Recent SIV-Macaque Trials:

A Reassessment of Immune Priming and Varying Infectability in Repeated Low-dose Challenge Studies

Short title: Repeated Low-dose SIV Challenges in Macaques

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Abstract

Nowadays, most preclinical HIV treatment trials use a protocol of administering repeated low-dose challenges (RLCs) of simian immunodeficiency virus (SIV) to macaques. Statistical analyses of treatment efficacy under the RLC protocol need to consider two confounding hypotheses, both pertinent biologically to HIV: (1) the non-infecting challenges may immunize animals against SIV; and (2) the animals may vary in intrinsic infectability ("frailty"). To explore the two hypotheses, a previous study (Regoes 2012) assembled a database from 7 articles with SIV-macaque treatment trials. With two explicable exceptions, Regoes concluded that the control data did not support either confounding hypothesis. Recent SIV-macaque trials present opportunities to evaluate the conclusions' robustness. Accordingly, the present article assembles from 24 articles an updated database containing net survival curves from both control and treatment arms in SIV-macaque treatment trials. Broad patterns of statistical significance (at $p<0.05$, uncorrected for multiple testing) made it difficult to dismiss the confounding hypotheses completely in the controls. Although statistical analysis has focused on defense against variable frailty, only one set of controls showed significant variable frailty, whereas many sets showed significant immunization. As trials progressed, changes in the probability of infection per challenge were significant in 8/28 trials (1/3 trials using oral challenges; 2/4 trials using vaginal challenges; and 5/21 trials using rectal challenges). The results suggest the possibility that vaginal challenges may immunize animals faster than rectal challenges, and they also bear on previous conclusions that repeated exposure to HIV without treatment may have no effect on infectability or may even reduce it.

Author Summary

Many preclinical trials of HIV treatments rely on repeatedly administering low-dose SIV challenges to macaques until infection occurs. The repeated low-dose protocol reuses macaques and is more sensitive to subtle therapeutic efficacies than a protocol administering a single large dose to each macaque. The animal reuse raises some pertinent biological questions, notably: (1) do macaques have intrinsically variable infectabilities? and (2) do the repeated SIV challenges immunize macaques against infection? A 2012 study collected a database of eight macaque trials, concluding that variable infectability and immunization were at most sporadic and readily explicable. I expanded the 2012 database to twenty-eight trials, discovering that the conclusions were not robust. Although only 1/28 SIV-macaque trials showed variable infectability, 7/28 showed immunization, with few ready explanations. Statistical analysis of SIV-macaque trials has focused on the confounding effects of variable infectability to the neglect of immunization, so the expanded database provides a rich empirical resource. The trials have general medical importance because they provide a model for analyzing animal trials of infectious disease therapies and other sparse trials, e.g., for breast cancer. My findings also indirectly suggest that repeated human exposure to HIV inconsistently immunizes and can foster either immune priming or tolerance.

Introduction

Preclinical experiments play a fundamental role in determining treatments for an infectious disease. In a modern re-interpretation, some of Koch's postulates specify reproducing the disease in an animal model and then reisolating the agent from an animal with signs resembling the human disease [1]. Historically, experimenters therefore tested vaccines or preventive treatment strategies against human-immunodeficiency virus (HIV) preclinically in non-human primates. They found that HIV infects in the chimpanzee animal model, although it does not consistently produce an AIDS-like disease.

Typically, preclinical HIV trials have control and treatment arms, and they infer therapeutic protection if treated animals are less easily infected than controls. In the 1980s, preclinical HIV trials were extremely sparing because of the cost of chimpanzees, e.g., one trial used 6 chimpanzees in a control arm and 2 chimpanzees in a treatment arm [2]. Such trials ensured infection of controls with a large viral challenge [3, 4]. The trials then applied a statistical test, e.g., the Fisher's exact test [5], to the resulting 2x2 tables of counts of animals in control/treated vs infected/uninfected groups. Unfortunately, with so few counts, the single high-dose protocol provided scant statistical power for detecting subtle protection against infection. Accordingly, experimenters relied heavily on surrogates of protection to detect a treatment effect, e.g., an increase in virus-specific immune responses from a vaccine treatment, or a decrease in the steady-state viral load (a.k.a. the viral set-point). The surrogates are chosen to correlate with protection, although they are not necessarily causal.

Since 2007, chimpanzees are an endangered species no longer bred for research [6], requiring other animal models for HIV infection and AIDS. Mice can receive transplanted components of a human immune system [7], and when applicable, the humanized mouse model is cost-effective.

78 The mouse model has, however, many serious limitations in cell number and volume, life span,
79 integrated operation of an immune system, etc. [8]. By contrast, over 40 different simian
80 immunodeficiency viruses (SIVs) infect primate species without such limitations [9], and some
81 do produce AIDS-like illnesses. Presently, the rhesus macaque and its SIV provide the most
82 common preclinical primate model of AIDS [10-33].

83 Although rhesus macaques provide less expensive preclinical data than chimpanzees, their
84 cost is still severe. Excluding the historical controls used in recent trials [26, 29, 32], macaque
85 trials have limited themselves on average to about 13 contemporary controls. Under such
86 limitations, if the trials used the single high-dose protocol, they would remain seriously
87 underpowered for detecting a subtle treatment effect [34].

88 Accordingly, preclinical HIV treatment trials have adopted a protocol using repeated low-
89 dose challenges (RLCs) [10-33]. As Table 1 of [18] helpfully illustrates, typically each animal is
90 challenged repeatedly at regular intervals with the same low viral dose, until either it is infected
91 or the experiment finishes. Experimenters usually challenge weekly, because the anesthetized
92 animal can then receive the current challenge after tests for infection from the previous week are
93 initiated. To limit the number of challenges required to infect controls, the challenge dose often
94 approximates the animal ID_{50} [4]. Some experiments challenge beyond infection (e.g., [10]), but
95 post-infection challenges are irrelevant to present purposes, and we ignore them.

96 The RLC protocol has several advantages [35], both biological and statistical [36]. First,
97 RLCs mimic human exposure to HIV, making the animal studies conceptually closer to
98 subsequent human studies. Second, they can in principle detect immunization of controls
99 statistically during repeated challenge, as follows. RLCs yield net survival curves for each
100 control or treatment arm (e.g., Figure 3 of [18]), where the curves count the uninfected animals
101 before and after each challenge. Non-infecting challenges might gradually immunize animals,

102 but statistical analysis of the fraction of controls infected after each challenge can detect, e.g.,
103 systematic decreases in the fraction over time. In effect, the statistical analysis defines
104 "infectability" as a probability of infection, with "immunization" as the corresponding change in
105 infectability. To paraphrase [35], the statistical definition of immunization presumes neither
106 knowledge nor measurement of the immune surrogates of protection, but instead specifies what
107 matters epidemiologically. In contrast, biological studies examining laboratory surrogates of
108 protection (e.g., [2]) implicitly rely on a strong but unproved assumption, that the laboratory
109 surrogates are causally linked to immune protection [37]. Though limited in other ways, a
110 statistical definition of immunization deftly avoids implicit assumptions about causality.

111 Third, RLCs can also expose intrinsic differences in infectability between subpopulations of
112 animals, their "frailties" in the terminology of survival analysis [35]. Immune surrogates have
113 demonstrated some specific subpopulation differences, e.g., the peak plasma RNA in
114 SIVsmE660 infection is about 10-fold reduced in rhesus macaques with the *Mamu-A*01* class I
115 allele [19]. On one hand, therefore, a study examining immune surrogates can draw specific
116 biological conclusions. On the other hand, a statistical analysis of infectability has the
117 complementary strength of generality, because it may detect immunization or variable frailty
118 regardless of specific biological mechanisms like alleles or restriction factors [38].
119 Unfortunately, because of a phenomenon known as "heterogeneity's ruse" [39] (see also the
120 Discussion and the SI), statistical analysis of a net survival curve does not dependably
121 distinguish variable frailty from immunization. Further biological analysis is often still required
122 to disentangle their effects [35].

123 The RLC protocol has already demonstrated substantial advantages over the single high-dose
124 protocol [36], but some advantages may remain unrealized. Presently, statistical analyses of
125 many SIV-macaque trials use a standard survival analysis with the logrank test [22-24, 26, 29,

126 32]. The standard logrank test requires a large-sample Gaussian approximation, whereas the
127 sparsity of SIV-macaque data suggests the need for an exact test. The development of an exact
128 test is eased if immunization, variable frailty, or other confounding effects do not occur in SIV-
129 macaque trials, because then every challenge in an RLC protocol presents the same hazard of
130 infection to every control animal [40, 41]. In 2012, therefore, Regoes collected a database of 7
131 articles with SIV-macaque treatment trials, concluding that most did not display immunization or
132 variable frailty [35].

133 In 2015, Nolen et al [42] pointed out that a Fisher exact test is valid in a single high-dose
134 challenge study, but not in a RLC study if it is applied to the total challenge counts in different
135 arms of the trial. They therefore suggested exact permutation tests based on exchangeability of
136 control and treatment animals under the null hypothesis of treatment having no effect. After a
137 careful theoretical analysis, they applied the permutation tests, demonstrating that large-sample
138 p-value approximations could yield misleading scientific conclusions in small trials. In passing,
139 however, observe that the single Ellenberger (2006) trial [17] mentioned explicitly in Section 5
140 of Nolen et al [42] was mildly atypical (see comments in the Results), and that the only
141 confounding hypotheses Nolen et al [42] simulated involved variable frailty. These two
142 observations partially motivated the present article by suggesting that statistical analysis might
143 profit from a comprehensive database of SIV-macaque trials.

144 Although the present article notes some technical points, it does not improve substantially on
145 Regoes' statistical analysis of immunization and variable frailty [35]. New SIV-macaque trials
146 contain many pertinent data, however. The present article therefore assembles a comprehensive
147 updated database from 24 articles containing the net survival curves for control and treatment
148 arms in SIV-macaque treatment trials. It has the purview of applying Regoes' statistical analysis
149 to a larger database to examine the robustness of his scientific conclusions.

150 The RLC protocol has regularities not available in clinical trials, notably compulsory follow
151 up and a possibility that between observations, all hazards to controls may be roughly the same,
152 i.e., all probabilities of infection per challenge are roughly equal. The database may therefore
153 provide useful biological examples for applications with data more irregular than SIV-macaque
154 treatment trials but still requiring the development of small-sample statistical tests, e.g., small
155 studies of breast cancer therapies [43]. As Regoes also pointed out [35], the database also
156 provides an animal model for exploring repeated low-dose exposure to HIV in humans without
157 treatment.

158 The present article is structured as follows. The Materials and Methods section gives the
159 criteria for trial data to enter our SIV-Macaque database of control results. The human effort in
160 assembly of the database and its subsequent manipulation was demanding, so the Materials and
161 Methods section discusses at length the various representations of data in the original articles,
162 during digitization of survival curves, and for the survival analysis [44]. It then gives Regoes'
163 models and two additional full models (Geometric priming and Delta frailty), promoting
164 continuity with Regoes' statistical analysis [35] by using his term "priming models" for
165 immunization. The Results section presents three tables pertinent to our SIV-Macaque database:
166 (1) the metadata; (2) the count of controls uninfected after each trial's maximum number of
167 challenges, with estimates of the infection probability per challenge under the assumption that
168 the probability is constant; and (3) p-values pertinent to the constancy of infection probabilities
169 per challenge. The three tables are drawn from an aggregate table in the SI. Finally, the
170 Discussion section explores the implications of the results.

171 Results

172 The comprehensive Excel file in the SI yielded all rounded numbers in Tables 1-3.

173 Table 1 presents the metadata of the SIV-Macaque database. Each row corresponds to a
174 single control arm and its trial. The columns indicate: (1) the citation in the present article; (2) its
175 first author; (3) its year of publication; (4) its ID in PubMed; (5) the source within it for each set
176 of controls (a citation may contain more than one trial and therefore more than one source of
177 data); (6) the virus used to challenge; (7) the species of macaque challenged; (8) the challenge
178 route; and (9) the number of weeks between the regular challenges. In the final 3 columns,
179 uncommon values are highlighted in yellow, e.g., the Ellenberger (2006) trial [12] used SHIV-
180 SF162P3 to challenge pigtailed macaques rectally every week, the Wilson (2009) trial [16] used
181 SIVsmE660 to challenge rhesus macaques rectally every 3 weeks, etc.

182 The first 5 columns of Table 1 appear in all tables, so the reader can recall references and still
183 find them immediately and unambiguously in the literature. In all tables, the rows are ordered on
184 the PubMed ID in Column 4; and the heavy borders enclose the Regoes' dataset [35].

185

186 **Table 1**: **Metadata for the SIV-Macaque database**

Cite	First Author	Year	PMID	Source	Virus	Macaque	Route	Weeks
[10]	Ma	2004	15564513	Table-1	SIVmac251	Rhesus	Vaginal	1
[11]	Van Rompay	2005	15671796	Figure-4A	SIVmac251	Rhesus	Oral	1
[12]	Ellenberger	2006	16725169	Figure-1	SHIV-SF162P3	Pigtailed	Rectal	1
[13]	Wilson	2006	16731926	Table-2	SIVmac239	Rhesus	Rectal	1
[14]	Garcia-Lerma	2008	18254653	Figure-2	SHIV-SF162P3	Rhesus	Rectal	1
[15]	Hansen	2009	19219024	Figure-4c	SIVmac239	Rhesus	Rectal	1
[16]	Wilson	2009	19403685	Table-2	SIVsmE660	Rhesus	Rectal	3
[17]	Hessell	2009	19525965	Figure-2	SHIV-SF162P3	Rhesus	Vaginal	0.5
[18]	Reynolds	2010	20592091	Table-1	SIVsmE660	Rhesus	Rectal	1
[19]	Letvin	2011	21543722	Figure-1A	SIVmac251	Rhesus	Rectal	1
[19]	Letvin	2011	21543722	Figure-1B	SIVsmE660	Rhesus	Rectal	1
[19]	Letvin	2011	21543722	Figure-1C	SIVsmE660	Rhesus	Rectal	1
[20]	Hansen	2011	21562493	Figure-S6	SIVmac239	Rhesus	Rectal	1
[21]	Barouch	2012	22217938	Figure-1e	SIVmac251	Rhesus	Rectal	1
[21]	Barouch	2012	22217938	Figure-3e	SIVmac251	Rhesus	Rectal	1
[22]	Pegu	2013	23175374	Figure-1B	SIVmac251	Rhesus	Rectal	1
[23]	Strbo	2013	23401588	Figure-3AB	SIVmac251	Rhesus	Rectal	1
[23]	Strbo	2013	23401588	Figure-3C	SIVmac251	Rhesus	Rectal	1
[24]	Roederer	2014	24352234	Figure-1a	SIVsmE660	Rhesus	Rectal	1
[25]	Radzio	2016	26743846	Figure-1B	SHIV-SF162P3	Pigtailed	Vaginal	1
[26]	Vaccari	2016	27239761	Figure-1bc	SIVmac251	Rhesus	Rectal	1
[27]	Jensen	2016	27252535	Figure-1C	SIVmac251	Rhesus	Oral	1
[28]	Butler	2016	27355414	Figure-1A	SHIV-SF162P3	Pigtailed	Vaginal	1
[29]	Gordon	2016	27591322	Figure-4E	SIVmac251	Rhesus	Rectal	1
[30]	Xu	2017	28302457	Figure-2c	SIVmac251	Rhesus	Rectal	1
[31]	Eudailey	2018	29359183	Figure-7A	SHIV-1157ipd3N4	Rhesus	Oral	1
[32]	Vaccari	2018	29785023	Figure-1b	SIVmac251	Rhesus	Rectal	1
[33]	Om	2020	32881968	Figure-1B	SHIV-1157ipd3N4	Rhesus	Rectal	1

187

188

189 Table 2 focuses on results pertinent to the Constant Hazard reduced model, which assumes
190 that every challenge has a constant probability of infection. Columns after Column 5 indicate: (6)
191 the number n of control animals; (7) the number of uninfected controls remaining after RLCs,
192 with non-0 values highlighted in yellow; (8) the number m of RLCs that uninfected controls
193 survived; (9) the MLE $\hat{p}$ of the probability p of infection in the reduced model; and the (10)
194 lower and (11) upper endpoint of the corresponding 95% confidence interval for p. Thus, Table
195 2 indicates for macaque trials the potential sparsity of the control arm, controls remaining
196 uninfected after RLCs, and an approximation to the infection probability p of each challenge.

197 For the Hansen (2009) trial [15], Regoes [35] gives the control number as $n=15$, whereas the
198 legend of Figure-4c, the source, gives $n=16$, yielding minor numerical discrepancies of no
199 scientific substance. In the Hansen (2011) trial [20], the single uninfected control eventually
200 showed signs of infection, but long after the 1-week window for establishing infection during the
201 trial. Its designation as "uninfected" is therefore somewhere arbitrary but at least consistent with
202 the intent of the RLC protocol. Some controls were historical compared to treated animals [26,
203 29, 32]. Both the Vaccari (2016) trial [26] and the Gordon (2016) trial [29] used 23 concurrent and
204 24 historical controls. The two control datasets yielded the same net survival curve. To make the
205 presentation consistent, the statistical analysis appears twice in all tables, once for each trial. The
206 Vaccari (2018) trial [32] used 6 concurrent and 47=23+24 historical controls.
207

Table 2: Constant Hazard reduced model for controls in the SIV-Macaque database

Cite	First Author	Year	PMID	Source	*n*	Not inf	m	p_hat	p_lo	p_hi
[10]	Ma	2004	15564513	Table-1	8	0	5	0.364	0.186	0.572
[11]	Van Rompay	2005	15671796	Figure-4A	5	0	25	0.312	0.125	0.555
[12]	Ellenberger	2006	16725169	Figure-1	14	1	13	0.206	0.119	0.317
[13]	Wilson	2006	16731926	Table-2	8	0	8	0.216	0.105	0.365
[14]	Garcia-Lerma	2008	18254653	Figure-2	18	1	14	0.205	0.128	0.300
[15]	Hansen	2009	19219024	Figure-4c	15	0	12	0.242	0.147	0.358
[16]	Wilson	2009	19403685	Table-2	8	2	5	0.250	0.108	0.443
[17]	Hessell	2009	19525965	Figure-2	4	0	40	0.400	0.146	0.700
[18]	Reynolds	2010	20592091	Table-1	8	1	10	0.318	0.151	0.525
[19]	Letvin	2011	21543722	Figure-1A	20	0	5	0.500	0.349	0.651
[19]	Letvin	2011	21543722	Figure-1B	25	3	12	0.200	0.133	0.281
[19]	Letvin	2011	21543722	Figure-1C	20	5	12	0.124	0.073	0.190
[20]	Hansen	2011	21562493	Figure-S6	28	1	25	0.156	0.107	0.215
[21]	Barouch	2012	22217938	Figure-1e	8	0	6	0.727	0.435	0.924
[21]	Barouch	2012	22217938	Figure-3e	8	0	6	0.533	0.291	0.765
[22]	Pegu	2013	23175374	Figure-1B	11	0	5	0.333	0.189	0.502
[23]	Strbo	2013	23401588	Figure-3AB	12	0	10	0.480	0.293	0.671
[23]	Strbo	2013	23401588	Figure-3C	12	0	6	0.324	0.189	0.483
[24]	Roederer	2014	24352234	Figure-1a	20	0	12	0.339	0.227	0.465
[25]	Radzio	2016	26743846	Figure-1B	5	0	16	0.313	0.125	0.555
[26]	Vaccari	2016	27239761	Figure-1bc	47	0	10	0.367	0.287	0.453
[27]	Jensen	2016	27252535	Figure-1C	6	0	9	0.240	0.103	0.428
[28]	Butler	2016	27355414	Figure-1A	11	5	20	0.045	0.018	0.089
[29]	Gordon	2016	27591322	Figure-4E	47	0	10	0.367	0.287	0.453
[30]	Xu	2017	28302457	Figure-2c	6	2	5	0.235	0.080	0.465
[31]	Eudailey	2018	29359183	Figure-7A	7	1	8	0.176	0.074	0.326
[32]	Vaccari	2018	29785023	Figure-1b	53	0	10	0.414	0.331	0.500
[33]	Om	2020	32881968	Figure-1B	16	7	3	0.237	0.122	0.386

211 Table 3 focuses on various full models nesting the Constant Hazard reduced model, and it
212 displays the p-values (uncorrected for multiple testing) from the corresponding LRTs. Columns
213 after the 5-th indicate the p-values for the following full models: (6) Arithmetic Priming (AP);
214 (7) Geometric Priming (GP); (8) Step Priming with l_{step}=1 (SP1); (9) Step Priming with l_{step}=2
215 (SP2); (10) Step Priming with l_{step}=3 (SP3); (11) Beta Frailty (BF); and (12) Delta Frailty (DF).
216 In the final 7 columns, any p-value less than 0.05 is highlighted in yellow.
217 Table 3 had 8/28 rows corresponding to trials displaying deviation from a constant infection
218 probability per challenge at statistical significance p<0.05, uncorrected for multiple testing. The
219 8 trials showing deviation included 1/3 trials using oral challenges, 2/4 trials using vaginal
220 challenges, and 5/21 using rectal challenges. For each full model of immune priming with a
221 significant LRT, the second of its two MLE parameters (given in the SI) indicates whether the
222 infection probability per challenge increased or decreased (tolerance or priming) as the trial
223 progressed. The direction of change, increase or decrease, was different in different trials.
224

225 **Table 3: Likelihood ratio test (LRT) p-values for the SIV-Macaque database**

Cite	First Author	Year	PMID	Source	AP	GP	SP1	SP2	SP3	BF	DF
[10]	Ma	2004	15564513	Table-1	0.084	0.060	0.395	0.054	0.250	1.000	1.000
[11]	Van Rompay	2005	15671796	Figure-4A	0.390	0.229	0.099	0.045	0.097	0.110	1.000
[12]	Ellenberger	2006	16725169	Figure-1	0.446	0.406	0.492	0.098	0.131	0.380	0.600
[13]	Wilson	2006	16731926	Table-2	0.017	0.024	0.035	0.036	0.428	1.000	1.000
[14]	Garcia-Lerma	2008	18254653	Figure-2	0.425	0.371	0.837	0.176	0.155	0.321	0.665
[15]	Hansen	2009	19219024	Figure-4c	0.856	0.834	0.799	0.307	0.992	1.000	1.000
[16]	Wilson	2009	19403685	Table-2	1.000	1.000	0.297	0.807	0.768	1.000	0.825
[17]	Hessell	2009	19525965	Figure-2	0.048	0.103	0.016	0.749	0.158	1.000	1.000
[18]	Reynolds	2010	20592091	Table-1	0.020	0.028	0.170	0.036	0.124	0.043	0.023
[19]	Letvin	2011	21543722	Figure-1A	0.810	0.769	0.204	0.489	0.677	0.696	1.000
[19]	Letvin	2011	21543722	Figure-1B	0.101	0.076	0.234	0.027	0.033	0.086	0.119
[19]	Letvin	2011	21543722	Figure-1C	0.214	0.222	0.705	0.407	0.706	0.238	0.217
[20]	Hansen	2011	21562493	Figure-S6	0.269	0.264	0.371	0.319	0.868	0.275	0.320
[21]	Barouch	2012	22217938	Figure-1e	0.910	0.898	0.785	0.412	1.000	1.000	1.000
[21]	Barouch	2012	22217938	Figure-3e	0.504	0.454	0.782	0.601	0.251	1.000	1.000
[22]	Pegu	2013	23175374	Figure-1B	0.006	0.007	0.025	0.022	0.183	1.000	1.000
[23]	Strbo	2013	23401588	Figure-3AB	0.877	0.869	0.847	0.568	0.584	1.000	1.000
[23]	Strbo	2013	23401588	Figure-3C	0.003	0.003	0.020	0.012	0.058	1.000	1.000
[24]	Roederer	2014	24352234	Figure-1a	0.966	0.962	0.898	0.652	0.506	1.000	1.000
[25]	Radzio	2016	26743846	Figure-1B	0.096	0.089	0.030	0.889	0.361	1.000	1.000
[26]	Vaccari	2016	27239761	Figure-1bc	0.267	0.268	0.388	0.258	0.449	1.000	1.000
[27]	Jensen	2016	27252535	Figure-1C	0.655	0.557	0.549	0.569	0.910	1.000	1.000
[28]	Butler	2016	27355414	Figure-1A	0.052	0.133	0.315	0.993	0.600	0.224	0.099
[29]	Gordon	2016	27591322	Figure-4E	0.267	0.268	0.388	0.258	0.449	1.000	1.000
[30]	Xu	2017	28302457	Figure-2c	0.181	0.257	0.488	0.442	0.121	0.305	0.222
[31]	Eudailey	2018	29359183	Figure-7A	0.476	0.386	0.417	0.912	0.364	1.000	1.000
[32]	Vaccari	2018	29785023	Figure-1b	0.178	0.180	0.282	0.246	0.267	1.000	1.000
[33]	Om	2020	32881968	Figure-1B	0.738	0.757	0.538	0.906	1.000	1.000	1.000

226

227

228 # Discussion

229 All p-values below are uncorrected for multiple testing.

230 In the tables, the rows inside the thick borders correspond to Regoes' database [35]. The
231 rows in Table 2 differ from Regoes' Table 2 in: (1) the subdivision of data from the Letvin
232 (2011) trial [19]; and (2) the number of controls in the Hansen (2009) trial [15]. Table 3 and
233 Regoes' Tables 3 and 4 have analogous differences. Otherwise, the present analysis agrees
234 broadly within numerical error on its overlap with Regoes' [35]. Regoes' Tables 3 and 4
235 displayed only a few anomalous rows with $p<0.05$, consistent with his conclusion that a constant
236 hazard of infection per challenge could explain most of his data [35]. The anomaly in the Wilson
237 (2006) trial was ascribed to possible misidentification of infecting challenges; the anomaly in the
238 Letvin (2011) Figure-1B trial, to the known biological effect of TRIM5 alleles on SIV infection
239 (e.g., [52]).

240 The trials in Table 3 outside Regoes' Tables 3 and 4 continue to display a broad pattern of
241 anomalies with $p<0.05$, however, making the anomalies harder to dismiss. Perhaps some
242 anomalies still reflect misidentification of the infecting challenges, but the misidentification then
243 becomes a persistent feature of the SIV-macaque trials. Other anomalies may depend on trials
244 using oral or vaginal challenge, which Regoes [35] excluded. The remaining anomalies may
245 reflect unknown and therefore interesting biological effects. The persistent pattern of anomalies
246 in Table 3 made Regoes' extensive analysis of the power of the LRTs against alternatives
247 unnecessary. By themselves, the anomalies clearly discourage any simplifying assumption of a
248 constant hazard of infection per challenge in SIV-macaque trials, particularly for oral or vaginal
249 challenges, particularly without specific evidence that supports the assumption's use as an
250 approximation.

The SIV-Macaque database and Table 3 noticeably enrich the empirical basis for the statistical analysis of sparse animal trials. Presently, permutation tests provide the leading candidates for exact tests. The power analysis of the permutation tests in [42], e.g., focused on confounding hypotheses of variable frailty, namely, the two frailty full models in the present article. The frailty models accurately reflect experimenters' concerns about the confounding effects of, e.g., sex [33] or the *Mamu-A*01* class I allele [19]. Table 3 suggests, however, that a statistical exploration of the two confounding hypotheses should probably be more concerned about immunization than variable frailty in [42].

The pattern of anomalies in Table 3 also has a notable feature. Except for the Hessell (2009) trial [17] and the Radzio (2016) trial [25], both of which challenged vaginally, whenever a full model in Table 3 displayed $p<0.05$, the full model SP2 for step priming after two challenges in the same row also displayed $p<0.05$. Under a weekly challenge, the full model SP2 could be interpreted as changes in control immunity occurring after 2 weeks, e.g., the recommended period for full immunity after COVID-19 vaccination. The immune changes were inconsistent: neither immune priming nor immune tolerance occurred consistently (see end of the Results and the SI). For the full model SP2, e.g., 3/6 changes indicated increased probability of infection per challenge (tolerance), rather than priming. Typically, however, net survival curves alone cannot distinguish variable frailty from immunization ("heterogeneity's ruse" [39]). The inconsistent immune interpretation of the SP2 full model does, however, bear on previous biological conclusions that repeated exposure without treatment may have no effect [35] or may even reduce [53] human infectability in HIV acquisition.

The exceptions above, the Hessell (2009) trial [17] and the Radzio (2016) trial [25], used vaginal challenge. Neither had full model SP2 displaying $p<0.05$, but instead had full model SP1

274 for step priming after one challenge displaying $p<0.05$, suggesting that immune priming in
275 vaginal challenge may occur faster than in rectal challenge.

276 The priming models do have some mild technical superiorities over frailty models, namely:
277 (1) in the context of net survival curves, priming models are more general than frailty models, at
278 least in one sense (see the SI); (2) they have strictly concave log-likelihoods (see the SI), slightly
279 simplifying numerical computations; and (3) they nest the reduced model in the interior of their
280 parameter space (see the Materials and Methods section), thereby satisfying the usual hypotheses
281 for theorems about the LRT [50]. Table 3 shows that for most trials, however, if one full model
282 achieves statistical significance at $p<0.05$, several others often follow. Typically, the underlying
283 SIV-macaque data are sparse, and the net survival curves are correspondingly nondescript. If
284 several models show significance, then, no specific biological interpretation can be considered
285 pre-eminent without further investigation.

286 If some controls remain uninfected after RLCs, e.g., it suggests the confounding hypothesis
287 that the controls may have an unusually low infectability. Fortunately, hyper-challenges with
288 increased viral doses after the RLCs can remove the doubt about low infectability [16, 31, 33],
289 although its cause, immunization or variable frailty, would remain unclear.

290 To summarize, then, the present article updated Regoes' database [35] with the SIV-Macaque
291 database in the SI. The updated database displays several trials where the controls appear not to
292 have a constant hazard of infection, and where full models of immune priming (or tolerance) or
293 variable frailty fit control data significantly better at uncorrected $p<0.05$ than the reduced model
294 positing a constant probability of infection per challenge. Given sparse animal data, exact tests
295 have advantages over large-sample tests like the standard logrank test that use Gaussian
296 approximations. Unfortunately, the present results show that any statistical test under
297 consideration should also be shown to be robust against confounding hypotheses about control

298 animals [54], notably both repeated low-dose immunization and variations in frailty.
299 Biologically, the data from SIV-macaque trials are consistent with the RLC protocol changing
300 the infectability of animals after about two weeks in rectal or oral challenge, and possibly even
301 earlier in vaginal challenge. Explanations other than immunization remain very possible,
302 however, particularly because changes in infectability were sometimes consistent with immune
303 priming, and sometimes with immune tolerance. Nonetheless, the results have some bearing on
304 previous biological conclusions that repeated exposure to HIV without treatment may have no
305 effect [35] or may even reduce human infectability [53].

306 Materials and Methods

307 **The SIV-Macaque database.** Our meta-analysis [45] permitted an article on an SIV-
308 macaque trial to contribute data to the SIV-Macaque database if it had: (1) at least 5 control
309 animals; (2) at least 3 repeats of the same challenge; (3) a regular challenge schedule; (4)
310 infection assigned to a specific challenge; and (5) publicly available data. Regoes [35] used more
311 stringent criteria than ours, including a restriction to rectal challenges, so his database has 7
312 articles [12-16, 19, 20] and is a subset of ours. Our SIV-Macaque database includes 2 articles
313 predating his; 2 articles contemporary with his; and 13 articles postdating his.

314 Table 1 in the Results section displays metadata for our SIV-Macaque database. To favor
315 completeness, our database included two borderline cases: (1) an article exploring the RLC
316 protocol with control animals only [10]; and (2) an article using (almost regular) twice-weekly
317 challenges [17]. The present article followed Regoes [35] in including the Wilson (2009) trial
318 [16], which followed its RLCs with viral hyper-challenges at greatly increased doses to test the
319 infectability of animals uninfected after Challenge 5. As in [35], our SIV-Macaque database
320 includes data corresponding to the RLCs, truncating the challenge data after the RLCs and before
321 any hyper-challenges. The Eudailey (2018) trial [31] also used hyper-challenges after Challenge
322 8. The Om (2020) trial [33] increased the challenge after Challenge 3, possibly just readjusting
323 the challenge ensure infection of controls, rather than testing their infectability with hyper-
324 challenges.

325 A reanalysis in the Letvin (2011) trial [19] regrouped some of its data to refine biological
326 conclusions about the *Mamu-A*01* allele. Similarly, the Om (2020) trial [33] regrouped their
327 data by sex to refine biological conclusions about vaccine protection by sex. The present article
328 avoided analyzing data twice, so it ignored the regroupings.

329 Each article yielded challenge data for its trial arms. If the data were figures with net survival
330 curves, the Windows® 10 Snip & Sketch tool captured the curves with a screen shot. Where
331 possible, two people independently digitized each net survival curve with the WebPlotDigitizer
332 (version 4.4) at https://apps.automeris.io/wpd/, later reconciling any discrepancies. No other data
333 format required digitization.

334 Some articles contained more than one set of control data. Within the articles, however,
335 every arm of every trial mapped to precisely one set of control data. All trial data in an article
336 were grouped by their control arm, and each group was stored as a DataFrame in a separate file
337 of comma-separated values (CSV), following the precepts of tidy data [46]. Each filename

338 corresponded to a composite key combining the article ID in PubMed and the source graphic(s)
339 or table(s) containing its "Control", anchoring all data unambiguously into the public literature.
340 In the Results section, the columns "PMID" and "Source" in each table implicitly display the
341 composite key for the relevant control.

342 The next sections detail the various representations used to assemble, retrieve, and analyze
343 the SIV-Macaque database. Flexible representation was indispensable in reducing human effort.

344 **Data Representation by Individuals.** To focus the following discourse, unless stated otherwise,
345 consider only the control arm of an RLC protocol in a specific trial. Following Regoes' notation
346 [35] where feasible, let $n = n_1$ count the controls, initially uninfected and indexed by
347 $j \in \{1,...,n\}$. Each Animal j receives serial challenges at regular intervals, e.g., weekly. The
348 challenges are numbered consecutively $t \in \{1,2,3,...\}$. Let Challenge c_j be the final challenge
349 to Animal j, with $m = \max_j c_j$ the largest challenge index among the controls, so $c_j \le m$. To
350 account for frequent right-censoring in general survival analysis [44], Regoes [35] carefully
351 permitted the possibility that the final Challenge c_j does not infect Animal j, even if $c_j < m$.

352 On one hand, if Animal j becomes infected, Criterion (4) above specifies that the index c_j
353 of the infecting challenge is known. Some trials continue to challenge infected animals, but for
354 present purposes, we ignore such challenges and consider c_j as the final challenge to Animal j.
355 On the other hand, the final Challenge c_j may not infect, so some animals may remain
356 uninfected at the end of the trial.

357 Consider, therefore, the final status $(\mathbf{c},\mathbf{i}) = \{(c_j, i_j) : j = 1,...,n\}$ of every Animal j, where i_j
358 indicates whether Challenge c_j infected Animal j: $i_j = 1$ indicates infection, and $i_j = 0$
359 otherwise. For a generic animal, drop the subscript j, so (c,i) denotes its final status. The final

status $(\mathbf{c},\mathbf{i})$ is particularly useful if an analysis uses multiple covariates (e.g., alleles) to distinguish individual animals, a rare circumstance indeed in sparse animal trials. We therefore summarize the final status $(\mathbf{c},\mathbf{i})$ with the statistics relevant to discrete-time hazards in analysis of net survival curves [44].

Hazards in Discrete Time. Given sampled Animals $j \in \{1,...,n\}$, assume a probability model where the corresponding components (c_j, i_j) of the final status $(\mathbf{c},\mathbf{i})$ are mutually independent. The hazard h_t gives the probability of infection of a randomly sampled control at Challenge t, conditional on previous challenges not having infected it. The Iverson bracket $[A]$ provides a standard notation for the indicator of the event A: $[A]=1$ if the event A occurs and $[A]=0$ otherwise [47]. The probability that a generic control has final status $(c,0)$ is $(1-h_1)...(1-h_{c-1})(1-h_c)$; final status $(c,1)$, $(1-h_1)...(1-h_{c-1})h_c$. In either case, the natural logarithm of the probability that a particular control has final status (c,i) is

$$\ln L = \sum_{t=1}^{m} \left\{ [t=c][i=1]\left(\ln h_t - \ln(1-h_t)\right) + [t \le c]\ln(1-h_t) \right\}, \qquad (1)$$

proved as follows. The terms on the right side are 0 for any Challenge t after Challenge c, so the sum on the right may be broken into terms for $t = 1,...,c-1$ and a term for $t = c$. Enumeration of two cases $[i=0]$ and $[i=1]$ then shows that Eq (1) gives the natural logarithm of the stated probability.

Let $n_t = \sum_{j=1}^{n} [t \le c_j]$ count the controls remaining in the trial at least to Challenge t; $d_t = \sum_{j=1}^{n} [t = c_j][i_j = 1]$, the controls infected by Challenge t. The sum over all controls for log-likelihood for the final status $(\mathbf{c},\mathbf{i})$ in Eq (1) can therefore be rewritten as

380
$$\begin{aligned}\ln L &= \sum_{j=1}^{n}\sum_{t=1}^{m}\left\{\left[t=c_j\right]\left[i_j=1\right]\left(\ln h_t-\ln\left(1-h_t\right)\right)+\left[t\le c_j\right]\ln\left(1-h_t\right)\right\}\\ &= \sum_{t=1}^{m}\left\{d_t\left(\ln h_t-\ln\left(1-h_t\right)\right)+n_t\ln\left(1-h_t\right)\right\} \qquad , \qquad (2)\\ &= \sum_{t=1}^{m} d_t\ln h_t+\sum_{t=1}^{T}\left(n_t-d_t\right)\ln\left(1-h_t\right)\end{aligned}$$

381 the standard expression from Kaplan-Meier survival analysis [44], put into the present context.

382 The representation by deaths $\left\{\left(n_t,d_t\right):t=1,\ldots,m\right\}$ quickly yields a net survival curve (e.g.,
383 Figure 3 of [18] or Fig 1 below). On one hand, Kaplan-Meier survival analysis derives its
384 generality from handling right-censoring due to incomplete patient follow-up. On the other hand,
385 the RLC protocol in animal trials typically makes follow-up compulsory, so every uninfected
386 control receives its next scheduled challenge. Challenge t then infects $d_t=n_t-n_{t+1}$ of the n_t
387 controls uninfected before Challenge t ($t=1,\ldots,m$), where n_{m+1} counts the uninfected controls at
388 the end of the trial (possibly, $n_{m+1}=0$). At the end of a typical trial, each of the n_{m+1} uninfected
389 controls have therefore survived m challenges. Assume below that animal trials have
390 compulsory follow up within each arm, yielding several compact alternatives to the
391 representation by deaths $\left\{\left(n_t,d_t\right):t=1,\ldots,m\right\}$.

392 **Alternative Data Representations** (see Fig 1). The representation by survivors $\left(n_1,n_2,\ldots,n_{m+1}\right)$
393 satisfies $n_1\ge n_2\ge\ldots\ge n_{m+1}$, and it permits challenge data from each arm in a trial to occupy one
394 column in a DataFrame. The SIV-Macaque database in the SI is a directory of CSV files, each a
395 DataFrame for one trial. Most trials enforce the same maximum m of challenges for each arm,
396 so most of the DataFrames are well-formed, with m rows in every column and 0s padding an
397 arm after infection of all animals in it. In fact, contrary to an assertion of plurality elsewhere
398 [42], only one trial in the SIV-Macaque database, Ellenberger (2006) [12], overtly permitted m

to vary with the arm. In the one trial [12], challenge was discontinued before complete infection of controls and before the end of the trial. The corresponding DataFrame therefore has NaNs padding the control arm, instead of 0s.

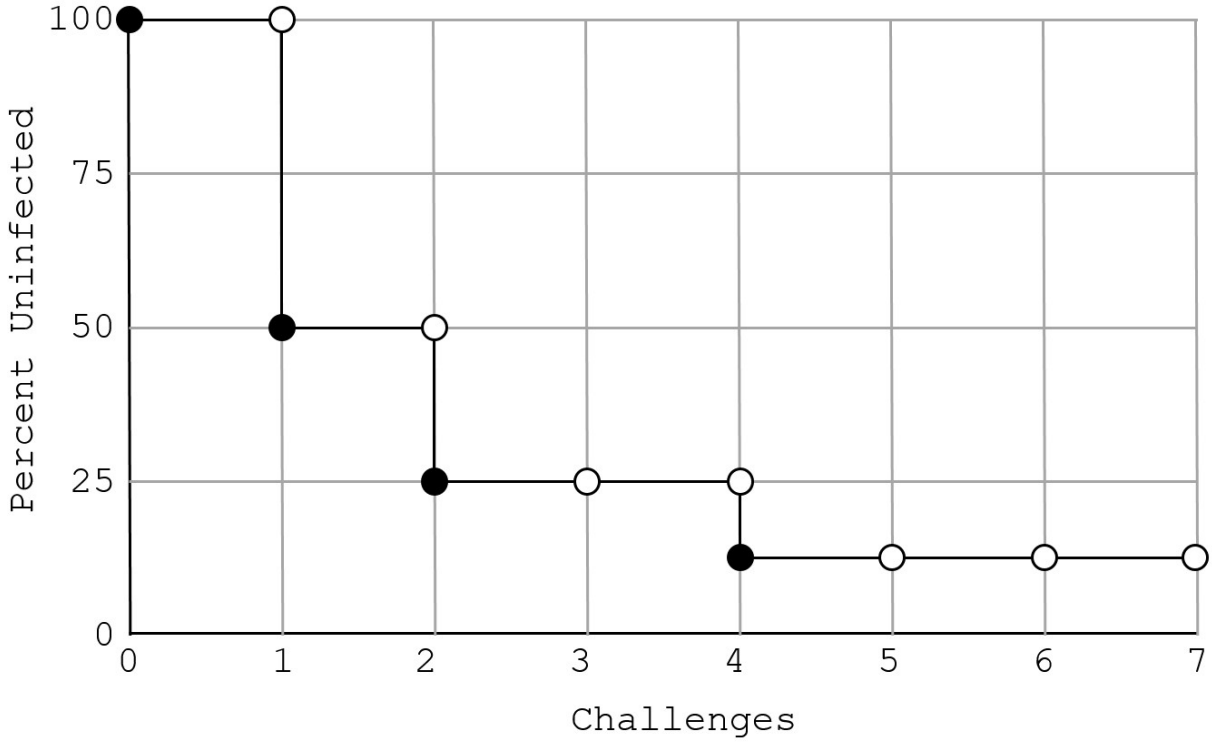

Fig 1. A hypothetical net survival curve for a control arm with 8 animals

Fig 1 plots percent uninfected animals against the number of challenges for a control arm with 8 animals. Typical net survival curves use percentages to facilitate comparison of results from trial arms with differing numbers of animals. At each Challenge $t = 1,\ldots,m$, the open point (t, n_t) gives the percentage of the originally uninfected animals receiving Challenge t; the closed point $(t, n_t - d_t)$ drops by d_t due to animals infected by Challenge t (if not obscured by an open point when $d_t = 0$). By convention, n_{m+1} counts the uninfected controls at the end of the trial, e.g., n_{m+1} =1 in Fig 1. Fig 1 also shows that m =7, so with n =8 animals in the arm, the representation by deaths $\{(n_t, d_t) : t = 1,\ldots,m\}$ is { (8,4), (4,2), (2,0), (2,1), (1,0), (1,0), (1,0) }. The representation by survivors $(n_1, n_2, \ldots, n_{m+1})$ uses only the open circles and n_{m+1}: (8,4,2,2,1,1,1,1). The minimal representation { ($\tau(i) - 1, n_{\tau(i)}$): $i = 1,\ldots,k$ } uses only the closed points and (m, n_{m+1}): { (0,8), (1,4), (2,2), (4,1), (7,1) }.

A minimal representation reduces the human effort in digitizing the net survival curves from SIV-macaque trials. In the representation by survivors, omit challenges where no infections occurred to yield $\left(n_{\tau(1)}, n_{\tau(2)}, \ldots, n_{\tau(k-1)}, n_{\tau(k)}\right)$, where $n_1 = n_{\tau(1)} > n_{\tau(2)} > \ldots > n_{\tau(k-1)} \geq n_{\tau(k)} = n_{m+1}$. Formally, start with $\tau(1) = 1$ and define $\tau(i+1) = \min\left\{t > \tau(i) : n_{\tau(i)} > n_t \text{ for } t \leq m\right\}$ recursively until the set is empty, say, after $\tau(k-1)$ has been defined. Then, define $\tau(k) = m+1$. The minimal representation $\left\{\left(\tau(i)-1, n_{\tau(i)}\right) : i = 1, \ldots, k\right\}$ compacts the representation by survivors without loss and greatly speeds digitization of net survival curves. Most experiments show the net survival curve for each arm as percent survival, as in Fig 1. Accordingly, we digitized only the points corresponding to the minimal representation, multiplied the percentages by $n = n_1$, and then rounded to integers to yield the minimal representation. If two people digitize and then disagree, the minimal representation facilitates a rapid reconciliation of their results.

Finally, a few trials (e.g., [13, 16]) use a representation $\left(t_j : j = 1, \ldots, n\right)$ by individual infecting challenges [35], where Challenge t_j infects Animal j. Retain our convention that $t_j = m+1$ if Animal j remains uninfected throughout the trial. Then, the data may be converted to $n_t = \#\left\{t_j \geq t : j = 1, 2, \ldots, n\right\}$ for $t = 1, \ldots, m$, where "#" counts the number of elements in the set.

Constant Hazard reduced model. The following nests a reduced model H_0, the Constant Hazard model, within several different full models, to detect deviation from H_0 with Likelihood Ratio tests (LRTs). The Constant Hazard model (Regoes' Geometric infection model [35]) posits that all challenges yield mutually independent results and have a constant probability p of

infecting a control. Every animal is equally infectable, so under the reduced model H_0, the hazard per challenge is the constant

$$H_0 : h_t = p . \tag{3}$$

The corresponding log-likelihood

$$\ln L_0 = \sum_{t=1}^{m} d_t \ln p + \sum_{t=1}^{m} \left(n_t - d_t \right) \ln \left(1 - p \right) \tag{4}$$

yields the maximum likelihood estimator (MLE)

$$\hat{p} = \sum_{t=1}^{m} d_t \Big/ \sum_{t=1}^{m} n_t , \tag{5}$$

a convenient initialization for maximizing likelihoods of the full models. Even if the reduced model is rejected, Eq (5) is still the fraction of all challenges that infected and provides a practical summary of the average potency of a challenge.

For brevity, to truncate a real number x to a probability $U\left(x\right)$ between 0 and 1, define the unit function $U\left(x\right) = x$ for $0 \le x \le 1$, with $U\left(x\right) = 0$ for $x < 0$ and $U\left(x\right) = 1$ for $x > 1$. Below, we characterize several full models $H_\bullet$, where "$\bullet$" is an arbitrary subscript, simply by specifying their hazard h_t. If required, Eq (2) then implicitly gives the corresponding likelihoods $L_\bullet$ and log-likelihoods $\ell_\bullet = \ln L_\bullet$. Our full models include all of Regoes' full models [35].

The first elaboration of the Constant Hazard model that Regoes [35] addressed was immune priming: even if the animals had the same infectability, their infectability might evolve during the trial because the RLCs provided immunization against the virus, either in the direction of immune priming or immune tolerance. In mathematical models, negative immune priming corresponds to immune tolerance.

Arithmetic priming full model (AP). Regoes [35] examined a full model equivalent to

$$H_{AP}: h_t = U\left(p + (t-1)\varepsilon\right). \tag{6}$$

H_{AP} reduces to H_0 if $\varepsilon = 0$. Priming corresponds to $\varepsilon < 0$; tolerance, to $\varepsilon > 0$.

Geometric priming full model (GP). Arithmetic priming has a hard boundary at 0 from the unit function. A geometric sequence avoids it:

$$H_{GP}: h_t = U\left(pr^{t-1}\right). \tag{7}$$

H_{GP} reduces to H_0 if $r = 1$. Priming corresponds to $r < 1$; tolerance, to $r > 1$.

Step priming full model (SP1, SP2, SP3). Following Regoes [35] (see also [48]), consider full models where the hazard is piecewise constant, changing from p to p_2 after Challenge l_{step}=1,2, or 3.

$$H_{SP}: h_t = \begin{cases} p, & t \le l_{step} \\ p_2, & t > l_{step} \end{cases}. \tag{8}$$

H_{SP} reduces to H_0 if $p_2 = p$. Priming corresponds to $p_2 < p$; tolerance, to $p_2 > p$.

The log-likelihoods for priming models are all strictly concave in their two variables (p, ε), (p, r), or (p, p_2) (see the SI), so hill-climbing methods always determine the MLE as the unique global maximum. Regoes [35] also addressed frailty models without priming, where animals might have different intrinsic infectabilities that remained constant during challenge [49]. The log-likelihoods for the two frailty models below are not concave in general (see the SI), mildly complicating numerical computation. In addition, the two frailty models nest the reduced model on the boundary of their parameter space, violating the hypotheses of standard statistical theorems [50]. The present article has the purview of examining the robustness of Regoes' scientific conclusions against expanding his database, making technically arduous statistical improvements peripheral.

Beta Frailty full model (BF). The Beta distribution with parameters (a,b) has probability density function

$$f_{BF}(x) = \frac{(a+b-1)!}{(a-1)!(b-1)!} x^{a-1}(1-x)^{b-1}, \tag{9}$$

where factorials replace the cumbrous Gamma function notation, even if a and b are not integers. The prefactor is the reciprocal of the Beta function

$$B(a,b) = \frac{(a-1)!(b-1)!}{(a+b-1)!} = \int_0^1 x^{a-1}(1-x)^{b-1}\,dx, \tag{10}$$

yielding the required normalization $\int_0^1 f_{BF}(x)\,dx = 1$.

Consider a frailty model where each challenge to Animal j has a probability x of infection, the infectability x being intrinsic to Animal j with the probability density function in Eq (9). The corresponding hazard, the probability of infection on Challenge t conditional on previous non-infection, is

$$H_{BF} : h_t = \frac{\int_0^1 x(1-x)^{t-1} f_{BF}(x)\,dp}{\int_0^1 (1-x)^{t-1} f_{BF}(x)\,dp} = \frac{\dfrac{a!(b+t-2)!}{(a+b+t-1)!}}{\dfrac{(a-1)!(b+t-2)!}{(a+b+t-2)!}} = \frac{a}{a+b+t-1}, \tag{11}$$

equivalent to animal frailty with a beta distribution (see the SI). Hence, the log-likelihood

$$\ln L_{BF} = \sum_{t=1}^{m} d_t \ln \frac{a}{a+b+t-1} + \sum_{t=1}^{m} (n_t - d_t) \ln \frac{b+t-1}{a+b+t-1}. \tag{12}$$

To nest H_0 within H_{BF}, Regoes [35] noted that the beta distribution in Eq (9) can be parametrized by its mean $p = a/(a+b)$ and variance $v = ab/(a+b)^2/(a+b+1)$. For $v > 0$, let $s = p(1-p)/v - 1$ so $a = sp$, $b = s(1-p)$. H_{BF} reduces to H_0 if $v \downarrow 0$ with p fixed, i.e., $a,b \to \infty$ with $p = a/(a+b)$ fixed.

Delta Frailty full model (DF) [34, 49]: In the context of a treatment trial, Hudgens et al [34] posit a frailty model for controls with parameters (θ, p). In their model, each control is independently susceptible with probability $1-\theta$. Only susceptible controls can be infected, and each challenge infects susceptible controls with probability p. The analog to Eq (9) is

$$f_{DF}(x) = (1-\theta)\delta(x-p) + \theta\delta(x), \tag{13}$$

where $\delta(x)$ symbolizes the Dirac delta, which satisfies $\int_0^1 h(x)\delta(x)dx = h(0)$ for functions h. Thus,

$$H_{DF} : h_t = \frac{\int_0^1 x(1-x)^{t-1} f_{DF}(x)dx}{\int_0^1 (1-x)^{t-1} f_{DF}(x)dx} = \frac{(1-\theta)p(1-p)^{t-1}}{\theta + (1-\theta)(1-p)^{t-1}}. \tag{14}$$

H_{DF} reduces to H_0 if $\theta = 0$.

Model Parameter Estimation. Animal data are sparse, so if the reduced model holds, we base a confidence interval for p on likelihood ratios [35, 51], rather than moments.

$$CI_{1-\alpha}(p) = \left\{x : 2\left[\ell_0(\hat{p}) - \ell_0(x)\right] \le \chi^2_{1,1-\alpha}\right\}, \tag{15}$$

where α is the desired significance level and $\chi^2_{1,1-\alpha}$ is the $(1-\alpha)$-percentile of the chi-squared distribution with one degree of freedom, i.e., for a random variate X with the distribution, $P\left(X \le \chi^2_{1,1-\alpha}\right) = 1-\alpha$. We take α=0.05 to yield 95% confidence intervals. To facilitate simulation of actual trials, the Excel file in the SI contains the MLE for each full model, along with the corresponding Fisher information matrix, if calculable numerically.

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References

1. Veazey RS, Lackner AA. Nonhuman Primate Models and Understanding the Pathogenesis of HIV Infection and AIDS. ILAR Journal. 2017;58(2):160-71.
2. Arthur LO, Bess JW, Jr., Waters DJ, Pyle SW, Kelliher JC, Nara PL, et al. Challenge of chimpanzees (Pan troglodytes) immunized with human immunodeficiency virus envelope glycoprotein gp120. J Virol. 1989;63(12):5046-53.
3. Spouge JL. Statistical-Analysis of Sparse Infection Data and Its Implications For Retroviral Treatment Trials in Primates. Proc Natl Acad Sci U S A. 1992;89(16):7581-5.
4. Roederer M. Parsimonious Determination of the Optimal Infectious Dose of a Pathogen for Nonhuman Primate Models. PLoS Pathog. 2015;11(8):e1005100.
5. Siegel S, Castellan NJ. Nonparametric Statistics for the Behavioral Sciences. 2 ed. New York: MacGraw-Hill; 1988.
6. Cohen J. NIH to End Chimp Breeding for Research. Science. 2007;316(5829):1265.
7. Marx JL. Progress reported on mouse models for AIDS. Science. 1988;242(4886):1638.
8. Marsden MD. Benefits and limitations of humanized mice in HIV persistence studies. Retrovirology. 2020;17(1):7.
9. Keele BF, Jones JH, Terio KA, Estes JD, Rudicell RS, Wilson ML, et al. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature. 2009;460(7254):515-9.
10. Ma Z-M, Abel K, Rourke T, Wang Y, Miller CJ. A Period of Transient Viremia and Occult Infection Precedes Persistent Viremia and Antiviral Immune Responses during Multiple Low-Dose Intravaginal Simian Immunodeficiency Virus Inoculations. 2004;78(24):14048-52.
11. Van Rompay KKA, Abel K, Lawson JR, Singh RP, Schmidt KA, Evans T, et al. Attenuated Poxvirus-Based Simian Immunodeficiency Virus (SIV) Vaccines Given in Infancy Partially Protect Infant and Juvenile Macaques Against Repeated Oral Challenge With Virulent SIV. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2005;38(2).
12. Ellenberger D, Otten RA, Li B, Aidoo M, Rodriguez IV, Sariol CA, et al. HIV-1 DNA/MVA vaccination reduces the per exposure probability of infection during repeated mucosal SHIV challenges. Virology. 2006;352(1):216-25.
13. Wilson NA, Reed J, Napoe GS, Piaskowski S, Szymanski A, Furlott J, et al. Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. J Virol. 2006;80(12):5875-85.
14. García-Lerma JG, Otten RA, Qari SH, Jackson E, Cong ME, Masciotra S, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. PLoS Med. 2008;5(2):e28.
15. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med. 2009;15(3):293-9.
16. Wilson NA, Keele BF, Reed JS, Piaskowski SM, MacNair CE, Bett AJ, et al. Vaccine-induced cellular responses control simian immunodeficiency virus replication after heterologous challenge. J Virol. 2009;83(13):6508-21.

559 17. Hessell AJ, Poignard P, Hunter M, Hangartner L, Tehrani DM, Bleeker WK, et al. Effective, low-
560 titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat Med.
561 2009;15(8):951-4.
562 18. Reynolds MR, Weiler AM, Piaskowski SM, Kolar HL, Hessell AJ, Weiker M, et al. Macaques
563 vaccinated with simian immunodeficiency virus SIVmac239Delta nef delay acquisition and control
564 replication after repeated low-dose heterologous SIV challenge. J Virol. 2010;84(18):9190-9.
565 19. Letvin NL, Rao SS, Montefiori DC, Seaman MS, Sun Y, Lim SY, et al. Immune and Genetic
566 Correlates of Vaccine Protection Against Mucosal Infection by SIV in Monkeys. Sci Transl Med.
567 2011;3(81):81ra36.
568 20. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound
569 early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature.
570 2011;473(7348):523-7.
571 21. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, et al. Vaccine protection against
572 acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature. 2012;482(7383):89-93.
573 22. Pegu P, Vaccari M, Gordon S, Keele BF, Doster M, Guan Y, et al. Antibodies with high avidity
574 to the gp120 envelope protein in protection from simian immunodeficiency virus SIV(mac251)
575 acquisition in an immunization regimen that mimics the RV-144 Thai trial. J Virol. 2013;87(3):1708-19.
576 23. Strbo N, Vaccari M, Pahwa S, Kolber MA, Doster MN, Fisher E, et al. Cutting edge: novel
577 vaccination modality provides significant protection against mucosal infection by highly pathogenic
578 simian immunodeficiency virus. J Immunol. 2013;190(6):2495-9.
579 24. Roederer M, Keele BF, Schmidt SD, Mason RD, Welles HC, Fischer W, et al. Immunological
580 and virological mechanisms of vaccine-mediated protection against SIV and HIV. Nature.
581 2014;505(7484):502-8.
582 25. Radzio J, Henning T, Jenkins L, Ellis S, Farshy C, Phillips C, et al. Combination Emtricitabine
583 and Tenofovir Disoproxil Fumarate Prevents Vaginal Simian/Human Immunodeficiency Virus Infection
584 in Macaques Harboring Chlamydia trachomatis and Trichomonas vaginalis. J Infect Dis.
585 2016;213(10):1541-5.
586 26. Vaccari M, Gordon SN, Fourati S, Schifanella L, Liyanage NP, Cameron M, et al. Adjuvant-
587 dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. Nat Med.
588 2016;22(7):762-70.
589 27. Jensen K, Nabi R, Van Rompay KKA, Robichaux S, Lifson JD, Piatak M, et al. Vaccine-Elicited
590 Mucosal and Systemic Antibody Responses Are Associated with Reduced Simian Immunodeficiency
591 Viremia in Infant Rhesus Macaques. Journal of Virology. 2016;90(16):7285-302.
592 28. Butler K, Ritter JM, Ellis S, Morris MR, Hanson DL, McNicholl JM, et al. A Depot
593 Medroxyprogesterone Acetate Dose That Models Human Use and Its Effect on Vaginal SHIV
594 Acquisition Risk. J Acquir Immune Defic Syndr. 2016;72(4):363-71.
595 29. Gordon SN, Liyanage NP, Doster MN, Vaccari M, Vargas-Inchaustegui DA, Pegu P, et al.
596 Boosting of ALVAC-SIV Vaccine-Primed Macaques with the CD4-SIVgp120 Fusion Protein Elicits
597 Antibodies to V2 Associated with a Decreased Risk of SIVmac251 Acquisition. J Immunol.
598 2016;197(7):2726-37.
599 30. Xu H, Andersson AM, Ragonnaud E, Boilesen D, Tolver A, Jensen BAH, et al. Mucosal
600 Vaccination with Heterologous Viral Vectored Vaccine Targeting Subdominant SIV Accessory Antigens
601 Strongly Inhibits Early Viral Replication. EBioMedicine. 2017;18:204-15.
602 31. Eudailey JA, Dennis ML, Parker ME, Phillips BL, Huffman TN, Bay CP, et al. Maternal HIV-1
603 Env Vaccination for Systemic and Breast Milk Immunity To Prevent Oral SHIV Acquisition in Infant
604 Macaques. mSphere. 2018;3(1).
605 32. Vaccari M, Fourati S, Gordon SN, Brown DR, Bissa M, Schifanella L, et al. HIV vaccine
606 candidate activation of hypoxia and the inflammasome in CD14(+) monocytes is associated with a
607 decreased risk of SIV(mac251) acquisition. Nat Med. 2018;24(6):847-56.

608 33. Om K, Paquin-Proulx D, Montero M, Peachman K, Shen XY, Wieczorek L, et al. Adjuvanted
609 HIV-1 vaccine promotes antibody-dependent phagocytic responses and protects against heterologous
610 SHIV challenge. Plos Pathogens. 2020;16(9).
611 34. Hudgens MG, Gilbert PB, Mascola JR, Wu CD, Barouch DH, Self SG. Power to detect the
612 effects of HIV vaccination in repeated low-dose challenge experiments. J Infect Dis. 2009;200(4):609-13.
613 35. Regoes RR. The role of exposure history on HIV acquisition: insights from repeated low-dose
614 challenge studies. PLoS Comput Biol. 2012;8(11):e1002767.
615 36. Regoes RR, Longini IM, Feinberg MB, Staprans SI. Preclinical assessment of HIV vaccines and
616 microbicides by repeated low-dose virus challenges. PLoS Medicine. 2005;2(8):798-807.
617 37. Shedlock DJ, Silvestri G, Weiner DB. Monkeying around with HIV vaccines: using rhesus
618 macaques to define 'gatekeepers' for clinical trials. Nature Reviews Immunology. 2009;9(10):717-28.
619 38. Thippeshappa R, Kimata JT, Kaushal D. Toward a Macaque Model of HIV-1 Infection:
620 Roadblocks, Progress, and Future Strategies. 2020;11(882).
621 39. Vaupel JW, Yashin AI. Heterogeneity's ruses: some surprising effects of selection on population
622 dynamics. Am Stat. 1985;39(3):176-85.
623 40. Hudgens MG, Gilbert PB. Assessing vaccine effects in repeated low-dose challenge experiments.
624 Biometrics. 2009;65(4):1223-32.
625 41. Kang C, Huang Y, Miller CJ. A discrete-time survival model with random effects for designing
626 and analyzing repeated low-dose challenge experiments. Biostatistics. 2015;16(2):295-310.
627 42. Nolen T, Hudgens M, Senb P, Koch G. Analysis of Repeated Low-Dose Challenge Studies. Stat
628 Med. 2015;34.
629 43. Heinze G, Gnant M, Schemper M. Exact log-rank tests for unequal follow-up. Biometrics.
630 2003;59(4):1151-7.
631 44. Kaplan EL, Meier P. Nonparametric-Estimation from Incomplete Observations. J Am Stat Assoc.
632 1958;53(282):457-81.
633 45. Nakagawa S, Noble DWA, Senior AM, Lagisz M. Meta-evaluation of meta-analysis: ten
634 appraisal questions for biologists. BMC Biology. 2017;15(1):18.
635 46. Wickham H. Tidy Data. Journal of Statistical Software. 2014;59(10):1-23.
636 47. Knuth DE. 2 Notes on Notation. American Mathematical Monthly. 1992;99(5):403-22.
637 48. Matthews DE, Farewell VT. On testing for a constant hazard against a change-point alternative.
638 Biometrics. 1982;38(2):463-8.
639 49. Longini IM, Halloran ME. A frailty mixture model for estimating vaccine efficacy. Journal of the
640 Royal Statistical Society Series C-Applied Statistics. 1996;45(2):165-73.
641 50. Self SG, Liang KY. Asymptotic Properties of Maximum-Likelihood Estimators and Likelihood
642 Ratio Tests under Nonstandard Conditions. J Am Stat Assoc. 1987;82(398):605-10.
643 51. Cox DR. Analysis of Binary Data. London: Methuen; 1970.
644 52. Wu F, Kirmaier A, White E, Ourmanov I, Whitted S, Matsuda K, et al. TRIM5α Resistance
645 Escape Mutations in the Capsid Are Transferable between Simian Immunodeficiency Virus Strains.
646 Journal of virology. 2016;90(24):11087-95.
647 53. Stranford SA, Skurnick J, Louria D, Osmond D, Chang SY, Sninsky J, et al. Lack of infection in
648 HIV-exposed individuals is associated with a strong CD8(+) cell noncytotoxic anti-HIV response. Proc
649 Natl Acad Sci U S A. 1999;96(3):1030-5.
650 54. MacKinnon DP, Krull JL, Lockwood CM. Equivalence of the mediation, confounding and
651 suppression effect. Prev Sci. 2000;1(4):173-81.

652

Supporting information

Supplementary Information.zip. This is a ZIP file of the following items.

README.txt. This is an index for the Supporting Information.

Supplementary_Methods.docx. This contains additional methods and a legend for Supplementary_Table.xlsx.

Supplementary_Table.xlsx. This table includes Tables 1-3 and other information in floating-point precision.

SIV-Macaque_Net_Survival_Curves/. This is a directory containing the CSV files, DataFrames containing the data from the net survival curves in the SIV-Macaque database.