2010

Inhibition of HIV-1 infection of peripheral blood mononuclear cells by a monoclonal antibody that binds to phosphoinositides and induces secretion of β-chemokines

Gary R. Matyas
Walter Reed Army Institute of Research

Lindsay Wieczorek
US Military HIV Research Program

Divya Bensal
US Military HIV Research Program

Agnes-Laurence Chenine
US Military HIV Research Program

Eric Saunders-Buell
US Military HIV Research Program

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/usarmyresearch

Part of the Operations Research, Systems Engineering and Industrial Engineering Commons

Matyas, Gary R.; Wieczorek, Lindsay; Bensal, Divya; Chenine, Agnes-Laurence; Saunders-Buell, Eric; Tovanabutra, Sodsai; Kim, Jerome H.; Polonis, Victoria; and Alving, Carl R., "Inhibition of HIV-1 infection of peripheral blood mononuclear cells by a monoclonal antibody that binds to phosphoinositides and induces secretion of β-chemokines" (2010). US Army Research. 138.

https://digitalcommons.unl.edu/usarmyresearch/138

This Article is brought to you for free and open access by the U.S. Department of Defense at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in US Army Research by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Authors
Gary R. Matyas, Lindsay Wieczorek, Divya Bensal, Agnes-Laurence Chenine, Eric Saunders-Buell, Sodsai Tovanabutra, Jerome H. Kim, Victoria Polonis, and Carl R. Alving
Inhibition of HIV-1 infection of peripheral blood mononuclear cells by a monoclonal antibody that binds to phosphoinositides and induces secretion of β-chemokines

Gary R. Matyas a, Lindsay Wieczorek b, Divya Bansal b, Agnes-Laurence Chenine b, Eric Sanders-Buell b, Sodsai Tovanabutrab a, Jerome H. Kim a, Victoria Polonis a, Carl R. Alving a,⇑

a Division of Retrovirology, Walter Reed Army Institute of Research, USA
b Henry M. Jackson Foundation for the Advancement of Military Medicine, US Military HIV Research Program, Rockville, MD 20850, USA

ABSTRACT

A murine IgG mAb, WR321, selected for the ability to bind to phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate, but an inability to bind to any of 17 other lipids, including phosphatidylinositol, was examined as a probe for studying interactions of HIV-1 with primary human peripheral blood mononuclear cells. The WR321 mAb broadly neutralized CCR5-tropic strains of HIV-1 to prevent infection of the cells. The mAb also exhibited direct interaction with cells in the culture, resulting in secretion of chemokines that interfered with the interaction of HIV-1 virions with CCR5, the coreceptor for HIV-1 on the susceptible cells, leading to inhibition of infection by HIV-1. Phosphoinositides that are recognized by WR321 do not exist on the external surface of cells, but are concentrated on the inner surface (cytoplasmic leaflet) of the plasma membrane. Murine anti-phosphoinositide mAbs similar to WR321 have previously been directly microinjected into a variety of cultured cells, resulting in important changes in the functions of the cells. The present results suggest that binding of a mAb to phosphoinositides, resulting in secretion of β-chemokines into the culture medium and neutralization of infection by CCR5-tropic HIV-1 of nearby susceptible cells, occurred by uptake and binding of the mAb at an intracellular location in the cultured cells that then led to secretion of HIV-1-inhibitory β-chemokines.

Published by Elsevier Inc.
phosphoinositides are known to be important chemical constituents of HIV-1 virions [7]. This is because phosphoinositides are functionally active both during the intracellular assembly of virions in host cells and localization of gag to the plasma membrane whereby PIP2 serves as a bridge between the matrix protein and the envelope of HIV-1 [8–11]. It was thus concluded that although both 4E10 and WR304 could neutralize HIV-1 in the PBMC assay without binding to any HIV-1 proteins, it seemed likely that the mAbs could bind to one or more HIV-1 lipids, even if the exact lipid binding sites, whether phosphoinositides or other lipids, remained unclear because of very broad lipid binding specificities of the mAbs.

Recently, a novel alternative neutralizing mechanism for anti-lipid mAbs has been proposed that does not require any direct binding of the anti-phospholipid antibodies to HIV-1. A neutralizing human mAb, CL1, was described as having binding capabilities for both cardioliadin and phosphatidylinerine, but no binding to HIV-1 envelope protein, and it inhibited proliferation of CCR5-tropic, but not CXCR4-tropic HIV-1 strains in a PBMC assay [12]. In this case, release of several β-chemokines, including MIP-1α and MIP-1β, was observed in the PBMC culture fluid. β-Chemokines are believed to be effectors secreted by many cells, including CD4+ and CD8+ lymphocytes, NK cells, and monocytes in the PBMC that inhibit infection by binding to CCR5, thus blocking infection with HIV-1 strains that utilize CCR5, but not those that utilize CXCR4, as a co-receptor [13]. Based on binding of CL1 to cells in the PBMC culture, it was hypothesized that CL1 bound to plasma membrane phosphophospholipid(s) and caused secretion of β-chemokines that interfered with binding of HIV-1 to CCR5-dependent, but not to CXCR4-dependent, viruses [12]. In the present study, we explore further the lipid binding specificities of CL1, including binding to phosphoinositides, and we describe a new murine neutralizing IgG mAb (WR321) which binds only to phosphoinositides PIP and PIP2 among 19 lipids tested, but which also causes release of HIV-1-inhibitory β-chemokines by PBMC even in the absence of HIV-1.

2. Materials and methods

2.1. Lipids, liposomes, proteins, and peptides

Recombinant, truncated HIV-1 gp41 (HXB2) (Swiss-Prot Accession Number P04578) containing amino acids 541–682 with 6 His attached to the carboxy terminus and expressed Pichia pastoris, was purchased from The Biotech Source (Franklin, MA). Mper23, LELDKWASLWNWFDITNWLWYIK, was synthesized in-house using Fmoc chemistry and standard solid-phase techniques with free amino termini [14]. Lipids and lipid antigens were obtained as described before [5]. Multilamellar liposomes containing DMPC:Chol:PIP (1:1:5:1) were prepared at a concentration of 50 mM phospholipid containing lipid A and mper23 at 200 μg/ml [3,15].

2.2. Monoclonal antibodies

Female BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were immunized intraperitoneally 6 times with 0.1 ml of liposomes at 3 week intervals. Three weeks after the last immunization, a mouse was boosted by the intravenous route and the spleen was removed 4 days after boosting. The spleen cells were fused with SP2/0 cells obtained from the American Type Culture Collection (ATCC), Chantilly, VA, and hybridomas were cloned using ClonaCell®-HY Hybridoma Kit (Stemcell Technologies, Vancouver, BC, Canada). WR321 was grown in serum-free media (Invitrogen, Carlsbad, CA), and purified using a protein G kit. The isotype of WR321 was determined as IgG2b with a kappa light chain using an isotype kit (Roche, Diagnostics Corp., Indianapolis, IN). TFTB-1 cells, which secrete a mouse IgG to the ricin B chain, were purchased from ATCC, and the mAb was grown and purified as described above. The human IgG anti-phospholipid mAb CL1 was kindly supplied as a gift by Drs. Pojen Chen (UCLA) and Barton Haynes (Duke University). Each of the WR321 and CL1 mAbs had very low levels of endotoxin.

2.3. ELISA

Individual lipids as indicated, gp41, and mper23 peptide, were each used as capture antigens, and assays using the antigens were performed as described for mouse antibodies [3] and human antibodies [5]. Assay background was determined by using wells lacking antigen, and values less than twice the background are considered negative. Values greater than twice background are indicated by asterisks in Fig. 1. Cell culture supernatant chemokines, MIP-1α, MIP-1β, and Rantes, and interferon-γ were quantified with ELISA kits (R&D Systems, Minneapolis, MN).

2.4. HIV-1 neutralization assay

PBMC neutralization assays were performed as previously described with replication component, Renilla reniformis luciferase
(LucR)-expressing HIV-1 reporter virus NL-LucR [3,16]. The clade B viruses, Bal and SF162, in T2A-ENV.ectoviruses were kindly provided by Christiana Ochsenbauer and John Kappes, University of Alabama at Birmingham. Viral envelope sequences from CRF01_AE CM235 and Clade C 16–29 were also cloned into LucR. T2A and are designated 01-pCM235-2-LucR. T2A (CM235) and C-ETH2220-ILB-LucR (16–29), respectively [17].

3. Results

3.1. Binding specificities of WR321 and CL1 to lipids

Representative experiments showing the relative binding of WR321 and CL1 to 19 different lipids as determined by ELISA is shown in Fig. 1. CL1 bound to 7 of the 10 phospholipids (PIP, PIP2, PI, PA, PG, CL, and PS); to cholesterol; to a sulfoglycolipid (sulfatide), but not to any other glycolipid; and it bound to lipid A. In contrast, WR321 bound only to PIP and to a lesser degree to PIP2. Neither of the mAbs bound to gp41 from HIV-1 or the mper (LucR), demonstrating that the lipid binding specificity of CL1 is relatively broad and is similar to the relatively broad lipid binding specificities of the previously reported murine mAb WR304 [5]. In contrast, we conclude that phosphoinositides represent the dominant, and probably the sole lipid binding specificities, of WR321.

3.2. Neutralization of HIV-1 by WR321 and CL1 for prevention of infection of PBMC

The potent broad neutralization properties of CL1 reported by Moody et al. [12] were confirmed with four different HIV-1 viruses in four representative experiments with four different PBMC donors (Fig. 2A–D). Similarly, WR321 also exhibited neutralization with each virus, but the neutralizing titers were lower than those of CL1. The relative potencies of WR321 and CL1 for 50% neutralization (IC50) of each of the viruses derived from 2 to 4 experiments is shown in Table 1. The negative control murine IgG mAb, TFTB, failed to exhibit significant neutralization.

3.3. Chemokine secretion elicited from PBMC by WR321 and CL1

As shown in Fig. 3, with two different PBMC donors, WR321 and CL1 each caused considerable secretion of the β-chemokines MIP-1α and MIP-1β. As indicated, the secretion by PBMC induced by WR321 occurred either in the presence or absence of HIV-1. The secretion of both of these cytokines by PBMC in the presence of WR321, as shown in Fig. 3, suggests that the secretion of the β-chemokines was partly, or possibly even completely, due to binding of the both the CL1 and WR321 mAbs to phosphoinositides. The data thus further suggest that phosphoinositides on (or in) the PBMC may be readily available for binding of antibodies that are highly specific for phosphoinositides.

4. Discussion

The murine mAbs WR301 and WR304 were originally created after immunization with liposomes containing PIP and lipid A, and the mAbs were then selected for binding and complement-dependent damage to liposomes containing PIP but not to liposomes lacking PIP [18]. This general method has also been employed by others for producing and/or selecting mAbs to phosphoinositides [19,20]. However, it is now apparent that considerable unexpected cross-reactivity of antibodies produced by this method can occur with other lipids [5,21]. Despite this, the observations in the present work of the lack of cross-reactivity of WR321 with other lipids, or with peptide or protein, and previous observations with similar anti-phosphoinositide mAbs [22], demonstrate that exquisite specificities of murine mAbs for phosphoinositides can be achieved. The present work further demonstrates that this can occur even when the liposomes used for immunization also contain a peptide antigen in addition to the phosphoinositide.

Based on the narrow specific binding characteristics of WR321, it appears reasonable to conclude that all of the observed secretion of β-chemokines in this study could have occurred in response to binding of the WR321 to one or more phosphoinositides, including PIP or PIP2. However, in view of the secretion of β-chemokines after incubation of intact cells with WR321 in the absence of HIV-1, this conclusion presents an interesting conundrum because of the complete absence of phosphoinositides on the outer surface of plasma membranes of non-permeabilized cells. In view of this, we propose the hypothesis that WR321 could have entered one or more of the cell types in the PBMC population, either by endocytosis, pinocytosis, or macropinocytosis, where it then exerted intracellular binding effects on the inner leaflet of plasma membranes or other intracellular organelles, resulting in the secretion of β-chemokines.

Table 1

Neutralization of four different strains of HIV-1.

| Virus     | Mean IC50 (μg/ml) ± SDa | WR321 | CL-1 | TFTB |
|-----------|-------------------------|-------|------|------|
| SF162     | 2.05 ± 1.24 (N = 3)     | 0.35 ± 0.19 (N = 3) | >25 (N = 2) |
| Bal       | 3.32 ± 2.27 (N = 4)     | 0.29 ± 0.10 (N = 3) | >25 (N = 3) |
| 16/19     | 1.70 ± 0.22 (N = 3)     | 0.24 ± 0.22 (N = 3) | >25 (N = 2) |
| CM235     | 4.33 ± 1.52 (N = 3)     | 0.26 ± 0.06 (N = 2) | >25 (N = 3) |

a IC50 was calculated from the antibody dose response curves as illustrated in Fig. 2. Assays were conducted in duplicate by two different operators. Values are mean IC50 of the number of independent experiments indicated.
It should also be pointed out that plus-strand RNA virus families (including picornaviruses, coronaviruses, and flaviviruses) are known to cause remodeling of intracellular organelles of infected host cells to promote PIP lipid-enriched uncoated membranes that are essential for viral RNA replication [28]. In view of the present work, it is theoretically possible that cytoplasmic uptake of antibodies that bind to PIP or other phosphoinositides might also have an impact on the intracellular replication of plus-strand RNA viruses.

Acknowledgments

This work was supported through Cooperative Agreement No. DAMD17-93-V-3004 between the Henry M. Jackson Foundation for the Advancement of Military Medicine and the US Army Medical Research and Material Command, working together with the Division of AIDS, National Institute for Allergy and Infectious Diseases, NIH, Bethesda, MD. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. The views and opinions expressed herein are the private opinions of the authors and do not necessarily reflect the views of the US Army or the US Department of Defense.

References

[1] V.R. Polonis, B.K. Brown, A. Rosas-Borges, S.Z. Olfa-Pazner, D.S. Dimitrov, M.Y. Zhang, B.W. Barnett, R.M. Ruprecht, G. Scarlatti, E.M. Fenyo, D.C. Montefiori, F.E. McCutchan, N.L. Michael, Recent advances in the characterization of HIV-1 neutralization assays for standardized evaluation of the antibody response to infection and vaccination, Virology 375 (2008) 315–320.
[2] B.K. Brown, N. Karasavas, Z. Beck, G.R. Matyas, D.L. Bix, V.R. Polonis, C.R. Alving, Monoclonal antibodies to phosphatidylinositol phosphate neutralize human immunodeficiency virus type 1: role of phosphate-binding subunits, J. Virol. 81 (2007) 2087–2091.
[3] G.R. Matyas, L. Wieczorek, Z. Beck, C. Ochsenbauer-Jambor, J.C. Kappes, N.L. Michael, V.R. Polonis, C.R. Alving, Neutralizing antibodies induced by liposomal HIV-1 glycoprotein 41 peptide simultaneously bind to both the 2F5 or 4E10 epitope and lipid epitopes, AIDS 23 (2009) 2069–2077.
[4] Z. Beck, N. Karasavas, J. Tong, C.R. Matyas, M. Rao, C.R. Alving, Calcium modulation of monoclonal antibody binding to phosphatidylinositol phosphate, Biochem. Biophys. Res. Commun. 354 (2007) 747–751.
[5] G.R. Matyas, Z. Beck, N. Karasavas, C.R. Alving, Lipid binding properties of 4E10, 2F5, and WR304 monoclonal antibodies that neutralize HIV-1, Biochem. Biophys. Acta 1788 (2009) 660–665.
[6] G. Di Paolo, P. De Camilli, Phosphoinositides in cell regulation and membrane dynamics, Nature 443 (2006) 651–657.
[7] R. Chan, P.D. Uchil, J. Jin, G. Shiu, D.E. Ott, W. Mothes, M.R. Wenk, Retroviruses human immunodeficiency virus and murine leukemia virus are enriched in phosphoinositides, J. Virol. 82 (2008) 11228–11238.
[8] A. Ono, S.D. Ablan, S.J. Lockett, K. Nagashina, E.O. Freed, Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane, Proc. Natl. Acad. Sci. USA 101 (2004) 14889–14894.
[9] J.S. Saad, J. Miller, J. Tai, A. Kim, R.H. Ghanam, M.F. Summers, Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly, Proc. Natl. Acad. Sci. USA 103 (2006) 11364–11369.
[10] V. Chukkapalli, B.H. Gogue, V. Boyko, W.S. Hu, A. Ono, Interaction between the human immunodeficiency virus type 1 Gag matrix domain and phosphatidylinositol-(4,5)-bisphosphate is essential for efficient gag membrane binding, J. Virol. 82 (5) (2008 Mar) 2405–2417.
[11] A. Ono, HIV-1 assembly at the plasma membrane, Vaccine 28 (Supp. 12) (2010) B55–B59.
[12] M.A. Moody, H.X. Liao, S.M. Alam, R.M. Scearce, M.K. Plonk, D.M. Kozink, M.S. Drinker, R. Zhang, S.M. Xia, L.L. Sutherland, G.D. Tomaras, I.P. Giles, J.D. Kappes, C. Ochsenbauer-Jambor, T.G. Edmonds, M. Scares, G. Barbero, D.N. Forthal, G. Landucci, C. Chang, S.W. King, A. Kavile, T.N. Denney, K.K. Hwang, P.F. Chen, P.E. Thorpe, D.C. Montefiori, B.F. Haynes, Anti-phospholipid human monoclonal antibodies inhibit CCR5-tropic HIV-1 and induce β-chemokines, J. Exp. Med. 207 (2010) 763–776.
[13] J.A. Levy, HIV and the Pathogenesis of AIDS, 3rd ed., ASM Press, Washington, 2007, pp. 296–298.
[14] N. Karasavas, Z. Beck, J. Tong, G.R. Matyas, M. Rao, F.E. McCutchan, N.L. Michael, C.R. Alving, Antibodies induced by liposomal protein exhibit dual binding to protein and lipid epitopes, Biochem. Biophys. Res. Commun. 366 (2008) 982–987.
[15] N.W. Wassef, C.R. Alving, R.L. Richards, Liposomes as carriers for vaccines, Immunomethods 4 (1994) 217–222.

[16] T.G. Edmonds, H. Ding, X. Yuan, Q. Wei, K.S. Smith, J.A. Conway, L. Wieczorek, B. Brown, V. Polonis, J.T. West, D.C. Montefiori, J.C. Kappes, C. Ochsenbauer, Replication competent molecular clones of HIV-1 expressing Renilla luciferase facilitate the analysis of antibody inhibition in PBMC, Virology (2010). doi:10.1016/j.virol.2010.08.038.

[17] A. Chenine, E. Sanders-Buell, L. Wieczorek, J. Kim, V. Polonis, S. Tovanabutra, Full-length IMC expressing Renilla luciferase for neutralization assay using PBMC, Retrovirology 6 (Suppl. 3) (2009) P361.

[18] N.M. Wassef, F. Roerdink, G.M. Swartz Jr., J.A. Lyon, B.J. Berson, C.R. Alving, Phosphate-binding specificities of monoclonal antibodies against phosphoinositides in liposomes, Mol. Immunol. 21 (1984) 863–868.

[19] A. Miyazawa, M. Umeda, T. Horikoshi, K. Yanagisawa, T. Yoshioka, K. Inoue, Production and characterization of monoclonal antibodies that bind to phosphatidylinositol 4,5-bisphosphate, Mol. Immunol. 25 (1988) 1025–1031.

[20] K. Fukami, K. Matsuoka, O. Nakanishi, A. Yamakawa, S. Kawai, T. Takenawa, Antibody to phosphatidylinositol 4,5-bisphosphate inhibits oncogene-induced mitogenesis, Proc. Natl. Acad. Sci. USA 85 (1988) 5057–5061.

[21] B.M. Alving, B. Raneri, W.E. Fogler, C.R. Alving, Lupus anticoagulant activities of murine monoclonal antibodies to liposomal phosphatidylinositol phosphate, Clin. Exp. Immunol. 69 (1987) 403–408.

[22] K. Matuoka, K. Fukami, O. Nakanishi, S. Kawai, T. Takenawa, Mitogenesis in response to PDGF and bombesin abolished by microinjection of antibody to PIP2, Science 239 (1988) 640–643.

[23] I. Uno, K. Fukami, H. Kato, T. Takenawa, T. Ishikawa, Essential role for phosphatidylinositol 4,5-bisphosphate in yeast cell proliferation, Nature 333 (1988) 188–190.

[24] J. Noel, K. Fukami, A.M. Hill, T. Capiod, Oscillations of cytosolic free calcium concentration in the presence of intracellular antibodies to phosphatidylinositol 4,5-bisphosphate in voltage-clamped guinea-pig hepatocytes, Biochem. J. 288 (Pt. 2) (1992) 357–360.

[25] A.P. Gilmore, K. Burridge. Regulation of vinculin binding to talin and actin by phosphatidylinositol-4,5-bisphosphate, Nature 381 (1996) 531–535.

[26] H. Yamaguchi, S. Yoshida, E. Muroi, M. Kawamura, Z. Kouchi, Y. Nakamura, R. Sakai, K. Fukami, Phosphatidylinositol 4,5-bisphosphate and PIP5-kinase Iα are required for invadopodia formation in human breast cancer cells, Cancer Sci. 101 (2010) 1632–1638.

[27] A.D. Luster, The role of chemokines in linking innate and adaptive immunity, Curr. Opin. Immunol. 14 (2002) 129–135.

[28] N.Y. Hsu, O. Ilnytska, G. Belov, M. Santiana, Y.H. Chen, P.M. Takvorion, C. Pau, H. van der Schaar, N. Kaushik-Basu, T. Balla, C.E. Cameron, E. Ehrenfeld, F.J. van Kuppeveld, N. Altan-Bonnet, Viral reorganization of the secretory pathway generates distinct organelles for RNA replication, Cell 141 (5) (2010) 799–811.