IMMUNOLOGIC FUNDAMENTALS

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Preface

The basic biologic concepts of immunology are fundamental to the practice of modern medicine. As a scientific discipline immunology includes immunochemistry and immunobiology. Being a biologic science, immunology includes developmental biology, genetics, biochemistry, microbiology, anatomy and medicine. The intact immune system is but one of many regulatory mechanisms functioning in an individual; it aids the individual in adaptation to environmental stimuli. The immune response is an adaptive response to external stimuli (e.g., microorganisms and pollen) as well as internal stimuli (e.g., dead or dying tissue cells, virus-altered cells, and possibly cancer cells). All of the genetic information, including that of the two-limbed immune system, is present in the fertilized ovum. The immune system evolves during embryogenesis; the capacity to recognize normal "self" stimuli is repressed or even destroyed while the ability to respond to foreign and altered "self" stimuli is emerging. The specific aim of this textbook is directed toward presenting the basic concepts of immunology in such a way that the medical student may form a mental image of the functioning immune system. Hopefully, this conceptual framework will serve the student as the basis upon which the rapidly emerging abundance of immunologic information may be placed so that the knowledge is clinically useful.

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To my students, thank you for being. It is for you that I wrote this textbook (hopefully, to ease the struggle in your comprehension of the subject matter).

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1 / Introduction to Host-Parasite Interactions

Host-parasite interactions are intimate cellular and even molecular (e.g., virus parasitisms) symbiotic relationships. Simply stated, *symbiosis* is the living together or close association of two dissimilar organisms. The normal microbial flora (microflora) of a human or animal host are considered as *commensals* and the relationship is termed *commensalism,* a relationship which is beneficial to the microorganism and (usually) not harmful to the host. Under certain conditions, the normal microflora may enter into a parasitic relationship with the host. In *parasitism,* the host is injured and the parasite is benefited.

VIRULENCE

Although the term virulence is generally used in describing the pathogenicity (capacity to produce disease) of a given microorganism, it actually describes the degree of pathogenicity of a specific microorganism in an individual host and is the result of the interaction between host and parasite. Some microorganisms possess the ability to invade the host. This capacity is referred to as *aggressin activity.* For example, encapsulated bacteria such as ***Diplococcus pneumoniae*** are able to resist ingestion by host phagocytes and thereby gain a temporal advantage in that they can multiply. Microorganisms that secrete toxic substances which are injurious to the host and its defense mechanisms gain an advantage in that they, too, can multiply without host interference long enough to produce an infection. Examples of this latter situation are seen in infections initiated by certain strains of group A, beta hemolytic ***Streptococcus pyogenes*** and strains of pathogenic ***Staphylococcus aureus*** in which host phagocytes are destroyed by toxic molecules elaborated by the bacteria.

Microorganisms which lack invasive properties may gain entrance into the host by accident. The clostridia, lacking aggressin activity, lead a saprophytic existence in the soil and are also present in the

gastrointestinal tract of man and animals. Certain of the clostridia or their endospores enter human or animal tissues via a wound or trauma. Tetanus (induced by *Clostridium tetani*) and gas gangrene (induced by several members of the genus Clostridium; e.g., *C. perfringens*) are disease processes in which a puncture wound or traumatized area of tissue is contaminated with soil and/or excrement. The strictly anaerobic environment essential for clostridial growth is thought to be initiated by the traumatic injury with resultant host cell necrosis and accumulation of injurious lactic acid. Aerobic microorganisms present in the wound then utilize the remaining available oxygen, thereby establishing an anaerobic crypt. Once anaerobic conditions are achieved the clostridial growth is initiated and a by-product of that growth is the excretion of potent exotoxins. Although these clostridial infections are usually localized at the site of injury, the toxins are able to gain entrance into the circulation and other tissues with serious consequences to the host. Another example of a localized infection and generalized toxemia is diphtheria. *Corynebacterium diphtheriae* initiates its focus of infection usually in the nasopharynx, but can, in rare instances, set up a focal infection at the site of a wound (wound diphtheria) with subsequent excretion of its exotoxin into the host. It is of interest here to note that the pseudomembrane characteristic of the diphtheritic lesion is the result of the host's inflammatory response to the bacteria and its toxic products. Although not included in the scope of this text, the process whereby *C. diphtheriae* and several strains of *Clostridium botulinum* gain the capacity to synthesize and secrete their potent exotoxins is under the genetic control of bacteriophage deoxyribonucleic acid (DNA). This process is termed phage or lysogenic conversion. Without a specific bacteriophage, each of these bacteria would be harmless. This is a peculiar type of synergism in which our parasites have their own molecular parasites.

Another way in which noninvasive microbial parasites gain entrance into human and animal hosts is through the bite of an insect or arthropod vector. Many exotic diseases are transmitted in this manner. The viral encephalitides are transmitted from animal to man and to other animals by insect and arthropod vectors. Yellow fever virus is transmitted by the female *Aedes aegypti* mosquito. Today, all of the virus parasites transmitted to man by insects (mainly mosquitoes) or arthropods such as ticks are classified as *arboviruses* (arthropod-borne viruses). All arboviruses appear capable of replicating in their vectors. Such infected vectors remain infected and asymptomatic during their lifespan. Most rickettsial diseases are vector-borne to man. Rickettsiae thrive selectively in the endothelial cells of the blood vessels, the sites

at which they are deposited and from which they disseminate to other endothelial cells. The plague bacillus, *Yersinia pestis,* is vector-borne to man by either rat flea or the human louse. The plague bacillus resides endemically in rodent hosts of South and Central America and in the southwestern United States as do appropriate vectors. Numerous protozoan diseases are vector-borne to man. Certain pathogenic protozoa undergo several asexual and sexual reproductive cycles in different host species; unfortunately, man can serve as one of these hosts. Undoubtedly, the most dramatic protozoan disease in humans is malaria. In malaria, man may serve as the host of the asexual life cycle (shizogony) of the Plasmodium species; i.e., man is their intermediate host. The sexual phase of the life cycle (sporogony) takes place in certain female mosquitoes, i.e., the definitive host.

For infectious diseases to occur, microorganisms must penetrate the host barriers (the intact skin, conjunctiva or mucous membranes). These barriers are routes or portals of microbial entry into the host. Except for vector-borne transmission, most microorganisms are transmitted either directly (by inhalation, ingestion or venereally) or indirectly through fomites (contaminated inanimate objects) between the diseased and susceptible hosts.

If the microbial invader is a virus it must survive the host microenvironment successfully until it reaches suitable host cells to which it may gain entrance and within which it may either survive or replicate itself. If the microbial invader is a procaryotic (e.g., bacteria) or eucaryotic (e.g., fungi and protozoa) microorganism, it, too, must survive long enough to multiply to numbers sufficient to cause disease (alteration of structure and/or function) in the host. In this survival of the invading parasite on the skin, conjunctiva or mucous membrane surfaces of the host, it must compete with the resident microflora for space and nutrients. Within hours following birth, the human infant may be colonized by staphylococci and within days to weeks by gram-negative enteric bacilli (e.g., *Escherichia coli*). The individual species which comprise the resident microflora appear stable over a period of time. For example, the resident staphylococcal microflora of the skin and mucous membranes, especially that of the anterior nares, in humans has been observed to aid in the prevention of colonization by other strains of staphylococcus. In mice, the presence of bacteroides in the gut appears to afford protection against experimental salmonellosis. The normal microflora thus establish a balance for space and nutrients among themselves and an equilibrium with their individual host. Certain antibiotics may disrupt the balance among the members of an individual's microflora present on the mucous mem-

branes of the mouth, gastrointestinal tract and vagina. Consequently, susceptible bacteria are temporarily eradicated and the fungal flora (not susceptible to the antibacterial agents) overgrow, and, in turn, cause disease. Reestablishment of the balance among the normal microflora eventually occurs.

THE HOST AS A FAVORABLE MICROENVIRONMENT

The host tissues supply the necessary optimal environment (e.g., nutrients, oxygen, carbon dioxide, pH, etc.) for microbial growth. Most bacterial pathogens are facultative anaerobes in their oxygen requirement and grow optimally at 37° C in the human host. Rather unique types of infection are produced by several species of mycobacteria whose optimal temperatures of growth are much less than 37° C. *Mycobacterium ulcerans* (grows slowly in primary culture at 30–33° C and will not grow at 37° C) and *Mycobacterium balnei* (also known as *Mycobacterium marinum*; grows at 31° C and not 37° C) only produce skin infections and ulcerations in areas on the host where the temperature is less than 37° C, e.g., the extremities such as the toes, nose, fingers, elbows, knees and legs. The latter microorganism (*M. balnei* or *M. marinum*), originally isolated from saltwater fish, has produced sporadic epidemics and involved individuals who swam in either saltwater or fresh water. There is evidence that *M. leprae* also grow optimally at temperatures less than 37° C.

It has been ingeniously demonstrated that the host serves as a rich source of essential nutrients for microbial parasites. Experiments with nutritional auxotrophs of pathogenic bacteria illustrate the significance of essential nutrients being present in the host to support the growth of certain bacterial pathogens. When these nutritional auxotrophs were injected into mice, no disease ensued. But, when the auxotroph was injected and the nutrient or metabolite required by it was simultaneously injected into, and in some instances fed to, the mice, then the bacteria multiplied and disease became evident. The Brucella species seem to be stimulated by polyerythritol, which is present in high amounts in fetal calves. *D. pneumoniae* lacks a catalase system and readily dies out and autolyzes in broth culture. Yet its natural host, the human, supplies the catalase that this bacterium requires for the inactivation of peroxides generated during its metabolism so that it may multiply and survive in the host.

Normal microflora may cause disease if they change their locale or habitat within the host or if the host's resistance is lowered (a compromised host). With regard to change of locale within the host,

Neisseria meningitidis is part of the normal flora of the nasopharynx of certain humans, but when it gains entrance in some manner into the central nervous system and/or circulation (see "endotoxin shock," Chapter 4) it can produce a rapidly fatal disease. *E. coli* is a normal inhabitant of the gastrointestinal tract of humans, but in the urinary bladder produces cystitis. Since the advent of the use of antibiotics and the accompanying widespread genetic exchanges of drug resistances among the enteric bacilli (Escherichia, Salmonella, Shigella, etc.), an increased incidence of aspiration pneumonia due to *E. coli* has occurred. Dental manipulations may be responsible for the entrance of microorganisms into the blood (bacteremia) and the relocalization within the host.

As examples of lowered host resistance and subsequent disease by normal microflora, none are more intriguing than the bacterial pneumonias which are sequelae to influenza virus infection usually occurring in certain debilitated or aging individuals. Among the normal microflora of the upper respiratory tract in some humans are types of *D. pneumoniae* or *Hemophilus influenzae.* Both of these bacteria are encapsulated pathogens and presumably are held in check normally by a combination of secretory antibody which enhances their ingestion by phagocytes in the mucous secretions (see Chapters 3 and 8). When the influenza virus is the primary infecting agent, the cells of the lung are heavily invaded and replicate virus. Although not killed, the host phagocytes may be seriously and temporarily maimed in their ability to generate energy from glucose. From in vitro studies the glycolytic pathway of such phagocytes was shown to be 90% inhibited by influenza virus. The action of the virus seemed to inhibit at the level of the phosphohexose isomerase; neither glucose nor glucose-6-phosphate was metabolized. If this occurs in vivo, then a likely explanation exists for the occurrence of secondary bacterial pneumonias seen in the wake of influenza epidemics. Similarly, *S. aureus* or beta hemolytic streptococci also may be the bacterial agent in these secondary pneumonias.

In general, the relationship between host malnutrition and infection is synergistic. Infections are more likely to produce more serious consequences in persons who are malnourished, and, in turn, infections have the capacity to convert borderline nutritional deficiencies into severe malnutrition. Occasionally, the undernourished host provides an antagonistic environment for certain viral and protozoan parasites. (Perhaps this is seen because viruses and some protozoan parasites reside inside host cells. If the host is severely malnourished, its cells are possibly deprived of certain essential nutrients and they, in

turn, would serve as a poor microenvironment for the replication of viruses or growth of protozoa.) Severe nutritional deficiency interferes with host functions (e.g., the integrity of the skin and mucous membrane barriers, the production of body fluids and enzymes antagonistic to microorganisms, phagocytic functions, antibody formation, and alterations in intestinal microflora).

A synergism among members of the normal microflora may produce disease in the human, usually in the oral cavity. This disease is called fusospirochetosis (Vincent's angina or "trench mouth"). The symbionts are usually normal microflora of the gingiva. The fusiform bacilli (Fusobacterium species) and the spirochetes (*Borrelia buccales, Borrelia vincentii, Treponema microdentium, Treponema mucosum,* and Bacteroides species) increase in number when local host resistance is reduced by trauma or vitamin deficiency. Other species of bacteria apparently are involved as symbionts in this synergism. For example, a Vibrio species in combination with an anaerobic species of streptococci and the fusiform-shaped *T. microdentium* are capable of reproducing this disease in guinea pigs. For infection to occur the proper microbial symbionts must be present in the host. Some evidence exists that a nutritional dependence occurs in which a by-product of one bacterium is an essential growth factor for another symbiont. It is extremely difficult to satisfy the cultural requirements in vitro of many of the contributory bacteria, especially the spirochetes. For example, the spirochete *Bacteroides melaninogenicus* cannot grow in the oral cavity without a vitamin K-like naphthaquinone supplied by diphtheroids. Also, anaerobic conditions, e.g., tissue trauma, must be established for growth of the symbionts. Fusospirochetal symbionts may serve as either primary or secondary invaders in infectious processes involving the upper and lower respiratory tracts, and, on occasion, even involve the skin, the genital areas and the gastrointestinal tract of man. In rare instances, metastases from local lesions may infect any tissue of the body. One of the host's major defense mechanisms against infection by anaerobes is the normal oxidation-reduction potential (Eh = +120 millivolts) of most normal human tissues. When lowering of the redox potential occurs, even in mouth and lungs, anaerobes may multiply. The lowering of tissue redox potentials may result from necrosis, impaired blood supply and prior growth of facultative anaerobes in a wound. The effectiveness of phagocytosis by polymorphonuclear neutrophils and other granulocytes is severely impaired by anaerobic environments.

Even the formation of dental caries is the result of attack by members of the normal microflora. Microorganisms which can both

withstand acid environments (aciduric) and ferment carbohydrates to generate acid (acidogenic) are involved in the initiation of caries formation. Yeast and three types of bacteria (streptococci, staphylococci and lactobacilli) are implicated. These microorganisms are present in the dental plaques which form on the enamel surface of teeth and generate the acid which is responsible for the demineralization of the enamel.

ESTABLISHING VIRULENCE AND MEASUREMENT OF VIRULENCE

Virulence of a given microorganism is the result of the host-parasite interaction. Early in the study of microbial disease, Koch established a set of criteria (*Koch's Postulates*) against which a specific microorganism was tested to *prove that the microorganism being examined was the actual etiologic agent of a specific infectious disease.* Koch's Postulates are as follows: that a specific microorganism must be isolated from all individuals with a specific infectious disease; that the microorganism be grown in pure culture; that the pure culture isolate be injected into a second susceptible host; that the same disease be reproduced in the second host from which the same microorganism is isolated and grown in pure culture.

Even today it is not possible to fulfill Koch's criteria for every microbial pathogen. For example, the obligately parasitic bacterial agents of syphilis (*Treponema pallidum*) and leprosy (*M. leprae*) cannot be cultivated in laboratory media following inoculation with material from human lesions.

Even though material from human syphilitic lesions injected into rabbit scrotal tissue may induce a local infection in the rabbit, there is doubt whether the rabbit-grown spirochetes are pathogenic for man. In addition, the growth of these spirochetes in the rabbit does not result in a disease comparable to human syphilis. It has only been in recent years, with improvements in enrichment of culture media and incubation environments, that the virulent form of *Neisseria gonorrhea* was even cultivated. Under these improved conditions, four colony types may be observed. Colonies designated as types 1 and 2 maintain virulence for the human and types 3 and 4 are avirulent.

For the measurement of virulence of a specific microorganism (whether virulence is the result of invasiveness, toxigenicity or a combination of these), standardized procedures are sought. For example, a standard susceptible animal host (age, weight, sex, etc.) is used. A standard route of inoculation, a standard time interval, and a

standard endpoint criterion (death or symptoms) are used. The measure is usually expressed in terms of a median dose (of bacteria or toxin) that will kill 50% of the animals inoculated within a stated time period. The number of bacteria which may accomplish this is referred to as a Lethal $Dose_{50}$ (LD_{50}).

Virulence can be measured more accurately by establishing LD_{50} dose levels than by measuring the dose which will kill 100% of the inoculated group.

DISSEMINATION OF MICROORGANISMS WITHIN THE HOST

Once the integrity of the host barriers is damaged, microorganisms may be disseminated by the blood stream, by the lymphatic system, within phagocytic cells, by continuity (continuous spreading of the infectious agent within the same tissue or organ) and by contiguity (contiguous dissemination by contamination of an organ or tissue contiguous (next) to the infected organ or tissue). ***Mycobacterium tuberculosis*** may be spread within the body by any or all of these means. Classically, the mere presence of bacteria in the blood is referred to as a ***bacteremia.*** Bacteremias ("bacterial showers") have been claimed to occur in healthy individuals periodically without ensuing disease. The presence of viruses in the blood stream is referred to as a ***viremia.*** The term ***septicemia*** is employed to describe the process in which microorganisms (usually bacteria) are present and actively metabolizing, multiplying and excreting toxic products in the blood stream.

HOST DEFENSE MECHANISMS

Basically, the three main lines of host defense against microorganisms consist of: the barrier effect of skin, conjunctiva and mucous membranes; the inflammatory response and phagocytosis; and the specific immune response.

The ***host barriers*** are classified as three anatomically distinct areas. The healthy intact skin is a barrier difficult for microorganisms to penetrate. However, the skin surface does support microbial growth. The fibrous protein keratin comprises the superficial cutaneous layer beneath which are the epidermal cells that die in the process of synthesizing keratin. The sweat glands of the skin provide a weak saline solution and small amounts of nitrogenous nutrient for microorganisms. Hair follicles in the skin exude sebum which is manufactured by the sebaceous glands. Sebum provides lipids and unsaturated fatty