Susceptibility of Human Peripheral Blood Dendritic Cells to Infection by Human Immunodeficiency Virus

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SUMMARY

Preparations of human peripheral blood dendritic cells have been infected with human immunodeficiency virus (HIV). After 5 days in culture they were examined by electron microscopy. Virus was observed budding from the plasma membrane of dendritic cells and mature virions were observed on the cell surface. In addition, a second cell type, similar in morphology to 'classical' dendritic cells but containing numerous cytoplasmic granules, was also found to support replication of the virus. We speculate that the growth of HIV in dendritic cells could cause immunosuppression by impairing antigen presentation.

The lytic infection of T4 helper lymphocytes by human immunodeficiency virus (HIV) is considered to cause the severe immunosuppression seen in patients with the acquired immune deficiency syndrome (AIDS) (for reviews, see Montagnier, 1986; Gallo et al., 1986). It is possible that the virus could mediate its immunosuppressive effects at different levels in the immune system. In addition to T4 helper lymphocytes, there is now evidence that the virus can infect macrophages (Levy et al., 1985; Koenig et al., 1986; Nicholson et al., 1986; Gartner et al., 1986), Epstein–Barr virus-transformed B lymphocytes (Montagnier et al., 1984) and lymph node follicular dendritic cells (Armstrong & Horne, 1984). These follicular dendritic cells arise in sites within the lymph nodes from fibroblastic reticulum cells (Villena et al., 1983) and are distinct from the bone marrow-derived dendritic cells of the T-dependent areas in the paracortex of lymph nodes (Steinman & Nussenzweig, 1980). The follicular dendritic cells are involved in memory B cell responses (Klaus et al., 1980) and the paracortical dendritic cells are potent antigen-presenting cells which are particularly important in initiating primary cellular and humoral immune responses.

Human peripheral blood dendritic cells have the properties of the bone marrow-derived, paracortical dendritic cells and are potent initiators of both T (Van Voorhis et al., 1982; Kuntzerow & Kunkel, 1982; Knight et al., 1986) and B cell (Inaba et al., 1983) responses. For this reason the susceptibility of these accessory cells to infection by HIV has been examined. As dendritic cell preparations invariably consist of a mixture of cell types which may be readily identified by electron microscopy, this technique was judged to be the most appropriate for this investigation.

Previously described procedures were used to obtain dendritic cell-enriched preparations from the peripheral blood of normal human volunteers (Knight et al., 1986). Preparations containing between $4 \times 10^5$ and $10^6$ cells were suspended in 0.9 ml of bicarbonate-buffered RPMI 1640 medium supplemented with 10% foetal calf serum, penicillin/streptomycin (100 IU ml) and L-glutamine (2 mM). They were infected with 0.1 ml of culture supernatant from an HIV persistently infected cell line, H9-IIIB (Popovic et al., 1984). After culturing for 5 days at 37°C the cells were pelleted by low speed centrifugation and fixed in 3% glutaraldehyde buffered with 0.1 M-sodium cacodylate (pH 7.2) containing 5% sucrose. Fixed preparations were processed for thin section electron microscopy by standard procedures.
Phenotype analyses of uninfected preparations indicate that, on average, one third of the cells are dendritic (Knight et al., 1986). The remainder are mainly macrophages, although up to 9% have been found to label with the natural killer cell marker Leu IIb. T and B lymphocytes represent less than 3% of the cell population. These data are borne out by the present morphological finding, in which the majority of contaminating cells were identified as macrophages. Dendritic cells lack the numerous vacuoles observed in macrophages, they have cytoplasm containing sparsely distributed elements of endoplasmic reticulum, their surface is relatively smooth and they frequently exhibit long extended processes.

On examination of dendritic cells 5 days after infection with HIV, virus was observed budding through the plasma membrane and mature virions were seen on the cell surface (Fig. 1). In four separate experiments between 3% and 17% of dendritic cells showed evidence of virus infection.

A second cell type, which was morphologically distinct from the dendritic cell described above, was also found to be infected by HIV in three of four experiments. It did not resemble cells of the macrophage lineage but seemed to be more closely related to dendritic cells. However, these cells were differentiated from the latter by the presence of numerous

Fig. 1. (a) A dendritic cell infected with HIV. Bar marker represents 5 μm. (b) Higher magnification of area indicated by arrows in (a). Virus particles are on the cell surface and a particle in the process of budding through the plasma membrane may be observed (arrow). Bar marker represents 500 nm.
cytoplasmic granules (Fig. 2). Virus was observed on the surface and also budding through the plasma membrane of these cells. In addition, granulated infected cells in mitosis were seen. These cells were rarely found in fresh preparations of uninfected dendritic cells taken from normal individuals. Infected cultures also contained cells with only a small number of granules and these appeared to be intermediate in morphology between the 'classical' dendritic and the granulated cell. In one experiment several lysed multinucleated giant cells were seen. Virus budding from macrophages was never observed, nor was virus ever found in uninfected preparations.
The data show that peripheral blood dendritic cells are susceptible to infection with HIV. It is thus possible that in AIDS patients HIV grows in dendritic cells and the immunological function of these cells is impaired. The notion that antigen-presenting cells are altered in AIDS is supported by the observation that patients show a reduction in the number of Ia-positive Langerhans cells, the epidermal antigen-presenting cells (Belsito et al., 1984). For these reasons functional studies on infected dendritic cells are now in hand.

There is evidence that the T4 molecule serves as the virus receptor on T helper lymphocytes (Klatzmann et al., 1984; Dalgleish et al., 1984). The identity of the HIV receptor on dendritic cells is unknown. It will therefore be important to establish whether T4 is present on dendritic cells and also to determine the effects of antibody to T4 on the initiation of infection.

Although macrophages are reported to be susceptible to HIV infection (Levy et al., 1985; Koenig et al., 1986; Nicholson et al., 1986; Gartner et al., 1986), electron microscopy provided no evidence of virus replication in these cells. Similarly Nicholson et al. (1986) found that when macrophage cultures were infected with HIV, low levels of infectious virus were produced but budding virus was not seen and assays for reverse transcriptase and detection of cytoplasmic antigens by immunofluorescence gave negative or only weakly positive results. These observations may be explained by different cell tropisms exhibited by various HIV isolates. Thus it was found that the H9-IIIB isolate used in these studies showed a 10000-fold lower ability to infect macrophages than T cells. On the other hand, isolates obtained from lung and brain macrophages of AIDS patients had a significantly higher ability to infect macrophages than T cells (Gartner et al., 1986). Our data suggest that the infectivity of the H9-IIIB isolate for dendritic cells more closely resembles that of T cells than of macrophages.

The significance of the granulated cells and whether their presence in infected cultures is due specifically to the virus or to conditions in vitro is not yet clear. In studies by Van Voorhis et al. (1982), the morphology of human peripheral blood dendritic cells was found to be unchanged after 4 days in culture. This finding suggests that the appearance of granulated cells in our experiments may not have been due to culturing alone. As the cells are susceptible to infection with HIV it will be important to establish their phenotypic profile and what role, if any, they play during infection. Regarding their identity, the observation of granulated cells in mitosis would argue against their being related to macrophages. However, a feature distinguishing mature dendritic cells from macrophages is that they arise from recently divided precursor cells (Pugh et al., 1983; Bowers & Berkowitz, 1986). Many dendritic cells from peripheral blood are smaller and have fewer veiled projections than those in afferent lymph (Knight et al., 1986) or from joint fluids of patients with arthritis (Tyndall et al., 1983) perhaps suggesting that peripheral blood contains immature dendritic cells. The similarity between some infected 'granular' cells and dendritic cells could result from infection of immature dendritic cells. The infection of follicular dendritic cells of the lymph node reported in AIDS patients (Armstrong & Horne, 1984) could suppress the development of immunological memory. Antigen in the context of the bone marrow-derived dendritic cells of the type described in this paper may be required for the initiation of primary immune responses. We are therefore considering the concept that in vivo infection of the two types of dendritic cells by the AIDS virus could compromise both primary and secondary immune responses.

Note added in proof: Recent experiments suggest that granulated cells can appear after culturing for 5 days in the absence of virus.

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REFERENCES


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