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Lipid binding properties of 4E10, 2F5, and WR304 monoclonal antibodies that neutralize HIV-1

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ABSTRACT

Two human mAbs (2F5 and 4E10), originally derived from HIV-1-infected patients, are important, but rare, mAbs that exhibit broad cross-clade neutralizing activities against HIV-1. In addition to peptide sequences on the gp41 envelope protein, both antibodies reportedly also bound specifically to several phospholipid antigens. However, the phospholipid binding property of 2F5 has been disputed and, because of uncertainly regarding phospholipid binding, the modeling of neutralizing mechanisms has been difficult. To explore this issue, we examined the binding of 4E10 and 2F5 to a broad range of lipid antigens by ELISA. 4E10 and 2F5 both bound to a variety of purified phospholipids, and 4E10 bound, but 2F5 did not bind, to cardiolipin. Both mAbs also bound to a sulfated glycolipid, sulfogalactosyl ceramide (sulfatide), and to two neutral glycolipids, galactosyl ceramide and glucosyl ceramide, but not to other galactosyl glycolipids. 4E10, but not 2F5, also bound to cholesterol, although both mAbs bound to squalene. Interestingly, 4E10, but not 2F5, exhibited striking binding to lipid A, the lipid moiety of Gram-negative bacterial lipopolysaccharide. The binding properties of 4E10 to phospholipids, sulfatide, cholesterol, squalene, and lipid A were similar to those of a neutralizing murine mAb (WR304) induced by liposomes containing phosphatidylinositol phosphate and lipid A, although WR304 did not bind to neutral glycolipids. The discovery of a binding specificity of 4E10 for lipid A, a widely used vaccine adjuvant, suggests that innate immunity stimulated by lipid A could have played a role for induction of multispecific antibodies that simultaneously recognize both HIV-1 protein and lipid antigens.

1. Introduction

The quest for an effective vaccine to HIV-1 is at a crucial, and perhaps historic, juncture in which analysis of available immunological models requires careful attention, particularly as they relate to induction of neutralizing antibodies [1]. A small number of human mAbs that broadly neutralize primary isolates of HIV-1 have served as important models of antibodies that have the types of binding properties that might be useful and effective in a prophylactic vaccine to HIV-1 [2]. 4E10, 2F5, and 2G12 human mAbs have been widely studied both because of their broad cross-clade neutralizing properties, and because their binding epitopes are relatively conserved, comprising either an oligomannose sequence on gp120 (2G12), or two different peptide sequences in the membrane proximal external region (MPER) of gp41 (4E10 and 2F5) (Fig. 1). A possible role of lipids as part of the antigen binding paratopes of the 4E10 and 2F5 mAbs was raised by reports that the mAbs not only bind to MPER of gp41, but also bind to phospholipids, particularly to 1,3-di(3-*sn*-phosphatidyl)-*sn*-glycerol (cardiolipin) (CL), and also to other phospholipids [3–5]. Despite this, the binding of 2F5 to phospholipids, including CL, has been a matter of considerable recent controversy [6]. Two laboratories failed to confirm any detectable binding to CL [7,8], although some slight binding to phosphatidylserine was observed at a high antigen concentration in one laboratory [8]. The lipid polar head group binding properties of 4E10 and 2F5 are therefore still uncertain.

It is well-known that phospholipids, cholesterol (Chol), and glycolipids all play important roles in the structure of the HIV-1 envelope, and all of these lipids participate dynamically in the intracellular assembly of the virion, in the budding process of the virion from the host cell, and in the fusion or entry process of HIV-1 with target cells [9]. The importance of phospholipids as potentially useful antigens for inducing neutralizing antibodies to HIV-1 was further suggested by the observation that a murine mAb (WR304), that was obtained after immunization with liposomes containing 3-*sn*-phosphatidylinositol-4-phosphate (PIP) and lipid A as an adjuvant, neutralized infection of human peripheral blood mononuclear cells (PBMC) [10]. Because 4E10 and 2F5 are both broadly neutralizing for HIV-1, identification of the exact lipid binding properties of these mAbs may provide insights for design of combined protein (or peptide) and lipid epitopes that could be included in a candidate prophylactic vaccine to HIV-1.

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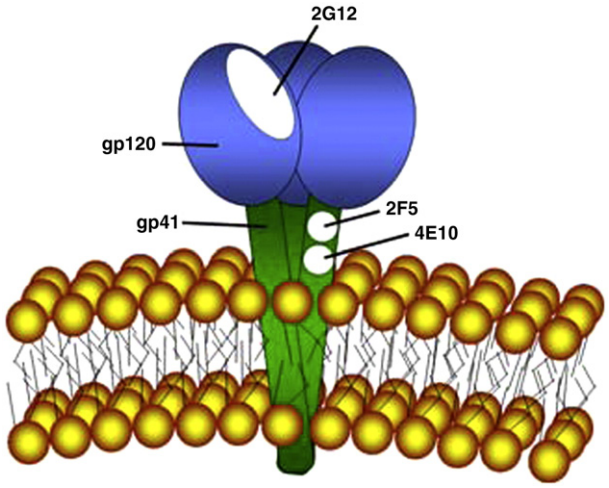

Fig. 1. Schematic illustration of binding sites of 2F5, 4E10 to peptide sequences on gp41, and of 2G12 to oligomannose on gp120 of HIV-1 envelope gp160.

Because of the importance of the polar head group region of the lipid bilayer of HIV-1, and of the lipid composition of the target or host cell lipid bilayer membranes that reflect the viral lipid composition, we have examined the binding of 4E10, 2F5, and 2G12 to purified phospholipids, and to other types of purified lipids, by ELISA. We confirm the binding of both 4E10 and 2F5 to a variety of purified phospholipids. However, we have discovered unexpected binding of 4E10 and 2F5 to several purified glycolipids, and also binding of 4E10 but not 2F5 to purified cholesterol. In addition, 4E10 but not 2F5 bound strongly to purified lipid A derived from Gram-negative bacterial lipopolysaccharide (LPS).

2. Materials and methods

2.1. Lipids, monoclonal antibodies, and ELISA materials

1,2 dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG); 3-*sn*-phosphatidylinositol (PI) (soybean); 1,2 dimyristoyl-*sn*-glycero-3-phosphate (DMPA); CL; 1,2 dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE); 1,2 dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS); PIP; 3-*sn*-phosphatidylinositol-4,5-phosphate (PIP2); (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-phosphocholine (sphingomyelin) (SM) (porcine brain); 1,2 dimyristoyl-*sn*-glycero-3-phosphate (DMPC); galactosyl ceramide (GalCer); lipid A; ceramide (Cer); and Chol were purchased from Avanti Polar Lipids. 3-*sn*-phosphatidylcholine purified from egg (Egg PC); Neu5Acα3-Galβ4GlcCer (GM3); sulfo3GalCer (sulfatide); squalene (SQE), gelatin; and BSA were purchased from Sigma Chemical Co. Glucosyl ceramide (GluCer); Galβ4GlcCer (LacCer); and Galα4Galβ4GlcCer (Gb3) were purchased from Matreya, LLC. Galβ3GalNAcβ4 (Neu5Acα3)Galβ4GlcCer (GM1) was purchased from Sialomed, Inc. 4E10, 2F5, and 2G12 mAbs were purchased from Polymun Scientific Immunbiologische Forschung GmbH. WR304 (formerly known as PIP4) was purified from ascites fluid as described [10]. Immulon 2HB "U" bottom ELISA plates were purchased from Thermolab Systems. Peroxidase-linked goat anti-mouse IgM (μ-chain specific) was purchased from Southern Biotech. Peroxidase-linked sheep anti-human IgG (γ-chain specific) was purchased from The Binding Site. 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate (ABTS) peroxidase substrate system was purchased from KPL, Inc.

2.2. Detection of binding of mAbs to lipids by ELISA

Chloroform, chloroform:methanol or methanol stock solutions of CL, DMPS, DMPA, sulfatide, GalCer, LacCer, PI, PIP, PIP2, GM1, or Gb3 were diluted in methanol, and lipid A, Chol, DMPC, Egg PC, DMPE, DMPG, GluCer, Cer, GM3, and SM were diluted in ethanol, to 10 nmol/ml, and 1 nmol/well was used as the antigen. Control wells contained 0.1 ml of the appropriate solvent to match the lipid plated. After evaporation overnight, the plates were blocked with 20 mM Tris–HCl, 154 mM sodium chloride, pH 7.4 (Tris-buffered saline) (TBS)-0.3% gelatin (except, where indicated with TBS-3% BSA) for 2 h. The ELISA was performed as described [11]. In brief, mAbs were diluted to 2 μg/ml in blocker and 50 μl/well were plated in 2-fold serial dilutions. Following incubation for 2 h at room temperature, the plates were washed with TBS, and 0.1 ml of peroxidase-linked secondary antibodies (diluted 1:1000) was added. Following a 1 h incubation and washing with TBS, 0.1 ml/well of ABTS substrate was added. After 1 h plates were read at A_{405}. The ELISA for SQE was adapted from the previously described method [12]. Sterile round bottom tissue culture plates (Corning) were coated with 5 nmol/well of SQE diluted in isopropanol. TBS-0.3% gelatin was used as the blocker and diluent and the ELISA was conducted as described above. It should be noted that preparation of TBS-0.3% gelatin was rigorously controlled in order to prevent large fluctuations between experiments in the background A_{405} of methanol- or ethanol-treated wells. TBS-0.3% gelatin was heated to 65 °C in a water bath and the gelatin was dissolved by swirling the flask. The solution was cooled to 37 °C in another water bath and then filtered through a 0.2 μm PES filter. It was stored at 4 °C and was used for up to 3 days.

3. Results

3.1. Binding of mAbs to phospholipids

Eleven different purified phospholipids were used as antigens for examination by ELISA of the qualitative binding characteristics of 4E10, 2F5, 2G12, and WR304 (Fig. 2). Among the human mAbs, 4E10 bound to nine of the tested phospholipids, 2F5 bound to three, and 2G12 bound to none of the phospholipids. Under the conditions employed, the strongest binding of 4E10 was observed with four anionic phospholipids: DMPG, followed by PI, DMPA, and CL. Binding of 4E10 also occurred with three other anionic phospholipids DMPS, PIP, and PIP_2. Interestingly, DMPE and egg PC, both neutral phospholipids, were also strongly bound by 4E10, but SM, a neutral ceramide phosphocholine-containing phospholipid, was not bound by 4E10.

In contrast to the results with egg PC, DMPC, a saturated PC, was not bound by 4E10 (Fig. 2). Thus, it appeared that the binding of 4E10, as determined by ELISA, to phospholipids containing phosphocholine headgroups can be strongly affected by the fatty acid composition of the lipid. It is possible that the physical structures of the phospholipid antigens adsorbed to the microtiter wells were in the form of a micelles or lipid bilayers, and the surface areas and packing characteristics of each would be strongly influenced by the fatty acyl composition of the lipid. Because of this, it should be noted that although the binding of the mAbs to different phospholipids, when it occurs, can be qualitatively determined by ELISA, the relative affinity of the antigen binding paratope of a given mAb for headgroups of different phospholipids cannot be quantitatively compared with different phospholipid antigens.

The 2F5 mAb bound unequivocally to DMPE, and to a lesser extent to egg PC and DMPS with gelatin as a blocker (Fig. 2). 2F5 also bound to PI and PIP, but not DMPE, when bovine serum albumin (BSA) was used as a blocker (data not shown). However, we did not observe binding of 2F5 to CL when either blocker was used. The previously reported binding of 2F5 to phospholipids [3,4] is therefore confirmed, but as also previously reported, the binding of 2F5 to phospholipids was weaker and sometimes technically difficult to observe [7,8]. We invariably saw binding of 2F5 to DMPE but, although binding to DMPS or egg PC was frequently observed,