

Host Defense Against the Pneumococcus in T-Lymphocyte-Deficient, Nude Mice

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Resistance to pneumococcal infection was tested in T-lymphocyte-deficient, nude (nu/nu) mice. Pneumococcal serum opsonizing activity, *in vivo* phagocytosis of the pneumococcus, and the mean lethal dose for the pneumococcus were all found to be the same in nude mice as in control (+/+) mice. T-lymphocytes do not appear to play a significant role in the host's defense against the pneumococcus.

Resistance to infection with the pneumococcus (*Streptococcus pneumoniae*) is a complex process known to involve a number of different aspects of immunity. Surface phagocytosis (12), opsonizing antibody (11), and the third (C3) (10) and fifth (C5) (8) components of complement have all been shown to participate in the host's defense against the pneumococcus. Information is incomplete, however, on the role of thymus-derived (T) lymphocytes in resistance to pneumococcal infections. Although the evidence obtained from patients with primary T-lymphocyte deficiencies suggests that, if T-lymphocytes play a role in the host's defense against the pneumococcus, it is a minor one, there is no direct experimental data on this point.

The nude mouse has a genetically determined absence of the thymus and a severe deficiency of T-lymphocyte functions (4-6). As a result, it provides the opportunity to study directly, and *in vivo*, the role of T-lymphocytes in the host's defense against pneumococcal infections.

Nude (nu/nu) mice were kindly supplied by James Hansen of the National Institutes of Health, Bethesda, Md. The nude mice were demonstrated to have 2% or less theta-bearing spleen lymphocytes by R. F. Mortensen (3). Swiss-Webster (+/+) mice were purchased from Microbiological Associates, Bethesda, Md. The colony of nude mice was maintained by mating nude (nu/nu) males with heterozygous (nu/+) females. Only male homozygous mice were used in experiments. Serum was obtained by bleeding the mice from their tails, pooled, and stored at -70 C.

Serum opsonizing activity is an important determinant in the host's defense against the pneumococcus. Accordingly, pneumococcal opsonizing activity was measured in the pooled serum of nude mice. Briefly, 1.25×10^6 exudate

leukocytes obtained from Swiss-Webster mice and 6.25×10^8 log-phase type 25 pneumococci were added to the desired dilution of pooled test serum and the mixture was rotated at 12 rpm at 37 C for 30 min (9). Serum opsonizing activity was determined by counting the percentage of polymorphonuclear leukocytes that contained pneumococci on a stained smear. The results are expressed as percentage of phagocytosis. As can be seen in Fig. 1, pneumococcal opsonizing activity was normal in the serum of nude mice when measured over a wide range of serum concentrations.

The phagocytosis of pneumococci was also measured *in vivo* in nude mice. Mice were injected intraperitoneally with 2 ml of a starch-gluten suspension (10). Eighteen hours later, the peritoneal exudates from either nude mice or normal mice were found to contain 2×10^7 leukocytes. Other animals were then injected intraperitoneally with 4×10^8 log-phase type 25 pneumococci. Thirty minutes later each animal was sacrificed, the exudate was recovered and stained, and the percentage of phagocytosis was determined. As can be seen in Table 1, the *in vivo* phagocytosis of pneumococci was the same in the nude mice as in the control mice.

The mean lethal dose (LD_{50}) for type 3 pneumococci, strain IIS-IR6, of intermediate virulence for the mouse (Pn 3-int), was also measured in nude mice. Groups of 10 mice, weighing 16 to 18 g each, were injected intraperitoneally with 10-fold dilutions of log-phase Pn 3-int. The mice were observed for 5 days. The LD_{50} was calculated by the Reed-Muench method (7) and the probability (*P*) value was determined as in reference 13. As can be seen in Table 2, the LD_{50} in the nude mice was the same as that in the control mice. It should also be noted that the cumulative mortality was the same in the two groups on each day of the LD_{50} study.

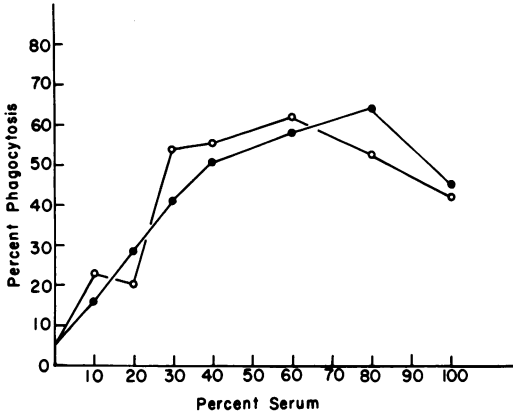


FIG. 1. *Pneumococcal opsonizing activity in pooled serum from nude (nu/nu) (○) and control (+/+) (●) mice.*

TABLE 1. *In vivo phagocytosis of Pn 25 in preformed peritoneal exudates of nude or normal mice*

Nude mice	Normal mice
33 ^a	24
45	43
50	58
52	62
60	67
Mean ^b 48	51

^a Percentage of phagocytosis. Each value represents a single mouse.

^b $P > 0.7$ by Student's *t* test.

TABLE 2. *LD₅₀ for Pn 3-int in nude mice and normal mice*

Dose of Pn 3-int	Nude mice	Normal mice
8×10^8	9/10 ^a	7/10
8×10^7	8/10	8/10
8×10^6	0/10	0/10
8×10^5	0/10	0/10
LD ₅₀ ^b	4×10^7	5×10^7

^a Dead/total.

^b $P > 0.7$.

Both clinical and laboratory observations have suggested that T-lymphocytes might play a role in antipneumococcal immunity. Although patients with primary deficiencies of T-lymphocyte function are most notably susceptible to viruses and fungi, they also may have

pneumococcal infections (2). In addition, studies have shown that the magnitude of the immune response to pneumococcal capsular polysaccharide is influenced by both "suppressor" and "amplifier" T-lymphocytes (1).

In the above studies, pneumococcal serum opsonizing activity and phagocytosis of the pneumococcus *in vivo*, both manifestations of antipneumococcal host defense, were found to be normal in nude mice. Most importantly, the LD₅₀ for the pneumococcus was the same in nude mice as in control mice. Thus, T cells do not appear to play a significant role in the host's defense against the pneumococcus.

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