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Title:

Has HIV evolved to induce immune pathogenesis?

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24 **Abstract**

25 **Human immunodeficiency virus (HIV) induces a chronic generalized activation of the**
26 **immune system, which plays an important role in the pathogenesis of AIDS. This ability**
27 **of the virus might either be an evolved (adaptive) trait or a coincidental side-effect of**
28 **jumping to a new host species. We argue that selection favours the ability of HIV to**
29 **induce immune activation at the local sites of infection (e.g. lymph follicles), but not at**
30 **the systemic level. Immune activation increases the supply of susceptible target cells;**
31 **however, mutations that increase systemic immune activation benefit all virus variants**
32 **equally, and are therefore selectively neutral. We thus conclude that the generalized**
33 **immune activation that is likely responsible for pathogenesis is probably not directly**
34 **under selection.**

35

35 **Introduction**

36 Accumulating evidence indicates that the pathogenesis of HIV infection is linked to the
37 ability of the virus to induce a chronic generalized hyperactivation of the immune system [1-
38 4]. Activation status is a strong predictor of disease progression [5-8], with consistent effect in
39 HIV-1 and HIV-2 in spite of different virus levels [9]; and the ongoing depletion of CD4⁺ T
40 cells has been associated with accelerated turnover due to chronic activation [2,4,10].
41 Understanding the proximate and ultimate (evolutionary) causes of HIV induced immune
42 activation may be the key to understanding pathogenesis. In this Opinion article, we
43 concentrate on the evolutionary background of this pathogenic mechanism.

44 HIV has its origin and closest relatives in the group of simian immunodeficiency viruses (SIV)
45 [11,12]. Remarkably, immune hyperactivation is absent in African primates naturally infected
46 with SIV, and these infections are indeed largely nonpathogenic [13-15]. From the
47 perspective of the virus, this raises the question of whether immune activation is an evolved
48 (adaptive) trait of HIV or an “unwanted” side-effect of jumping to a new host species, which
49 may be lost during subsequent evolution. Drawing on mathematical and simulation models,
50 we argue that it may actually be both: raising immune activation in a local microenvironment
51 provides selective advantage for the virus, but the induction of systemic immune activation
52 (associated with pathogenesis) is likely to reflect an evolutionary accident. We begin with a
53 discussion of how immune activation may benefit the virus, and then show when (and
54 whether) this benefit translates to a selective advantage that can drive evolution.

55

56 **How could immune activation be beneficial for HIV?**

57 Activating immune cells is generally thought to benefit HIV by increasing its supply of
58 susceptible target cells [16,17]. HIV replicates most efficiently in activated CD4⁺ T
59 lymphocytes, while quiescent cells that form the majority of this cell population are less
60 susceptible to productive infection [18-20], and produce less virus when infected compared
61 with activated cells [21,22]. HIV can activate and infect CD4⁺ T cells that recognize one of its
62 epitopes [23,24]; however, the majority of activated CD4⁺ T cells are not HIV-specific
63 [23,25]. Bystander activation of uninfected CD4⁺ T cells has been demonstrated to increase
64 susceptibility to infection and subsequent virus production *ex vivo* [17]. *In vivo*, the
65 percentage of activated cells (as measured by activation markers, e.g. KI67, HLA-DR or
66 CD25) increases during disease progression [7], which maintains the supply of target cells in
67 the face of a declining overall CD4 count. Finally, increased immune activation by
68 vaccination or unrelated infections generally increases the HIV virus load in infected patients
69 [26-30]. Activating CD4⁺ T cells thus seems to be clearly beneficial for the virus. However,
70 this benefit translates to a selective advantage only if it favours the activator virus against its
71 competitors in the diverse virus population of an infected host. This criterion is central to our
72 argument, and below we show why it is not necessarily fulfilled.

73

74 **Systemic vs. local immune activation**

75 The main mechanism of HIV induced (bystander) immune activation has not yet been
76 clarified, although there is no shortage of candidates: molecular mimicry of gp120 providing
77 false MHC type II signals [31], Nef-mediated lymphocyte activation [32], the effect of
78 abundant defective virus particles [16], altered cytokine production [33], dysregulation of
79 tumor necrosis factor receptor signalling [34], unchecked translocation of bacterial products
80 into the systemic circulation due to the disruption of the gut mucosa [35], or the induction of

81 Toll-like receptors by HIV RNA [36] have all been implicated, and may operate
82 simultaneously. Importantly, most of these effects can potentially spread beyond the
83 immediate neighbourhood of a virus-producing cell (by virus particles or shed viral proteins
84 entering the circulation, or by indirect stimuli mediated by cytokines or translocated bacterial
85 products), and are likely to contribute to the generalized immune activation that is associated
86 with pathogenesis. We therefore distinguish between systemic immune activation that, in the
87 simplest approximation, affects the baseline activation of all target cells, and local immune
88 activation, which is confined to the neighbourhood where the inducer virus was produced.
89 The two types of immune activation have drastically different evolutionary implications, and
90 whether they can be decoupled could be crucial for the future evolution of HIV virulence (the
91 severity of the disease). Note that real biological mechanisms of immune activation are likely
92 to fall somewhere in between the theoretical extremes (completely global or completely local
93 effect) that we analyze here: long-range effects tend to behave as our systemic model, while
94 short-range effects are better described by the local activation model.

95 **Mutations affecting systemic immune activation are selectively neutral**

96 Systemic immune activation increases the supply of susceptible target cells for the whole
97 virus population; however, it confers no selective advantage for the virus variants that induce
98 it. The reason for this is that the increased “global” availability of target cells benefits all virus
99 variants equally, irrespective of whether they contributed to immune activation or not. An
100 arising mutant with improved efficiency of systemic immune activation will therefore not be
101 able to increase in frequency, and the ability to induce systemic immune activation is not
102 expected to be under selection. This argument can be tested formally using mathematical
103 models (Box 1). A robust prediction of the models is that the induction of systemic immune
104 activation is indeed evolutionarily neutral for HIV, even though it is expected to increase the
105 total virus level. A population level benefit is thus not necessarily translated into a selective

106 advantage in competition within the virus population. The induction of systemic immune
107 activation can essentially be regarded as an “altruistic” trait for the virus, which benefits other
108 “individuals” (or viral lineages) in addition to the carrier of the trait. We will discuss this
109 interpretation in more detail later.

110 **Local immune activation confers selective advantage**

111 The lack of selection on systemic immune activation results from all viruses activating all
112 target cells equally. However, some of the activation effect is likely to be limited to the
113 neighbourhood of the inducer virus. HIV infects cells and is produced primarily in the
114 lymphoid tissues [37,38], which have a highly organized spatial structure. Virus samples
115 isolated from different sections of an infected spleen display markedly different genetic
116 composition even on a microscopic scale [39-42], which suggests that aggregates of target
117 cells can be colonized by a single or a few “founder” viruses and may serve as relatively
118 isolated local sites for several rounds of virus replication [4,43,44]. Phylogenetic analyses of
119 multiple virus isolates from individual patients have also hinted at a “metapopulation”
120 structure involving the dynamic establishment and extinction of distinct local HIV
121 subpopulations within a single patient [45-47]. Finally, localized activation by exogenous
122 antigens has been demonstrated to fuel a local expansion of SIV clones [48]. These data
123 suggest that HIV induced immune activation could partly be restricted to the neighbourhood
124 of the inducer virus, which might influence the evolution of this trait. The effect of such
125 spatial structure can be investigated in a simulation model involving a dynamic
126 metapopulation of local “bursts of infection” (Box 2). The simulations illustrate that, in
127 contrast to systemic activation, the ability to induce local immune activation provides
128 selective advantage to HIV (Box 3). This result arises because the benefit of locally increased
129 target cell supply is not shared equally by all virus types, but can be exploited by the virus

130 that induced it in a given microenvironment. We thus conclude that the evolution of HIV
131 favours the ability to induce local, but not systemic immune activation.

132 **Immune activation as an altruistic viral trait**

133 The different evolutionary outcome of local and systemic immune activation is consistent
134 with earlier work on the general evolutionary theory of altruistic traits. In particular, our
135 model of local immune activation resembles “structured deme models”, which were first
136 proposed by Wilson to explain how random group formation can facilitate the spread of
137 beneficial traits that would be excluded by “selfish mutants” in a homogeneous population
138 [49,50]. In our model, the ability to induce systemic immune activation provides a population
139 level benefit (increased overall virus load) for the virus, but activator mutants cannot spread in
140 the population. In fact, assuming any metabolic (or, in general, fitness) cost associated with
141 the ability of immune activation would result in competitive exclusion of activator viruses by
142 “selfish” non-activating mutants in the systemic model. The local structure of HIV infections
143 creates a “trait-group” structure *sensu* Wilson [49,50], and this structure indeed promotes the
144 selection for local immune activation in computer simulations. Structured deme models have
145 been used before to explore the evolution of multi-component viruses and defective
146 interfering virus particles [51], but this is their first application to the evolution of HIV
147 pathogenesis.

148 The evolution of “unselfish” viral traits (i.e. traits that confer advantage only at the population
149 level) has been addressed before in the context of viral strategies towards immune function
150 impairment [52]. We note that our argument can be extrapolated to speculate that “altruistic”
151 viral traits that contribute to the impairment of HIV specific immune responses could also be
152 selected within the host if their effect were confined to the local sites of infection.

153 **Implications for HIV evolution**

154 Our results have implications for the evolutionary history and future evolution of HIV
155 pathogenesis. While the mechanism of systemic immune activation in HIV infection is still
156 unclear, there is an indirect clue to its nature. Most observed cases of SIV transmission to
157 non-adapted hosts (humans and Asian macaques) have resulted in AIDS-like disease with
158 immune pathology [15], which suggests that the ability to induce systemic immune activation
159 in non-adapted hosts is a conserved “pre-adaptation” among SIV viruses. Evolutionary
160 conservation suggests maintenance by selection, and the ability to increase local target cell
161 supply may be a plausible candidate for this pre-adaptation. While we have demonstrated that
162 systemic immune activation *per se* is probably neutral for the virus, it may nevertheless arise
163 as a side-effect of the ability to induce target cell activation at the local sites of infection,
164 which is probably under selection. The example of nonpathogenic natural SIV infections
165 raises the hope that the two phenomena can be decoupled. The high virus load characteristic
166 of these infections suggests efficient local immune activation, while systemic immune
167 activation is absent from the infected hosts. How this decoupling occurs is currently unknown;
168 however, the observation that the same SIV strain can cause chronic activation and
169 pathogenesis in non-adapted, but not in adapted natural hosts [15] points out the role of host
170 factors. If systemic immune activation is indeed neutral for the virus, then selection acting on
171 the virus would neither drive nor oppose evolution towards the loss of systemic activation and
172 pathogenesis. Such evolution would, however, clearly benefit the host and could, in the
173 absence of opposing viral selection, be driven by the evolution of host factors [53].

174 We note that our models have concentrated on HIV evolution occurring within an infected
175 host; selection acting on transmission between hosts may add further complexity [52]. In
176 particular, if systemic immune activation is required to maintain the high virus loads that
177 ensure efficient transmission, then evolution may stabilize the efficiency of systemic
178 activation (and ensuing virulence) at an optimal level [54]. However, HIV evolution seems to

179 be largely dominated by the selection forces acting within hosts [55], which reduces the
180 strength of this constraint, while a decoupling of efficient transmission from systemic immune
181 activation (as in nonpathogenic SIV infections) would remove this selection constraint
182 entirely. In the latter case, the virus might even gain by losing systemic immune activation, if
183 the life span of infected hosts could increase without compromising their infectiousness.
184 However, even in this scenario, the selection pressure driving HIV towards decreasing
185 systemic immune activation might be weak, for two reasons. First, selection acting on the
186 transmission of HIV between hosts seems to be weak compared to selection acting within the
187 host [55], and we have demonstrated that systemic immune activation is likely to be neutral in
188 terms of the latter. Second, a large fraction of new infections seem to result from transmission
189 during acute infection [56], which further reduces the strength of selection acting on virus
190 traits that influence host survival. Therefore, we predict that attenuation of HIV might occur,
191 but the timescale of this process will be set by slow stochastic (neutral) evolution, rather than
192 by rapid selection against systemic immune activation. Evolution of hosts to resist systemic
193 activation might have accelerated (or entirely driven) the process of attenuation in natural SIV
194 infections. However, this evolutionary pathway is highly undesirable for the human disease of
195 HIV infection, since it could only proceed by the differential survival (and death) of infected
196 patients depending on their susceptibility to systemic activation.

197 Finally, we note that within-host evolution towards increasing immune activation could, in
198 principle, contribute to HIV disease progression [57,58], if two criteria are fulfilled. First,
199 local and systemic immune activation should remain coupled on the time scale of within-host
200 evolution so that evolution of the former would drive that of the latter. Second, the efficiency
201 of activation should be host-specific, so that its evolution could occur repeatedly and
202 independently in the infection of each individual. This could be plausible if the activation

203 signal depended on receptor-mediated lymphocyte activation, which is extremely variable due
204 to high MHC polymorphism and the random generation of the receptor repertoire.

205 **Implications for anti-activation treatment**

206 Future therapeutic strategies might target HIV induced systemic activation to reduce
207 pathogenesis. Such drugs will affect interactions between the virus and the immune system, or
208 the immune system alone, rather than directly inhibit a step in the viral lifecycle as current
209 drugs do. This raises the question whether the virus could evolve “resistance” against such
210 therapies. According to our results, drugs affecting systemic (but not local) immune activation
211 selectively would not put a selection pressure on the virus, and therefore the efficacy of the
212 drugs would not be jeopardized by the evolution of resistance. In contrast, drugs affecting
213 local immune activation might elicit viral evolution to restore the efficiency of local activation.
214 The spread of such “resistant” super-activator viruses could actually increase virulence at the
215 population level. The long-term success of this potential therapeutic approach might thus
216 depend on our ability to identify and selectively inhibit the viral mechanisms of systemic
217 immune activation.

218 **Conclusion**

219 We have argued that local, but not systemic immune activation is likely to provide selective
220 advantage to HIV within an infected host. Systemic immune activation and the resulting
221 immune pathogenesis are thus likely to reflect a coincidental side-effect of cross-species
222 transmission, rather than adaptation to a new (human) host. Systemic immune activation
223 might be lost during further evolution, but this might occur slowly or might require adaptation
224 of the human host. Further work should concentrate on elucidating the mechanisms of HIV
225 induced immune activation. Of particular interest is the range of the effects, which determines
226 their evolution; and whether short and long-range effects can be decoupled, which may shape

227 the future of HIV virulence and might also affect the success of future drugs that will target
 228 immune activation.

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371 **Box 1. Mathematical model of systemic immune activation**

372 Adapting the established framework of virus dynamics [59] to the scenario of systemic
373 immune activation yields the following set of equations:

$$\begin{aligned}
 \frac{dQ}{dt} &= \sigma - \delta_Q Q - (\alpha + kI)Q + rT \\
 374 \quad \frac{dT}{dt} &= (\alpha + kI)Q - (r + \delta_T)T - \beta IT && (1a-c) \\
 \frac{dI}{dt} &= \beta IT - \delta_I I \quad .
 \end{aligned}$$

375 The model comprises of quiescent CD4⁺ target cells, Q , activated target cells, T , and
376 productively infected cells, I . Quiescent CD4⁺ target cells arise at a constant rate σ , die at rate
377 $\delta_Q Q$ and become activated at an intrinsic (HIV-independent) rate αQ . HIV-dependent
378 bystander activation of quiescent cells occurs at rate kIQ , where the parameter k denotes the
379 efficiency of activation. Activated target cells, T , die at rate $\delta_T T$, revert to resting state at rate
380 rT and become infected at a rate proportional to the abundance of productively infected cells,
381 I , and the infection efficiency parameter β . Infected cells die at rate $\delta_I I$. For simplicity, we
382 only allowed for the infection of activated cells in the basic model, and did not represent the
383 level of free virus, which typically follows the level of virus-producing cells. This system is
384 structurally similar to some earlier models [58,60]. The scheme of the model is shown in
385 Figure Ia.

386 To investigate whether the ability to induce systemic immune activation is under selection, we
387 modelled competition between two virus variants (I_1 and I_2) that differed only in their
388 efficiency in activating quiescent CD4⁺ T cells (such that $k_1 > k_2$):

$$\begin{aligned}
\frac{dQ}{dt} &= \sigma - \delta_Q Q - (\alpha + k_1 I_1 + k_2 I_2) Q + rT \\
\frac{dT}{dt} &= (\alpha + k_1 I_1 + k_2 I_2) Q - (r + \delta_T) T - \beta(I_1 + I_2) T \\
\frac{dI_1}{dt} &= \beta I_1 T - \delta_I I_1 \\
\frac{dI_2}{dt} &= \beta I_2 T - \delta_I I_2 \quad .
\end{aligned}
\tag{2a-d}$$

390 A simplified scheme of the competition model is shown in Figure 1b. It can be shown
391 analytically that the ratio of the two virus variants remains constant over time irrespective of
392 the choice of parameters. This implies that when a new variant with altered activation
393 efficiency is introduced (by mutation or superinfection) into a chronic infection with a
394 resident virus, it will persist at its initial frequency, but will neither exclude nor be excluded
395 by the resident virus type. Mutations that affect systemic immune activation are thus
396 selectively neutral and the evolution of this trait is not expected to be driven by natural
397 selection. Importantly, this result is independent of the equations that describe the dynamics
398 of quiescent and activated target cells. The result is thus robust with respect to the processes
399 that affect these cell populations, and depends only on the assumption that both virus variants
400 have access to the same pool of activated cells (which is equivalent to all target cells being
401 equally affected by the overall level of systemic activation).

402

402 Box 2. Simulation model of local immune activation

403 Our simulation model consisted of a large number ($n=1000$) of spatially separated local sites
404 of infection (e.g. lymph follicles or aggregates of target cells) and a systemic pool (e.g. blood
405 or lymph) that received and mixed the output of all sites. Infection of each site (equivalent to
406 a “burst of infection”) was initiated with a defined number of uninfected quiescent and
407 activated target cells, and one or several “founder viruses” that were sampled randomly from
408 the systemic pool. To maintain consistency with the systemic model, the infection of each site
409 was simulated as an independent copy of the basic model (equation 2a-d). Considering that
410 the infection of local sites is probably short-lived compared to the dynamics of quiescent cells,
411 the influx rate of these cells was set to zero. All other parameter values were set to
412 biologically plausible values, and initial values were set to reflect the overall frequency (2%)
413 of activated lymphocytes. For simplicity, each site was terminated after 100 simulation steps
414 (corresponding to 10 days), by which time most target cells were typically exhausted. *In vivo*,
415 local bursts of infection may also be terminated by HIV specific cytotoxic lymphocytes that
416 infiltrate the site and kill infected cells. The initial age of local infections was randomized at
417 the beginning of a simulation, and new sites were initiated at each simulation step to maintain
418 the steady-state number of active sites. At each step, the overall ratio of the two virus variants
419 was computed by summing the two types of infected cells over all infected sites. New sites
420 were colonized according to a Poisson process, with the expected (mean) number of founder
421 viruses varied between simulations. The type of the founder viruses was determined randomly
422 (following a binomial distribution) according to the overall ratio of the two types. This
423 corresponds to assuming a systemic pool that mixes the output of all sites and serves as a
424 source of randomly sampled founder viruses (or infected cells) for the colonization of new
425 sites. The model thus combined continuous, deterministic virus dynamics within individual
426 sites and discrete, stochastic dynamics at the level of the metapopulation of all sites.

427 Considering that little quantitative data are available on the dynamics of the local sites of
428 infection, our model aimed at a qualitative description of the dynamics. The scheme of the
429 model is shown in Figure II.

430

430 Box 3. Mutations affecting local immune activation are selected

431 In typical simulations of the local activation model (Box 2), the virus variant with increased
432 efficiency of local immune activation (“activator type”) expands over time, and the variant
433 with weaker immune activation eventually goes extinct (Figure IIIa). Increased efficiency of
434 local immune activation thus provides selective advantage, even though within each site the
435 initial ratio of the two variants is preserved throughout the lifetime of the site. This result
436 arises because of two factors. First, random sampling of a small number of founder viruses
437 generates variation in the initial ratio of the two variants at newly initiated sites. E.g. sampling
438 two founder viruses per site from a systemic pool with 10% activator type mostly yields both
439 founders from the weakly activating type; however, some of the sites will have one, and about
440 1% will have both founders from the activator type. Second, the initial ratio of the activator
441 type influences virus production at a given site: more activator virus results in faster and more
442 profound activation and infection of the local stock of target cells. Thus, founder samples with
443 higher activator ratios experience stronger amplification, and therefore the frequency of the
444 activator type increases over time in the whole population. As long as these two criteria are
445 fulfilled, the result is very robust in terms of both the structure and the parameters of the
446 model. E.g., allowing for entry of new uninfected cells to a site during the lifespan of the site
447 can delay the extinction of the weakly activating type (by reducing the relative advantage of
448 the activator type gained from its faster initial expansion), but cannot prevent this outcome in
449 most simulations. Allowing for entry of further infected cells from the systemic compartment
450 in small numbers comparable to the initial founder sample has numerically negligible effect
451 on the outcomes.

452 To demonstrate the importance of the “founder effect”, we varied its strength by varying the
453 (mean) number of founders that colonize a new site. As expected, the time to the fixation of
454 the activator type (defined as the complete disappearance of the weakly activating type in a

455 simulation) increases with the number of founders (Figure IIIb), i.e. the selective advantage of
456 local immune activation decreases as the founder effect becomes weaker. In theory, the
457 founder effect is abolished completely if the number of founders approaches infinity, or if the
458 ratio of the activator type can assume any real number between 0 and 1 and is allowed to
459 follow the exact ratio in the systemic mixing pool. In this limit, the simulation model would
460 approximate the homogeneous systemic model that yielded no selective advantage for the
461 ability of immune activation. However, an analysis of HIV quasispecies indicated that local
462 sites of infection may be colonized by a single virus variant [47], which implies the strongest
463 possible founder effect.

464

464 **Box 4.** Outstanding questions

465 **What is the main mechanism of HIV induced immune activation?** At present, there are
466 many candidates [16,31-33,35,36], but no certain answer. Of particular interest for further
467 evolution is the range of the activation effect. While most candidate mechanisms could have
468 stronger local than systemic effects, this is not necessarily true for indirect mechanisms, like
469 bacterial translocation through the gut mucosa [35].

470 **Why are SIVs pre-adapted to induce systemic immune activation in non-adapted hosts?**

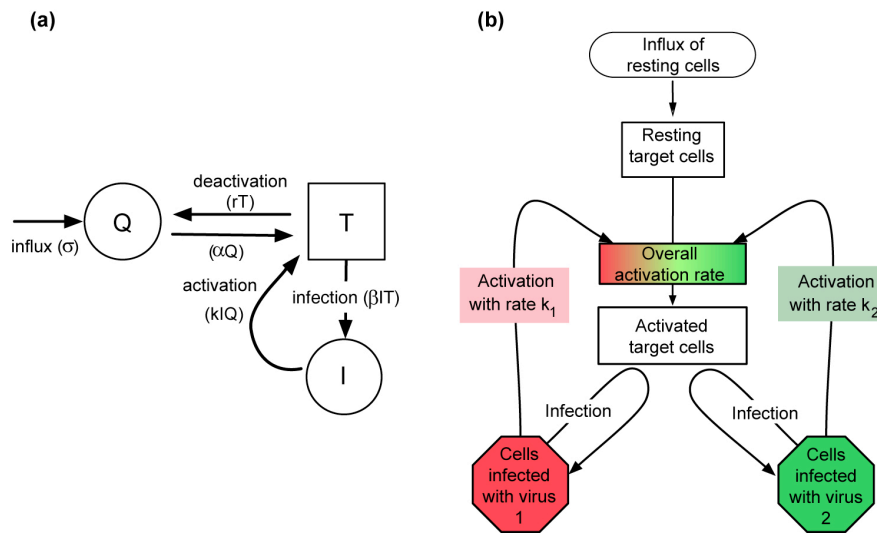
471 Our hypothesis suggests that SIVs may have evolved to induce local immune activation, and
472 it may be this mechanism that also inadvertently causes systemic activation in non-adapted
473 hosts. Understanding the original role of the mechanism in naturally infected primates may
474 provide important clues to why it goes awry in humans. Repeating the experiment of
475 Biancotto *et al.* [17] with lymphoid tissue isolated from natural SIV hosts and infected *ex vivo*
476 with species specific SIV might reveal whether the efficiency of local activation is indeed
477 important in natural SIV infections.

478 **Is systemic activation just the sum of local activation effects, or something more?**

479 Whether systemic activation is a side-effect of local activation, and if so, whether it can be
480 decoupled from the latter seems crucial for the future evolution of HIV induced immune
481 pathogenesis. Obligate coupling between the two types of activation could maintain current
482 levels of pathogenesis due to stabilizing selection acting on the efficiency of local immune
483 activation.

484 **What is the typical founder size?** The simulations suggest that the fragmentation of the virus

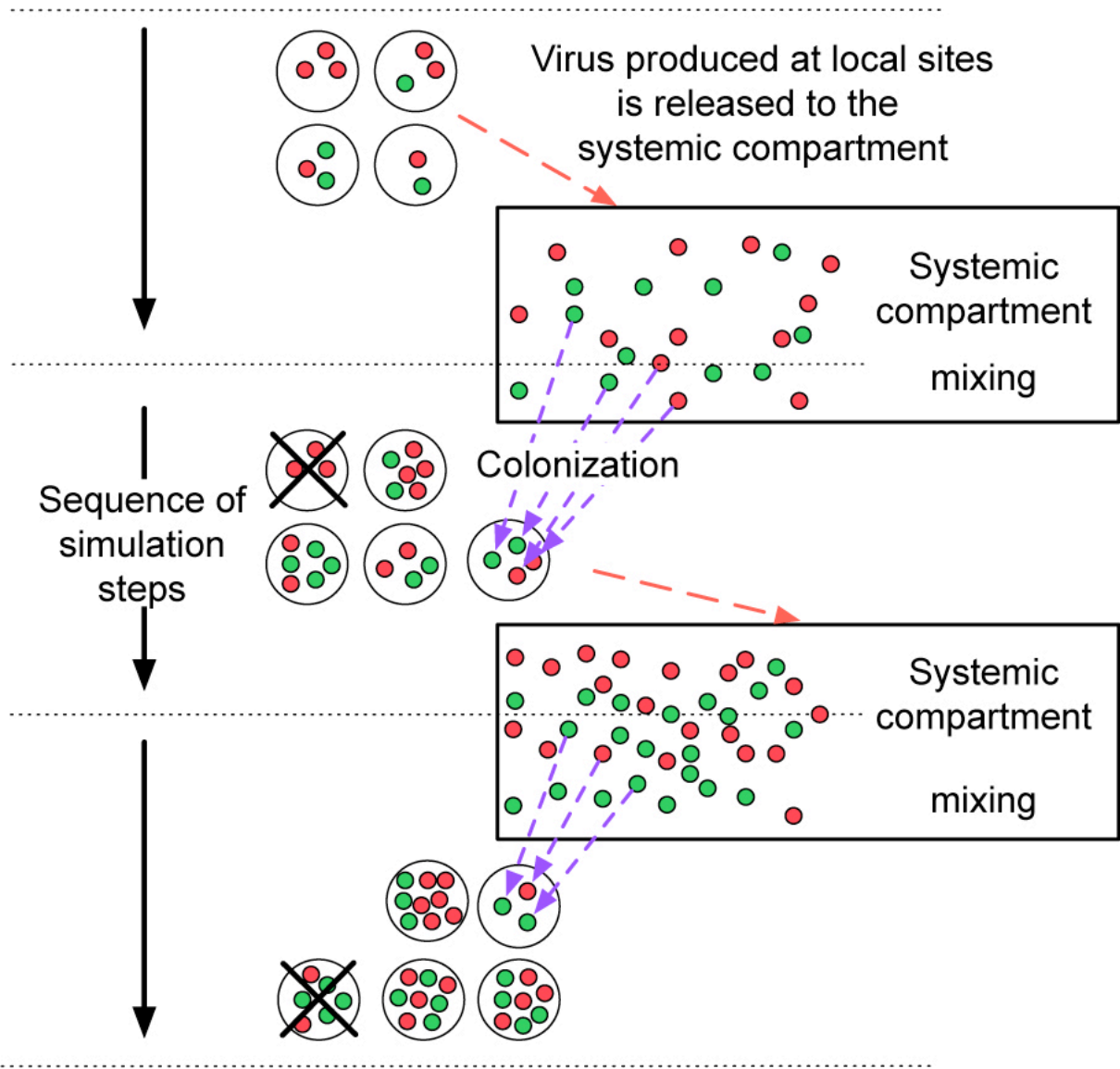
485 population within a host, in particular, the number of viruses that infect a local site, has a
486 marked effect on the strength of selection favouring (local) immune activation. More data are
487 needed to estimate this quantity.



488

489 **Figure I. (a)** Scheme of the basic model of systemic immune activation. **(b)** Simplified
 490 scheme of the extended model with two competing virus variants. The activation effect of
 491 both variants is summed up in the overall activation rate of target cells, and the two variants
 492 have equal access to the arising activated target cell pool. [TO BE INCLUDED IN BOX 1]

493

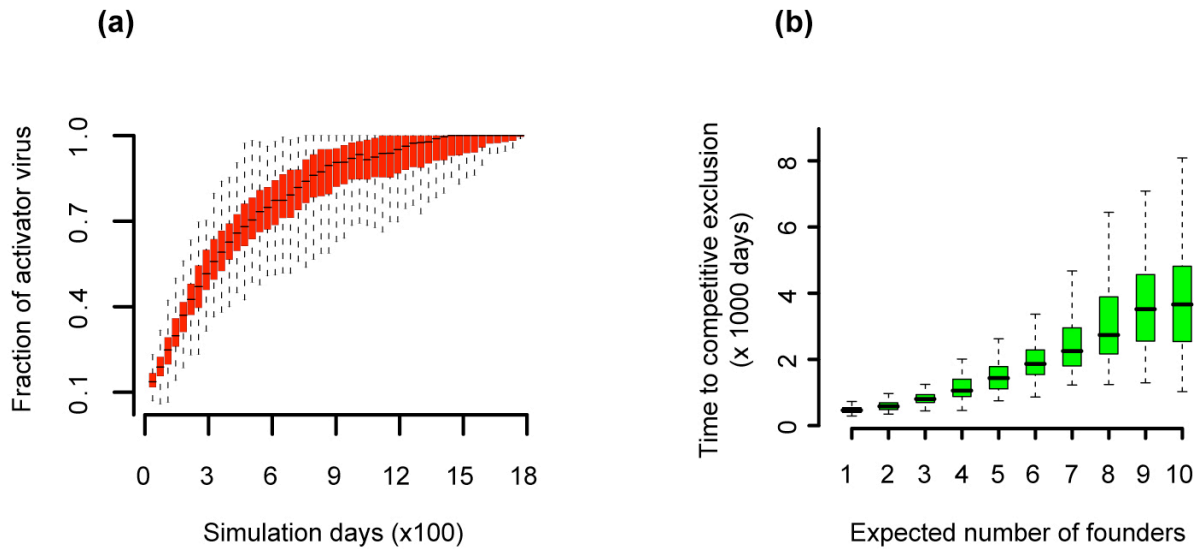


493

494 **Figure II.** Scheme of the simulation model of local immune activation [TO BE INCLUDED

495 IN BOX 2]

496



496

497 **Figure III. (a)** The activator type goes to fixation in the simulation of local immune

498 activation. The boxplot depicts the ratio of the activator type over time from 100 simulations.

499 Red boxes indicate the interquartile range of the ratio at each time point; median is indicated

500 by a horizontal line within the box, and whiskers extend to the farthest values that are not

501 more than 1.5 times the box width away from the box. The expected number of founders was

502 set to 5, and simulations were started with 10% activator type. **(b)** Time to fixation of the

503 activator type as a function of the expected number of founders in the simulation model. 100

504 simulations were performed for each value of the expected number of founders

505 (parameterizing the Poisson process of new site initialization); competitive exclusion was

506 registered when the variant with lesser activation efficiency was lost completely from the

507 system. The boxplot shows the median (horizontal lines), the interquartile range (green boxes)

508 and the farthest values that are not more than 1.5 times the box width away from the box

509 (whiskers) for the lengths of time that elapsed till competitive exclusion for each parameter

510 value. Weaker founder effect (more founders) increased the time to fixation indicating

511 decreasing selective advantage for the activator type. All simulations were started with 10%

512 activator type and shared the following parameters: $\alpha=0.012 \text{ d}^{-1}$, $\beta=0.1 \text{ d}^{-1}/\text{cell}$, $\delta_I=1 \text{ d}^{-1}$,

513 $\delta_T=0.2 \text{ d}^{-1}$, $\delta_Q=0.001 \text{ d}^{-1}$, $k_1=0.009 \text{ d}^{-1}/\text{cell}$, $k_2=0.003 \text{ d}^{-1}/\text{cell}$, $r=0.4 \text{ d}^{-1}$; initial values within

514 each site: $T(0)=20$, $Q(0)=1000$; number of sites: 1000, lifespan of sites: 10 d (see box 1 for
515 definitions). [TO BE INCLUDED IN BOX 3]