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Proceedings: Biological Sciences, Volume 246, Issue 1316 (Nov. 22, 1991), 141-146.

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A mathematical model of vaccination against HIV to prevent the development of AIDS

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SUMMARY

Vaccination and post-exposure immunization against the human immunodeficiency viruses (HIV-1 and HIV-2) faces the problem of the extensive genetic and antigenic variability of these viruses. This raises the question of what fraction of all possible antigen strains of the virus must be recognized by the immune response to a vaccine to prevent development of acquired immunodeficiency disease (AIDS). The success of a vaccine can depend on the variability of the target epitopes. The different HIV variants must be suppressed faster than new escape mutants can be produced. In this paper the antigenic variation of HIV during an individual infection is described by a stochastic process. The central assumption is that antigenic drift is important for the virus to survive immunological attack and to establish a persistent infection that leads to the development of AIDS after a long incubation period. The mathematical analysis reveals that the fraction of antigenic variants recognized by the immune response, that is induced by a successful immunogen, must exceed $1 - 1/R$, where R is the diversification rate of the virus population. This means that if each HIV strain can produce, on average, five new escape mutants, then more than 80% of the possible variants must be covered by the immunogen. A generic result of the model is that, no matter how immunogenic a vaccine is, it will fail if it does not enhance immune attack against a sufficiently large fraction of strains. Furthermore, it is shown that the timing of the application of post-exposure immunization is important.

1. INTRODUCTION

The human immunodeficiency virus (HIV) mutates rapidly during the course of an individual's infection. At any given time the virus population in an infected patient does not consist of a single uniform sequence but a distribution of different variants, generated by mutation and selection. During the reverse transcription of viral RNA into proviral DNA, errors occur at a rate greater than 10^{-4} per base per replication (Preston *et al.* 1988; Roberts *et al.* 1988). For a genome of 10^4 bases, this implies an average of more than one mutation per replication round (the probability of error-free replication of the whole genome is less than 0.4). Many of these mutations may be lethal or effectively neutral. A small minority, however, may give rise to new antigenic variants that escape from current immunological attack (escape mutants). This can happen if a mutation changes the structure of an epitope. It has been calculated that the error rate of the reverse transcriptase maximizes the probability to produce escape mutants (Nowak 1990).

The high genetic variability of HIV-1 has been demonstrated by virus isolation and sequencing, both sequentially from the same infected patient and from different patients (Hahn *et al.* 1986; Fisher *et al.* 1988; Saag *et al.* 1988; Meyerhans *et al.* 1989; Wain Hobson 1989; Balfe *et al.* 1990; Simmonds *et al.* 1990; Nowak *et al.* 1991). Sequence variation is not uniform throughout the genome. The *gag* and *pol* genes encoding,

respectively, for the core proteins and the reverse transcriptase, are more conserved than the *env* gene, which encodes for the envelope proteins gp120 and gp41. Within the envelope protein gp120 there is a pattern of five hypervariable regions, V1 to V5. Of particular interest is the immunodominant V3 loop, a region of about 30 amino acids, which can trigger neutralization phenomena in infected humans and chimpanzees (Goudsmit *et al.* 1988). However, this part of the envelope protein mutates rapidly. The change of a single amino acid in gp120 can apparently account for such clonal restriction of neutralizing activity (McKeating *et al.* 1989; Looney *et al.* 1988). There appears to be an association between the variability of a region and its immunogenicity (in the sense that a region that is recognized by a certain immune response is subject to selection pressure for variation). In this context the observed viral variability represents an adaptive response by HIV to evade the immune system. Recent studies have also demonstrated sequence variation in epitopes of the conserved *gag* gene that are recognized by cytotoxic killer cells (R. E. Phillips, D. F. Nixon, F. M. Gotch, J. G. Elvin, J. A. Rothbard & A. J. McMichael, unpublished results).

HIV-2 isolates exhibit biological and genome variability comparable to that observed for HIV-1 (Schulz *et al.* 1990). Antigenic and genetic variation appears to be a common feature of lentivirus infections. It has been documented for visna virus (Clements *et al.* 1980), equine infectious anaemia virus (Salinovich *et al.* 1986)

and caprine arthritis encephalitis virus (Ellis *et al.* 1987). A recent study (Burns & Desrosiers 1991) has demonstrated extensive sequence variation *in vivo* after infection of two rhesus monkeys with molecularly cloned simian immunodeficiency virus (siv), the closest relative of hiv. This work also suggests selection pressure for changes in distinct variable regions of the envelope protein.

The hiv population is very sensitive to selection pressure. Not only do natural factors such as immune responses and cell tropism, seem to drive variation, but drug treatment does too. A sequence of four amino acid substitutions in the (conserved) reverse transcriptase renders the virus less sensitive to AZT after about six months of treatment (Larder *et al.* 1989 *a, b*). AZT is the most important drug used in large-scale clinical treatment of hiv-infected patients. Would a vaccine face a similar problem?

The development of a successful vaccine that clears the virus infection or stops progression towards AIDS has to consider the problem of hiv's extensive antigenic variability. The obvious question is then: what fraction of hiv mutants must be recognized by the immune response to a vaccine to assure successful immunization?

In the following section we present a mathematical model for the stochastic appearance of escape mutants. At first, a simple model is used as an example to describe virus growth, the induction of an immune response and the production of mutants that can escape from this strain-specific response. By strain we mean a set of mutants all under the control of the same specific immune response. By an escape mutant we mean a mutant that is resistant to current immunological attack and which gives rise to a new strain. Each new strain grows initially; when the specific immune response against this strain is strong enough it will be suppressed again. Therefore each strain can survive immunological attack only for a finite time, but it gives rise to a number of 'offspring' strains that escape from its specific immune response. An individual hiv infection is described as a sequence of antigenically different hiv variants (= antigenic drift). A basic quantity is the diversification rate of the virus population, R , defined as the average number of escape mutants produced by any one virus strain. Only if R is larger than one are new variants produced faster than the immune system can suppress the old variants (it is assumed that a given variant is eradicated by the immune response after some time, and that the virus population as a whole can only survive if new mutants are produced). In this case the diversity of the hiv population will increase during the course of infection; the virus population will not be eliminated by the immune system and AIDS will be developed eventually. Thus the central assumption of this paper is that antigenic variation is necessary for the virus to survive immunological attack and to establish a persistent infection that leads to AIDS. It will be shown that the virus population as a whole can only survive if antigenic variation is fast enough such that the diversification rate, R , exceeds 1. We assume that an immunogen confers immunity to a certain fraction of the possible hiv

strains, and that it works in such a way that the strain-specific immune response is mounted faster against these strains. An immunogen, therefore, reduces the lifetime of some strains and the number of escape mutants they produce. The aim is to reduce the diversification rate of the whole virus population (averaged across recognized and not recognized strains) below one. Then each strain will produce, on average, less than one escape mutant, and the virus population cannot survive by antigenic variation. It will be shown that the critical fraction of virus strains that must be recognized by the immune response to a vaccine must be larger than $1 - 1/R$ to assure extinction of the virus population and, as a consequence, to prevent the development of the disease. (It is assumed that extinction of the virus population prevents progression to AIDS.) This result parallels epidemiological models for mass immunization. The model allows us to compare the effect of a vaccine that produces a strong immune response to a small number of strains with one that induces a weaker immune response to a larger number of strains. It is shown that a vaccine generating even extremely high immune responses, but only to a small number of strains, can fail if it does not reduce the population diversification rate below one. A third argument shows that immunotherapy is more successful if applied as early as possible after infection. The goal of this model is to explore the potential importance of antigenic variation for vaccination against hiv. We have taken a simplistic approach and neglected the detailed dynamics of different populations of immune cells (Cooper 1986; Reibnegger *et al.* 1987, 1989; Perelson 1989) or the synergistic interaction between hiv and other pathogens (McLean & Kirkwood 1990; McLean & Nowak 1991).

2. MATHEMATICAL BIOLOGY OF ANTIGENIC DRIFT

The replication dynamics of a single virus strain and its specific immune response can be written in its simplest form as follows:

$$\dot{v}_i = v_i(r - d - px_i); \quad (1)$$

$$\dot{x}_i = kv_i, \quad (2)$$

where v_i and x_i denote the density of the virus strain i and of immune cells ('immune agents') specific to this strain. In the absence of immunity, virus particles replicate with rate r . The constant death term, d , includes innate immune responses against all virus strains. The term $-pv_i x_i$ specifies killing of virus resulting from specific immune reactions directed at strain i . The rate constant, p , specifies the magnitude of immune cell-mediated virus killing. The specific immune cells x_i are produced at a constant rate proportional to the virus density, kv_i . In this simplest model we have assumed that the immune response against hiv is mounted at a rate proportional to the virus density. This is a caricature-like approach to the true complexity of the dynamics of immune responses, but it should be stressed that the results of this paper

are independent of this assumption and will hold for a more complex and realistic description of the immune system.

The trajectories of the differential equations (1) and (2) have the form (assuming $x_i(0) = 0$):

$$v_i(t) = v_i(0) + x_i(t)[r - d - (p/2)x_i(t)]/k.$$

The virus density, v_i , increases as long as $x_i < (r - d)/p$. After the immune response has overcome this threshold, the virus density decreases and goes to zero.

Here the total virus population can only survive if new resistant mutants are produced that escape from the specific immune response. The rate of production of escape mutants is proportional to the replication rate of the virus population. Therefore the probability that an escape mutant is produced in the time interval $[t, t + dt]$ is proportional to $v_i(t) dt$. Let R denote the average total number of escape mutants produced by one strain. We have (Nowak & May 1991)

$$R = c \int_0^\infty v_i(t) dt = cx_i(\infty)/k = 2c(r - d)/pk,$$

where c is a constant that includes the viral replication and mutation rate. It can be shown that the number of escape mutants produced from one strain follows a Poisson distribution with parameter R ,

$$P_i = R^i e^{-R}/i!$$

Each strain produces a total of i escape mutants with probability P_i .

If there are n_t different strains present at time t , the number of strains in the next 'generation', $t + 1$, is given by

$$n_{t+1} = \sum_{j=1}^{n_t} k_j,$$

where each k_j is a random variable with distribution $Prob(k_j = i) = P_i$. This generates a branching process (see for example Karlin & Taylor 1975), where the number of offspring is Poisson distributed. The probability generating function of this process is given by

$$F(s) = \sum_{i=0}^\infty P_i s^i = e^{R(s-1)}.$$

Starting this process with one single strain, the probability of eventual extinction is represented by the smallest positive root, s_0 , of the equation $s = F(s)$. In other words, $1 - s_0$ denotes the probability that the antigenic drift continues for ever, i.e. the virus infection is not cleared and AIDS is eventually developed. Figure 1 shows this probability as a function of R . If the branching process starts with n strains, then the probability of extinction is reduced to s_0^n . This means that the transition between extinction and persistence is sharpened around the critical bifurcation R smaller or larger than one. The initial number of strains characterizes the virus population diversity at the point of seroconversion. In this model a highly diverse virus population (large n) has a higher probability to survive immunological attack and to establish a persistent infection. The simple model - described by

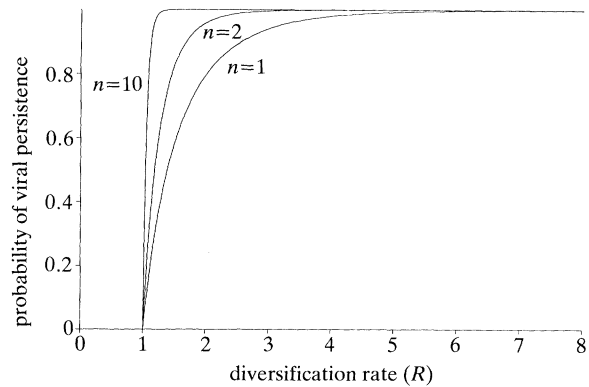


Figure 1. The probability of eventually developing AIDS as a function of HIV's diversification rate, R . This probability is defined by the branching process, which is used to model the antigenic variation of HIV during an individual infection. The diversification rate, R , represents the total number of escape mutants produced by one virus strain. There is a sharp transition between $R < 1$, where the probability that the virus population establishes a persistent infection and causes eventual development of AIDS is zero, and $R > 1$, where this probability quickly converges to one. Therefore each strain must produce at least one escape mutant (before being suppressed by its specific immune response) if the total virus population is to be maintained by antigenic variation. Three curves are shown for different values of the number of strains present at the start of the branching process ($n = 1, 2, 10$ strains).

equations (1) and (2) - serves as an example for the dynamics of virus replication and the induction of an immune response; it is shown how to calculate the diversification rate, R , for this particular model. But the result that $R > 1$ is a necessary condition for virus persistence by antigenic drift is independent of the details of equations (1) and (2).

3. IMMUNIZATION

In the context of a rapidly mutating virus, the purpose of post-exposure vaccination is to augment the specific immune response to such a degree that each strain produces, on average, less than one escape mutant. Suppose an immunogen induces a response that recognizes a fraction f of all possible virus strains. We specify the effect of immunization by increasing the specific immunity parameter k for those strains by a factor a . This means that the specific immune response will be mounted faster against recognized strains, therefore these strains will be present for a shorter time and produce relatively fewer escape mutants. Let R and R' denote the number of escape mutants produced by not recognized and recognized strains respectively. We have $R' = R/a$. The factor a characterizes the immunogenicity of an epitope and the efficiency of the immune response induced by the immunogen. The total virus population produces $\bar{R} = (1 - f)R + fR' = R[1 - f(1 - 1/a)]$ escape mutants on average. The condition $\bar{R} < 1$ leads to

$$f > f_{crit} = (1 - 1/R)/(1 - 1/a). \tag{3}$$

This is the critical fraction of strains that must be covered by the immunogen to assure extinction of the

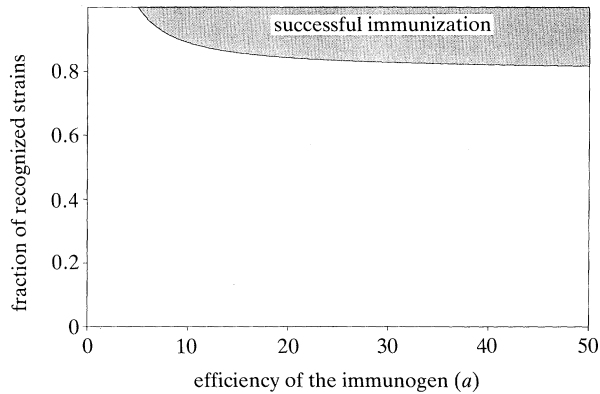


Figure 2. The critical fraction of variants that must be covered by a vaccine as a function of the immunogenicity, a , of the target epitopes. The parameter a is the factor by which the rate of mounting a specific immune response is increased as a result of vaccination. The figure shows that it is important to recognize a sufficiently large fraction of different variants, even if the efficiency of the vaccine, as characterized by the parameter a , is very high. A vaccine that greatly enhances the immune response to only a small proportion of strains can fail. The critical fraction of strains that must be recognized converges to $1 - 1/R$ for large values of a . The diversification rate in this example is $R = 5$.

virus population. Note that a must be greater than R to obtain $f_{\text{crit}} < 1$. This means that immunization can only be successful if recognized strains have a diversification rate lower than one ($R' < 1$). In the limit of very high immunogenicity ($a \rightarrow \infty$), we have

$$f_{\text{crit}} = 1 - 1/R.$$

This means that the minimal fraction of strains that must be recognized is at least $1 - 1/R$ (regardless of the magnitude of a). Suppose that strains which are not recognized have the ability to seed, on average, five resistant offspring strains (i.e. $R = 5$). In this case the immunogen must induce immunity to more than 80% of all strains to extinguish the virus population. Therefore the success of a vaccine depends critically on the diversification potential of the viral epitopes. Equation (3) also allows the study of the relative effect of having a strong immune response against fewer strains or a weak immune response against many strains. Figure 2 shows f_{crit} as a function of a .

4. THE TIMING OF POST-EXPOSURE IMMUNIZATION

In the context of a branching process with on average increasing antigenic diversity, it can be shown that immunotherapy would be more successful if applied as early as possible.

Suppose the HIV population has, in the absence of immunotherapy, a diversification rate $R > 1$. The average number of strains in generation t is given by R^t . Assume that immunization from the beginning results in an extinction probability, s , for the branching process. It is straightforward to show that the probability of extinction after generation t is given by

$$P(t) = s^{(R^t)}.$$

For s close to one there is a sharp transition with the critical time for successful immunization given by:

$$t < t_c = -(\log \log 1/s) / \log R \simeq -\log(1-s) / \log R.$$

This means that immunotherapy can be successful if applied early enough (when the virus diversity is still low). After a certain time, given by t_c , the probability to cure the infection by post-exposure immunization rapidly declines to zero (figure 3). This result may be important for the design of clinical trials for post-exposure immunization. Patients at an advanced stage of disease with a highly diverse virus population may be bad responders. This argument is only based on the dynamics of the viral population diversity during the course of infection, and not on the general decline of cross- or strain-specific responses as immunodeficiency is developed.

5. CONCLUSION

In a recent model (Nowak *et al.* 1990; Nowak & May 1991; Nowak 1991), antigenic variation of HIV was proposed as a major factor to drive progression towards AIDS. This theory is based on the following assumptions: (i) replication errors during reverse transcription can lead to new antigenic variants that escape from current immunological attack, and (ii) the virus impairs CD4+ cell function, which is important in mounting an immune response to the virus. The central idea is that the immune response is only able to control an HIV population whose antigenic diversity is below some threshold value. The long asymptomatic period between infection and collapse of the immune system is the time required for the virus population to evolve from an initially homogeneous state to a highly heterogeneous state just before the development of AIDS. The concept of the diversity threshold arises from the asymmetric pattern of killing that exists between immunologically distinct strains of HIV and their specific clones of T helper cells. Thus, whereas each different HIV strain can kill T helper cells of any specificity, each specific clone of T helper cells can only orchestrate the killing of one strain of HIV. A natural consequence of this asymmetry is a threshold on the number of immunologically distinct strains of HIV that can be controlled simultaneously. The theory therefore explains progression from HIV infection to AIDS as: an initial long period of accumulation of immunologically distinct HIV strains through the generation of escape mutants; this first stage ends with the breaching of the diversity threshold to enter the second phase of continuous growth of HIV and consequent destruction of the immune system. This implies that progression to disease (AIDS) will be foreshadowed by the accumulation of escape mutants. Experimental support for increasing genetic diversity comes from a study of two homosexual patients who were followed since seroconversion. Initially the virus population is genetically homogeneous in the immunodominant V3 loop, but diversity increases over time (Nowak *et al.* 1991).

In this paper we have used a simple branching process to describe the antigenic variation of HIV

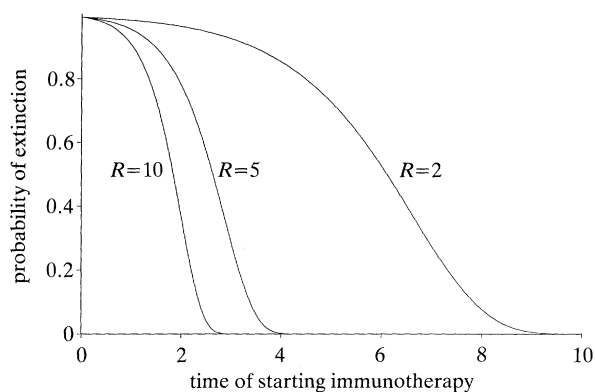


Figure 3. Successful immunotherapy (post-exposure immunization) may depend critically on the time when treatment is started. In this figure it is assumed that immunotherapy starting straight after infection leads to extinction of the virus population with probability 0.99. The probability of success decreases drastically as time progresses. Three curves are shown for different values of the diversification rate $R = 2, 5, 10$.

during the course of infection. The probability to generate a new escape mutant by erroneous replication is proportional to the total replication capacity of the virus population. Each new escape mutant will grow initially and will then be suppressed by the immune response (as specific immunity to this strain is developed). This allows the definition of a diversification rate, R , given by the average number of escape mutants produced by any one parental strain. This is a fundamental quantity to describe the antigenic variation capacity of a virus population. If R is lower than 1 then a virus population cannot persist by antigenic variation. The magnitude of R depends on the replication rate and accuracy of the virus population and on the rate of mounting an effective immune response against the virus. This last quantity could be increased by immunization. If the vaccine is able to generate a quicker immune response to a specific virus strain, then this strain will be suppressed faster and will seed fewer escape mutants. A simple calculation has shown that the fraction of all possible variants that must be recognized by an immunogen to extinguish the virus population must be larger than $1 - 1/R$. This means that the success of a vaccine based on strain-specific immunity (i.e. a vaccine using hypervariable but immunodominant epitopes) depends critically on the diversification potential of the virus population. If each HIV strain has the potential to seed ten new escape mutants, then more than 90% of all strains must be covered by an antigen cocktail. If the diversity of the virus population increases during the time of infection, as modelled by the branching process, then much can be gained by starting treatment as early as possible when the diversity is still low. The probability of driving the virus population to extinction depends on the number of antigenic variants that are present at the beginning of immunotherapy. Genetic diversity seems to be low at the time of seroconversion (Nowak *et al.* 1991).

Several arms of the immune system are found to be active against HIV; these include classic neutralizing

antibodies, cytotoxic T-lymphocytes (CTL) and antibody-dependent cell-mediated cytotoxicity (ADCC). The relative importance of these immune responses *in vivo* is unclear. Therefore it is an open question which of these immune responses should be stimulated by a potential vaccine. It appears to be a fascinating task for mathematical biology to present models that describe the dynamics and relative efficiency of these immune responses, and to provide a rationale for vaccine development.

We thank Bob May and Roy Anderson for discussion. Support by the Royal Society is gratefully acknowledged.

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Received 5 August 1991; accepted 27 August 1991