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Nonsterilizing Immunity in Avian Malaria: An Antibody-Independent Phenomenon (39186)

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The term nonsterilizing immunity or "premunition" is associated with malarial infections in which parasites remain in the tissues of the host long after the cessation of clinical disease (1). During this period the host is resistant to reinfection. Species exhibiting nonsterilizing immunity to malaria include man, monkeys, birds (2), and perhaps mice (3). Speculations regarding the immunologic basis for the development of "premunition" have been reviewed recently by Cohen and Butcher (4); however, the mechanisms involved remain to be determined.

With regard to the latter, it has been reported that bursectomized birds, which had been cured of acute malaria by means of chemotherapy or by passive immunization with convalescent sera were resistant to subsequent challenge infection with plasmodia (5, 6). Since the procedures employed did not eradicate exoerythrocytic parasites, these data suggest that either antibody-independent mechanisms were operative in nonsterilizing immunity in birds, or that antibodies were present and functioning in the rescued birds due to incomplete bursectomy. To investigate this question further, we have studied the capabilities of chickens rendered B-cell deficient by means of combined chemical bursectomy to resist reinfection with plasmodia following rescue from primary infection.

Materials and methods. Animals. White Leghorn-line WC chickens, which are homozygous at the B-2 locus, were purchased as fertile embryos from Hy-line Poultry Farms, Johnston City, Iowa and were reared in our facility. Agammaglobulinemia was induced by means of combined chemical bursectomy according to a modification of the procedure of Lerman and Weidanz (7). Briefly, this was accomplished by injecting 4 mg of testosterone propionate into the chorioallantoic cavity of 11-day-old embryonated eggs. Cyclophosphamide treatment ranging from 10 to 12 mg per bird was given over a period of 3 to 5 days. In all cases cyclophosphamide treatment was initiated on the day of hatch. Only those chickens assessed as being B-cell deficient by methods to be described below were employed for experimental purposes.

Assessment of B-cell function in bursectomized chickens. At 4 weeks of age, all bursectomized chickens were immunized by intravenous injection with 0.1 ml of a 10% sheep red-blood cell suspension (SRBC). One week after immunization, the sera of all birds were assayed for antibody to SRBC as previously described (8). The sera were also assessed for detectable IgM by means of gel diffusion analysis using a monospecific rabbit anti-chicken μ-chain antisera capable of detecting levels of IgM in excess of 10 μg/ml (9). It has been our experience, as well as the experience of others (10), that adult birds lacking IgM also lack IgG, whereas birds lacking detectable levels of IgG may or may not be deficient in IgM. All birds used in this study lacked both detectable IgM and antibody to SRBC after immunization with SRBC and were therefore considered agammaglobulinemic.

Experimental infection. The infection of chickens with *P. gallinaceum* was achieved with parasites and by methods described previously (11).

Chemotherapy. When the parasitemias of infected birds exceeded 40%, chloroquine diphosphate (20 mg/kg body weight of chloroquine base) was administered orally in the form of a 10% solution in distilled water. Treatment was continued for 3 consecutive days. Uninfected animals serving as controls received identical treatment.

Results. Experimental malaria in B-cell deficient chickens followed a fulminating course terminating in death as shown in Fig. 1. Eleven agammaglobulinemic and eight immunologically intact birds, 6 weeks old, were infected with 10^5 parasitized erythrocytes. Parasitemias became patent in both groups of birds several days after infection and increased at similar rates until the seventh day. Beyond that time, parasitemias in agammaglobulinemic birds exceeded those of their normal hatchmates. Whereas immunologically intact birds began to clear their parasites by the ninth day of infection, the parasitemias in agammaglobulinemic birds continued to rise. All of the B-cell deficient birds died by the eleventh day of infection. In contrast, none of the immunologically intact birds expired.

To determine if B-cell deficient chickens rescued from an otherwise lethal infection with *P. gallinaceum* were immune to reinfection, B-cell deficient birds were infected with *P. gallinaceum* and treated with chloroquine which destroyed the asexual forms of blood parasites. B-cell deficient chickens which had been rescued from primary infection by chemotherapy were resistant to challenge infection with *P. gallinaceum* initiated 4 days after the termination of chloroquine treatment even though they still lacked detectable levels of IgM (Table I). In contrast, typical disease resulted in B-cell deficient birds which had been treated with chloroquine but had not been exposed previously to malarial infection. In fact, five out of five such animals died within 12 days when infected with as few as 10^3 parasites. Prior to the termination of the experiment 30 days

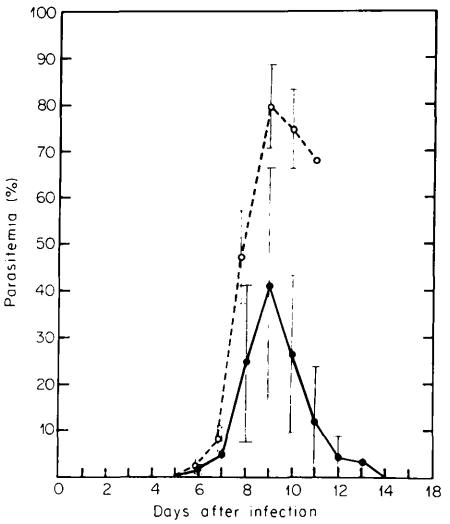

FIG. 1. Fulminating parasitemias in agammaglobulinemic chickens infected with *P. fallinaceum* (- -○- -, agammaglobulinemic birds; —●—, immunologically intact birds). Each point represents the mean ± SD of the parasitemias for the surviving birds. At Days 0, 8, 9, 10, and 11, there were 11, 11, 8, and 3 B-cell deficient birds, respectively. Only one B-cell deficient bird was alive 11 days after infection. All immunologically intact birds survived.

TABLE I. RESISTANCE OF RESCUED B-CELL DEFICIENT CHICKENS TO REINFECTION WITH *P. GALLINACEUM.*

Immunological status	Primary infection with 1×10^5 parasites	Chloroquine treated	Challenge infection (number of parasites)	Number with clinical disease[a]/ Number challenged
B-cell deficient	Yes	Yes	1×10^3	1/9
			1×10^5	0/10
			6.5×10^6	0/9
B-cell deficient	No	Yes	1×10^3	5/5
			1×10^5	5/5
			6.5×10^6	5/5
B-cell deficient	No	No	1×10^3	3/3
			1×10^5	3/3
			6.5×10^6	3/3
Normal	Yes	Yes	1×10^3	0/2
			1×10^5	0/2
			6.5×10^6	0/3
Normal	No	Yes	1×10^3	2/2
			1×10^5	2/2
			6.5×10^6	1/1

[a] Birds with increasing parasitemias in excess of 5% during an observation period of 30 days following challenge infection.

after infection, only 1 of 28 rescued birds succumbed to challenge infection with 10^3 parasites, and this bird remained free of disease for 21 days following challenge infection. Subinoculation of spleen cells from infected donors into B-cell deficient birds 30 days postinfection resulted in fulminating parasitemias and death.

Discussion. By using the parameter of resistance to reinfection as a criteria of nonsterilizing immunity, we have shown that "premunition" in avian malaria is a B-cell independent phenomenon. This interpretation of our data is justified by the demonstration that immunity to reinfection occurred in the absence of detectable B-cell function. The avian model system employed in the present investigation differs from those used in previous studies in that, with the possible exception of the model system described by Kincade *et al.* (12), it is the most uniformly severe experimental model of B-cell deficiency available (7, 13). Birds rendered B-cell deficient by means of combined chemical bursectomy lacked the ability to synthesize both immunoglobulins and antibodies even though they were immunized repeatedly. However, they did possess T-cell reactivity as measured by allograft rejection and tuberculin hypersensitivity (unpublished data).

The finding that rescued B-cell deficient birds were immune to reinfection may have special biological significance. This observation suggests that such animals possess a mechanism capable of preventing fulminating parasitemias at a time when small numbers of infectious parasites were still present in the host as evidenced by subinoculation. Though the mechanism by which antibody-independent immunity or nonsterilizing immunity occurs in the agammaglobulinemic chicken remains to be determined, the possibility exists that T-cell dependent macrophage activation which occurs in other intracellular infections (14) may be of significance. If this point is found to be valid, T-cell dependent macrophage activation could provide an effective means of dealing with small numbers of new antigenic variants emerging from exoerythrocytic sources at a time when the host is lacking protective antibodies specific for the new variant. This possibility is under active investigation in our laboratory.

Summary. Agammaglobulinemic chickens died with fulminating parasitemias following infection with *P. gallinaceum*. When rescued from otherwise fatal infection by means of chloroquine therapy, B-cell deficient chickens resisted challenge infection with the same parasite. The data suggest that nonsterilizing immunity to malaria in chickens is B-cell independent.

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