

Immunity to Coccidiosis: T-lymphocyte- or B-lymphocyte-Deficient animals

M. ELAINE ROSE* AND PATRICIA HESKETH

Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire PE17 2DA, England

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Comparisons were made between infections with *Eimeria* spp. in normal animals and in animals with functional deficiencies in either T-lymphocytes (athymic nude rats) or B-lymphocytes (bursectomized chickens). Approximately three times more oocysts of *E. nieschulzi* were passed during a primary infection in the nu/nu rats, and in contrast to the nu/+ rats, they were completely susceptible to reinfection. Nu/nu rats did not produce agglutinating antibodies to sporozoites, and injections of serum from immunized nu/+ rats caused a reduction of oocyst production during a primary infection in both nu/nu and nu/+ rats. In chickens, oocyst production in primary infections with *E. maxima* or with *E. acervulina* was increased 1.5 to 2-fold in the bursectomized (BX) groups, and the clinical effects of infection with *E. maxima* were greater than in controls. The BX birds were slightly more susceptible than the controls to challenge inoculations of oocysts, but nevertheless, they were very substantially immune. Tests for the functioning of B- and T-lymphocytes indicated that in the BX birds there was a severe deficiency of B-cell function, but near-normal T-cell function. The results show that T-lymphocytes are essential for immunity and that, although they may function partly as helper cells for immunoglobulin production, their major effect is exerted via some other mechanism.

Evaluations of the roles of T- and B-lymphocytes in immunity to infections with *Eimeria* spp. have mostly been carried out in thymectomized or bursectomized chickens (26). In these animals the results were equivocal, but only partial immune deficiencies, especially in T-lymphocytes (31) resulted from the procedures used. The availability of athymic (nude) mice and rats and of more efficient methods of bursectomy in chickens has now encouraged a reexamination of this subject. The inability of athymic mice (19) and rats (30) to become resistant to reinfection with *Eimeria falciformis* and *E. nieschulzi*, respectively, has already been reported. Here we describe further studies on infections with *E. nieschulzi* in athymic rats and with *E. acervulina* and *E. maxima* in chickens bursectomized by a combination of hormonal (in ovo) and chemical (neonatal) treatment. The results show that functioning T-cells are essential for the development of immunity and that a deficiency of B cells had a definite but very much smaller effect.

MATERIALS AND METHODS

Animals. Athymic nude (nu/nu) and heterozygous (nu/+) rats were bred at the Houghton Poultry Research Station (HPRS) from a homozygous (nu/nu)

male and heterozygous (nu/+) females obtained from the Laboratory Animals Centre, Carshalton, Surrey, England (6). They were of mixed sexes, maintained coccidia-free until used for the experiments when they weighed approximately 70 to 80 g. Chickens were HPRS Light Sussex of mixed sexes, kept coccidia-free until inoculated with oocysts. All chickens were injected with Marek disease vaccine (Marexine; Intervet, Cambridge, England) on the day of hatch.

Parasites. *E. nieschulzi* were originally obtained from Dawn Owen, Medical Research Council Laboratory Animals Centre, Carshalton, Surrey. *E. maxima* and *E. acervulina* were Houghton strains (15, 16). Methods for handling the parasites and measuring infections by estimations of oocyst output in 24-h fecal collections have been fully described previously (18). Sporozoites were excysted from sporulated, washed, sodium hypochlorite-treated oocysts by a modification of methods already described (17, 35). Oocysts in phosphate-buffered saline (PBS; pH 7.6) were cracked by agitation with glass beads (0.5 mm in diameter), and the released sporocysts were then incubated in 0.5% bile salts, 0.1% trypsin (0129-01 and 0152-15; Difco Laboratories, Detroit, Mich.) at 41°C for 5 to 10 min to excyst the sporozoites. After slow centrifugation (100 × g for 2 min) to remove heavier debris, oocysts, and sporocysts, sporozoites were deposited at 1,500 × g for 6 min, and the suspension was purified by passage over a 12-cm column of 0.15-mm-diameter glass beads in a 50-cm³ glass burette (38).

Serum. Blood was obtained from ether-anaesthe-

tized rats, by snipping the tails or by cardiac puncture, and from the wing veins of chickens. It was allowed to clot at room temperature, and the serum, collected by centrifugation, was used after a short period of storage at -20°C .

Bile. Birds were starved overnight, killed, and bile-aspirated from the gall bladder with a Pasteur pipette. After centrifugation to remove debris, the bile was stored at -20°C .

Measurements of antibodies. Agglutinins to sporozoites of *E. nieschulzi* were measured in rat serum and to human erythrocytes in chicken serum.

A 0.2-ml amount of a suspension of sporozoites (2.7×10^5 organisms) was added to an equal volume of doubling dilutions of rat serum (previously heated at 56°C for 30 min) in small agglutination tubes. After incubation for 60 min at 37°C the gently resuspended suspensions were examined microscopally at $\times 320$ magnification in a hemocytometer, and the number and size of clumps of sporozoites were noted.

The production of agglutinins to human erythrocytes was used to check on the efficacy of bursectomy. Chickens were injected intravenously with 0.3 ml of a 20% suspension of washed human erythrocytes, and hemagglutinin titers were measured in Perspex agglutination trays by adding 0.25 ml of a 1% suspension of erythrocytes in PBS (pH 7.2), containing 3% autologous human serum, to an equal volume of doubling dilutions of chicken serum. Incubation was at 41°C for 60 min, and the tests were read after allowing the cells to settle at 4°C overnight.

Bursectomy. This was done by combining hormonal treatment in ovo with injections of cyclophosphamide after hatching (12, 8). Embryonating chicken eggs were injected into the chorioallantoic cavity with antibiotics (2,500 U of penicillin and 1,850 U of streptomycin in 0.05 ml) on day 9 (experiment 3) or day 8 (experiment 4) of incubation. On days 12 or 11, respectively, they were similarly injected with 3.5 mg of testosterone propionate (T-1875; Sigma Chemical Co. St. Louis, Mo.) in 0.05 ml of liquid paraffin (BDH Chemicals Ltd., Poole, England) (BX groups), or with liquid paraffin alone (controls). Chicks which hatched from the BX groups were injected intraperitoneally with 4.0 mg of cyclophosphamide (CY; Ward, Blenkinsop and Co., Ltd., London) in 0.2 ml of sterile saline on the day of hatching and on each of the 2 (experiment 3) or 3 (experiment 4) succeeding days, i.e., a total of 12.0 (experiment 3) or 16.0 (experiment 4) mg of CY. Chicks hatching from the control groups of embryonating eggs were similarly injected with 0.2 ml of sterile saline.

Quantitation of immunoglobulins in chickens. To assess the efficacy of bursectomy, serum immunoglobulin G (IgG) and IgM and biliary IgA were measured by immunodiffusion in agar gel. Dilutions of serum or bile were reacted with rabbit antisera to IgG, IgM, and IgA (Miles Laboratories Ltd., Stoke Poges, Slough, England). Under the conditions of the test these antisera detected minimum concentrations of 0.002 mg of IgG per ml, 0.078 mg of IgM per ml, and 0.063 mg of IgA per ml, when tested against standards kindly provided by L. N. Payne and M. C. Rennie of this institute.

Measurements of cell-mediated immune responses in chickens. Measurements of cell-mediated immune response in chickens were made to check that the procedures used for bursectomy had no significant effect on the functioning of T-lymphocytes. Reactions of delayed-type hypersensitivity were measured in chickens given 1 mg of killed *Mycobacterium avium* suspended in 0.5 ml of liquid paraffin intramuscularly and tested approximately 3 weeks later in the wattle (28).

Statistical analysis. Student's *t* test was used for statistical analysis.

Design of experiments. For experiments with athymic rats, comparisons were made between groups of nu/nu and nu/+ animals in: (i) the extent of primary and secondary infections with *E. nieschulzi* as measured by the production of oocysts in the feces, (ii) the presence of agglutinating antibodies to sporozoites in serum samples taken on day 6 after a second inoculation of oocysts, and (iii) the effect of a series of intraperitoneal injections of serum obtained from immunized nu/+ animals on infection.

For experiments with bursectomized chickens, comparisons were made between groups of BX and control chickens in: (i) oocyst production resulting from primary and secondary infections with *E. maxima* (experiment 3) or the less immunogenic *E. acervulina* (experiment 4), and (ii) the effect of inoculation with oocysts of *E. maxima* on body weight gain (experiment 4). The efficacy of bursectomy was checked by the induction of antibodies to human erythrocytes and by measurements of serum IgG and IgM and of biliary IgA (experiment 4), and the normal functioning of T-lymphocytes in BX birds was confirmed by measurements of delayed hypersensitivity to tuberculin (experiments 3 and 4) and the response of peripheral blood lymphocytes to phytohemagglutinin (experiment 4).

RESULTS

Primary and secondary infections with *E. nieschulzi* in nu/nu and nu/+ rats. Figures for oocyst production resulting from primary and secondary inoculations of oocysts of *E. nieschulzi* in nu/nu and nu/+ rats, obtained in two similar experiments, are given in Table 1 (see also untreated groups, Table 3). These data show that in nu/nu rats primary infections were significantly enhanced (approximately three-fold) and that there was no resistance to a second inoculation of oocysts, the numbers of oocysts produced being even greater than in a previously uninfected nu/nu group given the same inoculum (experiment 1). In contrast, nu/+ animals were highly resistant to reinfection. In confirmation of previous reports (19, 30) the pattern of oocyst production and duration of patency was similar in nu/nu and nu/+ animals.

Sporozoite agglutinating antibodies in nu/nu and nu/+ rats. Titers of agglutinating antibodies in the serum of nu/nu and nu/+ rats

obtained 6 days after a second inoculum of oocysts are given in Table 2, and the appearance of sporozoites after incubation in serum from these rats is shown in Fig. 1. The serum of inoculated nu/nu rats, like that of uninfected nu/+ rats, did not agglutinate sporozoites of *E. nieschulzi* beyond dilutions of 1:10 to 1:20, whereas the inoculated nu/+ rats contained fairly high levels (titers of 1/2,560) of serum agglutinins.

Effect of administration of serum on oocyst production in nu/nu and nu/+ rats. Oocyst production was significantly lower in rats, with both nu/nu and nu/+ given injections of serum from immunized nu/+ (Table 3). Another experiment showed that the administration of serum from coccidia-free rats had no effect on oocyst production in either group (nu/nu, untreated = 181.1 ± 31.2 ; nu/nu, treated = 182.5 ± 35.2 ; nu/+, untreated = 59.3 ± 19.5 ; treated = 52.9 ± 23.9 ; values indicated are mean values for oocyst production, $\times 10^6$, per rat, with three or four rats per group).

Bursectomized chickens. The number of

TABLE 1. Oocyst production in nu/nu and nu/+ rats after inoculations of oocysts of *E. nieschulzi*^a

Expt no.	Group	No. of rats	Total oocyst discharge ($\times 10^6$) ^a	
			Primary (2,500 oocysts)	Secondary (5,000 oocysts)
1	nu/nu	4	231.0 ± 28.2^b	300.8 ± 72.7^b
	nu/+	4	62.2 ± 30.7	0 ± 0.3
	nu/nu	4	ND	245 ± 60.1^c
	nu/+	4	ND	85 ± 8.8
2	nu/nu	3	181.1 ± 31.2^b	308.2 ± 75.4^b
	nu/+	5	59.3 ± 19.5	0.04 ± 0.03

^a Each value indicates the mean of three, four, or five rats \pm standard deviation. Interval between inoculations was 4 weeks. ND, Not determined.

^b $P = 0.001$ by Student's *t* test. (Significance of differences between groups of nu/nu and nu/+ rats.)

^c $P = 0.01$. (See footnote b.)

TABLE 2. Sporozoite agglutinating antibodies in the serum of nu/nu and nu/+ rats

Expt	Group	No. of rats	Titer (reciprocal) of serum (mean \pm SD) ^a
2	nu/nu	3	12.6 ± 1.5
	nu/+	5	$2,560 \pm 1.6$
	Uninfected nu/+	6 (Pooled)	10
3	nu/nu	3 (Pooled)	<10
	nu/+	3 (Pooled)	2,560
	Uninfected	23 (Pooled)	10
	PVG/C		

^a Day 6 after the second inoculation of oocysts. Final dilutions of serum are given. SD, Standard deviation.

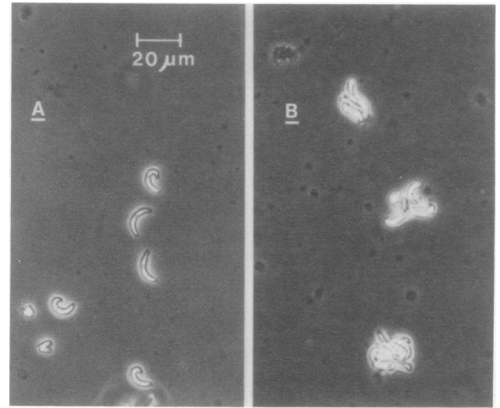


FIG. 1. Sporozoites of *E. nieschulzi* after incubation in an equal volume of 1:10 dilution of serum from nu/nu (A) or nu/+ (B) rats. Serum obtained 6 days after a second inoculum of oocysts (5,000) given 4 weeks after the primary inoculum of 2,500 oocysts.

TABLE 3. Effect of administration of antiserum on infections with 2,500 oocysts of *E. nieschulzi* in nu/nu or nu/+ rats

Group	Serum given ^a	Total oocyst discharge ($\times 10^6$) ^b
nu/nu	No	250.2 ± 60.8
nu/nu	Yes	141.1 ± 24.2
nu/+	No	80.4 ± 25.1
nu/+	Yes	38.0 ± 9.2

^a "Immune" serum obtained on day 7 after the second inoculum of oocysts (10,000) given 10 weeks after the initial dose of 5,000 oocysts. A 10-ml amount was given intraperitoneally in daily doses, 1 ml each. Challenge doses of oocysts were given on day of second serum injection.

^b Values indicate mean \pm standard deviation ($\times 10^6$) for three rats per group. $P < 0.05$ for both nu/nu and nu/+ rats.

chicks in the BX groups which hatched, and their subsequent survival, were related to the severity of the treatments given (Table 4). In experiment 4, of the 69 BX chicks which hatched, only 5 had visible remnants of bursa tissue, and 2 of these were among the 33 birds killed at the termination of the experiment when the birds were 13 weeks old.

Effect of BX on oocyst production in primary and secondary infections. The results obtained, showing the effect of BX on oocyst production in primary and secondary infections, are given in Tables 5 (for *E. maxima*) and 6 (for *E. acervulina*). The findings are similar and indicate that oocyst production in both primary and secondary infections was enhanced by bursectomy. In the BX groups, resistance to reinfection was not as great as in the controls, but nevertheless substantial (cf. groups 1A and 3 in

Tables 5 and 6). In three instances (groups 1 and 3 in Table 5 and group 1A in Table 6), the duration of patency was extended (but not significantly) in the BX birds, and in the case of secondary infection with *E. maxima* (Table 5), when the control group (2A) was completely resistant and did not pass oocysts the small number of oocysts passed by the BX group (1A)

appeared later than is usual for this species. In contrast to the control group, clinical signs of infection were apparent in BX birds given a primary inoculation of 1,000 oocysts of *E. maxima* (Table 5, group 3), and there was one death attributable to coccidiosis.

Effect of inoculation with oocysts of *E. maxima* on body weight gain. Birds from

TABLE 4. *Effect of bursectomy on survival of embryos and chicks*

Expt no.	Group	Treatment	Embryos treated	Chicks hatched	Alive at termination (weeks)
3	BX	Testosterone (3.5 mg) on day 12 of incubation; 4.0 mg of CY on days 0 to 2 after hatch (12.0 mg of CY, total)	110	90	57 (10)
	Control	Liquid paraffin and sterile saline ^a	50	44	43
4	BX	Testosterone (4.0 mg) on day 11 of incubation; 4.0 mg of CY on days 0 to 3 after hatch (16.0 mg of CY, total)	107	69 ^b	33 (13)
	Control	Liquid paraffin and sterile saline ^a	30	22	22

^a See text.

^b Five birds with visible bursal remnants.

TABLE 5. *Infections with E. maxima in BX and control chickens (experiment 3)*

Group no.	Birds		Inoculum		Oocyst production		Clinical effects	
	Age (days)	No.	Type	No. of oocysts given	No. ($\times 10^6$) ^a	Patency (days)	No. showing signs ^b	Deaths
1 (BX)	33	6	Primary	50	10.97 \pm 4.6 ^c	7.3 \pm 0.75	0	0
2 (Control)		12			4.35 \pm 2.5	6.5 \pm 1.87	0	0
1 (BX)	54	6	Secondary	1,000	0.15 \pm 0.19 ^d	2.5 ^e	0	0
2A (Control) ^f		6			0	NA ^g	0	0
3 (BX)	54	6	Primary	1,000	35.8 \pm 11.6 ^h	7.4 \pm 0.6	3	1
4 (Control)		6			21.7 \pm 8.0	6.3 \pm 1.5	0	0

^a Each value indicates the mean number per bird \pm standard deviation.

^b Appearance of birds and their feces was examined.

^c $P < 0.02$; $n = 5$.

^d In one bird oocysts were detectable by concentration method only (not included in calculation of mean).

^e Oocysts produced late in cycle, from day 8 onwards.

^f Six birds of group 2, chosen randomly.

^g NA, Not applicable.

^h Significance: $0.1 > P > 0.05$.

TABLE 6. *Infections with E. acervulina in BX and control chickens (experiment 4)*

Group	Birds		Inoculum		Oocyst production	
	Age (days)	No.	Type	No. of oocysts given	No. ($\times 10^6$) ^a	Patency (days)
1 (BX)	28	22	Primary	100	40.7 \pm 33.0 ^b	5.9 \pm 1.2
2 (Control)		12			24.5 \pm 20.3	6.2 \pm 0.6
1A (BX) ^c	49	6	Secondary	630	5.1 \pm 7.1	3.3 \pm 1.8
2A (Control) ^c		4			0.1 \pm 0.1	2.2 \pm 1.7
3 (BX)	49	4	Primary	630	39.4 \pm 17.8 ^b	6.5 \pm 1.0
4 (Control)		6			21.7 \pm 16.4	6.3 \pm 0.8

^a Each value indicates mean number per bird \pm standard deviation.

^b $P < 0.05$.

^c Taken at random from Group 1 or 2.

experiment 4 were given 5,000 oocysts of *E. maxima* when 70 days old (21 days after they had received the second inoculation of *E. acervulina*), and the effects on body weight were determined. Birds were not available to serve as uninoculated controls for weight gain in the absence of infection. A comparison of the mean weight gains for infected birds from day 0 to 10 postinoculation (Fig. 2) shows that BX birds lost weight between days 5 and 7, whereas birds in the control group failed to maintain growth during this period but did not lose weight. After day 7, growth was resumed in both groups.

Assessment of B-lymphocyte and T-lymphocyte activity. The hemagglutinin titers after injections of human erythrocytes (Table 7) indicate that B cell function in the BX groups was severely impaired, although there was a

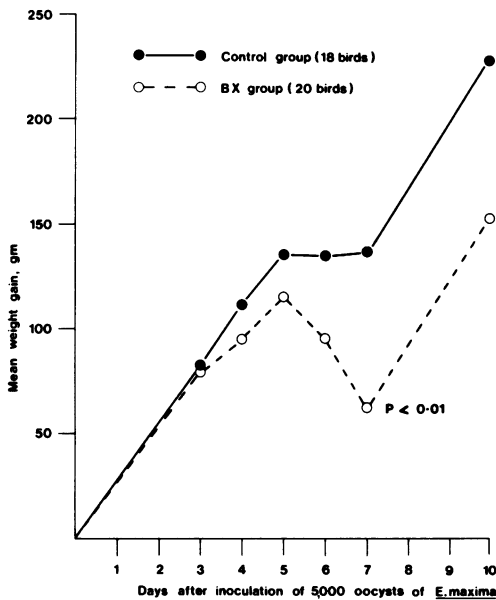


FIG. 2. Mean body weight gain of BX and control birds days 0 to 10 after the inoculation of 5,000 oocysts of *E. maxima* given at 10 weeks of age.

small and definite but belated response to the second injection of antigen. Table 8 gives values obtained for concentrations of immunoglobulins in birds of experiment 4 tested when they were 13 weeks old. The amounts in BX birds were severely reduced, being approximately 17.9, 0.3, and <0.9% of control values for IgM, IgG, and IgA, respectively.

Table 9 gives the results of tests for delayed hypersensitivity and shows that BX birds were competent in this respect, although the mean sensitivity indexes were lower (but not significantly so) than in the controls. Tests for the responsiveness of peripheral blood lymphocytes to incubation with phytohaemagglutinin carried out (10) on birds of experiment 4 when they were 13 weeks old, revealed no differences between BX and control groups, the mean stimulation indexes being 40.5 ± 15.5 ($n = 6$) and 44.7 ± 44.6 ($n = 3$), respectively.

Thus, the procedures used for bursectomy resulted in birds with severe defects in the functioning of B-lymphocytes, but had little effect on T-lymphocyte function when tested from 5.5 weeks.

DISCUSSION

It can safely be assumed that nu/nu rats and mice provide good models of T-lymphocyte deficiency, and therefore results obtained in experiments with these animals can be interpreted accordingly. In the case of bursectomized chickens as models for B-lymphocyte deficiency, it is necessary to establish that the procedures used have the desired effects on the functioning of B-lymphocytes while allowing T-lymphocytes to respond normally. The method used here, a combination of hormonal and chemical treatment, was chosen since, unlike either treatment alone (20) or surgical bursectomy on hatching (21), or even during early embryonic development (7, 13), it has been reported to be very effective in causing agammaglobulinaemia, the abolition of secretory antibody in tears and abla-

TABLE 7. Agglutinins to human erythrocytes (HRC) in BX and control groups

Expt no.	Group	Mean agglutination titer (reciprocal) ^a			
		Primary response after:		Secondary response after:	
		8 days	11 days	5 days	7 days
3	BX	<5 (9/24)	ND	ND	
	Control	40,960 (12/12)	ND	ND	
4	BX	<3 (7/24)	<1 (0/24)	17 (17/17)	37 (16/17) ^b
	Control	17,960 (12/12)	1,387 (12/12)	304,400 (12/12)	42,710 (12/12)

^a Primary injections of HRC were given at 4 weeks of age, and secondary injections (experiment 4) were given at 8 weeks. Values in parentheses indicate number of birds positive per number tested. Negative results were not included in calculations of the mean. ND, Not determined.

^b Response by 3 of 17 birds > 500.

TABLE 8. Concentrations of immunoglobulins in serum or bile at 13 weeks of age (experiment 4)^a

Group	Concn (mg/ml)		
	Serum		Bile (IgA)
	IgM	IgG	
BX	0.20 ± 0.09 (16/20)	0.013 ± 0.018 (17/19)	0.08 ± 0.03 (11/20)
Control	1.12 ± 0.12 (6/6)	4.65 ± 0.25 (6/6)	>8.6 (6/6)

^a Values indicate mean ± standard deviation, and those in parentheses indicate number of birds positive per number tested. The two birds negative for IgG were also negative for IgM. Negative results were not included in calculations of the mean. Minimum detectable concentrations (milligrams per milliliter) were: IgM, 0.078; IgG, 0.002; and IgA, 0.063.

TABLE 9. Delayed-type hypersensitivity to tuberculin

Expt no.	Group	No. of birds tested	Sensitivity index (mean ± SD) ^a
3	BX	11	2.90 ± 0.88
	Control	6	3.59 ± 1.10
4	BX	18	3.15 ± 0.84
	Control	12	3.40 ± 0.55

^a Sensitivity index = (thickness of wattle injected with purified protein derivative, in millimeters)/(thickness of wattle injected with saline, in millimeters), measured 48 h after injection of purified protein derivative. *M. avium* were injected at 7.5 (experiment 3) or 5.5 (experiment 4) weeks of age. Purified protein derivative was injected after an interval of 3 weeks. SD, Standard deviation.

tion of antibody production (8, 20). However, although CY is primarily a B-cell suppressant (12, 36, 37), it does have a transient effect on the functioning of T-cells, at least in chickens (5, 14, 32). Estimations of the duration of this effect vary, from 2 to 10 weeks, possibly due to genetic differences (34). The results of the present study would suggest that injection of BX birds with *M. avium* at 5.5 weeks induced full T-cell responses when tested at 8.5 weeks, and that the response of lymphocytes to phytohemagglutinin was normal at 10 weeks. The earliest age at which birds were given test infections with *Eimeria* spp. was 4 weeks, and although not tested for T-cell responses at this time, it is likely that they were substantially present. They were certainly fully restored by 10 weeks of age, when the birds were examined for the clinical effects of inoculation with a large dose of *E. maxima*. The responses of B-lymphocytes were very severely depressed, although not completely absent, in a large number of birds as shown by the very small quantities of immunoglobulins detected at 13 weeks of age and the depressed response to human erythrocytes in most of the birds, especially after the second injection of antigen. Thus, the majority of birds used here may be regarded as good subjects for a study of

infection in B-lymphocyte-depressed/T-lymphocyte-functional animals.

The results obtained from the experiments on infections with *E. nieschulzi* in nude rats further illustrated the essential role of T-lymphocytes in mediating resistance to reinfection with Eimerian coccidia (19, 30). T-lymphocytes may act by helping B cells to produce antibodies (helper T-cell activity) or by themselves producing antiparasitic effects (effector T-cell activity).

T-lymphocytes were shown here, as previously (19), to act as helper cells in antibody production to membrane antigens of sporozoites. Furthermore, transfers of serum from immunized nu/+ rats reduced test infections in nu/nu and nu/+ recipients. However, previous work has indicated that the protective effects of serum and of maternally transmitted yolk antibodies are limited (24, 25; W. Wittchow, Dr. Med. Vet. thesis, Freie Universität Berlin, Berlin, Federal Republic of Germany, 1972). Intestinally secreted IgA antibodies are likely to be of greater importance in immunity to *Eimeria* infections (3, 4), and although not examined here, it is likely that such antibodies were greatly reduced or absent in the nu/nu rats (2, 22). The results of the experiments on BX birds (of which almost 50% had no detectable biliary IgA, considered to be particularly relevant to immunity in the intestine [11]) indicate that Ig production does play a part in immunity to the *Eimeria*, but that it is a minor one. There was more parasite replication during primary and secondary infections in BX birds, and they suffered more severe clinical effects from infections with *E. maxima*; nevertheless, they were very substantially immune to reinfection.

Thus T-lymphocytes must be additionally involved in some manner other than as helper cells for immunoglobulin production. This could be as effector cells, possibly through the release of a soluble mediator able to act on the intracellular parasite in a similar fashion to that demonstrated in vitro with *Toxoplasma* infections (1, 33). If this is the case then the effect on the sporozoite would seem to be inhibition of devel-

opment rather than killing, since development may be resumed on transfer of parasites from immune to susceptible hosts (9, 29). The specificity of immunity to *Eimeria*, demonstrable against a heterologous species, even when this is introduced simultaneously with the homologous species (27), suggests that T-lymphocytes are not exerting their effect through a nonspecific inflammatory type of response, as may occur in some intestinal helminth infections (39). The action of T-lymphocytes within the intestine of *Eimeria*-infected animals requires further investigation.

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