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IMPERFECT VACCINES AND THE EVOLUTION OF PATHOGENS CAUSING ACUTE INFECTIONS IN VERTEBRATES

VITALY V. GANUSOV[1,2,3] AND RUSTOM ANTIA[3]
[1]*Theoretical Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*
[2]*E-mail: v.v.ganusov@bio.uu.nl*
[3]*Department of Biology, Emory University, 1510 Clifton Road, Atlanta, Georgia 30322*

Abstract.—A study by Gandon et al. (2001) considered the potential ways pathogens may evolve in response to vaccination with imperfect vaccines. In this paper, by focusing on acute infections of vertebrate hosts, we examine whether imperfect vaccines that do not completely block a pathogen's replication (antigrowth) or transmission (antitransmission) may lead to evolution of more or less virulent pathogen strains. To address this question, we use models of the within-host dynamics of the pathogen and the host's immune responses. One advantage of the use of this within-host approach is that vaccination can be easily incorporated in the models and the trade-offs between pathogen transmissibility, host recovery, and virulence that drive evolution of pathogens in these models can be easily estimated. We find that the use of either antigrowth or antitransmission vaccines leads to the evolution of pathogens with an increased within-host growth rate; infection of unvaccinated hosts with such evolved pathogens results in high host mortality and low pathogen transmission. Vaccination of only a fraction of hosts with antigrowth vaccines may prevent pathogens from evolving high virulence due to pathogen adaptation to unvaccinated hosts and thus protection of vaccinated hosts from pathogen-induced disease. In contrast, antitransmission vaccines may be beneficial only if they are effective enough to cause pathogen extinction. Our results suggest that particular mechanisms of action of vaccines and their efficacy are crucial in predicting longterm evolutionary consequences of the use of imperfect vaccines.

Key words.—Acute infection, evolution of virulence, immune response, imperfect vaccines, mathematical models, within-host dynamics.

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Vaccination is one of our most effective tools for the control of infectious diseases. A perfect vaccine should prevent vaccinated individuals from becoming infected on exposure to the pathogen. An imperfect vaccine, one that does not stop vaccinated individuals from becoming infected on exposure to the pathogen, may still be beneficial in a number of ways (Anderson and May 1991). The benefits conferred by imperfect vaccines may include: reducing the severity of the infection or decreasing the transmission of the pathogen from infected hosts, thus reducing the spread and prevalence of the disease in the community (herd immunity).

A recent study considered the consequences of pathogen evolution in response to the introduction of imperfect vaccines (Gandon et al. 2001). In particular, the authors suggested that introduction of vaccines that reduce the rate of pathogen replication within the host (antigrowth vaccines) may lead to the evolution of pathogens with increased virulence (as measured in unvaccinated hosts). In contrast, introduction of vaccines that reduce the rate of pathogen transmission from an infected host (antitransmission vaccines) may lead to the evolution of pathogens with reduced virulence.

Evolution of pathogens can be understood in terms of the basic reproductive number, R_0, of the infection caused by the pathogen (Anderson and May 1991). R_0 is defined as the number of secondary infections caused by the introduction of one infected host into a wholly susceptible population (Anderson and May 1991). Under a broad range of circumstances, for example, in the absence of intrahost competition (Levin and Pimentel 1981; Bonhoeffer and Nowak 1994; Nowak and May 1994; May and Nowak 1995; van Baalen and Sabelis 1995), pathogens evolve to maximize R_0 (Bremermann and Thieme 1989; Anderson and May 1991). For a directly transmitted pathogen, R_0 can be written as

$$R_0 = \frac{\beta S}{d + \alpha + \nu}, \quad (1)$$

where β is the average transmissibility of the pathogen, α and d are the rate constants for the pathogen-induced and natural host mortality, ν is the rate of host recovery from infection, and S is the density of susceptible hosts. The trade-offs between transmissibility β, host recovery rate ν, and virulence α determine the values of these parameters at which R_0 is maximum. While the existence of some of these trade-offs is intuitive, experimental data on the functional form for these trade-offs in vertebrates is relatively limited (Fenner et al. 1956; Schulman 1967; MeadBriggs and Vaughan 1975; Anderson and May 1982; Mackinnon and Read 1999).

Gandon et al. (2001) used this framework to investigate how the use of imperfect vaccines may affect the optimal level of virulence of a pathogen. The main difficulty with this approach is the uncertainty regarding the trade-offs between β, ν, and α, and how vaccination might affect these trade-offs.

Mathematical models of the within-host dynamics of pathogens and their transmission from infected hosts have been used to explore the trade-offs between β, α, and ν and the evolution of pathogens (Sasaki and Iwasa 1991; Antia et al. 1994; Ganusov et al. 2002; Gilchrist and Sasaki 2002; André et al. 2003; Ganusov and Antia 2003; André and Gandon 2006). These models can explicitly include the interaction between the pathogen and the immune response (Antia et al. 1994; Ganusov et al. 2002; André et al. 2003), and vaccination can be modeled by an appropriate change in the pa-

rameters describing the immune response. This allows us to use these within-host models to explore how immunization may affect the trade-offs between β, ν, and α, and consequently how vaccination may affect the level of virulence to which pathogens may be expected to evolve.

We first review the within-host dynamics and evolution of pathogens in unvaccinated hosts. We then examine how antigrowth and antitransmission vaccines may affect within-host dynamics and evolution of the pathogen following introduction of the vaccine. We then analyze how the trade-offs between pathogen transmissibility, host recovery, and virulence can be estimated from the model and how these trade-offs change with vaccination. Finally, we discuss the implications of our results and compare them with the results obtained from other models. Mathematical derivations and some additional results are summarized in the Appendix available online only at http://dx.doi.org/10.1554/05-504.1.s1.

Evolution of Pathogens in Unvaccinated Hosts

The model of the dynamics and the evolution of pathogens causing acute infections in vertebrates has been described previously (Antia et al. 1994; Ganusov et al. 2002), and the reader is referred to these publications for more detail. In short, we assume that new infections are initiated by a small inoculum, P_0, and the pathogen population, P, expands exponentially with the rate r. The presence of the pathogen induces the growth controlling immune response, X_1, expanding clonally from the density X_{10} in a pathogen-dependent manner and killing the pathogen at the per capita rate h_1P. The equations describing the dynamics of the pathogen and the immune response are:

$$\frac{dP}{dt} = (r - h_1X_1)P \quad \text{and} \tag{2}$$

$$\frac{dX_1}{dt} = \frac{sX_1P}{k + P}, \tag{3}$$

where s is the maximum rate of proliferation of the immune response, and k is the pathogen density at which this proliferation rate is half-maximum.

We assume that the pathogen kills the host when it reaches the lethal density D and that there is no transmission from a dead host. Acute infections are, thus, defined as infections of a short duration that result in either host's death or host's recovery and long-lived immunological memory to reinfection.

We let the rate of pathogen transmission from infected hosts, ζ, be proportional to the within-host density of the pathogen, P, $\zeta[P(t)] = cP(t)$. Then the total transmission $l(r)$ of the pathogen with the growth rate r during acute infection of duration Δ is

$$l(r) = \int_0^{\Delta} \zeta[P(t)]\,dt = c\int_0^{\Delta} P(t)\,dt. \tag{4}$$

Thus, in this model pathogens evolve their within-host growth rate r to maximize total transmission from infected hosts.

During acute infections of vertebrates both pathogen and immune response densities expand several orders of magnitude (Vitetta et al. 1991; Murali-Krishna et al. 1998; Blattman et al. 2002). To generate experimentally observed dynamics of the pathogen and large expansion of the immune response during acute infections, the model parameters must satisfy the following inequalities (Antia et al. 1994):

$$P_0 \ll k \ll D \quad \text{and} \tag{5a}$$

$$h_1X_{10} \ll r, s. \tag{5b}$$

In Figure 1A and B we plot the dynamics of the infection and the total transmission $l(r)$ for pathogens with different growth rates. We find that slowly growing pathogens are cleared before they reach high density, and thus achieve relatively little total transmission. Pathogens with an intermediate growth rate, r^*, which allows them to reach a maximum density just short of the lethal density before being cleared by the immune response, are able to generate the maximum total transmission. As shown in the Appendix (available online only) the optimal growth rate r^* is approximately the solution of the following equation:

$$\left(\frac{D}{k}\right)^s = \left(\frac{r^*}{ehX_{10}}\right)^{r^*}, \tag{6}$$

where e is the base of natural logarithm. Faster growing pathogens, which reach the lethal density D, kill the host rapidly and this limits their total transmission. These results suggest that selection will favor pathogens with an intermediate growth rate $r = r^*$, which are at the threshold of killing the host.

The next step is to describe virulence of pathogen strains with different growth rates. The most general definition of virulence of a pathogen is the reduction in host's fitness due to infection (Schall 2002). In acute infections, which are by definition infections of a short duration, whether the host survives the infection or not, is the most informative measure of the reduction in host's fitness (i.e., host's reproductive success). Therefore, the most appropriate measure of the virulence of pathogens causing acute infections is the case mortality (a probability that a host dies following infection with the pathogen). For persistent infections, however, a more appropriate measure of virulence may be the pathogen-induced host mortality rate, because it takes into account differences in the duration of the infection among different infected hosts. Note that the latter definition is the most commonly used measure of virulence in theoretical literature (Frank 1996) and that some predictions on the evolution of pathogens may depend on the definition of virulence used (Day 2002; Ganusov et al. 2002).

Because in this simple model all infections are identical and either all hosts survive (if $r \le r^*$) or all hosts die (if $r > r^*$) following infection, pathogen virulence defined as case mortality cannot be described in a satisfactory manner. This problem can be resolved by introducing stochastic heterogeneity in the model parameters (Ganusov et al. 2002). As we expect similar results with heterogeneity in any parameter (Ganusov et al. 2002), we introduce heterogeneity in the growth rate of the pathogen. Then $f(r', r)dr'$ is the probability that during a current infection, the pathogen will have the growth rate in the range $(r', r' + dr')$, where r is the average

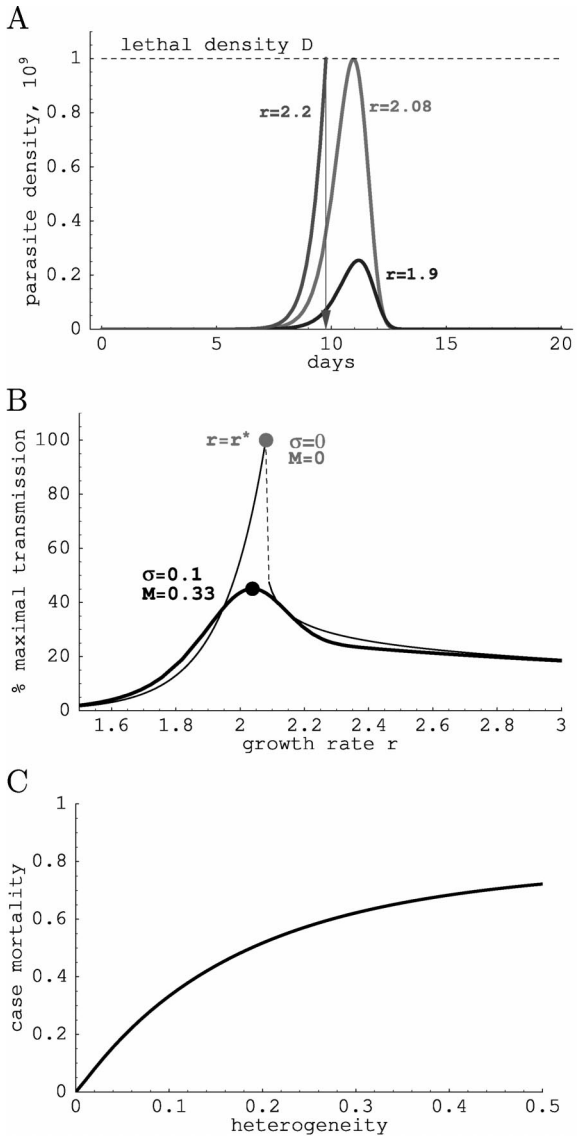

FIG. 1. Within-host dynamics (A), the total transmission (B) of pathogens with different growth rates and the evolutionarily stable pathogen virulence (C) in unvaccinated hosts. In (A) we show the dynamics of pathogens with different growth rates (growth rates are marked). An arrow at $r = 2.2$ denotes the time when a rapidly growing pathogen reaches the lethal density and kills the host. In (B) we show total transmission of pathogens with different growth rates in the absence (thin line, $\sigma = 0$) and presence (bold line, $\sigma = 0.1$) heterogeneity. Heterogeneity levels σ and corresponding case mortality M are marked. The total transmissions are normalized to the maximal value of transmission in the absence of heterogeneity. Dots denote maximal transmissions. Total transmissions in the presence of heterogeneity are calculated using an analytical approximation given in the Appendix (available online). Gamma distribution of the growth rate is used to describe heterogeneity. In (C) the standard deviation of the gamma distribution σ is on the x-axis. Parameters are: $P_0 = 1$, $X_{10} = 1$, $h_1 = 10^{-3}$, $s = 1$, $k = 10^3$, $D = 10^9$, $r^* = 2.08$. Rate parameters are given in day^{-1} units.

growth rate of the pathogen over all infections. Because different distributions for f are expected to give qualitatively similar results (Ganusov et al. 2002), we use a gamma distribution given by

$$f(r', r) = \frac{r/\sigma^2}{\Gamma(r^2/\sigma^2)}\left(\frac{r}{\sigma^2}r'\right)^{r^2/\sigma^2-1} \times \exp\left(-\frac{r}{\sigma^2}r'\right), \quad (7)$$

where $\Gamma(\cdot)$ is the Euler gamma function, r' is the current growth rate, r is the average growth rate, and σ is the standard deviation of the gamma distribution. Note that higher σ corresponds to higher heterogeneity levels. The average total transmission of the pathogen with the growth rate r in the presence of heterogeneity is then given by the integral

$$L(r) = \int_0^\infty l(r')\, f(r', r)\, dr'. \quad (8)$$

(In the Appendix, available online, we have derived an analytical approximation for average total transmission $L(r)$ used in our simulations.)

The evolutionary stable (ES) growth rate of the pathogen in the presence of heterogeneity is then found by maximizing the total transmission $L(r)$. All infections, during which pathogens replicate at a rate r' that is greater than the optimal r^* (given in eq. 6), will result in host's death. Therefore, for a pathogen with the average growth rate r the case mortality can be calculated as the fraction of infections resulting in pathogen replicating at the rate r'; greater than the critical rate r^*:

$$M(r) = \int_{r^*}^\infty f(r', r)\, dr'. \quad (9)$$

In Figure 1C we show that introducing heterogeneity in the pathogen's growth rate results in nonzero ES virulence of the pathogen. As has been shown before, we find this ES (or optimal) virulence increases with the increasing level of heterogeneity (Ganusov et al. 2002).

EVOLUTION OF PATHOGENS IN VACCINATED HOSTS

We now examine how vaccination may affect the optimal level of virulence of pathogens. As is shown in Figure 2, we consider two types of vaccines: vaccines reducing the rate of expansion of the pathogen population within the host (anti-growth vaccines) and vaccines reducing the rate of pathogen transmission from infected hosts (antitransmission vaccines). To describe the effects of vaccination with two different vaccines on the pathogen evolution, we assume that there are two immune responses controlling different stages of the pathogen's life cycle: replication (response X_1) and transmission (response X_2). Malaria is one example in which at least two immune responses specific to different pathogen stages can be generated (with merozoites representing a replicating stage and gametocytes representing a transmitting stage). Development of vaccines against both stages is an extensively explored area of current research (Dunachie and Hill 2003; Moingeon et al. 2003; Coban et al. 2004; Druilhe et al. 2005). In our analysis, for simplicity we consider only one pathogen stage (replicating and transmitting), and thus assume that expansion of both responses is driven by the same pathogen population. A more general model, involving two pathogen populations (replicating P_1 and terminally differentiated transmitting P_2) and two immune responses controlling replication of each pathogen population (X_1 and X_2,

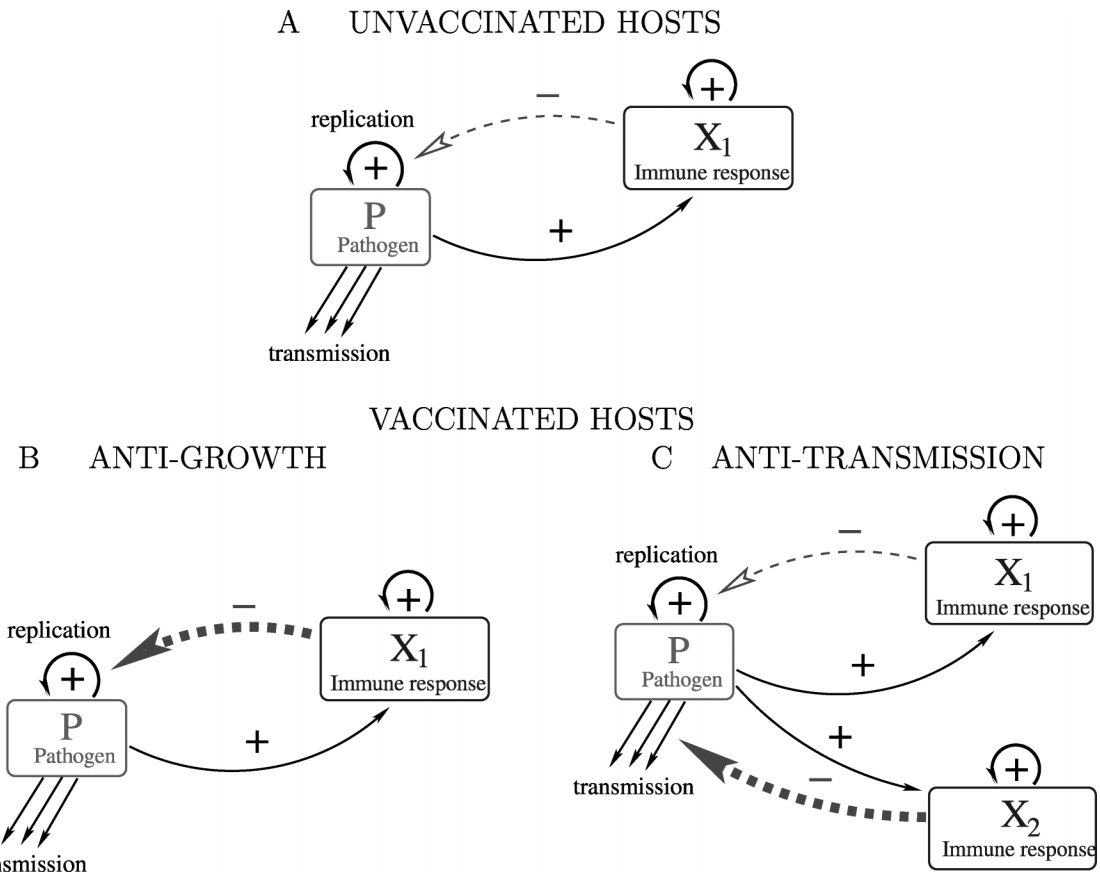

FIG. 2. Interactions between the pathogen and the host's immune responses in unvaccinated (A) and vaccinated (B, C) hosts. The antigrowth response X_1 reduces the rate of expansion of the pathogen population within the host (A, B), while the antitransmission response X_2 reduces the rate of pathogen transmission from infected hosts (C). To consider separately the effect of vaccination with two vaccines, we let only the X_1 response be present in unvaccinated or antigrowth vaccinated hosts. In antitransmission vaccinated hosts both immune responses are present. Arrows show positive (+) and negative (−) interactions with the line thickness denoting strength of the interaction.

respectively), gives qualitatively similar predictions as the simple model analyzed in the main text (see online Appendix for more detail).

The dynamics of the antigrowth response X_1 is given in equation (3); the dynamics of the antitransmission response X_2 is described similarly:

$$\frac{dX_2}{dt} = \frac{sX_2P}{k + P}, \tag{10}$$

where without losing generality we assumed identical parameters s and k for both immune responses. In the presence of the antitransmission immune response, the rate of pathogen transmission from infected hosts is reduced proportionally to the immune response density X_2; the total transmission of the pathogen over the course of acute infection of duration Δ then is

$$l(r) = \int_0^{\Delta} \zeta[P(t)]\, dt = \int_0^{\Delta} \frac{cP(t)}{1 + h_2X_2(t)}\, dt. \tag{11}$$

Experimental work suggests that vaccination generally results in a large increase in the number of immune cells specific to the pathogen and little or moderate changes in other parameters such as the rate of expansion of the immune response s or the activation parameters k (Vitetta et al. 1991; Flynn et al. 1999; VeigaFernandes et al. 2000; Grayson et al. 2002). In the following simulations, we therefore assume that vaccination leads to an increase in the precursor numbers X_{10} and X_{20} (i.e., the initial numbers of immune cells, which are specific to the pathogen). Because the dynamics of pathogen and its transmission are determined by the products h_1X_1 and h_2X_2, one can easily show that changes in the parameters h_1 and h_2 resulting after vaccination are equivalent to changes in the precursor numbers (results not shown). We have also found that changes in other parameters (such as increases in the expansion rate s or reduction in the activation threshold k resulting after vaccination) lead to qualitatively similar predictions as changes in the precursor numbers (results not shown).

Finally, to consider separately the effects of antigrowth and antitransmission vaccines on the evolution of pathogens, we let only the response X_1 be present in unvaccinated and antigrowth vaccinated hosts. In antitransmission vaccinated hosts, both immune responses X_1 and X_2 are present, but only the response X_2 has an increased precursor number X_{20} (Fig. 2). It is necessary to emphasize that we cannot exclude the X_1 response in antitransmission vaccinated hosts (by letting

$X_{10} = 0$), because in that case no immune response will control the pathogen growth and depending on the parameters either the host's death will be always assured or the infection will cease to be an acute infection of a short duration.

It is important to consider the changes in the precursor numbers of antigrowth (X_{10}) and antitransmission (X_{20}) immune responses occurring following vaccination. After immunization of mice with live or inactivated pathogens, the precursor numbers of, for example, CD4 and CD8 T cell responses, often increase 10^2–10^4 fold (Doherty and Christensen 2000; Homann et al. 2001; Blattman et al. 2002). However, for some infections such as influenza virus in mice, even such large increases in the precursor numbers do not lead to complete blocking of pathogen replication following re-exposure (Christensen et al. 2000; Doherty and Christensen 2000). In the simple models considered in this paper, even a moderate increase in the precursor number of the antigrowth response X_1 dramatically reduces the mortality due to the infection and the total pathogen transmission (shown in Fig. 5 by thin long-dashed lines). In these models, an effective vaccine that completely blocks pathogen replication must generate the precursor number $X_{10} > r/h_1$ at which the net initial rate of expansion of the pathogen population, $r - h_1X_{10}$, is negative. Therefore, for such models, to consider imperfect vaccines that only moderately reduce the transmission and virulence of pathogens, one should choose relatively small increases in the precursor number X_{10} resulting from vaccination. Imperfect vaccination would also correspond to a case when large increases of the precursor number X_{10} are compensated by a proportional decrease of the killing efficacy h_1 such that the product h_1X_{10} increases only moderately. To investigate the role of antigrowth vaccine efficacy on pathogen evolution, we therefore consider two levels of vaccine efficacy: low efficacy (when vaccination leads to only two-fold increase in the precursor number, from $X_{10} = 1$ to 2, i.e., imperfect vaccine) and high efficacy (when vaccination leads to 10-fold increase in the precursor number, from $X_{10} = 1$ to 10, i.e., a relatively perfect vaccine).

In contrast, increasing the precursor number of antitransmission response X_2 leads to a less dramatic reduction in the total transmission of pathogens while virulence is unaffected by such changes (shown in Fig. 5 by thin short-dashed lines). Therefore, even large increases in the precursor number X_{20} may still correspond to imperfect vaccination. In the paper, we thus consider the effects of low-efficacy antitransmission vaccines leading to generation of $X_{20} = 10$ precursors, on the pathogen evolution.

Antigrowth Vaccines

We first consider consequences of the use of antigrowth vaccines on the evolution of pathogens when all hosts in the population are vaccinated. In Figure 3A and B we plot the dynamics of the pathogen and the antigrowth immune response in unvaccinated and vaccinated hosts when the hosts are infected with the pathogen optimal in unvaccinated hosts in the absence of heterogeneity (i.e., with $r = r^*$). We find that in unvaccinated hosts the pathogen reaches high densities and has high total transmission (set to be 100%). Infection of antigrowth vaccinated hosts (with a higher precursor number $X_{10} = 2$) with the same pathogen results in a shorter infection, lower densities of the pathogen, and consequently, lower total transmission ($l \approx 24\%$). A similar decrease in total transmission is observed following infection of vaccinated hosts with the pathogen optimal in unvaccinated hosts in the presence of heterogeneity (see Figs. 1B and 4A). Increasing the efficacy of vaccination (by increasing the precursor number X_{10} in vaccinated hosts) results in further decline in pathogen's total transmission (shown by a thin long-dashed line in Fig. 5A). Importantly, vaccinated hosts also suffer lower mortality after infection with the pathogen optimal in unvaccinated hosts, especially at high vaccine efficacy (shown by a thin long-dashed line in Fig. 5C).

Because in antigrowth vaccinated hosts the immune response develops faster, the ES pathogen growth rate in vaccinated hosts is higher than the ES growth rate in unvaccinated hosts (Fig. 4A). In the absence of heterogeneity, the ES growth rate in vaccinated hosts can be calculated using equation (6) with the precursor number X_{10} generated after vaccination. In the presence of heterogeneity, the optimal growth rate of the pathogen is found by maximizing the pathogen's total transmission in vaccinated hosts (calculated using eq. 8). We find that the ES pathogen growth rate in vaccinated hosts monotonically increases with the increasing efficacy of vaccination (shown in Fig. 5B when $\sigma = 0.1$).

In Figure 4C we plot the changes in ES virulence of the pathogen in unvaccinated and vaccinated hosts the increasing heterogeneity level. Surprisingly, we find that the ES virulence in vaccinated hosts is slightly lower than the ES virulence in unvaccinated hosts. Furthermore, the ES virulence in vaccinated hosts decreases with increasing vaccine efficacy, although this decrease is relatively small (Fig. 5C). The pathogen adapted to vaccinated hosts, nevertheless, causes high mortality in unvaccinated hosts because of the increased ES growth rate.

We also find that the ES transmission of the pathogen in vaccinated hosts is lower than the ES transmission in unvaccinated hosts (cf. the dot and a bold long-dashed line in Fig. 5A). This mainly occurs because, while in both cases pathogens reach similar densities, infections are more rapid (and thus shorter) in vaccinated hosts. In summary, vaccination with antigrowth vaccines leads to selection of pathogen strains with higher ES growth rate, lower ES transmission, and lower ES virulence. Infection of unvaccinated hosts with such evolved pathogens results in high host mortality and in low pathogen transmission.

We now consider evolution of pathogens in a population when only a fraction of hosts $p = S_v/(S_v + S_u)$ is vaccinated (where S_v and S_u are the numbers of vaccinated and unvaccinated hosts, respectively). For the case when recovery from the infection leads to lifelong immunity (which is the case for most known acute infections), previous work has suggested that the total transmission of the pathogen in a partially vaccinated host population is simply a sum of pathogen transmission from vaccinated and unvaccinated hosts (Gandon et al. 2003; André and Gandon 2006). Then the average total transmission of a pathogen with the growth rate r infecting a host population where a fraction p of hosts is vaccinated is simply:

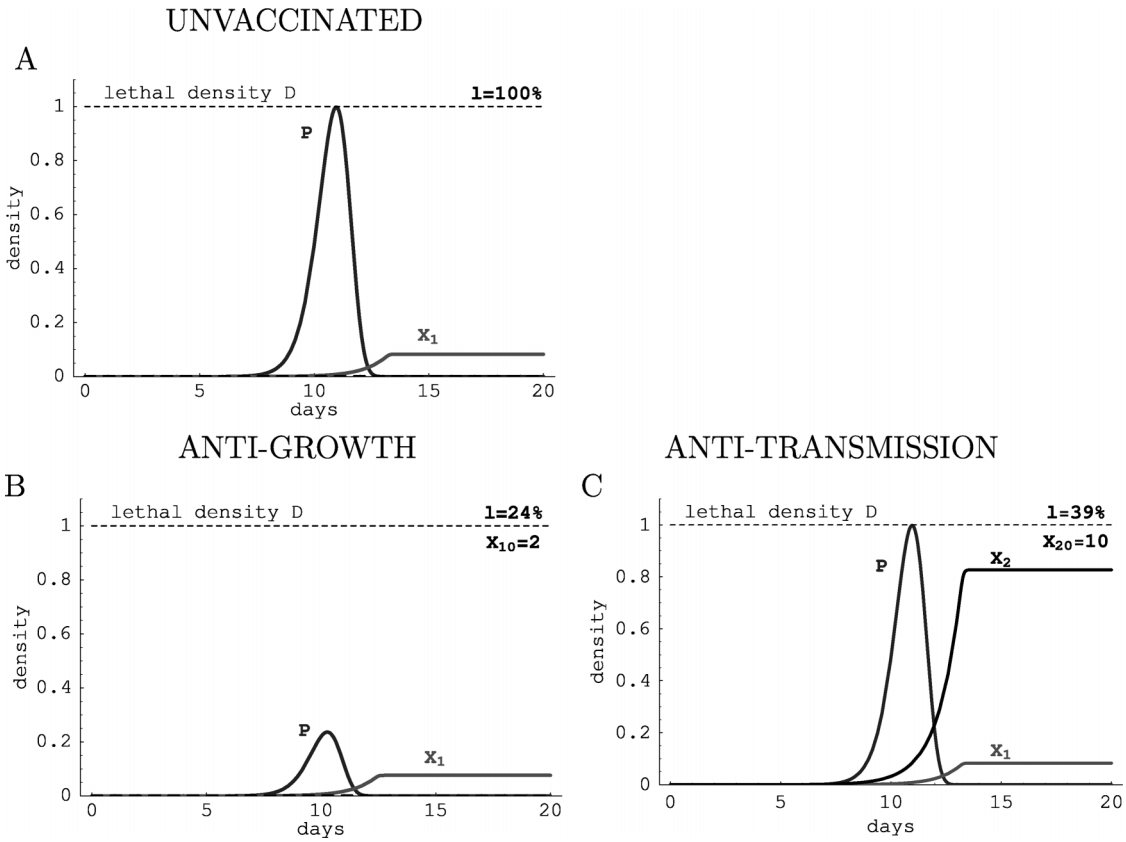

FIG. 3. Within-host dynamics of the pathogen and the host's immune responses in unvaccinated hosts (A; $X_{10} = 1$ and $X_{20} = 0$), in antigrowth vaccinated hosts (B; $X_{10} = 2$ and $X_{20} = 0$) and antitransmission vaccinated hosts (C; $X_{10} = 1$ and $X_{20} = 10$). Parameters are the same as in Figure 1, and $r = r^* = 2.08$ and $h_2 = 10^{-4}$. In all panels, the pathogen population is normalized to the lethal density D; density of both immune responses are multiplied by a factor 4×10^{-6} to fit on the same plot. The total transmission $l(r)$ was calculated using equation (11) and normalized to the maximal total transmission in (A). Rate parameters are given in day^{-1} units.

$$\bar{L}(r) = pL_v(r) + (1 - p)L_u(r), \tag{12}$$

where L_v and L_u is the total transmission of the pathogen from vaccinated and unvaccinated hosts, respectively. The average case mortality caused by the pathogen with the growth rate r is calculated in a similar way:

$$\bar{M}(r) = pM_v(r) + (1 - p)M_u(r). \tag{13}$$

In Figure 6 we plot how the total transmission of pathogens with different growth rates changes with the increasing fraction of vaccinated hosts p for low antigrowth vaccine efficacy. Changes in total transmission for high vaccine efficacy are shown in the Appendix (available online).

We find that when the fraction of vaccinated hosts p is small, the pathogen adapts to unvaccinated hosts by evolving a low growth rate which is the ES growth rate in these unvaccinated hosts (Figs. 6, 7). In this case, the pathogen obtains the maximum transmission from the most abundant host type. When the fraction of vaccinated hosts becomes intermediate, the pathogen evolves a higher growth rate yet it obtains a lower total transmission. In this case, the pathogen cannot optimize simultaneously to both host types (unvaccinated and vaccinated) and thus loses in transmission. As most hosts become vaccinated, the pathogen adapts to vaccinated hosts by evolving high growth rate that is optimal in vaccinated hosts (Figs. 6, 7).

Surprisingly, changes in the average ES virulence do not follow the changes in the ES growth rate (Figs. 7B, C). At first, the average case mortality of the optimal pathogen decreases. This occurs because with increasing p, more hosts become resistant to the infection while the ES growth rate of the pathogen changes only little (Fig. 7B). This initial decrease in average virulence is relatively small at low vaccine efficacy but becomes larger at high efficacy. At an intermediate fraction of vaccinated hosts, the ES virulence reaches its maximum. This occurs because the pathogen adapts to the resistant, vaccinated population and thus causes high mortality in unvaccinated hosts. When almost all hosts are vaccinated, the ES virulence again becomes relatively small because of the lower ES pathogen virulence in vaccinated hosts (see Figs. 4C, 5C).

Changes in the ES growth rate, transmission and virulence occurring with an increasing fraction of vaccinated hosts remain qualitatively similar at higher vaccine efficacy (Fig. 7). Importantly, however, at high vaccine efficacy, the total transmission may have two maxima, and depending on the fraction of vaccinated hosts, vaccine efficacy and degree of host heterogeneity, pathogens with high or low growth rate will obtain maximum transmission. The sharp increase in the ES growth rate and ES virulence shown in Figure 7 (by thin long-dashed lines) corresponds to such a case when a small

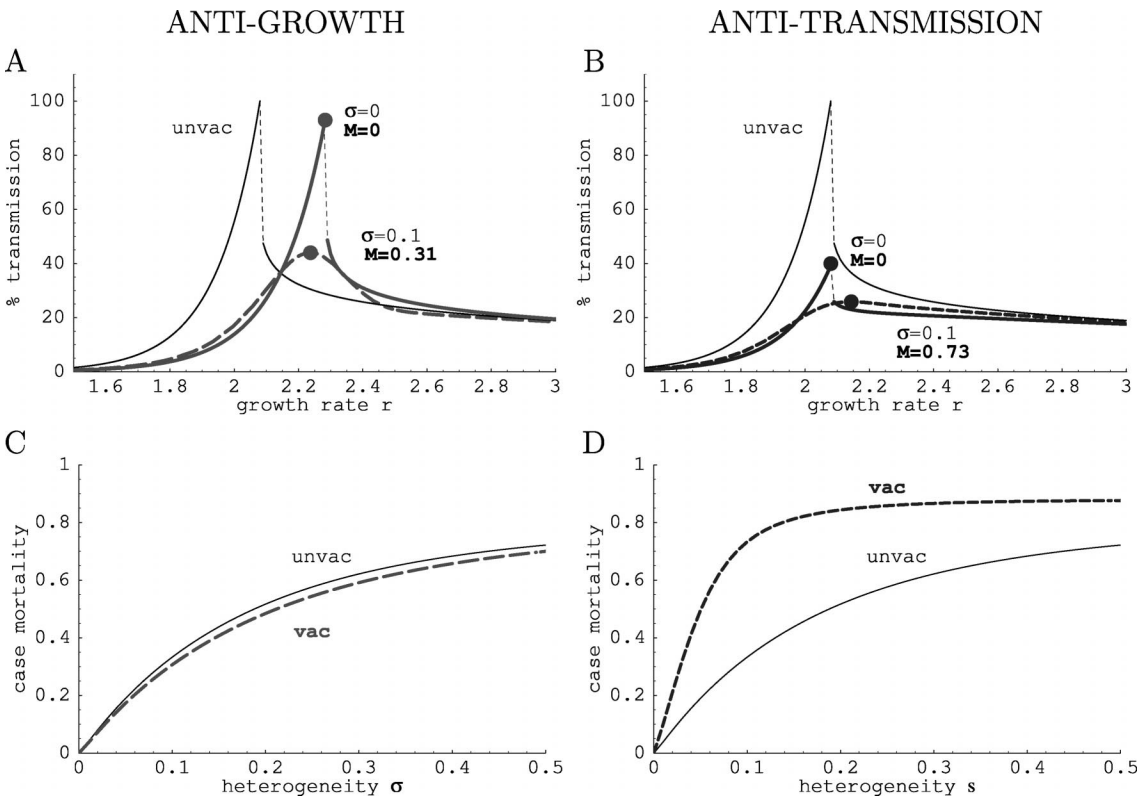

FIG. 4. Total transmission and virulence of pathogens evolved in unvaccinated, antigrowth (A, C) and antitransmission (B, D) vaccinated hosts. In (A, B) we plot the total transmission of pathogens as the function of the pathogen's growth rate r in the absence ($\sigma = 0$, solid lines) and presence ($\sigma = 0.1$, dashed lines) of heterogeneity. Dots denote the evolutionarily stable (ES) transmission and growth rate of the pathogen in vaccinated hosts. All total transmission are normalized to the ES pathogen transmission in unvaccinated hosts in the absence of heterogeneity. Changes in the total transmission of pathogens in unvaccinated hosts with the growth rate r are shown by solid thin lines. The heterogeneity level σ and the corresponding case mortality M are marked. In (C, D) we plot the ES pathogen virulence in unvaccinated (solid lines) and vaccinated (dashed lines) hosts as the function of the heterogeneity level. Parameters are the same as in Figures 1 and 3. In (C, D), the standard deviation of the gamma distribution σ is on the x-axis. Rate parameters are given in day^{-1} units.

change in the fraction of vaccinated hosts leads to pathogen switch from one maximum to another (for a more general discussion on pathogen evolution for the cases when pathogen transmission has two or more local maxima, see Gandon et al. 2003).

In summary, we find that: (1) antigrowth vaccination leads to selection of more rapidly growing pathogens that are more virulent in unvaccinated and less virulent in vaccinated hosts; (2) such evolved pathogens obtain lower total transmission in both vaccinated and unvaccinated hosts; and (3) vaccination of a fraction of the host population may lead to a dramatic increase in the average ES virulence at intermediate fractions of vaccinated hosts (i.e., coverage level), while it may also lead to a reduction of the average ES virulence at low and high vaccine coverage, especially when the vaccine efficacy is relatively high.

Antitransmission Vaccines

As above, we first consider consequences of the use of antitransmission vaccines on the evolution of pathogen when all hosts in the population are vaccinated. Vaccination with antitransmission vaccines leads to a generation of the second immune response, X_2, expanding from X_{20} precursors in a pathogen-dependent manner and inhibiting pathogen transmission (see Fig. 2C and eqs. 10, 11). In Figure 3C we plot the dynamics of the pathogen optimal in unvaccinated hosts in the absence of heterogeneity (with $r = r^*$) infecting antitransmission vaccinated hosts. We find that this dynamics is unchanged but the total pathogen transmission is reduced ($l \approx 39\%$). At these parameter values, the ES growth rate is the same in unvaccinated and vaccinated hosts, and the optimal pathogen is at the edge of killing the host (Fig. 4B, bold solid line at $\sigma = 0$). Importantly, however, pathogens with slightly higher than the optimal r^* growth rate suffer much smaller reduction in total transmission due to killing of the host than the pathogens killing unvaccinated hosts (cf. the drop in transmission for vaccinated and unvaccinated hosts at $r = r^*$ shown by vertical dashed lines in Fig. 4B). This difference in loss in transmission of rapidly replicating pathogens between unvaccinated and vaccinated hosts arises because the killing of the antitransmission vaccinated host early has an advantage for the pathogen. Killing the host very early prevents the development of antitransmission immune response, and this may augement transmission of rapidly growing pathogens in comparison with slowly growing pathogens. In fact, if antitransmission vaccination result in much

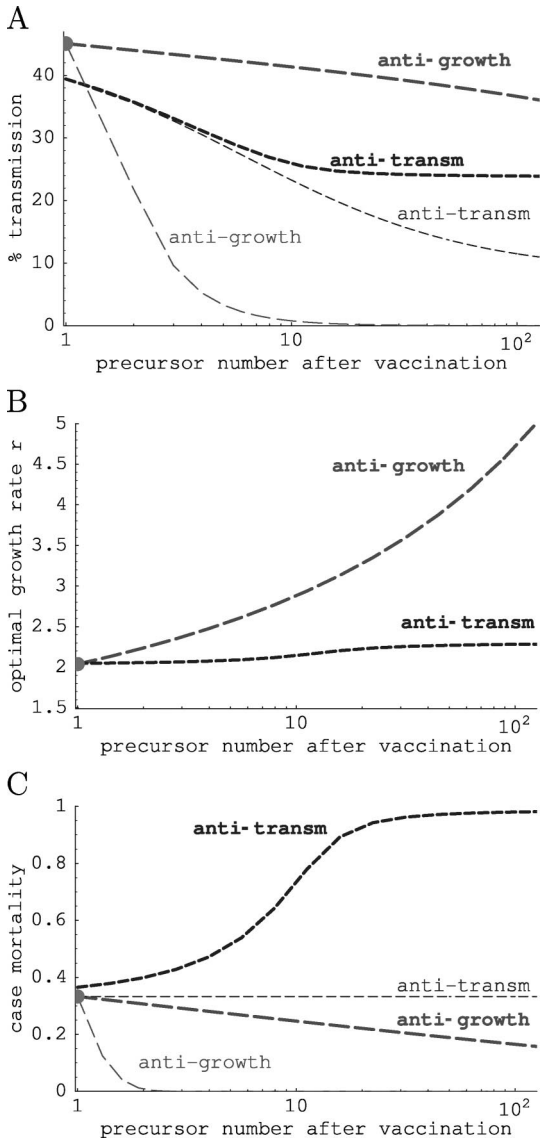

FIG. 5. Maximal total transmission (A), the evolutionarily stable (ES) growth rate (B) and virulence (C) of pathogens infecting antigrowth (long-dashed lines) or antitransmission (short-dashed lines) vaccinated hosts. Thin lines correspond to the case when pathogens optimal in unvaccinated hosts (with the ES growth rate $r \approx 2.038$) infect vaccinated hosts (i.e., before pathogen evolution in vaccinated hosts). Bold lines correspond to the case when pathogens optimal in vaccinated hosts (with the ES growth rate r given in B) infect vaccinated hosts (i.e., after pathogen evolution in vaccinated hosts). Dots in all panels correspond to ES characteristics of pathogens in unvaccinated hosts. In antigrowth vaccinated hosts there is no antitransmission immune response (i.e., $X_{20} = 0$), and the precursor number X_{10} generated following vaccination is on the x-axis. In antitransmission vaccinated hosts the antigrowth immune response is unchanged (i.e., $X_{10} = 1$), and the precursor number X_{20} generated following vaccination is on the x-axis. Total transmissions in (A) are normalized to the ES pathogen transmission in unvaccinated hosts in the absence of heterogeneity. Parameters are the same as in Figures 1 and 3 and $\sigma = 0.1$.

higher precursor numbers X_{20}, rapidly replicating pathogens, which kill the host early, may obtain higher transmission than the pathogens that are at the threshold of killing the host (results not shown).

Introducing heterogeneity in the pathogen's growth rate allows us to calculate the pathogen's total transmission and ES pathogen virulence. We find that infection of antitransmission vaccinated hosts with the pathogen optimal in unvaccinated hosts leads to a reduced pathogen transmission but, as expected, has no effects on host's survival (Figs. 5A, C). Surprisingly, in the presence of heterogeneity the ES pathogen growth rate is higher in antitransmission vaccinated hosts than that in unvaccinated hosts (Figs. 4B, 5B). This is the direct consequence of the smaller decline in total transmission for rapidly growing pathogens infecting vaccinated hosts (see above). The ES growth rate increases with increasing vaccine efficacy (measured by the precursor number X_{20} generated after vaccination), although this increase is much smaller than the increase in the ES pathogen growth rate in antigrowth vaccinated hosts (Fig. 5B). The ES pathogen virulence becomes higher than that in unvaccinated hosts (Figs. 4D, 5C). Because the antitransmission vaccine does not protect vaccinated hosts from pathogen-induced disease, even small changes in the ES pathogen growth rate will lead to large changes in host mortality. Increasing the vaccine efficacy leads to a further increase in the ES pathogen virulence (Fig. 5C). Infections of unvaccinated hosts with the pathogens adapted to vaccinated hosts will therefore result in high host mortality (in fact, similar to that in vaccinated hosts) and low pathogen transmission (due to early killing of the host).

We now consider the evolution of pathogens in a population where only a fraction of hosts is vaccinated. Changes in the total transmission of pathogens with different growth rates with the increasing fraction of vaccinated hosts p is shown in Figure 6. We find that there is a monotonic decrease in the ES transmission, increase in the ES growth rate and ES virulence as the fraction of vaccinated hosts increases (Fig. 7).

In summary, we find that: (1) antitransmission vaccination leads to selection of more rapidly growing pathogens that are more virulent in unvaccinated and vaccinated hosts; (2) such pathogens obtain lower total transmission in both vaccinated and unvaccinated hosts; and (3) the ES virulence increases with the increasing fraction of vaccinated hosts.

Trade-offs between Transmissibility, Host Recovery, and Virulence

As we mentioned in the introduction, previous work relied on the assumption that the trade-offs between pathogen characteristics such as transmissibility β, the rate of host recovery ν, and virulence α do not change with vaccination (Gandon et al. 2001). In the Appendix (available online) we demonstrate how these parameters for the epidemiological spread of the pathogen-induced disease can be estimated using models of the within-host dynamics of pathogen and the immune response. In Figure 8 we plot the trade-offs $\beta = \beta(\alpha)$, $\nu = \nu(\alpha)$, and $R_0 = R_0(\alpha)$ in unvaccinated and vaccinated hosts.

We find that, in contrast with previous suggestions for

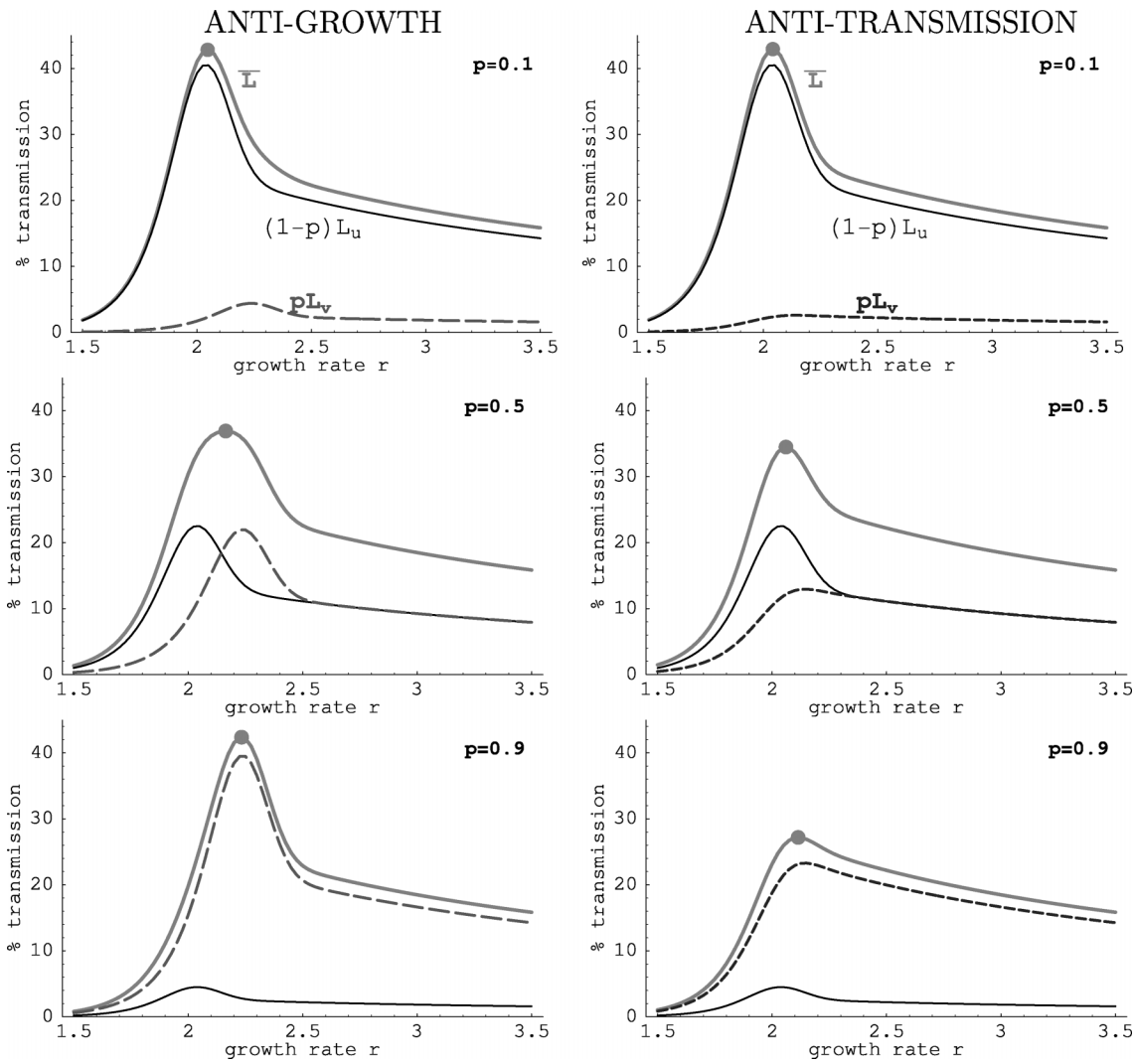

FIG. 6. Changes in the total transmission of pathogens with different growth rates occurring with the increasing fraction of vaccinated hosts p. Thin continuous lines denote the total transmission of pathogens from unvaccinated hosts, $(1 - p)L_u(r)$. Dashed lines denote the total transmission of pathogens from antigrowth (left panels) and antitransmission (right panels) vaccinated hosts, $pL_v(r)$. Bold continuous lines denote the total transmission of pathogens from the whole population $L(r) = pL_v(r) + (1 - p)L_u(r)$. Dots denote the maximum or evolutionarily stable total transmission. All total transmissions are normalized to the evolutionarily stable pathogen transmission in unvaccinated hosts in the absence of heterogeneity. In antigrowth vaccinated hosts, low vaccine efficacy is considered. The fraction of vaccinated hosts p is marked. Parameters are the same as in Figures 1 and 3 and $\sigma = 0.1$.

chronic infections (Gandon et al. 2001), for acute infections these trade-offs do change with vaccination. The changes, however, are relatively small at low levels of antigrowth vaccine efficacy (i.e., at small increases in the precursor number X_{10} following vaccination), but they may become quite large at high efficacy (Fig. 8). Both the pathogen's transmission rate and host recovery rate become higher after antigrowth vaccination for a given level of host mortality rate (Fig. 8A, C). These changes, however, cancel each other such as the ES host mortality rate α^* at which R_0 is maximal, is only slightly increased (Fig. 8E). Pathogen transmissibility becomes lower at low host mortality rates in antitransmission vaccinated hosts, while no changes in the recovery rate occur with vaccination (Fig. 8B, D). These changes result in a higher host mortality rate α^* at which R_0 is maximal (Fig. 8F).

DISCUSSION

While many vaccines can reduce the prevalence of the infection and disease in vaccinated populations, inefficient, imperfect vaccines may also affect the evolution of pathogens such as to increase or decrease their optimal virulence (Gandon et al. 2001). It has been suggested that antigrowth or antivirulence vaccines, by removing the cost of virulence, should select for pathogens with higher virulence (as measured in unvaccinated hosts). Moreover, vaccines, which block transmission of pathogens from infected hosts or infection of new hosts by reducing the strength of the intrahost competition between unrelated pathogen strains, should select for pathogens with lower virulence (Gandon et al. 2001, 2003). While these results point out potential problems that imperfect vaccines may elicit, the approach of Gandon et al.

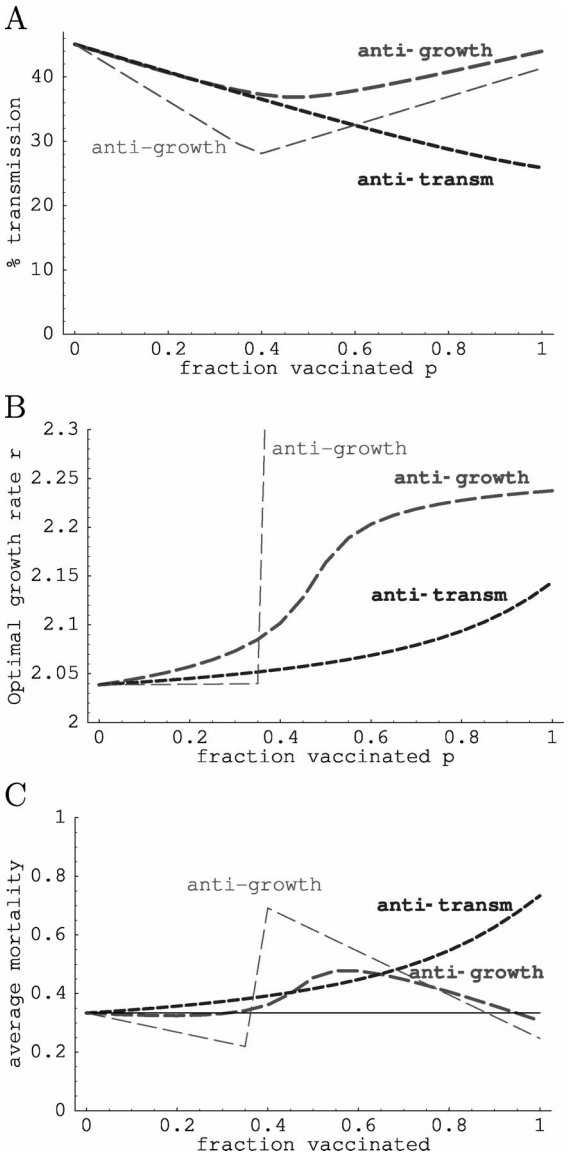

FIG. 7. The maximal average total transmission (A), the evolutionarily stable growth rate (B), and average virulence (C) of pathogens evolved in a partially vaccinated host population as a function of the fraction p of antigrowth (long-dashed lines) and antitransmission (short-dashed lines) vaccinated hosts. In antigrowth vaccinated hosts, two vaccine efficacy levels are considered: low efficacy (two-fold increase in the precursor number X_{10} following vaccination, shown by bold long-dashed lines) and high efficacy (10-fold increase in the precursor number X_{10} following vaccination, shown by thin long-dashed lines). In antitransmission vaccinated hosts the precursor number for the antitransmission immune response is increased to $X_{20} = 10$. A thin horizontal line in (C) denotes the evolutionary stable pathogen virulence in unvaccinated hosts. Other parameters are the same as in Figures 1 and 3 with $\sigma = 0.1$.

(2001) as well as the predictions have been criticized as being too general and inconsistent with some experimental observations (Smith 2002; Soubeyrand and Plotkin 2002; André et al. 2003; Ebert and Bull 2003). For example, the prediction that optimal virulence should increase during the use of an antivirulence (or antitoxin) vaccine is inconsistent the decline in prevalence of the toxin-producing *Corynebacterium diphtheriae* and *Bordetella pertussis* observed in countries with long and efficient diphtheria and pertussis antitoxoid vaccination programs (Schneerson et al. 1996; Taranger et al. 2001). Modifying the model by including the cost associated with the production of toxins has led to changed predictions suggesting that at high-efficacy antitoxin vaccination may select for less virulent pathogens (Gandon et al. 2002). This demonstrates that particular details of the within-host dynamics of pathogens may have dramatic qualitative effects on pathogen evolution.

In this paper, we have extended the study of Gandon et al. (2001) in a more specific way. We analyzed how imperfect vaccines may affect the optimal level of virulence to which pathogens, causing acute infections in vertebrate hosts, are expected to evolve. By focusing on acute infections we restricted our analysis to well-defined models of the within-host dynamics of pathogens controlled by the immune response(s); used biologically plausible scenarios of how vaccines may affect the strength of immune responses to pathogens; and, given the short duration of such infections, could to a first approximation neglect the intrahost competition between pathogen strains proven to affect the evolution of pathogens in different ways (Frank 1996; Brown et al. 2002).

Using this within-host approach we found the following. Antigrowth vaccines, by increasing the rate at which the immune response clears the pathogen, lead to selection of pathogens with an increased within-host growth rate. This result is a simple consequence of the arm race between the pathogen and immune response: stronger immune response drives the evolution of more rapidly replicating pathogens. Clearly, such rapidly replicating pathogens are highly virulent in unvaccinated hosts, as has been noted previously (Gandon et al. 2001), but by killing unvaccinated hosts early, they suffer a dramatic loss in total transmission. In fact, because of such a reduction in total transmission (i.e., in R_0), pathogens that are adapted to vaccinated hosts may not be able to spread in unvaccinated hosts. In addition, the ES pathogen virulence (measured by the case mortality) is lower in vaccinated hosts than the ES virulence in unvaccinated hosts.

We also found that if only a small fraction of the host population is vaccinated, then even imperfect antigrowth vaccines may be beneficial because, by adapting to unvaccinated hosts, pathogens evolve low growth rate and thus cause little harm in vaccinated hosts. This effect, however, may be quite small at low vaccine efficacy (Fig. 7C). At intermediate fractions of vaccinated hosts, antigrowth vaccines may be quite detrimental as the pathogen adapts to vaccinated hosts by evolving a high growth rate and causing high mortality in unvaccinated and intermediate mortality in vaccinated hosts.

Importantly, these predictions on the evolution of pathogens are likely to depend on the efficacy of the vaccine, that is, the increase in the precursor number resulting after vaccination. Increasing the precursor number of antigrowth immune response 10^2-fold or more dramatically reduces transmission of pathogens adapted to unvaccinated hosts following infection of vaccinated hosts (shown in Fig. 5A by a thin long-dashed line). This implies that at high enough efficacy, the vaccine may prevent pathogen transmission from vaccinated hosts and thus will not allow for pathogen evolution. Moreover, at high antigrowth vaccine efficacy, to obtain high

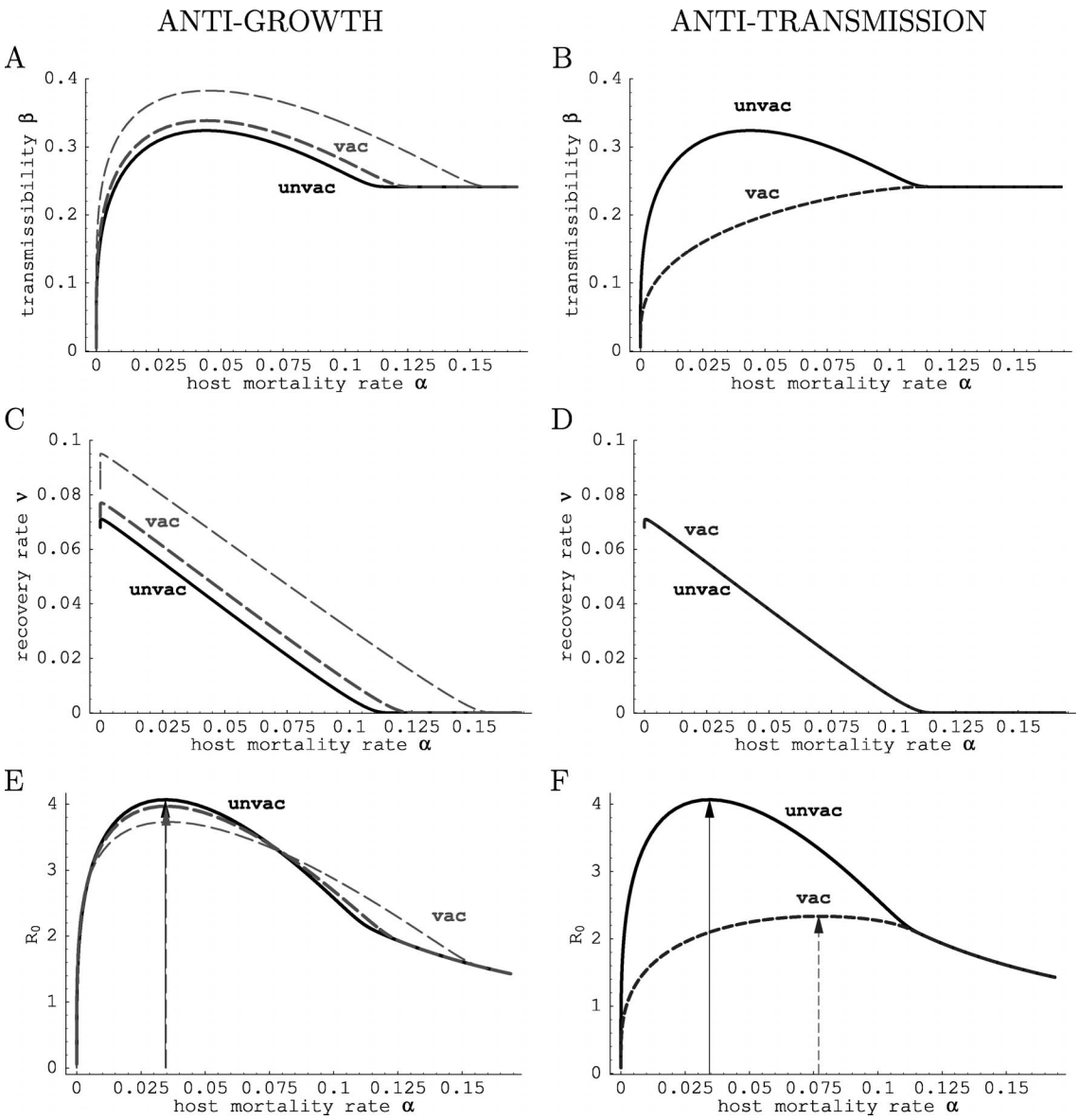

FIG. 8. Trade-offs between pathogen transmissibility β (A, B), host recovery rate ν (C, D), the basic reproductive number R_0 (E, F) and host mortality rate α in antigrowth (A, C, E) and antitransmission (B, D, F) vaccinated hosts. Trade-offs for pathogens infecting unvaccinated hosts are shown by solid lines. In antigrowth vaccinated hosts, two vaccine efficacies are considered: low efficacy (shown by thin long-dashed lines) and high efficacy (shown by bold long-dashed lines). Arrows denote the host mortality rate α* at which R_0 reaches its maximum in unvaccinated, antigrowth (with low vaccine efficacy) and antitransmission vaccinated hosts. Parameters are the same as in Figures 1 and 3 with $\sigma = 0.1$ and $u = 5 \times 10^{-9}$.

transmission the pathogens must evolve relatively high growth rate (shown in Fig. 5B by a bold long-dashed line). For many pathogens this may be impossible because there are physiological constrains on how rapidly pathogens can replicate.

Previous models have suggested that transmissionblocking vaccines by reducing the degree of the intrahost competition should select for pathogens with lower virulence (Gandon et al. 2001). Because in our model we do not consider the intrahost competition, there should be no effect of antitransmission vaccines on the optimal level of pathogen virulence. This is indeed the case if one considers a time-independent immune response that reduces pathogen's transmission rate (i.e., when $X_2[t]$ = constant). However, for an acute infection, a more biologically plausible scenario is when the immune response is time and pathogen dependent and is stronger in vaccinated hosts. In this case, pathogens that allow the antitransmission immune response to develop (by not killing the host) may suffer great losses in total transmission when compared with pathogens that kill the host early. This result demonstrates that timing of transmission may have important influence of pathogen evolution (see a more general discussion on this topic in Day 2003). Our analysis suggests that for acute infections transmission-blocking vaccines are also likely to select for more rapidly replicating pathogens, causing higher mortality in both vaccinated and unvaccinated

hosts. Vaccinating a fraction of hosts is detrimental and will lead to evolution of pathogens with high ES growth rate and high average virulence (Fig. 7C).

Are there any benefits of using a transmission-blocking vaccine? Clearly, a vaccine that only blocks transmission of the pathogen from infected hosts will not protect infected individuals from the pathogen-caused disease. However, because the total transmission of pathogens will be reduced in vaccinated hosts, there will be a lower probability for an uninfected host to become infected. Thus, at high enough efficacy, transmission-blocking vaccines may lead to pathogen extinction; however, if this extinction is not achieved, pathogens are expected to evolve to high virulence in both vaccinated and unvaccinated hosts.

In contrast with previous assumptions (Gandon et al. 2001), we have also found that vaccination generally changes the trade-offs between the parameters determining the epidemiological spread of the pathogen in the host population.

Interestingly, in a recent study no significant differences in the shape of these trade-offs after vaccination have been observed for *Plasmodium chabaudi* infection of laboratory mice (Mackinnon and Read 2003). Although the reasons of such discrepancy are not known, a better understanding of acquired immunity in malaria infection of mice and its changes with vaccination and the vaccine efficacy may be of particular importance.

Some of our results are in parallel with the work by André and colleagues, which showed that antigrowth vaccines may select for pathogens with higher virulence as measured by the host mortality rate α (André et al. 2003; André and Gandon 2006). In contrast with our lethal density model, the authors assumed stochastic host's survival during the infection dependent on the pathogen density (Sasaki and Iwasa 1991). While having stochastic host survival does not change our conclusions qualitatively (results not shown), it appears that for a range of biologically plausible parameters of such a model, selection will favor pathogens that are unrealistically virulent in their hosts as measured by the case mortality (e.g., in fig. 1b in André et al. [2003], the optimal pathogen has the case mortality above 0.95). Because for acute infections case mortality is a more adequate measure of virulence (than LD_{50} or host mortality rate α), we believe that the results of André et al. (2003) may be of a limited applicability. In particular, a later work of André and Gandon (2006) suggested that antigrowth vaccination reduces the average pathogen virulence (measured by the case mortality) for an intermediate fraction of vaccinated hosts p; this is in contrast with our conclusions. We have found that this result arises in part due to unreasonably high ES pathogen virulence in unvaccinated hosts. Modifying this model such as to include saturation in the pathogen transmission rate with pathogen density that allowed for low ES virulence in unvaccinated hosts leads to qualitatively different predictions, consistent with our results (results not shown). These differences in predictions of relatively simple models further demonstrate the importance of particular details in predicting pathogen evolution (Ganusov and Antia 2003).

Imperfect vaccines are defined as the ones resulting in a moderate decrease in the pathogen transmission and host mortality following infection of vaccinated hosts with the pathogens optimal in unvaccinated hosts. An important limitation of the considered mathematical models is that, for vaccines to be imperfect, changes in the precursor numbers of antigrowth and antitransmission immune responses resulting after vaccination must be relatively small. Larger increases in the precursor number of antigrowth immune response (10^2–10^4 fold as is observed for some infections of mice) will result in our model in almost complete blocking of pathogen replication, transmission, and host mortality. This is in contrast with some experimental observations. For example, vaccination of mice with one strain of influenza results in a large increase of the precursor numbers (Doherty and Christensen 2000), and yet infection of vaccinated mice with another strain of the virus does not prevent virus replication and transmission (Schulman 1970; Christensen et al. 2000). Developing more complex models that may account for these differences is required for a better understanding of the within-host dynamics and evolution of pathogens in response to vaccination.

While our approach takes into account some of the properties of the within-host dynamics of pathogens and immune responses during acute infections, many other details may also be important in predicting evolution of pathogens (Soubeyrand and Plotkin 2002). For example, we assume that pathogens evolve only by changing their within-host replication rate. Clearly, this is a simplification, and many pathogens may change their transmission and virulence by a variety of other means, while the within-host growth rate may remain unchanged. Taking into account different functional forms for the rate of pathogen transmission from infected hosts or/and the mechanisms of the pathogen-induced pathogenesis may also affect the optimal level of virulence to which pathogens are expected to evolve (Ganusov and Antia 2003). Our results, however, do emphasize that particular mechanisms of action of vaccines as well as their efficacy are crucial in predicting the long-term consequences of the use of imperfect vaccines in the evolution of pathogens.

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Literature Cited

Anderson, R. M., and R. M. May. 1982. Coevolution of hosts and parasites. Parasitology 85:411–426.

———. 1991. Infectious diseases in humans: dynamics and control. Oxford Univ. Press, Oxford, U.K.

André, J. B., and S. Gandon. 2006. Vaccination, within-host dynamics and virulence evolution. Evolution 60:13–23.

André, J., J. Ferdy, and B. Godelle. 2003. Within-host parasite dynamics, emerging trade-off, and evolution of virulence with immune system. Evolution 57:1489–1497.

Antia, R., B. R. Levin, and R. M. May. 1994. Within-host population dynamics and the evolution and maintenance of microparsite virulence. Am. Nat. 144:457–472.

Blattman, J. N., R. Antia, D. J. Sourdive, X. Wang, S. M. Kaech, K. Murali-Krishna, J. D. Altman, and R. Ahmed. 2002. Estimating the precursor frequency of naive antigen-specific CD8 T cells. J. Exp. Med. 195:657–664.

Bonhoeffer, S., and M. Nowak. 1994. Mutation and the evolution of virulence. Proc. R. Soc. Lond. B 258:133–140.
Bremermann, H. J., and H. R. Thieme. 1989. A competetive exclusion principle for pathogen virulence. J. Math. Biol. 27: 179–190.
Brown, S., M. Hochberg, and B. Grenfell. 2002. Does multiple infection select for raised virulence? Trends Microbiol. 10: 401–405.
Christensen, J., P. Doherty, K. Branum, and J. Riberdy. 2000. Profound protection against respiratory challenge with a lethal H7N7 influenza A virus by increasing the magnitude of CD8(+) T-cell memory. J. Virol. 74:11690–11696.
Coban, C., M. Philipp, J. Purcell, D. Keister, M. Okulate, D. Martin, and N. Kumar. 2004. Induction of *Plasmodium falciparum* transmission-blocking antibodies in nonhuman primates by a combination of DNA and protein immunizations. Infect. Immunol. 72:253–259.
Day, T. 2002. On the evolution of virulence and the relationship between various measures of mortality. Proc. R. Soc. Lond. B 269:1317–1323.
———. 2003. Virulence evolution and the timing of disease life-history events. Trends Ecol. Evol. 18:113–118.
Doherty, P. C., and J. P. Christensen. 2000. Accessing complexity: the dynamics of virus-specific T cell responses. Annu. Rev. Immunol. 18:561–592.
Druilhe, P., F. Spertini, D. Soesoe, G. Corradin, P. Mejia, S. Singh, R. Audran, A. Bouzidi, C. Oeuvray, and C. Roussilhon. 2005. A malaria vaccine that elicits in humans antibodies able to kill *Plasmodium falciparum*. PLoS Med. 2:e344.
Dunachie, S., and A. Hill. 2003. Prime-boost strategies for malaria vaccine development. J. Exp. Biol. 206:3771–3779.
Ebert, D., and J. Bull. 2003. Challenging the trade-off model for the evolution of virulence: Is virulence management feasible? Trends Microbiol. 11:15–20.
Fenner, F., M. Day, and G. Woodroofe. 1956. The epidemiological consequences of the mechanical transmission of myxomatosis by mosquitoes. J. Hyg. (Lond.) 54:284–303.
Flynn, K. J., J. M. Riberdy, J. P. Christensen, J. D. Altman, and P. C. Doherty. 1999. In vivo proliferation of naive and memory influenza-specific CD8(+) T cells. Proc. Natl. Acad. Sci. USA 96:8597–8602.
Frank, S. A. 1996. Models of parasite virulence. Q. Rev. Biol. 71: 37–78.
Gandon, S., M. J. Mackinnon, S. Nee, and A. F. Read. 2001. Imperfect vaccines and the evolution of pathogen virulence. Nature 414:751–756.
———. 2002. Antitoxin vaccines and pathogen virulence: a reply. Nature 417:610.
———. 2003. Imperfect vaccination: some epidemiological and evolutionary consequences. Proc. R. Soc. Lond. B 270: 1129–1136.
Ganusov, V., and R. Antia. 2003. Trade-offs and the evolution of virulence of microparasites: Do details matter? Theor. Popul. Biol. 64:211–220.
Ganusov, V. V., C. T. Bergstrom, and R. Antia. 2002. Within-host population dynamics and the evolution of microparasites in a heterogeneous host population. Evolution 52:213–223.
Gilchrist, M., and A. Sasaki. 2002. Modeling host-parasite coevolution: a nested approach based on mechanistic models. J. Theor. Biol. 218:289–308.
Grayson, J. M., L. E. Harrington, J. G. Lanier, E. J. Wherry, and R. Ahmed. 2002. Differential sensitivity of naive and memory CD8(+) T cells to apoptosis in vivo. J. Immunol. 169: 3760–3770.
Homann, D., L. Teyton, and M. Oldstone. 2001. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. Nat. Med. 7:913–919.
Levin, S., and D. Pimentel. 1981. Selection of intermediate rates of increase in parasite-host systems. Am. Nat. 117:308–315.
Mackinnon, M. J., and A. F. Read. 1999. Genetic relationship between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution 53:689–703.
———. 2003. The effects of host immunity on virulence-transmissibility relationships in the rodent malaria parasite *Plasmodium chabaudi*. Parasitology 126:103–112.
May, R. M., and M. A. Nowak. 1995. Coinfection and the evolution of parasite virulence. Proc. R. Soc. Lond. B 261:209–215.
Mead-Briggs, A. R., and J. A. Vaughan. 1975. The differential transmissibility of *Myxoma* virus strains of differing virulence grades by the rabbit flea *Spilopsyllus cuniculi* (Dale). J. Hyg. (Lond.) 75:237–247.
Moingeon, P., J. Almond, and M. de Wilde. 2003. Therapeutic vaccines against infectious diseases. Curr. Opin. Microbiol. 6: 462–471.
Murali-Krishna, K., J. Altman, M. Suresh, D. Sourdive, A. Zajac, J. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8+ T cells: a re-evaluation of bystander actiation during viral infection. Immunity 8:177–187.
Nowak, M. A., and R. M. May. 1994. Superinfection and the evolution of parasite virulence. Proc. R. Soc. Lond. B 255:81–89.
Sasaki, A., and Y. Iwasa. 1991. Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. Theor. Popul. Biol. 39:201–239.
Schall, J. J. 2002. Parasite virulence. Pp. 283–313 *in* E. E. Lewis, J. F. Campbell, and M. V. K. Sukhdeo, eds. The behavioural ecology of parasites. CAB International, New York.
Schneerson, R., J. Robbins, J. Taranger, T. Lagergard, and B. Trollfors. 1996. A toxoid vaccine for pertussis as well as diphtheria? Lessons to be relearned. Lancet 348:1289–1292.
Schulman, J. L. 1967. Experimental transmission of influenza virus infection in mice. IV. Relationship of transmissibility of different strains of virus and recovery of airborne virus in the environment of infector mice. J. Exp. Med. 125:479–488.
———. 1970. Effects of immunity on transmission of influenza: experimental studies. Prog. Med. Virol. 12:128–160.
Smith, T. 2002. Imperfect vaccines and imperfect models. Trends Ecol. Evol. 14:154–156.
Soubeyrand, B., and S. Plotkin. 2002. Microbial evolution: antitoxin vaccines and pathogen virulence. Nature 417:609–610.
Taranger, J., B. Trollfors, E. Bergfors, N. Knutsson, V. Sundh, T. Lagergard, L. Lind-Brandberg, G. Zackrisson, J. White, H. Cicirello, J. Fusco, and J. Robbins. 2001. Mass vaccination of children with pertussis toxoid-decreased incidence in both vaccinated and nonvaccinated persons. Clin. Infect. Dis. 33: 1004–1010.
van Baalen, M., and M. Sabelis. 1995. The dynamics of multipe infection and the evolution of virulence. Am. Nat. 146:881–910.
Veiga-Fernandes, H., U. Walter, C. Bourgeois, A. McLean, and B. Rocha. 2000. Response of naive and memory CD8+ T cells to antigen stimulation in vivo. Nat. Immunol. 1:47–53.
Vitetta, E., M. Berton, C. Burger, M. Kepron, W. Lee, and X. Yin. 1991. Memory B and T cells. Annu. Rev. Immunol. 9:193–217.

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