Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals

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Abstract

In addition to neutralization, antibodies mediate other antiviral activities including antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), as well as complement deposition. While it is established that progressive HIV infection is associated with reduced ADCC and ADCP, the underlying mechanism for this loss of function is unknown. Here we report considerable changes in FcR expression on the course of HIV infection on both mDCs and monocytes, including elevated FcγRII expression in acute HIV infection and reduced expression of FcγRIIa in chronic HIV infection. Furthermore, selective blockade of FcγRII alone was associated with a loss in ADCP activity, suggesting that FcγRII plays a central role in modulating ADCP. Overall, HIV infection is associated with a number of changes in FcR expression on phagocytic cells that are associated with changes in their ability to respond to antibody-opsonized targets, potentially contributing to a failure in viral clearance in progressive HIV-1 infection.

Introduction

In addition to their ability to neutralize pathogens, antibodies are able to mediate a number of additional antiviral functions following ligation of innate immune cells via an Fc receptor (fragment, crystallizable receptor, FcR) (Nimmerjahn and Ravetch, 2008; Oganesyan et al., 2008). These include stimulation of cytokine production, induction of cytolytic activity, antibody-mediated cellular-phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), or complement mediated cytotoxicity (CMC).

FcRs belong to the immunoglobulin superfamily and bind to the constant regions of antibodies (Nimmerjahn and Ravetch, 2007). Five different FcRs, with distinct antibody isotype binding properties, exist in humans including FcγR (IgA), FcγR (IgG), FcγR (IgM), FcεR (IgE), and FcεR (IgE). Among the IgG binding FcRs, 3 isoforms of FcγR with distinct functions have been identified: the FcγRI (high affinity), FcγRII (a and c, medium–low affinity), and FcγRIIIa (a and b, medium–low affinity) are activating receptors, whereas FcγRIIb delivers inhibitory signals (Siberil et al., 2007). All cells of the innate immune system, and a few non-innate immune cells, express FcRs including monocytes/macrophages, granulocytes, DC, and mast cells that express both inhibitory and activating FcγR, natural killer (NK) cells that express the activating receptor FcγRIIa, and B cells that only express the inhibitory receptor FcγRIIb (Anderson, 1989; Nimmerjahn and Ravetch, 2008).

Different combinations of FcRs on innate immune cells allow antibodies to induce a broad range of innate immune responses depending on the innate immune cell engaged, that may play a vital role in antiviral or antitumor immune responses. Importantly, ADCC and ADCP activity have been associated with better outcome in several diseases such as chronic viral infections (Shore et al., 1974), autoimmune diseases (Laszlo et al., 1986) and particular cancers (Dall’Ozzo et al., 2004; Natsume et al., 2009). In addition to NK cells that mediate potent ADCC via FcγRIIa, other innate immune cells, such as monocytes, DCs, and neutrophils, are also able to mediate ADCC as well as ADCP, and have equally been implicated as critical responders to antibody-opsonized material. Furthermore, unlike neutralizing antibodies, ADCC-inducing antibodies have been observed early in HIV infection, and correlate with better disease outcome (Aasa-Chapman et al., 2005; Forthal et al., 2001). Furthermore, results from the RV144 Thai trial suggest that additional antiviral humoral mechanisms, beyond neutralization, may play a critical role not only after infection but may also protect individuals from infection (Rerks-Ngarm et al., 2009).
While a great deal of work has focused on the role of ADCC activity in the control of HIV infection, little has been done to understand the role of other antibody-mediated effector functions, such as ADCP, which may also play a vital role in the effective clearance of immune complexes that are abundantly produced during progressive HIV infection (Tomaras et al., 2008). Thus robust ADCP-inducing antibodies could play a critical role in the rapid clearance of antibody-opsonized viral particles from the peripheral circulation where they may contribute to immune activation and disease progression (Tomaras et al., 2008).

Given the central role of FcRs in regulating the recruitment of innate immune responses, including ADCP, we speculated that changes in FcR expression may provide clues regarding the FcRs that are involved in viral clearance at different stages of HIV infection. Here we report that acute infection was associated with an increased expression of FcγRI, whereas chronic infection was associated with a decreased FcγRII and FcγRIII expression on a variety of myeloid cell subsets (mDC, pDC and monocytes). Phagocytic activity was significantly elevated in acute HIV infection while it was reduced in chronic infection, suggesting that changes in particular FcRs are associated with defective ADCP-inducing antibody function at different stages of HIV infection that may contribute to a differential capacity to clear immune complexes during HIV infection.

**Results**

Dramatic changes in the frequency and magnitude of FcR expression starting in acute HIV-1 infection

Chronic HIV infection is associated with an accumulation of dysfunctional NK cells (Alter et al., 2005; Alter et al., 2004; Eger and Unutmaz, 2004; Hu et al., 1995; Mavilio et al., 2003), and more recent work has shown that impaired NK-mediated ADCC activity is related to reduced expression of FcγRIIa on the surface of these cytolytic effector cells (Liu et al., 2009). Given the profound impact of reduced FcγR expression on NK cell mediated ADCC, it is plausible that changes in FcR expression on other cell subsets over the course of HIV infection may also modulate their capacity to respond to cytolytic antibodies. Thus we compared the frequency and magnitude of the expression of the 3 main Fcγ-receptors (FcγRI, FcγRII, FcγRIIIa) as well as FcεR and FcαR at different stages of HIV infection on several innate immune cell subsets, starting in acute HIV infection.

FcγRI is the sole high-affinity receptor for monomeric IgG that is constitutively expressed on monocytes, macrophages and neutrophils (Hulett and Hogarth, 1994; Ravetch and Kinet, 1991), and has been shown to play a central role in the induction of ADCC and ADCP (Diamond et al., 1978). HIV infection was associated with significant changes in the frequency of cells expressing FcγRI (Fig. 1A) and in the mean fluorescence intensity (MFI) of this FcR (Fig. 1B) on innate immune cells. In acute infection, we observed an increase in the frequency of FcγRI expression on mDCs (Fig. 1Ai) (p<0.001) and in the MFI of FcγRI expression on monocytes (Fig. 1Bi) (p<0.001) compared to chronically infected individuals and healthy controls. Similar to previous reports suggesting that pDCs do not express FcγRI (Bave et al., 2003), we did not observe FcγRI expression on pDCs (Fig. 1Bii). In summary, we found that only acute HIV infection is associated with a robust increase in the frequency of mDCs expressing FcγRI and an increased expression of FcγRI on monocytes, potentially suggesting that these innate immune cells may expand and/or upregulate the expression of this high-affinity FcγR preferentially at this early stage of the infection in response to the large burst of viral replication and/or the early cytokine cascade (Table 1).

In contrast to FcγRI, FcγRII has a low affinity for monomeric IgG and typically only binds multimerized IgGs found on immune complexes (Hulett and Hogarth, 1994; Ravetch and Kinet, 1991). FcγRII+ mDCs (Fig. 2Ai) and FcγRI+ monocytes (Fig. 2Aii) were slightly reduced in chronic untreated and chronic treated individuals, respectively, compared to controllers and negative controls. More

![Fig. 1](attachment:image): Acute HIV-1 infection is associated with changes in FcγRI expression on mDCs and monocytes. The dot plots depict changes in the frequency (A) and intensity (B) of FcγRI (CD64) expression on (i) mDCs, (ii) pDCs and (iii) monocytes derived from acute (●), chronic treated (▲), chronic untreated (▼), controllers (◆) and HIV-1 negative controls (●).
Interestingly, FcyRII expression was significantly reduced on monocytes in both chronic treated and untreated HIV-1 infected patients compared to controllers ($p<0.01$ and $p<0.05$, respectively, Fig. 2Biii). Similar patterns of FcyRII expression were observed on mDCs (Fig. 2Bi) and pDCs (Fig. 2Bii), suggesting that despite a reduction in viral replication with successful antiretroviral therapy, expression of FcγRs does not return to normal levels, which potentially results in a persistent reduction in ADCC/ADCP activity. These data suggest that FcyRII is significantly downregulated on the surface of multiple innate immune cell subsets in both chronic treated and untreated HIV-1 infection, potentially resulting in irreversibly reduced ADCC activity in progressive infection even in the absence of active viral replication (Liu et al., 2009).

Like FcyRII, FcγRIIa also binds antibodies with a weak affinity, and thus binds multimerized IgG complexes more readily (reviewed by Nimmerjahn and Ravetch, 2008). Previous work has shown that FcγRIIa expression is reduced on the surface of NK cells in chronic HIV infection, correlating with reduced ADCC activity (Liu et al., 2009). Interestingly, we also observed reduced FcγRIIa expression on mDCs in both acute and chronic untreated HIV-1 infection compared to HIV-1 negative controls ($p<0.001$, for both comparisons Fig. 3Bi). In addition, while FcγRIIa+ monocytes were present at higher frequencies in controllers than in all the other groups ($p<0.05$ for all comparisons, Fig. 3Aiii), its expression levels on monocytes were downregulated in all chronically infected individuals independent of treatment or spontaneous control compared to acute and HIV-negative controls ($p<0.001$, for both comparisons Fig. 3Bii). Thus similar to FcyRII, FcγRIIa expression is reduced on mDCs and monocytes in chronic HIV-1 infection, likely contributing to reduced ADCC/ADCP activity, whereas it is expressed at higher levels on innate immune cells in controllers, potentially allowing them to clear virus from the circulation more effectively.

Finally we examined changes in FcεR and FcεR expression, which bind to IgA and IgE, respectively. While the frequency of mDCs and pDCs expressing FcεR and FcεR was extremely low, we observed an enrichment in the frequency of FcεR+ monocytes at all stages of HIV-1 infection (Fig. 4). Acute HIV infection was associated with a trend towards an increase in the frequency of FcεR+ monocytes (Fig. 4Aiii), elevated FcεR+ pDCs (Fig. 4Aii), and a decreased frequency of FcεR+ monocytes compared to all groups ($p<0.001$ Fig. 4Ciii). Overall, both FcεR and FcεR were weakly expressed on innate immune cells; however, acute HIV infection was associated with a higher frequency of FcεR+ pDCs and monocytes, suggesting that FcεR expression may also be upregulated to contribute to clearance of immune complexes bound by antibodies of other isotypes during early HIV-1 infection.

**Impairment of phagocytic activity in untreated chronically HIV-infected individuals**

Previous reports that aimed at studying the underlying mechanism(s) accounting for alterations in phagocytic activity in progressive HIV infection (Kedzierska et al., 2002; Kedzierska et al., 2001) (and reviewed by Kedzierska et al., 2003) have shown that compromised ADCP activity is associated with a reduction in FcγRIIa mobilization to the cell surface.

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**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>Viral load (RNA copies/ml)</th>
<th>CD4 T cells (μl)</th>
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<tr>
<td>HIV-negative controllers</td>
<td>23</td>
<td>24,1562</td>
<td>538</td>
</tr>
<tr>
<td>Controllers</td>
<td>17</td>
<td>710</td>
<td>819</td>
</tr>
<tr>
<td>Acute</td>
<td>17</td>
<td>18,066,300</td>
<td>460</td>
</tr>
<tr>
<td>Chronic treated</td>
<td>20</td>
<td>&lt;50</td>
<td>716</td>
</tr>
<tr>
<td>Chronic untreated</td>
<td>24</td>
<td>41,562</td>
<td>538</td>
</tr>
</tbody>
</table>

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**Fig. 2.** FcγRII expression is strongly downregulated in chronic HIV infection. The frequency (A) and the intensity (B) of FcγRII (CD32) expression were compared among acute (●), chronic treated (■), chronic untreated (▲), controllers (▼) and HIV-1 negative controls (♦) on (i) mDCs, (ii) pDCs and (iii) monocytes.
potentially due to shedding (Webster et al., 2006), or due to reduced expression of the \( \gamma \) signaling subunit of Fc\( \gamma \)RIIIa in HIV-infected phagocytes (Kedzierska et al., 2002; Leeansyah et al., 2007). We did not observe any correlation between ADCP activity and CD4 counts or viral loads (data not shown); thus, we sought to determine whether changes in FcR expression at different stages of HIV infection on mDCs and monocytes may be associated with differences in their phagocytic activity. Therefore, we employed an opsonized, CFSE-labeled cell line, p815 cells, coated with p815-specific antibodies, to determine FcR-mediated phagocytic activity by the different innate immune effector cells by measuring CFSE

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**Fig. 3.** Fc\( \gamma \)RIIIa expression is downregulated on mDC and monocytes in chronic HIV-1 infection. The frequency \( (A) \) of Fc\( \gamma \)RIII+ cells and the expression \( (B) \) of Fc\( \gamma \)RIII on mDCs, pDCs, and monocytes were compared in subjects in HIV-infected subjects in acute (●), chronic treated (■), chronic untreated (▲), controllers (▼) and HIV-1 negative controls (♦).

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**Fig. 4.** Elevated frequencies of FcR+ monocytes in acute HIV-1 infection. The percentage and intensity FcεR (CD23) \( (A \text{ and } B) \) and FcαR (CD89) \( (C \text{ and } D) \) were compared on mDCs, pDCs, and monocytes derived from patients at different stages of the infection including cells derived from acute (●), chronic treated (■), chronic untreated (▲), controllers (▼) and HIV-1 negative controls (♦).
cell-labeled uptake by flow cytometry in the presence or absence of antibody coating. Our data demonstrate that mDCs and monocytes from acutely infected individuals possess an enhanced capacity to mediate ADCC, as these cells were able to take up elevated frequencies of antibody-coated CFSE+ p815 cells compared to cells from chronically infected individuals off therapy (p<0.1 and p<0.05 respectively, Fig. 5Ai), suggesting that elevated FcγRI expression observed on mDCs and monocytes in acute infection may contribute to an enhanced capacity to clear immune complexes early in infection. We also observed a reduced frequency of mDC mediated phagocytosis among untreated chronically infected patients compared to controllers, chronic treated and uninfected controls (Fig. 5Ai). This compromised frequency of phagocytic cells was also observed in monocytes in chronic untreated patients compared to uninfected controls (p<0.05, Fig. 5Aii). These data suggest that changes in FcR expression on innate immune cells over the course of HIV-1 infection modulate ADCC activity.

While the frequency of phagocytic cells provides insights into the ability of the total population of innate immune cells to mediate ADCC, we also aimed to determine whether on a per cell level, ADCC differed among the patient populations. Thus we compared the intensity of phagocytosis on a per cell level by determining the difference in the mean fluorescence intensity (dMFI) of CFSE+ mDCs or monocytes in the presence of non-coated or antibody-coated p815 cells. No difference was observed in phagocytosis of the uncoated p815 cells among the patient populations, demonstrating that this ADCC activity is specific to the presence of the antibody. Moreover, the level of antibody-mediated phagocytosis (dMFI) on a per cell level was reduced in chronic untreated subjects compared to the chronically treated individuals in mDCs (Fig. 5Bi) as well as in monocytes (p<0.05, Fig. 5Bii), suggesting that not only there are fewer phagocytic innate immune cells, but on a per cell level, phagocytic cells also mediated ADCC inefficiently.

Fig. 5. Impact of FcγRI, FcγRII and FcγRIla expression in FcR-mediated phagocytosis. The dot plots represent differences in FcR-mediated phagocytosis, measured as the proportion of phagocytic cells that have taken up an antibody-coated target cell (A) or the intensity of phagocytosis (B), in mDCs (i) and monocytes (ii) among a sub-group of acutely infected patients, chronic treated, chronic untreated, controllers and HIV-1 negative individuals. (C) The bars represent the percentage of reduction in phagocytosis in mDCs (i) or monocytes (ii) in 3 different donors in the presence of FcγR-blocking antibodies.
Given the dramatic changes in FcγR expression in HIV infection and their associated effect on compromising ADCP, we next sought to define whether changes in all or individual FcγRs modulated ADCP differentially using selective blocking reagents (Fig. 5C). As previously described in tumor cells (Richards et al., 2008), neither 10 μg/ml of an irrelevant murine IgG nor FcγRIIIa blocking antibody had an impact on the capacity of mDCs or monocytes to induce ADCP. However, selective blockade of either FcγRI or FcγRIIa alone or in combination with FcγRIIIa decreased ADCP in mDCs (Fig. 5Ci) and to a lesser extent in monocytes from healthy donors (Fig. 5Cii). Furthermore, the selective blockade of FcγRIIa alone also reduced ADCP activity in monocytes, albeit to a lesser extent than FcγRII. Moreover, triple blockade of FcγRI, FcγRII, and FcγRIIIA resulted in more robust inhibition of ADCP, suggesting that FcγRI may also contribute to the induction of phagocytosis (Fig. 5Ci). Thus overall, our data confirm that FcγRI and FcγRII expression on phagocytic cells play a central role in modulating ADCP over the course of HIV-1 infection. These data newly suggest that changes in FcγR expression over the course of HIV-1 infection, including an increase in FcγRI in acute infection and a decline in FcγRII expression in chronic infection, may differentially contribute to changes in ADCP function at these different stages of disease, altering the capacity of innate immune cells to clear and/or control HIV infection (Richards et al., 2008).

Discussion

Whereas ADCC has been shown to play a crucial role in better disease outcome in HIV-1 infection (Aasa-Chapman et al., 2005) (Forthal et al., 2001), little is known about additional non-neutralizing antiviral antibody functions, including ADCP which may play a central role in antiviral control through the rapid clearance of immune complexes. Furthermore, the recent results from the Thai trial suggest that non-neutralizing antibodies with the capacity to recruit the innate immune system through FcγR may have contributed to protection from HIV infection (Rerks-Ngarm et al., 2009); however, the precise effector mechanism of these non-neutralizing antibodies is not known. In this study, we show that HIV-1 infection is associated with significant alterations in the frequency of FcγR expressing cells, and that these changes occur as early as acute HIV-1 infection. In fact, acute infection was strongly associated with an increase in the frequency of FcγRI+ mDCs as well as an increase in FcγRI expression on monocytes. In contrast, as opposed to acute infection, chronic HIV infection was associated with a significant and persistent down-regulation of FcγRII expression, even following a reduction in viral replication with antiretroviral therapy. Interestingly, we did not observe any statistical differences in the FcγRII expression on innate immune cells between chronics and HIV-negative individuals, suggesting that additional factors, independent of the receptor expression, may also contribute to alterations in ADCP activity. Furthermore, selective blockade of either FcγRI or FcγRIIa alone or in combination with FcγRIIIa on monocytes resulted in decreased phagocytosis, suggesting that alterations in these specific FcRs may play a central role in modulating ADCP activity. These data strongly suggest that increased expression of FcγRI on monocytes in acute infection may help promote viral clearance of immune complexes, whereas decreased FcγRII expression in chronically infected individuals may dampen ADCP, resulting in compromised clearance of immune complexes in late disease. Overall, these results demonstrate that HIV-1 infection is associated with changes in the expression of specific FcRs on innate immune cells that are responsible for alterations in the capacity of these cells to mediate ADCP in chronic infection, potentially resulting in a failure of innate immune cells to efficiently clear immune complexes and therefore help control viral replication.

Several lines of evidence suggest that a number of myeloid cell activities are impaired following HIV-1 infection in vivo as well as in vitro including chemotaxis (Tas et al., 1988; Wahl et al., 1989), phagocytosis (Kedzierska et al., 2002; Kedzierska et al., 2000; Leeansyah et al., 2007; Webster et al., 2006), intracellular killing (Biggs et al., 1995), and cytokine production (Kedzierska et al., 2001). Several groups have speculated that the compromised phagocytic activity following HIV infection may partially contribute to the AIDS-associated pathogenesis, as the loss of these functions could result in poor antibody-mediated recruitment of innate immune cell associated viral control (Crowe, 1995; Crowe and Sonza, 2000; Kedzierska et al., 2003); however, the mechanism by which this activity is lost is unknown. Previous reports have demonstrated that HIV-1 infection of monocyte derived macrophages (MDM) results in a defect in phagocytic activity (Leeansyah et al., 2007) in the absence of changes in the surface expression of FcγRs in vitro (Kedzierska et al., 2002). However only a small fraction of monocytes and mDCs are infected in vivo, and alterations in innate immune cell mediated phagocytic activity extend far beyond this small subset of infected cells. Thus additional mechanisms must account for changes in ADCP activity in the larger majority of phagocytic cells. Here we show that changes in the frequency and expression levels of particular FcγRs over the course of HIV-1 infection, starting in acute infection, may contribute to variation in the capacity of these cells to mediate ADCP.

Changes in FcγR expression at different stages of HIV infection may provide critical insights into the receptors that may be centrally involved in the clearance of antibody-opsonized material. Along these lines, we observed a unique upregulation of FcγRI on monocytes in acute HIV infection, suggesting that at this early stage of infection, FcγRI expression may be critical for early clearance of immune complexes. Moreover, selective blockade of FcγRI on monocytes decreased ADCP activity, confirming its role in ADCP mediated clearance of antibody-opsonized material. In fact, FcγRI is the only high-affinity FcγR and is able to bind monoclonic antibodies (Hulett and Hogarth, 1994; Ravetch and Kinet, 1991). Upregulation of Fcγ RI expressing monocytes may provide these phagocytic cells with the capacity to respond to antibody-opsonized material with higher sensitivity, to aide in viral control. Previous studies have shown that FcγRI expression is altered on innate immune cells in other viral infections, driven by interferon-γ (IFN-γ) (Okayama et al., 2000), tumor growth factor-β (TGF-β) (Tridandapani et al., 2003) or other stimuli including LPS, associated with chronic immune activation in HIV as well as in SIV infection (Arend et al., 1987; Pricop et al., 2001) (Brenchley et al., 2006), supporting the fact that changes in the inflammatory milieu, due to cytokine cascades following infection, may drive alterations in FcγR expression at different stages of HIV infection, potentially modulating the capacity of innate immune cells to respond to antibody-opsonized material (Arend et al., 1987; Borouchv et al., 2005; Okayama et al., 2000; Tridandapani et al., 2003). Moreover, we show that FcγRII expression on mDCs and monocytes was reduced in chronic HIV infection and that alterations in the expression of this particular receptor contributed centrally to the reduced ADCP observed in chronic infection. Similarly, previous reports from the SLE model suggest that FcγRIIa expression also declines on monocytes in this inflammatory disease contributing to impaired ADCP clearance of immune complexes in the sera of these patients (Szucs et al., 1994), strongly suggesting that FcγRIIa expression is required for phagocytic clearance of immune complexes in autoimmunity as well as during infection.

While a great deal of research has focused on cross talk between inhibitory and activating FcRs (Holl et al., 2004), little has been done in the way of understanding how different activating FcRs may synergize with one another to modulate effector functions. Moreover, while the selective blockade of FcγRI and/or FcγRII alone or in combination with FcγRIIIa inhibited ADCP activity, the selective blockade of FcγRIIIa alone did not modify ADCP (Fig. 5Ci and ii). These data suggest that both FcγRI and II independently promote ADCP activity,
but also may synergize to additively mediate more robust ADCP when co-expressed on the same innate cell.

Finally, although the antibody-opsonized p815 assay does not directly measure the HIV-specific capacity of antibodies to trigger ADCP activity, it offers an indirect measure of the ADCP capacity of different innate immune cell subsets (Kondo et al., 1981; Caligiuri et al., 1993; Grazia Cifone et al., 1990). Thus this assay offers an indirect measure of the potential capacity of innate immune cells to mediate clearance of antibody-opsonized material during infection, that can be antibody-opsonized viral particles, HIV-infected cells, or other antibody-opsonized material. Thus it is also plausible that reduced ADCP activity in chronic HIV infection may also result in compromised clearance of other antibody-opsonized material that may contribute to reduced control/clearance of other opportunistic pathogens.

In conclusion, we show here that HIV-associated changes in ADCP activity in progressive infection may be directly related to changes in FcR expression. We highlight the potential involvement of particular FcRs in ADCP to help control viral infection at different stages of HIV infection, FcγRI in acute infection and FcγRII in viral control of infection. These data provide new insights into the mechanism of protective FcR-mediated ADCP activity and its loss over the course of HIV infection that may be critical for efficient antibody-mediated control that may make important new targets to enhance protection mediated by HIV-specific antibodies through vaccination.

Materials and methods

Subjects

A total of 101 subjects were recruited for this study, including 23 healthy HIV-1 negative control subjects; 24 untreated viremic HIV-1-infected subjects with an average viral load of 41,562 copies/ml of plasma (range, 1210–229,000 copies/ml) and an average of CD4 count of 538 cells/mm3 (range, 29–1041 cells/mm3); 20 HIV-1-infected subjects receiving highly active antiretroviral therapy (HAART), with undetectable viral loads (range, 310–517 cells/mm3). All samples were cryopreserved prior to analysis. The MGH institutional review board approved the study, and all subjects gave written informed consent for participation in the study (Perereya et al., 2008).

Phenotyping

Peripheral blood mononuclear cells (PBMCs) were obtained after Ficoll–Hypaque density gradient centrifugation of whole blood. FcR expression was assayed on three different cell subsets from cryopreserved PBMCs: mDCs (myeloid dendritic cells), defined as CD14+CD123− and CD11c+; pDCs (plasmacytoid dendritic cells), defined as CD3−CD14−CD123+ and CD11c−; and monocytes, defined as CD3−CD14+ and CD11c+. PBMCs were stained with blue viability dye (Invitrogen), and then with CD64-APC, CD23-biotin, CD16-APCCy7, CD89-PE, CD3-Alexa700, CD123-PECy5, CD14-PB (BD Biosciences) and CD11c-PECy5.5 (Invitrogen). Then the delta-mean fluorescence intensity (dMFI) was calculated as the percentage of effector cells that phagocytosed CFSE-labeled uncoated-target cells subtracted from the percentage of effector cells that took up CFSE-positive target cells from antibody-coated p815 cells. A minimum of 300,000 cells were acquired on a BD LSRII, and analysis was then performed using FlowJo software.

FcγR blockade assay

Blocking antibodies FcγRI (10 μg/ml, clone 10-1; BD Pharmingen), FcγRII (10 μg/ml, clone AT10; R&D Systems) and/or FcγRIIIa (10 μg/ml, clone 3G8; BD Pharmingen) were added to PBMCs for 1 h at 37 °C before the addition of either antibody-coated or non-coated CFSE-labeled p815 cells for 3h. Phagocytosis was evaluated as the proportion of mDCs and monocytes that took up fluorescent antibody-coated p815 cells by flow cytometry using the following markers: CD3-Alexa 700, CD14-APC, CD123-PECy5 (BD Biosciences) and CD11c-PECy5.5 (Invitrogen). Cells were acquired on a BD LSRII, and analysis was then performed using FlowJo software.

Statistics

Statistical analysis was performed using GraphPad Prism. Statistical comparisons of marker expression levels were made using one-way ANOVA. P values of pairwise comparisons were adjusted for multiple comparisons using Tukey’s method. Significant differences were noted when p<0.05. * represents p<0.05; ** represents p<0.001 and *** represents p<0.0001.

Conflict of interest

None reported.

Acknowledgments

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References


