An HIV-1 gp120 Envelope Human Monoclonal Antibody That Recognizes a C1 Conformational Epitope Mediates Potent Antibody-Dependent Cellular Cytotoxicity (ADCC) Activity and Defines a Common ADCC Epitope in Human HIV-1 Serum

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Among nonneutralizing HIV-1 envelope antibodies (Abs), those capable of mediating antibody-dependent cellular cytotoxicity (ADCC) activity have been postulated to be important for control of HIV-1 infection. ADCC-mediating Ab must recognize HIV-1 antigens expressed on the membrane of infected cells and bind the Fcγ receptor (FcR) of the effector cell population. However, the precise targets of serum ADCC antibody are poorly characterized. The human monoclonal antibody (MAb) A32 is a nonneutralizing antibody isolated from an HIV-1 chronically infected person. We investigated the ability of MAb A32 to recognize HIV-1 envelope expressed on the surface of CD4+ T cells infected with primary and laboratory-adapted strains of HIV-1, as well as its ability to mediate ADCC activity. The MAb A32 epitope was expressed on the surface of HIV-1-infected CD4+ T cells earlier than the CD4-inducible (CD4i) epitope bound by MAb 17b and the gp120 carbohydrate epitope bound by MAb 2G12. Importantly, MAb A32 was a potent mediator of ADCC activity. Finally, an A32 Fab fragment blocked the majority of ADCC-mediating Ab activity in plasma of subjects chronically infected with HIV-1. These data demonstrate that the epitope defined by MAb A32 is a major target on gp120 for plasma ADCC activity.

Antibodies (Abs) that bind to the Fcγ receptor (FcR) IIIα on the surface of natural killer (NK) cells can mediate antibody-dependent cellular cytotoxicity (ADCC) activity or antibody-dependent cellular viral infection (ADCVI) (4). In addition, binding of immunoglobulin (Ig) Fc to FcR can induce anti-human immunodeficiency virus type 1 (HIV-1) chemokine release (5, 29). These types of effector functions have been implicated in protective immune responses against HIV-1 (3, 12, 20). Several studies have reported that active and passive immunization provided protection from simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) infection in nonhuman primates. The mechanism of protection was related, at least in part, to ADCC- and ADCVI-mediating antibodies (11, 13, 16, 17, 35). Antibodies that mediate FcγR-dependent anti-HIV-1 activities that are nonneutralizing in conventional HIV-1 neutralizing assays have been postulated to be a correlate of protection in the Thai RV1144 gp120 vaccine efficacy trial (15, 26).

FcγR-mediated antibody activity is dependent on both the state of glycosylation of the Fc region (2, 23, 25) and on the specificity of the Fab region (i.e., the antibody must target epitopes on the surface of virus-infected cells). While the epitopes involved in mediating virus neutralization have been comprehensively profiled, HIV-1 epitopes that are capable of mediating ADCC activity and ADCVI in HIV-1 infection have not been adequately studied. Thus, we have begun to analyze existing neutralizing and nonneutralizing anti-Env human monoclonal antibodies (MAb) for their ability to bind to HIV-1-infected cells and to sensitize target CD4+ T cells for ADCC activity.

In this study, we report the ability of a human MAb (A32) to recognize a conformational epitope involving the C1 and C4 gp120 regions following Env binding to CD4 (22). We report that the A32 epitope is expressed on the surface of transmitted/ founder (T/F) virus-infected CD4+ T cells beginning at day 3 of in vitro infection and can mediate potent ADCC activity with both virus-infected and gp120-coated CD4+ T cells. Moreover, MAb A32 Fab blocks the majority of ADCC antibody activity in plasma of subjects chronically infected with HIV-1, indicating that the A32-binding site is highly recognized by the Ab
elicited during HIV-1 infection and might significantly contribute to the overall ADCC Ab responses.

MATERIALS AND METHODS

Monoclonal antibodies and IgG preparations. The A32, 2/11c, and 1/7b monoclonal antibodies utilized in this study were originally isolated by James Robinson (Tulane University, New Orleans, LA) (22). The 2G12 MAb was purchased from Polymun (Polymun Scientific Immunobiologische Forschung GmbH, Vienna, Austria). The b12 MAb was obtained through the NIH AIDS Research and Reference Reagent Repository from Dennis Burton and Carlos Barbas. VRC01 was kindly provided by John Mascola (Vaccine Research Institute, National Institutes of Health, Bethesda, MD) (36). The humanized monoclonal antibody IgG1(κ) directed to an epitope in the A antigenic site of the F protein of respiratory syncytial virus, palivizumab (Synagis; MedImmune, LLC; Gaithersburg, MD), was purchased from the manufacturer and used as a control. Human polyclonal anti-HIV-1 IgG preparation was used as a positive control from the NIH AIDS Research and Reference Reagent Repository (HIV immunoglobulin [HIVIG] lot114) (6). The A32, 1/7b, and 7B2 Fab fragments were produced by Barton Haynes.

Target cells. The CEM.NKRCCR line (31), chronically infected CEM.NKRCCR and SupT1 CD4+ T cells, and activated peripheral blood (PB) CD4+ T cells were used as target cells. PB CD4+ T cells were generated from cryopreserved PB mononuclear cells (PBMC) as follows. PBMC samples obtained from an HIV-seronegative donor were thawed and activated by 72-h culture at 37°C in 5% CO2 in RPMI 1640 medium (Invitrogen, Carlsbad, CA), supplemented with 20% fetal bovine serum (FBS) (Gemini Bio-Products, West Sacramento, CA) in the presence of 20 U/ml recombinant human interleukin-2 (iHL-2) (Peprotech, Rocky Hill, NJ) (R20–IL-2 medium) and containing anti-CD3 (clone OKT3; Biocsonics, San Diego, CA) and anti-CD28 (clone CD28.2; BD Biosciences, San Jose, CA) at 150 ng/ml. After activation, the CD4+ enriched population was obtained by removing CD8+ T cells by magnetic bead separation (Miltenyi Biotec). The same cells from the seronegative donors were obtained according to Institutional Review Board protocol.

Recombinant gp120 HIV-1 proteins. For experiments conducted with Env-coated target cells, CEM.NKRCCR cells were coated with recombinant gp120 HIV-1 protein from the WITO (gp120wito) early-infection Env clone (GenBank accession number AY835451) (Immunology Technology Corp, New York) or the recombinant gp140 cleavage-site deficient (C) oligomer from the transmitted/founder Env from subject CH040 (gp140C) (18). For each recombinant gp120 or gp140, the optimum amount to coat target cells was determined as that amount at which the CD4-Leu3A MAb target (BD Bioscience, San Jose, CA) could be competed by up to 50% of the binding of the anti-CD4 Leu3A MAb antibody (BD Bioscience, San Jose, CA). This competition was determined as the reduction of the mean fluorescence intensity (MFI) of Leu3A reactivity in gp120/gp140-coated CEM.NKRCCR cells compared to control cells.

Virus, IMCs, and chronically infected cells. The following replication-competent infectious molecular clones (IMCs) of clade B HIV-1 strains were used: X4-tropic NL-LucR.T2A-BaL.ecto, which expresses the BaL envelope (NL-LucR.T2A-BaL.ecto, which expresses the BaL envelope (GenBank accession number AY246110 in cis, in an isogenic, pNL4-3-based reporter virus backbone that preserves all viral open reading frames as described by Edmonds et al. (8); and recently generated full-length IMCs, pCH077, pCH040, c, and pCH058. (C. Ochsenbauer et al., unpublished data), representing the T/F virus strain from subjects CH077, CH040, and CH058, respectively (28). Virus stocks were generated by transfection of 293T cells with proviral DNA and titrated on TZM-bl cells. The A1953 virus was derived from the HX82 isolate selected for the inability to downregulate CD4 from the surface of chronically infected T-cell lines. It was subsequently shown to be defective in Nef expression. SupT1 and CEM.NKRCCR cells that are chronically infected with A1953 chronically express high levels of CD4 complexed with envelo

glycoprotein on the cell surface (J. A. Hoxie, unpublished data). Chronically infected A1953 SupT1CCR cells and A1953 CEM.NKRCCR cell lines were used to evaluate the binding capacity and ADCC activity of MAb A32 to the Env protein expressed on the surface of HIV-1-infected cells, respectively.

Effectors. PBMC obtained by leukapheresis from a seronegative donor were cryopreserved and subsequently used as effector cell populations or as the source of purified NK cells. Cryopreserved PBMC were thawed and then rested overnight at 2 × 106 cells/ml in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS (R10 medium). Cells were counted for viability and adjusted to the proper concentration to obtain an effector to target (E/T) ratio of 30:1. In experiments where infected CD4+ enriched cells were used as targets, NK cell populations were isolated from cryopreserved PBMC by negative selection with magnetic beads (Miltenyi Biotec). In these experiments, NK effector cells were used at an E/T ratio of 10:1.

Indirect surface immunofluorescence analysis of HIV-1-infected CD4+ T cells. Activated CD4+–enriched T cells and A1953 SupT1 cells were obtained as described above. CD4+ T cells were infected by spinoculation (1,200 × g for 2 h (24) with 0.33 50% tissue culture infective dose (TCID50)/cell of HIV-1 virus stock derived from the IMCs pNL4-3 (accession numbers M19921 and AF214493.2) (1) and pCH077 (Ochsenbauer et al., unpublished), representing an X4-tropic reference strain of HIV-1 and the T/F virus from subject CH077, respectively (28). Cells spinoculated in the absence of virus (mock infected) were used as a negative infection control. Following 72 h of infection in R20–IL-2 medium, CD4+–enriched T cells were washed in phosphate-buffered saline (PBS), dispersed in 96-well V-bottom plates at 1 × 104 viable cells/well, and stained with a vital dye (Live/Dead Fixable Aqua Dead Cell Stain; Invitrogen) to exclude nonviable cells from subsequent analyses. The cells were then washed twice with 250 μl/well of washing buffer ([WB] PBS–1% FBS) and incubated at 4°C for 25 min with the primary Ab or IgG preparation. After two washes, cells were stained with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG secondary Ab (KPL, Inc., Gaithersburg, MD) for 25 min at 4°C. Cells were subsequently washed three times and fixed with 1% formaldehyde–PBS. The samples were acquired on an LSRII instrument (BD Biosciences) within 24 h. A minimum of 10,000 total single events were acquired for each test to identify live events. Data analysis was performed using FlowJo, version 8.8.4, software (Tree- Star Inc., Ashland, OR).

Longitudinal Env expression assay. To assess the time course of Env antigen (Ag) expression on the membrane of CD4+–enriched T cells, PBMC were stimulated and cultured at a concentration of 1 × 106 cells/ml in R20–IL-2 medium supplemented with 0.05% Tween 20, 0.005% EDTA, 0.006 mg/ml gentamicin, and 100 ng/ml anti-CD28 (BD Biosciences). These cultures were incubated at 37°C and 5% CO2 for 72 h. CD4+ T cells were depleted from 10 × 106 stimulated PBMC via Miltenyi separation (Miltenyi Human CD8 Microbead Kit) using the AUTOMACS Deplete/Rinse program. CD4+–enriched T cells were then resuspended at 10 × 106 cells/ml in R20–IL-2 medium and bulk infected with 0.977 TCID50/cell of CH077 virus stock via spin inoculation at 1,200 × g for 2 h. Virus-infected cells were then split into the appropriate number of flasks for culture and incubated at 37°C and 5% CO2 for 3 to 7 days. At days 3 to 7 postinfection, viral envelope expression was analyzed by indirect immunofluorescence staining of virus-infected CD4+–enriched T cells (1 × 105 cells) with the appropriate antibodies at a final concentration of 10 μg/ml. Additionally, culture supernatants were collected, and virus infectivity was analyzed via TZM-bl assay.

ADCC assay. ADCC activity was detected according to modifications of the previously described GranToxiLux (GTL) procedure (19). Infected and uninfected target cells were counted, washed, resuspended in R10 medium at 1 × 106 cell/ml, and labeled with a fluorescent target cell marker (TFL4; OncoImmumun, Inc., Gaithersburg, MD) and a viability marker (NFL1; OncoImmumun, Inc.) for 15 min in a 37°C water bath, followed by two washes. Both effector and target populations were counted, and concentrations were adjusted to reach a final effector-to-target ratio of 30:1 and 10:1 when PBMC and NK cells were used, respectively. For the A32, 1/7b, and 7B2 Fab fragments, cells were plated, incubated for 15 min at 37°C, and fixed with a well of a 96-well V-bottom plate to reach a final GzB substrate concentration of 0.25×. After incubation for 5 min at room temperature (RT), 25 μl of the appropriate antibody dilutions were added to the target/effecto
was >5% and >8% for monoclonal Ab and plasma, respectively. These values were established as 3 standard deviations above the average percent GzB activity detectable in the negative controls.

RESULTS

Binding of MAb A32 to the surface of the SupT1 cell line chronically infected with HIV-1 A1953. We first asked if the A32 epitope was expressed on the surface of HIV-1-infected CD4+ T cells and evaluated the expression of the 17b and 2G12 epitopes for comparison. We observed saturation binding levels for each MAb between 5 to 20 μg/ml and a peak of mean fluorescence intensity (MFI) at 10 μg/ml on the A1953-infected SupT1 CD4+ T cell line. Most of the A1953-infected SupT1 CD4+ T cells were still positive for MAb A32 reactivity at a concentration of 0.25 μg/ml (Fig. 1).

Expression of the A32 binding site on the surface of HIV-1-infected PB CD4+ T cells. CD8-depleted PBMC obtained from HIV-1-seronegative healthy subjects were activated for 3 days using the anti-CD3/CD28 Ab combination (9) and infected with either an infectious molecular clone (IMC) of the transmitted/founder virus CH077.t or the laboratory-adapted HIV-1 strain, NL4-3, at a multiplicity of infection of 0.97 TCID50/cell. Mock-infected CD8-depleted cells from the same donor were used as uninfected cell controls. Cells were harvested, and MAb surface reactivity was determined at 24-h intervals starting 72 h postinfection and continuing through day 7. The A32, 17b, and 2G12 MAbs and controls were used at a 10 μg/ml saturating concentration (Fig. 2). Neither the three MAbs, nor the HIVIG preparation, nor the negative controls (palivizumab and intravenous immunoglobulin [IVIG]) bound to mock-infected cells. In contrast, the expression of Env antigens on HIV-1-infected cells was always detected with the A32 MAb before expression of both the CCR5 binding site (MAb 17b) and the gp120 carbohydrate epitope defined by MAb 2G12, and also before epitopes recognized by HIVIG. MAb A32-reactive CD4+ T cells infected with the R5-tropic T/F IMC, CH077.t, appeared on day 3 of in vitro infection, whereas PB CD4+ T cells infected with NL4-3 X4-tropic HIV-1 appeared on day 5. HIV-1-infected cells positive for staining with the 2G12, 17b, and HIVIG Abs were not detected at any time point from day 3 through 7 among the CH077-infected cells but were seen at days 6 and 7 in NL4-3-infected CD4+ T-cell cultures. These data suggested that HIV-1 envelope glycoproteins expressed on the surface of HIV-1-infected cells could be recognized by the A32 MAb earlier than by either the 17b or 2G12 MAb or by Env-specific Abs present in HIVIG. The differences observed between the binding to cells infected with transmitted/founder virus versus
the laboratory-adapted virus NL4-3 might be in part due to the
different kinetics of infections of these two isolates (data not shown).
Moreover, we analyzed the binding capacity of MAb A32 to the surface of CD4+ T cells infected with three R5-tropic T/F viruses and compared the intensity of staining to that measured on HIV-1-infected CD4+ T cells after incubation with no Ab (unstained), with the secondary Ab alone (black curve), and with primary and secondary Abs (green curve). A total of 100,000 to 200,000 CD4+ T cells were stained with 10 μg/ml of the MAbs A32, 17b, and 2G12 as well as with the IgG preparation HIVIG as a primary Ab source. Palivizumab and IVIG were used as negative controls for the MAb and IgG preparation, respectively. Max, maximum.

Monoclonal antibody A32 can mediate ADCC activity. It was originally reported that MAb A32 does not mediate HIV-1 neutralizing activity (22, 34). Similarly, in neutralization assays conducted in both TZM-bl cells and in PBMC cultures, we have also observed that MAb A32 did not neutralize HIV-1 (data not shown). We next asked if MAb A32 could mediate ADCC activity. We first utilized the CEM.NKRCCR5 cell line as a source of target cells after coating with recombinant Env proteins, or as a chronically infected cell line. The recombinant Env proteins utilized to coat the CEM.NKRCCR5 cells represented the gp120 and gp140 sequence, respectively, of the transmitted/founder HIV env from subjects WITO4160 and CH040 (gp120WITO and gp140CH040, respectively). MAb A32 was able to recognize gp120WITO and mediate ADCC activity, expressed as percent GzB activity (Fig. 3A). The maximum percent GzB activity detectable with MAb A32 was 52% ± 8% at 0.156 μg/ml. At this concentration, its activity was almost 6-fold higher than that observed with MAbs 2G12 (8% ± 5.8%) and 17b (9% ± 2%). We observed similar results when the gp140CH040-coated target cells were used (Fig. 3B). A32 was able to mediate a maximum of 40% GzB ADCC activity at a concentration of 0.156 μg/ml, which was 2.6-fold higher than that observed with MAb 17b at 40 μg/ml (15% GzB). In contrast to WITO gp120-coated targets, we did not detect any ADCC activity against the gp140CH040-coated target cells using MAb 2G12. It should be noted that in both settings the A32 ADCC activity displayed the previously described prozone phenomena with high concentrations of antibody inhibiting ADCC activity (21, 33).

MAb A32 is capable of mediating ADCC responses against HIV-1-infected target cells. Using the A1953_CEM.NKRCCR5 target cells, we determined that the peak of the ADCC activity mediated by MAb A32 was 42% GzB activity at 0.625 μg/ml.
This was a 4-fold higher activity than that observed with MAb 17b at a concentration of 40 μg/ml. The same potent ADCC activity was observed when the NL-LucR.T2A-BaL.ecto-infected CEM.NKRCCR5 cells were used as target cells. In this experiment, we observed that the A32 MAb mediates ADCC activity that is also 3-fold higher than that observed for the 2/11c, VRC01, and b12 MAbs (Fig. 3D). We have subsequently determined that the endpoint concentration for the A32-mediated ADCC activity against these two target cells is 1.3 ng/ml and 4.3 ng/ml, respectively (see Fig. S2 in the supplemental material). Moreover, ADCC activity mediated by MAb A32 was detected at concentrations of 50 and 5 μg/ml when NL-LucR.T2A-BaL.ecto-infected PB CD4 T cells obtained from a healthy HIV-1-seronegative subject were used to generate target cells, as described in Materials and Methods (Fig. 4). The NK-to-target cell ratio of 40:1 was required to detect an activity comparable to that observed when the HIVIG preparation was tested at 50 μg/ml. These levels of GzB release are comparable to those obtained using recombinant envelope protein-coated and chronically infected target cells and therefore indicate that higher concentrations of A32 and effector cells are required to eliminate HIV-1-infected CD4 T cells. Surprisingly, we could not detect significant ADCC-mediated MAb A32 activity when the T/F HIV-1-infected target cells were tested (data not shown).

Inhibition of ADCC-mediating Ab activity in plasma from infected individuals by the Fab fragment of A32 antibody. To determine whether the epitope recognized by MAb A32 was also targeted by ADCC responses in chronically infected individuals, we devised a strategy where target cells were preincubated with an A32 Fab fragment to prevent the subsequent binding and ADCC activity of A32-like Ab present in the plasma. FIG. 3. ADCC activity. gp120WITO-coated (A), gp140CH040-coated (B), A1953 chronically infected (C), and NL-LucR.T2A-BaL.ecto HIV-1-infected (D) CEM.NKRCCR5 cells were used to detect the ability of MAbs A32, 17b, 2G12, b12, VRC01, and 2/11c to mediate ADCC activity. Palivizumab was used as a negative control. Each MAb was tested starting at a concentration of 40 μg/ml using 4-fold dilutions. The results are reported as percent GzB activity after the background was subtracted. The results represent the average of triplicate experiments ± standard deviation. The black dotted line represents the cutoff for positive results (5% GzB activity). PBMC from a healthy HIV-1 seronegative donor were used as a source of effector cells with an effector-to-target cell ratio of 30:1 in all experiments.

FIG. 4. ADCC activity. Activated PB CD4 T cells from a healthy HIV-1-seronegative donor after a 3-day infection with the IMC NL-LucR.T2A-BaL.ecto were used as target cells. Autologous purified NK cells were used as effector cells at effector-to-target (NK:T) ratios of 10 and 40 to 1. The MAbs A32, HIVIG (positive control), and palivizumab (negative control) were tested at the concentrations indicated on the x axis. The results are reported as the average ± standard deviation of percent GzB activity observed in triplicate experiments. The black dotted line represents the cutoff for positive results (5% GzB activity).
plasma of HIV-1-infected subjects (Fig. 5). The preincubation of the target cells with 10 μg/ml of the A32 Fab fragment blocked 83 and 89% of MAb A32 ADCC activity when 10 and 1 μg/ml of A32 were used, respectively (Fig. 5A). The specificity of epitope blocking was shown by the absence of inhibition of ADCC activity mediated by MAb 2G12 following preincubation of the target cells with the A32 Fab fragment. Based on this experiment, we determined the ability of the A32 Fab fragment to inhibit ADCC activity of Ab present in the plasma of 14 HIV-1-infected individuals who had detectable virus load and neutralizing Ab activity limited to tier 1 isolates (Fig. 5B). We used a 10 μg/ml concentration of A32 Fab fragment to preincubate target cells in order to block A32 binding on the A1953_CEM.NKRCCR5 cells. The plasma was added to the target cells after this initial incubation at a dilution representing the peak ADCC reactivity for each sample. We observed a range of inhibitory activities for the peak of the response due to the A32 Fab fragment, i.e., from 14% to 87% (average ± standard deviation, 54% ± 23%). The ADCC activity in the plasma samples was significantly lower when the target cells were preincubated with the A32 Fab fragment (Wilcoxon matched-pairs signed rank test, P = <0.0006). A lower range of inhibition from 0 to 53% was observed when the target cells were incubated with a 17b Fab fragment (average ± standard deviation, 18% ± 16%; Wilcoxon matched-pairs signed rank test, P = <0.0066). The majority of ADCC activity (>50% GzB activity) was inhibited in 7 out of 14 patients (50%) by A32 and in 1 out of 14 (7%) patients by 17b. In each individual, A32 mediated a higher level of inhibition of the ADCC activity when the samples were tested at higher dilutions, with inhibition ranging from 48% to 96% (75% ± 16%) (data not shown). In any experiment, preincubation with 7b2 and control Ab did not significantly inhibit the observed level of ADCC.

**FIG. 5.** Inhibition of ADCC activity by A32 Fab fragment. The A1953_CEM.NKRCCR5 cell line was used as target cells, and PBMC from a healthy HIV-1-seronegative donor were used as a source of effector cells. The effector-to-target cell ratio was 30:1 in all experiments. The results are reported as percent GzB activity after background was subtracted. The values above the bars represent the percentage of inhibition when A32 Fab fragment, 17b Fab fragment, 7b2 Fab fragment, and palivizumab were used in the preincubation. (A) The target cells were preincubated with medium only (no pretreatment), with 10 μg/ml of the A32 Fab fragment, or with 10 μg/ml of palivizumab. The results represent the average of results from triplicate experiments. After being washed, the cells were incubated with 10 and 1 μg/ml of MAbs A32 and 2G12. (B) The plasma collected from 14 HIV-1-infected donors was added to A1953_CEM.NKRCCR5 target cells preincubated as previously described with medium only (no pretreatment), A32 Fab fragment, 17b Fab fragment, 7b2, or palivizumab. The MAbs A32 and 17b were used as positive controls.
activity (Wilcoxon matched-pairs signed rank test, $P = <0.4556$).

**DISCUSSION**

In this study we demonstrate that the HIV-1 Env gp120 MAb A32 binds to the surface of transmitted/founder HIV-1-infected CD4$^+$ T cells earlier in the course of *in vitro* infection than the gp120 Env MAbs 17b and 2G12. Moreover, MAB A32 was able to mediate ADCC activity that was 4- to 6-fold higher than that of the other two anti-gp120 MAbs when either gp120-coated or HIV-1-infected target CD4$^+$ cells were used. Finally, we demonstrate that antibodies that are blocked by A32 Fab comprise a majority of CD4-inducible (CD4i)-ADCC-mediating Ab responses elicited during the course of HIV-1 infection.

It has been previously reported that MAB A32 can be expressed on the surface of HIV-1-infected cells during later stages of HIV-1-mediated CD4$^+$ T cell line fusion (10). MAbs directed toward the C terminus, V3 loop, and CD4 binding domain of gp120 display a broad spectrum of binding capacity to the HIV-1 envelope glycoproteins expressed on the surface of infected cells (37). MAbs 17b and 2G12 were previously shown to stain the surface of a HeLa cell line that produced replication-defective HXB2 virions and to inhibit cell-to-cell fusion (10). In this current study, we utilized activated peripheral blood CD4$^+$ T lymphocytes in our experiments to more closely replicate what may occur in vivo. We observed that MAB A32 was capable of binding to CD4$^+$ T cells infected with both primary (transmitted/founder) and laboratory-adapted HIV-1 isolates before potent neutralizing MAbs 17b and 2G12 could bind. This was somewhat surprising and suggested that *in vitro* cell surface expression of gp120 Env epitopes may be differentially regulated. It was important to determine that A32 provided the earliest positive signal to identify envelope expression on the surface of HIV-1-infected cells regardless of the virus isolate, suggesting a relevant role in early recognition and elimination of HIV-1-infected cells. MAB A32 binds to a conformation gp120 determinant that involves the C1 region (34). A32 is nonneutralizing, and the reason for this has been attributed to the inability of A32 to bind to virions; i.e., it has been suggested that the antibody does not bind to the native trimer (34). Our work on PB CD4$^+$ T cells and the previous work of Finnegan et al. clearly demonstrate that MAB A32 does bind to the surface of the native trimer on the surface of HIV-1-infected cells. Moreover, these data suggest that the conformation of the Env trimer with regard to the C1 region is dramatically different between virions and virus-infected cells. We are currently investigating the mechanisms that could affect the ability of MAB A32 to bind and mediate ADCC activity against PB CD4$^+$ T cells infected with primary T/F HIV-1 isolates compared to activity against those infected with laboratory-adapted HIV-1. A relative decrease in the expression of the HIV-1 env on the surface of T/F compared to laboratory-adapted HIV-1-infected cells could be one of the factors responsible for this difference.

We further explored the ability of MAB A32 to mediate ADCC activity using a variety of target cell populations, including gp120-coated CD4$^+$ T cell line cells, chronically infected CD4$^+$ T cells using the A1953 isolate, and PB CD4$^+$ T cells infected with T/F and laboratory-adapted HIV-1 infectious molecular clones. Under our experimental conditions, the ADCC-mediating activity observed for MAB A32 was superior to that observed for any of the other MAbs included in this study. The ADCC-mediating activity of 2G12 using the gp120-coated target cells was comparable to that previously reported (32), and the paucity of ADCC reactivity observed for 17b confirms previous observations indicating the need for soluble CD4 (sCD4) triggering of some Env for exposure of the 17b CCR5-binding site epitope (30). The ability of A32 to mediate ADCC activity has also been confirmed in recent studies exploring the role of CD4i MAbs in ADCC responses (George Lewis and Anthony DeVico, personal communication). Importantly, we observed that MAB A32 was able to mediate 4- to 6-fold higher ADCC activity than MAbs 2/11c, 17b, 2G12, b12, and VRC01, comparable to ADCC activity mediated by HIV-positive immunoglobulin (HIVIG).

Most importantly, we determined the presence of A32-like Ab responses in the serum of subjects with chronic HIV-1 infection. We observed that an A32-like Ab mediated the majority of the total ADCC activity in 50% of the chronically infected subjects, whereas 17b-like Ab mediated the majority of ADCC activity in one subject only. The ability of 17b Fab fragment to also inhibit some of the ADCC activity mediated by the Ab responses in the chronically infected subjects suggests that CD4i Ab responses are a relevant component of the overall ADCC Ab response induced by HIV-1 infection.

Previous studies have demonstrated the frequent induction of CD4i antibody responses in acute (27) and chronic (7) HIV-1 subtype B infection and have shown that they contribute to the neutralizing activity in acute subtype C infection (14). These data, together with our observations of the ability of MAB A32 to recognize Env expression on the surface of HIV-1-infected cells earlier than 17b and 2G12, emphasize the importance of the A32 epitope as a candidate for the target of nonneutralizing gp120 antibodies that may participate in protection from HIV-1 acquisition. Our studies also demonstrate the importance of understanding what role A32-like antibodies may have in preventing HIV-1 acquisition in vaccine trials such as RV144 (26). Moreover, if MAB A32 is shown to mediate a component of prevention of acquisition in passive protection trials in nonhuman primates, then it will be important to design novel immunogens that could enhance the antigenicity of the gp120 region for MAB A32 binding in order to improve HIV-1 vaccine candidates.

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