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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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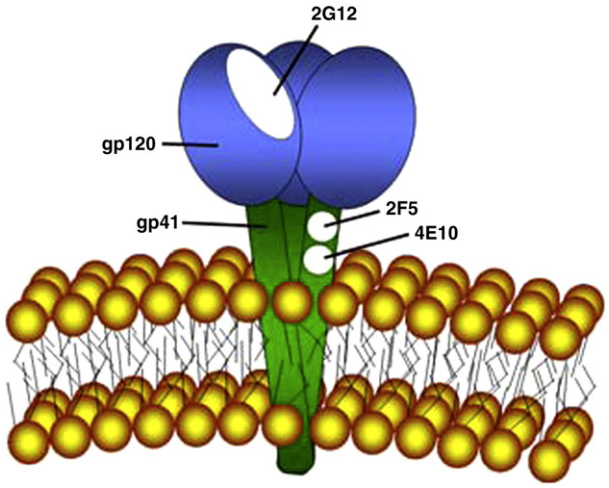

Fig. 1. Schematic illustration of binding sites of 2F5, 4E10 to peptide sequences on gp41, and of 2G12 to oligomannose on gp120 of HIV-1 envelope gp160.

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,

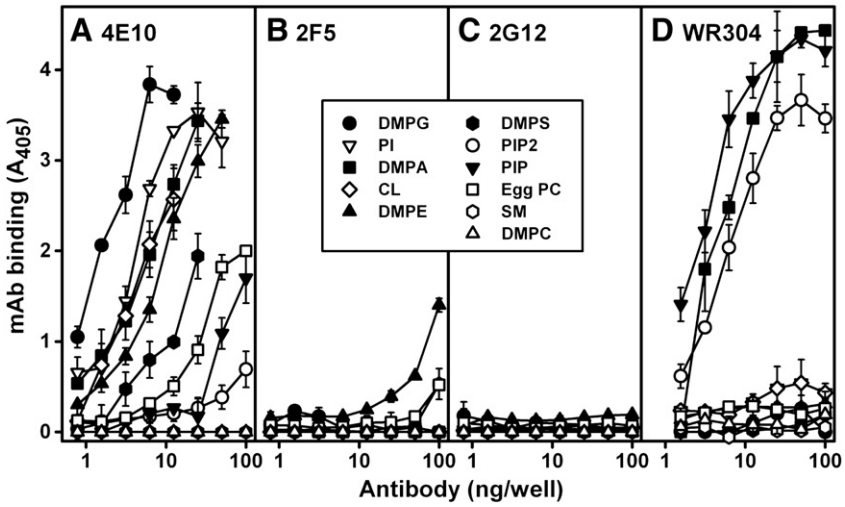

Fig. 2. Binding of mAbs to phospholipids. The wells were blocked with TBS-0.3% gelatin, except that TBS-3% BSA was used as the blocker and diluent for WR304 binding to egg PC, DMPC, DMPG and CL. Data are the mean of triplicate determinations (±SD) with background antibody binding to solvent treated wells lacking phospholipid subtracted. Data are representative of values obtained from 4 to 8 experiments. The data for binding of 2F5 to DMPS and Egg PC overlay each other in the figure. 2F5 binding to DMPS varied with 4 of 8 experiments yielding data similar to that shown and the other 4 indicating no binding of 2F5 to DMPS.

sometimes it was not found. We conclude that in the latter cases binding was probably obscured by unknown variable factors, and it is likely that the different biophysical states of phospholipid assemblies that were noted above played a large role in the quantitative binding characteristics of 2F5 to certain phospholipids.

WR304, a murine mAb, was originally obtained after immunization with liposomes containing PIP and lipid A as an adjuvant, and the clone was selected for specific binding to liposomes containing DMPC, Chol, and PIP, but an inability to react with the same liposomes lacking PIP [13]. In the present experiments WR304 bound to PIP and, as previously reported [14], it also cross-reacted with certain other phospholipids, including PIP2, DMPA, and CL, but not with DMPS (Fig. 2). Low level binding to CL (four times higher than background) also occurred, as previously reported [10].

3.2. Binding of mAbs to glycolipids

Among seven glycolipids tested, 4E10 and 2F5, both bound to GluCer, GalCer, and sulfatide, but did not bind to LacCer, Gb3, GM3, or GM1 (Fig. 3). WR304 also bound strongly to sulfatide.

3.3. Binding of mAbs to cholesterol and SQE

4E10 and WR304 both bound to Chol, but 2F5 and 2G12 did not exhibit such binding (Fig. 4A). The binding of both 4E10 and WR304 to Chol was unexpected, and this raises the possibility that binding of each of these mAbs to Chol might be important as a theoretical factor in the neutralization of HIV-1 by 4E10 and WR304. As mentioned above, although WR304 was obtained after immunization with liposomes containing PIP and Chol, the clone was selected for the inability to recognize liposomes containing Chol but lacking PIP. This is an example of a binding specificity of a mAb to purified Chol that is strongly influenced by interfering effects by the surrounding phospholipids in the lipid bilayer [15].

Binding of the mAbs to SQE, a triperpenoid alkene hydrocarbon lacking any polar groups, is shown in Fig. 4B. 4E10, 2F5, and WR304 all bound to SQE, but 2G12 did not bind. These results are compatible with the proposed hydrophobic interactions of 4E10, 2F5, and WR304 with membranes [4,11].

3.4. Binding of mAbs to lipid A

4E10 and WR304 both unequivocally bound to lipid A, but 2F5 and 2G12 did not exhibit such binding (Fig. 5).

3.5. Absence of binding of 2G12 to lipids

In contrast to 4E10 and 2F5, 2G12, another broadly neutralizing human IgG1 mAb, that recognizes an oligomannose epitope on gp120 of HIV-1, did not bind to any lipids in our studies. The absence of

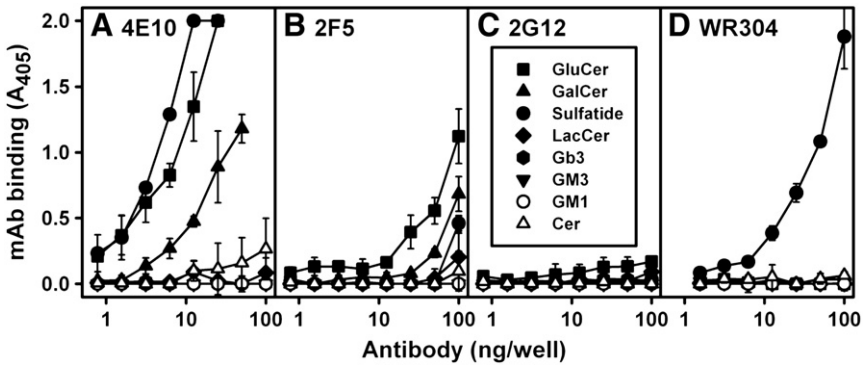

Fig. 3. Binding of mAbs to glycolipids. Data are the mean of triplicate determinations (±SD) with background antibody binding to solvent treated wells lacking glycolipid subtracted. Data are representative of values obtained from 4 to 8 experiments. A_{405} values >2.0 were assigned a value of 2.0 for graphing in order more effectively show lower levels of binding of glycolipids.

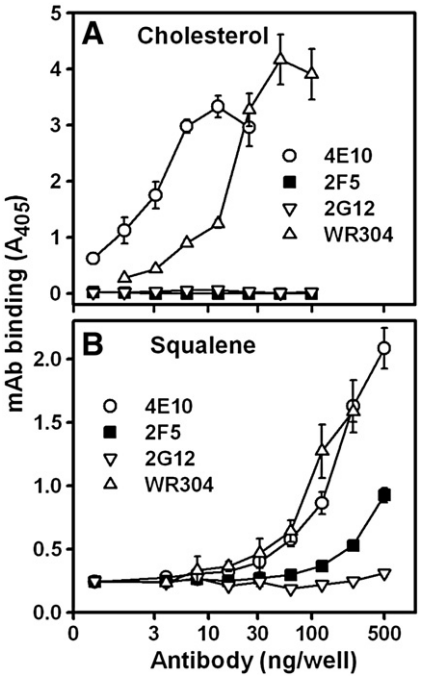

Fig. 4. Binding of mAbs to cholesterol and squalene. (A) Binding to cholesterol. Data are the mean of triplicate determinations (±SD) with background antibody binding to ethanol-treated wells lacking cholesterol subtracted. Data are representative of values obtained from 4 experiments. (B) Binding to squalene. Data are mean of triplicate determinations (±SD) with background binding to isopropanol-treated wells lacking squalene subtracted. Data are representative of values from 3 experiments.

binding of 2G12 to lipids is consistent with the location of gp120 epitope being at a considerable distance from the lipid bilayer of the HIV-1 virion [2] (Fig. 1). The lack of lipid binding by 2G12 also demonstrated that binding of 4E10 and 2F5 to lipids was not simply a function of nonspecific binding of IgG1 to lipids.

4. Discussion

In accordance with previous reports that 4E10 binds both to gp41 MPER epitope and to phospholipid, we have found strong and consistent binding of 4E10 with nine of the eleven anionic and neutral phospholipid antigens tested. However, as noted earlier (see Introduction) considerable controversy currently exists regarding phospholipid headgroup binding specificities, if any, of the 2F5 mAb [3–8]. The results of the present study strongly support the concept that the 2F5 mAb does exhibit distinctive binding to phospholipids. Among ten phospholipids tested, 2F5 consistently exhibited strong binding to DMPE. Weaker binding to egg PC and to DMPS was also generally observed (Fig. 2), but occasionally was not found in a given experiment. As noted below, with BSA as the blocker, 2F5 also bound to PI and PIP. In contrast to three reports [3–5] and in support of two others [7,8] we did not observe binding of 2F5 to either beef heart CL or synthetic CL (Suppl. Fig. 2).

In our experience, although specific binding of 2F5 to certain anionic and neutral phospholipids definitely occurs, it is also clear that binding by 2F5, as detected by ELISA, can be easily obscured by technical issues. For both 4E10 and 2F5, high background levels of absorbance to polystyrene microtiter wells sometimes occurred in the absence of antigen (Suppl. Fig. 1). Although the cause of the high background levels is unknown, whether due to specific recognition of polystyrene or to hydrophobic interactions, recognition of this phenomenon is important. We have also found that other technical issues, such as the type and particular lot of blocker used, and perhaps the orientation or self-assembly properties of the phospholipids adsorbed to microtiter wells (e.g., the arrangement of phospholipids in the form of multiple layers or monolayers, or lipid micelles, or vesicles) can sometimes reduce the resolution for consistent detection of binding of the 2F5 or 4E10 mAbs to lipids by ELISA. This might be partly due to a smaller or lower affinity lipid-binding region that may exist in the paratope of 2F5 when compared to the binding of the 2F5 paratope to its peptide epitope. It is also known that in the presence of water the type and charge of lipid headgroups, the number of fatty acyl groups, and the degree of fatty acyl unsaturation can all cause dramatic physical changes in the molecular volume and in the packing and orientation of the fatty acyl groups of phospholipids, all of which can result in different fluidity and orientation of the hydrated phospholipid molecules [16]. However, by immunizing with liposomes containing linear peptide epitopes that are present on gp41, murine mAbs have recently been produced that recognize unique lipid–peptide patterns [17]. In view of this it would not be necessarily surprising if different physical patterns presented by different types and physical states of lipid antigens could also represent important information for immunological recognition of a lipid pattern by lipid-binding antibodies such as 4E10, 2F5, or WR304.

It is clear that, just as is often found with antibodies to conformational states of proteins, the binding characteristics of antibodies to lipids in an ELISA cannot necessarily reproduce the exact conformational or pattern characteristics that may occur on the virion. Because of this, although the binding of a mAb to lipids by ELISA represents important qualitative or even semi-quantitative immunological information, the inability to control the particulate physical structures that may occur with different individual pure lipid antigens adsorbed to microtiter wells prevents rigorous differentiation of relative binding affinities of a given mAb to different lipids or mixtures of lipids and proteins as they may occur at the surface of the virion. In view of all of these issues, it is entirely reasonable that binding of a mAb to a phospholipid, such as 2F5 binding to CL, could exhibit different binding characteristics under different types of assays, such as by the use of fully hydrated lipids for inhibition of the mAb [4], or by surface plasmon resonance (SPR) analysis of anchored lipids or liposomes [18].

In extension of these studies, we have also discovered consistent and unequivocal binding of both 4E10 and 2F5 to other types of pure lipids, and in this respect we have made five novel observations. First, 4E10 and 2F5 mAbs both bound to two neutral glycolipids (GalCer and GluCer), but not to ceramide alone. Second, 4E10 exhibited strong binding to an anionic sulfated glycolipid, sulfatide, and 2F5 also consistently showed unequivocal, but low titer, binding to sulfatide. In both cases, the binding of the mAb to sulfatide might have occurred as

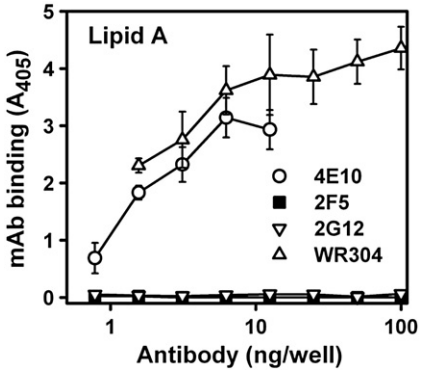

Fig. 5. Binding of mAbs to lipid A. Data are the mean of triplicate determinations (±SD) with background antibody binding to ethanol-treated wells lacking lipid A subtracted. Data are representative of values obtained from 13 experiments using 4E10, 2F5 and 2G12 and 3 experiments with WR304.

a cross-reaction of the phosphate-binding subsite in the paratope of the mAb with the sulfate of sulfatide [13]. Third, in addition to phospholipids and glycolipids, 4E10, but not 2F5, unexpectedly also bound with apparent very high affinity to Chol.

Fourth, 4E10 and 2F5 both bound to SQE, a hydrophobic alkene lacking any polar groups that serves as a precursor in the synthesis of Chol. Naturally-occurring antibodies to SQE have been observed in humans [12,19], and murine mAbs to SQE have been created [20]. It was previously hypothesized that the long hydrophobic Ig CDR3 groups of 4E10 and 2F5 might be responsible for interaction with the lipid bilayer [20,21], perhaps through a hydrophobic patch in the CDR3 groups [18]. In support of this concept, we have observed low level, but unequivocal, binding of 4E10, 2F5, and WR304 to SQE as an antigen. The binding of 4E10 and 2F5 to SQE is consistent with the hydrophobic patches present in the long CDR3 regions of those mAbs [4,21,22]. The absence of any detectable binding of 2G12 demonstrates that this was not due to nonspecific binding of human IgG1.

Fifth, and perhaps most interestingly, 4E10, but not 2F5 or 2G12, also exhibited strong binding to lipid A, a complex glycophospholipid derived from Gram-negative bacterial LPS [23]. Lipid A is the moiety that provides all of the adjuvant activity of LPS, and it is thought to serve as a major element of innate immunity [23]. Lipid A has also served as an adjuvant constituent for numerous candidate and registered vaccines, including a recently registered hepatitis B vaccine [24]. In view of the effectiveness of liposomes containing lipid A as a vaccine formulation [25], and in view of the ability of liposomes containing lipid A to model the properties of stabilized lipid rafts that could contain HIV-1 protein and lipid antigens [9], the intriguing possibility is raised that liposomes containing lipid A could induce antibodies that emulate the lipid binding properties and also the neutralizing activity of 4E10 or 2F5.

The results obtained with 4E10 and 2F5 were compared with a mAb (WR304) that was prepared after immunization of a mouse with liposomes containing PIP as a phospholipid antigen that was embedded in a membrane containing neutral and anionic bulk phospholipids, Chol, and lipid A as an adjuvant [13]. Although WR304 was selected for the ability to bind to liposomes containing both PIP and Chol but the inability to bind to the same liposomes lacking PIP, we have now found that WR304 binds to non-liposomal pure Chol and SQE by ELISA. Chol is a small antigen that is easily protected from binding to anti-Chol mAbs by steric hindrance from surrounding bulk phospholipids [15]. However, since 4E10 and WR304 both bind strikingly to PIP, Chol, SQE, and lipid A, and since both mAbs neutralize HIV-1 infection of peripheral blood mononuclear cells [10], the role of a lipid bilayer antigen containing lipid A as an inducing antigen for 4E10 seems theoretically possible. Recent studies have further extended this concept by demonstrating that immunization with liposomes containing a protein or peptide antigen that includes the 4E10 MPER epitope, and that also contain either phospholipid or glycolipid or Chol as an antigen, and lipid A as an adjuvant, can be used for immunization of mice to induce multi-specific mAbs that, like 4E10, simultaneously and specifically recognize both the MPER peptide and the associated lipid antigen in the lipid bilayer [17,26].

The question could be posed whether some of the broad neutralizing activities of 4E10 and 2F5 are dependent on the broad ranges of binding specificities that they exhibit toward different types of lipids. Based on studies of MPER peptide that was covalently conjugated with a linker to the outer surface of liposomes, it was proposed that binding of 4E10 or 2F5 to the MPER peptide proceeds through a two step (encounter-docking) conformational change reaction [18]. The concept of a two step reaction that involves simultaneous initial binding of 4E10 both to the peptide and to the adjacent lipid head group, followed by immersion of 4E10 into a lipid hydrophobic region was also proposed [11], and the present observation of binding of 4E10 to SQE is consistent with this latter hydrophobic docking step in the mechanism. Recently, a second possible mechanism to explain the two-stage binding properties of 4E10 was proposed that involves the MPER region itself. It was suggested that the MPER, because of its hydrophobic characteristics, is partially immersed in the hydrophobic region of the lipid bilayer, and that the initial binding of 4E10 to the MPER induces a conformation change in the gp41 protein antigen that results in the exposure of critical buried hydrophobic MPER amino acid epitopes that are required for neutralization by 4E10 [27]. However, this latter theory does not account for the rather dramatic polyreactivity of 4E10 and 2F5 across a range of different types of lipids, including phospholipids, glycolipids, Chol, and SQE. The theory of MPER conformation change [27] does not necessarily require binding of 4E10 to lipids, although it does not necessarily exclude such binding. It also does not explain the neutralizing ability of the murine anti-PIP mAb (WR304) that exhibits only a one-stage reversible binding mechanism to liposomal PIP [14]. If the broad neutralizing capabilities of 4E10 were related only to binding of the mAbs to the MPER peptide sequences, it should be possible to induce broadly neutralizing antibodies with pure protein or peptide antigens that contain the appropriate MPER sequences. However, despite much effort little success has been achieved by this approach [28].

Although the exact origins of 4E10 and 2F5, are unknown, they were both obtained from HIV-1-infected patients, and both bind specifically to well-defined peptide epitopes in the MPER of gp41 of HIV-1 [2]. In mammalian cells CL is normally found only on the inner membrane of mitochondria [29]. In contrast, CL, DMPG, DMPE, DMPS, and DMPA are all major constituents of the all of the membranes of Gram-negative bacterial cells, indeed of virtually all bacterial membranes [29], and lipid A is uniquely found only in Gram-negative bacterial cells where it covers the entire outer leaflet of the outer membrane of the cell [23,29]. The discovery of strong binding of 4E10 to lipid A, CL, DMPG, DMPE, DMPS, and DMPA, but the absence of binding of 4E10 to SM (which is not found in Gram-negative bacteria), therefore raises the intriguing possibility that an opportunistic Gram-negative infection of the HIV-1-infected patient from which 4E10 was derived might have played a role the origin of this unique antibody. It might be speculated that a Gram-negative bacterial cell could have served as a nonspecific carrier of HIV-1 during a Gram-negative bacterial infection, and the bacterial lipid A might have acted as an adjuvant for innate immunity to induce multi-specific antibodies that simultaneously recognized both the bacterial lipids and the HIV-1 envelope protein. This speculation would be consistent with the observation that murine mAbs having these same types of multispecific simultaneous binding properties to lipid and protein can be experimentally induced by liposomes containing both lipid A and HIV-1 protein or peptide antigen [17,26].

Acknowledgement and Disclaimer

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2008.11.015.

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