Type III (Arthus) allergic reactions and endotoxin toxicity in guinea-pigs[1]

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Summary. Lipopolysaccharide (endotoxin) from *E. coli* cells produced lethal effects in guinea-pigs. Endotoxin caused no visible dermal change in normal animals, but produced skin reactions characterized by specific Arthus-type (Type III immune hypersensitivity) vascular inflammation in immunized animals. It is concluded that Arthus allergic reactions were evoked by endotoxin, however, endotoxin lethal toxicity appears independent of this process.

Lipopolysaccharide complexes termed endotoxins may be derived from the cell walls of gram-negative bacteria by heat or by chemical methods[3]. These extracts produce a diverse range of biological effects when injected into mammals, including severe, frequently lethal, cardiovascular disorganization (shock)[4-7]. It is thought that these agents produce the shock state frequently associated with sepsis in man[8,9].

The mechanism of action of these endotoxins on the circulation remains unknown. Suggested mechanisms range from direct pharmacological effects on smooth muscle[10-12] and interactions with auticoid mechanisms[6,10,11] to immunologically mediated allergic reactions[13]. In the absence of knowledge of toxicity mechanisms, rationally based therapy is difficult to devise. This reflects in the high mortality rate associated with septic shock in humans[8,9]. The consensus of published reports is that endotoxins are unlikely to act through direct effects on components of the circulation[4-6]. The analysis of indirect effects cannot be separated from the question of the involvement of immunopharmacological reactions releasing vasoactive amines, peptides and lipids[14].

Immune toxicity (hypersensitivity) reactions may be divided into 4 classes of reaction, distinguished from each other by the type of immune mediators combining, and their modes of interaction[15]. Only the 2 classes exhibiting 'immediate' reactions[15] to antigen challenge are likely to be involved in endotoxin toxicity on the basis of congruity of time course[4,15] and capacity to release vasoactive mediators of the type thought to be involved in endotoxin-induced vascular changes[4,5,14]. These classes of reaction are termed Type I (anaphylactic) and Type III (immune-complex; Arthus) respectively.

It has been established that the toxicity in guinea-pigs is not dependent on anaphylactic reactions[16] leaving Type III mechanisms as the only immuno-pharmacological reactions likely to be involved. Accordingly the aim of these studies was to test for Type III reactivity to endotoxin in guinea-pigs using classically described dermal biopsy techniques[17] in parallel with toxicity testing.

Materials and methods. Test solutions of endotoxin were freshly prepared from distilled water stock solutions of a trichloracetic acid extract of *E. coli* 0111:B4 cells (Difco Labs., Detroit, Michigan). Toxicity testing was carried out using normal adult guinea-pigs. Animals were given 2 mg/kg doses of endotoxin by i.p. or i.v. injection. 6 adult guinea-pigs given 0.1 ml intradermal injections both of physiological saline and 2×10^{-4} g/ml solutions of endotoxin in physiological saline. Test sites on the skin surfaces were inspected at 4 and 8 h after injection, then animals sacrificed and injection sites autopsied. Tissues were fixed in formol-saline, then sections were cut using conventional histological techniques and stained with haematoxylin and eosin.

5 guinea-pigs were experimentally immunized with endotoxin. The footpads of each animal were injected with 0.4 ml of a 1:1 mixture of complete Freund's Adjuvant and a 2.5 mg/ml solution of endotoxin in distilled water. For 8 days from the 14th day after 'priming', the animals were 'boosted' every alternate day by 0.1 ml intradermal injections of a 2×10^{-4} g/ml solution of normal saline into the mantle region. Animals were then tested for dermal reactivity as described for controls. A pool of immune serum was made by bleeding animals immunized as above. Control animals were injected i.p. with 3.0 ml/kg the evening prior to intradermal challenge.

Results and discussion. All animals showed toxic reactions and deaths occurred in $^2/_5$ animals given i.p. injections and $^2/_2$ animals given i.v. injections. Intradermal challenge of normal guinea-pigs elicited no visible reactions at the injection sites at 4 or 8 h. A minimal cellular infiltrate was visible at endotoxin injection sites on microscopic examination, however, there was no evidence of vascular inflammation or tissue destruction typical of the Arthus phenomenon[15]. These negative findings indicated that Arthus (Type III) reactions are not involved in endotoxin toxicity in guinea-pigs. However, any final conclusion

1 Acknowledgment. Supported by a grant from the National Health and Medical Research Council of Australia.

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required a demonstration that the techniques used were adequate to detect Arthus phenomena i.e., that this result did not represent a false negative finding. The findings in experimentally immunized animals tended to exclude this possibility. In contrast to the findings in control animals, dermal inflammatory reactions with associated vasculitis and tissue necrosis were elicited in all actively immunized animals. The cells involved in the vascular inflammatory reaction were polymorphonuclear leukocytes. Challenge of passively immunized animals produced skin ulceration and association Arthus vasculitis as observed for actively immunized animals.

This inability to demonstrate Arthus reactivity to endotoxin is consistent with previous findings that this species does not appear to develop antibodies to *E. coli* antigens under natural conditions[18,19]. This lack of immune products to endotoxin possibly reflects the fact that guinea-pigs do not harbor *E. coli* among their natural bowel flora[20,21], and consequently are not normally exposed to *E. coli* antigens.

Bacteriological study of a variety of species ranging from cockroaches to elephants has shown that *E. coli* are present among the bowel flora of most animals; guinea-pigs, rabbits and gerbils represent isolated exceptions[20,21]. This pattern of flora appears to correlate with the development of circulating antibodies in most mammals studied[18]. Consequently the demonstration here that endotoxins may elicit Arthus reactions in immunized animals might have implications for the pathogenesis of at least a component of endotoxin toxicity in the majority of mammals. More importantly, however, it may be concluded from this study that there is an intrinsic toxicity mechanism activated by endotoxins which is independent of this major class of immune, hypersensitivity process. The combination of these findings and previous findings that Type I mechanisms are not involved[16] make it unlikely that immunoglobulin-dependent immune mechanisms play any role in endotoxin toxicity beyond potentiation of other, as yet undefined, toxic mechanisms.

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New antibiotics, trichopolyns A and B: Isolation and biological activity[1]

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11 July 1977

Summary. Polypeptide antibiotics, trichopolyns A and B, were isolated from the culture broth of *Trichoderma polysporum* (Link ex Pers) Rifai (TMI 60146). Assessment of biological activity of the antibiotics against microorganisms was made.

Lentinus edodes, one of the most popular edible mushrooms in Japan, is often prevented from growing by *Hypocrea*, *Trichoderma*, *Gliocladium* and *Cephalosporium*, which causes serious damage for cultivators[5–8]. Especially, *Trichoderma polysporum* (Link ex Pers.) Rifai (strain TMI 60146) has been known to have a strong inhibitory action against *Lentinus edodes*. Recently, the novel cyclic oligopeptide antifungal metabolites, cyclosporins A and C have been isolated from strain number NRRL 8044 of the same species, and their structures have been determined by chemical investigations[9] and X-ray analysis[10]. Our investigations on *Trichoderma polysporum*, strain TMI 60146, resulted in the isolation of 2 new metabolites trichopolyns[11] A and B, which strongly inhibit growth of hymenomycetes as well as other organisms. In this communication, preliminary studies on the structure and the biological activity of trichopolyns are reported.

Material and methods. The filtrate of the culture broth of *Trichoderma polysporum* was concentrated to about $^1/_5$ in volume at 45–50 °C under reduced pressure, and extracted with ether. The ether layer was dried over Na_2SO_4 and concentrated to dryness followed by chromatography over silicic acid. Elution with 10% methanol-acetone gave fractions having a strong inhibitory activity against *Lentinus edodes*. Further fractionation by column chromatography and preparative thin-layer chromatography (TLC) resulted in the isolation of 2 new peptide antibiotics, trichopolyn A as an amorphous solid which was shown to be homogeneous on TLC over silicic acid (R_f 0.29; solvent system, 70% dichloromethane-15% acetone-15% methanol) and trichopolyn B as crystals (m.p. 114–116 °C from dichloromethane-ligroin) whose R_f-value was 0.27 under the same conditions as trichopolyn A. Maximum yields (3–4 mg/l) of trichopolyns A and B were obtained from the culture medium which consisted of glucose (25 g), ammonium tartarate (4 g), KH_2PO_4 (2 g), $MgSO_4$ (1 g) and $FeCl_3$ (0.1 g) in 1000 ml of tap water at initial pH 3.5 under artificial ventilation for 4–5 days.

1 Acknowledgment. We are most grateful to Central Research Division, Takeda Chemical Industry Co. Limited, for determination of antifungal and antibacterial spectra.
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