

Serum Therapy for Tuberculosis Revisited: Reappraisal of the Role of Antibody-Mediated Immunity against *Mycobacterium tuberculosis*

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INTRODUCTION

Fifty years after the introduction of the first effective antimicrobial agents against *Mycobacterium tuberculosis* (11, 12, 62), this pathogen continues to be a tremendous public health problem. In 1990, approximately 1.7 billion humans were estimated to be infected worldwide (about one-third of the world's population) (91). In recent years, there has been a recrudescence of tuberculosis. The epidemic has been associated with a dramatic rise in strains which are multiply drug resistant and a large population of immunocompromised human immunodeficiency virus (HIV)-infected hosts, in whom tuberculosis (TB) is an aggressive and often incurable infection (91). For some strains of multidrug-resistant *M. tuberculosis*, this situation is similar to that which existed in the preantibiotic era, when no effective therapy was available. The inconsistent efficacy of the bacillus Calmette-Guérin (BCG) vaccine has further complicated these recent difficulties and renewed interest in the development of effective new vaccines against tuberculosis (82). Most vaccines under development against TB are intended to confer protection by stimulating cell-mediated immune responses to control the proliferation of mycobacteria after infection. This strategy differs from that used by most successful antimicrobial vaccines, which mediate protection by eliciting an antibody response that neutralizes the infecting inoculum (95).

Conventional thinking generally assumes that host defense mechanisms against *M. tuberculosis* rely exclusively on cellular defense mechanisms. A role for antibody-mediated immunity either in protection against *M. tuberculosis* infection or in the pathogenesis of infection is often discounted (2, 24, 35). To evaluate whether a potential vaccine against TB could prevent infection by eliciting a protective antibody response, we reviewed the history of antibody-mediated immunity against TB. We started with the classical experiments with serum therapy, followed the literature as technology advanced and allowed the detection of antibodies, and concluded with current data. We analyzed the implications of positive and negative results in light of what is known about other systems.

SERUM THERAPY

In the late 19th and early 20th centuries, a considerable amount of animal and human experimentation was performed in an attempt to identify therapeutic sera for TB. These studies were stimulated by the successful development of serum therapy against a variety of infectious diseases including those caused by *Streptococcus pneumoniae*, *Neisseria meningitidis*, and group A streptococci and several toxin-mediated diseases (18). Serum therapy consisted of the administration of animal or human sera for the treatment of infection. The majority of sera were obtained from animals immunized with the pathogen in question. The active agent in serum therapy was antibody.

Studies with Nonimmune Serum

Serum obtained from a nonimmunized dog was used in 1888 by Richet and Héricourt to treat TB (8). Dogs were selected because of their resistance to TB; it was reasoned that their serum may contain protective substances. The serum, administered by injection to rabbits, was reported to prevent TB after experimental infection and to arrest the disease process in animals previously inoculated with tubercle bacilli. Clinical improvement was reported in two-thirds of human patients treated with serum by injections or by mouth. Improvement was maintained in patients who were in the early stages of TB, but in most other cases the benefit was temporary and the disease process was delayed by only a few months. The authors concluded that the canine serum had a beneficial but noncurative effect against the tubercle bacillus. Similar results were reported when steer and sheep blood were used to treat TB. Serum of dogs immunized against TB was found to have a better prophylactic effect on rabbits but aggravated the infection when injected into a rabbit already ill with TB (8). A subsequent study by Ingraham in 1894 revealed no benefit after the administration of nonimmune mule serum to eight patients (54).

Studies with Immune Serum

Beginning in the 1890s, several studies reported results obtained with immune sera, generated by immunizing or infecting animals with mycobacteria and/or their products.

Maragliano. Starting in 1895, Maragliano reported the use of horse antiserum prepared by immunizing various animals with a mixture of two preparations of mycobacterial substances (Table 1) (69). Maragliano claimed that the serum had a prophylactic and protective effect in guinea pigs: administration of 1 g of serum per kg of guinea pig was reported to protect the animal against a lethal dose of tuberculin. Saving guinea pigs already sick from the dose of tuberculin required two to four times more serum. The serum was reported to be antitoxic and bactericidal. The bactericidal activity of the serum was evaluated by plating tubercle bacilli with heat-inactivated serum and observing the plate for colony growth. Tubercle bacilli did not grow in the presence of immune serum but grew in the presence of normal serum (69).

Administration of serum to 412 TB patients (with infection confirmed in most cases by demonstration of bacilli in the sputum) was associated with complete recovery in 16%, significant improvement in 40%, no change in 37%, and death in 11%. Tubercle bacilli disappeared from the sputum of 43% of patients treated with serum. Patients with circumscribed lesions appeared to benefit more from serum therapy than did patients with diffuse illness, having a recovery rate of 48% (70). Other physicians also reported successful results with Maragliano's serum (14, 15, 25).

In 1905, the Henry Phipps Institute published results of a trial with serum prepared by Maragliano's later method (Table 1) with a modification. In this study, cows were used instead of

TABLE 1. Preparations of immune sera made in the pre-antibiotic era

Investigator(s)	Yr ^a	Antibody preparation				Species studied	Route of administration	Reference(s)
		Immunizing agent	Species	Route(s)	Length of immunization			
Maragliano	1895	Liquid culture of <i>M. tuberculosis</i> , heated to 100°C, filtered, and concentrated; and liquid culture of <i>M. tuberculosis</i> , filtered and evaporated by vacuum at 30°C (a later protocol was modified by the addition of ground, heat-killed bacilli to the inoculum)	Horse	s.c., i.v.	6 mo	Guinea pigs, humans	NS ^b	69, 70
Henry Phipps Institute	1905	Maragliano's later protocol	Cow	s.c.	NS	Humans	Hypodermic injections (most); oral administration (few patients)	38
Viquerat	1894	Liquid cultures of tubercle bacilli	Donkey	s.c., i.v.	45 days	Guinea pigs Humans	NS ^b Hypodermic injections	3
Paquin	1895	Tuberculin and <i>M. tuberculosis</i> (in various states of alterations and dilutions)	Horse	NS	2–6 mo	Humans	Hypodermic injections	84, 86
Fisch	1897	T.R., T.O., and aqueous extracts of the nutrient agar	Horse	Injections	NS	Guinea pigs Monkeys Humans	Injections Injections Hypodermic injections	37
De Schweinitz and Dorset	1897	Attenuated bacilli (culture fluid was added when horses were used)	Cow, horse	NS	15 mo	Guinea pigs Humans	Injections Injections	31
Trudeau and Baldwin	1898	Tubercle bacilli (in different states of virulence) and tuberculin	Horse, cow, sheep, fowl, ass, rabbit	i.v., i.p., s.c.	Months	Guinea pigs	s.c., i.p.	112, 113
Marmorek	1903	Filtrates of early cultures of tubercle bacilli (the bacilli lacked a complete waxy coat and thus did not maintain acid-fast stain)	Horse	Injections	Few months	Rabbits Humans	i.v., s.c. NS	71
Josset	1924	Attenuated <i>M. tuberculosis</i>	Horse	i.v., s.c.	NS	Guinea pigs Humans	s.c. NS	14, 55–57
Vallée	1909	Low-virulence bacilli of equine origine, human bacilli, human bacilli cultures and "endotoxins"	Horse	i.v.	NS	Guinea pigs Humans	Injections NS	14, 115, 116
Spahlinger	1922	Various sera, each made with one of various "ectotoxins" and "endotoxins," and dead and live bacilli (the sera to the various toxins and the antibacillary sera were combined in various proportions)	Horse	NS ^b	NS	Humans	s.c. (mainly); other routes were used in special circumstances, e.g., p.o. ^c (for "abdominal TB"), injection into site of disease (for "surgical TB")	108
Reenstierna	1934	Tubercle bacilli in different stages of growth: acid fast and non-acid fast (the latter devoid of its "wax envelope")	Sheep	s.c.	4 mo	Humans	i.m.	50

^a Year of first report identified that describes the serum or its use.^b NS, not specified.^c p.o., orally.

horses to avoid the renal and pulmonary complications that developed in horses as a result of the immunization process (38). This study revealed little or no benefit of serum therapy. A total of 41 patients were treated, of whom 5 received Maragliano's original serum. Treatment lasted from 3 days to 7 months with an average length of 86 days. Overall clinical improvement was observed in 17 patients (41%). When categorized by the degree of sickness, improvement was observed in 53% of patients with a "favorable prognosis," 47% of patients with a "doubtful prognosis," and 23% of patients with an "unfavorable prognosis." After the report was prepared, one of the cows used for the preparation of the serum died of TB, raising the possibility of animal-to-human infection via serum (38).

Viquerat. In 1894, Viquerat reported the use of immune donkey serum (Table 1) for TB therapy (3). Viquerat reported one animal experiment involving an unknown number of guinea pigs in which all but one of the treated animals were "cured of" TB and resisted reinfection; no controls were reported. Of 25 patients treated with this serum by hypodermic injections, 15 had pulmonary TB, 5 had bone TB, and 5 had miscellaneous forms of TB. Of 13 patients who completed therapy, 12 (92%) were reported cured and were able to return to work. Of 12 patients who were still treated at the time of the report, 10 (83%) were reported to have an improvement in their condition. Most patients who were "cured" of pulmonary TB were in the early stages of disease. No details about clinical, bacteriological, or diagnostic parameters, length of treatment, or time elapsed from initiation of treatment to cure were provided. For these reasons, this study was strongly criticized at the time (3, 43).

Paquin. A more organized and somewhat better controlled study was reported by Paquin in 1895, using horse serum (Table 1) (84, 86). The initial treatment group consisted of 22 individuals with pulmonary TB confirmed by the presence of tubercle bacilli in their sputum (the method was unspecified). The patients did not receive any treatment in addition to the serum. After approximately 2 months of treatment, overall improvement was reported in 82% of the patients as manifested by decreased cough, reduction in the bacillary load in sputum, clearance of pulmonary infiltrates, reduction in hemoptysis, improved appetite, weight gain, and increase in vital capacity (method unspecified) (83, 84). Six months after initiation of treatment, all the patients were alive and more than half were discharged from the hospital (84). Patients from another TB ward in the same hospital, who received no serum treatment, served as controls. During the initial 4 months of the study, over 30 deaths occurred in the control group compared to none in the group receiving serum therapy. To reinforce that improvement was due to serum therapy and not to "mental influence," Paquin reports that nearly all patients were initially reluctant to accept the treatment and most were treated against their will. After an initial improvement was shown, the treatment was stopped for 2 weeks and the symptoms reappeared; reintroduction of serum therapy improved the patients' condition again (84). Other forms of TB, including laryngeal TB, were treated with Paquin's serum as well (65). Although an improvement was observed in a few patients, including objective findings on laryngeal examination, the number of patients with extrapulmonary TB was too small to form a basis for any conclusions.

In 1897, Paquin published results of 293 patients treated with his serum, of whom 226 had microscopic confirmation of their diagnosis (221 had pulmonary TB) (85). Of 252 patients available to follow-up, 57 (23%) recovered, 40 of whom were monitored for 1 to 3 years after serum therapy and were alive

and well; 3 (1%) recovered clinically but their cavitory lesions remained unchanged; 76 (30%) improved and were able to perform daily activities; 80 (32%) improved to a lesser extent; and 36 (14%) died. No controls were reported. Studies supporting the efficacy of Paquin's serum in treating pulmonary TB were published by other investigators (51, 89, 105). Less favorable results were obtained in small studies by Stubbert (110) and Lemen (63), who found Paquin's serum to be less effective than claimed by others. Fisch used Paquin's serum in animal experiments with disappointing results (37) (see the next section).

Fisch. In 1897, Fisch published the results of animal experiments performed with horse serum (Table 1) (37). Fisch's report is unique for that historical period in its completeness and apparent rigor. His experiments were described in detail, included controls, and examined multiple conditions. The first experiment examined the efficacy of serum administration before infection with *M. tuberculosis*. Seven guinea pigs were included in the experiment: five received multiple injections (route unspecified) of serum over a 30-day period; Three of these five animals were then challenged with a lethal dose of tubercle bacilli, and the other two animals served as controls, receiving serum alone. An additional two animals, which received tubercle bacilli alone, were also controls. Of the five animals challenged with tubercle bacilli, four received a subcutaneous (s.c.) injection and one received an intraperitoneal (i.p.) injection. The two animals that received tubercle bacilli without serum died after 21 and 24 days and had characteristic lesions of TB. The other animals remained healthy for 7 weeks and gained weight. One of the animals that received both serum and tubercle bacilli was killed and examined 6 weeks after infection and did not have any tuberculous lesions.

In a modification of the first experiment, Fisch studied the effect of serum dose on experimental TB. Five guinea pigs were pretreated with a lower total dose of serum than in the first experiment. Three animals were challenged (route not specified) with a lethal dose of tubercle bacilli; one died after 20 days (no autopsy reported), but the other two remained alive and did not lose weight after observation for 7 weeks despite having a local infection at the injection site. When the animals were treated with a higher total dose of serum, all the animals, in both the experimental and control groups, remained healthy for 2 months. The author concluded that the serum was effective against TB and that the effect was dose dependent.

In another experiment, Fisch examined the efficacy of simultaneous administration of serum and tubercle bacilli. Various volumes of serum were mixed with a lethal dose of tubercle bacilli, and the suspension was administered s.c. or i.p. Eleven animals that received suspensions containing 0.25 to 1 ml of serum remained healthy 2.5 months later and gained weight. Of three animals that received suspensions containing 0.1 ml of serum, two died after 37 and 54 days and the third had a "large infiltration" (organ not specified) and had lost weight. The controls, consisting of three animals received *M. tuberculosis* alone and 3 animals inoculated with a mixture containing Paquin's serum, all died 16 to 29 days after inoculation (the difference in time to death between the two groups was not statistically significant).

After establishing the effect of serum administered simultaneously, Fisch examined whether serum administration could modify the course of experimental tuberculosis in guinea pigs. These experiments involved the administration of serum to guinea pigs on day 4, 7 or 10 after challenge with a lethal dose of tubercle bacilli. Serum (0.25 ml) was thereafter administered on alternate days for 4 weeks and once a week after that.

Eighteen guinea pigs were used, six in each experiment. Sixteen animals remained alive 2.5 months after challenge, although one of them developed an extreme enlargement of the lymph nodes. Signs of illness such as ulcers or fever were reversed by serum treatment. The tuberculin test was negative in three animals that were tested after 6 weeks of treatment. Two animals that received serum beginning on day 10 of infection were killed at 5 weeks for autopsy. Scar lesions were observed in their livers, and the spleen of one animal was abnormally contracted. The pathological changes were described by Fisch as "attempts at restitution or at least at encapsulation"; encapsulation was particularly noted in the lymph nodes. Of three animals that were injected with serum starting on day 14 after challenge with tubercle bacilli, two became sick and the third animal died 7 weeks after challenge. Controls that received tubercle bacilli only (three animals) or that were treated with Paquin's serum beginning 4 days after challenge (three animals) died 20 to 28 days after infection (no significant difference in the time to death between the two control groups). Fisch concluded that his immune serum protected against and cured tuberculosis.

Fisch also performed two small experiments in monkeys. The first experiment was performed with two monkeys (genus *Cebus*, order *Platyrrhini*) challenged with tubercle bacilli i.p.; one was treated with serum on alternate days, starting the day after challenge, and the other served as an untreated control. The control monkey died of disseminated disease 50 days after infection, but the monkey receiving serum therapy was alive and healthy 75 days later, with a negative tuberculin test. A similar experiment was performed with intratracheal administration of tubercle bacilli. The control monkey died after 20 days with extensive disease of the larynx, lungs, liver and the lymphatic system. The serum-treated monkey remained healthy on day 50 of infection except for a small ulceration at the site of infection.

To investigate whether the serum had direct bactericidal properties, fresh anti-T.R. serum was mixed with tubercle bacilli and incubated for various times. An aliquot of the suspension was then injected i.p. into guinea pigs. Animals that received serum-bacillus mixtures (one animal per incubation time) that had been incubated for up to 3 h died 12 to 16 days after inoculation (three animals), while animals receiving mixtures that had been incubated for 5 to 24 h (four animals) were alive 6 weeks later. The serum antituberculin properties were demonstrated by injecting anti-T.R. serum and tuberculin into tuberculous guinea pigs. A 0.1-ml volume of serum was sufficient to inhibit the tuberculin reaction as manifested by an increase in body temperature. A volume of 0.5 ml of the same serum saved guinea pigs from a lethal dose of tuberculin. Twenty human patients with early TB (diagnosis made microscopically) were treated with daily hypodermic injections of 1 ml of serum; all were reported to show improvement within 6 to 8 weeks. Temperature decline, cessation of cough, expectoration, and night sweats, decreased respiratory rate and pulse, and weight gain were reported (37).

Holmes subsequently used Fisch's serum to treat 50 human patients who had pulmonary TB (53). The patients were divided into two groups; the first consisted of patients in the "earliest incipient stage," who had early symptoms of TB but no bacilli in their sputum (13 of them were tuberculin tested and found positive), and the second consisted of patients who had a productive sputum with bacilli (method unspecified). All 19 patients in the first group improved rapidly with serum therapy and were tuberculin negative at the end of treatment. The patients in the second group were divided into two subgroups: "incipient" and "advanced." Of 11 patients in the in-

ipient subgroup, 4 (36%) were cured, with elimination of bacilli from the sputa, and 7 (64%) showed significant improvement. Of 20 patients in the advanced subgroup, 4 (20%) showed significant improvement, 7 (35%) showed slight improvement, 3 (15%) showed no improvement, and 6 (30%) died. Many patients in the second group were treated for only a short time (the times not provided), although the author was aware of evidence suggesting that longer treatments gave better results. However, he concluded that serum was beneficial in early but not in advanced cases (53). A negative report about Fisch's antiserum was published in 1898 by Waxham, who administered serum to 10 patients, of whom only 3 showed clinical improvement but failed to clear the bacilli from their sputa (119). These patients had long-standing infections and were treated for a relatively short period of 3 to 10 weeks. Stubbart used this serum on six patients and obtained poor results (110). Fisch's serum was produced commercially by the John T. Milliken company of St. Louis (30).

De Schweinitz and Dorset. In 1897, De Schweinitz and Dorset reported the use of cow and horse serum (Table 1) (31). Protection experiments were done in guinea pigs that first received serum and then were challenged with tubercle bacilli. Guinea pigs that received cow serum were more resistant to infection and lived longer than did controls which received *M. tuberculosis* challenge alone, but the effect was observed only after administration of very large quantities of serum. This preparation of cow serum (Table 1) was more effective than another preparation made by immunizing a cow with tuberculin. However, no details were provided about the parameters by which efficacy was measured. When horse serum (Table 1) was used, the quantities required to achieve beneficial effects were smaller than those of the cow serum. Overall, serum-treated guinea pigs lived longer and had less organ involvement than did controls which received tubercle bacilli alone. Serum administration also prevented the rise in temperature and the reaction characteristic of tuberculin administration. The serum was used in humans by Stubbart, who treated 82 patients with De Schweinitz's antitubercle serum (most probably horse serum) in Loomis Sanitarium at Liberty, N.Y. (110). A few patients received Paquin's and Fisch's anti-TB serums. A significant improvement was reported in patients treated with De Schweinitz's serum (but not in those treated with Paquin's or Fisch's sera). The greatest improvement was observed in patients with early disease, and only mild side effects from the treatment were noted. Stubbart reported that some treatment failures were due to mixed infections with other bacteria. Unfortunately, these human studies did not include controls.

Trudeau and Baldwin. In 1898, Trudeau and Baldwin reported numerous animal studies that evaluated the efficacy of serum against TB (112, 113). Immune sera were produced in different animals by use of various immunization protocols (Table 1). Various passive immunization and infection protocols were used in addition to serum-bacillus suspensions that had been incubated together for several hours prior to injection. Overall, the serum administered did not affect survival, course of disease, or autopsy findings in treated guinea pigs relative to untreated controls. No effect on survival was noted even when serum prepared by others (sources not mentioned) was used. In vitro assays were performed with ass serum mixed with avirulent tubercle bacilli in the presence of medium (112). No effect was observed on the growth of bacilli compared to the growth in control tubes without serum. Despite their disappointing results, Trudeau and Baldwin concluded that serum therapy for TB was not hopeless but depended on further

knowledge about the "mechanism of immunity and antitoxin production by the body."

Marmorek. In 1903, Marmorek reported several animal experiments with horse serum (Table 1) in rabbits (71). Serum administered 3 days before infection was reported to be protective in rabbits subjected to intravenous (i.v.) challenge but no details about the number of animals, the use of controls, or parameters of serum protection were provided. When three groups of rabbits were injected with serum s.c. a few hours, 1 day, and 2 day after i.v. challenge with tubercle bacilli, the control rabbits that received no serum had multiple lung "tubercles" at autopsy 20 days after challenge whereas serum-treated rabbits had no lesions. Furthermore, serum-treated rabbits that were not killed for autopsy studies remained in good health.

In humans, the efficacy of Marmorek's serum varied depending on the type of TB (71). In pulmonary TB, the efficacy of the serum was dependent upon the duration of the illness rather than the extent of its anatomical involvement. Recent infections, even if extensive, were more likely to respond to serum than were long-standing infections, even if the latter were less extensive. Marmorek reported that some advanced cases of pulmonary TB did benefit from serum therapy. Cases of "surgical TB" were also reported to benefit from Marmorek's serum. As examples of the latter, Marmorek reported the complete cure of several patients with Pott's disease complicated by fistula, abscess, intestinal perforation, or lower-extremity palsy. Other cases of surgical TB such as adenitis, long-standing fistula, and skin granulomata that were refractory to other means of therapy were reported to improve significantly with serum therapy. Marmorek's serum was not effective for therapy of TB meningitis (71). Several studies supporting the use of Marmorek's serum, mainly in certain forms of surgical TB, were published. However, treatment failures, occurring primarily in patients with very advanced pulmonary TB, were also noted (7, 44, 52, 61, 75, 94).

Josset. Josset treated more than 800 patients with horse serum (14) (Table 1) over two decades and published his results in 1924 (55). Josset carried out extensive animal experiments before performing his clinical trials (56). In one experiment, 60 guinea pigs received s.c. injections of tubercle bacilli. The animals were separated into three treatment groups: one group received immune horse serum, one received serum from a nonimmunized horse, and the third group received no treatment. Five doses of serum were administered by injection at 5 day-intervals starting on day 5 after challenge. Guinea pigs given immune serum had lower fever and did not demonstrate signs of sickness following administration of tuberculin, in contrast to control animals, which became very ill or died within 48 h. Autopsy revealed additional differences between the groups: in guinea pigs treated with anti-TB serum, involvement of the lymphatic nodes was limited primarily to nodes that were contiguous to the site of bacilli injection (groin area), whereas untreated animals had more disseminated lymph node involvement. The total number of lesions was 3 to 12 in serum-treated animals and 12 to 21 in control animals (56).

Despite treating a large number of patients with this serum, Josset described only 15 cases. The serum was administered by injection in one or more doses; the duration of treatment and the clinical response varied from patient to patient. Josset concluded that since not all cases of TB responded to serum therapy, patients had to be carefully and appropriately selected. Cases of recently acquired tuberculosis were more likely to respond to serum therapy than were chronic cases (55, 57).

Vallée. In 1909, Vallée described the use of horse serum (Table 1) (14) that demonstrated antitoxic effects by preventing tuberculin-induced fever in guinea pigs injected with a mixture of heat-inactivated immune horse serum and tuberculin compared to controls that received a mixture containing nonimmune serum. However, the number of animals used was small (two per group) (115). Incubating the horse serum with serum from cows with TB produced a precipitation reaction which was interpreted as an antigen-antibody reaction. This effect was observed consistently with sera from 33 cows with TB but not with sera from healthy cows (116). Studies with humans receiving Vallée's serum gave encouraging results in approximately 20% of the patients, but details on the nature of the studies were not provided (14).

Spahlinger. Spahlinger believed that the symptoms of TB "intoxication" were due to different toxins and not to different manifestations of one toxin. He reported in 1922 the isolation of various "toxins" and divided them into "ectotoxins" (substances secreted by the bacilli in culture medium) and "endotoxins" (substances derived from the bacillary bodies) (108). Immune sera were prepared to each of these "toxins." Another serum was prepared by injecting a horse with dead bacilli followed by live bacilli (Table 1). The sera to the different toxins were combined in various proportions and used in different clinical settings. If a certain combination was not effective, another one was used until a clinical response was obtained. Multiple doses of serum were administered to patients at various intervals and for various durations, depending on the severity of disease. Spahlinger reported eight patients in whom the signs and symptoms of TB improved or resolved after combination serum therapy (108). Subsequently, two more patients were treated by others with Spahlinger's serum, with good results (109). Spahlinger's experience exemplifies the variability that can occur in serum treatment for tuberculosis. Some sera were reportedly more effective in certain patients; their beneficial effect ceased when they were replaced by another serum and returned when they were reinstated. Unfortunately, no details on the isolation and separation of the various "toxins" used for the preparation of sera and the relative proportion of each serum in the final formulations were provided. Criticism addressing these issues was published shortly after Spahlinger's report appeared (27).

Reenstierna. A large clinical trial of serum therapy was carried out by Hanson in the 1920s and 1930s with sheep serum (Table 1) produced by Reenstierna (50). The study included 132 patients with extrapulmonary TB (21 had lupus vulgaris, a form of cutaneous TB with characteristic nodular lesions on the face, particularly about the nose and ears; 52 had lymph node involvement; 7 had cutaneous TB; 11 had ocular TB; and 41 had osseous and articular involvement). The therapy consisted of four intragluteal injections of serum every other day for 8 to 10 days. If no effect was observed after the first series of injections, a second series was given 1 to 2 months later, and occasional patients required more serum. Most patients did not receive other forms of treatment while receiving serum. A rapid beneficial effect was reported in patients with lupus vulgaris, occasionally within hours of serum administration, and some patients were reportedly healed. A favorable effect was also reported in patients with lymph node and ocular disease. Some beneficial effect was reported in certain patients with osseous and articular TB, but no effect was found in those with cutaneous TB. Unfortunately, detailed data was provided for only a small number of the patients. Although patients were treated in a 500-bed sanatorium, which allowed the investigators to assess the effect of serum therapy relative to other patients under the same nutritional and environmental condi-

tions, no details were provided about the "controls" used in this study.

Calmette. The value of serum therapy for TB was reviewed by Calmette (14). In this review, the reported efficacy of some sera produced at the end of the 19th century and the beginning of the 20th century was assessed, with some samples being analyzed for antibody content. Calmette found that antibody content varied from serum to serum. For example, considerable amounts of antibodies were detected in Vallée's serum, but none were found in Maragliano's serum. Antibody content also varied in different lots of the same serum: for example, in some lots of Marmorek's serum, antibodies were not detected. Calmette thought that the sera produced by Maragliano and Marmorek acted as an "antigen" because they contained tuberculin and diluted bacillary products, but he provided no data to support this assertion. He found that Vallée's serum was devoid of "antitoxic power"; tuberculous guinea pigs that received tuberculin and immune serum died within few hours, as did controls who received normal serum. Hence, Calmette demonstrated major qualitative and quantitative differences between antituberculous sera used in prior studies.

Calmette recognized that the variability of immune sera depended upon several factors. Production of serum with high antibody titer was relatively easy, but different immunization protocols produced sera with different antibody titers. In addition, antibody-containing sera behaved differently: antibodies of some sera bound to soluble antigens in the medium or to antigens on the surface of bacilli, whereas antibodies from other sera bound to bacillus extracts or to antigens extracted from macerated bacilli. Calmette also reported that some sera containing the highest antibody titers were more likely to hasten the progression of disease in laboratory animals and cattle but provided no details on this important observation. Calmette further claimed that the antibodies given in the animal sera were useless because they did not add to the high titer of antibodies to *M. tuberculosis* in the patients sera. His criterion for determining the efficacy of any treatment was whether disease in animals already infected was modified. He concluded his review with the following words: "Up to now, therefore, it does not seem that specific serotherapy has realized the hopes which it aroused" (14).

Summary of Serum Therapy Studies

Serum therapy for TB was disappointing because no consistently effective serum formulation was ever developed. Most of the studies claimed some benefit to the administration of serum but lacked appropriate controls by present-day standards. Many studies provided little detail on the diagnostic criteria used, as well as on the preparation, administration, and effect of serum therapy. Comparisons between studies are difficult, because each investigator used different antigens for the preparation of anti-tuberculous sera (Table 1). The most thorough studies were those of Paquin and Fisch, both of whom reported favorable effects following serum administration.

Despite the limitations of these studies, there are some consistent themes in the experience with serum therapy for TB. Serum therapy appeared to be more effective in patients with early and localized cases of TB rather than long-standing, chronic cases (53, 55, 57, 70, 71, 110). Furthermore, long periods of treatment were often required to achieve a sustained therapeutic effect (53, 83, 84, 108).

The difficulties encountered in the development of serum therapy for TB were in contrast to the successful development of serum therapy for pneumococcal pneumonia. However, efforts to generate serum therapy for TB appears to have been

considerably smaller in scale than those to develop serum therapy for pneumococcal pneumonia. For pneumococcal pneumonia, hundreds of studies were done from 1890 to 1935 which eventually resulted in the development of several effective type-specific sera after considerable trial and error (17). In contrast, relatively few studies were done with anti-tuberculous sera, and there appears to have been no systematic study of the variables contributing to the serum efficacy. For example, the concentration of specific antibody in therapeutic sera was seldom determined. It is noteworthy that the development of serum therapy for pneumococcal pneumonia also encountered significant difficulties until fundamental discoveries were made, including the finding in the 1910s of the requirement for type-specific sera (17). For TB, lack of knowledge of the basic biology of the bacterium and slow growth of cultures may have contributed to the difficulty in developing effective sera. Furthermore, the pathogenesis of pneumococcal pneumonia is very different from that of TB in that pneumococcal pneumonia is an acute illness (79) whereas pulmonary TB is a chronic illness with a high rate of progression, dissemination, and death (47).

After the 1920s, reports of serum therapy for TB became increasingly scarce. This undoubtedly reflected the disappointing results observed during clinical trials of serum therapy and the powerful influence of Calmette and Trudeau, who did not believe in the usefulness of serum therapy for TB.

The inability of various investigators to consistently generate effective sera was almost certainly an important contributor to the present-day belief that antibody-mediated immunity plays a minor role at most in the outcome of *M. tuberculosis* infection. However, the majority of published experiments with the use of serum therapy for TB did produce some evidence of the efficacy of serum against *M. tuberculosis*. Unfortunately, the lack of appropriate controls, the minimal descriptions in many studies, and the lack of known antibody titer of the sera make much of the experience with serum therapy inconclusive with regard to the efficacy of antibody in protection against TB.

MODERN STUDIES

After the 1920s, there was little effort to develop serum therapy for TB. However, advances in immunology and microbiology continued and led to the "modern" period in antibody studies against *M. tuberculosis*. We define the modern period as the time between the 1930s and the present. This period differs from the past in that practically all studies of immune sera include a measurement of antibody concentration. Methods for fractionation of sera were developed, allowing investigators to study the effect of antibody-containing fractions. Furthermore, there was increased interest in the usefulness of serological studies to diagnose TB and predict outcome. Intellectually, this period saw a shift in interest from antibody-mediated protective mechanisms to cell-mediated immunity against *M. tuberculosis*. As a result, the literature on antibody studies is scattered over seven decades and is punctuated by several major studies which continued to provide evidence both for and against the importance of antibody-mediated immunity in TB. Hence, the role of antibody-mediated immunity in protection against TB has always been uncertain and somewhat controversial. Even as early as 1903, Marmorek was forced to resign his position at the Pasteur Institute over disagreements on the value of serum therapy for TB (71).

In this section we review some of the modern studies. Our aim is to be comprehensive but not exhaustive. Since many of these studies provide contradictory results on the efficacy of antibody-mediated immunity, we have categorized them as

providing supportive data and nonsupportive data with regard to the importance of antibody in host defense against *M. tuberculosis*.

Basics for Establishing the Role of Antibody-Mediated Immunity against Microbial Pathogens

An important role for antibody-mediated immunity against a given pathogen is usually established by fulfilling one of the following criteria: (i) correlating immunity to infection with the presence of specific-serum antibody; (ii) demonstrating that specific antibody administration mediates protection against the pathogen; and/or (iii) demonstrating an association between susceptibility to infection and a disorder of antibody-mediated immunity. Supportive data for a role of antibody in host defense can be provided by *in vitro* studies which demonstrate that specific antibody inhibits the pathogen, neutralizes a toxin elaborated by the pathogen, promotes antibody-dependent cellular cytotoxicity, serves as an opsonin, triggers the complement cascade, and/or prevents infection in tissue culture. Many of these parameters of antibody efficacy have been studied for *M. tuberculosis*.

Serological studies with humans. Human studies of antibody-mediated immunity in the postserum therapy era have been limited to measuring antibody titers in TB. The aim has usually been to use serological methods for diagnostic purposes. Here we have included studies that attempted to correlate the presence of serum antibodies with outcome of infection.

(i) Supportive data. (a) *Choucroun*. In 1949, Choucroun reported a correlation between the presence of serum antibodies to *M. tuberculosis* carbohydrate antigens and acquired immunity to disease (20). Sera of patients with active TB were compared to those of patients who had recovered from TB, by testing for the presence of antibodies to the carbohydrate fraction of the "tubercle bacillus" in a direct precipitin test. Active TB cases were classified as "rapidly progressive," "intermediate," and "regressive" depending on the state of infection. Persons without TB served as controls and included both healthy individuals and those ill with syphilis, pneumonia, or cancer. Antibodies were found in the sera of approximately 50% of the patients with active TB; however, the ratio of positive to negative sera was three times higher among patients with regressive disease than among patients with rapidly progressive disease. Almost equal numbers of positive and negative sera were found in patients with intermediate cases. Antibodies were found in about 30% of persons who recovered from TB: the ratio of positive to negative sera was 13 times higher in patients who had been disease free for less than 2 years than in patients with inactive disease for more than 2 years. Only 5% of controls had antibodies to mycobacterial carbohydrate antigen. The author concluded that antibodies to mycobacterial carbohydrate antigen were more prevalent in patients who were able to overcome TB than in those whose disease progressed rapidly and that antibodies persisted for a certain period in the sera of patients who recovered.

(b) *Smith and Scott*. In 1950, Smith and Scott reported the prevalence of serum antibodies to Old Tuberculin preparation, which was studied in 104 patients with active TB by using the Middlebrook-Dubos hemagglutination assay (106). Antibodies were found in the sera of 80% of patients with active TB. Of the 20% of patients with negative hemagglutinins, most had very advanced disease and a poor prognosis. Sera from 92% of healthy individuals, 82% of patients with syphilis, and 80% of patients who had been long recovered from TB, were negative for antibodies to Old Tuberculin. The explanation given by the

authors for the low antibody titers in patients with very advanced tuberculosis was that the antibodies were consumed by neutralization of excess antigen produced by the rapidly growing mycobacteria. However, another possible explanation is that patients with very low serum antibody titers were more likely to develop a progressive disease.

(c) *Daniel et al.* In 1981, Daniel et al. reported the use of an enzyme-linked immunosorbent assay (ELISA) to measure the humoral immune response in 65 patients with pulmonary TB to four mycobacterial antigens: a culture filtrate of *M. tuberculosis* H37Ra, arabinogalactan, and two protein preparations (29). Immunoglobulin G (IgG) geometric mean antibody titers to all the mycobacterial antigens tested were higher in patients with pulmonary TB who received 4 weeks or more of treatment than in those who received less than 4 weeks of treatment. In addition, anergic patients had slightly higher antibody titers to mycobacterial proteins but not to polysaccharides than did nonanergic patients. No conclusions were drawn by the authors regarding the potential effect of antibody-mediated immunity on the control of pulmonary TB. These data could be interpreted to suggest that therapy decreased the mycobacterial organ burden, allowing recovery of the immune system and humoral immunity. Alternatively, the antibody response may have been boosted by breakdown products of dead mycobacteria without necessarily playing a role in protection.

(d) *Costello et al.* In 1992, Costello et al. reported the measurement of serum IgG responses to mycobacterial antigens by ELISA and immunoblot analysis in 136 infants and children with clinical TB (26). Children with disseminated TB had a significantly lower IgG response to mycobacterial antigens and to lipoarabinomannan (LAM) than did those with localized disease. The authors concluded that a weak antibody response to LAM and other mycobacterial antigens before or in the early stages of infection increased the likelihood of dissemination.

(e) *Barrera et al.* In 1992, Barrera et al. reported the use of ELISA to measure the levels of IgG to purified protein derivative (PPD) antigens in patients at various stages of HIV infection (6). In patients with TB, the rate of detection of IgG varied with the degree of HIV disease. Among HIV-negative patients at risk for HIV infection, the prevalence of IgG to PPD was 50%, compared to 36% in HIV-positive patients and 5% in AIDS patients. The authors concluded that a progressive depression of the humoral responses to *M. tuberculosis* occurs during HIV infection. This study documented a defect in antibody response in patients with HIV infection who are at high risk for TB.

(f) *Da Costa et al.* In 1993, Da Costa et al. reported the use of ELISA to evaluate IgG responses to *M. tuberculosis* LAM in HIV-seropositive and -seronegative patients with TB (28). IgG2 was the predominant subclass found, being detected in 58% of HIV-negative tuberculous patients compared with 35% of HIV-seropositive tuberculous patients. IgG1 and IgG4 responses to LAM were detected in HIV-seropositive patients with TB but not in HIV-negative patients with TB. HIV-positive and -negative individuals without TB had no serum antibody to LAM. The authors suggested that antibodies may play a supplementary role in protective immunity to TB, possibly by enhancing or down-regulating cellular immune responses.

(ii) Nonsupportive data. (a) *Peterson et al.* In 1952, Peterson et al. reported the measurement of the hemagglutinating serum antibody titer in infants who received BCG in the neonatal period (87). These infants were reportedly protected against TB but had no measurable antibody response during the first year of life. The authors considered two interpretations of the data: (i) that hemagglutinating antibody titers had no signifi-

cance in the immunity established following BCG vaccination, or (ii) that antibody-mediated immunity was established but was below the level of detection. Although the authors held the view that antibodies detected by hemagglutination assays played no role in TB immunity, they were cautious on this point and suggested that the issue needed further study (87).

(b) *Lenzini et al.* In 1977, Lenzini et al. reported the cellular and humoral immune responses to mycobacterial antigens found in 66 patients with microbiological or histological evidence of TB (64). The antibody titer was measured by hemagglutination and double immunodiffusion assays with PPD antigen. Patients with advanced and disseminated disease were more likely to have serum antibodies to PPD antigens than were patients with localized TB. Antibodies to PPD were not detected in controls, regardless of their PPD skin test results. The authors concluded that these results do not support a role for antibodies in protection against TB, since the patients with high antibody titers were more likely to have advanced disease and to be unresponsive to therapy.

(c) *Kardito and Grange.* In 1980, Kardito and Grange reported the clinical and immunological features of 90 patients with smear-positive pulmonary TB as compared to those of 50 age-matched healthy controls (58). The total and *M. tuberculosis*-specific IgG and IgA levels were significantly elevated in patients with TB. Patients with advanced disease had higher levels of total IgG and IgA than did patients with less advanced disease. However, no correlation was found between the level of specific antibodies and the extent of disease.

Serological studies with experimental animals. (i) Supportive data. (a) *Lurie and Zappasodi.* In 1941, Lurie and Zappasodi reported the clinical course of experimental TB in various strains of rabbits and correlated their results with parameters of the immune response (67, 68). Twenty-eight rabbits from five strains which differed in their resistance to TB (four to six rabbits from each strain) were vaccinated with intracutaneous injections of heat-killed *M. bovis* Ravenal. Rabbits from the most resistant strains produced the most rapid and highest antibody titer, whereas rabbits from the most susceptible strain had the slowest and lowest antibody response. Rabbits from strains with intermediate resistance had intermediate antibody responses (67). Since the rabbit strains also differed in genetic background, cellular immunity, and acquisition of local immunity, the authors concluded that multiple factors were responsible for the variability in susceptibility of the different strains to TB (68).

(b) *Seibert et al.* In 1956, Seibert et al. reported the testing of rabbits for antibody responses after repeated BCG immunization (102). The titers of anti-mycobacterial protein and polysaccharide antigens were measured. The resistant rabbits developed high titers to both protein and polysaccharide antigens, whereas the susceptible rabbits produced mostly antibodies to mycobacterial protein antigens. The authors suggested that a balance between the various antibodies was important for protection against TB. Furthermore, they postulated that free mycobacterial polysaccharide, not counteracted by an antibody, interfered with a protective immune response. However, the small number of rabbits studied made it difficult to draw firm conclusions.

(c) *Seibert and Seibert.* In 1957, Seibert and Seibert (104) reported on a series of clinical and serological parameters evaluated under two experimental conditions to test the hypothesis raised in the previous citation (102). In the first experiment, survival and antibody responses were studied in four groups of three to five rabbits. The first group consisted of rabbits which were immunized repeatedly with BCG at weekly intervals, challenged with Ravenal strain (a virulent bovine

strain), and then given repeated weekly injections of dead BCG; the rabbits survived longer than controls and no signs of TB were found at autopsy. Serum from these animals contained precipitin antibodies to both mycobacterial protein and polysaccharide antigens. A second group of rabbits received no immunization with BCG, and most of them died. These animals had a moderate degree of TB at autopsy and no precipitin antibodies to either protein or polysaccharide antigen. In a third group, most rabbits that received weekly injections of mycobacterial protein and one injection of live BCG prior to challenge died. They had moderate to severe TB, and their serum contained antibodies to mycobacterial proteins but few or no antibodies to mycobacterial polysaccharide. In a fourth group, all rabbits that received only protein died of extensive TB, and their serum antibody profile was similar to that of the third group. In addition, rabbits with high levels of complement-fixing antibodies to PPD-S resisted infection while those with low titers had an extensive disease. The rabbits with high titers had received repeated immunizations with BCG, while the ones with low titers had received immunizations with mycobacterial protein. In a second experiment, rabbits were challenged with a higher dose of Ravenal strain. As in the first experiment, the inability of rabbits to mount anti-polysaccharide antibodies was associated with more extensive disease and earlier death than in animals that responded with both anti-protein and anti-polysaccharide antibodies. The authors concluded that rabbits capable of producing antibodies to both polysaccharide and proteins survived the longest.

Conclusions from serological studies. In both human and animal studies, a correlation between antibody titers and improved outcome of TB was demonstrated by some investigators (20, 26, 29, 67, 102, 104, 106) but not by others (58, 64, 87). Some of the studies that demonstrated a correlation between antibody and improved outcome also provided evidence for differences in efficacy, depending on the target antigen. Overall, the results suggest that antibodies to mycobacterial polysaccharides may affect the course of infection (20, 26, 29, 100, 102, 104). IgG antibodies to LAM were implicated in protection against dissemination in one study (26). Of particular interest are the results of serological studies in HIV-positive patients. HIV-positive patients have a higher rate of TB (107), which is thought to be due to impaired cellular immunity. However, since two studies (6, 28) documented abnormalities in the antibody response to mycobacterial antigens in these patients, such a deficit may contribute to their susceptibility to aggressive TB infection.

Passive antibody studies with animals. Serum from humans with TB and immunized animals has been used to study the effect of antibody administration on experimental TB. Like the results of serum therapy and serological studies, these studies provide data for and against a role for antibody-mediated immunity.

(i) **Supportive data.** (a) *Zitrin and Wasz-Höckert.* In 1957, Zitrin and Wasz-Höckert reported the effect of human antibody administered to mice that had undergone i.v. *M. tuberculosis* infection (124). Three groups of Webster Swiss Albino mice were given human serum or its fractions and challenged i.v. with *M. tuberculosis* Vallée. The serum was derived from TB patients who had been ill for 6 months or longer and were undergoing successful therapy. The serum was free of anti-TB medications. The following three serum preparations were used: fraction II-III, consisting of gamma and beta globulins; fraction IV, consisting of alpha and beta globulins; and commercial immune gamma globulin. Saline was used as a control. Each group of mice received a different treatment protocol consisting of daily intraperitoneal injections of whole serum or

its fractions. The first group received one dose of treatment before and six doses after challenge; the second group received four doses before and four doses after challenge; and the third group received four doses before and none after challenge. The mean and median survival times of the mice were used as a parameter of therapeutic effect. In the first group, survival time was the longest in mice receiving immune gamma globulin, followed by fraction IV, fractions II–III, and whole serum. In the second group, the longest survival time was in mice receiving fraction IV, followed by fractions II–III, fraction IV of nontuberculous tuberculin-positive serum, immune gamma globulin, and saline. In the third group, the longest survival time was in mice receiving fraction IV, followed by fractions II–III, saline, whole serum, and immune gamma globulin. The authors concluded that protective antibodies were found in fractions II–III and IV of human serum. They suggested that the difficulty of other studies to demonstrate protective humoral antibodies in TB may have been due to a low concentration of antibody in serum or to the fact that protective antibodies may have been bound to antigens and not available to mediate protection. Another possibility discussed was interference, or the inhibition of antibody in one fraction by substances in another. The protective effect of commercial immune gamma globulin was explained by the presence of protective antibodies from past infection. However, the investigators could not explain the protective effect of fraction IV, which contains primarily beta globulin.

(ii) Nonsupportive data. (a) *Reggiardo and Middlebrook.* In 1974, Reggiardo and Middlebrook reported their study of the effect of immune serum transfer in nonimmune rabbits infected with aerosolized *M. tuberculosis* (92). Antiserum was prepared by immunizing rabbits i.v. with live BCG and giving a booster dose at 4 weeks. Serum was obtained on days 7, 10, and 21 after the booster injection and pooled; nonimmune rabbit serum was used as the control. The antibody content in serum was not determined. The immune status of the donor rabbits was examined by aerosol administration of virulent *M. bovis* Ravalen. The lungs of rabbits immunized with BCG had no visible lesions and had 100 to 1,000 fewer CFU in the right lower lobe than did the lungs of rabbits that received saline. The recipient rabbits received serum 1 day before infection and were given four weekly injections after challenge. There were no differences in visible lung lesions or CFU in the right lower lobe between four rabbits that received immune serum and five rabbits that received nonimmune serum. The authors considered the possibility that the antibody content or the amount of an important antibody in the transferred serum was insufficient to mediate protection.

(b) *Forget et al.* In 1976, Forget et al. reported that administration of rabbit immune sera via i.p. injection enhanced infection by promoting the multiplication of BCG in the spleens of mice (42). Immune sera were produced in rabbits by repeated i.v. injections of live *M. tuberculosis* H37Rv (antiserum 1) or soluble extracts from *M. tuberculosis* H37Rv (antiserum 2). Both formulations were mixed with the adjuvant alum. Control sera consisted of nonimmune rabbit serum or immune serum absorbed with BCG. The sera were heat inactivated at 56°C. High mycobacterial hemagglutinating-antibody titers were detected in antisera 1 and 2 but not in sera from nonimmunized rabbits or in BCG-absorbed sera. Sera were injected i.p. 6 h before and 24 h after i.v. challenge with BCG. No CFU were detected in the spleens of mice that received normal serum, while they were detected in the spleens of mice that received antiserum 1. In a second experiment, absorbed and unabsorbed forms of antiserum 2 were used. The number of CFU recovered from the spleens of mice treated with un-

absorbed antiserum was significantly larger than that recovered from the spleens of mice treated with absorbed antiserum. The authors concluded that the results of the experiment were consistent with a disease-enhancing effect of antimycobacterial antibodies.

Other animal studies. (i) Supportive data. (a) *Tsuji et al.* In 1957, Tsuji et al. reported the effect of humoral factors on the growth of mycobacteria in vivo by using a chamber made with a membrane filter and a watch glass (114). Bacilli were smeared and fixed inside the watch glass, and the chamber was placed inside the peritoneal cavities of rabbits, such that body fluids without cells could pass through the membrane filter. Chambers with three different membranes were used: chamber O, with a membrane permeable to both high- and low-molecular-weight components; and chambers S and K, with membranes permeable only to low-molecular-weight components. Chambers O and S contained physiologic saline solution, and chamber K contained medium with 10% serum. The role of humoral factors in nonimmunized rabbits was evaluated first. The nonpathogenic mycobacteria *M. smegmatis* and *Mycobacterium* strain 607 proliferated in chamber K but not in chamber O, suggesting the presence of a “growth-inhibiting function” against nonpathogenic mycobacteria in the higher-molecular-weight component of body fluid. On the other hand, the virulent mycobacteria *M. tuberculosis* H37Rv, Ayoma B, and bovine RM, as well as BCG, grew well in chamber O, did not grow in chamber S, and showed restricted growth in chamber K. These data were interpreted to suggest that the inhibiting factor against virulent and attenuated mycobacteria was located in the lower-molecular-weight fraction of body fluid. Growth in chamber O was attributed to antagonism by whole-body proteins. The virulent avian tubercle bacilli grew well in all three chambers, suggesting that none of the serum components affected the viability of this strain.

Next, Tsuji et al. evaluated the efficacy of humoral factors in rabbits that were immunized with either the human Ayoma B or the heat-killed bovine RM strain. At 30 days after immunization, chambers O, K, or S containing the bovine RM strain or *M. tuberculosis* H37RV were implanted in the rabbit peritoneal cavity; nonimmunized rabbits were used as controls. Little or no proliferation of bacilli was observed in O chambers placed in immunized rabbits, whereas proliferation was observed in the chambers placed in nonimmunized rabbits. In K chambers placed in both immunized and nonimmunized rabbits, little or no growth was observed, and no growth was observed in the S chambers placed in either immunized or nonimmunized rabbits. Similar results were observed in rabbits vaccinated with BCG. The authors concluded that a “powerful bacteriostatic effect” developed in the high-molecular-weight fraction of the rabbits’ body fluid following immunization, but they attributed the effect to reduction or loss in the high-molecular-weight fraction of “growth promoting function” such as albumin, whose level is known to decrease in TB. Nevertheless, these results could also be interpreted as being due to the presence of growth-inhibiting antibodies in the immune sera.

(b) *Gheorghiu et al.* In 1985, Gheorghiu et al. reported their evaluation of mice selected for high (H) or low (L) antibody responsiveness to TB by measuring the rate of multiplication of *M. tuberculosis* H37Rv in their organs (45). The CFU of *M. tuberculosis* H37Rv administered i.v. increased in a similar manner in the spleens of H, L, and F₁ hybrid mice during the first 2 weeks after infection. Beyond that time, the CFU decreased significantly in H and F₁ mice. All L mice died within 22 days, suggesting that the CFU continued to increase in these mice. While a 100% mortality was noted in the L mice, there was only a 20% mortality in H and F₁ mice. In the

spleens of control H, L, and F₁ mice, vaccinated with BCG 5 months before infection, no increase in CFU occurred in the first 2 weeks. A decrease in CFU occurred by day 28 in all three lines of mice. The authors concluded that during a mycobacterial infection, different defense mechanisms play successive roles.

(c) *Vordermeier et al.* In 1996, Vordermeier et al. found that B-cell-deficient (μ -chain knockout) mice that had received an i.v. injection of *M. tuberculosis* H37Rv had significantly higher splenic CFU at 3, 4, and 6 weeks after infection than did non-B cell-deficient controls (118). At 6 weeks after infection, the CFU were also significantly higher in the livers and lungs of the B-cell-deficient mice than in the controls. Similar results were observed with *M. bovis* BCG infection. However, mortality was not increased during the 18 weeks after infection with *M. tuberculosis*. The authors thought that B cells may play a role in T-cell regulatory function but did not rule out a direct effect of antibodies.

(ii) **Nonsupportive data.** (a) *Lurie.* In 1942, Lurie reported his evaluation of the efficacy of peritoneal mononuclear phagocytes from normal and immunized rabbits in the presence of normal and immune serum (66). *M. tuberculosis* was incubated with washed peritoneal mononuclear phagocytes and with immune and nonimmune sera. After centrifugation, the supernatant was removed and exchanged for fresh serum (normal or immune according to the serum originally used). The new mixtures were injected into the eye chambers of albino rabbits. After 14 to 20 days, the CFU of bacilli in each chamber fluid and iris was determined and correlated with the number of colonies from the original inoculum. The results demonstrated that phagocytes from immune rabbits inhibited the multiplication of *M. tuberculosis* in their cytoplasm more effectively than did phagocytes derived from nonimmune rabbits. The results were similar regardless of whether immune or normal serum was added. Lurie concluded that immune serum did not have a constant bacteriostatic effect on the growth of tubercle bacilli in normal mononuclear cells.

(b) *Raffel.* In 1946, Raffel reported the effects on immunity of various mycobacterial fractions (90). Guinea pigs were immunized repeatedly over several months with each of various mycobacterial preparations and antigens, including BCG, defatted bacilli, carbohydrates, protein, phosphatide, and acid-fast wax, and then infected with virulent bacilli. Acquired resistance was demonstrated only in animals immunized with live BCG. Antibody responses to protein antigens were more frequent than responses to wax or phosphatide antigens. Antibodies to mycobacterial polysaccharides were found in only 1 of 200 immunized animals. No correlation was found between antibodies and resistance. The author concluded that, "The important issue is not whether antibodies reacting with the whole cell have been produced, but whether there is antibody to the particular component of the cell which is responsible for inducing acquired immunity."

Conclusions from animal studies. Although animal experiments performed in the modern era showed inconsistent results, advances in technology allowed investigators to examine the effects of different serum or body fluid fractions. The studies done by Zitrin and Wasz-Höckert (124), as well as by Tsuji et al. (114), demonstrated that certain serum or body fluid fractions were beneficial in prolonging the survival of mice or affecting the growth of mycobacteria. Although Zitrin and Wasz-Höckert (124) demonstrated a protective effect of serum gamma globulins from TB patients, they found that fractions containing alpha and beta globulins from the same patients had a protective effect as well. These fractions are not known to contain antibodies; however, it is worth noting that an in-

hibitory effect on the growth of *Cryptococcus neoformans* by both alpha 2 and gamma globulin fractions of normal human serum has also been reported (93). Some investigators who did not demonstrate antibody-mediated protection nevertheless pointed out that antibodies important for protection may have been missing from their preparations (90, 92). A disease-enhancing effect of antibodies was found by one investigator (42). The studies with B-cell-deficient mice (118) and those with high and low antibody responsiveness (45) produced results consistent with a role for antibody-mediated immunity in protection against TB.

In vitro effect of antibodies on *M. tuberculosis.* Several investigators have sought to obtain data for or against antibody-mediated immunity against *M. tuberculosis* by studying the effect of antibody on mycobacteria in vitro.

(i) **Supportive data.** (a) *Clawson.* In 1936, Clawson reported the contribution of immune sera to the lysis of BCG in mononuclear cells by using sera from rabbits immunized with BCG (22). Equal amounts of mononuclear cells, mycobacterial suspension, and diluted serum were mixed and incubated at 37°C for 1 h. One drop was then placed on each slide, dried, fixed, and acid-fast stained, and the percentage of lysed organisms was calculated. Incubation with immune sera produced lysis of 68% of bacilli, whereas normal sera had no effect. The results were the same whether the cells originated from immunized or control rabbits. Incubation of bacilli with serum alone did not cause lysis. A dose response was demonstrated for the efficacy of antibody-mediated lysis of *M. tuberculosis*. There was no correlation between the lytic power of serum and the degree of tuberculin reactivity of the animals. Clawson concluded that both leukocytes and immune serum are necessary for the lysis of bacilli and that lysis correlated with the antibody titer.

(b) *Kreidler and Nissler.* In 1939, Kreidler and Nissler studied the effect of patient sera on the growth of *M. tuberculosis* in vitro (60). Sputum and serum were obtained from 23 patients with pulmonary TB, 22 of whom had advanced disease. *M. tuberculosis* isolated from the sputum samples was mixed with serum at various dilutions and incubated at 37°C in the presence of mycobacterial medium. Controls consisted of medium without serum and medium with normal serum. Growth was evaluated qualitatively after 1 month. Sera from eight patients produced moderate inhibition, sera from another eight patients produced slight inhibition, and sera from seven patients produced no inhibition. When these results were correlated with clinical outcome, it was found that none of the patients whose sera produced moderate growth inhibition died, 71.4% of patients whose sera produced no growth inhibition died, and 50% of patients whose sera produced slight growth inhibition died. Improvement was observed in 62.5% of patients whose sera produced moderate growth inhibition, 12.5% of patients whose sera produced slight growth inhibition, and none of the patients whose sera produced no growth inhibition (the patients were observed for 2 years or longer). The authors believed that this effect had prognostic value in patients with TB.

(c) *Seibert and Nelson.* In 1943, Seibert and Nelson showed that the gamma globulin fraction of antiserum as well as the whole immune serum from rabbits immunized with a PPD preparation could precipitate tuberculous protein antigens, agglutinate live tubercle bacilli, and inhibit the growth of tubercle bacilli in a test tube (103). The non-gamma-globulin fraction of the immune serum and normal rabbit serum had no activity.

(d) *Emmart and Seibert.* In several experiments reported in 1945, Emmart and Seibert assessed the effect of serum and its gamma globulin fraction on the growth of *M. tuberculosis* (36). A mixture of equal parts of *M. tuberculosis* suspension and serum or its fractions was incubated overnight (no temperature

was provided) and plated, and the number of colonies was counted after 4 weeks. Reduction in the number of colonies was caused by serum from tuberculous and PPD-immunized rabbits as well as by the gamma globulin fraction of the immunized serum compared to normal serum; the differences were statistically significant. The next experiment examined the effect of serum and its fractions on the growth of tubercles on chicken chorioallantoic membranes. The average number of tubercles per membrane and the number of membranes with tubercle growth were smaller in membranes inoculated with immune serum, serum from tuberculous rabbits and humans, or their gamma globulin fractions than in membranes inoculated with normal rabbit serum and sensitized rabbit serum devoid of gamma globulins. The differences were statistically significant, except for the case of gamma globulin from human sera, possibly due to the small number of embryos that survived. These effects were maintained irrespective of the time when the serum or its fractions were applied: 1 day before, simultaneously with, or after incubation with *M. tuberculosis*. The sizes of the tubercles were also affected by the immune rabbit serum and by infected rabbit serum, being smaller when these sera were used than when normal sera were used. The authors concluded that the results were suggestive of an inhibitory antibody effect on *M. tuberculosis*. The mechanism suggested for the antibody-mediated effect was neutralization and/or agglutination.

(e) *Seibert*. In 1958, Seibert reported that antibodies did not have a direct static or cidal effect on BCG but, rather, appeared to have an indirect effect that was mediated by antibodies to the mycobacterial polysaccharide (101). Both normal rabbit serum and the sera of rabbits immunized with live BCG, when mixed with dispersed BCG, caused more growth inhibition in test tubes than did the gamma globulin fraction of tuberculous serum. The addition of polysaccharide to the suspension containing immune serum led to further inhibition of growth. When the gamma globulin fractions of immune and nonimmune rabbit sera were compared, the nonimmune serum fractions caused the greatest inhibition. The addition of polysaccharide to the gamma globulin fraction from nonimmune sera stimulated growth, while the addition of polysaccharide to the gamma globulin fraction of immune sera inhibited growth. On the other hand, sera which contained no antibodies or contained antibodies to proteins stimulated increased BCG growth upon addition of polysaccharide. The explanation suggested by the authors was that antibody binding to free polysaccharide allowed other natural antimicrobial agents to exert their action on the pathogen.

(f) *Bluhm*. In 1952, Bluhm reported the effect of sera from tuberculin-positive and tuberculin-negative persons on oxygen consumption of *M. tuberculosis* (9). Sera from tuberculin-positive and -negative persons were mixed with a clinical virulent strain of *M. tuberculosis*, and each sample was divided between two manometers of a Warburg apparatus, which was used to measure oxygen consumption by *M. tuberculosis*. In 91% of the experiments, *M. tuberculosis* mixed with sera from tuberculin-positive individuals consumed less oxygen than did *M. tuberculosis* mixed with sera from tuberculin-negative individuals. In almost 50% of these experiments, there was a 20 to 40% difference in oxygen consumption. In 9.2% of the experiments, the opposite results were obtained, but the difference was negligible. The effect was independent of patient age. The author concluded that a specific "respiration inhibiting factor" existed in the sera of tuberculin-positive donors. Another possibility that was considered was a bacteriostatic and/or bactericidal effect of the serum.

(g) *Tsuji et al.* In 1957, Tsuji et al. reported the effect of

serum on the growth of tubercle bacilli cultivated on glass slides. Immune serum was obtained from rabbits vaccinated with *M. tuberculosis* H37Rv (114). When immune serum diluted with saline was used, there was little or no growth of mycobacteria. In contrast, when nonimmune serum was used, mycobacteria proliferated. When serum was diluted with mycobacterial medium, mycobacteria grew under the conditions where both immune and control sera were used. A bacteriostatic effect was observed with serum from rabbits that were immunized with a high dose of a heat-killed strain of bovine mycobacteria. The results were qualitative. When interpreting the results, the authors considered the possibility that the "bacteriostatic" effect was due to reduction in the amount of a "growth promoting" factor such as albumin, which is known to have decreased levels in TB. Alternatively, the results could be interpreted as being consistent with antibody-mediated bacteriostasis.

(h) *Zitrin and Wasz-Höckert*. In 1957, Zitrin and Wasz-Höckert reported the effect of serum and serum fractions (described in "Passive antibody studies in animals," above) from patients with TB of 6 months duration on the growth of *M. tuberculosis* H37Rv (124). Bacilli were incubated with serum or serum fractions and diluted serially in mycobacterial medium. Inhibition of growth occurred in the presence of whole tuberculous sera, fractions II and III (containing gamma and beta globulins), fraction IV (containing alpha and beta globulins), and commercial immune gamma globulin. The greatest inhibition was caused by fractions II and III, which contain gamma globulin. No inhibition was observed when sera from tuberculin-negative individuals or bovine albumin were used. The authors concluded that these observations and the animal experimental data (see above) supported a protective role for antibodies in TB.

(i) *Fong et al.* The role of monocytes and serum was examined in a series of experiments by Fong et al. published in 1956 to 1959 (39-41). In the first study, the investigators established that when incubated in vitro with normal serum, *M. tuberculosis* H37Rv induced degeneration of normal and immune monocytes (from rabbits immunized with BCG) (40). In the presence of serum from rabbits immunized with BCG, immune monocytes were protected from degeneration but normal monocytes were not. In a second study, the specificity of sera in protection against mycobacteria was evaluated (41). The results showed that homologous antiserum (from rabbits vaccinated with BCG) and heterologous antiserum (from animals immunized with *Salmonella rutgers* or with a solution of crystalline albumin) protected both immune and normal monocytes from degeneration. In the presence of normal serum, 38 and 11% of normal and immune infected monocytes, respectively, had degenerated at 24 h. After exposure to the immune homologous or heterologous sera, 0 to 4% and 0 to 2% of normal and immune infected monocytes, respectively, had degenerated at 24 h. The third study in their series examined the conditions under which the serum exerted its effect (39). Absorption of anti-BCG serum with heat-killed *M. tuberculosis* H37Rv and absorption of anti-*Salmonella* serum with formalin-killed *S. rutgers* did not change the protective characteristics of the respective sera. Immune globulin from BCG-immunized rabbits delayed the onset of monocyte degeneration compared to globulin from normal serum and/or whole normal serum, although whole immune serum was much more effective than the immune globulin fraction. Heating to 60 to 70°C did not alter the protective effect of serum from BCG-immunized rabbits. Incubation of *M. tuberculosis* with immune serum before the experiments did not affect the ability of bacilli to induce monocyte degeneration or to multiply. The protective effect of

the serum was not affected by heating to 56°C, thus excluding complement participation. The authors concluded that a protective effect of serum factors did exist, although it was non-specific because similar effects were demonstrated with anti-BCG and anti-*Salmonella* serum in some experiments.

(j) *Armstrong and Hart*. In 1975, Armstrong and Hart reported a significant increase in phagosome-lysosome fusion in macrophages infected with *M. tuberculosis* H37Rv pretreated with serum prepared by immunizing rabbits with BCG (4). The treatment did not affect the viability or multiplication of mycobacteria. Nevertheless, the authors thought that in an in vivo system exposed to the complex immune system, where macrophages are able to kill *M. tuberculosis*, increased phagosome-lysosome fusion can play a role in host defense.

(ii) **Nonsupportive data.** (a) *Hanks and Brockenbrough*. In 1940, Hanks and Brockenbrough reported their studies which examined whether immune or nonimmune rabbit serum enhanced the bactericidal effect of leukocytes from normal, immunized, or infected rabbits (49). No significant or consistent reduction in mycobacterial viability was caused by incubating *M. tuberculosis* in serum-leukocyte mixtures. Experiments with *M. phlei* also failed to demonstrate an effect of blood or serum from normal or immunized animals on mycobacterial viability. The authors concluded that serum-leukocyte mixtures did not mediate a bactericidal effect upon *M. tuberculosis* regardless of whether immune or control sera were used.

(b) *Suter*. In 1953, Suter reported his studies of the effect of immune and nonimmune serum on phagocytosis by using mononuclear cells and serum from guinea pigs and rabbits immunized with BCG (111). Intracellular multiplication of virulent and attenuated forms of bacilli (including BCG) was delayed or inhibited by monocytes from immunized animals relative to those from control animals. Immune serum had no effect on phagocytosis of attenuated forms of bacilli by monocytes from immunized or normal animals (the experiment was not performed with virulent strains). In this study, the immunization was done with BCG, the length of time from vaccination to serum extraction was not provided, and the antibody concentration in serum was not measured. Another potential problem with this study was that the mononuclear cells were not washed before the experiments but were used in the form of an "exudate," raising the possibility that "serum factors" from the donor animal participated in the in vitro effects and obscured possible differences between the conditions.

Conclusions from in vitro studies. In vitro studies suggest potential mechanisms by which antibody may exert effects against TB. Immune sera could enhance effector cell function against TB by increasing killing (22) or promoting phagosome-lysosome fusion (4). Direct effects of antibody on *M. tuberculosis*, such as neutralization, agglutination, bacteriostasis, and bacteriolysis, were suggested by other studies (9, 36, 103). In several studies, these effects were observed with the gamma globulin fractions of immune sera (36, 103, 124). Antibodies directed against mycobacterial polysaccharide appeared to mediate an indirect effect in one study (101). In summary, many in vitro studies provided evidence which supports the ability of antibody-mediated immunity to modify the course of infection.

Effects of antibodies on skin test reactivity. The effects of antibodies on skin test reactivity are probably not directly related to the role that they play in host defense against tuberculosis. Nevertheless, these studies demonstrate the potential effects of antibody-mediated immunity on skin test reactivity and suggest the potential for interactions between antibodies and cell-mediated immunity.

(i) *Cole and Favour*. In 1955, Cole and Favour reported the effect of antibody administration on PPD reactivity (23).

Plasma was obtained from guinea pigs that were immunized s.c. with autoclaved *M. tuberculosis* H37Rv over a period of 3 weeks to 5 months and whose skin-test reaction to PPD or Old Tuberculin was greater than 1 cm. The plasma was fractionated, and the individual fractions were administered i.p. to guinea pigs with a negative baseline tuberculin skin test. Each plasma fraction was then tested for the presence of antibodies to mycobacterial polysaccharide and protein by the Middlebrook-Dubos hemagglutination method (73) and "Boyden test" (10), respectively. Antibodies to the polysaccharide component were found in fraction II of the plasma, correlating with the gamma globulin fraction, and antibodies to proteins were found in fraction IV-10, a subfraction of alpha globulin. After injection of fraction IV-10, the animals developed a delayed-type hypersensitivity (DTH) skin reaction to PPD that was not observed after administration of fraction IV-10 preadsorbed with Boyden-sensitized cells. However, injection of a combination of fractions II and IV-10 resulted in inhibition of DTH to PPD. The authors concluded that antibodies to "tuberculo-polysaccharide" found in Cohn fraction II (gamma globulin) inhibit passive transfer of delayed-type skin reactivity (23).

(ii) *Drexhage et al.* In 1980, Drexhage et al. reported the effect of immune sera on skin test reactivity (33). Rats were immunized in the footpads and the neck area with *M. tuberculosis* H37Ra suspended in Freund's complete adjuvant, whereas control rats received injections of Freund's incomplete adjuvant. Two weeks later, the rats received one of two batches of immune serum i.v. and were skin tested with soluble PPD, aggregated PPD, or *M. tuberculosis* H37Ra. A reduction in the skin test reactivity to mycobacterial antigen was noted in the rats that received immune serum. The effect was seen in the rats sensitized with *M. tuberculosis* or with aggregated PPD but not in those that received soluble PPD. When the same experiment was performed in guinea pigs, immune serum had no effect on skin test reactivity. Increasing the amount of immune serum administered to the rats caused increased swelling at the site of skin testing after 5 h, presumably due to an Arthus-type reaction. Human sera with high antibody titers to *M. tuberculosis* reduced skin test reactivity in rats sensitized with *M. tuberculosis*, whereas sera with low titers had no effect. The differences were statistically significant, and there was a positive correlation between antibody titer and skin test reduction. To confirm that the effect was due to antibodies, aliquots of two human sera with high titers were absorbed with *M. tuberculosis*. The absorbed sera did not inhibit the skin test reactivity, but the unabsorbed sera did. The authors interpreted their results as demonstrating an interaction between antibodies, cell mediated immunity, and the infectious agent.

(iii) *Kardito and Grange*. In 1980, Kardito and Grange reported their study which examined the correlation between serum titers to mycobacterial antigen and skin reactivity to PPD (58). A group of 90 patients with smear-positive pulmonary TB were compared with 50 age-matched controls with respect to clinical, radiographic, and laboratory parameters. A positive correlation was found between the level of IgG to *M. tuberculosis* and the skin test diameter measured at 48 h; there was no correlation between the level of serum antibodies and clinical parameters.

Effects of antibody on other mycobacteria. Although the role of antibody in protection or pathogenesis of other mycobacterial diseases is unclear, certain experiments performed with other mycobacteria may suggest a role for antibodies in tuberculosis.

(i) *M. leprae*. *M. leprae* is a pathogenic mycobacterium, which causes leprosy; like *M. tuberculosis*, it is an intracellular microorganism.

TABLE 2. Potential mechanisms by which antibody could modify the outcome of mycobacterial infection

Effect	Reference(s)
Opsonization.....	4
Prevention of attachment.....	21, 99
Inhibition of DTH	23, 33
Enhancement of phagosome-lysosome fusion.....	4
Toxin neutralization.....	31, 37, 56, 69, 115

(a) Choudhury et al. In 1989, Choudhury et al. reported that monoclonal and polyclonal antibodies to polysaccharide and lipid components of *M. leprae* inhibited the adherence of *M. leprae* to dissociated Schwann cells in vitro (21). Monoclonal antibodies (MAbs) to LAM and phenolic glycolipid I (PGL-I) of *M. leprae* inhibited the adherence of this organism to Schwann cells, while MAbs to cytoplasmic or surface protein epitopes did not. Sera raised in rabbits against a *M. leprae* sonicate, delipidified cell wall fraction, or *M. bovis* BCG also reduced the adherence of *M. leprae* to Schwann cells. Serum rich in antibodies against PGL-I, pooled from patients with active lepromatous leprosy, significantly reduced the adhesion of *M. leprae*, while serum from patients with tuberculoid leprosy, low in anti-PGL-I antibodies, did not. The authors concluded that antibodies could play an important role in inhibiting the adhesion of *M. leprae* to Schwann cells.

(b) Schlesinger and Horwitz. In 1994, Schlesinger and Horwitz reported that nonimmune serum, containing natural antibodies to mycobacterial surface carbohydrates, mediated complement C3 fixation to *M. leprae* and C1q binding to PGL-I (98). In another study, they found that complement receptors on mononuclear phagocytes mediated phagocytosis of *M. leprae* (97). These studies suggest that antibody may mediate phagocytosis of *M. leprae* via complement.

(ii) *Mycobacterium w.* *Mycobacterium w.* is a nonpathogenic, rapidly growing, atypical mycobacterium. *Mycobacterium w.* vaccine is one of the antileprosy vaccines under investigation (122).

(a) Band et al. The effect of immunized rabbits sera on the interaction of *Mycobacterium w.* with rat schwannoma culture was reported by Band et al. in 1987 (5). Preincubation of *Mycobacterium w.* with hyperimmune sera led to an almost complete inhibition of bacterial uptake by the schwannomas. Phagocytosis by macrophages, however, was not affected.

POTENTIAL MECHANISMS FOR ANTIBODY-MEDIATED EFFECTS AGAINST *M. TUBERCULOSIS*

While acknowledging that the role of antibody-mediated immunity in protection against *M. tuberculosis* remains uncertain, several mechanisms by which certain antibodies could modify the outcome of infection are considered (Table 2). While it is commonly believed that antibody-mediated immunity is not effective against intracellular pathogens, an examination of the available data on antibody-mediated protection against other microbes does not support this view (16a). Antibodies can protect against many viruses, all of which are obligate intracellular pathogens. The IgA class MAb has been shown to neutralize viruses intracellularly (72). Antibodies have also been shown to protect against more complex intracellular microbes. For example, a MAb has been described which inhibits the replication of *Toxoplasma gondii*, an obligate intracellular parasite (74), and antibody-mediated immunity has been shown to protect against the intracellular fungus *C.*

neoformans (34, 77, 96). Hence, the assertion that antibody-mediated immunity is not likely to be important against *M. tuberculosis* because this microbe is often found inside cells is not tenable in view of what is known about other organisms.

Interference with Adhesion

Microbial adhesion to host tissues is an important step in the colonization of the host and establishment of infection. Antibodies to surface determinants of *M. tuberculosis* could interfere with mycobacterial adhesion to host tissues. Venisse et al. demonstrated that mannose-capped LAM (ManLAM) from *M. bovis* BCG can bind macrophages and granulocytes (117). The binding occurs either via mannose receptors of macrophages or mannan binding proteins that are present in serum, permitting selective uptake of mannosylated bacteria by granulocytes. Schlesinger et al. demonstrated that ManLAM from *M. tuberculosis* Erdman binds to human macrophages via mannose receptors and that *M. tuberculosis* attachment to macrophages was inhibited after preincubation with an anti-LAM Mab (99). Antibodies that interfere with adherence could, in principle, affect the outcome of infection.

Promotion of Phagosome-Lysosome Fusion

M. tuberculosis infection of phagocytic cells is believed to be accompanied by interference with phagosome-lysosome fusion (4). Lysosomes contain a variety of microbicidal substances that could potentially kill or inhibit *M. tuberculosis*. Presumably, interference with phagosome-lysosome fusion is a mechanism that allows *M. tuberculosis* to survive and replicate inside macrophages. Armstrong and Hart demonstrated that antibody-mediated phagocytosis promoted phagosome-lysosome fusion (4). If this phenomenon occurred in vivo, it could enhance the microbicidal efficacy of phagocytic cells.

Toxin Neutralization

The ability of antibodies to bind and neutralize microbial products that are harmful to the host is a classical function of antibody-mediated immunity. Mycobacterial infection is accompanied by the release of microbial products such as LAM that could potentially affect host immune function. LAM is a scavenger of oxygen free radicals and can downregulate the oxidative burst by inhibiting protein kinase C (19). Furthermore, LAM can inhibit the transcriptional activation of gamma interferon-inducible genes (19). Hence, antibodies that bind to LAM may be beneficial to the host. In this regard, the presence of IgG to LAM has been associated with a reduced likelihood of dissemination in one study (26).

Studies in the preantibiotic era (31, 37, 56, 69, 115) demonstrated that immune serum could protect experimental animals against the toxic effects of mycobacterial antigen preparations. Presumably, antibody in immune sera bound to and neutralized mycobacterial toxins. Antibodies to these antigens could contribute to the protection against infection.

Other Mechanisms

Antibody molecules are multifunctional molecules that can play many roles in enhancing host defense and protecting against pathogens. For example, antibodies can serve as opsonins, mediate antibody-dependent cellular cytotoxicity, activate complement, promote cytokine release through Fc receptor cross-linking, and possibly enhance antigen presentation. Antibodies may exert their action independently or augment and possibly direct cellular immune mechanisms, which are

TABLE 3. Comparison of several characteristics between *M. tuberculosis* and *C. neoformans*

Characteristic	Value for:	
	<i>M. tuberculosis</i>	<i>C. neoformans</i>
Class	Mycobacterium	Fungus
Size	2–4 µm by 0.2–0.6 µm	4–6 µm (diameter)
Reservoir	Humans	Environment
Microbial surface	Polysaccharides and lipids	Polysaccharide capsule
Major surface antigen	LAM	GXM ^a
Mode of infection	Inhalation	Inhalation
Tissue reaction	Granuloma formation	Granuloma formation
Tissue cellular location	Intracellular and extracellular	Intracellular and extracellular
Protective cytokine response	Th1-associated cytokines	Th1-associated cytokines
Latency	Yes	Yes
Extrapulmonary dissemination	Yes	Yes
Role of antibody-mediated immunity	Uncertain	Uncertain

^a GXM, glucuronoxylomannan.

important in the host defense against *M. tuberculosis*. Although these functions have not yet been demonstrated in regard to *M. tuberculosis*, they remain potential mechanisms by which antibody-mediated immunity could modify the course of infection with *M. tuberculosis*.

THE FUNGAL PARADIGM

Recent observations on antibody-mediated immunity to several invasive fungi provide a number of precedents that may be relevant to *M. tuberculosis*. Like *M. tuberculosis*, the efficacy of antibody-mediated immunity against the fungi was uncertain for decades, despite considerable efforts to establish a case for or against antibody-mediated immunity in host defense (16). The problem was that experiments with polyclonal sera produced inconsistent results (16). Some investigators were able to transfer protection with immune sera, whereas others were not. Also arguing against an important role for antibody-mediated immunity in protection against fungi were the difficulty in associating immunity with the antibody titer in serum and the fact that individuals with Ig deficiencies were not at particular risk for fungal infection.

A breakthrough in the understanding of the potential efficacy of antibody immunity against fungi came from the use of MAbs to study questions of humoral immunity. In 1987, Dromer et al. reported that administration of an IgG1 MAb to *C. neoformans* significantly prolonged survival in lethally infected mice (34). CN is a pathogen with many similarities to *M. tuberculosis* (Table 3). Like *M. tuberculosis*, its primary site of infection is the lungs and the effective tissue response is marked by granulomatous inflammation. Furthermore, *C. neoformans* can be an intracellular pathogen, and both *M. tuberculosis* and *C. neoformans* have polysaccharide surface antigens that are virulence factors (13, 19).

In contrast to previous work with polyclonal serum, the use of MAbs in antibody protection experiments against *C. neoformans* produced consistent results. Unlike polyclonal antibody preparations, which are heterologous, MAbs are antibody preparations with a single specificity and isotype. The observation that an IgG1 anti-*C. neoformans* MAb modified the course of experimental *C. neoformans* infection was confirmed in two other laboratories with different MAbs (77, 96). These studies provided insight into why the results with polyclonal sera were not consistent: the protective efficacy of murine MAbs to *C. neoformans* depended on their isotype (77, 78, 81, 96, 121) and epitope specificity (76, 80). A striking observation was that IgG3 MAbs were nonprotective or disease enhancing

(81, 121), even though IgG3 is a subclass frequently elicited by T-cell-independent antigens such as the *C. neoformans* capsular polysaccharide. Antibody efficacy was also influenced by the location of antibody binding on the capsule (76, 80). The mechanism of antibody-mediated protection against *C. neoformans* is not fully understood (88), and protective and nonprotective antibodies can be opsonic in vitro (78). An additional level of complexity is illustrated by the fact that the ability of an antibody to mediate protective or deleterious effects is dependent on the T cells of the host (120). Protective antibodies were ineffective in the absence of CD4⁺ T cells or IFN-γ, and, remarkably, disease-enhancing IgG3 MAbs were protective in mice without CD8⁺ T lymphocytes (120).

The existence of protective and nonprotective MAbs against *C. neoformans* has now been confirmed with another yeast, *Candida albicans*. Han and Cutler described two agglutinating MAbs, one protective and the other nonprotective (48). A protective MAb has also been described for the unusual fungus *Pneumocystis carinii* (46). Hence, there are now three fungi for which protective MAbs have been described, even though antibody-mediated protection has not been consistently demonstrated with polyclonal serum preparations. For each of these fungi, protective antibodies have been discovered despite continuing questions about the protective efficacy of heterologous antibody responses.

The observations with *C. neoformans* have led to the hypothesis that some pathogens, and fungi in particular, elicit mixtures of protective, nonprotective, and disease-enhancing antibodies (16). With these pathogens, the efficacy of polyclonal preparations such as immune serum is critically dependent on the relative proportion of each type of antibody (16). This working model predicts that some antibody responses will be protective whereas others could make the host more susceptible to infection. If the antigens that elicit protective antibody responses can be identified and developed into vaccines, it may be possible to protect a host through antibody-mediated immunity alone, even if this arm of the immune system plays little or no role in protection against natural infection. This principle has already been successfully applied to *C. neoformans*. A highly immunogenic conjugate vaccine has been shown to elicit strong antibody responses to *C. neoformans*, which can protect mice against a lethal inoculum (32). Furthermore, protective antibodies to the *C. neoformans* capsular polysaccharide are being developed for passive immunotherapy (123).

By applying the observations with *C. neoformans* to *M. tuberculosis*, one might explain the difficulties involved in establishing a role for antibody immunity against TB by postulating

TABLE 4. Summary of available data on the role of antibody-mediated immunity to *M. tuberculosis*

Effect or parameter	Experimental result	References with evidence for effect	References with evidence against effect
Level of antibody in serum correlates with protection against infection and/or improved prognosis	There is evidence for and against the benefit of serum antibody to mycobacterial antigens	Human studies: 20, 26, 29, 106 Animal studies: 67, 68, 102, 104	Human studies: 58, 64, 87
Passive antibody administration modifies infection to the benefit of the host	Antibody administration has been associated with protection, no protection, or enhancement of infection	Human studies: 3, 7, 14, 15, 25, 37, 44, 50–53, 55, 57, 61, 65, 70, 71, 75, 84–86, 89, 94, 105, 108–110 Animal studies: 3, 31, 37, 56, 69, 71, 115, 124	Human studies: 38, 63, 110, 119 Animal studies: 42, 92, 112, 113
Infection associated with conditions of impaired antibody-mediated immunity	There is no evidence that tuberculosis is more prevalent or severe in classical antibody deficiencies	None	None
Antibody inhibits mycobacteria in vitro	There is experimental evidence for and against an effect of antibody against <i>M. tuberculosis</i> in vitro	9, 36, 60, 69, 101, 103, 114, 124	112
Antibody enhances cell-mediated immunity against <i>M. tuberculosis</i>	There is experimental evidence for and against the efficacy of antibody in enhancing cellular function against <i>M. tuberculosis</i>	4, 22	49, 111

that antibody response to mycobacteria contain useful, useless, and harmful antibodies. Since polyclonal serum to *M. tuberculosis* consists of antibodies with multiple specificities and isotypes, its efficacy against *M. tuberculosis* would be a function of its composition. Hence, the observations that some antibody preparations modified the course of *M. tuberculosis* infection probably reflected the occasional generation of preparations that were rich in protective antibodies. In this regard, it is noteworthy that some investigators associated protective effects with antibodies to mycobacterial polysaccharides (20, 26, 102, 104) but not to protein antigens (102, 104). Similarly, the observations that patients with advanced disease often had high antibody titers in serum to mycobacterial antigens raises the possibility that they had deleterious or disease-enhancing antibodies.

The experience with fungi strongly suggests the need for a reevaluation of the potential of antibody-mediated immunity to *M. tuberculosis* by using MAbs. If there are protective, non-protective, and disease-enhancing antibodies to *M. tuberculosis*, polyclonal sera are unlikely to demonstrate protection to *M. tuberculosis* consistently. However, protection experiments performed with MAbs will be able to define the protective efficacy of antibodies with known isotype and antigen specificity. This could permit a precise determination of the potential of the different mycobacterial antigens to elicit biologically useful antibodies.

SUMMARY STATEMENTS

Our review of the published literature on the role of antibody-mediated immunity against *M. tuberculosis* over the past 100 years, demonstrates that the studies that indicate a role for or against antibody-mediated immunity have been performed rigorously and their results are credible. Since both studies for and against a role for antibody-mediated immunity are credible, the question at hand is how best to reconcile their results (Table 4). Our review supports the following statements. (i) Before the discovery of antibodies, as well as their structure and function, immune serum was shown to prevent and modify the course of TB in some cases. (ii) With the discovery of antibodies and the availability of fractionation methods, specific antibodies and antibody-containing fractions of immune serum were demonstrated to be the mediators of beneficial biological effects against *M. tuberculosis* in some cases. (iii) Antibodies directed against mycobacterial polysaccharides have been associated with beneficial effect in some studies. (iv) The availability of MAb techniques allowed the discovery of antibodies with diverse functions that include protective, non-protective, and enhancing (the fungal paradigm), despite continuing uncertainty about the protective efficacy of natural antibody responses.

A PROPOSAL

We propose that the historical difficulties encountered in establishing a role for antibody-mediated immunity in protec-

tion against *M. tuberculosis* are a consequence of the complexity of the antibody response to mycobacterial antigens. We propose that protective, nonprotective, and disease-enhancing antibodies to *M. tuberculosis* exist and that the conflicting results obtained over the past 100 years are the results of studies with different polyclonal sera containing different proportions of these antibodies. This proposal can provide an explanation for the fact that the literature contains many rigorous studies that simultaneously argue for and against an important role for antibody-mediated immunity in protection against *M. tuberculosis*. According to this concept, some immunization techniques elicit primarily protective antibodies, and studies with these sera produced results suggesting the importance of antibody-mediated immunity against *M. tuberculosis*. Conversely, some immunization techniques elicit nonprotective or disease-enhancing antibodies, and studies that used these preparations suggest that antibody-mediated immunity is either irrelevant or deleterious against *M. tuberculosis*. Considering that antibody responses are highly variable depending on the antigen, the route of immunization, the species used, the timing of vaccination and serum collection, etc., the difficulties involved in consistently duplicating results should not be surprising. The inability to produce consistently beneficial results with antibody preparations against *M. tuberculosis* in the past may have resulted in part from technological limitations.

The validity of our proposal can be tested by generating MAbs to mycobacterial antigens and testing each individually for their protective efficacy. Based on the experience with fungi, we predict that some MAbs to *M. tuberculosis* will be protective, others will have no effect on the course of infection, and yet others will enhance infection. The demonstration that some antibodies are protective would not necessarily imply that antibody-mediated immunity was important in protection against infection, since antibodies of that type may not occur or may not be abundant during the natural polyclonal response. By the same reasoning, the demonstration that some MAbs are not protective does not imply that all antibodies are ineffective. However, studies with MAbs will provide unequivocal evidence for or against the efficacy of individual antibody molecules to defined antigens in modifying the course of infection.

THE CASE FOR REAPPRAISAL

After more than 100 years of study, the role of antibody-mediated immunity to *M. tuberculosis* remains uncertain. Nevertheless, there is sufficient evidence in the literature to conclude that antibody-mediated immunity can modify the course of infection in certain situations. All studies to date have been performed with polyclonal antibody preparations that almost certainly include antibodies of different specificities, affinities, and isotype compositions. The difficulty in establishing an unequivocal role for antibody-mediated immunity in protection, enhancement, and/or pathogenesis of *M. tuberculosis* infection with polyclonal antibody preparations strongly suggests that a new approach is needed to resolve this important question. For the fungi, a similar uncertainty about the role of antibody-mediated immunity led to studies with MAbs, which revealed unexpected complexity in the relationship between antibody structure and function.

Considering the tremendous health problem posed by *M. tuberculosis* to humankind, it would seem very important to clarify the role of antibody-mediated immunity against this pathogen. Historically, the contradictory results have led to controversy and a general neglect of this area of investigation. Vaccines which elicit protective antibodies to *M. tuberculosis* may protect against infection, even if the role of natural

antibody immunity is uncertain or detrimental to the host. Conversely, a better understanding of whether antibodies contribute to the pathogenesis of infection may allow the identification of individuals who are more likely to succumb to TB. The time is ripe to dissect the role of antibody-mediated immunity against *M. tuberculosis* by systematic testing of MAbs to defined mycobacterial antigens.

APPENDIX

Definitions and abbreviations. (i) **Tubercle bacillus.** Tubercle bacillus is a term used in the early literature to refer to the organism responsible for tuberculosis. The term "tubercle bacillus" includes two species of the family *Mycobacteriaceae*: *M. tuberculosis* and *M. bovis* (47). In this review, we have used this term if it is used in the original literature and additional information about its source or identification was not provided.

(ii) **Tuberculin.** Tuberculin, which is also occasionally referred to as Old Tuberculin, is a crude preparation of antigens of *M. tuberculosis* and was initially described by Koch (59). Tuberculin was prepared from 6- to 8-week-old *M. tuberculosis* cultures that were grown in a glycerol-containing medium, heat inactivated, filtered, and concentrated. Tuberculin consisted of antigens and bacterial products present in growth media. It was believed to have immunizing and therapeutic properties against *M. tuberculosis* (1, 25, 59).

(iii) **Tuberculin T.R.** Tuberculin T.R., a modified form of tuberculin, also described by Koch, was made by crushing dehydrated *M. tuberculosis*, mixing this material with distilled water, and centrifuging it to collect the precipitate, which was then preserved in a solution of 20% glycerol (25). This preparation was believed to include "toxins" contained in the bacilli (37).

(iv) **Tuberculin T.O.** Tuberculin T.O. is the supernatant which remained from the T.R. preparation (25). It was believed to contain traces of "toxins" from the culture media, as well as those that were extracted easily from the membrane of the bacilli (37).

(v) **Toxicity.** Toxicity is a nonspecific term found in the early literature, which probably refers to a general systemic reaction observed after the administration of tuberculin. The reaction started several hours after tuberculin administration and consisted of high fever, chills, pain in the extremities, nausea, vomiting, and fatigue (25). Calmette defined an antitoxic serum as follows: "serum of which 1 cc protects 100 g of normal guinea pig against a fatal dose of bacilliary extract is said to contain 100 antitoxic units" (14).

(vi) **PPD-S.** PPD-S is a purified protein derivative that was produced by Florence B. Seibert and later chosen as the international standard of tuberculin (1).

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