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Failure of Passive Serum Transfer of Immunity Against Aerogenic Tuberculosis in Rabbits (37771)

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There is a want of direct, noncircumstantial scientific evidence reported in the literature that supports the conclusion, repeatedly asserted in contemporary literature (1–3), that immunity against infection with virulent tubercle bacilli can be passively transferred with cells and not with serum. Indeed the few experiments on cell transfer which have been suggestive of this conclusion, as carried out by Lurie in the anterior chamber of the rabbit eye (4), and by Suter in allogeneic mice (5), were evaluated by those investigators as not excluding a role for humoral factors synthesized by the transferred cells.

On the other hand, there is only one unconfirmed report which has provided evidence that immunity against tuberculosis cannot be transferred by serum and that was carried out in only one animal species, the guinea pig (6).

The purpose of this report is to present results of the effect of transfer of serum from well-immunized rabbits to nonimmune rabbits on their response to aerogenic challenge with small numbers of a virulent strain of *Mycobacterium bovis* (Ravenel).

In addition to the fact that passive serum transfer experiments have not been previously reported in rabbits, there are three fundamental considerations which led to the choice of this experimental model—aerogenic challenge infection of rabbits—for reinvestigating the possibility of serum transfer of immunity against tuberculosis: (a) the aerogenic route is the natural route of infection of man; (b) a minimally active immune mechanism could have a maximal opportunity for expression against small numbers of individual bacterial cells deposited "micromiles" apart, as it were, in the host's tissues; and (c) rabbits, in our experience, manifest a degree of antibacterial immunity after BCG vaccination which is more strikingly impressive than in the other experimental animals (7), guinea pigs and mice, in which such passive serum transfer experiments have been tried.

Materials and Methods. New Zealand albino rabbits weighing about 2.5 kg were used. They were immunized with 1.7×10^6 viable units of BCG (Research Foundation and University of Illinois, Chicago, IL) by the intravenous route. Four weeks later they received a booster injection of 3×10^3 viable units of BCG by the same route. Seven days after the booster injection the rabbits were aerogenically challenged with *M. bovis* Ravenel (TMC No. 401 from the Trudeau Mycobacterial Culture Collection, Saranac Lake, NY) or streptomycin-resistant Ravenel (SM-R) in an airborne infection apparatus (7). The single cell suspensions of Ravenel used in the nebulizer were prepared as described by Grover *et al.* (8). Six weeks after infection rabbits were sacrificed by intravenous injection of pentobarbital, and autopsy was performed. The number of lung surface lesions was counted; the right lower lobe of the lungs of each animal was ground in a Teflon grinder in 0.2% bovine serum albumin in distilled water and serial dilutions were inoculated onto 7H10 medium (Difco Laboratories, Detroit, MI) in order to establish the number of viable units of Ravenel present in this organ. When SM-R was the challenging agent, 7H10 containing 5 μg of streptomycin/ml was used, a concentration sufficient to inhibit the growth of BCG but not the SM-R.

The immune rabbit serum was prepared by

TABLE I. BCG Vaccination of Rabbits Against Aerogenic Infection with *M. bovis* (Ravenel, SM–R).[a]

Rabbit no.	Received	No. of lung lesions[b]	No. of viable units in right lower lobe
61	BCG	0	<16
62		0	4×10^2
63		0	5×10^3
68		0	<16
64	Saline	24	6.3×10^5
65		24	1.9×10^5
66		36	1.3×10^6
67		39	3.4×10^5

[a]Aerogenic challenged 5 wk after first BCG injection.

[b]Autopsy performed 6 wk after challenge.

immunizing rabbits with living BCG as described above. They were bled 7, 10 and 21 days after the booster injection. Sera from the different bleedings were pooled and sterilized by filtration through a 0.22 μm millipore filter and stored at −20° until use. Normal rabbit serum was obtained from nonimmunized rabbits and processed in the same fashion. Serum was passively transferred by the intraperitoneal route for rapid absorption into the blood (9). Each rabbit received 10 ml of serum the day before infection and 10 ml each on Days 5, 11, 18 and 25 after infection, a total of 50 ml of serum per rabbit.

TABLE II. Passive Transfer of Immune Serum to Rabbits Aerogenically Infected with *M. bovis*

No. of rabbits	Received[a]	Av no. of lung lesions[b]	Av no. of viable units in right lower lobe
4	Immune rabbit serum	27	7.7×10^6
5	Normal rabbit serum	33	9.6×10^6

[a]Each rabbit received 10 ml of serum the day before challenge and 10 ml each on Days 5, 11, 18 and 25 after infection.

[b]Autopsy performed 6 wk after challenge.

Results and Discussion. In Table I are presented the results of preliminary experiments in which it is shown that the method employed to immunize rabbits was effective in establishing a high degree of antibacterial immunity in the donor animals. The absence of any grossly visible lesions on the lungs and of detectable, culturable organisms in the right lower lobes of two of the four vaccinated donors bespeaks a level of acquired resistance much higher than is seen with similar challenge doses in guinea pigs (7). Nevertheless, as seen in Table II, no gross or bacteriologic evidence was obtained that passive transfer of serum from highly immune donors had modified the response of recipient rabbits. This confirms and extends the results of earlier experiments by Raffel (6) with guinea pigs challenged by an extrapulmonary route and evaluated by organ pathology.

It cannot be conclusively established by such experiments, however, that humoral mechanisms are not operative in the expression of antibacterial immunity in tuberculosis. For example, they may be necessary but not sufficient; or the amount of some critically important antibody or other humoral factor may be inadequate in the transferred serum, *e.g.*, a macrophage-cytophilic antibody. Studies by Rowley, Turner and Jenkin (10) have demonstrated that the specific aspect of cellular immunity in an experimental mouse typhoid infection is dependent on a specific antibody passively adsorbed onto macrophages. Furthermore, although the donor rabbits in this experiment were highly resistant at the time their serum was obtained, this time interval after BCG immunization may not be favorable for the revelation of humoral mechanisms of antibacterial immunity.

Observations by Lurie (11) confirmed by Tsuji, Ito and Oshima (12) provided evidence for an extracellular, humoral, antibacterial activity against tubercle bacilli in immunized guinea pigs and rabbits. Fong, Schneider and Elberg (13) have observed that a rabbit serum factor can nonspecifically pro-

tect immune rabbit macrophages *in vitro* against the necrotizing action of phagocytosed virulent *M. tuberculosis*. Evidently such humoral factors were either not present in the immune rabbit serum or were unable to express their activity in the experiments described here.

Summary. Passive transfer of serum from highly immunized rabbits failed to protect the recipient rabbits against aerogenic challenge with *Mycobacterium bovis*.

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