Gamma Globulin Potentiation of Proteus Lipopolysaccharide Effect on Sarcoma 180*

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SUMMARY

γ-Globulin in combination with Proteus vulgaris lipopolysaccharide inhibited tumor growth to a much greater extent than did the lipopolysaccharide alone. Combined treatment frequently caused regression and disappearance of the tumor.

The presence of a blood-tumor barrier was considered, as was also its alteration by lipopolysaccharide.

The nonspecificity of the combined γ-globulin-lipopolysaccharide effect on tumor growth is discussed.

It has been reported that γ-globulin potentiates the Shwartzman phenomenon (6). It has also been found that substances which contain Shwartzman preparatory factors1 also produce hemorrhage into and/or regression of tumors (2–5, 11, 17, 19, 20, 24–27). In view of these facts an investigation of the effect of γ-globulin in combination with a Shwartzman active substance (4) on Sarcoma 180 was undertaken.

MATERIALS AND METHODS

A lipopolysaccharide (LPS) preparation extracted from Proteus vulgaris was used in these experiments (4). It was suspended in pyrogen-free saline and placed in a boiling water bath for 15 minutes. The final opalescent solution contained 50 µg in a 0.2-cc. volume or 250 µg in a 0.5-cc. volume.

The γ-globulin, of human origin, was prepared by the Cohn low temperature alcohol fractionation method from outdated blood plasma from various Red Cross Regional Centers.2 The solution used was a 16 per cent concentration of pure γ-globulin powder in 0.3 M glycine with the pH adjusted to about 6.8.

Over 200 C57BL mice, 4–5 months of age, and weighing 21–27 gm., inbred for many generations, were used in this investigation. They were kept in metal cages, housed in an air-conditioned room at 75°F., and fed on Purina Laboratory Chow and water ad libitum. Each mouse received approximately 1 mm. implant (by trocar) from a single freshly dissected Sarcoma 180 free of necrosis in the right axillary region. Therapy was instituted after the tumor had grown to measurable size, usually in a week. The tumors were measured at least every other day to determine the cross-sectional size. (Measurements of the longest diameter of the tumor and of a diameter perpendicular to it were taken through the wet skin by means of vernier calipers.)

When combined γ-globulin-LPS treatment was employed, γ-globulin was administered at least 2 hours before or more than 6 hours after LPS administration, because of the pronounced toxicity with shock, prostration, and often death which occurred when the interval between the two injections was shorter (7).

Experiment I.—Forty female mice, divided into four groups of ten each, were used in this experiment. Therapy was instituted the 7th day after tumor implantation. One group served as control receiving 0.2 cc. normal saline intraperitoneally every 3d day; the second group received 0.2 cc. γ-globulin intraperitoneally every 3d day; the
third group received 50 µg. LPS intraperitoneally every 3d day, and the last group received 50 µg. LPS every 3d day and 0.2 cc. γ-globulin after this injection. The mice were sacrificed 16 days after the tumor implantation (9 days after therapy was initiated), and the tumors were dissected free of surrounding tissues and weighed.

Experiment II.—Forty female mice were divided into four groups of ten each. On the 8th day after tumor implantation one group was given injections intraperitoneally of 0.5 cc. of normal saline (control), another group of 0.5 cc. of γ-globulin, a third group of 250 µg. of LPS, and the last group of 0.5 cc. of γ-globulin, followed by 250 µg. LPS. The mice were sacrificed 17 days after tumor implantation, and the tumors were dissected free of surrounding tissues and weighed.

Experiment III.—Thirty male mice in three groups of ten each were used. On the 8th day after tumor implantation one group was given injections intraperitoneally of 0.5 cc. of normal saline and served as control, another group of 250 µg. of LPS, and the third group of 250 µg. of LPS followed by 0.5 cc. of γ-globulin. The animals were sacrificed 14 days after tumor implantation, and the tumors were dissected free of surrounding tissues and weighed.

Experiment IV.—Twenty-seven female mice in three groups of nine each were employed. The 8th and 10th day after tumor implantation one group received, intraperitoneally, 0.5 cc. of normal saline and served as control; another, 0.5 cc. of γ-globulin; and the third group 250 µg. of LPS. On the 11th day after tumor implantation each group was subdivided at random into two subgroups of three to five mice. One of the subgroups in each group received a third injection of the substance originally administered, while the other received intraperitoneally 0.5 cc. of γ-globulin following or preceding an intraperitoneal injection of LPS. In total, thirteen mice received this combined LPS-globulin treatment. The mice were not sacrificed and were used for other experiments.

Experiment V.—Forty female mice were divided into four groups of ten each. The 6th day after implantation 0.5 cc. of normal saline was injected intraperitoneally into one group that served as control, 0.5 cc. of γ-globulin into another group, 250 µg. of LPS into a third group, and 0.5 cc. of γ-globulin followed by 250 µg. of LPS into the last group. On the 10th day after transplantation the control, the γ-globulin, and the LPS-treated groups were subdivided into two subgroups of four to six animals. One subgroup in each group received a second injection of the substance originally administered, while the other received 0.5 cc. γ-globulin intraperitoneally preceding or following an intraperitoneal injection of 50 µg. LPS. In total, 22 mice received this combined LPS-globulin treatment. The mice were not sacrificed and were used for other experiments.

RESULTS

Experiment I.—Measurement of the cross-sectional size of the tumors at the beginning, during the course of, and at the end of therapy showed clearly that the tumors in control mice and those treated with γ-globulin alone grow rapidly. In some of the latter mice the rate of tumor growth was faster than in the control group. The LPS by itself inhibited tumor growth, but this was moderate in comparison with LPS combined with γ-globulin which produced striking regression of the tumor (Table 1). Chart 1 shows that, during the period of therapy, all tumors in the saline control and the γ-globulin-treated group increased in size; in the LPS-treated group six of ten tumors increased in size, three remained relatively stationary, and one receded slightly; whereas in the combined γ-globulin-LPS-treated group all the tumors decreased in size, four regressed completely, and none increased in size. Table 2 shows that at termination of the experiment, after 9 days of therapy (10 days after tumor implantation), the average tumor weight in the control group was 685 ± 132.9 mg., in the γ-globulin group, 300 ± 96.3 mg.; the LPS group, 316 ± 66.7 mg.; and in the γ-globulin-LPS group, 64 ± 17.7 mg. Statistical analysis reveals that the differences between the control group and the γ-globulin-LPS group are highly significant (0.001 > P). The differences between the LPS group and the combined γ-globulin-LPS groups are also within accepted levels of confidence (0.02 > P > 0.01).

Experiments II and III.—The rate of tumor growth and the tumor weights in each group at termination of the experiments are summarized in Tables 1 and 2.

Experiments IV and V.—During the first phase of these experiments all the tumors in the control and γ-globulin groups grew rapidly. In the LPS-treated group there was some inhibition of tumor growth, but no regression was noted. In the second phase each group was subdivided into two subgroups. The subgroups in which the original treatment was continued showed no change in the rate of tumor growth. In contrast to this, most of the tumors regressed in the subgroups which received the combined LPS-γ-globulin treatment. Table 1 shows the comparative effect of LPS alone and γ-globulin.
TABLE 1

**EFFECT OF LIPOPOLYSACCHARIDE ALONE AND IN COMBINATION WITH \( \gamma \)-GLOBULIN ON GROWTH OF SARCOMA 180**

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Lipopolysaccharide</th>
<th>Lipopolysaccharide and ( \gamma )-globulin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total treated</td>
<td>Decrease in tumor size</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>In all exp.</td>
<td>42</td>
<td>10</td>
</tr>
</tbody>
</table>

All arabic figures indicate number of mice.

* In the control mice and those treated with \( \gamma \)-globulin alone, all the individual tumors steadily increased in size during the course of the experiment.

combined with \( \gamma \)-globulin on the rate of tumor growth.

The sex difference had no significance, which is in accord with the previous observation that Sarcoma 180 grew equally in both sexes after implantation from the same batch of donor tumor (18). During the course of these experiments most of the mice lost weight. The average loss in net body weight (original body weight before tumor growth minus body weight after tumor removal at termination of experiment) was 4.25 gm. in the control (tumor) group, 2.9 gm. in the \( \gamma \)-globulin-treated group, 3.9 gm. in the LPS group, and 2.5 gm. in the LPS-\( \gamma \)-globulin group. The tumor weights from the five separate experiments cannot be compared with one another, since these experiments were carried out at different times and with different donor tumors.

From the over-all results of the five experiments

TABLE 2

**EFFECT OF LIPOPOLYSACCHARIDE, \( \gamma \)-GLOBULIN, OR COMBINATION OF BOTH ON GROWTH OF SARCOMA 180**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Average tumor weights* at termination of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>I</td>
<td>685±138</td>
</tr>
<tr>
<td>II</td>
<td>828±200</td>
</tr>
<tr>
<td>III</td>
<td>1055±80</td>
</tr>
</tbody>
</table>

* Average tumor weights are given in mg. with their standard error.
it is apparent that combined LPS-γ-globulin treatment caused regression of the tumor in the majority of the animals (in 73 per cent), whereas LPS alone had this effect only in a limited number (in 23 per cent). No regressions were noted in the control and γ-globulin-treated groups. It may be added that in the LPS-γ-globulin group the tumor regressed to more than 50 per cent of the tumor size at the initiation of treatment, and in four cases the tumor disappeared. In the LPS group few tumors regressed more than 50 per cent, and none regressed completely.

DISCUSSION

From the data herein reported, it is apparent that the inhibiting effect of the LPS on tumor growth is potentiated by γ-globulin.

The actual mechanism of globulin potentiation of the LPS effect remains obscure. A possibility to be considered is interaction of γ-globulin components with the LPS, since toxicity of the LPS is so strikingly increased by γ-globulin (7), nephotoxic sera (13), and in animals previously given injections of brucella species (1). In all these instances interaction between circulating immune globulins and LPS might be responsible for the exaggerated toxicity. Such interaction between the two substances already has been considered in the potentiation of "endotoxin"-produced fever (15). The tumor-retarding effect of LPS alone likewise might be due to interaction of LPS with the normally circulating γ-globulin. It has been reported that γ-globulin contains tumor-inhibiting components (TIP) (14). It is possible that it also contains growth-enhancing factors as other serum proteins do (21), in balance with the tumor-inhibiting components. The LPS interacting with γ-globulin may upset this balance with a resulting predominance of tumor-inhibiting factors.

Also to be considered is the presence of a blood-tumor barrier (7, 10), which prevents the globulin-inhibitory factors from reaching the tumor cells proper. The LPS may break down this barrier, just as trypsin and other proteolytic enzymes break the blood-brain barrier, or they may be effective through activation of bradykinin, a nonapeptide, known to increase capillary permeability. The postulated barrier is not necessarily located between intravascular and interstitial fluid compartments, since selective impermeability of the intact tumor cell could also act as a functional barrier.

The combined γ-globulin-LPS effect is probably nonspecific, since lipopolysaccharides derived from unrelated materials such as tumors (5), bacteria, and normal mammalian tissues (4, 11, 24) are all capable of producing the Shwartzman phenomenon as well as hemorrhage into tumor. This nonspecificity is also in accord with the finding that the local Shwartzman phenomenon is more pronounced in rabbits with extensive coccidiosis or other chronic infections (6, 12), the antigens of which bear no known antigenic relationship to Shwartzman-active substances.

REFERENCES

5. - . The Use of Tumor Extracts in the Production of the Shwartzman Phenomenon. Ibid., pp. 334-37.
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