Infection of Cord Blood Monocyte-Derived Macrophages with Human Immunodeficiency Virus Type 1

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We have investigated the susceptibility of cord blood monocyte-derived macrophages to human immunodeficiency virus type 1 (HIV-1) infection in vitro. Cord blood monocytes were maintained in vitro for 10 to 15 days and then infected with HIV-1. Syncytia were observed 14 days after infection by light microscopy. Viral proteins were detected by immunofluorescence assay. Electron microscopic examination demonstrated typical lentivirus particles within cytoplasmic vacuoles. The supernatants from the HIV-1-infected cultures also contained significant reverse transcriptase activity and p24 antigen. Like adult monocyte/macrophages, cord-derived monocyte/macrophages expressed the CD4 receptor molecule. Pretreatment with blocking antibody prior to infection with HIV-1 Bal significantly reduced or blocked infection of cord monocyte/macrophages. When cord and adult monocyte/macrophages were infected with HIV-1 Bal or Ada-M and directly compared, higher reverse transcriptase activities and p24 antigen expression were obtained with cord monocyte/macrophages. However, no significant difference was found between adult and cord monocyte/macrophages infected with HIV-1 IIIB. These observations suggest that cord monocyte-derived macrophages may be important in the pathogenesis of pediatric AIDS and that the increased susceptibility of cord monocyte/macrophages to HIV-1 infection in vitro may be relevant to the enhanced susceptibility of neonates to HIV-1 diseases in vivo.

Children are among the growing number of individuals who are infected with human immunodeficiency virus type 1 (HIV-1). The majority of these children acquire the infection from the mother in utero or at birth. Children with HIV-1 infection have clinical sequelae different from those observed in adult patients. In infants with AIDS, encephalopathy, bacterial infections, and a unique type of lymphocytic pneumonia occur more frequently than in adults (2, 6, 26, 32, 34). The immunopathogenesis of pediatric AIDS may be partially explained by relative immaturity of the immune system in early infancy, particularly in the neonatal period. Moreover, perinatal HIV-1 disease has a more rapid and fatal course than does HIV-1 disease which occurs in older children or adults. Most infants become symptomatic within the first month of life; however, a subset of infants remains asymptomatic with laboratory evidence of immune abnormalities for years (1, 18, 24, 31). The basis for these differences between pediatric and adult AIDS is unknown. Different strains of the AIDS virus infect not only T lymphocytes but other cells of the immune system, particularly monocytes and their mature form, the macrophages (9, 10, 16, 27). Infection of monocyte/macrophages (M/M) is of major importance because these cells are relatively refractory to the cytopathic effects of HIV-1 and may serve as a major reservoir for the virus as well as a vehicle for disseminating the virus to various tissues, including the central nervous system. Furthermore, by expression of major histocompatibility complex class II antigens, HIV-1-infected monocytes may actually deliver virus to the CD4-positive T lymphocytes during normal antigen presentation (7).

In vitro investigations using adult M/M have demonstrated the important role that these cells play in the immunopathogenesis of HIV-1 infection (4, 5, 11, 21, 29). These cells may contribute to latency of the virus by acting as a viral reservoir. Studies with monocyte-tropic strains of HIV-1 have shown HIV-1 replication within monocyte-derived macrophages (MDM), those with higher virus titers forming large multinucleated giant cell syncytia (4, 29). Once infected in vitro, cytoplasmic intravascular virion accumulation and slow release of viral particles may continue in the cultured MDM for 2 months. Additionally, different HIV-1 strains have been found to be heterogenous in their replication patterns within the MDM.

Much information related to the immunopathogenesis of AIDS has been gained from studies of primary adult MDM for HIV-1 infection in vitro. The role of the MDM in immunopathogenesis of pediatric AIDS has not, however, been determined. The only reported data (abstract) indicate that cord blood macrophages are more readily infected with HIV-1 (JR-FL and JR-CSF strains) than are adult cells (35). We have therefore assessed the replication of HIV-1 in cord blood M/M by using several criteria for viral permissiveness. The results indicate that monocytes derived from cord blood are permissive for HIV-1 infection and that monocyte-tropic strains replicated more efficiently than T-lymphocyte-tropic strains in cord M/M, similar to M/M from adult blood. In an attempt to explain the enhanced susceptibility of human neonates to disease induced by HIV-1, we have also directly compared cord and adult M/M for their susceptibility to HIV-1 infection.

Umbilical venous heparinized cord blood (20 to 50 U/ml) was obtained from the placentas of term newborn infants, immediately after delivery following uncomplicated pregnancies, normal labor, and vaginal deliveries. All cord blood

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TABLE 1. Flow cytometric study of adult and cord M/M^a

Surface marker	% Positive cells			
	Adult M/M		Cord M/M	
	0 day	7 day	0 day	7 day
IgG2a (control)	0.92	0.88	0.72	0.29
IgG2b (control)	0.95	0.99	0.85	0.97
Leu-M3 (CD14)	96.5	81.2	99.2	82.3
OKM1 (CD11b)	97.9	85.6	98.7	88.1
Ia (HLA-DR)	52.5	38.6	49.8	37.8
OKT3 (CD3)	0.72	0.97	0.12	0.25
NKH1 (CD56)	0.41	0.12	0.12	0.14
MY10 (CD34)	0.51	0.3	0.10	0.11
B1 (CD20)	0.71	0.12	0.67	0.20
OKT4 (CD4)	8.74	1.21	7.88	2.12
OKT4A (CD4)	9.21	2.09	8.76	1.21

^a Monocytes were characterized immediately after isolation from PBMC (0 day) and after culture in Teflon vials for 7 days. Binding of MAbs to the surface markers was assessed by flow cytometry.

samples were identified as HIV-1 antibody negative, by enzyme-linked immunosorbent assay (ELISA; Abbott), in an anonymous fashion. Cord monocytes were purified according to our previously described techniques (14, 15). Briefly, blood was layered over LSM lymphocyte separation medium (Teknika Corp., Durham, N.C.) and centrifuged at 1,500 rpm (International Portable refrigerated centrifuge model PR-2; International Equipment Co.) for 45 min at room temperature. The gradient of peripheral blood mononuclear cells (PBMC) was obtained above the Ficoll layer, carefully aspirated, and then transferred to gelatin-coated flasks for adherence. After 45 min in 5% CO₂ at 37°C, all flasks were washed 8 to 10 times with Dulbecco's modified Eagle medium (DMEM; GIBCO Laboratories) to eliminate nonadherent cells. Cells were then exposed to 10 mM EDTA in DMEM containing 20% fetal calf serum. Monocytes were recovered by centrifugation for 10 min at 4°C. The pellet was then resuspended in DMEM containing 20% fetal calf serum (Hyclone Laboratories, Logan, Utah) with penicillin (50 U/ml) and streptomycin (100 U/ml). Cells were plated at a density of 2.5×10^5 per well in 48-well plates (Costar) or 5.0×10^5 per well in 24-well plates (Corning). The cells were cultured in 5% CO₂ at 37°C for 10 to 15 days until the monocytes demonstrated characteristics of macrophages, including morphological changes (spreading and elongation). The medium was changed approximately every 5 days. Following the initial purification, >97% of the cells were monocytes, as determined by morphology and fluorescence-activated cell sorting (FACS) analysis using monoclonal antibody (MAb) Leu-M3 and low-density lipoprotein (LDL) specific for M/M (8, 12). After maintenance of the cells for 10 to 15 days in culture, >99% of the cells were determined to be M/M by the same criteria, and T lymphocytes were undetectable. Peripheral blood was also obtained from HIV-1 ELISA-negative adult volunteers, and monocytes were isolated as described above.

To evaluate the phenotype and purity of the cord MDM preparation, flow cytometric analysis was used to determine the expression of both M/M, T-lymphocyte, natural killer cell, and B-lymphocyte markers on the surface of cord M/M. Similar to adult-derived cells, these cells expressed high levels (greater than 90%) of M/M-specific markers, as detected by MAbs Leu-M3, OKM1, Ia, and My4, and only very low levels (less than 1%) of T-lymphocyte, B-lympho-

FIG. 1. Purity and surface characteristics of cord monocytes immediately after isolation from PBMC. Flow cytometry profiles represent the entire population of cells isolated. Monocytes were stained with MAbs for monocyte markers (My4, Leu-M3, and OKM1), major histocompatibility complex class II expression (I2), CD4 expression (OKT4 and OKT4A), and T-lymphocyte markers (OKT3 and OKT11). Isotype-matched control antibodies are IgG2a and IgG2b.

cyte, and natural killer cell markers, as detected by MAbs OKT3, OKT11, MY10, and B1 (Table 1). These cells also expressed low levels of CD4 markers, as detected by OKT4 and OKT4A (Table 1; Fig. 1). In addition, virtually 100% of cord MDM were stained positive for acetylated LDL as demonstrated by Dil-Ac-LDL assay on living adherent MDM at the time of infection (data not shown).

HIV-1 Bal (9) was isolated from human infant lung tissue, human T-cell leukemia virus type IIIB (HTLV-IIIB) (28) was isolated from peripheral blood or bone marrow, and HIV-1 Ada-M (10, 11) was isolated from peripheral blood lymphocytes. HIV-1 Bal and IIIB were provided by R. C. Gallo's laboratory, and HIV-1 Ada-M was provided by H. Gendelman. All three strains were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Health, Bethesda, Md. Virus stocks (HTLV-IIIB) were prepared in H9 cells. The infectious titers were determined by serial dilutions on H9 cells and then adjusted to 8.0×10^5 50% tissue culture infective doses per ml. HIV-1 Bal and Ada-M stocks were prepared in normal peripheral blood-

FIG. 2. (A and B) Light microscopy of mock-infected (A) and HIV Bal-infected cord M/M (25 days postinfection) showing syncytia (B) ($\times 250$). (C and D) Detection of HIV-1 antigens in mock-infected (C) and HIV-1 Bal-infected (D) adherent cord MDM culture by immunofluorescence assay ($\times 250$). Cord MDM cultured in 24-well plates containing 12-mm coverslips *in vitro* for 12 days were infected with HIV-1 Bal (2×10^6 cpm of RT activity per 5×10^5 cells), fixed 25 days later, and stained for HIV-1 antigens. (E and F) Electron photomicrographs of HIV-1 Bal-infected cord MDM 25 days after infection (E, $\times 12,000$; F, $\times 95,000$).

derived M/M and contained 8.0×10^5 cpm of reverse transcriptase (RT) activity per ml.

The morphology of uninfected cord blood-derived monocytes cultured in 24- or 48-well plates was observed to change over a 2- to 3-week observation period. Shortly after plating, the primary monocytes started to spread and fused into small syncytia. After 10 to 15 days in culture, three predominant morphological cell types were observed: rounded, spindle-shaped, and large multinucleated cells (Fig. 2A). In contrast, HIV-1 Bal-infected companion cultures demonstrated cell death and large syncytium formation (Fig. 2B). The first syncytia were usually observed in the cord MDM culture 2 weeks postinfection, their size and number increased with duration of culture. Syncytia-containing virus particles in cord MDM were also observed by electron microscopy (Fig. 2E and F), with the numerous virions budding from a vesicular complex in the interior of the cell as well as at the cell membrane, similar to HIV-1-infected adult MDM. The various stages of virus budding from the cell membrane were observed infrequently by electron microscopy (data not shown).

To determine the proportion of HIV-1-infected cells and provide direct evidence of HIV-1 antigen expression, HIV-1 Bal-infected adherent cord MDM cells were examined for

viral antigens by immunofluorescence using the antibody (immunoglobulin G [IgG] purified from HIV-seropositive human serum) against HIV-1 antigens 20 to 25 days after infection. At the time of fixation, approximately 30 to 50% of the cord MDM were positive by immunofluorescence for viral antigens (Fig. 2D). These antigens were observed in the cytoplasm and not in nuclei of both mononucleated and multinucleated cells and were usually concentrated in multiple focal accumulations. All three predominant morphological cell types (small round, spindle-shaped, and multinucleated giant cells) were observed to contain viral proteins. Mock-infected MDM showed no fluorescence staining (Fig. 2C).

To determine whether virus particles were released from cord MDM infected with the HIV-1 strains (Bal, Ada-M, and IIB), the supernatants were collected at different time points (every 4 days) postinfection and tested for the presence of p24 viral antigen, using a commercially available ELISA (Coulter, Hialeah, Fla.). Levels of RT activity present in HIV-1-infected cord MDM culture supernatants were determined by the methods of Willey et al. (38) with modification. Significant RT activity and p24 antigen expression were observed 8 to 12 days postinfection with HIV-1 Ada-M or Bal. HTLV-IIIb infection of cord M/M did not

FIG. 3. RT activity and ELISA for the p24 antigen of HIV-1 in supernatants of cord M/M infected with HIV-1 Bal, Ada-M, and IIIB. Supernatants were harvested at the indicated times and assayed for RT activity (A) and p24 antigen expression (B) as determined by ELISA.

show measurable RT activity or p24 antigen until 16 days postinfection (Fig. 3). The maximal RT activity and p24 expression were reached between days 24 and 32. HIV-1 Bal and Ada-M produced much higher levels of RT activity and p24 antigen than did HTLV-IIIB (Fig. 3).

To determine whether HIV-1 utilized the CD4 receptor to infect cord M/M as it does in T lymphocytes and adult M/M (22, 29, 33), we performed blocking experiments with MAb Leu-3a, which recognizes CD4 epitopes corresponding to the binding site for the HIV-1 cell attachment glycoprotein gp120 (25, 33). Ten-day-cultured cord- and adult blood-derived M/M cultures in 48-well plates were treated with or without the anti-CD4 MAb (0.2 to 10 μ g/ml; Leu-3a from Becton Dickinson) for 45 min and then incubated with 100 μ l of HIV-1 Bal at 37°C in the continued presence of Leu-3a. Blocking experiments were carried out at 4°C to inhibit capping and internalization of the CD4 antibody complexes (25). The Leu-3a antibody was kept in the cultures throughout the experiment in order to maintain its effect on viral replication. As a control, we used an IgG2a antibody which is an isotype-matched MAb. Productive infection of both cord and adult M/M cultures by the macrophage-adapted

strain HIV-1 Bal was markedly inhibited by preincubation of the cultures with 2 μ g of Leu-3a per ml and completely prevented by 10 μ g of Leu-3a per ml, as assayed by RT activity in the supernatant fluids (Fig. 4). No significant blocking was seen with Leu-3a at less than 0.8 μ g/ml (Fig. 4). Thus, the efficiency of blocking productive HIV-1 Bal infection was dependent on the concentration of anti-CD4 antibody used during preincubation. Similar blocking patterns were observed with both cord and adult M/M cultures.

To directly compare MDM from cord and adult blood for their susceptibility to HIV-1 infection, simultaneous infections of cord and adult M/M with both HIV-1 lymphocyte-tropic and monocyte-tropic strains were performed. Both adult and cord blood M/M were isolated on the same day and seeded into plates at the same density (5×10^5 cells per well for 24-well plates; 2.5×10^5 cells per well for 48-well plates). The purities of isolated cord and adult M/M were comparable (Table 1). After 10 to 12 days in culture, the M/M derived from both adult and cord blood were infected with HIV-1 strains (Bal, Ada-M, and IIIB) at the same multiplicity of infection (RT activity of 8×10^5 cpm/ml). Higher RT activities and p24 antigen expression were obtained with the

FIG. 4. Inhibition of HIV-1 Bal replication in cord and adult MDM by MAb Leu-3a, which blocks HIV-1 gp120-CD4 binding. Both cord and adult MDM cultured in vitro for 12 days were pretreated with various concentrations of MAb Leu-3a or IgG2a (0.2, 0.8, 2, 5, and 10 μ g/ml) for 45 min at 4°C and then challenged with HIV-1 Bal for 90 min. After the cultures were washed and refed, Leu-3a was replaced and cultures were maintained as described in Materials and Methods. The supernatants were harvested for HIV-1 RT activities 14 days postinfection. Mean percentage inhibition for two experiments is given. Control IgG2a murine MAb was ineffective in inhibiting HIV-1 Bal replication. HIV-1 RT activity in the absence of pretreatment with the MAbs was 12×10^3 cpm/ μ l.

FIG. 5. Comparative studies of HIV-1 infection in cord and adult MDM. Cord and adult monocytes were isolated on the same day and infected with HIV-1 Bal (A and C) or Ada-M (B and D) at the same titer as described in Materials and Methods. The culture supernatants were periodically tested for HIV-1 RT activity (A and B) and p24 antigen expression (C and D). The results shown are means of triplicate cultures and are representative of five independent experiments (five different donors).

cord M/M infected with HIV-1 Bal and Ada-M (Fig. 5) than with the adult blood-derived cells.

In studies of primary cultures of human adult monocytes, several laboratories have demonstrated that monocyte-tropic strains of HIV-1 replicate in these cells *in vitro* (4, 10, 11, 16, 29). It has not, however, been determined whether primary cultures of human neonatal monocytes are susceptible to HIV-1 infection and whether there is any difference in the susceptibility to HIV-1 infection between adult and cord monocyte-derived macrophages. Using HIV-1 Bal and Ada-M strains, well-characterized macrophage-adapted isolates, we efficiently infected cord M/M as determined by RT and p24 antigen assays. Viral antigen expression was observed both intracellularly and in the culture supernatant. Immunofluorescence assays showed that a large proportion of cells were positive for HIV-1 antigens and that infection is associated with significant syncytium formation. In addition, HIV-1 virions were demonstrated within the infected cells by electron microscopy. Similar to results for adult MDM, HIV-1 infection with different strains showed monocyte tropism in cord MDM. The lymphocyte-tropic strain, HTLV-III_B, had the capacity to infect cord MDM, but a high multiplicity of infection was required and the level of virus expression was at least 10- to 15-fold lower than with monocyte-tropic strains (Fig. 3), which is consistent with previous reports of studies using adult M/M (9). Similar to adult M/M, freshly isolated cord monocytes did not initially support efficient HIV-1 replication; however, when cultured *in vitro* for 10 to 15 days, the cord M/M produced high levels of virus 7 to 10 days after HIV-1 Bal or Ada-M infection. Direct comparison between cord MDM and adult MDM demonstrated that cord MDM showed greater susceptibility

to HIV-1 infection with monocyte-tropic strains HIV-1 Bal and Ada-M, which may be related to enhanced susceptibility of neonates to disease induced by HIV-1 *in vivo*. However, no significant differences (RT activity or p24 antigen expression) were found between cord MDM and adult MDM when the cells were infected with HTLV-III_B (data not shown).

Cord M/M are similar to M/M isolated from adults in some of the specific surface markers (Table 1; Fig. 1). The percentage of monocyte-bearing CD14, CDIIb, HLA-DR, and LDL is equivalent to the percentage of adult peripheral blood-derived monocytes exhibiting these receptor markers. However, Kelley et al. reported that HLA-DR, -DP, and -DQ markers were expressed in lower percentages on cord monocytes than on adult peripheral blood monocytes and that the intensity of staining is less on the individual monocytes when analyzed by FACS (23). The cell purity obtained was also comparable to that for adult-derived monocytes, with less than 1% contaminating T lymphocytes, natural killer cells, or early blood precursor cells and B lymphocytes (Table 1). Both cord- and adult-derived M/M showed decreased expression (down regulation) of several markers following suspension culture in Teflon vials for 7 days (CD14, CDIIb, HLA-DR, and CD4; Table 1).

The major cellular receptor involved in the attachment of HIV-1 to cord M/M is the CD4 molecule. HIV-1 infects T lymphocytes through the association of its surface gp120 with CD4. Human adult-derived M/M also express CD4, although various laboratories have reported from 2 to 33% CD4 positive cells (3, 5, 39). Both lymphocyte- and macrophage-tropic strains of HIV-1 utilize the CD4 receptor as an entry pathway for the infection of adult M/M (3, 13, 25, 29, 33). In addition, infection of brain microglial cells by

HIV-1 is CD4 dependent (19). We have shown that the anti-CD4 MAb Leu3a blocked infection of cord M/M by a monocyte-tropic strain (Bal) and that the pattern of such blocking was similar in both adult and cord M/M, although only small percentages of both adult and cord M/M cells showed CD4 positivity by flow cytometry assay (Table 1; Fig. 1). Consistent with previous reports (11, 22, 37), CD4 expression on both cord and adult M/M was decreased at the time (7 to 10 days) of HIV-1 infection. Apparently, susceptibility to infection of both cord and adult M/M is not directly correlated with the level of CD4 expression. Our results, however, do not exclude the possibility that under certain circumstances, HIV-1 may enter macrophages by other pathways as well, such as antibody- or antibody- and complement-mediated entry via Fc or complement receptors, either in conjunction with or independent of CD4 (17, 20, 30, 36). Because HIV-1 strains vary widely in their genetic and biological characteristics, it is important to show that the CD4 receptor entry pathway can be demonstrated with a variety of HIV-1 isolates for cord M/M. In this study, we tested only HIV-1 Bal, which is a well-characterized macrophage-adapted isolate.

The data indicate that monocytes from human cord blood show a high degree of permissiveness for HIV-1 monocyte-tropic strain infection, supporting the concept that monocytes may play an important role in the pathophysiology of congenital AIDS. However, similarities or differences between the cord-derived and adult blood-derived monocytes in HIV-1 permissiveness may be related not to an intrinsic cell property but to the in vitro culture state, and it is possible that the findings do not directly relate to in vivo conditions. At the cellular level, however, these studies offer a basis for further research into the mechanisms of HIV-1 infection in the newborn. Also, HIV-1 infection of cord M/M potentially offers an in vitro model for the evaluation of various modalities of prevention and treatment of pediatric AIDS.

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REFERENCES

- Auger, I., P. Thomas, V. Degruittola, D. Morse, R. Williams, B. Truman, and C. E. Lawrence. 1988. Incubation periods for pediatric AIDS patients. *Nature (London)* 336:575-577.
- Belman, A. L., G. Diamond, D. Dickson, D. Horouplan, J. Llana, G. Lantos, and A. Rubinstein. 1988. Pediatric acquired immunodeficiency syndrome: neurologic syndromes. *Am. J. Dis. Child.* 142:29-35.
- Collman, R., B. Godfrey, J. Cutilli, A. Rhodes, N. F. Hassan, R. Sweet, S. D. Douglas, H. Friedman, N. Nathanson, and F. Gonzalez-Scarano. 1990. Macrophage-tropic strains of human immunodeficiency virus type 1 utilize the CD4 receptor. *J. Virol.* 64:4486-4476.
- Collman, R., N. F. Hassan, R. Walker, B. Godfrey, J. Cutilli, H. Friedman, S. D. Douglas, and N. Nathanson. 1989. Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). *J. Exp. Med.* 170:1149-1163.
- Crowe, S., J. Mills, and M. S. McGrath. 1987. Quantitative immunocytofluorographic analysis of CD4 surface antigen expression and HIV infection of human peripheral blood monocyte/macrophages. *AIDS Res. Hum. Retroviruses* 3:135-145.
- Epstein, L. G., L. R. Sharer, J. M. Oleske, E. M. Connor, J. Goudsmit, L. Bagdon, M. Robert-Guroff, and M. R. Koenigsberger. 1986. Neurological manifestations of HIV infection in children. *Pediatrics* 78:678-687.
- Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* 239:617-620.
- Fogelman, A. M., I. Schechter, J. Seager, M. H. H. Kom, J. S. Child, and P. A. Edwards. 1980. Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophages. *Proc. Natl. Acad. Sci. USA* 77:2214-2218.
- Gartner, S., P. Markovits, D. M. Markovits, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 233:215-219.
- Gendelman, H. E., J. M. Orenstein, L. M. Baca, B. Weiser, H. Burgers, D. C. Kalter, and M. S. Meltzer. 1989. The macrophage in the persistence and pathogenesis of HIV infection. *AIDS* 3:475-495. (Editorial review.)
- Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, M. Mitra, T. Phipps, L. Wahl, H. C. Lane, A. S. Fauci, O. S. Burke, D. Skillman, and M. Meltzer. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J. Exp. Med.* 167:1428-1434.
- Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA* 76:335-337.
- Harbison, M. A., J. M. Gillis, P. Pinkston, R. A. Byan, R. M. Rose, and S. M. Hammer. 1991. Effects of recombinant soluble CD4 (rsCD4) on HIV-1 infection of monocyte/macrophages. *J. Infect. Dis.* 161:1-6.
- Hassan, N. F., D. E. Campbell, and S. D. Douglas. 1986. Purification of human monocytes on gelatin-coated surfaces. *J. Immunol. Methods* 95:273-276.
- Hassan, N. F., J. R. Cutilli, and S. D. Douglas. 1990. Isolation of highly purified human blood monocytes for in vitro HIV-1 infection studies of monocytes/macrophages. *J. Immunol. Methods* 130:283-285.
- Ho, D. D., R. T. Rota, and M. S. Hirsch. 1986. Infection of monocyte/macrophages by human T lymphotropic virus type III. *J. Clin. Invest.* 77:1712-1715.
- Homsy, J., M. Meyer, M. Tateno, S. Clarkson, and J. A. Levy. 1989. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection of human cells. *Science* 244:1357-1360.
- Johnson, J. P., P. Nair, S. E. Hines, S. W. Sieden, L. Alger, D. R. Renie, K. M. O'Neil, and R. Hebel. 1989. Natural history and serologic diagnosis of infants born to human immunodeficiency virus-infected women. *Am. J. Dis. Child.* 143:1147-1153.
- Jordan, C., B. A. Watkins, C. Kufta, and M. Dubois-Dalcq. 1991. Infection of brain microglia cell by human immunodeficiency virus type 1 is CD4 dependent. *J. Virol.* 65:736-742.
- Jouault, T., F. Chapuis, R. Oliver, C. Parravicini, E. Bahraoui, and J. C. Gluckman. 1989. HIV infection of monocyte cells: role of antibody-mediated virus binding to Fc-gamma receptors. *AIDS* 3:125-133.
- Kalter, D. C., M. Nakamura, J. A. Turpin, L. M. Baca, D. L. Hoover, C. Dieffenbach, P. Ralph, H. E. Gendelman, and M. S. Meltzer. 1991. Enhanced HIV replication in macrophage colony-stimulating factor-treated monocytes. *J. Immunol.* 146:298-306.
- Kazazi, F., J. M. Mathijs, P. Foley, and A. L. Cunningham. 1989. Variation in CD4 expression by human monocytes and macrophages and their relationship to infection with the human immunodeficiency virus. *J. Gen. Virol.* 70:2661-2672.
- Kelley, V. E., W. Fiers, and T. B. Strom. 1984. Cloned human interferon- γ , but not interferon- β or - α , induces expression of HLA-DR determinants by fetal monocytes and myeloid leukemic cell lines. *J. Immunol.* 132:240-245.
- Lampert, R., J. Milberg, R. O'Donnell, A. Kristal, and P. Thomas. 1989. Life table analysis of children with acquired immunodeficiency syndrome. *Pediatr. Infect. Dis.* 5:374-375, 1986.
- McDugal, J. S., J. K. A. Nicholson, G. D. Cross, S. P. Cort, M. S. Kennedy, and A. C. Mawle. 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule:

- conformation dependence, epitope mapping, antibody inhibition and potential for idiotypic mimicry. *J. Immunol.* 137:2937-2944.
26. Oleske, J., A. Minnefore, R. Cooper, K. Thomas, A. della Cruz, H. Ahdieh, I. Guerrero, V. V. Foshi, and F. Desposito. 1983. Immune deficiency syndrome in children. *JAMA* 249:2345-2349.
 27. Popovic, M., and S. Gartner. 1987. Isolation of HIV-1 from monocytes but not T-lymphocytes. *Lancet* ii:916-918.
 28. Popovic, M., M. G. Sarngaharan, E. Read, and R. C. Gallo. 1984. Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224:497-500.
 29. Potts, B. J., W. Maury, and M. A. Martin. 1990. Replication of HIV-1 in primary monocyte cultures. *Virology* 175:465-476.
 30. Robinson, W. E., D. C. Montefiori, and W. M. Mitchell. 1988. Antibody-dependent enhancement of human immunodeficiency virus type 1 infection. *Lancet* i:790-794.
 31. Rogers, M. F., P. A. Thomas, E. T. Starcher, M. C. Noa, T. J. Bush, and H. W. Jaft. 1987. Acquired immunodeficiency syndrome in children: report of the Centers for Disease Control natural surveillance, 1982-1985. *Pediatrics* 79:1008-1014.
 32. Rubenstein, A., M. Sicklick, A. Gupta, L. Bernstein, N. Klein, E. Rubenstein, I. Spigland, N. Litman, H. Lee, and M. Hollander. 1983. Acquired immunodeficiency with reversed T4/T8 ratios in infants born to promiscuous and drug addicted mothers. *JAMA* 249:2350-2356.
 33. Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverly. 1986. Epitopes of the CD4 antigen and HIV infection. *Science* 234:1120-1123.
 34. Scott, G. B., B. E. Buck, J. B. Leterman, F. L. Bloom, and W. P. Parks. Acquired immunodeficiency syndrome in infants. *N. Engl. J. Med.* 310:76-81. 1984.
 35. Sperduto, A. R., Y. J. Bryson, and I. S. Chen. 1990. Effect of HIV-1 infection of neonatal macrophages. *Int. Conf. AIDS* 6:137 (abstr. FA247).
 36. Takeda, A., C. U. Tuazon, and F. A. Ennis. 1988. Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. *Science* 242:580-583.
 37. Valentin, A., S. Matsuda, and B. Asjo. 1990. Characterization of the in vitro maturation of monocytes and susceptibility to HIV infection. *AIDS Res. Hum. Retroviruses* 6:977-978.
 38. Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. Capon, and M. A. Martin. 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J. Virol.* 62:139-147.
 39. Wood, G. S., N. L. Warner, and R. A. Warnke. 1983. Anti-leu-3T4 antibodies react with cells of monocyte/macrophage and langerhans lineage. *J. Immunol.* 131:212-216.