S and *I* represent the proportion of the population susceptible and infectious, respectively. *U* represents the proportion dead and recovered, with the number dead dependent on the specific case fatality rates given in the data sets. The contacts of cases are divided into the following classes: E_n the number of untraced latent individuals in the population, E_i the number of traced latent contacts, and C_i the number of traced uninfected contacts. The final class of contacts are those untraced and uninfected and so effectively remaining in *S*. *Q* represents the proportion in quarantine and *V* the proportion protected by vaccination. The average rate at which latent individuals become infectious[13,21] is $\alpha = (\text{latency period})^{-1} = 0.0685\ \text{days}^{-1}$ and the rate at which infectious individuals in the community recover or die[22] is $\gamma = (\text{infectious period})^{-1} = 0.116\ \text{days}^{-1}$. Two states of quarantine are defined: the first for the traced contacts successfully vaccinated and released into the community at a rate χ_1, and the second for the infectious cases, which enter *U* at a rate χ_2. Different vaccine efficacies are assumed for those uninfected, ϵ_1, and infected, ϵ_2. The proportion of contacts found through contact tracing is ρ and the daily rate at which infectious individuals enter quarantine from the community is θ. The proportion of contacts infected is defined as φ. The rate at which potentially infected contacts occur is defined as β, as in equation (2), and *N* is the size of the population in which the epidemic occurs.

$$\beta = \frac{R_0\gamma}{\varphi N} \tag{2}$$

Additional assumptions are that no transmission occurs from those quarantined, dead or recovered and the background mortality rate was assumed to be negligible over the time periods examined.

For the Boston, Burford, Warrington and Chester data sets, $\rho = \theta = 0$, which effectively reduces equations (1) above to a simple SEIR model[9]. Intervention parameters were only required when equations (1) was fitted to the data from Kosovo. Here, interventions were implemented 31 days after the onset of symptoms in the index case[1] with the associated parameters shown in Table 2. The number of potentially infected contacts per case was determined as 50 (ref. 1). Values of R_0 were derived for each outbreak by minimizing the mean square error between the mortality data and the predictions of mortality from the model, while applying the outbreak-specific case fatality rates to *U* and adjusting R_0 and time of onset of symptoms in the index case. In the case of Kosovo, equations (1) were fitted more simply to the reported number of cases rather than deaths. All the other parameters required for equations (1) were obtained independently from the published source(s) given in Table 1. For epidemics in London, R_0 was roughly calculated from the interepidemic interval, $T = 2\pi[L(D + D')/(R_0 - 1)]^{1/2}$, where *L* is life expectancy between 1840 and 1870 adjusted for excess births over deaths, equal to 25 years, and $D + D'$ is latent + infectious period, equal to 0.063 years[9].

Estimation of current vaccination coverage

Given that smallpox vaccination ceased in industrialized countries in the mid to late 1970s (ref. 13), a crude estimate of the immunity of the contemporary UK population was calculated, on the basis of 50% having been vaccinated as infants up to 1972, and estimating that about 60% of these would be alive today from current population statistics. Of these only about 60% would still be protected by the vaccinations done on average 50 years previously, calculated by extrapolating from data on secondary attack rates, which increased from 4 to 12% over 10 years following vaccination[23]. This suggests that the level of herd immunity may be about 18%, which will continue to decrease with time.

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1. Fenner, F., Henderson, D. A., Arita, I., Jezek, Z. & Ladnyi, I. D. *Smallpox and its Eradication* (World Health Organization, Geneva, 1988).
2. World Health Organization. *Future Research on Smallpox Virus Recommended* Press Release WHO/77 (World Health Organization, Geneva, 1999).
3. US sounds alarm over smallpox weapon threat. *Nature* **399,** 628 (1999).
4. Henderson, D. A. *et al.* Smallpox as a biological weapon: medical and public health management. *J. Am. Med. Assoc.* **281,** 2127–2137 (1999).
5. Henderson, D. A. Bioterrorism as a public health threat. *Emerg. Infect. Dis.* **4,** 488–492 (1999).
6. Henderson, D. A. The looming threat of bioterrorism. *Science* **283,** 1279–1282 (1999).
7. O'Toole, T. Smallpox: an attack scenario. *Emerg. Infect. Dis.* **4,** 488–492 (1999).
8. Meltzer, M. I., Damon, I., LeDuc, J. W. & Millar, J. D. Modeling the potential responses to smallpox as a bioterrorist weapon. *Emerg. Infect. Dis.* **7** (in the press; also available on http://www.cdc.gov/ncidod/eid/vol7no6/meltzer.htm).
9. Anderson, R. M. & May, R. M. *Infectious Diseases of Humans: Dynamics and Control* (Oxford Univ. Press, Oxford, 1992).
10. Creighton, C. *A History of Epidemics in Britain* Vol. 2 (Cambridge Univ. Press, Cambridge, 1891).
11. Moody, J. The Burford small-pox outbreak of 1758. *Tolsey Pap.* No. 1 (The Tolsey Museum, Burford and Burford School, 1980).
12. Registrar General. *Annual Report of the Registrar General* 1869–74 (Her Majesty's Stationery Office (HMSO), London, 1870–75).
13. Mack, T. M. Smallpox in Europe, 1950–1971. *J. Infect. Dis.* **125,** 161–169 (1972).
14. World Health Organization. *Health Aspects of Chemical and Biological Weapons* (WHO, Geneva, 1970).
15. Duncan, C J., Duncan, S. R. & Scott, S. Oscillatory dynamics of smallpox and the impact of vaccination. *J. Theor. Biol.* **183,** 447–454 (1996).
16. Dietz, K. & Heesterbeek, J. A. Bernoulli was ahead of modern epidemiology. *Nature* **408,** 513–514 (2000).
17. Dixon, C. W. *Smallpox* (Churchill, London, 1962).
18. Arita, I., Wickett, J. & Fenner, F. Impact of population density on immunization programmes. *J. Hyg. Cambridge* **96,** 459–466 (1986).
19. Wehrle, P. F., Posch, J., Richter, K. H. & Henderson, D. A. An airborne outbreak of smallpox in a German hospital and its significance with respect to other outbreaks in Europe. *Bull. World Health Organ.* **4,** 669–679 (1970).
20. Henderson, D. A. & Fenner, F. in *Vaccines* 2nd edn (eds Plotkin, S. A. & Mortimer, E. A.) 13–40 (Saunders, Philadelphia, 1994).
21. Gelfand, H. M. & Posch, J. The recent outbreak of smallpox in Meschede, West Germany. *Am. J. Epidemiol.* **94,** 234–237 (1971).
22. Koplan, J. P., Azizullah, M. & Foster, S. O. Urban hospitals and rural village smallpox in Bangladesh. *Trop. Geogr. Med.* **30,** 355–358 (1978).
23. Mack, T. M., Thomas, D. B., Ali, A. & Khan, M. M. Epidemiology of smallpox in West Pakistan: I. Acquired immunity and distribution of disease. *Am. J. Epidemiol.* **95,** 157–168 (1972).
24. Franz, D. R. *et al.* Clinical recognition and management of patients exposed to biological warfare agents. *J. Am. Med. Assoc.* **278,** 399–411 (1997).

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Competing interests statement

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Correspondence and requests for materials should be addressed to S.L. (e-mail: steve.leach@camr.org.uk).

Imperfect vaccines and the evolution of pathogen virulence

Sylvain Gandon*†, Margaret J. Mackinnon*†, Sean Nee* & Andrew F. Read*

* *Institute of Cell, Animal and Population Biology, The University of Edinburgh, Edinburgh EH9 3JT, UK*
† *These authors contributed equally to this work*

Vaccines rarely provide full protection from disease. Nevertheless, partially effective (imperfect) vaccines may be used to protect both individuals and whole populations[1–3]. We studied the potential impact of different types of imperfect vaccines on the evolution of pathogen virulence (induced host mortality) and the consequences for public health. Here we show that vaccines designed to reduce pathogen growth rate and/or toxicity diminish selection against virulent pathogens. The subsequent evolution leads to higher levels of intrinsic virulence and hence to more severe disease in unvaccinated individuals. This evolution can erode any population-wide benefits such that overall mortality rates are unaffected, or even increase, with the level of vaccination coverage. In contrast, infection-blocking vaccines induce no such effects, and can even select for lower virulence. These findings have policy implications for the development and use of vaccines that are not expected to provide full immunity, such as candidate vaccines for malaria[4].

Previous studies on the evolution of vaccine resistance have focused on the spread of 'escape' mutants that display epitopes different to those in the vaccine, thereby escaping immune recognition[5–7]—this has already happened for polio[8] and hepatitis B[9]. New vaccines may eventually get around this problem by, for example, targeting conserved epitopes or multiple epitopes simultaneously. Here we study an alternative counter-adaptation to vaccination involving pathogen life-history traits, namely virulence (induced host mortality) and transmission rate. To address this issue we incorporated standard evolutionary theory for virulence evolution[10–12] into an epidemiological framework[1].

We begin with an analysis of the evolution of parasite virulence

(the disease-induced mortality rate of the host) in a homogeneous host population. We do this by studying the ability of a rare mutant, with virulence denoted α^*, to invade a population of resident parasites with virulence α (the asterisk distinguishes the mutant's trait from the resident's). The evolutionarily stable (ES) pathogen virulence can be found by maximizing the mutant pathogen's $R_0[\alpha^*,\alpha]$ at $\alpha = \alpha^*$. (See equation (1) below.) When the host population is homogeneous and has reached epidemiological equilibrium (as denoted by the circumflex accent ('hat' symbol) throughout), the expression for the mutant's fitness is given by the expected number of secondary cases produced by a single host infected by this mutant over its entire infectious period[10–13]:

$$R_0[\alpha^*,\alpha] = \frac{\beta^*(\hat{x} + \sigma\hat{y})}{\delta + \alpha^* + \chi^* + \sigma\hat{h}} \quad (1)$$

where x and y are the densities of uninfected and infected hosts, respectively, β is the pathogen's transmission rate, $h = \beta y$ is the rate at which hosts acquire new infections (termed 'the force of infection'), χ is the pathogen's clearance rate (rate at which the host becomes non-infectious), δ is the host's natural mortality rate, and σ is the efficiency with which the pathogen invades an already infected host (superinfection) relative to invading an uninfected host[13,14]. The superinfection parameter, σ, can also be modelled as a function of virulence[13], but here, for simplicity, we assume it to be a constant. It is assumed that superinfecting parasites immediately replace the strain already present in the host: thus σh is the rate at which the pathogen is cleared from the host due to arrival of another strain. Note that $\hat{h}$ is determined by the resident pathogen strain. Thus, by setting the density of infected hosts to zero, we recover the classical definition of R_0, which allows us to tell whether the mutant pathogen can invade a fully susceptible host population[1].

Here we assume, as in classical models of the evolution of virulence[10–14], that the pathogen fitness function in equation (1) includes trade-offs involving pathogen virulence—that is, virulence has beneficial, pleiotropic effects on other pathogen life-history traits that offset the fitness cost of host death (which prematurely ends the infectious period). Two types of virulence benefits have been proposed. First, transmission rate is assumed to be an increasing function of pathogen virulence. Second, clearance rate is assumed to be slower with higher virulence. The net result of these negative and positive influences on pathogen fitness is that there is an intermediate optimum level of virulence that maximizes fitness. Although there are few data testing these assumed fitness relationships in pathogens, they are generally supportive[10,15,16]. The exact nature of these relationships will depend on the biology of each particular host–pathogen interaction, but here we define these trade-offs in simple forms by:

$$\beta = \beta[\alpha] = b_1\alpha^{b_2}$$
$$\chi = \chi[\alpha] = c_1\alpha^{-c_2} \quad (2)$$

where the coefficients with subscripts are constants that determine the shape of the trade-offs, and hence the value of α that maximizes fitness.

The question now is how does host immunity (or 'resistance') change the optimum virulence relative to that in a completely non-immune ('susceptible') host population? Still assuming a homogeneous host population, we consider four different forms of immunity, with efficacies denoted r_1, r_2, r_3 and r_4, which independently affect different stages of the pathogen's life cycle (Fig. 1). The first is anti-infection immunity, which decreases the probability that a host becomes infected. The second is anti-growth-rate immunity, which directly reduces virulence and concomitantly affects transmission rate and host recovery. The third is transmission-blocking immunity, which only decreases parasite transmission. The fourth is anti-toxin immunity which directly reduces virulence but, contrary to anti-growth-rate immunity, does not affect parasite transmission and host recovery rates. This yields:

$$\alpha' = (1 - r_2)(1 - r_4)\alpha$$
$$\beta' = (1 - r_3)\beta[(1 - r_2)\alpha]$$
$$\chi' = \chi[(1 - r_2)\alpha] \quad (3)$$
$$h' = (1 - r_1)\beta' y'$$

where the prime pertains to immune hosts. Assuming that only the trade-off between virulence and transmission is operating (clearance rate, χ, is a constant), yields the ES virulence:

$$\alpha^* = \frac{b_2(\delta + \chi + \sigma h(1 - r_1)(1 - r_3))}{(1 - b_2)(1 - r_2)(1 - r_4)} \quad (4)$$

Note that, throughout, virulence is measured as induced host mortality in susceptible (non-immune) hosts. Equation (4) implies that anti-growth-rate and anti-toxin immunity (modelled by r_2 and r_4) always select for higher virulence. This is because they reduce the risk of host death and hence selection against more virulent mutants. Indeed, evolution will restore the virulence observed in a uniform population of resistant hosts, as well as the force of infection, to that observed in a uniform population of susceptible hosts by increasing intrinsic virulence (that is, virulence as measured in susceptible hosts). Thus a pathogen following a strategy that would generate optimal virulence in a resistant host will induce a higher-than-optimal virulence in a susceptible host[17]. In contrast, anti-infection (r_1) and transmission-blocking (r_3) immunity select for lower virulence whenever there is superinfection, and leave it unchanged otherwise. They act indirectly on the evolution of parasite virulence via the force of infection through their effects on the rate at which an infection is prematurely ended by the arrival of a superinfecting pathogen[14,17]. Although equation (4) defines the ES virulence as a function of the force of infection, which is itself a function of virulence, the results discussed above can be rigorously proven using implicit differentiation analysis (not shown).

If we alternatively assume that there is only a trade-off between virulence and recovery rate, the ES virulence is:

$$\alpha^* = \frac{(c_1 c_2/(1 - r_4))^{\frac{1}{1+c_2}}}{1 - r_2} \quad (5)$$

In this case, anti-growth and anti-toxin immunity increase virulence, and the other two forms of immunity have no effect. When both trade-offs are included, the conclusions derived from equation

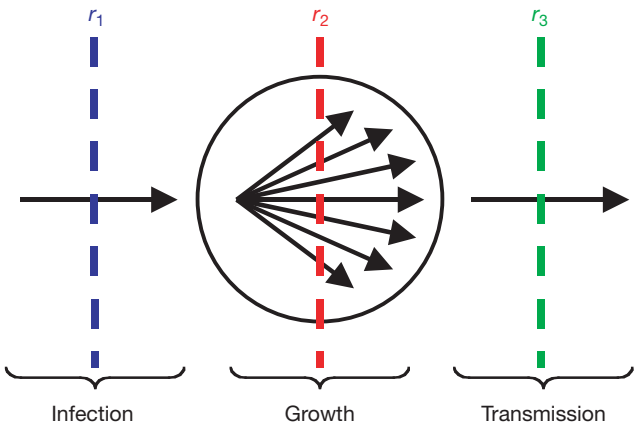

Figure 1 Schematic representation of the action of different types of host resistance at different stages of the pathogen's life cycle. r_1, anti-infection resistance; r_2, anti-growth-rate resistance; r_3, transmission-blocking resistance. A fourth type of resistance—anti-toxin resistance, r_4—is not shown because it only acts upon host death rates.

(4) do not qualitatively change.

What, then, if the pathogen faces a heterogeneous population of susceptible and resistant hosts, as would happen if a vaccination programme was implemented? On the one hand, we have shown that the consequences of vaccinating individual hosts is to select for higher levels of intrinsic virulence in the case of anti-growth-rate and anti-toxin vaccines. On the other hand, immunized hosts will transmit less, die less and recover more quickly than non-immune hosts, thus reducing the overall level of disease in the population. We now allow this epidemiology to feed back into the pathogen's virulence evolution, and vice versa, in order to determine the overall impact of vaccination programmes on the health of the population. The epidemiological model that we used was a modified version of the standard susceptible-infected model[1] with two classes of hosts—those that are fully susceptible to the pathogen, and those that are partially immune. In addition, we assume a continuous vaccination procedure which provides imperfect but life-long immunity. It is written as:

$$\begin{aligned} dx/dt &= (1-f)\lambda - (\delta + h)x + \chi y \\ dx'/dt &= f\lambda - (\delta + h')x' + \chi' y' \\ dy/dt &= hx - (\delta + \alpha + \chi)y \\ dy'/dt &= h'x' - (\delta + \alpha' + \chi')y' \end{aligned} \qquad (6)$$

where λ is a constant rate of flow (which covers both reproduction and immigration) of uninfected hosts into the population, among which a fraction f are resistant, that is, vaccinated, and the forces of infection on susceptible and resistant hosts become $h = \beta y + \beta' y'$ and $h' = (1 - r_1)h$, respectively. Note that there are no terms for superinfection in equation (6) as these cancel out. For simplicity, we assume that resistant hosts do not lose immunity to become susceptible, and, except by vaccination, susceptible hosts do not acquire immunity. This latter assumption is relaxed in our malaria example below.

As for the simple homogeneous case, the ES parasite virulence is found by maximizing at $\alpha = \alpha^*$:

$$R_0[\alpha^*, \alpha] = \frac{\beta^*(\hat{x} + \sigma\hat{y})}{\delta + \alpha^* + \chi^* + \sigma\hat{h}} + \frac{\beta'^*(1 - r_1)(\hat{x}' + \sigma\hat{y}')}{\delta + \alpha'^* + \chi'^* + \sigma\hat{h}'} \qquad (7)$$

which can be seen as a weighted average of the per-host transmission factors[18] on susceptible and resistant hosts (see Supplementary Information). Equations (6) and (7) can then be solved jointly to yield the ES virulence and population prevalence of disease once evolution has occurred.

A numerical example shows that as the efficacy of anti-growth-rate and anti-toxin vaccines increases, there is a marked increase in virulence (Fig. 2a). An exception occurs for very high efficacy anti-growth-rate vaccines, because the contribution to fitness from vaccinated individuals becomes very small (see Supplementary Information). ES virulence always increases with the efficacy of anti-toxin vaccines, because this type of vaccine removes the cost of virulence (increased mortality) without affecting its benefit (increased transmission). Consequently, the fitness contribution of vaccinated hosts always increases with the efficacy of an anti-toxin vaccine. In contrast, as the efficacy of anti-infection and anti-transmission vaccines increases, pathogens will evolve lower levels of virulence if superinfection occurs (Fig. 2a). This is because these vaccines reduce superinfection rates. When pathogens are less likely to be competitively excluded, the benefits of keeping the host alive are greater. The reduction in virulence is weaker for transmission-blocking vaccines than for infection-blocking vaccines because, with transmission-blocking vaccines, vaccinated hosts are fully susceptible to infection. Consequently, the force of infection is higher and this selects for higher virulence levels. When vaccines are fully effective ('perfect'), all except the anti-toxin vaccines share the same ES virulence. When superinfection occurs, this level falls below the virulence reached when vaccines are never used (or are totally ineffective). This is because, with a perfect vaccine, vaccinated hosts do not transmit the disease and, through the decrease of the force of infection, serve to indirectly favour lower virulence[17,19]. A perfect anti-toxin vaccine (a vaccine that completely removes the deleterious effects of the parasite on its host) does not decrease transmission and always selects for extreme intrinsic virulence (Fig. 2a).

In addition to virulence consequences, vaccination changes disease prevalence. Figure 2b shows that as vaccination coverage increases, anti-infection and anti-transmission vaccines always reduce disease prevalence when pathogen evolution occurs, and can sometimes eliminate the disease. Anti-growth-rate vaccines, on the other hand, have hardly any effect on prevalence because of a balance between two forces that act in different directions—

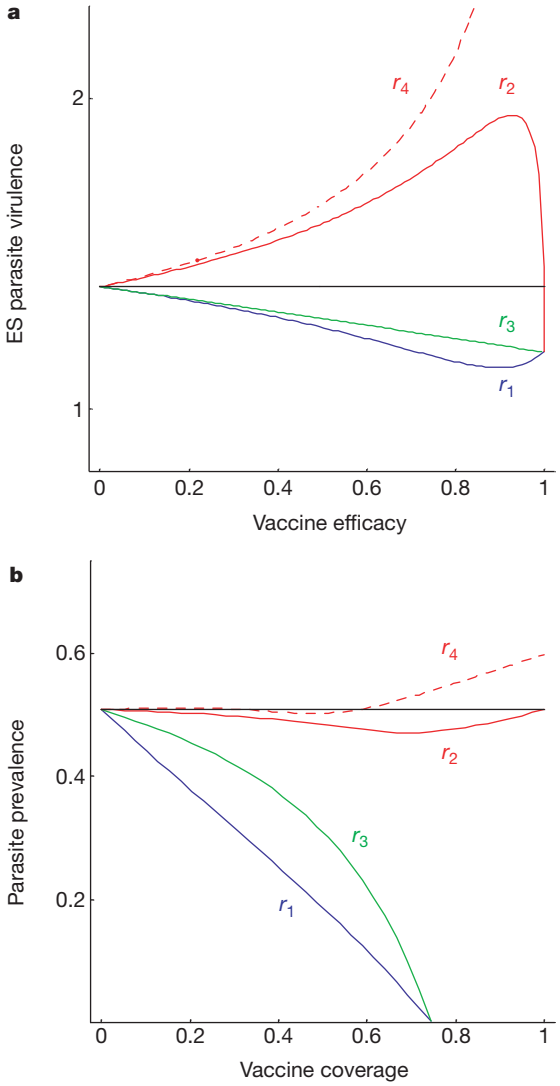

Figure 2 Evolutionary and epidemiological consequences of using different types of vaccines. The different coloured solid curves represent the first three types of vaccines (see Fig. 1), and the red dashed line is for anti-toxin vaccines. **a**, Evolutionarily stable (ES) parasite virulence (measured on susceptible hosts) plotted against the efficacy of the vaccine. **b**, Parasite prevalence (fraction of infected hosts) plotted against the proportion of vaccinated hosts. The horizontal black lines show the outcome in the absence of vaccination. In **a**, $b_1 = 0.5$ and $f = 0.2$ (see text for definitions of b_1 and f). In **b**, $b_1 = 0.2$ and efficacy (r_1, r_2, r_3 or r_4) is 0.9. Other parameter values (see text for definitions) are: $\lambda = 25$, $\delta = 1$, $\sigma = 1$, $b_2 = 0.2$, $c_1 = 0$ (that is, no relationship between recovery rate and virulence).

reduced transmission due to the direct effect of the vaccine and increased transmission through the evolution of higher virulence in vaccinated hosts. Anti-toxin vaccines may be even worse. Because these vaccines do not reduce transmission rate, increased vaccination coverage can increase pathogen prevalence above pre-vaccination levels.

The above evolutionary analysis assumes that hosts and pathogens are in population dynamic equilibrium. Extensive numerical simulations indicate that our model generates simple dynamics of rapid approaches to a stable point equilibrium. Many diseases, however, have more complex epidemiological dynamics, which may exhibit cycles or chaotic behaviour. Even if there is a point attractor, the transient dynamics following vaccination may be so pronounced or so long-lasting that an analysis based on equilibrium conditions may be irrelevant. In these situations, the selective pressures would vary both in space (from one host to another) and time, and the evolutionary analysis would need to take into account the effects of such variability on the invasion exponent of a mutant parasite[20].

Although a single epidemiological equilibrium exists, the evolutionary analysis of our model revealed that there could be different evolutionary outcomes depending on the initial conditions. Such evolutionary bistability emerges when the parasite can take an evolutionary route that leads to specialization on either susceptible or resistant hosts (leading to low or high virulence, respectively). However, our predictions regarding the effects of imperfect vaccines are not qualitatively altered by evolutionary bistability. This result has potentially important implications for the evolution of specialization[21,22] and for the evolution of multihost pathogens[23], but further exploration of this phenomenon falls outside the scope of the present Letter.

Can the above general theory for a virtual pathogen contribute to the rational design of vaccines against real pathogens such as malaria parasites? Current efforts to develop a malaria vaccine are focused on three different stages of the parasite's life cycle—the pre-erythrocytic stages (sporozoites and liver-stage parasites), asexual blood-stage parasites (merozoites and infected erythrocytes) and the mosquito-stage parasites (gametocytes, gametes, ookinetes)[4]. Immunity against these three stages corresponds to the anti-infection, anti-growth-rate and transmission-blocking forms of resistance studied here. Anti-toxin malaria vaccines are also being explored[24]. Using a modified form of the general model to incorporate two important features of malaria epidemiology—naturally acquired immunity and vector transmission (see Supplementary Information)—we evaluated the public health consequences of using various vaccines.

The model was parameterized using values typical of year-round endemic *Plasmodium falciparum* malaria in a high transmission area. Figure 3a–e shows that, as for the general model, the malaria model predicts that anti-growth-rate and anti-toxin vaccines select for higher virulence, while anti-infection vaccines select for lower parasite virulence. In the malaria model, however, transmission-blocking vaccines may favour slightly higher virulence (Fig. 3c). This vaccine reduces transmission and, consequently, the reproduc-

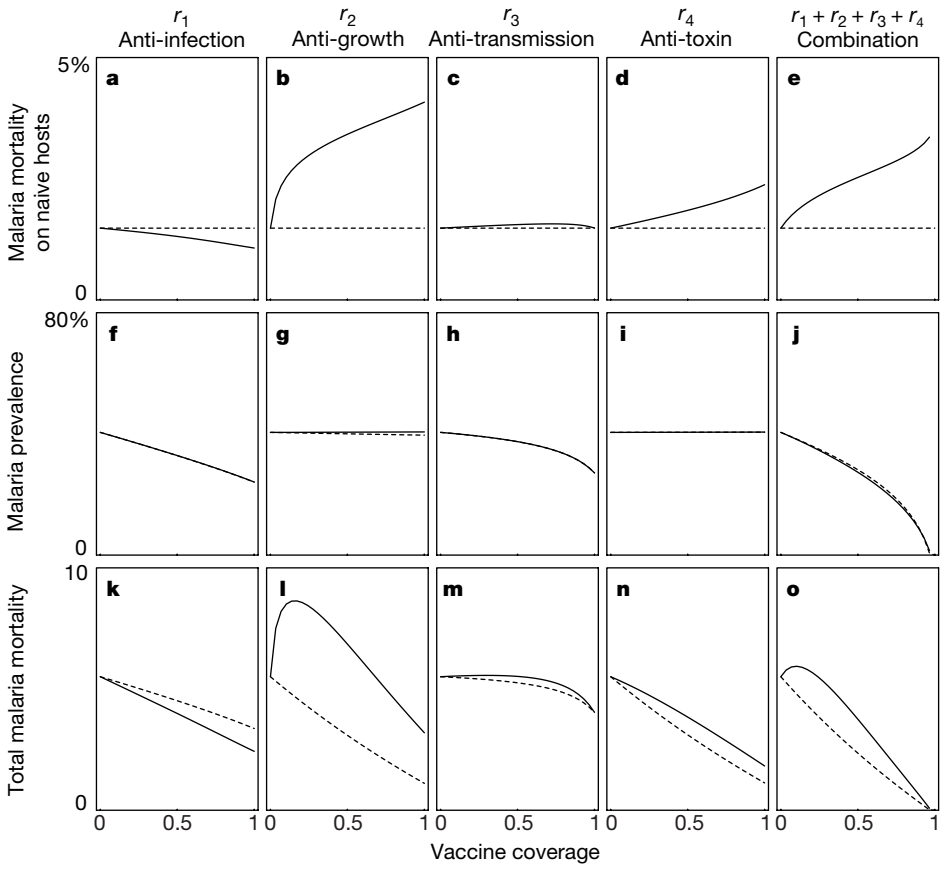

Figure 3 Predicted effects of anti-malaria vaccination coverage on virulence. **a**–**e**, The probability of dying due to malaria in naive hosts, $\alpha_N/(\alpha_N+\chi_N+\delta)$. **f**–**j**, the prevalence of malaria ($y_N+y_I+y_V$). **k**–**o**, The total (population-wide) disease-induced mortality (population average virulence weighted by prevalence of the different host types, given in number of deaths per thousand per year). These results are for an example of endemic malaria; predictions are shown not allowing (dashed line) and allowing (full line) parasite evolution. We show the effects of single type vaccines and a combination vaccine under the assumption that the different types of vaccines have the same level of efficacy as that provided by natural immunity: $r_1=\rho_1=0.8$, $r_2=\rho_2=0.8$, $r_3=\rho_3=0.8$, $r_4=\rho_4=0.8$. See Supplementary Information for symbol definition and further details on the malaria model.

tive value of parasites infecting vaccinated hosts. In this case, evolution becomes mainly driven by the selective pressures occurring in naturally immune hosts. This explains the increase in virulence in spite of the indirect effect of superinfection acting in the opposite direction (equation (4)). We also examined the evolutionary consequences of a combination of different types of vaccines. The use of a vaccine combining the four different types also favours higher pathogen virulence despite the beneficial effect of anti-infection vaccines (Fig. 3e).

With or without evolution of the pathogen, vaccination is expected to affect the prevalence of malaria. As in the general model (Fig. 2b), the use of anti-infection and transmission-blocking vaccines reduces the force of infection and consequently malaria prevalence (Fig. 3f, h). In contrast, anti-growth-rate and anti-toxin vaccines have hardly any effect on prevalence (Fig. 3g, i). A combination vaccine, via the anti-infection and transmission-blocking effects, is the most efficient in reducing malaria prevalence, and could even lead to eradication for extreme vaccine coverage (Fig. 3j).

The total number of deaths due to malaria depends on both malaria virulence and on the prevalence of infection in the different types of hosts. Figure 3k–o presents the consequences of vaccination at the level of the whole host population. In the absence of pathogen evolution, not surprisingly, all the different types of vaccines decrease the total disease mortality. However, with anti-growth-rate, anti-toxin and transmission-blocking vaccines, evolution towards higher virulence (Fig. 3b–d) erodes the overall benefits of vaccination (Fig. 3l–n). In contrast, when anti-infection vaccines are used, evolution towards lower virulence (Fig. 3a) may increase the population-level benefits of vaccination (Fig. 3k). At high vaccination coverage, a vaccine that incorporates all four types of vaccines would be the most efficient, even when evolution occurs (Fig. 3o). This result supports the development of multivalent, multi-stage vaccines which, it is hoped, will provide greater overall protection than single-target vaccines[4]. Our finding that anti-infection vaccines may have favourable effects on virulence evolution also strongly supports the use of other partially effective control methods (for example, bed nets, mosquito control) to enhance the long-term benefits of vaccination.

How long might such virulence evolution take? Given that genetic variation exists for pathogen virulence[10,15,16,25,26], one might expect that, like the evolution of vaccine and drug resistance, the evolution of virulence would occur on timescales that are relevant to public health (decades or less). As an explicit example, we used the malaria model to track the spread of a virulence mutant through time following the start of a vaccination campaign. At 90% vaccine coverage with an anti-growth-rate vaccine of 80% efficacy, it took 38 years for a mutant more than twice as virulent to increase from 1% to 50%, after which it spread towards fixation very rapidly (see Supplementary Information). Numerical simulations, however, indicate that the speed of invasion is very sensitive to the shape of the trade-off function. Accurate predictions of both the invasion dynamics of a virulence mutant and of the evolutionary outcome require further investigation of the shape of these trade-off functions.

Purely epidemiological models have demonstrated that vaccines which are protective for individuals in clinical trials can nonetheless generate unwelcome consequences for a population as a whole[2,3]. Our incorporation of evolution into the analysis shows that clinically detrimental or beneficial evolution can also occur. The direction of virulence evolution depends critically on the type of vaccine, with several types promoting evolution that increases mortality risk to individual hosts. However, virulence management (an application of darwinian medicine[27,28]) requires specific models to answer questions regarding specific biological systems. Using malaria as an example, we found that the wide-scale use of even reasonably effective anti-growth-rate and transmission-blocking vaccines may ultimately do little to relieve community disease levels in malaria-endemic areas. Moreover, the evolution prompted by anti-growth-rate and anti-toxin vaccines can substantially increase the risk for non-immune individuals, such as unvaccinated children and non-immune travellers. The widespread use of such vaccines thus raises difficult ethical issues. Nevertheless, it is probable that anti-disease vaccines (anti-growth-rate and anti-toxin) will be used widely for their short-term beneficial effect at the individual level.

Like drug resistance, the clinically detrimental evolution that we are discussing here will occur on timescales longer than those of clinical trials. Marked increases in virulence of some viral diseases have already followed widespread use of anti-growth-rate vaccines in the chicken industry[29]. When human populations become uncontrolled experimental systems, we recommend that at the very least, intrinsic virulence of the pathogen population (or more realistically, putative virulence determinants such as *in vitro* multiplication rates[30]) be closely monitored. □

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1. Anderson, R. M. & May, R. M. *Infectious Diseases of Humans* (Oxford Univ. Press, Oxford, 1991).
2. McLean, S. A. & Blower, S. M. Imperfect vaccines and herd immunity to HIV. *Proc. R. Soc. Lond. B* **256,** 9–13 (1993).
3. Halloran, M. E., Struchiner, C. J. & Spielman, A. Modeling malaria vaccines II: Population effects of stage-specific malaria vaccines dependent on natural boosting. *Math. Biosci.* **96,** 115–149 (1989).
4. Hoffman, S. L. *Malaria Vaccine Development: A Multi-immune Response Approach* (American Society for Microbiology Press, Washington DC, 1996).
5. McLean, A. R. Vaccination, evolution and changes in the efficacy of vaccines: a theoretical framework. *Proc. R. Soc. Lond. B* **266,** 389–393 (1995).
6. Gupta, S., Ferguson, N. M. & Anderson, R. M. Vaccination and the population structure of antigenically diverse pathogens that exchange genetic material. *Proc. R. Soc. Lond. B* **266,** 1435–1443 (1997).
7. Lipsitch, M. Vaccination against colonizing bacteria with multiple serotypes. *Proc. Natl Acad. Sci. USA* **96,** 6571–6576 (1997).
8. CDC. Public health dispatch: outbreak of poliomyelitis—Dominican Republic and Haiti, 2000. *Morb. Mortal. Wkly Rep.* **46,** 1094 (2000).
9. Zuckerman, A. J. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* **356,** 1382–1384 (2000).
10. Anderson, R. M. & May, R. M. Co-evolution of hosts and parasites. *Parasitology* **86,** 411–426 (1982).
11. Levin, S. A. & Pimentel, D. Selection of intermediate rates of increase in parasite-host systems. *Am. Nat.* **116,** 308–315 (1981).
12. Frank, S. A. Models of parasite virulence. *Q. Rev. Biol.* **76,** 37–78 (1996).
13. Gandon, S., Jansen, V. A. A. & van Baalen, M. Host life-history and the evolution of parasite virulence. *Evolution* **56,** 1056–1062 (2001).
14. Nowak, M. A. & May, R. M. Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B* **256,** 81–89 (1994).
15. Lipsitch, M. & Moxon, E. R. Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* **6,** 31–36 (1997).
16. Mackinnon, M. J. & Read, A. F. Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* **56,** 689–703 (1999).
17. Gandon, S. & Michalakis, Y. Evolution of parasite virulence against qualitative or quantitative host resistance. *Proc. R. Soc. Lond. B* **266,** 985–990 (2000).
18. van Baalen, M. & Sabelis, M. W. The dynamics of multiple infection and the evolution of virulence. *Am. Nat.* **146,** 881–910 (1995).
19. May, R. M. & Nowak, M. A. Superinfection, metapopulation dynamics, and the evolution of diversity. *J. Theor. Biol.* **176,** 95–114 (1994).
20. Caswell, H. *Matrix Population Models: Construction, Analysis, and Interpretation* (Sinauer, Sunderland, 2001).
21. Regoes, R. R., Nowak, M. A. & Bonhoeffer, S. Evolution of virulence in a heterogeneous host population. *Evolution* **56,** 64–71 (2000).
22. Ronce, O. & Kirkpatrick, M. When sources become sinks: migrational meltdown in heterogeneous habitats. *Evolution* **56,** 1520–1531 (2001).
23. Woolhouse, M. E. J., Taylor, L. H. & Haydon, D. T. Population biology of multihost pathogens. *Science* **296,** 1109–1112 (2001).
24. Tachado, S. D. *et al.* Signal transduction in macrophages by glycophosphatidylinositols of *Plasmodium*, *Trypanosoma*, and *Leishmania*: activation of protein tyrosine kinases and protein kinase C by inositolglycan and diacylglycerol moieties. *Proc. Natl Acad. Sci. USA* **96,** 4022–4027 (1997).
25. Read, A. F. & Taylor, L. H. The ecology of genetically diverse infections. *Science* **296,** 1099–1102 (2001).
26. Ebert, D. Experimental evolution of parasites. *Science* **286,** 1432–1435 (1998).
27. Williams, G. & Nesse, R. M. The dawn of Darwinian medicine. *Q. Rev. Biol.* **66,** 1–22 (1991).
28. Dieckmann, U., Metz, J. A. J., Sabelis, M. W. & Sigmund, K. (eds) *Virulence Management: the Adaptive Dynamics of Pathogen-host Interactions* (Cambridge Univ. Press, Cambridge, 2001).
29. Witter, R. L. Avian tumor viruses: persistent and evolving pathogens. *Acta Vet. Hung.* **46,** 251–266 (1997).
30. Chotivanich, K. T. *et al.* Parasite multiplication potential and the severity of falciparum malaria. *J. Infect. Dis.* **186,** 1206–1209 (2000).

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Correspondence and requests for materials should be addressed to S.G. (e-mail: Sylvain.Gandon@ed.ac.uk).

Drosophila Toll is activated by Gram-positive bacteria through a circulating peptidoglycan recognition protein

Tatiana Michel, Jean-Marc Reichhart, Jules A. Hoffmann & Julien Royet

Institut de Biologie Moléculaire et Cellulaire, UPR 9022 du CNRS, 15 rue René Descartes, 67084 Strasbourg cedex, France

Microbial infection activates two distinct intracellular signalling cascades in the immune-responsive fat body of *Drosophila*[1,2]. Gram-positive bacteria and fungi predominantly induce the Toll signalling pathway, whereas Gram-negative bacteria activate the Imd pathway[3,4]. Loss-of-function mutants in either pathway reduce the resistance to corresponding infections[4,5]. Genetic screens have identified a range of genes involved in these intracellular signalling cascades[6–12], but how they are activated by microbial infection is largely unknown. Activation of the transmembrane receptor Toll requires a proteolytically cleaved form of an extracellular cytokine-like polypeptide, Spätzle[13], suggesting that Toll does not itself function as a *bona fide* recognition receptor of microbial patterns. This is in apparent contrast with the mammalian Toll-like receptors[14] and raises the question of which host molecules actually recognize microbial patterns to activate Toll through Spätzle. Here we present a mutation that blocks Toll activation by Gram-positive bacteria and significantly decreases resistance to this type of infection. The mutation *semmelweis* (*seml*) inactivates the gene encoding a peptidoglycan recognition protein (PGRP-SA). Interestingly, *seml* does not affect Toll activation by fungal infection, indicating the existence of a distinct recognition system for fungi to activate the Toll pathway.

To isolate new genes implicated in the *Drosophila* immune response, we screened the first chromosome for mutations that inactivate genes involved in the control of the challenge-induced expression of the antimicrobial peptides Diptericin and Drosomycin. *diptericin* expression is controlled by the Imd pathway, whereas induction of *drosomycin* is dependent on the Toll pathway[4,5]. From 2,500 independent lines, we isolated a mutant line in which the ability to express *drosomycin* in response to infection by Gram-positive bacteria (*Micrococcus luteus*, *Streptococcus faecalis*, *Bacillus thuringiensis*) was abolished or severely reduced (Fig. 1a and data not shown). We named this mutation *semmelweis* (*seml*) after Ignaz Philipp Semmelweis, a Hungarian physician who was a pioneer in the field of antiseptic treatments and discovered the cause of puerperal fever[15]. Expression of *diptericin* by Gram-negative bacteria (*Escherichia coli*, *Erwinia carotovora carotovora* and *Enterobacter cloacae*; Fig. 1b and data not shown) was unaffected in *seml* mutant flies but abolished in two mutants of the Imd pathway[7,8], *key* and *dredd* (Fig. 1b). These phenotypes of *seml* in response to challenges by various microorganisms are similar to those of loss-of-function mutants of the Toll pathway[5,6] (*spz*, *Dif*) but distinct from mutations affecting the Imd pathway.

In addition to its Toll-dependent induction by Gram-positive bacteria, *drosomycin* expression in the fat body is also activated by fungal infection in a Toll-mediated process[5]. When challenging *seml* flies with the entomopathogenic fungus *Beauveria bassiana*, we noted that *drosomycin* expression was wild type, in contrast to the results obtained with *spz* and *Dif* mutants, in which fungal induction of *drosomycin* was nearly abolished (Fig. 1c). *seml* is therefore the first described *Drosophila* mutation that specifically impairs the Toll-dependent induction of *drosomycin* by Gram-positive bacteria without affecting that induced by fungal infection.

We next analysed the resistance of *seml* mutants to infections by various microorganisms. We compared the data with those obtained with *spz* and *key* mutants. The results (Fig. 2) show that *seml* mutants are highly susceptible to Gram-positive infection (*Bacillus megaterium*, *S. faecalis*), but are as resistant as wild-type flies to fungal (*B. bassiana*) and Gram-negative bacterial infection (*E. coli, E. c. carotovora*). As expected, in these experiments *key* flies were susceptible only to Gram-negative infection[8], and *spz* mutants both to fungal and Gram-positive infections[5].

The similarities between the antibacterial responses in *seml*, *spz* and *Dif* mutants prompted us to study the epistatic relationship between *seml* and the Toll pathway components. We first tested whether *seml* was genetically upstream or downstream of *Toll*, using the $Toll^{10b}$ gain-of-function allele, which leads to a challenge-independent expression of *drosomycin*. The levels of *drosomycin* transcription in $Toll^{10b}$ and *seml; *$Toll^{10b}$ flies were similar (Fig. 3a), indicating that *seml* is genetically upstream of *Toll*. We used the same strategy to analyse the relationship between *seml* and the serine protease inhibitor *nec*. A loss-of-function mutation in the *nec* gene results in challenge-independent expression of *drosomycin* and in the formation of melanotic spots on the cuticle[13]. Both phenotypes are mediated by *spz* and *Toll*. Both *nec* and *seml; nec* flies express *drosomycin* in the absence of immune challenge and exhibit melanotic spots (Fig. 3b, c). These results suggest that the *seml* mutation inactivates a protein acting upstream of the Toll receptor and of the protease cascade necessary to activate its ligand Spz through proteolytic cleavage. In contrast to Toll-pathway mutants, homozygous *seml* females are fertile. Therefore *seml* does not seem

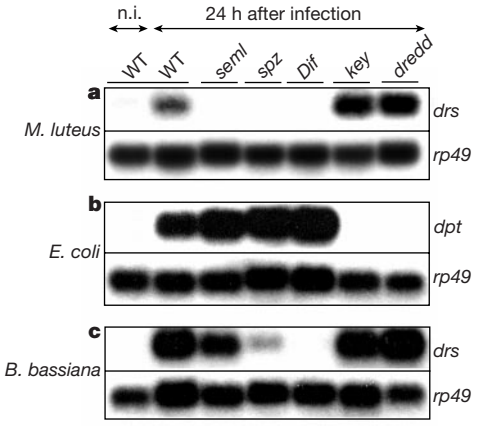

Figure 1 Expression of antimicrobial peptides in different mutant backgrounds after infection by fungi, Gram-positive or Gram-negative bacteria. Northern blots were performed with total RNA from wild type (WT) flies, *seml* mutant flies, or flies mutant for genes in the Toll signalling pathway (spz^{rm7}, Dif^{2}) and in the Imd pathway (key^{4}, $dredd^{D55}$). The flies were infected with *M. luteus* (**a**), *E. coli* (**b**) or *B. bassiana* (**c**) and incubated for 24 h at 25 °C before RNA preparation. *rp49* is used as an RNA loading control. n.i., not induced; *drs*, *drosomycin*; *dpt*, *diptericin*.