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Expression patterns of Fcγ receptors, HLA-DR and selected adhesion molecules on monocytes from normal and HIV-infected individuals

CH. LOCHER, G. VANHAM*, L. KESTENS*, M. KRUGER†, J. L. CEUPPENS†, J. VINGERHOETS* & P. GIGASE* *Department of Tropical Medicine, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, *Laboratory of Pathology and Immunology, Institute of Tropical Medicine, Antwerp, and †Laboratory of Clinical Immunology, Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium*

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SUMMARY

The expression and co-expression profiles of functionally important monocyte surface markers were compared between control and HIV^+ individuals using combined physical gating and dim CD4 expression to delineate the monocytes. The FcγRII (CD32), the MHC class II antigen HLA-DR and the adhesion molecules CD11a (LFA-1α), CD18 and CD54 (ICAM-1) showed an unimodal distribution. Of these markers, CD11a and HLA-DR were up-regulated in the HIV^+ subjects compared with controls. The expression levels of the adhesion molecules correlated with each other in both patients and controls. The CD11b (CR3-α), CD14, FcγRI, and FcγRIII markers were bimodally distributed. Compared with controls, monocytes from seropositives contained fewer $CD14^{bright+}$ cells, an equal proportion of $Fc\gamma RI^{bright+}$ cells, but twice as many $Fc\gamma RIII^+$ cells. The expression level of FcγRI and CD11b within their brightly positive subset increased as CD4 T cells decreased. Both in patients and controls, co-expression of bright CD11b, CD14 and FcγRI was shown, whereas the $Fc\gamma RIII^+$ cells were negative or dim positive for the former triad. We conclude that the expression of two FcγR (I and III), of the adhesion molecules CD11a and CD11b and of HLA-DR showed particular alterations on monocytes from HIV^+ subjects. The relationship of these phenotypic observations with altered cytokine profiles and altered monocyte function is discussed.

Keywords HIV monocyte Fcγ receptor adhesion molecules flow cytometry

INTRODUCTION

Whereas CD4 T cell loss and dysfunction are the most striking immunological abnormalities associated with HIV infection, the role of monocyte/macrophage and dendritic cells is increasingly recognized in the multifactorial pathogenesis of immunodeficiency [1]. Monocytes are infected by HIV via their CD4 receptor, and are important in the persistence and pathogenesis of HIV [2]. HIV-infected monocytes are disseminated throughout the body, including the central nervous system [3]. Impairment of monocyte/macrophage function may directly cause organ dysfunction and may induce T cell non-responsiveness [4]. Therefore, it is necessary to develop a better understanding of HIV and monocyte interactions, and to investigate changes in their phenotype and function, in relation to disease progression.

A number of reports have analysed surface antigens of monocytes in order to identify potential correlations with AIDS pathogenesis. However, differences in experimental methodologies may have rendered contradictory reports. For example, the surface expression of the MHC class II antigen HLA-DR was shown to have increased [5,6], decreased [7,8] or not changed [9] after HIV infection. In addition, the influence of HIV on monocyte-expressed CD11a [7,10], CD11b [6,7], FcγRI [9,11], and transferrin receptor [6,9] remains unclear. In each of these studies, monocytes from controls and HIV^+ individuals were either isolated or defined by CD14 expression. Both of these approaches have drawbacks, since isolation of monocytes induces phenotypic changes, and CD14 is expressed by only 76–90% of all monocytes [12–14].

The purpose of this study was to re-evaluate expression and co-expression of functionally important monocyte membrane markers such as the IgG Fc receptors (FcRγI, FcRγII, and FcRγIII); the HLA-DR and the lipopolysaccharide (LPS) receptor CD14. Since lymphocyte function antigen–intercellular adhesion molecule (LFA–ICAM) and other adhesion molecule interactions are thought to play a major role in monocyte–lymphocyte communication and in HIV transmission [15–17], we also measured CD11a (the α chain of

Correspondence: Guido Vanham MD, Laboratory of Pathology and Immunology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium.

LFA-1 or LFA-1α); CD11b (complement receptor 3 α chain or CR3-α) and their common β chain (CD18) as well as CD54 (ICAM-1). Disturbing influences of washing and/or separation techniques were avoided: the monocytes were stained in whole blood, using directly conjugated MoAbs, and the monocyte population was carefully delineated during analysis, using a combination of physical characteristics and CD4 expression.

SUBJECTS AND METHODS

Subjects and samples

HIV seropositivity was screened using an ELISA (Abbott Diagnostics, Wiesbaden, Germany) and confirmed using an immunoblot (Dupont de Nemours, Singapore). Whole blood samples were taken from 53 HIV^+ individuals at the Institute of Tropical Medicine Outpatient Clinic, Antwerp, Belgium. Only patients without acute illness at the time of sampling were included. They were classified into four groups based on $CD4^+$ T cell percentages. The control group consisted of 29 HIV^- blood donors recruited from the Blood Transfusion Centre at the University of Antwerp Hospital.

In both patients and controls, the blood was taken between 9 a.m. and 12 a.m. in polypropylene tubes containing K^3EDTA (final concentration 1·6 mg/ml blood). The samples were stored at room temperature until 3 p.m., when the immunostaining procedure was started.

Immunophenotyping

During preliminary experiments to determine the effect of three washes on the physical characteristics of monocytes, as is used in the indirect immunofluorescence method, the number of monocytes was found to decrease selectively by up to 50%, and their physical characteristics were altered, probably due to aggregation. These results indicated that the indirect labelling method was not suitable to phenotypically characterize monocytes. All subsequent experiments were performed using directly conjugated MoAb reagents, thus requiring only one wash step.

The MoAb anti-FcγRI (clone 32.2) and anti-FcγRII (clone IV.3), conjugated to FITC, were purchased from Medarex (W. Lebanon, NH) and MoAb anti-CD4 (clone RPA-T4), conjugated to Cy-Chrome, was purchased from Pharmingen (San Diego, CA). All other MoAbs were purchased from Becton Dickinson (Erembodegem, Belgium). In the two-colour experiments, anti-CD4 (Leu-3a; clone SK3) PE-conjugated was combined with the following FITC-conjugated MoAbs: anti-FcγRI, anti-FcγRII, anti-CD14 (Leu-M3; clone MoP9), anti-HLA-DR (clone L243), anti-CD11a (clone G-25.1) or anti-CD18 (clone L130). In other two-colour experiments, anti-CD4 (Leu-3a) FITC was combined with the following PE-conjugated MoAbs: anti-FcγRIII (Leu-11c; clone B73.1), anti-CD11b (Leu-15; clone D12) or anti-CD54 (Leu-54; clone LB-2). Isotype-matched FITC- or PE-labelled MoAbs of irrelevant specificity were used as negative controls. In three-colour experiments, anti-CD4 Cy-Chrome was combined with anti-CD14 FITC or PE, anti-CD11b PE, anti-FcγRI FITC, and anti-FcγRIII PE. In the experiment represented in Fig. 1, anti-CD4 PE was combined with anti-CD3, conjugated to peridynin chlorophyl protein.

Blood (50 μl) was incubated at 4°C for 30 min in polypropylene tubes with saturating amounts of MoAb. Afterwards, the samples were washed once, the erythrocytes were lysed with Becton Dickinson's lysing solution, and the leucocytes were fixed with 0·5% paraformaldehyde. Five thousand 'events' from each sample were acquired in a FACScan (Becton Dickinson), by live gating on the monocyte region as defined by forward and side scatter (FSC, SSC). The acquisition modes of FSC and SSC were linear, those of the three fluorescent colours were logarithmic. Within the physical 'monocytic' gate, dimly $CD4^+$ cells were selected for analysis of the secondary markers. The expression of these markers was investigated in the histogram mode, using the LYSYS I software. For unimodally distributed markers, results were expressed as the mean fluorescence intensity (MFI) on the logarithmic fluorescence scale. The MFI value is represented as a channel number between 1 and 256: channel 64 corresponds to 10 arbitrary fluorescence units, channel 128 to 100 units, and so on. For bimodally distributed markers, the limit between negative/dim and bright expression was determined for each individual marker, and it was kept at the same level throughout the study. The results on each bimodal monocyte marker were described in two ways: first, the proportion of brightly positive cells within the monocytes, and next the MFI of the brightly (+) subset were compared between patients and controls.

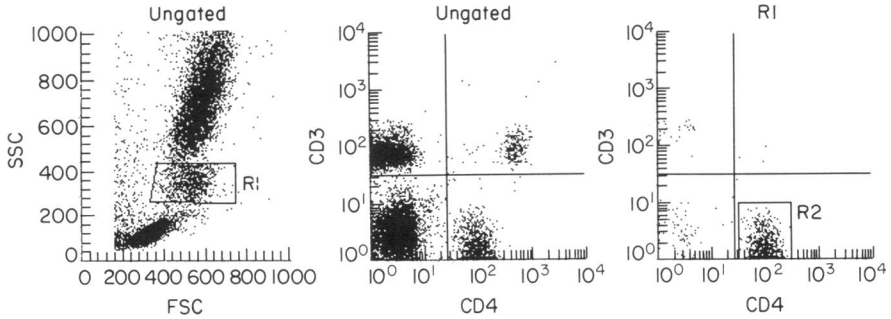

Fig. 1. Physical and fluorescence characteristics of monocytes. Whole blood from an HIV^+ subject was stained with anti-CD3 peridinin chlorophyll protein conjugate and anti-CD4 PE. The leucocytes were acquired in the flowcytometer as described in Subjects and Methods. The forward and side scatter of the ungated leucocytes and the definition of the 'physical' monocytic gate (R1) are shown in the left dot blot. The expression profile of CD3 (ordinate) and CD4 (abscissa) within the ungated leucocytes (middle dot blot) and within the R1 gate (right dot blot) are represented. The R2 gate delineates the dim $CD4^+$ cells within the 'physical' monocytic gate.

All samples were acquired within 3 weeks, and each day of the study patients and controls were included. The FACScan was calibrated daily with the Calibrite beads, using the Autocomp program (Becton Dickinson). The instrument proved to be very stable over the study period and the settings were kept unchanged.

Statistical analysis

The results were summarized and analysed using non-parametric methods, since the distribution of most data was non-Gaussian. The significance of differences between two and more than two groups was calculated with the Mann–Whitney *U*-test and the Kruskal–Wallis test, respectively. Correlations were calculated using Spearman's rank test [18]. All statistics were calculated using the Epi Info V Program (Centre for Disease Control, Atlanta, GA).

RESULTS

Patterns of surface antigens on monocytes

A typical scattergram and two-colour staining with anti-CD3 and anti-CD4 is shown (Fig. 1). It is evident that, after careful physical gating, the 'monocytic gate' (R1) mainly contained $CD3^-$ $CD4^{dim+}$ cells, but also 10–20% of cells with other fluorescence characteristics. In a series of preliminary experiments, we showed that cells lacking CD4 expression but having monocyte-like physical characteristics could be identified either as lymphocytes or granulocytes with specific MoAbs. Sometimes a low number of brightly $CD4^+$ cells was present in the physical monocytic gate. Since these co-expressed CD3, they were CD4 T cells. The dimly $CD4^+$ cells always lacked typical lymphocyte markers, including CD3 (T), CD19 (B) and CD56 (natural killer (NK)). Conversely, these cells always expressed HLA-DR (present on monocytes, but not on granulocytes) [19,20]. Therefore we concentrated on the dimly $CD4^+$ cells within the physical monocyte gate for further analysis.

Within the selected monocyte population, the FcγRII marker and the adhesion molecules CD11a, CD18 and CD54 showed a unimodal distribution by histogram analysis, and usually had a narrow peak. The distribution of HLA-DR was also unimodal, although broad, compared with the others. Representative histograms of CD11a and HLA-DR on monocytes from a control and an HIV^+ subject are shown (Fig. 2a).

By contrast, the histograms of FcγRI, FcγRIII, CD14 and CD11b showed a bimodal fluorescence distribution with negative/dimly (+) and brightly (+) subsets. Representative examples of CD11b and CD16 (FcγRIII) distributions are shown in Fig. 2b.

Up-regulation of HLA-DR and CD11a on monocytes from HIV^+ subjects

The expression of markers with a unimodal distribution was studied using the logarithmic MFI values. Comparison of this parameter between monocytes from patients and controls showed that HLA-DR and CD11a were selectively up-regulated in the patients (Table 1a).

To determine the possible association between marker expression and immune status, the HIV^+ subjects were classified according to strata of CD4 T cell percentages. The enhanced expression of CD11a was restricted to the patient groups with intermediate CD4 T cell levels (5–25%); the up-regulation of HLA-DR was most pronounced in the same groups (Table 1b).

Increased proportion of bright FcγRIII expression in seropositives

The proportions of monocytes brightly expressing the bimodally distributed markers were compared between HIV^+ and HIV^- subjects. About twice as many monocytes from seropositives displayed FcγRIII compared with controls (Table 2a). In contrast, the proportions of monocytes brightly expressing CD14 or CD11b were decreased in HIV^+ and the proportion of FcγRI bright cells was unchanged.

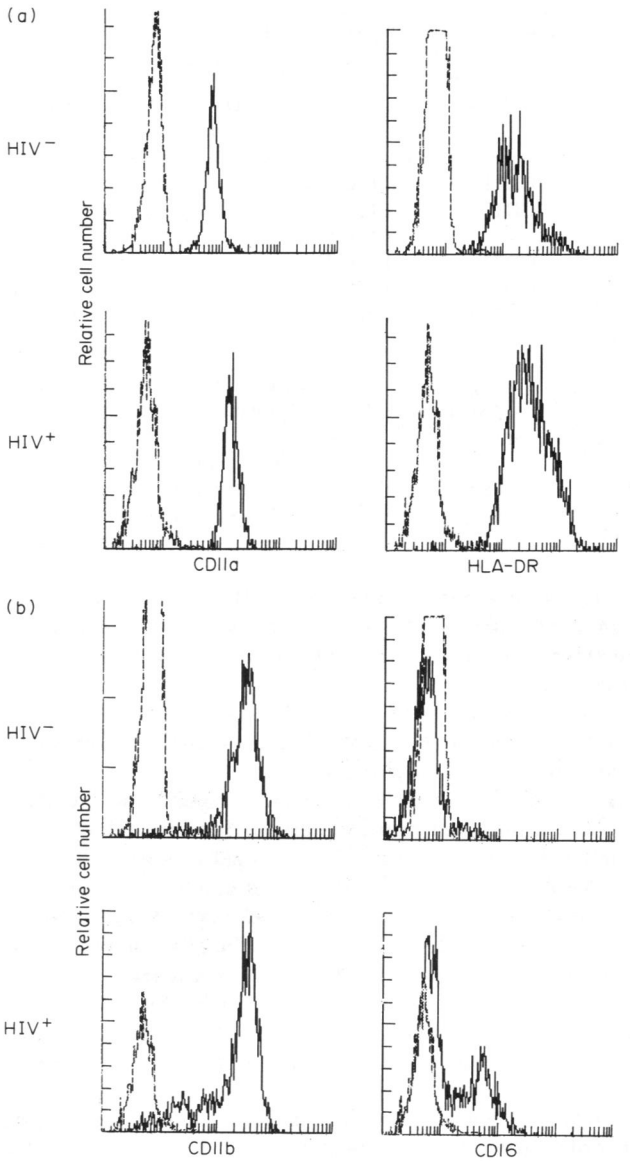

Fig. 2. Representative histograms of phenotypic markers with a unimodal (a) or bimodal (b) expression on monocytes from control and HIV^+ subjects. The fluorescence intensity (abscissa) is expressed on a logarithmic scale. Monocytes were selected on the basis of their dim CD4 expression and physical characteristics. The grey histograms represent the binding of the isotypic control MoAb; the black histograms represent the binding of the specific MoAb.

Table 1. Unimodally expressed monocyte markers in HIV^- and HIV^+ individuals

a. Comparison according to serostatus

	Antigen	HIV^- ($n = 29$)	HIV^+ ($n = 53$)	P*
CD11a	LFA-1α	123·1 ± 5·3†	126·1 ± 6·8	0·02
CD18	LFA-1β CR3-β	141·9 ± 4·6	144·2 ± 6·9	NS
CD32	FcγRII	126·6 ± 16·4	130·9 ± 7·1	NS
CD54	ICAM-1	118·3 ± 5·2	117·6 ± 8·3	NS
MHC II	HLA-DR	141·9 ± 6·7	148·2 ± 11·3	0·002

b. Comparison between stratified HIV^+ subjects‡

		CD4 T < 55% ($n = 15$)	CD4 T 5–15% ($n = 19$)	CD4 T 15–25% ($n = 10$)	CD4 T > 25% ($n = 9$)	P§
CD11a	LFA-1α	121·7 ± 9·3	125·3 ± 5·1	128·1 ± 3·7	121·7 ± 5·2	0·02
CD18	LFA-1β CR3-β	147·1 ± 8·1	143·9 ± 4·9	143·9 ± 7·5	140·3 ± 6·2	NS
CD32	FcγRII	132·1 ± 6·2	128·3 ± 8·6	133·0 ± 3·1	132·5 ± 7·8	NS
CD54	ICAM-1	116·4 ± 9·1	119·0 ± 5·9	121·5 ± 9·6	113·2 ± 8·6	NS
MHC II	HLA-DR	143·3 ± 14·5	149·8 ± 9·3	155·9 ± 8·3	146·0 ± 6·9	0·04

* Significance of differences determined using the Mann–Whitney U-test. NS, Not significant.

† Median ± 95% confidence interval of the mean fluorescence intensities as determined by histogram analysis of a given monocyte surface antigen.

‡ Stratification based on per cent $CD4^+$ T cells within the lymphocyte gate.

§ Significance determined using the Kruskal–Wallis test.

ICAM-1, Intercellular adhesion molecule-1.

The proportion of brightly $Fc\gamma RIII^+$ monocytes was highest and the proportion of brightly $Fc\gamma RI^+$ monocytes was lowest in patients with intermediate CD4 T cell counts (Table 2b).

The MFI of the brightly $Fc\gamma RI^+$ subset is inversely correlated with CD4 T cells in the patients

The densities of the CD11b, CD14, FcγRI and FcγRIII markers within their respective brightly positive subsets were compared between seropositives and controls, using the MFI parameter. The density of FcγRI was significantly higher on monocytes from patients compared with controls ($P < 0·001$), and increased as CD4 T cells decreased (Fig. 3). The MFI of CD11b within the brightly positive subset from the patients was slightly, but significantly increased ($P = 0·02$); whereas the MFI of CD14 and FcγRIII in their respective brightly positive subsets was similar in patients and controls (data not shown).

Correlations between monocyte markers in patients and controls

In order better to understand the relationship between the different surface markers on monocytes, the correlation coefficients were calculated amongst the proportions of the brightly (+) subsets from bimodally expressed markers and the MFI of the unimodally expressed markers.

In control monocytes, proportional CD11b, CD14 and CD64 (FcγRI) expression correlated positively with each other, but proportional CD64 expression correlated inversely with CD16 (FcγRIII). The MFI of FcγRII expression correlated with proportional CD14 expression. The MFI of CD11a (LFA-1α) and of CD18 (LFA-1β) correlated with each other (Table 3).

Several of these correlations were also found in monocytes from HIV^+ subjects, including the direct relationship between FcγRI and CD14, between FcγRII and CD14, and between CD11a and CD18, as well as the inverse relationship between FcγRI and FcγRIII. In the patients, however, proportional FcγRIII expression correlated also inversely with CD11b and CD14, whereas proportional CD14 expression and the MFI of FcγRII both correlated directly with the MFI of CD18. In addition, the MFI of FcγRII correlated directly with CD11a, and the MFI of CD54 correlated with CD11a, CD18 and HLA-DR (Table 3).

Co-expression of CD14, FcγRI, and CD11b but not FcγRIII

Three-colour immunofluorescence was used to determine further the relationships between the expression of the bimodally expressed markers. Monocytes which were brightly positive for CD14 also strongly co-expressed FcγRI and CD11b. The FcγRIII-negative monocytes belonged to the FcγRI and CD14 brightly positive subset. The FcγRIII-positive monocytes, in contrast, were largely negative or dimly positive for FcγRI and CD14. Similar associations were found in five seronegative and five seropositive samples. The co-expression profiles in control monocytes are shown (Fig. 4).

Table 2. Bimodally expressed monocyte markers in HIV$^-$ and HIV$^+$ individuals

a. Comparison according to serostatus

	Antigen	HIV$^-$ ($n = 29$)	HIV$^+$ ($n = 53$)	P*
CD11b	CR3-α	88·9 ± 17·5†	82·5 ± 7·8	0·03
CD14	LPSR	87·4 ± 5·0	81·8 ± 10·3	0·01
CD16	FcγRIII	9·7 ± 3·4	16·2 ± 6·9	0·0001
CD64	FcγRI	87·6 ± 5·1	86·0 ± 11·9	NS

b. Comparison between stratified HIV$^+$ subjects‡

		CD4 T < 5% ($n = 15$)	CD4 T 5–15% ($n = 19$)	CD4 T 15–25% ($n = 10$)	CD4 T > 25% ($n = 9$)	P§
CD11b	CR3-α	81·8 ± 7·7†	81·4 ± 6·8	82·2 ± 5·6	85·7 ± 11·6	NS
CD14	LPSR	83·4 ± 14·8	80·0 ± 10·3	82·0 ± 4·0	83·0 ± 5·1	NS
CD16	FcγRIII	15·4 ± 8·8	18·9 ± 5·3	17·3 ± 7·0	11·5 ± 4·1	0·04
CD64	FcγRI	87·3 ± 14·1	81·7 ± 9·4	85·0 ± 5·0	92·3 ± 15·3	0·02

* Significance determined using the Mann–Whitney U-test.
† Median ± 95% confidence interval of the percentages of monocytes brightly expressing a specific antigen.
‡ Stratification based on per cent CD4$^+$ T cells.
§ Significance determined using the Kruskal–Wallis test.

DISCUSSION

The present study compared functionally important markers on monocytes from control and HIV$^+$ subjects. Amongst the five unimodally distributed markers, CD11a and HLA-DR were up-regulated in the HIV$^+$ group, with maximal levels in the subjects having intermediate CD4 T cell depletion. The expression levels of the unimodal markers were clearly positively correlated with each other, especially in the patients.

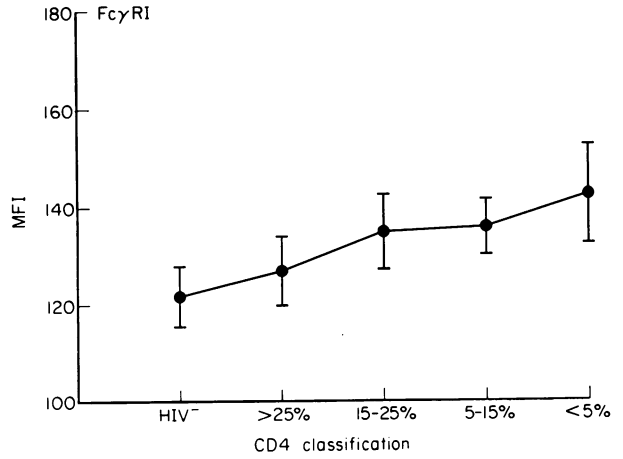

Fig. 3. FcγRI is progressively up-regulated on monocytes as CD4$^+$ T cells decrease. The mean fluorescence intensities (MFI) of the brightly FcγRI$^+$ monocyte subset were compared between HIV$^-$ controls ($n = 29$) and HIV$^+$ persons having >25% ($n = 9$), 15–25% ($n = 10$), 5–15% ($n = 19$), and <5% ($n = 15$) CD4$^+$ T cells. Median values ± confidence intervals are represented for each group.

The bimodally expressed markers showed complex changes after HIV infection. These included a doubling of the FcγRIII$^+$ and a reduction of the FcγRI$^{bright+}$ subset in patients with an intermediate CD4 T cell depletion. At the same time, however, a progressively increasing density of FcγRI within the brightly positive subset was associated with decreasing CD4 T cell counts. According to calculated correlations and to co-expression experiments in both patients and controls, it was shown that CD11b, CD14 and FcγRI were brightly co-expressed on 80–90% of the monocytes, and FcγRIII was preferentially expressed on those monocytes, which were dim positive or negative for the former triad.

To our knowledge, the present study is the first to describe this particular bimodal co-expression pattern on peripheral blood monocytes from control and HIV$^+$ subjects. Some of the differences of monocyte phenotype between HIV$^-$ and HIV$^+$ subjects, we observed, are novel (e.g. decreased proportional CD14 and CD11b expression), others were controversial (e.g. up-regulation of CD11a and HLA-DR) [7–10], whereas our data on increased proportional FcγRIII expression confirm earlier reports [21]. It was also previously shown that the FcγRI density was increased on monocytes from HIV$^+$ subjects [11]. We show here in addition that the proportion of FcγRI$^{bright+}$ monocytes is actually decreased in patients. Inconsistencies and missed observations in previous studies may be the consequence of (i) the use of CD14 as a marker for monocytes, which may account for as little as 76% of the monocyte population [12]; (ii) indirect labelling techniques, which may induce altered characteristics and a loss of monocytes during the washing process, as we observed in this study; (iii) the use of frozen blood samples resulting in cell lysis [9]; and (iv) partial purification using density gradients or adherence of monocytes

Table 3. Correlation between the different monocyte markers within the HIV^+ and the HIV^- group

	CD11b	CD14	CD16	CD64	CD11a	CD18	CD32	CD54	MHC II
CD11b*									
CD14*	(0·56**)‡§								
CD16*	−0·36**	−0·33**							
CD64*		0·62****	−0·54****						
	(0·47**)	(0·64**)	(−0·53**)						
CD11a†									
CD18†		0·41***			0·77****				
					(0·64***)				
CD32†		0·37**			0·36**	0·53****			
		(0·67**)							
CD54†					0·47***	0·44***			
MHCII†								0·58****	

* For the bimodally expressed markers, the proportions of the brightly positive subsets were considered.
† For the unimodally expressed markers, the mean fluorescence intensity (MFI) was taken.
‡ The correlation coefficients were calculated using the Spearman's rank test.
§ The correlation coefficients of the HIV^- group are represented between parentheses.
** $P < 0{\cdot}05$; *** $P < 0{\cdot}01$; **** $P < 0{\cdot}001$. The non-significant correlation coefficients are not represented.

[7,8], which alters some phenotypical characteristics, as recently shown [14]. Most of these pitfalls could be avoided by staining freshly drawn whole blood with directly conjugated MoAb and by carefully delineating the monocytic population, using a combination of physical gating and dim CD4 expression.

The phenotypic changes on monocytes after HIV infection are certainly not a direct effect of cellular infection with HIV, since only a small percentage of circulating monocytes contain proviral DNA and still fewer produce the virus, even in advanced stages [22]. Indirect effects, such as the perturbation of the cytokine network, are more probably responsible. Interferon-gamma (IFN-γ) mediates up-regulation of FcγRI

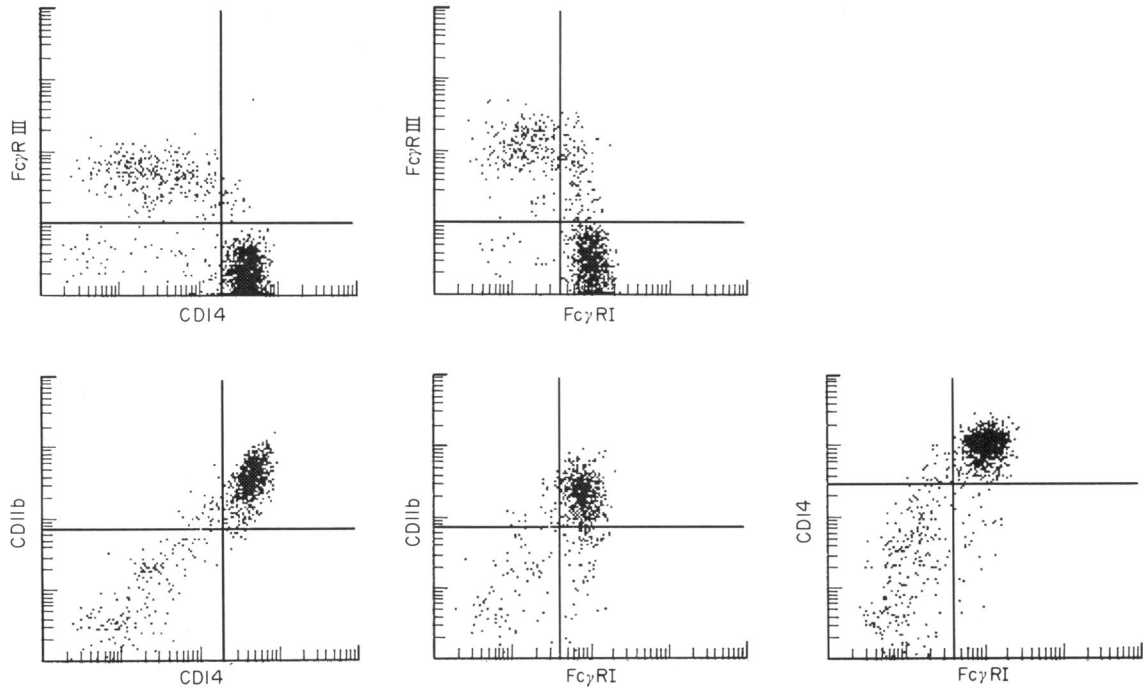

Fig. 4. Co-expression profiles of CD11b, CD14, FcγRI and FcγRIII on control monocytes. The monocytes were selected on the basis of physical characteristics and dim CD4 expression (using anti-CD4 conjugated to Cy-Chrome). They were further stained with a combination of MoAbs directly conjugated to FITC (abscissa) and PE (ordinate). The limit of discrimination between $Fc\gamma RIII^-$ and $Fc\gamma RIII^+$ cells was at a relative fluorescence of 10; the limit between FcγRI dim and bright was at 4×10; the limit between CD11b dim and bright was at 7×10. To discriminate between CD14 dim and bright the limit was 2×10^2 using the FITC-labelled MoAb and 3×10^2 using the PE-labelled MoAb.

on normal monocytes [23,24]. Mainly tumour necrosis factor-alpha (TNF-α), IL-2, and to a lesser extent TNF-β induce increased density of CD11a, CD11b and HLA-DR, but also CD18 on normal monocytes [25]. Elevated levels of neopterin, an IFN-γ-induced monocytic metabolite, have been consistently found in the serum and urine from seropositive subjects [26,27]. Increased mRNA levels and serum concentrations of TNF and IFN-γ have been shown in mononuclear cells and serum from seropositive subjects [28–32]. In addition, Capsoni and co-workers showed an association between elevated IFN-γ serum levels and increased FcγRI expression on neutrophils and monocytes from HIV^+ individuals [11]. Finally, elevated serum levels of transforming growth factor-beta (TGF-β) have been suggested as the reason for enhanced FcγRIII expression on monocytes from seropositive subjects [21].

Defective accessory function of monocytes from seropositive subjects has often been described [33,34]. Bender and co-workers observed AIDS-associated decreased antibody-dependent cellular cytotoxicity and complement-mediated phagocytosis *in vivo*, and reproduced these phenomena *in vitro* in monocytes from AIDS patients [35]. Under slightly different experimental conditions and/or in other patient groups, however, accessory, phagocytic and cytotoxic functions were normal or sometimes even enhanced compared with control monocytes [36–39]. Although again controversial, the basal cytokine expression in monocytes from seropositives is usually increased, whereas the production after stimulation is rather decreased [29,40–42]. In view of the functional deficiencies, suggested by several authors, it is remarkable that in our study several receptors and adhesion molecules were either unchanged or up-regulated. Of particular interest in the context of phenotype–function relations, Ziegler-Heitbrock *et al.* showed that the small subset of normal monocytes co-expressing CD14 dimly and CD16 brightly have a lower phagocytic capacity and a lower stimulated cytokine production [43]. On the other hand, Capsoni *et al.* showed a decreased antibody-mediated phagocytosis by monocytes from AIDS patients, despite an increased density of FcγRI, an increased proportional expression of FcγRIII, and elevated levels of IFN-γ [44].

Using careful techniques to stain and identify monocytes in whole blood, complex surface marker changes were observed after HIV infection. These could be interpreted as a relative expansion of monocytes, positive for FcγRIII, but with only low levels of FcγRI, CD14 and CD11b. Globally increased HLA-DR and CD11a expression as well as selectively enhanced density of FcγRI and CD11b in the bright positive subsets add to the complexity. A combined action of HIV-induced TNF-α, TGF-β and/or IFN-γ could be responsible for altered surface marker expression. In order to identify those phenotypic changes typical for HIV infection, it is necessary to investigate the same surface characteristics with the same technique in other pathological conditions. Most importantly, the relation between altered monocyte phenotype and altered monocyte function has to be further explored.

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