

1 Pegivirus avoids immune recognition but does not attenuate acute-phase
2 disease in a macaque model of HIV infection

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22 **One Sentence Summary:**

23 Pegivirus avoids immune recognition but does not attenuate acute-phase disease in a macaque
24 model of HIV infection.

25

26 **Short Title:**

27 Pegivirus and AIDS-virus co-infection

28 **Abstract:**

29 Human pegivirus (HPgV) protects HIV+ people from HIV-associated disease, but the
30 mechanism of this protective effect remains poorly understood. We sequentially infected
31 cynomolgus macaques with simian pegivirus (SPgV) and simian immunodeficiency virus (SIV)
32 to model HIV+HPgV co-infection. SPgV had no effect on acute-phase SIV pathogenesis – as
33 measured by SIV viral load, CD4+ T cell destruction, and immune activation – suggesting that
34 HPgV's protective effect is exerted primarily during the chronic phase of HIV infection. We also
35 examined the immune response to SPgV in unprecedented detail, and found that this virus
36 elicits virtually no activation of the immune system despite persistently high titers in the blood
37 over long periods of time. Overall, this study expands our understanding of the pegiviruses – an
38 understudied group of viruses with a high prevalence in the global human population – and
39 suggests that the protective effect observed in HIV+HPgV co-infected people occurs primarily
40 during the chronic phase of HIV infection.

41

42 **Accessible Summary:**

43 People infected with HIV live longer, healthier lives when they are co-infected with the human
44 pegivirus (HPgV) – an understudied virus with a high prevalence in the global human
45 population. To better understand how HPgV protects people with HIV from HIV-associated
46 disease, we infected macaques with simian versions of these two viruses (SPgV and SIV). We
47 found that SPgV had no impact on the incidence of SIV-associated disease early during the
48 course of SIV infection – a time when SIV and HIV are known to cause irreversible damage to
49 the immune system. Oddly, we found that the immune system did not recognize SPgV; a finding
50 that warrants further investigation. Overall, this study greatly expands on our understanding of
51 the pegiviruses and their interaction with the immune system.

52

53

54 **Introduction:**

55 Human pegivirus (HPgV) – formerly known as GB virus C (GBV-C) and also as Hepatitis G
56 Virus (HGV) – is a positive-sense, single-stranded RNA virus in the Pegivirus genus of the
57 *Flaviviridae* family (1). HPgV infects one out of six humans globally and is frequently transmitted
58 via blood products (2). Little is known about the molecular biology of pegiviruses and the natural
59 course of HPgV infection is poorly understood. However, HPgV causes persistent, high-titer
60 viremia without eliciting symptoms or overt signs of disease (3, 4). Interestingly, epidemiological
61 studies have found that people infected with human immunodeficiency virus (HIV) experience
62 reduced disease when they are co-infected with HPgV. Specifically, HIV-infected individuals co-
63 infected with HPgV are protected from HIV-induced CD4 T cell depletion (5-8) and pathological
64 immune activation (9-12). These individuals also experience a 2.5-fold reduction in all-cause
65 mortality relative to HIV+ individuals not co-infected with HPgV (see (13) for a meta-analysis
66 and (2) for a review). However, the timing and mechanistic underpinnings of this protective
67 association are not known, in part because most data on HIV+HPgV co-infection comes from
68 cross-sectional studies performed during the chronic phase of HIV infection. In particular, the
69 impact of HPgV infection on acute phase HIV infection – a period during which pathologic
70 changes in the HIV-infected host are most dramatic (14) – has not been studied. As such, the
71 impact of HPgV co-infection on the natural course of HIV infection, and the mechanism(s) by
72 which HPgV attenuates HIV disease *in vivo*, remain uncharacterized.

73 Macaque monkeys infected with simian immunodeficiency virus (SIV) exhibit several
74 features of progressive HIV disease in humans, including CD4+ T cell depletion and
75 pathological immune activation. As such, macaques infected with SIV are a valuable model for
76 investigating the pathogenesis of HIV *in vivo* (15). We recently discovered simian pegiviruses
77 (SPgVs) infecting wild baboons in Africa (16) and used blood from an olive baboon (*Papio*

78 *anubis*) sampled in Kibale, Uganda to infect captive cynomolgus macaques (*Macaca*
79 *fascicularis*) with SPgV. This resulted in the first laboratory-animal model of HPgV infection (17).
80 Notably, SPgV infection causes persistent, high-titer viremia in macaques without eliciting signs
81 of disease, recapitulating several defining features of HPgV infection in humans.

82 Here, we used SPgV and SIV infection in Mauritian cynomolgus macaques to model
83 HPgV and HIV co-infection in humans. We compared SIV disease parameters in four
84 SPgV+SIV co-infected macaques to four macaques infected with SIV-only, with the hypothesis
85 that SPgV would attenuate SIV pathogenesis during the acute phase of SIV infection, or result
86 in improved recovery from acute insult of SIV infection, as is observed in natural simian hosts of
87 SIV which are often co-infected with their own species-specific SPgVs (18, 19).

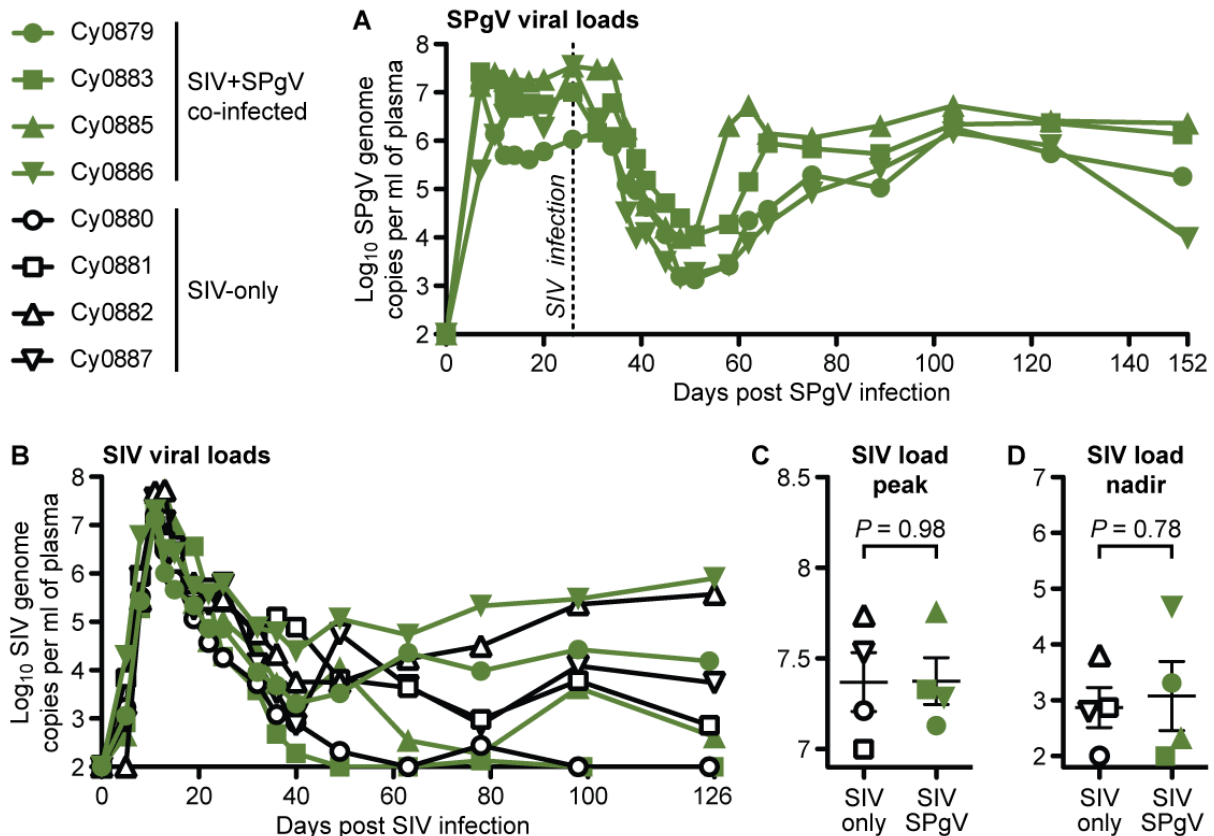
88

89 **Results:**

90 ***SPgV pre-infection does not impact acute-phase SIV viremia.*** For this study, eight female
91 cynomolgus macaques (*Macaca fascicularis*) with identical major histocompatibility complex
92 (MHC) class I haplotypes (M3/M4) were randomized to receive SPgV or no intervention prior to
93 SIV infection (Fig S1, Table S1). Four macaques received intravenous inoculations of plasma
94 containing 2.29×10^7 genome copies (gc) of SPgV measured by quantitative RT-PCR, as done
95 previously (17). These infections achieved initial peak titers between 7.30×10^6 and 2.66×10^7
96 gc/ml of plasma between 7 and 14 days post SPgV infection (Fig. 1A), similar to what had been
97 observed previously. By 26 days post-SPgV infection viral loads had established a high-titer
98 steady-state (average of 2.02×10^7 gc/ml) and all eight macaques were inoculated intra-rectally
99 with 7,000 tissue-culture-infectious-dose (TCID)₅₀ of SIVmac239.

100 SIV plasma viral loads followed a typical trajectory during acute phase, reaching peak
101 titers between 1.01×10^7 and 5.25×10^7 gc/ml of plasma between days 11 and 13 post-SIV
102 infection in all eight macaques (Fig. 1B). No differences in peak SIV plasma titer or post-peak
103 nadir were observed between the SIV+SPgV co-infected and SIV-only groups (Fig. 1C). To

104 determine whether SPgV impacted subsequent SIV viral load trajectory, we followed the
105 macaques for 126 days after SIV infection. Within each group, we observed a wide range of
106 viral load set-point titers. However, there was not a significant difference in SIV viral loads
107 between the SIV+SPgV group and the SIV-only group at any time point (Fig 1D).



108

Figure 1. SPgV and SIV viral loads in infected macaques. Titers for each virus were measured from plasma using highly sensitive virus-specific quantitative RT-PCR assays. (A) SPgV titers in the four macaques infected with SPgV+SIV. (B,C,D) SIV titers in the four macaques infected with SPgV+SIV (green) and the four macaques infected with SIV-only (black). P values are from a two-tailed unpaired t test with error bars representing the SEM. The symbols used for each animal in this figure are used consistently throughout the manuscript.

109

110

111 **Acute SIV infection reduces SPgV viremia.** Beginning as early as day 5 of SIV infection (day
112 31 of SPgV infection), we observed a significant drop in SPgV plasma viral loads in all
113 SPgV+SIV co-infected macaques. The decline in SPgV viral loads reached a nadir of 1.36×10^3 -

114 1.11×10^4 gc/ml of plasma between day 22 and 25 of SIV infection (day 48-51 of SPgV
115 infection), then gradually rebounded to a new set-point that was approximately $1.5 \log_{10}$ lower
116 than the pre-SIV viremic set-point by day 40-49 of SIV infection (66-75 of SPgV infection).
117 Previously, we showed that SPgV accumulates little-to-no sequence variation over time in
118 infected macaques. Therefore, we deep sequenced the SPgV genome from each animal before
119 the decline (day -6 of SIV infection; day 20 SPgV infection) and after recovery of high-titer SPgV
120 viremia (day 49 of SIV infection; day 75 SPgV infection) to look for unique signatures of immune
121 pressure on SPgV that may have been triggered by SIV infection. While SPgV from two
122 macaques accumulated 1-3 protein-coding (*i.e.* non-synonymous) mutations over this period,
123 SPgV from the other two macaques revealed no protein-coding mutations (Table S2),
124 suggesting that an SPgV-specific immune response and subsequent mutational escape was not
125 responsible for the measured decrease in SPgV viral loads.

126 We hypothesized that the reduction in SPgV viral load during acute SIV infection was the
127 result of inflammation induced by SIV, which has been reported to occur secondary to microbial
128 translocation from the gut lumen (14). Thus, we infected eight macaques intravenously with
129 2.29×10^7 gc of SPgV and treated four of these macaques with dextran sulfate sodium (DSS) on
130 day 26 post-SPgV infection to induce microbial translocation (20, 21). DSS treatment had no
131 significant impact on SPgV viral loads, suggesting that inflammation caused by microbial
132 translocation during acute SIV infection was not responsible for the observed decline in SPgV
133 viral loads (Table S3).

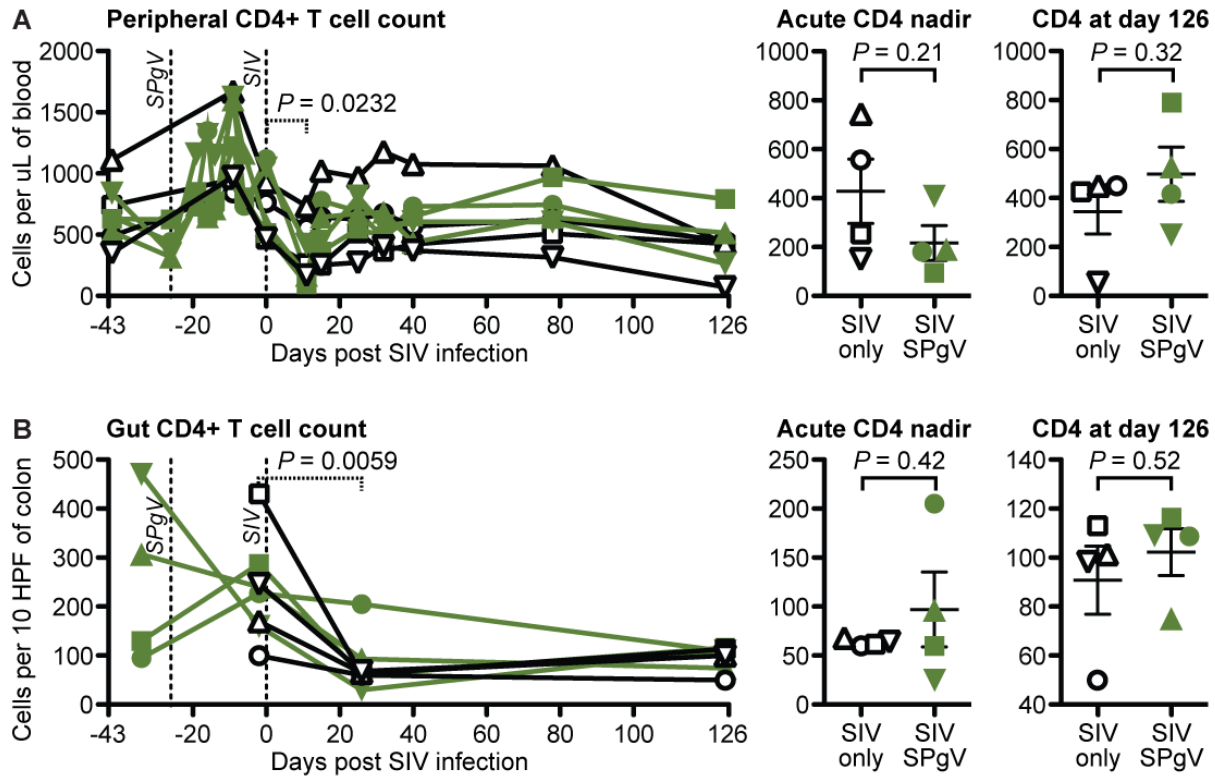
134
135 ***SPgV pre-infection does not prevent loss of peripheral CD4+ T cells.*** The absolute number
136 of circulating CD4+ T cells remains the most clinically-relevant marker of HIV/SIV disease
137 progression, and some studies of HIV+ human patients have shown a modest association
138 between HPgV co-infection and higher peripheral CD4+ T cell counts (5-8). Therefore, we
139 followed blood CD4+ T cell counts in both the SIV-only and SIV+SPgV groups. We observed an

140 initial drop in the circulating CD4+ T cells that corresponded with peak SIV viremia in all eight
141 animals which then recovered to near pre-SIV levels. However, there were no statistically
142 significant differences in the CD4+ T cell count between the SIV-only and SIV+SPgV groups
143 (Fig. 2A). An increase in the absolute number of circulating CD4+ T cells was observed prior to
144 SIV infection in the macaques infected with SPgV, although a concomitant increase was also
145 noted in the SPgV-naïve macaques during this time period, suggesting that this increase was
146 not due to SPgV infection.

147

148 ***SPgV pre-infection does not prevent loss of gut CD4+ T cells.*** The early loss of CD4+ T
149 cells in the gastrointestinal tract is a hallmark of HIV/SIV pathogenesis (14, 22); yet the effect of
150 HPgV/SPgV co-infection on gut CD4+ T cell depletion has never been examined. To see if
151 SPgV pre-infection protected gut CD4+ T cells from SIV-mediated destruction, we collected
152 colon pinch-biopsies from animals pre- and post-SIV infection, then analyzed the abundance of
153 lamina propria CD4+ cells using immunohistochemistry (IHC). As expected, SIV infection led to
154 an acute loss of gut-resident CD4+ cells, but the loss in the SIV+SPgV group was not
155 statistically different compared to the SIV-only group (Fig. 2B,C).

156



C Gut CD4+ T cell staining

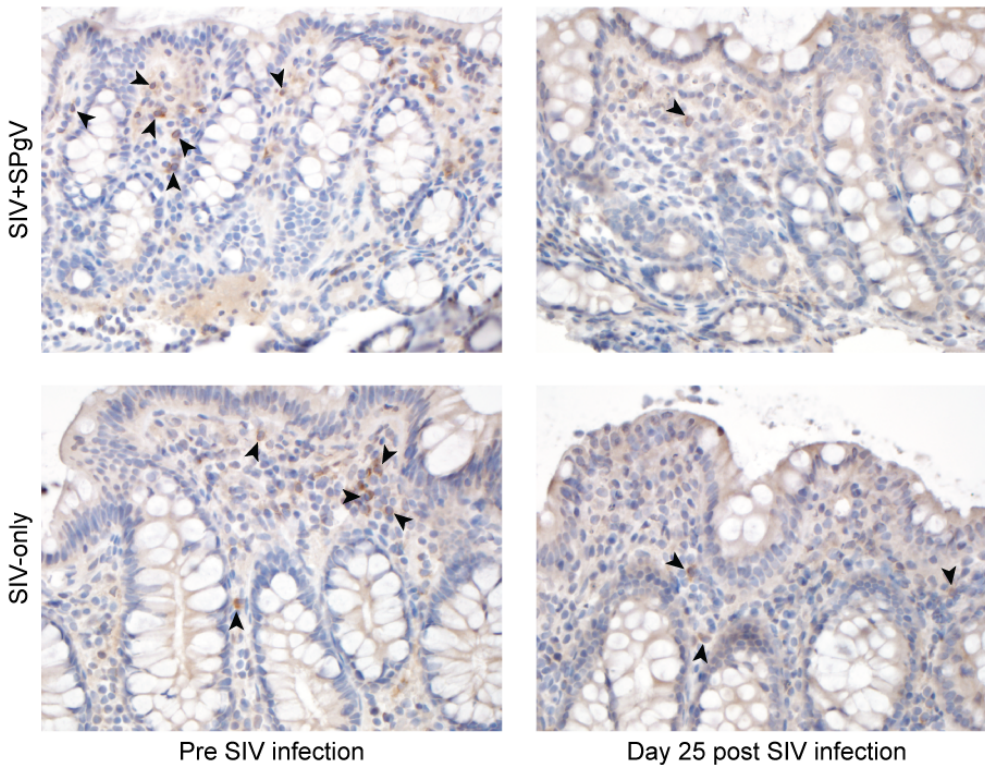


Figure 2. SIV pathogenesis in SIV-only vs. SIV+SPgV infected macaques. (A) Peripheral CD4+ T cell counts were obtained by multiplying absolute lymphocyte counts by the percentage of lymphocytes that were CD3+ CD4+ CD20- CD8- (see Figure 3 for gating strategy details). (B) Gut CD4+ T cells were stained within sections of colonic tissues via IHC with an anti-CD4 antibody and manually quantified. Significant differences between the SIV-only and SPgV+SIV groups were analyzed using a two-tailed unpaired t test (solid line) with error bars representing the SEM. Significant changes in all animals over the course of acute SIV infection were quantified using a two-tailed paired t test (dashed line). (C) A representative set of colonic tissue from Cy0883 (SIV+SPgV) and Cy0887 (SIV-only) are shown pre and post SIV infection at 400x for comparison, with arrows highlighting cells with particularly strong membranous staining for CD4.

158

159

160 **SPgV pre-infection does not reduce pathological immune activation during acute SIV**

161 **infection.** Several studies have demonstrated a correlation between HPgV infection and

162 reduced immune activation in HIV-infected people (9-12). However, none of these studies have

163 examined the effect of HPgV pre-infection on the trajectory of HIV disease during acute HIV

164 infection, a time when HIV is known to cause irreversible damage to the immune system.

165 Therefore, we trended changes in peripheral immune cell activation after SIV infection using

166 flow cytometry. For each immune cell subset examined (CD3+CD4+ T cells, CD3+CD8+ T cells,

167 and CD3-CD8+ natural killer [NK] cells) we chose a combination of activation markers that most

168 clearly delineated a positive and negative population (Fig 3A). While the timing and magnitude

169 of activation following SIV infection varied by cell subset, we did not detect a significant

170 difference in the magnitude of peak activation, the time to peak activation, or the post-peak

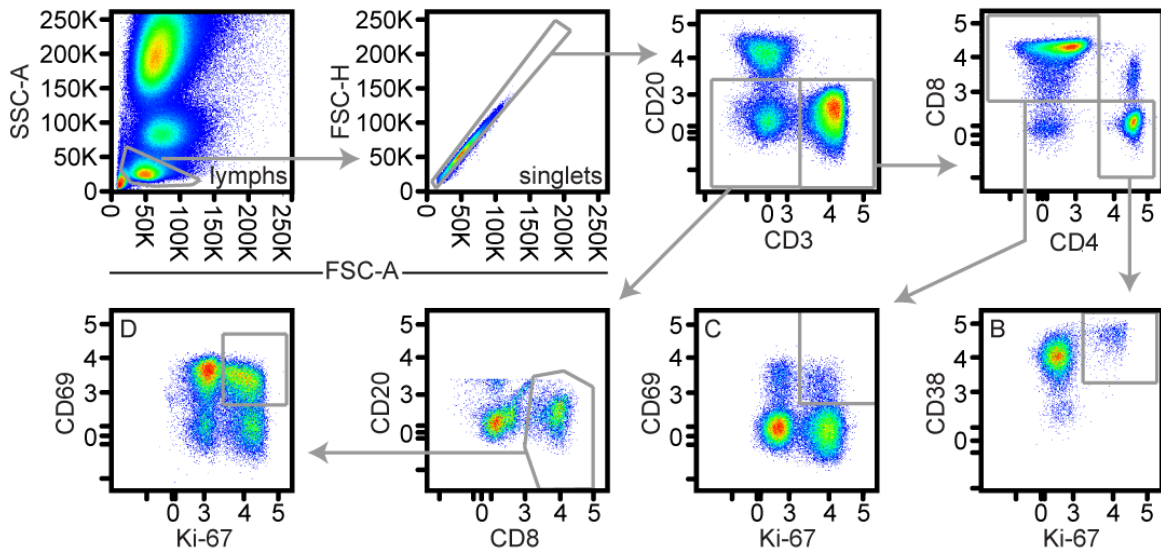
171 nadir of activation between the SIV-only and SIV+SPgV groups within any subset (Fig. 3B-D).

172 Activation of immune cells in the gut and in lymph nodes, as measured by IHC staining for the

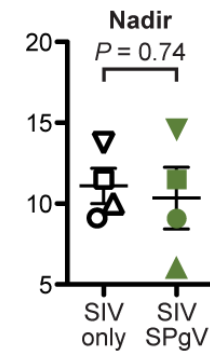
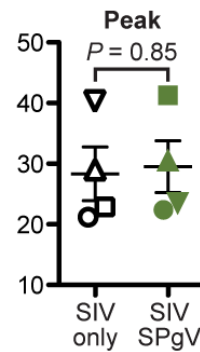
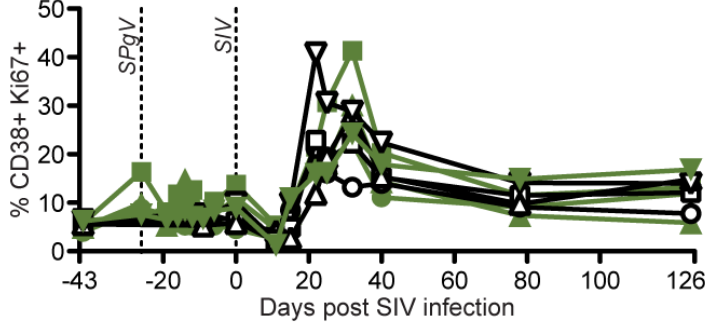
173 proliferation marker Ki67, showed a similar pattern (Fig. 4).

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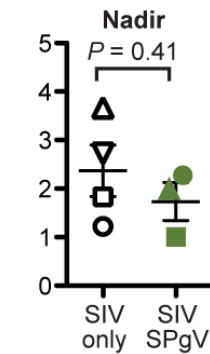
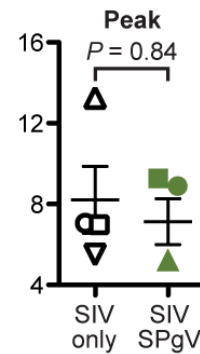
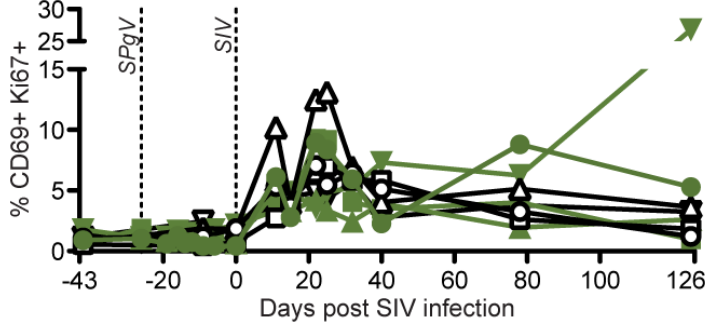
A Flow cytometry gating strategy



B Activation of CD3+ CD4+ T cells



C Activation of CD3+ CD8+ T cells



D Activation of CD3- CD8+ natural killer cells

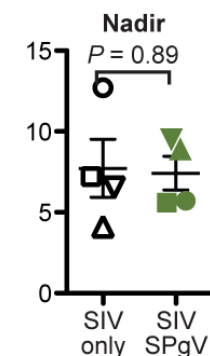
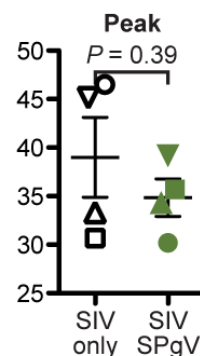
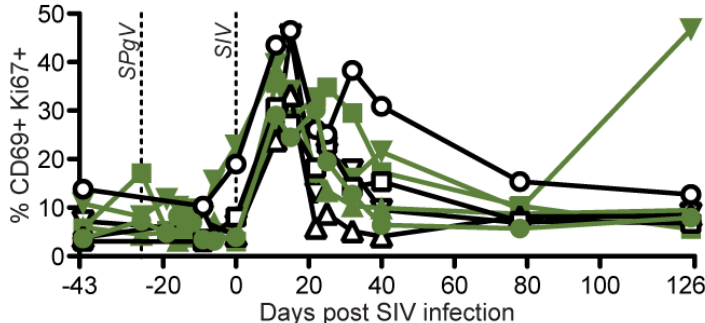


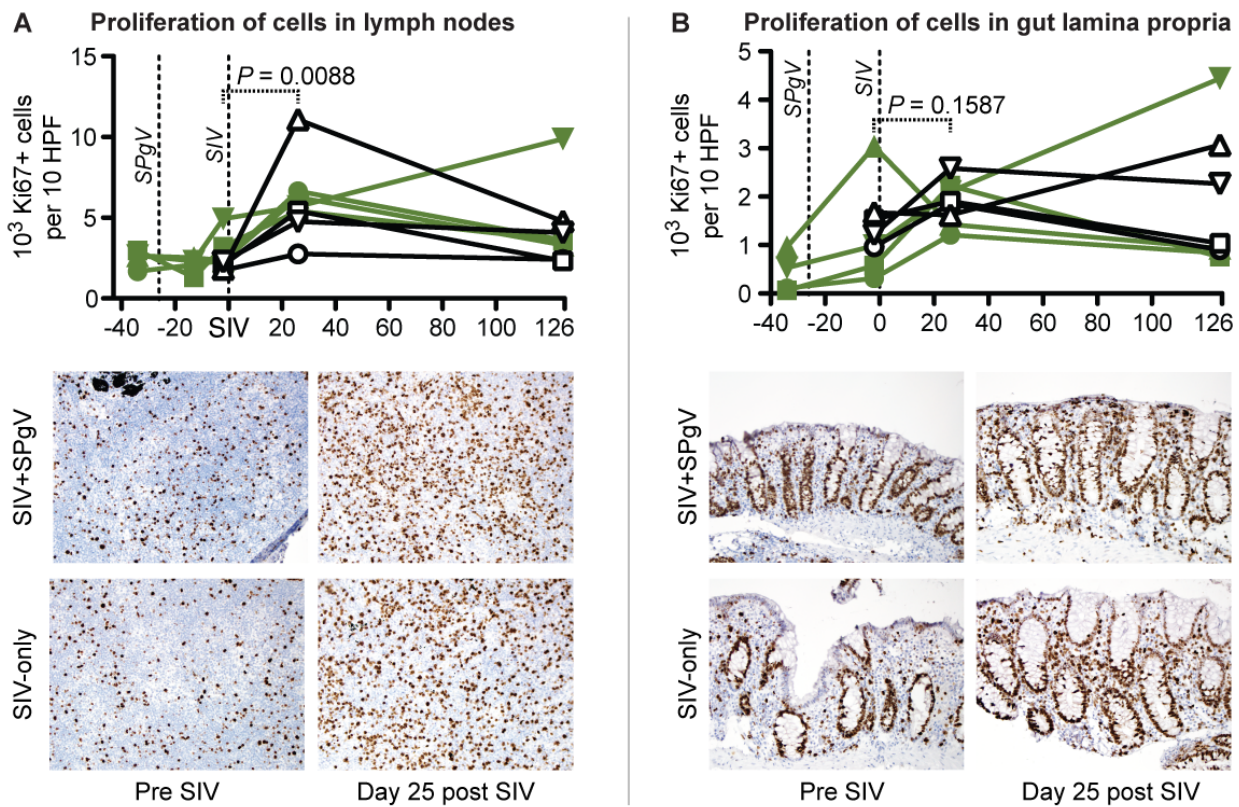
Figure 3. Peripheral immune activation in SIV-only vs. SIV+SPgV infected macaques.

Fresh whole blood was used for staining and flow cytometry at each timepoint, with gating and analysis performed in the same way on all samples at the end of the experiment. *P* values are from a two-tailed unpaired t test with error bars representing the SEM. Note: Cy0886 did not exhibit a distinct peak or nadir of CD69+ Ki67+ expression in the CD3+ CD8+ T cell population, and so is not included in these analyses.

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Figure 4. Activation of immune tissues in SIV-only vs. SIV+SPgV infected macaques.

Proliferating cells were stained within sections of lymph nodes (A) and colon (B) via IHC with an anti-Ki67 antibody and manually quantified. Significant changes in over time were quantified using a two-tailed paired t test (dashed line). A representative set of lymph node staining from Cy0883 (SIV+SPgV) and Cy0881 (SIV-only) is shown from pre and post SIV infection at 400x for comparison in (A). A representative set of colon tissue staining from Cy0886 (SIV+SPgV) and Cy0887 (SIV-only) is shown from pre and post SIV infection at 400x for comparison in (B).

180

181

182 **SPgV infection does not induce activation of the immune system.** Systemic viral infections
 183 typically elicit a Th1-type immune response characterized by the activation of lymphocytes. To
 184 examine the immune response to acute pegivirus infection, we trended markers of immune cell
 185 activation by flow cytometry for 26 days following SPgV infection. Oddly, we saw no significant
 186 changes from pre-infection baseline in the total number or activation state of circulating
 187 lymphocytes during this time period despite high titers of SPgV. This is in stark contrast to SIV
 188 infection, which elicited a robust increase in immune activation during the same timeframe (Fig.
 189 5).
 190

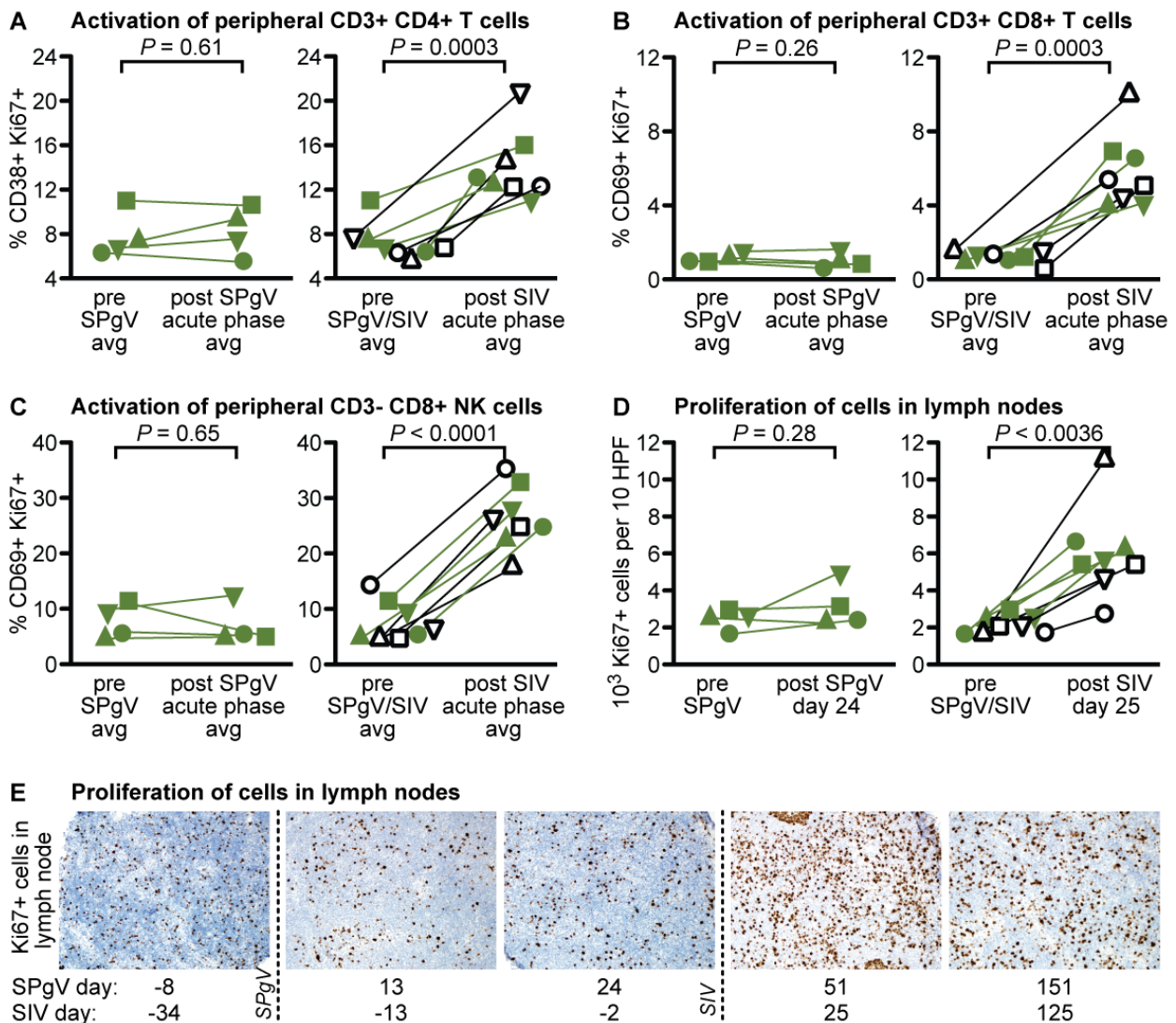


Figure 5. Immune activation following SPgV vs. SIV infection. (A-C) Fresh whole blood was used for staining and flow cytometry at each timepoint, with gating and analysis performed in the same way on all samples at the end of the experiment. *P* values are from a two-tailed paired *t* test that compare the pre-infection average to the post-SPgV or post-SIV infection average within the first 26 days post-infection for each virus, respectively. (D) Proliferating cells were stained within sections of lymph nodes via IHC with an anti-Ki67 antibody and manually quantified. Significant changes in over time were quantified using a two-tailed paired *t* test. (E) A representative set of lymph node tissue from Cy0885 is shown at 400x.

192

193

194

195 **Discussion:**

196 HIV+ people experience slower disease progression and reduced mortality when co-infected
197 with HPgV, but the mechanisms by which HPgV mediates this protective effect are not known.
198 Here, we utilized a recently-developed macaque model to study SPgV+SIV co-infection and
199 found that pre-infection with SPgV had no effect on acute-phase SIV pathogenesis as measured
200 by SIV viral loads, peripheral and gut-resident CD4+ T cell depletion, or SIV-induced immune
201 activation. One interpretation of these findings could be that, unlike HIV+HPgV co-infection in
202 humans, SPgV does not protect macaques from SIV disease. While this is possible, studies of
203 SPgVs in non-human primates have shown that the biology of these viruses closely mirrors
204 HPgV in humans (1, 16, 17, 19, 23). The progression of SIV infection in macaques also closely
205 mirrors that of HIV infection in humans, and so it appears that SIV+SPgV infection in macaques
206 is a close approximation of HIV+HPgV in humans. Therefore, our data suggest that HPgV does
207 not exert a protective effect on HIV pathogenesis during the acute phase of HIV infection, but
208 rather alters HIV/AIDS disease during the chronic phase. This conclusion is supported by some
209 of the epidemiologic data on HIV+HPgV co-infection (8, 13). Unfortunately, confirming and
210 studying an effect on this timescale (*i.e.* years) is intractable in macaques. Nonetheless, a
211 protective effect in the chronic phase of HIV infection may ultimately prove to be of greater
212 clinical value, should a HPgV-inspired anti-HIV therapy ever come to fruition.

213 Our study resulted in the first-ever, highly-detailed analysis of the immune response to
214 acute pegivirus infection. Previous studies of persistent pegivirus infection showed that these
215 viruses accumulate very few mutations over time, which is in stark contrast to other persistent
216 RNA viruses like HIV/SIV, hepatitis C virus, and simian arteriviruses (17, 24-26). These viruses
217 rapidly acquire mutations that alter protein structure, allowing them to escape adaptive host
218 immune responses targeting viral proteins (27-29). Thus, the lack of sequence changes
219 observed in the pegivirus genome over the course of infection alludes to an uncharacteristically
220 weak or absent host immune response. To investigate this further, we measured the activation
221 of immune cells following SPgV infection. While SIV infection resulted in robust activation of all
222 cell subsets examined, we found that SPgV infection elicited no detectable activation of the
223 immune system. This is the first direct *in vivo* evidence demonstrating the absence of an anti-
224 pegivirus immune response. It remains to be determined whether pegiviruses inhibit activation
225 of the immune system or simply avoid immune detection, but answering this question will
226 require the development of additional virus-specific reagents and assays. Interestingly, either
227 scenario would require that these viruses employ a unique mechanism to maintain high-titer,
228 persistent infection in the primate host.

229 Although pegivirus infection does not appear to elicit a substantial immune response, the
230 significant drop in SPgV plasma viral loads that we observed upon co-infection of macaques
231 with SIV suggests that pegivirus replication may be restricted by the immune activation which
232 occurs as a result of acute SIV infection. One of the major drivers of immune activation during
233 early SIV/HIV infection is the translocation of microbial products from the gut lumen into
234 systemic circulation, which occurs as a result of the destruction of gut-resident CD4+ T cells. To
235 determine whether this impacted SPgV replication, we treated SPgV infected macaques with
236 DSS, a chemical known to induce microbial translocation. These macaques experienced no
237 decrease in SPgV plasma viral loads, in contrast to SPgV+ monkeys infected with SIV. This
238 possibly suggests that antiviral innate immune factors induced by SIV were responsible for

239 decreased SPgV replication during the acute-phase of SIV infection. Alternatively, SPgV and
240 SIV could share the same target cell-type, and the destruction of these cells by SIV could
241 explain the temporary reduction in SPgV plasma viral loads. This hypothesis warrants further
242 investigation, and studies designed to determine the tissue tropism of the pegiviruses and
243 SIV/HIV are ongoing. We are hopeful that future pegivirus studies will provide a deeper
244 understanding of the biology of these enigmatic viruses, and ultimately the mechanisms by
245 which HPgV protects humans from HIV-associated disease.

246

247 **Materials and Methods:**

248 ***Care and use of animals.*** All macaque monkeys used in this study were cared for by the staff
249 at the Wisconsin National Primate Research Center in accordance with the regulations and
250 guidelines outlined in the Animal Welfare Act and the Guide for the Care and Use of Laboratory
251 Animals. Details of this study (UW-Madison Animal Care and Use Protocol No. G00707) were
252 approved by the University of Wisconsin Institutional Animal Care and Use Committee, in
253 accordance with recommendations of the Weatherall Report.

254

255 ***Selection of animals.*** To control for host genetic factors to the extent possible, we used
256 cynomolgus macaques from the island of Mauritius, where there is an inbred macaque
257 population due to a recent founder effect. All animals selected for this study were female and
258 were major histocompatibility complex (MHC)-matched: all animals were heterozygous with an
259 M3/M4 combination of MHC haplotypes. Unlike other defined MHC haplotypes found in
260 Mauritian cynomolgus macaques (e.g. M1), spontaneous control of SIV infection is not known to
261 be associated with the M3 or M4 haplotype (30).

262

263 ***Virus inoculations.*** A SPgV stock was created for this study by aliquoting plasma collected
264 from a macaque (cy0500) inoculated intravenously with plasma from an SPgV+ olive baboon

265 (*Papio anubis*) sampled in Kibale National Park, Uganda (GenBank accession: KF234530), as
266 described in detail previously (16, 17). Macaques infected with SPgV in this study were
267 inoculated intravenously with 700 μ L of cy0500 plasma containing 2.29×10^7 genome copies of
268 SPgV. SIV infections were achieved using a single intrarectal inoculation of 7,000 tissue-culture-
269 infectious-dose (TCID)₅₀ of the molecularly cloned SIVmac239 virus (GenBank accession:
270 M33262).

271

272 **Chemical induction of microbial translocation.** Microbial translocation was induced as
273 described previously (21). Briefly, a 0.5% solution of dextran sulfate sodium (DSS) was
274 prepared by resuspending colitis-grade DSS (MPBio, Santa Ana, CA) in sterile drinking water
275 and stored at 4°C. Animals were treated once per day for 5-consecutive days with 200 mL of the
276 DSS-containing drinking water, administered by gavage. Animals were monitored for clinical
277 signs of colitis and gastrointestinal distress, and received palliative and clinical care at the full
278 discretion of WNPRC veterinarians.

279

280 **RNA extraction from plasma for sequencing and viral loads.** RNA was extracted from 300
281 μ L of EDTA-treated plasma using the Viral Total Nucleic Acid Purification Kit (Promega,
282 Madison, WI) on a Maxwell 16 MDx instrument and eluted in 50 μ L of DNase/RNase free water.

283

284 **SPgV Viral loads.** A Taqman quantitative RT-PCR (qRT-PCR) assay was used to quantify viral
285 RNA for SPgV (forward primer: 5'-CGGTGTTTCATGGCAGGTAT-3'; reverse primer: 5'-
286 CAGTTACAGCCGCGTGTTT-3'; probe: 5'-6FAM-ATGCACCCTGATGTAAGCTGGGCAA-
287 BHQ1-3'), as described previously (16). Reverse transcription and PCR were performed using
288 the SuperScript III One-Step qRT-PCR system (Invitrogen, Carlsbad, CA) on a LightCycler 480
289 instrument (Roche, Indianapolis, IN). Reverse transcription was carried out at 37°C for 15
290 minutes and then 50°C for 30 minutes followed by 2 minutes at 95°C, and then 50 cycles of

291 amplification as follows: 95°C for 15 sec and 60°C for 1 minute. The 20 µL reaction mixture
292 contained 5 µL of extracted RNA, MgSO₄ at a final concentration of 3.0 mM, with the two
293 amplification primers at a concentration of 500 nM and probe at a concentration of 100 nM. RNA
294 copy number was calculated using a standard curve that was sensitive down to 10 copies of
295 RNA transcript per reaction.

296

297 **SIV viral loads.** A Taqman qRT-PCR assay was used to quantify viral RNA for SIV (forward
298 primer: 5'-GTCTGCGTCATCTGGTGCATTC-3'; reverse primer: 5'-
299 CACTAGCTGTCTCTGCACTATGTGTTTTG-3'; probe: 5'-6FAM-
300 CTCCTCAGTGTGTTTCACTTTCTTCTTGCG-BHQ1-3'), as described previously (31).
301 Cycling conditions were: 37°C for 15 min, 50°C for 30 min, and 95°C for 2 min, followed by 50
302 amplification cycles of 95°C for 15 sec and 62°C for 1 min with ramp times set to 3°C/sec. The
303 final reaction mixtures (20 µL total volume) contained 0.2 mM dNTPs, 3.5 mM MgSO₄, 150 ng
304 random hexamer primers (Promega, Madison, WI), 0.8 µL SuperScript III One-Step qRT-PCR
305 enzyme mix, 600 nM of each amplification primer and 100 nM of probe.

306

307 **Amplicon-based sequencing of SPgV.** SPgV was amplified with the Qiagen OneStep RT-
308 PCR kit and five overlapping ~2.5kb amplicons. Primers were designed using Primer3 (32) in
309 Geneious R9 (Biomatters, Auckland, NZ) (Table S4). Cycling conditions were: 50°C for 30
310 minutes and 94°C for 2 minutes, followed by 40 cycles of 94°C for 15 seconds, 55°C for 30
311 seconds, and 68°C for 2.5 minutes, followed by a final extension at 68°C for 5 minutes.
312 Amplicons were fragmented and sequencing adaptors were added using the Nextera DNA
313 Sample Preparation Kit (Illumina, San Diego, CA). Deep sequencing was performed on the
314 Illumina MiSeq, and sequence data were analyzed using Geneious Pro R9 (Biomatters,
315 Auckland, NZ). Low quality (<Q40, Phred quality score) and short reads [<100 base pairs (bp)]
316 were removed, and coding-complete genome sequences for SPgV were acquired by mapping

317 reads to the reference sequence for the SPgVkob inoculum (Genbank ID KF234530) using the
318 Geneious alignment tool at medium-low sensitivity. Consensus SPgV sequences from each
319 animal at each timepoint were compared to the inoculum using a ClustalW alignment with an
320 IUB cost matrix, gap open cost of 15, and gap extend cost of 6.66.

321

322 ***Immune cell activation and CD4+ T cell counts.*** Staining for flow cytometry was performed
323 on EDTA-anticoagulated whole blood as described previously (Pomplun, 2015). Briefly, 0.1 mL
324 of EDTA-anticoagulated whole-blood was incubated for 15 min at room temperature in the
325 presence of a mastermix of antibodies against CD38 (clone AT1, FITC conjugate, 20 μ l), CD69
326 (clone TP1.55.3, ECD conjugate, 10 μ L), CD3 (clone SP34-2, Alexa Fluor 700 conjugate, 3 μ l),
327 CD25 (clone M-A251, Brilliant Violet 421 conjugate, 5 μ l), CD8 (clone SK1, Brilliant Violet 510
328 conjugate, 2.5 μ l), CD20 (clone 2H7, Brilliant Violet 650 conjugate, 2 μ l), CD4 (clone L200,
329 Brilliant Violet 711 conjugate, 5 μ l) antigens. All antibodies were obtained from BD BioSciences
330 (San Jose, CA, USA), except the CD69-specific antibody, which was purchased from Beckman
331 Coulter (Brea, CA, USA) and the CD38-specific antibody, which was purchased from Stemcell
332 Technologies (Vancouver, BC, Canada). Cells were also stained with LIVE/DEAD Fixable Near-
333 IR during this time (Invitrogen, Carlsbad, CA). Red blood cells were lysed using BD Pharm
334 Lyse, after which they were washed twice in media and fixed with 0.125 mL of 2%
335 paraformaldehyde for 20 min. After an additional wash the cells were permeabilized using Bulk
336 Permeabilization Reagent from Invitrogen (Carlsbad, CA, USA). The cells were stained for
337 15 min with an antibody against Ki-67 (clone B56, Alexa Fluor 647 conjugate, 5 μ L) while the
338 permeabilizer was present. The cells were then washed twice and resuspended in 0.125 mL of
339 2% paraformaldehyde for 20 min. After a final wash and resuspension with 125 μ L PBS
340 supplemented with 10% fetal bovine serum (fluorescence-activated cell sorting [FACS] buffer),
341 all samples were run on a BD LSRII Flow Cytometer within 24 hrs. Flow data were analysed
342 using Flowjo version 9.8.2. Absolute CD4+ T cell counts were determined by multiplying the

343 absolute number of lymphocytes obtained by complete blood count by the percentage of
344 lymphocytes that stained positive for CD3 and CD4 and negative for CD20 and CD8 by flow
345 cytometry.

346

347 ***Gut and lymph node histology.*** Tissues were collected from anesthetized macaques, fixed in
348 10% formalin, then embedded in paraffin (FFPE). Five micron thick sections were cut from
349 FFPE blocks and mounted on charged slides. To remove paraffin, slides were baked at 80°C,
350 treated with xylene (5 min x 3), and hydrated through graded alcohols to deionized water. Heat-
351 induced epitope retrieval was performed in pH 9.0 Tris-EDTA solution (10mM tris base, 1 mM
352 EDTA, 0.05% tween-20) for 3 minutes (Ki67) or 1 minute (CD4) in a Decloaking Chamber
353 (Biocare Medical, Concord, CA). Slides were then rinsed with PBS and blocked with 0.3% H₂O₂
354 in PBS for 10 min at room temperature followed by serum [10% goat serum (Sigma, St. Louis,
355 MO) in PBS] for 1 hr at room temperature. Slides were incubated with primary antibody in PBS
356 with 1% goat serum and 0.1% Triton X-100 overnight at 4°C in dilutions of 1:800 for anti-Ki67
357 (BD Pharmingen, 556003) or 1:100 for anti-CD4 (Leica, NCL-L-CD4-368). Slides were then
358 rinsed thrice with PBS and treated with Signal Stain Boost IHC Detection Reagent (HRP,
359 Mouse) (Cell Signaling Technology, Beverly, MA) for 30 min at room temperature. Slides were
360 then rinsed three times with PBS and treated with DAB substrate (Cell Signaling Technology,
361 Beverly, MA) for 1 min and Mayer's hematoxylin (Sigma, St. Louis, MO) for 1 min. Slides were
362 then rinsed in H₂O and dehydrated through graded alcohols to xylene.

363

364 Immunohistochemical (IHC) stains for Ki67 and CD4 were scored by a pathologist who was
365 blinded to study design and treatment assignment. Quantification was reported as the average
366 of 10 high-power fields (600X). In the lymph nodes, Ki67 positive cells were counted within the
367 parafollicular region only, since germinal centers were universally positive. In the colon both
368 Ki67 and CD4 were quantified within the lamina propria and crypt epithelial cells were excluded.

369

370 **Statistical analysis.** Information on statistical tests used to determine significance can be found
371 in corresponding figure legends. All statistical analyses were performed in Graphpad Prism
372 software version 6.0h (GraphPad Software, Inc., La Jolla, CA).

373

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431

432 **Author contributions:**

433 ALB, CRB, and DHO conceived of the experimental design. CRB collected tissues and
434 performed all flow cytometry experiments and analysis with assistance from ALB and MM. MB
435 and LMS assisted with tissue collection. CRB performed the sequencing analysis. AJE helped
436 prepare the SIVmac239 inoculum.. EJP, KGB, and SCIII provided clinical support for infected
437 animals, performed tissue biopsies, and administered virus inocula. HAS processed tissues for
438 histopathological analysis. DM and DTY performed the histopathological analyses. ALB wrote
439 the manuscript with edits, input, and approval from all authors.

440

441 **Competing interests:**

442 The authors declare no competing interests.

443

444 **Data and materials availability:**

445 The coding-complete genome sequence for the SPgV strain used in this study can be found in
446 the Genbank database under accession number KF234530.

447

448 **Figure legends:**

449 **Figure 1. SPgV and SIV viral loads in infected macaques.** Titers for each virus were
450 measured from plasma using highly sensitive virus-specific quantitative RT-PCR assays. (A)
451 SPgV titers in the four macaques infected with SPgV+SIV. (B,C,D) SIV titers in four macaques
452 infected with SPgV+SIV (green) and four macaques infected with SIV-only (black). *P* values
453 reflect a two-tailed unpaired t-test and error bars represent SEM. The symbols used for each
454 animal in this figure are consistent throughout the manuscript.

455

456 **Figure 2. SIV pathogenesis in SIV-only vs. SIV+SPgV infected macaques.** (A) Peripheral
457 CD4+ T cell counts were obtained by multiplying absolute lymphocyte counts by the percentage
458 of lymphocytes that were CD3+ CD4+ CD20- CD8- (see Figure 3 for gating strategy details). (B)
459 Gut CD4+ T cells were stained within sections of colonic tissues via IHC with an anti-CD4
460 antibody and manually quantified. Significant differences between the SIV-only and SPgV+SIV
461 groups were analyzed using a two-tailed unpaired t-test (solid line) with error bars representing
462 SEM. Significant changes in all animals over the course of acute SIV infection were quantified
463 using a two-tailed paired t-test (dashed line). (C) A representative set of colonic tissue from
464 Cy0883 (SIV+SPgV) and Cy0887 (SIV-only) are shown pre and post SIV infection at 400x for
465 comparison. Arrows highlight representative cells with membranous CD4 staining.

466

467 **Figure 3. Peripheral immune activation in SIV-only vs. SIV+SPgV infected macaques.**
468 Fresh whole blood was used for staining and flow cytometry at each timepoint. *P* values
469 represent a two-tailed unpaired t-test with error bars reflecting SEM. Note: Cy0886 did not
470 exhibit a distinct peak or nadir of CD69+ Ki67+ expression in the CD3+ CD8+ T cell population,
471 and so is not included in these analyses.

472

473 **Figure 4. Activation of immune tissues in SIV-only vs. SIV+SPgV infected macaques.**

474 Proliferating cells were stained within sections of lymph nodes (**A**) and colon (**B**) via IHC with an
475 anti-Ki67 antibody and manually quantified. Significant changes over time were quantified using
476 a two-tailed paired t-test (dashed line). A representative set of lymph nodes from Cy0883
477 (SIV+SPgV) and Cy0881 (SIV-only) is shown at 400X pre and post SIV infection for comparison
478 in (**A**). A representative set of colon tissues from Cy0886 (SIV+SPgV) and Cy0887 (SIV-only) is
479 shown pre and post SIV infection at 400x for comparison in (**B**).

480

481 **Figure 5. Immune activation following SPgV vs. SIV infection. (A-C)** Fresh whole blood was

482 used at each timepoint. *P* values are from a two-tailed paired t-test comparing pre-infection
483 average to post-SPgV or post-SIV average within the first 26 days post-infection for each virus,
484 respectively. (**D**) Proliferating cells were stained within sections of lymph nodes via IHC with an
485 anti-Ki67 antibody and manually quantified. Significant changes in over time were quantified
486 using a two-tailed paired t-test. (**E**) Representative set of lymph node tissue from Cy0885 is
487 shown at 400x.

488

489 **Supplementary Materials:**

490 Supplemental figure: Fig S1

491 Supplemental Tables: Tables S1-S4