Brief Definitive Report

Human Immunodeficiency Virus Infection of Eosinophils in Human Bone Marrow Cultures

By Andrew R. Freedman, Frances M. Gibson,* Simon C. Fleming, Christopher J. Spry,‡ and George E. Griffin

*From the Divisions of Communicable Diseases, *Haematology, and ‡Immunology, Department of Cellular and Molecular Sciences, St. George's Hospital Medical School, London SW17 0RE, United Kingdom*

Summary

Normal human bone marrow, cultured in vitro with interleukin 5 to promote eosinophil production and maturation, was inoculated with cell-free isolates of human immunodeficiency virus type 1 (HIV-1). CD4 expression by eosinophil precursors, determined by immunocytochemistry, was found to be greatest early in their maturation with a rapid decline after 28 d in culture. Productive HIV infection of eosinophil precursors was detected 14 d after inoculation, by a combination of immunostaining for HIV-1 p24 and gp41/160 and in situ hybridization for viral RNA, together with assay of culture supernatants for p24 antigen and reverse transcriptase activity. Thus, eosinophils are susceptible to productive HIV-1 infection in vitro and may be an important reservoir for the virus in vivo.

Human immunodeficiency viruses (HIV-1 and HIV-2) are known to infect in vivo cells bearing the CD4 glycoprotein surface receptor (1). These include both T helper lymphocytes and also cells of the monocyte/macrophage series. Infection of these cells is believed to be the major cause of immune deficiency seen in the later stages of HIV infection. However, several other cell types are known to express membrane CD4 and are, therefore, potential targets for HIV infection. For example, eosinophils isolated from peripheral blood of patients with eosinophilia have been shown to express CD4 on their surface, to which HIV-1 gp 120 will bind in vitro (2), although HIV infection of these cells has not previously been reported.

Eosinophils are granulocytic leukocytes derived from bone marrow stem cells and, like macrophages, they migrate rapidly from peripheral blood into tissues, where they may survive for several weeks (3). Although in vivo functions of eosinophils are not fully understood, their numbers, both in blood and tissues, increase greatly in response to parasitic infections (4). Thus, the possibility that eosinophils are infected by HIV in vivo would have important implications for the pathogenesis of HIV disease, particularly in tropical countries where both HIV and parasitic infections are widespread. For this reason we have investigated the potential for eosinophils to support HIV infection by attempting to infect normal human eosinophil precursors with HIV-1 strains in vitro.

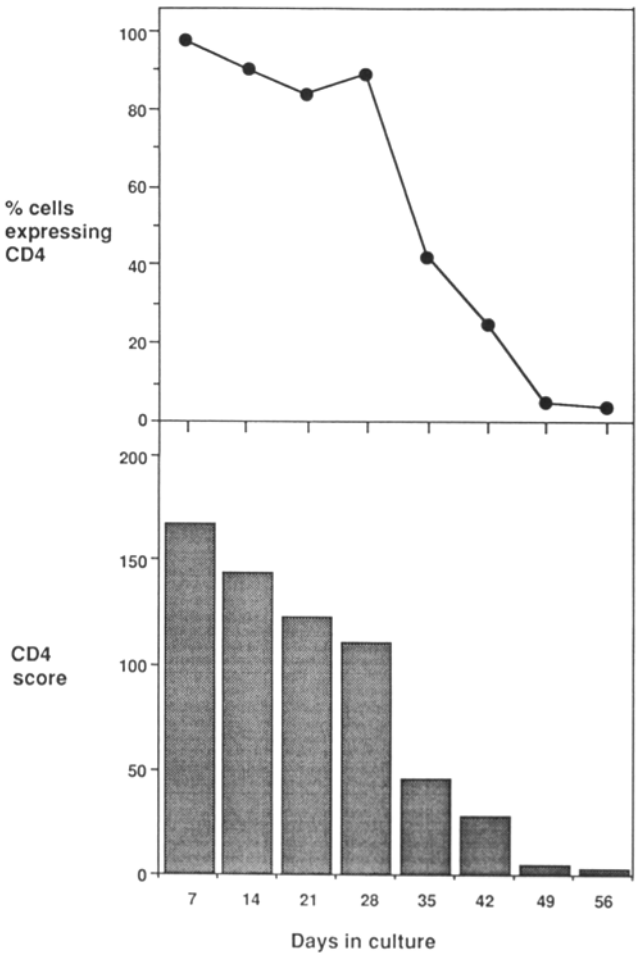
Materials and Methods

Bone Marrow Culture and Infection with HIV-1. Normal human bone marrow samples were obtained, with informed consent, from five healthy volunteers by iliac crest aspiration. Mononuclear cells were separated by centrifugation over Ficoll and suspended in IMDM containing 15% FCS, at 10^6 cells/ml. Human rIL-5 (Glaxo Group Research Ltd., Greenford, UK) was added at 2 ng/ml to promote eosinophil differentiation (5). Cells were cultured for up to 56 d in 96-well microtitre plates (10^5 cells in 100 μl/well), at 37°C in 95% air/5% CO_2. To infect the cultured cells with HIV, cell-free supernatants of the RF, RUT, and IIIB strains of HIV-1 (MRC AIDS Directed Programme) were added at a concentration of 10^4 tissue culture infectious doses (TCID)/ml, 7–21 d after the start of the cultures.

Detection of Eosinophil CD4, HIV Antigens, and RNA. Antigen expression by the maturing eosinophil precursors was detected by immunostaining of cytospin slides prepared at 7-d intervals, using the alkaline phosphatase anti-alkaline phosphatase technique with fast red substrate and hematoxylin counterstain (6). Separate slides at each time-point were stained with mAbs to CD4 (Dakopatts, High Wycombe, UK), and HIV p24 core protein (MRC EH12) and gp41/160 envelope glycoprotein (MRC ADP330). The percentage of maturing eosinophils expressing CD4 was determined as a function of time in control, uninfected cultures by examining 100 cells on each slide. CD4 expression was quantitated by scoring each cell on an arbitrary scale from 0 to 3 according to the intensity of staining by anti-CD4 antibody. HIV-1 RNA was detected in infected cells by in situ hybridization using a specific ^{35}S-DNA probe (BH10), with carbol chromotrope treatment to block nonspecific staining of eosinophils (7).

Detection of Viral Replication. 50-μl aliquots of culture supernatant was removed from each well at weekly intervals and replaced with fresh medium. Supernatants were stored at −70°C and subsequently assayed for reverse transcriptase (RT) activity (8), for HIV p24 antigen level measured by ELISA (Coulter Immunology, Luton, UK) and for infectivity, assessed by coculture with Jurkat T cells. Supernatants from the cocultures were assayed for RT activity at day 5.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/12/1661/04 $2.00
Volume 174 December 1991 1661–1664



Results

Bone Marrow Culture and Eosinophil CD4 Expression. After 21 d in culture, >95% of surviving cells were eosinophil precursors. These cells developed gradually from early promyelocytes seen at day 7, through to mature, terminally differentiated eosinophils after 56 d in culture, as assessed morphologically. CD4 expression by control, uninfected eosinophil precursors was maximal in terms of both percentage and intensity during the first 28 d in culture, with a gradual decline to almost undetectable levels by 56 d (Fig. 1).

HIV Antigen and RNA Expression by Eosinophils. Fig. 2 shows a 35-d-old culture, 21 d after infection with HIV-1 RF strain, stained as described above for p24 antigen. Infected eosinophils expressed p24 predominantly on their cell membrane and a similar pattern was observed when stained for gp41/160. In situ hybridization confirmed the presence of HIV RNA in infected eosinophils (Fig. 3). Control, uninfected cultures were negative for both HIV antigen and RNA expression (not shown). Eosinophil precursors first showed evidence of infection 14 d after the addition of viral inoculum. A maximum of 4% of the cells stained positively for either antigen or RNA. Infection of eosinophils was observed in two cultures infected with HIV-1 RF strain and one with

Figure 1. CD4 expression by uninfected eosinophil precursors in culture. Normal human bone marrow was cultured in the presence of IL-5, and CD4 expression was assessed at weekly intervals by staining cytospin slides with anti-CD4 mAbs. (*Top*) The percentage of maturing eosinophils expressing CD4 plotted against days in culture. The CD4 score (*bottom*) was derived by scoring 100 cells at each time-point on a scale from 0 to 3 according to the intensity of staining.

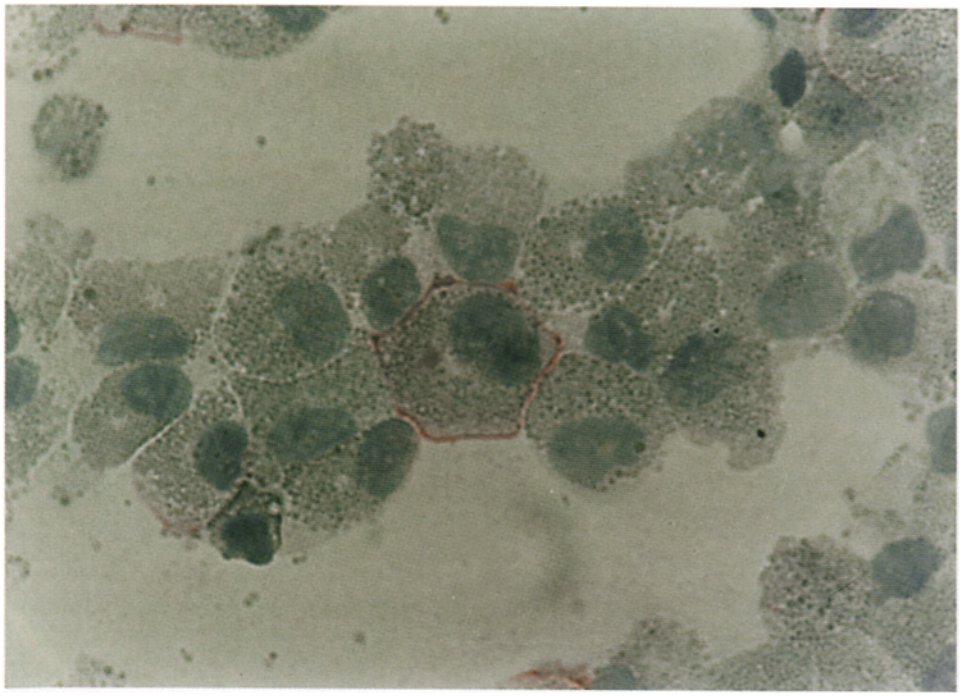


Figure 2. P24 antigen expression by HIV-1 RF-infected eosinophils. Normal human bone marrow was cultured in the presence of IL-5 and inoculated with HIV-1 RF supernatant after 14 d. Cells were harvested 21 d later and stained with anti-p24 mAbs using the APAAP technique with fast red substrate to stain positive cells red. An infected eosinophil is shown in the center of the picture.

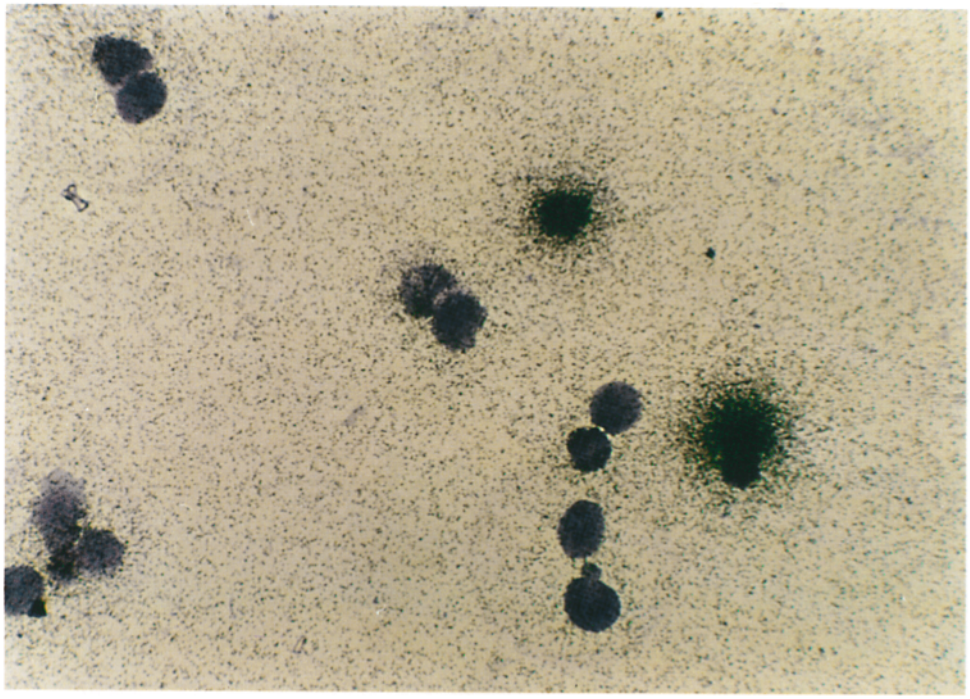


Figure 3. HIV-1 RNA expression by HIV-1 RF-infected eosinophils. Normal human bone marrow was cultured in the presence of IL-5 and inoculated with HIV-1 RF supernatant after 14 d. Cells were harvested 14 d later and subjected to in situ hybridization with ^{35}S-DNA HIV-1 probe (BH10) as described. Two infected eosinophils are shown together with uninfected cells.

HIV-1 RUT, but in neither of two cultures inoculated with HIV-1 IIIB.

Viral Replication. Productive infection of eosinophil cultures was confirmed by rising levels of RT activity in the supernatant, beginning 7 d after viral inoculation and continuing up to 49 d after inoculation, by which time all surviving eosinophils were morphologically mature (Fig. 4). No RT activity was detected in control culture supernatant. Parallel rises in p24 antigen levels were found in the infected culture supernatants (data not shown). Jurkat coculture experiments confirmed the presence of infectious virus in the supernatants for up to 42 d after infection.

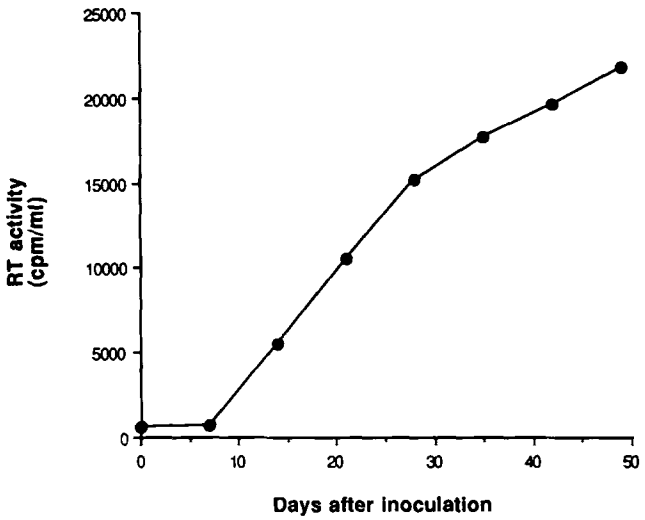


Figure 4. RT activity in eosinophil culture supernatant after infection with HIV-1. Normal human bone marrow was cultured in the presence of IL-5 and inoculated with HIV-1 RF supernatant after 14 d. Culture supernatant was withdrawn at weekly intervals and assayed for RT activity as described. RT activity (cpm/ml) is plotted against days after inoculation.

Discussion

These studies have shown for the first time that eosinophils can be infected by HIV-1 and support active replication of the virus, thus extending the work of Lucey et al. (2). We chose to use IL-5-stimulated bone marrow cultures as a source of actively dividing eosinophil precursors, in view of the difficulties associated with obtaining purified peripheral blood eosinophils from normal individuals in sufficient quantities (9). We carried out preliminary experiments on peripheral blood eosinophils isolated from hyper-eosinophilic subjects, but were unable to infect such cells with HIV or to transfect them with HIV-LTR CAT constructs. In addition, the presence of IL-5 in the culture medium has been found to activate eosinophils (10, 11) and, by analogy with macrophages (12), this would be expected to enhance HIV replication within them.

We found that CD4 expression by eosinophil precursors was greatest early in their maturation, with only very weak expression after 28 d in culture. This decline in CD4 expression occurred in the absence of HIV which, itself, is known to downregulate CD4 expression on infected cells (13). We, therefore, inoculated the cultures with HIV within the first 21 d in order to maximize the chance of achieving infection, although in vitro infection by HIV-1 of a purified $CD34^+/CD4^-$ myeloid progenitor population has been reported (14, 15), suggesting that CD4 expression may not be necessary. Although only up to 4% of eosinophils in culture expressed

HIV antigens or RNA, none of the other contaminating cells, which comprised <5% of the total, were infected, confirming that the viral activity detected in the culture supernatants was due to viral replication within eosinophils. Typical bilobed nuclear morphology was observed in all eosinophils after day 49 in culture. The increase in RT and p24 activity within the culture supernatant between days 49 and 63 in culture (days 35–49 after inoculation) provides strong evidence of continuing HIV replication in such mature cells.

The ability of RF and RUT, but not IIIB, strains of HIV-1 to infect eosinophils suggests that, as for macrophages (16) and megakaryocytes (17), there may be strains of HIV with tropism for eosinophils. The latter is a laboratory adapted T lymphocyte tropic strain, which failed to infect $CD34^+$ myeloid precursors in vitro (15).

Eosinophils are known to have many similarities to macrophages, including their tropism for the skin and mucosal tissues and their activation by cytokines (18). Like macrophages, they may also be a major reservoir for HIV, but further studies will be required to elucidate their role in vivo.

We thank Glaxo Group Research Ltd. (Greenford, UK) for the gift of recombinant human IL-5, Dr. S. Patterson (CRC, Harrow, UK) for the HIV-1 BH10 probe, and the MRC ADP for the supply of HIV-1 isolates, mAbs and p24 antigen assay kits. We thank Dr. Gary Nabel for helpful discussion and advice.

This work was supported by a grant from the Medical Research Council AIDS Directed Programme (MRC ADP). G. E. Griffin acknowledges support from the Wellcome Trust, F. M. Gibson is supported by the Marrow Environment Fund, and C. J. Spry by the British Heart Foundation.

Address correspondence to Andrew Freedman, Division of Communicable Diseases, St. George's Hospital Medical School, London SW17 0RE, UK.

Received for publication 22 July 1991 and in revised form 19 September 1991.

References

1. Dalgleish, A.G., C.L. Beverley, P.R. Clapham, M. Crawford, F. Greaves, and R.A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for AIDS retrovirus. *Nature (Lond.).* 312:763.
2. Lucey, D.R., D.I. Dorsky, A. Nicholson-Weller, and P.F. Weller. 1989. Human eosinophils express CD4 protein and bind human immunodeficiency virus 1 gp120. *J. Exp. Med.* 169:327.
3. Sheff, D.M., W.F. Owen, Jr., and K.F. Austen. 1991. Eosinophil phenotypes and LTC4 generation in vitro and in hypereosinophilic states. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 21B:481.
4. Conrad, M.E. 1971. Hematologic manifestations of parasitic infections. *Semin. Hematol.* 8:267.
5. Clutterbuck, E.J., E.M.A. Hirst, and C.J. Sanderson. 1989. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. *Blood.* 73:1504.
6. Cordell, J., B. Falini, W. Erber, A. Ghosh, Z. Abdulaziz, S. Macdonald, K. Pulford, H. Stein, and D. Mason. 1984. Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase anti-alkaline phosphatase (APAAP). *J. Histochem. Cytochem.* 32:219.
7. Patterson, S., J. Gross, and A.D.B. Webster. 1989. DNA probes bind non-specifically to eosinophils during in situ hybridization: carbol chromotrope blocks binding to eosinophils but does not inhibit hybridization to specific nucleotide sequences. *J. Virol. Methods.* 23:105.
8. Hoffman, A., B. Banapeur, and J. Levy. 1985. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. *Virology.* 147:326.
9. Hansel, T.T., J.D. Pound, and R.A. Thompson. 1990. Isolation of eosinophils from human blood. *J. Immunol. Methods.* 127:153.
10. Rothenberg, M.E., J. Petersen, and R.L. Stevens. 1989. IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype using 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. *J. Immunol.* 143:2311.
11. Fujisawa, T., R. Abu-Ghazaleh, H. Kita, C.J. Sanderson, and G.J. Gleich. 1990. Regulatory effect of cytokines on eosinophil degranulation. *J. Immunol.* 144:642.
12. Griffin, G.E., K. Leung, T.M. Folks, S. Kunkel, and G.J. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. *Nature (Lond.).* 339:70.
13. Stevenson, M., X. Zhang, and D.J. Volsky. 1987. Downregulation of cell surface molecules during noncytopathic infection of T cells with human immunodeficiency virus. *J. Virol.* 61:3741.
14. Folks, T.M., S.W. Kessler, J.M. Orenstein, J.S. Justement, E.S. Jaffe, and A.S. Fauci. 1988. Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. *Science (Wash. DC).* 242:919.
15. Kitano, K., C.N. Abboud, D.H. Ryan, S.G. Quan, G.C. Baldwin, and D.W. Golde. 1991. Macrophage-active colony-stimulating factors enhance human immunodeficiency virus type 1 infection in bone marrow stem cells. *Blood.* 77:1699.
16. Cordonnier, A., L. Montagnier, and M. Emerman. 1989. Single amino acid changes in HIV envelope affect viral tropism and receptor binding. *Nature (Lond.).* 340:571.
17. Sakaguchi, M., T. Sato, and J.E. Groopman. 1991. Human immunodeficiency virus infection of megakaryocytic cells. *Blood.* 77:481.
18. Weller, P.F. 1991. The immunobiology of eosinophils. *N. Engl. J. Med.* 324:1110.