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The effect of preadministration of *Corynebacterium parvum* on the protection afforded by heat-killed and acetone-killed vaccines against experimental mouse typhoid

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SUMMARY

Mice given an intraperitoneal injection of 0·5 mg. *Corynebacterium parvum* (dry weight) before vaccination with heat-killed (HK) or acetone-killed (AK) *Salmonella typhimurium* vaccine and later challenged intraperitoneally with *S. typhimurium* strain 1566, showed a statistically significant increase in mortality when compared with mice that had received only *C. parvum* but no vaccine. They also showed a higher mortality rate than mice receiving only HK or AK vaccine or mice that had received no vaccine before challenge. Prior administration of *C. parvum* to mice that are vaccinated with HK or AK vaccine appears to make them more susceptible to an intraperitoneal challenge with *S. typhimurium*. This was more apparent with HK vaccine than with AK vaccine.

INTRODUCTION

In the last decade considerable interest had been focused on the ability of heat-killed suspensions of *Corynebacterium parvum* to stimulate the immune defence mechanisms of experimental animals. Halpern *et al.* (1964) demonstrated an intense and prolonged stimulation of phagocyte activity of the reticulo-endothelial system after injecting *C. parvum* into mice by the intravenous or intraperitoneal route. These mice were found to have enlarged livers and spleens and histological studies revealed proliferation of existing elements and lympho-histiocytic infiltration of these organs. The administration of *C. parvum* also leads to an increase in the production of antibodies to specific antigens (Neveu, Branellec & Biozzi, 1964) by increasing not only the number of antibody-producing cells but also the output of antibody by each cell (Biozzi *et al.* 1966). Pinckard, Weir & McBride (1967*a*, *b*) administered *C. parvum* strain 10387 to rabbits 6 days before challenge with a weak antigen and demonstrated a considerable increase in the production of antibodies. The binding capacity of the antisera produced was considerably augmented and there was an increase in the affinity of the antibodies for the antigen. These workers concluded that the adjuvant effect of *C. parvum* was not entirely due to lymphoreticular proliferation and increased phagocytosis but possibly also to stimulation of nonspecific immunity

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by affecting the 'recognition' and 'triggering off' stages of the immune response. Siskind & Howard (1966) showed that *C. parvum* was able to augment protective immunity to pneumococci in mice. They injected *C. parvum* into the animals before administration of pneumococcal polysaccharide and demonstrated a prolonged survival time of the mice upon subsequent challenge with live pneumococci.

Sterne & Trim (1970) investigated the ability of calcium alginate to enhance the protection conferred on mice by heat-killed *Salmonella typhi* vaccine. These workers suggested that the enhanced potency of typhoid vaccine when mixed with calcium alginate and injected intraperitoneally into mice was due to the stimulation of nonspecific immunity in the peritoneum by the calcium alginate rather than a specific immune mechanism. In the present study a heat-killed suspension of *C. parvum* was administered intraperitoneally to mice in an attempt to augment the immunity conferred by heat-killed (HK) and acetone-killed (AK) vaccine against mouse typhoid.

MATERIALS AND METHODS

Animals

Male Swiss white mice weighing 19–22 g. were used.

Bacterial strains

Salmonella typhimurium strain 1566 was used for the preparation of vaccines and for challenge.

Vaccines

Heat-killed (HK) and acetone-killed (AK) *S. typhimurium* vaccines were prepared as described in a previous publication (Cronly-Dillon, 1972*a*). A dose of 10^3 HK or AK organisms was administered to each mouse as a single subcutaneous injection at the root of the tail. This dose was known to be non-protective against an intraperitoneal challenge with 100 *S. typhimurium* strain 1566 (Cronly-Dillon, 1972*b*) and was used in this experiment so that any possible potentiation of vaccine activity would be more apparent.

Challenge inoculum

The challenge organisms were suspended in 0·1 M phosphate buffer at pH 8 made by adding 5·3 ml. of 0·2 M solution of monobasic sodium phosphate to 94·7 ml. of 0·2 M solution of dibasic sodium phosphate and diluting to 200 ml. Preliminary tests had shown that this medium does not impair the viability of the organism. The challenge dose of 100 organisms was administered intraperitoneally in a volume of 0·1 ml. The actual dose administered was determined by a surface viable count (Miles & Misra, 1938).

Preparation of C. parvum

C. parvum strain 10387 was grown and killed by heat at 70° C in a water bath for 1 hr. as described by Pinckard *et al.* (1967*a*). The sterility of the preparation

was tested and strictly confirmed before use. The suspension was diluted in physiological saline and a volume of 0·1 ml. containing 0·5 mg. (dry weight) of the *C. parvum* was injected intraperitoneally into each mouse. This dose appeared to be non-toxic to the animals since mean daily weights recorded over a period of 28 days were not altered when compared with those of controls. Histological studies on the livers and spleens of four of the test mice killed 11 days after injection of *C. parvum* showed no obvious changes and individual weights of livers and spleens of these mice were not significantly altered when compared with those of controls.

Design of the study

Sixty mice were each given an intraperitoneal injection of 0·1 ml. of *C. parvum* suspension containing 0·5 mg. (dry weight) of the organism. Six days later, 20 of these mice and 20 untreated mice were given a single subcutaneous injection of 10^3 HK vaccine organisms. Another group of 20 mice that had received *C. parvum* and 20 untreated mice were given 10^3 AK vaccine organisms subcutaneously. The third group of 20 mice that had received *C. parvum* and 20 untreated mice remained as the control groups. On the 15th day after vaccination, all the animals were challenged intraperitoneally with a dose of 10^2 organism of *S. typhimurium* strain 1566. The mice were then observed daily for 28 days and deaths were recorded every day. The liver and spleen of each mouse that died was cultured in nutrient broth to establish the presence of *S. typhimurium*. On the 28th day after challenge all the survivors were killed and examined in the same way to test for the presence of *S. typhimurium* in their organs.

Statistical analysis

Results were analysed by the χ^2 test of probability using a formula that makes allowances for small numbers. When the value for P was 0·05 or less the result was regarded as statistically significant.

RESULTS

The detailed results are shown in Table 1.

In comparison with mice that did not receive *C. parvum*, those mice that received *C. parvum* 6 days before vaccination with 10^3 HK or AK organisms were not more resistant to intraperitoneal challenge with 100 *S. typhimurium* 15 days after vaccination. In fact there was a statistically significant reduction in protection, i.e. an increased mortality rate, among mice that had received *C. parvum* before vaccination with HK organisms ($0{\cdot}025 > P > 0{\cdot}01$) or AK organisms ($P = 0{\cdot}05$) when compared with the group of mice that had been given *C. parvum* alone. The mortality rates among mice primed with *C. parvum* and subsequently vaccinated with HK or AK organisms were slightly higher than in the untreated control group and in those mice vaccinated with HK or AK vaccine alone, but these results were not statistically significant. Although the mortality among mice primed with *C. parvum* and then vaccinated with

Table 1. *The observed mortality rates, mean times to death, and infectivity rates in groups of* 20 *test mice treated in various ways before intraperitoneal challenge with* 10^2 *organisms of* S. typhimurium *strain* 1566

Immunization procedure		No. of deaths due to *S. typhimurium*	Mortality (%)	Mean time to death of fatal cases (days)	Infectivity (%)
Initial treatment	Vaccine given s.c. on day 6 after initial treatment				
C. parvum	Heat-killed vaccine	17/18*	94	13	100
Nil	Heat-killed vaccine	13/20	65	12	100
C. parvum	Acetone-killed vaccine	16/20	80	12	100
Nil	Acetone-killed vaccine	14/20	70	13	100
C. parvum	Nil	9/20	45	12	100
Nil	Nil	14/20	70	12	100

Mice were given 0·5 mg (dry weight) killed *C. parvum* intraperitoneally (IP). Six days later the animals were given a single subcutaneous (SC) injection of 10^3 HK or AK vaccine organisms. All the animals including controls were challenged on the 15th day after vaccination. The experiment was terminated on the 28th day after challenge.

* Two mice died before challenge.

HK organisms was not significantly higher than that of mice receiving only HK organisms, the χ^2 test showed a border-line value ($0{\cdot}1 > P > 0{\cdot}05$).

The dose of 10^3 HK or AK vaccine organisms on their own did not confer any protection against the intraperitoneal challenge with 10^2 *S. typhimurium*. This result was expected.

Mice given only *C. parvum* before challenge showed the lowest mortality rate (45%) in the experiment. However, this result was not statistically significant when compared with the mortality of untreated controls (70%) although it was significantly lower than in mice that had been primed with *C. parvum* before vaccination with HK or AK organisms.

The infectivity rates were 100% in all the groups, and mean survival times of those mice that died were not significantly altered in any of the groups.

DISCUSSION

The adjuvant effect of administering *Corynebacterium parvum* to mice before immunization has been shown to increase the protective potency of the immunogen to a virulent challenge with pneumococci (Siskind & Howard, 1966). The results of the present study with *Salmonella typhimurium* show the reverse. Not only does prior administration of *C. parvum* fail to protect mice vaccinated with HK or AK vaccines against an intraperitoneal challenge with *S. typhimurium*, but it seems to make the challenge more lethal for the vaccinated animals. Thus the mortality was considerably higher in mice primed with *C. parvum* and immunized with HK vaccine than in those mice that had received *C. parvum* alone or HK vaccine alone. This trend was also reflected in the groups of mice receiving AK vaccine in that mice given *C. parvum* and then immunized with AK vaccine showed a significantly increased mortality when compared with mice that had received *C. parvum* alone. This combined form of immunization was definitely less protective against challenge in comparison with the mortality among the

animals given each component on its own. HK vaccine seems to be slightly worse in combination than AK vaccine in combination with *C. parvum* but the difference was not statistically significant.

It is unlikely that an inflammatory reaction resulting from the initial *C. parvum* injection could be responsible for the adverse reactivity to the intraperitoneal challenge injection since those mice given *C. parvum* alone showed significantly fewer deaths after challenge than those given the combined treatment. It is also interesting to note that the group of mice given *C. parvum* alone showed a lower mortality rate than the untreated control group. Although this result was not statistically significant it may indicate that administration of *C. parvum* alone tends to exert a slightly protective rather than adverse effect upon mice challenged intraperitoneally.

Histology of liver and spleen of mice given an intraperitoneal injection of *C. parvum* alone failed to show conclusive evidence of lymphoreticular hyperplasia when studied 11 days after challenge. This is in accord with work done concurrently in this department that demonstrated that the particular strain of *C. parvum* used in the present study was capable of inducing only slight histological changes in the lymphoreticular system 6 days after injection into rabbits (Pinckard, Weir & McBride, 1967*b*, 1968).

The antibody titres of the animals were not followed in the present study since the original object was to achieve protection in terms of survival or a decreased infectivity rate, and it has already been demonstrated that in mouse typhoid antibody titres do not correspond with the degree of immunity (Hobson, 1957*a*, *b*).

The increased mortality noted upon challenge of vaccinated mice that had previously received *C. parvum* is very difficult to explain. A possible explanation worthy of further study is based on the idea of blocking or enhancing antibody (Hellström & Hellström, 1970). Immunity to mouse typhoid is now generally believed to be largely cell-mediated (Mitsuhashi, Sato & Tanaka, 1961; Mitsuhashi & Saito, 1962). It is probable that those animals who survive the experimental infection do so because they develop specific cell-mediated immunity to *S. typhimurium* during the course of the infection. Perhaps the previous administration of *C. parvum* so modifies the humoral response both qualitatively and quantitatively as to result in an 'enhanced' state – the induced humoral factor now blocking the potentially beneficial effects of cell-mediated immunity. In other words, the blocking antibody combines with the organism and prevents direct contact with sensitized lymphocytes, thus enhancing the virulence of the *S. typhimurium* in much the same manner as the enhancement effect noted in studies of tumours and graft versus host reactions (Hellström & Hellström, 1970).

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