AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

VACCINES AND ANTIVIRAL AGENTS

Broadly Neutralizing Antibodies Display Potential for Prevention of HIV-1 Infection of Mucosal Tissue Superior to That of Nonneutralizing Antibodies

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ABSTRACT Definition of the key parameters mediating effective antibody blocking of HIV-1 acquisition within mucosal tissue may prove critical to effective vaccine development and the prophylactic use of monoclonal antibodies. Although direct antibody-mediated neutralization is highly effective against cell-free virus, antibodies targeting different sites of envelope vulnerability may display differential activity against mucosal infection. Nonneutralizing antibodies (nnAbs) may also impact mucosal transmission events through Fc-gamma receptor (FcγR)-mediated inhibition. In this study, a panel of broadly neutralizing antibodies (bnAbs) and nnAbs, including those associated with protection in the RV144 vaccine trial, were screened for the ability to block HIV-1 acquisition and replication across a range of cellular and mucosal tissue models. Neutralization potency, as determined by the TZM-bl infection assay, did not fully predict activity in mucosal tissue. CD4-binding site (CD4bs)-specific bnAbs, in particular VRC01, were consistent in blocking HIV-1 infection across all cellular and tissue models. Membrane-proximal external region (MPER) (2F5) and outer domain glycan (2G12) bnAbs were also efficient in preventing infection of mucosal tissues, while the protective efficacy of bnAbs targeting V1-V2 glycans (PG9 and PG16) was more variable. In contrast, nnAbs alone and in combinations, while active in a range of cellular assays, were poorly protective against HIV-1 infection of mucosal tissues. These data suggest that tissue resident effector cell numbers and low FcγR expression may limit the potential of nnAbs to prevent establishment of the initial foci of infection. The solid protection provided by specific bnAbs clearly demonstrates their superior potential over that of nonneutralizing antibodies for preventing HIV-1 infection at the mucosal portals of infection.

IMPORTANCE Key parameters mediating effective antibody blocking of HIV-1 acquisition within mucosal tissue have not been defined. While bnAbs are highly effective against cell-free virus, they are not induced by current vaccine candidates. However, nnAbs, readily induced by vaccines, can trigger antibody-dependent cellular effector functions, through engagement of their Fc-gamma receptors. Fc-mediated antiviral activity has been implicated as a secondary correlate of decreased HIV-1 risk in the RV144 vaccine efficacy trial, suggesting that protection might be mediated in the absence of classical neutralization. To aid vaccine design and selection of antibodies for use in passive protection strategies, we assessed a range of bnAbs and nnAbs for their potential to block *ex vivo* challenge of mucosal tissues. Our data clearly indicate the superior efficacy of neutralizing antibodies in preventing mucosal acquisi-

Received 1 September 2016 **Accepted** 4 October 2016

Accepted manuscript posted online 19 October 2016

Citation Cheeseman HM, Olejniczak NJ, Rogers PM, Evans AB, King DFL, Ziprin P, Liao H-X, Haynes BF, Shattock RJ. 2017. Broadly neutralizing antibodies display potential for prevention of HIV-1 infection of mucosal tissue superior to that of nonneutralizing antibodies. J Virol 91:e01762-16. https://doi.org/10.1128/JVI.01762-16.

Editor Guido Silvestri, Emory University

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tion of infection. These results underscore the importance of maintaining the central focus of HIV-1 vaccine research on the induction of potently neutralizing antibodies.

KEYWORDS HIV vaccines, human immunodeficiency virus, mucosal immunity, neutralizing antibodies, nonneutralizing antibodies

The induction of broadly neutralizing antibodies (bnAbs) remains a key focus of human immunodeficiency virus type 1 (HIV-1) vaccine research; however, this goal has yet to be realized. Classical neutralization is thought to require binding of the antibody to the trimeric envelope spike, blocking key epitopes on the surface of the virus, inhibiting engagement with cell receptors, and preventing conformational change required for viral fusion and entry (1). During the natural course of HIV-1 infection, ~50% of HIV-1-infected individuals develop neutralizing antibodies (nAbs) capable of inhibiting more than 50% of viral isolates (2), with ~10% developing high levels of bnAbs capable of inhibiting 90% of viruses. While both bnAbs and nAbs require a period of months to years to develop (3), nonneutralizing antibodies (nnAbs) are found in all HIV-1-infected individuals from the acute stage of infection onwards (4). These nnAbs are thought to target a diversity of envelope structures in addition to functional trimers that include noncleaved trimers, dimers, and monomers, as well as gp41 stumps that have shed gp120. The extent to which these structures are expressed on infectious virus and/or infected cells may prove critical to any potential antiviral activity (5, 6). Nevertheless, binding of the Fc region of immunoglobulin G (IgG) to Fc-gamma receptors (FcγRs) can engage a range of effector cells capable of mediating potent antiviral activity. The extent to which Fc-effector functions can impact on initial events determining mucosal infection may prove critical to vaccine design.

Two key observations are cited in support of the potential role for Fc-effector functions contributing to mucosal protection. The first was the observation that the solid passive protection mediated by the b12 nAb against vaginal simian-human immunodeficiency virus (SHIV) challenge in the nonhuman primate model (NHP) was reduced by introducing the LALA mutation that impaired its binding to FcγRs (7). These data suggest that binding to FcγRs may augment, but is not essential for, the protective activity of nAbs. The extent to which FcγR-mediated engagement of mucosal effector cells versus the role of FcγR extending the half-life of CD4-binding site (CD4bs) antibodies contributed to the protective efficacy of b12 has yet to be resolved (8). The second key observation was that the marginal protection (31%) provided by the RV144 Thai phase III trial correlated with high concentrations of anti-V1-V2 nnAbs and an absence of nAbs against circulating viral strains (9). Although mechanistic correlates remain elusive, reduced risk correlated to Fc-mediated effector functions of nnAbs targeting the V1-V2 region of the HIV-1 envelope.

The Fc-mediated effector functions of both nabs and nnAbs are dependent upon the engagement of Fc receptors leading to activation of effector cells and further downstream activities, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular viral inhibition (ADCVI), or antibody-dependent cellular phagocytosis (ADCP). ADCC requires engagement of FcγRs by effector cells (natural killer [NK] cells, neutrophils, and macrophages) capable of eliminating infected cells following their recognition by binding antibodies. Importantly, ADCC targets infected cells, cells that have bound virus, and cells binding shed viral envelope (5, 10, 11). The inverse correlation with infection risk and high levels of serum V1-V2 loop antibodies capable of mediating ADCC (12–14) has driven speculation that protection in RV144 was partially due to ADCC-mediating antibodies (15). ADCVI and ADCC activities likely overlap; however, ADCVI measures the additional contribution of noncytolytic mechanisms, such as FcγR-triggered production of β-chemokines that can also contribute to viral inhibition of cell-free virus (16). ADCP targets cell-free virus and prevents infection of antigen-presenting cells (macrophages and dendritic cells [DC]) through FcγR-dependent phagocytosis of opsonized viral particles (17). This may have particular relevance given the potential role of mucosal antigen-presenting cells in the uptake

and subsequent presentation of HIV-1 to $CD4^+$ target cells (18–21), facilitating dissemination through *cis*- and *trans*-infection pathways (22). Additionally, phagocytosis of opsonized virions may itself reduce the probability of successful infection of tissue resident $CD4^+$ T cells, the primary targets of mucosal infection in humans and macaques, by reducing the half-life of infectious virus (23–25).

To date, little is known about the critical parameters mediating effective antibody blocking of HIV-1 acquisition within mucosal tissue. To bridge this gap, we assessed the relative antiviral potencies of a panel of neutralizing and nonneutralizing antibodies, including those associated with protection in the RV144 vaccine trial, across a range of tissue and cellular models designed to mimic the initial events required to establish mucosal infection. To assess the relative efficacy of bnAbs, we chose representative monoclonal antibodies (MAbs) targeting four major sites of envelope vulnerability: the CD4-binding site (b12, VRC01, and CH31), membrane-proximal external regions (MPERs) (2F5 and 4E10), V1-V2 glycan Env regions (PG9 and PG16), and outer domain glycans (2G12). To assess the potential of nnAbs to block infection and/or onward transmission from mucosal tissue, we selected two individual nnAbs targeting the C1 region of gp120 (A32) and cluster I of gp41 (4B3) previously reported to show high levels of ADCC and ADCP in *in vitro* assays (26, 27). In addition, we assembled three nnAb combinations. Combination 1 was 7B2/CH58/CH90, targeting the principal immunodominant domain (PID) of gp41 (7B2), the V2 region of gp120 (CH58), and the CD4-induced (CD4i) cluster 1 region (CH90); all are known to display ADCC activity in a range of *in vitro* models (15, 28), and 7B2 in combination with CH58 shows enhanced capacity to capture of infectious virions (29). Combination 2 was 7B2/CH58/CH22, combining 7B2 and CH58 with CH22 targeting the V3 region of gp120, also with known ADCC activity and limited tier 1 neutralization (30). Combination 3 was F240/M785-U1/N10-U1, all focused on different epitopes within the C1 region of gp41 and previously shown to exhibit ADCC activity (31, 32).

RESULTS

TZM-bl and peripheral blood mononuclear cell (PBMC) assays differentiate FcR-dependent function. Initial studies assessed the ability of antibodies to block HIV-1_{BaL} infection using an indicator cell line (TZM-bl) devoid of FcR. Known bnAbs VRC01, CH31, b12, PG9, and PG16 demonstrated significant reduction in infection (Fig. 1A and Table 1). The inhibitory activity of CH31 was reduced when presented as monomeric IgA2 (mIgA2) or dimeric IgA2 (dIgA2) compared to IgG (Table 1). In contrast, MPER bnAbs failed to demonstrate significant inhibition in the absence of FcR engagement, while 2G12 provided only a modest reduction in infection at the highest concentration tested (50 μg/ml). None of the nnAbs or HIV-IG preparations demonstrated inhibition in the absence of FcR.

bnAbs active in the TZM-bl assay were also active in PBMC (Fig. 1B) and, although individual antibodies showed some differences in potency between the two assays (Table 1), there was no evidence that the presence of FcR in the PBMC assay had a major impact on activity. In contrast, the activity of MPER bnAbs 2F5 IgG and 4E10 and glycan-specific 2G12 showed enhanced activity in PBMC cultures. Strikingly, the nnAb combinations, nnAb A32, and both HIV-IG B and C pools demonstrated measurable levels of HIV-1_{BaL} inhibition in PBMC cultures (Fig. 1B and Table 1), although only HIV-IG C generated a 90% inhibitory concentration (IC_{90}).

Antibody inhibition in macrophage and dendritic cell cultures. To further investigate the ability of the antibodies to block HIV-1_{BaL} infection in FcR-positive cells, subsequent experiments were performed in macrophage and dendritic cell models, as previously described (24). Fc receptor expression was determined for the different cellular models (Fig. 2). All IgG bnAbs displayed potent inhibition of macrophage infection (Fig. 3A). Interestingly, IgA forms of CH31 were less effective than IgG, while 2F5 dIgA was completely inactive. With the exception of A32, all nnAbs and both HIV-IG preparations were effective against macrophage infection. A similar pattern was evident for direct infection of dendritic cells. However, the difference in activity between

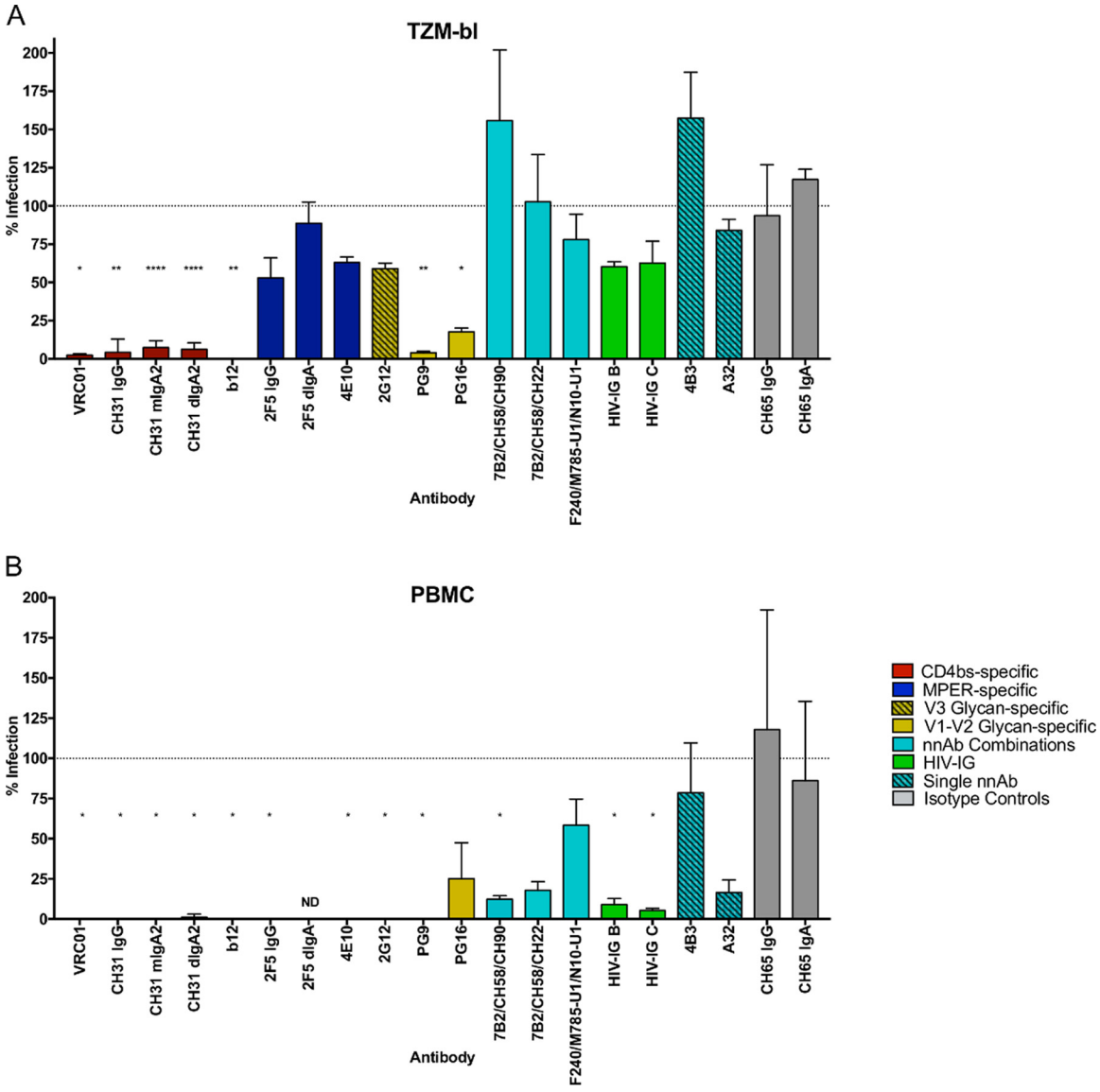


FIG 1 Inhibition of single antibodies and antibody combinations in TZM-bl cells and PBMC. Shown are results for inhibition of HIV-1_{BaL} by antibody panels (50 μg/ml of single antibodies; 25 μg/ml of each in combinations) in the direct infection of TZM-bl ($n = 3$) (A) and PBMC ($n = 3$) (B). Data are presented as percent infection compared to the HIV-1_{BaL}-positive control. One-way ANOVA with Dunnett's multiple-comparison test followed by an unpaired *t* test was used to compare the antibodies with the CH65 isotype controls. ND, not done. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

CH31 IgG and both mIgA2 and dIgA2 was more pronounced, while the activity of some of the nnAbs failed to reach significance (4B3 and 7B2/CH58/CH90) (Fig. 3B). To complement these studies, the ability of antibodies to inhibit *trans*-infection from DC to $CD4^+$ T cells was also assessed. All IgG bnAbs were effective against *trans*-infection. IgA versions were also active in this assay (Fig. 3C), reflective of their activity in TZM-bl cells (Fig. 1A). In contrast, nnAbs and HIV-IG B failed to show significant inhibition, the exception being HIV-IG C, which reduced *trans*-infection by 89.5% (standard deviation [SD], 9.2; $P < 0.01$ [Table 2]).

Inhibitory activity in mucosal tissue explants. To model the activity of antibodies at the mucosal portals of infection, their potential to inhibit direct HIV-1_{BaL} infection of mucosal tissue explant cultures (penile glans, ectocervical, and colorectal) was assessed (24). The location, phenotype, and number of cells expressing the range of FcR and their relative levels of expression across these three different tissue models are described in reference 33. All CD4bs bnAbs were active across the three explant models

TABLE 1 Summary of HIV-1_{BaL} neutralization data in TZM-bl cells and PBMC[a]

Antibody	Antibody Specificity	IC50		IC80		IC90	
		TZM-bl	*PBMC*	*TZM-bl*	*PBMC*	*TZM-bl*	*PBMC*
VRC01 IgG	CD4-binding site	0.015	0.062	0.042	0.465	0.087	1.253
CH31IgG		0.322	0.060	1.621	0.444	6.125	1.643
CH31 mIgA2		2.867	0.439	14.052	6.133	35.205	15.528
CH31 dIgA2		2.671	1.076	11.506	13.287	23.082	27.912
b12 IgG		0.013	0.167	0.036	1.243	0.070	3.638
2F5 IgG	Membrane-Proximal External Region (MPER)	X	20.219	X	23.236	X	25.204
2F5 dIgA		X	ND	X	ND	X	ND
4E10 IgG		X	2.253	X	13.963	X	24.416
2G12 IgG	V3 Glycan	X	8.731	X	18.289	X	24.735
PG9 IgG	V1-V2 Glycan	0.007	0.001	0.019	0.007	0.036	0.019
PG16 IgG		0.011	0.002	0.056	0.536	0.243	9.274
7B2/CH58/CH90	Various (IgG)	X	1.159	X	11.324	X	X
7B2/CH58/CH22		X	1.018	X	18.775	X	X
F240/M785-U1/N10-U1		X	X	X	X	X	X
HIV-IG B	Polyclonal	X	2.984	X	24.393	X	X
HIV-IG C		X	1.573	X	12.045	X	31.537
4B3 IgG	gp41	X	X	X	X	X	X
A32 IgG	CD4i Cluster 1	X	28.256	X	X	X	X
CH65 IgG	Flu Haemagglutinin	X	X	X	X	X	X
CH65 IgA		X	X	X	X	X	X

[a]IC_{50}s, IC_{80}s, and IC_{90}s (in micrograms per milliliter) were determined from neutralization data of HIV-1-specific antibodies in TZM-bl cells (n = 3) or PBMC (n = 3). X, no neutralization seen at 25 μg/ml (antibody combinations) or 50 μg/ml (single antibodies). ND, not done.

(Fig. 4), with statistical significance in penile and cervical tissues. The lack of significance in colorectal tissue, despite major reductions in HIV-1_{BaL} infection, reflects the high variability in levels of infection in the positive controls (Fig. 4 and Table 2). MPER-specific 2F5 IgG also displayed potent inhibition across the different tissue models, as previously described (34); however, 2F5 dIgA failed to show activity in the penile and cervical models. MPER-specific 4E10 appeared to be active only in colorectal tissue (Fig. 4C). The activity of glycan-specific bnAbs was more mixed. PG9 demonstrated good

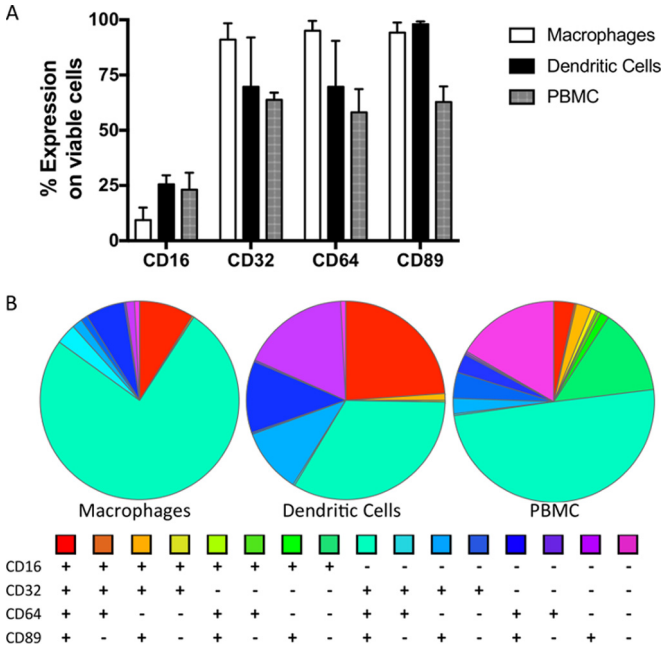


FIG 2 Fc receptor phenotyping of macrophages, dendritic cells, and PBMC used in the cellular inhibition assays. (A) Flow cytometry analysis of the percent expression of CD16, CD32, CD64, and CD89 FcR expression on total viable macrophages, dendritic cells, and PBMC used in the cellular inhibition assays (n = 3). (B) Boolean gating analysis of the FcR expression on total viable cells to show the combinatorial variability of the Fc receptors.

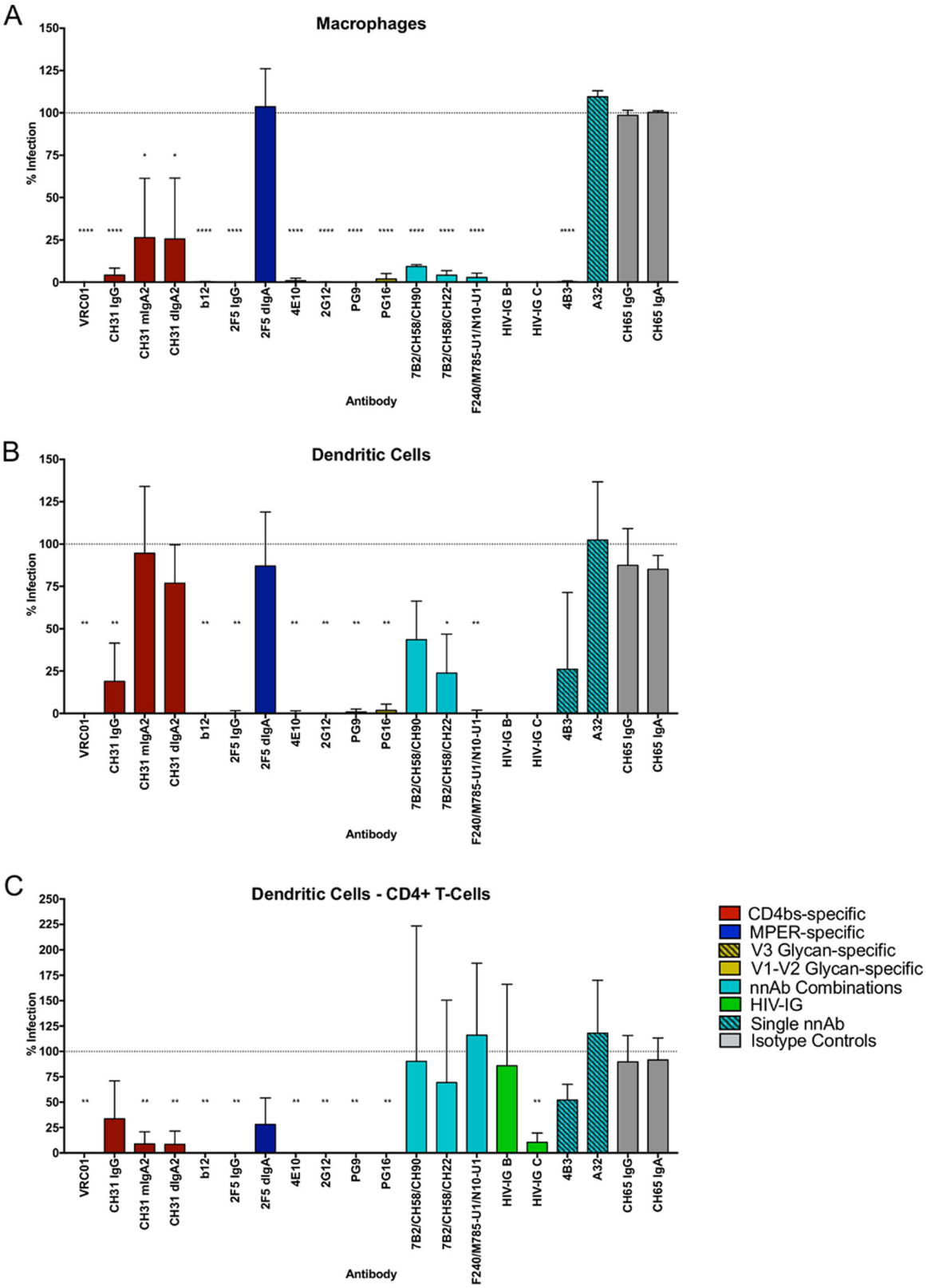
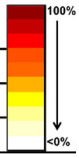


FIG 3 Inhibition of single antibodies and antibody combinations in macrophages, dendritic cells, and DC-to-CD4 T-cell inhibition assays. Shown are results of inhibition of HIV-1_{BaL} by antibody panels (50 μg/ml of single antibodies; 25 μg/ml of each in combinations) in the direct infection of macrophages ($n = 3$) (A) and dendritic cells ($n = 3$) (B) and *trans*-infection of $CD4^+$ T cells from dendritic cells ($n = 3$) (C). (For HIV-IG B and C, $n = 1$ in panels A and B.) Data are presented as percent infection compared to the HIV-1_{BaL}-positive control. One-way ANOVA with Dunnett's multiple-comparison test followed by an unpaired *t* test was used to compare the antibodies with the CH65 isotype controls. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

TABLE 2 Summary of percentage HIV-1_{BaL} inhibition for all inhibition assays[a]

Antibody	Antibody Specificity	Cellular										Tissue						Tissue Migratory Cells-CD4+ T-Cells					
		TZM-bl		*PBMC*		*Macrophages*		*Dendritic Cells*		*DC-T Cells*		*Penile Glans*		*Ectocervical*		*Colorectal*		*Penile Glans*		*Ectocervical*		*Colorectal*	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
VRC01	CD4-binding site	**97.5**	± 0.7	**100.0**	±0.0	**100.0**	±0.0	**100.0**	±0.0	**100.0**	±0.0	**92.5**	±12.9	**95.0**	±5.4	**86.0**	±12.2	**100.0**	±0.0	**100.0**	±0.0	**97.8**	±3.8
CH31IgG		**95.8**	±8.7	**100.0**	±0.0	**95.8**	±4.0	**81.1**	±22.6	66.3	±37.2	**85.4**	±19.3	**92.5**	±13.7	76.1	±41.9	**98.6**	±2.7	**99.7**	±0.4	**100.0**	±0.0
CH31 mIgA2		**92.5**	±4.5	**100.0**	±0.0	73.6	±35.0	5.4	±39.4	**91.0**	±11.8	**84.3**	±15.7	60.7	±33.9	**97.6**	±1.7	**89.4**	±23.8	**100.0**	±0.0	**100.0**	±0.0
CH31 dIgA2		**93.8**	±4.3	**100.0**	±0.0	**74.5**	±36.0	23.1	±22.7	**91.4**	±13.0	78.2	±21.9	62.3	±35.7	69.6	±48.3	**95.7**	±9.6	**99.9**	±0.3	**100.0**	±0.0
b12		**100.0**	±1.0	**98.9**	±1.9	**99.8**	±0.4	**100.0**	±0.0	**100.0**	±0.0	**100.0**	±0.0	**100.0**	±0.0	47.6	±83.9	**100.0**	±0.0	**100.0**	±0.0	**100.0**	±0.0
2F5 IgG	Membrane-Proximal External Region (MPER)	47.0	±13.0	**100.0**	±0.0	**100.0**	±0.3	**100.0**	±2.2	**100.0**	±0.0	**91.8**	±6.3	**84.0**	±11.7	**94.0**	±4.3	**82.3**	±30.7	**86.1**	±12.1	**100.0**	±0.0
2F5 dIgA		11.3	±13.9	ND	ND	-3.7	±22.5	13.0	±31.8	71.9	±26.1	9.3	±46.7	-6.0	±40.9	**91.3**	±7.7	1.1	±13.0	4.0	±64.6	31.6	±50.6
4E10		37.0	±3.6	**100.0**	±0.0	**99.1**	±1.6	**100.0**	±1.8	**100.0**	±0.0	43.3	±87.0	30.2	±50.2	**91.7**	±13.6	28.2	±24.5	55.3	±57.0	-14.6	±112.8
2G12	V3 Glycan	41.0	±3.6	**100.0**	±0.0	**99.9**	±0.1	**100.0**	±0.0	**100.0**	±0.0	41.8	±8.4	71.0	±14.1	**94.3**	±6.2	61.2	±42.2	**86.4**	±16.5	73.7	±27.5
PG9	V1-V2 Glycan	**96.0**	±1.0	**100.0**	±0.0	**100.0**	±1.3	**99.2**	±1.8	**100.0**	±0.0	**90.5**	±18.7	**96.3**	±6.1	35.7	±62.2	-5.5	±60.1	**95.2**	±5.6	28.5	±43.0
PG16		**82.3**	±2.5	75.0	±22.5	**98.1**	±4.1	**98.1**	±3.6	**100.0**	±0.1	30.4	±37.2	45.5	±25.1	11.9	±83.5	-9.4	±9.6	54.2	±53.2	28.5	±54.2
7B2/CH58/CH90	Various	-55.8	±46.2	**87.7**	±2.1	**90.7**	±1.0	56.5	±22.8	9.8	±133.2	6.4	±35.1	11.5	±42.2	10.2	±10.4	6.7	±42.1	-23.6	±19.7	12.9	±45.8
7B2/CH58/CH22		-2.8	±30.7	**82.2**	±5.3	**95.8**	±2.6	**76.2**	±23.0	30.7	±81.2	49.8	±44.6	32.8	±39.0	45.2	±50.0	8.8	±47.7	5.1	±14.3	16.1	±46.9
F240/M785-U1/N10-U1		22.0	±16.6	**41.7**	±16.4	**97.2**	±2.7	**100.0**	±2.4	-16.0	±70.8	45.3	±30.4	51.3	±43.1	**89.7**	±1.9	14.2	±42.1	-26.0	±10.2	25.3	±39.4
HIV-IG B	Polyclonal	**39.7**	±3.2	**91.0**	±3.7	**100.0**	N/A	**100.0**	N/A	14.1	±80.2	**62.5**	±19.5	52.1	±59.5	**96.7**	±4.7	42.8	±32.6	6.4	±7.6	47.8	±41.3
HIV-IG C		**37.3**	±14.3	**94.7**	±1.3	**100.0**	N/A	**100.0**	N/A	**89.5**	±9.2	**59.2**	±25.0	**65.1**	±15.7	**76.1**	±27.4	22.4	±27.1	**33.5**	±5.4	13.7	±28.1
4B3	gp41	-57.3	±30.1	21.4	±31.1	**99.5**	±0.7	73.9	±45.3	47.9	±48.7	6.9	±19.3	**41.8**	±13.3	51.4	±28.8	1.1	±10.6	-18.7	±25.2	-31.7	±47.1
A32	CD4i Cluster 1	**16.0**	±7.2	**83.5**	±7.8	-9.5	±37.1	-2.4	±34.3	-18.0	±52.0	42.3	±26.8	57.7	±40.5	77.5	±49.2	22.2	±30.5	-5.2	±8.6	12.8	±58.6
CH65 IgG	Flu Haemagglutinin	6.3	±33.2	-17.9	±74.6	1.5	±3.0	12.6	±21.7	10.2	±25.9	0.4	±41.4	9.2	±22.9	-9.3	±74.8	7.7	±44.1	-5.1	±9.1	7.5	±27.0
CH65 IgA		-17.3	±6.7	13.8	±49.3	-0.2	±1.1	14.9	±8.2	8.4	±21.6	**41.0**	±19.7	5.3	±4.6	23.9	±51.0	22.2	±11.8	-28.8	±39.2	12.6	±13.3



[a]Percent inhibition of single antibodies (50 μg/ml) and antibody combinations (25 μg/ml each). Means and SDs are included. Highlighted cells indicate inhibition of >2 SD. ND, not done; N/A, not available ($n = 1$).

activity in penile and cervical tissues but poor activity in colorectal explants. In contrast, 2G12 was active only in colorectal tissue, while PG16 was ineffective at preventing infection in any of the tissue models. nnAbs failed to demonstrate significant inhibition in any of the mucosal tissue models (Fig. 4). However, a major reduction of infection was observed for the F240/M785-U1/N10-U1 combination (89.7% ± 1.9% [Table 2]). HIV-IG B and C displayed a range of reductions in infection across the models, reaching significance in cervical (B and C) and colorectal (B only) tissues.

Inhibition of *trans*-infection by mucosal tissue emigrants. A critical step in HIV-1 transmission may be the dissemination of infection beyond the initial foci of primary infection. Using an established model designed to mimic these events (24), cells migrating out of tissue explants following HIV-1_{BaL} viral exposure were collected and cocultured with $CD4^+$ target cells in the presence of antibody (Fig. 5). Across all tissue models, CD4bs-specific antibodies were very efficient at blocking onward transmission of HIV-1_{BaL} to the $CD4^+$ target cells, with levels of inhibition ranging between 89.4 and 100% for IgG and IgA forms. The MPER-specific antibodies were less efficient at inhibiting infection, with only 2F5 IgG demonstrating any significant ability to block onward infection of HIV-1_{BaL} across the three models. Glycan-specific antibodies showed a more diverse ability to inhibit onward infection, where significant reduction was observed only for 2G12 and PG9. nnAbs and HIV-IG B and C failed to inhibit onward infection in any of the migratory tissue cell models (Fig. 5).

DISCUSSION

This study set out to investigate the functional activity of neutralizing and nonneutralizing antibodies in a series of cellular and tissue explant models designed to mimic the early transmission events at the mucosal portals of infection.

CD4bs-specific bnAbs were efficient at inhibiting HIV-1_{BaL} infection across all models. There was little difference in activity between PBMC and TZM-bl assays, suggesting that inhibitory activity was not dependent upon of FcγR engagement. Monomeric and dimeric IgA versions of CH31, unable to engage FcγR, were also active across the models. Furthermore, all CD4bs bnAbs were active against cell-cell transmission in both cellular and tissue models. The observation that VRC01 provided the most consistent inhibition across all infection models underscores the potential importance of the conserved CD4bs as a target for vaccine design (35) and monoclonal prevention strategies (36). These data mirror passive-infusion experiments with nonhuman primates (NHPs) demonstrating potent efficacy against mucosal challenge (36).

V1-V2 glycan-specific bnAbs, PG9 and PG16, demonstrated good levels of inhibition across all cellular models but more variable results in tissue models. PG9 demonstrated good levels of inhibition in penile glans and ectocervical tissues but was less effective

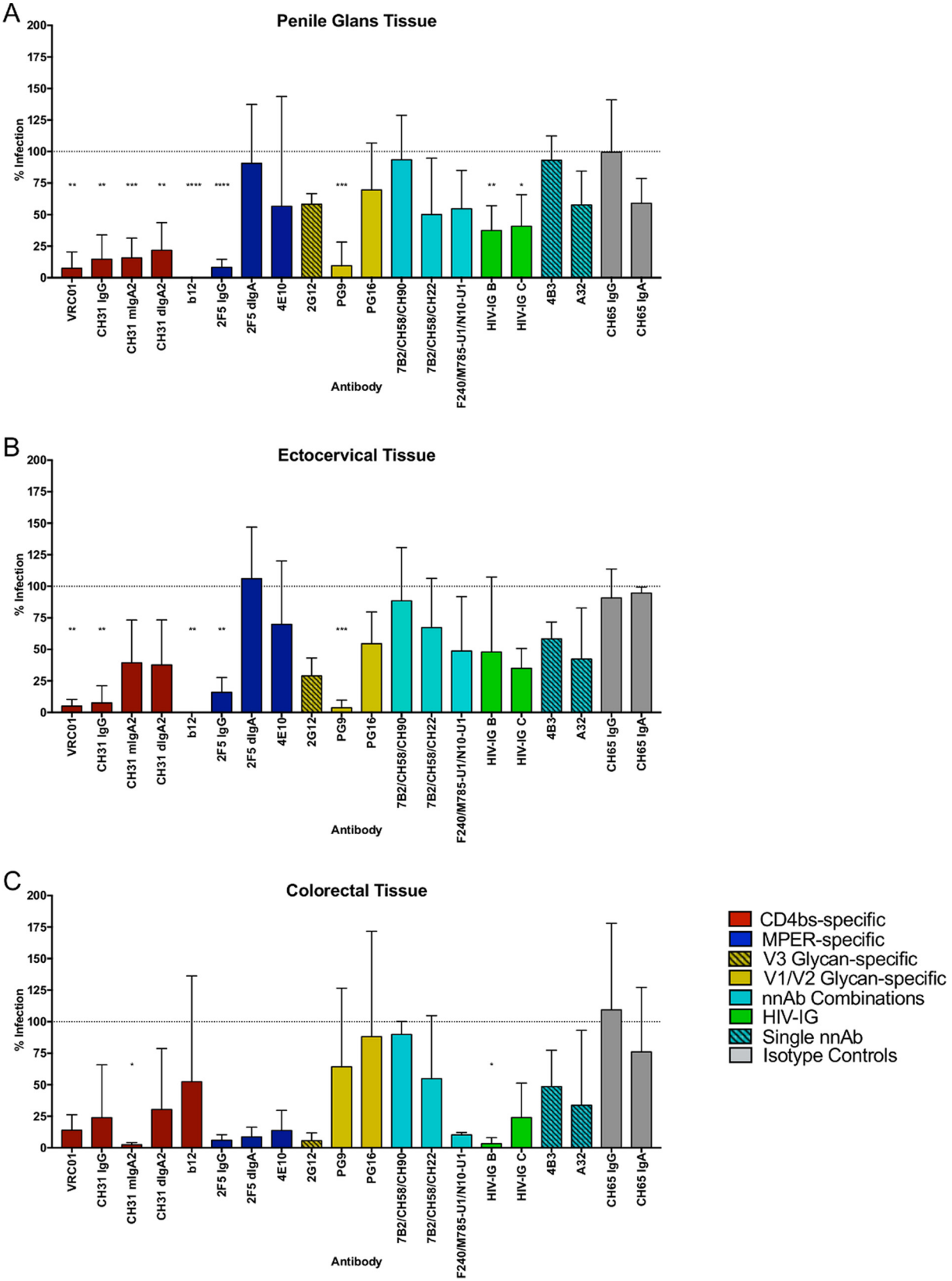


FIG 4 Inhibition by single antibodies and antibody combinations in mucosal tissue explants. Shown are results of inhibition of HIV-1_{BaL} by antibody panels (50 μg/ml of single antibodies; 25 μg/ml of each in combinations) in the direct infection of penile glans ($n = 3$) (A), ectocervical ($n = 2$ for HIV-IG B and C; $n = 3$ for remaining Abs) (B), and colorectal ($n = 3$) (C) tissues. Data are presented as percent infection compared to the HIV-1_{BaL}-positive control. One-way ANOVA with Dunnett's multiple-comparison test followed by an unpaired *t* test was used to compare the antibodies with the CH65 isotype controls. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

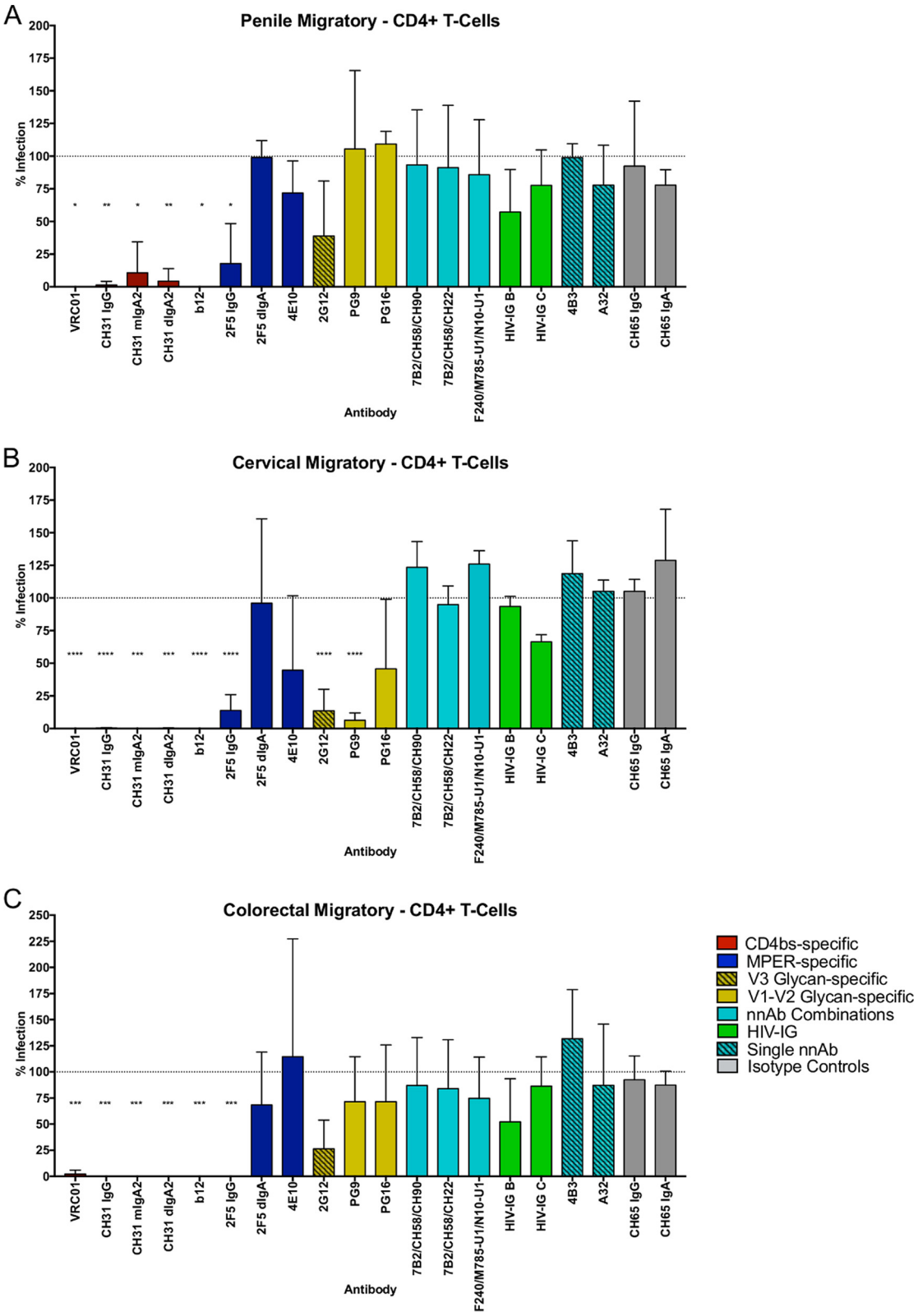


FIG 5 Inhibition by single antibodies and antibody combinations of HIV-1_{BaL} infection from mucosal migratory cells to $CD4^+$ target cells. Shown are results of inhibition of HIV-1_{BaL} by antibody panels (50 μg/ml of single antibodies; 25 μg/ml of each in combinations) in preventing the onward infection of $CD4^+$ T cells by migratory cells isolated from penile glans ($n = 3$) (A), ectocervical ($n = 2$ for HIV-IG B and C; $n = 3$ for remaining Abs) (B) and colorectal ($n = 3$) (C) tissues. Data are presented as percent infection compared to the HIV-1_{BaL}-positive control. One-way ANOVA with Dunnett's multiple-comparison test followed by an unpaired *t* test was used to compare the antibodies with the CH65 isotype controls. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

against direct infection of colorectal tissue and was active only against onward transmission by ectocervical migratory cells. PG16, in contrast, was ineffective against HIV-1_{BaL} infection in all tissue models. Differences across tissues may reflect potential reactivity with mammalian carbohydrates where PG16 binds complex-type glycans more tightly than Man5GlcNAc2, while PG9 prefers Man5GlcNAc2 (37); alternatively, this may reflect known differences in trimer dependence (38, 39): PG9 is able to weakly bind monomeric gp120 in addition to trimeric Env, but PG16 is able to bind only the latter. An alternative *ex vivo* study using colorectal and ectocervical tissue explants demonstrated sustained inhibition of viral replication by PG9 and PG16 in an ectocervical tissue model but loss of viral control within the colorectal tissue after 21 days in culture (40). Significant differences in methodology are likely to explain the variance in the observed levels of inhibition; nevertheless, viral rebound in colorectal tissue reflects the lack of inhibition observed in our study. Heterogeneity in glycosylation of HIV-1 Env leaves these antibodies vulnerable to viral escape (41, 42). The higher levels of HIV-1 replication in colorectal tissue likely enhance the chance of observing viral outgrowth by the proportion of virions not recognized by these glycan-dependent antibodies. The lower potency of PG9 relative to VRC01 mirrors that seen in NHP passive-infusion studies (36).

Interestingly, the V3 glycan-specific antibody 2G12, although performing poorly for classical neutralization in the TZM-bl assay, performed well across all inhibition assays, with the notable exception of penile glans tissue, and exerted similar levels of inhibition against onward transmission by migratory cells across the tissue types. Lack of activity in TZM-bl cells likely reflects the kinetics of 2G12 neutralization (43). The potency of 2G12 in mucosal tissue explants concords with that seen in NHP passive-protection studies (44).

The MPER-specific bnAb 2F5 IgG also performed well across all cellular and tissue models (Table 2), consistent with the results of *in vivo* NHP challenge experiments (34, 45), but was ineffective in the TZM-bl assay. Interestingly, 2F5 dIgA was ineffective in the majority of assays, with the notable exception of colorectal tissue. These observations confirm previous studies showing reduced activity for polymeric dIgA and pIgM isotypes (46, 47) and likely reflect the known influence of FcγR engagement on the neutralizing activity of 2F5 IgG (48). In contrast, MPER-specific 4E10, despite demonstrating good levels of inhibition across cellular models, was only able to inhibit viral infection in colorectal tissue and was inactive against onward transmission by migratory cells. The inhibitory activity of 4E10 in the colorectal tissue is in concordance with NHP studies demonstrating that 4E10 protects against intrarectal challenge with $SHIV_{BaL}$ (45). The weak activity of 4E10 against onward dissemination of virus reflects earlier reports of poor activity against cell-to-cell spread of HIV-1 (49). The lack of activity in the cervical and penile glans tissues is in concordance with similar observations in an alternative cervical tissue model (40). The predictive nature of these observations is unclear given that 4E10, as a single monoclonal antibody, has not been tested against vaginal challenge in the SHIV model. It is also possible that potential polyreactivity for mammalian cells could have influenced activity in these different mucosal models (50).

To assess the potential of Fc-mediated effector functions to block infection and/or onward transmission from mucosal tissue, we selected two individual nnAbs (4B3 and A32) previously reported to show high levels of ADCP and ADCC in *in vitro* assays (26, 27). 4B3 displayed inhibitory activity only in macrophages, as previously described (27), while A32 was active only in PBMC cultures. Critically, neither antibody displayed potent inhibition in any of the mucosal tissue models.

The three nnAb combinations demonstrated variable levels of inhibition in PBMC cultures, good levels of inhibition in macrophage cultures, and again more variable levels in dendritic cell models. These antibodies were less active when assessed individually (data not shown), supporting previous observations that specific antibody combinations synergize for increased antiviral activity (51). Both macrophages and dendritic cells can mediate ADCP, ADCC, and ADCVI (reviewed in reference 52). These

data extend previous observations of the inhibitory potential of 7B2, CH22, and F240 in macrophage and dendritic cell models (28, 53) and likely reflect the high levels of FcγR expression on these *in vitro*-derived cells (Fig. 2). In contrast, none of the nnAb combinations were effective at preventing onward cell-cell transmission by *in vitro*-derived DC or tissue migratory cells. Furthermore, the nnAb combinations were unable to significantly inhibit infection across the three tissue models, with one notable exception in which the third combination (F240/M785-U1/N10-U1) was able to reduce infection of colorectal explants by 89.7% ($\pm$1.9%).

The trend for increased activity with increased polyclonality was also evident for the two polyclonal HIV-IG sera (B and C). While both sera displayed little activity with respect to classical neutralization in the TZM-bl assay, both demonstrated robust activity across PBMC, macrophage, and dendritic cell cultures and variable levels of inhibition in the three tissue models, providing the highest levels of inhibition in colorectal tissue. However, both were ineffective in blocking onward transmission by tissue migratory cells to $CD4^+$ T cells.

These data demonstrate some important features. First, activity in the TZM-bl assay showed a moderate correlation to activity in penile and cervical tissue models ($R^2 = 0.64$ and $P = 0.0001$ and $R^2 = 0.54$ and $P = 0.0007$, respectively). However, there was no correlation ($P < 0.05$; not significant) with activity in the colorectal model. Furthermore, the TZM-bl assay was not fully predictive of inhibition for some specific antibodies, specifically 2F5 and 2G12 being more active and PG9 and PG16 less active in tissue versus TZM-bl cells. Second, activity of nnAbs in PBMC, macrophage, and dendritic cell assays did not translate to equivalent activity in tissue, and there was no correlation for any of these cellular models with activity in tissue. There was, however, an apparent trend for increased inhibition with nnAb polyclonality (HIV-IG > MAb combinations > individual MAbs). Nevertheless, activity of nnAbs in tissue was low to absent, with the notable exceptions of HIV-IG (B and C) and a MAb combination (F240/M785-U1/N10-U1) in colorectal tissue. It is, however, possible that these modest effects might contribute to activity of bnAbs in the context of polyclonal response to vaccination; assessment of bnAbs in combination with nnAbs merits further investigation.

ADCC, ADCP, and ADCVI have been proposed as potential mechanisms by which antibodies (both nAb and nnAbs) might impact transmission events. It is clear from the current study that individual nnAb with known ADCC activity (26), while active in PBMC cultures, were ineffective in mucosal tissues. This likely reflects the very low numbers of resident ADCC effector cells within these tissues (33). Likewise, nnAbs with known potent ADCP and ADCVI activity (26, 27) were also ineffective at protecting mucosal tissue from infection. Again, these results reflect low FcR expression in penile and cervical tissues, while the increased activities of the two HIV-IGs mirror the trend for a higher number of effector cells in colorectal tissue as reported in our recent characterization of FcR expression across these different models (33). Interestingly, the trend for increased antiviral activity in colorectal tissue was also observed for 4E10 IgG and 2F5 dIgA. These data, combined with our earlier studies (33), suggest that resident effector cell numbers and low FcγR expression limit the potential of nnAbs to prevent the initial foci of infection within these mucosal tissue sites. This is perhaps unsurprising given that the predominant targets of infection are $CD4^+$ T cells that lack FcγR expression (23–25). This is compounded by the inability of nnAbs or HIV-IG to block onward cell-cell transmission by migratory cells emigrating from these tissue sites, likely essential in rapid dissemination of infection (54). These data reflect previous cellular studies that demonstrate that nnAbs are ineffective against cell-cell transmission (55). The observed inferiority of nnAbs to block initial mucosal infection is in concordance with the reported inability of nnAb and HIV-IG to prevent mucosal SHIV acquisition in NHP studies (27, 28, 56, 57), contrasting with the sterile protection mediated by a number of bnAbs (58).

A number of limitations should be considered when interpreting this study. First, experiments were performed with a single viral isolate ($HIV\text{-}1_{BaL}$) known to replicate efficiently in primary cells (CD4 T cells, macrophages, and dendritic cells) while univer-

sally targeting CD4 T cells in mucosal models (23, 59, 60). Furthermore, HIV-1_{BaL} is a tier 1B neutralization-sensitive virus; therefore, while the observed superiority of bnAbs in preventing HIV-1 infection will certainly be generally applicable, the activity of individual monoclonal antibodies is likely to vary with different viral isolates and their neutralization sensitivities. Nevertheless, the approach described in this publication provides an important benchmark for evaluation of additional antibodies and viral strains. Second, the high variability in viral replication between tissue donors means that lower, but potentially meaningful, levels of inhibition may not be apparent in this model; further, the maximal concentration of antibody used (50 μg/ml) does not preclude potential inhibition with higher concentrations. Third, all IgG antibodies were expressed within a common recombinant IgG1 isotype. We cannot exclude that expression within a different isotype backbone, in particular IgG3, with a greater potential to engage FcR effector cells (61, 62) might have generated different results. Finally, and perhaps most importantly, the tissue models used in this study assess only the impact of resident effector cells on the establishment of mucosal infection. This precludes the potential influx of effector cells, such as neutrophils, natural killer cells, macrophages, and dendritic cells, in response to the chemotactic signals induced by HIV-1 infection (63). The potential of infiltrating FcγR effector cells to modulate the establishment of infection warrants further investigation. It is of interest to note that R. Astronomo et al. have obtained results similar to those reported here in explant cultures *in vitro* and have confirmed limited protection of nonneutralizing antibodies *in vivo* with high-dose intrarectal challenges (unpublished data). Furthermore, previous NHP studies have suggested that while nnAbs have an ability to limit the number of transmitter/founder viruses (28) and reduced the viral set point postinfection (27), they are inferior to bnAbs in preventing acquisition of infection.

This study provides important mechanistic insight on the differential activities of bnAbs and nnAbs in preventing infection at the mucosal tissue level. The solid protection provided by bnAbs, in particular those targeting the CD4bs, clearly demonstrates their superior potential over nonneutralizing antibodies for preventing HIV-1 infection at the mucosal portals of infection.

MATERIALS AND METHODS

Antibodies and reagents. Antibodies b12, 4E10, 2F5 IgG and dIgA, PG9, PG16, 4B3, and 2G12 were obtained from Polymun Scientific, GmbH (Austria). Expression plasmids for antibody VRC01 were obtained from Dennis Burton, Scripps Research Institute (La Jolla, CA), and antibodies were produced as recombinant IgG1 in 293T cells. A32, 7B2, CH22, CH31, CH58, and CH90 were produced as recombinant IgG1 (also CH31 mIgA2 and dIgA2 [64]) in CHO cells, as previously described (28, 61). A32, 7B2, CH90, and CH22 contained the AAA mutations (S298A, E333A, and K334A) optimized for binding to FcγRIIIa (CD16) and to augment antibody ADCC activity (65). The CH65 isotype control is an IgG1 bnAb recognizing influenza virus hemagglutinin (66). F240, M785-U1, and N10-U1 IgG1 were kindly provided by George Lewis (Institute of Human Virology, Baltimore, MD). HIV-IG B and C were kindly provided by David Montefiori, Duke University. The following reagents were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl cells were from John C. Kappes, Xiaoyun Wu, and Tranzyme Inc; PM-1 cells were from Paulo Lusso and Robert Gallo; and HIV-1_{BaL} was donated by Suzanne Gartner, Mikulas Popovic, and Robert Gallo.

Tissue samples. Ectocervical tissue was acquired from women undergoing planned therapeutic hysterectomy at St. Mary's Hospital (London, United Kingdom). Penile glans tissue was acquired from men undergoing elective gender reassignment surgery at Charing Cross Hospital (London, United Kingdom). Surgically resected specimens of colorectal tissue were collected at St. Mary's Hospital from patients undergoing rectocele repair and colectomy for colorectal cancer. Only healthy tissue obtained 10 to 15 cm away from any tumor was employed.

Ethics statement. Written informed consent was obtained from all donors. All tissues were collected under protocols approved by the Imperial College NHS Trust Tissue Bank and the National Research Ethics Committee in accordance with the Human Tissue Act 2004. Approval for this project was granted by the Imperial College Healthcare Tissue Bank, under their HTA research license, and ethics thus conveyed through this process by the Multi Research Ethics Committee (MREC), Wales.

Cell lines. TZM-bl cells (NIH AIDS Reagent Program) were cultured in complete Dulbecco modified Eagle medium (DMEM) in a 95% humidified incubator with 5% CO_2 at 37°C. PM-1 cells (NIH AIDS Reagent Program) were grown in suspension in complete RPMI medium in a 95% humidified incubator with 5% CO_2 at 37°C.

TZM-bl neutralization assay. TZM-bl cells were utilized to assess the potential neutralizing ability of antibodies to HIV-1_{BaL}. Test antibodies were titrated 1:4 from a starting concentration of 50 μg/ml.

Positive control (virus plus cells only) and negative control (cells only) wells were included in the assay setup. The cells were incubated in a 95% humidified incubator with 5% CO_2 at 37°C for 36 to 72 h.

Postincubation, all supernatant was removed from the cells, which were washed once with 200 μl of phosphate-buffered saline (PBS). Luciferase lysis buffer was diluted 1:5 with distilled water (dH_2O), and 100 μl was added to all wells. Plates were placed at −80°C for at least 2 h to allow for full lysis. Postlysis, the plates were thawed and 50 μl of the lysate was transferred to a white 96-well high-binding plate. Luciferase substrate was reconstituted by adding 10 ml of luciferase buffer to the lyophilized substrate. Fifty microliters of luciferase substrate was added to the lysate and mixed well. Plates were read immediately in relative light units (RLU) using a FLUOstar Omega plate reader (BMG Labtech, United Kingdom). Ninety percent, 80%, and 50% inhibitory concentrations (IC_{90}, IC_{80}, and IC_{50}, respectively) were calculated according to linear regression of the antibody titration using GraphPad Prism7.

PBMC. Peripheral blood mononuclear cells (PBMC) were obtained from leukocyte cones (NHS Blood and Transplant, Collingdale, United Kingdom). Leukocytes were separated by Histopaque (Sigma, United Kingdom) gradient centrifugation. Before HIV-1 infection, PBMC were activated with 5 μg/ml of phytohemagglutinin (PHA; Sigma, United Kingdom) and 10 U/ml of interleukin-2 (IL-2; Novartis, United Kingdom) in complete RPMI medium for 3 days.

Monocyte-derived macrophages (MDM). Freshly isolated PBMC were washed and resuspended at 3×10^6/ml in serum-free AIM-V medium containing 20 ng/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF). Cells were seeded at 3×10^5 in 100 μl in flat-bottomed high-binding 96-well plates and incubated at 37°C (5% CO_2). After 3 to 4 days, fresh serum-free AIM-V medium supplemented with 20 ng/ml of GM-CSF was added and the cells were cultured for a total of 7 days.

Monocyte-derived dendritic cells (MDDC). Freshly isolated PBMC were used to separate mononuclear cells by CD14 positive selection using an AutoMacs separation system. Twenty microliters of CD14 MicroBeads was added per 10^7 cells and incubated for 20 min at 4°C. Cells were washed once with AutoMacs running buffer by centrifugation and resuspended in 2 ml of AutoMacs running buffer for magnetic separation. CD14-positive cells were resuspended in 40 ml of complete RPMI medium supplemented with 30 ng/ml of IL-4 and 25 ng/ml of GM-CSF and cultured for 3 to 4 days at 37°C (5% CO_2). Medium was replaced after 3 to 4 days with fresh complete RPMI medium supplemented with 30 ng/ml of IL-4 and 25 ng/ml of GM-CSF and incubated for a further 3 days at 37°C (5% CO_2).

Flow cytometry staining. MDM, MDDC, or PBMC were stained using a multicolored flow cytometry panel designed to determine Fc receptor expression. Cells were stained with CD3 V450 (UCHT1), CD14 Qdot 605 (T[u]K4) (Invitrogen), CD16 Pacific Orange (3G8) (Invitrogen), CD11c A700 (B-ly6), CD123 phycoerythrin (PE)-Cy5 (9F5), CD32 allophycocyanin (APC) (FLI8.26), CD64 APC H7 (10.1), CD89 PE (A59), and CD19 fluorescein isothiocyanate (FITC) (HIB19). Unless otherwise specified, all antibodies were sourced from BD Biosciences. Dead cells were excluded from analysis through staining with Aqua viability dye (Invitrogen).

Flow cytometry acquisition and analysis. Samples were acquired using an LSRIIFortessa fluorescence-activated cell sorter (FACS) (BD Biosciences) and analyzed using FlowJo (Tree Star, Ashland, OR) and PESTLE and SPICE (National Institute of Allergy and Infectious Diseases, USA). Compensation matrices were created on FlowJo using single-stained anti-mouse Ig, κ/negative-control compensation beads (BD Biosciences).

MDM inhibition assay. Antibodies were prepared at 100 μg/ml of AIM-V medium and incubated 1:1 with cell-free HIV-1_{BaL} (10^4 50% tissue culture infective doses [$TCID_{50}$]) for 1 h at 37°C. The virus-antibody suspension was added to 7-day-old macrophage cultures and incubated for 2 h at 37°C. Postincubation, cells were washed 3 times with PBS and antibodies at 50 μg/ml in 200 μl of AIM-V medium plus 20 ng/ml of GM-CSF. The cells were further incubated for 7 days in a 95% humidified incubator with 5% CO_2 at 37°C. All assays were performed in triplicate and included controls: medium-only, virus-only, and antibody isotype controls at the same concentration as the test antibodies.

MDDC inhibition assay. Antibodies were prepared at 100 μg/ml in complete RPMI medium and incubated 1:1 with cell-free HIV-1_{BaL} (10^4 $TCID_{50}$) for 1 h at 37°C. A total of 4×10^4 MDDC were added in 100 μl of complete RPMI medium to the virus-antibody suspension and incubated overnight 37°C. Postincubation, cells were washed 3 times with PBS by centrifugation and antibodies were added at 50 μg/ml in 200 μl of complete RPMI medium plus 30 ng/ml of IL-4 and 25 ng/ml of GM-CSF. The cells were further incubated for 7 days in a 95% humidified incubator with 5% CO_2 at 37°C. All assays were performed in triplicate and included controls: medium-only, virus-only, and antibody isotype controls at the same concentration as the test antibodies.

MDDC–PM-1 T cell *trans*-infection inhibition assay. MDDC at 2×10^4 per well were incubated with cell-free HIV-1_{BaL} (10^4 $TCID_{50}$) for 1 h at 37°C. Postincubation, MDDC were washed 3 times with PBS by centrifugation and 50 μg/ml of antibody was incubated with the cells for 30 min at 37°C. Postincubation, 4×10^4 PM-1 T cells were added per well and the cells were further incubated for 7 days in a 95% humidified incubator with 5% CO_2 at 37°C. All assays were performed in triplicate and included controls: medium-only, virus-only, and antibody isotype controls at the same concentration as the test antibodies.

Mucosal tissue explant inhibition assay. Antibodies were prepared at 100 μg/ml of complete RPMI medium and incubated 1:1 with cell free HIV-1_{BaL} (2×10^4 $TCID_{50}$) for 1 h at 37°C. Tissue explants were cut to 3 mm^3 and added to the virus/antibody suspension for overnight incubation at 37°C. Postincubation, tissues were washed 3 times with PBS and antibodies were added at 50 μg/ml in 200 μl of complete RPMI medium. The tissues were further incubated for a total of 21 days in a 95% humidified incubator with 5% CO_2 at 37°C, with feeding every 3 to 4 days. All assays were performed in triplicate and included controls: medium-only and antibody isotype controls at the same concentration as the test antibodies.

Mucosal migratory cells. Post-overnight infection, migratory cells were collected from the tissue explants and washed three times with PBS by centrifugation (67, 68). The cells were further incubated with 50 μg/ml of antibody and 4×10^4 PM-1 T cells for 7 days in a 95% humidified incubator with 5% CO_2 at 37°C.

Detection of p24. p24 content in culture supernatant was measured using an enzyme-linked immunosorbent assay (ELISA) (AALTO, IRE) or by a high-sensitivity RETROTEK p24 ELISA kit (Gentaur), where lower levels of p24 were produced.

Statistical analysis. Graphs show mean values with standard deviation error bars. One-way analysis of variance (ANOVA) followed by unpaired *t* test with Dunnett's correction was used to compare the different antibodies with the CH65 isotype control antibodies. All statistical analyses were performed using Prism 7 (GraphPad Software, Inc., La Jolla, CA).

ACKNOWLEDGMENTS

We acknowledge the generosity of Dietmar Katinger (Polymun, Scientific, Vienna, Austria) for providing monoclonal antibodies. We thank George Lewis and Yongjun Guan (Institute of Human Virology, Maryland) for provision of M785-U1/N10-U1 MAbs. We thank D. Burton for supplying various antibody expression constructs. We thank David Montefiori for HIV-IG B and C. We thank genitourinary surgeons Tina Rashid, Phillip Thomas, and Ronald Morley (Charing Cross Hospital, London, United Kingdom) for providing the penile glans tissue.

Research in this publication was supported by the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Division of AIDS (DAIDS), the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, grant number UM1-AI100645-01, and Bill and Melinda Gates Foundation grants 1033098 and 1040758. We gratefully acknowledge an equipment grant from Dormeur Investment Service Ltd. that provided funding to purchase equipment used in these studies.

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REFERENCES

1. Pantophlet R, Burton DR. 2006. GP120: target for neutralizing HIV-1 antibodies. Annu Rev Immunol 24:739–769. https://doi.org/10.1146/annurev.immunol.24.021605.090557.
2. Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT. 2014. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS 28:163–169. https://doi.org/10.1097/QAD.0000000000000106.
3. Klein F, Diskin R, Scheid JF, Gaebler C, Mouquet H, Georgiev IS, Pancera M, Zhou T, Incesu RB, Fu BZ, Gnanapragasam PN, Oliveira TY, Seaman MS, Kwong PD, Bjorkman PJ, Nussenzweig MC. 2013. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. Cell 153:126–138. https://doi.org/10.1016/j.cell.2013.03.018.
4. Alter G, Moody MA. 2010. The humoral response to HIV-1: new insights, renewed focus. J Infect Dis 202(Suppl 2):S315–S322. https://doi.org/10.1086/655654.
5. Richard J, Veillette M, Ding S, Zoubchenok D, Alsahafi N, Coutu M, Brassard N, Park J, Courter JR, Melillo B, Smith AB, III, Shaw GM, Hahn BH, Sodroski J, Kaufmann DE, Finzi A. 2016. Small CD4 mimetics prevent HIV-1 uninfected bystander CD4+ T cell killing mediated by antibody-dependent cell-mediated cytotoxicity. EBioMedicine 3:122–134. https://doi.org/10.1016/j.ebiom.2015.12.004.
6. Stieh DJ, King DF, Klein K, Aldon Y, McKay PF, Shattock RJ. 2015. Discrete partitioning of HIV-1 Env forms revealed by viral capture. Retrovirology 12:81. https://doi.org/10.1186/s12977-015-0207-z.
7. Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, Lanigan CM, Landucci G, Forthal DN, Parren PW, Marx PA, Burton DR. 2007. Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449:101–104. https://doi.org/10.1038/nature06106.
8. Gautam R, Nishimura Y, Pegu A, Nason MC, Klein F, Gazumyan A, Golijanin J, Buckler-White A, Sadjadpour R, Wang K, Mankoff Z, Schmidt SD, Lifson JD, Mascola JR, Nussenzweig MC, Martin MA. 2016. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. Nature 533:105–109. https://doi.org/10.1038/nature17677.
9. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 361:2209–2220. https://doi.org/10.1056/NEJMoa0908492.
10. Veillette M, Richard J, Pazgier M, Lewis GK, Parsons MS, Finzi A. 2016. Role of HIV-1 envelope glycoproteins conformation and accessory proteins on ADCC responses. Curr HIV Res 14:9–23.
11. von Bredow B, Arias JF, Heyer LN, Moldt B, Le K, Robinson JE, Zolla-Pazner S, Burton DR, Evans DT. 2016. Comparison of antibody-dependent cell-mediated cytotoxicity and virus neutralization by HIV-1 Env-specific monoclonal antibodies. J Virol 90:6127–6139. https://doi.org/10.1128/JVI.00347-16.
12. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 366:1275–1286. https://doi.org/10.1056/NEJMoa1113425.
13. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, Bonsignori M, Moody MA, Fong Y, Chen X, Poling B, Nicholson CO, Zhang R, Lu X, Parks R, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Gilbert PB, Kim JH, Michael NL, Montefiori DC, Haynes BF. 2013. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc Natl Acad Sci U S A 110:9019–9024. https://doi.org/10.1073/pnas.1301456110.
14. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang ZY, Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter A, Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD. 2014. Vaccine-induced Env V1-V2 IgG3 correlates with

lower HIV-1 infection risk and declines soon after vaccination. Sci Transl Med 6:228ra239.
15. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, Gilbert PB, Huang Y, Gurley TC, Kozink DM, Marshall DJ, Whitesides JF, Tsao CY, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Kim JH, Michael NL, Tomaras GD, Montefiori DC, Lewis GK, DeVico A, Evans DT, Ferrari G, Liao HX, Haynes BF. 2012. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 86:11521–11532. https://doi.org/10.1128/JVI.01023-12.
16. Forthal DN, Landucci G, Daar ES. 2001. Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. J Virol 75:6953–6961. https://doi.org/10.1128/JVI.75.15.6953-6961.2001.
17. Sips M, Krykbaeva M, Diefenbach TJ, Ghebremichael M, Bowman BA, Dugast AS, Boesch AW, Streeck H, Kwon DS, Ackerman ME, Suscovich TJ, Brouckaert P, Schacker TW, Alter G. 2016. Fc receptor-mediated phagocytosis in tissues as a potent mechanism for preventive and therapeutic HIV vaccine strategies. Mucosal Immunol https://doi.org/10.1038/mi.2016.12.
18. Wu L. 2008. Biology of HIV mucosal transmission. Curr Opin HIV AIDS 3:534–540. https://doi.org/10.1097/COH.0b013e32830634c6.
19. Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, McElrath MJ. 2007. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity 26:257–270. https://doi.org/10.1016/j.immuni.2007.01.007.
20. Harman AN, Kim M, Nasr N, Sandgren KJ, Cameron PU. 2013. Tissue dendritic cells as portals for HIV entry. Rev Med Virol 23:319–333. https://doi.org/10.1002/rmv.1753.
21. Rinaldo CR. 2013. HIV-1 trans infection of CD4+ T cells by professional antigen presenting cells. Scientifica 2013:30.
22. Turville SG, Santos JJ, Frank I, Cameron PU, Wilkinson J, Miranda-Saksena M, Dable J, Stossel H, Romani N, Piatak M, Jr, Lifson JD, Pope M, Cunningham AL. 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood 103:2170–2179. https://doi.org/10.1182/blood-2003-09-3129.
23. Peters PJ, Gonzalez-Perez MP, Musich T, Moore Simas TA, Lin R, Morse AN, Shattock RJ, Derdeyn CA, Clapham PR. 2015. Infection of ectocervical tissue and universal targeting of T-cells mediated by primary non-macrophage-tropic and highly macrophage-tropic HIV-1 R5 envelopes. Retrovirology 12:48. https://doi.org/10.1186/s12977-015-0176-2.
24. King DF, Siddiqui AA, Buffa V, Fischetti L, Gao Y, Stieh D, McKay PF, Rogers P, Ochsenbauer C, Kappes JC, Arts EJ, Shattock RJ. 2013. Mucosal tissue tropism and dissemination of HIV-1 subtype B acute envelope-expressing chimeric virus. J Virol 87:890–899. https://doi.org/10.1128/JVI.02216-12.
25. Stieh DJ, Matias E, Xu H, Fought AJ, Blanchard JL, Marx PA, Veazey RS, Hope TJ. 2016. Th17 cells are preferentially infected very early after vaginal transmission of SIV in macaques. Cell Host Microbe 19:529–540. https://doi.org/10.1016/j.chom.2016.03.005.
26. Ferrari G, Pollara J, Kozink D, Harms T, Drinker M, Freel S, Moody MA, Alam SM, Tomaras GD, Ochsenbauer C, Kappes JC, Shaw GM, Hoxie JA, Robinson JE, Haynes BF. 2011. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. J Virol 85:7029–7036. https://doi.org/10.1128/JVI.00171-11.
27. Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, Van Ham G, Heyndrickx L, Van Dorsselaer A, Katinger D, Vcelar B, Zolla-Pazner S, Mangeot I, Kelly C, Shattock RJ, Le Grand R. 2014. Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. Mucosal Immunol 7:46–56. https://doi.org/10.1038/mi.2013.23.
28. Santra S, Tomaras GD, Warrier R, Nicely NI, Liao HX, Pollara J, Liu P, Alam SM, Zhang R, Cocklin SL, Shen X, Duffy R, Xia SM, Schutte RJ, Pemble Iv CW, Dennison SM, Li H, Chao A, Vidnovic K, Evans A, Klein K, Kumar A, Robinson J, Landucci G, Forthal DN, Montefiori DC, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Robb ML, Michael NL, Kim JH, Soderberg KA, Giorgi EE, Blair L, Korber BT, Moog C, Shattock RJ, Letvin NL, Schmitz JE, Moody MA, Gao F, Ferrari G, Shaw GM, Haynes BF. 2015. Human non-neutralizing HIV-1 envelope monoclonal antibodies limit the number of founder viruses during SHIV mucosal infection in rhesus macaques. PLoS Pathog 11:e1005042. https://doi.org/10.1371/journal.ppat.1005042.
29. Liu P, Williams LD, Shen X, Bonsignori M, Vandergrift NA, Overman RG, Moody MA, Liao H-X, Stieh DJ, McCotter KL, French AL, Hope TJ, Shattock R, Haynes BF, Tomaras GD. 2014. Capacity for infectious HIV-1 virion capture differs by envelope antibody specificity. J Virol 88:5165–5170. https://doi.org/10.1128/JVI.03765-13.
30. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, Moody MA, Bonsignori M, Ochsenbauer C, Kappes J, Tang H, Greene K, Gao H, LaBranche CC, Andrews C, Polonis VR, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Self SG, Berman PW, Francis D, Sinangil F, Lee C, Tartaglia J, Robb ML, Haynes BF, Michael NL, Kim JH. 2012. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. J Infect Dis 206:431–441. https://doi.org/10.1093/infdis/jis367.
31. Ding S, Veillette M, Coutu M, Prevost J, Scharf L, Bjorkman PJ, Ferrari G, Robinson JE, Sturzel C, Hahn BH, Sauter D, Kirchhoff F, Lewis GK, Pazgier M, Finzi A. 2016. A highly conserved residue of the HIV-1 gp120 inner domain is important for antibody-dependent cellular cytotoxicity responses mediated by anti-cluster A antibodies. J Virol 90:2127–2134. https://doi.org/10.1128/JVI.02779-15.
32. Pollara J, Bonsignori M, Moody MA, Pazgier M, Haynes BF, Ferrari G. 2013. Epitope specificity of human immunodeficiency virus-1 antibody dependent cellular cytotoxicity [ADCC] responses. Curr HIV Res 11:378–387. https://doi.org/10.2174/1570162X113116660059.
33. Cheeseman HM, Carias AM, Evans AB, Olejniczak NJ, Ziprin P, King DF, Hope TJ, Shattock RJ. 2016. Expression profile of human Fc receptors in mucosal tissue: implications for antibody-dependent cellular effector functions targeting HIV-1 transmission. PLoS One 11:e0154656. https://doi.org/10.1371/journal.pone.0154656.
34. Klein K, Veazey RS, Warrier R, Hraber P, Doyle-Meyers LA, Buffa V, Liao HX, Haynes BF, Shaw GM, Shattock RJ. 2013. Neutralizing IgG at the portal of infection mediates protection against vaginal simian/human immunodeficiency virus challenge. J Virol 87:11604–11616. https://doi.org/10.1128/JVI.01361-13.
35. Georgiev IS, Gordon Joyce M, Zhou T, Kwong PD. 2013. Elicitation of HIV-1-neutralizing antibodies against the CD4-binding site. Curr Opin HIV AIDS 8:382–392. https://doi.org/10.1097/COH.0b013e328363a90e.
36. Pegu A, Yang ZY, Boyington JC, Wu L, Ko SY, Schmidt SD, McKee K, Kong WP, Shi W, Chen X, Todd JP, Letvin NL, Huang J, Nason MC, Hoxie JA, Kwong PD, Connors M, Rao SS, Mascola JR, Nabel GJ. 2014. Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. Sci Transl Med 6:243ra288.
37. Pancera M, Shahzad-Ul-Hussan S, Doria-Rose NA, McLellan JS, Bailer RT, Dai K, Loesgen S, Louder MK, Staupe RP, Yang Y, Zhang B, Parks R, Eudailey J, Lloyd KE, Blinn J, Alam SM, Haynes BF, Amin MN, Wang LX, Burton DR, Koff WC, Nabel GJ, Mascola JR, Bewley CA, Kwong PD. 2013. Structural basis for diverse N-glycan recognition by HIV-1-neutralizing V1-V2-directed antibody PG16. Nat Struct Mol Biol 20:804–813. https://doi.org/10.1038/nsmb.2600.
38. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, Wrin T, Simek MD, Fling S, Mitcham JL, Lehrman JK, Priddy FH, Olsen OA, Frey SM, Hammond PW, Kaminsky S, Zamb T, Moyle M, Koff WC, Poignard P, Burton DR. 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326:285–289. https://doi.org/10.1126/science.1178746.
39. Sanders RW, Derking R, Cupo A, Julien JP, Yasmeen A, de Val N, Kim HJ, Blattner C, de la Pena AT, Korzun J, Golabek M, de Los Reyes K, Ketas TJ, van Gils MJ, King CR, Wilson IA, Ward AB, Klasse PJ, Moore JP. 2013. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog 9:e1003618. https://doi.org/10.1371/journal.ppat.1003618.
40. Scott YM, Park SY, Dezzutti CS. 2016. Broadly neutralizing anti-HIV antibodies prevent HIV infection of mucosal tissue ex vivo. Antimicrob Agents Chemother 60:904–912. https://doi.org/10.1128/AAC.02097-15.
41. McCoy LE, Falkowska E, Doores KJ, Le K, Sok D, van Gils MJ, Euler Z, Burger JA, Seaman MS, Sanders RW, Schuitemaker H, Poignard P, Wrin T, Burton DR. 2015. Incomplete neutralization and deviation from sigmoidal neutralization curves for HIV broadly neutralizing monoclonal antibodies. PLoS Pathog 11:e1005110. https://doi.org/10.1371/journal.ppat.1005110.
42. Huskens D, Van Laethem K, Vermeire K, Balzarini J, Schols D. 2007. Resistance of HIV-1 to the broadly HIV-1-neutralizing, anti-carbohydrate

antibody 2G12. Virology 360:294–304. https://doi.org/10.1016/j.virol.2006.10.027.
43. Platt EJ, Gomes MM, Kabat D. 2012. Kinetic mechanism for HIV-1 neutralization by antibody 2G12 entails reversible glycan binding that slows cell entry. Proc Natl Acad Sci U S A 109:7829–7834. https://doi.org/10.1073/pnas.1109728109.
44. Hessell AJ, Rakasz EG, Poignard P, Hangartner L, Landucci G, Forthal DN, Koff WC, Watkins DI, Burton DR. 2009. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog 5:e1000433. https://doi.org/10.1371/journal.ppat.1000433.
45. Hessell AJ, Rakasz EG, Tehrani DM, Huber M, Weisgrau KL, Landucci G, Forthal DN, Koff WC, Poignard P, Watkins DI, Burton DR. 2010. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. J Virol 84:1302–1313.
46. Wolbank S, Kunert R, Stiegler G, Katinger H. 2003. Characterization of human class-switched polymeric (immunoglobulin M [IgM] and IgA) anti-human immunodeficiency virus type 1 antibodies 2F5 and 2G12. J Virol 77:4095–4103. https://doi.org/10.1128/JVI.77.7.4095-4103.2003.
47. Tudor D, Yu H, Maupetit J, Drillet AS, Bouceba T, Schwartz-Cornil I, Lopalco L, Tuffery P, Bomsel M. 2012. Isotype modulates epitope specificity, affinity, and antiviral activities of anti-HIV-1 human broadly neutralizing 2F5 antibody. Proc Natl Acad Sci U S A 109:12680–12685. https://doi.org/10.1073/pnas.1200024109.
48. Perez LG, Zolla-Pazner S, Montefiori DC. 2013. Antibody-dependent, FcgammaRI-mediated neutralization of HIV-1 in TZM-bl cells occurs independently of phagocytosis. J Virol 87:5287–5290. https://doi.org/10.1128/JVI.00278-13.
49. Malbec M, Porrot F, Rua R, Horwitz J, Klein F, Halper-Stromberg A, Scheid JF, Eden C, Mouquet H, Nussenzweig MC, Schwartz O. 2013. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. J Exp Med 210:2813–2821. https://doi.org/10.1084/jem.20131244.
50. Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, Kepler TB, Alam SM, Liao H-X, Cain DW, Spicer L, VandeBerg JL, Haynes BF, Kelsoe G. 2013. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. J Exp Med 210:241–256. https://doi.org/10.1084/jem.20121977.
51. Pollara J, Bonsignori M, Moody MA, Liu P, Alam SM, Hwang KK, Gurley TC, Kozink DM, Armand LC, Marshall DJ, Whitesides JF, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Robb ML, O'Connell RJ, Kim JH, Michael NL, Montefiori DC, Tomaras GD, Liao HX, Haynes BF, Ferrari G. 2014. HIV-1 vaccine-induced C1 and V2 Env-specific antibodies synergize for increased antiviral activities. J Virol 88:7715–7726. https://doi.org/10.1128/JVI.00156-14.
52. Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. 2014. The function of Fcγ receptors in dendritic cells and macrophages. Nat Rev Immunol 14:94–108. https://doi.org/10.1038/nri3582.
53. Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, Aubertin AM, Moog C. 2006. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. J Virol 80:6177–6181. https://doi.org/10.1128/JVI.02625-05.
54. Gummuluru S, Pina Ramirez NG, Akiyama H. 2014. CD169-dependent cell-associated HIV-1 transmission: a driver of virus dissemination. J Infect Dis 210(Suppl 3):S641–S647.
55. Su B, Xu K, Lederle A, Peressin M, Biedma ME, Laumond G, Schmidt S, Decoville T, Proust A, Lambotin M, Holl V, Moog C. 2012. Neutralizing antibodies inhibit HIV-1 transfer from primary dendritic cells to autologous CD4 T lymphocytes. Blood 120:3708–3717. https://doi.org/10.1182/blood-2012-03-418913.
56. Dugast AS, Chan Y, Hoffner M, Licht A, Nkolola J, Li H, Streeck H, Suscovich TJ, Ghebremichael M, Ackerman ME, Barouch DH, Alter G. 2014. Lack of protection following passive transfer of polyclonal highly functional low-dose non-neutralizing antibodies. PLoS One 9:e97229. https://doi.org/10.1371/journal.pone.0097229.
57. Nakane T, Nomura T, Shi S, Nakamura M, Naruse TK, Kimura A, Matano T, Yamamoto H. 2013. Limited impact of passive non-neutralizing antibody immunization in acute SIV infection on viremia control in rhesus macaques. PLoS One 8:e73453. https://doi.org/10.1371/journal.pone.0073453.
58. Moldt B, Le KM, Carnathan DG, Whitney JB, Schultz N, Lewis MG, Borducchi EN, Smith KM, Mackel JJ, Sweat SL, Hodges AP, Godzik A, Parren PW, Silvestri G, Barouch DH, Burton DR. 2016. Neutralizing antibody affords comparable protection against vaginal and rectal simian/human immunodeficiency virus challenge in macaques. AIDS 30:1543–1551. https://doi.org/10.1097/QAD.0000000000001102.
59. Shen R, Meng G, Ochsenbauer C, Clapham PR, Grams J, Novak L, Kappes JC, Smythies LE, Smith PD. 2011. Stromal down-regulation of macrophage CD4/CCR5 expression and NF-kappaB activation mediates HIV-1 non-permissiveness in intestinal macrophages. PLoS Pathog 7:e1002060. https://doi.org/10.1371/journal.ppat.1002060.
60. Merbah M, Arakelyan A, Edmonds T, Ochsenbauer C, Kappes JC, Shattock RJ, Grivel JC, Margolis LB. 2012. HIV-1 expressing the envelopes of transmitted/founder or control/reference viruses have similar infection patterns of CD4 T-cells in human cervical tissue ex vivo. PLoS One 7:e50839. https://doi.org/10.1371/journal.pone.0050839.
61. Tay MZ, Liu P, Williams LD, McRaven MD, Sawant S, Gurley TC, Xu TT, Dennison SM, Liao HX, Chenine AL, Alam SM, Moody MA, Hope TJ, Haynes BF, Tomaras GD. 2016. Antibody-mediated internalization of infectious HIV-1 virions differs among antibody isotypes and subclasses. PLoS Pathog 12:e1005817. https://doi.org/10.1371/journal.ppat.1005817.
62. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daeron M. 2009. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113:3716–3725. https://doi.org/10.1182/blood-2008-09-179754.
63. Haase AT. 2010. Targeting early infection to prevent HIV-1 mucosal transmission. Nature 464:217–223. https://doi.org/10.1038/nature08757.
64. Zhang R, Alam SM, Yu JS, Scearce R, Lockwood B, Hwang KK, Parks R, Permar S, Brandtzaeg P, Haynes BF, Liao HX. 2016. Novel monoclonal antibodies for studies of human and rhesus macaque secretory component and human J-chain. Monoclon Antib Immunodiagn Immunother 35:217–226. https://doi.org/10.1089/mab.2016.0014.
65. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B, Fox JA, Presta LG. 2001. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem 276:6591–6604. https://doi.org/10.1074/jbc.M009483200.
66. Whittle JR, Zhang R, Khurana S, King LR, Manischewitz J, Golding H, Dormitzer PR, Haynes BF, Walter EB, Moody MA, Kepler TB, Liao HX, Harrison SC. 2011. Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. Proc Natl Acad Sci U S A 108:14216–14221. https://doi.org/10.1073/pnas.1111497108.
67. Fletcher P, Kiselyeva Y, Wallace G, Romano J, Griffin G, Margolis L, Shattock R. 2005. The nonnucleoside reverse transcriptase inhibitor UC-781 inhibits human immunodeficiency virus type 1 infection of human cervical tissue and dissemination by migratory cells. J Virol 79:11179–11186. https://doi.org/10.1128/JVI.79.17.11179-11186.2005.
68. Hu Q, Frank I, Williams V, Santos JJ, Watts P, Griffin GE, Moore JP, Pope M, Shattock RJ. 2004. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. J Exp Med 199:1065–1075. https://doi.org/10.1084/jem.20022212.