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HIV-1 infection of monocyte-derived macrophages reduces Fc and complement receptor expression

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SUMMARY

Fc receptor (FcR) and complement receptor (CR) expression on HIV-infected monocyte-derived macrophages may be an important determinant of immune function. We studied the effects of HIV-1 infection of macrophages *in vitro* on FcR and CR expression. Macrophages were infected with HIV-1_{DV} 7 days following isolation, and the expression of FcγRI–III and CR3 were measured at intervals thereafter by flow cytometry. We found a reduction in receptor expression with the percentage of cells expressing FcRI 14 days post infection declining from 77% to 13%, FcRII fell from 96% to 85%, FcRIII from 45% to 9%, and CR3 from 91% to 67% 14 days following infection. As these receptors are important for macrophage function, their down-modulation may contribute to the pathogenesis of HIV-related disease.

Keywords HIV-1 monocytes macrophages Fc receptors complement receptors

INTRODUCTION

Human monocyte-derived macrophages (MDM) have surface receptors for the Fc portion of immunoglobulins (FcR). These FcR mediate a number of immune processes. MDM express FcγRI, a high-affinity receptor for IgG which facilitates phagocytosis of opsonized microorganisms as well as antibody-dependent cellular cytotoxicity. FcγRII is a low-affinity receptor expressed on human MDM, which is necessary for the initiation of the oxidative burst. MDM, but not monocytes, express FcγRIII, a low affinity Fc receptor which mediates both phagocytosis and the clearance of immune complexes [1–6]. For example, the phagocytosis of *Toxoplasma gondii* is in part dependent on the presence of functional FcR [7,8]. Receptors to complement components are essential for the phagocytosis of complement-coated organisms [9]. Complement receptor 3 (CR3) and CR4 bind the complement component C3bi, are expressed abundantly on MDM, are involved in adhesion of monocytes to plastic surfaces and endothelium, and augment the phagocytosis of organisms such as *Mycobacterium tuberculosis* and *Myco. avium* complex [10–13].

MDM are susceptible to HIV infection both *in vitro* and *in vivo* [14,15]. MDM are likely to function as a major reservoir for HIV *in vivo*, as they are capable of latent and/or chronic infection and do not develop marked cytopathic effects following HIV infection, although syncytial formation may be evident [15]. The functional capacity of MDM following HIV infection has been reported to be depressed [16–18]. Not all of the immune deficiency and opportunistic infections (OI) that occur in HIV-infected individuals can be explained solely by T cell depletion or dysfunction [19,20]. HIV-infected patients frequently develop reactivation of intracellular organisms, including *T. gondii, Myco. tuberculosis* and *avium* complex and *Histoplasma capsulatum* [21]. In the immunocompetent host, these organisms are normally controlled at least in part by activated MDM.

We hypothesize that HIV infection of MDM may down-regulate expression of important surface receptors. The resulting MDM dysfunction then contributes to the pathogenesis of HIV-related disease.

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MATERIALS AND METHODS

Isolation of monocytes

Monocytes were isolated from HIV$^-$ buffy coats (donated by Red Cross Blood Bank, Melbourne, Australia) by density gradient centrifugation and glass adherence, as previously described [14]. Briefly, 25 ml of buffy coat diluted 1:3 with PBS was underlaid with 15 ml of Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) and centrifuged at 650 *g* for 20 min at 20°C. The leucocyte layer was aspirated, washed with PBS and incubated on 150-mm Petri dishes (Greiner, Frickenhausen, Germany) for 2 h at 37°C to allow adherence. The cell layer was washed with PBS to remove non-adherent cells and then gently scraped off with a cell lifter (Costar, Cambridge, MA). Washed monocytes were suspended in Teflon-coated pots (Savillex, Minnetonka, MN) at a concentration of 10^6 monocytes/ml in RPMI 1640 (Flow Labs, Sydney, Australia), supplemented by

10% heat-inactivated human AB+ serum (kind donation of Sydney Red Cross Blood Bank), L-glutamine 2 mM (Flow) and pencillin 50 U/ml and streptomycin 50 μg/ml (Flow). Media were changed weekly. MDM were also cultured adherent to plastic using six-well plates (Costar). All media and materials were tested and found to contain less than 0·10 EU/ml lipopolysaccharide (LPS) by the limulus amoebocyte lysate colorimetric assay (Whittaker, Walkerville, MD). By trypan blue exclusion, the viability of monocytes was greater than 98%. By morphology, >95% of cells had the appearance of MDM after 21 days in culture. The proportion of MDM staining for the macrophage marker CD11c was tested after 21 days in culture. Purity of monocytes was greater than 88% by non-specific esterase staining (Sigma, St Louis, MO).

Measurement of receptors

The percentage of macrophages expressing Fcγ receptors I–III (FcRI–III) and complement receptor CR3 was measured by staining 5×10^5 cells using an indirect two-stage labelling technique [14,22] followed by flow cytometric analysis (Becton Dickinson FACStar plus, San Jose, CA). The FACStar plus was aligned on a daily basis with 2 μm fluorescein-labelled microbeads (Polysciences Inc., Warrington, PA) using the same amplification and photomultiplier tube (PMT) voltage settings to ensure consistent performance of the instrument. The PMT voltage was adjusted so that the peak of the isotype control of the uninfected sample was contained within the first of a 4-decade logarithmic scale. Once set, the PMT voltage was kept constant for the other samples from the same donor where cells had been in culture for the same period of time. The specificities of the murine MoAbs used were as follows: FcγRI (CD64) 32.2 (Medarex, West Lebanon, NH), 50 μl of a 50 μg/ml concentration per test; FcγRII (CDw32) 8.7 (kindly donated by Dr M. Hogarth, University of Melbourne) using 50 μl of ascites preparation; FcγRIII (CD16) 3G8 (kindly donated by Dr S. Clarkson, Rosalind Russell Arthritis Research Laboratories, San Francisco, CA) 50 μl of a 100 μg/ml concentration; CR3 (CD11b) (Becton Dickinson) 10 μl of a 0·5 mg/ml concentration, OKM1 and OKM10 10 μl of ascites preparations (kindly supplied by Dr D. Gordon, Flinders Medical Centre, Adelaide, Australia); HLA-DR (Becton Dickinson) 20 μl of 25 μg/ml concentration; intercellular adhesion molecule-1 (ICAM-1) 10 μl of ascites preparation (WeCAM, kindly provided by Dr A. Boyd, Walter and Eliza Hall Institute, Melbourne, Australia); Leu-M5 (CD11c) 10 μl of 10 μg/ml concentration (Becton Dickinson). MDM were washed with PBS supplemented with 1% heat-inactivated fetal calf serum (FCS; Flow) and 0·1% sodium azide (PBS/FCS/Az), then incubated with MoAb to the above receptors for 45 min at 4°C. MOPC 21 (IgG1; Organon Teknika, Westchester, PA) and MOPC 141 (IgG2b; Bionetics, Charleston, SC) were used as immunoglobulin subtype-specific control MoAbs. The cells were then rewashed with PBS/FCS/Az and incubated with 50 μl of 0·91 μg/ml of goat $F(ab')_2$ anti-mouse fluorescein conjugate (FITC-GAM) (Tago, Burlingame, CA) for 45 min before being rewashed and fixed with 1% formaldehyde in PBS/FCS/Az. In specific experiments involving adherent MDM, cells were placed on ice for 10 min and gently scraped from plastic wells using a cell lifter (Costar), then treated as above. The relative density of fluorescence was examined in addition to the per cent cells staining positive using the formula: (% cells positive using specific MoAb × mean fluorescence) − (% cells positive using control MoAb × mean fluorescence).

HIV infection

On day 7 post-isolation, half of each MDM culture was infected with $HIV\text{-}1_{DV}$, an amphotropic isolate of HIV-1 (kindly supplied by Dr G. Reyes, Genelabs, Redwood City, CA) as previously described [14]. Briefly, freshly thawed virus stock was incubated with MDM at a multiplicity of infection of approximately 1·7 $TCID_{50}$ (1·0 infectious particles) per cell. After 48 h of incubation at 37°C the cells were washed three times and fresh medium was added, and supernatants collected for HIV-1 p24 quantification.

HIV infection of MDM was documented 14 days after infection by flow cytometric detection of intracellular HIV p24 antigen as previously described [14]. Briefly, MDM were fixed with 3% formaldehyde in PBS/FCS/Az, washed with 0·1 M glycine in PBS/FCS/Az twice, then permeabilized with 1 ml of 1% Triton X-100 in PBS/FCS/Az for 60 s before being rewashed. The cells were then stained with anti HIV-1 p24 MoAb (Olympus, Lake Success, NY), 50 μl of 100 μg/ml for 45 min, using MOPC 21 as a control MoAb, then rewashed and stained with FITC-GAM. Quantification of p24 antigen by enzyme immunoassay (Abbott Laboratories, Abbott Park, IL) was performed on serial supernatants of all cultures to confirm HIV infection.

To measure the proportion of cells which were positive for p24 which were also expressing FcRI, cells were initially stained by indirect fluorescence using anti-HIV p24 followed by FITC-GAM as above. Cells were then stained for FcRI (or isotype control) using MoAb directly conjugated to PE.

RESULTS

Following HIV infection, a mean of 28% of MDM expressed p24 antigen ($n=6$, s.d. 7%, range 18–45%) as detected by flow cytometric analysis. p24 antigen in culture supernatant rose from a mean of 211 pg/ml 2 days after infection to a mean of 76000 pg/ml 2 weeks post-infection in HIV-infected MDM cultures. Viability remained above 91% in all cultures during the course of experiments, and the proportion of cells expressing the macrophage marker CD11c after 3 weeks in culture was 92%.

Expression of the receptors was documented weekly over 3 weeks and each experiment was repeated at least six times to allow for donor variability. Expression of FcRI, II and III all declined following HIV infection of MDM ($P<0·001$ using Student's t-test) (Fig. 1a, b, c). The proportion of cells expressing FcRI 14 days post-infection declined markedly from a mean of $77 \pm 10·2\%$ in the uninfected cultured to $13 \pm 6·6\%$ in the infected cultures (Fig. 1a). FcRII expression showed a less dramatic decline, from $96 \pm 2·5\%$ to $85 \pm 5\%$ (Fig. 1b); the fall in FcRIII was from $45 \pm 16\%$ to $9 \pm 2\%$ (Fig. 1c). This decline in receptors was maximal 2 weeks following infection. Similarly, the proportion of cells expressing CR3 fell from $91 \pm 3·4\%$ in the uninfected culture to $67 \pm 13\%$ in the infected culture 2 weeks after infection ($P<0·02$) (Fig. 1d). With both FcRI–III and CR3, significant decreases in expression were not seen until 7 days post-infection, and were maximally observed 14 days post-infection (Fig. 1a–d). The decline in CR3 detected with CD11b MoAb (Becton Dickinson) was detected equally with MoAbs OKM1 and OKM10 (data not shown). There were no differ-

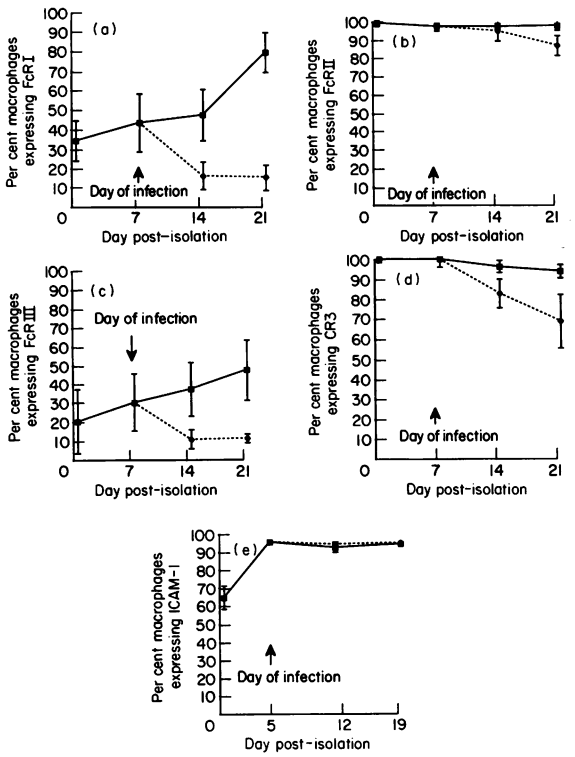

Fig. 1. Expression of FcRI–III, CR3 and intercellular adhesion molecule-1 (ICAM-1) on uninfected macrophages (——) and macrophages from same donors infected with HIV-1 (– – –), as determined by flow cytometry. (a) FcRI expression. (b) FcRII expression. (c) FcRIII expression. (d) CR3 expression. (e) ICAM-1 expression.

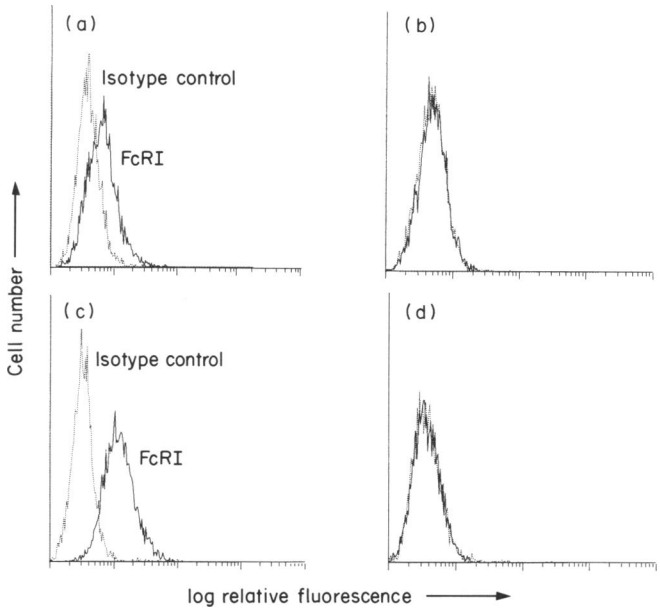

Fig. 2. Representative histograms for HIV-infected and -uninfected monocyte-derived macrophages (MDM) stained with anti-FcRI (——) or isotype-matched controls (· · · · ·). (a) Uninfected cells day 14 of culture. (b) Infection cells day 14 of culture (7 days post HIV infection). (c) Uninfected cells day 21 of culture. (d) Infected cells day 21 of culture (14 days post HIV infection).

ences in expression of FcRI–III and CR3 when MDM in suspension were compared with those adherent to plastic (data not shown). Representative histograms of cells from a single donor stained with FcRI (or isotype control) from days 7 to 14 post-infection are shown in Fig. 2. The decline in receptor expression was unimodal (Fig. 2).

The density of fluorescence of FcRI at 14 days post-infection declined from 91·2 ± 10·4 units in the uninfected cultures to 55·0 ± 9·78 units in the infected cultures. FcRII declined from 141·1 ± 13·6 units in the uninfected cultures to 128·9 ± 14·7 units in the infected cultures, FcRIII declined from 93·0 ± 10·9 units in the uninfected cultures to 49·1 ± 6.23 units in the infected cultures, and CR3 fell from 166·4 ± 18·3 units in the uninfected cultures to 139·2 ± 14·2 units in infected cultures.

To ascertain whether the observed decline in receptor expression reflected a generalized down-regulation of surface receptors, cells were additionally stained with a MoAb directed to both ICAM-1 and HLA-DR or isotype-matched controls. Surface expression of HLA-DR was 74·9 ± 9·6% on uninfected MDM and 80·6 ± 9·6% on HIV-infected MDM 8–14 days following HIV infection. Similarly, there was no alteration in ICAM-1 expression on MDM following HIV infection. ICAM-1 expression was 92·0 ± 1·0% on uninfected MDM and 92·5 ± 0·5% on HIV-infected MDM from two individual donors 14 days following HIV infection. A time course of ICAM-1 expression is shown in Fig. 1e.

To determine the proportion of HIV-infected MDM which were expressing FcRI, double-labelling experiments with both anti-HIV p24 and FcRI were performed. Within the infected culture of MDM 10% of cells dually labelled for both FcRI and HIV p24 and 72% of cells were FcRI-expressing and p24-negative. In the control uninfected MDM from the same donor, 77% of cells expressed FcRI.

To determine whether FcR expression could still be induced by interferon-gamma (IFN-γ) on MDM following HIV infection, we exposed MDM infected with HIV-1 for 14 days and paired uninfected cells from the same donor to 100 U/ml of IFN-γ (Boehringer, Mannheim, Germany) or to media alone. FcγRI was measured 48 h later. There was a rise in FcRI expression from 75% to 91% in the uninfected culture, and from 15% to 37% in the infected culture.

DISCUSSION

We have documented a decline in expression of FcR and complement receptor CR3 on human macrophages following infection with HIV-1. There was a decline in both the percentage of cells expressing the receptor and the density of fluorescence seen. Further, in double-labelling experiments, the decline in FcRI in bulk-infected cultures was in large part (but not exclusively) due to the decline in FcRI on the MDM, in which intracellular HIV p24 expression could be demonstrated. The decline was most marked with FcRI and III, but was also noted with FcRII and CR3. We were careful to exclude the presence of LPS in our materials, as this causes a decline in FcR expression ([23] and Crowe, unpublished studies). The effect of HIV-1 on FcR and CR3 was not due to a general decline in receptor expression, as no loss of HLA-DR or ICAM-1 expression was seen. Additionally, we demonstrated that the HIV-1-infected MDM were still responsive to the up-regulating effect on FcRI

by IFN, even though the concentration of IFN we used could have had an antiviral effect [24].

The expression of surface FcR is known to vary depending on the state of cellular maturation (i.e. monocytes to MDM). FcγRIII is induced as monocytes mature into macrophages, whilst FcRI and II and CR3 are present on both monocytes and macrophages [25]. It is known that there is a decline in FcRI–III and CR3 when healthy MDM are maintained *in vitro* for greater than 14–21 days [26]. Our results are consistent with the above observations.

There is a precedent for viral infection of monocyte-macrophages and other cells resulting in alteration of Fc and complement receptor expression. Cytomegalovirus (CMV) infection of human embryonic lung fibroblasts results in increased expression of an FcR-like molecule, rather than a known FcR [27]. Indirect evidence that viral infection can reduce FcR and CR expression on both monocyte-macrophages and other cells is also available. Dengue virus infection of murine peritoneal macrophages resulted in decreased adherence and phagocytosis of immunoglobulin-coated sheep erythrocytes [28]. Murine peritoneal macrophages infected with murine CMV bound immunoglobulin-coated sheep erythrocytes less efficiently than uninfected cells, implying a loss of FcR [29]. Bovine rhinotracheitis virus infection of bovine alveolar macrophages caused a progressive reduction in the binding and ingestion of IgG and complement opsonized sheep erythrocytes as well as reduced phagocytosis of *Candida parasilopsis*, thereby implying a loss of Fc and complement receptors [30]. Infection of bovine granulocytes with bovine rotaviruses and herpesviruses results in a decreased attachment of haemolysin-coated sheep erythrocytes and phagocytosis of *Staphylococcus aureus* [31].

Evidence exists that monocyte and MDM functions which are mediated through FcγR and CR are defective in HIV-1-infected individuals. Reduced clearance of IgG and complement-coated cells *in vivo*, and reduced Fc-mediated phagocytosis of monocytes and MDM *in vitro* have been reported [17,18,32–34]. Monocyte HLA-DR expression in AIDS patients has been reported to be decreased [35], although more recently HLA-DR expression was reported to be increased on a subgroup of large monocytes of HIV^+ patients [36]. A reduction in FcRIII (CD16) has been reported in a variety of cells from HIV-infected patients, including peripheral blood lymphocytes and neutrophils, and in cells from bronchoalveolar lavage fluid [37–39]. The expression of FcR on monocytes isolated from HIV-infected individuals, however, has not been found to be depressed [40,41]. Indeed, FcRIII was expressed at higher amounts on circulating monocytes of AIDS patients than of healthy controls, which correlated with increased activation of monocytes by transforming growth factor-beta [42].

The study of freshly isolated monocytes or cultured MDM from HIV-infected persons largely reflects indirect consequences of HIV infection on monocyte/macrophage function, as *in vivo* very few of the circulating monocytes are infected with HIV [43]. This is in contrast to tissue MDM, where the limited data available suggest a higher proportion of MDM may be HIV-infected [44,45]. In our study, the defect in FcRI expression in infected cultures was largely confined to the p24-expressing cells, although there was a degree of down-modulation of this receptor in the p24-negative cells within the infected culture. The reasons for the down-regulation of receptor expression and dysfunction of uninfected monocytes or MDM from HIV-infected patients or in HIV-infected cultures may be either cytokine-mediated, due to effects of soluble gp120 and subsequent interactions with MDM (e.g. binding CD4), which has been reported to interfere with chemotaxis and differentiation [46], or due to defective maturation of monocytes to MDM [47]. Indeed, these direct consequences of HIV infection on monocytes or MDM may be multifactorial in origin. Although monocyte function is abnormal in HIV-infected patients, the reasons for which are unclear, tissue MDM constitute the bulk of the monocyte-macrophage system, and loss of FcR and CR expression on HIV-infected tissue MDM may be an important aspect of the immunopathogenesis of HIV infection.

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