THE INFLUENCE OF ZYMOSAN AND ITS FRACTIONATION PRODUCTS ON THE LEVEL OF PROPERDIN IN THE BLOOD AND ON THE MORPHOLOGY OF ORGANS AND TISSUES

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The biological activity of the polysaccharide zymozan is related to its influence on the properdin system of the blood. Also it is known that the most characteristic feature of the properdin-zymosan (PZ) complex, that of inactivating the third component (C'_3) at 37° is not manifested in experiments in vivo. Furthermore the formation of the complex PZ in the blood-stream is itself subject to doubt because the effectiveness of zymosan does not increase with increase of dosage. All these facts indicate that the mode of action of the foreign high-molecular polysaccharide zymosan may be different according to whether it is injected into the organism or acts in vitro.

In the present investigation we have used both zymosan and its fractionation products, which are partially or completely inactive with respect to the properdin system. Before and at various times after the injection of the polysaccharides we investigated the properdin blood level and in addition we made a histological study of the condition of the organs and tissues.

EXPERIMENTAL METHOD

For the experiment we used 750 mice weighing up to 20 g. For the injection we used zymosan (series No. 46) obtained from compressed baker's yeast, and containing an admixture of brewer's yeast, as well as zymosan (series No. 1) separated from a pure culture of Saccharomyces cerevisiae. Both zymosans were prepared by the method of Pillimer and Ecker [9], modification No. 2 of Rutberg [6]. In one experiment we used as a control the zymosan of Fleishman, which was used by Pillimer et al. [11] and which was investigated by A. A. Poznanskaya and E. L. Rozenfel'd [3].

The glucan (insoluble) and glucomannic (soluble) fractions were obtained by E. L. Rozenfel'd, A. A. Poznanksya, and N. K. Rudakova by alkaline hydrolysis of the zymosans of the series we have mentioned [4].

As our experiments showed zymosan separated from compressed yeast is able to bind properdin and to inactivate C'_3 at 37°; zymosan obtained from a pure culture of Saccharomyces cerevisiae also effectively binds properdin, but is much less effective in inactivating C'_3; glucan scarcely affects the titer of C'_3 from human serum, while glucomannan is completely inactive with respect to the properdin system.

To obtain an even suspension of zymosan the insoluble fraction was triturated in a mortar with 0.9% physiological saline and homogenized for 20 min in a homogenizer. The resulting suspension was poured into ampoules which were sealed, and then autoclaved for 30 min at 1.2 atmospheres.

The zymosan and its fractions were given into the tail vein of mice as a single injection of 0.2 ml containing 5, 25, or 125 mg/kg of the substance. In three experiments three injections of zymosan were given at intervals of one day, and in another experiment the interval was three days. The mice were killed at various times up to 12 days after the injection (after 1, and 4 h, and 1, 2, 3, and 4 days etc.). For each test blood was collected from five mice;

Titration of Properdin

First stage (1 h at 37°)					Second stage (30 min at 37°)		
No. of sample	serum (in dilution 1:4)	barbital buffer	suspension of zymosan (10 mg/ml)	RP (serum without properdin)	number of test	R_3 (of serum without C'_3)	sensitized sheep erythrocytes
1	0.4	–	0.1	0.1	1	0.05	0.5
2	0.2	0.2	0.1	0.1	2	0.05	0.5
3	0.1	0.3	0.1	0.1	3	0.05	0.5
4	0.05	0.35	0.1	0.1	4	0.05	0.5
5	0.025	0.37	0.1	0.1	5	0.05	0.5

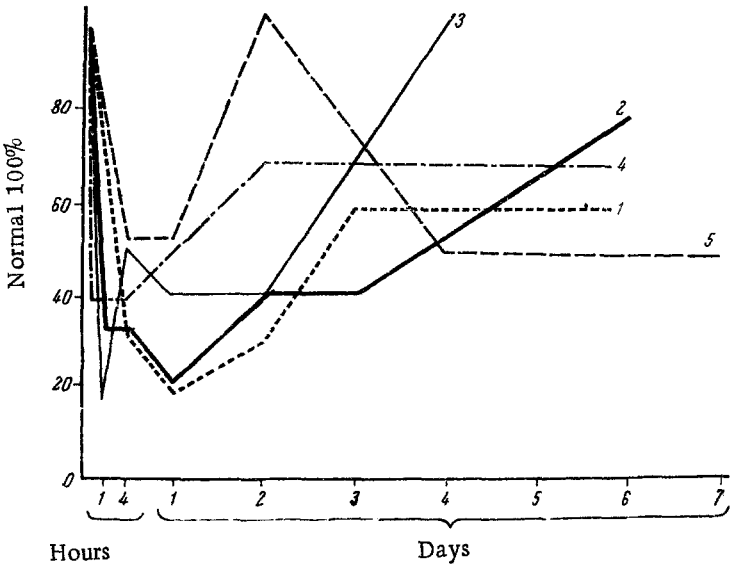

Fig. 1. Titer of blood properdin after the injection of zymosan and its fractions. 1) Injection of zymosan (series No. 46), obtained as a result of 1 injection of 5 mg/kg of compressed baker's yeast; 2) three injections of the same amount; 3) injection of the same dose of insoluble fraction; 4) injection of the soluble zymosan fraction (series No. 1) separated from a pure culture, single dose of 125 mg/kg; 5) single dose of 25 mg/kg.

a post mortem examination was carried out on one of the mice; histological studies were made of the liver, lungs, kidneys, lymph nodes, spleen, and bone marrow.

As a control we used the blood of five healthy mice decapitated on the day of the experiment. Titration of properdin was carried out on all tests simultaneously at the end of any set of experiments. Before titration the samples were preserved at −16°. Properdin was titrated by the method of Pillemer et al. [10] or of Isliker and Linder [8] as modified by us (R. A. Rutberg) (see table).

After incubation and centrifugation the tube showing 50% hemolysis was identified; in the first tube there was 1 unit/ml of properdin serum, in the second 2 units/ml, etc.

RP was prepared from human blood serum (mixture of 3-4 sera) by twice repeated incubation with zymosan (3 mg/ml at 15°).

Before the second incubation fresh guinea pig serum was added until the original titer of complement was restored (approximately 1/10 of the volume).

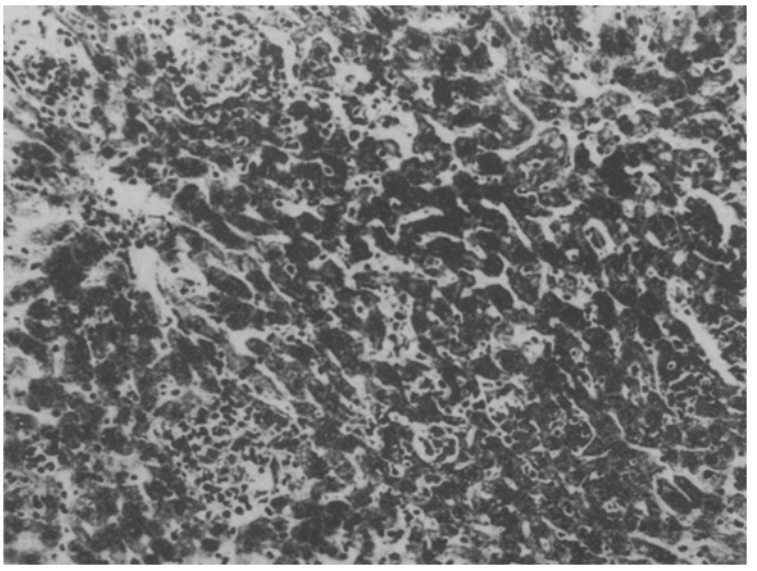

Fig. 2. Numerous necroses in the liver with marked cellular proliferation around them. Micrograph.

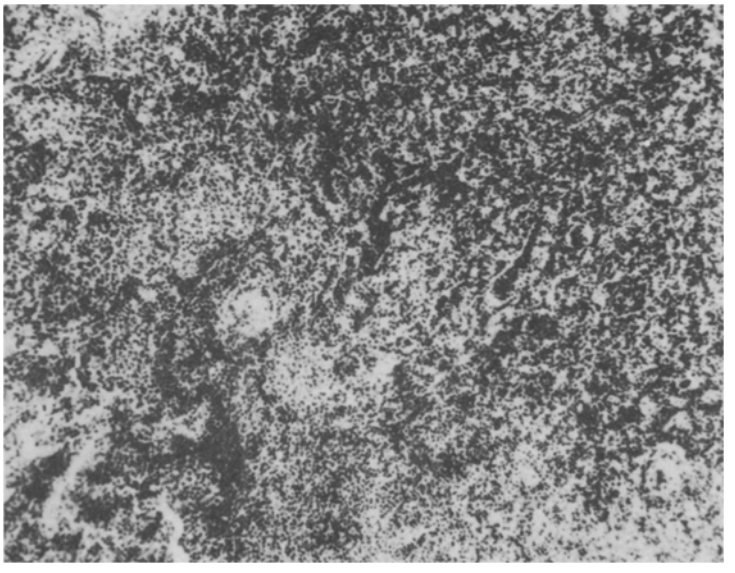

Fig. 3. Proliferation of the reticular cells in the lymphatic node. Micrograph.

The source of R_3 was guinea pig serum treated with zymosan for 1 h at 37° (4 mg/ml). Before the experiment the R_3 was diluted 1 : 8 with barbital buffer.

EXPERIMENTAL RESULTS

The injection into the animal of zymosan (series No. 46) consisting of approximately 50% carbonhydrates, just as in the case of pure carbohydrates in the form of glucomannic (soluble) and glucan (insoluble) fractions leads to quite a rapid reduction in the titer of properdin in the serum, and to subsequent increase to a new level, which however does not exceed the original one (Fig. 1). Similar results were obtained also in the experiment with the Fleishman zymosan (5 mg/kg). The effect occurred when the zymosan was diluted three times at intervals of one or three

days. Despite the more prolonged phase of suppression of properdin activity, which occupied the whole of the period of the zymosan injection (from 3 to 7 days), the nature of the recovery of the normal level of properdin was approximately the same as when a single zymosan injection was given; in this case we were unable to show any increase of titer of properdin beyond normal limits.

The complex PZ which we obtained with optimal saturation of zymosan with properdin was injected into mice as a dose of 50 mg/kg after it had been washed free from serum. The reduction of properdin level was the same as was obtained with the pure zymosan.

In a post mortem study of 97 mice killed at various times after the zymosan and its fractions had been injected, it was found that the changes in the organs were of the same type, and depended chiefly upon the survival time of the animal. When a single zymosan injection had been given (5.25 or 125 mg/kg), no pathological changes occurred within one or four hours; only in certain experiments was it found that there had been some accumulation of zymosan in the cells of the reticuloendothelial system of the spleen, lover, and lungs. Subsequently we were unable to find zymosan in any organs, probably on account of its absorption by the cellular elements.

In animals killed after one day, a swelling of the cells of the liver could be seen, and there was a proliferation of the reticulo-endothelium; in the spleen and lymphatic nodes there was a hyperplasia of the reticular cells. After two days, in some of the experiments small necrotic areas appeared in the liver. After three days the necrotic zones were numerous in some of the experimental series, but occurred only occasionally in others. Proliferation of the reticular cells in the lymphatic nodes and spleen was well marked. Subsequently (on the 4th and 5th days) a cellular reaction was observed in the necrotic areas of the liver (Fig. 2).

By the 7th day an increase of granulation tissue was recorded. Proliferation of the reticular cells was somewhat less than it was on the 10-12th days. At this period in the liver small zones of histiocytic proliferation occurred (Fig. 3). The bone marrow underwent no changes in the majority of experiments.

In the experiments with three injections of either small or large doses of zymosan the proliferative reaction was more marked, and the general picture of changes in the liver was rather reminiscent of what is observed in experimental leucoses in mice. By contrast with leukosis, no diffuse leukemic infiltration was observed anywhere but in the liver.

After 10-12 days, in some experiments a marked hyperplasia was found in the bone marrow.

In parallel studies on control animals killed without previous injection of zymosan no changes could be observed in the viscera.

The changes we have described followed a single injection of either small or large doses of zymosan. The dystrophic changes in the liver which took the form of a swelling of the cells began to be apparent on the first day, a time which coincided with the maximum reduction in the blood properdin level. The most marked necrotic changes occurred on the third day when the blood properdin level began to return to normal. At this time a strong reaction could be observed, and consisted of a vigorous proliferation of cells both in the necrotic areas and outside them.

When the mice were injected with zymosan and with soluble and insoluble fractions, or with Fleishman's zymosan, the anatomical changes in the viscera were identical.

When a comparison is made of our results with those reported elsewhere [1, 2, 10], the following fact stands out. Injection of zymosan separated by various methods and differing in their content of protein and carbohydrate, and in the products of the fractionation of zymosan, which appeared to be almost pure carbohydrates, the primary reaction in all animals (dogs, mice, and rats) was the same, and consisted of a fall in the properdin titer and stimulation of the reticuloendothelial system. At the same time the nature of the recovery of the original blood properdin level was subject to variation. Thus in small concentrations zymosan obtained by the classical method of Pillemer and Ross, [10] or separated by the method of S. L. Karmanova [2] by enzymatic hydrolysis and extraction in phenol and water, caused an increased titer of properdin to ensue immediately, after a transitory fall, and then to return to normal. However zymosan as obtained by us exerts no stimulating influence on the blood properdin system.

At the same time the zymosans we have described were active with respect to the properdin system in vitro. The divergences to which we have drawn attention are not to be attributed to the methods of titration of properdin,

because the results differed amongst almost all the authors we have quoted. The impression is gained that the variability of the responses to the injection of zymosans obtained by different methods is chiefly related to the structure of these high-molecular polysaccharides.

According to Riggi and Luzio [12] the stimulating influence on the reticulo-endothelial system is associated with the glucan fraction of the zymosans, whose chemical basis depends upon β-1−3-glucocide linkages. At the same time E. L. Rozenfel'd et al., have shown that activity with respect to the properdin system in vitro is manifest only when the β-1−6-linkages in the zymosan or in its fractionation products are preserved [5].

SUMMARY

Intravenous injection into mice of zymosan and fractions isolated from it led to a reduction of properdin titer and to dystrophic changes mainly in the liver. By the time the blood properdin titer had returned to normal a considerable proliferative reaction of the reticulo-endothelial cells had occurred. These changes were of the same type whether zymosan, glucan, or glucomannic fractions, all partially or completely inactive with respect to the properdin system in vitro were administered.

LITERATURE CITED

1. G. A. Gankevich, Z. P. Karmanova, T. A. Krotova et al., The Pathogenesis, Clinical Management, Therapy, and Prophylaxis of Radiation Sickness [in Russian], Leningrad (1957), p. 40.
2. Z. P. Karmanova, In the book: Problems of the Leucoses and Immunohematology [in Russian], Leningrad (1960), p. 304.
3. A. A. Pozenznanskaya and E. L. Rozenfel'd, Biokhimiya, No. 4 (1960), p. 624.
4. E. L. Rozenfel'd, A. A. Poznanskaya, and N. K. Rudakova, Dokl. AN SSSR, Vol. 125, No. 4 (1959), p. 928.
5. E. L. Rozenfel'd and M. E. Preobrazhenskaya, Biokhimiya, No. 2 (1962), p. 214.
6. R. A. Rutberg, Dokl. AN SSSR, Vol. 125, No. 4 (1959), p. 931.
7. N. L. Samoilina, Probl. gematol., No. 1 (1961), p. 23.
8. H. C. Isliker and E. Linder, Vox Sang. (Basel), Vol. 3 (1958), p. 23.
9. L. Pillemer and E. E. Ecker, J. biol. Chem., Vol. 137 (1941), p. 139.
10. L. Pillemer and O. A. Ross, Science, Vol. 121 (1955), p. 732.
11. L. Pillemer, L. Blum, I. H. Lepow et al., J. exp. Med Vol. 103 (1956), p. 1.
12. S. J. Riggi and N. R. Luzio, Fed. Proc., Vol. 20, Pt. 1 (1961), p. 265.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.