Antibody-dependent cellular cytotoxicity and neutralization of human immunodeficiency virus type 1 by high affinity cross-linking of gp41 to human macrophage Fc IgG receptor using bispecific antibody

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Human monocytes/macrophages, which express Fc receptors for IgG are involved in human immunodeficiency virus type 1 (HIV-1) infection and pathogenesis. These receptors are known to mediate numerous immunological functions including cell-mediated killing and possibly targeting of HIV to the lysophagosome monocyte-derived macrophage (MDM) entry route for virus neutralization. To study both activities in HIV-1 infection, MDM FcRI and virus neutralization. To study both activities in HIV-1 infection, MDM FcRI was specifically selected using bispecific antibody (Bs-Ab) containing whole human monoclonal antibody against gp41 and the Fab' fragment of murine anti-FcγRII 22.2 antibody. Bs-Ab was found to mediate potent antibody-dependent cellular cytotoxicity and virus neutralization.

Human monocytes and macrophages are involved in a variety of physiological and pathogenic events in human immunodeficiency virus type 1 (HIV-1) infections (Barré-Sinoussi et al., 1983; Roy & Waimberg, 1988; McElrath et al., 1989). They express on their membranes receptors for IgG (FcγRs), among many other molecules. The fundamental functions of these receptors in mediating clearance of immune complexes, phagocytosis, secretion of biological effector molecules and cell-mediated killing have previously been described (Anderson et al., 1986). Evidence of Fc receptor involvement in antibody-dependent cellular cytotoxicity (ADCC) against HIV-infected cells has been also reported in HIV-1-seropositive individuals (Ljunggren et al., 1988). Moreover, these receptors have been suspected to be involved in macrophage infection (Takeda et al., 1988; Homsy et al., 1989; Juoault et al., 1991). These specific FcγRs have been demonstrated to be an alternative route of infection for opsonized virus with bispecific antibody (Bs-Ab) without leading to cellular infection (Connor et al., 1991; Mabondzo et al., 1992). These previous reports indicated that the nature of these interactions between HIV-1 and macrophage surface molecules might determine the outcome of HIV binding, namely virus killing or infection. Although ADCC and neutralization (Robert-Guroff et al., 1985; Weiss et al., 1985; Bernard et al., 1990) have been described in HIV-1-infected patients, they do not seem to prevent the development of AIDS. Numerous hypotheses have been described to explain this failure (Saag et al., 1988). Therefore, the development of a virus-specific agent which could specifically interfere with virus replication by mediating virus clearance and eliminating infected cells may help to slow down the fatal course of HIV infection.

In this paper, we report the development of a new bispecific antibody (Bs-Ab) and present the results of ADCC and virus neutralization studies. To produce a Bs-Ab, a whole human monoclonal antibody (Mab) against HIV-1 glycoprotein gp41 (anti-gp41 Mab; epitope amino acids 827 to 843) (Desgranges et al., 1988; Madaule et al., 1991; Boyer et al., 1991) was covalently cross-linked to an Fab' fragment of the murine anti-FcγRII 22.2 (which we shall refer to as 22.2 Fab') (Guery et al., 1989) directed against an external epitope of FcγRI according to a procedure described elsewhere (Connor et al., 1991). Bs-Ab was then purified by HPLC. It binds simultaneously to an epitope outside the IgG ligand binding site on FcγRI and also to this site. Isolation of human monocytes from healthy donors was performed by a countercurrent centrifugal elutriation technique as described elsewhere (Fidgert et al., 1983). Monocyte-derived macrophages (MDM) were obtained after 7 days culture in 24-well microtitre plates (Becton Dickinson). Cell culture maturation was monitored by flow cytometry analysis using macrophage-specific staining with anti-CD11b, FcγRI and MAX1 antibodies (Andreesen et al., 1986). To determine whether this Bs-Ab is able to trigger killing of HIV-1-infected cells, we performed a 51Cr
release assay as described elsewhere (Ljunggren et al., 1987) with varying parameters (incubation time for target cell labelling, effector:target ratio, final antibody concentration, and incubation time for yielded maximum $^{51}$Cr release). The assays were carried out in quadruplicate. The 8E₅ cell line (Folks et al., 1986) (kindly provided by Professor H. Fleury, Bordeaux, France) and fresh elutriated human monocytes were used as target and effector cells respectively. The 8E₅ cell line, infected with the HIV-1/LAV strain, was selected for its membrane HIV envelope glycoprotein expression. As assessed by flow cytometry, our human anti-gp41 MAb or murine anti-gp120 MAb 110.4 (Genetic System) recognizes the infected 8E₅ cell line (data not shown). The uninfected CEM T cell line (kindly provided by Dr F. C. Barré-Sinoussi, Institut Pasteur, Paris, France), from which the 8E₅ cell line was derived, was used as a control target in these studies to assess the specificity of antibody-mediated cytotoxicity. Heat-inactivated serum from seropositive individuals, which exhibit ADCC, were included in the test as a positive control. As shown in Fig. 1, Bs-Ab mediates ADCC against the infected target cell line. Maximum killing activity (42%) was observed with 0.2 µg/ml of Bs-Ab at a 10: effector: target cell ratio. This ADCC activity was higher than anti-gp41 MAb alone (Fig. 1a), 22.2 Fab' alone (Fig. 1b), or anti-gp41 MAb mixed with 22.2 Fab' (Fig. 1c) (ANOVA test significant at 99.99%; non-parametric Mann-Whitney test, $P = 0.0495$). Moreover this ADCC activity was higher than our positive control (Fig. 1d). In contrast, anti-gp41 MAb alone, 22.2 Fab' alone, or anti-gp41 MAb mixed with 22.2 Fab' did not induce any ADCC activity.
activity. Nor was reactivity seen when Bs-Ab was tested against the uninfected CEM T cell line control. These data demonstrate that Bs-Ab is a potent mediator of ADCC activity when pure monocytes are used as effector cells.

The ability of Bs-Ab to neutralize HIV-1 infectivity in vitro was further monitored by measuring HIV-1 p24 antigen at day 10 post-infection (p.i.) and by analysing unintegrated and integrated viral DNA by PCR within the first hours following HIV-1 infection. For all neutralization experiments, clinical isolate HIV-1/DAS (Boussin et al., 1987) and the macrophage-tropic HIV-1 strain HIV-1/Bal (Gartner et al., 1986) were used. Virus stocks were produced by passage of the virus in MDM cultures and titrated on phytohaemagglutinin-stimulated cord blood mononuclear cells. For the PCR assay, in order to remove any contamination by HIV DNA, the virus stock was treated prior to infection with RNase-free DNase (Promega), at 10 U/ml and MgCl₂ at 1 mM for 45 min at 25 °C. These conditions have been shown not to affect infectivity as reported elsewhere (Eron et al., 1992) since viral production in HIV-1/DAS-infected peripheral blood lymphocytes is identical with or without pretreatment with RNase-free DNase (data not shown).

Heat-inactivated virus (56 °C for 45 min), pretreated with RNase-free DNase was used to check whether our virus stock gave a rise in the PCR background level. A new frozen virus preparation was used for each new set of experiments.

In the HIV-1 p24 antigen inhibition assay, 10⁶ TCID₅₀ of the clinical HIV-1/DAS isolate or HIV-1/Bal was incubated with sequential dilutions of Bs-Ab. The mixtures were incubated 30 min at 37 °C and then layered on 10⁵ human MDM overnight. Cells were then washed extensively with PBS to remove residual virus. Supernatants were harvested every 2 or 4 days and HIV-1 p24 antigen was measured by HIV-1 ELISA (Abbott) or by reverse transcriptase (RT) activity (Rey et al., 1984). The assays were performed in triplicate. As shown in Fig. 2, virus production in HIV-1/DAS-infected human MDM was markedly reduced throughout the 10 days of observation. Inhibition ranged from 50 to 80% at Bs-Ab concentrations of 2 to 38 µg/ml. This inhibition of virus production was also seen in HIV-1/Bal-infected MDM (Fig. 3) in the presence of Bs-Ab. No enhancement of HIV infection was observed even at low doses of Bs-Ab (20 to 2 ng/ml). Our experimental system did not detect enhancing antibodies with MDM using sera from seropositive individuals (Gras et al., 1993). Otherwise, in contrast, anti-gp41 MAb alone or 22.2 Fab' did not induce any neutralizing activity (Fig. 2), nor was the neutralization seen when a mixture of anti-gp41 MAb and 22.2 Fab' was used. Bs-Ab was investigated in the same manner as described above for HIV-1 neutralization on PBLs which do not express FcγRI. Virus production was monitored by HIV-1-specific RT activity or by the HIV p24 assay at day 6 following HIV infection, and no neutralizing activity was detected in the presence of Bs-Ab with pure PBLs (Table 1). Taken together, these data suggest that high affinity binding of Bs-Ab on FcγRI through the FcγRI pathway is required for virus neutralization following binding by the anti-gp41 MAb. Results showing that anti-gp41 MAb/anti-FcγRI antibody (Bs-Ab) neutralizes the macrophage infection, whereas the anti-gp41 MAb alone does not,
could be due to the fact that cross-linking FcγRI allows endocytosis to take place. This hypothesis is strengthened by the fact that neutralization with Bs-Ab occurs only with cells bearing FcγRI.

For the PCR assay, DNA was extracted from in vitro infected human MDM within the first hours following HIV-1 infection to yield unintegrated and integrated viral DNA according to a modified Hirt method (Hirt, 1967). Briefly, 2 × 10⁶ infected human MDM were lysed in a buffer containing 0.6% SDS, 25 mM-Tris-HCl pH 7.5, 10 mM-EDTA. After 1 h at room temperature, 200 µl of 5 M-NaCl was added to the sample and incubation was performed for 16 h at 4 °C. Unintegrated and integrated viral DNA were then separated by centrifugation at 30000 g at 4 °C. Nucleic acids were then extracted twice with phenol-chloroform. The DNA fraction was resuspended in 10 mM-Tris-HCl pH 8, 1 mM-EDTA pH 8. Amplification by PCR for both unintegrated and integrated DNA was then performed using specific primers from the HIV gag gene SK38/39 (Ou et al., 1988) and/or HIV pol gene P4/P3 (Laure et al., 1988). Aliquots of the PCR products were analysed by Southern blotting, hybridized with a specific ³²P-labelled HIV probe (purified gag and pol PCR products).

Experiments were first carried out to assess the kinetics of appearance of unintegrated and integrated viral DNA in HIV-1-infected MDM. As shown in Fig. 4(a), unintegrated viral DNA appeared 1 h p.i. and a maximum amount of unintegrated viral DNA was reached at 4 h p.i. and then decreased at 6 and 24 h p.i. This decrease was associated with the progressive appearance of integrated viral DNA (Fig. 4b). These data are consistent with those published by Munis et al. (1992), who reported a significant increase of HIV-1 DNA during the first cycle of infection. No PCR products were detected in the MDM infected with heat-inactivated virus stock pretreated with RNase-free DNase (data not shown). The virus stock did not give rise to a positive PCR background. We then examined whether Bs-Ab affects the appearance of unintegrated viral DNA at 4 h following HIV-1 infection on MDM. As shown in Fig. 4c, unintegrated viral DNA was not yet detectable at 4 h p.i. when infection was performed in the presence of Bs-Ab (38 µg/ml), suggesting that Bs-Ab may block or modulate a phase of the viral cycle before the reverse transcription process in human MDM. The ability of Bs-Ab to neutralize seems to be dose-dependent since weak detectable specific HIV-1 hybridization could be seen after 24 h of autoradiography at low doses (9 to 2 µg/ml) of Bs-Ab and an unvarying concentration of anti-gp41 MAb + 22.2 Fab'. No detectable viral DNA was seen at 4 h after exposition.

### Table 1. Effects of Bs-Ab on HIV-1/DAS and HIV-1/Bal infection of pure PBLs

<table>
<thead>
<tr>
<th>Bs-Ab concentration (µg/ml)</th>
<th>RT activity (c.p.m./ml)*</th>
<th>HIV-1/DAS</th>
<th>HIV-1/Bal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34560 ± 174</td>
<td>345800 ± 24120</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>ND</td>
<td>326100 ± 6432</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>ND</td>
<td>246000 ± 47680</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3170 ± 293</td>
<td>337600 ± 2020</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>35140 ± 554</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>39260 ± 560</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* RT activity in the supernatant of HIV-1/DAS- or HIV-1/Bal-infected PBLs was measured at day 6 p.i. Each value represents the mean of three determinations.

† ND, Not determined.
Bs-Ab with two distinct binding specificities brings theoretical advantages for clinical therapy. The choice of a non-immunodominant epitope on a constant domain of HIV avoids competitive binding to the virus by endogenous anti-HIV antibodies. The anti-FcγRI antibody used in this bispecific construct binds outside the Fc binding site of the Fc receptor, allowing access to the FcγRI whether or not this receptor is already occupied by the Fc portion of any endogenous antibody.

We are currently investigating the intracellular degradation pathway to mediate virus neutralization. If FcγRI-mediated neutralization involves endocytosis and intracellular degradation of virus such an approach could be used to target antigenic viral components to the macrophage, and may improve viral processing for antigenic presentation.

These data emphasize the importance of understanding the subcellular routes of infection and virus degradation in designing new immunological approaches for AIDS prevention and/or therapy.

We are grateful for the assistance of the Centre de Transfusion Sanguine des Armées (Clamart, France) and Centre de Transfusion Sanguine (Critteil, France). This work was supported by Agence Nationale pour la Recherche sur le SIDA and the Fondation Européenne pour la Recherche sur le SIDA.

References


Short communication


(Received 9 August 1993; Accepted 13 December 1993)