



Opinion

Cellular Superspreaders: An Epidemiological Perspective on HIV Infection inside the Body

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Introduction

Worldwide, more than 250 people become infected with HIV every hour [1], yet an individual's chance of becoming infected after a single sexual exposure, the predominant mode of HIV transmission, is often lower than one in 100 [2]. When sexually transmitted HIV-1 infection does occur, it is usually initiated by a single virus, called the founder strain, despite the presence of thousands of genetically diverse viral strains in the transmitting partner [3]. Here we review evidence from molecular biology and virology suggesting that heterogeneity among CD4+ T cells could yield wide variation in the capability of individual cells to become infected and transmit HIV to other cells. Using an epidemiological framework, we suggest that such heterogeneity among CD4+ T cells in the genital mucosa could help explain the low infection-to-exposure ratio and selection of the founder strain after sexual exposure to HIV.

During sexual transmission, founder viral strains preferentially infect CD4+ T cells using the CCR5 coreceptor [4,5]. At the time of initial exposure to HIV, these CD4+ T cells exhibit baseline heterogeneity due to stochasticity in cellular gene expression [6] and dynamic variation in immunological status (activated, resting, etc.) [7]. In addition, because CD4+ T cells are mobile, they are heterogeneously distributed in the genital mucosa, with varying degrees of clustering and contact [8–11]. In other contexts, it is well-known that heterogeneity among isogenic cells inside the body can affect many cellular behaviors and outcomes, including infection dynamics [12,13].

Epidemiological analyses of disease outbreaks among people indicate that heterogeneity in the ability of individuals in a population to spread disease can have a significant impact on whether a local outbreak becomes an epidemic [14]. Heterogeneity among a population of

CD4+ T cells may play a similarly critical role in the establishment and spread of HIV in the genital mucosa after sexual exposure.

Basic and Individual Reproductive Number

To quantify the spread of infectious disease, epidemiologists use the basic reproductive number, R_0 , which describes the average number of secondary infections that arise from one infected individual in an otherwise totally susceptible population [15]. The basic reproductive number can be approximated as the product of the following: (1) the average number of susceptible individuals contacted by an infected individual during the infectious period (the “number of contacts”) and (2) the average probability that a susceptible individual will become infected by a single infected individual during its infectious period (the “shedding potential”). Thus,

$$R_0 \approx \text{Number of contacts}$$

$$\times \text{Shedding potential.}$$

The number of secondary infections caused by a specific individual throughout the time that the individual is infectious is called the “individual reproductive number” [14]. For any disease within a given

population, there exists a distribution of individual reproductive numbers, of which R_0 is the mean [14]. In populations of homogeneous individuals, the distribution of individual reproductive numbers will be clustered around the population average value of R_0 , and thus, this average value will more accurately predict the likelihood of transmission from each infected to each susceptible individual. If $R_0 > 1$, then an outbreak is likely to become an epidemic, and if $R_0 < 1$, then an outbreak will not spread beyond a few initially infected individuals [15,16].

In heterogeneous populations, however, the population average value of R_0 is less predictive of transmission dynamics [14]. For example, in populations with highly right-skewed distributions of individual reproductive numbers, most individuals infect few, if any, others, but a few individuals infect many others. In such populations, there is a high probability that a disease outbreak will not be sustained in the population and will instead go extinct [14]. In some cases, however, those rare individuals in the tail of the distribution with a much higher-than-average individual reproductive number while they are infected, known as “superspreaders” [15], can have a significant impact on whether an outbreak becomes an epidemic or goes extinct. Epidemiological outbreak investigations, which track the spread of disease by a technique called contact tracing, have

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identified the existence of superspreaders in many well-known infectious disease outbreaks, including typhoid fever, measles, smallpox, Ebola, and severe acute respiratory syndrome (SARS) [14,17,18]. These rare individuals often make a significant, sometimes deciding, contribution to the dynamics of disease spread (Table 1).

During the 2003 SARS outbreak in Singapore, for example, the majority of individuals who became infected spread the virus either to no one else or to only one other [14]. Five infected individuals, however, were superspreaders, each infecting at least 20 others (Figure 1) [19]. In this example, R_0 , which is an average population value, did not adequately describe the dynamics of SARS because it did not capture the heterogeneity among individuals in their ability to spread disease or the key contribution made by superspreaders to establishment and spread of the virus [14].

Individual Cellular Reproductive Number

For a population of HIV-infected cells inside the body, the basic reproductive number, R_0 (a population average), has been quantified [20]. As with individual humans inside a population, however, empirical evidence indicates that individual human cells inside a population are also heterogeneous [12], varying in their contact with one another, their ability to become infected (permissivity), and also in whether and to what extent they transmit infectious virus to other cells during their infectious period. Such heterogeneity

among CD4+ T cells in the genital mucosa of a single individual could generate a skewed distribution in the individual *cellular* reproductive number, or ICRN, in the context of HIV infection. Here we review evidence for heterogeneity among CD4+ T cells that could lead to wide variation in ICRN and possibly give rise to cellular superspreaders.

Number of Contacts

CD4+ T cells exhibit considerable heterogeneity in activation status (e.g., resting or activated) and expression of surface molecules important for HIV infection (including the HIV coreceptors CCR5 and CXCR4) in human penile [21], foreskin [22,23], cervical [24,25], and rectal [26] tissue. In addition, various studies that stain for CD4+ T cells in uninfected genital mucosal tissue, such as cervical tissue [27] and human foreskin [22], indicate that T cells vary in their spatial distribution and the extent to which they form clusters. Cell density and spatial arrangement have been identified as important sources of heterogeneity among cells that can affect virus spread in vitro [8,28–30]. Indeed, imaging studies exploring the dynamics of virus spread in a model of sexual transmission to female nonhuman primates indicate that virus spreads unevenly among clusters of cells in the endocervix [9]. Cells in these clusters tend to be in close proximity, and if cell-to-cell transmission is far more efficient than cell-free transmission, as some studies suggest [31], then a cell that is physically touching its neighbors could generate more secondary infections than a cell that

is not close enough to others to transmit virus by direct contact [8]. Thus, heterogeneity in cell distribution and clustering inside the body could generate wide variation in the efficiency of virus transmission from cell to cell and in ICRN.

Transmission of virus from an infected to a susceptible cell also depends on a cell's permissivity to productive infection. The level of surface expression of CD4 and CCR5 (the predominant coreceptor utilized during acute infection [32]) varies widely among CD4+ T cells [25,33], even in a single individual [34], and affects cellular permissivity to HIV [35,36]. Indeed, low expression of CD4 or CCR5 can completely inhibit infection of CD4+ T cells by certain viral strains [37]. A recent multiparameter analysis of HIV entry efficiency at the level of single cells indicated large cell-to-cell variation in expression of CD4, CCR5, and the coreceptor CXCR4, which subsequently influenced permissivity of individual cells to HIV binding and entry [38]. In addition, CD4+ T cells isolated from rectal and cervical tissue exhibit considerable heterogeneity in expression of the surface integrin $\alpha 4\beta 7$, which can specifically bind the V2 loop of the HIV envelope protein gp120 and may improve cell-to-cell spread by activating other cell-surface molecules in the viral synapse [39]. Experiments in vitro indicate that HIV preferentially infects cells expressing high levels of CCR5 and that infection can be further enhanced by high levels of surface $\alpha 4\beta 7$ expression in some individuals [40,25]. Heterogeneity among CD4+ T cells in expression of specific cell surface receptors can thus affect permissivity and

Table 1. Superspreading events during infectious disease outbreaks.

Disease	Location (year)	(R_0) ^a	SSE ^b	References
EBOLA	Congo (1995)	1.83	21+, 28–38	[85,86]
MEASLES	Greenland (1951)	16	250	[15,87]
	US (1985)	16	69,84	[15,88]
	Canada (2011)	16	678	[15,17]
PNEUMONIC PLAGUE	China (1946)	1.3	32	[89,90]
SARS	Hong Kong (2003)	3	187	[91,92]
	Vietnam (2003)	3	20	[91,93]
	Singapore (2003)	1.6	12,21,23,23,40+	[14,19]
	Canada (2003)	3	19,12–24	[91,94]
SMALLPOX	Yugoslavia (1975)	5.5	38	[95,96]

^a R_0 : The average number of secondary cases caused by an infected individual during the outbreak; here, R_0 is reported either for a specific outbreak, when available, or as a measure calculated based on multiple past outbreaks.

^bSSE: Superspreading events—number of infections caused by a single individual during an outbreak; number of infections caused by multiple superspreading events during the same outbreak are separated by commas.

This table is adapted from the supplementary material from reference [14].

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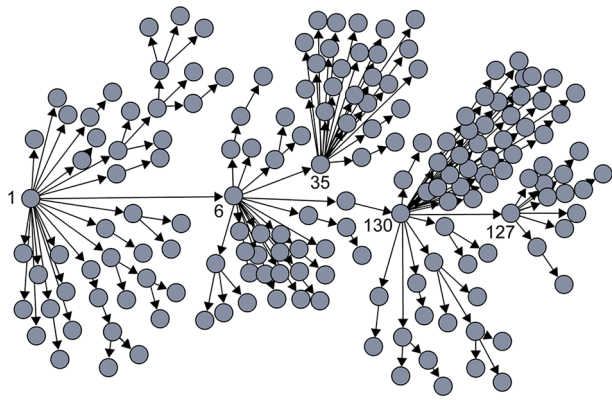


Figure 1. Contact tracing of SARS in Singapore showed that most people (gray circles) transmitted the virus to very few others, while a few individuals acted as “superspreaders,” infecting many more people than average. Patient numbers corresponding to those individuals who were identified as superspreaders are shown. All cases trace back to patient 1 [18].

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cell-to-cell transfer of HIV infection in the genital mucosa.

Permissivity of cells to productive HIV infection can also be affected by intracellular proteins called restriction factors, which block the progress of HIV through the cell [41]. Expression of some cellular restriction factors has been shown to vary among different human populations [42] and even between different types of CD4+ T cells within the same individual [43]. Notably, an intracellular host restriction factor known as SAMHD1 (sterile alpha motif [SAM] and histidine/aspartic acid [HD] domain-containing protein 1) blocks reverse transcription in resting—but not activated—CD4+ T cells, inhibiting HIV replication. These findings help to explain the inability of resting CD4+ T cells to produce infectious virus [44]. HIV combats the effects of some cellular restriction factors with its own viral proteins, but the success of these proteins in overcoming cellular resistance and facilitating virus production depends on their quantity within the cell, which can also vary depending on viral gene expression levels [41].

Together, these data suggest that among CD4+ T cells in the genital mucosa, significant heterogeneity may exist in the number of contacts that become infected by any given infected cell even within the same host, potentially leading to a skewed distribution of ICRN.

Shedding Potential

Release of infectious viral particles from an infected CD4+ T cell, or shedding potential, can be influenced by many factors in both the cell and the virus.

Variation in virus gene expression at the level of individual cells has been demonstrated *in vivo* in a mouse model of cytomegalovirus infection [45], and heterogeneity among individual cells in the production of virus particles, or virus shedding, has been shown through analysis of cells isolated from simian immunodeficiency virus (SIV)-infected nonhuman primates, in which rare activated CD4+ T cells were shown to individually release large quantities of virus [10]. Here we focus on variability in gene expression and its potential impact on an individual cell’s capability to release infectious virus as a possible source of heterogeneity in shedding potential.

Stochasticity in cellular gene expression is a common phenomenon [6] and has also been observed in viral gene expression, including HIV. In populations of genetically identical cells infected with HIV, viral genes tended to be expressed at either high or low levels but were also rarely expressed at intermediate levels, varying from cell to cell [46].

The site of virus integration also affects viral gene expression. HIV viral DNA preferentially integrates at sites of active cellular gene expression although not at any specific site or in any specific gene [47–49]. Some viral integration events occur at sites of much higher gene expression than others [47], and gene expression can vary significantly depending on the site of integration, even in genetically identical cells [50]. Since the majority of HIV-infected cells in lymph nodes and peripheral blood contain a single virus [51], while cells in splenic tissue contain one to eight integrated proviruses (mean 3.2) [52], viral integration site can be an important factor in determining viral

gene expression and likely also subsequent virus shedding potential from any given cell.

Within the population of CD4+ T cells in the genital mucosa, therefore, a wide range of ICRN may exist due to stochastic and/or infection-driven variations in viral gene expression and viral particle production, arising from potentially wide heterogeneity in virus shedding potential among infected cells.

Cellular Superspreaders

Experiments in a nonhuman primate model of HIV infection have demonstrated that the vast majority of CD4+ T cells in the genital mucosa of a healthy, uninfected individual are resting cells, which outnumber activated cells 70:1 (Figure 2A) [9]. Activated CD4+ T cells express higher levels of CCR5 [33] and $\alpha 4\beta 7$ [40] than do resting cells. In addition, experiments in nonhuman primates indicate that infected, activated cells contain five times more viral RNA, release 10-fold more viral particles to their surrounding environment, and tend to form larger cell clusters than resting cells [9]. Notably, although the nonhuman primate model has long been used to study many facets of HIV infection [53], the virus used in these experiments, SIV, expresses a protein that allows it to productively infect resting CD4+ T cells. In contrast, HIV-1 does not express this protein and is thus unable to generate productive infection in resting CD4+ T cells [54]. Resting human CD4+ T cells in the human genital mucosa are therefore even less likely to be able to produce and spread HIV than are resting CD4+ T cells in a nonhuman primate model.

Together, these data suggest that upon infection with HIV the majority of CD4+ T cells in the genital mucosa would spread the virus to few if any others and that only rare cells would have the capacity to release large quantities of HIV to their nearest neighbors. These rare cells may be activated CD4+ T cells, which have been described as “amplifiers” that can cause additional cells to become infected with HIV [10,55]. Such rare cells may exhibit a specific set of traits that facilitate establishment of HIV inside the body. For example, experiments done *in vitro* indicate that CD4+ Th17 cells, which express high levels of CCR5 as well as the chemokine receptor CCR6 and the integrin $\alpha 4\beta 7$, are preferentially targeted during early infection, and analysis of samples from female sex workers who are infected with HIV indicate that CD4+ Th17 cells are selectively depleted from

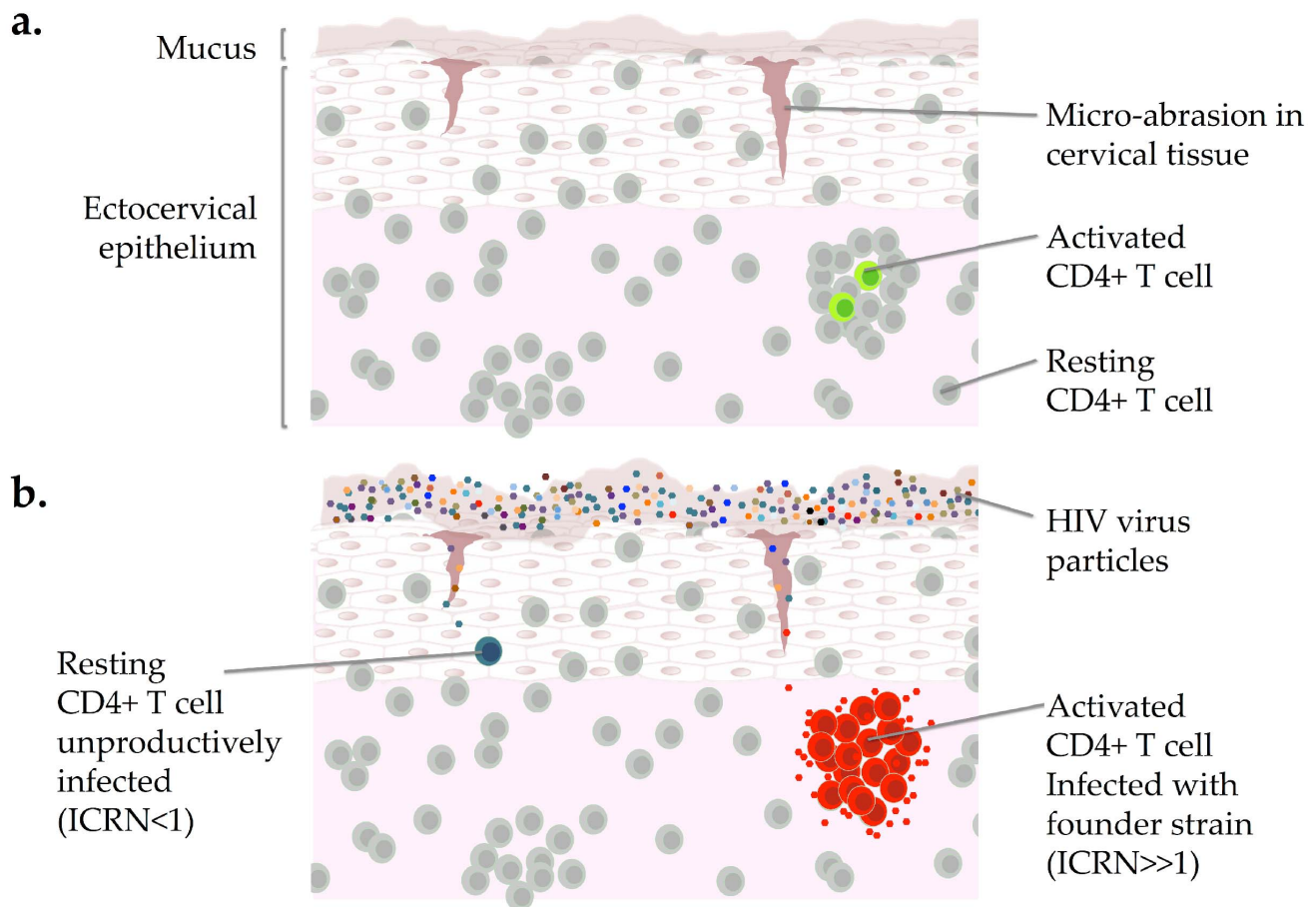


Figure 2. Heterogeneity among CD4+ T cells in the genital mucosa and the HIV founder strain. (A) The majority of CD4+ T cells in the female genital mucosa of an uninfected individual are resting cells; rare cells are activated. (B) Most virus particles remain trapped in the mucus that coats the cervical epithelium though a few can enter through microabrasions. Resting CD4+ T cells can become infected with HIV but do not produce infectious virus. Infection of activated CD4+ T cells, which tend to form clusters, have higher levels of gene expression than resting cells, and produce infectious virus, may be the superspreading event that establishes the HIV founder strain after sexual transmission. ICRN: individual cellular reproductive number. Based on data from references [3] and [10]. Images of cervical epithelium by OpenStax College [CC-BY-3.0 (<http://creativecommons.org/licenses/by/3.0/>)] via Wikimedia Commons. doi:10.1371/journal.ppat.1004092.g002

the cervix during HIV infection [25,56–58]. In addition, given that the majority of HIV infections begin with a single founding strain [59] and most infected cells in peripheral blood and lymph node tissue contain a single copy of HIV DNA (although these may or may not be representative of HIV integration in mucosal lymphatic cells) [51], infection of a single cell has the potential to establish HIV infection inside the genital mucosa after a given exposure. Infection of a rare CD4+ T cell with very high ICRN could thus be the superspreading event that both establishes HIV infection in the genital mucosa and selects a single founder strain (Figure 2B).

Such a founding superspreader infection event would parallel, for example, the dynamics that governed the SARS outbreak in Singapore, giving rise to an epidemic despite a low individual

reproductive number for most individuals. As shown in Figure 1, infection of a single superspreading individual (labeled as “1”) triggered the SARS outbreak in Singapore in 2003.

Implications of Cellular Superspreaders in HIV Infection

A highly skewed distribution of individual reproductive numbers in a population, in which most individuals infect few if any others but a tiny minority are superspreaders, has two important implications when applied to CD4+ T cells in the genital mucosa. First, since the majority of the cells would have a low ICRN, most, if not all, of the viral strains that successfully overcome physiological barriers during exposure are likely to infect cells that have an ICRN less than one. Provided that none of these cells becomes latently

infected, which has been shown to occur within days after infection in vitro [60,61] but has yet to be confirmed in vivo, a “local outbreak” inside the body would go extinct. In this case, infection of most cells immediately after sexual exposure would not lead to sustained infection, which could explain the very low infection-to-exposure ratio for sexual exposure to HIV.

Second, such short-lived “local outbreaks” of HIV within an individual could still yield a low level of virus production, even if the initial outbreak of HIV infection ultimately goes extinct. Among a group of nonhuman primates exposed to a low physiological dose of SIV, some animals experienced initial low levels of viral replication and immune response without ever proceeding to full infection or seroconversion, a phenomenon called occult infection [62–64]. In addition, some HIV-exposed, seronegative humans who

continue to engage in high-risk sexual behavior exhibit immunological markers that indicate a prior immune response to HIV infection even though they remain seronegative, supporting the idea that local infections may have occurred in these patients [65–67]. Finally, analysis of the unsuccessful HIV vaccine STEP trial suggested that a large portion of the exposed individuals may have experienced occult infection, implying that this phenomenon could be more widespread than previously suspected [68]. There is, however, no direct experimental evidence for occult infection in humans.

If infection of a rare superspreader CD4+ T cell establishes the founder strain, then the few features of founder viral strains that have been observed might in fact confer a selective advantage during early infection. These features include shorter envelope glycoproteins with fewer N-linked glycosylation sites [69,70] as well as preferential infection of CD4+ T cells expressing high levels of CCR5. Some founder viral strains have also exhibited high affinity for the $\alpha 4\beta 7$ integrin receptor [71,72] though this has not been universally observed [73,5]. The founder virus, which more closely resembles the ancestral founder strain than it does the predominant strain in the transmitting partner [74], may be selected by its ability to infect a subtype of CD4+ T cell: a cellular superspreader. This specific efficiency could explain why HIV founder strains have not shown a consistent infectivity advantage in CD4+ T cells over strains from chronic infection *in vitro* [5,75,76], as these experiments likely do not fully replicate the cellular heterogeneity of the *in vivo* environment [32,77,78], where the founder viruses might have an advantage in infecting the rare superspreader cells.

Conclusions

Here we have applied two key concepts from epidemiology: R_0 , which is the approximated product of number of contacts and shedding potential throughout the infectious period, and individual reproductive number, to suggest that a skewed distribution of individual cellular reproductive number among CD4+ T

cells in the genital mucosa gives rise to cellular superspreaders that may drive establishment of HIV infection inside the genital mucosa after sexual transmission.

The definition of R_0 provided here implicitly integrates transmission over the time that an index cell was infected, meaning that we have incorporated duration of infectiousness into our overall definition of R_0 . Thus, a CD4+ T cell could theoretically become a superspreader either by spreading a large amount of infectious virus to other cells in a short amount of time, or by spreading a smaller amount of virus to other cells for a comparatively longer period of time, or through some combination of the two. Though any of these mechanisms are possible in early infection [79], studies in nonhuman primates suggest that establishment of infection in the genital mucosa typically occurs within 3–7 days after male-to-female sexual exposure [62,80]. The lifespan of a productively infected CD4+ T cell is on average 2.2 days [81]. Thus, in the specific case of HIV infection in the genital mucosal tissue, if infection becomes established via a superspreading event, it is likely to occur within the first few days of exposure and be driven by a relatively short-lived, productively infected cell that generates a much higher-than-average number of secondary infections due to a high shedding rate, or a high contact rate, or both. Notably, as in other superspreading events, establishment of HIV by infection of a cellular superspreader could occur even if the basic reproductive number for the entire population of susceptible cells is low.

If cellular HIV superspreaders do exist, and if they are the cellular culprits driving the establishment of HIV infection inside the body, then the most successful strategy for preventing the infection from becoming established in the body is to block or remove these cells before or shortly after infection in order to drive a local, within-host outbreak to extinction [13,82]. Such cells may have specific traits, such as high expression of surface receptors including CCR5 and possibly also $\alpha 4\beta 7$, that allow them to be identified and targeted by novel therapies to prevent establishment of infec-

tion. Several recent studies suggest that Th17 cells, a subset of CD4+ T cells, are preferentially infected by early viral strains and selectively depleted from the cervix during HIV infection [58,25]. We are not aware of *in vivo* data that explicitly support the existence of cellular superspreaders; nevertheless, the data reviewed here suggest their existence, warranting further empirical research. Identification and targeting of cells most likely to become superspreaders could facilitate the development of preexposure or immediate postexposure therapies that could prevent a local outbreak of HIV inside the genital mucosa from becoming a within-host epidemic that spreads throughout the body [9,83].

In this review, we have applied epidemiological concepts of disease spread specifically to explore unsolved questions regarding establishment of the HIV founder strain and the low infection-to-exposure ratio of infection after sexual transmission. We suggest that these concepts may also be applied more broadly to explain the documented existence of HIV founder strains after transmission via injection drug use and from mother to child [59] since CD4+ T cells in the blood of healthy, uninfected individuals are also heterogeneous, with only a very small subset exhibiting an activated or replicating phenotype [84].

Since heterogeneity among cells has been acknowledged as an important factor in a variety of cellular processes, including certain viral infections [12], we suggest that the epidemiological framework described here may also be applicable to the establishment and spread of other cellular diseases inside the body, including not only infections but perhaps also certain cancers. The impact of cellular heterogeneity may be particularly profound if the distribution of individual cellular reproductive numbers is highly skewed, yielding cellular superspreaders.

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