Influence of *B. pertussis* on Host Survival Following S-180 Implantation*

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SUMMARY

Prior conditioning with *B. pertussis*, a lipopolysaccharide fraction obtained therefrom, and the lipopolysaccharide of *S. marcescens* definitely inhibits successful transplantation of the mouse tumor, Sarcoma 180. The lipoid A component of the endotoxin of *E. coli* 0111 and the lipide fraction from Sarcoma 180 were without effect.

The lethality of Sarcoma 180 (S-180) for mice has been well documented. However, it was reported recently that definite protection following implantation resulted if the animals were previously infected with Bacillus Calmette-Guerin (11). This effect has been attributed to the enhancement of the activity of the reticuloendothelial system (R.E.S.) (1, 2) and the subsequent increase in natural resistance to infection which follows the administration of endotoxins, zymosan, products of the tubercle bacillus, etc. (4). This latter phenomenon has been observed also for *Bordetella pertussis* (3). Since we have had the opportunity to investigate many aspects of the mechanism of *B. pertussis* action (9), the effectiveness of its vaccine and certain related agents in depressing S-180 “takes” was studied.

MATERIALS AND METHODS

Mice.—Adult, female, Swiss-Webster mice of at least 85 days in age and weighing initially about 80 gm. were used throughout.

S-180.—The source of infectious inoculum was either an actively growing, subcutaneous nodule of tumor in a CAF1 mouse or the product of one intraperitoneal (I.P.) passage in a Swiss-Webster mouse, harvested after 10 days of growth. The tumor was macerated with sterile saline in a mortar with pestle; the inoculum was given intraperitoneally in a volume of 0.5 ml. and passed through a 20-gauge needle.

*B. pertussis*.—A vaccine of phase I organisms in a 0.5-ml. volume containing about 6 X 10^9 cells was given intraperitoneally at varying intervals prior to tumor inoculation.

*B. pertussis lipopolysaccharide*.—This water-soluble, lipopolysaccharide fraction of *B. pertussis* was prepared and supplied by Dr. Otto Westphal and Dr. A. Wander Forschungsinstitut, Freiburg, Germany, from the lyophilized organisms. The mice each received 200 μg. intraperitoneally 12 days prior to tumor inoculation.

*Serratia marcescens lipopolysaccharide*.—This material was purchased from Difco Laboratories, Inc., Detroit, Mich. A solution of 200 μg. in 0.5 ml. saline was injected intraperitoneally 12 days prior to tumor inoculation.

*Lipoid A*.—This fraction represents the lipide moiety of endotoxin. The preparation used in these experiments was generously supplied by Dr. Harry J. Robinson of Merck Institute for Therapeutic Research, Rahway, N.J., as MK-335 and represents the lipide fraction isolated from the lipopolysaccharide of *E. coli* 0111. The sterile solution as received was diluted so that each mouse received 140 μg. intraperitoneally 12 days prior to tumor inoculation.

*S-180 lipide*.—A lipide extract from homogenized S-180 was prepared according to the procedure of Folch et al. (5) as modified by Rapport et al. (14). Each mouse received the equivalent of 10 mg. wet tissue as the lipide emulsified in saline and given intraperitoneally 12 days prior to tumor inoculation.
RESULTS

*B. pertussis.*—Control mice, receiving no prior treatment, succumbed uniformly and predictably to inoculation with tumor. On day 20 following the challenge, 6/6 and 5/6 animals were dead, in parallel experiments. The simultaneous inoculation of *B. pertussis* and tumor homogenate had no retardative effect on the lethal effects of the tumor. At 20 days there were 6/6 and 4/6 dead. However, when the tumor was inoculated 7 days after treatment with *B. pertussis*, a decided influence was noted on tumor growth. In three parallel experiments, after 20 days, 0/6, 0/6, and 0/6 were dead; after 30 days, 0/6 and 1/6 were dead; and after 80 days 2/6 were dead. This retardation was still markedly evident if tumor inoculation followed treatment with *B. pertussis* by 12 days.

After 20 days in parallel experiments there were 0/6 and 0/6 deaths; after 30 days, 1/6; and after 80 days, 3/6. Essentially the same degree of retardation was noted if an 18-day interval was allowed to elapse between *B. pertussis* and tumor inoculation. After 20 days, there were 1/6 and 0/6 deaths; after 30 days, 1/6; and after 80 days, 2/6.

With a 24-day interval between challenge inoculations, the retardation effect began to wane; at 20 days there were 3/12 deaths, after 30 days 5/12, and after 80 days, 6/12. This effect was somewhat magnified if a 30-day interval was allowed to elapse between the *B. pertussis* and tumor inoculations. In this experiment, 7/12 deaths were noted after 20 days, 8/12 after 30 days, and 9/12 after 80 days. The results are also depicted in Table 1.

| DAYS BETWEEN | EXP. 1 | EXP. 2 | EXP. 3 |
| B. pertussis | 16 | 20 | 30 | 80 | 16 | 20 | 30 | 80 |
| S-180 inoculations | | | | | | | | |
| Control* | 6/6† | 2/6 | 5/6 | 5/6 | 0/6 | 6/6 | 0/6 | 0/6 | 0/6 |
| 0 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 7 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 12 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 18 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 24 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 30 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |

* No pertussis.
† Mortality = no. animals dead/no. animals injected.

Lipide and lipopolysaccharide fractions.—These results, which are recorded in Table 2, indicate that protection is afforded by the lipopolysaccharides but not by the lipides. The challenge with tumor was made 12 days after inoculation with the corresponding lipopolysaccharide or lipide and resulted after 20 days in 1/12, 2/12, 3/6, and 6/6 deaths, respectively; after 30 days in 5/12, 2/12, 6/6, and 6/6 deaths; and after 80 days in 7/12 and 4/12 deaths.

Autopsy results.—Animals which died as a result of tumor growth showed almost identical pictures. The peritoneal cavity was filled with ascitic fluid, usually hemorrhagic, and there was a similar fluid present in the pleural cavity. The lungs were collapsed and, together with the liver, were markedly hemorrhagic. Nodules were found on the walls of the peritoneal cavity and on the surface of the organs which were frequently cemented to the walls by tumor.

TABLE 1

MORTALITY FOLLOWING S-180 IMPLANTATION IN B. pertussis-TREATED MICE

| DAYS BETWEEN | 16 | 20 | 30 | 80 |
| B. pertussis and S-180 inoculations | | | | |
| Control* | 0/6 | 1/6 | 0/6 | 0/6 |
| 0 | 0/6 | 0/6 | 0/6 | 0/6 |
| 7 | 0/6 | 0/6 | 0/6 | 0/6 |
| 12 | 0/6 | 0/6 | 0/6 | 0/6 |
| 18 | 0/6 | 0/6 | 0/6 | 0/6 |
| 24 | 0/6 | 0/6 | 0/6 | 0/6 |
| 30 | 0/6 | 0/6 | 0/6 | 0/6 |

* No pertussis.
† Mortality = no. animals dead/no. animals injected.

TABLE 2

MORTALITY FOLLOWING S-180 IMPLANTATION IN LIPOPOLYSACCHARIDE- OR LIPOIDE-TREATED MICE

| AGENT GIVEN | 16 | 20 | 30 | 80 |
| B. pertussis lipopolysaccharide | 1/12* | 1/12 | 5/12 | 7/12 |
| Serratia marcescens lipopolysaccharide | 2/12 | 2/12 | 2/12 | 4/12 |
| Lipoid A | 0/6 | 0/6 | 0/6 | 0/6 |
| S-180 lipide | 0/6 | 0/6 | 0/6 | 0/6 |

* Mortality = no. animals dead/no. animals injected.
In the animals which survived the experimental period and were sacrificed there was little to be found. No ascitic or pleural fluid was noted. Relatively few mice displayed any tumor tissue, grossly. If present, it usually consisted of a small solitary nodule which had to be identified microscopically as being tumor.

**DISCUSSION**

Essentially all the literature relative to any alteration of the regression rate of the mouse tumor, S-180, by external agents is concerned with their effects on tumor more-or-less established in the host. The above described experiments indicate that conditioning of the mouse with *B. pertussis* prior to transplantation can effectively influence tumor "takes." Similar results were obtained by prior treatment with the lipopolysaccharide fraction from *B. pertussis*, and the lipopolysaccharide of *S. marcescens*. Favorable results were reported, also by Old et al. (11), who attempted to transplant S-180 into mice previously infected with B.C.G.

There is no direct evidence for the mechanism of the above as yet, but a hypothesis can be discussed in the light of other related experimental results. It has been noted that certain agents such as bacteria, endotoxins, zymosan, colloidal dyes, products of the tubercle bacillus, etc., stimulate the activity of the R.E.S. cells, thereby enhancing their capacity to respond to antigenic stimulation. There are ample reports which attest to this effect in several species of animals; the enhancement of antibody formation in the mouse and rabbit (7, 9), the enhanced resistance to natural infections in the mouse (3), the enhanced anaphylactic death rate in the mouse and rat (8), etc., which have been found to follow prior pretreatment with *B. pertussis*, the endotoxin of *S. typhosa*, etc. The marked lethal effects of Sarcoma 180 for mice may be due to a lack of natural resistance or failure to stimulate host defenses. Antibody production is recognized as an important mechanism for the latter. The failure of mice to reject tumor cells may result from their poor antigenicity or the inherent inability of this species of animal to react profoundly to antigenic stimulation. The mode of rejection of Sarcoma 180 may be through an antibody-antigen reaction, a result of *B. pertussis*-stimulated R.E.S. activity, with consequent enhanced antibody response, on the assumption that the tumor cells act as an antigen.

An alternate, and conflicting, hypothesis may be derived from the apparent need for the maintenance of the integrity of R.E.S. under certain conditions. With "blockade" of these cells by thorium dioxide, trypan blue, colloidal carbon, etc., a decrease in the resistance of the host occurs. This can be demonstrated by the enhanced response to parenterally administered endotoxins (6, 15). Similarly, increased susceptibility to experimental bacterial infection has been found to follow the administration of *B. pertussis* vaccine (12, 13). Thus, as conditioning of endotoxin or bacteria by the R.E.S. may be a prerequisite for their removal or detoxification, so conditioning of tumor cells may be a requirement for viability, with subsequent successful implantation and multiplication.

It is interesting to note that *Serratia marcescens* lipopolysaccharide is effective in necrotizing established S-180 (16) as well as retarding the pattern of normal growth as observed in this study.

From the above experiments and others a similar effect has been observed among the lipopolysaccharides of unrelated bacteria. Indicating, as it has, the presence of a common moiety, the lipid component of lipopolysaccharide (endotoxin) of *B. pertussis*, lipoid A, was studied but was found to be without effect in retarding "takes." In addition, a lipide, unrelated to the lipopolysaccharides and obtained from S-180, was without effect in altering the growth pattern of the parent tumor. A recent report purports to demonstrate the necrotizing effect of lipoid A on established tumor (10); the effect of S-180 lipide should merit a similar study.

**REFERENCES**


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