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Immunosuppressive Effects of Experimental Infection with *Plasmodium gallinaceum* (38618)

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(Introduced by A. Bondi)

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Immunodepression secondary to experimental infection with malarial parasites was first reported by Salaman *et al.* who observed a decreased antibody response to sheep erythrocytes in mice infected with *Plasmodium berghei yoelii* (1). This finding has since been confirmed and extended by others (2, 3) who demonstrated that the immunological deficiency resulting from malarial infection was of a selective nature. Whereas the antibody response to certain antigens, e.g., sheep erythrocytes, human gamma globulin, and tetanus toxoid, was suppressed by *P. berghei yoelii* infection (2–4), the response to the bacteriophage, ϕX 174, and hemocyanin remained normal (2, 3).

Data regarding the effect of experimental malaria on cellular immunity have been conflicting. Sengers *et al.* (5) reported that heterograft rejection was delayed in infected mice; however, Greenwood *et al.* (2) observed that neither allograft rejection nor contact hypersensitivity was impaired in mice infected with *P. berghei yoelii.* It is of interest to note that malarial infection has been reported to decrease resistance to infection by assorted microbial agents including *Salmonella typhimurium* (6), Moloney leukemia virus (7), and Toxoplasma (8).

Although Greenwood *et al.* (9) demonstrated that aggregated human gamma globulin failed to reach germinal centers in the spleens of infected mice and suggested that *P. berghei* infection might interfere with antigen processing, the mechanism of malarial-induced immunodepression remains to be determined. Since the well-delineated lymphoid system of the chicken has increased our understanding of immunological deficiency diseases in general, it was thought that the avian host could be employed with special advantage in future studies aimed at defining the mechanism of malaria-induced immunodepression provided that avian malaria could indeed suppress the immune response to selected antigens. In view of this, the present investigation was initiated to determine if immunodepression would occur in chickens infected with *P. gallinaceum.*

Materials and Methods. Chickens. White Leghorn, line 96 cockerals weighing approximately 1 kg were used throughout this study with one exception to be noted later. The birds were reared from fertile embryos purchased from Hyline International, Inc., Des Moines, IA.

Experimental infection. Chickens were injected intravenously with approximately 10,000 erythrocytes parasitized with *P. gallinaceum.* The parasites used in these experiments were obtained originally from Dr. K. Powers and Dr. J. Finerty, NIH, Bethesda, MD. Parasitemias were determined on Giemsa-stained peripheral blood smears. Hematocrit values were determined on heparinized blood drawn from the alar vein.

Antibody formation. Chickens were immunized by injecting intravenously 0.5 ml of a 20% suspension of sheep erythrocytes (SRBC) in saline containing 25 μg of *E. coli* 0127 lipopolysaccharide (LPS) or 0.5 ml of the SRBC suspension alone. The number of direct plaque-forming cells (PFC) in the spleens of immunized birds was determined 3 days after immunization by a modification of the procedure of Jerne and Nordin (10), employing avian complement and SRBC or viable *E. coli* 0127 as target cells. For tabulation purposes, the numbers of PFC/10^7 cells were converted to base 10 logarithms. Differences in mean

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values were tested for statistical significance by the Student's t test (11).

Allograft rejection. When parasitemias in infected birds reached 15–71%, both parasitized and normal uninfected control birds were treated with chloroquine, 20 mg base/kg daily for 3 consecutive days. One day later, birds in each category were grafted with skin obtained from 3 wk-old histoincompatible line 94 White Leghorn donors according to the method described by Sato and Glick (13). Beginning the fifth day after grafting, skin grafts were read daily and scored as rejected when a black eschar was observed in the graft site.

Results. Experimental malarial infection. Parasitemias became patent 5–6 days after injection of approximately 10,000 parasites and reached peak values between the 10th and 12th days of infection. In most instances, parasites could not be detected in blood films obtained 16–20 days after the initiation of infection. Occasionally, parasite numbers remained relatively high for several days after peak parasitemia had been attained. Such infections were usually fatal. In contrast, the typical infection experienced by most birds was characterized by massive red cell destruction as indicated by a sharp drop in hematocrit values which coincided with the development of peak parasitemia. After crisis, these birds made an uneventful recovery.

Suppression of antibody formation. The antibody response to sheep erythrocytes as measured by localized hemolysis in gel was markedly suppressed in the spleens of chickens immunized with the same mixture of antigens. As shown in Table I, the geometric mean PFC response against SRBC in normal birds was 60-fold greater than that of infected birds. The mean spleen weight for normal birds was 1.9 ± 0.4 g and the mean spleen weight for the infected birds was 5.8 ± 1.6 g. This difference in PFC between the normal and infected birds could not be accounted for by the splenomegaly evident in the infected birds, i.e., a significant difference ($P < 0.001$) in mean PFC to SRBC between infected and noninfected birds remained after adjusting the PFC data to account for the increased numbers of cells in the spleens

TABLE I. SUPPRESSION OF PFC RESPONSE IN CHICKENS INFECTED WITH *P. gallinaceum*

Group	Treatment	No. responding[a] / No. immunized	PFC/10^7 spleen cells[b] SRBC	PFC/10^7 spleen cells[b] *E. coli*
A	Infected[c]	8/9	1.0458 ±0.3952 (11)	0.6851 ±0.3305 (5)
B	Normal	6/6	2.7795 ±0.7460 (602)	1.7247 ±0.5326 (53)
			$P < 0.001$	$P < 0.001$

[a] Responding birds had one or more PFC/10^7 spleen cells.

[b] Log_{10} PFC/10^7 spleen cells ± SE; geometric means are given in parentheses.

[c] Birds were infected with approximately 10,000 parasites.

of infected birds. The PFC response to endotoxin was also depressed by malarial infection; however, the degree of suppression was less significant ($P < 0.025$) when the 3-fold increase in spleen weights of infected birds was taken into consideration. Few plaque-forming cells specific for either antigen ($\leq 1/10^7$ spleen cells) were detected in the spleens of unimmunized birds.

Duration of malarial-induced immunodepression. Birds immunized with SRBC at various intervals of time subsequent to infection with *P. gallinaceum* responded in a manner either comparable with or deficient to normal birds immunized with the same antigen. The magnitude of the response was directly related to the time of immunization after infection. As shown in Fig. 1, maximal suppression resulted when the birds were immunized with SRBC 12 days postinfection. At this time, peak parasitemia occurred. The immune response remained depressed for a short time after peak parasitemia but returned to normal as evidenced by the fact that birds immunized 32 days after infection responded by producing significant numbers of PFC.

Cellular immune response. As shown in Table II, allograft rejection occurred at approximately the same time in both uninfected and infected birds. Whereas graft

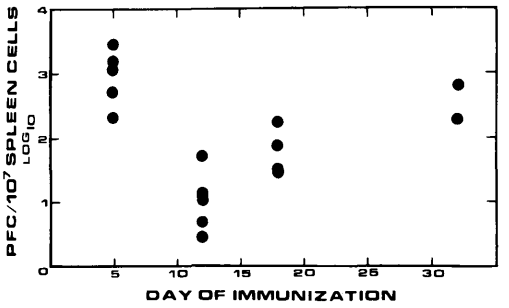

FIG. 1. The immunosuppressive effect of experimental malaria on the PFC response to SRBC in chickens immunized at various intervals of time after infection. Each point represents one bird. The mean number of PFC (Log_{10}) for five uninfected birds immunized with SRBC was 3.1326 ± 0.6692.

rejection was not evident in any of the birds on the 6th day after grafting, all the birds had rejected their grafts by the 10th day.

Discussion. The results of the present investigations demonstrate that experimental infection with *P. gallinaceum* has a pronounced immunosuppressive effect on the host. Whereas uninfected chickens produced significant numbers of plaque-forming cells when stimulated with sheep erythrocytes, infected birds responded poorly to this antigen.

Immunosuppression was also evident when *E. coli* (LPS) was employed as antigen. While the immune response to LPS was minimal in both infected and uninfected animals, the numbers of plaque-forming cells in the spleens of infected birds were significantly less than those observed in the spleens of uninfected animals even when the increased spleen size of the infected birds was considered. Statistical analysis of the data indicate that the degree of suppression with LPS was not as marked as that for SRBC based on the observation that the level of significance decreased when splenomegaly was accounted for in the response to LPS but not in the response to SRBC.

Malarial infection failed to enhance allograft survival. Skin grafts from histoincompatible donors were rejected at approximately the same time by infected as well as by uninfected birds. Similar findings have been reported by Greenwood who utilized a murine model system (2). These data suggest that T-cell immunity as measured in this manner remains intact during malarial infection.

TABLE II. THE EFFECT OF MALARIAL INFECTION ON ALLOGRAFT REJECTION

Group	Treatment[a]	Day of rejection[b] 6	8	10
A	Infected	0/8	5/8	8/8
B	Normal	0/7	7/7	7/7

[a] Birds were infected with approximately 10,000 parasites and treated with chloroquine (20 mg/kg) daily over 3 consecutive days when parasitemias ranged between 15% and 71%. Normal birds were treated with the same regimen of chloroquine.
[b] No. rejection/no. grafted.

In contrast to the observation of Lourie (13) and Corredetti *et al.* (14), who failed to demonstrate immunosuppression in rats infected with *P. berghei*, our results indicate that the phenomenon of malarial-induced immunosuppression may be more general in occurrence than has previously been reported. It is conceivable that other species both mammalian and nonmammalian, may demonstrate malarial-induced immunosuppression provided that proper timing between infection and immunization is employed and sensitive assays for antibody formation are utilized. Also, our findings as well as those of others (1, 2) employing malaria-infected mice demonstrate that the period of suppression is finite and, therefore, its detection may depend largely upon experimental design.

Regarding a mechanism which would account for the immunosuppressive effects of malarial infection, the data reported by Greenwood *et al.* (9) and Loose *et al.* (15) indicate that defective antigen handling or processing may be involved. The precise means by which this occurs remains to be determined and it is for this purpose that the avian model of malaria-induced immunosuppression described in the present study may have special value. The compartmentalization of the immune response was first described in this host (16) and it is now well established that both T and B lymphocyte systems can be manipulated with relative ease. Employing this model system it should be possible to pinpoint

more precisely which cell populations involved in the immune response are affected by malarial infection. Further, studies using the avian model will serve to complement those employing the murine system and may lead to the elucidation of the mechanism(s) of malaria induced immunosuppression.

Summary. Experimental infection of chickens with *P. gallinaceum* markedly suppressed the splenic PFC response to SRBC. Suppression was most pronounced when birds were immunized at the time of peak parasitemia. The PFC response to *E. coli* LPS was of low magnitude in both normal and infected chickens; however, it, too, was suppressed in infected birds, but not to the same degree as observed in response to SRBC. Cellular immunity as evidenced by allograft rejection was not influenced by infection.

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