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Immunity to *Toxoplasma* and *Listeria* induced by homologous and heterologous organisms

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Summary

Cross protection of animals against various organisms have been shown for many years. This type of resistance to phylogenetically unrelated organisms might be attributed to certain immunological phenomena such as non-specific macrophage activation. In this report the cross-protective effect of some organisms against *Toxoplasma gondii* RH strain and *Listeria monocytogenes* is described.

Groups of mice were immunized with BCG, *Toxoplasma* lysate antigen, viable cysts of *T. gondii* Tehran strain and heat killed *L. monocytogenes*. Seventeen days after initial immunization, the animals were tested for delayed hypersensitivity by a skin test. The hypersensitive animals in each group were challenged with either lethal doses of *T. gondii* RH strain or 5×10^5 viable *L. monocytogenes*.

Among the animals challenged with *T. gondii*, it was observed that complete protection was achieved only in those mice immunized with viable cysts of *T. gondii* Tehran strain. Although all other immunized mice eventually died after infection, they did show some degree of resistance as their deaths were delayed considerably as compared to non-immunized animals.

In animals which were infected with 5×10^5 *L. monocytogenes*, complete resistance was observed only in BCG immunized mice. The other antigens including *L. monocytogenes* induced partial resistance as evidenced by their survival times and the multiplication of the bacteria in various internal organs.

Key words: Listeria monocytogenes; Toxoplasma gondii; mouse; RH *Toxoplasma* lysate antigen; BCG; cross protection.

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Introduction

Non-specific cell-mediated immunity has been shown to occur in animals infected with bacteria (Ruskin and Remington, 1968; Ruskin et al., 1969; Swartzberg et al., 1975), protozoa and fungi (Gentry and Remington, 1971; Hoff and Frenkel, 1974; Marra and Balish, 1974). Mackaness (1964) showed that mice infected with *Brucella abortus* are immune against subsequent *Listeria* challenge if the latter is given during the peak of the brucella infection. Cellular mechanism has been postulated to be involved in the resistance against phylogenetically related and unrelated organisms. It has been shown experimentally that peritoneal cells of the animals infected or immunized with one type of microorganism were significantly resistant to another type in vitro (Mackaness, 1964; Remington et al., 1972; Krahenbuhl and Remington, 1971).

Cross protection among organisms is usually not equally expressed, for instance *Listeria* infection confers greater resistance to protozoa than to cryptococcus (Gentry and Remington, 1971).

The present investigation was undertaken to study the degree of resistance of mice infected or immunized with various related or unrelated antigens to *T. gondii* and *L. monocytogenes*. The criteria used to evaluate the degree of resistance of challenged animals were: 1. prolonged survival of the mice, and 2. in the case of *Listeria*-infested animals, the quantity of bacteria in various organs such as the liver, spleen and kidney, at various time intervals.

Materials and methods

Animals. Outbred white mice, Charles Rivers, Swiss mice, aged 8–10 weeks were used in all experiments. Twenty mice were used for immunization with each antigen: *Toxoplasma* lysate antigen (TLA), *Listeria monocytogenes*, BCG and *Toxoplasma* Tehran strain (T strain).

Microorganisms. Two strains of *Toxoplasma* were used throughout this investigation. The RH strain of *Toxoplasma* was obtained from the School of Veterinary Medicine, Tehran, Iran. This strain of *Toxoplasma* which was lethal for mice was maintained in the peritoneal cavity of mice and passaged every 72–96 h. The Tehran strain (T strain) of *Toxoplasma* was originally isolated from the lymph nodes of a patient with lymphadenitis. This strain of *Toxoplasma* was not lethal for the experimental mice, even after one year of infection, but it did cause chronic infection in our strain of mice.

L. monocytogenes. No. 7973 received from the National Collection of Type Cultures, Central Public Health Laboratory, London, N.W.9, was maintained in a tryptic soy agar. The virulence of the bacterium was maintained by periodically injecting the bacterial suspension into the peritoneum of normal mice.

BCG was originally obtained from the Pasteur Institute of Paris and subcultured in Dubos broth base containing 10% Dubos albumin (Difco).

Preparation of TLA and Listeria antigens. The trophozoite form of *Toxoplasma* RH strain obtained from the peritoneal cavities of infected mice was used to prepare the TLA. The lysate antigen was prepared by the method of Remington et al. (1972) using hypotonic shock on a preparation containing 1×10^7 *Toxoplasma* per ml.

L. monocytogenes grown in tryptic soy broth was used for the preparation of the *Listeria* antigen. The *Listeria* grown in tryptic soy broth for 18–24 h were centrifuged at 5000 rpm for 20 min.

The resulting bacterial sediment was resuspended and washed two times with sterile saline. The number of bacteria was assessed by measurement of the O. D. of the suspension. In order to prepare lysed *Listeria* antigen, the organism was adjusted to 1×10^{20} with saline and after heat inactivation the cells were lysed by means of hypotonic shock and freezing and thawing.

Immunization of mice. Immunization with TLA was performed by two intraperitoneal injections each of 0.25 ml of TLA at 7 day intervals.

For vaccination with *Toxoplasma* T strain, a brain homogenate of mice containing 3–5 cysts of the T strain was injected in 0.1 ml volumes into the peritoneal cavities of normal mice. If after 15–20 days a sample of injected mice was found to contain brain cysts, the entire group of mice was considered to be chronically infected.

For immunization with *L. monocytogenes*, animals received two intraperitoneal injections of 0.1 ml of a suspension containing 1×10^{10} per ml dead *Listeria* at 7-day intervals.

Mice were immunized with live BCG by two intraperitoneal injections of 5 mg wet weight of this microorganism at 7-day intervals.

Skin testing. Seventeen days post vaccination or immunization animals were skin-tested in their right foot pads by injecting the specific antigens (TLA, PPD and *Listeria* antigen). The left hind foot pad was injected with sterile saline. A difference in foot pad thickness of 0.2 mm after 24 h was considered a positive reaction.

In vivo challenge of animals. Twenty-one days after the initial immunization, mice showing skin reactivity to the specific antigens were challenged intraperitoneally with either *L. monocytogenes* or *T. gondii* RH strain.

For the survival studies a challenge dose of either 5×10^5 *Listeria* or a lethal dose of *Toxoplasma* RH containing at least 10 organisms per inoculum were employed (Ruskin et al., 1969). For the enumeration of bacteria in internal organs a challenge dose of 2.5×10^5 *Listeria* was used so that the organ studies were possible.

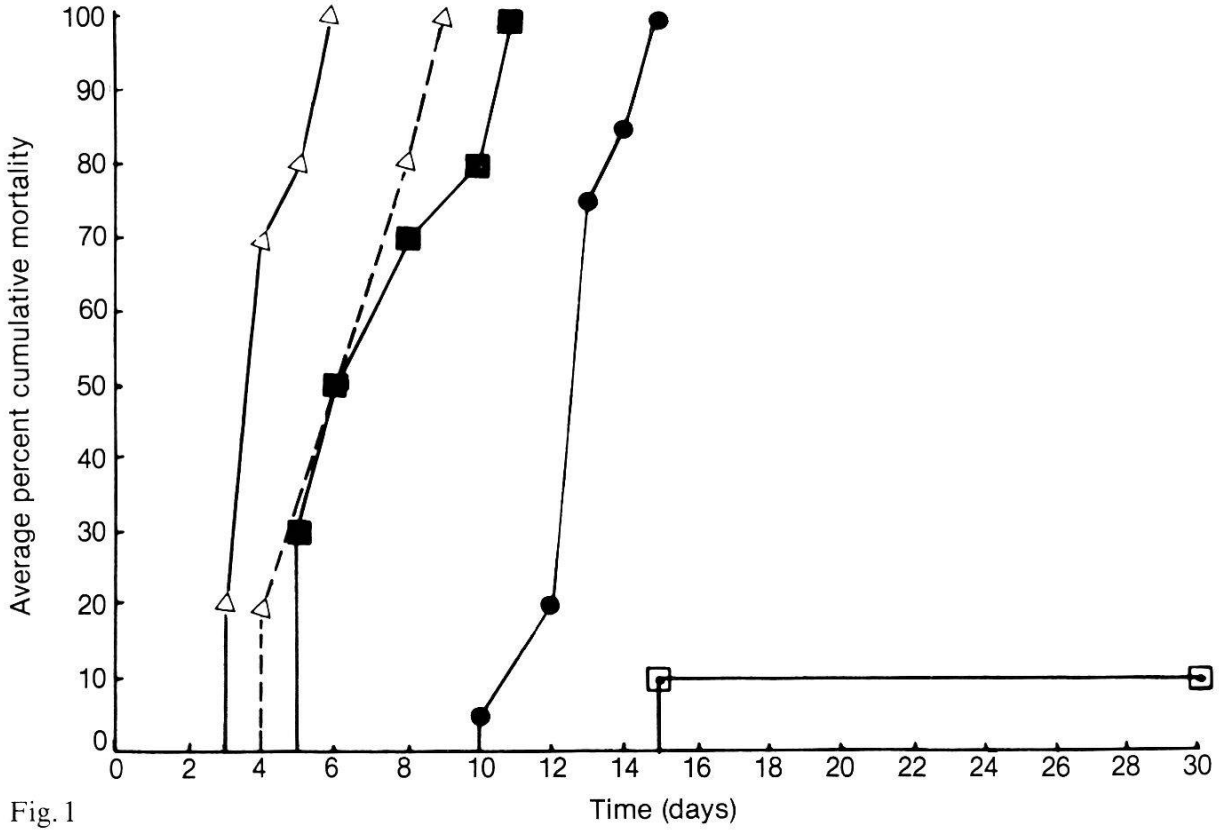
Enumeration of viable Listeria in different organs. At one hour and various time intervals after infection, the livers, spleens and kidneys of two of the mice immunized with various immunogens were taken out aseptically and homogenized separately in a tissue grinder containing 5 ml saline. The suspensions were serially diluted in saline and the bacteria quantitated by the pour plate method.

Results

Resistance of normal and immune mice to T. gondii RH strain. Groups of mice which had been immunized with TLA, viable cysts of *Toxoplasma* T strain, BCG or *L. monocytogenes* and showed skin reactivity, to the specific antigen were challenged with the trophozoite form of *Toxoplasma* RH strain. Following challenge, the deaths of the mice were recorded daily in the immune and control sets of animals (mice which had not received any immunogen). From these data the survival times of the mice were determined and the average percent cumulative mortalities for each group over a period of 30 days were calculated and are presented in Fig. 1.

Ninety percent of the animals which had received *Toxoplasma* T strain as the immunogen, survived for over 30 days after challenge, whereas all control animals died within 6 days. The other immunogens only induced partial protection in these animals as their deaths were delayed in comparison to normals.

Resistance of normal and immunized mice to L. monocytogenes. Animals which had been immunized with TLA, *Toxoplasma* T strain, BCG and *L. mono-*


Average percent cumulative mortality
100
90
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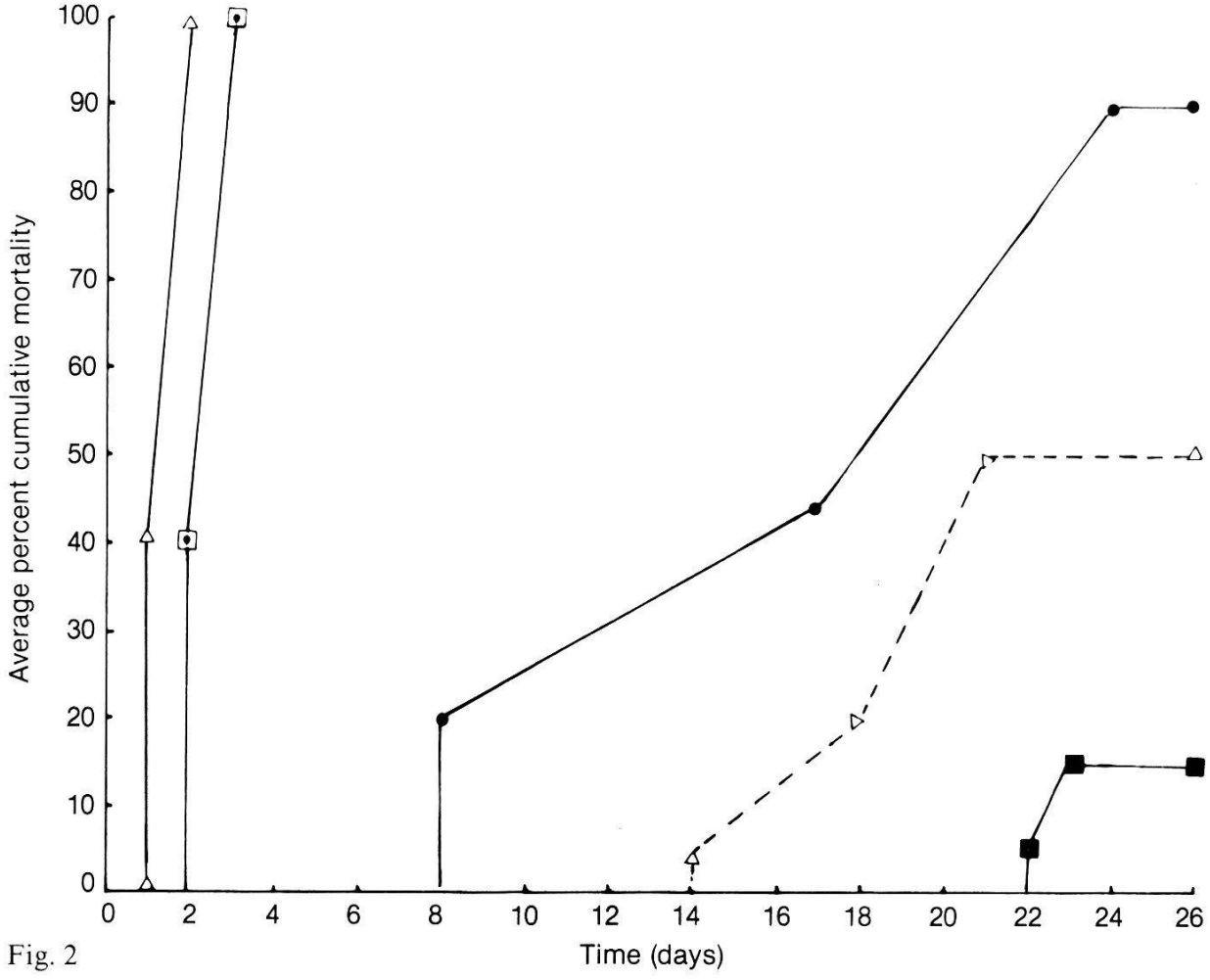
Time (days)

Fig. 1


Average percent cumulative mortality
100
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Time (days)

Fig. 2

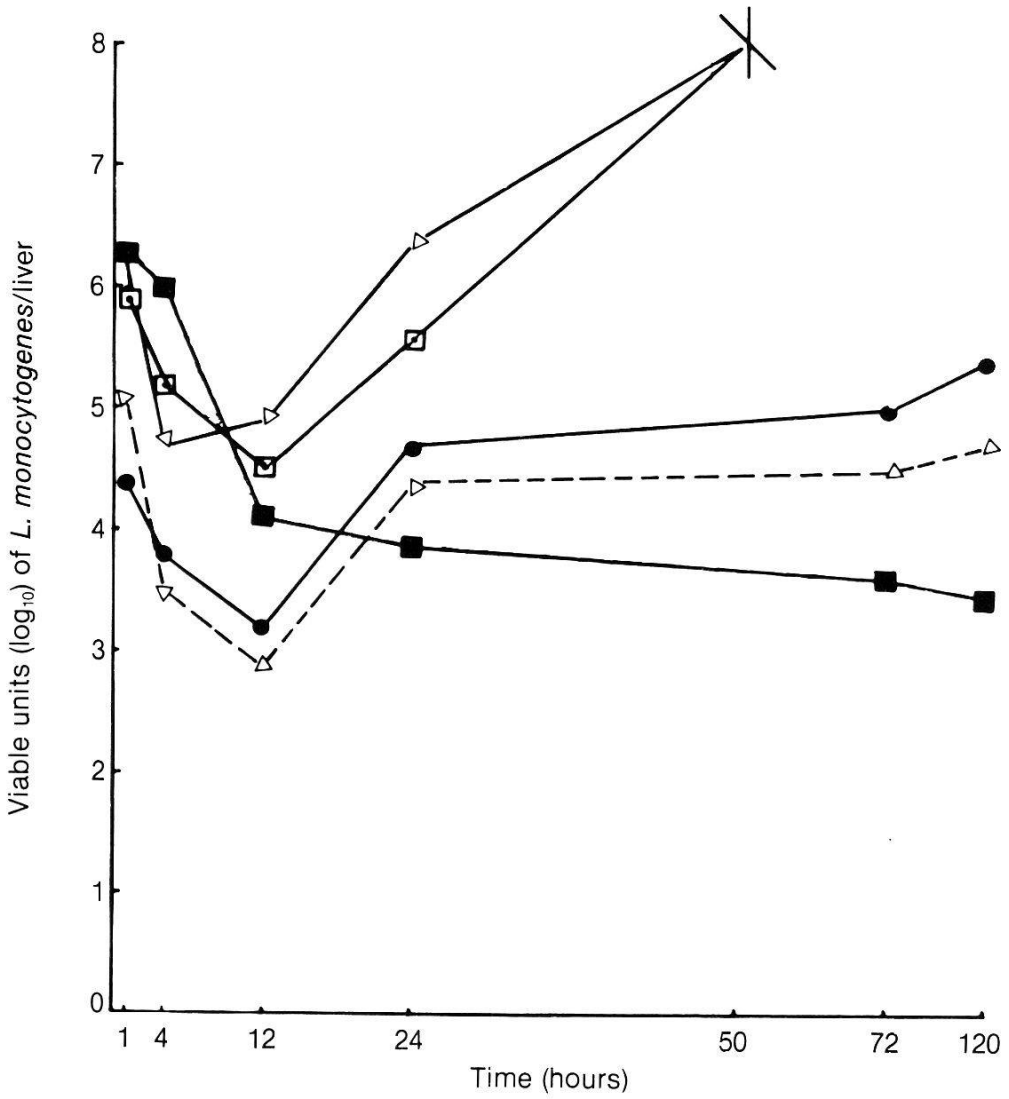

Fig. 3. Multiplication on *L. monocytogenes* in the livers of immunized and normal mice. Each point represents the mean number of viable *L. monocytogenes* in the livers of the animals following the intraperitoneal challenge of 2.5×10^5 bacteria. *Toxoplasma* T-immune and normal mice died (X) about 50 h after challenge (△———△ Normal, □———□ *Toxoplasma* T-immune, △----------△ *Listeria*-immune, ●----------● TLA-immune, and ■———■ BCG-immune).

cytogenes and showed skin reaction to the specific antigen were challenged with 5×10^5 *Listeria* 21 days after the initial injection. As before, the deaths of the mice were recorded. From these data the average percent cumulative death for each group for a period of 25 days were calculated and are presented in Fig. 2.

Fig. 1. Mortality following the challenge of immunized and normal mice with *T. gondii* RH strain. 20 mice were used in each group. Each point represents the cumulative percent mortality of immune and normal mice following the intraperitoneal challenge with a lethal dose of *T. gondii* RH strain (△———△ Normal, □———□ *Toxoplasma* T-immune, △----------△ *Listeria*-immune, ●———● TLA-immune, and ■———■ BCG-immune).

Fig. 2. Mortality following the challenge of immunized and normal mice with *L. monocytogenes*. Each point represents the cumulative percent mortality of immune and normal mice following the intraperitoneal challenge with 5×10^5 *Listeria* (△———△ Normal, □———□ *Toxoplasma* T-immune, △----------△ *Listeria*-immune, ●———● TLA-immune, and ■———■ BCG-immune).

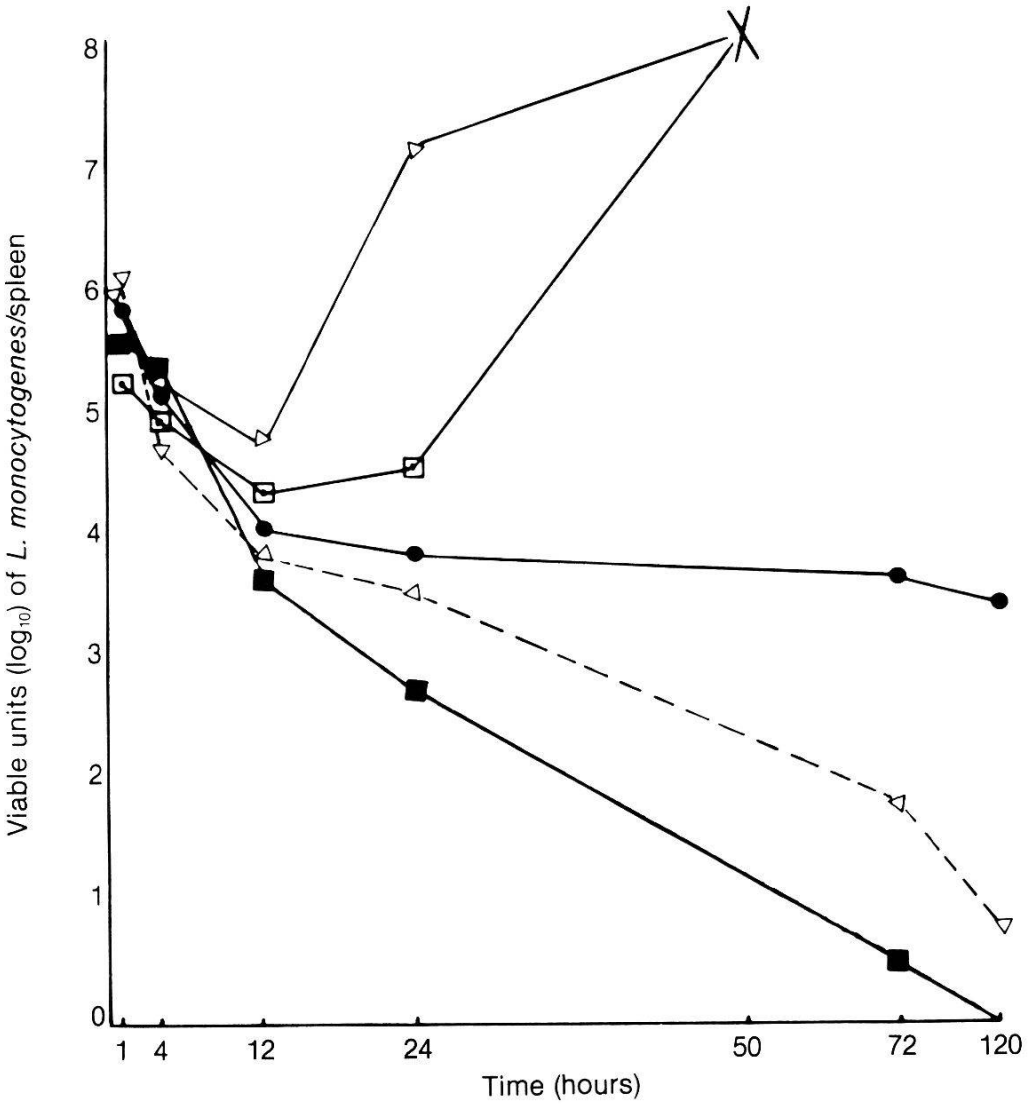

Fig. 4. Multiplication of *L. monocytogenes* in the spleens of immunized and normal mice. Each point represents the mean number of viable *L. monocytogenes* in the spleen of two animals following the intraperitoneal challenge of 2.5×10^5 bacteria. *Toxoplasma* T-immune and normal mice died (X) about 50 h after challenge (△——△ Normal, □——□ *Toxoplasma* T-immune, △----------△ *Listeria*-immune, ●——● TLA-immune, and ■——■ BCG-immune).

Although all normal animals died two days after infection, 85% of the BCG-immune mice and 50% of the *Listeria*-immune mice survived for at least 25 days after challenge. Immunization with TLA delayed the deaths of most of the mice but chronic infection with *Toxoplasma* T strain conferred no resistance on the challenged animal.

Quantitation of viable L. monocytogenes in the internal organs of normal and immune mice. Animals immunized as before were challenged with 2.5×10^5 *Listeria*. At one hour and various time intervals thereafter the livers, spleens and kidneys of two mice were homogenized and the bacteria in the resulting suspension were quantitated.

The abilities of the differently-immunized animals to clear *Listeria* from the liver varied considerably as shown in Fig. 3. After the first 4 h, the number of

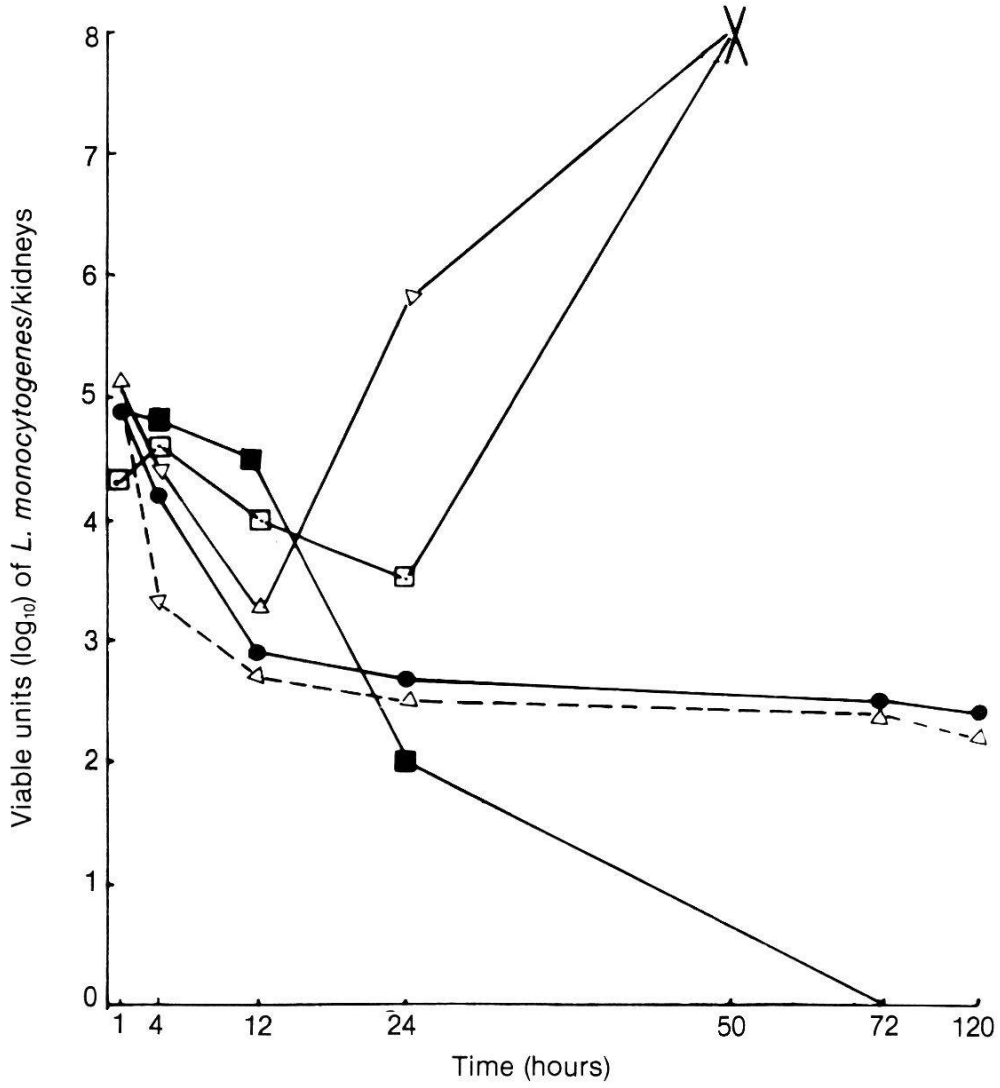

Fig. 5. Multiplication of *L. monocytogenes* in the kidneys of immunized and normal mice. Each point represents the mean number of viable *L. monocytogenes* in the kidneys of two animals following the intraperitoneal challenge of 2.5×10^5 bacteria. *Toxoplasma* T-immune and normal mice died (X) about 50 h after challenge (△——△ Normal, □——□ *Toxoplasma* T-immune, △----------△ *Listeria*-immune, ●——● TLA-immune, and ■——■ BCG-immune).

bacteria in the livers of all animals including normals had dropped. Twelve hours later, however, the number of *Listeria* in the livers of the normal animals had begun to rise and continued to increase until the animals died at 50 h. The number of bacteria in the livers of all the immune animals, however, continued to drop until 120 h.

With the exception of animals immunized with BCG in which the number of *Listeria* continued to decline, after 12 h the quantity of *Listeria* in other immunized animals rose at different rates to different levels. The ability of the *Toxoplasma* T-immune animals to clear the liver of *Listeria* injected mice was the least, as the number of bacteria continued to rise rapidly until the mice died, and the BCG-immune animals were, on the other hand, the most capable in inhibiting the growth of the bacteria in the liver.

The clearance pattern in the spleen was similar to the liver except that the numbers of bacteria in the spleens of TLA-immune and *Listeria*-immune animals continued to decline progressively over the 120-h test period. Again the BCG-immune animals were the most capable in clearing the spleen of *Listeria* and the *Toxoplasma T-immune* animals were nearly as incapable as the normal animals in inhibiting the growth of bacteria (Fig. 4).

A study of the kidneys of these *Listeria*-challenged animals again showed a similar inhibition pattern. BCG was by far the most effective immunogen and *Toxoplasma* T strain was the least in inducing in vivo anti-*Listeria* activity.

Interestingly, at 120 h in the BCG-immunized animals, the spleens and kidneys were completely free of *Listeria*, whereas the livers at this time contained still 5×10^3 organisms (Fig. 5).

Discussion

The present study was designed to compare the degree of resistance induced in mice towards infection with *Toxoplasma* or *Listeria* by specific and non-specific immunogens. Chronic infection with an avirulent strain of *Toxoplasma* conferred complete immunity to 90% of the mice challenged with a lethal dose of *Toxoplasma* RH strain, whereas immunization with BCG, *Listeria* and TLA merely delayed the deaths of the challenged mice. When similarly immunized groups of mice were challenged with *L. monocytogenes*, however, the mice vaccinated with BCG showed the highest degree of resistance and were the most capable of clearing *Listeria* from the kidneys, spleens and livers. These results indicated that immunization with *Toxoplasma* T strain did not confer protection to *L. monocytogenes*. Ruskin and Remington (1968), however, found variable degrees of resistance of *Toxoplasma*-infected mice when challenged with *L. monocytogenes*. The difference between our results and those of Ruskin and Remington's could be due to differences in strains of mice (Cheers and McKenzie, 1978), strains of infecting *Toxoplasma*, and possibly virulence of *L. monocytogenes*. Recently, Swartzberg et al. (1975) have also reported that immunization with *Corynebacteria parvum*, a potent non-specific stimulator confers protection against infection with *L. monocytogenes* or an avirulent strain of *T. gondii* but not against challenge with the RH strain of *Toxoplasma*. On the other hand, mice which were chronically infected with *Toxoplasma* resisted infection with the RH strain. On the basis of this work and our own results it appears that in some cases a non-specific immunogen is capable of stimulating resistance to one pathogen but not another.

Mackaness showed that cellular immunity against intracellular microorganisms depends upon the interaction of lymphoid cells and macrophages. First lymphoid cells interact with an antigen and then in turn interact with macrophages which are then made capable of destroying non-specifically a wide variety of pathogenic microorganisms. It was observed in our in vitro studies on

immune macrophages (manuscript in preparation) that the peritoneal exudate cells from BCG immune mice were more effective in killing both *Listeria* and *Toxoplasma* than the macrophages obtained from mice immunized with the specific antigen. The in vivo protection induced by BCG against *Listeria* correlates well with the activity of the BCG-immune macrophages in vitro. A similar correlation was not observed when *Toxoplasma* RH was used as the challenge organism; in this case chronic infection with the avirulent strain of *Toxoplasma* conferred better protection than BCG. This dichotomy remains to be explained: Why, in animals with a population of macrophages highly activated to kill *Toxoplasma* in vitro, is there relatively poor protection when these same animals are challenged with *Toxoplasma* in vivo? One cannot rule out the role of antibody in the in vivo protection. A similar dichotomy between the in vitro macrophage activation and in vivo protection was also observed by Swartzberg et al. (1975) when *C. parvum* was used as the non-specific immunogen and *Toxoplasma* as the challenge pathogen.

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