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Live Simian Immunodeficiency Virus Vaccine Correlate of Protection: Local Antibody Production and Concentration on the Path of Virus Entry

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We sought design principles for a vaccine to prevent HIV transmission to women by identifying correlates of protection conferred by a highly effective live attenuated SIV vaccine in the rhesus macaque animal model. We show that SIVmac239 nef vaccination recruits plasma cells and induces ectopic lymphoid follicle formation beneath the mucosal epithelium in the rhesus macaque female reproductive tract. The plasma cells and ectopic follicles produce IgG Abs reactive with viral envelope glycoprotein gp41 trimers, and these Abs are concentrated on the path of virus entry by the neonatal FcR in cervical reserve epithelium and in vaginal epithelium. This local Ab production and delivery system correlated spatially and temporally with the maturation of local protection against high-dose pathogenic SIV vaginal challenge. Thus, designing vaccines to elicit production and concentration of Abs at mucosal frontlines could aid in the development of an effective vaccine to protect women against HIV-1.

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Although there have been substantial and continuing advances in preventing HIV-1 infection, an effective HIV-1 vaccine is still critically needed, particularly to prevent transmission to the young women who bear the brunt of infection in the pandemic’s epicenter in Africa (1, 2). To that end, we have been seeking design principles to guide HIV-1 vaccine development by identifying correlates of the robust protection afforded by the live attenuated SIVmac239 nef vaccine (3–7) against high-dose vaginal challenge in the SIV rhesus macaque (RM) model of HIV-1 transmission to women.

To identify correlates of protection in this animal model in vivo within the female reproductive tract (FRT) tissues, we investigated the very early stages of infection and the maturation of SIVmac239 nef-associated protection for two reasons. First, exist-
ing immune responses or responses that can be deployed rapidly in early infection would be working at favorable odds against the small infected founder populations established at the portal of entry (1). Thus, a correlate of protection might be recognizable as local innate or adaptive immune mechanisms that could inhibit the establishment and expansion of these infected founder populations. Second, SIV\textsubscript{mac239}Δnef vaccination’s protective effects mature over time. Animals are not significantly protected if challenged at 5 wk following vaccination, whereas there is sterilizing protection or attenuated infection with challenges at $\geq 15$ wk after vaccination (8). Thus, protection would be expected to correlate with immune responses that increase between 5 wk and later challenge. We show in this study that one striking correlate of the maturation of protection at the portal of entry between 5 and 20 wk postvaccination is a vaccine-induced system to locally produce IgG Abs reactive with the SIV envelope glycoprotein gp41 and to concentrate these Abs on the path of virus entry by the neonatal FcR (FcRn) (9) operating in mucosal epithelium.

**Materials and Methods**

**Animals, vaccination, and vaginal challenge**

FRT tissue correlates of protection were analyzed in tissues collected and archived in a cross-sectional serial necropsy study of 19 SIV\textsubscript{mac239}Δnef-vaccinated female RM monkeys (*Macaca mulatta*); 8 of the 19 animals were necropsied at 5 and 20 wk postvaccination (n = 4 animals/group) to assess the maturation of protection in FRT tissues. The remaining 11 animals were necropsied at 4, 5, 7, or 11 d post–high-dose vaginal challenge at 20 wk postvaccination. The postchallenge archived tissues from the 11 vaccinated animals were compared with FRT tissues from 11 unvaccinated animals that were collected and archived from days 4 to 10 following vaginal challenge (10). Protection also was evaluated in a separate longitudinal study (R.K. Reeves, manuscript in preparation) in which vaccinated animals that were collected and archived from days 4 to 10 post–high-dose vaginal challenge mature over time. Animals are not significantly protected if challenged at 5 wk following vaccination, whereas there is sterilizing protection or attenuated infection with challenges at $\geq 15$ wk after vaccination (8). Thus, protection would be expected to correlate with immune responses that increase between 5 wk and later challenge. We show in this study that one striking correlate of the maturation of protection at the portal of entry between 5 and 20 wk postvaccination is a vaccine-induced system to locally produce IgG Abs reactive with the SIV envelope glycoprotein gp41 and to concentrate these Abs on the path of virus entry by the neonatal FcR (FcRn) (9) operating in mucosal epithelium.

**Real-time RT-PCR to determine SIV\textsubscript{mac239}Δnef and wild-type SIV\textsubscript{mac251} viral loads in tissues and plasma**

RT-PCR assays were performed, as described, with specific primers to determine the levels of SIV\textsubscript{mac239}Δnef and SIV\textsubscript{mac251} but with modifications for tissue RNA to accommodate the higher amounts and complexity of input RNA (14).

**Isolation of Abs from tissues and cervical vaginal fluids, IgG and IgA determinations, and Western blot analysis**

Snap-frozen tissues were homogenized in T-PER tissue protein extraction reagent with 1$\times$ Halt Protease Inhibitor, and the protein layer was collected by centrifugation. Cervical vaginal fluids were extracted from Weck-Cel sponges by eluting with 0.25% BSA and 10% IGEPAL (15). IgG and IgA in sera and tissue extracts were measured using ELISA commercial kits (Immunology Consultants Laboratory, Portland, OR). After subtracting diluent samples in the ELISA plate, the plates were washed, incubated with anti-human IgG/IgA-HRP, washed again, and developed with tetramethylbenzidine substrate; the absorbance was measured at 450 nm. SIV-specific Abs were detected in Western blot (WB) strips, normalized for total protein in the tissue extracts and sera of individual animals, and developed under conditions that maximized visualization of SIV-specific Ags with the least background. SIV-specific bands were quantified using the Bio-Rad GS-700 densitometer system, expressed as a percentage of the SIV+ control band assayed concurrently, and normalized for the IgG concentrations of the tissue Abs.

**Solvable trimeric gp41**

The recombinant gp41 ectodomain (Supplemental Fig. 2) was expressed in 293F cells from a phCMV plasmid that included gp160 residues 554–676 (SIV\textsubscript{mac239} numbering), which was flanked N-terminally by an IgG signal sequence to target the protein to the endoplasmic reticulum for glycosylation, disulfide bond formation, and secretion and C-terminally by a strep tag for affinity purification from Freestyle 293 media using Strep-Tactin resin. Avidin was added to the filtered media to block biotin before loading onto the column. The column was washed with PBS, and protein was eluted with 2.5 mM dethiobiotin. The eluent was loaded on a Superdex 200 column to purify a 66-kDa protein from higher molecular mass aggregates ($\sim$1 mg from 2 l). This size is consistent with a trimeric quaternary structure, likely the six-helix bundle, which was solved previously by X-ray crystallography and nuclear magnetic resonance spectroscopy (16) (inset, Supplemental Fig. 2b).

**Reverse immunohistochemistry**

For reverse immunohistochemistry staining to detect gp41-specific Ab in cells, the heat step for Ag retrieval was omitted to avoid nuclear staining artifacts. After deparaffinization and rehydration, tissue sections were blocked overnight at 4°C with SNIPER Blocking Reagent and then with an Avidin/Biotin Blocking Kit to minimize binding to endogenous biotin. Sections were incubated with Strep-tagII–labeled gp41 Ag, followed by incubation with anti-Strep-tagII mAb conjugated to HRP. Sections were then stained using DAB as substrate, counterstained with Harris hematoxylin, and mounted in Permount. As specificity controls, the Strep-tagII mAb alone did not stain plasma cells or reserve epithelium and, in combination with the Strep-tagII–labeled gp41, it did not stain these cells in FRT tissues from animals not infected with SIV.

**Neutralization assays**

Protease inhibitors and the nondenaturing detergents in the tissue extracts were removed by ultrafiltration (100 kDa) and replaced by RPMI 1640 containing 10% normal rhesus sera. Tissue extracts and sera were serially diluted (10-fold) in RPMI 1640 containing 10% normal rhesus sera as a source of complement, which was reported to increase neutralization activity in sera from RMs vaccinated with SIV\textsubscript{mac239}Δnef (17). Diluted samples were coincubated with tissue culture laboratory-adapted (TCLA) SIV\textsubscript{mac239}Δnef, SIV\textsubscript{mac251} 32H , or SIV\textsubscript{mac251} vaginal challenge stock at 37°C for 1 h before the addition of C8166-45-SEAP reporter cells (multiplicity of infection = 0.05). The secreted alkaline phosphatase activities were measured after 72 h of incubation (18).
In vitro culture model assessment of potential protective mechanisms

The SIV transcytosis assay was modified from Shen et al. (19). A total of $10^6$ HEC-1A cells was seeded on 24-Transwell polycarbonate permeable membranes to establish a tight, polarized monolayer of HEC-1-A cells, and monolayers were used only when the transepithelial electrical resistance was $\approx 700 \, \Omega \cdot \text{cm}^2$. Ab-mediated blockade of transcytosis was measured in cervical tissue extracts from vaccinated RMs; sera from vaccinated RMs or a chronically infected RM; uninfected RM sera; serum from the chronically infected RM plus staphylococcal protein A (SA) as a competitive inhibitor of FcRn-mediated transport of IgG (20, 21); and the rhesus monoclonal 4.9c Ab that reacted with oligomeric gp41 identically to the Abs in the cervical tissues. Dilutions were added to the basolateral...
FIGURE 2. Increased SIV-gp41 IgG Abs correlate with the temporal maturation of protection. Each lane in the WBs represents an individual animal. (a, c, and d) Increases, between 5 and 20 wk, in IgG Abs in cervical vaginal tissue extracts or fluids reacting with oligomeric Env gp41 Ags - gp160, gp80, and two other glycoproteins of lower molecular mass. Lanes marked α-SIV indicate positive-control polyclonal Ab; serum control indicates that sample was loaded. (b) Rhesus mAbs to gp41 and gp120 identify prominent Env Ag bands as oligomeric gp41. The band at ~31 kDa is thought to be a truncated species of gp41. The WB lane with a rhesus mAb 4.9C, which reacts identically to cervical tissue Abs to oligomeric gp41, is indicated by a red box and arrow. (e) Similar increases and SIV reactivities in IgG in serum. (f) Cervical vaginal tissue and serum controls in tissues not infected with SIV.
and apical compartments of the Transwell and incubated with HEC-1-A cells for 24 h. Thereafter, SIV mac251 32H was added to the apical chamber of the Transwell. Transcytosis was assessed after 6 and 24 h by measuring p27 Ag in the basal chamber with a SIV p27 ELISA kit. Inhibition of SIV transcytosis was expressed as the percentage of p27 Ag recovered in the basal chamber in the presence of Abs from different sources compared with the amount of p27 Ag recovered in the presence of uninfected RM serum (defined as 100% transcytosis efficiency). To measure intracellular
SIV p27, HEC-1-A cells were washed extensively with PBS and lysed, and SIV p27 in the intracellular extracts was measured with the SIV p27 ELISA kit. For the analysis of intracellular Ab location, HEC-1-A cells on the transmembrane were fixed briefly with SafeFix, permeabilized with 1% Triton X-100, and immunofluorescently stained for IgG and TOTO-3. The transmembranes with the HEC-1-A cells were harvested and mounted on microscope slides using Aqua Poly/Mount. Immunofluorescent micrographs were taken using an Olympus FV1000 FluoView confocal microscope; images were acquired, and mean fluorescence intensity was analyzed using Olympus FluoView software (version 1.7a).

Statistical methods

To test for differences between groups, pairwise two-sample, equal variance t tests were conducted after logarithmically transforming the data (because errors for intensity measurements are typically multiplicative) and adding the value 1 to all observations (because some measurements are 0). Differences were deemed significant at $p < 0.05$. All computations were done using statistical software R, version 2.10.1. Figs. 6 and 8 were created in GraphPad Prism. For Fig. 8g, colocalization of pixels was measured with Pearson correlation coefficients, as computed using software for the confocal microscope.

Results

Vaccination effects in the early stages of infection

SIVΔnef vaccination was associated with inhibition of the establishment and local expansion of small founder populations of SIV RNA+ cells in the TZ and adjoining endocervix (10–12). In naive unvaccinated controls, SIV RNA+ cells were shown to be detectable as early as 3 or 4 d following high-dose vaginal exposure to wild-type (WT) SIVmac251. These founder populations greatly expand between days 7 and 10–14, at the peak of local replication (Fig. 1a, 1c) (11, 12). In striking contrast, SIV RNA+ cells were detected only rarely in the vaccinated animals at days 4 and 5 postvaginal exposure to high doses of WT SIVmac251 at 20 wk postvaccination, and there was no evidence of local expansion (Fig. 1b, 1d). We quantified these differences from exponential expansion to peak replication by PCR determinations of VLs and found that VLs in cervix and vagina were reduced from an average of $5.8 \times 10^5$ copies/μg tissue RNA in four unvaccinated animals (10) to undetectable levels in the cervical tissues of 6/6 vaccinated animals and in the vaginal tissues of 3/6 vaccinated animals and an average of 50 copies/μg in 3/6 animals (Fig. 1e, 1f).

IgG Abs to oligomeric gp41 correlate with maturation of protection

We hypothesized that SIV-specific Abs at the portal of entry prior to challenge are at the “right place and time” to account for the rapid inhibition of establishment and expansion of infected founder populations just described; therefore, we looked for prechallenge Abs reactive with gp41t at 20 wk in cervical reserve epithelium, plasma cells, and ectopic follicles. Sgp41t (Supplemental Fig. 2) with a Strep-tag for detection was used in RHIC to stain cells with Abs reacting with sgp41t. (a and b) Brown-stained reserve epithelium beneath lining epithelium (arrows). Scale bars, 200 μm (a), 50 μm (b). (c) Brown-stained gp41+ Ab+ reserve epithelium (double-headed arrows) and plasma cells (circled). Scale bar, 20 μm. (d) High-power view of stained cervical reserve epithelium (arrow) just below the columnar epithelium lining the cervix. Scale bar, 20 μm. (e and f) Background staining in two naive controls. Arrows point to cervical reserve epithelium. Scale bars, 50 μm.
Abs that increased from 5 to 20 wk as a correlate of the maturation of the protective effects of vaccination. Consistent with this hypothesis, we detected predominantly IgG Abs reactive with SIV oligomeric gp41 in WBs prior to WT SIVmac251 vaginal challenge that increased significantly ($p < 0.01; 5$-fold) between 5 and 20 wk in cervical tissues, vaginal tissues, and cervical vaginal tissues and fluids (CVF) (Fig. 2a, 2c, 2d). The gp160 and gp80 bands with which the Abs reacted most strongly in CVF and in serum (Fig. 2e) were shown, two decades ago, to be oligomeric complexes of gp41 (22–24). This conclusion was further supported by reactivity with rhesus mAbs to gp41, but not gp120 (25) (Fig. 2b) and, indeed, identical reactivity with oligomeric gp41 in WBs of mAb 4.9C and Abs in the FRT (red box, Fig. 2b). Cervical gp41-IgA Abs reacting mainly with oligomeric gp41 also increased between 5 and 20 wk in the cervical vaginal tissue extracts, CVF, and serum (data not shown). However, because IgA concentrations were 150–200-fold lower than IgG concentrations (Supplemental Table I), we subsequently focused on the role that IgG Abs might be playing in the temporal maturation of protection.

Cervical IgG Ab production and delivery

We initially stained sections to see whether IgG Abs might be detectable at the tissue sites where the inhibition of viral replication had been observed. We discovered, by this simple approach, that the numbers of IgG$^+$ cells in the cervix increased in the vaccinated animals compared with unvaccinated SIV$^-$ controls and between 5 and 20 wk in the vaccinated animals (Fig. 3a, 3b; Supplemental...
Fig. 1a). The IgG+ cells in the submucosa were identified as plasma cells by their morphology and by staining for a plasma cell marker, CD138 (26) (data not shown), whereas the IgG+ cells just beneath the columnar epithelium lining the cervix were identified as epithelial reserve cells (27) by their epithelial morphology, location in the TZ and adjoining endocervix (Fig. 3b), and the colocalization of IgG staining with the reserve cell marker (28), cytokeratin 5 (CK5) (Fig. 3c, 3d).

The images of the location of IgG in the reserve epithelium struck us as somewhat similar to the images of FcRn in murine and human uterine and vaginal epithelium (29). Therefore, we hypothesized that the RM cervical reserve epithelium might express FcRn+, and we tested this hypothesis by staining cervical sections with Ab to FcRn. We found that IgG and FcRn colocalized in CK5+ cervical reserve epithelium (Fig. 3e–h). IgG+-staining plasma cells increased ~2.5-fold and cervical reserve epithelium increased ~10-fold between 5 and 20 wk (Fig. 3i).

Local trimeric gp41 Ab production and delivery

Thus, we had visualized a potentially generic system for local production and concentration of IgG Abs in the TZ of the cervix. To show that such a system concentrated gp41-specific IgG Abs at the portal of entry, we used reverse immunohistochemistry (RIHC) (30) to stain cells containing gp41 Abs. For RIHC staining, we generated a soluble trimeric gp41 (sgp41t) ectodomain recombinant protein, which was designed to mimic in vivo Ags, such as trimeric gp41 (gp41t) six-helical bundles (“stumps”) left in the viral membrane following gp120 shedding (31). The sgp41t protein retained cluster I and II immunodominant epitopes (32, 33) but lacked the hydrophobic transmembrane domain, the membrane proximal external region, and fusion peptide regions of gp41 (Supplemental Fig. 2). With this reagent, we found that gp41t Abs were readily detectable in plasma cells and FcRn+ cervical reserve cells (Fig. 4 a–d) in 4/4 animals at 20 wk but only faint staining was seen compared with naive controls (Fig. 4e, 4f) in four animals at 5 wk (Fig. 5). The increased staining of reserve epithelium at 20 wk compared with 5 wk is consistent with the increased gp41t+ plasma cells in the submucosa, increased Abs to gp41t present in the tissues and CVF (Fig. 2), and maturation of protection.

Vaginal IgG Ab production and delivery

We next showed that there is a system in the vagina that locally produces and concentrates IgG Abs comparable to the one in the endocervix. In the vagina, IgG+ cells beneath the epithelium increased in the vaccinated animals compared with unvaccinated SIV– controls (Supplemental Fig. 1b) between 5 and 20 wk (Fig. 6a, 6b). The IgG+ cells were identified as plasma cells by their morphology and by staining with the plasma cell marker CD138 (Supplemental Fig. 3). In addition, ectopic tertiary lymphoid...
follicles in the vagina, which contained IgG+ and gp41t Ab+ plasma cells (Fig. 6c, 6d), increased in number by 9-fold between 5 and 20 wk (Fig. 6e). Because murine vaginal epithelial cells are a known source of chemokines that recruit CTLs to the FRT (34), we asked whether epithelium might be a source of chemokines to recruit plasma cells in RMs. We found that expression of CXCL10 at 20 wk in vaginal multilayered squamous epithelium (Fig. 6f) was associated with recruitment of CXCR3+ plasma cells to vaginal submucosa and into follicles (Fig. 6g, 6h). Similarly, CXCL10 expression increased in endocervical epithelium between 5 and 20 wk as a mechanism to recruit virus that has traversed the mucosal barrier to that depth.

**Potential protective mechanisms for gp41t Abs in the FRT**

Thus, the gp41t Abs are located at the right time and right place to intercept virus at entry and immediately contain infection by a number of mechanisms. These include classical extracellular neutralization (35) and intracellular neutralization (36), in which virus captured by IgG bound to FcRn is diverted from the early endosome pathway and degraded in lysosomes, as well as virus binding and capture (37). We investigated neutralization in vitro in assays using unheated sera as a source of complement (17). The 20-wk sera neutralized TCLA SIVmac251 at a higher titer than did the 5-wk sera (Fig. 7a). However, activity against the somewhat more neutralization-resistant SIVmac251-32H strain or the challenge stock of SIVmac251 was weaker and did not differ significantly between 5 and 20 wk. We interpret reductions in viral infectivity of only 50% over a broad range of dilutions at 20 wk as plateau effects of a neutralization-resistant fraction of virus (Fig. 7b, 7c).

Virus capture and intracellular neutralization were assayed in FcRn+ HEC-1-A cells in a Transwell system (19) to mimic, in vitro, these activities in cervical reserve and vaginal epithelium (Fig. 8a). We tested sera from a chronically infected animal as a positive control in this assay, as well as the rhesus mAb 4.9C, because of the similar reactivity of that Ab and anti-oligomeric gp41 Abs in WBs (Fig. 2b). IgG and FcRn colocalized in an early endosomal pathway in this assay system (Fig. 8b), as did IgG from the positive-control sera and SIV (Fig. 8c). A significantly greater fraction of the SIV captured by positive-control sera compared with sera from uninfected animals was diverted to LAMP1+ lysosomes for degradation (Fig. 8d, 8g), as described for intracellular neutralization (36). Positive-control sera, 20-wk sera and cervical tissue extracts, and mAb 4.9C all significantly decreased transcytosis in this system, whereas sera from uninfected animals and 5-wk sera and tissue extracts did not (Fig. 8e, 8f). We showed that the FcRn-translocation pathway was involved in capture and transcytosis by reversing these activities with SA binding of IgG, which acts as a competitive inhibitor of FcRn-mediated transport (Fig. 8e, 8f) (20, 21).

**Discussion**

As one of the interfaces with the external environment, the mucosa of the FRT has evolved multiple mechanisms to protect against pathogens. In this article, we describe a spatial and temporal correlate of the maturation of protection against high-dose vaginal challenge conferred by vaccination with an effective live attenuated vaccine.

We found that vaccination with SIVΔnef induced an organized system of local Ab production and concentration that correlated with reduction in VLs in the FRT tissues to undetectable or very low.
levels in vaccinated animals compared with unvaccinated animals. In the cervix, local production of gp41t IgG Abs by plasma cells underlying FcRn+ cervical reserve epithelium acts as a system to deliver and concentrate these Abs at a critical site and time where establishment and expansion of infected founder populations precede production of sufficient virus and infected cells to disseminate and rapidly establish a robust systemic infection (1, 10–12). In the vagina, Ab production and delivery by plasma cells and ectopic follicles underlying FcRn+ basal epithelium would similarly concentrate gp41t Abs as a second line of defenses to prevent viruses traversing the multilayered squamous epithelial barrier from infecting target cells in the underlying submucosa. There are four important components of this Ab correlate of local protection induced by SIVΔ nef vaccination (Fig. 9): recruitment of
plasma cells and formation of ectopic follicles; local production of gp41t Abs; FcRn-mediated mechanisms in cervical reserve and basal vaginal epithelium concentrate SIV-specific IgG Ab to intercept virus on entry and inhibit the necessary interactions with target cells to establish infection at the portal of entry; and an active role for the mucosal epithelium lining the cervix and vagina in recruiting plasma cells and increasing ectopic follicles, as well as concentrating Abs by FcRn-mediated mechanisms.

One critical feature of this system is locating a production source in close proximity to the delivery system. In principle, this feature overcomes diffusion constraints for Abs coming from the circulation to achieve the high local concentration of Abs to intercept virus at entry by mass action mechanisms we next describe. We also note in this study that direct visualization of gp41t staining of reserve epithelium is consistent with the idea that we can see IgG Abs in the cells because the high local concentrations exceed the normal FcRn flux rate for IgG of multiple specificities.

Ab production and concentration in the FRT could likely protect through multiple cooperative, complementary, and nonexclusive mechanisms. We think that the most important of these mechanisms is an example of the general principle of concentration-dependent protection at mucosal frontlines. This concept provides a theoretical framework to understand the increased efficacy of protection against acquisition by passive immunization with more potent, new-generation mAbs against HIV (38), as well as the importance of the FcRn in protecting mice against HSV-2 infection (29). For SIV\_D\_nef, we think that this principle applies through the cooperative “mass action” effects of the high local concentrations of reagents—Ab and virus—in the small volume where they would first interact at the portal of entry. These concentration-dependent effects might amplify in vivo what appear, from in vitro assays, to be relatively weak extracellular neutralization, and they might similarly amplify protective mechanisms related to virus binding and trapping to prevent access to susceptible target cells (39).

FIGURE 9. Model of local production and FcRn-mediated delivery of Abs and effects of anti-gp41t Abs at the cervical (upper panel) and vaginal (lower panel) mucosal borders. In the TZ and endocervix, IgG Abs to gp41t are produced by plasma cells. These Abs are collected efficiently because of their spatial proximity to FcRn\(^+\) cervical reserve epithelium and, thereby, are concentrated on the path of virus entry. The concentrated Abs can interfere by several potential mechanisms, with the establishment and expansion of infected founder populations. In both the vagina and cervix, mucosal epithelial expression of CXCL10 recruits CXCR3\(^+\) plasma cells. In the vagina, ectopic follicles are induced, and Abs are produced by plasma cells in the submucosa and follicles. FcRn expression in the basal epithelium concentrates these IgG Abs on the path of virus that has traversed the multilayer epithelium lining the vagina and ectocervix to prevent infection of target cells in the submucosa.
What are the similarities and differences between this system of local Ab production and concentration as a correlate of SIV\textsuperscript{\Delta nef}’s robust protection and other potential mechanisms of protection? First, vaginal IgG Abs with neutralizing or Ab-dependent cell-mediated cytotoxicity (ADCC) activity were shown to protect against vaginal simian HIV challenge (40). However, these Abs were elicited by immunization with a gp41-\textsuperscript{peptide} from which the immunodominant cluster I epitope had been deleted. In contrast, IgG Abs to gp41\textsuperscript{peptide} elicited by SIV\textsuperscript{\Delta nef} are more reminiscent of the HIV-1 mAb F240 (35), which strongly binds to the cluster I epitope. Moreover, ADCC activity and NK cell and macrophage numbers as ADCC effectors in cervical vaginal tissues do not correlate with the maturation of SIV\textsuperscript{\Delta nef} protection (M. Alpeter, D. Evans, et al. unpublished observations; 41).

Second, the FcRn pathway has been used to enhance Ag presentation and the B and T cell responses in the mouse to HIV-Gag, as well as deliver passively transferred Abs that increased protection against HSV-2 infection (29, 42, 43). However, there is no evidence that these strategies reproduced the SIV\textsuperscript{\Delta nef} system of local Ab production and concentration. Lastly, a new chemokine “pull” strategy for recruiting T cells to the genital tract was shown to local protection against HSV-2 in mice (44). In contrast, we do not find an increase in SIV-specific CD8 T cells shown to enhance local protection against HSV-2 in mice (44). In contrast, we do not find an increase in SIV-specific CD8 T cells shown to enhance local protection against HSV-2 in mice (44). In contrast, we do not find an increase in SIV-specific CD8 T cells shown to enhance local protection against HSV-2 in mice (44).


