The Interplay of Defense Mechanisms Against Infectious Diseases*

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Defense systems against infectious agents can be subdivided into two broad categories involving humoral and cellular mechanisms. Specific humoral systems comprise the antimicrobial and antitoxic effects of at least three classes (IgG, IgM and IgA) of immunoglobulins. These immunoglobulins in varying patterns may (a) neutralize toxins, (b) neutralize viral infectivity and (c) kill certain gram-negative microorganisms in concert with the complement system. A few microorganisms having "deficient" cell walls, such as mycoplasma, may be inhibited by specific antibodies without the aid of complement. Since IgA does not fix complement, IgG and IgM must play the dominant role in complement mediated reactions. A fourth (d) category of specific immunoglobulin activity involves enhancement of phagocytosis or opsonic action, which illustrates an important interplay between a specific humoral mechanism and phagocytic mechanisms. It is probable that IgG and IgM also play the dominant role in opsonization, because IgA has been reported to lack opsonizing activity (Quie, Messner and Williams, 1968). The major functions of IgA must be to neutralize toxins and the infectivity of viruses.

In addition to specific humoral mechanisms, several nonspecific humoral defense mechanisms exist. Two such factors are β-lysin, which is released during blood clot formation (Tew, Hess and Donaldson, 1969), and lysozyme, which is probably secreted and/or released from several cell types. It is significant that lysozyme can act in concert with the antibody-complement system and augment the bactericidal and lytic effects of this system against certain gram-negative microorganisms (Amano et al., 1954). Lysozyme is usually present in lysosomes of phagocytic cells and may be secreted by mononuclear cells (Heise and Myrvik, 1967). Other potentially active nonspecific factors secreted from living cells or released from disrupted and necrotic cells include cationic substances, lactate, interferon, viral inhibitors and a recently described antibacterial system involving peroxidase, thiocyanate and H_2O_2 (Hirsch, 1960; Klebanoff, Clem and Luebke, 1966; Wolstenholme and O'Connor, 1967; Zeya and Spitznagel, 1968).

If a microorganism is not inactivated by the humoral mechanisms present in the microenvironment at the site of infection, phagocytic cells promptly come into play, and, if conditions permit, the infecting microorganisms will be ingested. The polymorphonuclear (PMN) cell is usually the first line of phagocytic cells to engage microorganisms. However, if the infection continues and is not contained by the PMNs, mononuclear phagocytes (macrophages)

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eventually engulf the parasite and, if necessary, can enter into a long-term engagement with the infectious agent. During this interaction both nonspecific and specific humoral factors may participate prior to and perhaps even conjointly with the intracellular events. It is generally considered that the phagosomes in PMNs and macrophages receive antibacterial factors and hydrolytic enzymes from lysosomes as the result of a fusion process between lysosomes and the membrane surrounding the phagocytized particle (Hirsch, 1962).

It would be expected that the least virulent of all microorganisms might be highly susceptible to the nonspecific humoral systems of defense. Such microorganisms would be represented by *Sarcina lutea* (sensitive to lysozyme) and *Bacillus subtilis* (sensitive to β-lysin). The next hierarchy of pathogenic microorganisms to evolve would be expected to be resistant to the humoral mechanisms and capable of resisting phagocytosis unless specific opsonizing antibody is present. *Diplococcus pneumoniae* is a representative of this type of host-parasite relationship. However, *D. pneumoniae* is fully susceptible to the normal intracellular bactericidal mechanisms once the organism has been ingested. In this case there is no evidence that specific opsonizing antibody contributes significantly to the normal intracellular bactericidal and digestive activities of PMNs and macrophages except for mediating phagocytosis.

The ultimate evolutionary step reached by microorganisms has involved not only resistance to the humoral systems of defense, but also resistance to the normal baseline cellular systems of defense. *Mycobacterium tuberculosis* is a classic example of a parasite reaching this stage of host-parasite evolution. In fact, all of our so-called chronic granulomatous diseases (tuberculosis, brucellosis, listeriosis, histoplasmosis, tularemia, etc.) are chronic and granulomatous because of a persisting intracellular infection of the phagocytic cells of the reticuloendothelial system. Characteristically the infectious lesion in these host-parasite relationships is composed primarily of macrophages, epithelioid cells, giant cells and lymphoid cells.

Macrophages in a "status quo" nonimmunized animal are essentially incapable of resisting the intracellular growth of virulent tubercle bacilli. Furthermore, the PMNs fail, in all probability, because their life span is too short to effectively bring to bear their bactericidal and digestive systems against an organism with such challenging substrates and biochemical armor as the virulent tubercle bacillus. Accordingly, the ultimate in parasite evolution has resulted in microorganisms with complex cell walls and "capsular" substrates that are refractory to the normal bactericidal and/or digestive processes of the phagocytic cell systems. An alternative to this possibility could involve toxic moieties secreted or released intracellularly by a pathogenic microorganism which could disrupt the chain of events leading to an effective phagosome or block differentiation to an increased immunologic potential.

In this regard, PMNs are quite capable of digesting *Mycobacterium smegmatis* within three hours, which illustrates their potential for killing an avirulent species of mycobacteria (Leake and Myrvik, 1966). Consequently, virulent mycobacteria are biochemically structured in a way which allows them to survive the intracellular potential of the PMN and makes them candidates ultimately for phagocytosis by macrophages. Normal macrophages appear to undergo a form of non-immunologic mediated differentiation and can kill *M. smegmatis* after about four to six days of intracellular residence. However, macrophages can cope only with virulent mycobacteria after they have differentiated *in vivo* under the blanket of an immunologic stimulus. Accordingly, there is good evidence to support the concept that normal macrophages are relatively immature cells and can differentiate upon demand by either stimulation with "substrate" or some specific immunological mechanism (Evans and Myrvik, 1968). In addition, these forms of stimulation may lead to mitosis, resulting in a more favorable host cell-parasite ratio. Undoubtedly, macrophages activated by a specific immunologic mechanism develop a population of "immune" phagocytes sooner than by way of the "substrate" principle of stimulation. For example, sodium stearate will develop a characteristic epithelioid cell granuloma which has the same rate of formation regardless of the number of injections. In contrast, repeated injections of mycobacteria illustrate the development of so-called accelerated or allergic granulomas (Myrvik, Leake and Oshima, 1962).

The PMN normally emerges from the bone marrow as a mature cell with a constitutive load of antibacterial and digestive factors packaged in lysosomes. It thus appears that opsonizing antibody is the main specific immunologic component that interplays directly with the PMN. It is also feasible in certain instances for bactericidal antibody plus complement to interplay with lysozyme and, possibly, other antibacterial agents that are transferred to the phagosome. In addition, certain components of complement have been demonstrated to exert positive chemotactic effects on PMNs (Müller-Eberhard, 1968). However, chemotaxis of PMNs also can be prompted by components resulting from tissue damage apart from specific antigen-antibody-complement reactions or other specific sensitivity responses. There is no evidence that PMNs proliferate in tissues as a result of a specific immunologic stimulus.

Therefore, mobilization to any lesion is dependent on blood-borne transport.

In contrast, macrophages commonly circulate or exist in tissues as relatively undifferentiated cells with a sparse number of lysosomes and a low level of hydrolases as well as a baseline level of metabolic activity (Heise, Myrvik and Leake, 1965; Myrvik and Evans, 1967; Leake and Myrvik, 1968). It is of interest that normal alveolar macrophages, even of a low order of activation, can readily kill and digest avirulent strains of *Listeria monocytogenes* but not virulent strains of this organism (Evans, 1968). However, BCG-(*Mycobacterium bovis*) activated alveolar macrophages are capable of killing virulent strains of *L. monocytogenes*. These findings indicate that, once a macrophage is activated by interaction with one type of microorganism, it also has an increased potential to contain and destroy certain unrelated microorganisms. It should be emphasized that this generalization does not hold when BCG-activated macrophages are presented with virulent *Pasteurella tularensis*. On the other hand, macrophages from *P. tularensis*-vaccinated animals are capable of handling *P. tularensis* (Nutter and Myrvik, 1966).

Whereas the role of specific antibodies and their interplay with PMNs is reasonably well understood, our concepts concerning the interplay of specific antibodies and acquired immunity to facultative intracellular parasites (the ultimate in evolution of microbial pathogens) is obscure. Speculation is our only *modus operandi* for discussing this facet of cellular immunity. There is compelling evidence that with most facultative intracellular parasites opsonizing antibody is not the limiting factor. For example, there appears to be an adequate concentration of opsonins in normal sera for mycobacteria and listeria. These opsonins could be the result of long-term contact with similar or related organisms in the environment. Accordingly, opsonins (if needed with these organisms) are usually normally present, and, thus, phagocytic uptake by macrophages is not the limiting step following primary infection with these organisms. This does not exclude the possibility that phagocytosis of some facultative intracellular parasites may be dependent on antibodies present only in the specifically immune host. Nevertheless, the key point in our understanding of the host-parasite relationship in the so-called granulomatous diseases rests on the fact that normal macrophages are incapable of suppressing the intracellular parasite even if sufficient time is allowed for "substrate" activation. This is exemplified in the case of tuberculosis, where the generation time of the parasite is probably two to four days *in vivo*. In this host-parasite relationship, it is quite apparent that only immune macrophages can significantly suppress the growth of tubercle bacilli.

Assuming that the macrophages which mediate immunity to tuberculosis are derived as the result of some specific immunologic activation process, an interplay of specific and nonspecific factors in cellular immunity must be postulated.

Current knowledge in this area supports the concept that immune lymphocytes are activated following contact with the corresponding antigen. As a result, these lymphocytes undergo transformation and secrete either specific antibody with cytophilic properties for macrophages or a product (Granger's lymphotoxin?) which can activate macrophages (Granger and Kolb, 1968; Heise, Han and Weiser, 1968). If cytophilic antibody is involved, the macrophages passively receive a recognition system (cytophilic antibody) which could function to specifically activate macrophages when they have made contact with the appropriate antigen. On the other hand, if a nonspecific secretion product of the specifically activated immune lymphoid cells is responsible, specificity of the response would not exist on the macrophage level. However, it is unlikely that the effector cell (macrophage) of antibacterial cellular immunity is totally devoid of specific components. Cytophilic antibodies on macrophages could function in an "antitoxic" capacity on the intracellular level. This possibility is best exemplified by the lack of immune expression of BCG-activated (immune) macrophages against *P. tularensis*. In contrast, macrophages derived from *P. tularensis*-immune animals express definite cellular immunity against *P. tularensis*. The possibility that certain types of antibody could function on the intracellular level to protect the macrophage against toxic products secreted by intracellular pathogens warrants investigation.

In transplantation immunity it appears likely that immune lymphoid cells act directly as effector cells. In this case, macrophages may act only as a secondary participant, which is in contrast to the primary role macrophages play in antibacterial cellular immunity. The case for a lymphotoxin being an effector agent in transplantation immunity is attractive and deserves further study.

Summary

The total complex of immune expression is an interplay between nonspecific antimicrobial humoral systems plus specific antibodies and accessory factors. These systems are backstopped by the phagocytic functions of PMNs. If these fail, mononuclear phagocytes respond as a second line of defense to carry out chronic engagements. In addition to a direct activation process by "substrate," macrophages may be activated and mobilized by a lymphocyte-mediated immunologic reaction which probably in-

volves either a "lymphotoxin" and/or a specific antibody cytophilic for macrophages. Immunologically activated lymphocytes appear to be the primary effector cells of antitissue (transplantation) cellular immunity, whereas immunologically activated macrophages appear to be the primary effector cells of antibacterial cellular immunity.

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