Title:

Has HIV evolved to induce immune pathogenesis?

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Abstract

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

Introduction

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

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

How could immune activation be beneficial for HIV?

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

Systemic vs. local immune activation

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

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

Mutations affecting systemic immune activation are selectively neutral

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

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

Local immune activation confers selective advantage

The lack of selection on systemic immune activation results from all viruses activating all target cells equally. However, some of the activation effect is likely to be limited to the neighbourhood of the inducer virus. HIV infects cells and is produced primarily in the lymphoid tissues [37,38], which have a highly organized spatial structure. Virus samples isolated from different sections of an infected spleen display markedly different genetic composition even on a microscopic scale [39-42], which suggests that aggregates of target cells can be colonized by a single or a few "founder" viruses and may serve as relatively isolated local sites for several rounds of virus replication [4,43,44]. Phylogenetic analyses of multiple virus isolates from individual patients have also hinted at a "metapopulation" structure involving the dynamic establishment and extinction of distinct local HIV subpopulations within a single patient [45-47]. Finally, localized activation by exogenous antigens has been demonstrated to fuel a local expansion of SIV clones [48]. These data suggest that HIV induced immune activation could partly be restricted to the neighbourhood of the inducer virus, which might influence the evolution of this trait. The effect of such spatial structure can be investigated in a simulation model involving a dynamic metapopulation of local "bursts of infection" (Box 2). The simulations illustrate that, in contrast to systemic activation, the ability to induce local immune activation provides selective advantage to HIV (Box 3). This result arises because the benefit of locally increased 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**

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

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

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

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

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

Implications for anti-activation treatment

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

Conclusion

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

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

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References

1 Ascher, M.S. and Sheppard, H.W. (1988) AIDS as immune system activation: a model for pathogenesis. *Clin Exp Immunol* 73, 165-167

2 Hazenberg, M.D. *et al.* (2000) T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol* 1, 285-289

3 Lawn, S.D. *et al.* (2001) Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. *Clin Microbiol Rev* 14, 753-777, table of contents

4 Grossman, Z. *et al.* (2002) CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat Med* 8, 319-323

5 Giorgi, J.V. *et al.* (1999) Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis* 179, 859-870

6 Leng, Q. *et al.* (2001) Immune activation correlates better than HIV plasma viral load with CD4 T-cell decline during HIV infection. *J Acquir Immune Defic Syndr* 27, 389-397

7 Hazenberg, M.D. *et al.* (2003) Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS* 17, 1881-1888

8 Deeks, S.G. *et al.* (2004) Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 104, 942-947

9 Sousa, A.E. *et al.* (2002) CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol* 169, 3400-3406

10 McCune, J.M. (2001) The dynamics of CD4+ T-cell depletion in HIV disease. *Nature* 410, 974-979

11 Gao, F. *et al.* (1992) Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 358, 495-499

12 Gao, F. *et al.* (1999) Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature* 397, 436-441

13 Rey-Cuille, M.A. *et al.* (1998) Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J Virol* 72, 3872-3886

14 Silvestri, G. *et al.* (2003) Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity* 18, 441-452

15 Hirsch, V.M. (2004) What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Rev* 6, 40-53

16 Finzi, D. *et al.* (2006) Defective virus drives human immunodeficiency virus infection, persistence, and pathogenesis. *Clin Vaccine Immunol* 13, 715-721

17 Biancotto, A. *et al.* (2007) HIV-1-induced activation of CD4+ T cells creates new targets for HIV-1 infection in human lymphoid tissue ex vivo. *Blood*

18 Zack, J.A. *et al.* (1990) HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* 61, 213-222

19 Bukrinsky, M.I. *et al.* (1991) Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* 254, 423-427

20 Chiu, Y.L. *et al.* (2005) Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. *Nature* 435, 108-114

21 Zhang, Z. *et al.* (1999) Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science* 286, 1353-1357

22 Zhang, Z.Q. *et al.* (2004) Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection. *Proc Natl Acad Sci U S A* 101, 5640-5645

23 Douek, D.C. *et al.* (2002) HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 417, 95-98

24 Brenchley, J.M. *et al.* (2006) Preferential infection shortens the life span of human immunodeficiency virus-specific CD4+ T cells in vivo. *J Virol* 80, 6801-6809

25 Pitcher, C.J. *et al.* (1999) HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. *Nat Med* 5, 518-525

26 O'Brien, W.A. *et al.* (1995) Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood* 86, 1082-1089

27 Staprans, S.I. *et al.* (1995) Activation of virus replication after vaccination of HIV-1-infected individuals. *J Exp Med* 182, 1727-1737

28 Bush, C.E. *et al.* (1996) A study of HIV RNA viral load in AIDS patients with bacterial pneumonia. *J Acquir Immune Defic Syndr Hum Retrovirol* 13, 23-26

29 Goletti, D. *et al.* (1996) Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation. *J Immunol* 157, 1271-1278

30 Mole, L. *et al.* (1997) The impact of active herpes simplex virus infection on human immunodeficiency virus load. *J Infect Dis* 176, 766-770

31 Ascher, M.S. and Sheppard, H.W. (1990) AIDS as immune system activation. II. The panergic imnesia hypothesis. *J Acquir Immune Defic Syndr* 3, 177-191

32 Swingler, S. *et al.* (1999) HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat Med* 5, 997-103

33 Biancotto, A. *et al.* (2007) Abnormal activation and cytokine spectra in lymph nodes of people chronically infected with HIV-1. *Blood* 109, 4272-4279

34 Herbein, G. and Khan, K.A. (2008) Is HIV infection a TNF receptor signalling-driven disease? *Trends Immunol* 29, 61-67

35 Brenchley, J.M. *et al.* (2006) Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 12, 1365-1371

36 Meier, A. *et al.* (2007) MyD88-dependent immune activation mediated by human immunodeficiency virus type 1-encoded Toll-like receptor ligands. *J Virol* 81, 8180-8191

37 Embretson, J. *et al.* (1993) Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 362, 359-362

38 Pantaleo, G. *et al.* (1993) HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 362, 355-358

39 Delassus, S. *et al.* (1992) Nonhomogeneous distribution of human immunodeficiency virus type 1 proviruses in the spleen. *J Virol* 66, 5642-5645

40 Cheynier, R. *et al.* (1994) HIV and T cell expansion in splenic white pulps is accompanied by infiltration of HIV-specific cytotoxic T lymphocytes. *Cell* 78, 373-387

41 Gratton, S. *et al.* (2000) Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. *Proc Natl Acad Sci U S A* 97, 14566-14571

42 Dumaurier, M.J. *et al.* (2005) The majority of human immunodeficiency virus type 1 particles present within splenic germinal centres are produced locally. *J Gen Virol* 86, 3369-3373

43 Grossman, Z. *et al.* (1998) Multiple modes of cellular activation and virus transmission in HIV infection: a role for chronically and latently infected cells in sustaining viral replication. *Proc Natl Acad Sci U S A* 95, 6314-6319

44 Grossman, Z. *et al.* (2006) Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* 12, 289-295

45 Frost, S.D. *et al.* (2001) Genetic drift and within-host metapopulation dynamics of HIV-1 infection. *Proc Natl Acad Sci U S A* 98, 6975-6980

46 Potter, S.J. *et al.* (2006) Genetic analyses reveal structured HIV-1 populations in serially sampled T lymphocytes of patients receiving HAART. *Virology* 348, 35-46

47 Shriner, D. *et al.* (2006) Evolution of intrahost HIV-1 genetic diversity during chronic infection. *Evolution Int J Org Evolution* 60, 1165-1176

48 Cheynier, R. *et al.* (1998) Antigenic stimulation by BCG vaccine as an in vivo driving force for SIV replication and dissemination. *Nat Med* 4, 421-427

49 Wilson, D.S. (1975) A theory of group selection. *Proc Natl Acad Sci U S A* 72, 143-146

50 Wilson, D.S. (1977) Structured Demes and the Evolution of Group-Advantageous Traits. *Am Nat* 111, 157-185

51 Szathmary, E. (1992) Natural selection and dynamical coexistence of defective and complementing virus segments. *J Theor Biol* 157, 383-406

52 Bonhoeffer, S. and Nowak, M.A. (1994) Intra-host versus inter-host selection: viral strategies of immune function impairment. *Proc Natl Acad Sci U S A* 91, 8062-8066

53 Müller, V. and De Boer, R.J. (2006) The integration hypothesis: an evolutionary pathway to benign SIV infection. *PLoS pathogens* 2, e15

54 Fraser, C. *et al.* (2007) Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. *Proc Natl Acad Sci U S A* 104, 17441-17446

55 Rambaut, A. *et al.* (2004) The causes and consequences of HIV evolution. *Nat Rev Genet* 5, 52-61

56 Wawer, M.J. *et al.* (2005) Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. *J Infect Dis* 191, 1403-1409

57 De Boer, R.J. and Perelson, A.S. (1998) Target cell limited and immune control models of HIV infection: a comparison. *J Theor Biol* 190, 201-214

58 Yates, A. *et al.* (2007) Understanding the slow depletion of memory CD4+ T cells in HIV infection. *PLoS medicine* 4, e177

59 Nowak, M.A. and May, R.M. (2000) *Virus Dynamics: Mathematical Principles of Immunology and Virology*, Oxford University Press

60 Wodarz, D. *et al.* (1999) Dynamics of macrophage and T cell infection by HIV. *J Theor Biol* 196, 101-113

Box 1. Mathematical model of systemic immune activation

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

$$\frac{dQ}{dt} = \sigma - \delta_Q Q - (\alpha + kI)Q + rT$$

$$\frac{dT}{dt} = (\alpha + kI)Q - (r + \delta_T)T - \beta I T \qquad \text{(1a-c)}$$

$$\frac{dI}{dt} = \beta I T - \delta_I I \quad .$$

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

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

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

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

Box 2. Simulation model of local immune activation

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

Box 3. Mutations affecting local immune activation are selected

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

To demonstrate the importance of the "founder effect", we varied its strength by varying the (mean) number of founders that colonize a new site. As expected, the time to the fixation of 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

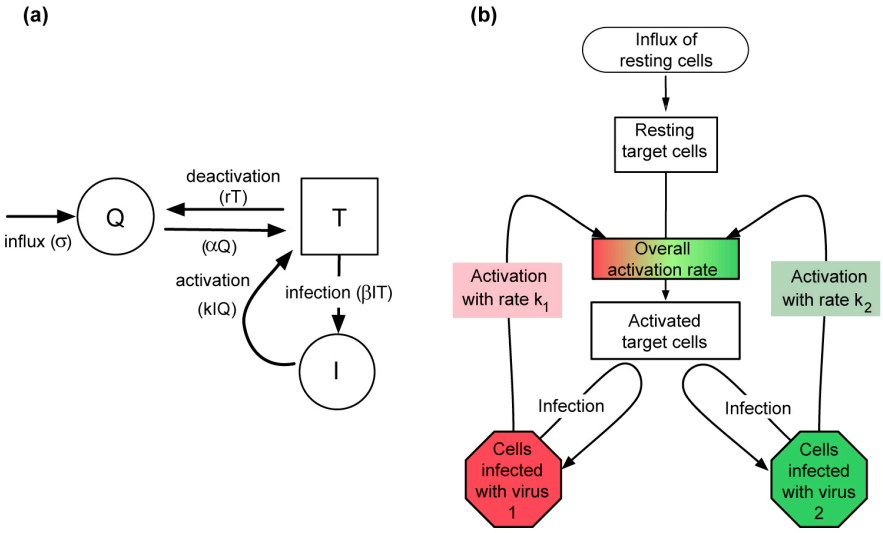
Box 4. Outstanding questions

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

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

Is systemic activation just the sum of local activation effects, or something more? Whether systemic activation is a side-effect of local activation, and if so, whether it can be decoupled from the latter seems crucial for the future evolution of HIV induced immune pathogenesis. Obligate coupling between the two types of activation could maintain current levels of pathogenesis due to stabilizing selection acting on the efficiency of local immune activation.

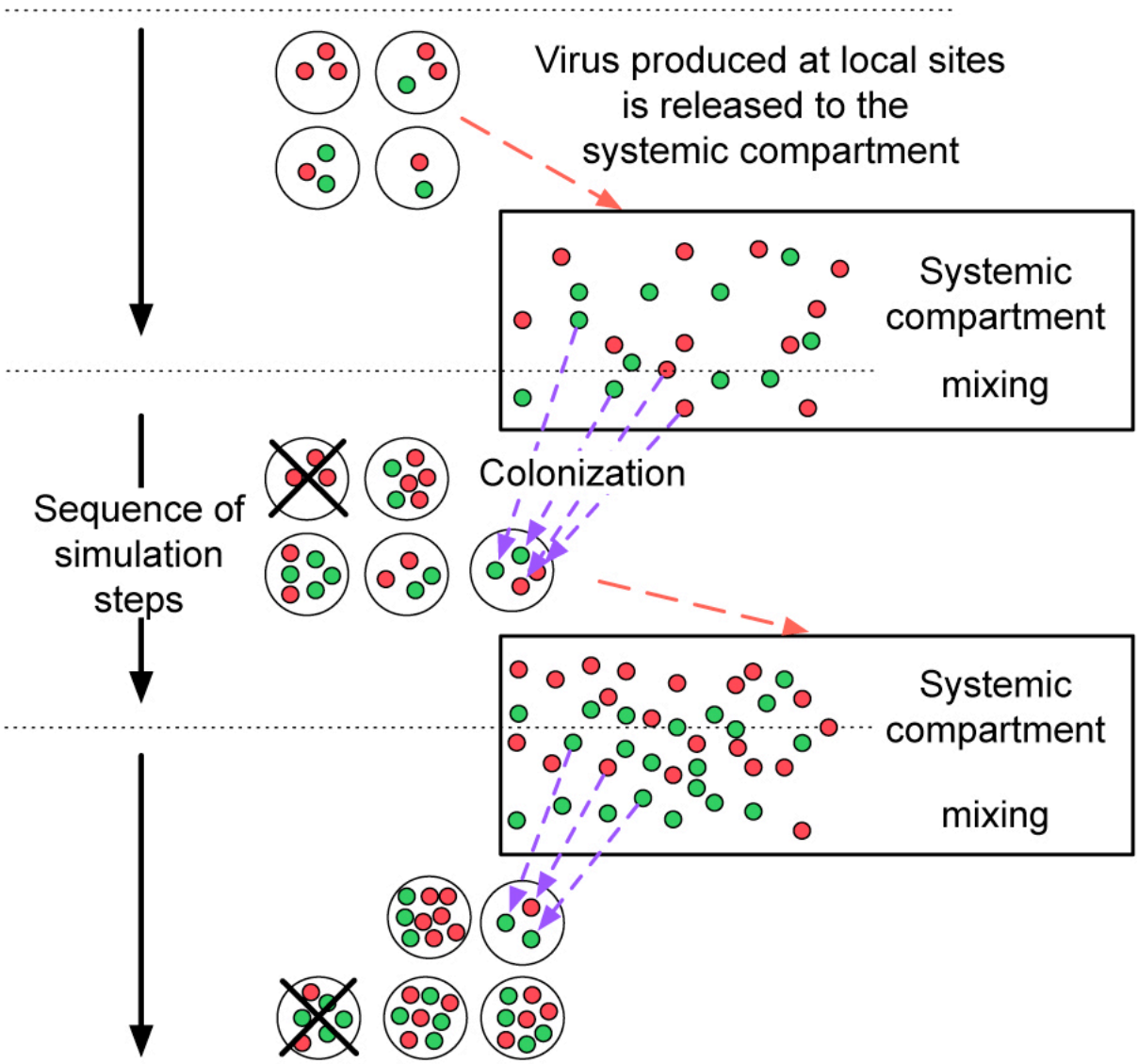
What is the typical founder size? The simulations suggest that the fragmentation of the virus population within a host, in particular, the number of viruses that infect a local site, has a marked effect on the strength of selection favouring (local) immune activation. More data are needed to estimate 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

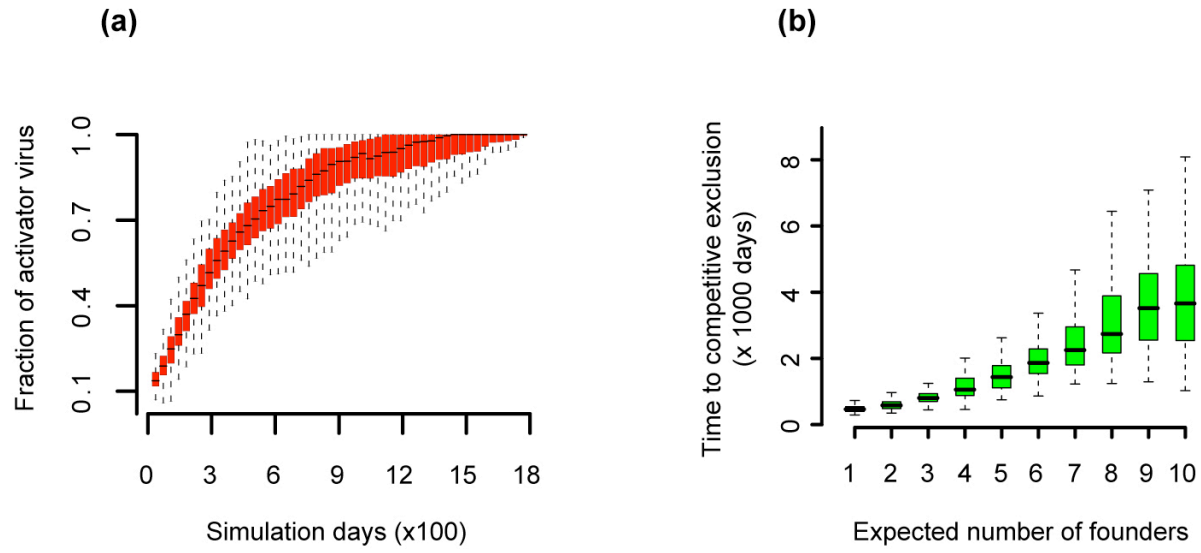


493

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: α=0.012 d^{-1}, β=0.1 d^{-1}/cell, δ_I=1 d^{-1},
513 δ_T=0.2 d^{-1}, δ_Q=0.001 d^{-1}, k_1=0.009 d^{-1}/cell, k_2=0.003 d^{-1}/cell, r=0.4 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]