# MATHEMATICAL MODELS OF COLONIZATION AND PERSISTENCE IN BACTERIAL INFECTIONS

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After decades of research focusing on infected patients and experimental animals, most modern research on microbial pathogenesis takes place at the level of cellular and biochemical mechanisms governing host-parasite interaction; however, studies at many scales will undoubtedly be needed for a deeper understanding of infectious diseases. For example, linking pathogen-specific information to that on the immune system will be critical for understanding the dynamics of most bacterial infections. Components of host-pathogen systems are sufficiently numerous and their interactions sufficiently complex that intuition alone is insufficient to fully understand the dynamics of the interactions. Here, mathematical modeling becomes an important experimental tool. In this chapter, we will focus on mathematical models of colonization and persistent bacterial infections. We will review the modeling method and the state of the field and then focus on three key areas where modeling has, and will continue to have, an impact: the ecology of the indigenous microflora and its plasmids, *Helicobacter pylori* colonization, and host-pathogen interactions with *Mycobacterium tuberculosis*. This is by no means a complete list of bacterial pathogens that have been explored with modeling; models of other bacterial infections will certainly emerge over the next decade and beyond.

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## MODELING PRINCIPLES

In many infectious diseases, particularly those arising from persistent infections with pathogens such as *M. tuberculosis* and *H. pylori,* we are far from understanding the mechanisms of disease progression. The strength of the modeling process is that it can lend insight and clarification to existing data and theories. Mathematical models thus provide a unique approach to representing and studying the integrated behavior of complex biological systems. The use of mathematical models also enables us to compare and contrast existing theories of the dynamic interactions in a complex system.

Mathematical models of host-pathogen dynamics are formulated on the basis of specific assumptions regarding the system's components and their interactions. In the same way that an experimental animal model can play a key role in our understanding of a human biological system (allowing for comparative biology), a mathematical model can lend valuable insights into complex interactions and reveal key governing parameters. An important dis-

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tinction to make is that unlike statistical methods that rely solely on the analysis of empirical data, mathematical models of host-pathogen interactions are based on assumptions about the host-bacterial dynamics and use data to estimate the rate constants that govern the interactions. Host-pathogen models are based on mechanistic assumptions and can therefore be used effectively to compare and contrast alternative hypotheses concerning mechanisms of pathogenesis. Thus, it should be understood that the main purpose of mathematical modeling is to determine the interplay among specific interacting factors in infection and not merely to achieve correlations within empirical data. A good model, therefore, uses parameters that represent defined biological entities (e.g., growth rates, nutrient uptake, etc.) rather than numerically derived values that merely serve to align the model solutions with experimental data. Also, a key strength of modeling is that it reveals various sensitivities to the parameters and initial conditions involved in the model, indicating which processes and interactions are dominant in the dynamics. For example, the outcome of an infection initiated with an inoculum of 10 bacteria might be shown to be qualitatively different from that of one initiated with $10^4$ bacteria, thus illustrating a sensitivity to this parameter. The choice of mathematical method is based on several considerations about the system being studied. For example, whether the time frame of a study is short or long, whether the population sizes are large or small, whether the system has randomness present or is strictly determinable, and/or the types of questions being posed about the system all determine the modeling technique that is most appropriate.

As in experimentation, modeling research develops by iterative refinement; thus, the models can progressively incorporate greater detail as it becomes available. A criticism of modeling is that the models are only as good as the knowledge, data, and assumptions which they are based on. This point actually highlights their strength in that specific hypotheses can be tested and compared. A successful mathematical model will not necessarily answer a question but instead will pose questions about the system. It also should suggest experiments that can be conducted to clarify understanding of the system. Once a host-pathogen system can be reliably described with a mathematical model, it becomes possible to explore the effects of perturbing elements of the system that may be problematic, or even impossible, to address experimentally. If the predictions of a mathematical model are incompatible with experimental data and the underlying theory, it proves conclusively that the theory is incomplete or faulty. However, if the predictions of a mathematical model agree with the data, this represents strong evidence for the correctness of the theory but does not itself constitute conclusive proof of its validity.

Some of the earliest mathematical modeling, of population growth, was done in 1798 by Malthus. The simple idea he used was that of exponential growth. Exponential-growth models assume that the rate of change of a population at time $t$, namely, $P(t)$, is proportional to itself, and this can be represented mathematically as

$$\frac{dP(t)}{dt} \quad kP(t) \qquad 1)$$

where $k$ is the growth rate constant of that change. The mathematical solution to this differential equation, where $P_0$ represents the initial population size, is $P(t) = P_0 e^{kt}$. The graph of this function is shown on a log scale in Fig. 1B.

Although Malthus was attempting at the time to predict how the human population was growing, this proportionality assumption could be applied to other populations, such as those of bacteria. Of course for any population, this model of exponential growth cannot hold true over a long time frame. For example, the actual growth curve of bacteria is given in Fig. 1A. Thus, the model should be modified to include greater complexity about the system to better capture known dynamics. This modification elaborates a key step in the modeling processes—that of iteration. Finer as well as

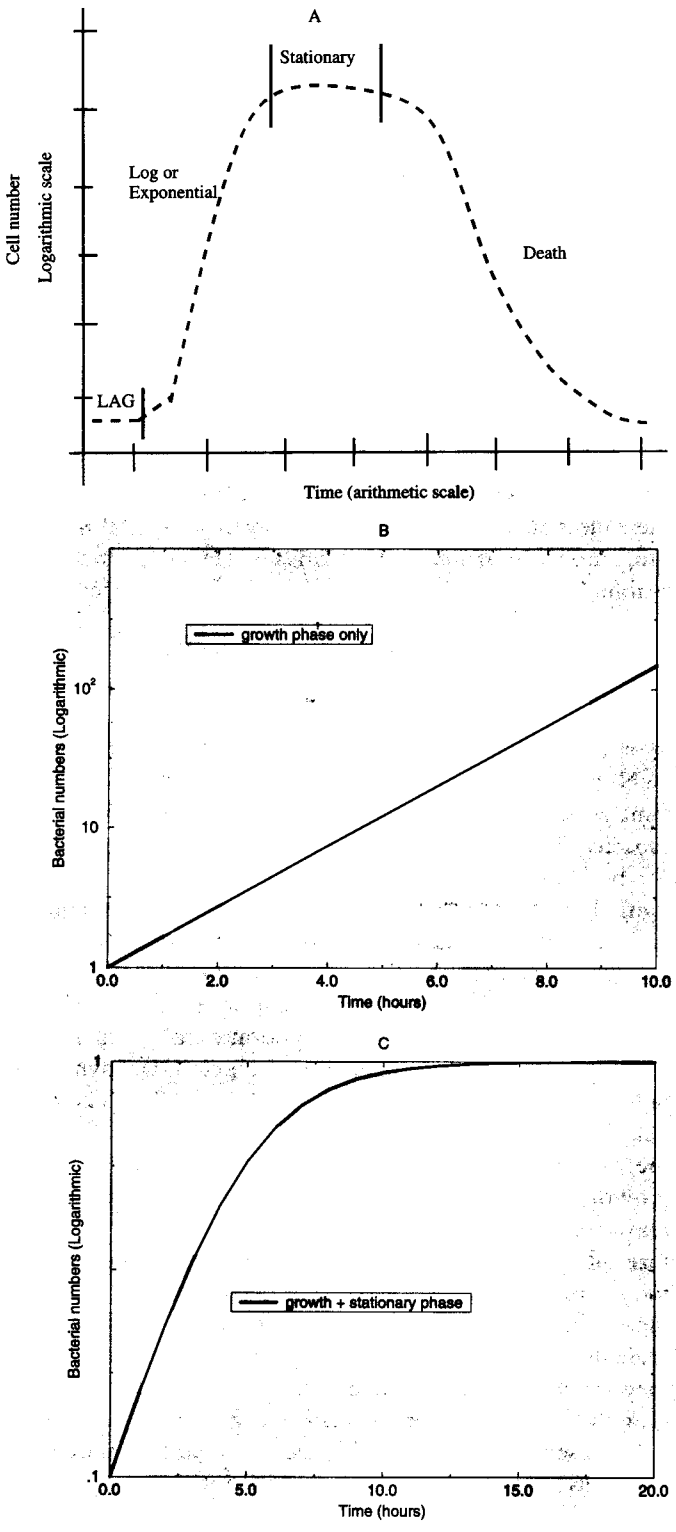


**FIGURE 1** (A) Classical complete growth curve for bacteria. (B) Model of exponential growth phase only. (C) Model of exponential and stationary phases of growth.

broader details can be included in the system to better capture its behavior. To that end, in our example of population growth, we consider the concept of a population carrying capacity, defined as $K$. This is a general term that can encompass various elements affecting the growth of the population. For example, consider the situation of bacteria growing in culture. These bacteria are limited by, among other things, the volume of the culture (which could moderate toxic effects) as well as the availability of oxygen and nutrients in the medium (which could account for competition between bacteria). The parameter $K$ would then be a measure of these elements in the particular experimental system. To introduce these ideas mathematically, we multiply the existing model, equation 1, by a term, $1 - (P/K)$, as follows:

$$\frac{dP(t)}{dt} = kP(t)\left(1 - \frac{P(t)}{K}\right) \qquad (2)$$
$$= kP(t) - \frac{k(P(t))^2}{K}$$

Notice that the growth term remains the same as before (i.e., $kP$), but now the carrying capacity, $K$, and interaction between bacteria, $P^2$, both inhibit that growth (via the minus sign). The mathematical solution of the differential equation in equation 2 is not as tractable as our first model (equation 1); this is due to the now nonlinear nature of the equation (i.e., the $P^2$ term). The graph of the solution function can, however, be obtained through computer simulation and is given in Fig. 1C. Notice that through this simple modification to the system we are able to predict both the growth and stationary phases of the well-known bacterial growth curve. To fully capture all the stages (including lag and death) shown in Fig. 1A, we would again have to modify the model. While the equations can accurately trace the bacterial growth curve, they are not yet useful as models to understand growth, because the parameters $k$ and $K$ were not chosen as functions of basic biological mechanisms. This could be improved, however, by expressing $k$ as a function of nutrient concentration, temperature, rates of nutrient uptake, etc., and $K$ as a function of the accumulation of toxic metabolites, oxygen concentration, etc.

Testing and validation are other elements of the modeling process. A key method for addressing these lies in the comparison of the model output with experimental and/or clinical data. The typical goal of the modeling method is to determine if the assumptions about the interactions of the elements of the system lead to the dynamics seen either clinically or experimentally. This would then indicate that the interactions included in the model sufficiently capture some of the key biological dynamics. It should be noted that many times if data are not available with which to test the model, the model itself can suggest which experiments are needed.

No other modeling of population growth was done until the mid- to late 20th century. Recently, models of host interactions with microbes have begun to appear, including relatively few models that explore bacterium-host-level interactions (9, 11, 26, 27, 38, 39, 50, 51). We will discuss in detail below the key findings of some of these models. The models by Lipsitch and Levin (50, 51) focus on antimicrobial chemotherapy, while the one by Gordon and Riley (27) is a first work on urinary tract bacterial infections. Two other models by Antia et al. (7, 8) explored mycoparasite immune dynamics. The first of these (8) considered the dynamics of parasites during acute infections. The model incorporates a generic population of parasites together with an immune response. The investigators assumed that the virulence of the organism is proportional to its growth rate in the host. Their results indicated that optimal transmission of parasites would result if the parasite had an intermediate rate of growth (not high as in *Escherichia coli* or low as in *M. tuberculosis*), and they argue that this would result in the evolution and maintenance of an intermediate level of parasite virulence. Their second model (7) considered a different set of hypotheses for the dynamics of persistent mycobacterial infections. This model predicted that

the initial persistence of the parasite may be achieved by very-slow-growing parasites or by parasites having a refuge that is inaccessible to the immune response. They also suggested that escape from immune control at a later time might be a consequence of two processes: antigen deletion of T cells in the thymus and the presence of a limit to the maximal number of divisions a T cell can undergo (i.e., a "Hayflick limit"). In their scenario, the persistent parasite antigens prevent the generation of new parasite-specific cells from the thymus and the existing parasite-specific cells are eventually eliminated as they reach the Hayflick limit.

Epidemic models of infectious diseases have been developed since the middle of the 1900s. Hundreds of mathematical models have been published exploring the effects of both bacterial and viral pathogens on different subgroups of human populations. Many of the results have defined paradigms in epidemiology, such as the notion of a core population in sexually transmitted diseases (31) as well as ways to determine herd immunity levels for vaccination policies (3). Relating to persistent bacterial infections, key pathogens that have been studied are *Neisseria gonorrhoeae* (31), *M. tuberculosis* (13, 14, 16, 17, 58), and *Treponema pallidum* (10). Such important issues as drug resistance, rate of spread of infection, trends of the epidemics, and the effects of treatment and vaccination all have been insightfully addressed through these modeling approaches.

Models of persistent viral infections, namely, human immunodeficiency virus (HIV)-host models, also have a successful recent history. Many of the key results that have shaped our understanding of the T-cell and viral dynamics in HIV disease have come from mathematical modeling approaches (32, 68, 75). Many others have provided insight into HIV-immune system dynamics as well as disease progression (1, 4–6, 40–46, 54, 55, 63, 64, 67). For example, a recent model developed by one of us (D.E.K.) examined the role of the thymus in pediatric HIV type 1 infection (41). Until this work, there was no clear explanation for the different disease progressions in pediatric versus adult HIV infections. The model was able to show that infection in the thymus not only can supplement peripheral infection but can help explain the faster progression in pediatric cases, as well as the early and high viral burden. This is based on the fact that the thymus is most active in children and involutes in adulthood. Subsequent clinical data have confirmed that the thymus does play a key role (60).

## COLONIZATION BY BACTERIA IN THE LARGE INTESTINE

Two studies by one of us (R.F.) lend themselves to illustrating that some problems in persistent infections (or, for that matter, in any area of microbiology) can be studied most effectively by integrating experimental or clinical observations with mathematical modeling. In the following discussion, we demonstrate some of the unique contributions mathematical modeling can make to the study of complex problems in host-microbe interactions. Space does not permit a detailed recounting of the experimental details of each investigation; these are available, however, in the original publications (23, 25, 26).

The indigenous microflora of the mammalian large intestine is a stable ecosystem, comprising more than 400 different kinds of bacteria, most of them strict anaerobes. The study of the indigenous microflora represents a subspecialty of ecology—the science that considers the relations and interactions of organisms with their environment and with each other. The microflora is usually in the climax stage of ecological succession, meaning that it will prevent colonization by exogenous bacteria, including potential pathogens, entering from the environment. Because of this colonization resistance, the microflora forms a host defense mechanism in the intestine that is even more effective than the much better understood immunological mechanisms. Colonization is the first step in the pathogenesis of persistent (as well as most other) infections, and for this rea-

son, insight into the principles underlying colonization by the indigenous flora is a necessary step toward an eventual understanding of the main topic of this volume. In the absence of a generally accepted definition, we define colonization here as the state in which the population size of the colonizing microorganism in (or on) the host remains constant, i.e., when the number of microorganisms that are killed or otherwise removed from a given site is precisely compensated for by multiplication of the remaining microorganisms.

Colonization of the large intestine by several hundred different kinds of bacteria is obviously a complex process involving many parameters, such as microbial multiplication rates, nutrient concentration, rates of adhesion to the gut wall, rate of removal by intestinal peristalsis, etc. Critical to the performance of natural ecosystems is the manner in which these various elements interact. Such interactions are usually difficult to appreciate when the individual microbial populations, and the ecological mechanisms controlling them, are studied in isolation rather than under the physiological and ecological conditions existing in a complex environment harboring these diverse populations. For example, under the different conditions prevailing in various natural environments, such as the lumen or the wall of the large intestine, a parameter that is potentially able to control the population size of an indigenous bacterium may be quantitatively most important or it may be partially or totally eclipsed in effectiveness by other mechanisms. For this reason, colonization is still imperfectly understood, with most investigators studying individual mechanisms.

In the first study to be discussed, we used an in vitro model as well as a mathematical model. The in vitro model was an anaerobic continuous-flow (CF) culture of the entire flora of a mouse cecum. Such a culture had been shown to duplicate bacterial interactions as they occur in the mouse large intestine (23). The mathematical model of this system made the following assumptions.

1. A resident strain of *E. coli* colonizes the large intestine (or CF culture). An invader strain is ingested once, and in large numbers.
2. Resident and invaders have exactly the same properties.
3. Both strains compete for the same adhesion sites on the wall of the intestine or CF culture.
4. Offspring of adherent strains occupy additional sites or, when most sites are filled, are shed into the lumen.
5. Adhesion is reversible, and adherent bacteria are slowly shed into the lumen.
6. Both resident and invader strains compete for the same limiting nutrient. (Thus, the relations between growth rates and limiting-nutrient concentrations were modeled by classical Monod kinetics.)

In a typical experiment, such as that shown in Fig. 2, normal mice were inoculated with a culture of the *E. coli* invader strain (marked with streptomycin resistance). Inoculation was directly into the stomach by means of a blunt feeding needle. At intervals thereafter, the ani-

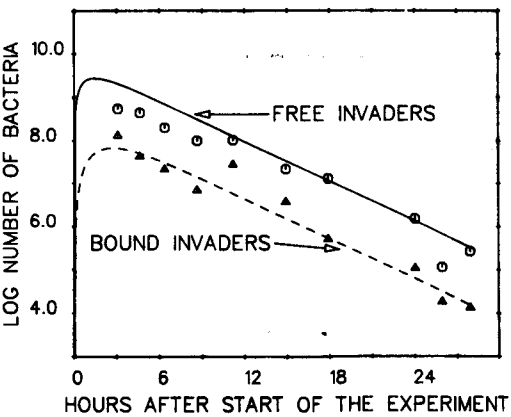


**FIGURE 2** Passage of *E. coli* invaders through mouse cecum. The symbols represent experimental data: the circles are the bacteria suspended in the lumen, and the triangles are the adherent population. The curves represent the best-fit estimates generated by the mathematical model for each of the two experimental populations. (Reprinted from *Microecology and Therapy* [25] with permission from publisher.)

mals were euthanized and the number of invaders in the lumen or adherent to the wall of the large intestine was determined by culture of the homogenized specimens. In Fig. 2, these experimental data are represented by symbols. The mathematical model of this system was also employed. The model system was solved, and its output was run through a computer program that incrementally varied the parameters of interest (e.g., the rate constants of adhesion and elution) until an optimal fit to the data was obtained (shown by the curves in Fig. 2).

The parameter estimates thus obtained (e.g., the rate constants of adhesion and elution, flow through the system, multiplication, etc.) were then incorporated into the mathematical model. The output of the model was then studied to answer some of the original questions we posed. For example, the major feature of the indigenous flora is that it confers colonization resistance. This is seen in Fig. 2, where the population size of the invaders decreases without their ever being able to colonize (i.e., to achieve a stable population size). This is in spite of the fact that the resident *E. coli* bacteria in these experiments (not shown) formed stable populations (which is part of the definition of a normal mouse). In light of the importance of this feature for human and animal health, numerous hypotheses have been proposed over the past decades to explain the elimination of invaders by the indigenous flora, e.g., the production of toxic substances or competition for nutrients or for adhesion sites. Unfortunately, none of these hypotheses were able to account for the observation that whatever mechanism caused the invaders to be eliminated had no effect on a physiologically identical resident. The explanations furnished by the mathematical model are shown in Fig. 3 (top).

As may be seen in Fig. 3, when an invader strain is introduced at the 50-h mark, it is eliminated rapidly from the mouse intestine. This is in spite of the fact that the rate of elimination of bacteria from the large intestine is lower than the optimal growth rate of the bacteria. In other words, in the absence of an indigenous

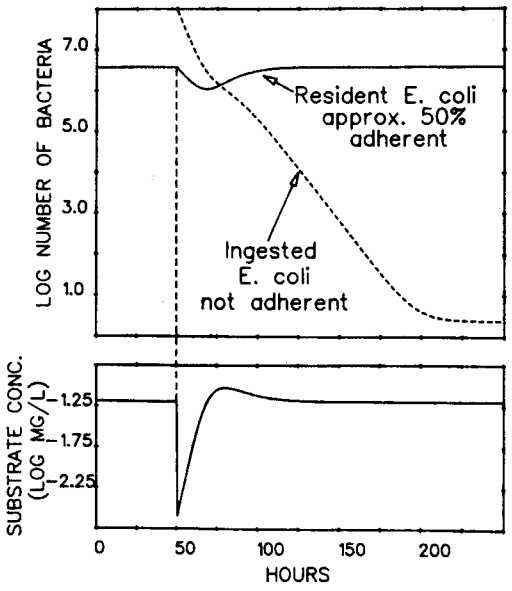


**FIGURE 3** (Top) Prediction by the mathematical model of the fate of an *E. coli* strain that invades the large intestine of an animal that already harbors an adherent *E. coli* resident strain. (Bottom) Concentration of limiting nutrient in the system. (Reprinted from reference 24 with permission.)

flora, the invader strain can form large stable populations in the lumen, even without adherence to the gut wall. Moreover, as mentioned above, resident and invader strains have the same properties (in paired experiments in mice one can exchange the strains used as residents and invaders without changing the finding that it is the invader that is always eliminated). Why then is the invader strain at such a striking disadvantage? The mathematical model shows that the large number of invaders causes a temporary decrease in the concentration of the limiting nutrient (Fig. 3, bottom), but when the nutrient concentration quickly returns to normal, the invader population is still decreasing. The mathematical model furnishes an explanation for this phenomenon. In a CF culture, or the large intestine, a large portion of the resident strain adheres to the wall. In the adherent state, its rate of elimination by the flow of nutrient through the gut or through

the CF culture is lower than that of bacteria suspended in the lumen. Consequently, the growth rate that an adherent strain must achieve in order to maintain a constant population (i.e., to have a growth rate equal to its rate of elimination) must be lower than that required for a suspended, nonadherent population. According to classical CF culture theory, the concentration of limiting nutrient that will establish itself in the culture will be exactly that which will allow the resident strain to maintain a growth rate equal to its rate of elimination. However, when an invader strain having the same properties as the resident invades, it will initially be suspended in the lumen and, consequently, have a higher rate of elimination. For this reason it would require a higher concentration of nutrient to maintain its population at a constant level. Not finding an adequately high nutrient concentration, the invader strain will grow too slowly and will be eliminated. Elimination will continue until the small remaining invader population has found adhesion sites and has subsequently achieved the lower rate of elimination typical of adherent bacteria (at about 200 h [Fig. 3]). Consequently, it will then be able to maintain a constant population at the prevailing nutrient concentration. The mathematical model indicated that this slow adhesion was not due to a lack of free adhesion sites but rather was a function of the relatively low rate constant of adhesion of the invader strain. In other words, the rate of adhesion of the invader was so low that most of its population had already been eliminated by the time it could form significantly large adherent populations.

The mathematical model further indicated that in the absence of adhesion on the part of the indigenous microflora, there would be no resistance to colonization by invading bacteria. Thus, adhesion of bacteria in the large intestine is not required for colonization because of the low rate of elimination of contents from this organ (in contrast to colonization of the small intestine), but adhesion in the large intestine is required for the protective function of the indigenous flora against colonization by invading bacteria.

This theory was developed with the aid of mathematical modeling. Was the mathematical model essential? In retrospect, it is not entirely impossible that the relationship among adhesion, nutrient concentration, and resistance to colonization could have been derived through experimentation alone. However, the constant guidance obtained through mathematical modeling made the study much more efficient.

## PLASMID TRANSFER AMONG BACTERIA IN THE LARGE INTESTINE

The second example to illustrate the role of a mathematical model is a study of plasmid transfer among bacteria in the large intestine. As in the first study, it involves CF cultures as in vitro models, in vivo animal experiments (which gave essentially similar results), and published data from human experiments. Genes for drug resistance and virulence factors of bacteria are often located on plasmids, as are sequences inserted by recombinant DNA techniques. Plasmids may then transfer to other bacteria, thereby increasing the genetic complements of their new host microorganisms. When plasmids specify resistance to antibiotics or virulence factors, there is a strong possibility that plasmid-bearing bacteria may transfer such genetic components in vivo to normally saprophytic members of the indigenous flora of the large intestine, with potentially disastrous results. Because of this, a constant stream of studies concerning plasmid transfers in vitro and in vivo were published until about the mid-1980s. The resulting conclusions were consistent but rather confusing. Nevertheless, interpretation of that literature clearly permits the following generalizations to be made. Even among pairs of bacteria that readily permit plasmid transfers in vitro, very little or no plasmid transfer occurs in the normal gut, i.e., one that is colonized by an undisturbed microflora. In contrast, when the microflora is absent, as in germfree or newborn animals, or when it is incomplete or disturbed, as in the very young or in antibiotic-treated animals, then plasmid transfer can be observed as readily as during in vitro matings.

The fact that a normal intestine does not readily permit plasmid transfer has given rise to numerous speculations about the reason, such as that inhibitors of plasmid transfer are produced by organisms of the indigenous microflora, especially short-chain fatty acids; that the anaerobic conditions prevailing in the large intestine may be nonconducive; that the growth phases of donor and recipient bacteria may be different; and that the inherent efficiency with which potential recipients can accept a plasmid, the demonstrable negative effect of some plasmids on the growth rate of their host bacteria, and fragmentation or segregation of the plasmid in vivo are all relevant (see reference 26 for a review and further references). Contradictory experimental results made it impossible to make definitive choices among these various hypotheses. The major obstacle to progress was the lack of a rational method of describing the fertility of a given pair of donor and recipient strains for a given plasmid. A major step forward was made by Levin and Rice (47), who developed a mathematical model based on mass-action kinetics and determined the transfer rate constants for various plasmids in mixed static and CF cultures containing only the recipient and donor strains. The transfer rate constant ($\gamma$) was then taken by these authors as a measure of the fertility of a given mating. Thus, the transfer rate constant, $\gamma_1$, was determined as $dN_*(t)/dt = \gamma_1 N_+(t)N(t)$, with $N_*(t)$ denoting the concentration of transconjugants, $N_+(t)$ representing the concentration of the original donors, and $N(t)$ being the concentration of recipient bacteria. In the pure-culture experiments by Levin et al. (48), plasmid transfer occurred quite rapidly, so that these authors could neglect the contribution from recent transconjugants. In contrast, transfers in the presence of the indigenous microflora were slow, and the contribution of recent transconjugants became significant. That was particularly true with those plasmids whose fertility is increased for a few generations after transfer. Accordingly, a second transfer rate constant was defined by us (24) as $dN_*(t)/dt = \gamma_2 N_*(t)N(t)$. The concentration of transconjugants in a CF culture of mouse intestinal flora or in the mouse gut itself was then described as a combination of the two models, namely, $dN_*(t)/dt = \gamma_1 N_+(t)N(t) + \gamma_2 N_*(t)N(t) + (\psi_* - \rho)N_*(t)$, with $\psi_*$ representing the rate constant of multiplication of the transconjugants and $\rho$ representing the flow rate of contents through the CF culture or the gut.

The mathematical-modeling experiments were conducted in a manner analogous to the one described above, and the best-fit transfer rate constants were determined. This model was not very efficient in matching the experimental data points to those calculated. Much better results were obtained when additional terms were introduced to account for the depression of fertility of transconjugants for a few generations after transfer of the plasmid, the loss of donor population to transconjugants, and the segregation of the plasmids. In a total of 68 experiments in CF cultures and 5 in vivo experiments in the mouse gut, the most surprising result was that fertility, defined above as the transfer rate constants for a given mating, was not appreciably different in CF cultures free of indigenous flora, in CF cultures of normal mouse flora, and in normal mice. These results imply that the environment of the gut, contrary to common intuitive assumptions, did not impair plasmid transfer at all. The low plasmid transfer rates in the gut were entirely due to the kinetics of the gut environment, i.e., low concentrations of donors and/or recipients. As a final test, data published by E. S. Anderson (2) on human volunteers were inserted into the mathematical model (Fig. 4). Anderson had fed plasmid-bearing *E. coli* bacteria to volunteers and was able to recover transconjugants only on the first day after ingestion of the donor strain by the volunteers. Our model shows that this would be expected if transfer in the human gut were analogous to that in the mouse or in CF cultures of mouse flora.

The sensitivity of the culture method employed by Anderson was 10 bacteria per ml (or g) of feces, a concentration which was found only once, on day 1. For subsequent days, the mathematical model postulated lower numbers

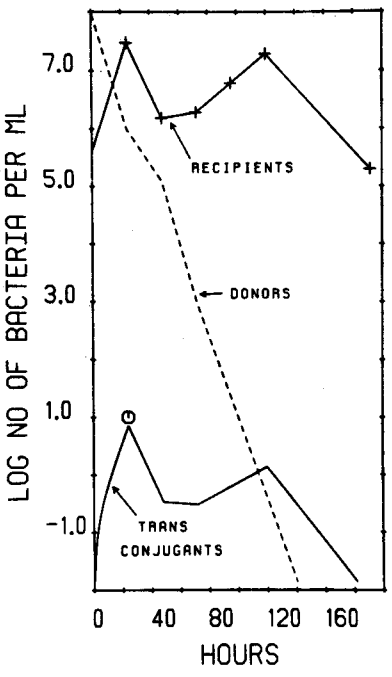


**FIGURE 4** Modeling of plasmid transfer in the human gut, based on data published by Anderson (2). The symbols represent Anderson's data; the lines were calculated by the mathematical models based on parameters derived from computer-generated best-fit estimates for mice and from CF cultures of mouse intestinal floras. (Reprinted from reference 26 with permission.)

of transconjugants in the stool. These were not detected by the culture methods used, prompting the earlier authors to assume that all transconjugants had disappeared. This and other reconstructions of human experiments reported in the literature are consistent with the conclusion that the quantitative aspects of fertility and plasmid transfer in the human gut are similar to those in our mice and CF cultures. It appears that plasmid transfer occurs consistently in the human gut but that the resulting transconjugant populations are too small to be detected with the culture methods available to earlier investigators.

The latter study is an example where the availability of a mathematical model was a necessary condition for success. There was no other way to have arrived at the meaningful parameters and the transfer rate constants without the mathematical model or the computer to perform the tedious calculations.

## DYNAMICS OF *H. PYLORI* COLONIZATION

*H. pylori* is a bacterial pathogen of the gastrointestinal tract that persists for decades. *H. pylori* induces chronic gastric inflammation that results in peptic ulcer disease or gastric cancer in a small set of infected persons (29). *H. pylori* is tropic for the acid-rich stomach, which is essentially sterile, and the immune response, although present (22), appears to be ineffective (19). A key question, then, is how can *H. pylori* colonize this environment in the face of peristalsis and very low pH?

To address this issue, we posed a regulatory feedback system based on both bacterial and host characteristics as a mechanism enabling *H. pylori* to colonize. We then created a mathematical model to explore this theoretical construct. It indicates that the proposed feedback network produces the observed colonization as well as ruling out other conceptual models of persistence (39). A summary of the modeling results is presented below.

### Model of Colonization and Persistence

Adherence is a virulence attribute for many pathogenic bacteria, and in particular, for gastrointestinal pathogens that must evade peristalsis or sloughing. For *H. pylori*, adherence plays a key role in survival, since the mucus layer in which most *H. pylori* organisms reside is washed away multiple times per day (62). *H. pylori* adheres to the gastric epithelium lining the lower stomach and forms adherence pedestals (30, 73). These epithelial cells are also sloughed, although at a lower rate than mucus is shed (49). We assume that the adherent phenotype is more advantageous than the free-swimming phenotype because of proximity of the adherent bacteria to nutrients, a lower washout rate, and the fact that the pH at the epithelial cells is in the range for bacterial growth. Thus, the model incorporates migra-

tion of *H. pylori* from the mucus compartment to adhere to the cell surface. Since the adherent *H. pylori* cells divide, and the carrying capacity of the tissue is most likely near saturation, most of the new daughter cells must migrate back into the mucus layer. Thus, in our model of *H. pylori* colonization, the small portion of the bacterial population that adheres to the epithelial cells is crucial to persistence. In comparing these ideas with the situation in the colon, it has been assumed that the indigenous colonic bacteria associate with the mucus gel and epithelium; however, they do not serve as a reservoir for the mucus-living bacteria, as is specified in the *H. pylori* model. Further, microbial colonization of the colon and stomach is dissimilar in environmental pH, nutrient sources, and interspecies competition, among other factors. Thus, this model of colonization is unique to *H. pylori*.

Recent estimates of the population size of colonizing *H. pylori* in the mucus gel range from $10^4$ to $10^5$ per $mm^3$ (62). In the same study, histological assays indicated that the *H. pylori* population on the epithelial layer ranges from $10^1$ to $10^3$ per $mm^3$. Thus, we assume that free-living and adherent *H. pylori* cells represent 99 and 1% of the *H. pylori* populations, respectively. This high ratio of mucus-living to adherent bacteria, although characteristic, is not necessary for colonization, as low concentrations of *H. pylori* may be present in the mucus during persistence. We show with our model that it is the adherent population that serves to sustain colonization by acting as a core population (where a small proportion of the population serves to sustain an epidemic, as in sexually transmitted diseases) and that the mucus population acts both to replenish the adherent population and for transmission to new hosts.

## The Theoretical Construct

To describe the complex interactions between *H. pylori* and the host, we propose a model in which these colonizing organisms together with the host regulate their responses in both a positive and a negative autoregulatory fashion (Fig. 5).

In this model, bacteria release proinflammatory effectors (such as urease), increasing the pH of the local environment and provoking a host response that leads to tissue damage (via inflammation) with subsequent nutrient release; the bacteria then grow in response to this growth-limiting nutrient. However, in the long term, uncontrolled inflammation may be deleterious for *H. pylori*, since its niche would be lost (33, 36). Experimental observation indicates that *H. pylori* surface molecules, such as lipopolysaccharide, have low proinflammatory activities (57). Thus, we assume that *H. pylori* can down-regulate effector production. Released host nutrients may also activate *H. pylori* signal transduction pathways that repress synthesis of bacterial proinflammatory effectors (12) and nitrogen repression (20) of cloned *H. pylori* urease; this is consistent with our hypothesis, since urease and its products have proinflammatory activities (52, 72). Inflammation may be damaging when infection cannot be eradicated, leading to impairment of tissue structure and function. Experimental data show that the cellular response to *H. pylori* infection appears to be suppressed even in the early stages of colonization (37, 70). The elements of this highly regulated feedback model are summarized in a schematic diagram in Fig. 5.

Using this proposed feedback model for *H. pylori* colonization, we created a mathematical model that examined these interactions and described the sensitivity of the system to changes of the interaction rates.

## THE MATHEMATICAL MODEL

We define four populations and describe their interactions by using differential equations that monitor their rates of change, where $M(t)$ is the concentration of *H. pylori* cells in the mucus gel per cubic millimeter at any time and $A(t)$ is the concentration of *H. pylori* cells adherent to the epithelial cells per cubic millimeter. We also define $N(t)$, representing the nutrient concentration (assumed proportional to inflamma-

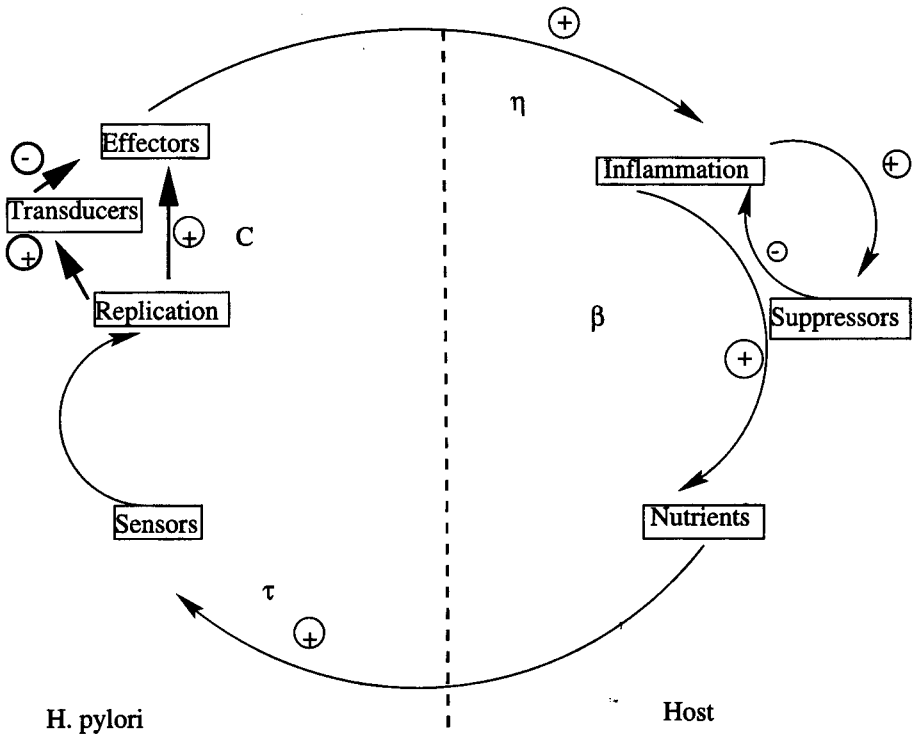


**FIGURE 5** Theoretical model describing interactions of *H. pylori* with the host, incorporating positive and negative feedback regulation. Effectors released by *H. pylori* interact with the mucosa and induce inflammation. Inflammation leads to the release of nutrients that are taken up by *H. pylori*, allowing replication and further release of effectors. The bacteria sense inflammation indicators and down-regulate effector production, while the host also down-regulates the inflammatory response. The interactions within this system are governed by the four parameters $\tau$, $C$, $\beta$, and $\eta$, which are not presently measurable. Therefore, mathematical modeling can play the unique role of elaborating these host-pathogen interactions. (Adapted from reference 1.)

tion), and $E(t)$, the total effector concentration released by *H. pylori* that leads to inflammation and nutrient release. A schematic representation of the mathematical model is given in Fig. 6.

## PARAMETER ESTIMATION

To complete the development of a mathematical model, we must define values for the parameters and initial conditions for the rate constants in the model. This is a key place where experimental results are incorporated into the models. We chose millimeters$^{-3}$ as the units marking the volume of population concentrations and measured time in days. Note that the model is robust with small changes in the choices of these parameter values. We illustrate the process of estimation for some of the key parameters in the model. As mentioned above, during colonization *H. pylori* density ranges from $10^4$ to $10^5$ per mm$^3$ in the mucus gel and from $10^1$ to $10^3$ per mm$^3$ on the epithelial layer (62); thus, we select the initial population size of mucus-living *H. pylori* to be $10^5$/ml and that of the adherent bacteria to be 500/ml. Epithelial cells slough every 2 to 3 days (37); thus, the rate ($\mu_A$) is 0.3/day. Estimating that the mucus sheds at a rate at least two to three times higher than that of the epithelial cells, $\mu_M$ is 0.85/day. The growth of *H. pylori* can be determined from the doubling time based on logistic growth (see equation 2). If we assume the in

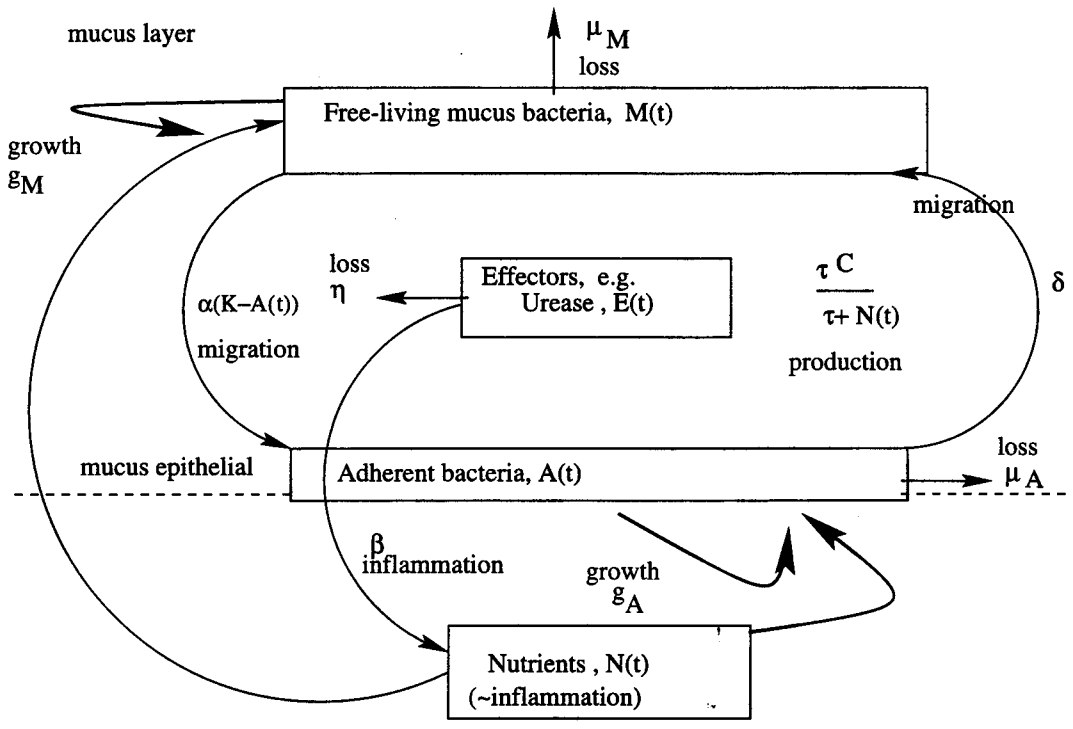


**FIGURE 6** Mathematical model describing the interaction of *H. pylori* and host. Mucosal bacteria, $M(t)$, grow proportionally to nutrient at rate $rg_M$ and are cleared continuously by peristalsis at the rate $\mu_M$. They also migrate to the adherent sites [at rate $\alpha(K - A(t))$] and gain in numbers due to migration from the adherent sites (at rate $\delta$). Adherent bacteria, $A(t)$, follow a similar dynamic, with opposite migration. Nutrients, $N(t)$, are produced proportionally to effector amounts (at rate $\beta$) and are taken up by the adherent and mucosal populations (at rates $g_M$ and $g_A$, respectively). Effectors are produced by both mucosal and adherent bacteria [at rate $\tau C/\tau + N(t)$] and degrade nonspecifically at rate $\eta$.

vivo doubling time of *H. pylori* is 1 h ($D$ = 0.0416 day), then using the formula $r = \ln 2/D$, the growth rate ($r$) is 16.66/day.

The four parameters, $C$, $\beta$, $\eta$, and $\tau$, defined in the feedback system (Fig. 5) play a key role in the dynamics of the system; values for these parameters are not presently known and cannot be experimentally measured. Except for the parameter $\tau$, they are each bifurcation parameters, i.e., changes in their values can cause significant change in the resulting dynamics. This is not surprising, since a fine-tuned feedback system may be crucial for the unprecedented survival of *H. pylori* in the human stomach.

To study the model, we numerically solve the complex mathematical system that describes the scheme in Fig. 6; the time-series solution, showing the system immediately going into steady state, is shown in Fig. 7. Thus, the bacteria have completely colonized the system and are in equilibrium (c.f. reference 39).

This model can now be tested for a variety of different influences, such as competition between strains, host perturbations, and other biological variations. For example, we study the question of competition between different *H. pylori* strains. Clinical studies indicate that humans may be simultaneously colonized with (at least) two different strains of *H. pylori*. For example, *cagA*$^+$ and mutant *cagA* (34, 74) have been shown to be associated with different outcomes of infection (34, 74). We found with the mathematical model of these interactions

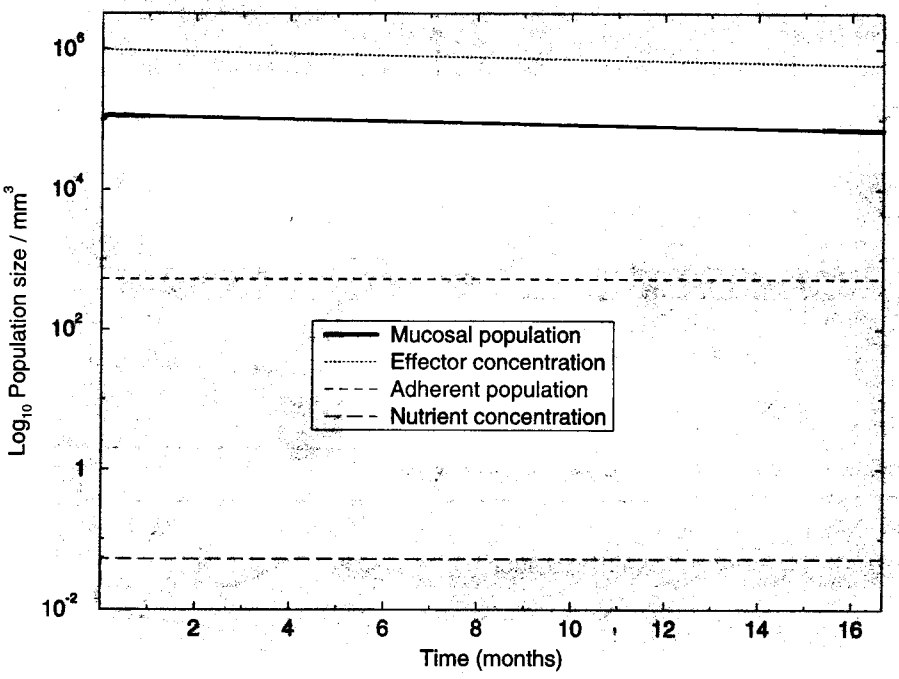


**FIGURE 7** Simulation of colonization model showing *H. pylori* persistence. The four populations shown are the mucosal bacteria, the adherent bacteria, and the effector and nutrient concentrations. Notice that within a year the populations enter a steady-state in which they will remain indefinitely unless there is some perturbation in the system.

that only where genotypic differences did not alter the apparent phenotype of the strains with respect to growth, acquisition of nutrients, etc. (i.e., differences in one or more of the parameters), could there be persistent coexistence. If the phenotypes varied to any measurable degree based on known characteristics, then coexistence was only temporary and eventually the strain with the advantage competitively excluded the other over a time frame that was inversely correlated with the magnitude of the phenotypic difference (11, 39). Therefore, a clinical biopsy may reveal the presence of multiple strains within a given host; however, their concentration levels and long-term existence patterns may be very different.

## The Effect of a Developing Host Response on Colonization

The system discussed above was designed to model the steady-state condition that exists for the majority of *H. pylori* cells resident in the human stomach, where we assumed that any host response was already in a down-regulated steady state. During infection, however, there are a variety of host responses to *H. pylori*. For example, there are both humoral and cellular immune responses (19, 22), although both appear to be ineffective in clearing the bacteria and preventing colonization in most individuals. Our present model of colonization does not account for the initial events in infection, just after an inoculum is introduced into a naive host. In a second study (11), we examined the initial events by including a generalized host response.

To elaborate the early dynamics of *H. pylori* colonization, we developed a new model that incorporates the role of the developing host response. The model allows us to examine both the initial events following *H. pylori* introduction into a naive host and the development of

colonization. This model also allows us to predict the effects of host perturbations on the *H. pylori* populations and the resulting consequences. Incorporation of the dynamic host response into a model of *H. pylori* colonization is critical if we are to understand the initial features of the interactions between microbe and host, as well as the phenomena that permit persistence to develop. Thus, we extended our earlier model to incorporate characteristics of the host-microbial interaction that had not been addressed previously. In the new model, the major role of the host response is to down-regulate tissue inflammation and its exudate into the gastric lumen, which we have assumed to be the major nutrient source for *H. pylori*. We were able to show that the strength of the host response plays a key role in deciding whether persistent infection can be established.

We describe a new population reflecting the intensity of the host response to *H. pylori* ($H_R$) (11). The growth rate of the host response is reflected by $k_1$. The host response initially grows as a function of the bacterial population, but this growth has a limited capacity, which is represented by $k_2$ (69). In this model, we assume that adherent *H. pylori*, ($A$), will have a greater impact on the host response than will the mucus-living *H. pylori*, ($M$), due to its proximity to host epithelial cells. The new equation is

$$\frac{dH_R(t)}{dt} = k_1(M(t) + k_3A(t))(k_2 - H_R(t)). \quad (3)$$

The equation marking the change in nutrients (proportional to inflammation) in the earlier model is now altered to reflect the host's developing response to introduction of *H. pylori*. The source of nutrients now represents the proportional relationship between effectors, $E$, and the limiting effects of the host response, $H_R$: if $H_R$ is small, the term acts as it did in the previous model, but if it is large, then the production rate of the nutrients is limited.

## Exploring the Host Response Model

If we solve the new system of equations together with the new initial starting values that reflect beginning at conditions reflecting the inoculum, we see that the model yields two qualitatively different outcomes (Fig. 8). For small values of $k_2$, the host response capacity, the model predicts that the system will develop into persistent colonization (Fig. 8A). For large values of $k_2$, the model predicts the system will undergo transient colonization and the bacterial population will eventually be cleared (Fig. 8B). Notice in Fig. 8A and B how different values for the parameter $k_2$ alter the host response curves. This indicates that under certain host responses, the bacteria either can be cleared or will establish persistence. This model can also be used to test how other variations in the host response will affect the predicted model outcomes.

## DYNAMICS OF THE HOST IMMUNE RESPONSE AND *M. TUBERCULOSIS*

Tuberculosis (TB) has been a leading cause of death in the world for centuries. Today it remains the number one cause of death by infectious disease worldwide—3.1 million deaths per year. TB is not only one of our oldest microbial disease enemies, it remains one of the most formidable: an estimated one-third of the world population has latent TB. Thus, there is a great need to elucidate the mechanisms of TB progression. Key issues are to understand the immunologic mechanisms that are involved in establishing and maintaining latent infection (resolution) and those that lead to reactivation of *M. tuberculosis* and development of active disease. There exists an enormous body of literature regarding the individual elements of both pathogenic mechanisms and the immune response to *M. tuberculosis*; however, little is known about combined interactions or the balance among these processes. This lack of knowledge is reflected in the limited number of antibiotic therapies that are currently effective against multidrug-resistant strains. The therapy limitations, coupled with the emergence of multidrug resistance, make the development of alternative therapeutic approaches even more pressing. Preliminary efforts in one

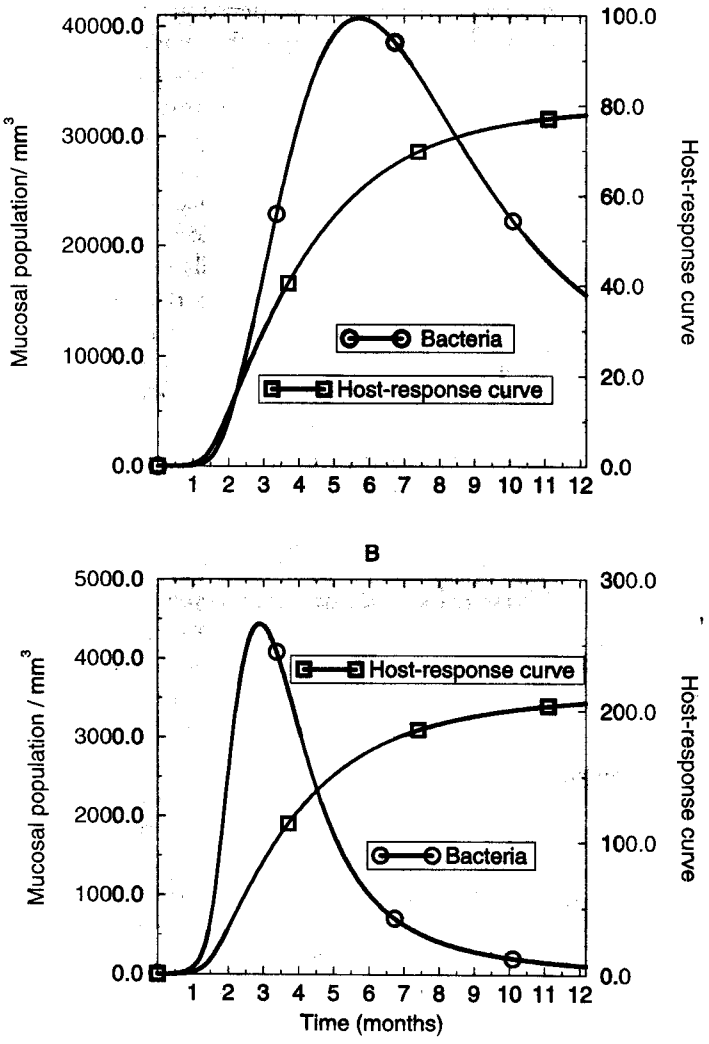


**FIGURE 8** Model dynamics. (A) Initial transient dynamics and development of persistent colonization. The results are obtained by numerically solving the modified model system. (B) Transient colonization that results from a much larger host response (due to an increase in the host carrying capacity). Note how this larger host response causes a timely elimination of the bacteria.

of our laboratories (D.E.K.) focus on elucidating the mechanisms of disease progression through investigation of the regulation of the immune response to *M. tuberculosis* infection. Our long-term goal is to explain the establishment and maintenance of latency in *M. tuberculosis* infection. TB is a unique disease in that 90% of persons establish a latent infection; however, 5% progress rapidly and 5% progress slowly to active disease over their lifetimes (18, 71). Our particular focus is to predict why some individuals clear *M. tuberculosis* infection while others develop latent infection essentially for life, and why still others develop active disease, via either a fast or slow disease course. The immune mechanisms that are involved in these alternative disease trajectories are crucial, and these mechanisms are the focus of our present and continuing work.

We have shown above the detailed elements involved in various approaches to modeling. Therefore, in the interest of brevity and because this work is still very much in progress, we will only outline our preliminary results in this section.

During infection, immune cells secrete cy-

tokines that modulate the immune response in both a positive and a negative fashion. Cytokines have been shown to be involved in the dynamics of *M. tuberculosis* infection, specifically by influencing the differentiation of $CD4^+$ T cells into either TH1 or TH2 cells. Mosmann and colleagues (56) discovered that, upon stimulation, $CD4^+$ T cells further differentiate into TH1 and TH2 subsets that are distinguished on the basis of the cytokine profiles they produce. TH1 cells are not only responsible for stimulating macrophages to engulf and kill foreign particles but also for activating $CD8^+$ T cells, which then differentiate into cytotoxic T lymphocytes that can kill infected cells directly. TH2 cells down-regulate the cell-mediated response while up-regulating the humoral (antibody) response.

Models that qualitatively and quantitatively characterize the balance of the TH1- and TH2-type response can be useful in delineating the mechanisms of disease progression in *M. tuberculosis* infection. During infection, there are two types of cellular immune responses: an activated macrophage response (governed by a TH1 response), leading to a delayed-type hypersensitivity (DTH) reaction that on its own is not able to effect resolution, and a T-cell-regulated, macrophage-suppressing response resulting in the down-regulation of the DTH reaction (governed by a TH2 response) that can effect resolution.

Although the events in the immunology and pathology of *M. tuberculosis* infection are not well characterized, numerous factors that regulate immune processes have been implicated in the development of TB. These processes include cell-pathogen and cell-cell interactions as well as the production and action of cytokines that facilitate these interactions. In particular, the progression of disease may depend upon the dominant cytokine phenotype. The initiation of a TH1 cytokine response by an inciting agent may result in a vigorous DTH response with the expression of gamma interferon (IFN-$\gamma$) and interleukin 12. An effective response spearheaded by elevated levels of IFN-$\gamma$ usually will clear the inciting agent and ensure that granuloma formation takes place. On the other hand, TH2 cytokines result in a cessation of the TH1 response, which may ultimately prevent the lung environment from being destroyed by an overly active DTH response. We devised a hypothesis that the stages of TB depend on the balance of TH1 and TH2 cytokines that are generated during the expression of disease. We are testing this hypothesis through mathematical modeling.

Primary TB, the response following the first exposure to *M. tuberculosis*, usually develops in the alveoli of the lung at the peripheral midzone after droplets containing the bacteria are inhaled. The bacteria are then ingested by resident alveolar macrophages and begin to multiply (15). These cells are poor at destroying their occupants because *M. tuberculosis* has the ability to prevent the phagosome-lysosome fusion in insufficiently activated macrophages (53, 59). Eventually, an infected macrophage either bursts due to the large number of bacteria multiplying within or it circulates out through the lymphatic ducts to the lymph nodes (transporting bacteria and antigen), where the specific immune response is initiated. Here, $CD4^+$ T cells are activated to become TH1 and TH2 cells. TH1 cells, which are thought to be the dominant type in the immune response to TB infection (28), must migrate to the site of infection, activate macrophages to facilitate killing the ingested bacteria, orchestrate the DTH response, kill (or stimulate cytotoxic T lymphocytes to kill) macrophages that are unable to destroy their ingested bacteria.

Many recent studies explore the role of cytokine profiles in infection with *M. tuberculosis* (e.g., references 21, 35, 61, 65, and 66). The relevant immune responses involve a complex interplay of cellular immune processes and cytokine mediators. To distinguish among the cellular immune processes for modeling, we first define the tissue-damaging, DTH response that occurs due to IFN-$\gamma$ activation of macrophages as dominated by a TH1-type response. Second, we define the immune response resulting in macrophage deactivation as dominated by a TH2-type response. We are inter-

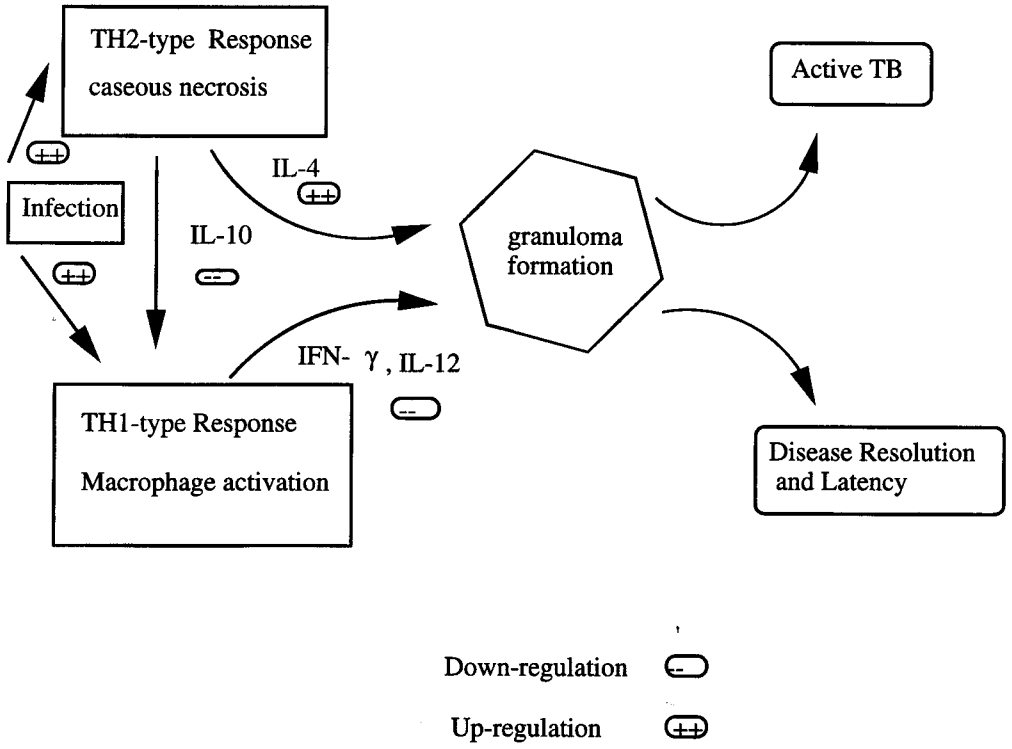


**FIGURE 9** Our hypothetical cytokine-mediated immune response network in *M. tuberculosis* infection. The progression of disease to either active or latent TB may depend on the balance of the TH1 and TH2 cytokines that are generated during the expression of disease.

ested in the interactions between these two processes and in the balance between them that allows for the establishment and maintenance of the latent state. We present a scheme for this interaction in Fig. 9.

Because we hypothesize that it is the balance between the TH2-type and the TH1-type immune responses that allows for the establishment of latency in *M. tuberculosis* infection, we developed a model of the cytokine control network during infection. In an effort to identify interactions among the immune mechanisms that are critical for the establishment of this balance, we explored the effects of perturbing different factors in the model, including cytokines, T cells, macrophages, and bacteria, and relevant interactions among these populations. Our initial results indicate that the TH1-TH2 cytokine response is indeed a key factor in the different disease trajectories of TB. Thus, therapies that enhance or depress certain aspects of either response may have potential use for treating *M. tuberculosis* infections.

## CONCLUDING REMARKS

Although mathematical-modeling approaches have been widely used in the study of virus-host interactions, they have been applied less frequently to the study of bacterium-host interactions. If we consider long-term associations between bacteria and humans a question of bacterial ecology, such as persistent infections or the homeostasis of an indigenous microflora, it becomes more logical to consider mathe-

matical approaches to understanding these associations, as modeling has long been used by ecologists.

This chapter reviews studies which illustrate how complex problems in host-microbe interactions are unlikely to have been solved without mathematical modeling. Very often, models appear to confirm what the experimentalists may already surmise about a system. Properly understood, however, the model can be seen as a starting point for designing crucial experiments to test those assumptions. In cases when the biological system is experimentally intractable, a representative mathematical model may offer the sole means of testing key hypotheses. On other occasions, a model may illuminate testable aspects of the system that had not occurred to the experimentalist.

As we accumulate more and more detailed data, mainly on the molecular level, through experimental techniques of increasing sophistication, it is clear that there is a strong need for an integrative understanding of the complex processes of host-pathogen interactions. Mathematical modeling offers a unique method for achieving this integration and thus will be an increasingly important tool in understanding these processes.

## REFERENCES


1. **Agur, Z.** 1989. Clinical trials of zidovudine in HIV infection. *Lancet* **ii:**1400–1401.
2. **Anderson, E. S.** 1975. Viability of, and transfer of a plasmid from *E. coli* K12 in the human intestine. *Nature* **255:**502–504.
3. **Anderson, R. M.** 1982. Transmission dynamics and control of infectious disease agents, p. 149–176. *In* R. M. Anderson and R. M. May (ed.), *Population Biology of Infectious Diseases.* Springer-Verlag, Berlin, Germany.
4. **Anderson, R. M., and R. M. May.** 1987. Transmission dynamics of HIV infection. *Nature* **326:**137–142.
5. **Anderson, R. W.** 1996. How adaptive antibodies facilitate the evolution of natural antibodies. *Immun. Cell Biol.* **74:**286–291.
6. **Anderson, R. W., M. S. Ascher, and H. W. Sheppard.** 1998. Direct HIV cytopathicity cannot account for CD4 decline in AIDS in the presence of homeostasis: a worst-case dynamical analysis. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **17:**245–252.
7. **Antia, R., J. C. Koella, and V. Perrott.** 1996. Models of the within-host dynamics of persistent mycobacterial infections. *Proc. R. Soc. Lond.* **1:** 257–263.
8. **Antia, R., B. Levin, and R. B. May.** 1994. Within-host population dynamics and the evolution and maintenance of microparasite virulence. *Am. Nat.* **144:**457–472.
9. **Asachenkov, A., G. Marchuk, R. Mohler, and S. Zuev.** 1994. *Disease Dynamics.* Birkhauser Boston, Cambridge, Mass.
10. **Bailey, N. T. J.** 1975. *The Mathematical Theory of Infectious Diseases,* 2nd ed. Hafner, New York, N.Y.
11. **Blaser, M. J., and D. E. Kirschner.** 1999. Dynamics of Helicobacter pylori colonization of the human stomach in relation to the host immune response. *Proc. Natl. Acad. Sci. USA* **96:**8359–8364.
12. **Blaser, M. J., and J. Parsonnet.** 1994. Parasitism by the "slow" bacterium H. pylori leads to altered gastric homeostasis and neoplasia. *J. Clin. Investig.* **94:**4–8.
13. **Blower, S. M., A. R. McLean, T. C. Porco, P. M. Small, P. C. Hopewell, M. A. Sanchez, and A. R. Moss.** 1995. The intrinsic transmission dynamics of tuberculosis epidemics. *Nat. Med.* **1:** 815–821.
14. **Blower, S. M., P. M. Small, and P. C. Hopewell.** 1996. Control strategies for tuberculosis epidemics: new models for old problems. *Science* **273:** 497–500.
15. **Canetti, G.** 1955. *The Tubercle Bacillus in the Pulmonary Lesion in Man.* Springer Publishing Co., New York, N.Y.
16. **Castillo-Chavez, C., and Z. Feng.** 1997. To treat or not to treat: the case of tuberculosis. *J. Math. Biol.* **35:**629–659.
17. **Castillo-Chavez, C., and Z. Feng.** 1998. Global stability of an age-structure model for TB and its applications to optimal vaccination strategies. *Math. Biosci.* **151:**135–154.
18. **Comstock, G. W.** 1982. Epidemiology of tuberculosis. *Am. Rev. Respir. Dis.* **125:**8–16.
19. **Crabtree, J. E., J. D. Taylor, and J. L. Wyatt.** 1991. Mucosal IgA recognition of Helicobacter pylori 120 kDa protein, peptic ulceration, and gastric pathology. *Lancet* **338:**332–335.
20. **Cussac, V., R. L. Ferrero, and A. Labigne.** 1992. Expression of *Helicobacter pylori* urease genes in *Escherichia coli* grown under nitrogen-limiting conditions. *J. Bacteriol.* **174:**2466–2473.
21. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S.**

**Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted inteferon gamma genes. *Science* **259:**1739–1742.

22. **Dooley, C. P., P. L. Fitzgibbons, H. Cohen, M. Appleman, M. Bauer, G. J. Perez-Perez, and M. J. Blaser.** 1989. Prevalence of H. pylori infection and histologic gastritis in asymptomatic persons. *N. Engl. J. Med.* **321:**1562–1566.
23. **Freter, R.** 1983. Human intestinal microflora in health and disease, p. 33–54. *In* D. J. Hentges (ed.), *Mechanisms that Control the Microflora in the Large Intestine.* Academic Press, Inc., San Diego, Calif.
24. **Freter, R.** 1984. Factors affecting conjugal plasmid transfer in natural bacterial communities, p. 105–114. *In* M. J. Klug and C. A. Reddy (ed.), *Current Perspectives in Microbial Ecology.* American Society for Microbiology, Washington, D.C.
25. **Freter, R., H. Brickner, and S. J. Temme.** 1986. An understanding of colonization resistance of the mammalian large intestine requires mathematical analysis. *Microecol. Ther.* **16:** 147–155.
26. **Freter, R., R. R. Freter, and H. Brickner.** 1983. Experimental and mathematical models of *Escherichia coli* plasmid transfer in vitro and in vivo. *Infect. Immun.* **39:**60–84.
27. **Gordon, D. M., and M. A. Riley.** 1992. A theoretical and experimental analysis of bacterial growth in the bladder. *Mol. Microbiol.* **6:**555–562.
28. **Hahn, H., and S. H. E. Kaufmann.** 1981. Role of cell-mediated immunity in bacterial infections. *Rev. Infect. Dis.* **3:**1221–1250.
29. **Hentschel, E., G. Brandstatter, B. Dragoisics, A. M. Hirschl, H. Nemec, K. Schutze, M. Taufer, and H. Wurzer.** 1993. Effect of ranitidine and amoxicillin plus metronidazole on the eradication of H. pylori and the recurrence of duodenal ulcer. *N. Engl. J. Med.* **328:** 308–312.
30. **Hessey, S. J., J. Spencer, J. Wyatt, G. Sobola, B. J. Rathbone, A. T. Axon, and M. F. Dixon.** 1990. Bacterial adhesion and disease activity in H. pylori associated chronic gastritis. *Gut* **31:**134–138.
31. **Hethcote, H. W., and J. A. Yorke.** 1984. *Gonorrhea: Transmission, Dynamics and Control.* Springer-Verlag, Berlin, Germany.
32. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV1 infection. *Nature* **373:** 123–126.
33. **Ihamaki, T., M. Kekki, P. Sipponen, and M. Siurala.** 1985. The sequelae and course of chronic gastritis during a 30- to 34-year bioptic follow-up study. *Scand. J. Gastroenterol.* **20:**485–491.
34. **Jorgensen, M., P. Daskalopoulos, G. Warburton, V. Mitchell, and S. L. Hazell.** 1996. Multiple strain colonization and metronidazole resistance in helicobacter pylori-infected patients: identification from sequential and multiple biopsy specimens. *J. Infect. Dis.* **174:**631–635.
35. **Jouanguy, E., F. Altare, S. Lamhamedi, P. Revy, J. F. Emile, M. Newport, M. Levin, S. Blanche, E. Sebourn, and A. Fischer.** 1961. Interferon gamma receptor deficiency in an infant with fatal Bacille Calmette-Guerin infection. *N. Engl. J. Med.* **335:**1956–1961.
36. **Karnes, W. E., Jr., I. Samloff, M. Siurala, M. Kekki, P. Sipponen, S. W. Kim, J. H. Walsh, and J. L. Casanova.** 1991. Positive serum antibody and negative tissue staining for H. pylori in subjects with atrophic body gastritis. *Gastroenterology* **101:**167–174.
37. **Karttunen, R.** 1991. Blood lymphocyte proliferation, cytokine secretion and appearance of T cells with activation surface markers in cultures with Helicobacter pylori: comparison of the responses of subjects with and without antibodies to H. pylori. *Clin. Exp. Immunol.* **83:**396–400.
38. **Kirschner, D.** 1999. Dynamics of co-infection with M. tuberculosis and HIV-1. *Theor. Popul. Biol.* **55:**94–109.
39. **Kirschner, D., and M. J. Blaser.** 1995. The dynamics of H. pylori infection of the human stomach. *J. Theor. Biol.* **176:**281–290.
40. **Kirschner, D., S. Lenhart, and S. Serbin.** 1997. Optimizing chemotherapy of HIV infection: scheduling, amounts and initiation of treatment. *J. Math. Biol.* **35:**775–792.
41. **Kirschner, D., R. Mehr, and A. Perelson.** 1998. The role of the thymus in pediatric HIV-1 infection. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **18:**95–108.
42. **Kirschner, D., and A. Perelson.** 1995. A model for the immune system response to HIV: AZT treatment studies. *Math. Popul. Dyn.* **1:**296–310.
43. **Kirschner, D., and G. F. Webb.** 1996. A model for treatment strategy in the chemotherapy of AIDS. *Bull. Math. Biol.* **58:**367–390.
44. **Kirschner, D., and G. F. Webb.** 1997. A mathematical model of combined drug therapy of HIV infection. *J. Theor. Med.* **1:**25–34.
45. **Kirschner, D., and G. F. Webb.** 1997. Qualitative differences in HIV chemotherapy between resistance and remission outcomes. *Emerg. Infect. Dis.* **3:**273–283.

46. **Kirschner, D., and G. F. Webb.** 1997. Understanding drug resistance in the monotherapy treatment of HIV infection. *Bull. Math. Biol.* **59:** 763–785.
47. **Levin, B. R., and V. A. Rice.** 1980. The kinetics of transfer of nonconjugative plasmids by mobilizing conjugative factors. *Genet. Res.* **35:** 241–259.
48. **Levin, B. R., F. M. Stewart, and V. A. Rice.** 1979. The kinetics of conjugative plasmid transmission: fit of a simple mass action model. *Plasmid* **2:**247–260.
49. **Lipkin, M., B. Sherlock, and B. Bell.** 1963. Cell proliferation kinetics in the gastrointestinal tract of man. *Gastroenterology* **46:**721–735.
50. **Lipsitch, M., and B. R. Levin.** 1997. The population dynamics of antimicrobial chemotherapy. *Antimicrob. Agents Chemother.* **41:**363–370.
51. **Lipsitch, M., and B. R. Levin.** 1998. Population dynamics of tuberculosis treatment. *Int. J. Tuberc. Lung Dis.* **2:**187–199.
52. **Mai, U. E., G. Perez-Perez, J. B. Allen, S. M. Wahl, M. J. Blaser, and P. D. Smith.** 1992. Surface proteins from H. pylori exhibit chemotactic activity for human leukocytes and are present in gastric mucosa. *J. Exp. Med.* **175:**517–525.
53. **McDonough, K. A., Y. Kress, and B. R. Bloom.** 1993. Pathogenesis of tuberculosis: interaction of M. tuberculosis with macrophages. *Infect. Immun.* **61:**2763–2773.
54. **McLean, A., and M. Nowak.** 1991. Interactions between HIV and other pathogens. *J. Theor. Biol.* **155:**69–86.
55. **Merrill, S. J.** 1987. AIDS: background and the dynamics of the decline of immunocompetence, p. 59–75. *In* A. S. Perelson (ed.), *Theoretical Immunology. Part 2.* Springer-Verlag, Berlin, Germany.
56. **Mosmann, T. R. H., H. Cherwinski, and M. W. Bond.** 1986. Two types of murine T cell clones. I. Definition according to profiles of lymphokine activity and secreted proteins. *J. Immunol.* **136:**2348–2357.
57. **Muotiala, A., I. M. Helander, L. Pyhala, T. U. Kosunen, and A. P. Moran.** 1992. Low biological activity of *Helicobacter pylori* lipopolysaccharide. *Infect. Immun.* **60:**1714–1716.
58. **Murray, J. D.** 1980. *Mathematical Biology.* Springer-Verlag, Berlin, Germany.
59. **Myrvik, Q. N., E. S. Leake, and M. J. Wright.** 1984. Disruption of phagosomal membranes of normal alveolar macrophages by the H37 Rv strain of M. tuberculosis. *Am. Rev. Respir. Dis.* **129:**322–328.
60. **Nahmias, A. J., W. S. Clark, A. P. Kourtis, F. K. Lee, G. Cotsonis, C. Ibegbu, D. Thea, P. Palumbo, P. Vink, R. J. Simonds, and S. R. Nesheim.** 1998. Thymic dysfunction and time of infection predict mortality in HIV-infected infants. *J. Infect. Dis.* **178:**680–685.
61. **Newport, M. J., C. M. Huxley, S. Huston, C. M. Hawrylowicz, B. A. Ostra, R. Williamson, and M. Levin.** 1996. A mutation in the interferon gamma receptor gene and susceptibility to mycobacterial infection. *N. Engl. J. Med.* **335:**1941–1949.
62. **Nowak, J. A., B. Forouzandeh, and J. A. Nowak.** 1997. Estimates of *H. pylori* densities in the gastric mucus layer by PCR, histological examination and CLOtest. *Anat. Pathol.* **108:** 284–288.
63. **Nowak, M. A., and C. R. M. Bangham.** 1996. Population dynamics of immune responses to persistent viruses. *Science* **272:**74–79.
64. **Nowak, M. A., R. M. May, and R. M. Anderson.** 1990. The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease. *AIDS* **4:**1095–1103.
65. **Orme, I. M.** 1998. The immunopathogenesis of tuberculosis: a new working hypothesis. *Trends Microbiol.* **6:**94–97.
66. **Parrish, N. M., J. D. Dick, and W. R. Bishai.** 1998. Mechanisms of latency in M. tuberculosis. *Trends Microbiol.* **6:**107–112.
67. **Perelson, A., D. Kirschner, and R. De Boer.** 1993. The dynamics of HIV infection of CD 4+ T-cells. *J. Math. Biosci.* **114:**81–125.
68. **Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho.** 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life span and viral generation time. *Science* **271:**1582–1586.
69. **Roos, M. T., F. Miedema, M. Koot, M. Tersmette, W. P. Schaasberg, R. A. Coutinho, and P. T. Schellekens.** 1995. T cell function in vitro is an independent progression marker for AIDS in human immunodeficiency virus-infected asymptomatic subjects. *J. Infect. Dis.* **171:** 531–536.
70. **Sharma, S. A., G. G. Miller, G. Perez-Perez, R. S. Gupta, and M. J. Blaser.** 1994. Humoral and cellular immune recognition of H. pylori proteins are not concordant. *Clin. Exp. Immunol.* **97:** 126–130.
71. **Styblo, K.** 1986. Respiratory medicine. *Adv. Respir. Med.* **1:**77–108.
72. **Suzuki, M., S. Miura, M. Suematsu, D. Fukumura, I. Kurose, H. Suzuki, A. Kai, Y.**

**Kudoh, M. Osashi, and M. Tsuchiya.** *Helicobacter pylori*-associated ammonia production enhances neutrophil-dependent gastric mucosal cell injury. *Am. J. Physiol.* **263:**G719.

73. **Thomsen, L. L., J. B. Gavin, and C. T. Jones.** 1990. Relation of *H. pylori* to the human gastric mucosa in chronic gastritis of the antrum. *Gut* **31:** 1230.
74. **vanderEnde, A., E. A. Rauws, M. Feller, C. J. J. Mulder, G. N. J. Tytgat, and J. Dankert.** 1996. Heterogeneous Helicobacter pylori isolates from members of a family with a history of peptic ulcer disease. *Gastroenterology* **111:**638–647.
75. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsh, J. D. Lifsoh, S. Bonhoeffer, M. A. Nowak, B. H. Hahn, et al.** 1995. Viral dynamics in HIV virus 1 infection. *Nature* **373:**117–122.