Effect of Cyclophosphamide on Experimental Infection of Mice against Clostridium chauvoei

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Clostridium chauvoei is a spore-forming anaerobic bacterium, which causes blackleg, a disease with economic impact in cattle, sheep and sometimes other ruminants. Several experimental animals such as the guinea pig and the mouse have been utilized for studies of pathogenesis of this organism and potency tests of the vaccines. Most of these infection models are, however, not clinically relevant because injection of a huge number of the organisms is necessary to cause infection. Calcium chloride is often added to the inoculum because it enhances the pathogenicity of the organisms by stimulating germination of the spores and producing local tissue damage at the inoculation site [1, 5]. The mechanism involved in the natural resistance of such experimental animals to C. chauvoei infection is still not fully understood.

Phagocytic cells such as macrophages and polymorphonuclear leucocytes (PMN) are believed to protect against early infection of bacteria as an effector cell [6, 7]. In the present study, the involvement of such cells in natural resistance of mice was analysed with phagocyte-depleted mice.

The strain of C. chauvoei used in this study was OKINAWA, which is used for vaccine production and the potency tests in mice in Japan [1]. The minimal lethal dose of this organism for mice when injected intramuscularly with a 3% calcium chloride solution was approximately 10 spores.

Outbred female ddY mice aged 6 weeks were inoculated on the middle part of the right thigh muscles with either $2.3 \times 10^2$ spores suspended in 0.25 ml of a 3% calcium chloride solution or $2.3 \times 10^4$ spores suspended in distilled water of the same volume. At different times after inoculation, four mice were sacrificed each time to count the organisms in the thigh muscle of the injected side and the liver as an index of local infection and that of systemic infection, respectively. Each tissue homogenate was prepared with nine volumes of anaerobic solution A [4] with a glass homogenizer. Tenfold serial dilutions were made with the same solution and 0.05 ml of each dilution was spread on TF medium (Eiken) supplemented with 5% defibrinated sheep blood. Colonies were counted after culture at 37°C for 24 hr under anaerobic conditions.

Mice were treated with either carrageenan (CG) or cyclophosphamide (CY) as follows: CG (Sigma, type II) was dissolved in distilled water and injected intraperitoneally (200 mg/kg) 24 hr before infection. CY (Sigma) was dissolved also in distilled water and injected intraperi-
toneally (150 mg/kg) 72 hr before infection.

The effects of addition of calcium chloride to the inoculum on experimental C. chauvoei infection in mice are shown in Fig. 1. Although the mice resisted completely intramuscular inoculation without calcium chloride, all of those inoculated with calcium chloride died within 24 hr as previously reported [1, 5]. Viable bacteria in the infected muscle and the liver were counted at various times after inoculation. In the mice inoculated with calcium chloride, the organism markedly increased to more than $10^7$ g in 12 hr after infection in the injected muscle and to approximately $10^9$ g in the liver. In the mice inoculated without calcium chloride, on the other hand, the organisms in the injected site progressively decreased to an almost undetectable level within 48 hr and no bacterium was detected in the liver during the period.

To analyze the possibilities of different roles of PMN and macrophages in the natural resistance, CG- and CY-treatments of mice were done respectively 24 hr and 72 hr before inoculation of the intact spores without addition of calcium chloride. The fatality and the number of viable organisms in both the muscle and the liver are shown in Fig. 2. Most CY-treated mice (96%) died within 48 hr, whereas all CG-treated mice survived. In the muscle of the CG-treated mice, bacterial growth was observed for 6 hr after inoculation, but elimination occurred thereafter. No bacterium was detected in the liver of CG-treated mice at any time during the experiment as was the case in non-treated mice. On the other hand, progressive growth of the inoculated bacteria was observed in the CY-treated mice. The bacterial growth curves in the injected muscle and the liver were similar each other in non-treated mice inoculated with calcium chloride and the activated spores. The organisms markedly increased to $10^8$ g for 24 hr in the injected muscle and approximately $10^9$ g in the liver.

In general, mice are naturally resistant to C. chauvoei infection, but addition of
calcium chloride to the inoculum enhances the pathogenicity of the organisms. Thus, the chemical compound has been added in experimental clostridial infection for many years. The enhancing effect was attributed to the direct stimulation of germination of the inoculated spores and to the production of localized tissue damage which provides an ideal anaerobic condition for germination of the spores and multiplication of the vegetative cells [5]. These previous findings were confirmed by the present studies. In the mice inoculated with the spores suspended in a calcium chloride solution, the bacteria grew in the injected site but not in the liver before 6 hr after inoculation. Thereafter, the organisms spread into the systemic organs as the viable organisms were detected in the liver (Fig. 1). Therefore, as speculated previously [5], calcium chloride seems to act as an activator in local infection of C. chauvoei. Establishment of the local infection is only an early event. In such an early stage, such phagocytic cells as PMN and macrophage have more important roles in elimination of the inoculated spores than do immunologically reactive cells [6, 7]. CY is a famous drug depleting PMN and macrophage as well as immune lymphocytes [2, 3]. On the other hand, CG is used for selective depletion of the macrophage series [6, 7]. The CY treatment in the present study dramatically increased the fatality of the mice inoculated with the intact spores but the CG treatment did not affect the fatality at all. The death caused by the CY treatment was a result of bacterial growth in the systemic organs (Fig. 2). Therefore, it seems that PMN rather than macrophages are more important effector cells in the protection against infection of this organism.

In addition, these findings suggest another possibility that calcium chloride protects the organisms from elimination by preventing the migration of PMN into the inoculation site. Further studies on the role of PMN in natural resistance of mice are necessary to assess this speculation.

REFERENCES


要約

マウスの Clostridium chauvoei 実験感染におけるサイクロホスファマイドの効果 (短報): 田村 豊・田中正三（農林水産省動物病原検査所）——動物の Clostridium chauvoei に対する自然抵抗性機構を解析するため、サイクロホスファマイド (CY) およびカラギーナンで食細胞活性を抑制したマウスで攻撃菌の推移を観察した。その結果、CY 処理マウスの胸腔発症が認められ、ほとんどのマウスは死亡した。このことから、マウスの本菌に対する防御にはマクロファージより多形核白血球が重要な役割を果していますと考えられた。