

Cellular Immunity to *Listeria monocytogenes* Induced by Sensitization and Challenge with Bovine Gamma Globulin

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Resistance to *Listeria monocytogenes* was demonstrated in guinea pigs undergoing systemic delayed-type hypersensitivity reactions to bovine gamma globulin.

Cellular immunity arises during infections caused by intracellular parasites; it is expressed in macrophages as an enhanced capacity to kill bacteria which would normally multiply within them. This bactericidal activity is effective not only against the primary infecting organism, but also against completely unrelated intracellular microorganisms (7, 9); the continued presence of the primary infection is necessary for its expression (7). On the basis of temporal relationships (5) and transfer by lymphoid cells (6) but not serum (8), Mackaness has suggested that cellular immunity is causally related to a systemic delayed-type hypersensitivity reaction to the primary infecting organism (6). This reaction could be mediated by materials released from actively allergized cells on specific challenge, as is the macrophage migration inhibition reaction (2, 4). This paper presents evidence indicating that resistance to an intracellular pathogen accompanies a systemic delayed reaction to bovine gamma globulin (BGG) in the absence of a primary infection.

Listeria monocytogenes NCTC 7973 was maintained and cultured on Brain Heart infusion agar (Difco) throughout. Bacterial counts were performed by using tryptone-soy-agar. Random-bred Dunkin-Hartley guinea pigs were used for these experiments and were sensitized to BGG by injection into each footpad of 0.05 ml of an emulsion of equal parts Difco Freund's complete adjuvant (FCA) and a solution (10 mg/ml) of BGG in saline. A control group was injected with an emulsion of FCA and saline only. After 3 to 4 weeks, when sensitized animals invariably show strong dermal delayed hypersensitivity to BGG, they were tested for resistance to *Listeria* infection.

Half the BGG-sensitized animals received an intraperitoneal challenge of 100 μ g BGG in

saline, and the remainder were untreated. The FCA-treated controls were also "challenged" with BGG. At 3 to 4 hr later, all groups were given 10^8 viable *Listeria* intraperitoneally and a group of normal animals was similarly infected. Growth of the organism was followed in the spleens of the infected animals, by using the methods described by Mackaness for mice (5). On days 1 to 3 after infection, 5 animals from each group were killed and their spleens were removed and homogenized in distilled water, by using a Teflon-glass homogenizer. The amount of water was adjusted to give 5 ml of homogenate for each spleen. Appropriate dilutions of the suspension were plated out on well-dried agar, bacterial counts were recorded, and the number of *Listeria* per spleen was calculated for each animal.

Figure 1 represents pooled data from four replicate experiments: spleen content of *Listeria* is expressed relative to the value for normal animals on day 2 of each experiment, when counts were most consistent. Due to the variability of individual counts, ranking methods were used to analyze these results and *P* values were estimated by the application of the binomial theory over the whole experimental series. It is evident that BGG-sensitized guinea pigs specifically challenged before infection are more resistant to *Listeria* infection than are normal animals ($P = 0.01$) or sensitized but unchallenged animals ($P = 0.04$), as measured by the growth of the organism in the spleen. Unchallenged but sensitized animals and FCA-treated controls which received a "challenge" dose of BGG show a significant, but lesser resistance to *Listeria* infection, but there is no detectable difference between these two control groups. This effect may be due to nonspecific activation of the macrophage

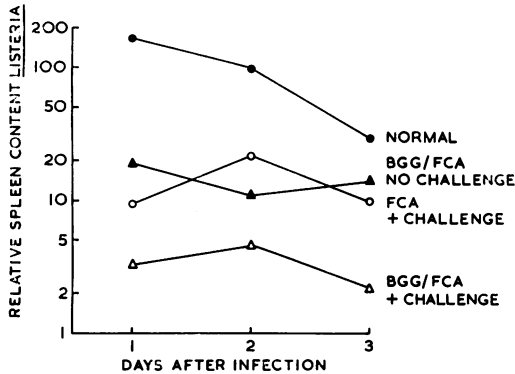


FIG. 1. Relative growth of *Listeria monocytogenes* in spleens of normal guinea pigs (●); animals immunized with FCA alone and "challenged" with 100 μ g of BGG (○); animals immunized with BGG-FCA but not challenged (▲); animals immunized with BGG-FCA and challenged with 100 μ g of BGG (△).

population by the mycobacterial constituents of the adjuvant (1, 3).

Washed lymphoid cell suspensions from the spleens and regional lymph nodes of BGG-sensitized guinea pigs were transferred to normal animals, each receiving about 3×10^8 viable lymphoid cells and, by using the experimental design described above, these passively sensitized recipients were examined for resistance to *Listeria*. Preliminary results indicate that when specifically challenged, such recipients acquired resistance similar to that found in actively sensitized animals in 5 out of 7 experiments, whereas no effect was seen when serum was transferred (Dodd, *in press*).

Thus, specific systemic challenge of BGG-sensitized guinea pigs will induce a degree of resistance to *Listeria monocytogenes*. Preliminary studies on the passive transfer of the effect suggest that the response is cell-mediated, although the participation of antibody is not completely excluded. This system may be a valid model for cellular immunity as described by Mackaness, and, if so, the theory that it is induced by a systemic delayed-type hypersensitivity reaction is favored.

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