2

Aging and Altered Resistance to Infection

All in all, my fellow pathogens, Homo is the opportunity that ultimately can benefit us all. Aside from their prevalence in numbers, they show all the weaknesses that maximize our effective potential. Although they themselves deny that there is such a thing as a free lunch, we know better. There is a free lunch, and it is them.

—Thomas Eisner and Paul R. Ehrlich, New world pathogen strategy disclosed, Science 2000; 292, Editorial.

Altogether, microbial and parasitic diseases constitute the leading cause of morbidity and mortality worldwide. They affect preferentially the very young and the elderly, the two age groups that are deficient in immunological competence. This chapter is a review of some of the organisms that are particularly devastating to the elderly. A portion of the chapter deals with the remarkable variability that those microorganisms are capable of manifesting in order to ensure their adequacy to reproduce in their hosts.

Optimally, a pathogen should be sufficiently virulent to thwart the defenses of its host without overwhelming it. A host that is quickly ravaged is unsuitable for the pathogen, which has the single objective of perpetuating itself. Upon infection, a struggle develops between host and pathogen with the advantage going first to one adversary and then to the other. Microbial pathogens are, of course, capable of much more rapid variation than are their hosts. Therefore, it is in the pathogen's self-interest to utilize sparingly the weapons of virulence in its arsenal so that there is opportunity to reproduce and allow progeny to move on to new hosts. When a microbial pathogen (or any parasite) quickly overwhelms its host, it probably indicates that an adaptive equilibrium has not been achieved. That is sure to be the case when hosts that are immunodeficient are involved, i.e., hosts that are very young, those suffering from immunodeficiency diseases or being treated with immunosuppressive agents, and the elderly.

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Table 2-1
Some Important Geriatric Infectious Diseases and Their Relative Mortality Rates

Infection	Relative mortality rate (compared with young adults)
Pneumonia	3
Urinary tract infection	5–10
Bacterial meningitis	3
Tuberculosis	10[a]
Sepsis	3

[a]Excluding HIV-infected young adults.
Adapted from ref. *1*.

RELATIVELY COMMON BACTERIAL INFECTIONS OF AGING HUMANS

Some important infectious diseases and their relative mortality in elderly subjects are shown in Table 2-1 *(1)*. As expected, that list reflects the fact that there are three principal routes of infection: respiratory, urinary, and gastrointestinal (GI). The most compelling explanations of the prevalence of those diseases in the elderly are: 1) age-associated changes in the structure and function of the respiratory, urinary, and gastrointestinal organs; 2) underlying pathological changes resulting from existing disorders (comorbidity); and 3) age-associated decline (dysregulation) in innate (natural) and acquired (adaptive) imunological competence.

Respiratory and Urinary Tract Infection

Table 2-2 provides a list of organisms found most often in respiratory and urinary tract infections of the elderly. The most common respiratory infection is bacterial pneumonia. In about half of the community-acquired pneumonia (CAP) cases, the etiologic agent remains unidentified *(2)*. It is estimated that 20%–30% of all CAP infections are caused by *Streptococcus pneumoniae* and most of the remaining cases by the other bacteria listed in Table 2-2.

Upper respiratory viral infections were studied in a group of 533 subjects ages 60 to 90 years living in England *(3)*. In that group of patients, 52% of the infections were associated with rhinoviruses, 26% with coronaviruses, 9.5% with influenza A or B, and 7% with respiratory syncytial virus, and the remainder with other agents.

In the case of urinary tract infections (UTIs) in the elderly, two independent studies, separated by an interval of 12 yr, gave very similar results. One study was performed in Sweden in 1986 on a group of 1966 subjects having a mean

Table 2-2
Pathogens Found Frequently in Elderly Subjects with Respiratory or Urinary Tract Infections

Organ system	Pathogen found frequently
Respiratory tract (upper and lower)	Bacteria
	Streptococcus pneumoniae
	Hemophilus influenza
	Legionella pneumophila
	Chlamidia pneumoniae
	Viruses
	Rhinoviruses
	Coronaviruses
	Influenza
	Respiratory syncytial
Urinary tract	Bacteria
	Escherichia coli
	Proteus
	Klebsiella
	Pseudomonas aeruginosa
	Enterococci

age of 70 years *(4)*. The majority of those subjects were not in hospitals or institutions. The other study occurred in England in 1998 on a group of 3119 subjects all of age greater than 65 years *(5)*. The results of the two studies agreed that *Escherichia coli* was the most common organism in UTIs. *Klebsiella*, *Proteus*, *Pseudomonas aeruginosa*, and *enterococci* were found less frequently but in significant numbers of subjects. The study performed in 1998 *(5)* included comparisons of the organisms found in bacteremic patients with respect to: 1) where the infection was acquired, i.e., in the community or in the hospital; and 2) the patients' ages, over 65 years or in the range 18–64 years. In both age groups, *E. coli* was the dominant organism in more than 70% of the community-acquired infections. In the case of hospital-acquired infections, *E. coli* was the principal organism in approx 40% of the patients, regardless of age. Various other organisms (*Klebsiella*, *Proteus*, *P. aeruginosa*) were dominant in about 60% of the hospital-acquired infections. The list of organisms associated with UTIs reflects the fact that a major portion of UTIs is caused by pathogens derived from the patients' colonic flora that enter the bladder by the "ascending route," i.e., via the perineum, urethra, vagina, or prostate. Viral infections of the bladder are rare.

The purpose of presenting the lists in Tables 2-1 and 2-2 is to provide a general indication of the types of microorganisms with which the elderly must contend. Geriatric infectious diseases *per se* are not discussed here; they are the topic of a major, recent publication edited by Yoshikawa and Norman *(6)*.

Gastrointestinal Infections

Many factors can influence the GI flora; e.g., diet, medications, malabsorption, deficient intestinal motility, lumenal pH. Several of these may be altered with age as is discussed below. There are important reasons for giving special attention to intestinal microbial flora in the elderly. First, the gut is a likely source of pathogens that cause illnesses of high mortality in older patients (e.g., infective endocarditis, cholecystitis, sepsis); second, the importance of diet (caloric restriction) on longevity (discussed in a later chapter); third, the rather common problems of malnourishment, malnutrition, and dietary deficiencies (e.g., vitamins) in the elderly; and fourth, the translocation of microbial components and products from the gut to the circulation and the adverse effects on the health of the elderly.

There is no known intestinal microbial pattern that distinguishes young adult from elderly. Given that there are more than 400 bacterial species in the colonic flora of a single individual *(7)*, it is unlikely that a catalog of intestinal flora would be a useful biomarker of senescence. However, it is possible that one or a few species might be characteristically different in the young adult and the elderly. Apparently, this possibility has not been explored.

The number of bacteria and the spectrum of species in normal adults varies with the segment of the intestine, as displayed in Table 2-3 *(8)*. There are relatively few bacteria in the stomach and jejunum; those that are present are predominantly aerobes or facultative aerobes. In contrast, the colon is lushly endowed with bacteria, as revealed by fecal examination, the majority of which are anaerobes. Only small numbers of fungi, or protozoa, are present, even in the colon.

The number and variety of bacteria in the gut remain rather constant in the healthy individual and are controlled primarily by gastric acid secretion and normal intestinal motility. In the healthy, well-nourished, elderly subject, the intestinal flora appears to be similar to that of the young adult. However, there is much more variation among the elderly for reasons that are considered below.

As far as is known, there are no microbial pathogens that uniquely infect the elderly. Rather, the heightened susceptibility to infections associated with aging may be viewed in the following way *(9)*: "Diminished physiologic reserve secondary to both biologic changes of aging and coexisting chronic diseases contributes to the higher mortality and morbidity rates observed for serious infection in older compared with younger persons."

Table 2-3
The Normal Gastrointestinal Flora of Humans

Total bacterial count	Stomach $0–10^3$	Jejunum $0–10^5$	Ileum $10^3–10^7$	Feces $10^{10}–10^{12}$
Aerobic or facultative anaerobic bacteria				
Enterobacteria	$0–10^2$	$0–10^3$	$10^2–10^6$	$10^4–10^{10}$
Streptococci	$0–10^3$	$0–10^4$	$10^2–10^6$	$10^5–10^{10}$
Staphylococci	$0–10^2$	$0–10^3$	$10^2–10^5$	$10^4–10^7$
Lactobacilli	$0–10^3$	$0–10^4$	$10^2–10^5$	$10^6–10^{10}$
Fungi	$0–10^2$	$0–10^2$	$10^2–10^3$	$10^2–10^6$
Anaerobic bacteria				
Bacteroides	Rare	$0–10^2$	$10^3–10^7$	$10^{10}–10^{12}$
Bifidobacteria	Rare	$0–10^3$	$10^3–10^5$	$10^8–10^{12}$
Gram-positive cocci[a]	Rare	$0–10^3$	$10^2–10^5$	$10^8–10^{11}$
Clostridia	Rare	Rare	$10^2–10^4$	$10^6–10^{11}$
Eubacteria	Rare	Rare	Rare	$10^9–10^{12}$

[a]Includes Peptostreptococcus and Peptococcus.
From ref. *8*.

Age-Associated Changes in Anatomical–Functional Relationships

The "diminished physiologic reserve" referred to in the preceding quotation includes anatomical and functional changes associated with aging of the respiratory, urinary, and gastrointestinal systems. In the case of the respiratory system, it is well established that pulmonary function deteriorates with age *(2)*. Some of the anatomical changes that contribute to loss of function include: (a) decreased mean broncheolar diameter; (b) increased diameter of the alveolar sacs, which become shallower; (c) decrease in elastic fibers and increase in type III collagen. Those anatomical changes contribute to the following functional changes: (a) decrease in elastic recoil; (b) decrease in oxygen diffusion capacity; (c) small airway closure resulting in air trapping; (d) decreased expiratory flow rates. Spirometric changes include decreased inspiratory reserve volume, decreased expiratory reserve volume, and decreased vital capacity. In addition, the mucociliary clearance is substantially decreased in older subjects. The net effect of these changes is an increased probability of being unable to expire or clear infectious organisms that enter the lungs. The normal oropharygeal flora is a mixture of aerobic and anaerobic bacteria and may account for a significant number of cases of CAP. In fact, it has been estimated that aspiration of oral flora is second only to *S. pneumoniae* in causing CAP *(10)*.

The healthy bladder is quite resistant to colonization by bacteria. Emptying of the bladder is the most effective way of preventing bacteria from colonizing. The elasticity of the bladder diminishes with age, which makes effective emptying more difficult. Whereas among mature adults the incidence of bacteria is much greater in females, in males and females over age 65 the incidence of bacteria is almost equal. The principal contributing factors are 1) obstructive uropathy from prostatic disease in males, 2) impaired emptying of the bladder with residual urine in females, and 3) urethral catheters and associated paraphernalia in both *(5)*.

As long as the physiological condition of the individual remains good, there are no changes in the GI system that become threatening. That is not to say that there are no changes in the GI system, rather that what changes may occur are of no serious consequence. This point was made by Saltzman and Russell *(11)*, who wrote: "The multiorgan system that composes the gastrointestinal tract has a large reserve capacity, and thus there is little change in gastrointestinal function because of aging in the absence of disease." That can accurately be said about many organ systems with respect to aging. Consider the large functional reserve of the liver, the necessity for only one kidney, the reserve capacity of the lungs, or the large excess capacity of the bone marrow for hematopoiesis. Certainly, there is a large excess of immunological potential in the young adult that gradually diminishes with advancing age as is discussed in Chapters 3 and 4. Indeed, it can be argued that the gradual diminution in potential declines to a point approaching the level that must be expressed to deal with an acute need or an emergency; beyond that point the effects of aging are manifested.

There are functional changes that occur in the GI system with age, beginning with the fact that gastric acid secretion diminishes resulting in an increase in pH in the proximal small intestine and the potential for bacterial overgrowth. In addition, normal intestinal motility may not be maintained, a factor that also disposes to bacterial overgrowth. The latter condition can cause histological changes in the mucosa of the small bowel such as hypertrophy of villi and crypts, vesiculation of the cytoplasm of mucosal cells, swollen mitochondria, and dilated cisternae of the endoplasmic reticulum *(12–14)*.

SELECTED EXAMPLES OF AGE-ASSOCIATED SUSCEPTIBILITY TO BACTERIAL INFECTIONS

Mycobacterium tuberculosis

Worldwide, tuberculosis (TB) is a major cause of morbidity and mortality. In the more-developed countries, during the early 20th century, TB gradually declined and by midcentury was not considered a significant public health problem. That changed in the 1970s with the onslaught of HIV-1 infections and the associated immunodeficiency. The incidence of TB rose significantly over the next 20 years or more. Prior to 1970, it was already recognized that there was a

clear association between advancing age and the susceptibility to TB. For example, in 1970 the incidence in the United States among persons 65 and under was approx 7 per 100,000 population and among persons over 65 about 35 per 100,000. In 1992, it was reported *(15)* that slightly over half of all TB cases in the United States were found in people over 65 who, at that time, constituted about 14% of the population.

Research concerned with TB was at a low ebb during much of the 20th century. In the 1980s there was a resurgence of research prompted by the recognition that (a) TB was a prominent opportunistic infection among AIDS victims and (b) many cases of TB were caused by antibiotic-resistant organisms. Much has been learned in the last decade. There has been some debate concerning which experimental animal serves as a suitable model of human TB; and, further, as to whether or not aging experimental animals are more susceptible to *Mycobacterium* infections than young adults. It was reported that old mice were no more susceptible than young adults to *M. tuberculosis (16)*. The levels of bacteria in target organs and the frequency of death from infection were reported to be essentially the same in young and aged mice. Systematic studies by Orme and associates have shown that there is a difference in the way mice (young and old) cope with *M. tuberculosis* infection depending on the route of infection and the dose (number) of bacteria provided to the animals *(17–19)*. Aged mice were definitely more susceptible than young when a relatively high number of bacteria was given intravenously. However, when a much smaller number of bacteria was provided aerogenically (modeling a realistic human exposure) the course of infection in the lungs of young and aged mice was similar. Nevertheless, there remained important differences between young and aged mice with respect to elements of the immune system involvement in the infection. For example, T cells collected from infected aged mice failed to confer adoptive immunity on recipient mice whereas T cells from infected young mice did. In the lungs, the levels of mRNA specific for several cytokines, especially IL-12 and IFN-γ, were severalfold lower in aged than in young adult mice. In this regard, it was found that *M. tuberculosis* infections progressed unabated in interferon (IFN)-γ knockout mice *(18)*. Recent work has shown that components of *M. tuberculosis* can block IFN-γ-induced, STAT-1 mediated gene transcription in macrophages *(20)*. (STAT is the acronym for "signal transducer and activator of transcription.")

The dissemination of live bacteria from the lungs to form granulomas in livers of aged mice was much greater than in young mice. Orme and associates concluded that there exist (unidentified) mechanisms in the aged animals that can compensate for the impaired immune control of *M. tuberculosis* infection. Their finding suggested that CD4+ T cells, which play a pivotal role in the control of infection, are affected by aging.

Recent work has revealed that T cells other than the CD4+ subset can afford protection against *M. tuberculosis* and probably other intracellular infections *(21)*. T cells were isolated and cell lines generated that were CD4–CD8– (double negative) or CD4–CD8+ and were CD1-restricted. Those T-cell lines possessed αβ T-cell receptors and responded to *M. tuberculosis* lipid and lipoglycan antigens when the latter were presented by CD1+ macrophages. Both the double negative and the CD8+ lines could affect lysis of *M. tuberculosis*-infected macrophages. However, the mechanisms of lysis by the two types of T cells were entirely different. Lysis achieved by the double-negative cells was mediated by way of interaction of Fas on the infected target cells and Fas ligand on the T cells and, therefore, was an apoptotic event. Lysis by the CD8+ cells involved exocytosis of granules containing the lytic factors, perforin and granzymes, in typical cytotoxic T lymphocyte (CTL) fashion. Only the CD8+ cells were able to destroy the intracellular *M. tuberculosis* organisms.

Thus, CD1-restricted, CD8+ T cells are candidates for the mechanism postulated by Orme and associates that compensates in the old mice for senescent CD4+ T cells. Of course, there are other candidates. It should be informative to analyze the effects, if any, of senescence on CD1-restricted T cells.

Listeria monocytogenes

This bacterium is a Gram-positive, human pathogen. The natural portal of entry is oral, leading to invasion of mucosal surfaces of the small intestine. However, *L. monocytogenes*, which is a facultative intracellular organism, can invade and replicate inside a variety of mammalian cells including those that are, and are not, typical phagocytes. Once ingested, the bacteria are incorporated into phagosomes from which they escape by lysing the phagosomal membrane. The bacteria replicate in the cytoplasm and spread from cell to cell often without becoming extracellular. Thus, they become sheltered from the humoral immune response of the host. Immune defense against *L. monocytogenes* is cell mediated and involves both activated phagocytic cells, especially IFN-γ-activated macrophages, and cytotoxic T cells (*see* Chapters 3 and 4). Foci of infection may be seen in various organs, such as the liver and spleen where they appear as granulomas.

One of the early reports that aged animals are more susceptible to infections than young adults, was a study of *L. monocytogenes* in mice *(22)*. When mice were inoculated intravenously with a moderate number of *L. monocytogenes*, the course of infection was similar in young and old animals as judged by the numbers of bacteria in livers and spleens. However, when a larger inoculum was used, the numbers and persistence of bacteria in livers and spleens were substantially greater in the case of the aged mice. Moreover, adoptive immunity conferred on recipients by transfer of either spleen cells or enriched T cells

from immunized donors was much more effective with cells from young compared to old donors. The results of this very interesting study were challenged by a report of a very similar investigation performed in the same location utilizing the same strain and ages of mice and the same strain of *Listeria (23)*. The conclusion was drawn that aging was without detriment on the ability of mice to generate T-cell immunity to *L. monocytogenes*. It was found that the numbers of bacteria surviving in the livers and spleens of aged mice were considerably lower than in young mice over the first day following intravenous inoculation of the same number of bacteria. Therefore, some nonimmunological mechanism that destroyed the bacteria in aged mice prevented an optimum dose of antigen from reaching immunological tissues. When a significantly larger number of bacteria was provided to the old than to the young mice (to compensate for those destroyed), it was now found that the T-cell responses in aged mice were equivalent to those in the young. Thus, the apparent defect in T-cell responses in aged mice was in reality a matter of inadequate antigen reaching sites of immune response. It was argued that destruction/sequestration of bacteria by the more-active monocytes/macrophages of aged mice prevented antigens from stimulating the immune response.

The discrepancies between the findings in the two reports (*see* refs. *22* and *23*) remain unexplained. Whatever the explanation may be, it is clear that aged mice in the experiments of Lovik and North *(23)* required a larger inoculum of *L. monocytogenes* to generate a T-cell response equal that in the young mice. Considerably more bacteria were retained in the livers of old than of young mice. The condition of the bacteria in the livers of aged mice was not determined. It is now well-known that macrophages vary in the way ingested *L. monocytogenes* are handled; they may be killed or they may be retained in viable condition *(24)*. They may not have been killed but, rather, retained alive in the Kupffer cells as occurs, for example, for 24–48 hours after intravenous inoculation of the parasite, *Leishmania donovani (25)*. If those entrapped bacteria were subsequently released by the Kupffer cells, a large bolus of antigen might arrive at sites of immune response just in time to drive an anamnestic response. Thus, the response reported in ref. *23* might not have been an assessment of the competence of aged mice for a true primary immune response. The question of why the livers of aged mice retained bacteria more effectively than livers of young mice is a separate matter. The effects of senescence on macrophages and their ability to deal with microorganisms are discussed in Chapter 3.

The need to provide aged mice with 10- to 50-fold more *L. monocytogenes* to obtain a T-cell response equal to that of the young, as found by Lovik and North, could be a reflection of inefficient antigen processing by dendritic cells of old mice, or a reflection of a requirement for more intense processed-anti-

gen stimulation of senescence-altered T cells. The effects of aging on dendritic cells (DCs) and T cells are discussed in Chapter 4. At this point, it need only be mentioned that dendritic cells are the critical antigen-presenting cells that prepare microbial antigens for triggering immune responses.

It appears that more attention to the effects of aging on immune responses to *L. monocytogenes* could be rewarding. Much is now known about the mechanisms of natural and acquired immunological resistance to this organism *(26)* but that knowledge has not been applied to understanding the possible effects of senescence.

Salmonella typhimurium

In the preceding paragraphs, the finding *(23)* that intravenous *L. monocytogenes* are trapped effectively by livers (and spleens) of aged compared to young mice was discussed. That is also true of liver (and spleen) of aged rats inoculated with *S. typhimurium (27)*. Perhaps that is the case generally for intracellular microorganisms. If so, it is important to determine why this is so and investigate the influence of macrophage entrapment of the microbes on the immune response to their antigens. Macrophages themselves are not efficient microbial antigen-presenting cells. However, after ingestion of bacteria, macrophages may undergo apoptosis, and components of bacteria picked up by immature dendritic cells. The latter may thus acquire the bacterial antigens, which they then present to T cells *(28)*. Uptake of apoptotic material can induce maturation of the dendritic cells and expression of new surface molecules that allow the cells to migrate to lymphoid sites where they interact with T cells *(29)*. Studies of these events in aged mice and other animals is likely to provide much new insight into the effects of senescence on immune responses.

Before leaving this discussion of *S. typhimurium* infections, it should be mentioned that this pathogen typically enters the body by the oropharyngeal route. It traverses the intestinal barrier by invading epithelial cells and membranous epithelial (M) cells, which overlie the lymphoid follicles (*see* Chapter 4). After passing through the M cells, the bacteria encounter a network of macrophages and dendritic cells where the events described in the preceding paragraph can occur. However, there is an alternative process, which involves transmigration of the macrophages bearing live *S. typhimurium* from the intestine into the circulation and subsequent dissemination to sites where humoral antibodies can be generated *(30)*. This is discussed in more detail in Chapters 3 and 4.

BACTERIAL INTERACTIONS WITH MUCOSAL SURFACES

Whether it be in the lungs, the urinary bladder, or the intestine, the flourishing of bacteria depends upon their attachment to, and successful interac-

tions with, mucosal surfaces. The interactive processes in which various types of bacteria engage include: attachment and effacement, translocation across epithelial or endothelial barriers either between cells (pericellular route) or through cells (transcellular route), and invasion of host cells. Only in the last decade have these various events been elucidated. Most of the studies have been done in model, in vitro systems or in young, experimental animals. At present, little is known about how the various interactive events might differ in the case of mucosal surfaces of aged hosts. There follows a series of brief descriptions of the interactive events as currently understood.

Bacterial Attachment

The attachment to host cells is required for bacterial proliferation, colony formation, invasion of host cells, or translocation across endothelial or epithelial host cell layers. Both the bacteria and the host cells may be altered as a consequence of activation of genes in both. Adherence allows the bacteria to resist host defensive processes such as mucociliary sweeping. There is a clear correlation between the ability of a pathogen to adhere to host cells and the susceptibility of the host to that pathogen. For example, among individuals who experience recurring UTIs, adherence of *E. coli* to epithelial cells of the subjects may be as much as five times greater than in the case of subjects who remain free of infections *(31)*.

Pathogens, including bacteria, employ a variety of mechanisms for adhering to host cells. In several, well-studied cases, known adhesion molecules are involved *(32)*. For example, outer membrane molecules of several bacteria (*Yersinia* spp., *Bordetella pertussis*), protozoa (*Leishmania mexicana*), and even viruses (echovirus 1, adenovirus) have been found to bind directly to integrins present on model host cells in vitro. Either β1or β2 integrins may be utilized. Several studies have revealed that in some cases bacteria such as *Streptococcus* spp., *P. aeruginosa*, and *Staphylococcus aureus* bind first to host cell molecules such as laminin, collagen, and fibronectin, which then associate with integrin receptors. Other pathogens such as *Legionella pneumophila* may bind selectively to the complement component, C3bi, which is a ligand for α_{mac} β2 integrin.

B. pertussis display several adhesive molecules of which two have been fairly well studied, *viz.*, the filamentous hemagglutinin (FHA) and the pertussis toxin (PT) (reviewed in ref. *33*). Although not a pathogen of elderly humans, what has been learned about adherence of *B. pertussis* to human cells is broadly instructive. Ciliated cells and macrophages are the host cells to which *B. pertussis* binds. Mutant strains of *B. pertussis* have been prepared that lack either FHA or PT or both *(34)*. When tested in normal rabbits, wild-type strains localized to cilia in the respiratory tract and produced lesions.

Mutants lacking both FHA and PT were cleared without inducing pathology. Mutants lacking either adhesin failed to attach to cilia but managed to pass into the alveoli where they caused pathological changes *(33)*.

FHA is a functionally complex molecule that displays several domains capable of interacting with complementary sites on host cells. These domains include: an N-terminal lectin domain that binds sialic acid and is required for hemagglutination; a lectin domain for ciliated host cells; a domain containing an arginine-glycine-asparagine (RGD) sequence that binds to the leukocyte integrin CR3 (CD11b/CD18) and two regions that resemble sites on factor X of the coagulation mechanism that also bind to leukocyte CR3 *(33)*. This complexity allows FHA to interact with a variety of receptors on host ciliated cells, erythrocytes, and leukocytes.

The PT protein is a hexamer. One monomeric subunit bears the catalytic site that affects adenosine 5'-diphosphate (ADP) ribosylation of guanine nucleotide proteins involved in host cell signal transduction and thus exerts the toxic effect of PT. The pentameric region of PT displays the binding sites that promote binding to host cells and intracellular delivery of the toxin. Those binding sites include lectin subunits (S2 and S3) of the pentamer, which are responsible for PT binding to glycoconjugates on cilia (S2) and the association of *B. pertussis* with macrophages (S3). Studies on the binding specificities of the S2 and S3 subunits, especially their recognition of the Lewis "a" and "x" blood group determinants, suggested that they are selectins. They possess significant sequence homology to stretches of known selectins *(35)* and structural studies have revealed that those stretches of homology are superimposable in the crystal structures of S3 and E selectin *(36)*. It has been demonstrated that FHA and PT resemble natural ligands to the extent that they can act as competitive inhibitors of integrins and selectins, respectively. As a consequence these components of *B. pertussis* can interfere with neutrophil adherence and endothelial transmigration. This is an example of how bacterial components might exacerbate infection by interfering with a host defense mechanism. Although whooping cough is not a problem in the elderly, the preceding discussion of *B. pertussis* adherence to host cells highlights an important point; namely, that very little work has been done to understand whether or not adhesion receptors and ligands change with age and, if certain of them do, the consequences of such changes. That is an important consideration in the following discussion of infections with *S. pneumoniae*.

S. pneumoniae is responsible for several localized and systemic infections such as otitis media, meningitis, sepsis, and pneumonia. There is a well-established relationship between pneumococcal bacteremia associated with pneumonia and mortality of aging subjects *(38)*. Among patients with bacteremia, the fatality rate has been related to age as follows: 17–18% among

young adults, 43% among those of age 60–69, 48% of those aged 70–79, and 60% among patients 80 years or older. *S. pneumoniae* enters the body by the nasopharyngeal route and pneumonia results from inspiration of the bacteria into the lower respiratory tract. Bacteria may be found in the alveoli from which they gain access to the circulation by crossing the endothelium of the alveolar capillaries.

S. pneumoniae attaches primarily to cells of the nasopharynx, vascular endothelium, and other cells of the lung. The local inflammation that they induce is triggered by components of the cell wall. In fact, the pathogenesis of inflammation can be induced experimentally by mixtures of cell wall components *(39,40)*. Those components are able to activate the complement system via the alternative pathway giving rise to leukocytosis, vascular permeability, secretion of interleukin-1 (IL-1) by macrophages, and other effects *(33)*.

The pneumococci bind to glycoconjugate moieties on host cells. Cells of the nasopharynx display glycoconjugate receptors of the neolactose type containing GlcNAc β1-3 Gal. The latter is also a component of the ABH, Lewis, and Ii blood group antigens and is present in human colostrum. In fact, colostrum can inhibit pneumococcal adherence to nasopharyngeal cells *(41)*. The receptors on type II pneumocytes and vascular endothelial cells responsible for attachment of pneumococci are of two types; both of them differ from the receptor on nasopharyngeal cells. Saccharides that can competitively inhibit the adherence of *S. pneumoniae* to pneumocytes and vascular endothelial cells help to define those receptors. They include mannose, GalNAc, Gal, the glycoconjugates asialo-GM1 and GM2, and the Gal NAcβ1-3 Gal-containing Forssman glycolipid *(33)*. It should be mentioned here that the exposure of type II pneumocytes and vascular endothelial cells to the inflammatory cytokines TNFα and IL-1 significantly elevate the glycoconjugate receptors for pneumococci *(33,42)*. As a consequence, adherence of *S. pneumoniae* is markedly enhanced. Enhanced adherence entails a new receptor, *viz.*, that for the platelet-activating factor (PAF). As pointed out *(33)*, this is an example of an important principle of bacterial adherence; *viz.*, the initial attachment of bacteria to resting host cells may involve one set of sugar specificities leading to activation of the cells and the expression of a new or altered receptor specificity. A virulent organism must parallel this change in the host cell by expressing a new cognate adhesion molecule. It has been emphasized that: "This action-and-reaction scenario underlies the success of virulent pathogens and illustrates the dynamic nature of the response of both partners in an adherence interaction" *(33)*. It appears that little, if anything, is known about the possible changes that might occur in, for example, pneumocytes or endothelial cells with age that would affect attachment and transmigration of pneumococci or other pneumonia-causing pathogens.

The ability to adhere to host enterocytes is a major determinant of virulence in the case of enteric pathogens. Most of those pathogens express adhesins that function as lectins *(43)*. The adhesins may or may not be present on bacterial fimbriae. Adhesins present on rigid fimbriae are maintained at a distance from bacterial surface components that might interfere with adherence to host cells. Adhesins present on flexible fimbriae are allowed spatial freedom in binding to cognate receptors.

Many enteric bacterial adhesins interact with and agglutinate erythrocytes from various species. They can be characterized by the spectrum of species of erythrocytes that they agglutinate. By this test, families of lectins have been identified *(43)*; these include galactoside-specific, sialic acid-specific, and N-acetylglucosamine-specific lectins. Some adhesins fail, however, as hemagglutinins (perhaps because the saccharide moieties that they recognize on erythrocytes are inaccessible) but do interact with cells of the intestinal tract. Intestinal mucous contains numerous, potentially inhibitory saccharide moieties that may block bacterial adherence. Whether or not this occurs depends on a number of factors including the quantity and rate of formation of mucous. The wide variety of glycoconjugates with which bacterial lectins interact is shown in Table 2-4. That variety ensures the ability of many enteric bacteria to adhere at optimum sites and niches within the intestine.

Intestinal *E. coli* strains can be categorized into several types depending on their attachment and invasive properties. Those categorized as enteropathogenic *E. coli* (EPEC) adhere to gut epithelial cells through intimin molecules. The latter are ligands for the bacterial complementary receptor known as Tir (translocated intimin receptor) *(44,45)*. Following the initial adherence of EPEC to host cells, the bacteria introduce Tir, along with several other proteins, into the host cells via a type III secretion mechanism. The expression of Tir significantly enhances the adherence and, thus, promotes the secretion of effacing factors into the host cells. This is presumably a key event in the initiation of diarrhea. What is particularly interesting about this example of adherence is the fact that the bacterium provides to the host cell the receptor (Tir) to which it strongly binds. Additional interest in this interaction arises from the recent finding *(46)* that the ensuing inflammatory reaction that leads to pathology of the colon is dependent on the involvement of type 1 T helper (Th1) cells (CD4+) and the IFN-γ that they secrete. It appears that intimin not only interacts with Tir but also with β1 integrin molecules on T cells. The resulting hyperplastic changes in the gut provide further opportunities for EPEC colonization. This is an excellent example of the diversion of host immune defenses (T cells and their cytokines) into paths that promote the welfare of the bacterial pathogen. There are other outstanding cases such as that of *S. typhimurium (47)*. In this case, the bacterium secretes proteins via a type III mechanism that

Table 2-4
Some Interactions of Bacterial Lectins-glycoconjugates

Bacteria	Lectin type	Carbohydrate specificity
Klebsiella pneumoniae	Type 1 & 3	mannose
Mycobacteria	Mycotin	mannose, mannan
Salmonella		mannose
Serratia marcescens		mannose
Shigella flexneri		α-mannoside
Vibrio cholerae		fucoside
Streptococcus pyogenes		galactose
Escherichia coli (urinary pathogens)	P-related (F7–F16)	Galα1-4Gal in
	G-I	globotriaosylceramide
	G-II	globotetraosylceramide
	G-III	globopentaosylceramide (Forssman antigen)
	AFA	Dr blood group antigen
Clostridium difficile	(enterotoxin)	Galα1-3Galβ1-4GlcNAc
Escherichia coli	G	N-acetylglucosamine
Streptococcus pneumoniae		GlcNAcβ1-3Gal
Escherichia coli (sepsis pathogen)	S	NeuAc α2-3Gal β1-3GalNAc & NeuAcα2-3Galβ1-3(NeuAcα2-6) GalNAc
Escherichia coli (urinary pathogen)		NeuAcα2-3Galβ1
Vibrio cholerae	(enterotoxin)	GM 1
Helicobacter pylori		GM3 (NeuAcα2-3 Gal β1–4Glc-cer)So3-GM3; Leb-group blood antigen
Mycoplasma galliseptum; *M. pneumoniae*		NeuAc α2–
Candida albicans		Lewis (a) antigen
Haemophilus influenzae		Lewis (a) antigen
Staphylococcus aureus		Lewis (a) antigen
Bordetella pertusis	PHA	Sulfated glycolipids, heparin
Borrelia burgdorferi (Lyme disease)		Gal-Cer, Lac-cer; GD1a, GD1b, GM2, GM3

Modified from ref. *43*.

activates signaling pathways in the host epithelial cells leading to production of IL-8 and other proinflammatory cytokines, e.g., TNF (tumor necrosis factor) α, and GM-CSF *(48–50)*.

Bacterial Type III Secretion Mechanism

There are four different mechanisms utilized by bacteria to export synthesized products, especially virulence factors *(51)*. Types I and II lead to secre-

tion of materials directly into the surrounding milieu. In type I secretion, the mechanism employs three proteins that form a channel through the inner and outer bacterial membranes. The type II mechanism has been studied extensively in *Vibrio cholerae (52)*. There are at least 12 proteins that appear to create a pore that bridges the inner and outer membranes and through which bacterial products are secreted. The type IV system was discovered fairly recently and the mechanism is under scrutiny *(53)*. Among other products, the secretion of immunoglobulin A (IgA) proteases occurs via the type IV system.

The type III secretion system is of particular interest owing to its complexity and the fact that it is employed by a number of Gram-negative pathogens to introduce virulence- related substances directly into the cytosol of host cells *(54)*. Some substances are secreted into the extracellular environment rather than translocated into a host cell. The type III system is employed by both animal and plant pathogens. Among the animal pathogens are *Shigella* spp., *Yersinia* spp., *Chlamydia* spp., *P. aeruginosa*, EPEC, enterohemorrhagic *E. coli*, and *S. typhimurium*. The type III secretion mechanism involves a structure termed the needle complex, which has been isolated from interacting *S. typhimurium* and host cells *(55)*. Electron microscopy showed that the structure resembles a long hollow tube attached to a cylindrical base that anchors the structure to the bacterial inner and outer membranes. Other bacteria may induce the formation of pedestals or cuplike structures between themselves and host cells. The formation of such structures is initiated upon contact and association of bacterium and host cells facilitated by adhesion molecules. Some bacteria, e.g., EPEC, induce extensive cytoskeletal reorganization in host cells leading to pedestals that contain cytoskeletal proteins (e.g., actin, α-actinin, talin). Those cytoskeletal rearrangements result in effacement, which includes loss of microvilli and the resulting diarrhea.

Bacterial Invasion of Host Cells

Some bacteria are obligate intracellular pathogens (e.g., *Chlamydia* spp.), many important pathogens are facultative intracellular organisms (e.g., *Salmonella* spp., *L. pneumophila*, *Mycobacterium* spp., *L. monocytogenes*), and others are predominantly extracellular (e.g., enterotoxigenic *E. coli*, *Haemophilus influenzae*, *V. cholerae*). Pathogens that penetrate epithelial barriers survive by invading and replicating in host cells. Tight junctions (zona occludens) that normally prevent penetration of epithelial cell layers also divide the epithelial cells into apical (lumenal) and basolateral surfaces. Some pathogenic bacteria such as *Salmonella* invade host cells from the apical surface whereas others (*Yersinia*, *Shigella*) interact with and invade through the basolateral surface.

The invasion of host cells by *S. typhimurium* has attracted the attention of several groups of researchers *(55–57)*, whose findings are quite significant.

The adherence of the bacteria triggers some pronounced cytoskeletal and membrane modifications of the host cells. Those modifications are triggered by the introduction (via a type III secretion mechanism) of bacterial proteins including Sop E, which activates GTPases of the Rho subfamily, and Sip A, which binds to actin and prevents depolymerization of actin filaments. The formation of actin filaments is required for bacterial internalization. Other proteins involved in cytoskeletal changes include α-actinin, talin, and ezrin. Associated with those changes is an intracellular Ca2+ shift and activation of "mitogen-activated protein" (MAP) kinase. These and other events, still to be elucidated, in a signal transduction pathway lead to membrane ruffling, macropinocytosis, and thus to internalization of the bacteria by a host cell.

The preceding descriptions of attachment, type III secretion, and invasion of bacteria ,vis-à-vis, epithelial cells are fundamental to future studies of epithelial cells that have been altered by senescence. To underscore the importance of such studies, it is worth stressing the growing evidence (discussed later) that aging results in some considerable changes in the composition and structure of membranes.

As noted above, pathogens such as *Yersinia* and *Shigella* invade epithelial cells via the basolateral surface. *Salmonella* can also take this as well as the apical approach. How do bacteria that can only penetrate the basolateral surface gain access to that surface? This question is particularly relevant to enteric pathogens. The answer appears to be that they are transported indiscriminately through specialized M cells overlying Peyer's patches that are scattered throughout the small intestine *(56)*. M cells lack all but a thin layer of mucous and are nearly devoid of villi. It is agreed that the transport of macromolecules and particulate matter from the intestinal lumen through the M cell brings the transported material into proximity with macrophages and lymphocytes located in "pockets" on the antilumenal side of M cells. In this manner bacteria can reach the basolateral surfaces of epithelial cells. *Shigella* employ an alternate method of crossing the barrier. They can induce chemotaxis of phagocytic cells, especially polymorphonuclear leukocytes. As the latter migrate toward the bacteria they open spaces in the tight junctions through which the bacteria pass across the epithelial surface *(58)*. It is interesting to note that *S. typhimurium* is representative of several bacteria that can cross the intestinal barrier either via M cells or by traversing enterocytes *(56)*.

The entrance and fate of bacteria that enter into host macrophages and dendritic cells are discussed in some detail in Chapter 3. As mentioned previously, the case of *L. monocytogenes* may be considered prototypic. Some macrophages readily destroy ingested *L. monocytogenes* whereas others lack that ability *(24)*. Bacteria replicate in the latter and may be passed from cell to cell without becoming accessible to the host's immune system.

Biofilms and Quorum Sensing

Individual bacteria that lodge in alveoli or attach to the epithelium of the intestine or urinary bladder are in a precarious situation. The hostility of the environment makes it unlikely they will survive for long. Therefore, in order to outmaneuver host defenses, the bacteria replicate rapidly and form microcolonies. Many species of bacteria, including a number of pathogens, form organized communities termed "biofilms."

Only in the last decade has a clear understanding of the mechanisms and significance of biofilm formation begun to emerge *(59)*. For example, it was realized that particles of biofilm formed by *L. pneumoniae* and circulating in building air ducts were responsible for the notorious outbreak of Legionnaires disease in Philadelphia in 1976. In 1993–1994, hundreds of asthmatic individuals received albuterol that, although drawn from a disinfected processing tank, was contaminated with particles of *P. aeruginosa* biofilm *(60)*. It is now well-known that the lungs of cystic fibrosis patients are sites where biofilms of *P. aeruginosa* are formed.

The importance of biofilms in infectious diseases associated with aging is suggested by the contents of Table 2-5. In addition to the infections shown in that table, there is growing evidence that biofilms may develop and complicate bacterial pneumonia and intestinal infections (bacterial overgrowth) and may perpetuate the durable infections of *M. tuberculosis* often associated with the recurrence of tuberculosis in the elderly. The recent realization that bacteria present in biofilms are notoriously resistant to antibiotics and are protected from both humoral and cell-mediated immunity of the host are of major concern in treating infections of the elderly.

The community of bacteria in biofilms is protected by an extracellular matrix of polysaccharide (termed "glycocalyx"). The chemical nature of the latter varies with the species of bacteria and whether the community is mixed or of a single species. Moreover, the community organization allows functional heterogeneity and regional specialization much like an organized tissue. Individual bacteria (called "planktonic") may leave the biofilm and disperse to other sites somewhat resembling metastasis of tumor cells. Planktonic bacteria are susceptible to antibiotics and host immune response.

The formation of biofilms by *P. aeruginosa* has received considerable attention because it is a critical event in the devastating disease, cystic fibrosis *(59,61)*. This same bacterium is found in most healthy individuals in whom it causes no disease. It is an opportunistic organism that only becomes pathogenic in compromised individuals. A brief account of biofilm formation by *P. aeruginosa* on the epithelial linings of the lungs of cystic fibrosis patients provides a concept of the process. Attachment factors are present on hairlike appendages of the bacteria called type IV pili *(62)*. Those pili allow a twitching

Table 2-5
Some Human Infections Involving Biofilm Formation

Infection or disease	Common biofilm bacterial species
Dental caries	Gram-positive cocci (e.g., *Streptococcus*)
Periodontitis	Gram-negative anaerobic oral bacteria
Biliary tract infection	Enteric bacteria (e.g., *E. coli*)
Osteomyelitis	Several bacterial and fungal species
Bacterial prostatitis	*E. coli* and other Gram-negative bacteria
Native valve endocarditis	Viridans group *Streptococci*
Nosocomial infections	
ICU Pneumonia	Gram-negative rods
Sutures	*Staphylococcus epidermidis* and *S. aureus*
Contact lens	*P. aeruginosa* and Gram-positive cocci
Urinary catheter cystitis	*E. coli* and other Gram-negative rods
Hickman catheters	*S. epidermidis* and *C. albicans*
Vascular grafts	Gram-positive cocci
Biliary stent blockage	Various enteric bacteria and fungi
Orthopedic devices	*S. aureus* and *S. epidermidis*

Modified from ref. *59*.

motion that aids the bacteria in assembling into colonies. *P. aeruginosa* utilizes a type III secretion system to secrete toxin into epithelial cells, which among other effects interferes with ciliary sweeping thus further aiding microcolony formation. The attached bacteria proliferate, and as their number reaches a certain minimum density, sets of genes become activated the products of which include both virulence factors (e.g., toxin A, exoenzyme S) and other substances that promote bacterial cell wall remodeling and glycocalyx formation. In the case of *P. aeruginosa*, the principal glycocalyx material is the polysaccharide, alginate. At this point, the bacteria embedded in the glycocalyx are protected from antibodies, cell-mediated immunity, and antibiotics. Antigens are released by bacteria in the biofilm as well as planktonic forms and high levels of immunity may prevail in the host, but to no avail.

As noted in the preceding paragraph, when bacteria in the developing colony reach a certain number (density) activation of a new set of genes occurs. This event reflects the recognition of a threshold concentration of organisms termed "quorum sensing." It was first noted in bioluminescent marine bacteria in which the intensity of luminescence increased dramatically at a certain bacterial density.

What is the mechanism of quorum sensing? There appear to be several mechanisms employed by different species of bacteria as well as certain fungi

and possibly protozoa. The best understood mechanism, at present, operates in several Gram-negative human pathogens and has been analyzed extensively, again utilizing *P. aeruginosa (61,63)*. As the density of the proliferating bacteria increases, so does the local concentration of small molecules that the bacteria synthesize known as acylhomoserine lactones (acyl-HSL) [e.g., N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) and N-butyryl-L-homoserine lactone (but-HSL)]. The acyl-HSL molecules are the quorum-sensing signals. Two quorum-sensing systems have been identified in *P. aeruginosa*: one is the lasR-lasI system and the other the rhlI-rhlR system. The product of the lasI gene directs the synthesis of 3OC12-HSL and the product of lasR, in the presence of a sufficient concentration of 3OC12-HSL, activates a set of virulence genes that includes *rhlI* and *rhlR*. The gene, *rhlI*, is responsible for a product that directs the synthesis of but-HSL, which is involved in the activation of virulence genes.

Progress in understanding quorum sensing and the variety of systems that participate in the phenomenon has been rapid (*see*, e.g., ref. *64*). Some biofilms comprise a single species of bacteria in which case the signals utilized by that species must be distinguished from those of other species. Other biofilms are composed of mixed species and in this case organisms may need to recognize several signals but obviously not all. Signal sensing among various species may help a given species, or group of related species, to avoid competition, ascertain an appropriate niche, or perhaps produce an antimicrobial substance to minimize or eliminate local competitors.

From the perspective of bacterial infections in aging subjects, biofilms would appear to be of great importance. First and perhaps foremost, biofilms render many pathogens safe from antibiotics and immune attack. Second, it is likely that biofilm formation by various bacteria that are nonpathogenic in healthy, young adults may lead to serious infections in immunocompromised elderly or those already afflicted with some disorder. Third, the widespread use of urinary catheters, the high prevalence of prostatic disease among elderly males, and the frequency of bone and joint repair and replacement in the elderly offer to microbial pathogens a range of opportunities for clinical biofilm formation. Finally, it seems important to stress that biofilms is a subject that has received very little attention in relation to the susceptibility of the elderly to infections. Are conditions in aging tissues more or less favorable for the formation of biofilms? Or unchanged? Consider the mounting evidence that changes in the cytoplasmic membranes accompany senescence of various cells; does such a change occur in epithelial cells and might that influence bacterial adherence and biofilm formation? Do senescing tissues offer more and better-sheltered niches for bacterial colonization? An opportunity seems to exist for research that could have quite significant consequences.

ANTIBIOTIC RESISTANCE AND BACTERIAL VARIATION

Resistance to antibiotics is increasing rapidly among human pathogens as pointed out by many authors (e.g., refs. *65* and *66*). The reasons center around one problem: the failure to control the human use of antibiotics. Numerous studies have shown that in medicine antibiotics are frequently prescribed unnecessarily or inappropriately. For example, it was estimated that in 1992, 12 million adults who presented bronchitis or upper respiratory infections received prescriptions for antibiotics that offered little or no benefit *(67)*. Similar studies of inappropriate antibiotics usage have focused on Canada, Europe, and Japan. In developing countries, antibiotics usage has been poorly regulated, patient compliance has been poorly monitored, and much of the supply of antibiotics is of low quality. The common use of antibiotics in veterinary medicine and in agriculture has contributed to the problem to an extent that is difficult to determine but likely to be considerable.

Geriatric medicine has certainly contributed to the growing problem of microbial antibiotic resistance. As a group, elderly patients in hospitals and long-term care facilities (LTCFs) are the major recipients of antibiotics. It has been estimated that among residents of LTCFs approx 40% of prescribed drugs for systemic use are antibiotics *(68)*. A distressing result is the promotion and spread of antibiotic-resistant microorganisms *(69)*.

The types and origins of antibiotic-resistant pathogens in LTCFs have been carefully reviewed in a recent publication *(70)*. Information about antibiotic resistance in the population of elderly who reside in LTCFs is probably the most reliable available. As stated in the review article *(70)*, the antibiotic resistant bacteria of greatest concern to geriatricians are: 1) β-lactam resistant organisms, especially penicillin-resistant pneumococci and aerobic Gram-negative bacilli resistant to third-generation cephalosporins; 2) vancomycin-resistant enterococci; and 3) quinolone-resistant Gram-negative and Gram-positive bacteria.

As recently as 1997, it was reported that more than one-third of *S. pneumoniae* isolates analyzed in broad survey were resistant to penicillin and more than 13% of them were highly resistant *(71)*. Relatively resistant isolates have been found in many regions of the world. Altered penicillin-binding proteins (enzymes involved in the final stage of bacterial cell wall formation), which have low affinity for penicillin, are responsible for resistance to that antibiotic. Other organisms noted for β-lactam antibiotic resistance are certain species of *Staphylococcus* (some resistant to all penicillins, cephalosporins, and carbapenems) and *Enterococci*, which are resistant to all cephalosporins because they lack significant penicillin-binding proteins.

Enzymes known as β-lactamases are largely responsible for the ability of bacteria to resist the cephalosporins. Considerable effort to produce enzyme-

resistant derivatives of those antibiotics has been expended leading to so-called third-generation cephalosporins that showed considerable promise. However, a number of enzymes have now been reported (called "extended spectrum β-lactamases") that can cleave advanced-design cephalosporins and penicillins.

Many of the extended spectrum β-lactamases (ESBLs) are encoded by genes borne on plasmids such as those encoding TEM-1 associated with enteric bacteria. TEM-1 is responsible for close to three-fourths of plasmid-borne β-lactamase resistance world wide *(72)*. The TEM group of enzymes is encoded by the TnA transposon, which probably accounts for the β-lactamases present in more than one-third of *H. influenzae* isolates in the United States. Several pathogenic Gram-negative bacilli (*Enterobacter*, *Citrobacter*, *Pseudomonas*) produce β-lactamases that are encoded by a chromosomal gene. Those AmpC enzymes are able to inactivate all cephalosporins. Several AmpC β-lactamases are now known to be conveyed by mobile, conjugative plasmids in *E. coli* and in *Klebsiella* species. The presence of those enzymes can result in resistance to penicillins, cephalosporins, cephamycins, and β-lactamase inhibitors *(73)*. It should be noted that resistance to β-lactam antibiotics can occur as a result of restricted entry of the antibiotics as well as low binding affinity to the penicillin-binding proteins and destruction by β-lactamases.

Drug efflux pumps, which restrict antibiotic entrance into bacteria, have become a major problem in antibiotic therapy. They result in inadequate accumulation of antibiotics inside bacterial cells to be effective. The formation of transport proteins, which bind and inactivate antibiotics or escort them out of the bacterial cell, prevents the antibiotics from reaching critical targets. Some of these efflux pumps are drug-specific such as Tet B in enteric bacteria and *H. influenzae*; others act in a broad, "multidrug" pattern. Tet B is plasmid encoded, although chromosomally mediated tetracycline resistance occurs in some bacteria such as *Proteus*. Tetracycline resistance conveyed by plasmids is situated near insertion sites and as a consequence those plasmids rather readily acquire other genetic information, which results in broadening the specificity of the resistance. It is likely that the widespread use of tetracyclines in animal feed is responsible, in part, for the existence in many regions of the world of resistant *Enterobacteriaceae*.

The quinolone antibiotics such as nalidixic acid and ciprofloxacin bind to DNA gyrase (type II topoisomerase) in Gram-negative bacteria, and to topoisomerase IV in Gram-positive bacteria thus interfering with DNA replication. Some of the later quinolones have other actions in addition to their effects on topoisomerases and display a broad spectrum of antimicrobial activity *(74)*. Resistance to these antibiotics may emerge as a result of mutations in bacterial topoisomerases, diminished membrane penetrability in Gram-negative bacteria, or active efflux transporter proteins. Such changes are generally

caused by alterations in the chromosomal DNA such as point mutations in the A subunit of DNA gyrase *(74)*.

Several species of *Staphylococci* and *Enterococci* are notorious for being nosocomial infections. The antibiotic, vancomycin, has been widely used to combat these infections because for many years no resistance to this substance was reported. Resistance of entercocci was first reported in 1986, which resulted in considerable effort to elucidate the causes *(75,76)*. Vancomycin is a glycopeptide that interferes with cell wall formation of Gram-negative bacteria. It interacts with D-alanine at the C-terminus of precursors of peptidoglycans. This creates a complex from which the precursor substances cannot be transferred by transglycosidases to the growing peptidoglycan cell wall.

Resistance to vancomycin appears as a consequence of expression in bacteria of transposable genes, which encode cell-wall-synthesizing enzymes that alter the C-terminus of the peptidoglycan precursors from D-alanine to D-lactate. This change allows cell wall construction to continue even in the presence of vancomycin. There are four known phenotypes of vancomycin-resistant enterococci of which two (Van A and Van B) are associated with moderate to high resistance to the antibiotic *(70)*. Both of those phenotypes are readily transferred on plasmid and transposon elements. The Van B phenotype is also associated with a chromosomal complex that closely resembles the organization of the Van A transposable element.

The importance of vancomycin resistance to the geriatrician is the common usage of that antibiotic to treat UTIs and infected pressure ulcers, which are relatively common in LTCFs *(70)*. Vancomycin-resistant enterocci are introduced most often into LTCFs by accepting patients who have acquired resistant organisms in hospitals.

To complete this brief discussion of antibiotic resistance, a word about transposons is in order. Those genetic elements, conveyed by resistance plasmids (R-plasmids), are responsible for much of the current microbial resistance to antibiotics. There are collections of bacteria, assembled in the preantibiotic era, that display recognizable plasmids; but most of those plasmids lack antibiotic-resistance elements. This must mean that current bacterial pathogens displaying antibiotic resistance harbor familiar plasmids that have become R-plasmids by acquiring resistance transposons. That is a consequence of the excessive use of antibiotics, which has given selective advantage to bacteria possessing R-plasmids. Among the latter, those that convey multiple antibiotic resistance vary with respect to the transposons they contain. Some have a single transposon composed of multiple resistance determinants. Some have several transposons located in different sites. In some cases, there is present a complex element in which one transposon has become integrated into another. Apparently, there has been no effort to evaluate the R-plasmids present in bacterial

isolates from older persons, especially those residing in LTCFs, to determine whether or not the plasmids and the resistance transposons differ from isolates obtained from young adults. If a significant difference exists, such information should be useful in planning judicious antibiotic therapy.

The extreme variability, versatility, and adaptability of bacteria arise from two processes that are not found in eukaryotic organisms: (a) horizontal (lateral) transfer of genetic material *(77)*; and (b) hypermutability associated with "mutator" strains *(78,79)*. There are three mechanisms for delivering exogenous DNA into recipient bacteria: (a) transformation, which involves bacterial uptake of naked, ambient DNA; (b) transduction, in which new DNA is delivered by bacteriophages; and (c) conjugation, which requires physical contact between donor and recipient cells and, most frequently, transfer of a plasmid. Once inside the recipient cell, the DNA must become assimilated either as a stable episome or by integration into the recipient's genome if it is to be expressed. Hypermutation, exemplified by mutator strains, appears to arise from mutations in genes that affect the synthesis, modification, or repair of DNA. A recent study *(78)* of the presence of mutator strains of *P. aeruginosa* isolates from cystic fibrosis patients has provided strong evidence for the concept that the emergence of mutator strains is a mechanism employed by bacteria for rapid adaptation to nonoptimal, even hostile conditions in their hosts. This concept (*see* comments in ref. *79*) could be of major significance with regard to infections in the elderly in whom microenvironmental conditions in organs such as the lung, urinary bladder, and GI system may differ considerably from those in younger subjects.

VIRAL INFECTIONS IN AGING HUMANS

Viruses are small, composite particles of nucleic acid and protein, and are obligate, intracellular parasites; i.e., they cannot replicate outside a host cell. An individual virus contains only one type of nucleic acid, either RNA or DNA, which is protected by the associated protein from destruction by hostile substances, such as nucleases, present in its environment. Viral proteins serve two other crucial functions; first, they are responsible for attachment of virions to host cells and, second, they include a minimal array of enzymes that are necessary to cajole the host cell machinery into synthesizing new virions. Together, the nucleic acid and associated protein form the nucleocapsid. Some viruses are encased in a lipid bilayer, derived from host cell membrane, termed the "envelope." It is often studded with outward-protruding, complex molecules of glycoprotein. Viral nucleic acid (genome) may be either RNA or DNA arranged in linear or circular fashion. The nucleic acid may occur in single-stranded or double-stranded form. If the genome is single-stranded RNA, it is considered to be in the positive (plus) sense orientation if it can serve as its

own mRNA and in negative (minus) sense orientation if a copy must first be made by a viral RNA transcriptase, which then serves as mRNA. Most DNA viruses display a linear, double-stranded genome although a few families are characterized by linear single-stranded (parvoviruses), circular double-stranded (papovaviruses), or even circular single-stranded (circoviruses) DNA. Comprehensive introductions to viral genomes and virus replication are provided in many excellent texts (e.g., ref. *80* and *81*).

Viral infection begins with attachment of virions to host cells. Specific attachment that leads to virion penetration generally depends upon complementary interaction between viral protein (counter-receptor or anti-receptor) and specific receptors on host cells. Viruses may display a single species of protein counter-receptor or multiple species of counter-receptors in the case of some complex viruses such as herpes simplex. Whether or not a host cell is susceptible to a given virus depends upon the cell having receptors. Cells lacking receptors are not susceptible. If a host cell supports the complete reproduction of a given virus, it is termed "permissive." Some host cells can be shown to be permissive but not susceptible because they lack the appropriate receptors.

The cellular receptors for viruses are generally glycoproteins. Some of the receptors are familiar molecules known to be involved in other functions. Table 2-6 is a list of a few of those receptors. The ability of viruses to usurp surface molecules designed for some other purpose as receptors for themselves is well illustrated by human and simian immunodeficiency viruses. Those viruses utilize members of the chemokine superfamily (CXCR4, CCR5) along with CD4 as coreceptors for entrance into T cells and monocytes (reviewed in ref. *82*). Chemokine receptors have been appropriated also by other viruses; e.g., myxoma virus can utilize CCR1, CCR5, CXCR4 for entrance into host cells *(83)*. Myxoma virus is a poxvirus the receptors for which have been difficult to identify. The epidermal growth factor receptor is utilized by vaccinia virus another poxvirus.

A poxvirus that has the human as primary host is the molluscum contagiosum virus (MCV), which causes persistent, benign, skin neoplasms in children and severe opportunistic infections in AIDS victims. Both children and AIDS victims are immunodeficient (to much different degrees, of course). Elderly individuals are immunodeficient. It seems natural, therefore, to wonder whether or not the elderly are also susceptible to MCV. If so, it has not been reported (to our knowledge). Perhaps factors other than immunodeficiency are involved in rendering subjects susceptible to MCV. It would be useful to know.

The complexity of viruses is exemplified by their nomenclature, which comprises some 8 major families of DNA viruses and 14 families of RNA viruses (with more to come, no doubt). Of those 22 families, 20 include members that have medical importance (*see* ref. *84* for a concise overview). The first five of

Table 2-6
Some Familiar Cell Membrane Receptors for Viruses

Virus	Receptor	Representative important cell infected
Adenovirus	Integrin ($\alpha 5\beta 3$)	Respiratory epithelium
Epstein-Barr	Complement type 2 receptor (CD 21)	B lymphocytes
Herpes simplex	Proteoglycans (heparin sulfate moieties)	Oral and genital epithelium
Influenza A, B	Glycoproteins of 5Ac Neu	Oropharyngeal cells
Respiratory syncytial	Hemagglutinin glycoprotein	Respiratory epithelium
Rhinoviruses	Intercellular adhesion molecule (ICAM)	Nasal epithelium
HIV-1, -2	CD4, galactosyl ceramide, chemokine receptors	T lymphocytes

six families listed in Table 2-7 contains members that cause respiratory disorders any of which can progress to pneumonia in the elderly. The influenza viruses of family Orthomyxaviridae are of the greatest concern because the elderly are so susceptible and because each new flu season may bring an antigenic variant arising from the "drift" and "shift" in antigenic types that are so typical of influenza viruses.

The far-right column of Table 2-7 is headed "Persistence." Two families (four types) of viruses are listed to illustrate persistence. There are three types of persistence, termed "chronic" (diffuse or focal), "latent," and "slow." Here, we are interested in latent persistence. Latency refers to the fact that some viruses may integrate into the genomes of host cells where they persist for extended periods without replicating or killing host cells and without causing disease. An outstanding example of latency in a bacterial infection is that of the *Mycobacteria*, which cause tuberculosis. The four viruses listed in the table are representative of persistence. *This phenomenon may be of unrecognized importance in aging humans.* It should be stressed that (to our knowledge) there is no irrefutable evidence that persistent viruses afflict the elderly inordinately. However, it should be stressed equally that (to our knowledge) there have been no systematic studies of that possibility. In the following brief paragraph, evidence is marshalled to support the idea that more attention should be focused on assessing persistent viral infections in aging humans.

Table 2-7
Significant Viruses of Aging Humans

Family	Virus	Disorder	Persistence
Coronaviridae	Coronaviruses (two major types)	Common cold (sinusitis)	No
Orthomyxoviridae	Influenza (A,B,C)	Influenza (pneumonia)	No
Paramyxoviridae	Respiratory syncytial virus	Respiratory infections (upper and lower)	No
Picornoviridae	Rhinovirus (>100 serotypes)	Respiratory infections (common cold)	No
Adenoviridae	Adenovirus (numerous serotypes) gastroenteritis	Respiratory infections (colds, pneumonia)	Yes
Herpesviridae	Herpes simplex	Gingivostomatitis, genital herpes, herpetic keratitis, encephalitis	Yes
	Cytomegalovirus	Mononucleosis (multiple organ infection in immunocompromised)	Yes
	Varicella-zoster Virus	Herpes zoster (shingles)	Yes

Consider, first, the adenoviruses. There are at least six subgenera and numerous serotypes of human adenoviruses. The principal targets of the viruses are the respiratory tract, ocular tissues and, less frequently, the GI system. The ability of a few types of human adenoviruses to induce tumors in hamsters and transform human and animal cell lines has attracted attention for many years although there is little evidence that they are oncogenic in humans. The adenoviruses present a classical example of latency. The viruses or their genomes are found in tonsils. Cells of the tonsils of individuals who have experienced infections but have been symptom-free for extended times may have whole or partial virus genomes integrated in their own genomes. It is uncertain how long the virus genomes may continue to replicate in individuals who remain symptom-free. Whether or not latent adenoviruses may be reactivated under certain conditions in aging subjects is a question that seems not to have been addressed.

The establishment of latency generally involves integration of viral genomes into the host cell genome or occasionally an episome. Integration of adenovirus DNA has been demonstrated in transformed human cells and in virus-induced tumors in hamsters; and integrated viral DNA may persist for long

periods in human tonsil cells. What restricts the viral replication in those cells, and the events or factors that trigger reactivation, are unknown. Clearly, this emergence from latency deserves careful study on the hypothesis that adenovirus and other viruses may be reactivated in the immunodeficient elderly and precipitate disease.

Three of the viruses in the family of Herpesviridae that are well known for their latency are listed in Table 2-7. First, there is herpes simplex, which exists as two closely related types (HSV-1 and -2). The former is primarily responsible for gingivostomatitis in young children, the latter for genital herpes in adults. HSV-1 is the principal cause of focal, sporadic encephalitis, which in the United States occurs in approximately 1 in 150,000 population. Second is the cytomegalovirus (CMV), which when acquired congenitally (approx 1% of live births in the United States) causes severe disease in infants and young children. In adults and older children, CMV may cause a mononucleosis which resembles that caused by Epstein-Barr virus. The third herpes virus known for latency is varicella-zoster virus (VZV), the causative agent of chickenpox, which may occur in children or adults. Reactivation of VZV may produce herpes zoster ("shingles"), which appears in about 1% of individuals over age 50.

HSV-1 and -2 infections occur preferentially at mucocutaneous sites. As the infection and accompanying inflammation progress, the viruses ascend peripheral sensory nerves to reach dorsal root ganglia. The viruses replicate in nervous tissue and then migrate in retrograde fashion along axons to reach other mucosal and epithelial surfaces thus spreading the infection. Latency is established in cells of the dorsal root ganglia. Herpes simplex encephalitis affects preferentially the temporal lobe of the brain and can be initiated by reactivated viruses in addition to viruses of the primary infection.

Primary CMV infections occur most efficiently in salivary glands and kidneys. Persistent infections are found in those tissues and in breast, endocervix, seminal vesicle tissues, and peripheral blood leukocytes. Patients with deficient immune systems, such as bone marrow transplant recipients and those with immunodeficiency diseases, are at risk of primary or reactivated CMV infections. In those patients, infection may involve the lungs, GI system, liver, and other organs/tissues, and often becomes life-threatening. It would be interesting, and probably quite significant, to determine whether, and how frequently, CMV-induced respiratory and GI disorders occur in the aging population as a consequence of reactivation of host cell-integrated viral genomes.

Similar to HSV-1 and -2, VZV assumes latency in the dorsal root ganglion. Herpes zoster appears as a result of reactivation of latent virus. An important fact about herpes zoster stands out; *viz.*, acute neuritis is characteristic in most patients whereas the frequency of postherpetic neuralgia occurs in about half of the adults, but not in juveniles, and the frequency seems to increase in older

patients. As noted above, herpes zoster occurs with a frequency of about 1% in adults over 50.

All four of the described latent viral infections are serious problems in immunocompromised individuals in whom multiple organ sites are involved. It is well established that aging humans (and laboratory animals) are deficient in one or more aspects of immunity and that T-lymphocyte-dependent antiviral immunity is one such aspect (Chapters 3 and 4). We are not so foolish as to suggest that an elderly subject is similar to an AIDS victim or an individual under treatment with an immunosuppressive drug; however, we do suggest that lessons learned from those patients may be applicable to the elderly. Under conditions of good health and environmental circumstances, most elderly persons retain sufficient immunological potential to cope effectively with acute infections. However, when the immune potential is further reduced by illness, injury, stress, or severe xenobiotic (pollutant) exposure, many elderly subjects may become vulnerable to microbial pathogens. Those are precisely the insults and injuries that are known to activate latent viruses.

The need for effective, safe antiviral drugs will continue with increasing urgency in the years ahead. One reason it has been difficult to find or develop antivirals is because viruses utilize so much of the host cell machinery for their own fabrication. Another reason is the extreme ingenuity displayed by viruses to defend and protect themselves as shown in Table 2-8 *(85)*. A new, promising direction toward antivirals is that of interfering with, or redirecting, viral association with cellular receptors and is based on detailed structural knowledge. For example, the counter-receptor site ("knob domain") in association with the binding domain of the cognate receptor (the Coxsackie and adenovirus receptor, or CAR) has been crystallized and analyzed to the 2.6 A resolution level *(86)*. Whether or not the extensive viral use of receptors involved in key host cell functions will allow the development of discriminating antivirals remains to be discovered.

PROTOZOAN PARASITES IN AGING SUBJECTS

There are no reliable data on the relative susceptibility of aging humans to animal parasites (i.e., parasites other than microbial) or on the relative severity of parasitic infections in aged compared to young or middle-aged subjects. The principal reasons for this dearth of information are (a) parasitic infections are largely restricted to tropical climates in underdeveloped regions of the world where public health records are limited, and (b) where parasites abound, the majority of the population carries chronic infections acquired in childhood or young adulthood. Similarly, there are few data concerned with parasitic infections relative to age in natural animal populations. An example of the few published studies in animals reported an analysis of cattle infected with the parasite,

Table 2-8
Intracellular Defense Strategies Used by Viruses

Type of Virus	Host cell antivirus mechanism	Virus counter strategies
Epstein-Barr	Apoptosis (cell death)	Homologs of bcl-2
Rabbit pox		Serpins
Simian virus 40		p 53 binding protein
Herpes virus	Intracellular signaling	Tyrosine kinase modulation
Myxoma virus		Receptor mimicry
Adenovirus Cytomegalovirus	Viral antigen presentation	MHC Class I suppression
Molluscum contagiosum	Oxidative stress response	Antioxidant selenoprotein

Modified from ref. *85*.

Onchocerca ochengi (87). Those cattle lived in an area of high endemicity in the Cameroon and 71% of those studied were infected. Although there was no difference in the prevalence of infection among the three age groups studied (1.5–2.5 years, 3–5 years, >8 years of age), the parasite burden ("worm load") was significantly greater in the group >8 years of age. In contrast, there was a significantly lower number of the immature forms (microfilariae) in the older compared to the younger cattle. Whether or not this latter finding reflected more effective immunity or some other, inimical physiological change with age could not be determined. It is necessary, therefore, to extrapolate from experimental studies in laboratory animals to gain insight concerning the abilities of elderly humans to cope with parasites.

The earliest study (of which we are aware) was of infections of rats of different ages with the nematode, *Trichinella spiralis (88)*. The data suggested that the severity of infection (parasite burden) was significantly greater in the oldest animals. Apparently, there have been no other studies with parasites other than protozoa.

The work of Gardner and Remington has shown clearly that aged mice develop significantly worse infections with *Toxoplasma gondii* than do younger mice *(89,90)*. *T. gondii* is not a natural human parasite but can infect normal infants and young children in whom it may cause serious central nervous system disorders. *T. gondii* is one of the major opportunistic, protozoan infections in AIDS victims. The susceptibility of aged mice was shown to be, in part, the

result of decreased antibody production against the parasite in both the acute and chronic phases of the infection. However, of greater importance was the finding of a pronounced difference in the activation of macrophages of young and aged mice during the acute phase of infection. *T. gondii* is an intracellular parasite and, therefore, immunological resistance is primarily a cell-mediated process. The depressed activity of macrophages, which play key roles in natural/innate resistance and immunity to intracellular parasites, was considered responsible for the heightened infections found in aged mice. The effects of senescence on macrophages are discussed in Chapters 3 and 4. However, T cells (both CD4+ and CD8+) play roles in immunity to *T. gondii (91)* and those cells are significantly altered by senescence, as is discussed later.

The protozoan, *Trypanosoma musculi*, is a natural parasite of mice. It infects all of a number of inbred strains of mice; however, the severity of infection, judged by the parasite burden, varies over a 20-fold range (approximately) among different strains *(92)*. Regardless of the strain, however, aged mice develop significantly worse infections. This is illustrated in Figure 2-1, where the course of infection in young and aged mice of strain A is depicted. *T. musculi* organisms live extracellularly in the bloodstream of mice and the parasite burden can be assessed by determining the level of parasitemia, i.e., counting the numbers of parasites in blood samples. *T. musculi* infections are self-limiting; i.e., after a prolonged period of about 3 weeks in young adult mice the infections terminate. Thereafter the cured mice are permanently immune to reinfection. As Figure 2-1 shows, both the parasite burden (parasitemia) and the duration of infection (time before the cure) are markedly extended in aged compared to young adult mice.

To demonstrate that the elevated parasitemia in aged mice was a reflection of a deficient immune response, the technique of adoptive conferral ("transfer") of immunity was employed. The conferral of immunity to *T. musculi* on irradiated, immunologically incompetent mice by the transfer of a predetermined, optimum number of spleen cells from normal, infected, or cured donor mice was evaluated. After receiving the donor spleen cells, the irradiated recipient mice were inoculated with viable *T. musculi* and the course of infection monitored. The results of such a study, in which equivalent numbers of spleen cells were transferred from young or aged infected donor mice into irradiated young-adult recipients, are depicted in Figure 2-2. Irradiated mice, lacking a competent immune system, that were inoculated with *T. musculi* but given no donor spleen cells died from overwhelming *T. musculi* infection (Fig. 2-2A).The transfer of spleen cells from young donor mice on day 7 of their infection was able to protect irradiated recipients and cure their infection in about three weeks (Fig. 2-2B). In contrast, the same number of spleen cells from aged donors on day 7 of infection conferred no protection on the irradiated

recipients (Fig. 2-2B). On day 14 of infection, spleen cells from both young and aged donor mice were able to protect irradiated, young recipients from lethal *T. musculi* infection. However, the cells from young donors were much more efficient than those from aged donors as shown by the marked differences in levels of parasitemia and duration of infection in the recipient mice (Fig. 2-2C). Finally 21 days after initial infection, cells from aged donors were able to protect aged recipients but only after a prolonged infection (Fig. 2-2D).

The two preceding examples of the relative inability of aged mice to cope with protozoan infections provide compelling evidence that senescence cripples the immune system. In both cases, there is considerable understanding of the nature of the immune response against the parasites in young adults as is discussed in Chapters 3 and 4. It should be stressed here that the two parasites, *T. gondii* and *T. musculi*, are quite different in their life cycles and in other aspects. *T. gondii* are intracellular parasites whereas *T. musculi* are extracellular. Immunity to *T. gondii* is a cell-mediated process whereas immunity to *T. musculi* is dependent on specific antibodies, probably of IgG2a isotype (mouse) *(93)*. *T. gondii* will establish infection in several hosts (cats, mice, humans) whereas *T. musculi* is strictly a mouse-specific parasite. Considered together, studies of these two protozoa suggest that the ability of aged mice to generate both humoral and cell-mediated immunity to pathogens is impaired.

FUNGAL INFECTIONS IN AGING SUBJECTS

There have been few attempts to evaluate the frequency or severity of fungal infections in aging subjects. On the other hand, it is well-established that fungal infections are rather common in other immunodeficient individuals such as AIDS victims, persons being treated with immunosuppressive drugs, patients on antibiotic therapy or suffering from burns, diabetes, or malnutrition *(94)*. One study in particular, strongly indicates that more attention should be given to fungal infections in the aging *(95)*. In that study, the frequency of mortality as a consequence of systemic infections with bacteria (bacteremia) or fungi (fungemia) was assessed from the medical records of 500 patients identified as having true bacteremia or fungemia. The parameters relevant to the present discussion that were considered included: 1) mortality associated with both bacteremia and fungemia; 2) the primary site of the infection; 3) body temperature; and 4) the degree of leukopenia. There was a substantial increase in the risk of death of subjects over age 50 and deaths were more frequent in males than females. The risk of death was significantly greater when the primary site of infection was a surgical wound, a burn or even untraumatized skin, an abscess, or the respiratory tract compared to other sites. There was a markedly greater (about fivefold) frequency of mortality of patients whose body temperature was less than 36°C compared to those whose temperature was over 40°C. A peripheral leukocyte count

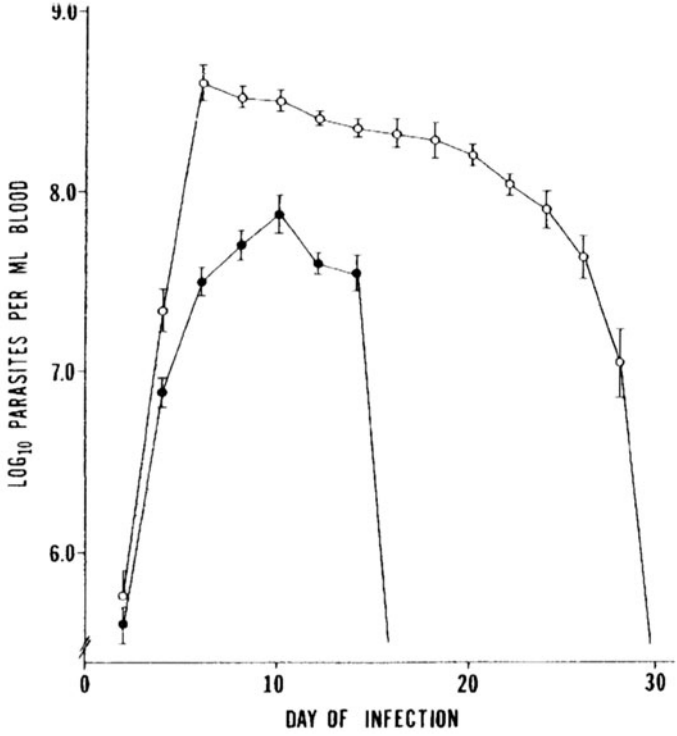

Fig. 2-1. Course of parasitemia in young (●) and aged (○) A/He mice following inoculation with *T. musculi*. Four or five samples per point. Bars represent 1 S.E.M. (From Albright JW, Albright JF. Mech Ageing Dev 1982;20:315–330.)

of less than 4000/μL, or a granulocyte count of less than 1000/μL, both correlated with substantially higher mortality. Given that (a) injury and trauma (i.e., stress) significantly alter immune responses in the elderly (see later), (b) the skin and respiratory systems of the elderly are sites of common fungi that are benign in younger individuals (e.g., ref. *96*), and (c) that elderly humans are less disposed to run fevers *(97)*, it is difficult to avoid the conclusion that fungal infections are significant problems in the elderly. Emerging, opportunistic fungi *(98)* and drug-resistant fungi *(99)* will begin to compound the problems for the elderly in the near future.

CHAPTER SUMMARY

There are no known microorganisms that uniquely infect elderly humans. Overt diseases caused by some pathogens (e.g., tuberculosis, pneumonia, influenza, UTIs, sepsis) are clearly more common in the elderly. The reasons

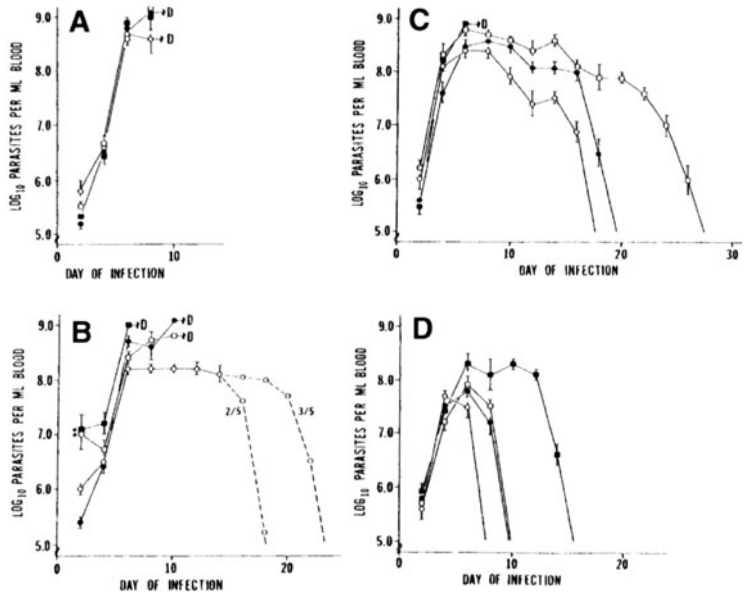

Fig. 2-2. Course of parasitemia in irradiated A/He recipients of 3×10^7 spleen cells from : **(A)** uninfected donors; **(B)** donors on day 7 after trypanosome inoculation (*indicates parasites at this time largely transferred as contaminants of donor spleen cell preparations); **(C)** donors on day 14 after trypanosome inoculation; **(D)** donors on day 21 after trypanosome inoculation. Donor-recipient combinations: (○) young donor-young recipients; (●) young donors-aged recipients; (□) aged donors-young recipients; (■) aged donors-aged recipients. Data from one of two replicated experiments, 5–6 samples per point. Bars represent 1 SEM. (D means all were dead). (From Albright JW, Albright JF. Mech Ageing Dev 1982;20:315–330.)

for this include (a) functional and anatomical changes in organs (e.g., respiratory, urinary), (b) comorbidity, and (c) decline in immunological competence. With regard to the latter, research with experimental animals has provided compelling evidence that immunological resistance to infection declines with age. Such is the case of infections with *M. tuberculosis*, *S. typhimurium*, *T. gondii*, *T. musculi*, and probably *L. monocytogenes*.

Bacterial infections begin with attachment of the microorganisms to host cells (epithelial, endothelial) followed by secretion of toxins and other virulence factors both outside and inside (type III secretion) host cells. Attachment involves a number of well-known cell adhesion receptors and counter-receptors as well as some that are restricted to microorganisms. In some cases, bacteria may secrete their own receptors into host cells. In order to survive in the hostile environment of the host, microorganisms often engage in colonial orga-

nization. To form organized colonies, called biofilms, bacteria reproduce rapidly and upon reaching a certain density, lay down a matrix that protects them from host defenses, including both humoral and cell-mediated immunity. Those bacteria that can form biofilms (many, perhaps most, species can) are able to sense population density and to distinguish between self and other species, an ability termed quorum sensing. The formation of biofilms also renders colonized bacteria impervious to antibiotics. Is it possible that biofilm formation by bacteria (or fungi that also appear capable) that have been selected for resistance in the elderly is a major factor in the elevated morbidity and mortality that characterize the aging?

At present, very little is known about bacterial adherence and the efficiency of secretion of virulence factors into host cells of aging subjects. There are limited data that suggest that intercellular adhesion is altered in aging humans, perhaps as a consequence of the effect of senescence on cytoplasmic membranes. If so, it is likely that changes occur in bacterial adherence to host cells. Similarly, very little attention has been given to date to evalulating the relationships between susceptibility of aging humans to infection and the formation of biofilms in aging subjects.

Both persistent (latent) bacterial and persistent viral infections in the elderly require attention. Tuberculosis appearing as a result of reactivated *Mycobacteria* is a classical example of bacterial latency. It is probable that bacterial latency is much more important in the pathogenesis of infectious diseases than has been realized (*see* ref. *100*). Herpes zoster (shingles) is one of several well-known examples of viral latency that result in diseases in the elderly. It is likely that the decline in immunological potential contributes in a major way to the reactivation of latent infections in aging subjects. However, this has not been afforded rigorous proof. If it is true, it becomes important to ask which of numerous other latent infections reappear in the elderly. Has sufficient attention been given to this question? To what extent are the well-known opportunistic infections in immunocompromised individuals paralleled in less severe degree in the elderly? Has this question received adequate attention?

Finally, it should be pointed out that both parasitic (especially protozoan) and fungal infections in the elderly deserve much more concern than they have received to date. Both environmental pollution and global warming are likely to precipitate a significant increase in the prevalence of infections by those organisms in the years ahead. And in the case of both protozoa (e.g., malaria) and fungi (e.g., *Candida* spp.) drug-resistant forms are already with us.

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