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Jana Blazkova, … , Susan Moir, Tae-Wook Chun

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Concise Communication **AIDS/HIV** **Immunology**

A number of highly potent and broadly neutralizing antibodies (bNAbs) against the human immunodeficiency virus (HIV) have recently been shown to prevent transmission of the virus, suppress viral replication, and delay plasma viral rebound following discontinuation of antiretroviral therapy in animal models and infected humans. However, the degree and extent to which such bNAbs interact with primary lymphocytes have not been fully delineated. Here, we show that certain glycan-dependent bNAbs, such as PGT121 and PGT151, bind to B, activated T, and natural killer (NK) cells of HIV-infected and -uninfected individuals. Binding of these bNAbs, particularly PGT121 and PGT151, to activated $CD4^+$ and $CD8^+$ T cells was mediated by complex-type glycans and was abrogated by enzymatic inhibition of N-linked glycosylation. In addition, a short-term incubation of PGT151 and primary NK cells led to degranulation and cellular death. Our data suggest that the propensity of certain bNAbs to bind uninfected/bystander cells has the potential for unexpected outcomes in passive-transfer studies and underscore the importance of antibody screening against primary lymphocytes.

Glycan-dependent HIV-specific neutralizing antibodies bind to cells of uninfected individuals

Jana Blazkova,[1] Eric W. Refsland,[1] Katherine E. Clarridge,[1] Victoria Shi,[1] J. Shawn Justement,[1] Erin D. Huiting,[1] Kathleen R. Gittens,[2] Xuejun Chen,[3] Stephen D. Schmidt,[3] Cuiping Liu,[3] Nicole Doria-Rose,[3] John R. Mascola,[3] Alonso Heredia,[4] Susan Moir,[1] and Tae-Wook Chun[1]

[1]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases (NIAID), [2]Critical Care Medicine Department, Clinical Center, and [3]Vaccine Research Center, NIAID, NIH, Bethesda, Maryland, USA. [4]Institute of Human Virology, University of Maryland, Baltimore, Maryland, USA.

A number of highly potent and broadly neutralizing antibodies (bNAbs) against the human immunodeficiency virus (HIV) have recently been shown to prevent transmission of the virus, suppress viral replication, and delay plasma viral rebound following discontinuation of antiretroviral therapy in animal models and infected humans. However, the degree and extent to which such bNAbs interact with primary lymphocytes have not been fully delineated. Here, we show that certain glycan-dependent bNAbs, such as PGT121 and PGT151, bind to B, activated T, and natural killer (NK) cells of HIV-infected and -uninfected individuals. Binding of these bNAbs, particularly PGT121 and PGT151, to activated $CD4^+$ and $CD8^+$ T cells was mediated by complex-type glycans and was abrogated by enzymatic inhibition of N-linked glycosylation. In addition, a short-term incubation of PGT151 and primary NK cells led to degranulation and cellular death. Our data suggest that the propensity of certain bNAbs to bind uninfected/bystander cells has the potential for unexpected outcomes in passive-transfer studies and underscore the importance of antibody screening against primary lymphocytes.

Introduction

Recent advances in antibody cloning technologies have led to the isolation of a number of highly potent and broadly neutralizing antibodies (bNAbs) against the human immunodeficiency virus (HIV). Some of these bNAbs have been shown in animal models and human studies to prevent transmission of virus (1–3), suppress viral replication (4–6), eliminate persistent viral reservoirs (7), enhance antigen presentation via formation of immune complexes (8, 9), and delay plasma viral rebound in infected individuals upon discontinuation of antiretroviral therapy (ART) (10, 11). Given that HIV cannot be eradicated by ART alone (12) and the consequent challenges associated with life-long treatment, the use of bNAbs represents a therapeutic alternative for achieving sustained virologic remission in the absence of antiretroviral drugs (13).

Some of the most potent bNAbs evaluated to date, that are considered appropriate for use in clinical trials, include the CD4 binding site–specific antibodies VRC01 and 3BNC117, as well as the V3 loop N332 glycan supersite-specific antibodies PGT121 and 10-1074 (14). These antibodies have recently been used in clinical trials involving passive transfer of single bNAbs (10, 11) or in combination (15) in HIV-infected individuals. Despite moderate to significant delays in time to plasma viral rebound following discontinuation of ART compared with historical controls, preexisting and/or emergent bNAb-resistant HIV necessitate the use of more potent and/or combinations of bNAbs that target multiple sites on HIV Env (10, 11, 15). In this regard, it is desirable to use a combination of bNAbs that does not react with HIV-uninfected lymphocytes in order to maximize their anti-HIV potentials and to prevent unintended antibody-mediated effector functions in vivo. It has been shown that some anti-HIV antibodies exhibit auto-/polyreactivity against cytoplasmic or nuclear self-antigens (16–20). Although the recent generation of HIV-specific bNAbs, including those that have been administered to humans, have not shown discernable auto-/polyreactivity when tested against a panel of self-antigens and cell lines (17), they have not been extensively screened for their capacity to bind to various subsets of lymphocytes derived from infected and uninfected individuals in the absence or presence of cellular activation and potential physiologic consequences. We conducted the present study to address these issues.

Results and Discussion

In order to characterize the binding capacity of bNAbs to various cell types, we first conducted flow cytometric analyses using an uninfected (CEM-NKR-CCR5) and an HIV-infected (CEM-IIIB) cell line. We used HIV-specific antibodies directed against various HIV Env epitopes, including the V3 loop N332 glycan supersite (PGT121 and 10-1074), CD4 binding site (VRC01 and 3BNC117), V1V2 apex (PG9 and PG16), as well as the gp41 membrane proximal external region (10E8). As shown in Figure 1A, all HIV-specific antibodies tested exhibited strong binding to HIV Env expressed on CEM-IIIB cells (73.0%–97.9%) compared with minimal binding to CEM-NKR-CCR5 cells (0.4%–4.6%). We then examined binding of bNAbs to B and T cell lines and nonlymphocytic primary cells/

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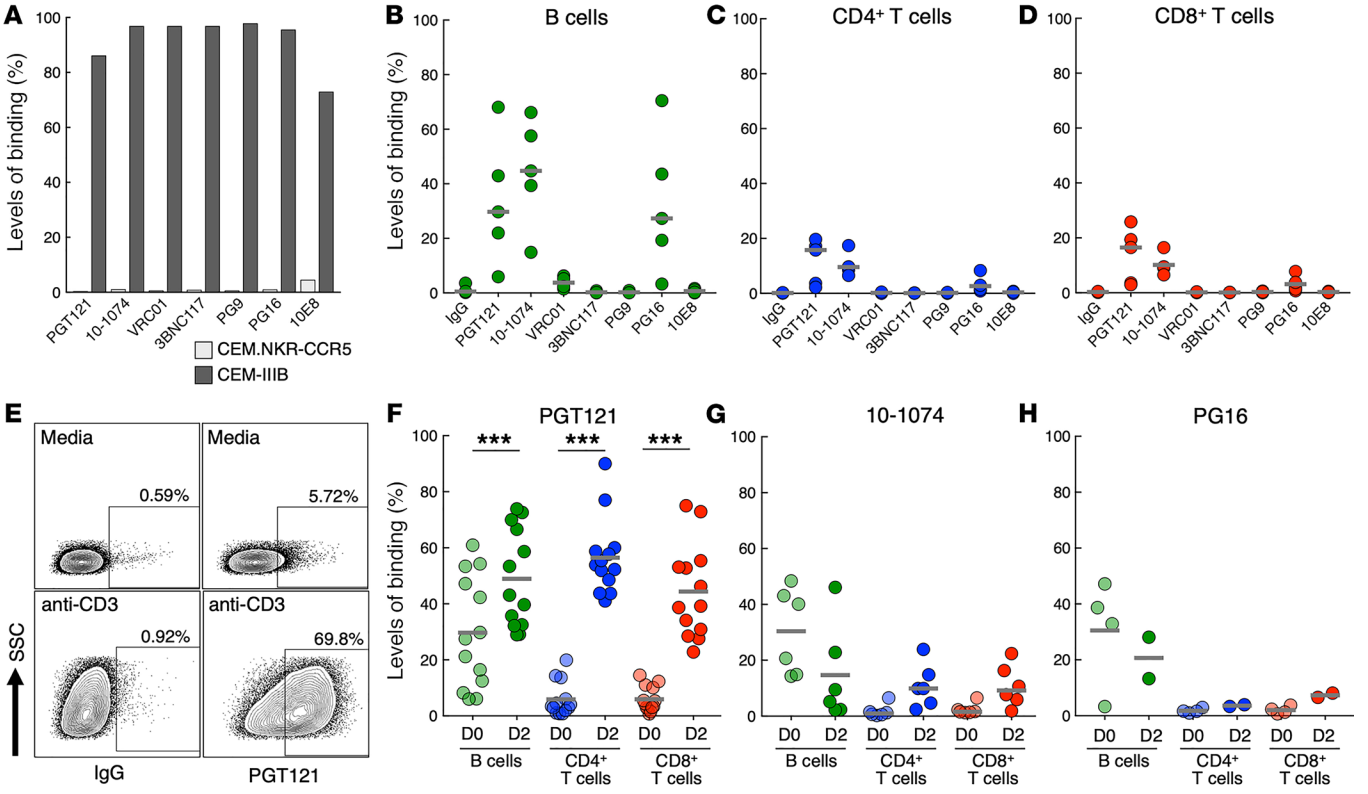

Figure 1. Binding of bNAbs to a chronically HIV-infected cell line and lymphocytes of uninfected individuals. (**A**) Binding of bNAbs to an uninfected (CEM-NKR-CCR5, light gray) and a chronically HIV-infected cell line (CEM-IIIB, dark gray). Levels of binding of bNAbs and IgG (negative control) to B cells (**B**), CD4+ T cells (**C**), and CD8+ T cells (**D**) in PBMCs from HIV-negative donors. (**E**) IgG and PGT121 binding to unstimulated and anti-CD3–stimulated CD4+ T cells. Binding of PGT121 (**F**), 10-1074 (**G**), and PG16 (**H**) to B cells, and CD4+ and CD8+ T cells in PBMCs from HIV-uninfected individuals prior to (day 0, D0) and 2 days following stimulation with anti-CD3 (D2). The median values are shown as gray bars. Statistical significance was tested with Wilcoxon's matched-pairs signed-rank test. ***$P < 0.001$.

cell lines. PGT121 bound to Jurkat and MT-2 (T cell lines) and Raji (B cell line) cells, endothelial and epithelial cell lines, and human foreskin fibroblast cells (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI125955DS1). To further characterize the binding properties of the aforementioned antibodies, we performed the same analyses on unstimulated peripheral blood mononuclear cells (PBMCs) isolated from HIV-uninfected individuals (Figure 1 and Supplemental Figure 2). Unexpectedly, we observed binding of 3 of the bNAbs tested, namely PGT121 (median 29.7%), 10-1074 (44.7%), and PG16 (27.3%) to B cells of HIV-uninfected individuals (Figure 1B and Supplemental Figure 2A). In contrast, the other bNAbs tested (PG9, VRC01, 3BNC117, and 10E8) exhibited minimal binding (0.03%–6.3%) to the B cells of the same donors (Figure 1B and Supplemental Figure 2A). Further analyses revealed low, yet detectable binding of PGT121 to unstimulated CD4+ (median 15.8%) and CD8+ (median 16.5%) T cells (Figure 1, C and D, and Supplemental Figure 2A). A similar pattern was observed when 10-1074 was incubated with unstimulated CD4+ (median 9.6%) and CD8+ (9.4%) T cells, whereas the other bNAbs tested showed minimal binding to T cells (Figure 1, C and D, and Supplemental Figure 2A).

We then determined whether binding properties of bNAbs were affected by cellular activation. As shown in Figure 1, E and F, and Supplemental Figure 2B, we observed a significant increase in PGT121 binding to the CD4+ ($P = 0.0002$) and CD8+ ($P = 0.0002$) T cells following anti-CD3 antibody stimulation. Of note, binding of PGT121 to the B cells increased after stimulation of PBMCs with anti-CD3 antibody ($P = 0.0002$, Figure 1F), potentially due to activation of B cells via soluble factors released by activated T cells and/or cell-cell interactions. In contrast, as shown in Figure 1G, binding of 10-1074 to CD4+ ($P = 0.125$) and CD8+ ($P = 0.125$) T cells remained relatively low despite cellular stimulation, while binding to B cells decreased ($P = 0.051$). The latter pattern was also observed with PG16 (Figure 1H). These results suggest a significant increase of PGT121 binding to T and B cells following T cell–dependent cellular activation irrespective of HIV status of the donor.

Next, we examined the kinetics of PGT121 binding to B cells and T cells of HIV-infected and -uninfected individuals following cellular stimulation. An increase of PGT121 binding to B cells occurred on day 1 and remained the same after anti-CD3 antibody stimulation of PBMCs (Figure 2A). In contrast, as shown in Figure 2, B and C, PGT121 binding to CD4+ and CD8+ T cells increased over time, with maximal levels of binding occurring on day 3. We then compared the effect of B cell–specific (anti–human IgM/A/G) and T cell–specific (anti-CD3) stimulation on binding of PGT121 to corresponding cell types. When compared with the unstimulated condition (media alone), 2-day stimulation of B cells led to a

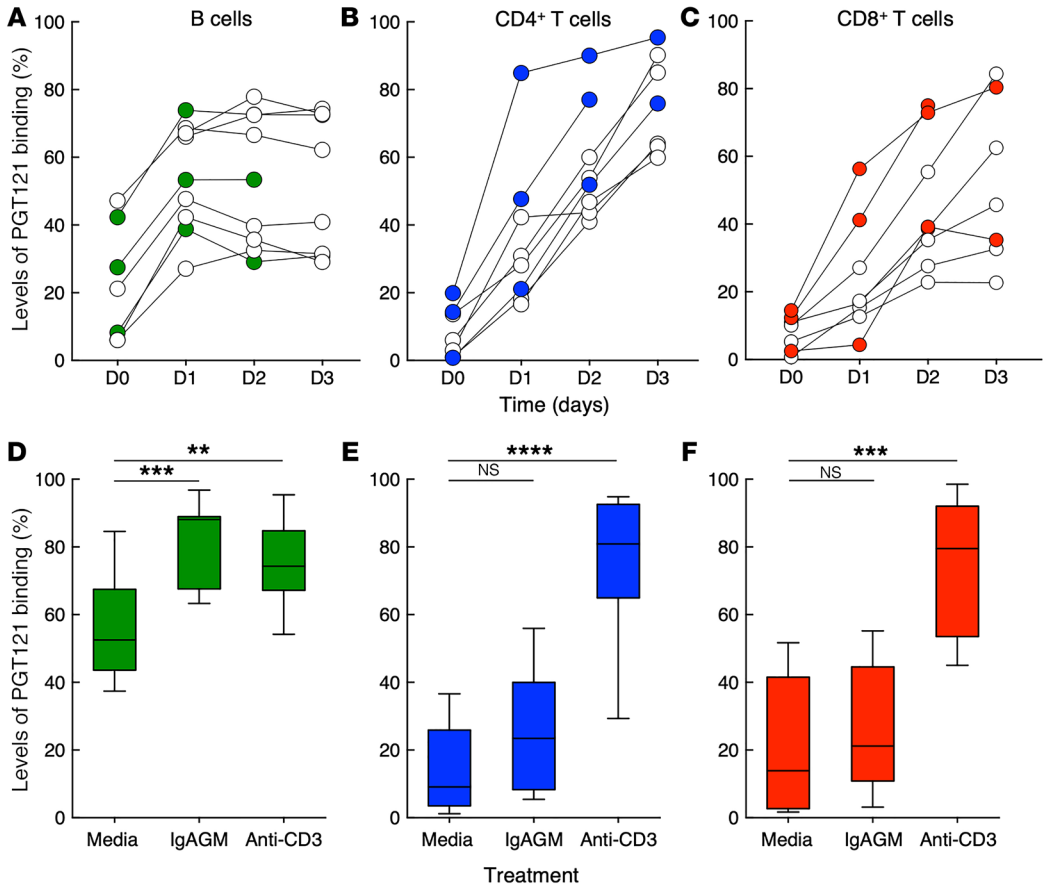

Figure 2. Time course of PGT121 binding to B cells and CD4⁺ and CD8⁺ T cells of HIV-infected and -uninfected individuals following cellular activation. Levels of PGT121 binding to B cells (**A**), CD4⁺ T cells (**B**), and CD8⁺ T cells (**C**) in PBMCs from HIV-infected (filled circles) and -uninfected (empty circles) individuals, at days 0, 1, 2, and 3 after stimulation with anti-CD3. Levels of PGT121 binding to B cells (**D**), CD4⁺ T cells (**E**), and CD8⁺ T cells (**F**) following 2-day stimulation of PBMCs of HIV-uninfected individuals using B cell–specific (IgAGM) and T cell–specific (anti-CD3) stimulation. Cells incubated without any stimulus (media) served as baseline. Statistical significance was tested with Friedman's ANOVA. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. NS, not significant.

significant increase in PGT121 binding, whether the stimulus was anti-IgM/A/G ($P = 0.0003$) or anti-CD3 antibody ($P = 0.0073$; Figure 2D). In addition, while PGT121 binding to T cells was low in the absence of cellular activation or with the B cell stimulus, stimulation with anti-CD3 antibody significantly increased the binding of PGT121 to CD4⁺ ($P < 0.0001$) and CD8⁺ ($P = 0.0001$) T cells (Figure 2, E and F). These data suggest that expression of the ligand(s) for PGT121 on B and T cells could be modulated by direct and, to a certain extent, indirect cellular activation.

In order to further delineate the binding properties of PGT121 on T cells, we evaluated different subsets of CD4⁺ T cells and activation markers prior to and following stimulation of enriched CD4⁺ T cells with anti-CD3 antibody. As shown in Supplemental Figure 3A, the lowest binding of PGT121 was observed on naive cells, and the highest on CD4⁺ T cells with effector phenotypes. Furthermore, PGT121 binding was associated with expression of activation marker CD25 (86.6% of PGT121⁺ cells) and CD69 (67.6% of PGT121⁺ cells) (Supplemental Figure 3B), further supporting the observation that PGT121 preferentially binds to activated CD4⁺ T cells.

PGT121 has previously been shown to bind HIV Env gp120 in a complex-type N-glycan–dependent manner (21), a finding that prompted us to investigate the glycan dependency of PGT121 binding to the B and T cells of HIV-uninfected individuals. First, we used EBV-transformed B cell lines derived from HIV-infected or uninfected individuals as well as from 2 siblings with a congenital disorder of glycosylation type IIb (CDG-IIb), a genetic disease associated with mutations in mannosyl-oligosaccharide glucosidase (22). While PGT121 bound to a median of 17.6% of EBV-transformed B cells from donors without CDG-IIb, it only bound to 2.51% of the EBV-transformed B cells from the CDG-IIb donors ($P = 0.0256$, Figure 3A), suggesting that N-linked glycosylation is involved in the binding of PGT121 to B cells. In order to further evaluate the role of glycans in PGT121 binding to T cells, we stimulated CD4⁺ T cells of HIV-uninfected individuals in the absence and presence of kifunensine, an α-mannosidase I inhibitor that blocks processing of glycoproteins resulting in an accumulation of high-mannose rather than complex glycans. As shown in Figure 3B, binding of PGT121 to activated CD4⁺ T cells was significantly decreased in the presence of kifunensine ($P = 0.0156$), suggesting that PGT121 interacts with complex-type glycans expressed on the surface of uninfected targets. Similarly, stimulation of PBMCs in the presence of kifunensine led to a substantial decrease of PGT121 binding to B and CD4⁺ and CD8⁺ T cells (Figure 3C). Of note, the binding of 10-1074 on B and T cells increased in the presence of kifunensine (Supplemental Figure 4A), confirming preferential binding of the antibody to high-mannose glycans (21). Binding of PG16 was not affected by the presence of kifunensine (Supplemental Figure 4B). Collectively, these results demonstrate that PGT121 recognizes complex-type glycans on the surface of B and activated T cells of HIV-uninfected individuals and that its binding can be modulated by an agent that alters the composition of glycans following cellular activation.

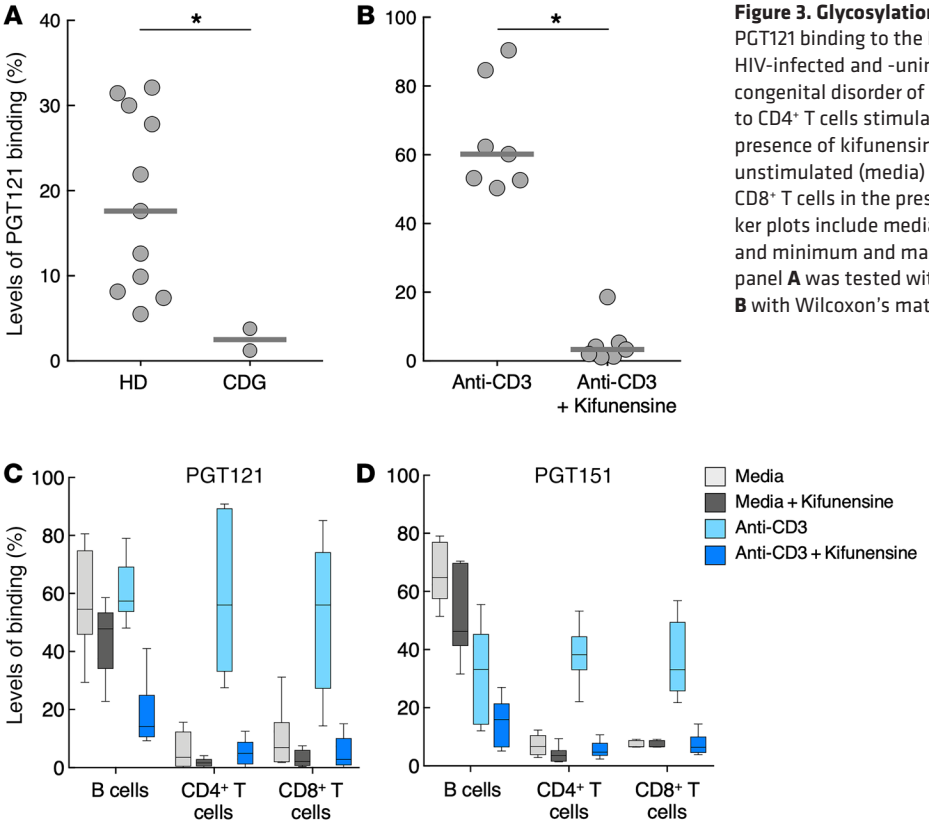

Figure 3. Glycosylation-dependent PGT121 binding. (**A**) Levels of PGT121 binding to the EBV-transformed B cell lines derived from 11 HIV-infected and -uninfected individuals and from 2 donors with a congenital disorder of glycosylation (CDG). (**B**) Levels of PGT121 binding to CD4+ T cells stimulated with anti-CD3 antibody in the absence or presence of kifunensine. (**C**) Binding of PGT121 and (**D**) PGT151 to the unstimulated (media) and stimulated (anti-CD3) B cells, CD4+, and CD8+ T cells in the presence or absence of kifunensine. Box-and-whisker plots include median (n = 6) with 25th and 75th percentile borders and minimum and maximum whiskers. Statistical significance for panel **A** was tested with the Mann-Whitney test, *P = 0.0256; and for **B** with Wilcoxon's matched-pairs signed-rank test, *P = 0.016.

To extend the observation that certain HIV-specific antibodies bind to complex-type glycans on lymphocytes of HIV-uninfected individuals, we investigated the binding property of 3 other bNAbs (PGT151, VRC26.09, and 35O22) with similar specificities involving complex-type glycans of HIV Env (23, 24). As shown in Supplemental Figure 5, A–C, PGT151 (median 18.4%) and VRC26.09 (58.1%) but not 35O22 (2.3%) bound to B cells, and to a lesser extent to CD4+ and CD8+ T cells of HIV-uninfected individuals in the absence of activating stimuli. Anti-CD3 antibody stimulation of PBMCs enhanced binding of PGT151 and VRC26.09 to CD4+ (median 48.4% and 27.9%, respectively) and CD8+ T cells (median 42.8% and 36.2%, respectively) (Supplemental Figure 5, D–F). Of note, the binding of PGT151 to B and CD4+ and CD8+ T cells significantly decreased when cells were stimulated in the presence of kifunensine (Figure 3D). These data suggest a propensity for certain complex-type glycan-dependent bNAbs to interact with uninfected/bystander lymphocytes.

To evaluate whether glycan-dependent HIV-specific bNAbs, particularly PGT151, bind to T cells in vivo, we used humanized mice as described in the Supplemental Methods. We observed significantly higher levels of PGT151 binding to $CD3^+CD4^+$ (P = 0.0079) and $CD3^+CD8^+$ (P = 0.0079) T cells when compared with the mice injected with IgG (Supplemental Figure 6, A and B), demonstrating the binding of PGT151 to human T cells in vivo.

We then investigated whether these antibodies bind to NK cells. Highly enriched NK cells derived from HIV-infected and -uninfected individuals were pretreated with an Fc-receptor blocker, followed by incubation with several individual bNAbs. As shown in Figure 4, A and B, stronger binding compared with human IgG was observed when freshly isolated NK cells were incubated with PGT121 (P = 0.0006) or PGT151 (P = 0.0006) but not 3BNC117 (P > 0.9999) (Figure 4B). Inclusion of the Fc-receptor blocker did not completely abrogate binding of PGT121 and PGT151 to NK cells, suggesting that these glycan-dependent antibodies interact with antigens expressed on targets (Supplemental Figure 7). We further investigated the functional consequence of binding of PGT121 and PGT151 to NK cells. NK cell degranulation (CD107a) and death (staining with a nucleic acid dye, Sytox) were measured following short periods of incubation (2 and 6 hours, respectively). Binding of PGT151 to NK cells resulted in the highest level of degranulation (median 15.8%) compared with human IgG (P = 0.00018), PGT121 (P = 0.00018), and 3BNC117 (P = 0.00018) (Figure 4, A and C). Of note, the subset of NK cells undergoing degranulation upon interaction with PGT151 showed phenotypes associated with higher cytolytic activities (Supplemental Figure 8). Additionally, incubation of PGT151 with NK cells led to a marked increase in cell death (median 17.6%) compared with human IgG (P = 0.0003), PGT121 (P = 0.0003), and 3BNC117 (P = 0.0003) (Figure 4, A and D). Collectively, these data suggest that binding of certain bNAbs, such as PGT151, to bystander/uninfected cells could potentially lead to abnormalities/impairment of immune cells.

A major emphasis of current efforts in HIV therapeutic research is to achieve ART-free virologic remission in infected individuals. These efforts include the use of HIV-specific bNAbs to delay or prevent plasma viral rebound and to allow

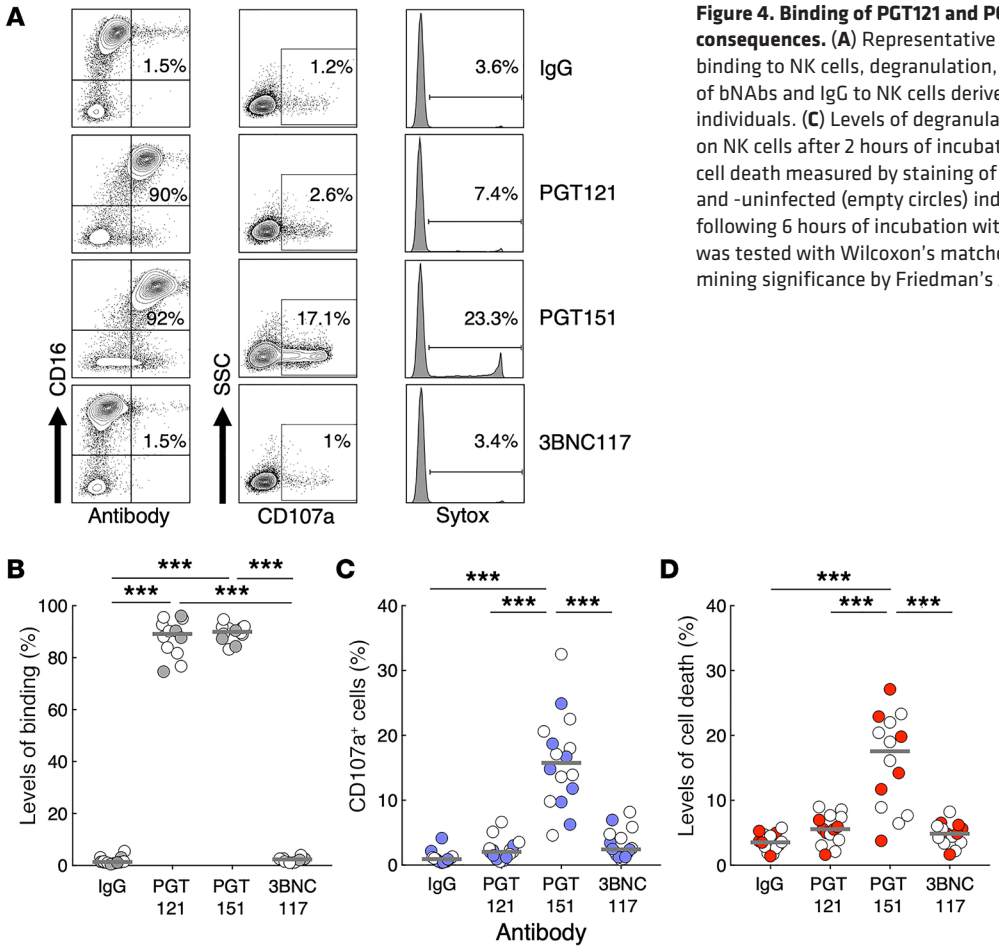

Figure 4. Binding of PGT121 and PGT151 to NK cells and immunologic consequences. (**A**) Representative FACS plots showing levels of bNAb binding to NK cells, degranulation, and cell death. (**B**) Levels of binding of bNAbs and IgG to NK cells derived from HIV-infected and -uninfected individuals. (**C**) Levels of degranulation measured by expression of CD107a on NK cells after 2 hours of incubation with bNAbs or IgG. (**D**) Levels of NK cell death measured by staining of NK cells of HIV-infected (filled circles) and -uninfected (empty circles) individuals with the nucleic acid dye Sytox following 6 hours of incubation with bNAbs or IgG. Statistical significance was tested with Wilcoxon's matched-pairs signed-rank test after determining significance by Friedman's ANOVA. ***$P < 0.001$.

long-term control of viral replication in infected individuals following discontinuation of ART (8). It is critical to choose antibodies that do not interact with host antigens in order to maximize HIV neutralization and to prevent potentially detrimental bystander antibody-mediated effects. In the present study, we demonstrated that certain glycan-dependent bNAbs bind to various resting and activated lymphocyte populations of HIV-infected and -uninfected individuals. In particular, PGT121 and PGT151 strongly bound to $CD4^+$ and $CD8^+$ T cells following cellular activation. Of note, T cell development and activation have been shown to be accompanied by upregulation of complex N-glycans on the cell surface (25, 26). Our data suggest that complex glycans are naturally expressed and available on the surface of lymphocytes for targeting by certain HIV-specific bNAbs, such as PGT121 and PGT151, especially after cellular activation.

Potentially autoreactive properties have been previously described for certain bNAbs, including 2F5, 4E10, and VRC01 (16, 17, 19, 20). Such bNAbs with reactivity against host molecules are thought to arise in HIV-infected individuals, at least in part, due to the breakdown in tolerance that would otherwise lead to deletion and/or inactivation of these virus-specific B cells (18, 27, 28). These findings underscore the potentially deleterious effects that autoreactive antibodies can pose, including those that react with glycans.

Despite evidence of strong binding of complex-type glycan-dependent bNAbs to various lymphocyte populations in both HIV-infected and -uninfected individuals, the outcomes may not necessarily be deleterious (i.e., induction of antibody-dependent cell-mediated cytotoxicity, ADCC). Indeed, we were not able to demonstrate NK cell–mediated ADCC against activated $CD4^+$ or $CD8^+$ T cells despite high levels of PGT121/PGT151 binding ex vivo (Supplemental Figure 9). However, our data demonstrated that incubation of primary NK cells with PGT151 rapidly led to autodegranulation and cell death, suggesting that certain bNAbs could potentially mediate deleterious effector functions against immune cells ex vivo.

Our findings have potentially important implications for the use of bNAbs in the treatment and prevention of HIV infection. Although the auto-/polyreactivity of bNAbs is typically determined by their capacity to bind a panel of nuclear and cytoplasmic self-antigens, more physiologically relevant targets, such as those expressed with or without activation on lymphocytes of healthy donors, should be considered. The potential unintended consequences of passive transfer of such antibodies should be evaluated in preclinical studies prior to the clinical use of current and newly developed HIV-specific bNAbs.

Methods

Please see the Supplemental Methods for detailed experimental procedures.

Study subjects. Leukapheresed products were collected from HIV-infected and -uninfected individuals in accordance with clinical protocols approved by the Institutional Review Board (IRB) of the NIH. All study subjects provided informed consent.

Author contributions

JB, SM, and TWC designed the research. JB, EWR, KEC, VS, JSJ, EDH, KRG, AH, SM, and TWC performed the research. XC, SDS, CL, NDR, and JRM contributed research materials. JB, SM, and TWC analyzed the data. JB, SM, and TWC wrote the manuscript.

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Address correspondence to: Tae-Wook Chun, National Institutes of Health, Building 10, Room 6A33, 9000 Rockville Pike, Bethesda, Maryland 20892, USA. Phone: 301.496.0890; Email: twchun@nih.gov.

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