suggests an initial frequency of infection of 1 of 500 to 1 of 1,000 monocytes.

The adherent cells were indeed macrophages, as confirmed by the specific binding of the lectin RCA-1 to their surfaces (Fig. 3F). Although they initially expressed high levels of esterase activity, this diminished with culture, as did levels of the marker identified with the Leu-M1 antibody. A small percentage of adherent cells were CD4 positive when first isolated, but expression of this marker also decreased over time (data not shown). Attempts were made to determine whether the production of virus was coupled to DNA synthesis. Pooled HIV-seropositive macrophages were cultured with [^{3}H]thymidine during the final week, removed from the dish, plated on slides, stained for HIV p24, dipped in NTB2 emulsion, and developed 6 days later. Cells overlaid with grains could be identified (indicating either host or proviral DNA synthesis), as could cells staining for p24 (red) (Fig. 3G and H). Although a rare double-labeled cell was identified, many ^{3}H-labeled cells showed no evidence of p24, and those expressing HIV p24 often were not labeled with ^{3}H. Macrophages labeled by ^{3}H made up 3% of the monolayer. From these preliminary results, it would appear that (i) host cell DNA synthesis is not a requirement for the induction of HIV expression in macrophages and (ii) this assay is not sufficiently sensitive to detect proviral DNA synthesis. However, further investigation of this topic is clearly necessary.

Requirements for macrophage-T-cell contact for induction of HIV. The role of activated T cells and the type of interaction required to induce HIV expression in monocytes is of great interest. Experiments were designed to determine whether direct contact between the macrophages and the stimulated nonadherent cells was required for induction of HIV. Such experiments were possible since adherent and nonadherent populations (donor cells were mixed to induce allogeneic response) which were cocultured for only 2 h showed no induction of HIV in the adherent population at 2 weeks, whereas 24 h of coculture induced significant HIV replication in the adherent cell fraction, as previously shown. Therefore, in the assays shown in Fig. 4, the nonadherent populations were removed from the adherent cells at 2 h rather than 24 h and the attached cell layer was washed twice with saline. Fresh PBMC were added either directly to the adherent monolayer or in a permeable well above, but not in contact with, the adherent cells. All nonadherent cells (including those in the permeable well and the well itself) were removed at 24 h, and the adherent cells were cultured for 2 weeks and assayed for HIV p24 production. Data are shown for pooled (6) and three individual HIV-seropositive donors.

The first experiment, testing pooled cells from HIV-seropositive donors, confirmed that with only 2 h of contact between the adherent and nonadherent cells, no p24 is produced. However, 24-h coculture results in significant levels of p24 at 14 days. The pooled nonadherent cells from the HIV-seropositive donors did produce p24 (<260 pg/ml) when assayed 14 days later, indicating that they did contain HIV. When the pooled HIV-positive nonadherent cells were removed at 2 h and then replaced in a permeable well above the nonadherent cells, no p24 was induced in the adherent cells derived from a seropositive donor. Since the activated T cells in the upper well were unable to initiate HIV replication in the monocytes in the lower well, this result indicated a requirement for cell contact. This conclusion was supported by the observation that supernatants from allogeneic or ConA-stimulated T cells failed to initiate HIV expression in the adherent population (results for HIV-seropositive donor 1186, in picograms of HIV p24 per milliliter: medium, 0; ConA, >1,000; allogeneic supernatant, 0; ConA supernatant, 0). The same protocol was used for the three individual seropositive donors in Fig. 4 (186, 744, and 1769), the results confirming that cell contact was necessary for HIV induction in the adherent monolayer.

The additional observation that HIV-seropositive donor 1769 expressed p24 only when his own nonadherent cells

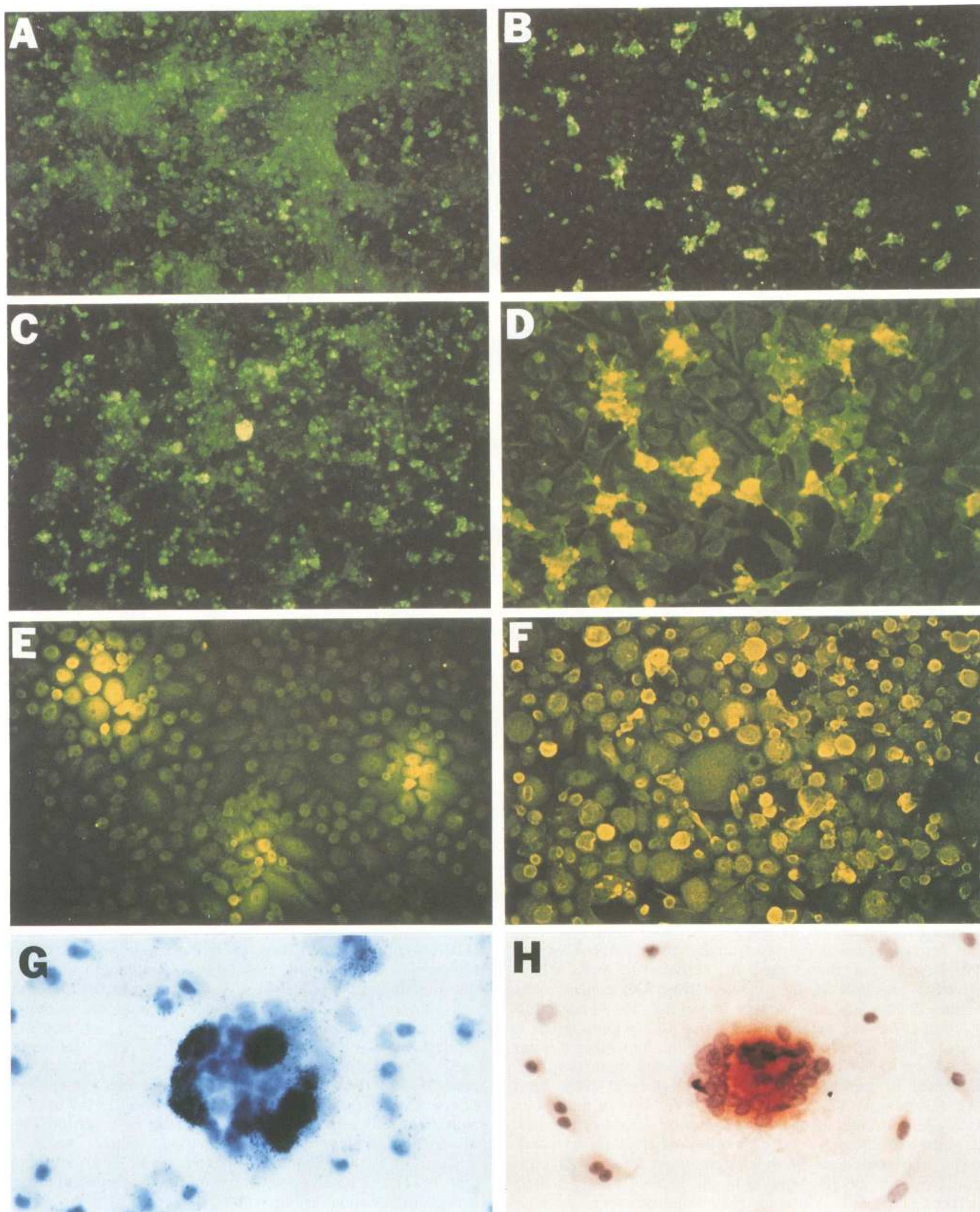

FIG. 3. (A) Monocytes from an HIV-seropositive donor (843) were cultured alone. (In all cultures, nonadherent cells were removed at 24 h.) In panels A to D, adherent cells were removed from the dish at 2 weeks, plated on CD4-positive HeLa cell monolayers for 1 week, and stained with human anti-HIV serum. (B) Pooled monocytes from HIV-seropositive donors 842 and 843 (cells from 842 cultured alone were identical to those in panel A). (C) Monocytes from seropositive donor 842 pooled with two HIV-seronegative-donor cell populations. (D) Monocytes from HIV-seropositive donor 843, cultured with ConA for initial 24 h. (E) Macrophage monolayers derived from a cell pool (two HIV seropositive, one seronegative for panels E to H) stained directly with antibody to HIV p24. (F) Macrophage monolayers stained with RCA-1 lectin. (G) Macrophage monolayers labeled with $[^3H]$thymidine but not expressing HIV proteins (red label). (H) A multinucleated giant cell expressing HIV p24 but no $[^3H]$thymidine uptake.

were present as well as those of the HIV-negative donor 251 suggested that either a seropositive T cell or autologous T cells were required for adherent cells to produce p24. The final result that the pooled, HIV-positive, nonadherent cells were inferior to autologous cells suggests the latter. It should be noted that in the final column for donor 1769 (Fig. 4), the pooled, nonadherent, HIV-seropositive cells contained a small fraction (one-sixth) of autologous cells, while in the other combinations where the donor is listed, that donor represents half of the nonadherent cell population.

Together, the data indicate that efficient production of p24 in macrophages from HIV-seropositive donors requires that (i) there be direct contact between the adherent and nonadherent cells (likely to be monocytes and T cells) and (ii) at least a portion of both cell populations be derived from the same donor. Such requirements and the cell types involved implicate HLA restriction mapping to class II. As can be noted for Fig. 4, treatment of cells with anti-DR blocked the induction of HIV; however, this could occur either at the level of initiation of allogeneic recognition or at the induction of virus expression. Similarly, it was possible, though unlikely within the time frame, that the virus derived from the T cells and that transfer of the virus from the nonadherent to the adherent population was genetically restricted.

Induction of HIV from seropositive-donor macrophages by HIV-seronegative nonadherent cells semicompatible at the HLA DR locus. An investigation as to whether the virus derives from the macrophages themselves or from the activated T cells is clearly necessary for an understanding of HIV pathogenesis. To examine this particular issue without extensive genetic analysis, we chose to limit our study to the induction of HIV expression in monocytes by the addition of nonadherent cells derived from HIV-seronegative donors who were semicompatible (sharing one of the two alleles) at the DR locus. As immune recognition is clonally distributed, the differences would provide the basis for the allogeneic response, while the compatibility could allow for restricted interaction. Since we have shown that removal of autologous HIV-positive nonadherent cells at 2 h yields no virus, it follows that any HIV induced following culture with HIV-negative nonadherent cells must be derived from the HIV-seropositive monocytes.

The data in Table 1 are the results of several experiments designed to determine whether nonadherent cells of HIV-seronegative donors (99 and 98) could induce HIV expression in the monocytes from seropositive donors. Cultures from HIV-seropositive macrophage donors 292 and 209 were both able to express HIV following culture with partially DR-compatible, HIV-negative nonadherent cells. Macrophages from donor 224 could not be induced to express HIV when cultured with the nonadherent cells from donor 292 (DR noncompatible). However, virus could be activated by ConA treatment of 224 monocytes with autologous nonadherent cells, indicating that the lack of expression was not due to an absence of HIV in the monocyte population. Also, p24 was produced in the culture which included both 224 and 292 (adherent and nonadherent) cells during the first 24 h. In contrast to the response triggered by the allogeneic reaction, mitogen-induced induction of HIV in macrophages does not require DR compatibility. Adherent cells from donor 224 could be activated to produce HIV by ConA-stimulated cells from seronegative donors 99 and 98 (>454 pg of p24 was produced with each). Collectively, the data suggest that the HIV expressed by macrophages from HIV-seropositive donors is derived from the monocytes and can be induced by T cells undergoing an allogeneic or mitogenic response.

Cells in Transwell a.	media	media	pHIV+	media	media	media	media	186+&pHIV-	media	media	744+&pHIV-	media	media	media	media
Transwell barrier															
Non adherent cells b.	media	pHIV+	media	pHIV- f.	pHIV+	media	186+& pHIV-	media	media	744+&pHIV-	media	pHIV-	251- h.	1769+&251-	pHIV+
Adherent cells c.	pHIV+e.	pHIV+	pHIV+	pHIV+	pHIV+	186+ g.	186+	186+	744+	744+	744+	1769+	1769+	1769+	1769+
					&anti DR										
p24 in adherent cells d.	9	**115**	15	16	9	11	**37**	17	5	**124***	9	19	22	**74**	33

FIG. 4. Contact between adherent and nonadherent cells is required for induction of HIV. Single-letter abbreviations: a, PBMC, not in direct contact with adherent layer, for 2 to 24 h; b, PBMC in direct contact with adherent layer for 2 to 24 h; c, PBMC which adhered 0 to 2 h, with nonadherent cells removed; d, HIV p24 (in picograms) in adherent cells assayed at 2 weeks; e, pooled HIV-infected-donor PBMC; f, pooled uninfected-donor PBMC; g, individual infected donor; h, individual uninfected donor. Numbers in bold type indicate significant levels of HIV p24; *, off the scale.

DISCUSSION

We have shown that blood monocytes (RCA-1 positive, esterase positive, no CD4 detected) from most (74%) HIV-seropositive donors can be induced to produce HIV by activated T cells. Contact between the monocytes and T cells is necessary, but only for the first 24 h (2 h is insufficient) of culture, after which the nonadherent cells can be removed and HIV expression by the differentiated macrophages can be detected within 2 weeks. The biphasic kinetics, showing maximal p24 production with either a 24-h or 1-week coculture interval, may result from a primary activation from within macrophages followed by a second wave of virus expression representing infection of adherent cells from T cells which became activated. The reduced expression in the wells where nonadherent cells were removed between 48 h and 1 week may relate to the observation that following the initial attachment, many cells detached, only to reattach by 1 week.

We determined that the expression of p24 by the macrophages represents complete viral replication, not simply *gag* protein production. Cultures containing T-cell-activated macrophages could form infectious centers on CD4-transfected HeLa cell monolayers. Also, the confluent macrophage cultures could be immunostained directly, and infectious foci could be identified. Cytopathic effects at the center of the plaque suggested that some macrophages could be eventually destroyed by the infection. The comparative numbers of these foci in each culture correlated well with the pattern of p24 as detected by antigen capture. In the seven different pools of HIV-positive macrophages stained to date, six have shown infectious centers as shown in Fig. 3. Frequency has ranged from 107 to 390 foci in a 35-mm well, and calculations suggest that the frequency of infected monocytes was (initially) 1 in 500 to 1 in 1,000. Furthermore, the appearance of discrete plaques indicates that transmission of infection was mediated primarily by cell contact, and the spread of virus to all contiguous cells within these foci implies that viral replication was not limited to a subpopulation of macrophages. No CD4 was detected on these infected macrophages. Whether this indicates that a different receptor is utilized or that the requirement for a specific receptor is bypassed when transmission is accomplished by cell-cell contact remains to be determined.

The capacity of macrophages to proliferate and the ability of viruses to replicate in nondividing cells are both controversial issues. Experiments in which [^{3}H]thymidine was added to the medium indicated that a subpopulation of monocyte/macrophages were undergoing DNA synthesis. However, the appearance of tritium grains was not exclusively associated with presence of HIV proteins as detected by immunocytochemistry. Also, given the long labeling interval of 4 days to 1 week, it is possible that a subpopulation of macrophages undergo high levels of DNA repair synthesis in certain stages of differentiation. Future experiments include more extensive analysis of this issue.

Experiments which demonstrated that activated T cells could induce macrophages to produce HIV utilized either allogeneic cells or the mitogen ConA. Preliminary evidence suggests that specific antigens can initiate the same effect, and thus it would appear that the consistent success of the allogeneic and ConA stimulation is quantitatively rather than qualitatively unique. The finding that the allogeneic reaction can trigger HIV replication may have an in vivo correlate, since modes of transmission (intravenous and sexual) of this virus often involve the transfer of intact allogeneic cells.

TABLE 1. Induction of HIV in macrophages with nonadherent cells from uninfected donors

Cell populations[a]		HIV p24 in adherent cells at 2 wk (pg)[b]
Adherent	Nonadherent	
209+	209+	18
209+	99− (pc)	**454**[c]
292+	292+	16
292+	99− (pc)	**454**[c]
292+	98− (pc)	**454**[c]
224+ and 292+	224+ and 292+	**438**
224+	292+ (nc)	9
224+	224+	16
224+	224+ and ConA	**454**[c]

[a] +, HIV-infected donor; −, uninfected donor. Nonadherent cells were PBMC in contact with the adherent cells from 2 to 24 h. pc, Partially compatible at the HLA DR with the donor of adherent cells; nc, not compatible. Adherent cells were PBMC which adhered 0 to 2 h with nonadherent cells removed.

[b] Boldfaced values indicate significant levels of HIV p24.

[c] Off the scale.

There appear to be some mechanistic differences in induction by the allogeneic cells versus ConA, since some donor T cells and macrophages could be induced to express p24 when activated with both allogeneic cells and ConA whereas other donors produced HIV only in response to one or the other. These individual patterns may result from differential impairment of certain pathways of activation or depletion of selective T-cell subpopulations in different infected patients. Future studies will include an evaluation of T-cell (and macrophage) competence and number and disease progression with ability to reactivate HIV in monocyte/macrophages.

Monocyte-T-cell contact was necessary for the induction of HIV expression, as shown by experiments in which nonadherent cells were placed either directly over or in a permeable well above the adherent monolayer. Our earlier results demonstrated that a 2-h coculture of the mixed autologous adherent and nonadherent cells was insufficient for virus induction. Thus, the nonadherent population could be removed and replaced with any desired population at 2 h prior to the 2- to 24-h period required for T cells to trigger virus replication in the monocyte/macrophages. Attempts at using a variety of allogeneically activated nonadherent cells to induce virus in macrophages indicated that the presence of autologous cells was usually necessary. In addition, cultures in which cells from numerous (more than five) donors were mixed (reducing the chances for autologous contact) tended to generate less HIV p24 than did cultures consisting of cells from two to three donors.

The requirement for autologous cells implied HLA class II restriction of the monocyte-T-cell interaction, and we were then limited to using HIV-seropositive nonadherent cells. The use of nonadherent cells from HIV-infected donors raised the possibility that the restricted event could be either the transfer of very small amounts of HIV or the differentiation and activation signal from the T cell to the latently infected monocyte. Clearly, this is an important distinction, and we chose to determine the source of the virus by standardizing the HLA class II compatibility to sharing of one of the two alleles at the DR locus. The results demonstrated that nonadherent cells from a semicompatible, HIV-seronegative donor (coculture from 2 to 24 h) could induce the expression of HIV in adherent cells from HIV-seropositive donors, implying that the adherent cell was the source of the virus.

Requirements for direct, HLA DR-restricted, T-cell-macrophage interaction are a fundamental feature of the immune system, specifically T-cell helper function and T-cell-induced, macrophage-mediated delayed hypersensitivity. However, it is generally thought that the restriction functions at the level of antigen presentation to the T cell, and therefore, the observation that contact and genetic restriction might control a signal from the T cell to the macrophage is unusual. It is of interest that preliminary results suggest that although ConA-mediated induction also involved direct contact between the monocytes and T cells, it did not require HLA compatibility (data not shown). We have also observed two donor monocyte populations which produced virus without T-cell contact, but these unusual cultures also expressed significant levels of HIV p24 in the absence of any activated T cells, suggesting that the monocytes may have been preactivated in vivo (data not shown). Macrophages from the eight other donors tested in this protocol did not produce HIV when cultured individually but did when in direct contact with activated T cells.

While induction of HIV requires direct interaction between the monocyte/macrophage and T cells, it is possible that soluble factors are involved as well. A variety of cytokines secreted by activated T cells cause macrophage differentiation, bacteriostatic activity, and formation of multinucleated giant cells (13, 19, 20). Some of these cytokines (such as interferon and interleukin-4) may have opposing effects (16), and future experiments will involve the addition of immune T cells or T-cell products to determine the effect on macrophages which have previously received the signal to replicate HIV. Future studies will also involve investigation of the host metabolic changes which make virus replication possible.

Monocyte/macrophage differentiation is complex. The term macrophage activation implies a common stimulus and endpoint and thus is an inadequate description of the multitude of stimuli which influence macrophage functions as well as the versatility in adaptation to different tissues. It is likely that the emergence of the virus as well as the direct consequences of infection are influenced by both host and viral regulatory elements. Evidence from several groups indicates that effector functions of monocyte/macrophages are decreased following HIV infection. Wahl et al. (33), Poli et al. (24), and Smith et al. (29) have reported that chemotactic activity is diminished in monocyte/macrophages from HIV-infected patients. Murray et al. (17, 18) have also reported that macrophage capacity is diminished by HIV but that it is a secondary effect due to the reduced production of cytokines (primarily gamma interferon) by T cells.

It has been reported in other virus systems that monocyte/macrophages (and other cells) carrying silent virus may undergo metabolic changes which permit virus replication, and the conditions appear to vary with the virus under study. Visna virus, also a lentivirus, replicates in macrophages treated with lipopolysaccharide (6), but this mitogen does not initiate HIV replication (12). Both murine cytomegalovirus and T-cell leukemia virus can reportedly be induced during an allogeneic reaction (9, 22). Crowe and colleagues report that a high level (70%) of monocytes can be infected in vitro (3). Expression of HIV in macrophages can be induced by macrophage colony-stimulating factor, as reported by Gendelman et al. (8). Ho et al. (10) and Gartner et al. (4) have also reported that HIV can be recovered from the monocyte fraction from certain HIV-infected patients without additional factors. However, examination of culture protocols in many of these reports reveals that activated T cells were often added to the HIV-positive monocytes. Therefore, it is possible that the virus was reactivated under conditions similar to those described here but that the role of the allogeneic cells in the induction of HIV expression in macrophages was not investigated.

The requirement for T cells in the induction of viral expression in macrophages also implies that previous assumptions as to the source and mechanisms of recovery of HIV in lymphocyte cultures might be reassessed. In certain reports, the requirement for activated T cells in the recovery of HIV from macrophages has been interpreted as an indication that macrophages could not replicate the virus themselves and that therefore T cells were necessary for rescue of HIV (15). Reinterpretation in the light of the evidence presented here suggests that the addition of activated T cells could have provided the trigger for the cryptically infected macrophages, which were then able to replicate HIV (although T cells appear to produce quantitatively more extracellular HIV once activated) (21). Since macrophages are necessary for T-cell activation and activated T cells markedly affect the function of macrophages, there are obviously opportunities for the exchange of virus, and therefore, the direction of transmission must be carefully investigated. Our results show that T-cell and macrophage interaction in the context of an immune response can initiate virus expression in both cells. Unfortunately, the acquired immunodeficiency syndrome virus undermines the effectiveness of two limbs of the immune response usually involved in the clearance of cell-associated virus. Thus, any immune regulation attempted must take into account the possible positive effects on HIV production as well as the anticipated virus destruction.

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