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Vaccination by Delayed Treatment of Infection

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Abstract

Two medical interventions allow us to combat infectious diseases: vaccination which can be administered well in advance of exposure, and antimicrobials which are most often administered contemporaneously with exposure. In this paper we show how they can, in principle, be combined – with infection followed by treatment being used as a form of vaccination. We use mathematical models to examine how appropriately administered antimicrobial treatment following natural infection can be used to reduce the pathology caused by the infection, and also generate long-lasting immunological memory to the pathogen. The models explore the tradeoff between reduction in pathology and strength of immunization. This tradeoff suggests a limited treatment window during which antimicrobial treatment can be started and provide both amelioration of disease symptoms and long-term immunity. This approach may be particularly well suited to combat the emergence of novel pandemic influenza infections particularly for individuals such as medical healthcare professionals at greatest risk for exposure during the initial stages of a pandemic.

Keywords

Modeling; biology; treatment; vaccine

1. Introduction

The control of infectious diseases is performed with prevention and treatment. Prevention can come in two forms, immunizations and antimicrobial chemoprophylaxis. Vaccination, though when available can be administered well in advance of exposure, also requires lead-time for development and production, and that time is not always available. For influenza the lead-time for vaccine production can exceed six months [1]. In the case of epidemics like the recent H1N1 outbreak, where the spread of the disease is more rapid than the rate of vaccine production, the disease must be controlled by other measures. These measures include the use of antimicrobial drugs both prophylactically and therapeutically [1]. Under certain conditions administration of antimicrobial drugs concomitantly with infection can stimulate the immune system to an extent that it provides long-term immunity [2, 3, 4, 5].

While infections can lead to longterm immunity, antimicrobial drug use following exposure often interrupts the development of immune memory [6]. There are exceptional cases where antimicrobial treatment enhances the generation of immunity above what would be found

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through an untreated infection (e.g., treatment exposing previously hidden antigens [7, 8]) however we restrict our attention to the more common cases where drug treatment limits the development of immunity.

There are many other drawbacks associated with antimicrobial medication such as side-effects, scarcity, and the evolution of drug resistant strains [9]. As chemoprophylaxis and treatment combined with exposure do not necessarily induce immunity, the abatement of disease during prolonged epidemics would require frequent re-administration. This will compound consumption, side-effects, and selection of drug resistant strains, making this type of control less than optimal [6, 9].

To understand the conference of immunity through pathogen exposure combined with antimicrobial drug use we examine the tradeoff between the two primary effects of antimicrobial treatment: the reduction in pathology, and the reduction in the magnitude of the immune response [10, 11]. To one extreme there is a natural, untreated infection, which generates long term immunity at the high cost of the pathology of the infection. The other extreme is pre-exposure chemoprophylaxis, which can eliminate any pathology but also offers little or no immunological protection against future infections [6]. Treatment at intermediate times can offer both immunological protection and reduced pathology [12, 13]. In this paper we examine how the tradeoff between generation of immunity and reduction in pathology can be exploited such that infection followed by treatment can act as a vaccine.

The subject of this paper is motivated by experimental observations in parasitic protozoa infection [2, 3, 4, 5], bacterial infection [12], and viral infection [13]. These studies present evidence that live-unattenuated pathogen can be used in combination with antimicrobial drug use as a vaccine. This provides a reduction in the pathology of the natural infection and long-term immunity.

In protozoa infection, both *Theileria* and *Plasmodium* in combination with antimicrobial drug use have been shown to act as vaccines. Vaccination against *Theileria* is commonly performed in cattle by inoculation with live-unattenuated parasite and simultaneous administration of tetracycline giving long lasting immunity [2, 3]. Similarly, inoculation with *Plasmodium* in mice being treated with either antibiotic [4] or chloroquine [5] gives immunity to malaria.

A study of a bacterial infection with delayed treatment [12] showed that delayed treatment could reduce bacterial density while having little effect on lymphocyte numbers presumably resulting in immunity. Ruprecht et al. [13] performed a similar experiment with a viral infection where they delayed treatment of mice infected with Rauscher murine leukemia virus by 96 hours. This treatment delay was able to both rescue the mice from otherwise certain death and provided them with immunity against re-challenge.

These experimental studies establish that antimicrobial treatment can under certain conditions be used as a vaccine. They do not however explore the tradeoff between abatement of disease and long-term immunity, or the robustness of this vaccination technique.

In this paper we use mathematical models of the within-host dynamics of infection and immune responses. The models are used to explore the tradeoff between treatment and immunity, and the robustness of this vaccination technique. The specifics of this tradeoff and the robustness of the technique are studied through both timing of antimicrobial administration and by differing forms of the generation of immune responses. The immune response models differ by inclusion or omission of two factors: programmed division, and stimulation by killed pathogen.

Our results suggest that with proper timing, delayed treatment can lead to both adequate reduction in pathology and to immunity. Our results also show that in disease systems with programmed division or stimulation by killed antigen, successful treatment strategies become less sensitive to timing.

2. Materials and Methods

We begin with a “basic” model for the dynamics of pathogen, antimicrobial drugs, and the immune response. For lymphocyte dynamics we focus on a typical CD8 T cell response. The basic model includes naive, effector and memory lymphocytes with division and differentiation in the continued presence of live pathogen.

This basic model is presented as a null model in order to compare with models in the following subsections that include two additional biological effects. These additional effects are lymphocyte stimulation by killed pathogen, and “programmed responses”. The programmed response models are based on experimental and theoretical studies, which have shown that a short stimulation of CD8 T cells with antigen results in subsequent antigen-independent proliferation, and differentiation into memory cells [12, 14, 15, 16]. The terms for the expansion of immune responses are based on previous models for the dynamics of CD8 T cell responses [16, 17, 18, 19, 20].

2.1. Basic model

In the basic model, for simplicity, we let the pathogen grow exponentially in the absence of an immune response. Changing this to logistic growth does not qualitatively alter our results and conclusions (provided, of course, that the carrying capacity is high compared with the pathogen density required to stimulate the immune response). Pathogen death in the model can be a result of antimicrobial killing or of killing by lymphocytes specific for the pathogen. Both types of killing are modeled with mass-action terms dependent on pathogen density and either antimicrobial concentration or lymphocyte density. When the killing rate exceeds the growth rate the pathogen will decay. Antimicrobial concentration is modeled as zero until time τ_1 when treatment is started and is a constant value A_m after that. At time τ_2 after the primary infection is cleared, treatment is stopped and $A(t)$ returns to zero.

Lymphocytes in the model are divided into three categories, naive, effector and memory cells. Naive cells in the model kill pathogen and are stimulated to divide and differentiate into effector cells when pathogen density is high. Those effector cells then continue to kill and will divide as long as pathogen is present. Once pathogen is cleared the majority of effector cells die by apoptosis while a fraction f differentiate into memory cells which remain indefinitely.

The model equations for the basic model are:

$$\text{pathogen } \frac{dP}{dt} = rP - hP(N+E+M) - aPA(t) \quad (1)$$

$$\text{antimicrobial } A(t) = \begin{cases} 0 & \text{if } t < \tau_1 \\ A_m & \text{if } \tau_1 \leq t \leq \tau_2 \\ 0 & \text{if } t > \tau_2 \end{cases} \quad (2)$$

$$\text{naive cells } \frac{dN}{dt} = -\sigma N \frac{P}{k+P} \quad (3)$$

$$\text{effector cells } \frac{dE}{dt} = (2\sigma N + \sigma E) \frac{P}{k+P} - dE \left(1 - \frac{P}{k+P}\right) \quad (4)$$

$$\text{memory cells } \frac{dM}{dt} = f dE \left(1 - \frac{P}{k+P}\right). \quad (5)$$

Lymphocyte division in this basic model requires stimulation by live pathogen, (this restriction is relaxed below to include stimulation with killed pathogen and programmed division). The dependence of lymphocyte stimulation on pathogen density in the basic model is assumed to be a monotonically increasing, saturating function. To satisfy these assumptions we use a Hill function with Hill coefficient 1 and stimulation coefficient k (roughly the density of pathogen that stimulates lymphocytes to divide and differentiate). In this model lymphocyte death has a similar dependence on pathogen density, such that effector cells don't begin to decay until pathogen has been removed from the system.

We have a mass action term for the clearance of the pathogen by the immune response. In previous papers typically only effector cells were capable of killing pathogen. In our model we let all antigen-specific cells (naive, effector and memory) kill pathogen. We do so because recent experiments have shown that both effector and memory cells have similar rates of killing [21]. At present it is not known whether naive cells can kill pathogen before becoming activated. However, because there are very few naive cells, their ability to control pathogen is very limited and the model is robust as to whether naive cells are capable of killing pathogen.

Details on implementation of the model such as initial conditions for simulating primary and secondary exposures, parameter values, caveats for model usage and numerical routine are included in Appendix A.

2.2. Killed Pathogen

Two of the models studied in this paper contain stimulation by killed pathogen (or equivalently continued antigen presentation), one where it is added alone to the basic model and one added in conjunction with programmed response. To incorporate the stimulation of lymphocytes by killed pathogen we add an additional differential equation for killed pathogen density to the system:

$$\frac{dR}{dt} = hP(N+E+M) + aPA(t) - \Delta R \quad (6)$$

The killing terms from the pathogen equation (Eq. 1) feed directly into this equation which also includes a decay of killed pathogen from the system with rate Δ . The lymphocyte equations are also modified by replacing P with $P + R$ which simulates stimulation by both pathogen and killed pathogen (see Appendix A). Lymphocytes in these models are adequately stimulated when the density of both live and killed pathogen exceeds the stimulation coefficient ($P + R \gg k$). This mechanism provides additional stimulation giving a more rapid and prolonged response, and an increase in memory cells and conferred protection.

2.3. Programmed Lymphocyte Divisions

To incorporate programmed cell division we modify the basic model above so that once stimulated, naive cells differentiate to effector cells and proceed through n divisions. To implement this we replace Eq. 4 with n equations:

$$\frac{dE_1}{dt} = 2\sigma N \frac{P}{k+P} - \sigma E_1 \quad (7)$$

$$\frac{dE_2}{dt} = 2\sigma E_1 - \sigma E_2 \quad (8)$$

$$\begin{array}{c} \vdots \\ \vdots \\ \frac{dE_i}{dt} = 2\sigma E_{i-1} - \sigma E_i \\ \vdots \\ \vdots \end{array} \quad (9)$$

$$\frac{dE_n}{dt} = 2\sigma E_{n-1} - \sigma E_n, \quad (10)$$

and $E = \sum E_i$ equals the total number of effector cells. The full model is presented in Appendix A.

A stimulated naive cell in this model expands into a population of 2^n effector cells regardless of the dynamics of the pathogen after the stimulation has occurred. In our simulations we use $n = 18$ divisions.

The numerical values of model parameters used in the simulations presented in the following sections are found in Appendix A Table A.1.

2.4. Correlates of Disease and Protection

As a measure of pathology we look at the maximum pathogen density (max-pathogen density). Max-pathogen density is a correlate of more complex quantities such as disease pathology and transmission. Other correlates of pathology such as time above threshold for pathogen or integral of the pathogen curve over time (accumulated pathogen), yield qualitatively similar results to what is presented in this paper and correlate well with max-pathogen density. For correlates of protection [22] we look at both accumulated memory cells and the projected max-pathogen density for a secondary infection.

3. Results

The results of the four models considered are qualitatively similar and we illustrate the features of the output with the killed pathogen model in Fig. 1. This figure shows primary (1° -solid red curve) and secondary (2° -dashed red curve) infections under three different treatment strategies: no treatment (A), prophylactic treatment (B), and delayed treatment (C). The lymphocytes (blue curve) are the sum of the naive, memory and effector cells. The infective dose of pathogen is identical in all six infections.

In the 1° exposure without treatment (Fig. 1A), the pathogen grows exponentially, stimulates lymphocyte expansion, and is then cleared. After pathogen clearance the lymphocytes decay into a stable population of memory cells. The number of memory cells

can be taken from the asymptotic value of the lymphocytes curve. The memory cells are abundant and provide excellent protection upon 2° exposure.

In Fig. 1B we see that prophylactic treatment prevents pathogen growth and no immunity is generated. Consequently there is no protection to a subsequent infection.

While exposure during prophylactic treatment gives negligible immunity, Fig. 1C shows the effect of delaying treatment for four days after exposure. On the left we see the effect of the treatment on the initial exposure. Once treatment begins the pathogen immediately declines. The max-pathogen density is reduced by nearly a factor of one thousand from a natural infection (Fig. 1A left). We also see a moderate accumulation of memory cells after the pathogen clearance.

The secondary exposure in Fig. 1C shows the likely outcome if the system is later re-exposed to the pathogen without treatment. In this case the max-pathogen density is again reduced by a factor of one thousand from the max-pathogen of an untreated 1° exposure (Fig. 1A). Thus the infection with delayed treatment has provided some protection against a possible future exposure.

We next performed a set of simulations similar to the one shown in Fig. 1C, varying the delay in the start of treatment. From these simulations we examined the amount of memory produced after the 1° exposure is cleared as a function of the start of treatment, and the max-pathogen densities for the 1° and 2° exposures. This was done for four different models, the basic model, a model with stimulation by killed pathogen, a model with programmed lymphocyte division, and a model with both programmed lymphocyte division and stimulation by killed pathogen.

Killed pathogen can stimulate the immune system until the antigenically active molecules decay or are removed from the system. This prolongs lymphocyte stimulation, generating greater numbers of memory cells and can function as a vaccine [23]. Not all disease systems will have appreciable amounts of killed pathogen when treatment is started and the antigens in some disease systems may decay more rapidly. The persistence of killed pathogen is typically more relevant for bacterial infections.

Programmed division is an important factor in CD8 T cell dynamics [12, 14, 15, 16]. When a CD8 T cell is stimulated it will proceed through several rounds of division even if the stimulating antigen is removed from the system. The programmed cell division models in this paper (Eqs 7–10) have a stimulated lymphocyte go through 18 rounds of division yielding 2^{18} effector cells for every stimulated naive cell. For other lymphocyte cells, such as B cells and CD4 T cells, programmed division may not be as important of an effect.

Fig. 2 shows the memory generation as a function of the delay in the start of treatment. The memory production is a monotonically increasing function of the delay in the start of treatment for all models, with an asymptotic value of memory production for long delays equal to the memory produced for an infection without treatment. The simulations show that all models are capable of generating memory under delayed treatment but that the longer we wait to begin treatment, the more memory will be produced.

Stimulation by killed pathogen gives an increase in memory production particularly for later treatment. This effect is due to the increased amount of killed pathogen when its density is high.

In the programmed division model memory production continues after the pathogen is cleared with treatment. The optimal memory creation strategy in disease systems with a

strong programmed response is to provide enough pathogen and time to stimulate many naive cells, then remove the pathogen with treatment.

Fig. 3 shows the tradeoff between pathology for a 1° exposure and a 2° exposure. This result was obtained from the same set of simulations used in Fig. 2. The figure shows the max-pathogen densities taken from those simulations for the 1° and 2° exposures as a function of the delay.

Early treatment (prophylaxis) gives a low max-pathogen density for the 1° exposure but a large max-pathogen density for the 2° exposure, whereas late treatment gives the opposite relationship. The values for late treatment are in correspondence with the values for no treatment, as treatment begun after the immune system has cleared the pathogen is ineffectual. For intermediate delays the tradeoff depends more heavily on the details of the model.

We can see from Fig. 3 that all four models have tradeoffs between 1° and 2° max-pathogen density that allow for treatment with a significant reduction in both. In relation to the max-pathogen density of an untreated 1° infection, both the 1° and 2° max-pathogen densities can be reduced by more than a factor of 100 (two-log reduction). To guide the eye we plotted the numerical value of the two-log reduction from an untreated primary infection (blue dotted line).

The window for when treatment can be started to reduce both the 1° and 2° max-pathogen densities with at least a two-log reduction, is shaded in blue in Fig. 3 for each of the four models. The models show us that when stimulation by killed pathogen is important, treatment can be started earlier and the window is wider. We also see that when there is a programmed response the treatment window is much wider and earlier than for the basic model. This corresponds to treatment strategies being more robust when these factors contribute to memory generation.

The immune system can also cause pathology. This is a result of cytotoxic T cells killing infected target cells and secreting toxic cytokines. In the Appendix we show that reduction in accumulated immunopathology has a very similar time dependence to the reduction in max-pathogen density. Treating to reduce max-pathogen density with a two log reduction also yields at least a two-log reduction in immunopathology.

The 2° max-pathogen density for the killed pathogen model (Fig. 3B) has a small increase around day 5. This is an effect of the approximations of the model and is discussed in Appendix A.

4. Discussion

Medical management of the symptoms of pathogenic infections typically takes two forms: immunizations and antimicrobial treatment. These are typically viewed as mutually exclusive, vaccination is given pre-exposure to prevent infection and treatment is given post-exposure to ameliorate the symptoms of infection. Occasionally however, vaccination can be used as a mechanism of treatment and treatment can play a role for vaccination. Post-exposure vaccination is used to treat rabies infection rapidly after the bite of a rabid animal [24] – it works because the vaccination induces protective immunity prior to the natural infection migrating from the site of the bite to the central nervous system. Post-exposure vaccination can also be used to treat smallpox if taken within a few days of exposure [25]. Infection with otherwise lethal doses of live *Theileria parva* pathogen followed by tetracycline antimicrobial treatment is used as a form of vaccination against Theileriosis [3].

The simulations of the previous section showed that delayed treatment could act as a vaccine, providing immunity and reducing pathology. For all models studied there was a tradeoff between reduction of 1° and 2° max pathogen densities that allows significant reduction in both. The four models each predict adequate treatment windows where delayed treatment gives a two-log reduction in both 1° and 2° pathogen density.

The additional factors incorporated into the models, stimulation by killed pathogen and programmed lymphocyte division, aid in the generation of memory. This increases the window of time where delayed treatment can reduce both the 1° and 2° max pathogen densities adequately. These mechanisms make treatment strategies more robust, going from a 24 hour treatment window without these factors, to a nearly four day window when both factors are prevalent. These predictions are not meant to be precise but rather to illustrate the importance of these factors. The extent to which these mechanisms are utilized in generating immunity may vary between different infectious diseases.

Standard antimicrobial treatment regimens are typically either pre-exposure prophylaxis or as soon as possible post-exposure [6]. This type of treatment is not likely to confer immunity and during an epidemic would require constant re-treatment. Delaying treatment acts as a vaccine and would presumably not require future attention. It may even prove advantageous for people to purposefully expose themselves, when a highly effective antimicrobial is available, in order to safely immunize in the absence of a vaccine.

This method of vaccination also has generality where it could work for any micro-parasite (bacteria and viruses) that stimulates adaptive memory and to which there exists an antimicrobial treatment. All that is required is a tradeoff between 1° and 2° pathologies that allows adequate reduction of both.

This vaccination technique would likely have the greatest utility in emerging epidemics where a vaccine is not yet available. The conditions and epidemiology of this type of scenario, such as an emerging influenza pandemic, are presently being researched by the authors. A key factor in the utility of the technique is the likelihood of re-exposure.

Those people with the highest likelihood of re-exposure would have the greatest benefit from this technique. Medical personnel for instance, who would be in frequent contact with infected persons throughout a pandemic, might be better protected and avoid the side effects of prolonged prophylactic treatment, with a vaccination by delayed treatment.

It is likely that much treatment already does come with some delay providing sufficient immunity. A better understanding of this dynamic will prevent unnecessary re-treatment. Testing can be performed to ascertain where a person is in the course of an infection and better predict when treatment should be performed. Medical personnel, who have the highest potential benefit from delayed treatment, also have the greatest access to frequent testing, being able to utilize this personalized approach. Medical personnel are also less likely to miss the treatment window and suffer the pathology of the untreated infection.

This study looks at treatment of acute infections. The use of this technique in a chronic virus infection is the subject of much current experimental and modeling research. During chronic virus infection T cells become dysfunctional and their population can fall [26]. The equations for the dynamics of immune responses during chronic infections are more complex than those presented here as they need to include immune exhaustion [27]. We also do not consider the tradeoffs between treatment and the generation of immunity in complex helminth infections where the parasite may have a complex life cycle with several stages. It is worth noting however that treatment of helminth infections can accelerate the development of immune responses [7, 8].

The simple models used in this paper are illustrative [28]. While we have attempted to use biologically reasonable parameter values, the precise pathology-immunity tradeoff can be sensitive to the parameters. The predictions on treatment window are therefore not meant to be precise but illustrate the dependence on factors such as persistent antigen and programmed division. The use of simple models presents a first step in the development of using treatment as a vaccine. While many of the results of this paper are simple and intuitive, the models show us the robustness of this type of vaccination. The models also illustrate the importance of characterizing the tradeoff between primary and secondary pathology to establish the robustness.

We now require measurements to determine the pathology-immunity tradeoff for delayed treatment. While this method could be used broadly to treat a range of infectious diseases, each disease would likely require a different set of parameters. Additionally we have used maximum pathogen density as a correlate of disease symptoms, but the exact relationship between the two is not known. Maximum pathogen is likely a good measure of transmissibility from one individual to another and limiting max-pathogen with this type of vaccination should limit transmission. Testing needs to be performed not only to establish the actual reduction in maximum pathogen density, but also how that relates to the symptoms of disease.

The concept of using delayed treatment as a vaccine is a generalization of the normal vaccine concept. It provides a different way of thinking about vaccination. More broadly a vaccine must provide immunity with minimal pathology. Killed pathogen does this by being low in quantity to limit immunopathology. Attenuated live vaccine is unlikely to grow large enough in number to cause pathology. With vaccination by delayed treatment we limit pathology by externally controlling the pathogen.

Highlights

We model how delayed treatment of infections can work as a vaccination >> Increasing the delay between infection and treatment leads to increasing the extent the vaccination provides protection but decreases the reduction in pathology >> We explore how this "tradeoff" between vaccination and reduced pathology depends on the features of the pathogen and immune response.

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Appendix A

Model Details

Immunopathology

The immune system itself can be a source of pathology as the cytotoxic T cells kill infected target cells during an immune response. We have modeled accumulated immunopathology as the accumulated loss of target cells (proportional to the accumulated amount of cytokine secreted) [27]. This quantity is given by the integral of the lymphocyte killing:

$$\int_0^{\tau_2} hP(t) [N(t)+E(t)+M(t)]dt. \quad (\text{A.1})$$

Fig. A.4 shows the results of simulations for accumulated immunopathology for the four models used in this paper. As in Fig. 3 we have performed a set of simulations like the one in Fig. 1C varying the start of treatment from day 0 to day 7. The set of times for starting treatment and reducing immunopathology for both the primary and secondary exposures, with a two-log reduction from the maximum immunopathology, are shown with the diagonal hash lines.

Figure A.4: Accumulated immunopathology for 1° and 2° infections from the four models. A set of simulations like Fig. 1C were performed using each model while varying the delay in the start of treatment. The diagonally hashed regions indicate treatment windows where both the 1° and 2° exposures have at least a two-log reduction (blue dotted line) in immunopathology. The reduction of immunopathology in the four models has very similar properties to the reduction of max-pathogen density shown in Fig. 3. We have included the treatment window for two-log reduction in both 1° and 2° max-pathogen density from Fig. 3 to show that treating to reduce max pathogen also treats to reduce immunopathology.

The dotted lines in each plot show the numerical value of a two-log reduction in immunopathology from that of an untreated infection. These are provided to guide the eye in our choice in treatment window for minimizing immunopathology. We have also included the treatment windows from Fig. 3 (Blue) for comparison that indicate the treatment windows for minimizing the maximum pathogen density. The figure shows that the treatment windows for minimizing immunopathology by two-log reduction are similar to

those for minimizing maximum pathogen density. As can be seen in the figure, treatment within the two-log reduction window for max-pathogen results in at least a two-log reduction in immunopathology.

Model Equations

The equations for the basic model are given in the text as Eq.s 1–5. The model equations for the model with killed pathogen are:

$$\text{pathogen } \frac{dP}{dt} = rP - hP(N+E+M) - aPA(t) \quad (\text{A.2})$$

$$\text{killed pathogen } \frac{dR}{dt} = hP(N+E+M) + aPA - \Delta R \quad (\text{A.3})$$

$$\text{antimicrobial } A(t) = \begin{cases} 0 & \text{if } t < \tau_1 \\ A_m & \text{if } \tau_1 \leq t \leq \tau_2 \\ 0 & \text{if } t > \tau_2 \end{cases} \quad (\text{A.4})$$

$$\text{naive cells } \frac{dN}{dt} = -\sigma N \frac{P+\alpha R}{k+P+\alpha R} \quad (\text{A.5})$$

$$\text{effector cells } \frac{dE}{dt} = (2\sigma N + \sigma E) \frac{P+\alpha R}{k+P+\alpha R} - dE \left(1 - \frac{P+\alpha R}{k+P+\alpha R} \right) \quad (\text{A.6})$$

$$\text{memory cells } \frac{dM}{dt} = fdE \left(1 - \frac{P+\alpha R}{k+P+\alpha R} \right). \quad (\text{A.7})$$

Here the killing terms from the pathogen equation feed directly into the killed pathogen equation which also includes a decay of killed pathogen from the system. The stimulation of lymphocytes is now a function of both the live and killed pathogen $P+\alpha R$, where the constant α is added to account for stimulatory differences between live and killed pathogen.

Setting $\alpha = 0$ gives a model where killed pathogen has no stimulative effect on the immune system. A non-zero value for α yields additional antigenic stimulation, generating a more rapid response and prolonged proliferation of lymphocytes. This gives rise to an increase in memory cell production and conferred protection after the infection is cleared.

Neither Eq.s 1–5 nor Eq.s A.3–A.7 have a memory recruitment term needed for activation of memory cells during a secondary exposure. This is discussed below under Secondary Exposures.

The programmed division model equations are:

$$\text{pathogen } \frac{dP}{dt} = rP - hP(N+E+M) - aPA(t) \quad (\text{A.8})$$

$$\text{killed pathogen } \frac{dR}{dt} = hP(N+E+M) + aPA - \Delta R \quad (\text{A.9})$$

$$\text{antimicrobial } A(t) = \begin{cases} 0 & \text{if } t < \tau_1 \\ A_m & \text{if } \tau_1 \leq t \leq \tau_2 \\ 0 & \text{if } t > \tau_2 \end{cases} \quad (\text{A.10})$$

$$\text{naive cells } \frac{dN}{dt} = -\sigma N \frac{P+\alpha R}{k+P+\alpha R} \quad (\text{A.11})$$

$$\text{gen.1 Effectors } \frac{dE_1}{dt} = 2\sigma N \frac{P+\alpha R}{k+P+\alpha R} - \sigma E_1 \quad (\text{A.12})$$

$$\text{gen.2 Effectors } \frac{dE_2}{dt} = 2\sigma E_1 - \sigma E_2 \quad (\text{A.13})$$

$$\text{gen.i Effectors } \frac{dE_i}{dt} = \begin{matrix} \vdots \\ 2\sigma E_{i-1} - \sigma E_i \\ \vdots \end{matrix} \quad (\text{A.14})$$

$$\text{gen.n Effectors } \frac{dE_n}{dt} = 2\sigma E_{n-1} - \sigma E_n \quad (\text{A.15})$$

$$\text{memory cells } \frac{dM}{dt} = f dE_n, \quad (\text{A.16})$$

where $E = \sum E_i$. Here we have included the killed pathogen term. The model with programmed division but not stimulation by killed pathogen can be obtained from these equations by setting $\alpha = 0$.

In this model, once a naive cell is stimulated to divide into 2 first-generation effector cells it is committed to another $n - 1$ cell divisions giving 2^n cells for every stimulated naive cell. The pathogen clearance rate is proportional to the total density of CD8 cells ($N + E + M$).

Model Parameters

The parameters used in the simulations of this paper are provided in the Table A.1.

Parameter	Symbol	Value	Units
Pathogen Growth	r	3.3	day ⁻¹
Lymphocyte Killing	h	1×10^{-5}	μl/cell/day
Antimicrobial Killing	a	1	kg/mg/day
Antimicrobial Conc.	A_m	5	mg/kg
Killed Pathogen Decay	Δ	7	day ⁻¹

Parameter	Symbol	Value	Units
Naive Cell Recruitment	σ	2	day ⁻¹
Stimulation Coefficient	k	1×10^5	cell/ μ l
Killed Pathogen Coef.	α	0 or 1	–
Effector Decay Rate	d	0.35	day ⁻¹
Memory Conversion	f	0.1	–
Programmed. Divisions	n	18	–
Initial Path. Dens.	$P(0)$	10	cell/ μ l
Initial Naive Cells	$N(0)$	200	cell/ μ l

Table A.1: Numerical values of model parameters. There are four models studied determined by the incorporation of two factors: stimulation by killed pathogen, and programmed lymphocyte division. Models that have stimulation by killed pathogen have the parameter $\alpha = 1$, and models where killed pathogen is inert have $\alpha = 0$.

Simulations

Primary Exposures—In simulating a primary exposure we set the initial number of memory cells and effector cells to zero. The initial values for naive cells and the infective dose of pathogen are found in Table A.1. The numerical integration of these equations was performed in Matlab using the ode45 routine.

Secondary Exposures—Throughout this paper we simulate secondary infections to ascertain the degree of protection provided by the memory cells generated during the primary response. Equations 1–5 do not include a memory recruitment term like the naive recruitment term in Eq. 3. This prevents the re-recruitment of memory cells during an immune response, which is known to not occur. Biologically the maturation of memory takes longer than the time of an immune response. To simulate a secondary infection we take the number of memory cells at the end of the simulation of the primary infection and add that number to the initial naive cell number found in Table 1.

This scheme gives equivalent results to a model with recruitment from memory, a source of naive cells and a naive cell decay term (that replenishes the naive population after the immune response), and an additional cell type that effector cells differentiate into, that slowly differentiate into memory.

Model Caveats—In continuous ODE models of immune responses the pathogen density will never reach identically zero. This is due to the nature that continuous models allow fractions of a single pathogen. It is frequently necessary in such models to truncate the results such that when pathogen density is reduced below a given threshold it is set to zero to reflect the discrete nature of the pathogen. In this paper all pathogen densities that descend to less than 10^{-1} are truncated to zero.

The basic and killed pathogen models (Eq.s 1–5 and Eq.s A.3–A.7) will have oscillations around a fixed point value for pathogen and lymphocyte densities. This is shown in Fig. A.4. In this case the oscillations occur around the fixed point of lymphocyte numbers $\approx r/h = 3.3 \times 10^5$, $P(t) \approx dk/\sigma = 1.75 \times 10^4$. For the parameters used in this paper the pathogen density during primary infections peaks much higher than this stable point and then descend below the threshold for extinction of the pathogen in the host (truncation of the pathogen density in the model).

For secondary infections with the basic model the number of naive and memory cells can give a max-pathogen density near the quasi-equilibrium value. In this case we get oscillations because the pathogen density does not fall to below the threshold for extinction. In these cases we limit our analysis to the first peak. These oscillations are not observed experimentally or with the more realistic programmed division models.

This effect produces the slight increase in 2° max-pathogen density Fig. 3B on day 5.

Figure A.5: Example of oscillations in basic model when lymphocyte numbers are approximately equal to r/h .

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Figure 1.

Primary (1°, left) and secondary (2°, right) infections simulated using the killed pathogen model for: natural infection without treatment (A), prophylactic treatment in the primary exposure (B), and delayed treatment in the primary exposure (C). In the untreated 1° infection (A) we reach a large pathogen density. This stimulates adequate memory production (right hand asymptote of blue curve) such that in the 2° exposure the pathogen is controlled. Under prophylaxis (B) the treatment during the 1° exposure prevents pathogen growth but this results in no accumulation of memory cells. The 2° exposure is then unprotected resulting in peak pathogen density equal to an untreated 1° infection (A). For delayed treatment in a 1° exposure (C) the treatment reduces the max-pathogen density and

a moderate number of memory cells accumulate. This provides limited protection against 2° exposures.

Figure 2.

Memory accumulation for four models. A set of simulations like Fig. 1C were performed using each model while varying the delay in the start of treatment. The density of memory cells following the primary infection is plotted as a function of the treatment delay. As the delay is increased the density of memory increases. The asymptotic value corresponds to the density of memory cells following an untreated infection. When killed pathogen can stimulate lymphocytes more memory is generated. Under programmed division lymphocytes continue to divide after pathogen is removed giving more memory for early treatment times.

Figure 3.

Maximum pathogen densities for 1° and 2° infections from four different models. A set of simulations like Fig. 1C were performed using each model while varying the delay in the start of treatment. Early treatment reduces 1° max-pathogen, but late treatment generates memory (Fig. 2) and reduces 2° max-pathogen. The blue regions indicate treatment windows where both the 1° and 2° exposures have at least a two-log reduction (blue dotted line) in max-pathogen density. When killed pathogen is included in the model the treatment window starts earlier because of the additional memory generated during early treatment. Programmed lymphocyte division gives an earlier and much wider treatment window as stimulated lymphocytes will continue to divide after antigen is removed.