Potent Cross-Group Neutralization of Primary Human Immunodeficiency Virus Isolates with Monoclonal Antibodies—Implications for Acquired Immunodeficiency Syndrome Vaccine

Flavia Ferrantelli,1,2 Moiz Kitabwalla,1,2 Robert A. Rasmussen,1,2 Chuanhai Cao,6 Ting-Chao Chou,7 Hermann Katinger,7 Gabriela Stiegler,7 Lisa A. Cavacini,2,3 Yun Bai,4 Joseph Cotropia,1 Kenneth E. Ugen,4 and Ruth M. Ruprecht1,2

1Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, 2Department of Medicine, Harvard Medical School, and 3Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts; 4Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa; 5Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, New York; 6BioClonetics, Philadelphia, Pennsylvania; 7Institute of Applied Life Sciences, University of Agriculture, and Polymun Scientific Immunobiological Forschung GmbH, Vienna, Austria

Human immunodeficiency virus type 1 (HIV-1) is phylogenetically classified into groups and clades (or subtypes). Human neutralizing monoclonal antibodies (nMAbs), originally isolated from individuals infected with HIV-1 group M–clade B, neutralized not only primary HIV-1 clade B isolates in vitro but also primary isolates of other group M clades (A, C, D, E, and F). This corrected the previously held notion that primary HIV-1 isolates are resistant to neutralizing antibodies. Here we show that anti–HIV-1 group M–clade B nMAbs potently neutralized primary isolates of the phylogenetically distant HIV-1 group O. We and others have previously shown that passive immunization with human nMAbs protected adult or neonatal primates against infection with simian-human immunodeficiency virus strains encoding HIV-1 group M–clade B envelope genes. The in vitro cross-group neutralization shown here underscores the broad potential of these nMAbs against divergent virus variants and the relevance of their epitopes in the design of acquired immunodeficiency syndrome vaccines.

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Human immunodeficiency virus type 1 (HIV-1) is highly variable and has been classified into 3 groups (M for “major,” N for “new,” and O for “outlier”). Group M is subdivided into at least 8 subtypes, or clades (A–H), each comprising strains isolated from different geographic sites worldwide. Phylogenetic variability is extended further by intragroup and intraclade variability and, within the same infected individual, by the emergence of viral quasi species with time and interclade recombinants with superinfection. Consequently, effective prevention or treatment of HIV-1 infection should be broadly protective against highly divergent viral variants. Here we show that human neutralizing monoclonal antibodies (nMAbs), originally isolated from HIV-1 clade B (group M)–infected individuals, potently neutralized several primary isolates of the phylogenetically distant group O in vitro.

Materials and methods. The nMAbs used—lgG1b12 (b12), F105, 2G12, 2F5, 4E10, F424, and Clone 3 (CL3)—recognize conserved epitopes on the HIV-1 envelope (Env) glycoproteins. b12 and F105 are specific for the CD4 binding site on the HIV-1 gp120 subunit [1, 2]. F424 recognizes an unknown epitope that is also on gp120. nMAbs 2F5, 4E10, and CL3 bind Env-gp41 linear epitopes ELDKWA, NWFDIT, and GCSGKLICTT, respectively [3–8]. 2F5, 2G12, and 4E10 were provided by Polymer Scientific (Vienna, Austria); b12 was produced in engineered CHO cells, as described elsewhere [9]. F105 and F424 were purified from tissue culture supernatant by protein G chromatography. CL3 was provided by BioClonetics (Philadelphia). Primary HIV-1 group O isolates BCF02, BCF06, BCF07, BCF11, BCF13, and MVP-5180, which were randomly chosen for inclusion in the present study from all group O isolates available, were obtained from the National Institute of Health AIDS Reagent and Reference Program (Rockville, MD) [10–12].

Neutralization assays were performed in triplicate in human peripheral blood mononuclear cells, as described elsewhere [13]. Results obtained with HIV-1 strain MVP-5180 were also confirmed in an MT-2 cell–based assay, as reported elsewhere [14]. In all of our neutralization assays, nMAbs were not washed away but rather were diluted 1:1 with fresh medium daily, starting on day 3 after the beginning of the experiment. Because of this distinction within our neutralization assays, neutralization titers may differ slightly from titers measured by other neutralization assays, as discussed by Wei et al. [15]. Antibody-mediated neutralization is expressed as percentage of neutralization of virus infectivity; antibody concentrations during the preincubation of nMAbs with virus are provided and, when
Figure 1. Neutralization of primary human immunodeficiency virus group O isolates by human neutralizing monoclonal antibodies (nMAbs) in human peripheral blood mononuclear cell cultures. Results of 1 of 2–5 independent experiments are shown. A, Neutralization of BCF02, BCF11, BCF13, and MVP-5180, with human nMAbs used as single agents and human IgG1 as a negative control. B, Neutralization of BCF02, BCF06, BCF07, BCF11, and BCF13, with the quadruple combination of IgG1b12 (b12), 2G12, 2F5, and 4E10, at a ratio of 1:1:1:1. The effective nMAb concentrations giving 50% or 90% neutralization (EC50 or EC90, respectively) for each combination, considered as a single neutralizing agent, are shown in table 1. C, Neutralization of isolates BCF02, BCF11, and BCF13, with 6 different nMAb combinations (each containing nMAbs at a ratio of 1:1) and IgG1 negative control. CL3, Clone 3.
referring to antibody combinations, indicate the sum of single nMAb concentrations.

**Results.** When used as single agents, nMAbs 4E10, 2F5, CL3, and b12 were the most potent antibodies against the HIV-1 group O isolates tested; 4E10 effectively neutralized all 3 virus strains tested, even at low concentrations (figure 1A and data not shown). The nMAb combinations evaluated here (including the quadruple combination of b12, 2F5, 2G12, and 4E10, which was previously found to protect neonatal macaques against oral challenge with a pathogenic simian-human immunodeficiency virus [SHIV] [16]), neutralized all 6 isolates tested by 62%–97%, at a total nMAb concentration of 40 μg/mL (figure 1B and 1C and data not shown).

**Discussion.** We and others have previously shown that anti–HIV-1 Env nMAbs elicited against HIV-1 clade B neutralized not only various primary HIV-1 clade B isolates in vitro but also primary isolates of clades A, C, D, E, and F [5, 6, 13, 17–19], thus invalidating the formerly held belief that primary HIV-1 isolates are neutralization resistant. Here we have shown that the potent reactivity of these nMAbs extends beyond clades and is, in fact, cross-group.

Interestingly, within the 6 group O strains evaluated here, the linear epitope recognized by nMAb 2F5 is represented as ELDEWA, with a single amino acid substitution with respect to the epitope mapped on HIV-1 clade B gp41 (table 1). nMAb CL3 is specific for a linear epitope containing 2 cysteines that form an intrachain disulfide bridge. The latter generates a loop that could be important for the folding of gp41 during the fusion of the virus with the target cell [21]. This epitope is represented as GCXGXXXCXT in the 6 HIV-1 group O isolates analyzed here (table 1); the 2 cysteines generating the loop and 3 other amino acids are conserved, thus indicating that this epitope is shared by HIV-1 isolates of different groups as well. The linear epitope of 4E10 is 100% conserved in 3 of the 6 group O strains and is present with a single/double amino acid substitution in the others (table 1). Therefore, despite HIV-1 variability, Env neutralization–sensitive epitopes that are shared among HIV-1 groups do exist.

This finding stresses the potential of human nMAbs to neutralize widely different viral variants, a finding that is relevant for passive, as well as active, immunization against HIV-1. In primate models, passive immunization with human nMAbs prevented infection in most animals given mucosal challenge with SHIV strains encoding HIV-1 group M–clade B envelope genes [16, 22] (reviewed in [23]). Moreover, nMAbs F105, 2G12, 2F5, and 4E10 were safe in phase 1 clinical trials and had long half-lives (H. Katinger, unpublished data, and reviewed in [23]). Together, these results suggest that passive immunization with human nMAb combinations may be a powerful tool against transmission of HIV-1 strains that are genetically divergent.

Our finding of intergroup and interclade neutralization by human nMAb combinations, given their success in passive immunization of primates, is also important for the design of AIDS vaccines. Since these nMAbs recognize broadly conserved epitopes, the latter are ideal targets for AIDS vaccine–induced humoral immunity. AIDS vaccines should be constructed, perhaps through epitope mimetics, to preferentially induce neutralizing antibody responses against these and similarly conserved Env epitopes. Since viral escape mutants can overcome the suppression of viremia by virus-specific cytotoxic T lymphocytes (CTLs) [24], the presence of broadly reactive neutralizing antibodies could minimize the possibility of vaccine failure as a result of CTL escape mutants and/or superinfection [25].

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References


Table 1. Human neutralizing monoclonal antibody (nMAb) linear epitopes in the human immunodeficiency virus group O isolates tested.

<table>
<thead>
<tr>
<th>Epitope</th>
<th>4E10</th>
<th>2F5</th>
<th>CL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapped (group M–clade B)</td>
<td>NWFDIT</td>
<td>ELDKWA</td>
<td>GCGKLICCT</td>
</tr>
<tr>
<td>Group O isolate</td>
<td>NWFDIT</td>
<td>ELDEWA</td>
<td>GCKGRVICYT</td>
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<tr>
<td>BCF02</td>
<td>NWFDIT</td>
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<td>NWLGT</td>
<td>ELDEWA</td>
<td>GCKRLTCYT</td>
</tr>
<tr>
<td>BCF13</td>
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<td>ELDEWA</td>
<td>GCKGLICYT</td>
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<tr>
<td>MVP-5180</td>
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<td>ELDEWA</td>
<td>GCGKLICCT</td>
</tr>
</tbody>
</table>

**NOTE.** Conserved amino acids are underlined.