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Pavlovian conditioning with cyclosporin enhances survival from infectious peritonitis

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In this experiment, mice were exposed to a distinctive environment and either the immunosuppressant drug cyclosporin (Csp) or saline. For some Csp-treated mice, exposure to the distinctive environment coincided with Csp injections (paired or conditioned animals). For others, Csp injections occurred 24 h after exposure to the distinctive chamber (unpaired or nonconditioned animals). The study was terminated after only four injections, because of unexplained deaths in 17% of the animals. Postmortem examinations suggested that these deaths may have been due to pathogen(s) introduced from a contaminated injection. The surprising observation was that these deaths occurred mostly in unpaired animals and not in either saline control or paired animals. Because paired animals had received the same immunosuppressant drug and dose from the same contaminated drug supply as had the unpaired animals, the differential mortality observed in these two groups suggests that conditioning aided the survival of paired animals. This interpretation is consistent with recent demonstrations of compensatory conditioning of immune function.

Much interest has been aroused by recent demonstrations that Pavlovian conditioning can modify immune function (Ader & Cohen, 1975, 1985; Ader, Cohen, & Bovbjerg, 1982; Dyck, Greenberg, & Osachuk, 1986; Gorczynski, MacRae, & Kennedy, 1984; Krank & MacQueen, 1988; MacQueen & Siegel, 1989). These studies have all demonstrated that an animal exposed to an arbitrary conditioned stimulus (CS) paired with a drug unconditioned stimulus (US) acquires a conditioned response (CR) that affects immunological functioning. In each case, the CR to future exposure to the CS is some modification of immunological function. There is currently some controversy about the nature of the CR in such studies, but it is clear that Pavlovian conditioning can perform a major regulatory function in the immune system (Ader, Felten, & Cohen, 1990; Siegel, Krank, & Hinson, 1987).

The potential importance of immunological conditioning to life-threatening conditions was demonstrated by Ader and Cohen (1982). They showed that conditioning with cyclophosphamide (Cy) was capable of modifying mortality in a specialized population of mice (New Zealand, NZBX, NZW, F1 mouse) that is susceptible to systemic lupus erythematosus (SLE). SLE is an auto-immune disease that can be treated with immunosuppressant drugs such as Cy. Ader and Cohen showed that survival in these animals was enhanced by conditioning procedures that were known to produce conditioned suppression of immune responses. Siegel, Hinson, Krank, and McCully (1982) have similarly demonstrated that drug-induced death may be modified by conditioning procedures. We report here a similar contribution of conditioning with the drug cyclosporin (Csp) to death caused by infectious agents accidentally introduced into the peritoneal cavity by injection.

Csp is a very specific suppressant of T-cell-mediated cellular immunity and is commonly used to prevent rejection of organ and bone marrow transplants. Its major side effects include hepato-toxicity and increased susceptibility to infection and the development of lymphomas (see Calabresi & Parks, 1985, pp. 1298-1299). Csp is of particular interest in conditioning studies, because of its specificity of action. This immunosuppressant drug exerts its effects by interfering with T-helper cell activity and markedly reduces production of the proliferative factor, interleukin-2. Because the unconditioned effects of Csp are so discrete, conditioning studies with this drug would help to determine the relationships of immune CRs to the underlying changes in immunological function induced by the drug US.

The present study was intended to investigate the effects of Csp conditioning on the production of antibodies to sheep red blood cells. Unfortunately, unexpected mortality in drug-treated animals, probably caused by the inadvertent injection of a pathogen, interfered with the completion of the study. Nevertheless, the pattern of impact of this pathogen suggests an important conditioning ef-

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fect that should be noted: deaths due to infection occurred mainly in animals that received unpaired injections of Csp; they rarely occurred in animals that received paired injections of Csp. These findings suggest an as yet unspecified compensatory conditioned response that may have been responsible for the enhanced survival of animals that received a good signal for the Csp.

METHOD

Subjects

The subjects were 72 mice, CD 1 strain, weighing 18-22 g at the start of the experiment. The mice were obtained from Charles River Canada in St. Constant, Quebec. They were housed 6 per cage, with 2 animals from each group in each cage. The animals were housed in a constantly dark environment to reduce the contribution of the diurnal cycle as a signal for drug injections. Food and water were freely available except as described below.

Procedure

At the start of the experiment, animals were assigned to three groups: paired, unpaired, or controls. These groups differed according to (1) whether or not they received Csp, and (2) the relationship between injections and experience with a distinctive context. Once every 5-7 days, all animals were exposed to a distinctive context for 2 h. Two environmental contexts were used. For half of the animals, the distinctive context involved moving individual animals from their normal home cages into wire-mesh cages in a different room with overhead illumination. The other half of the animals had access to a 0.1% (w/v) saccharin solution during the stay in the distinctive context. The animals that received the context-plus-taste exposure were water-deprived 48 h prior to each trial.

During each exposure to the distinctive context, the animals were injected after 30 min and then returned for the remaining 90 min. Each animal was also injected 24 h after exposure to the distinctive context. Paired animals received injections of Csp in the distinctive context and saline 24 h later. Animals in the unpaired group received a saline injection after 30 min rather than a Csp injection. Unpaired animals received saline injections in the distinctive context and Csp injections 24 h later. Control animals received saline injections both in the distinctive context and 24 h later. Although the experiment originally called for five training trials, training was terminated after the fourth conditioning trial.

The dosage of Csp used was 30 mg/kg. The drug was dissolved in 100% ethanol and suspended in a vehicle solution of olive oil (5% ethanol v/v). The drug was injected in a volume of 1 cc/100 g. All drug and saline injections were intraperitoneal.

Twelve days after their last injection, all animals were inoculated with a .5-ml injection of 1% thrice-washed solution of sheep red blood cells and again exposed to the distinctive context. Three days later, the animals were again exposed to the distinctive context. Three days later, the animals were sacrificed and subjected to necropsy by a veterinarian (G.F.), who determined the presence of lesions in the peritoneal cavity. The extent of lesions was graded from 0 to 3, with 0 being *no evidence* of and 3 being *intensive evidence* of peritonitus. These evaluations were conducted with no prior knowledge of the group assignment of the subjects. Lesions were subsequently examined histologically and bacteriologically.

RESULTS

During the course of the experiment, 15 of the 72 animals died. Only 1 animal in the paired group and 1 animal in the control group died prior to the end of the experiment. This level of loss (4%) is not uncommon in such experiments. Moreover, these animals died from an acute reaction to a drug injection. However, 13 of 24 unpaired animals died during the same period (54%). Twelve of these showed a marked weight loss and deterioration of health prior to death. Only 1 died acutely following an injection. The type of context (taste cue vs. no taste cue) did not appear to be related to this pattern: 8 animals died in the context-only groups, and 7 animals died in the context-plus-taste-cue groups. A simple chi-square analysis on group membership indicated that survival was related to the training condition [$\chi^2(2, N = 72) = 24.3, p < .001$]. The training phase was terminated when it became evident that the deaths were related to Csp injections.

Nine of the remaining animals sacrificed at the end of the experiment showed evidence of fibrous peritoneal adhesions around small abscesses. Histological findings indicated that all lesions were similar and included a focal necrosis surrounded by neutrofils, fibroblasts, and macrophages. Special tissue stains revealed negative results for acid-fast organisms. However, multiple branching gram-variable, rod-like organisms were present. Bacteriological culture revealed several organisms suggestive of Arachnea sp. or Actinomyces sp. The postmortem findings suggested that an organism had been introduced into the injected drug supply by accident, with peritonitus as the consequence.

Identification of animals at necropsy allowed specification of the degree of peritonitus present at the time of sacrifice according to training condition. Five of the remaining 11 unpaired animals (45%) showed evidence of peritonitus. Only 3 of the remaining 23 paired animals (13%) and none of the control animals showed such evidence. The average severity of the infection (indicated by the 4-point scale) for the unpaired animals was 1.09, for the paired animals 0.22, and for the control animals, 0. A simple one-way ANOVA revealed a significant group effect [$F(2,54) = 8.57, p < .001$].

DISCUSSION

The primary finding of this experiment was the differential mortality occurring in groups of animals that received equivalent exposure to the immunosuppressant drug Csp. Paired animals, although they received the drug from the same suspect drug supply as did unpaired animals, did not show very much evidence of peritonitus. Unpaired animals, on the other hand, were markedly affected by peritonitus and showed a weakened ability to survive its effects. This weakening of resistance in the unpaired animals is an expected natural side effect of Csp administration. The relative ability of paired animals to survive is the surprising observation, and it suggests some form of tolerance to the immunosuppressive effects of Csp. Because the only difference between these groups was the conjunction of the drug injection with environmental and taste cues, these observations suggest that conditioning influenced the effects of Csp on disease resistance.

The present findings are reminiscent of an earlier controlled study on heroin overdose death (Siegel et al., 1982). In this study, conditioning manipulations enhanced the ability of rats to survive a potentially lethal dose of heroin. The enhanced survival of animals in the heroin overdose experiment was attributed to the presence of a compensatory response that served to compensate for the respiratory depression normally caused by the opiate. The present findings suggest a similar compensatory conditioning effect in the immune system. Moreover, the present results suggest that such compensatory conditioning may be crucial in resistance to potentially fatal diseases.

These findings are also consistent with recent demonstrations involving the drug Cy (Krank & MacQueen, 1988; MacQueen & Siegel, 1989) and the drug poly IC (Dyck et al., 1986) of compensatory conditioning of immune functions. The present study provides little information about the mechanism of this effect, but its magnitude implicates a major component of cell-mediated immunity. It should be acknowledged that without direct evidence of a CR to the signal for Csp, our conclusions are tentative. Alternative explanations based on factors such as biological rhythms induced by drug injections or stress-drug interactions may be viable (cf. Gorczynski et al., 1984). Nevertheless, the clearest and most parsimonious explanation is that there was a compensatory conditioning effect.

The practical implications of these findings are quite important, because Csp is commonly given to transplant patients and because reduced disease resistance is an important side effect of such treatment (Calabresi & Parks, 1985). Obviously, the first line of defense against such side effects is to reduce exposure to disease agents. Nevertheless, the present study suggests that certain drug injection procedures may help to minimize the risk of Csp compromise of infection resistance. It remains to be seen whether conditioning will also contribute to a reduction in the effectiveness of the drug's antirejection properties.

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