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Induction of Host Resistance in Different Mouse Strains (38247)

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Enhancement of host resistance to infectious agents is a major objective of preventive medicine. One area of investigation of this objective is the influence of genetics on specific immunological host responses (1–10). For instance the amount of antigen needed to induce antibody synthesis (3) and the types of antibody produced (5, 7, 8, 10) are under genetic control. Hence an important factor in the evaluation of vaccine potency is not only the species but also the strain of animal used in the tests. Another area of investigation explores ways to induce a nonspecific enhancement of the immunological response (11–21). A wide variety of substances have been used both as adjuvants, to increase specific humoral responses, and as general reticuloendothelium stimulants. For instance: Vitamin E as a supplement in diets has been shown to increase the IgG produced in response to tetanus toxoid (11). *Bordetella pertussis* vaccine and beryllium sulfate have been shown to be potent adjuvants for increasing antibody response via their effect on macrophages (13). Hyperphagocytic activity of the reticuloendothelial system by glucan increased the primary and secondary immune responses of mice (15). Zymosan (17–20) and bacterial endotoxins share certain chemical and biological properties and both have been used to increase phagocytosis and humoral responses to many different infections. However, zymosan does not have the toxic properties of endotoxin and hence its use in the studies of nonspecific host resistance is less complicated. Methanol extract of the tubercle bacilli (24, 25) (referred to as MER) has the interesting property of prophylactically protecting not only against the tubercle bacilli, from which it is derived, but against such widely different bacterial infections as those caused by staphylococci and *Klebsiella*. Moreover, mice are protected for as long as 12 weeks after injection with MER. The mechanism by which endotoxins, zymosan and MER induce an increase in nonspecific host resistance has not yet been deciphered although as mentioned above phagocytic studies have indicated that the macrophages stimulated by these substances may be the prime mediators in raising the level of host resistance. This report suggests that a suitable assay for compounds that nonspecifically increase host resistance can be constructed using two mouse strains and measuring two parameters of host resistance.

The zymosan (Standard Brands Inc., Lot #7B-3401) used in these studies (to induce host resistance) was suspended in pyrogen free distilled water (300 mg/25 ml) and autoclaved at 121°/15 min. Doses of 6, 3 and 1 mg were injected intraperitoneally into mice. Three and 4 days later the mice were challenged intraperitoneally with a 6 hr culture of *Klebsiella pneumoniae* (MB-3123) grown in a casamino acid medium (22). Survival was used as one of the parameters of host resistance.

The other parameter of host resistance measured was the phagocytic response. For this, the *Klebsiella* challenge culture was grown overnight in the casamino acid medium containing ^{32}P phosphate (0.02 mCi/μg P). The labelled culture was washed twice with saline, the cells were resuspended in the medium and 0.2 ml was injected intravenously into zymosan-treated and control mice. Ten and 30 min after injection mice were bled from the heart

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TABLE I. Effect of Mouse Strain on a *Klebsiella* Challenge Given 3 Days after Zymosan Treatment.

	Percent survivors 10 days postchallenge with					
	4×10^4 cells		850 cells		85 cells	
Zymosan mg/mouse	C[a]	T[a]	C	T	C	T
6	60	0	100	60	ND	ND
3	60	0	100	70	ND	ND
1	40	0	90	50	ND	ND
0	0	20	10	0	30	0

[a] C = Charles River #1 Mice, T = Taconic Farm Mice (10 mice per treatment).

then killed by neck dislocation and the liver, spleen, kidney and lungs were excised. Blood and tissues were counted in a gamma scintillation spectrometer (Packard). Despite the low efficiency in counting a hard beta in a gamma counter this rapid method gave the same relative distribution of counts as with the method reported earlier (23). Moreover, counts for controls and test sample were consistent from mouse to mouse and on repeated assays. Viable bacterial counts on both intraperitoneal and intravenous challenge cultures were done on brain heart infusion agar, and both cultures were titrated for virulence in tenfold steps.

These two parameters of host resistance, survival and phagocytic response, to virulent *Klebsiella,* were measured in Taconic Farm and Charles River female mice (17–20 g).

Table I shows that both mouse strains are equally susceptible to *Klebsiella,* but after zymosan treatment, the Charles River mice were resistant to both a high and low challenge dose of organisms. In contrast, zymosan increased host resistance in Taconic Farm mice only against a low challenge dose of *Klebsiella.*

Table II reinforces the data in Table I and shows further that if the challenge is given 4 days after the induction of host resistance by zymosan rather than 3 days (Table I) the zymosan is even more effective in decreasing the susceptibility of Charles River mice to *Klebsiella.* Increasing the time between induction and challenge also increased the ability of Taconic Farm mice to withstand a larger challenge dose.

Experiments with MER (24, 25) (a nonsoluble residue from a methyl alcohol extraction of phenolized BCG organisms) showed that like zymosan, better host resistance could be induced in Charles River mice than in Taconic Farm mice and that

TABLE II. Effect of Mouse Strain on a *Klebsiella* Challenge Given 4 Days after Zymosan Treatment.

	Percent survivors 10 days postchallenge with					
	8×10^3 cells		850 cells		85 cells	
Zymosan mg/mouse	C[a]	T[a]	C	T	C	T
6	90	50	100	100	ND	ND
3	90	60	100	90	ND	ND
1	50	0	100	50	ND	ND
0	0	0	0	30	0	40

[a] C = Charles River #1 Mice, T = Taconic Farm Mice (10 mice per treatment).

TABLE III. ^{32}P in Blood, Liver and Spleen after a Labeled *Klebsiella* Challenge Given 4 Days after Zymosan Treatment.

Zymosan mg/mouse	Average[a] counts/min in ml blood and 10 mg organ							
	Charles River Mice				Taconic Farm Mice			
	Blood	Spleen	Liver	S:L[a]	Blood	Spleen	Liver	S:L[a]
	10 Min after challenge							
6	3,623	47	78	0.6	797	50	100	0.5
1	2,897	104	62	1.7	1,684	86	85	1.0
0	4,892	107	36	3.0	3,551	127	47	2.7
	30 Min after challenge							
6	335	47	78	0.6	739	56	82	0.7
1	665	104	62	1.7	1,610	210	55	3.8
0	457	234	46	5.1	1,756	180	48	3.8

[a] Counts corrected for background were averaged from 10 mice whose spleen to liver values ranged within ±10%. Mice received an ip challenge of 10^7 cells containing 10^4 counts/min.

the nonspecific host resistance is at a higher level when challenge comes 4 days rather than 3 days after a MER treatment.

Table III shows the phagocytic response as measured in blood, liver and spleen of the 2 mouse strains. As discussed in an earlier report (23) the ratio of the *g*-negative challenge cells found in the spleen to that found in the liver is an index of susceptibility, i.e., normal mice have a higher number of labeled challenge cells in their spleen per mg of tissue than in their liver. In contrast, mice nonspecifically and specifically sensitized to *g*-negative organisms have a lower number of challenge cells per mg of spleen than per mg of liver.

The data in Table III substantiate the above thesis. Both strains of mice receiving 6 mg of zymosan 4 days before challenge are less susceptible to the infection as shown by a decreased spleen to liver ratio of challenge cells per mg of tissue, and by an increased percent survival over control mice (Table II). The data on mice receiving 1 mg of the zymosan is equivocal in that Taconic Farm mice measured at 10 min postchallenge show a low spleen to liver ratio while the 30 min measurement shows a spleen to liver ratio that equals that of the control animals. The survival figures for these mice (Table II) show that the mice cannot withstand a challenge of 8×10^3 cells and only 50% of the mice survive a challenge of 850 cells. In contrast, the spleen to liver ratio for the zymosan-treated Charles River mice is lower than for control mice at both 10 and 30 min postchallenge and the survival data (Table II) show that the resistance of these mice has been considerably heightened.

The phagocytic response of the blood at 10 min postchallenge does not correlate with the survival data obtained with the two mouse strains. From the phagocytic blood data it would appear that Taconic Farm mice were more resistant than the Charles River mice and this is not substantiated by the survival data. This observation requires further experimentation since it appears at variance with past knowledge (22). However, the marked drop in blood count from 10 to 30 min postchallenge found in the Charles River mice versus that found in Taconic Farm mice reflects the increased host resistance induced by zymosan in Charles River mice compared to that induced in Taconic Farm mice.

Similar experiments with *Salmonella typhimurium* have so far been negative. In neither mouse strain was host resistance induced by either zymosan or MER. However, the timing of host resistance induction

to challenge may be more critical in *Salmonella typhimurium* because mice survive longer to a virulent challenge of *Salmonella typhimurium* than to a virulent challenge of *Klebsiella.*

From the data presented, an assay to measure nonspecific induction of host resistance can be constructed. Zymosan or MER used to induce host resistance in a high and low responding mouse strain establishes positive and negative control levels of host resistance. Measuring the ratio of labelled cells found per mg of spleen to mg of liver provides a rapid method for evaluating compounds for their effect on nonspecific host resistance.

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