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Infection-Immunity in Tularemia: Specificity of Cellular Immunity

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The relationship between hypersensitivity and cellular resistance to infection with facultative intracellular parasites was studied in mice by using infection-immunity in tularemia as a model system. Delayed hypersensitivity to antigenic fractions of *Francisella tularensis* was first detected 6 to 7 days after immunization with viable *F. tularensis* vaccine, at which time immunity against challenge infection developed. Both immunity and delayed-type sensitivity reached maximal levels by 9 to 10 days. Immediate hypersensitivity occurred after immunization with both viable and nonviable tularemia vaccines but could not be correlated with resistance since nonviable antigens were not protective. Attempts to relate resistance to *F. tularensis* with nonspecific immunity factors were unsuccessful. Immunization of mice with BCG vaccine stimulated protection against infection with *F. novicida* and *Salmonella typhimurium* but provided no protection against infection with *F. tularensis*. Moreover, viable tularemia vaccine, while inducing marked protection against challenge with specific organisms, afforded no protection against infection with *S. typhimurium* or *S. enteritidis*. It is concluded that cellular immunity in tularemia involves an immunologically specific component.

Arguments for a concept of cellular immunity to infections with intracellular parasites are strengthened by observations of the concomitant occurrence of both resistance and delayed hypersensitivity. Mackaness observed a consistent association between the development of resistance and delayed hypersensitivity in listeriosis (15) and salmonellosis (9). A correlation between resistance and delayed hypersensitivity has also been demonstrated in tuberculosis (27) and brucellosis (16). In these infections, the time of development of delayed-type sensitivity coincides with the appearance of immune macrophages. Both these responses are passively transfered with spleen cells (9, 17), and both are susceptible to the effects of mitomycin C and antilymphocyte serum (19).

Although an association between the onset of resistance and delayed hypersensitivity is apparent and serves as an attractive hypothesis in explaining cellular immunity (10), the existence of a direct relationship has not yet been adequately demonstrated. Much of the problem arises from attempts to determine whether immunity results from nonspecific or specific factors, or both. At least some of this difficulty can be averted by studying host-parasite relationships in tularemia, since, as will be shown, virtually no nonspecific resistance occurs.

The causative agent, *Francisella tularensis*, is a facultative intracellular parasite of the reticuloendothelial system (RES). Resistance of mice to infection with fully virulent strains of the organism depends upon immunization with viable vaccines (11, 12), though some protection is afforded by an ether-extracted whole-cell vaccine (4, 14). Passive transfer of specific immune serum does not protect mice against lethal challenge (2, 29), and serum antibodies, though demonstrable (14, 29), do not allow prediction of the immune state. A cellular immune mechanism residing in an activated macrophage population is generally considered to be the prime mediator of protection (13, 20, 28).

The purpose of this paper is to examine the specificity of resistance to infections with *F. tularensis* and to attempt to correlate the development of resistance with both immediate and delayed hypersensitivity to cellular antigens of tularemia bacilli. Data are presented to show that resistance to tularemia is directly associated with the development of delayed hypersensitivity and that resistance develops only after specific immunization.

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MATERIALS AND METHODS

Organisms. Strains of *F. tularensis* and *F. novicida* were obtained from C. Owen, Rocky Mountain Laboratory, U.S. Public Health Service, Hamilton, Mont., and maintained on Difco glucose-cystine heart agar (CHA) supplemented with whole sheep blood at a final concentration of 5%. *F. tularensis* strain Schu, which has a median lethal dose (LD_{50}) of one organism for the mouse, rabbit, and guinea pig, was used as the challenge organism in protection tests. *F. tularensis* RV15R (blue variant) was the source of live vaccine and of most nonliving antigens. This variant has an LD_{50} for the mouse of 10^6 to 10^7 organisms. *F. novicida*, an organism which shares some antigenic similarities with *F. tularensis* (21), is fully virulent for mice.

Salmonella typhimurium and *S. enteritidis* were obtained in the lyophilized state from J. Rudbach, Rocky Mountain Laboratory, and each had an LD_{50} for the mouse of 5 to 50 organisms. After two passages in mice, they were maintained on Difco brain heart infusion agar (BHI).

Immunizing antigens. Viable tularemia vaccine (LVS) was prepared from a 24-hr culture of *F. tularensis* RV15R grown on CHA. A suspension of cells in sterile physiologic saline (SS) was adjusted to an appropriate concentration by using a Klett-Summerson photoelectric colorimeter. The number of viable organisms was determined by plating serial decimal dilutions of organisms on CHA plates. A heterogeneous, cellular fraction released from intact tularemia organisms by treatment with ether and designated EEA was prepared by using the procedure of Larson (14). The preparation contained 33% protein, as determined by micro Kjeldahl analysis, and 26% reducing substances (measured as glucose), as determined by the anthrone test (25). A polysaccharide fraction (NP) which showed two precipitin arcs after immunoelectrophoretic analysis was extracted by the method of Alexander (1). Both EEA and NP were stored at −20 C in the lyophilized state and reconstituted in SS on the basis of their dry weight.

Viable BCG vaccine was supplied by Research Foundation, Sol Rosenthal, Director, Chicago, Ill.

Mice and immunization procedures. Swiss-Webster mice between 5 and 7 weeks of age obtained from the colony at Rocky Mountain Laboratory were used in all experiments.

Viable tularemia vaccine and immunizing antigens derived from tularemia bacilli were administered to mice subcutaneously (sc) in single or multiple doses contained in 0.2 ml of SS. In some experiments, NP and EEA were given sc in complete Freund's adjuvant (90 ml of Bayol F, 10 ml of Arlacel A, and 40 mg of mycobacteria). Viable BCG vaccine was inoculated intravenously (iv) at a dose of 300 μg (wet weight).

Protection tests. Protection against a challenge infection was measured in terms of survival after sc injection of mice with serial decimal dilutions of organisms. Immunized or control mice in groups of 6 to 10 were inoculated sc with 0.1 ml of each dilution. Deaths in challenged mice were followed daily for 14 (tularemia) or 28 (typhimurium) days, at which time the LD_{50} values (22) and mean time to death (MTD) were calculated. The degree of protection afforded by the particular test immunogen was determined by subtracting the log LD_{50} value of the control group from that of the experimental group.

Test for hypersensitivity. Groups of 10 mice each were injected in one hind footpad with EEA dissolved in 0.03 ml of SS. The same volume of saline alone was injected into the contralateral footpad. At 4, 24, 48, and 72 hr, foot thicknesses were measured with a dial-gauge calipers (Schnelltäster, Kroplin). Significance of the differences in thickness of saline- and EEA-inoculated feet in each group was calculated by the Student's *t* test.

RESULTS

Comparison of viable and nonviable vaccines for prevention of tularemia in mice. Prior to studying delayed hypersensitivity and its relationship to protective capacity, cellular antigens from tularemia bacilli were tested for their ability to stimulate resistance to fully virulent tularemia organisms. Groups of 40 to 100 mice were immunized with the antigens outlined in Table 1. Antigens were administered sc in each inguinal region. Fourteen days after immunization with viable organisms or with antigens in saline and 35 days after immunization with antigens in adjuvant (Groups IV and VII), the mice in each group were challenged. These experiments were performed at different times during a 6-month period. Because the mice immunized with killed or extracted antigens and challenged with large numbers of Schu organisms died at rates similar to control mice, only the survival rate and increase in MTD at lower infecting doses are shown. Mice immunized with 10^2 to 10^4 LVS (Group I) resisted infection with more than $10^{3.5}$ to $10^{6.3}$ LD_{50} doses of Schu organisms. None of the procedures employing killed organisms or cellular antigens, regardless of the strain of *F. tularensis* from which they were obtained, size of the immunizing dose, or length of immunization, produced significant protection.

Footpad reactivity. Mice were immunized with either 10^2 or 10^3 viable LVS, and groups of 10 mice each were tested 14 days later by injection of various concentrations of EEA and NP into their footpads. Immunization with LVS sensitized mice to tularemia antigens (Table 2). Injection of 0.5 μg of EEA produced a significant but transient 24-hr reaction. Injection of 1.0, 5, and 10 μg of EEA elicited strong reactions at 24 hr which persisted for at least 72 hr. Both immediate and delayed hypersensitivity reactions developed after injection of 20 μg of EEA. Higher concentrations of EEA produced a pronounced delayed response, but central necrosis of the lesion prevented accurate measurement of the reaction and obscured differentiation of 4-hr and 24-hr responses. The polysaccharide fraction (NP) produced a definite

TABLE 1. *Efficacy of viable and nonviable vaccines prepared from Francisella tularensis strain RV15R in inducing resistance to subcutaneous infection with F. tularensis strain Schu*

Exptl group	Immunizing[a] antigen	Immunization protocol	Logs[b] protection	Differences in MTD[c] at infection dose		
				10^3 Viable units	10^2 Viable units	10^1 Viable units
I	LVS	1.7×10^4 Viable units, single dose	>6.3	-- (6/6)[d]	-- (6/6)	-- (6/6)
	LVS	1.7×10^3 Viable units, single dose	4.6	-- (6/6)	6 (5/6)	-- (6/6)
	LVS	1.7×10^2 Viable units, single dose	3.5	8 (5/6)	-- (6/6)	-- (6/6)
II	Heat-killed organisms	1 mg, Single dose	<1	3.4	4	6.8
III	EEA[e]	10–500 μg, Single or multiple doses	<1	2.4	2.5	3.6
IV	EEA[e]	100 μg in CFA[f], single dose	<1	4.5 (1/20)	3.4 (1/20)	5.2 (3/20)
V	EEA[e]	5 mg in CFA + 3 weekly injections of 1.2 mg of antigen in saline	<1	5.0	5.6 (1/10)	6.8 (2/10)
VI	NP[e]	10–500 μg, Single or multiple doses	<1	2–3	2–3	3–5
VII	NP[e]	100 μg in CFA, single dose	<1	2.7	4.8	5.0 (2/20)

[a] Abbreviations: LVS, viable tularemia vaccine; EEA, ether-extracted antigen; NP, Nicholes' polysaccharide.

[b] Difference in log LD_{50} between immunized and unimmunized mice challenged with 10^0 to 10^7 Schu organisms.

[c] Difference in mean time to death (MTD) between immunized and unimmunized mice dead after subcutaneous challenge with lower challenge doses.

[d] Dashed lines indicate MTD > 14 days. Numbers in parentheses indicate number of surviving mice per total infected.

[e] Some experiments employed EEA or NP which were extracted from strain Schu organisms instead of strain RV15R.

[f] Freund's adjuvant.

TABLE 2. *Footpad reactivity of mice immunized subcutaneously with viable Francisella tularensis strain RV15R (LVS) and tested 14 days later with cellular fractions[a] of tularemia bacilli*

Immunizing dose of LVS (viable units)	Eliciting antigen and dose (μg)[b]	Increase in footpad thickness (mm)[c]			
		4 Hr	24 Hr	48 Hr	72 Hr
2.3×10^2	EEA, 0.5	0.02 ± 0.01[d]	0.17 ± 0.03	0.08 ± 0.02	0.02 ± 0.01[d]
2.3×10^2	EEA, 1.0	0.02 ± 0.01[d]	0.27 ± 0.04	0.18 ± 0.04	0.08 ± 0.01
2.3×10^2	EEA, 5.0	0.05 ± 0.01[d]	0.49 ± 0.05	0.24 ± 0.03	0.12 ± 0.02
2.3×10^2	EEA, 10.0	0.05 ± 0.01[d]	0.49 ± 0.06	0.26 ± 0.04	0.16 ± 0.03
2.3×10^2	EEA, 20.0	0.11 ± 0.03	0.83 ± 0.08	0.38 ± 0.03	0.17 ± 0.02
2.3×10^2	NP, 15.0	0.23 ± 0.03	0.07 ± 0.02[d]	0.03 ± 0.01[d]	ND
2.3×10^3	EEA, 10.0	0.22 ± 0.04	0.48 ± 0.08	ND	ND
2.3×10^3	NP, 15.0	0.63 ± 0.1	0.14 ± 0.05	0.07 ± 0.03[d]	ND
None	EEA, 20.0[e]	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
None	NP, 15.0	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	ND

[a] Ether-extracted antigen (EEA) or Nichole's polysaccharide (NP) prepared from *F. tularensis* strain RV15R.

[b] Dry weight of antigen in 0.03 ml of saline.

[c] Mean ± standard deviation in groups of 10 mice. ND, not determined.

[d] Not significant ($P > 0.05$) when compared with unimmunized mice inoculated with corresponding dose of EEA or NP.

[e] Since no unimmunized mice injected with any dose of EEA or NP responded, only data involving higher antigen doses are provided.

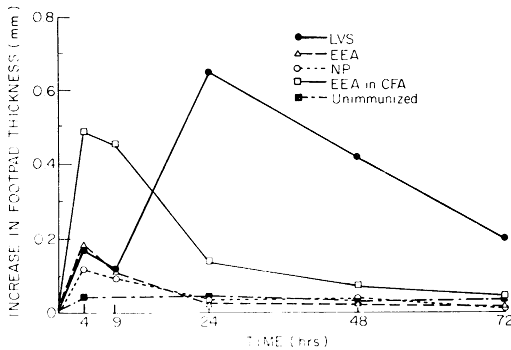

FIG. 1. *Time course of footpad reactivity in mice immunized with either 600 viable F. tularensis (LVS), 100 μg of ether-extracted antigen (EEA) in saline or Freund's adjuvant (CFA), or 100 μg of Nicholes' polysaccharide (NP) in saline. Each point represents mean of 10 mice after injection of 0.03 ml of saline containing 20 μg of EEA.*

lesion measurable at 4 hr but not at 24 hr. Because of the consistency with which both immediate and delayed hypersensitivity reactions were elicited with 20 μg of EEA, this dose was chosen for subsequent experiments. Histologic examination of footpad lesions of LVS-sensitized mice tested with 20 μg of EEA revealed an infiltrate at 4 hr composed primarily of polymorphonuclear cells. By 24 to 48 hr mononuclear cells predominated in the reaction site. Normal animals footpad-tested with 20 μg of EEA exhibited a mild and transient infiltrate composed primarily of polymorphonuclear cells.

Effect of different immunizing antigens on induction of footpad reactivity. Four groups of 10 mice each were immunized with either 600 viable LVS (Group I), 100 μg of EEA (Group II), or 100 μg of NP (Group III) in saline, or 100 μg of EEA in complete Freund's adjuvant (Group IV). One group of 10 unimmunized mice served as controls. All mice were tested for footpad responses to 20 μg of EEA at the same time, although this was 14 days after immunization for Groups I to III and 21 days after immunization for Group IV. The results are shown in Fig. 1. Significant immediate and delayed responses were provoked by immunization with LVS. The response to immunization with EEA and NP was characterized by a moderate but significant 4-hr reaction ($P < 0.001$), but this diminished rapidly and was not observed at 24 hr. Immunization of mice with EEA in Freund's adjuvant stimulated a pronounced immediate reaction (0.48 mm) and a weak 24-hr reaction.

Correlation between footpad reactivity and resistance. To determine the relation of footpad reactions to resistance, the experiment summarized in Table 3 was performed. Eight days after the last injection of antigen, all mice and a group of 10 uninoculated controls were skin-tested in the footpad and challenged with 200 virulent strain Schu organisms. All mice receiving injections of EEA or NP developed demonstrable swelling of the footpad evident 4 hr after injection of EEA. In all instances, the intensity of the reactions declined rapidly and was indistinguishable from background levels at 24 hr and thereafter. There were no survivors when mice receiving nonviable antigens were challenged with Schu organisms. Mice immunized with LVS developed typical immediate and delayed hypersensitivity reactions and were completely immune to the challenge dose of Schu.

To substantiate the above findings, the experiment shown in Fig. 2 was performed. Groups of 120 mice each were immunized sc with either 860 LVS organisms, 100 μg of EEA, or 100 μg of NP. At specified times after immunization, 10 mice in each group were tested for footpad sensitivity to 20 μg EEA. Six hours later, the same mice were challenged with 1,200 virulent Schu organisms. At each time interval, a group of 10 unimmunized mice was tested and challenged in the same way as the experimental groups. The results are shown in Fig. 2. Only mice immunized with LVS developed significant delayed hypersensitivity reactions to EEA which first became evident 7 days after immunization and persisted for at least 19 days. No significant delayed reactions or resistance were observed in mice injected with NP or EEA. Protection afforded by LVS was present as early

TABLE 3. *Footpad reactivity and resistance to infection with virulent Francisella tularensis strain Schu in mice immunized subcutaneously with viable and nonviable vaccines*

Group	No. of mice	Immunizing[a] antigen	Footpad test (mm[b])		Per cent survival after challenge with 200 Schu
			4 Hr	24 Hr	
I	10	1 Mg of EEA in CFA + 0.5 mg in saline	0.42	0.05	0
II	10	1 Mg of NP in CFA + 0.5 mg in saline	0.26	0.02	0
III	10	1.5 Mg of EEA in saline	0.33	0.05	0
IV	10	600 Units of LVS	0.21	0.52	100

[a] Injected subcutaneously, mice in groups I and II received antigen in saline 2 weeks after receiving antigen in complete Freund's adjuvant (CFA). EEA, ether-extracted antigen; NP, Nicoles' polysaccharide; LVS, viable tularemia vaccine.

[b] Mean increase in footpad thickness in groups of 10 mice each after injection of 20 μg of EEA (dry weight) 8 days after immunization.

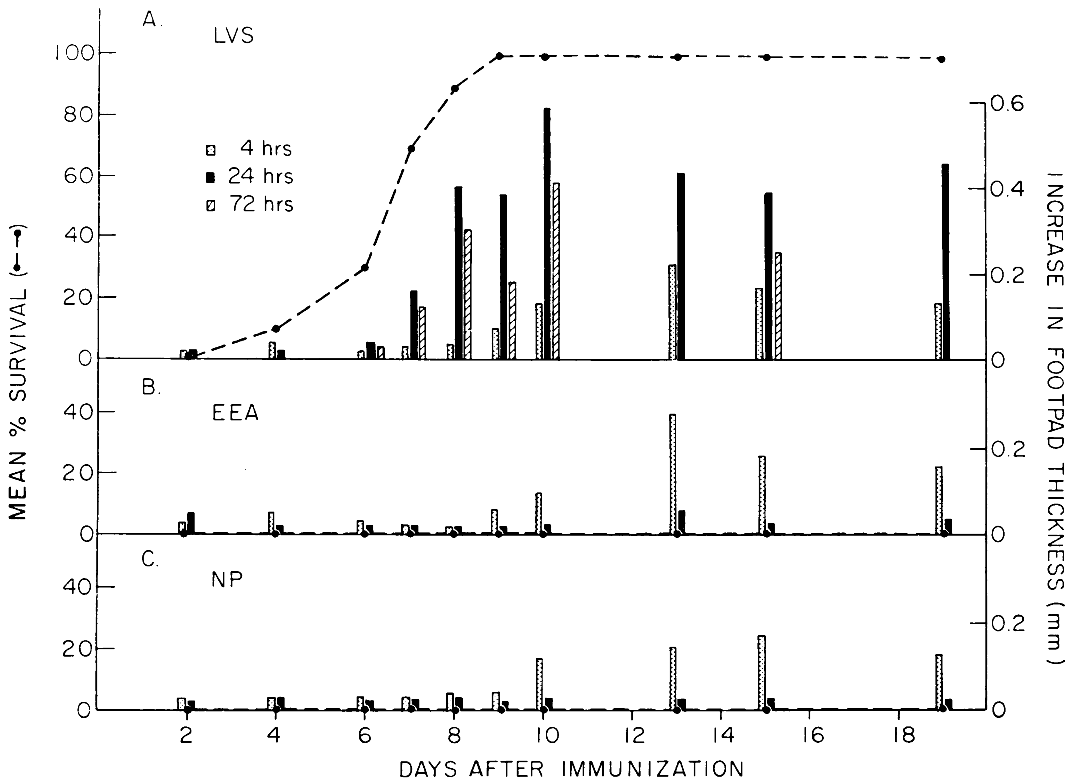

FIG. 2. *Footpad reactivity and resistance to infection with virulent F. tularensis strain Schu in mice immunized subcutaneously with viable and nonviable vaccines. Mice were immunized with 860 LVS or with 100 μg of ether-extracted antigen (EEA) or Nicholes polysaccharide (NP) before skin-testing with 20 μg of EEA and subcutaneous infection with 1200 virulent Schu organisms.*

as 4 to 6 days after immunization but was not complete until day 9.

Except for slight (0.2 to 2.2 days) extensions in survival time, no mice immunized with NP or EEA showed any resistance to challenge with Schu at any test interval.

All three experimental groups of mice developed significant ($P < 0.001$) immediate hypersensitivity reactions (4 hr) first detectable 10 days after administration of immunogen. Arthus reactions did not correlate with protection.

Resistance of mice to tularemia by BCG vaccination. An experiment was conducted to determine whether resistance to infection with *F. tularensis* strain Schu in specifically immunized mice could be associated with nonspecific immunity. One hundred twenty mice were inoculated iv with 300 μg (wet weight) of viable BCG vaccine. After a period of 3 weeks, when nonspecific resistance was at its height (D. Lodmell and C. L. Larson, *unpublished data*), separate groups of mice were challenged with serial 10-fold dilutions of either strain Schu, *F. novicida*, or *S. typhimurium*. The results shown in Table 4 demonstrate that, although immunization with BCG led to enhanced resistance to *F. novicida* and *S. typhimurium*, no protection was conferred against infection with *F. tularensis*. In the *F. tularensis*-challenged groups, both the mortality ratios and average survival times were the same in immunized and unimmunized groups of mice.

A final series of experiments was performed to determine whether immunization with live tularemia vaccine (LVS) induced cross-protection to an antigenically unrelated intracellular parasite. Fourteen days after immunization with 700 viable LVS organisms, groups of immunized and unimmunized control mice were challenged with serial 10-fold increments of strain Schu, *S. typhimurium*, or *S. enteritidis* organisms. The results (Table 5) demonstrated that protection against more than 10^5 LD_{50} doses was obtained in specifically challenged mice, whereas no significant protection (< 1 log) was afforded against infection with *S. typhimurium* or *S. enteritidis*. In the latter two groups, LVS-immunized mice survived at best 1 day longer than control mice.

TABLE 4. *Failure of BCG immunization to provide nonspecific protection to Francisella tularensis strain Schu*[a]

Challenge dose[b] (viable units)	BCG immunized, infected with			Unimmunized control, infected with		
	Francisella tularensis	*F. novicida*	*Salmonella typhimurium*	*F. tularensis*	*F. novicida*	*S. typhimurium*
10^5	6/6[c]	6/6	7/8	6/6	6/6	8/8
10^4	6/6	3/6	5/8	6/6	6/6	7/8
10^3	6/6	1/6	2/8	6/6	6/6	8/8
10^2	6/6	0/6	2/8	6/6	6/6	6/8
10^1	6/6	0/6	1/8	6/6	4/6	4/8
10^0	5/6	0/6	0/8	1/6	1/6	1/8
LD_{50}	$<10^0$	$10^{3.84}$	$10^{3.45}$	$10^{0.39}$	$10^{0.65}$	$10^{1.22}$
Logs protection	0	3.19	2.23			

[a] Mice were challenged subcutaneously with *F. tularensis* strain Schu., *F. novicida*, or *S. typhimurium* 3 weeks after intravenous immunization with BCG vaccine (300 μg wet weight, 5.6×10^6 viable units).

[b] Actual challenge dose of 10^5 organisms was 2.2×10^5 for Schu, 1.5×10^5 for *F. novicida*, and 9.1×10^4 for *S. typhimurium*.

[c] Deaths/total.

TABLE 5. *Resistance of mice immunized (14 days previously with Francisella tularensis strains RV15R to subcutaneous infection with F. tularensis strain Schu, Salmonella typhimurium, or S. enteritidis*

Exptl group	Immunized[a]	Challenge organism	LD_{50} (log_{10})	LD_{50} protection[b] (log_{10})
I	+	*Francisella tularensis*	5.58	5.45
	−	*F. tularensis*	0.13	
II	+	*Salmonella typhimurium*	2.23	−0.10
	−	*S. typhimurium*	3.33	
II	+	*S. typhimurium*	2.42	0.45
	−	*S. typhimurium*	1.97	
III	+	*S. enteritidis*	1.68	0.39
	−	*S. enteritidis*	1.29	

[a] Mice were immunized subcutaneously with 700 viable units of *F. tularensis* strain RV15R (LVS).

[b] Difference in LD_{50} between immunized and unimmunized mice challenged with 10^0 to 10^7 organisms.

DISCUSSION

Successful induction of resistance to tularemia in man and most experimental animals depends on immunization with viable, attenuated strains of *F. tularensis* (11, 12). Nonviable antigens afford considerable protection in mice against infection with moderately virulent bacilli, such as strain 425 F4G (4, 14). However, no nonliving vaccine protects mice against fully virulent strains such as Schu. Experimental studies reported here corroborate these findings. A limited degree of immunity can be induced by EEA and NP, but this amounts to only a slight extension of the survival time. Viable vaccine, by contrast, induces complete protection.

From the present data, it is apparent that, in mice, the increased bactericidal capacity of the immunized host for virulent *F. tularensis* organisms is dependent upon specific immunization rather than upon nonspecific stimulation of the RES. Three lines of evidence support this contention. (i) Immunization of mice with BCG, a potent stimulator of enhanced macrophage activity (5), induces no protection to infection with *F. tularensis*. This is in sharp contrast to previous studies with other facultative intracellular parasites (16) showing that stimulation of the RES with BCG induces marked resistance to a wide variety of antigenically unrelated organisms. Immunization with BCG did however confer marked resistance to infections with *F. novicida* and *S. typhimurium*. (ii) Mice immunized with LVS are resistant to challenge with *F. tularensis* but show no augmented resistance to an unrelated organism such as *S. typhimurium* which is normally susceptible to nonspecific factors of immunity. (iii) In separate studies, mice immunized with LVS and unimmunized mice show no appreciable differences (based on phagocytic indices K and α) in their abilities to remove carbon particles from their circulating blood (J. L. Claflin, Ph.D. thesis, Univ. of Montana, Missoula, 1970). Although this latter measure of

RES activity may not fully reflect the enhanced microbicidal activity of free macrophages, it has provided a useful means of determining the increased phagocytic properties in fixed RES cells and is commonly used for assaying nonspecific cellular immune factors (5).

Effective antibacterial immunity induced by LVS is concurrent with a state of delayed hypersensitivity to antigens of *F. tularensis*. Although immediate hypersensitivity also occurs in mice immunized with LVS, this is not associated with resistance. Nonresistant mice immunized with nonviable vaccines NP and EEA, show immediate responses. The lack of correlation between immediate hypersensitivity and immunity is not surprising in view of the numerous reports that passive transfer of antibody (even from resistant LVS-immunized mice) fails to protect mice against virulent strains of *F. tularensis* such as strain Schu (2, 13, 29; J. L. Claflin, Ph.D. thesis, Univ. of Montana, Missoula, 1970).

A correlation between cellular immunity and delayed hypersensitivity has been adequately demonstrated for other facultative intracellular parasites, and the causality of the infection-immunity relationship has been discussed (18). The basic thesis is that a state of delayed hypersensitivity produces sensitized lymphocytes and macrophages, and the latter have the capacity to destroy invading bacteria. Cellular immunity considered from this aspect is not an immunologic phenomenon, since once developed, it is effective against many microorganisms. It has been suggested by Mackaness (17) that a possible mediator influencing macrophage function could be a lymphokine, though not necessarily macrophage inhibition factor. While more research is necessary in order to substantiate this suggestion, the mechanism of lymphocyte-macrophage interaction put forth by Mackaness bears serious consideration as a means of explaining nonspecific resistance. It can even be assumed that resistance to most facultative intracellular parasites is entirely nonspecific in nature, a position which has, in fact, been strongly advocated by Mackaness (17) and more recently by Blanden (6) and by Simon and Sheagren (26).

However, certain features of infection-immunity to intracellular parasites suggest that resistance may have an immunologically specific component. Data from in vivo challenge experiments on cross-protection between bacteria and protozoa (24) and on cross-protection between different species of *Salmonella* (8) and *Mycobacteria* (7) indicate that resistance is best against the homologous organisms. As shown in the present study of infection-immunity in tularemia, nonspecific altered cellular states per se (such as those induced by injection of BCG) play virtually no role in resistance. Furthermore, only tularemic infections are affected by immunization with LVS. Thus, in murine tularemia, cell-mediated immunity is clearly specific.

In understanding immunity to tularemia, one must consider both the specificity and the preponderant evidence that resistance ultimately resides in the macrophage (13, 20, 28). Specific antibody with high avidity for macrophages and high serum turnover might explain why only tularemia organisms are affected. Although not yet demonstrated in mouse tularemia, cytophilic antibody is strongly implicated in resistance in salmonellosis (23). Alternatively, committed lymphocytes may, upon stimulation with specific antigens of *F. tularensis*, secrete a soluble substance which interacts with macrophages, resulting in enhanced bactericidal activity to tularemia bacilli. That some soluble substance secreted by lymphocytes, other than antibody, may fulfill this role is suggested by a recent report of Amos and Lachmann (3). They found that macrophage inhibition factor requires specific antigen not only for its synthesis but also for its inhibitory activity on macrophages. The possibility also exists that resistance to tularemia is not dependent on any specific humoral factor but is related to an inducible enzyme of macrophages which attacks some substrate peculiar to tularemia organisms.

The problem of cell-mediated immunity is a complex one, and appropriate model systems are needed to determine the factors, specific and nonspecific, involved in mechanisms of resistance to intracellular parasites of the RES. While numerous examples are available for studying nonspecific immunity, few examples exist in which the specific factors of cellular resistance can be studied separately. The present study of infection-immunity in tularemia defines a model system which offers this opportunity.

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