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## Live SIV vaccine correlate of protection: immune complex-inhibitory Fc receptor interactions that reduce target cell availability

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### Abstract

Principles to guide design of an effective vaccine against HIV are greatly needed, particularly to protect women in the pandemic's epicentre in Africa. We have been seeking these principles by identifying correlates of the robust protection associated with SIV<sub>mac239</sub> nef vaccination in the SIV-rhesus macaque animal model of HIV-1 transmission to women. We have identified one correlate of SIV<sub>mac239</sub> nef protection against vaginal challenge as a resident mucosal system for SIV-gp41 trimer antibody production and neonatal Fc receptor (FcRn)-mediated concentration of these antibodies on the path of virus entry to inhibit establishment of infected founder populations at the portal of entry. Here we identify as a second protection correlate, blocking CD4<sup>+</sup> T cell

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recruitment to inhibit local expansion of infected founder populations. Virus-specific immune complex interactions with the inhibitory Fc $\gamma$ RIIb receptor in the epithelium lining the cervix initiate expression of genes that block recruitment of target cells to fuel local expansion. Immune complex-Fc $\gamma$ RIIb receptor interactions at mucosal frontlines to dampen the innate immune response to vaginal challenge could be a potentially general mechanism for the mucosal immune system to sense and modulate the response to a previously encountered pathogen. Designing vaccines to provide protection without eliciting these transmission-promoting innate responses could contribute to developing an effective HIV-1 vaccine.

## Introduction

While there have been advances in preventing HIV-1 infection (1-3), an effective vaccine is urgently needed, particularly to prevent infection in the high-risk populations of women in sub-Saharan Africa that continue to drive the pandemic's progress. To that end, we have been seeking design principles to guide development of such a vaccine by identifying correlates of the robust protection afforded by live-attenuated SIV vaccines in the SIV-rhesus macaque model of HIV-1 transmission to women (4-6). In the accompanying paper, we identified one such correlate of protection conferred by SIV<sub>mac239</sub> nef vaccination in an organized system of antibody production and concentration at mucosal frontlines on the path of virus entry. We found that vaccination induces production by plasma cells in the submucosa and ectopic follicles of IgG antibodies reactive with a trimeric form of the viral envelope gp41 glycoprotein (gp41t). These antibodies are concentrated by neonatal Fc receptor (FcRn)-mediated mechanisms as the spatial and temporal correlate of preventing or restricting the establishment of founder populations of infected cells as the antecedents for a robust, systemic infection.

In this paper, we show that a second Fc receptor, the inhibitory Fc receptor for IgG, Fc $\gamma$ RIIb, also plays an important role in the protection conferred by SIV<sub>mac239</sub> nef vaccination. Virus-specific immune complexes (ICs), formed by reaction of challenge virus with vaccine-induced antibodies, interact with the inhibitory Fc $\gamma$ RIIb receptor in the columnar epithelium lining the cervix. This interaction elicits expression of genes that down-regulate the innate immune and inflammatory response to the viral inoculum, which in turn disrupts the outside in signaling that in naïve animals recruits CD4<sup>+</sup> T cells to the portal of entry where they then fuel local expansion of any foci of infected cells initially established there. Thus, a second correlate of SIV<sub>mac239</sub> nef IgG antibody protection is reducing target cell availability through engagement of Fc $\gamma$ RIIb-mediated inhibitory pathways.

## Materials and Methods

### Animals, vaccination, and vaginal challenge

The 9 SIV<sub>mac239</sub>-Nef vaccinated female rhesus macaque monkeys (*Macaca mulatta*) described in this study were housed at the New England Primate Center in accordance with the regulations of the American Association of Accreditation of Laboratory Animal Care and the standards of the Association for Assessment and Accreditation of Laboratory

Animal Care International. Animals were vaccinated by infecting intravenously with SIV<sub>mac239</sub>-Nef supplied by Dr. Ronald C. Desrosiers. At 20 wk post vaccination, these animals were challenged vaginally twice in the same day (separated by 4 h) with 10<sup>5</sup> TCID<sub>50</sub> of SIV<sub>mac251</sub> supplied by Dr. Christopher J. Miller. The 3 naïve, uninfected animals and 5 unvaccinated controls for the same time points were from a previous study<sup>8</sup> that had been similarly exposed vaginally with an identical stock of SIV<sub>mac251</sub>.

### Tissue collection and processing

At the time of euthanasia, tissues were collected and fixed in 4% paraformaldehyde or SafeFix II and embedded in paraffin for later sectioning. To examine the critical anatomical niches in the female reproductive tract, the uterus, cervix, and vagina were dissected en block. The relevant region of cervix was dissected away from most of the uterus and vagina and then further divided into four quadrants. Tissue pieces from each quadrant were either snap-frozen, fixed as described, or used unfixed for other assays.

### RNA extraction, synthesis of biotin-labeled cRNA probes, and microarray hybridization

These methods were performed as previously described (7).

### Microarray data analysis

The R package “affy” was used to generate robust multi-chip average (RMA) values for all arrays based on the CEL files produced by the Affymetrix data analysis platform. Some samples had multiple arrays; hence the mean RMA values for those samples were computed and used in the subsequent analysis. The RMA algorithm produces a summary of gene expression on a log scale; we used this log scale for all analyses. To test for differences across groups, weighted least squares with an effect for group was used (weights were used because some RMA values were the mean of multiple measurements). The resulting p-values were converted to q-values using the q-value package in R in order to look for genes of interest. Genes were selected based on their associated q-values (< 0.2) and fold change in expression (> 1.2).

### Immunohistochemistry

Immunohistochemistry was performed as previously described (8). The following antibodies with the indicated specificities were used: FCGR1A/CD64 EPR4623 (LifeSpan LS-B6707); FCGR2A/CD32a (Sigma HPA010718); FCGR2A/CD32a 19C10 (Novus Biologicals NBP1-47740); FCGR2B/CD32b (Abcam, catalog # ab45143, rabbit mAb, lot # GR32165-2; Abnova, catalog # H00002213-B01P, mouse pAb, lot # 08338 WULZ); FCGR2C/CD32c (Abgent AP8992b); CD16 2H7 (Novocastra NCL-CD16) These and other antibodies used are described in more detail in Supplemental Table 1.

### Ex vivo organ culture

FRT tissue was removed from healthy, uninfected, adult Rhesus macaques (Worldwide Primates Inc., Miami, FL) and shipped overnight on wet ice. Cervix was isolated and dissected into small tissue pieces (5-10 mm in diameter × 5-10 mm in thickness) that were placed, mucosal surfaces exposed, atop hydrated collagen sponges (hydrated in RPMI-1640

supplemented with 10% FCS and penicillin/streptomycin) in individual wells of 6-well tissue culture plates. Most experiments required explants to be incubated for 24 h at 37°C / 5% CO<sub>2</sub>. For experiments requiring longer incubation times, fresh complete medium was added drop-wise to hydrate tissue pieces twice a day. At the conclusion of the experiment, tissue pieces were fixed in SafeFix II before embedding in paraffin.

For immune complex formation,  $1.2 \times 10^5$  Infectious Units of SIV<sub>mac251 32H</sub> were combined with excess (~ 7µg) antibody (gp41 monoclonal antibody 4.9C or serum from SIV<sub>mac239</sub>-Nef-vaccinated animals) and incubated for 60 min at room temperature before application to explants. As a control,  $1.2 \times 10^5$  Infectious Units of SIV<sub>mac251 32H</sub> was similarly admixed with serum from unvaccinated animals that had not been infected with SIV. For blocking FcγRIIb, explants were pre-incubated with 5 µg each of two FcγRIIb blocking antibodies (Abcam, catalog # ab45143, rabbit mAb, lot # GR32165-2; Abnova, catalog # H00002213-B01P, mouse pAb, lot # 08338 WULZ) for 30 min at 37°C / 5% CO<sub>2</sub> before addition of immune complexes.

### Statistics

Associations between continuous variables involving the four animal groups [uninfected, unvaccinated, vaccinated (prior to challenge), and challenge] were estimated using Pearson's correlation coefficient (after transforming to the log scale to stabilize the variance), and tests were conducted using the usual t test for a correlation. Differences in gene activity between vaccinated and unvaccinated animals were tested using a 2-sample t test for proportions (after transforming to the logarithmic scale to stabilize the variance). All calculations were conducted using the statistical software R, version 2.10.1.

### Accession number

The microarray data sets were submitted to the GEO repository according to Minimum Information about a Microarray Experiment standards (GEO accession number: GSE33251). The URL for the data sets is <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33251>.

## Results

### Disrupted outside-in signaling and CD4<sup>+</sup> T cell recruitment in vaccinated animals

In unvaccinated animals, the transition zone of ectocervix and adjoining endocervix are consistently sites where small founder populations of infected cells are first detected, and where these populations rapidly expand, fueled by an influx of CD4<sup>+</sup> target cells. These target cells are recruited by an outside-in signaling circuit that begins with increased expression of MIP3-α in the lining epithelium (Fig. 1a,c,e, and model at bottom of the figure). MIP3-α then recruits CCR6<sup>+</sup> plasmacytoid dendritic cells (pDCs), and the β-chemokines such as MIP1-β that the pDCs elaborate recruit CD4<sup>+</sup> T cells to fuel local expansion (10-12). In vaccinated animals, this CD4<sup>+</sup> T cell recruitment and outside-in signaling cascade was disrupted (Fig. 1b,d,f). There was no detectable influx of CD4<sup>+</sup> T cells as a correlate of the inhibition of establishment and local expansion of infected founder populations at the portal of entry in vaccinated animals shown in the accompanying paper.

## Mucosal epithelial system of expression of genes that inhibit the innate immune response to vaginal challenge

We sought clues to the mechanism of disrupted signaling and target cell recruitment comparing the transcriptional profiles of the responses in unvaccinated and vaccinated animals to high dose SIV vaginal challenge. To generate these comparisons, we determined changes in transcript levels between animals that had not been exposed to SIV (n = 3) and unvaccinated animals exposed intra-vaginally to WT SIV<sub>mac251</sub> (n = 5) (transcriptional profile I, Fig. 2) and then compared vaccinated animals before WT SIV challenge (n = 4) with vaccinated animals after intra-vaginal challenge (n = 5) (transcriptional profile II, Fig. 2). We identified 405 transcripts significantly altered 4-5 days post exposure in the cervix of unvaccinated animals infected with WT SIV<sub>mac251</sub> while only 246 transcripts were altered in SIV<sub>mac239</sub><sup>-</sup> Nef-vaccinated animals when challenged with the same virus and exposure (Fig. 2). Remarkably, there was almost no overlap in host response between unvaccinated and vaccinated animals exposed vaginally to WT SIV (~ 1% overlap, 5 transcripts in common, Fig. 2).

In examining the proportion of RNA transcripts ascribed to a particular biological/functional category, we found that in the vaccinated animals there was significant enrichment of host response genes involved in moderating innate immunity and inflammation (Fig. 3a). These genes encode proteins involved in inhibiting production of type-I IFN (i.e., PCBP2) (13,14) and inflammatory cytokines and chemokines (i.e., COMMD1, COMMD10, PPM1B, SPRED1, RORA, IKBA, ELOB) via distinct intracellular mechanisms: e.g., SPRED1 (sprouty-related, EVH1 domain containing 1) negatively regulates the RAS/MAPK pathway (15,16); COMMD1 and COMMD10 (copper metabolism MURR1 domain containing 1) disrupt NF- $\kappa$ B binding of target genes in the nucleus (17,18); and RORA (RAR-related orphan receptor A) sequesters NF- $\kappa$ B in the cytoplasm (19,20). Expression of these inhibitory genes increased early after WT SIV exposure in the cervix of vaccinated animals, whereas expression of these genes remained unchanged or even decreased in the unvaccinated setting (Fig. 3b).

The spatial and temporal expression patterns of these genes was at the right place and time to dampen the inflammatory and chemotactic signaling cascade engendered by the host's immune system that facilitates early virus propagation and spread (12). These inhibitory proteins were expressed at higher levels in the vaccinated animals compared to the controls predominantly in the single layer of columnar epithelial cells lining the mucosal surface of the endocervix (Fig. 3c). Consistent with the notion that a concerted inhibitory response within the FRT might be dampening the early chemotactic and inflammatory signalling cascade, we found that expression of numerous inhibitory genes negatively correlated with markers of inflammation in SIV<sub>mac239</sub><sup>-</sup> Nef-vaccinated animals (Fig. 4).

## Immune complex interactions with Fc $\gamma$ R11b in cervical epithelium mediates inhibition of signaling and CD4<sup>+</sup> T cell recruitment

SIV<sub>mac239</sub><sup>-</sup> Nef vaccination thus induces coordinated expression in mucosal epithelial cells of genes associated with inhibition of the transmission-facilitating innate response in unvaccinated animals. Why would exposure to the viral inoculum elicit such a profoundly



different response? In seeking an answer to this question, we focused on SIV-specific antibodies acting at the mucosal surface, because in the accompanying paper we had found gp41t antibodies were concentrated at the mucosal border in the FRT by the FcRn, and concentrated gp41t antibodies at that site correlated with prevention/constriction of infected founder populations in the cervix during this same timeframe. Since these antibodies might form ICs with virus in the inoculum, we hypothesized that the ICs so-formed might interact with the inhibitory Fc receptor for IgG, Fc $\gamma$ RIIb to then trigger an anti-inflammatory program, based on the ample precedents for such a mechanism (21).

We tested the main prediction of the hypothesis that Fc $\gamma$ RIIb would be in the cervical lining epithelium where it could interact with IC by staining cervical sections with antibodies to Fc $\gamma$ RIIb, and documented expression in the cervical lining epithelium in both naïve (not shown) and vaccinated animals following vaginal exposure to SIV (Fig. 5a). We only detected expression of the inhibitory Fc $\gamma$ RIIb receptor in cervical epithelium, but not the activating receptors, Fc $\gamma$ RIIa, c; Fc $\gamma$ RI; and Fc $\gamma$ RIII (Supplemental Figure 1). We then showed that Fc $\gamma$ RIIb co-localized with SIV antibodies and the major SIV p27 protein in the cervical epithelium of vaccinated animals (Fig. 5b,c), but not in unvaccinated animals, where neither viral antibodies nor p27 antigen were detectable (not shown).

These results thus support the hypothesis that antibodies form ICs with virus in the inoculum that then interact with and trigger an anti-inflammatory program mediated by Fc $\gamma$ RIIb. To test mechanistic predictions of this hypothesis that we could not test in vivo, we used an explant system (24) derived from cervixes of naïve, uninfected rhesus macaques in which we could examine Fc-receptor expression and effects of immune complexes on the innate signaling system (MIP-3 $\alpha$ ), inhibitory mediators SPRED1 and COMMD1, and migration of pre-existing CD4<sup>+</sup> T cells in the explants.

In this ex vivo system, we first documented expression of the inhibitory Fc $\gamma$ RIIb-receptor in the epithelium, but not the activating receptors, Fc $\gamma$ RIIa,c; Fc $\gamma$ RI; and Fc $\gamma$ RIII (Fig. 6). We then reproduced the anti-inflammatory effects and inhibition of target cell recruitment observed in vivo with SIV-specific ICs and Fc $\gamma$ RIIb (Fig. 7): (1) exposure of the endocervical epithelium for 24 h to media alone (uninfected control) did not elicit increased expression of the innate signaling system (MIP-3 $\alpha$ ), the inhibitory mediators SPRED1 and COMMD1, or increase the numbers of CD4<sup>+</sup> T cells in the submucosa (Fig. 7a); in contrast, exposure to WT SIV<sub>mac251 32H</sub> (+SIV) did increase expression of MIP-3 $\alpha$  but not SPRED1 and COMMD1, and CD4<sup>+</sup> T cell density increased (Fig. 7b); (2) exposure of the endocervical epithelium for 24 h to SIV-specific ICs (formed by pre-incubating SIV<sub>mac251 32H</sub> with serum from vaccinated animals, or with the gp41 rhesus monoclonal antibody 4.9C (25) that reacts with the trimeric form of gp41 in Western blots identically to the antibodies elicited by SIV<sub>mac239- Nef</sub> vaccination, increased expression of SPRED1 and COMMD1, while MIP-3 $\alpha$  and CD4<sup>+</sup> T cell density were essentially unchanged from untreated samples (Fig. 7c,d); (3) intact, SIV-specific ICs were needed to elicit these innate inhibitory proteins in the mucosal epithelium as antibody alone (similar to untreated, data not shown), disruption of antibody-antigen complexes using a mild acid-base treatment (data not shown) or a non-specific antibody<sup>+</sup> virus (Fig. 7e) did not prevent increased expression of MIP-3 $\alpha$  and increased CD4<sup>+</sup> T cell density, and /or increase expression of SPRED1 and



COMMD1; and (4) blocking Fc $\gamma$ RIIb increased expression of MIP-3 $\alpha$ , decreased expression of SPRED1 and COMMD1, and increased CD4<sup>+</sup> T cell density (Fig. 7f).

## Discussion

In these studies of protection associated with SIV<sub>mac239</sub>- Nef vaccination, we have identified two spatial and temporal correlates at the portal of entry (Fig. 8). In the accompanying paper, we provide evidence that Correlate 1 in the cervix is an organized system of local production of antibody to gp41t, concentrated on the path of virus entry by FcRn-mediated mechanisms, as a correlate of preventing establishment or constraining the size of founder populations of infected cells. Here we provide evidence that Correlate 2 is inhibition of target cell recruitment to fuel local expansion of any foci of infected cells. In this case, a second Fc receptor, the inhibitory Fc $\gamma$ RIIb receptor mediates the inhibition.

The two correlates are interrelated because the concentrated antibodies at mucosal frontlines form immune complexes with incoming virus that can then interact with the inhibitory Fc-receptor, Fc $\gamma$ RIIb, appropriately positioned in the lining epithelium. While we have not identified the proximate signaling events, we show that the co-localization of SIV-ICs and Fc $\gamma$ RIIb is associated with a coordinated gene expression program that inhibits the innate and inflammatory response to vaginal exposure to the SIV inoculum. Collectively these genes block the outside in signaling system and CD4 T cell recruitment that in naïve animals facilitates transmission by providing cellular substrates to expand infection at the portal of entry.

What we find quite fascinating by this initially surprising and counter intuitive response in vaccinated animals to vaginal challenge is the possibility that this system is representative of a general way for the immune system to immediately distinguish at mucosal frontlines between a pathogen that it has or has not previously seen. By localizing the sensing and inhibitory system in the mucosal epithelium and utilizing the inhibitory Fc-receptor, Fc $\gamma$ RIIb, the immune system can “see” ICs formed by antibodies induced by prior antigen exposure, and modulate the initial response to exposure to a response that does not engender a counterproductive inflammatory response.

There are parallels between the association of protection in SIV<sub>mac239</sub>- Nef-vaccinated animals with inhibition of outside-in signaling, CD4 T cell recruitment and local expansion, and protection against high dose vaginal challenge afforded by the microbicide, glycerol monolaurate (GML). GML also inhibits MIP-3 $\alpha$  production in the rhesus macaque FRT, and is thought in this way to block the outside in signaling that facilitates transmission. Thus, inhibiting innate and inflammatory responses to SIV, and, by extension, to HIV-1 may be an important general component of preventive strategies to prevent transmission to women.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations used

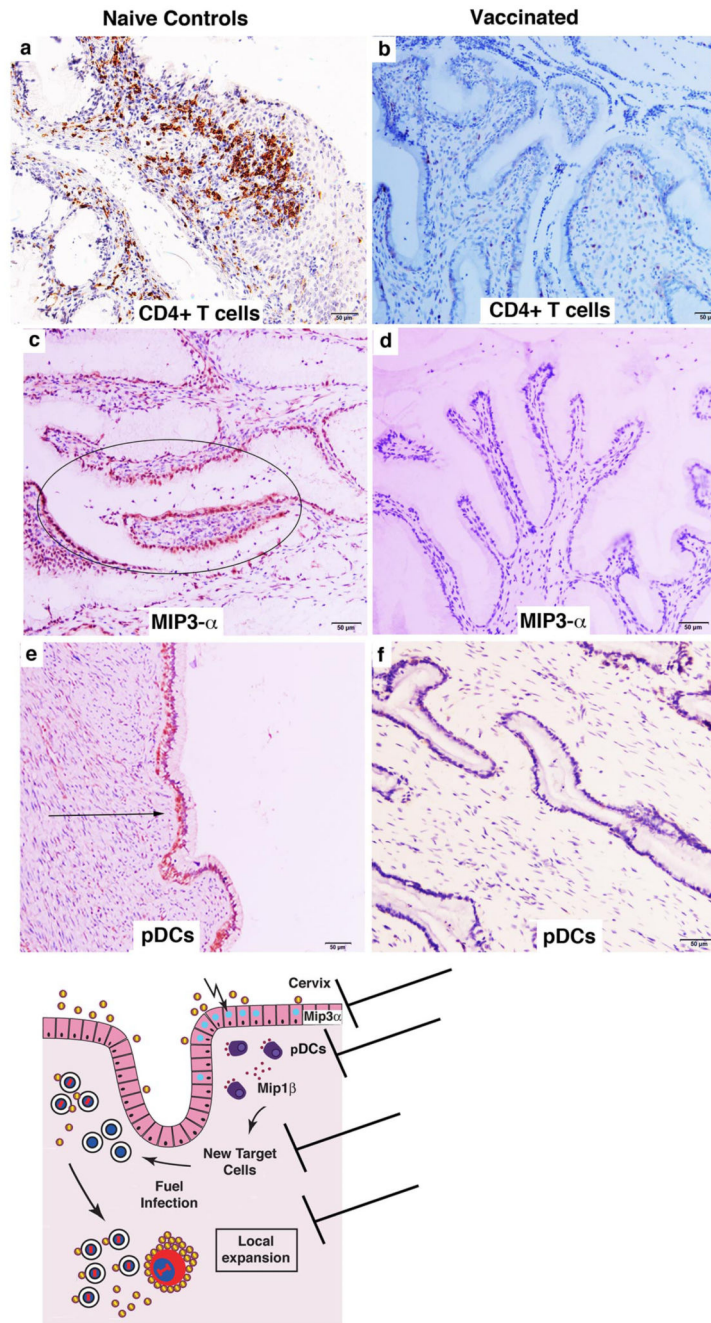
<b>COMMD1</b>	copper metabolism MURR1 domain containing 1
<b>FcRn</b>	neonatal Fc receptor
<b>FRT</b>	female reproductive tract
<b>gp41t</b>	trimeric form of the viral envelope glycoprotein
<b>IC</b>	immune complex
<b>RMA</b>	robust multi-chip average
<b>RORA</b>	RAR-related orphan receptor A
<b>SIV</b>	simian immunodeficiency virus
<b>SPRED1</b>	sprouty-related, EVH1 domain containing 1
<b>VL</b>	viral load

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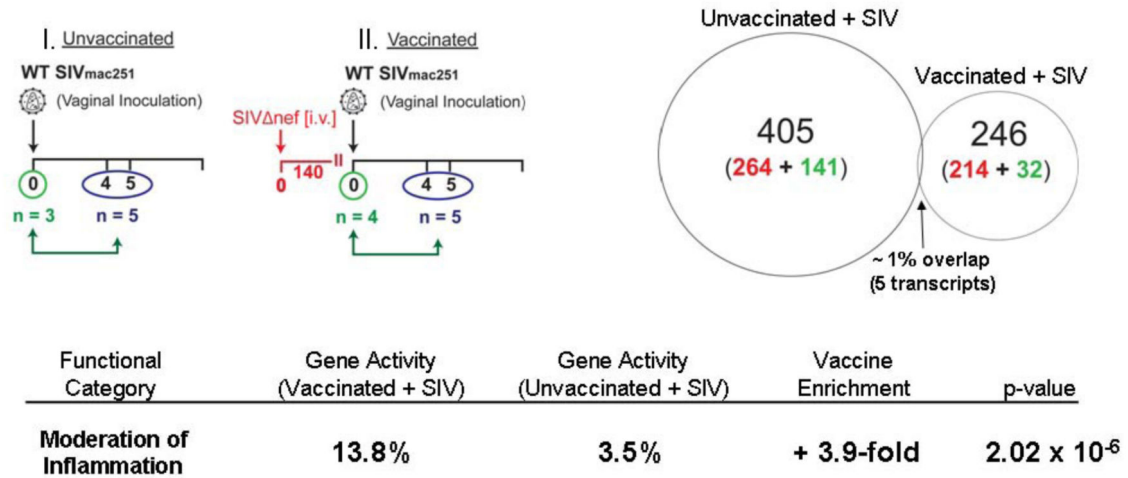
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**Figure 1.**

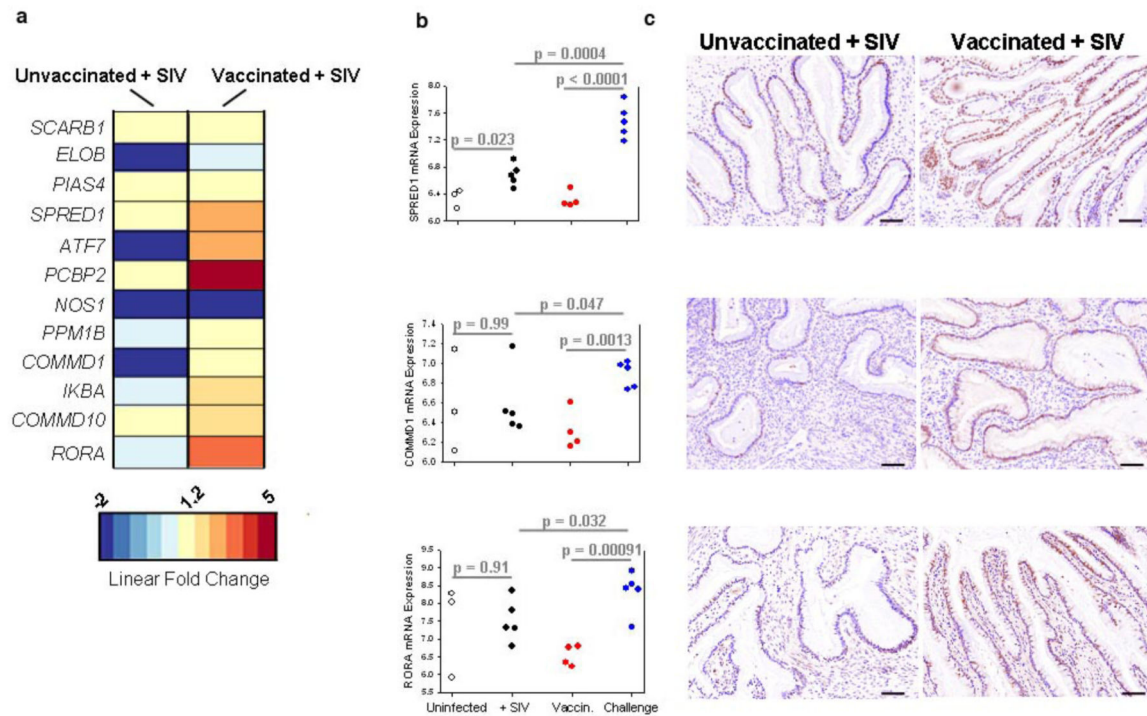
Inhibition of CD4 T cell recruitment and outside in signaling in *SIV<sub>mac239</sub>-Nef*-vaccinated animals. In naïve animals, brown-stained CD4 T cells (1a), MIP3a (circle, 1b) and pDCs (arrow in 1c) increase after vaginal exposure to the SIV inoculum. By contrast, no increases are evident 4 days post high dose vaginal challenge with *SIV<sub>mac251</sub>* in the vaccinated animals (Fig. 1b,d,f). A model of outside in signaling in naïve animals, and inhibition in vaccinated animals (indicated by the blocked symbols) is shown at the bottom of Fig. 1, revised from ref. 9.



**Figure 2.**

Host responses of unvaccinated and SIV<sub>mac239</sub><sup>-</sup> Nef-vaccinated animals to vaginal WT SIV<sub>mac251</sub> exposure. The transcriptional response of unvaccinated and SIV<sub>mac239</sub><sup>-</sup> Nef-vaccinated animals to vaginal inoculation of WT SIV<sub>mac251</sub> was determined by comparing transcript levels between uninfected, naïve animals (n = 3) with unvaccinated animals 4-5 days post WT SIV<sub>mac251</sub> exposure (transcriptional profile I) and between vaccinated animals before challenge (140 days post vaccination) (n = 4) with vaccinated animals 4-5 days post WT SIV<sub>mac251</sub> exposure (n = 5) (transcriptional profile II). (b) Venn diagram indicating minimal overlap of altered transcripts between unvaccinated animals exposed intra-vaginally to WT SIV<sub>mac251</sub>. The size of each region is proportional to the number of altered transcripts. Green and red numbers indicate, respectively, decreased and increased expression. Inflammation-moderating genes are significantly increased about 4 fold in the vaccinated animals after vaginal exposure to SIV.

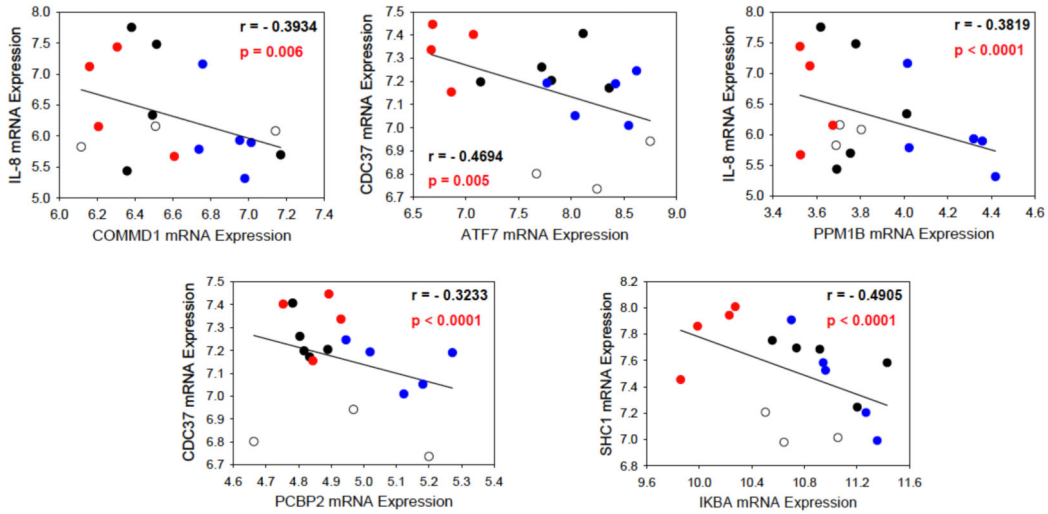




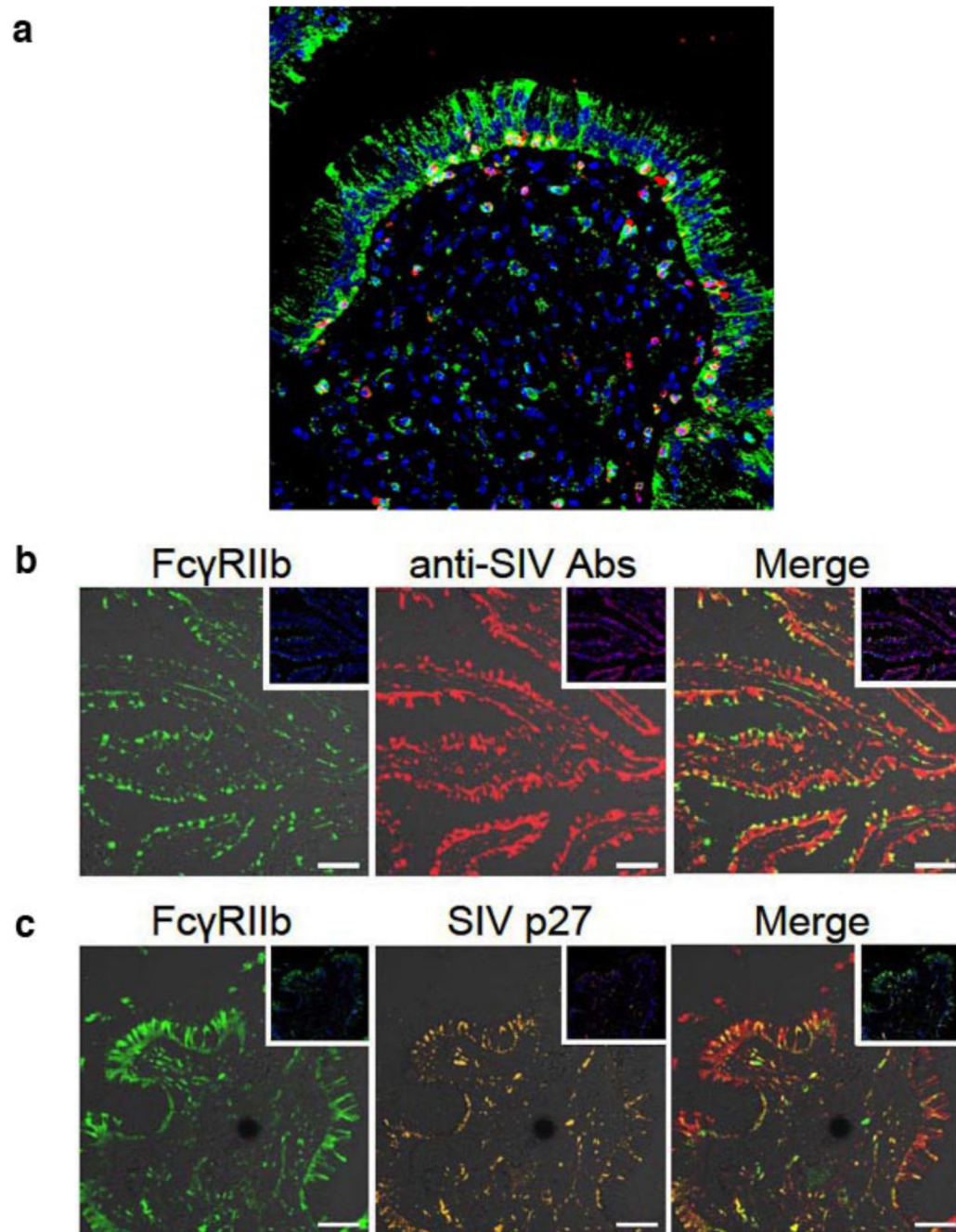
**Figure 3.**

Increased expression of inflammatory cytokine and chemokine inhibitors in the mucosal epithelium of the cervix in *SIV<sub>mac239</sub><sup>-</sup>* Nef-vaccinated animals. **(a)** Heat map of selected inhibitory genes with altered expression in the cervix of unvaccinated and *SIV<sub>mac239</sub><sup>-</sup>* Nef-vaccinated animals exposed vaginally to WT *SIV<sub>mac251</sub>*. A color scale is shown at bottom and ranges from 2-fold down-regulation to 5-fold up-regulation. **(b)** mRNA levels and corresponding protein expression of various genes in the cervix of unvaccinated and *SIV<sub>mac239</sub><sup>-</sup>* Nef-vaccinated animals 4-5 days post WT *SIV<sub>mac251</sub>* exposure. *SPRED1*, *COMMD1*, and *RORA* mRNA levels are significantly increased compared to unvaccinated animals ( $p = 0.0004$ ,  $p = 0.047$ ,  $p = 0.032$ , respectively). **(c)** The respective proteins are predominantly expressed in the endocervical epithelium. Original magnifications:  $\times 200$ ; scale bars: 50  $\mu\text{m}$ .





**Figure 4.** Inhibitors of inflammation whose expression increased in the cervix of *SIV<sub>mac239- Nef</sub>*-vaccinated animals inversely correlate with expression of genes mediating inflammation. COMMD1 = copper metabolism MURR1 domain containing 1; IL-8 = interleukin 8; ATF7 = activating transcription factor 7 (ATF7 transcriptionally represses innate immune genes); CDC37 = cell division cycle 37 (CDC37 acts as co-chaperone in activating the IFN pathway); PPM1B = protein phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup> dependent, 1B (PPM1B plays an important role in termination of cytokine-mediated NF-κB activation as well as inhibits IL-1-induced inflammation); PCBP2 = poly(rC) binding protein 2 (PCBP2 inhibits RIG-I and MDA-5 innate signaling pathway); IKBA = inhibitor of κB, α (IKBA sequesters NF-κB in the cytoplasm, preventing NF-κB-mediated transcription of inflammatory genes); SHC1 = SHC (Src homology 2 domain containing) transforming protein 1 (SHC1 is involved in IL-5- and IL-6-mediated inflammation). White, black, red, and blue circles represent respectively uninfected animals, unvaccinated animals infected intra-vaginally with WT *SIV<sub>mac251</sub>* for 4-5 days, *SIV<sub>mac239- Nef</sub>*-vaccinated animals, and *SIV<sub>mac239- Nef</sub>*-vaccinated animals infected intra-vaginally with WT *SIV<sub>mac251</sub>* for 4-5 days.

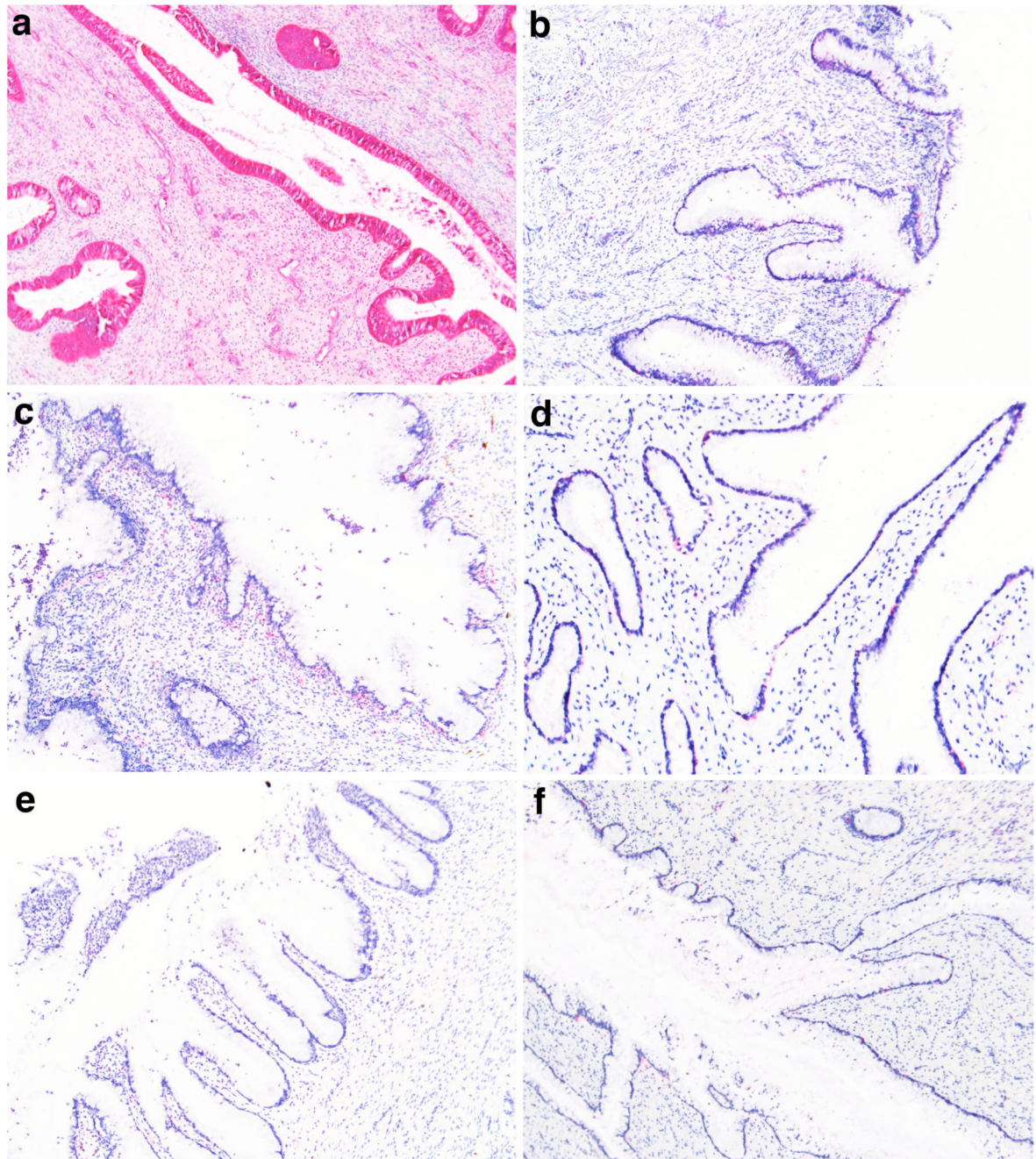


**Figure 5.**

Fc $\gamma$ RIIb (Fc fragment of IgG, low affinity IIb, receptor) expression is increased in the cervical epithelium of SIV<sub>mac239</sub>- Nef-vaccinated animals following vaginal challenge with WT SIV, and spatially co-localizes with SIV protein and SIV-specific antibodies. **(a)** Green-stained Fc $\gamma$ RIIb<sup>+</sup> columnar epithelium lining the endocervix. Sections have been stained with antibodies to the FcRn to show that FcRn is not expressed in the lining epithelium in rhesus macaques. The red-stained FcRn<sup>+</sup> cells in the merged confocal micrograph are mainly in the submucosa. **(b,c)** Fc $\gamma$ RIIb (green) co-localizes with SIV-specific antibodies

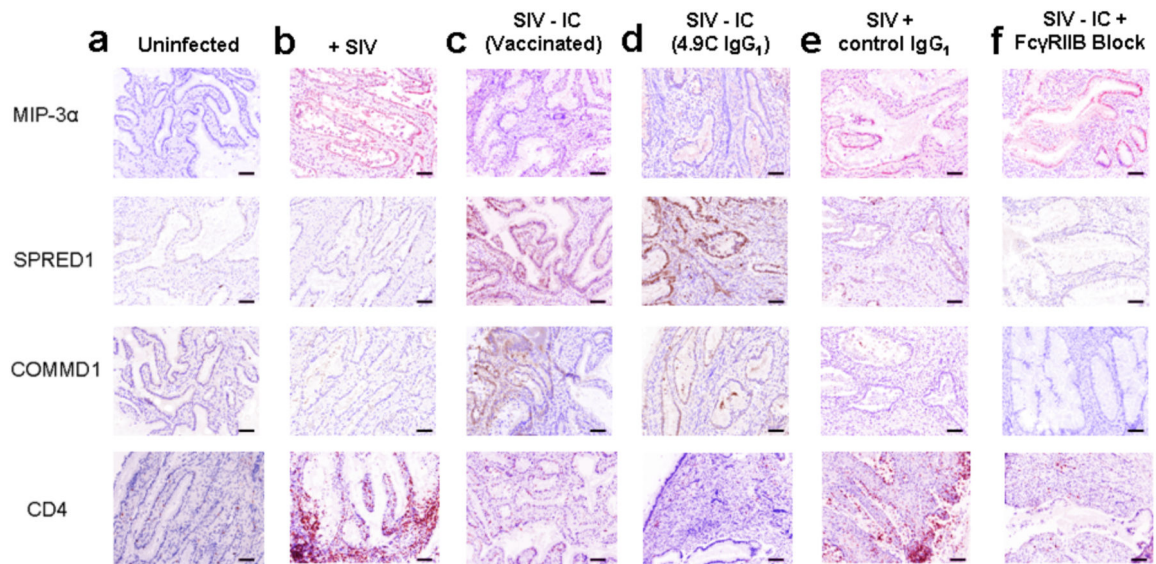
[red, stained using a monoclonal antibody against a collection of public idiotypes of antibodies to HIV-1 and SIV (22,23)] (b) and the major virion p27 protein (orange) (c) along the mucosal surface of the endocervix in SIV<sub>mac239</sub>- Nef-vaccinated animals challenged with WT SIV<sub>mac251</sub>. The insets show the total number of cells (cell nuclei appear blue) in each image. Original magnifications: X200; scale bars: 50  $\mu$ m.





**Figure 6.**

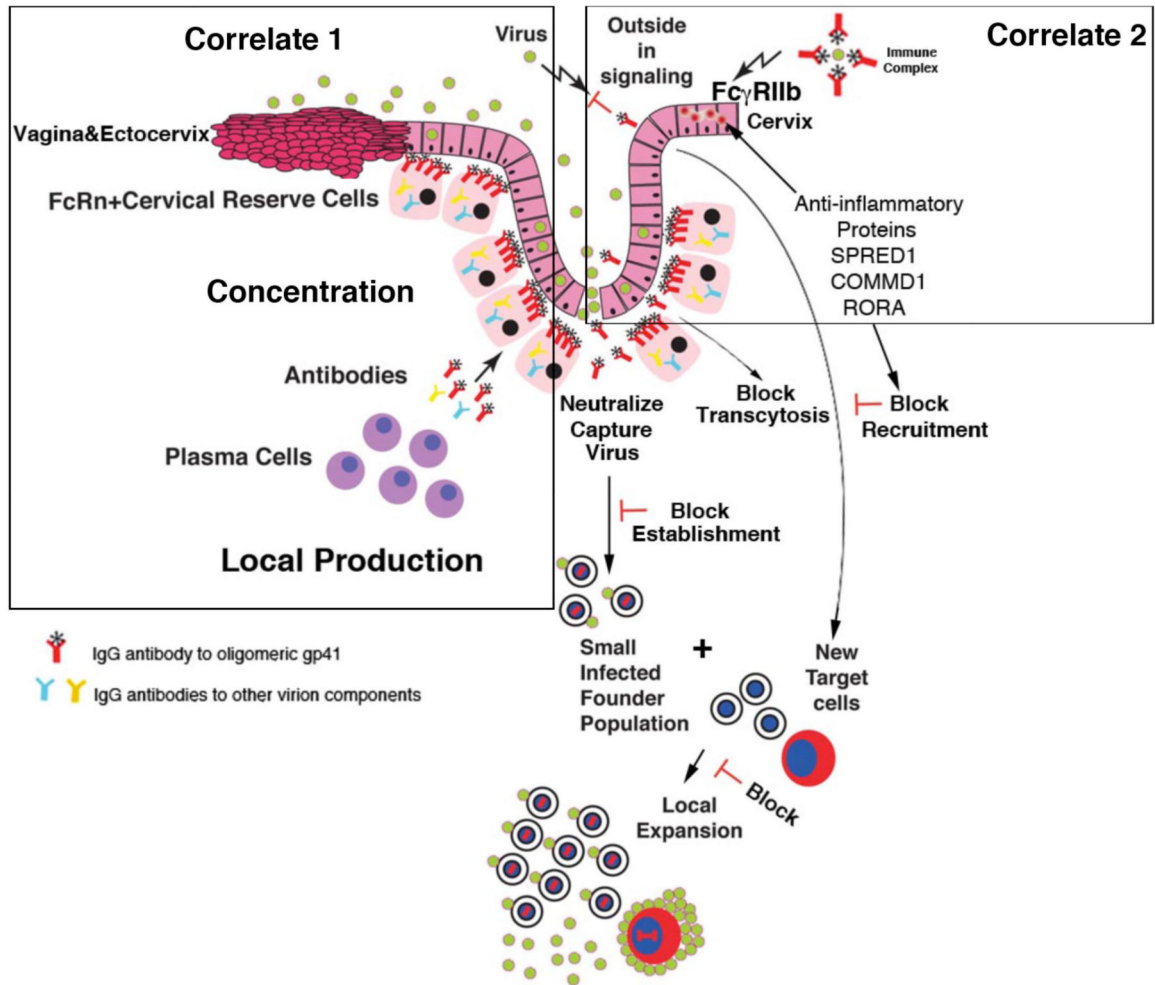
Only  $Fc\gamma RIIb$  is expressed in columnar epithelium lining the endocervix in primary cervical explants from vaccinated rhesus macaques. (a)  $Fc\gamma RIIb+$  red-stained epithelium. Antibodies to  $Fc\gamma RIIa$  and c (b), (c)  $Fc\gamma RI$  (d) and  $Fc\gamma RIII$  (e) do not stain endocervical epithelium in explants. (f). Isotype staining control. Original magnifications, 10 $\times$ .



**Figure 7.**

SIV-specific immune complexes interact with Fc $\gamma$ RIIb in primary cervical explants from rhesus macaques to dampen outside in signaling and submucosal increases in CD4<sup>+</sup> T cell density through increased expression of inhibitory genes (a) Exposure of the endocervical epithelium to media alone (uninfected control) did not elicit increased expression of MIP-3 $\alpha$ , innate response moderators (SPRED1, COMMD1) or increase CD4<sup>+</sup> T cell density; (b) Exposure to WT SIV<sub>mac251 32H</sub> (+SIV) did increase expression of MIP-3 $\alpha$  (red-stained epithelium) but not SPRED1, COMMD1; density of red-brown-stained CD4<sup>+</sup> T cells increases. (c,d) Exposure to SIV-specific ICs (formed by pre-incubating SIV<sub>mac251 32H</sub> with serum from vaccinated animals, or with the gp41 rhesus monoclonal antibody 4.9C<sup>20</sup> that reacts with the trimeric form of gp41 in Western blots identically to the antibodies elicited by SIV<sub>mac239</sub>- Nef vaccination), increased expression of SPRED1 and COMMD1 (brown-stained epithelium), while MIP-3 $\alpha$  and CD4<sup>+</sup> T cell density were essentially unchanged from untreated samples. (e) SIV<sub>mac251 32H</sub><sup>+</sup> nonspecific antibody effects were indistinguishable from SIV alone. (f) SIV-specific immune complexes do not up-regulate SPRED1 and COMMD1 or increase MIP-3 $\alpha$ , or CD4<sup>+</sup> T cell density in explants pre-treated with Fc $\gamma$ RIIb blocking antibodies.





**Figure 8.**

Model of correlates of protection against vaginal challenge in the cervix conferred by SIV<sub>mac239</sub>-Nef vaccination. In the accompanying paper, Correlate 1 was an organized system to locally produce antibodies to gp41 and concentrate these antibodies on the path of virus entry by FcRn-mediated mechanisms operating in cervical reserve epithelium underlying the epithelium lining the transition zone and adjoining endocervix. These antibodies by a number of mechanisms could then prevent or restrict establishment of infected founder populations. In this paper, Correlate 2 was inhibition of target cell recruitment to fuel local expansion. This inhibition was mediated by immune complexes engaging the inhibitory receptor, Fc $\gamma$ RIIb, with subsequent expression of an anti-inflammatory program that suppresses outside-in signaling and the recruitment of CD4 T cells.