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Interaction of Human Immunodeficiency Virus Type 1, Human T-Cell Leukemia/Lymphoma Virus Type I (HTLV-I), and HTLV-II with In Vitro-Generated Dendritic Cells

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Although it is known that impairment of dendritic cells (DC) plays a role in the pathogenesis and immunosuppression of retrovirus-associated diseases, it is not clear whether, or to what extent, these antigen-presenting cells themselves become infected. The realization that the cells can be generated in vitro in larger numbers than can be isolated from circulating blood or bone marrow raised the possibility that they could be used for therapeutic purposes. Therefore, we investigated whether DC generated in vitro from CD34 precursors are susceptible to infection when cocultured with human immunodeficiency virus type 1- or human T-cell leukemia/lymphoma virus-infected cell lines. While there appears to be a remarkable affinity of the viruses for the plasma membranes of the DC, interiorization or budding was not observed in 30 experiments carried out under a variety of conditions.

Dendritic cells (DC) and their variants, such as the Langerhans cells in the skin, constitute a widely distributed system of antigen-presenting cells, which play a critical role in the immune response (for a review, see references 40 and 41). Under physiologic conditions, lymphocytes are often seen to cluster around DC, and in vitro, the cells are able to activate heterologous lymphocytes in the absence of an antigen (10, 19, 44). A very small number of DC is needed to effect such responses as allograft rejection, T-cell cytotoxicity, contact sensitivity, and formation of antibodies to thymus-dependent antigens (10, 17). DC are also efficient in the defense against viruses (16, 18). It is thus not surprising that the recognition of human retrovirus infections and their concomitant life-threatening immunosuppression has spurred widespread interest in this cell lineage.

To date, most of the studies concerned with the relationship of DC and human retroviruses have dealt with human immunodeficiency virus type 1 (HIV). Early in the AIDS epidemic, it had been noted that HIV-infected patients have decreased numbers of epithelial Langerhans cells (2, 11, 43), and HIV infection of these cells was confirmed subsequently (3, 14). Whether circulating blood DC of AIDS patients are infected is much less clear. While some observers have reported that DC obtained from healthy individuals are susceptible to infection with HIV in culture (23, 33), a very carefully conducted study carried out with freshly isolated cells put these observations in question (4). Despite these controversies, few investigators doubt that DC play a major role in the pathogenesis and evolution of AIDS. The cells are known to entrap viruses in their long processes, and they may thereby promote the rate of activation and infection of clustering T lymphocytes. This is believed to be responsible for the high virus load in lymphoid tissue, even during the clinically latent phase of the infection (9, 12, 30, 31, 39). In addition, there are studies suggesting that the DC of AIDS patients are functionally impaired (8, 25). This could explain the defective immune response to recall antigens of HIV-infected individuals in the presence of normal CD4 T-cell counts.

The DC of patients infected with human T-cell leukemia/lymphoma virus type I or II (HTLV-I or -II, respectively) have received much less scrutiny. While the involvement of Langerhans cells in mycosis fungoides, a condition recently proven to be an HTLV-I-associated disease (28, 29), has been pointed out repeatedly (1, 26, 35), whether the cells are actually infected is still controversial. However, it is well recognized that asymptomatic carriers of HTLV-I, as well as patients who have developed diseases caused by this virus, are severely immunocompromised (15, 45).

The studies reported here were undertaken to explore whether DC generated in vitro from normal hematopoietic precursors are susceptible to infection with human retroviruses. If they are not, it may be possible to use such cells therapeutically, e.g., to boost the immune responses of infected patients suffering from intercurrent opportunistic infections. This paper conveys our observations in 30 experiments in which in vitro-generated DC were cocultured with either HIV- or HTLV-infected cell lines. While the viruses were shown to have a remarkable affinity for these antigen-presenting cells, interiorization or budding was never observed.

MATERIALS AND METHODS

Isolation of cells. Neonatal cord blood was collected into sterile heparinized containers at the time of delivery. It is important to mention that these specimens should not be refrigerated to prevent undue clumping of cells. The blood was diluted 1:1 with Ca- and Mg-free phosphate-buffered saline (PBS) and subjected to Ficoll-Hypaque centrifugal fractionation as described before (48). The cells at the interphase were washed twice with PBS containing 1% fetal calf serum (FCS). The final pellet was resuspended in the same medium with the cell concentration ranging from 10^6 to 10^8 per ml and placed on ice. Murine monoclonal antibody to human CD34 cells, at 30 μl/ml (HPCA-I; Becton Dickinson, Bedford, Mass.), was added for 30 min with frequent gentle agitation. This was followed by two washes with Hanks' balanced salt solution containing 1% FCS and resuspension of the cells in balanced salt solution plus 1% FCS with the addition of 50 to 75 μl of sheep anti-mouse immunoglobulin G1-coated magnetic beads (Dynabeads; Dynal, Great Neck, N.Y.) for 45 min at 4°C with intermittent agitation. The beads with attached cells were removed with a magnet. They were then sedimented and resuspended in Iscove's modified Dulbecco's medium (Gibco-BRL Life Technologies, Gaithersburg, Md.) containing 20% FCS in a final volume of 1 ml. This was followed by incubation overnight at 37°C in one well of a six-well tissue culture plate, which sufficed to permit detachment of the

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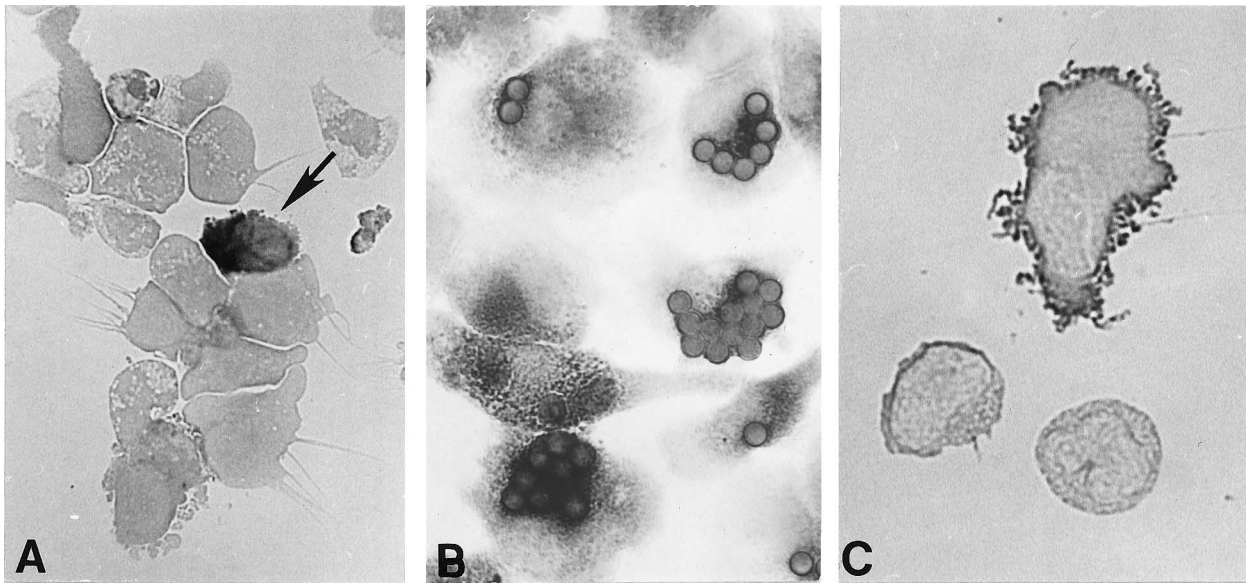

FIG. 1. Identification of DC generated from CD34 precursors. (A) Cell staining for α-naphthylbutyrate esterase (as described in reference 46) characterizes the monocyte (arrow), whereas DC (note the long processes) are negative. (B) Monocytes have phagocytosed magnetic beads and show the reaction product for α-naphthylacetate esterase, whereas DC did not phagocytose or stain. (C) DC stained for factor XIIIa by a peroxidase-antiperoxidase method (6). The smaller, smooth-surfaced mononuclear cells are negative.

$CD34^+$ cells from the beads. The specimen was then resuspended in 15 ml of medium and reexposed to the magnetic field several times to remove most of the beads. The detached cells were cultured in a 35-mm-diameter petri dish in a medium consisting of 3 ml of RPMI medium, 10% FCS, 200 U of granulocyte-macrophage colony-stimulating factor (Collaborative Bioproducts, Bedford, Mass.) per ml, and 75 ng of tumor necrosis factor alpha (Genzyme, Cambridge, Mass.) per ml, essentially as recommended for the generation of DC by a previously published method (5, 36, 37). The cultures did not require feeding for the first 3 or 4 days, after which half of the complete medium, which contained granulocyte-macrophage colony-stimulating factor, was gently replaced at 3-day intervals. The cells were used for analyses and/or coculture with virus-infected cell lines 8 to 14 days after their isolation. Although the day on which the largest number of typical DC had been generated varied from specimen to specimen, the optimal day for harvesting DC seemed to be day 10 after isolation of $CD34^+$ cells.

Virus-infected cell lines. The prototypic HTLV-I- and HTLV-II-infected cell lines C91PL (34) and MoT (38) have been maintained in this laboratory for use in this and other studies (47). Experiments with HIV were carried out with the prototypic HIV-infected cell lines H9 and CEM, which are maintained in the laboratory of Fred Valentine at our institution. In addition, HIV isolate 615, a syncytium-inducing isolate which had originally been obtained from a symptomatic patient with a $CD4^+$ T-lymphocyte count of 19, was expanded in phytohemagglutinin-stimulated normal peripheral blood mononuclear cell cultures, producing virus "factories" in which 45 to 65% of the cells were gp120 positive by fluorescence-activated cell sorter (FACS) analysis. On two occasions, cell-free HIV particles derived from an acute serum converter were prepared for us by Y. Cao at the Aaron Diamond Center.

Coculture of viruses with DC. Coculture of DC with virus-infected cell lines was done on 30 different occasions: 12 with HTLV-I, 8 with HTLV-II, and 10 with HIV. Because of the high growth rate of C91PL cells, they were cocultured with DC at a ratio (C91PL to DC) of 1:10, whereas the MoT/DC ratio was 1:3 and the ratio of DC to HIV-infected cells was 1:1. The cultures were maintained in complete medium for 24 to 72 h. In some instances, the incubation time was extended to 96 h. At the end of the culture period, the specimens were exposed to monoclonal anti-CD1a at 4°C. It should be noted that the CD1a epitope is very sensitive to fixatives and must, therefore, be reacted with the antibody prior to fixation. The method used for immunoelectron microscopy has been described in detail before (47). All labelling steps were performed with unfixed cell suspensions at 4°C. Cultured DC were washed in 1% bovine serum albumin in PBS (BSA-PBS) and then resuspended in a 1:20 dilution of anti-CD1a monoclonal antibody (Biosource International) and incubated for 30 min with agitation on ice. Following three washes in BSA-PBS, the cells were incubated with goat anti-mouse immunoglobulin G-coated 15-nm-diameter colloidal gold particles (E. Y. Laboratories, San Mateo, Calif.) for 30 min with agitation on ice and then washed once in BSA-PBS and fixed in 3% glutaraldehyde for routine electron microscopy. Histochemical stains for α-naphthylacetate esterase and α-naphthylbutyrate esterase have been described elsewhere (24, 46).

RESULTS

Characterization of cultured cells. The following criteria were used to identify DC. First, it was ascertained that the morphology of the cultured cells concurred with that of DC by light and electron microscopy (Fig. 1 and 2). In contrast to monocytes/macrophages also present in the cultures, the DC were shown to be negative for nonspecific esterase (α-naphthylacetate esterase staining kit from Sigma Diagnostics, St. Louis, Mo.) (Fig. 1A). The cells were neither adherent nor phagocytic, as evidenced by their inability to phagocytose Dynal beads, which had not been removed by the magnet and which were avidly phagocytosed by macrophages, as had also been observed by others (36) (Fig. 1B). As reported elsewhere (5), between 20 and 40% of the cells were positive for CD1a, as shown by indirect immunofluorescence microscopy and FACS analysis with a mouse monoclonal antibody (data not shown). In addition, the cells were shown to be positive for factor XIIIa (Fig. 1C), a prominent characteristic of DC in normal as well as inflamed skin (36). The identity of the cells was further confirmed on the ultrastructural level (Fig. 2), by the immunogold technique (Fig. 3A and B) and by the occasional presence of Birbeck granules (Fig. 3A and B).

The remarkable interaction of the virus particles with DC is illustrated in Fig. 3. Although the particles were seen in close apposition to the plasma membrane of the DC within 24 h of incubation, interiorization of the particles or budding was not observed, even when the cells were cocultured for 96 h. Further prolongation of the culture period was not deemed useful, as DC start to lose several of their characteristics, and 48 h is considered adequate for the infection of other mononuclear cell types with these viruses.

DISCUSSION

After having generated DC from 30 different cord blood samples and having cocultured the DC with HIV- or HTLV-releasing cell lines as well as with cell-free HIV particles for

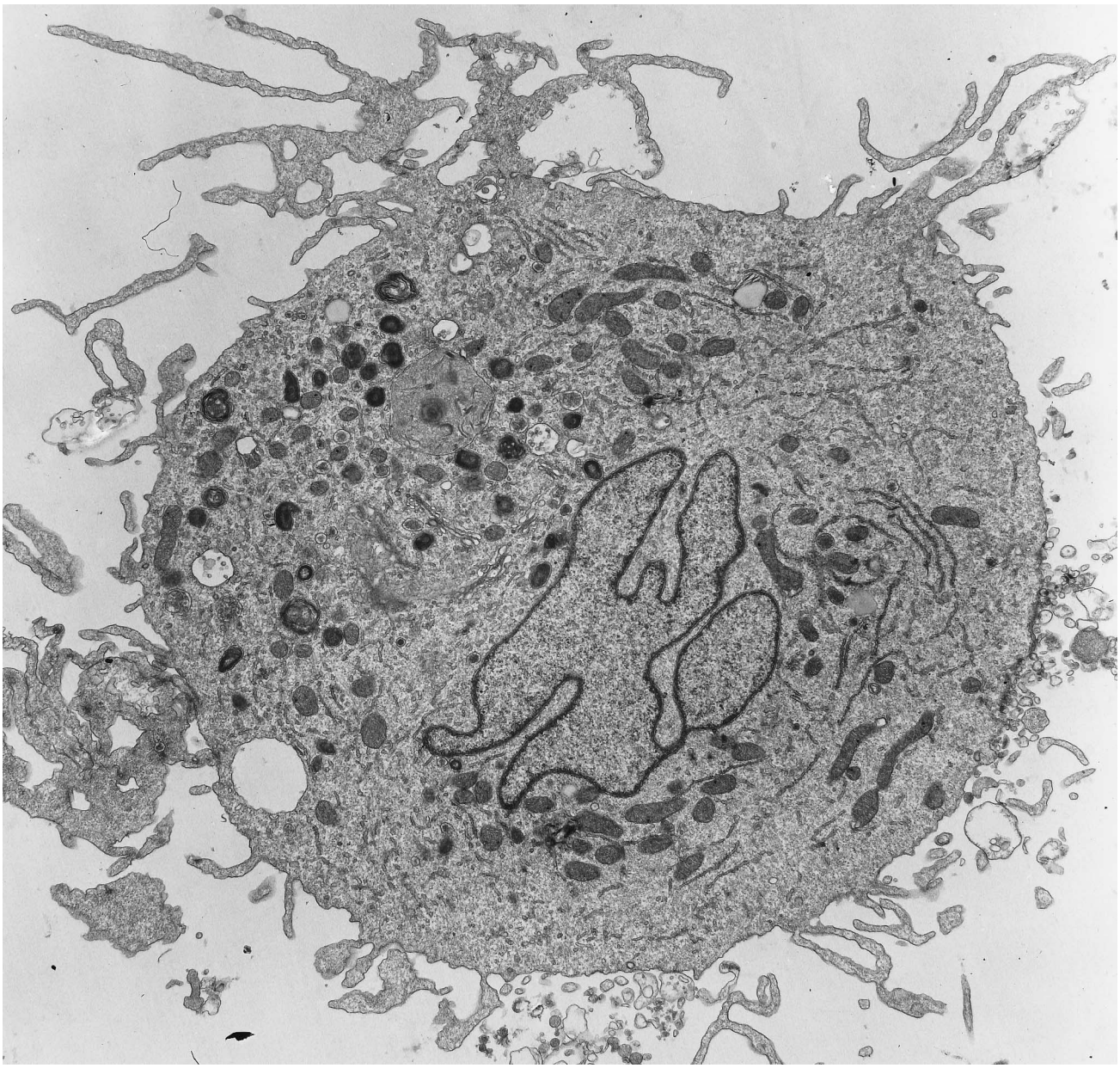

FIG. 2. Ultrastructure of a typical DC. Magnification, ×9,000.

time intervals ranging from 24 to 96 h without having seen any evidence of virus uptake, it may be valid to conclude that the cells are not susceptible to infection under these conditions. Moreover, no reverse transcriptase was detected in the supernatants of DC cultures which had received cell-free HIV particles. On the other hand, the number of particles seen closely associated with the plasma membrane of the DC was impressive (Fig. 3A to C). It is almost redundant to mention that peripheral blood mononuclear cells obtained from healthy donors are easily infected when cocultured with HIV- or HTLV-producing cell lines under the same conditions. Although the results derived from these experiments must be considered negative, the information obtained from them may be very important. It has become clear that the loss or reduction in the number of $CD4^+$ T lymphocytes cannot entirely account for the immunologic defect(s) exhibited by HIV- or HTLV-infected patients. Opportunistic infections are often seen in HIV-seropositive individuals who have normal $CD4^+$ cell counts (7). In addition, the recrudescence of tuberculosis in patients with AIDS does not necessarily correlate with the decrease in their $CD4^+$ T-cell counts (20). Evidence for subtle immune suppression is often manifested by asymptomatic carriers of HTLV-I, as exemplified by their low level of response to purified protein derivative (42), the occurrence of infectious dermatitis in Jamaican children (21), and dissemination of parasitic infestations such as strongyloidiasis (27, 32). The severe immunologic impairment of patients with adult T-cell leukemia/lymphoma is generally recognized. Because in many of these situations a reduction in the number or function of $CD4^+$ T lymphocytes cannot be demonstrated, it has been proposed that the afferent arm of the immune response may be dysfunctional (8, 25). As reviewed in the introduction, DC are

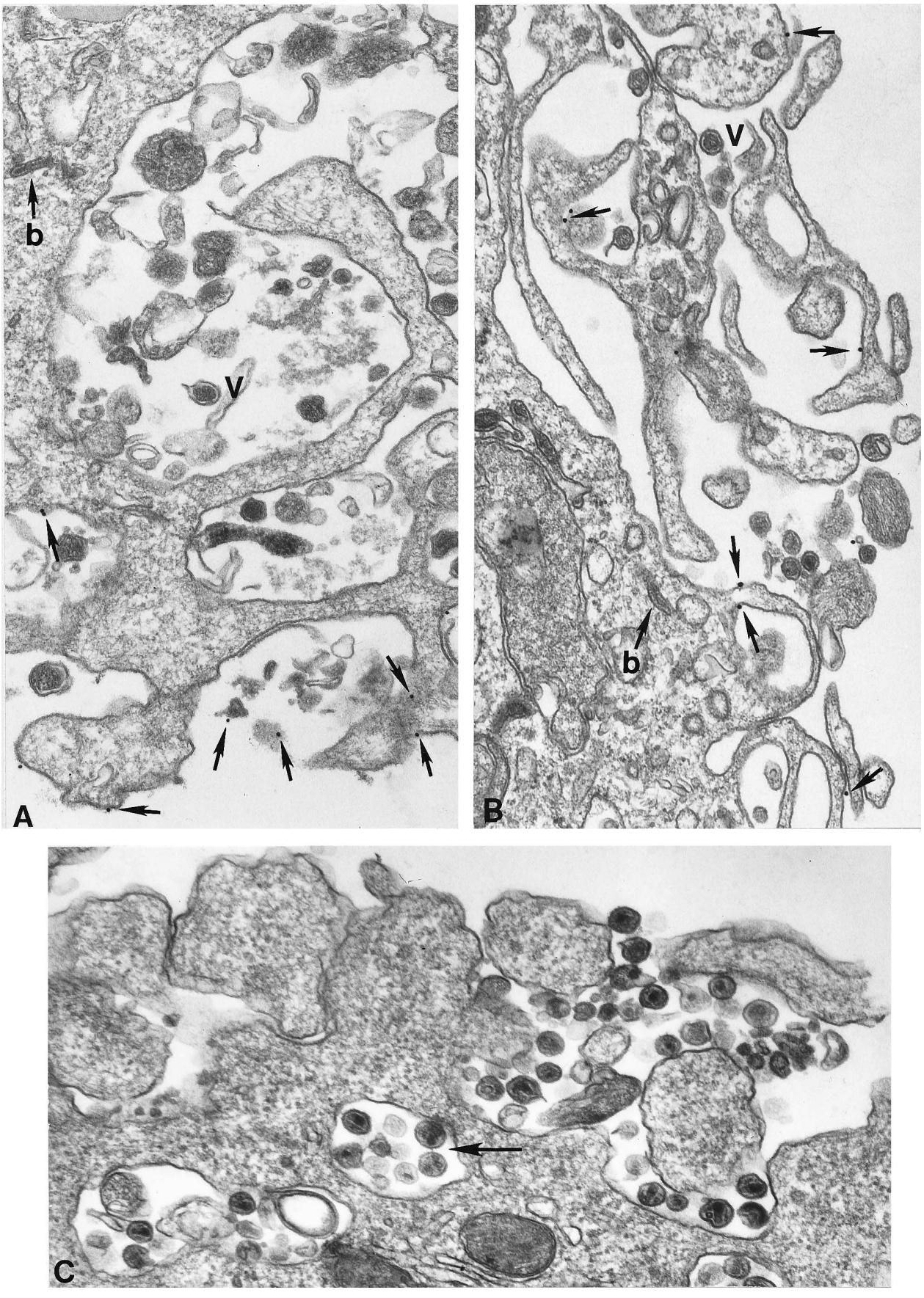
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FIG. 3. Association of virus particles with the surface membrane and processes of DC. (A and B) Details of the surfaces of two different DC which had been incubated with HTLV-I for 48 h. Small arrows indicate gold particles labelling the CD1a epitope. V, virus particles; b, Birbeck granule. Magnifications, ×41,400 (A) and ×36,800 (B). (C) Detail of a DC from a specimen cocultured with HIV-infected cells. The particles are trapped in DC processes. A serial section of the area indicated by the arrow would show that the space in which the particles are seen communicates with the exterior. Magnification, ×42,320.

the most powerful representatives among antigen-presenting cells, and their restoration in retrovirus-infected patients may be crucial. Since DC represent only 0.1 to 1% of peripheral blood or bone marrow cells, their isolation and use for replacement therapy in DC-depleted patients would be a formidable undertaking. The present studies as well as those by others (5, 36) have now shown the feasibility of generating DC from $CD34^+$ precursors in vitro. It may be possible to obtain similar results with a patient's autologous blood or bone marrow $CD34^+$ cells. The majority of data have failed to show that $CD34^+$ progenitors are infected in vivo. Admittedly, the benefit to be derived from an infusion of freshly generated DC into patients who continue to harbor replicating viruses would probably be short-lived. However, it would be possible to use autologous DC to more efficiently deliver an antigen pulse to patients who have lost the ability to respond to primary antigens. In this regard, it has been shown that extracorporeal priming of DC—even with soluble antigens—can elicit a memory type of response, i.e., it may induce T-helper lymphocytes, uncommitted in lymphokine pattern, to differentiate into TH-1-type lymphocytes (13). Such primed cells have been demonstrated to support the clonal expansion of naive T cells (22). Therefore, in vitro pulsing of autologous DC with a specific antigen relevant to a particular ongoing opportunistic infection may be a way to induce an immune response in a patient who has lost the ability to respond to such an antigen spontaneously despite the presence of an adequate number of circulating $CD4^+$ cells. The studies reported here may serve to stimulate further work in this direction.

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REFERENCES

1. **Bani, D., N. Pimpinelli, S. Moretti, and B. Giannotti.** 1990. Langerhans cells and mycosis fungoides—a critical overview of their pathogenic role in the disease. Clin. Exp. Dermatol. **15:**7–12.
2. **Belsito, D. V., and G. J. Thorbecke.** 1984. Reduced Ia-positive Langerhans cells in AIDS. N. Engl. J. Med. **311:**857–858.
3. **Braathen, L. R., G. Ramirez, R. O. F. Kunze, C. Mork, and O. Strand.** 1991. Latent infection of epidermal Langerhans cells in HIV-positive individuals. Res. Virol. **142:**119–121.
4. **Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba, and R. M. Steinman.** 1992. Dendritic cells exposed to human immunodeficiency virus type-I transmit a vigorous cytopathic infection to $CD4^+$ T cells. Science **257:**383–387.
5. **Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau.** 1992. GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. Nature (London) **360:**258–261.
6. **Cerio, E., C. E. M. Griffiths, K. D. Cooper, B. J. Nickoloff, and J. T. Headington.** 1989. Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. Br. J. Dermatol. **121:**421–431.
7. **Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. R. Lucey, C. S. Via, and G. M. Shearer.** 1993. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic human immunodeficiency virus-seropositive patients. J. Clin. Invest. **84:**1892–1899, 1989.
8. **Eales, L.-J., J. Farrant, M. Helbert, and A. J. Pinching.** 1988. Peripheral blood dendritic cells in persons with AIDS and AIDS-related complex: loss of high intensity class II antigen expression and function. Clin. Exp. Immunol. **71:**423–427.
9. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature (London) **362:**359–362.
10. **Flechner, E. R., P. S. Freudenthal, G. Kaplan, and R. M. Steinman.** 1988. Antigen-specific T lymphocytes efficiently cluster with dendritic cells in the human primary mixed leukocyte reaction. Cell. Immunol. **111:**183–195.
11. **Flotte, T. J., T. A. Springer, and G. J. Thorbecke.** 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections and epidermal sheets. Am. J. Pathol. **111:**112–124.
12. **Fox, C. H., K. Tenner-Racz, P. Racz, A. Firpo, P. A. Pizzo, and A. S. Fauci.** 1991. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type I RNA. J. Infect. Dis. **164:**1051–1057.
13. **Francotte, M., and J. Urbain.** 1985. Enhancement of antibody response by mouse dendritic cells pulsed with tobacco mosaic virus or with rabbit anti-idiotypic antibodies raised against a private rabbit idiotype. Proc. Natl. Acad. Sci. USA **82:**8149–8152.
14. **Giannetti, A., G. Zambruno, A. Cimarelli, A. Marconi, M. Negroni, G. Giromoloni, and U. Bertazzoni.** 1993. Direct detection of HIV-I RNA in epidermal Langerhans cells of HIV-infected patients. J. Acquired Immune Defic. Syndr. **6:**329–333.
15. **Hanaoka, M.** 1982. Progress in adult T cell leukemia research. Acta Pathol. Jpn. **32:**171–185.
16. **Hengel, H., M. Lindner, H. Wagner, and K. Heeg.** 1987. Frequency of herpes simplex virus-specific murine cytotoxic T lymphocyte precursors in mitogen and antigen-driven primary *in vitro* T cell responses. J. Immunol. **139:**4196–4204.
17. **Inaba, K., J. P. Metlay, M. T. Crowley, and R. M. Steinman.** 1990. Dendritic cells pulsed with antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ. J. Exp. Med. **172:**631–640.
18. **Kast, W. M., C. J. P. Boog, B. O. Roep, A. C. Voordouw, and C. J. M. Melief.** 1988. Failure or success in the restoration of virus-specific cytotoxic T lymphocyte response defects of dendritic cells. J. Immunol. **140:**3186–3193.
19. **Knight, S. C., and S. E. Macatonia.** 1991. Effect of HIV on antigen presentation by dendritic cells and macrophages. Res. Virol. **142:**123–128.
20. **Kornbluth, R. S., and J. A. McCutchan.** 1993. Skin test responses as predictors of tuberculous infection and of progression in HIV-infected persons. Ann. Intern. Med. **119:**241–243.
21. **La Grenade, L., B. Hanchard, V. Fletcher, B. Cranston, and W. Blattner.** 1990. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. Lancet **336:**1345–1347.
22. **Langhoff, E., and R. M. Steinman.** 1989. Clonal expression of human T lymphocytes initiated by dendritic cells. J. Exp. Med. **169:**315–320.
23. **Langhoff, E., E. F. Terwilliger, H. J. Bos, K. H. Kalland, M. C. Poznansky, O. M. L. Bacon, and W. A. Haseltine.** 1991. Replication of human immunodeficiency virus type 1 in primary dendritic cell cultures. Proc. Natl. Acad. Sci. USA **88:**7998–8002.
24. **Li, C. Y., K. W. Lam, and L. T. Yam.** 1973. Esterases in human leukocytes. J. Histochem. Cytochem. **21:**1–12.
25. **Macatonia, S. E., R. Lau, S. Patterson, A. J. Pinching, and S. C. Knight.** 1990. Dendritic cell infection, depletion and dysfunction in HIV-infected individuals. Immunology **71:**38–45.
26. **MacKie, R. M.** 1981. Initial event in mycosis fungoides of the skin is viral infection of epidermal Langerhans cells. Lancet **ii:**283–285.
27. **Newton, R. C., P. Limpuangthip, S. Greenberg, A. Gam, and F. A. Neva.** 1992. Strongyloides stercoralis hyperinfection in a carrier of HTLV-I virus with evidence of selective immunosuppression. Am. J. Med. **92:**202–208.
28. **Pancake, B. A., and D. Zucker-Franklin.** 1993. HTLV tax and mycosis fungoides. N. Engl. J. Med. **239:**580.
29. **Pancake, B. A., D. Zucker-Franklin, E. E. Coutavas, and E. Wassef.** 1995. The cutaneous T cell lymphoma, mycosis fungoides, is an HTLV-associated disease: a study of 50 patients. J. Clin. Invest. **95:**547–554.
30. **Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV-infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature (London) **362:**355–358.
31. **Pantaleo, G., C. Graziosi, and A. S. Fauci.** 1993. The role of lymphoid organs in the pathogenesis of HIV infection. Semin. Immunol. **5:**157–163.
32. **Patey, O., A. Gessain, J. Breuil, A. Courillon-Mallet, M. T. Daniel, J. M. Miclea, A. M. Roucayrol, F. Sigaux, and C. Lafaix.** 1992. Seven years of recurrent severe strongyloidiasis in an HTLV-I-infected man who developed adult T-cell leukemia. AIDS **6:**575–579.
33. **Patterson, S., and S. C. Knight.** 1987. Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. J. Gen. Virol. **68:**1177–1181.
34. **Popovic, M., P. S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann,**

J. Minowada, and R. C. Gallo. 1983. Isolation and transmission of human retrovirus (human T-cell leukemia virus). Science **219:**856–859.
35. **Rowden, G., and M. G. Lewis.** 1976. Langerhans cells: involvement in the pathogenesis of mycosis fungoides. Br. J. Dermatol. **95:**665–672.
36. **Santiago-Schwarz, F., F. Belilos, B. Diamond, and S. E. Carsons.** 1992. TNF in combination with GM-CSF enhances the differentiation of neonatal cord blood stem cells and macrophages. J. Leukocyte Biol. **52:**274–281.
37. **Santiago-Schwarz, F., N. Divaris, C. Kay, and S. E. Carsons.** 1993. Mechanisms of tumor necrosis factor-granulocyte-macrophage colony-stimulating factor-induced dendritic cell development. Blood **82:**3019–3028.
38. **Shimotohno, K., Y. Takahashi, N. Shimizu, T. Gojobori, D. W. Golde, I. S. Y. Chen, M. Miwa, and T. Sugiyama.** 1985. Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: an open reading frame for the protease gene. Proc. Natl. Acad. Sci. USA **82:**3101–3105.
39. **Spiegel, H., H. Herbst, G. Niedobitek, H.-D. Foss, and H. Skin.** 1992. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type I in lymphoid tissues facilitating infection of CD_4^+ T-helper cells. Am. J. Pathol. **140:**15–22.
40. **Steinman, R. M.** 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. **9:**271–296.
41. **Steinman, R. M., G. Schuler, N. Romani, and G. Kaplan.** 1988. Dendritic cells, p. 361–377. *In* D. Zucker-Franklin et al. (ed.), Atlas of blood cells: function and pathology, 2nd ed. Lea & Febiger, Philadelphia.
42. **Tachibana, N., A. Okayama, J. Ishizaki, T. Yokota, E. Shishimi, K. Murai, S. Tsuda, M. Essex, and N. Mueller.** 1988. Suppression of tuberculin skin reaction in healthy HTLV-I carriers from Japan. Int. J. Cancer **42:**829–831.
43. **Tschachler, E., U. Groh, M. Popovic, D. Mann, K. Konrad, B. Safai, L. Eron, F. Verenose, K. Wolf, and G. Stingl.** 1987. Epidermal Langerhans cells—a target for HTLV III/LAV infection. J. Invest. Dermatol. **88:**233–237.
44. **Van Voorhis, W. C., J. Valinsky, E. Hoffman, J. Luban, L. S. Hair, and R. M. Steinman.** 1983. Relative efficacy of human monocytes and dendritic cells as accessory for T cell replication. J. Exp. Med. **158:**174–191.
45. **Welles, S. L., N. Tachibana, A. Okayama, S. Shioiri, S. Ishihara, K. Murai, and N. E. Mueller.** 1994. Decreased reactivity to PPD among HTLV-I carriers in relation to virus and hematologic status. Int. J. Cancer **56:**337–340.
46. **Zucker-Franklin, D., G. Grusky, and A. Marcus.** 1978. Transformation of monocytes into "fat" cells. Lab. Invest. **38:**620–628.
47. **Zucker-Franklin, D., B. A. Pancake, and A. E. Friedman-Kien.** 1994. Cutaneous disease resembling mycosis fungoides in HIV-infected patients whose skin and blood cells also harbor proviral HTLV type I. AIDS Res. Hum. Retroviruses **10:**1173–1177.
48. **Zucker-Franklin, D., J. S. Yang, and G. Grusky.** 1992. Characterization of GpIIb/IIIa positive cells in human umbilical cord blood: their potential usefulness as megakaryocyte progenitors. Blood **79:**347–355.