BCG VACCINE: TUBERCULOSIS-CANCER

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Foreword

BCG vaccination against tuberculosis has expanded greatly around the world since 1957, the year when the first edition of this book appeared.* BCG is mandatory by law for the entire population, or a segment of it, in 26 countries and is optional but recommended in 19 others.

The WHO-UNICEF has assisted in establishing BCG laboratories or vaccination campaigns in over 45 countries and accounts for over 200 millions vaccinated. The worldwide number of persons vaccinated is estimated at 500 million or more.

Notwithstanding the worldwide acceptance of BCG, vaccination against tuberculosis in the United States has had only token acceptance. Theodore L. Badger, Clinical Professor Emeritus at Harvard, stated:

> The United States Public Health Service has assumed a defeatist attitude toward BCG. The persuasiveness of its arguments against the use of the vaccine, formed on the basis of rather unconvincing facts and evidence, stems from its position of prestige in our nation.

The discovery that BCG is a potent stimulator of the reticuloendothelial system (Rosenthal 1936), that neoplasms may be caused by a foreign agent that may be repressed by immunologic means

*BCG Vaccination against Tuberculosis, Little, Brown & Co.

(Old and Clarke 1959, Halpern 1959), and that BCG prolonged the remission time of acute lymphatic leukemia (Mathé 1969) extended widely the use of BCG in cancer and leukemia around the world.

Basically, the entire book has been rewritten. Guérin, who is the "G" of BCG, wrote on the history of BCG: Weill-Hallé, who was the first to vaccinate human subjects with BCG orally, the first method of administration for BCG vaccination, wrote the section on oral vaccination; Wallgren was the first to introduce BCG intradermally, and he and Dahlström have written the section on the intradermal method.

A new section has been added on the immunotherapy and immunoprophylaxis of cancer and leukemia. Only a fraction of the vast literature on BCG could be cited. The results of BCG vaccination in leprosy, mycobacterium ulcerans, Crohn's disease (regional ileitis), and so forth are not yet definitive and shall not be considered in this volume.

The author founded the BCG laboratory in Chicago which continues to be the first and only one in the United States licensed to produce and distribute BCG in the United States. The laboratory and clinic have had the support of the University of Illinois at the Medical Center, Cook County Hospital, the Chicago Board of Health, and Research Foundation, all of Chicago. More recently, the laboratory and clinic have been taken over by the University of Illinois at the Medical Center in Chicago. They distribute the vaccine. Research Foundation has been instrumental in perpetuating the use of BCG in the United States. Distribution licenses have been issued to foreign BCG laboratories (Glaxo and Connaught Laboratories).

The text of both editions was edited by Dr. William H. Oatway, Jr., formerly medical director of La Vina Sanitorium. He has long been devoted to the BCG cause, beginning at the University of Wisconsin, where he vaccinated students of medicine and nursing by the multiple puncture method, and continuing at the Barlow Sanitorium, University of Southern California, and La Vina Sanitorium.

I wish to thank PSG Publishing Company for their consideration and help, especially Marie Goldstein, Editor, in publishing the book, as well as members of my laboratory and clinical staff at the University of Illinois, Research Foundation, my wife, Lucy Donna, and children Sara Lough and Sol Roy Rosenthal, Jr. All helped to make this book possible.

Contents

CHAPTER 1

Immunity in Tuberculosis

Man has a great capacity to develop resistance against the tubercle bacillus, and there can be no doubt of it.[1,2] The occasional writer in the United States who denies this fact[3] undermines the potential beneficial effect of BCG vaccination.

Acquired resistance against species-specific strains of tubercle bacilli is not absolute, nor is it for any other organism. Resistance to most known infectious diseases can be increased in certain individuals with sufficient exposure under certain conditions. The degree and duration of acquired resistance produced by various organisms differ markedly. A degree of acquired resistance to tuberculosis can be developed which in a relatively high percentage of cases will protect the individual against subsequent exposures to the bacillus. As stated by Rich,[1]

> It is an example of the treachery of terms that the statement on the part of some authoritative writers that there is no acquired immunity in tuberculosis has led many to believe that a tuberculous infection confers no protection at all in spite of the great mass of evidence to the contrary.

The problem of determining the degree of acquired immunity in tuberculosis is unique. First, no experimental animal responds exactly as man does to selected strains of the tubercle bacillus. The animals most commonly used for determining resistance have been the guinea

pig and the mouse and, to a lesser extent, the rabbit and the monkey (see Chapter 13). For example, as few as four or five organisms will regularly cause progressive disease in the guinea pig,[4] yet when primary infection was almost universal among human beings, as in the recent past, the incidence of disease was roughly only 1%. Second, because tuberculosis is considered to be mainly a cellular and not a humoral disease, humoral tests for antibodies, such as agglutinins, precipitins, complement-fixation, etc., cannot be used to determine the degree of immunity produced by acquired or native infection. Third, there are no skin tests which will accurately determine the amount of resistance. A positive tuberculin reaction is only presumptive evidence that an associated resistance has been acquired.

It has also been difficult clinically to compare the degree of resistance in tuberculosis (whether native or acquired) with other communicable diseases because the time of exposure varies greatly. Strict isolation is rigidly utilized in most communicable diseases; in tuberculosis it is only partially used. Patients who are considered "closed" active cases are often allowed to be ambulatory, and at times are allowed to lead normal, active lives with few restrictions. This nonisolation and the fact that an estimated two-thirds of the active cases of tuberculosis in the United States are recognized and under treatment leave many foci for dissemination of the disease, placing an even greater burden upon native or acquired resistance to tuberculosis than for any other disease.

Since it may help to better understand acquired resistance by vaccination with BCG, some of the salient features of native and acquired immunity against tuberculosis are discussed below. For more detailed studies see Rich,[1] Raffel,[5] Lurie,[6] Prigge and Heymann,[7] Youmans,[8] and Mackaness.[9]

Native Resistance

A striking example of native resistance to tuberculosis is the species-specificity of the tubercle bacillus. Very early in the study of tuberculosis Theobald Smith[10] showed that there were bovine and human strains of the tubercle bacillus and that the human strain was less virulent in cattle than the bovine strain and vice versa. One of the accepted methods for differentiating the human from the bovine strain is inoculation of the strains into guinea pigs and rabbits. The rabbit is less susceptible to the human strain of tubercle bacillus than to the bovine strain, whereas the guinea pig is equally susceptible to both strains. The rat and the mouse are known to be resistant to almost all strains of the tubercle bacillus although progressive tuberculosis may be produced by adequate doses.

Differences in native resistance exist among individuals in a given species. Thus in guinea pigs certain strains are more resistant to tuberculosis than others.[11] Similarly, highly resistant and highly susceptible strains of rabbits have been described.[12]

Differences among the various human races in reaction to the human virulent tubercle bacillus are notable; for example, the Eskimo, the American Indian, and the Senegalese Negro are more susceptible to tuberculosis than are Caucasians. In the United States mortality from tuberculosis is three or four times greater in blacks than in whites. This perhaps may result from a relatively low socioeconomic status with its concomitant closeness of contact and thus larger size of infecting dose rather than greater native susceptibility to infection. It has been found, however, that the pathology in black adults is of a more exudative and disseminative character[13] than in whites, although in both it is of the adult type. In contrast to American blacks, the adult Senegalese Negro usually develops a

childhood type of tuberculosis with caseation of the hilar lymph nodes and dissemination. Natural selection may account for some of the differences in the susceptibility to tuberculosis of American blacks and Sudan Negroes.

Differences in susceptibility to tuberculosis also have been found in individuals of the same race. A study of twins showed that the type of tuberculosis morbidity which developed in identical twins was similar to 70 in 87% of the cases whereas in fraternal twins it was similar in only 25 to 30%.[14 15] In a review of the literature by Verscheur of 600 pairs of twins, 74% of the identical twins had similar types of tuberculosis, as did 28% of the fraternal twins.[16 17]

Native resistance also varies within a given species among the organs of the host. There is evidence to show that the lung and kidney have less resistance than the liver, spleen, and bone marrow.[6] It also varies with age and sex.

The faculty of developing acquired resistance is dependent in great measure upon the native resistance of the individual. Lurie has shown in his rabbit studies that animals with only slight susceptibility to tuberculosis develop a greater resistance to the disease by immunization than those with greater susceptibility. This is also true for their individual organs. A similar situation may exist in human beings.

Lurie states that genetic resistance to tuberculosis in the rabbit may be controlled by the hormone balance. He believes that it is not a single hormone or a group of hormones which in themselves account for resistance or susceptibility to infection, but rather the interaction of many hormones and numerous other forces which are integrated in the response of a given organism to tuberculosis and other stressful states. The mode of integration may vary in resistant and susceptible strains.[18-20]

Summary

Native resistance plays a dominant role in resistance or immunity to tuberculosis. This resistance may approach the absolute to strains of the tubercle bacillus which are not specific for the species. Variations in native resistance occur in a species as well as in the organs of certain species, whether animal or man. The nature of the mechanism by which native resistance plays its role—genotypic, environmental, by natural selection, or by variations in the hormone balance, metabolism, or physiology of the host—is not known. It is of interest that those who are relatively resistant to the tubercle bacillus also have a greater capacity to develop acquired resistance than do those with less resistance to the organism.

Resistance Acquired by Natural Means

Long before the tubercle bacillus was discovered it was shown experimentally that if animals were inoculated with treated sputum of tuberculosis patients, they developed a more chronic type of disease when reinfected at later periods with untreated sputa and lived longer than those not previously treated.[21] Laboratory studies[22-24] made possible by the discovery of the tubercle bacillus helped to demonstrate clearly that reinfection was distinctly different in character from primary infection. If the primary infection did not by itself produce progressive disease, reinfection after a suitable time would be followed by rapid localization of the organisms at the site of inoculation and draining lymph nodes and by a severe local reaction in which many of the organisms were destroyed or exuded in a necrotic slough (Koch phenomenon). It was shown later that spread from the site of the inoculation was retarded,[23-25] that the multiplication of the organisms was inhibited,[26] that the disease was more chronic (as evidenced by increased

fibrosis and proliferation rather than exudation), and that the life of the animal was prolonged[1] (see Chapter 13).

Soon after the discovery of the tubercle bacillus Marfan noted that the incidence of pulmonary tuberculosis in human beings was much less frequent in individuals who had had tuberculous lymphadenitis which had healed before adolescence than in those who had not had this type of lesion,[27] observations disputed by some who felt that there was not sufficient statistical evidence.[28] However, it was not the impression of those who had observed this phenomenon that the immunity was absolute. The South African Institute for Medical Research confirmed Marfan's view in a study of South African Negro miners.[29 30]

Studies of young adult students of medicine and nursing show that those who react to tuberculin when they enter training develop fewer cases of tuberculosis during their training period than those who are nonreactors on entrance[31-35] (see Chapter 14). Badger et al.[36] followed nurses by questionnaire after their training was completed and found that the incidence of tuberculosis in the tuberculin positives was less than in those who were tuberculin negative during their training. Among the individuals who were followed over a period of 15 years, however, the difference became less apparent. Badger did not take into account the fact that during the period of training at Boston City Hospital, where exposure to tuberculosis was great, the majority of the negative reactors developed primary infection and became tuberculin positive during their training. Similarly at Cook County Hospital, Rosenthal[37] found that the nurses who became naturally "vaccinated" had an increased resistance to tuberculosis.

The study of Bates and Davey at the University of Michigan[38] clearly demonstrates the resistance to tuberculosis of tuberculin reactors among students of nursing and medicine. In a ten-year observation period there were 20 cases of tuberculosis, ranging from minimum to moderately advanced with cavity formation, all 20 of whom had to be hospitalized for ten months or more. All had been negative reactors to tuberculin on admission to school when two dilutions of PPD (purified protein derivative) with doses up to 0.005 mg (100 TU) were used for testing. There were no cases of tuberculosis in the students who had a positive tuberculin reaction at the start of training.

The situation is somewhat different in children, for whom the prognosis of tuberculosis varies with age and is poorest in the very young. It is known that differences in native immunity at the various age levels exist. An infant or a child first seen with a positive tuberculin reaction may still have active progressive disease although there may be no x-ray evidence of tuberculosis. The J.A. Myers group[39] studied children from a few months to 19 years of age who had contact with tuberculous patients for periods averaging ten years. The tuberculous persons were moved in many instances, but the children were not completely isolated since they were visited in their homes by the patients. Of the 446 tuberculin-positive children, 15% developed tuberculosis, whereas only 1.68% of the 772 tuberculin-negative children did so. There were 22 deaths among the tuberculin positives but only one death among the tuberculin negatives. Friedman et al,[40] in contrast, followed about the same number of children (409 tuberculin-positive reactors) for a similar length of time (ten years), but the children were isolated in a preventorium; only 2.2% developed tuberculosis, and two died. These findings indicate the seriousness of primary tuberculosis in childhood, especially when contact is not completely broken.

A striking example of acquired resistance is the experience of Papworth Village in England. Here parents with tuberculosis were allowed to live a normal communal life with their children under

more or less ideal conditions (food, housing, security, etc.).[41] Of the 108 children born in the village since 1921, the director Dr. R.R. Trail wrote in 1955[42]

> We do not know of any proved case of clinical pulmonary tuberculosis to date, and yet we know that all these children were tuberculin positive in childhood, the majority by the age of two and a half. Seventy of the boys were captured in Singapore and worked on the Death Railway of Siam; none of them developed tuberculosis.

Adolescents with no known cases of tuberculosis in their homes who had negative x-rays had an annual incidence of tuberculosis over a two and one-half year period of 0.75 per 1000 among those positive only to 1:100 old tuberculin (OT) as compared to 1.94 per 1000 in the completely negative group. Among those who were initially positive to 1:3000 OT the rate was 1.75 per 1000[43] (see Chapter 14).

Reinfection tuberculosis in human beings is usually localized to one organ, the lung—dissemination to other organs is uncommon—and the type of lesion is more proliferative and fibrotic.[1] In recent times the degree of tuberculosis infection of the population has been reduced, and the disease which is found in young adults who were known to be tuberculin negative resembles the so-called reinfection type. The question arises if this is actually infection which progresses[36 44 45] or whether a subminimal primary infection has occurred with a transitory tuberculin conversion or none at all. Natural resistance increases with age and this may account for the resemblance of a progressive primary infection to reinfection tuberculosis in young adults.

Summary

As we have seen, a healed primary infection in human beings in the lung or elsewhere confers an increased resistance against virulent infection, attested to by a decreased incidence of disease, a greater localization, and a better prognosis. Paradoxically, however, the possibility that viable organisms may produce active disease at a later date is present, since in many instances they may remain viable in the body. It is for this reason that artificial immunization with a well-standardized attenuated organism would be highly desirable, when its potentialities as a vaccine, though not as great, approach those of a virulent organism.

Resistance Acquired by Artificial Means

Artificial immunization which could produce an acquired resistance in animals (Chapter 13) and in man (Chapter 14) was attempted even before the discovery of the tubercle bacillus. Attempts at immunization have been made with dead organisms or their products as well as with organisms of related or unrelated species. The conclusions drawn from these studies have been that, although a certain degree of immunity may be produced with dead organisms, significant resistance is accomplished only by the use of live bacilli[1 2] (see Chapter 2). The best vaccine to date has been the bacillus of Calmette and Guérin (BCG) as it has retained its capacity to increase the bodily resistance of animal and man and has the great advantage over primary infection by virulent organisms that there is no danger of producing progressive disease in the host. Dr. Geoffrey Edsall, former editor of the *Journal of Immunology*, states:

> It is interesting to note that more convincing statistically significant evidence has been accumulated for the efficacy of such a bacterial vaccine, as for example the BCG strain of *Mycobacterium tuberculosis*, or pertussis vaccines, than it has been possible to collect even for diphtheria or tetanus toxoids.[46]

The Mechanism of Immunity in Tuberculosis

Native or acquired resistance is manifested in the body to a greater or lesser extent by the following:[6]

1. Localization or fixation of the infecting organisms at the site of entrance or adjacent lymph nodes
2. Inhibition of growth and possibly destruction of some or many of the organisms at the site of inoculation
3. Inhibition of the spread of the organisms throughout the body
4. Limitation of the multiplication of the organisms locally or in distant organs
5. Response of the organs with a more productive or fibrotic type of lesion
6. Prolongation of the life of the host (see Chapter 7 and review by Lurie)

The mechanism by which a host resists the deleterious effects of the tubercle bacillus is not clearly understood, but it is believed to be based on cellular hypersensitivity. The part played by the humoral and cellular constituents and the tissue environment as well as the effect of a hypersensitive state on immunity have been discussed in great detail by many authors.[1,5,6,47] No attempt will be made to review the literature in its entirety, but some of the pertinent studies are relevant to the role of artificially induced acquired resistance.

The Humoral Factor in Immunity The presence of detectable antibodies in both animals and man was noted early in the history of tuberculosis. The passage of serum with high titers of these antibodies to animals with tuberculous disease was generally reported to be without effect. Only occasionally were positive results reported.[1] The general conclusion was that serum antibodies do not play a major role in resistance to tuberculosis. Rich[1] questioned the validity of this conclusion because he felt that special precautions had to be taken to effect an artificial passage of resistance. First, the recipient animal should be continuously under the influence of the test serum during the time when the infection is becoming established and preferably throughout its course. Second, it is especially important that the transferred serum be homologous for the test animal lest potentially protective effects be vitiated by the formation of antibodies against serum proteins.

Raffel and his group[48] performed extensive experimentation in guinea pigs in which these precautions were taken. The serum donors comprised one group of 235 guinea pigs vaccinated over a period of six months with BCG and a second equal-sized group of untreated animals.

Sample animals from the vaccinated donor group were tested for immunity at the beginning and at the end of the experiment and on both occasions proved to have increased resistance to virulent tubercle bacilli. Serum from the vaccinated group was injected subcutaneously in the challenged group within 24 hours after collection from the donors in amounts of 2 ml daily throughout the two months of each experiment, beginning two days before challenge infection. Similar studies in other groups of animals were done using whole blood from immune and from normal animals injected into challenge animals. The results of these experiments seemed to be unequivocal—animals receiving either immune serum or immune whole blood under the circumstances outlined, derived no benefit from these transfer substances. These studies strongly indicate that it is not possible to transfer immunity passively, either by serum or by whole blood. As will be discussed below, sera of immunized animals do not transfer cellular or skin sensitivity.

A correlation between the existence of antibodies and the immune state was tested also by Raffel and his group. As antigens the authors used BCG, bacilli killed by various mechanical means, tuberculoprotein, wax, and polysaccharides and phosphatides, alone or mixed with tuberculoprotein in guinea pigs. The antibody responses (current serologic techniques) were shown to be almost entirely limited to the sera of those groups of animals which received whole bacilli or mixtures of components in which protein was a constituent. Resistance to a virulent infection followed vaccination with BCG and, in lesser degrees, the variously killed bacillary suspensions (in water-oil suspen-

sions), although the antibody titers with some of these antigens were often higher than those following BCG vaccination[5 48-51] (see Chapter 2). It is possible that the techniques for testing the type of antibody which is responsible for increased resistance in immunized animals is not known and thus has not been tested for. Sera of immunized rabbits (BCG) protected macrophages of these rabbits against the necrotizing action of virulent tubercle bacilli. This action, however, was nonspecific since the sera of rabbits immunized with totally unrelated organisms (*Salmonella, Brucella,* ovalbumin) had the same effect.[52-56] Thus, immune sera may combat some of the untoward effects of hypersensitization.

Some of the early studies of Lurie[57 58] showed that if virulent tubercle bacilli were embedded in agar and injected subcutaneously, or contained in cellophane bags and placed intraperitoneally, in animals previously immunized with BCG, they became clumped and reduced in number and failed to multiply to the extent noted in nonimmunized animals. No visible cells entered these foci, and Lurie explained the action on a humoral basis. These studies were criticized by Rich,[1] who claimed that an increased amount of fibrin would be formed around these foreign masses and that there would be a blocking of the lymphatics in the allergic inflammatory reactions, all of which interfered with the nutrition and growth of the organisms. Dubos[59] also took issue with Lurie's conclusions since he believed that the accumulation of organic acids at the site of inflammation in immune hypersensitive animals is great and that such acids inhibit the growth of the tubercle bacillus. Raffel,[48] who used semipermeable capsules implanted in the body for the study of immune mechanisms, stated that he was unable to find any distinction between the multiplication of bacilli in capsules implanted in the peritoneal cavity of immune animals and subjected to its tissue fluids and those of normal animals.

The present concept of immunity caused by facultative intracellular parasites is that sensitized lymphocytes (T or thymus derived) when stimulated by a specific antigen release a series of factors (lymphokines) involved in the immune state. Some of these are:

1. Chemotactic factor—which attracts cells to the site of the invading antigen[59]
2. Macrophage-inhibiting factor MIF—which inhibits mobility of monocytes[60 61]
3. Transfer factor—which may provide passive immunity against infectious agents by creating the essential sensitization of lymphocytes[62]
4. Cytotoxin factor—which may be involved in caseation[63]
5. Interferon-stimulating factor—which may inhibit virus multiplication[64]

Summary

The classical serum antibodies that develop in the course of natural or artificial infection with the tubercle bacillus do not directly protect the host from the disease. Cellular or skin hypersensitivity is not transferable by serum. The sera may inhibit the degree of sensitivity developed and thus reduce the degree of necrosis and caseation. Tissue fluids may have bacteriostatic and bacteriocidal properties.

The Role of the Cells in Immunity Against Tuberculosis Lurie has shown by a variety of experiments that macrophages play an active role in resistance to the tubercle bacillus. He allowed virulent tubercle bacilli to be phagocytosed by macrophages from immunized and nonimmunized animals, in vitro or in vivo. These cells were then injected into the cornea of normal rabbits' eyes. The cells or fragments were aspirated from the eyes 10 to 20 days later and cultured quantitatively, as well as observed histologically. The tubercle bacilli contained in normal cells continued to multiply but in the cells of the immunized animals they were reduced in number[6 65] These results were somewhat similar to those in earlier studies of

Manwaring et al., who demonstrated a decrease in the number of tubercle bacilli when they were cultured with pieces of omentum from an immune animal.[66] Woodruff also showed[67] that virulent tubercle bacilli would grow freely in 92% of normal animals when they were inoculated intraperitoneally; they were never observed in animals which had been previously immunized and were reactive to tuberculin. Phagocytosis and supression of growth of tubercle bacilli were also noted by Jensen et al. in the macrophages of animals immunized with BCG which had been challenged by tracheal route with virulent tubercle bacilli.[68]

Before the role of the lymphocyte was well understood there were many studies reported pro[48 69] and con[70 73] on the in vitro inhibition of intracellular growth of virulent tubercle bacilli in the macrophage of immunized and nonimmunized animals. The evidence now indicates that when certain antigens of intracellular infections are introduced into a host, they activate the macrophages of the reticuloendothelial system* which then engulf the antigens, break them up (probably enzymatically), and both retain and excrete the products thereof.[74 76] Some determinants of the latter are taken up by receptors of small lymphocytes, probably derived from the thymus (T cells). These are activated to large blast forms which in turn propagate into smaller, specifically sensitized lymphocytes (see Chapter 7). These so-called T cells represent a large proportion (80%) of the circulating lymphocytes and are involved in cellular immunity or delayed hypersensitivity.[64 77 78] These T cells are able to kill target cells (killer cells) bearing antigen on their surfaces (cell-mediated cytotoxicity) or inhibit the further immune responses to the same antigen (suppressor cell). Some T lymphocytes are required to assist B cells in responding to antigen and are called "helper cells." The bone marrow derived lymphocytes (B cells) develop into plasmalike cells capable of antibody formation.[74 79] The relative number of helper, killer, and/or suppressor cells that are produced will determine the nature and extent of an immune reaction.[75]

Lymphocytes of animals which have been sensitized to intracellular parasites and are then exposed to these antigens release substances which activate macrophages capable of suppressing the growth of the specific bacteria as well as other unrelated ones.[47 78] The stimulus required to provoke the activated cells is a specific one but the antibacterial capacity is nonspecific. Thus when BCG was injected into an animal previously sensitized with a small sensitizing dose of BCG, its macrophages changed rapidly with increases in size, processes, lysosomal and mitochondrial enzymes, etc.[80] (see Chapter 7), and the host became resistant not only to this organism but also to *Listeria monocytogenes, Brucella abortus,* and *Salmonella typhimurium.*

In vitro experiments of Patterson and Youmans[81] demonstrated that lymphocytes from mice immunized with viable attenuated mycobacteria (H37Ra) or RNA therefrom[81 82] when mixed with viable tubercle bacilli elaborated a filterable substance which, when added to a suspension of normal mouse intraperitoneal macrophages, would enhance to a great extent the capacity of such cells to inhibit the intracellular growth of virulent tubercle bacilli (H37Rv). It is of interest that the lymphocytes of mice infected with heat-killed H37Ra did not elaborate or generate a bacillary inhibitory factor.[47 78 81]

Summary

There is evidence to indicate that the sequence of reactions of the macrophages to intracellular parasites consists of phagocytosis of the antigen followed by partition, retention, and excretion of substances capable of specifically sen-

*Lympho-reticuloendothelial system (LRES) should replace reticuloendothelial system (RES) wherever it occurs in the text.

sitizing lymphocytes (T cells) and later antibody forming lymphocytes (B cells) and plasma cells. The specifically sensitized lymphocytes in turn have the capacity, in the presence of specific antigen, to liberate substances which greatly activate macrophages to phagocytize and inhibit or destroy a wide range of invading antigens, as will be discussed below. Cellular hypersensitivity (specific) and immunity (nonspecific) are intimately related.

The role of the specific tubercle and the nonspecific stimulation of the reticuloendothelial system (RES) is discussed in Chapter 7.

The Hypersensitivity State and Its Relation to Immunity in Tuberculosis There has been a considerable change in thinking regarding the role of the hypersensitive state and immunity against tuberculosis. Earlier studies[22-24] demonstrated that the hypersensitive (or so-called "allergic") state in animals developed concomitantly with the immune state and were closely allied. The question of the dependency of one upon the other has been explored in great detail.

Rich and his co-workers[1 83] presented experimental evidence that the hypersensitive state is separate and distinct from the immune state. This group inoculated guinea pigs with attenuated tubercle bacilli (R_1 strain); after the skin test had become positive to tuberculin they desensitized the animals by injecting graduated doses of tuberculin until the animals failed to respond to 1 mg of concentrated OT. If such animals were injected with virulent tubercle bacilli at the same time as the vaccinated sensitized animals, the retardation of disease was equal in both groups.

The work was criticized[84] because many of the animals died during desensitization, leaving the more resistant ones alive, and also because the animals were sacrificed at 65 days rather than being allowed to die naturally. When the experiment was repeated and the animals were allowed to die, the desensitized animals did not live as long as the sensitized ones, and a peculiar type of pneumonia developed in the desensitized animals in which innumerable organisms were found in the lungs. This latter phenomenon, however, was present, though to a lesser extent, in animals injected with glycerin or saline without desensitization.[85] Wilson pointed out[86] that complete desensitization in guinea pigs is extremely difficult to achieve and if it is done, about 90% of the animals die. Only a relative desensitization occurs since the animals quickly develop reactivity to tuberculin if the injections of tuberculin are stopped. Wilson interpreted the experiment on desensitization as showing that the allergic response was of value if properly controlled.

Raffel reported that the wax of tubercle bacilli plus protein injected into guinea pigs produces delayed type of tuberculin sensitivity of the skin[51] which is indistinguishable from that found in vaccinations with killed organisms. Such animals developed no resistance to tuberculosis. Cellular hypersensitivity in these animals was not established.

Youmans has shown that cytoplasm, or an extract of tubercle bacilli (RNA), produced immunity in mice without producing a hypersensitive state, as shown by tuberculin injected into the footpads of the animals (see Chapter 2). Cellular hypersensitivity in such animals was later established: cellular immunity was dependent upon hypersensitivity of lymphocytic cells (T cells) and cellular hypersensitivity can exist without skin sensitivity.[47 79 86 87] The immune state is demonstrated only following hyperergic stimulation by antigen.[47 79 81]

The hypersensitivity state develops in animals about the same time as the immune one.[47 88-90] The disappearance of the hypersensitive state more or less parallels that of the immunologic one, although immunity may outlast detectable hypersensitivity.[91] Clinically, Hart has reported that in 85% of the children in a BCG vaccination program in Great Britain

the immunity and the hypersensitivity paralleled each other, but in 15%, although the hypersensitivity had disappeared, immunity persisted.[91 92] As mentioned above, skin sensitivity does not always parallel the cellular sensitivity, and there may be antigens other than the present-day tuberculin which are involved in the hypersensitivity state.

The present thinking is supported by numerous workers, beginning with Chase[93] who demonstrated that the lymphocyte transfers delayed hypersensitivity, and Mackaness et al. who showed the relative roles of the lymphocytes and the macrophages in the hypersensitive and immunity states.[47 78 79] These attest to the prominent role of delayed hypersensitivity in immunity. Cellular resistance depends upon antigenic activation of cells sensitized to a specific microbial antigen. Thus in mice vaccinated with BCG and challenged intravenously with virulent tubercle bacilli (H37Rv), inhibition of the growth of the latter is delayed, supposedly due to the time necessary for the specific antigen to activate the specifically sensitized cells.[94] Cells of donors immunized with BCG transferred intravenously to normal recipients conferred no measurable protection against *Listeria monocytogenes* given intraperitoneally, even though the donors themselves were resistant to this organism. However, if such animals were given an eliciting dose of BCG intravenously, they then developed a statistically significant immunity against *L. monocytogenes*. It appears that a nonspecific mechanism of resistance developed during a specific immunological reaction, and that a cell-mediated form of hypersensitivity is the underlying process responsible for this form of acquired immunity.[47]

It is believed that the altered state of the host macrophages may be due to an interaction of antigen and a specific antibody adsorbed to the lipoprotein surface layer of these mobile cells,[95] and that the antibody involved in the reaction is perhaps identical with the antibody which confers the state of delayed type of hypersensitivity.[47]

Transfer of tuberculin sensitivity has been accomplished not only by sensitized lymphocytes,[60 93 96] but also by RNA extracts of such cells, using the capillary tube migration inhibition assay as a measure of delayed hypersensitivity.[61 97] The so-called macrophage inhibiting factor (MIF) is generated when sensitized lymphocytes, or RNA extract of same, are incubated with nonsensitive peritoneal exudate cells. The migration of these cells from capillary tubes is specifically inhibited when placed in contact with the sensitizing antigen.[60 96] This reaction is highly specific.[61 96]

As discussed above, the ability to inhibit multiplication of virulent tubercle bacilli within normal macrophages is dependent upon a factor liberated by a mechanism similar to that in the release of MIF.[81]

Delayed skin hypersensitivity may also be transferred by sensitive lymphocytes in animals and man.[98-101] Subcellular extracts have also been reported to have this capacity[102] although the work has not been fully verified.[103 104] However, when RNA from sensitized lymphocytes was mixed with normal lymphocytes and the mixture injected into guinea pigs, transfer of skin sensitivity was accomplished. This phenomenon was highly specific.[97]

Summary

The finite answer to the relationship between hypersensitivity and immunity is still not at hand, but with more refined techniques it appears that cellular hypersensitivity plays an important role in immunity. Hypersensitivity and immunity are intimately related and develop almost simultaneously. Cellular immunity and hypersensitivity can be passively transferred in vitro and in vivo by lymphoid cells or RNA fractions thereof and not by peritoneal macrophages or serum. Such