Interleukin 4 and human immunodeficiency virus stimulate LFA-1–ICAM-1-mediated aggregation of monocytes and subsequent giant cell formation

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The effects of recombinant interleukin 4 (IL-4) on cell cluster and multinucleated giant cell (MGC) formation from human immunodeficiency virus (HIV)-infected and uninfected monocytes were examined. Human blood monocytes were isolated by centrifugal elutriation and monoclonal antibody-complement-dependent lysis of residual T cells, and infected with low passage HIV strains. Monocytes were exposed to recombinant IL-4 (1 to 20 ng/ml), continuously after inoculation with HIV. Monocyte expression of ICAM-1 but not LFA-1 was significantly enhanced by IL-4 although substrate adherence was a more potent stimulus. Monocyte cluster and MGC formation was quantified after fixation and staining with Giemsa. Clusters of HIV-infected and uninfected monocytes were consistently and significantly increased at 4 to 7 days after IL-4 stimulation. The combination of HIV and IL-4 was more stimulatory than either treatment alone. In two out of five uninfected and three out of seven HIV-infected monocyte cultures, MGC formation was also markedly increased at 10 to 14 days after stimulation. Incubation with anti-LFA-1 (anti-CD11a, anti-CD18) and anti-ICAM-1 (anti-CD54) monoclonal antibodies reduced IL-4-stimulated aggregation in HIV-infected and uninfected monocytes and subsequently reduced MGC formation. Anti-ICAM-1 was not as effective as anti-CD11a or anti-CD18 in inhibiting aggregation of HIV-infected monocytes and in these cultures anti-ICAM-2 was also inhibitory. Extracellular HIV antigen concentrations were not consistently reduced by anti-CD11a or anti-ICAM-1. Hence IL-4 markedly enhanced monocyte aggregation in both HIV-infected and uninfected monocytes, probably through enhanced LFA-1–ICAM-1 interactions in all cultures and LFA-1–ICAM-2 interactions in infected monocytes, leading subsequently to MGC formation in some cultures.

Recently we and others (Kazazi et al., 1991; Novak et al., 1990) showed that interleukin 4 (IL-4) could stimulate replication of human immunodeficiency virus (HIV) in human monocytes cultured in vitro without exogenous macrophage colony-stimulating factor (M-CSF), but only when added after HIV inoculation. Addition of IL-4 prior to HIV inoculation had either no effect [48 h, moderate concentrations (Kazazi et al., 1991)] or inhibited HIV replication [5 days, high concentrations (Schuitemaker et al., 1992)]. In our experiments the magnitude of the stimulatory effect varied markedly according to the low passage strain of HIV and donor cells used, but maximum stimulatory effects were comparable with other cytokines such as IL-2 and granulocyte–macrophage colony-stimulating factor (GM-CSF). We also observed an increase in clusters of HIV-infected monocytes in the in situ hybridization preparations after incubation with IL-4 (Kazazi et al., 1991). IL-4 has previously been reported to enhance the formation of multinucleated giant cells (MGCs) from murine bone marrow macrophages (McInnes & Rennick, 1988). Culture supernatants from concanavalin A-stimulated mononuclear cells, containing a mixture of cytokines, have also been shown to stimulate the coalescence of human monocytes into MGCs. No single cytokine had an equivalent stimulatory effect, although interferon (IFN)-γ slightly enhanced MGC formation (Most et al., 1990). IL-4 does not directly increase monocyte migration and actually reduces secretion of chemotactic factors (Te Velde et al., 1988) but does have marked effects on monocyte membrane proteins, down-regulating some (CD14; Lauener et al., 1990) and up-regulating others (HLA-DR, CD23; Lauener et al., 1990; Littman et al., 1989), including ICAM-1, according to a recent report (Valent et al., 1991). Therefore, increased adhesion was thought to be the most likely mechanism for IL-4-stimulated monocyte clustering. As many lymphocyte–lymphocyte and lymphocyte–monocyte functional interactions involve reciprocal binding of cell adhesion molecules, especially LFA-1–ICAM-1, on
the cell surface (Springer, 1990), we sought evidence for involvement of these molecules in monocyte cluster formation.

In this report we show that IL-4 consistently stimulates both uninfected and HIV-infected monocytes to form cell clusters, which appear to be the temporal precursors of MGCs, and that this was mediated by enhanced LFA-1-ICAM-1 interaction. These results complement recent reports, which have shown that the LFA-1-ICAM-1 interaction was involved in the formation of syncytia or MGCs from HIV-infected lymphocytes (Hildreth & Orentas, 1989; Valentin et al., 1990; Pantaleo et al., 1991a, b).

Aliquots of blood (150 ml) were obtained from healthy HIV-seronegative volunteers. Monocytes were separated by countercurrent elutriation and by complement-dependent lysis using monoclonal anti-CD3 antibody as previously described. Monocytes and macrophages were grown in RPMI-1640 medium containing 10% human or fetal calf serum. This regularly tested negative for the presence of endotoxin by the Limulus lysate chromogenic assay. Plastic-adherent macrophages did not produce IL-1 or macrophage pro-coagulant activity spontaneously under normal growth conditions (Kazazi et al., 1989, 1991).

Monoclonal antibodies to cell surface antigens and their sources were as follows: Leu M3 (anti-CD14; Becton Dickinson), OKT3 (anti-CD3; Ortho Diagnostics), anti-LFA-1 (anti-CD11a, anti-CD18; Immunotech; Becton Dickinson) anti-ICAM-1 (anti-CD54; Immunotech; Becton Dickinson). All fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies were used in excess at concentrations of 1 μg/ml. Monoclonal antibodies to β2-microglobulin, VLA-4 and VCAM were obtained from Immunotech. Anti-ICAM-2 was a generous gift of Dr P. Nortamo (Nortamo et al., 1991). Neutralizing polyclonal antibody to IL-4 was obtained from Endogen. This antibody had been previously demonstrated to reverse IL-4 stimulation of monocyte CD23 expression. Purified monocytes were stained with monoclonal antibodies to the cell adhesion molecule followed by goat anti-mouse F(ab)2 conjugated to FITC and quantified by flow cytometry (Epics Coulter) as previously described (Kazazi et al., 1989, 1991).

Twenty recent, low (one) passage HIV-1 isolates from Australian patients with asymptomatic HIV infection, AIDS-related complex or AIDS were tested for their ability to replicate in human monocytes. Seven strains, from patients who had not been recently exposed to antiviral agents, that replicated well in monocytes were chosen for use in this study. HIV isolates were passaged once or twice in phytohaemagglutinin (PHA)-stimulated donor peripheral blood mononuclear cells (PBMCs) to increase titres before use. Most studies were performed with viral inocula that were concentrated by ultracentrifugation (45000 g, 18 h) to remove any residual contaminating mitogens or cytokines. HIV strains were quantified by estimation of TCID₅₀ in the THP-1 (macrophage-like) cell line and in PHA-stimulated PBMCs. With the monocytotropic virus strains used here, titres estimated in THP-1 cells were 0·5 to 1·0 TCID₅₀ higher than in PBMCs.

Recombinant IL-4, IL-2, GM-CSF, M-CSF and tumour necrosis factor (TNF-α) were obtained from Amersham and IFN-γ from Genzyme. Cytokines were used at the following concentrations: IL-4 at 1 to 20 ng/ml, IL-2 at 10 to 500 units/ml, GM-CSF at 500 to 1500 units/ml, M-CSF at 500 to 1500 units/ml and IFN-γ at 10, 100 and 1000 units/ml. ELISA kits for IL-4, GM-CSF and TNF-α were obtained from Genzyme.

Adherent monocytes or macrophages (10⁶) were cultured in 24-well tissue culture plates (Costar) or in Labtek dual-chambered slides in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and 10% human group AB positive serum. The cells were inoculated with cell-free HIV isolates at 10⁴ to 10⁶ TCID₅₀ in THP-1 cells (0·01 to 0·1 TCID₅₀/monocyte) and allowed to adsorb for 2 h. The medium was aspirated, the monocytes washed with RPMI-1640 at 37 °C and fresh medium added. Most (90%) of the medium was replaced every 3 days and the supernatants stored for quantification of HIV. Cell culture supernatants were assayed at 3 day intervals for HIV antigen. (HIV antigen assay; Abbott Laboratories). This assay detects HIV p24. The limit of detection of HIV p24 antigen was 9 pg/ml. Results were confirmed by reverse transcriptase assays and, as previously, found to correlate with HIV antigen concentrations (Kazazi et al., 1991). Population means and s.d.s were compared with the Student's t-test, adjusted for unequal variances.

Our results showed that recombinant IL-4 at concentrations of 1 to 20 ng/ml consistently enhanced the formation of monocyte clusters within 3 to 6 days. These concentrations were below those that readily induce monocyte detachment from substrate (50 ng/ml; Elliott et al., 1991). The clusters were easily visible by phase contrast microscopy but could be better defined and quantified by fixation and staining with Giemsa (Fig. 1). The clusters were of variable size, consisting of 4 to 10 distinct cells that were attenuated in morphology. As shown in Fig. 1(c), further incubation of the cultures for 10 to 14 days resulted in the formation of MGCs in two out of five experiments. Incubation of parallel cultures of monocytes with IL-1 (10 to 100 ng/ml), IL-2 (1 to 10 ng/ml), TNF-α (10 to 50 ng/ml), GM-CSF (500 to 1500 units/ml), M-CSF (500 to 1500 units/ml) or IFN-γ (10 to 1000 units/ml) for 12 days did not significantly
enhance monocyte clustering or MGC formation (data not shown). No stimulation of cluster formation after pre-incubation of IL-4 with anti-IL-4 neutralizing polyclonal antibody for 1 h at room temperature was observed and assays of culture supernatants for IFN-γ and TNF-α were negative, thus excluding the effects of contaminating endotoxin or secondarily induced cytokines (data not shown). Addition of IL-1 (40 to 100 ng/ml), TNF-α (10 to 40 ng/ml) or IFN-γ (10 to 1000 units/ml) simultaneously or at 4 days after addition of IL-4 had no additional effect on monocyte clusters or MGC formation above that seen with IL-4 alone (data not shown).

Monocyte clusters (Fig. 2a) and MGCs (Fig. 2b) were quantified by Giemsa staining and light microscopy in uninfected monocyte cultures incubated with IL-4 with or without monoclonal antibodies against LFA-1 (anti-CD18 and anti-CD11a) and ICAM-1 (anti-CD54). Medium was used as the control for IL-4 and a monoclonal antibody against canine thymocytes was used as an isotype control for the specific monoclonal antibodies. In two representative experiments, cell cluster formation markedly and significantly increased from 13 (±3) to 45 (±5) and 15 (±4) to 35 (±2) clusters/5 high-power fields (HPF) under the microscope after 6 days incubation with IL-4 at 10 ng/ml (P < 0.01; Fig. 2a and b, respectively). However, MGC were prominent in only two out of five IL-4-stimulated monocyte cultures.

There was marked and significant inhibition of both cell clustering and MGC formation with anti-CD11a,
Stimulation of cell cluster formation by IL-4 and inhibition by monoclonal antibodies to LFA-1 (CD11a, CD18) and ICAM-1. (b) Cluster (■) and MGC (■) formation using similar treatments to those in (a).

Monoclonal antibodies to LFA-1 (anti-CD11a and anti-CD18) produced a marked and significant inhibition of cluster formation (94 ± 5 to 35 ± 5; 32 ± 4 to 6 ± 1 and 10 ± 3 respectively, P < 0.01; Fig. 3a, b). The effects of monoclonal antibodies to ICAM-1 were consistently less marked (94 ± 5 to 56 ± 5 and 32 ± 4 to 22 ± 2 respectively; P < 0.01; Fig. 3a, b) than those of monoclonal antibodies to CD18 or CD11a (30 to 50% less inhibition). The pattern of inhibition of subsequent MGC formation was similar (Fig. 3c). Monoclonal antibodies to CD11a and CD18 from Becton Dickinson were slightly less inhibitory than those from Immunotech. A graded effect of increasing concentrations of these monoclonal antibodies was again observed; some inhibition occurred at 1 μg/ml and this reached a maximum at 10 to 20 μg/ml.

Monoclonal antibodies to LFA-1 and ICAM-1 in HIV-infected monocyte cultures, the effect of CD11a and CD18 from Becton Dickinson were less inhibitory than those from Immunotech. An inhibitory effect of these monoclonal antibodies was observed at a concentration of 1 μg/ml but reached a maximum at 10 to 20 μg/ml.

Monocyte cluster and MGC formation was again quantified after fixation and Giemsa staining of HIV-infected cells, incubated with and without IL-4 for 4 to 6 and 10 to 14 days respectively. As shown in Fig. 3(a and b), monocyte cluster formation was significantly increased by HIV infection, although not to the same extent as IL-4 alone (18 ± 1 to 30 ± 7 and 53 ± 5 clusters/5 HPF compared with 9 ± 2 to 18 ± 2 and 24 ± 3 clusters/5 HPF; P < 0.01). The combination of HIV infection and IL-4 (added after HIV inoculation) resulted in a markedly enhanced formation of monocyte clusters, which was significantly greater than either effect alone (up to 94 ± 5, P < 0.01, Fig. 3a; up to 32 ± 4, P < 0.02, Fig. 3b). The size and morphology of the clusters were similar to those shown in Fig. 1(b). The stimulatory effects of HIV and IL-4 on subsequent MGC formation were similar (Fig. 3c). As with uninfected monocyte cultures, IL-1, TNF-α and IFN-γ did not stimulate cluster formation. When incubated together with IL-4, none of these cytokines enhanced clustering or MGC formation above that observed with IL-4 alone.

Although monocyte cluster formation was consistently enhanced after IL-4 stimulation of HIV-infected monocytes (in seven out of seven experiments with four different HIV isolates and six different donors), progression to MGC formation was variable (three of seven experiments; Fig. 3b). MGC formation did not correlate with extracellular HIV concentrations as measured by an antigen assay (for example, 0.5 and 8 ± 5 MGCs/5 HPF; 87 ± 9 and 58 ± 7 pg/ml respectively).

In view of the unequal inhibition of cell cluster formation by monoclonal antibodies to LFA-1 and ICAM-1 in HIV-infected monocyte cultures, the effect of...
incubating anti-ICAM-2 monoclonal antibody alone and in combination with anti-ICAM-1 with HIV-infected and uninfected monocytes was assessed. Anti-ICAM-2 did not significantly inhibit uninfected monocyte aggregation but inhibited both HIV-infected monocyte aggregation and MGC formation to the same extent as anti-ICAM-1. However, consistent additive or synergistic effects of anti-ICAM-1 and anti-ICAM-2 could not be demonstrated (Fig. 3c).

Monoclonal antibodies to CD11a and ICAM-1 in concentrations that inhibited aggregation (1 to 10 μg/ml) had no significant effect on extracellular concentrations of HIV antigen in IL-4 stimulated, infected monocyte supernatants. Incubation of uninfected monocytes adhered to plastic for 3 to 5 days consistently resulted in markedly enhanced ICAM-1 expression and addition of IL-4 at 10 to 20 ng/ml for 3 to 5 days resulted in further significant increases in membrane expression of ICAM-1. The proportion of monocytes expressing ICAM-1 increased from 5% to 30% and 79% respectively; the mean fluorescence intensity increased from 60 to 271 and 322 fluorescence units, respectively. HIV infection alone had no consistent effects on LFA-1 or ICAM-1 expression by monocytes. Incubation of HIV-infected monocytes with IL-4 for 3 to 5 days had similar effects to uninfected monocytes (30% increasing to 80% of monocytes expressed ICAM-1; the increase in mean fluorescence intensity was 26 to 37 fluorescence units). There was no consistent or significant effect of IL-4 on LFA-1 (CD11a or CD18), VCAM, VLA-4 or β2-microglobulin (control) expression by HIV-infected or uninfected monocytes.

IL-4 secretion from HIV-infected MT4 cells could not be detected. In these experiments, 2 × 10⁶ MT4 cells were inoculated with 10⁶ TCID₅₀ of two freshly isolated strains of HIV. Supernatants were collected at days 1, 3 and 5 and tested for HIV antigens and IL-4 by ELISA. The MT4 cells were infected (peak extracellular HIV antigen concentration of 254±30 pg/ml) but no IL-4 was detected. These experiments were repeated with HIV-infected purified monocytes and supernatants collected on days 1, 3 and 5. Peak HIV antigen con-

![Fig. 3. Stimulation of cell cluster formation (a to c) and MGC formation (c) by IL-4, and inhibition by monoclonal antibodies to LFA-1 (CD11a and CD18), ICAM-1 (CD54) and ICAM-2 (CD102) in three different combinations of donor monocytes infected with HIV strains. (a) A synergistic effect of HIV and IL-4 on monocyte cluster formation was usually observed. (b) A graded inhibitory effect of different concentrations of anti-CD11a, anti-CD18 and anti-ICAM-1 (CD54) monoclonal antibodies was observed. The concentrations of monoclonal antibodies used, in μg/ml, are indicated. (c) The stimulatory effects of IL-4 and HIV on monocyte cluster and MGC formation and inhibition by monoclonal antibodies to CD11a, CD11b, ICAM-1 and ICAM-2 were similar.](image-url)
centration was 120 pg/ml. No IL-4 was detected in any of these supernatants.

These experiments have demonstrated that IL-4 is capable of stimulating cell clustering and MGC formation in both HIV-infected and uninfected human monocytes, cultured without the addition of recombinant M-CSF. HIV infection alone stimulated clustering and MGC formation but both were markedly enhanced by IL-4. Furthermore, most of the cells in the cluster were infected by HIV, as demonstrated by in situ hybridization at 7 days after HIV inoculation (Kazazi et al., 1991; F. Kazazi et al., unpublished results). MGC formation was always preceded by the formation of monocyte clusters. Clusters were invariably induced by IL-4. However, the effect of IL-4 in stimulating MGC formation in both HIV-infected and uninfected monocytes was variable, occurring in only 40% of uninfected or infected cultures by day 14. Hence it appears that monocyte cluster formation is a precursor to MGC formation and that additional factors may influence their subsequent development (see below). Furthermore, monoclonal antibodies to LFA-1 (either anti-CD11a or anti-CD18) and ICAM-1 from several sources can markedly reduce the formation of monocyte clusters (and subsequently MGC formation) in uninfected and HIV-infected monocyte cultures.

In uninfected monocyte cultures the effects of monoclonal antibodies to LFA-1 and ICAM-1 were approximately equal, suggesting that ICAM-1 is the major ligand for LFA-1 in these IL-4-stimulated cultures. These observations complement those of Most et al. (1990), which demonstrated that supernatants from concanavalin A-stimulated lymphocytes induced the formation of MGCs from normal (uninfected) human monocytes in vitro. However, they found that IFN-γ was capable of inducing only a low level of MGC formation and that other cytokines, including IL-4, produced no effect. Furthermore, treatment of the concanavalin A supernatants with monoclonal antibody to IFN-γ inhibited MGC but not cluster formation in their system. Conversely, McNnes et al. (1988) reported that IL-4 consistently stimulated MGC formation from bone marrow-derived murine macrophages. The variability in the effects of IL-4 on MGC formation in our system may account for these discrepancies. The response of some human monocyte functions to IL-4 declines with increasing duration of in vitro culture and treatment with GM-CSF (Hart et al., 1993). Hence, differences in the effect of IL-4 may also occur in monocyte culture systems using exogenous M-CSF. It seems likely that IL-4 is the major cytokine inducing monocyte cluster formation but that MGC formation may require the participation of other cytokines. Candidates include IFN-γ, IL-1 and TNF-α, all of which have been documented to enhance ICAM-1 expression (Springer, 1990). However, in our culture system IFN-γ, TNF-α or IL-1 did not stimulate cell cluster formation nor did they enhance MGC formation when added to IL-4-stimulated, HIV-infected or uninfected monocytes, in contrast to the effects of TNF-α on HIV-infected lymphoblastoid cells (Matsuyama et al., 1989).

Cytokines could enhance cluster formation by stimulating monocyte migration or adhesion. However, IL-4 has been reported not to increase monocyte mobility or chemotaxis (Te Velde et al., 1988) although it decreases monocyte adherence to substrate at high concentrations (Kazazi et al., 1991; Elliott et al., 1991). The involvement of LFA-1–ICAM-1 interactions in monocyte adhesion, cell cluster and subsequent MGC formation is not surprising in view of their importance in many other lymphocyte–lymphocyte or monocyte–lymphocyte interactions (Springer, 1990).

Monoclonal antibodies to ICAM-1 from two sources were consistently less effective in inhibiting monocyte cluster formation than the anti-CD11a and anti-CD18 antibodies in HIV-infected cells. These results suggested that another ligand, such as ICAM-2 or ICAM-3, may also be involved in these interactions (Nortamo et al., 1991; De Fougerolles & Springer, 1992) and are similar to recent reports using HIV-infected lymphoid cells (Pantaleo et al., 1991 a, b). Further monoclonal antibody inhibition experiments suggested that both ICAM-1 and ICAM-2 participated in IL-4-stimulated, HIV-infected monocyte clustering and MGC formation but ICAM-1 was much more important in the uninfected monocytes. Failure of anti-ICAM-1 and anti-ICAM-2 to inhibit these changes to the same degree as anti-LFA-1 monoclonal antibodies suggests involvement of further ligands (for example ICAM-3) or HIV-induced changes in ICAM-1 conformation. These experiments also demonstrated that IL-4 had a more potent effect in inducing monocyte cluster formation than HIV infection alone and suggested that the local production of IL-4 in vivo could have a significant role in intercellular transmission of HIV by the formation of HIV-infected MGCs from monocytes or macrophages.

The dissociation between the inhibition of monocyte aggregation by anti-LFA-1 and ICAM-1 and the lack of significant inhibition of HIV production was similar to that observed by Pantaleo et al. (1991b) in HIV-infected lymphoblastoid cell lines. Although Kalter et al. (1991) reported inhibition of cell-free HIV infection of monocytes by anti-LFA-1 monoclonal antibodies, they indicated that this was epitope-specific and less effective against transmission from infected cells. These observations suggest that significant HIV transmission between monocytes can still occur via extracellular fluid, independently of the formation of cell clusters.
The effects of IL-4 on the up-regulation of ICAM-1 confirm the recent report of Valentin et al. (1991), who showed IL-4-stimulated ICAM-1 expression on mast cells and other cell types, including monocytes and tissue macrophages. We used purified monocytes from several donors to confirm that this is a consistent and significant effect. However, adherence to plastic and subsequent differentiation is the most potent stimulus to enhancement of monocyte or macrophage ICAM-1 expression, as also demonstrated by Most et al. (1992), and may contribute to the basal cell cluster formation shown in Fig. 3. The marked effect of IL-4 on cell clusters is therefore unlikely to be solely due to ICAM-1 up-regulation. Although up-regulation of LFA-1 was not demonstrated, this integrin has been shown to undergo rapid conformational changes, which play an important role in transient enhancement of intercellular adhesion (Springer, 1990). The monoclonal antibodies used in this study do not recognize the activation state of LFA-1 but one such antibody has recently been described (Dransfield et al., 1992).

The marked and consistent effect of IL-4 on cluster formation strongly suggests a supporting role for this cytokine in enhancing MGC formation (or syncytial formation) from monocytes in vivo. Such effects would be particularly important in the brain, where HIV infection occurs early and the predominant infected cell types, microglia cells and macrophages, fuse to form the typical MGCs (Wiley et al., 1986). IL-4 is released from activated T lymphocytes, not usually from monocytes (Te Velde et al., 1988) and activated T cells have also been found in perivascular infiltrates in HIV-infected brain tissue. In this study, HIV infection alone did not induce IL-4 secretion from lymphoblastoid cells in vitro. Recently we have demonstrated the presence of IL-1β, TNF-α, IFN-γ and IL-4 RNA in lymphocytes adjacent to, but not within, MGC in sections of brain from four HIV-infected patients, using oligonucleotide and riboprobe in situ hybridization (J. M. Mathijs et al., unpublished results). These findings suggest that IL-4 may play an important role in the pathogenesis of MGC in the brain.

Several factors may be important in the progression from adherent clusters of HIV-infected monocytes or macrophages to formation of MGCs, via cell membrane fusion in vitro and in vivo. In HIV-infected lymphoblastoid cells these factors include the concentration of HIV gp160 and CD4 at the cell surface (Lifson et al., 1986), strain variation in HIV, particularly in the V3 loop (Tersmette et al., 1988; De Jong et al., 1992), variation in the state of the host cell membrane (Ashorn et al., 1990; Mohagheghpour et al., 1991) and effects of other cytokines (Matsuyama et al., 1989). Although expression of gp160 and CD4 in adjacent cells is necessary for fusion in this system, it is not sufficient alone. Marked variability in syncytium forming ability by strains of HIV has been linked to replicative capacity and amino acid substitutions in the V3 loop (De Jong et al., 1992). There may be additional undefined factors present within human cell membranes necessary for cell fusion. Early activation events in T lymphocyte membranes, involving protein kinase C, were also essential for optimum syncytial formation. In the in vitro systems described to date it has been difficult to distinguish enhancement of HIV replication and subsequent gp160 expression from other effects on the cell membrane.

In HIV-infected monocytes, where the expression of both CD4 and gp160 at the cell surface is much lower than in T lymphoblastoid cells, other factors may be still more important in apposing cell membranes of adjacent cells or contributing to induction of fusion pores via the viral fusion peptide (White, 1992). For example, human placental macrophages express CD4 at very low levels in vitro and yet can be infected with HIV and readily form syncyta (Kesson et al., 1993). The lack of correlation between MGC formation and extracellular HIV concentrations produced by infected monocytes in this study suggests that variability in the effects of cytokines on these target cells may be important in vitro and perhaps also in vivo. This concept is strongly supported by the variability of MGC formation in IL-4-stimulated uninfected monocytes. Hence, the relative contributions of cytokines (especially IL-4), host cell factors and the level of gp160 expression at the monocyte membrane are now being investigated in detail.

We thank Claire Wolczak and Barbara Dupond for typing the manuscript; Dr Alison Kesson for critically reading it and Dr P. Nortamo for his generous gift of anti-ICAM-2 monoclonal antibody. This work was supported by the Commonwealth AIDS Research Grant scheme of Australia.

**References**


monocytes differ in their response to IL-4. *Journal of Immunology* **151**, 3370-3380.


(Received 21 April 1994; Accepted 10 May 1994)