Immunogenicity of Toxic and Detoxified Endotoxin Preparations (32790)

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Preparation and biological properties of chemically detoxified endotoxins (endotoxoids) have been previously reported (1, 2). It was shown that rabbit and mouse lethality and pyrogenicity were abolished or reduced, while adjuvant effect and enhancement of nonspecific resistance were not impaired (3). Immunogenicity of the preparations was also investigated, and it was found that some of the detoxifying processes did not seem to affect the antigenicity of the materials. Similarities between some endotoxic effects and hypersensitivity reactions (4–7, 11) indicated that toxicity of endotoxin may be related to its immunogenicity. Therefore, it became necessary to conduct investigations on the immunoglobulins produced by the nontoxic endotoxoid preparations.

Materials and Methods. Preparation of endotoxin. Serratia marcescens cells were extracted with trichloroacetic acid. The extracts were purified as described earlier, using precipitation with ethanol and sucrose density gradient centrifugation (8). The preparations obtained were dialyzed and lyophilized.

Preparation of endotoxoid-2. Detoxification of the above preparation was achieved by potassium methylate (CH_3OK) treatment, as described earlier (1). Determination of toxicity was carried out by measuring mouse LD_{50}, pyrogenicity, and local Shwartzman reactivity (3).

Immunization. New Zealand white rabbits weighing 4–6 lb were used in all experiments. Five rabbits were injected with each dose of each preparation. Two types of immunization schedule were used: (a) Intravenous injection of the preparation every 3–4 days, in increasing doses from 20 to 800 μg, for 4 weeks. Before the injections were given, blood samples were withdrawn from the rabbits' ear veins on the same day. Three different antigens were used in this experiment: toxic endotoxin, endotoxoid-2, and heat killed whole cells of the same strain. (b) Rabbits received a single i.v. injection of 10, 1, or 0.1 μg doses of the antigens. Approximately 4 weeks after the first immunization, a second injection was given applying the same dose of the same material. The booster injection was repeated once more, approximately 8 weeks after the first injection.

Titration. Blood samples were taken in 3–4-day intervals and circulating antibody titers were determined by the passive hemagglutination method (9).

2-Mercaptoethanol (2-Me) resistance of the antibodies was determined as follows: To 0.2 ml of serum, 0.2 ml of 2% 2-Me was added, mixed, and allowed to stand at room temperature for 60 min. By adding 1.6 ml of saline to this mixture, a 10-fold serum dilution was achieved. The antibody titer of the samples was determined by passive hemagglutination, in the same manner as the titer of the untreated sera.

Results. If rabbits were injected every 3–4 days for 4 weeks, as shown in Fig. 1, with heat-killed whole cells or with toxic endotoxin, a rapid increase of total antibody titer could be observed. The rate of increase and the final titer obtained were identical. The production of 2-Me resistant immunoglobulins started simultaneously with the production of 2-Me sensitive immunoglobins.

The antibody production elicited by the same amount of endotoxoid-2 showed a different picture. The total antibody production had a slower initial rate, but after several repeated injections, it reached a titer similar to that elicited by toxic endotoxin or by whole cells. The antibodies produced in the first 10 days consisted almost exclusively of 2-Me sensitive immunoglobulins. The production of resistant antibodies became measurable only after this time. These results are shown in Fig. 1.

In the second type of immunization, shown in Fig. 2, these differences became even more obvious. A single injection of 0.1, 1, or 10 μg

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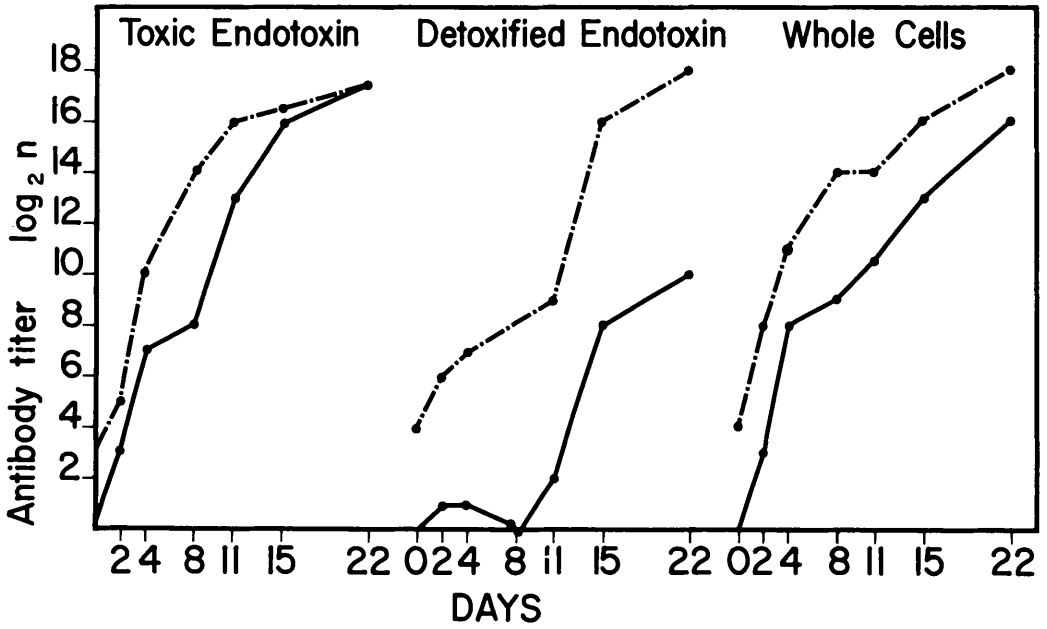

FIG. 1. Antibody production by endotoxin, endotoxoid, and whole cells of *Serratia marcescens*, effect of several times repeated injection in increasing doses. (—●—) total Ig; (-) 2-Me resistant Ig.

of toxic endotoxin resulted in the usual antibody production, which reached a peak in 13–17 days, then started to decline slowly. The injection of the same amount of endotoxoid-2 produced a much lower total antibody level. A second injection of the toxic material showed a slight further elevation, and a third injection a month later did not give a significant further increase. In contrast to this, the booster endotoxoid-2 injection elevated the antibody titer rapidly and after the third injection, a further increase could be observed, which brought the total Ig level elicited by the two preparations close to each other. Lower doses of toxic or nontoxic preparations resulted in a lower antibody production.

The production of 2-Me resistant antibodies was practically zero after one endotoxoid-2 injection, and it became somewhat elevated but still remained relatively low in the booster response.

Discussion. It is reasonable to assume that the chemical detoxification reaction is incomplete, a few percent or less of the toxophore groups may remain unaltered during the treatment. Complete detoxification would require such aggressive treatment of the substance as to introduce unwanted destruction of other structural parts of the macromolecule. Therefore, it had to be considered that the retention of certain biological properties, such as the adjuvant effect, stimulation of nonspecific resistance, or antigenicity, is due to residual endotoxins present in the endotoxoids.

In order to test whether residual toxic endotoxin is responsible for the immunogenicity of endotoxoid-2, different concentrations were used for immunization, as shown in Fig. 2. If the endotoxoid-2 contained 1 or 10% residual toxicity, 10- or 100-fold amounts of this preparation should give antibody production comparable to toxic doses. The results obtained do not substantiate this possibility. Primary antibody production of 10 μg of endotoxoid-2 is still much lower than that of 0.1 μg of toxic endotoxin. This means that if the endotoxoid 2 contains residual toxic endotoxin, it must be much less than 1%.

Comparison of the antibody response elicited by toxic and detoxified endotoxin preparations reveals a qualitative difference. Whether the lack of 2-Me resistant antibody production is a consequence of lack of toxic

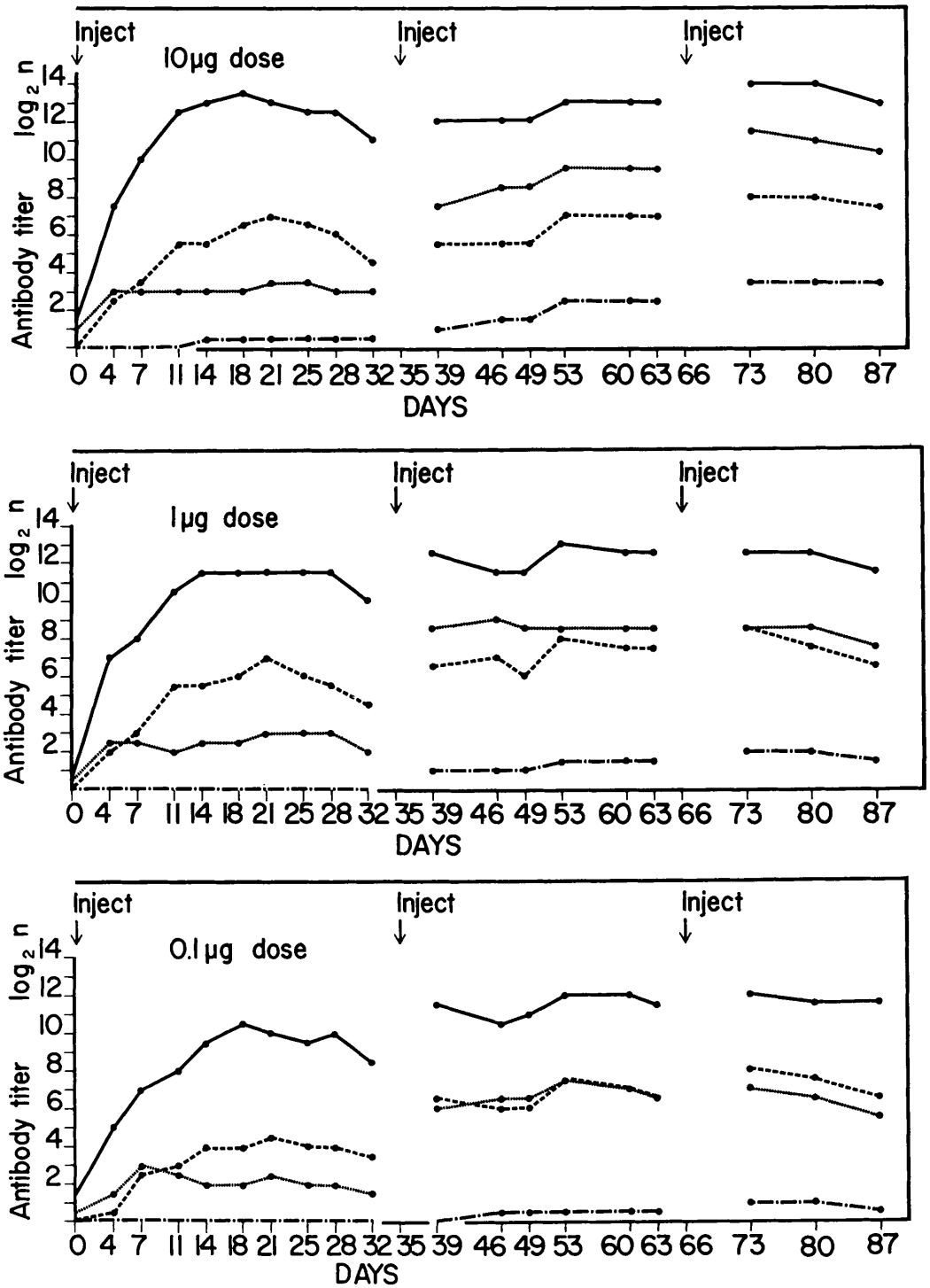

FIG. 2. Antibody production by endotoxin and endotoxoid. Effect of single injections in different doses and effect of booster injections. Injected with toxic endotoxin: (–) total Ig; (- - -) 2Me resistant Ig. Injected with detoxified endotoxin: (. . .) total Ig; (——) 2Me resistant Ig.

structural subunits or these phenomena merely coincide is the subject of current investigations.

In connection with the fact that primary antibody response to endotoxoid-2 is very low, recent results of this laboratory should be mentioned (10). It was shown that while toxic endotoxin is taken up very rapidly by RES cells, endotoxoids are not taken up and remain in the circulation for a longer time. This was measured in normal BRVR mice. If the mice were immunized by toxic endotoxin and the RES uptake of different endotoxoids was measured, a rapid uptake could be observed. This explains the significant effect of the booster injection. This observation is also in agreement with findings reporting that several-times repeated injections of endotoxoid-2 result in an antibody titer comparable to the toxic preparation.

Summary. Several-times repeated injections of heat-killed cells, toxic endotoxins, or CH_3OK detoxified derivative thereof (endotoxid-2) result in a comparable antibody titer after 8–10 injections. The antibody titer produced by toxic preparations shows a rapid initial increase, while endotoxoid-2 produces a lower titer of circulating antibodies in the first 10 days. While toxic preparations produce both 2-Me resistant and sensitive antibodies from the beginning of the immunization, endotoxoid-2 produces almost exclusively 2-Me sensitive antibodies in the first 10 days. Simultaneous with the appearance of 2-Me resistant antibodies, the level of total antibodies rises rapidly. A single injection of toxic endotoxin produces a rapid antibody production which starts to decline after approximately 2 weeks. Booster injections bring this antibody level back to previous highs, but hardly any additional effect can be observed. A single injection of endotoxoid-2 gives a very slow output of antibodies, which seems to remain stable for 1 month. Booster injection of endotoxoid-2 results in a strong increase of antibody production.

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The Effect of Reserpine Administration on Vanillylmandelic Acid Excretion in Man (32791)

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Previous studies have shown that the acute administration of reserpine in man is associated with an increase in the urinary excretion of 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA) in both normal and schizophrenic males (1, 2). This finding is consistent with those in animal studies which show that acute reserpine administration induces a depletion of catecholamines in all body tissues (3, 4) and an increase in blood