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# Dendritic cell infection, depletion and dysfunction in HIV-infected individuals

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## SUMMARY

Immune responses in resting T cells are initiated by the presentation of antigen by bone marrow-derived dendritic cells (DC). Normal DC are susceptible to infection with human immunodeficiency virus (HIV) *in vitro* (Patterson & Knight, 1987) and this blocks their capacity to stimulate T-cell responses to other antigens (Macatonia, Patterson & Knight, 1989a). To study the relationship between HIV and DC in patients and its relevance to the pathogenesis of disease, DC have been isolated from the blood of individuals in the different clinical categories, counted, examined for the presence of virus genome and their antigen-presenting capacity measured. Infection, depletion and impaired function of DC occur in early HIV infection. HIV seropositive patients who were asymptomatic and those with symptoms of disease had significantly reduced numbers of DC, but patients with persistent generalized lymphadenopathy had normal numbers. Between 3% and 21% of DC, identified as large low-density cells not bearing monocyte, lymphocyte or natural killer cell markers, were infected with HIV, as indicated by *in situ* hybridization. Less than 0·12% of the lymphocytes or monocytes were infected. The DC from infected individuals were poor at enhancing responses to the mitogen concanavalin A (Con A). They also caused low levels of stimulation in allogeneic lymphocytes in mixed leucocyte cultures. By contrast, T cells from asymptomatic patients gave normal T-cell responses to uninfected allogeneic DC, although those from acquired immunodeficiency syndrome (AIDS) patients did show reduced responsiveness. Defects in DC thus precede both the appearance of symptoms and changes in T cells and may be instrumental in the development of AIDS. Furthermore, since DC numbers and function differ at different stages of disease, monitoring these may contribute to clinical assessment and lead to new therapeutic approaches.

## INTRODUCTION

Immunosuppression in acquired immunodeficiency syndrome (AIDS) is believed to result from the infection and destruction of CD4-positive T lymphocytes (Ho, Pomerantz & Kaplan, 1987). However, human immunodeficiency virus (HIV) may produce immunological effects through infection of other cell types. Infection of macrophages, particularly after treating cells from HIV-positive patients with granulocyte–macrophage colony-stimulating factor, has been reported (Meltzer & Gendelman, 1988). In late disease, infection of some follicular dendritic cells in the B-cell areas of spleens and lymph nodes has been reported in germinal centres that are being destroyed during the infection (Armstrong & Horne, 1984). Infection of these cells might contribute to depression of secondary immune responses. However, a defect in bone marrow-derived dendritic cells (DC) could block recruitment of T cells into immune responses.

During development of immune responses, particularly on primary exposure to antigen, bone marrow-derived DC in the tissues (e.g. Langerhans' cells in the skin) acquire antigens, travel as veiled cells in afferent lymph to T-dependent areas of lymph nodes (Silberberg-Sinakin *et al.*, 1980) and cause clustering and activation of T cells; presentation of antigen in the context of these DC may be a prerequisite for activation of resting T cells (Steinman & Inaba, 1983; Steinman *et al.*, 1986; Macatonia *et al.*, 1989b). In AIDS, Langerhans' cells are infected with HIV (Tschachler *et al.*, 1987) and depletion of Langerhans' cells or loss of major histocompatibility complex (MHC) class II molecules from these cells is suggested, as there are fewer MHC class II-positive cells in the skin (Belsito *et al.*, 1984). The proportion of low density mononuclear cells with

Abbreviations: AIDS, acquired immunodeficiency syndrome; APAAP, alkaline phosphatase–anti-alkaline phosphatase; Con A, concanavalin A; DC, dendritic cells; HIV, human immunodeficiency virus.

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high levels of class II MHC molecules in the peripheral blood in AIDS is also reduced (Eales *et al.*, 1988). In one report, a low number of HIV-infected peripheral blood DC was suggested in a single HIV seropositive patient (Ranki *et al.*, 1987). DC from blood of normal individuals are also susceptible to infection with HIV *in vitro* (Patterson & Knight, 1987; Knight & Patterson, 1990; Knight & Macatonia, 1988), possibly through their expression of CD4 antigen (Knight & Patterson, 1990). These infected cells present HIV antigens to autologous T cells, causing *in vitro* production of primary proliferative and cytotoxic responses to virus (Macatonia *et al.*, 1989b; S. E. Macatonia, S. Patterson and S. C. Knight, manuscript in preparation). However, the infection of the DC with HIV also blocks the capacity of these cells to induce responses to other antigens *in vitro* (Macatonia *et al.*, 1989a). In this study, cells were isolated from individuals at different stages of HIV infection and the numbers of DC counted, the presence of viral genome in these cells assessed and their functional capacity tested. We show depletion, infection and dysfunction of these cells, which precedes any detectable T-cell abnormalities, and suggest that this may be instrumental in the development of the immunosuppression seen in AIDS.

## MATERIALS AND METHODS

*Patient material*

DC were isolated from peripheral blood of homosexual men with HIV infection but without symptoms (Group II) (Center for Disease Control, 1986), with persistent generalized lymphodenopathy (Group III), those with AIDS/ARC (Group IV) and HIV seronegative individuals from a cohort of homosexual men at risk of infection (Weber *et al.*, 1986) (Group 0). Normal laboratory staff were used as a control group.

*Cell separations*

Human peripheral blood mononuclear cells, isolated using Ficoll gradients, were washed in medium (RPMI-1640, Dutch modification with 100 IU penicillin, 100 μg/ml streptomycin and 10% fetal calf serum). Enriched DC were isolated from non-adherent cells that had been cultured overnight on Petri dishes, by centrifuging at 600 ***g*** for 10 min on metrizamide gradients (13·7% w/v). These cells in normals were around 30% DC and 70% monocytes with $<2\%$ contamination with lymphocytes, as previously reported (Knight *et al.*, 1987). The DC and monocytes in this mixed population were initially identified using light microscopy of live cells at 37° by their 'cotton wool' appearance, indistinct cellular outline and frequent moving veiled projections. This contrasted with cells of the macrophage lineage, which have clearer nuclear and cell-surface definition and spreading cytoplasm. The cell identification was confirmed in this work by cell staining with antibodies to monocytes, lymphocytes and natural killer (NK) cells, which did not stain the DC but identified the other cell populations (see below). In previous studies, cell identification of these low-density cells was confirmed from electron microscope pictures (Knight *et al.*, 1987), expression of MHC class II molecules, monocyte/macrophage markers, enzyme-staining patterns, phagocytic ability and expression of C3 and Fc receptors (Knight *et al.*, 1986, 1987).

The cells from the pellets of the metrizamide gradients, depleted of DC and macrophages, were diluted from the hypertonic metrizamide by drop-wise addition of medium, washed and analysed for infected cells or used in lymphocyte stimulation assays.

*Identification of HIV-infected cells*

Cell populations were identified by reactivity with a cocktail of monoclonal antibodies against CD19, CD14, CD16 and CD3, which recognize B cells, monocytes, NK cells and T cells, respectively. Cells were labelled with antibodies for 30 min on ice prior to adsorbtion onto poly-L-lysine-coated slides, then fixed with paraformaldehyde and antibody binding was detected by APAAP staining (Mason, 1985). Cells with characteristic DC morphology remain unstained. In the same preparations HIV-infected cells were detected by *in situ* hybridization using a HIV lambda BH10 probe (Hahn *et al.*, 1984) incorporating $^{35}$S-labelled ATP and CTP by nick translation (Rigby *et al.*, 1977). Labelled probe was hybridized to viral RNA and DNA (Schrier, Nelson & Oldstone, 1985). On each slide 10,000 cells were assessed for APAAP staining and positivity by *in situ* hybridization. Only cells markedly above background labelling with silver grains were counted as positive, as demonstrated in Fig. 2. In some experiments cells were labelled by a monoclonal antibody to P24 of HIV (provided through the MRC-AIDS Directed Programme) and labelled using the APAAP technique.

*Lymphocyte stimulation*

Cells isolated from peripheral blood were cultured in 20 μl hanging drops in Terasaki plates (Knight, 1988). In each experiment lymphocytes from one to three controls and between one and four HIV-infected individuals were isolated and proliferative responses of lymphocytes to 1000 autologous or allogeneic DC and to 0·1, 1 or 10 μg/ml of the mitogen Con A (Sigma, Poole, Dorset) in the presence or absence of 1000 autologous DC were studied using a range of responder lymphocyte concentrations (6000–100,000). Uptake of [$^3$H]thymidine (1 μg/ml, 2 Ci/mM; Amersham International, Amersham, Bucks) in a 2-hr pulse on Day 3 (Con A) or on Day 5 (allogeneic DC) was measured. Highest responses were seen with the highest numbers of lymphocytes and these values only are shown for ease of comparison in some experiments. Lymphocyte stimulation was assessed in nine patients in disease Group II, 14 in Group III, in seven patients with AIDS/ARC (Group IV), 20 laboratory controls and in four homosexual HIV seronegative 'at risk' individuals. Statistical significance at $P = <0{\cdot}01$ was measured using analysis of variance and Student's *t*-test to identify differences greater than replication variability.

## RESULTS

### Numbers of DC

In HIV seropositive individuals (Group II; Center for Disease Control, 1986) who were asymptomatic, the number of DC in peripheral blood was initially assessed from their morphology at 37° in low-density DC-enriched populations. This was significantly lower than in normal controls (Fig. 1a). In patients with persistent generalized lymphadenopathy (PGL; Group III; Center for Disease Control, 1986), the numbers were in the normal range, providing further evidence of differences between subjects in Groups II and III (Center for Disease Control, 1986). In patients within each category of AIDS (Group IV), the

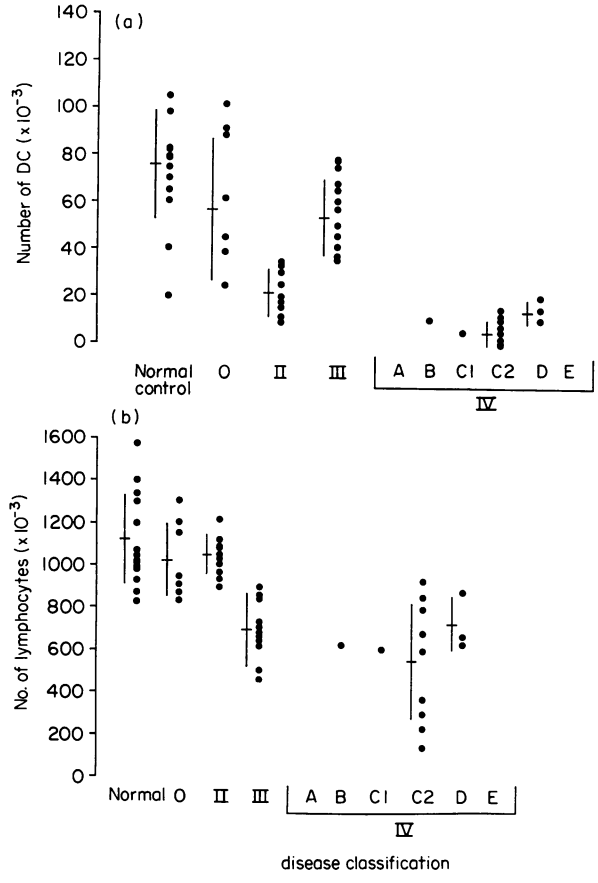


**Figure 1.** Numbers of cells isolated from peripheral blood. (a) Numbers of dendritic cells; (b) numbers of lymphocytes. 0, seronegative (high risk); (I, acute infection, no patients) II, seropositive, asymptomatic; III, persistent generalized lymphadenopathy; IV, other disease. Subgroup A, constitutional disease; B, neurological disease; C1, specific secondary infectious disease (listed in Center for Disease control surveillance definition for AIDS); C2, other specific secondary infectious diseases, D, secondary cancers; E, other conditions.

numbers of DC were below the normal values. The reduction in DC in Group II contrasted with the unchanged lymphocyte numbers in these same blood samples (Fig. 1b). T-cell subsets were not recorded in these samples but changes in lymphocyte numbers within these same groups of patients correlated with reduced CD4-positive cells (Weber *et al.*, 1986). Reduced T-cell numbers were only found in Groups III and IV. The reduced DC numbers thus preceded changes in the lymphocyte numbers. Several individuals in the group of HIV seronegative homosexual men had low DC numbers (Fig. 1a), although these were not outside the normal range.

The number of DC present in the samples was confirmed from studies with antibodies and APAAP labelling. As there is no single marker specific for DC, cells were labelled with antibodies to other cells, including lymphocytes, monocytes and NK cells. When purified DC were added to DC-depleted lymphocytes there was a corresponding increase in the numbers of large, APAAP-negative cells, as described previously (Macatonia *et al.*, 1989a), supporting the validity of this method of negative staining for identification of DC. The lower numbers of DC in Groups II and IV of disease was shown in the lower percentages of the low-density metrizamide-separated cells that were APAAP negative (Table 1). In the AIDS patients only 0–5% of the cells were APAAP negative. This represented a real decrease in DC, since there was no evidence of greater numbers of DC passing through the metrizamide gradients into the lymphocyte-rich pellets in these samples.

### Infection of different cell types

Low-density cells enriched for DC that were taken from HIV seropositive individuals showed some APAAP positivity when labelled with a monoclonal antibody to the P24 core protein of HIV. The percentage of labelled cells remained below 0·1%, but a higher proportion of low-density cells was labelled than of pellet lymphocytes. Labelled cells were frequently of a morphology suggestive of DC, but it was not possible to decide clearly whether these cells were monocytes or DC from this single labelling technique. The cells were therefore again labelled with APAAP using monoclonal antibodies to lymphocytes, monocytes and NK cells. Large cells of DC morphology remained unlabelled. These samples were then labelled for HIV genome using *in situ* hybridization. The low-density cells consisting of DC and monocytes were labelled separately from the lymphocytes in the pellets from the metrizamide gradients. This gave samples with sufficiently large numbers of DC and monocytes so that it was practical to scan 10,000 cells per sample for APAAP labelling and positivity for virus. In the low-density cell population APAAP-negative HIV-positive cells were of 'fluffy' DC-type morphology (Fig. 2) and rare APAAP-positive cells, also positive for viral DNA, were monocytes from their morphology. In the pellet cells the vast majority of cells present were lymphocytes, which were APAAP positive and these only rarely showed positivity for HIV DNA.

Table 1 shows that in cells from the asymptomatic HIV seropositive individuals and from patients with persistent generalized lymphadenopathy, viral genome was detected in more than half the samples studied. In those that were positive, the number of DC with virus was two orders of magnitude greater than the labelling seen in other cell populations. In AIDS patients all samples were positive for HIV and, where DC were detectable, a high proportion was labelled. In all laboratory controls there was no evidence of labelling of any cells with the HIV probe. One individual from the 'high risk' homosexual HIV seronegative group showed occasional positive cells by *in situ* hybridization and his lymphocytes showed some evidence of syncytia formation. Confirmation of this finding and further studies of this group are required since monitoring DC numbers and infection could provide early indications of exposure to virus.

### Lymphocyte stimulation studies

Two tests of the function of lymphocytes and DC from individuals in different stages of disease were performed. In the first, moderate Con A responses seen in lymphocytes from the pellet of metrizamide gradients were enhanced by the addition of small numbers of DC. As previously described, in normal cells this enhancement was reproducible and highly significant (Figs 3 and 4) ($P = < 0{\cdot}001$) (Macatonia *et al.*, 1989a). In Fig. 3 the results from a representative experiment showing the whole range of cell concentrations and Con A doses is given. Further

**Table 1.** HIV-infected cells detected by *in situ* hybridization

| | Clinical assessment | | | | |
|---|---|---|---|---|---|
| | C* | 0† | II‡ | III§ | IV¶ |
| No. of patients with HIV-infected cells | 0/3 | 1/3 | 4/7 | 5/8 | 3/3 |
| Low-density cells (monocytes + DC) | | | | | |
| Dendritic cells (%)** | 22–40 | 10–30 | 11–23 | 17–25 | 0–5§§ |
| HIV infected dendritic cells (%)†† | 0 | 0·21 | 5–11 | 3–15 | 14,21¶¶ |
| HIV infected non-DC (%)‡‡ | 0 | 0–0·07 | 0·05–0·09 | 0·01–0·05 | 0·04–0·008 |
| Lymphocytes (DC + monocyte-depleted) | | | | | |
| HIV-infected non-DC (%) | 0 | 0·02 | 0·01–0·05 | 0·04–0·12 | 0·03–0·07 |

Monocytes and lymphocytes were identified by alkaline phosphatase anti-alkaline phosphatase (APAAP) staining. DC remained unstained. Preparations were subsequently hybridized with the BH10 probe to detect HIV nucleic acid.

* C, control, laboratory staff.

† 0, seronegative homosexual men.

‡ II, seropositive asymptomatic individuals.

§ III, seropositive persistent generalized lymphadenopathy.

¶ IV, AIDS/ARC.

** Range of purity of DC in low-density cell preparations.

†† Range of percentage of HIV infected DC.

‡‡ Range of percentage of HIV-infected non-DC in mononuclear cell population.

§§ This represents a real decrease in DC as DC did not appear in other cell fractions during separation procedures.

¶¶ DC were not detected in the third patient.

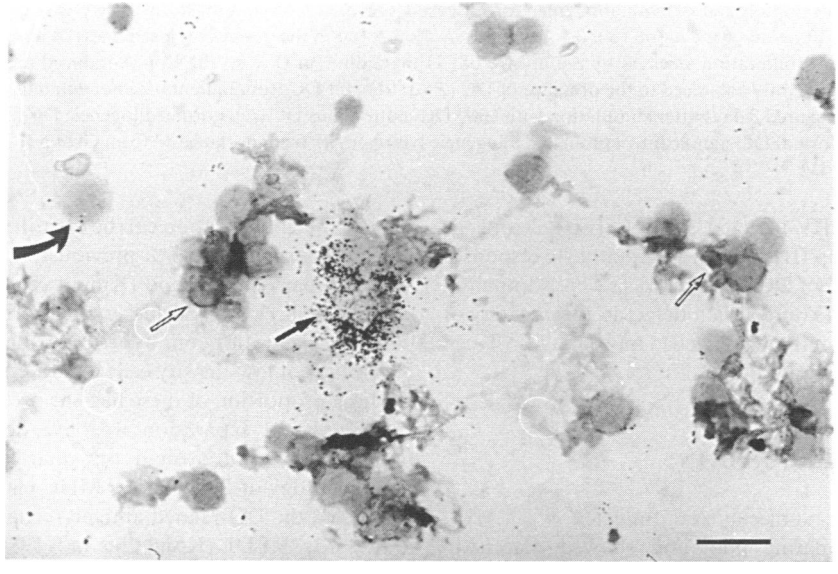

**Figure 2.** Combined *in situ* hybridization and immunolabelling by the APAAP technique of enriched DC preparations from an HIV seropositive individual. Filled straight arrows show pale unstained DC infected with HIV. Curved arrows represent uninfected DC. Non-DC indicated by open arrows are stained and HIV negative. Bar represents 20 $\mu$.

examples (Fig. 4) show the results at the highest cell concentrations only using 1 $\mu$g/ml Con A, to make comparisons easier. Lymphocytes from HIV-positive, asymptomatic patients (Group II) or from those in Group III responded normally to the Con A. Those from AIDS patients showed low responses. DC from all HIV-positive subjects failed to enhance the Con A responses.

The second functional study was to investigate the responsiveness of T cells to stimulation with allogeneic DC in a mixed leucocyte reaction. It was thus possible to separate the responding ability of T cells from the stimulatory ability of DC. In patients with AIDS (Group IV), as suggested previously (Eales *et al.*, 1988), both the responsive capacity of T cells and the stimulatory effects of DC were defective (Figs 3 and 4). Nine HIV seropositive subjects without symptoms (Group II) had T cells which responded normally to allogeneic DC from uninfected individuals. However, DC from seven of these nine Group II subjects caused little stimulation of allogeneic lympho-

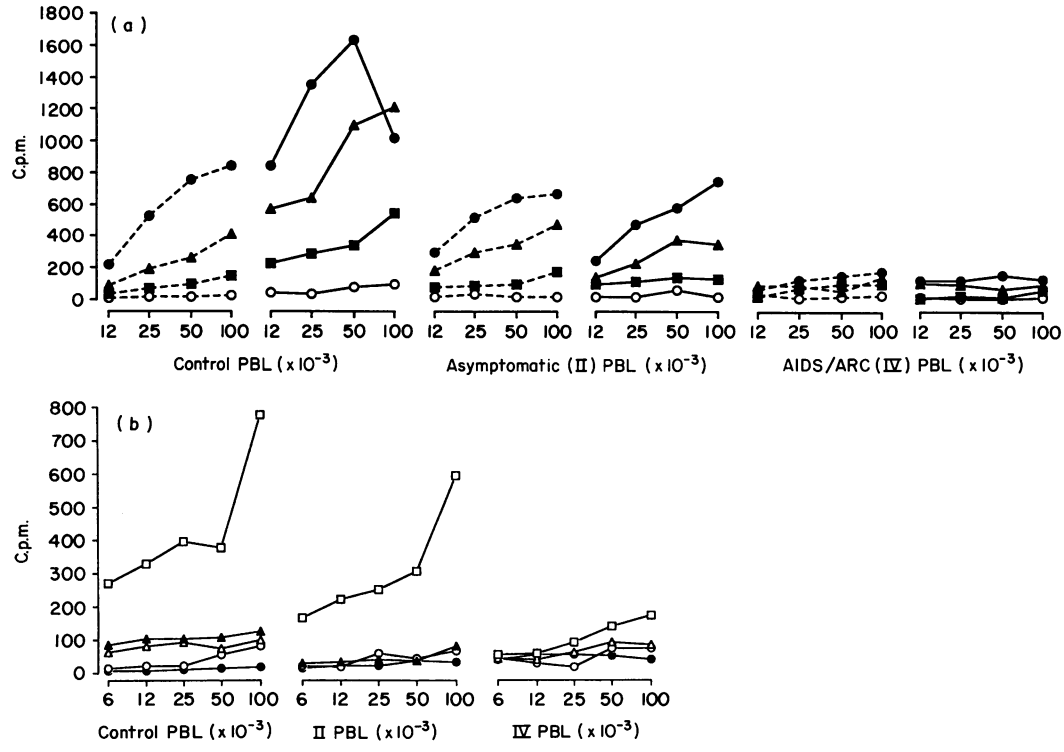


**Figure 3.** Functional defects in stimulatory capacity of DC. Different numbers of lymphocytes, in 20 $\mu$l hanging drops from a normal control, a Group II asymptomatic individual (Center for Disease Control, 1986), and a Group IV (AIDS/ARC) patient. (a) Cells cultured with Con A 10 $\mu$g/ml (●), 1 $\mu$g/ml (▲), 0·1 $\mu$g/ml (■) no Con A (○) in the presence (closed lines) or absence (dashed lines) of 1000 autologous DC. Proliferation assessed by the uptake of [$^3$H]thymidine on Day 3. The Con A-induced proliferation of normal lymphocytes was significantly enhanced in the presence of DC ($P < 0{\cdot}01$), but DC from patients did not enhance responses. (b) Mixed leucocyte reaction measured 5 days after stimulation with 1000 DC, autologous DC (○), normal allogeneic DC (□), Group II DC (△), Group IV DC (△). Normal DC significantly stimulated allogeneic lymphocytes from normal and from Group II individuals ($P < 0{\cdot}01$) but not from Group IV.

cytes from normal or HIV-infected subjects (Figs 3 and 4). Patients with PGL (Group III) showed T-lymphocyte responses in the low normal range, but DC function was severely impaired (Fig. 4). Therefore, in HIV infection a defect in the capacity of DC to present antigen or mitogen preceded any change in T-cell responsiveness to these stimuli.

## DISCUSSION

DC from HIV-positive subjects were infected with HIV, depleted from the circulating pool and showed defects in antigen-presenting function before the appearance of other signs and symptoms of AIDS. The identification of DC in human peripheral blood is difficult because there is no single marker identifying the cells. In this study the cells were identified in two ways. Firstly, they were enriched and then studied by light microscopy at 37° and their characteristic form and movement used to distinguish them from the monocytes co-separating with them. Secondly, the monocytes and lymphocytes were labelled with specific antibodies and stained with APAAP and the cells of dendritic morphology remained unlabelled. We can thus say categorically that large low-density cells which did not label for the monocyte marker CD14 represented the majority of the cells containing specific HIV DNA. After a 24-hr culture period, up to 4% of the mononuclear cell population can be identified as DC, which is higher in number than the 1% previously reported to be present in human peripheral blood (Knight *et al.*, 1986; Young & Steinman, 1988; Van Voorhis *et al.*, 1983). The 24-hr incubation before separation on hypertonic metrizamide increased the numbers of low-density cells separated from human blood, and a high proportion of these had the properties of DC. Using this technique of separation we have demonstrated by electron microscopy that around one-quarter of the cells have the morphology of DC, express MHC class II molecules DR, DP, DQ and the DQ-related antigen recognized by the monoclonal antibody RFDI (Knight *et al.*, 1987), do not phagocytose particles or reduce the dye nitroblue-tetrazolium to formazan, do not express Fc or C3 receptors and do not label significantly for non-specific esterase (Knight *et al.*, 1986). The RFDI highly positive CD14-negative cells have also been separated from the CD14-positive cells in double-labelled samples on the fluorescence-activated cell sorter. The RFDI-positive cells stimulated mixed leucocyte cultures and enhanced Con A responses, whereas the CD14-positive cells had little or no effect (S. C. Knight, unpublished data). All this information supports the view that there are significant numbers of DC or their precursors in the populations of large, low-density mononuclear cells from human peripheral blood.

The specificity of the *in situ* hybridization technique was initially established using HIV-infected or non-infected cell

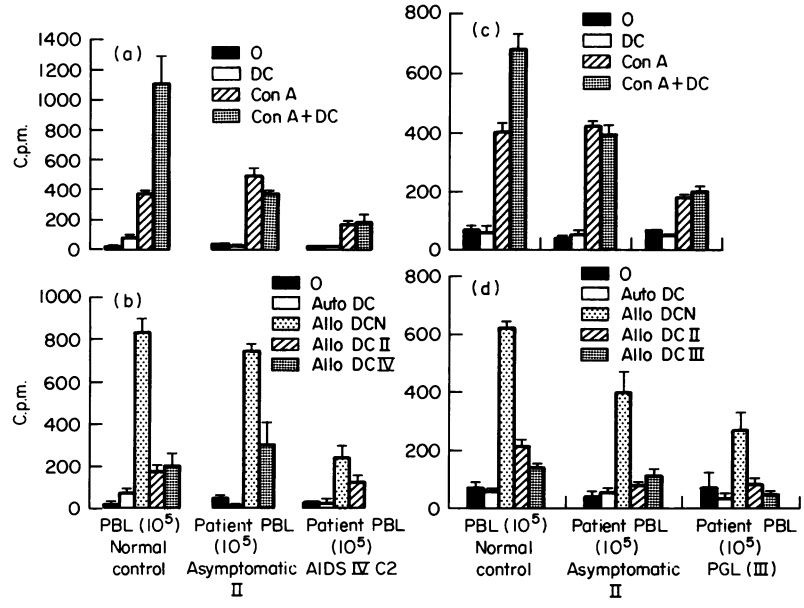


**Figure 4.** Functional defects in dendritic cells. Results are taken from two experiments of the type shown in Fig. 3, giving values from the highest cell concentration and a single dose of Con A only. (a) and (c) The addition of Con A (1 μg/ml) to 20 μl hanging drop cultures stimulated proliferation of human peripheral blood lymphocytes (PBL) ($10^5$/well) from normal controls, asymptomatic seropositive individuals (II) or from PGL (III) or from AIDS/ARC patients (IV). Untreated autologous DC (1000 markedly enhanced proliferation in the presence of Con A using normal cells, but failed to induce further proliferation in cells from individuals in Groups II, III and IV. (b) and (d) One-thousand normal allogeneic DC (DCN allogeneic) stimulated peripheral blood lymphocytes ($10^5$ well) from normals or from individuals with HIV infection (asymptomatic II, III) but the DC from individuals in these clinical categories failed to stimulate normal lymphocytes. Low or negative stimulation resulted on addition of 1000 autologous DC (auto DC).

lines. It was then used to assess the infection in mononuclear cells isolated from non-infected individuals that were exposed to HIV *in vitro* (Macatonia *et al.*, 1989a). After 5 days in culture, around 25% of DC were positive for viral DNA using this probe. In cultures of infected DC added to lymphocytes and stimulated with Con A, more than 50% DC became infected and less than 2% lymphocytes were positive (Macatonia *et al.*, 1989a). When comparing *in situ* hybridization for viral DNA and RNA, higher numbers of DC were labelled for DNA than RNA. This contrasted with studies of infected H9 cells, which gave identical labelling values for viral DNA and RNA (S. Patterson *et al.*, manuscript in preparation). The increased percentage of DNA-labelled DC suggests the possibility of a latent infection with virus in these cells. This higher labelling for DNA than RNA may also account for the higher percentage labelled by this method than by labelling for P24 antigen in our studies and for the fact that the total percentage of cell labelling in this report exceeds that seen in many earlier studies. The APAAP staining did not inhibit the *in situ* hybridization reaction as persistently infected cell lines showed equivalent numbers of HIV-positive cells, whether prestained with APAAP or not. Similarly, in these studies, APAAP-positive HIV-positive cells were also detected, albeit rarely.

The reduction in DC numbers is particularly marked in asymptomatic subjects and patients with AIDS. DC in Group III patients, however, are in the normal range. This is further evidence that the asymptomatic patients and those with persistent generalized lymphadenopathy should be considered as separate disease states (Rogers, Forster & Pinching, 1989). It is not yet clear whether a reduction in DC numbers and function can also occur during other viral infections contributing to the transient suppression in lymphocyte function observed (Denman, Bacon & Pelton, 1983). However, in preliminary studies, DC within the 'normal' range have been isolated from Tropical Spastic Paraparesis patients, positive for the retrovirus HTLV-1 (S. E. Macatonia *et al.*, manuscript in preparation). Therefore the reduction in numbers of DC in this study may reflect a specific effect of HIV infection. Since DC may be required to initiate responses of resting T cells which are not responsive to interleukin-2 (Steinman *et al.*, 1986) a DC defect may be instrumental in producing alterations in T-cell populations *in vivo*.

*In vitro* studies of DC infection with HIV have already shown that infection blocks immune responses of T cells in two ways—by infection of the DC themselves and by allowing DC to act as a reservoir of virus, causing secondary infection of T lymphocytes (Macatonia *et al.*, 1989a), probably during the close clustering between these cells during lymphocyte activation.

The infection of the bone marrow-derived DC lineage has been suggested from the reports of infected Langerhans' cells in the skin (Tshachler *et al.*, 1987) and from changes in the function of DC in peripheral blood (Eales *et al.*, 1988). Lower levels of MHC class II molecules were reported on the low-density cells from peripheral blood of AIDS patients compared with those from normals (Eales *et al.*, 1988). From our studies, the lower MHC class II expression probably reflects a loss of DC from the low-density cells from peripheral blood. Similarly, the low mixed leucocyte reactions initiated by low density cells from patients (Eales *et al.*, 1988) could reflect the loss of DC. In this

work, equivalent numbers of DC from patients and normals were added as stimulants. There was still a marked defect in the responses, indicating that both loss of DC and malfunction of the DC present may cause the inhibition. One report also suggested that cells of DC morphology were infected in a single patient (Ranki *et al.*, 1987). In this work we first showed that low-density cells enriched for DC contained more cells labelling with antibody to P24 than seen in lymphocyte populations. Although the morphology of these cells was very suggestive of DC, as reported in the earlier individual (Ranki *et al.*, 1987), confirmation of this was sought from labelling studies. The *in situ* hybridization showed that the non-CD14-positive cells contained the majority of virus-positive cells, so confirming the early single labelling observation. The labelling of the DC in the peripheral blood with HIV suggests that the infection may occur, not in the periphery, but may be present already in cells entering the blood, perhaps from the bone marrow.

DC exposed to influenza virus show no infection, as judged by electron microscopy. They cause stimulation of responses to mitogens and to the influenza virus itself (Knight & Macatonia, 1988). They can also act as targets for cytotoxic T-lymphocyte responses (Macatonia *et al.*, 1989b; S. E. Macatonia, P. Taylor, S. Patterson, B. Askonas and S. C. Knight, unpublished data). HIV infection of DC *in vivo* might produce an efficient presentation of HIV antigen and initiate primary cytotoxic responses leading to destruction of persistently infected DC. If cells are already infected in the peripheral blood before entering peripheral tissues, this could have a particularly dramatic effect on their numbers, distribution and function. This would not significantly affect any on-going immune responses, since many cells bearing MHC class II antigens can promote stimulation of already activated lymphocytes (Steinman & Inaba, 1983; Macatonia *et al.*, 1989b), although some impairment of secondary responses could result with the additional infection of some macrophages (Meltzer & Gendelman, 1988) and follicular dendritic cells (Armstrong & Horne, 1984; Tenner-Racz *et al.*, 1986). However, the individual would be particularly vulnerable to any new infection (or re-infection where no on-going lymphocyte response to these antigens was present) because of a requirement for DC to activate resting T cells. This concept of the DC as a critical target for HIV infection suggests that production of an efficient immune response which eliminates infected cells is a double-edged sword. Further infection of DC and elimination of infected DC may both reduce antigen-presenting capacity and block development of immune responses. A hope for counteracting this immunosuppressive effect may be in more precise definition and circumvention of the defect in antigen-presenting cells.

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