Eperythrozoon coccoides

I. Effect on the Interferon Response in Mice

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Eperythrozoon coccoides is a common blood parasite of rodents and the etiological agent of a chronic infection present in many mouse colonies. After primary infection, mice develop a parasitemia and anemia followed by a chronic, latent infection. During the acute phase of infection, mice manifest a striking suppression of interferon production in response to induction with Newcastle disease virus, Chikungunya virus, and poly I:C. These data suggest that the reticuloendothelial system involvement with this agent is associated with impairment of the interferon response. The enhanced susceptibility of E. coccoides-infected animals to certain viral infections may be related to this suppression of interferon production.

Eperythrozoon coccoides is a rodent parasite which shares certain characteristics with the family Bartonellaceae (14). In mice, E. coccoides produces an acute infection manifested by a transient parasitemia, anemia, and splenomegaly. After clearance of organisms from the blood, animals develop a chronic, latent infection which may be activated by splenectomy, superinfection with rodent malaria, or X-irradiation (15, 20). Present evidence suggest that transmission within mouse colonies is by ectoparasites (5, 9). E. coccoides has been demonstrated to alter host resistance to a number of murine viruses, including mouse hepatitis virus (8, 13), lymphocytic choriomeningitis virus (17), lactic dehydrogenase agent (16), and Semliki Forest virus (22). Although enhanced susceptibility of E. coccoides-infected mice to both mouse hepatitis virus and lymphocytic choriomeningitis virus has been associated with more rapid multiplication and higher final titers of virus in the livers of infected animals, the mechanism of the altered pattern of pathogenicity has never been defined.

Involvement of the reticuloendothelial system by E. coccoides is suggested by the occurrence of splenomegaly (16, 19) during the acute phases of infection, the observation of increased phagocytic index (7), and the synergistic effect of lactic dehydrogenase agent and E. coccoides on the levels of lactic dehydrogenase in plasma of infected animals. The latter effect would appear to be the result of decreased processing of the enzyme by reticuloendothelial system cells. This involvement of the reticuloendothelial system by E. coccoides in conjunction with the enhanced susceptibility of infected animals to a number of viral infections suggests that this experimental infection might affect interferon production.

The purpose of this report is to define the effect of E. coccoides infection on interferon production in mice after its induction with representative viral and nonviral inducing agents.

MATERIALS AND METHODS

E. coccoides. E. coccoides was obtained by separation from a stock preparation of Plasmodium berghei contaminated with the organism. Blood was obtained from animals jointly infected with both parasites by cardiac puncture 48 to 72 hr after infection when smears of peripheral blood showed maximum parasitemia with E. coccoides. Samples were diluted 1:2 in phosphate-buffered saline containing 40 units of heparin per ml and centrifuged at approximately 1,500 × g for 10 min. The P. berghei organisms were sedimented with the red blood cells, whereas E. coccoides remained in the serum. The supernatant fluid was used to infect mice, and the procedure was repeated until only evidence of E. coccoides was found in infected animals. Infection with E. coccoides was documented by determination of daily hemocrits by using the standard clinical microhematocrit technique and counting parasites in peripheral blood smears stained with Giemsa stain. Experimental animals were inoculated with a 1:5 or 1:10 dilution of serum collected from infected animals at the time of peak parasitemia and stored at −70 °C.

Interferon inducers. Newcastle disease virus (NDV),

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Herts strain, was grown in the amniotic cavity of 11-day-old embryonated hens' eggs. Stock virus pools were assayed in chick embryo fibroblasts and contained approximately 10^9 plaque-forming units (PFU)/ml.

Chikungunya virus (CV) was a standard reference strain obtained from the Walter Reed Medical Center, Arbovirus Unit. Stock virus was prepared from the brains of suckling mice infected by the intraperitoneal (ip) route, made into a 10% suspension, and assayed by the plaque method in primary chick embryo cells. Virus pools titered approximately 5 × 10^6 PFU/ml. 

E. coli 0111 B4 endotoxin (Westphal) was obtained from Difco. Animals were inoculated with 100 μg by the ip route.

Polyinosinic:cytidylic acid (poly I:C) was obtained from P-L Laboratories, Milwaukee, Wis. Animals were inoculated with 100 μg by the ip route.

**Interferon assay.** Serum samples were diluted 1:5 with minimal essential medium, adjusted to pH 2 with concentrated HCl. After 4 days at 4°C, the pH was returned to 7 with NaOH and stored at −20°C until assayed. Assays were carried out on L-cell monolayers with vesicular stomatitis virus (VSV; Indiana strain from the American Type Culture Collection) as the challenge, and all samples from one experiment were run simultaneously. The concentration of interferon was defined as the reciprocal of the dilution which produced a 50% plaque inhibition of the standard VSV inoculum. All assays were standardized by simultaneous titration of an internal laboratory mouse serum which contained approximately 2,000 (range 1,500 to 2,000) units of interferon per ml. The titer of the laboratory standard was determined by comparison with the international standard mouse interferon preparation obtained from the National Institutes of Health (NIH). The NIH standard containing 500 units/ml titered between 350 and 400 units/ml in our assay system.

**Animals.** Six-week-old female albino mice, Swiss Webster strain, were obtained from Blue Spruce Farms, and strain A mice were obtained from the Defned Flora Colony at the University of Utah College of Medicine.

**RESULTS**

Infection of 6-week-old Swiss Webster or strain A mice in our laboratory was characterized by the rapid onset of a parasitemia which peaked at 48 to 72 hr and was cleared by 72 to 96 hr. A second transient parasitemia of lesser magnitude was frequently observed between 5 and 7 days after infection. A striking anemia was observed to follow the parasitemia, with the hematocrit decreasing from 50 to 55% to 20 to 30% (Fig. 1).

The mechanism of clearance of *E. coccoides* from the blood has never been adequately defined. Although animals appear to develop immunity, specific antibody has never been demonstrated. It would seem reasonable to postulate that phagocytic cells of the reticuloendothelial system are involved in the clearance process. The development of splenomegaly (16, 19) in infected animals and evidence of an increased phagocytic index (7) support this concept, although definitive proof is lacking. In strain A mice infected with *E. coccoides*, spleen weights were observed to increase within 48 hr of infection and to weigh 500 to 600 mg within 1 week. The average weights of spleens from three animals sacrificed at 2-, 3-, 4-, and 7-day intervals after infection are compared with those of normal animals in Table 1. Histological sections obtained from infected and control mice were stained with Giemsa and hemotoxylin and eosin strains. The response to
infection with *E. coccoides* was characterized by progressive hypertrophy of lymphoid follicles, an increase in the number of mitotic figures, lymphoid cell proliferation, and a proportional decrease in the red pulp. The proliferation of lymphoid cells was evidenced by the histological signs of increased metabolic activity, including trabeculation of chromatin in the nuclei, increase in cytoplasm, and increased staining of Golgi apparatus. A striking increase in plasma cells was observed in areas of lymphoid proliferation. No organisms were detected either intra- or extracellularly in any of the sections.

Because of the altered patterns of susceptibility of *E. coccoides*-infected animals to a number of murine viruses and the evidence of reticuloendothelial system involvement during infection, experiments were designed to determine if interferon production is affected by infection with this common parasite of laboratory rodents.

Six-week-old female mice were infected by the ip route. Infection was documented by the presence of organisms in Giemsa stain peripheral blood smears 48 to 72 hr after infection. This screening of animals before utilization in experiments was necessary because mice carrying a chronic infection with *E. coccoides* are resistant to secondary challenge. At the present time, there is no satisfactory test other than susceptibility to infection for identifying such chronically infected animals. Groups of mice were challenged with an interferon inducer 48 to 72 hr after infection, at the time of peak parasitemia, and heparinized samples were obtained by an orbital bleed 3, 6, and 24 hr after infection. Serum from each group was pooled, acid-treated, and frozen at −20 °C until assays could be carried out for interferon activity.

**NDV.** Serum interferon levels produced in response to infection with $2 \times 10^8$ to $4 \times 10^8$ PFU of NDV were strikingly suppressed. Results from one of four similar experiments are illustrated in Fig. 2. Within 3 hr after infection in control animals, interferon levels of 800 to 900 units/ml were observed in the serum, increasing to 10,000 to 12,000 by 6 hr and dropping to 900 to 1,000 at 24 hr. In contrast, *E. coccoides*-infected mice were found to have detectable quantities of interferon only at the expected time of maximal interferon response at 6 hr after inoculation. The highest level observed in the serum of these animals was approximately 900 units/ml, a greater than 90% decrease when compared with controls.

**CV.** A second viral inducer, CV, was selected because of previous studies indicating that the interferon response to CV may involve different cell populations than induction by NDV (3, 4, 10; L. A. Glasgow, Fed. Proc., 1971, *in press*). In mice receiving 650 r of whole-body X-irradiation, we found that the interferon response to NDV was strikingly suppressed, whereas only a 10 to 25% reduction was observed when CV was utilized as the inducing agent. These data suggested that, in contrast with NDV, the more radiosensitive cell population made a lesser contribution to the total interferon response of the host when CV was the inducer. *E. coccoides*-infected mice, however, manifested a suppression of the interferon response to CV as well as to NDV. Three hours after infection, the mean serum interferon level in control animals from five experiments was approximately 300 units/ml at a time when no antiviral activity was detectable in infected mice. By 6 hr, 1,250 units/ml were found in the serum from control animals compared with 300 units in the experimental group. The results from one representative experiment are illustrated in Fig. 3.

**Synthetic polynucleotides.** In a third series of experiments, the interferon response to poly I:C was determined. Mice were inoculated with 100 μg of poly I:C by the ip route, and serum samples were obtained 2 to 3 and 5 to 6 hr later. The data from one of four experiments are presented in Fig. 4. In normal mice, poly I:C induced a rapid response with 1,800 units of interferon per ml in the serum at 2 to 3 hr and 1,300 units/ml at 5 to 6 hr. In contrast, interferon production was barely detectable in *E. coccoides*-infected animals with maximum levels of only 110 units/ml in the serum 6 hr after infection.

**Endotoxin.** Finally, endotoxin was utilized to
reduce the release of preformed interferon in *E. coccoides*-infected and control animals. In four separate experiments, mice were inoculated with 100 μg of *E. coli* 0111 B4 endotoxin. The data from one representative experiment are illustrated in Fig. 5. In contrast with the decreased production of interferon in response to viruses and synthetic polynucleotides, a three- to fourfold enhancement in serum interferon levels was observed in animals infected with *E. coccoides*.

**Interferon production by *E. coccoides***. A wide variety of organisms have been found to induce interferon in vivo. The striking effects observed on interferon production during *E. coccoides* infection suggested that interferon-producing cells were affected and that interferon synthesis might be a product of this interaction. Strain A mice were infected with 0.2 ml of serum obtained from infected animals at the time of maximum parasitemia. Based on counts of the organism compared with the number of red blood cells in the peripheral smear, the inoculum contained an estimated $4 \times 10^4$ to $7 \times 10^4$ organisms. Samples of serum were obtained from groups of five mice at intervals after inoculation, pooled, and assayed for interferon activity. An interferon-like inhibitor was found in the serum within 18 hr after infection, reached peak levels at 24 hr, and declined over the next 24 hr. Maximum levels varied from

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**Fig. 3.** Serum interferon levels in *Eperythrozoon coccoides* (EC)-infected and control mice after intraperitoneal inoculation of 10^6 PFU of Chikungunya virus.

**Fig. 4.** Serum interferon levels in *Eperythrozoon coccoides* (EC)-infected and control mice after intraperitoneal inoculation of 100 μg of poly I:C.

**Fig. 5.** Serum interferon levels in *Eperythrozoon coccoides* (EC)-infected and control mice after intraperitoneal inoculation of 100 μg of bacterial endotoxin.

**Fig. 6.** Serum interferon levels in mice after intraperitoneal inoculation with *Eperythrozoon coccoides* (EC).
150 to 700 units/ml in different experiments (Fig. 6). The lack of a reasonable technique for quantitating the number of organisms present in the inoculum was probably a major factor in the range of variation in the level of the interferon response in separate experiments. By 48 hr after infection with *E. coccoides*, interferon levels were either undetectable or had fallen to 100 to 200 units/ml. In view of the fact that determination of the effect of this organism on the interferon response to other inducing agents was determined between 48 and 72 hr after infection, it is possible that some of the interferon detected in the experiments reported above could be attributed to *E. coccoides* rather than the secondary inducer. These results confirm the work of Rytel and co-workers, who found 64 to 256 units per 2 ml of interferon in the serum over the 1-week period after infection with *E. coccoides* (M. W. Rytel, P. Suntharasamai, and P. D. Marsden, personal communication).

**DISCUSSION**

The present study demonstrates the striking degree of suppression of interferon production in response to NDV, CV, and poly I:C during the acute phase of infection with *E. coccoides* in mice. The degree of suppression is particularly striking in that it is a more general inhibition than observed with whole body X-irradiation at levels up to 1,000 r (3, 4, 10; L. A. Glasgow, Fed. Proc., 1971, *in press*). Although X-irradiation produced a similar level of suppression when NDV was used to induce interferon, only moderate reductions in the interferon response were noted with CV and no effect could be detected with poly I:C. De Maeyer, De Maeyer-Guignard, and Jullien (3, 4, 10) have interpreted their data to indicate that different cell populations, with various degrees of radiosensitivity, may be involved in the synthesis or release of interferon in response to different inducing agents. If this interpretation in fact obtains, then the results of the present study suggest that multiple cell populations are either involved in the infection with *E. coccoides* or have their metabolic functions altered by the organism. At the present time, there is only fragmentary evidence identifying the cell population involved in *E. coccoides* infection, although the data do suggest that both phagocytic cells of the reticuloendothelial system and lymphoid cells are affected. It has been documented previously that the phagocytic index of infected animals is increased (7), suggesting stimulation of reticuloendothelial cell activity, and, in this report, evidence of lymphoid cell proliferation is described. The mechanism of the inhibition of interferon formation remains undefined. On the basis of the data presented, it does not appear to be due to cell destruction nor does the increased phagocytic index suggest reticuloendothelial system blockade.

Data have been previously reported which suggest that the interferon produced in response to endotoxin is preformed or at least does not require new protein or ribonucleic acid synthesis (21). This release of interferon appears to be enhanced by *E. coccoides* infection. It is interesting to note that certain other stimulants of RE system activity, such as BCG and Freund's adjuvant, have also been found to increase the interferon response to endotoxin (23). In general, various metabolic functions are increased in activated macrophages (11), and the enhanced release of interferon in response to endotoxin may be one of these functions. This concept is supported by recent evidence that activated peritoneal exudate cells produced more interferon than control nonstimulated cells after exposure to an inducing agent in vitro (2, 12). The effect of *E. coccoides*, however, is clearly more complex as evidenced by the concomitant effects of this agent on the interferon response to NDV, CV, and poly I:C.

The most important implications of the present observations, however, are related to the ubiquitous distribution of *E. coccoides* in experimental animal colonies combined with capacity of the organism to affect host resistance and interferon production. If a latent *E. coccoides* infection can be activated by splenectomy or superinfection, then X-irradiation, immunosuppressive drug regimens, antilymphocyte sera, thymectomy, and other techniques for altering host resistance could similarly trigger an unrecognized *E. coccoides* infection. This potential raises the important question of the possibility that exacerbation of an unrecognized *E. coccoides* infection in studies which have been designed to determine the effect of these various techniques or procedures on the interferon response or on host resistance to subsequent virus infections could also affect the interferon-producing capacity of the host.

An example of a situation in which the role of *E. coccoides* should be considered is presented by the conflicting evidence concerning the effect of splenectomy on interferon production in rodents (21). In mice, Borecky and Lakovic (1) reported the induction of interferon in response to viruses was not affected by splenectomy. In contrast, Subrahmanyan and Mims (18) and Fruitchstone et al. (6) found significant reduction in interferon levels after removal of the spleen. These differences could be explained if latent *E. coccoides* infections were activated by the splenectomy in mice from some colonies but not others. The evi-
idence presented suggests that is is incumbent upon an investigator to define the presence or absence of this organism in any population of experimental animals used in studies of the effect of procedures which may potentially exacerbate a latent infection with *E. coccoides*.

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**LITERATURE CITED**