Diverse HIV-1 Isolates

Antigen on HIV-1 Particles and Neutralize

GB Virus Type C Envelope Protein E2 Elicits

Antibodies That React with a Cellular

Emma L. Mohr, Jinhua Xiang, James H. McLinden, Thomas M. Kaufman, Qing Chang, David C. Montefiori, Donna Klinzman and Jack T. Stapleton

J Immunol 2010; 185:4496-4505; Prepublished online 8 September 2010;
doi: 10.4049/jimmunol.1001980
http://www.jimmunol.org/content/185/7/4496

References

This article cites 65 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/185/7/4496.full#ref-list-1

Why The JI? Submit online.

• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Fast Publication! 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
GB Virus Type C Envelope Protein E2 Elicits Antibodies That React with a Cellular Antigen on HIV-1 Particles and Neutralize Diverse HIV-1 Isolates

Emma L. Mohr,*†‡ Jinhua Xiang,*†‡ James H. McLinden,* Thomas M. Kaufman,* Qing Chang,* David C. Montefiori,‡ Donna Klinzman,* and Jack T. Stapleton*†

Broadly neutralizing Abs to HIV-1 are well described; however, identification of Ags that elicit these Abs has proven difficult. Persistent infection with GB virus type C (GBV-C) is associated with prolonged survival in HIV-1–infected individuals, and among those without HIV-1 viremia, the presence of Ab to GBV-C glycoprotein E2 is also associated with survival. GBV-C E2 protein inhibits HIV-1 entry, and an antigenic peptide within E2 interferes with gp41-induced membrane perturbations in vitro, suggesting the possibility of structural mimicry between GBV-C E2 protein and HIV-1 particles. Naturally occurring human and experimentally induced GBV-C E2 Abs were examined for their ability to neutralize infectious HIV-1 particles and HIV-1–enveloped pseudovirus particles. All GBV-C E2 Abs neutralized diverse isolates of HIV-1 with the exception of rabbit anti-peptide Abs raised against a synthetic GBV-C E2 peptide. Rabbit anti–GBV-C E2 Abs neutralized HIV-1–pseudotyped retrovirus particles but not HIV-1–pseudotyped vesicular stomatitis virus particles, and E2 Abs immune-precipitated HIV-1 gag particles containing the vesicular stomatitis virus type G envelope, HIV-1 envelope, GBV-C envelope, or no viral envelope. The Abs did not neutralize or immune-precipitate mumps or yellow fever viruses. Rabbit GBV-C E2 Abs inhibited HIV attachment to cells but did not inhibit entry following attachment. Taken together, these data indicate that the GBV-C E2 protein has a structural motif that elicits Abs that cross-react with a cellular Ag present on retrovirus particles, independent of HIV-1 envelope glycoproteins. The data provide evidence that a heterologous viral protein can induce HIV-1–neutralizing Abs. The Journal of Immunology, 2010, 185: 4496–4505.

Human immunodeficiency virus-1 vaccine development has relied primarily on the use of HIV-1 proteins as immunogens in an attempt to elicit either neutralizing Abs or cellular immune responses to prevent or modify HIV-related disease (reviewed in Refs. 1 and 2). Because of the high replicative rate of HIV-1 and the error-prone RNA-dependent DNA polymerase, neutralization and T cell escape mutations are generated on a daily if not hourly basis in infected individuals. Broadly neutralizing human mAbs (hmAbs) have been isolated from HIV-infected individuals (3), including hmAbs directed against gp120 that interfere with CD4 binding (e.g., 2G12) or that react with the membrane proximal ectodomain region (MPER) of gp41 (e.g., 2F5 and 4E10) (3, 4). These Abs also react with a 36-residue peptide that overlaps with the MPER called T-20 (5). T-20 inhibits HIV replication by preventing virus envelope fusion with the cell membrane, and T-20 is an effective and licensed antiretroviral treatment (Fuzenon) (5, 6). 2F5 and 4E10 Abs are polyspecific and cross-react with cellular Ags including several lipids (7–12). Although Ags that interact with these Abs have been identified, active immunization with gp41, MPER, or T-20 does not elicit broadly neutralizing HIV Abs (13, 14). Clearly, new approaches to HIV-1 vaccines are needed (1, 2).

GB virus type C (GBV-C) is a common human infection that is not clearly associated with any disease. The virus replicates in B and T lymphocytes including CD4+ and CD8+ T cell subsets (15; reviewed in Ref. 16). Because of shared modes of transmission, the prevalence of GBV-C in HIV-infected people is high (17–42%) (17). Several studies and a meta-analysis of studies including 1294 HIV-infected individuals found that persistent GBV-C infection is associated with prolonged survival (18–22). GBV-C infection is also associated with decreased maternal-to-child HIV-1 transmission (23, 24). Abs to GBV-C are usually not detected during viremia; however,

Received for publication June 14, 2010. Accepted for publication August 3, 2010.

Abbreviations used in this paper: AZT, azidothymidine; BHK, baby hamster kidney; CHO, Chinese hamster ovary; GBV-C, GB virus type C; GBVpp, GBV-C enveloped retrovirus pseudoparticle; HIVpp, HIV-enveloped retrovirus pseudoparticle; HIVmenv- HIV-encoding HIV-1 pseudovirus pseudoparticle; hmAb, human mAb; HOS, human osteocarcoma; I, IgG; IC, isotype control; IP, immune precipitation; IPV, the amount of input virus used in an immune-precipitated experiment; M, the lane containing the m.w. marker; MPER, membrane proximal ectodomain region; MV, mumps virus; NA, not applicable (not obtained from NARRRP); NARRRP, National Institutes of Health AIDS Research and Reference Reagent Program; NS5A, GBV-C nonstructural protein 5A; P, positive; Pep, rabbit anti–GBV-C E2 peptide IgG; Post, postimmune rabbit IgG; R5, CCR5 tropic; RLU, relative light unit; S, serum; Sup, supernatant; UIVC, isolate obtained from University of Iowa Virology Clinic; VS, vireostat; VSV, vesicular stomatitis virus; VSVmenv- VSV-enveloped retrovirus pseudoparticle; X4, CXCR4 tropic; YFV, yellow fever virus.

1Division of Infectious Diseases, Department of Internal Medicine, Iowa City Veterans Affairs Medical Center; 2Interdisciplinary Program in Molecular and Cellular Biology, University of Iowa, Iowa City, IA 52242; and 3Department of Surgery, Duke University, Durham, NC, 27708

*E.L.M. and J.X. contributed equally to this work.

*Division of Infectious Diseases, Department of Internal Medicine, SW54, General Hospital, 200 Hawkins Drive, Iowa City, IA 52242. E-mail address: jack.stapleton@uiowa.edu

†Affiliation Medical Center; ‡Interdisciplinary Program in Molecular and Cellular Biology, *Division of Infectious Diseases, Department of Internal Medicine, SW54, General Hospital, 200 Hawkins Drive, Iowa City, IA 52242. E-mail address: jack.stapleton@uiowa.edu
following clearance of GBV-C, Abs specific for the envelope glycoprotein (E2) are identified. Consequently, GBV-C E2 Ab serves as a marker of prior infection (reviewed in Ref. 16). Although persistent GBV-C viremia is associated with the best survival in epidemiological studies (25), one study found that subjects without viremia who have GBV-C E2 Abs survived longer than those without E2 Abs (20). Human GBV-C E2 Abs and all but one of characterized GBV-C E2 murine mAbs are conformation dependent (26). One mAb (M6) recognizes a linear epitope on E2 (27); however, the interaction is complex. M6 binds to six amino acids within E2 if there are four or eight amino acids added to the C or N termini, respectively, suggesting that there is a size and sequence requirement for interaction (26). A GBV-C E2 peptide encompassing this epitope has been proposed to be involved in GBV-C–cell membrane fusion, based on findings that it forms an amphipathic helix in the presence of lipids and model membranes (28, 29). In addition, another E2 peptide that overlaps the putative fusion peptide prevents oligomerization of the HIV-1 gp41 fusion peptide and membrane fusion in an in vitro model (30). Finally, incubation of PBMCs or CD4+ T cell lines with the GBV-C envelope glycoprotein E2 competitively inhibits HIV-1 entry in vitro (31, 32), raising the possibility that there is structural mimicry between GBV-C E2 and HIV-1 particles, or between E2 and a cell surface molecule carried on the HIV particle that is involved in HIV-1 entry. If this is true, Abs directed against GBV-C E2 may interfere with HIV attachment or fusion and potentially modify HIV-1 disease progression.

Table 1. Characteristics of HIV isolates used in neutralization assays

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Isolate Name</th>
<th>Tropism</th>
<th>Derivation*</th>
<th>Clade</th>
<th>Catalog No.</th>
<th>Cell Types Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV-1 92UG031</td>
<td>R5</td>
<td>Clinical</td>
<td>A</td>
<td>1741</td>
<td>PBMC</td>
</tr>
<tr>
<td>2</td>
<td>AZT intermediate†</td>
<td>R5-X4</td>
<td>Clinical</td>
<td>B</td>
<td>1073</td>
<td>PBMC</td>
</tr>
<tr>
<td>3</td>
<td>HIV-1 92UG029</td>
<td>X4</td>
<td>Clinical</td>
<td>A</td>
<td>1650</td>
<td>MT-2; PBMC</td>
</tr>
<tr>
<td>4</td>
<td>HIV-1xH9</td>
<td>X4</td>
<td>Laboratory</td>
<td>B</td>
<td>394</td>
<td>PBMC</td>
</tr>
<tr>
<td>5</td>
<td>HIV-1xH9</td>
<td>X4</td>
<td>Laboratory</td>
<td>B</td>
<td>398</td>
<td>MT-2; PBMC</td>
</tr>
<tr>
<td>6</td>
<td>HIV-1xH9</td>
<td>X4</td>
<td>Laboratory</td>
<td>B</td>
<td>2969</td>
<td>PBMC</td>
</tr>
<tr>
<td>7</td>
<td>HIV-1xH9</td>
<td>X4</td>
<td>Laboratory</td>
<td>D</td>
<td>2521</td>
<td>MT-2; PBMC</td>
</tr>
<tr>
<td>8</td>
<td>NFN-HAS(pNL4-3)</td>
<td>X4</td>
<td>Laboratory</td>
<td>B</td>
<td>NA</td>
<td>MT-2; PBMC</td>
</tr>
<tr>
<td>9</td>
<td>NFN-SX-HSA(pNL4-3)</td>
<td>R5</td>
<td>Laboratory</td>
<td>B</td>
<td>NA</td>
<td>PBMC</td>
</tr>
<tr>
<td>10</td>
<td>UIVC</td>
<td>R5</td>
<td>Clinical</td>
<td>B</td>
<td>NA</td>
<td>MT-2; PBMC</td>
</tr>
<tr>
<td>11</td>
<td>UIVC</td>
<td>R5/X4</td>
<td>Clinical</td>
<td>B</td>
<td>NA</td>
<td>MT-2; PBMC</td>
</tr>
</tbody>
</table>

*Derivation refers to whether HIV isolate is a laboratory-adapted isolate or a clinical isolate.
†NARRRP catalog number. Isolate name from NARRRP data sheet.
‡Isolate was initially mixed tropic (R5-X4), but following amplification in MT-2, cells reverted to CXCR4 tropic.
NA, not applicable (not obtained from NARRRP); R5, CCR5 tropic; UIVC, isolate obtained from University of Iowa Virology Clinic; X4, CXCR4 tropic.

FIGURE 1. Ab to the GBV-C envelope glycoprotein E2 neutralizes HIV-1 in vitro. Human serum (S) diluted 1/100 obtained from GBV-C E2 Ab-positive (P) subjects (n = 3; SP1, SP2, and SP3), IgG (I) purified from these sera (I-P1, I-P2, and I-P3; 10 μg/ml), murine mAbs [M1(VS) and M6], and human HIV-1 mAbs (2G12, 2F5, and 4E10) (5 μg/ml for all mAbs) neutralized CCR5- and CXCR4-tropic HIV-1 isolates in primary human PBMCs compared with no Ab controls, whereas E2 Ab-negative human sera (SN1 and SN2), IgG (I-N1 and I-N2), or murine isotype control (IC) Ab did not (A). HIV p24 Ag concentration was measured 5 d postinfection. The dose relationship between the human IgG preparations is shown (B).

FIGURE 2. GBV-C E2 protein elicits HIV-1–neutralizing Abs in mice. Mouse sera (diluted 1/5000) from two of three mice immunized with rGBV-C E2 protein reacted with CHO cell culture supernatant containing rE2 but not supernatant (Sup) from CHO cells not expressing E2. Pooled preimmune murine sera diluted 1/5000 did not react with E2 (A). There was insufficient serum from the third mouse to test by immunoblot. The concentrations of HIV p24 Ag produced in MT-2 cells 5 d following incubation with preimmune sera (1/1000) or postimmune (GBV-C E2 Ab positive) sera are shown in B. Data represent the average HIV p24 concentration released by cells following neutralization with the three mouse serum samples performed at the dilutions shown. The dashed line shows the amount of HIV-1 p24 Ag produced in the no Ab control cells.
We examined naturally occurring human and experimentally induced murine and rabbit GBV-C E2 Abs and found that they crossed-reacted with and neutralized diverse HIV-1 isolates. They also precipitated pseudotyped HIV-1 gag particles independent of the viral envelope used. In contrast, the Abs did not neutralize or precipitate mumps virus or yellow fever virus (YFV), and they did not neutralize vesicular stomatitis virus (VSV) particles, suggesting that the Ag is of cellular origin and that it is present on HIV-1 gag particles. Thus, immunization with GBV-C E2 protein broadly neutralizing HIV Abs, a long-sought-after goal in HIV-1 vaccine development.

Materials and Methods

Ags and Abs

GBV-C E2 protein truncated to remove the C-terminal transmembrane domain (nt 1167–2162 based on the infectious clone isolate; GenBank number AF121950) (33) was expressed in Chinese hamster ovary (CHO) cells as described previously (26). The CHO cells were adapted for serum-free growth, and supernatants containing GBV-C E2 protein were concentrated on an Amicon CP10 concentrator (Millipore, Billerica, MA). Three BALB/c mice were immunized with 25 μg GBV-C E2 protein mixed with IFA, followed by two immunizations of E2 in IFA at 2-wk intervals. Rabbits were immunized with 50 μg E2 in IFA (Invitrogen, Carlsbad, CA) and boosted 6 and 12 wk later by the Iowa State University Hydromina Facility (Ames, IA). Sera were collected before immunization (preimmune) and at various intervals after immunization for analysis and IgG purification as described previously (26). A synthetic peptide representing GBV-C E2 sequences (GGAGLTGGFYEPLVRRC) was provided by Dr. O. Sharma (National Institutes of Health [NIH] AIDS Research and Reference Reagent Program [NARRRP], Rockville, MD). Rabbit sera were immunized with KLH (National Institutes of Health [ NIH ] AIDS Research and Reference Reagent Program [NARRRP], Rockville, MD). Sera were immunized with KLH conjugated E2 peptide (100 μg) in IFA and boosted 2, 7, and 9 wk later.

HIV-, HCV- and HBsAg-negative sera were obtained from healthy blood donors. Anti-E2 Ab status was assessed by ELISA (μPlate anti-HIVenv test; provided by Dr. G. Hess, Roche Diagnostics, Mannheim, Germany). Murine anti–GBV-C E2 mAb M1 (virostat [VS]) was purchased (Virostat, Portland, ME), whereas M6 and M11 (Roche Laboratories, Penzberg, Germany) were provided by Dr. A. Engel (Roche Diagnostics). Isotype control Abs were purchased from Sigma-Aldrich (St. Louis, MO), M1(VS) and M11 recognize conformation-dependent epitopes, whereas M6 recognizes a linear epitope on GBV-C E2 (26). E2 immunoblot were performed as described previously (26). A synthetic peptide representing GBV-C E2 sequences (GGAGLTGGFYEPLVRRC) was provided by Dr. O. Sharma (National Institutes of Health [NIH] AIDS Research and Reference Reagent Program [NARRRP], Rockville, MD). Sera were immunized with KLH conjugated E2 peptide (100 μg) in IFA and boosted 2, 7, and 9 wk later.

HIV-, HCV- and HBsAg-negative sera were obtained from healthy blood donors. Anti-E2 Ab status was assessed by ELISA (μPlate anti-HIVenv test; provided by Dr. G. Hess, Roche Diagnostics, Mannheim, Germany). Murine anti–GBV-C E2 mAb M1 (virostat [VS]) was purchased (Virostat, Portland, ME), whereas M6 and M11 (Roche Laboratories, Penzberg, Germany) were provided by Dr. A. Engel (Roche Diagnostics). Isotype control Abs were purchased from Sigma-Aldrich (St. Louis, MO), M1(VS) and M11 recognize conformation-dependent epitopes, whereas M6 recognizes a linear epitope on GBV-C E2 (26). E2 immunoblot were performed as described previously (26). HIV-neutralizing hmAbs 2F5, 2G12, and 4E10 were obtained from NARRRP (catalog numbers 1475, 1476, and 10091, respectively).

Cells and viruses

PBMCs were isolated from blood obtained from healthy donors. All human subjects provided written informed consent, and the project was approved by the University of Iowa Institutional Review Board. CD4+ cell lines (MT-2 and human osteosarcoma [HO5] cells expressing human CD4, CCR5, and CXCR4) were maintained as described previously (34–36). The HOS cells were obtained from NARRRP and also contained GFP under the control of HIV-1 long terminal repeat (catalog numbers 3685 and 3944). Baby hamster kidney (BHK) 21 cells were maintained in DMEM containing 10% FCS and antibiotics. Table I summarizes the infectious HIV-1 isolates used in these studies. Briefly, using the nomenclature described in the data sheets for
HIV-1 isolates obtained from NARRRP; we studied the following viruses: R5 isolates—HIV-1 92UG031 (clade A; catalog number 1741), HIVIRCSF (clade B; catalog number 394), and NFN-SX-heat stable Ag (HSA) (clade B); and X4 isolates—HIV-1 92UG029 (clade A; catalog number 1650), azidothymidine (AZT) intermediate (clade B; catalog number 1073), HIV1-R5/H9 (clade B; catalog number 398), HIVxBRU-JF (clade B; catalog number 2969), HIVELI (clade D; catalog number 2521), and NFN-HSA (pNL4-3 backbone; clade B). Infectious cDNA clones of two HIV-1 isolates were provided by Dr. B. Jamieson (University of California, Los Angeles, CA); NFN-HSA (derived from pNL4-3 and expressing murine HSA [also known as CD24]) and NFN-SX-HSA (pNL4-3 env replaced with an R5-tropic env gene (37, 38). Two clinical isolates were obtained and characterized from the University of Iowa HIV/AIDS Clinic (Iowa City, IA). HIV stock viruses were prepared in MT-2 cells (X4 isolates), PBMCs (X4 and R5 isolates), or HOS cells as indicated in Results. YFV (vaccine strain, 17D; Sanofi-Pasteur, Swiftwater, PA) was propagated in BHK21 cells or MT-2 cells. The mumps virus was propagated in Vero Cells or MT-2 cells.

Pseudotype virus production

293T cells were cotransfected with pNL4-3-LucR-E− (luciferase reporter inserted into pNL4-3 nef gene) and a plasmid that expresses the envelope glycoproteins of VSV (VSV-G), HIV (pHXB2-env), GBV-C E1-E2 (nt 555–2479), or no viral envelope protein using CaCl2 as described previously (39). Particles were collected 72 h posttransfection, filtered with a 0.45-μm filter, and concentrated by centrifugation at 12,000 rpm for 20 h at 4°C. Alternatively, 293T cells were transfected with pHXB2-env-expressing plasmid and transduced with defective VSV particles with a GFP reporter gene provided by Dr. W. Maury (University of Iowa). Pseudotyped VSV particles were collected from supernatants and filtered with a 0.45-μm filter prior to use.

Viral infections and neutralization assays

The HIV-1 inocula (2 ng/ml p24 Ag per infection) or defective HIV or VSV particles (produced in 293T) were incubated with a range of concentrations of GBV-C E2 Ab-positive sera, IgG preparations or mAbs for 1 h at 37°C prior to adding to CD4+ T cell or HOS cell cultures. GBV-C E2 Ab-negative sera, E2 Ab-negative IgG preparations, and isotype control mAbs served as the negative control Abs. Inocula were removed, CD4+ cells (PBMCs or MT-2 as described in Results) were washed after 4 h, and culture supernatants were collected postinfection for measurement of HIV-1 replication by measuring HIV-1 p24 Ag (ELISA) or reporter gene expression 72 h posttransduction as described previously (19, 35). Pseudotyped particle transduction was determined by measuring luciferase by the Bright-Glo Luciferase Assay System (Promega, Madison, WI) or GFP expression by flow cytometry. The concentration of each Ab preparation (micrograms per milliliter) required to reduce HIV-1 p24 Ag release into culture supernatants by 50% was calculated (IC50). YFV and mumps virus infectious titers (tissue culture infective dose 50%) were determined by terminal dilution in BHK21 cells or Vero cells, respectively (40, 41), and YFV and mumps viral RNA concentrations were measured by real-time PCR as described previously (42, 43).

Table III. Interassay variation in GBV-C E2 Ab neutralization

<table>
<thead>
<tr>
<th>Ab</th>
<th>MN</th>
<th>SS196.01</th>
<th>QA692.42</th>
<th>PAVO 0.04</th>
<th>REJO 0.67</th>
<th>THRO 0.18</th>
<th>Du123 0.06</th>
<th>Du156 0.12</th>
<th>Du172 0.17</th>
<th>Du422.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 (VS)</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>M5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>M6</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>rPep-1</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>rPep-2</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>I-N1</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>I-N2</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>I-P1</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>I-P2</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>I-P3</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

IC50 results >5 μg/ml for mAbs or >30 μg/ml for polyclonal Abs are in bold.

Values represent concentration (micrograms per milliliter) at which RLU’s were reduced 50% compared with virus control wells (no test sample). All HIV-1 isolates, except MN, are molecularly cloned pseudoviruses produced by transfection in 293T cells. M1(VS), M5, and M6 are murine anti–GBV-C E2 mAbs. rPep-1 and rPep-2 are rabbit anti-E2 peptide Abs (see Materials and Methods). I-N1 and I-N2 are two human IgG preparations from blood donors without E2 Ab, and I-P1, I-P2, and I-P3 are human IgG preparations from three blood donors with E2 Ab.
All neutralization experiments were performed in triplicate and independently repeated at least once with consistent results.

For some experiments, the standardized neutralization of HIV envelope-pseudotyped HIV particles was assessed after a single round of infection in TZM-bl cells as described previously (36). A total of 200 tissue culture infective dose 50% of virus was inoculated with serial dilutions of test samples for 1 h at 37°C in 96-well flat-bottom culture plates. Freshly trypanized cells were added to each well. No Ab control and cell only controls served as the background controls. Cells were lysed, and luminescence was measured 48 h after transduction using a Victor 2 luminometer. The serum concentration or IgG concentration that resulted in a 50% reduction in relative light units (RLUs) compared with virus control wells after subtraction of background RLUs was calculated (36).

HIV-1 and SIV envelopes used to pseudotype HIV-1 retroviral particles included SS1196.01, QH0692.42, PA VO.04, 6535.03, REJO.67, THRO.18, Du123.06, Du156.12, Du172.17, Du422.01, and control HIV, and SIV isolates included HIVMN (propagated in H9 cells) and SIVmac239/CS generated in PBMCs. In addition, HIV isolate SF162.LS was propagated in PBMCs and used in one TZM-bl experiment.

**Immune precipitation**

HIV-1, YFV, mumps virus, or defective HIV-1 pseudoparticles were mixed with Abs at various concentrations and incubated while mixing overnight at 4°C. All three viruses (HIV-1, YFV, and mumps) were prepared in PBMCs and pseudoparticles were prepared in 293T cells. Heat-killed, formalin-fixed *Staphylococcus aureus* cells with a coat of protein A (Pansorbin; Calbiochem, San Diego, CA) were added and incubated for 2 h at 4°C. Cells were pelleted, washed three times in TBS or PBS containing 0.02% Tween 20, and resuspended in PBS, and viral particle precipitation was measured (p24 Ag ELISA or real-time PCR for YFV and mumps RNA as above). For the E2 competition assay, GBV-C E2 or E2-negative cell supernatants were incubated with E2 Ab for 1 h at 37°C prior to incubating the Ab with virus particles.

**ELISA**

GBV-C E2 Ab reactivities with GBV-C E2 were assessed using a GBV-C mAb E2 capture assay. Plates were coated with E2-specific mAb (M5) in carbonate buffer (pH 9.6) overnight at 4°C, washed with PBS containing 0.02% Tween 20 (PBST), and blocked with PBS containing 1% BSA (PBSA) at 4°C overnight or 1 h at 37°C. E2 Ag was applied at 1 μg/ml in PBSA and allowed to adsorb for 1 h at 37°C. Plates were washed with PBST, and serial dilutions of Abs in PBSA were applied and incubated at 37°C for 1 h. Following washing, bound rabbit Abs were detected using alkaline phosphatase-labeled goat anti-rabbit Fc Ab (Sigma-Aldrich). Plates were washed, p-nitrophenylphosphate substrate was added, and the absorbance (405 nm) was measured on a Bio-Rad model 680 microplate reader. Previous studies found that human anti–GBV-C E2 Abs bound to E2 that was generated in PBMCs and allowed to adsorb for 1 h at 37°C in 96-well flat-bottom culture plates. Freshly trypanized cells were added to each well. No Ab control and cell only controls served as the background controls. Cells were lysed, and luminescence was measured 48 h after transduction using a Victor 2 luminometer. The serum concentration or IgG concentration that resulted in a 50% reduction in relative light units (RLUs) compared with virus control wells was calculated (36).

**Results**

**GBV-C E2 Abs neutralize HIV-1 infection**

Heat-inactivated sera from healthy blood donors with and without GBV-C E2 Ab were tested for HIV-1–neutralizing activity. E2 Ab-positive, but not E2 Ab-negative, sera (diluted 1/100) neutralized a CCR5-tropic HIV isolate (R5, isolate 1) (Table I) and a CXCR4-tropic HIV isolate (X4, isolate 2) (Table I) in a PBMC-based neutralization assay. The percent inhibition compared with no Ab control is shown in Fig. 1A. Sera were maintained in culture media (1/100). None of these healthy blood donor sera reacted with denatured HIV-1 proteins in Western blot analyses (data not shown).

To determine whether the effect was due to soluble factors in serum or whether it was due to serum Abs, IgGs purified from the serum samples were tested for HIV-1 neutralization activity in PBMCs (concentration 10 μg/ml) (Fig. 1A). The three human GBV-C E2 Ab-positive IgG preparations neutralized HIV-1 in MT-2 cells in a dose-dependent manner (Fig. 1B). Three murine GBV-C E2 mAbs M1(VS), M5, and M6 also inhibited HIV-1 isolates (concentration 5 μg/ml) in the PBMC-based assay (Fig. 1A). Three broadly neutralizing human HIV-1 mAbs (2G12, 2F5, and 4E10; 5 μg/ml) (Fig. 1A) served as positive control Abs.

**GBV-C E2 immunogenicity**

Mice and rabbits were immunized with rGBV-C E2 protein to determine whether it elicited HIV-1–neutralizing Abs. Murine serum obtained 10 d after the third E2 immunization demonstrated anti-E2 reactivity in a mAb capture ELISA, whereas the preimmune sera did not (data not shown). Sufficient sera were available to test two of the three mice by immunoblot assay, and both reacted with rE2 (Fig. 2A). All three murine sera neutralized the infectivity of HIV-1 (isolate 2) (Table I) in MT-2 cells compared with preimmune sera, as measured by HIV-1 p24 Ag release into culture supernatants 5 d post-HIV infection (Fig. 2B).

<table>
<thead>
<tr>
<th>Sample</th>
<th>SF162.LS Clade B</th>
<th>HIV-1 R5 Isolate 1</th>
<th>HIV-1 X4 Isolate 2</th>
<th>HIV-1 R5 Isolate 1</th>
<th>HIV-1 X4 Isolate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-N2</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
</tr>
<tr>
<td>I-P1</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>45</td>
</tr>
<tr>
<td>I-P2</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>9</td>
</tr>
<tr>
<td>I-P3</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>14</td>
</tr>
<tr>
<td>M1(VS)</td>
<td>2.6</td>
<td>2.6</td>
<td>3.2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>M5</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>M6</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>17</td>
<td>&gt;50</td>
</tr>
<tr>
<td>TriMAb</td>
<td>0.04</td>
<td>&gt;25</td>
<td>0.58</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

1IC50 results >5 μg/ml for mAbs or >30 μg/ml for polyclonal Abs are in bold. HIV isolates 1 (R5) and 2 (X4) refer to isolates described in Table I.

2Values represent the Ab concentration (micrograms per milliliter) at which RLUs were reduced 50% compared with virus control wells (no test sample).

3Values represent the Ab concentration (micrograms per milliliter) at which p24 Gag Ag synthesis was reduced by 50% relative to virus control wells (no test sample).

4TriMAb is a positive control of three HIV-1 human neutralizing mAbs.
GBV-C E2 protein and the GBV-C 17-aa E2 peptide were immunogenic in rabbits as well, as sera obtained from all rabbits reacted with E2 in a mAb capture ELISA (Fig. 3A) and by immunoblot analysis (Fig. 3B), whereas preimmune sera did not. Postimmune anti–GBV-C E2 IgG (n = 4 rabbits) significantly inhibited HIV-1 (isolate 2) (Table I) replication in MT-2 cells 3 and 6 d postinfection (Fig. 4A). In contrast, preimmune rabbit IgG, IgG purified from rabbit sera following immunization with a different GBV-C protein (NS5A) (46), or polyclonal goat IgG containing Ab to hepatitis B surface Ag did not inhibit HIV-1 infection compared with the no Ab control (Fig. 4A). All polyclonal Abs were used at a concentration of 10 μg/ml, and the Ab was maintained in the media following HIV-1 inoculation of cells.

To ascertain whether the rabbit IgG inhibited the HIV-1 p24 Ag ELISA, HIV-1 was incubated with no Ab or pre- or postimmune rabbit IgG (10 μg/ml each), and the concentration was assessed. There was no difference between the no Ab control (11,107 pg/ml) and the pre-(11,219 pg/ml) and postimmune (11,106 pg/ml) (ANOVA; p = 0.99). In addition, incubation of MT-2 cells and PBMCs with rabbit preimmune or anti-E2 IgG did not alter cell number or viability during 6 d in culture (data not shown). HIV-1 inhibition was diminished when Abs were not maintained in the cell culture media (Fig. 4B).

Specifically, HIV replication was inhibited by >99% in MT-2 cells if E2 Ab was maintained in the media (p < 0.01); however, HIV-1 replication inhibition was diminished when Ab was removed after inoculation. Initial infection appears to be incompletely inhibited by the Abs, and HIV-1 cell-to-cell spread is suppressed in the presence of GBV-C E2 Abs.

Single cycle replication assays allow assessment of viral envelope specificity and provide information regarding which step(s) of HIV replication is inhibited. HIV-1 envelope protein (gp160) was used to pseudotype defective HIV-1 (gag) particles or defective VSV particles generated in 293T cells, and rabbit anti–GBV-C E2 IgG was assessed for neutralizing activity in a HOS cell-based assay. Anti–GBV-C E2 IgG preparation neutralized HIV-1–pseudotyped retrovirus particles in a dose-dependent fashion; however, this Ab did not neutralize HIV-1–pseudotyped VSV particles (Fig. 4C). Preimmune rabbit IgG did not neutralize either particle type. These data suggest that the anti–GBV-C E2 Abs neutralize HIV-1 particles independently from the HIV-1 envelope proteins.

**HIV-1 neutralization by GBV-C E2 Abs is assay dependent**

The breadth and potency of HIV-1–neutralizing activity present in GBV-C E2 Ab preparations was examined in virus replication assays using a diverse panel of HIV-1 isolates including clinical and laboratory derived R5 and X4 isolates and representing clades A, B, and D (Table I). The Ab concentration (micrograms per milliliter) required to inhibit HIV replication by 50% (IC50) for human polyclonal anti-E2 Ab, rabbit anti-E2 Ab, rabbit anti-E2 peptide Ab, and murine mAbs M1(VS), M5, and M6 is shown in Table II. All anti-E2 Abs possessed neutralizing activity against all isolates tested except the rabbit anti-E2 peptide Ab (<50% reduction at 50 μg/ml Ab concentration). Three HIV-1–neutralizing hmAbs served as control Abs (2F5, 4E10, and 2G12). Because endotoxin contamination of Ab may lead to non-specific-neutralizing activity against HIV-1 (47), all of the rabbit IgG preparations were tested for endotoxin (assay; Cape Cod Associates, Woods Hole, MA) and were found to be endotoxin free (<0.25 EU/ml).

Some HIV-neutralizing Abs perform differently in neutralization assays that use different cell types (48, 49). To see whether this were true for the GBV-C E2 Abs, a standardized TZM-bl assay was used to examine neutralization of a panel of HIV-enveloped pseudoparticles (36, 50, 51). Differences in neutralization in the TMZ-bl cells and the PBMC-, MT-2-, and HOS-based assays were observed (Table III). The murine mAb M5 did not neutralize any of the isolates tested in TZM-bl cells, and M6 mAb neutralized only one HIV envelope (Du224.01) at concentrations of <5 μg/ml (Table III). In contrast, the murine M1(VS) mAb was broadly neutralizing and neutralized all viruses HIV-1 envelopes studied, except REJO.67 in the TZM-bl cell assay (Table III). The mAb M1 (VS) was also active against an SIV macaque envelope (SIVmac 239/CS), further suggesting that the interaction is independent of HIV-1 envelope glycoproteins. Although the rabbit anti-peptide
IgG and E2-positive human IgG neutralized some HIV and SIV isolates at high concentrations (generally >20 μg/ml) (Table III), the specificity of this is unclear, because the E2-negative IgG also inhibited some isolates at high concentration (Table III). One of the HIV-negative blood donor human anti-E2 IgG preparations (I-P2) neutralized three HIV isolates at <10 μg/ml, indicating a more narrow neutralization effect on HIV-1 envelope-containing particles in the TZM-bl cell neutralization assay (Table III). Because these are polyclonal human Abs, the relative concentration of Ab that reacts with E2 is considerably lower than the HIV-neutralizing IC50 concentration.

To determine whether the differences were related to the neutralization system used or to the viruses studied, human anti-E2 IgG and the three murine anti–GBV-C E2 mAbs were retested at Duke University (Durham, NC) in PBMC-based assays using HIV-1 isolates 1 and 2 (Table I). Two of the three human GBV-C E2 Ab-positive IgG preparations neutralized the R5 and ×4 isolate at <20 μg/ml (I-P2 and I-P3), and the three murine E2 mAbs neutralized HIV-1 in PBMC assays (Table IV). The TriMAb control (2G12, 4E10, and 2F5) did not neutralize the HIV-1 R5 isolate (Table I, isolate 1) in TZM-bl cells but did in PBMCs. Of the anti–GBV-C E2 mAbs, only M6 did not neutralize well in PBMCs, with a very high IC50 value for the isolate 1 (17 μg/ml) and no activity against the ×4 isolate number 2.

GBV-C E2 Abs precipitate HIV-1 particles

As noted above, naturally occurring human GBV-C E2 Abs are conformation dependent (26), although the rabbit anti-E2 Abs recognized denatured E2. The human GBV-C E2 Abs and the rabbit anti-E2 Abs did not react with denatured HIV-1 proteins (Refs. 52–54 and data not shown). In contrast, the rabbit anti-E2 IgG preparations precipitated infectious HIV-1 particles prepared in HOS cell lines (Fig. 5A), suggesting that the interaction is conformation dependent. Immune precipitation was inhibited by CHO cell culture supernatant fluid containing rE2 protein in a dose-dependent manner but not CHO supernatant without E2 (Fig. 5A).

Rabbit anti–GBV-C E2 IgG precipitated retrovirus particles displaying HIVenv, GBV-Cenv, or VSV envelope glycoprotein significantly more than preimmune IgG or no Ab control (Fig. 5B). In addition, defective HIV particles with no viral envelope protein were precipitated by the rabbit anti–GBV-C E2 Abs (Fig. 5B), indicating that precipitation did not involve the viral envelope proteins. GBV-C–enveloped HIV pseudoparticles (GBV-Cpp) were precipitated to a significantly greater extent than the other defective particles by anti-E2 Ab, suggesting that, in addition to the gag particle Ag recognized by these Abs, interactions with the GBV-C E2 protein contributed to the immune precipitation (Fig. 5B). The murine mAb M6 and the human anti-gp120 (2G12) mAb precipitated HIV-1–pseudotyped gag particles (HIVpp) more than the IgG2a and human polyclonal IgG control Abs (data not shown). However, the percentage of input of defective HIVpp and GBV-Cpp precipitated by all of these mAbs was very low (<2% of input virus), suggesting that their reactivity with gag particles is significantly weaker than the rabbit polyclonal anti–GBV-C E2 Ab.

The rabbit GBV-C E2 Abs did not precipitate or neutralize YFV or mumps virus prepared in MT-2 cells (Fig. 5C). Like HIV-1, the mumps virus has a class I envelope glycoprotein. YFV prepared in Vero cells, and mumps prepared in BHK cells were also not neutralized or precipitated by the E2 Abs (data not shown). Taken together, the data suggest that the anti-E2 Ab interactions are not dependent on HIV-1 or other viral envelope glycoproteins and that the antigenic site is not exposed on flavivirus or orthomyxovirus particles.

**FIGURE 6.** Anti–GBV-C E2 Abs do not inhibit HIV-1 by interacting with cell plasma membrane but interfere with HIV-1 cellular attachment. HIV replication was not altered when MT-2 cells were preincubated with no Ab (NA), pre- (Pre), or post-E2 (Post) rabbit IgG (10 μg/ml) (A). Incubation of HIV-1 particles with rabbit postimmune (anti–GBV-C E2 positive) IgG inhibited HIV-1 particle attachment to MT-2 cells compared with no Ab or preimmune IgG controls in a dose-dependent manner (B; see Materials and Methods) (+p < 0.01 compared with preimmune IgG; †p < 0.05). Surface staining of intact MT-2 cells and PBMCs by the murine anti-E2 M6 mAb is shown in C and E, respectively. M6 reactivity increased with both MT-2 cells and PBMCs following fixation and permeabilization (D and F, respectively). IC, IgG2a isotype control Ab.
**Anti-GBV-C E2 Abs interfere with HIV-1 particle binding**

To determine whether GBV-C E2 Abs neutralized HIV-1 by binding to cell plasma membrane Ags and blocking either HIV-1 attachment or entry, MT-2 cells were incubated with no Ab, E2-negative rabbit IgG (preimmune; 10 μg/ml), or anti-GBV-C E2 rabbit IgG for 2 h at 4°C (postimmune; 10 μg/ml). Cells were washed, and HIV-1 was added prior to warming the cells to 37°C. HIV replication was monitored. Preincubation of cells with rabbit GBV-C E2 Ab did not inhibit HIV-1 replication compared with controls (Fig. 6A). Thus, if the Ag interacting with GBV-C E2 Abs is of cellular origin, it is either not exposed on the surface of MT-2 cells, or it is expressed in a different confirmation.

To determine the step of HIV replication that is inhibited by GBV-C E2 Abs, HIV-1 was mixed with rabbit E2 Abs for 1 h prior to addition to PBMCs for 2 h at 4°C. Cells were washed to remove nonspecifically bound HIV-1 particles, and the amount of HIV-1 bound to the cell was measured by p24 Ag ELISA. Rabbit anti-GBV-C E2 Ab blocked HIV-1 binding in a dose-dependent manner, whereas preimmune IgG did not (Fig. 6B). The murine mAb 6 did not interact with intact MT-2 cells or PBMCs as measured by flow cytometry (Fig. 6C, 6E). However, following fixation and permeabilization, M6 reacted with MT-2 cells and PBMCs (Fig. 6D, 6F). Similarly, M6 did not react with intact CHO cells but did follow fixation and permeabilization (data not shown). These data indicate that the E2 Abs react with cellular Ags that are not highly exposed on the cell surface.

To determine whether the anti-GBV-C E2 Abs inhibited HIV entry in addition to inhibiting HIV attachment, HIV-1 was incubated with cells for 2 h at 4°C to allow attachment. Following attachment, preimmune rabbit IgG, rabbit anti-E2, or rabbit anti-E2 Fab fragments were added to the cells at 4°C and cells were warmed to 37°C. One hour later, the medium was replaced with medium with or without the rabbit IgG or Fab fragments. In cells maintained in anti-E2 IgG or Fab fragments prepared from the polyclonal anti-E2 IgG preparation, HIV-1 replication was inhibited in a dose-dependent manner (Fig. 7A). HIV inhibition was not related to steric interactions between the Fc portion of the anti-E2 IgG. Similar to what was observed when anti-E2 IgG was removed from the media (Fig. 4B), inhibition was lost when anti-E2 Fab fragments were removed from the media. These data cannot distinguish between inhibition of entry during initial infection or subsequent inhibition of HIV-1 cell-to-cell spread.

To assess this in a single cycle of infection, HIV-1 envelope pseudotyped gag particles generated in 293T cells were added to HOS cells expressing human CD4, CXCR4, and CCR5. Pre- or postimmune (anti-E2) rabbit IgG was added to the cells following viral attachment for 2 h at 4°C, and the cells were warmed to 37°C to allow viral entry. Luciferase activity was measured 72 h later, and HIV-1 transduction was not inhibited (Fig. 7B). Thus, once HIV particles bound to CD4, access to the cross-reacting Ag was blocked, and the Abs were not able to block subsequent entry steps.

**Discussion**

Naturally occurring GBV-C E2 Abs from HIV-negative blood donors and experimentally induced GBV-C E2 Abs neutralized HIV-1 infection in vitro. The Abs inhibited HIV-1 isolates obtained from patients from geographically diverse regions and representing three separate HIV-1 clades. Neutralization was independent of entry coreceptor usage (CCR5 or CXCR4), and similar to other characterized Abs, neutralization differed when neutralization assays used different cellular substrates. VSV particles displaying HIV-1 envelope glycoproteins, YFVs, and mumps viruses were not neutralized by the anti-GBV-C E2 Abs. Anti-GBV-C E2 Abs also precipitated infectious HIV-1 particles and defective HIV-1 particles but not YFV or mumps viruses. Immune precipitation was independent of the viral envelope, because retrovirus particles with HIV-1, VSV, GBV-C, or no envelope protein were precipitated.

Anti-GBV-C E2 Abs interacted with cellular Ag(s) following cell permeabilization, suggesting that anti-E2 Abs cross-react with an Ag(s) that is not exposed on the cell surface. Together with the neutralization and immune precipitation data, these data indicate that the anti-E2 Abs neutralize and precipitate HIV-1 particle interactions with a cellular Ag that is present or enriched on HIV-1 gag particles. The Abs also react with a variety of phosphatidylinositols and lipids (10). Studies are under way to further characterize the cellular Ag recognized by the anti-GBV-C E2 Abs (J.H. McLinden, J. Xiang, E.L. Mohr, T.M. Kaufman, Q. Chang, and J.T. Stapleton, manuscript in preparation).

HIV-1 neutralization required incubation of anti-E2 Abs with the viral particles before interaction with the cell surface, because the Abs did not bind to cells, and they did not block entry following viral attachment in single cycle infections. Preincubation of the E2 Abs with HIV-1 particles reduced virus-cell binding; thus, HIV-1 replication is inhibited at least in part by decreasing HIV-1 attachment (Fig. 6B). Of note, the GBV-C E2 peptide recognized by the M6 mAb did not elicit HIV-1-neutralizing Abs nor neutralize HIV-1 infectivity, whereas the rE2 protein did, illustrating that the peptide is insufficient to induce neutralizing HIV-1 Abs. Previous studies found that immunization with the MPER peptide sequence or T-20 is not immunogenic unless the peptide is presented as part of a structural particle (e.g., viral particles or liposomes) (9, 11, 55, 56).

![FIGURE 7. GBV-C Abs do not block HIV-1 entry following HIV-1 attachment to cells. Following attachment of HIV particles to MT-2 cells for 2 h at 4°C, rabbit anti-GBV-C E2 Ab or Fab fragments were added to cells for 1 h. Following washing, Abs were included in the media (maintain) or not (remove). Cells were warmed to 37°C, and HIV-1 p24 Ag release was measured 3 d later (A). HIV envelope pseudotyped defective HIV-1 particles were similarly analyzed in HOS cells. Following attachment at 4°C, pre- or postimmune rabbit GBV-C E2 IgG was added at the concentrations indicated (B). Cells were warmed to 37°C, and transduction (luciferase activity) was measured 72 h later and compared to the virus only control (B).](http://www.jimmunol.org/)
GBV-C E2 protein inhibits HIV-1 entry in vitro through an unknown mechanism (31, 32). Thus, either viremia with GBV-C or the presence of E2 Ab may influence HIV-1 replication. Viremia appears to have the greatest impact on survival in HIV-infected people (20), perhaps because of a dose effect. GBV-C concentration in infected humans is typically >50 million genome equivalents/ml plasma (57), and GBV-C is produced by B lymphocytes and both CD4+ and CD8+ T lymphocytes (15). Because each virus particle is predicted to have multiple copies of E2 protein on the surface, lymphocytes are constantly exposed to low concentrations of E2 protein. In contrast, the concentration of GBV-C E2 Abs in human varies over time and may drop to levels below the limit of detection during longitudinal follow up (20, 53). It is likely that the E2 Ab concentration is an important variable in determining the magnitude of any potential clinical effect of GBV-C E2 Abs.

No significant amino acid sequence homology between GBV-C E2 and either HIV-1 or cellular proteins was identified in a protein-protein basic local alignment search tool search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Although several studies have identified HIV-1–neutralizing Abs in ~20% of humans with HIV-1 infection and a lower percentage in HIV-uninfected people (58–64), there are few data suggesting a beneficial clinical course in those with HIV-neutralizing Abs compared with those without. In contrast, a modest association between GBV-C E2 Ab and prolonged survival was observed by Tillmann et al. (20). There are clearly neutralizing Abs present in HIV-1–infected people that are not due to GBV-C E2 Abs; thus, measuring survival, on the basis of the presence of HIV-1–neutralizing Abs independent of the presence of GBV-C E2 Ab will confound the analysis. It will be interesting to determine what proportion of HIV-1–neutralizing Abs in HIV-infected individuals are directed against the GBV-C E2 protein, and studies to address this question are under way.

One concern related to the use of non–HIV-1 Ags to invoke HIV-1–neutralizing Abs is that these Ags may induce autoantibodies. Because ~10% of healthy U.S. blood donors have Ab to GBV-C E2 protein (52, 65, 66), it appears that GBV-C E2 Abs do not induce any ill effects in humans. Furthermore, the GBV-C E2 Ab appears to recognize cellular proteins carried on HIV-1 particles that are not displayed on the surface of cell plasma membranes.

Currently, no commercial GBV-C E2 mAbs or hybridoma cell lines producing GBV-C E2 mAbs are available (67). Consequently, the development of additional GBV-C E2 mAbs to allow further characterization of the Ag(s) that elicits the HIV-1–cross-reacting neutralizing Abs is needed. Characterization of the immunogenic domain(s) on the GBV-C E2 protein and its interactions with HIV particles may provide novel HIV-1 candidate vaccines.

Because a high proportion of HIV-1–infected people have GBV-C E2 Abs, it is unlikely that this Ab will, by itself, prove highly protective. Nevertheless, identification of immunogens that elicit HIV-1–neutralizing Abs against diverse isolates may contribute to protection induced by a multivalent HIV-1 vaccine, or they may delay or modify HIV-1 disease progression. Nevertheless, characterization of the E2 antigenic structure responsible for eliciting HIV-1–neutralizing activity may identify conserved targets for drug design. Our data suggest that testing of GBV-C E2 protein as a candidate HIV-1 vaccine Ag appears to be warranted.

Acknowledgments

We thank Dr. Beth Jamieson (University of California, Los Angeles) for providing NFN-HAS and NFN-SX-HSA HIV-1 isolates; Dr. Wendy Maury (University of Iowa) for providing defective VSV particles; and NARRRP, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, for the following HIV-1 isolates: HIV-19UG031 and HIV-19UG029 from the UNAIDS Network for HIV-1 Isolation and Characterization, AZT intermediate isolate from Dr. Douglas Richman, HIV19U-CIF from Dr. Irvin Chen, HTLV-IIIB/H9 from Dr. Robert Gallo, HIVxEru-JF from Dr. John Mellors and Chaufo Shu, and HIV 323 from Dr. Jean-Henry Thierry, Marie Bechet and Dr. Luc Montagnier. Catalog numbers are shown in Materials and Methods. We also thank Dr. Opendra Sharma (NARRRP) for providing the GBV-C synthetic peptides and Drs. George Hess and Alfred Engle (Roche Diagnostics and Laboratories) for providing GBV-C E2 ELISA kits and M6 mAb. We also thank Shawn Rouach (University of Iowa) for assistance with the figures.

Disclosures

The authors have no financial conflicts of interest.

References
