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IMMUNITY TO BABESIA IN MICE
III. THE EFFECTS OF CORTICOSTEROIDS AND ANTI-THYMOCYTE SERUM ON MICE IMMUNE TO BABESIA RODHAINI

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ABSTRACT

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BALB/c mice, immunized against Babesia rodhaini by an amicarbalide controlled infection, were exposed to selective immunosuppressive treatment with corticosteroids and anti-thymocyte serum (ATS) respectively. Hydrocortisone acetate, 100 mg/kg, given i.p. six times during the three weeks after challenge inoculation caused a rising parasitaemia and high mortality (6/7). Dexamethasone in the drinking water at 20 mg/l or 10 mg/l for 22 days had a similar suppressive effect on the protection against B.rodhaini. Mortality, 100% at the dose rate of 20 mg/l and 50% at 10 mg/l, occurred both in challenged and in carrier animals after the reappearance of parasites in the bloodstream. All the ATS-treated immune mice demonstrated parasitaemia after challenge, although at a lower level than did the corticosteroid treated mice. Seven out of 9 animals died.

Corticosteroid-sensitive macrophages together with T-lymphocytes are considered to play an important role in protection against B.rodhaini in specifically induced immunity in mice.

INTRODUCTION

Laboratory strains of the intraerythrocytic protozoan parasite Babesia rodhaini induce acute lethal infections in mice. Immunization by means of a drug-cured infection (Cox & Young, 1969; Mitchell, 1977; Zivkovic et al., 1984a) results in a protection against lethal effects of this parasite.

Protection of mice against Babesia spp. has also been induced

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by pretreatment with agents such as live Bacillus Calmette Guérin (BCG) (Clark et al., 1976), Corynebacterium parvum (Clark et al., 1977a), Cord factor, Zymosan, Glucan and COAM (=Chloride-oxidized oxyamylose) (Clark, 1979), all with macrophage-activating and interferon-inducing potentials. For the induction of such non-specific protection the presence of T-lymphocytes does not seem to be essential, as shown by Clark et al. (1976), who succeeded in inducing protection against Babesia microti in congenitally athymic "nude" mice using BCG.

In contrast, immunisation against B.rodhaini by a drug-cured infection failed in athymic nude mice, indicating the importance of T-lymphocytes for the induction of specific immunity (Zivkovic et al., 1984b). The essential role of T-lymphocytes in immunity against B.microti has been established by Ruebush and Hanson (1980). Protection of mice against B.microti could be transferred to naive mice with spleen cells from immune animals, but this protective activity was abolished by preincubation of the spleen cells with anti-θ serum and complement.

To obtain information about the role of different cell populations in the effector phase of the defence against B.rodhaini, we have studied the effect of various immunosuppressive treatment on the protection of immune mice against challenge infections. Neither cyclophosphamide (300 mg/kg) nor 400 R total body γ-radiation could decrease the resistance of immune mice to challenge infections with B.rodhaini (Zivkovic et al., 1984a). Even after a lethal irradiation of 875 R followed by bone marrow reconstitution, the animals still showed a considerable degree of resistance. Since such a high level of radioresistance is known to be characteristic of macrophages (Anderson and Warner, 1976) and a small fraction of T-lymphocytes (Kataoka and Sado, 1975) an involvement of these cells in the effector phase of the immune response against B.rodhaini is indicated.

In the present study the role of T-lymphocytes and macrophages in the specific immunity to Babesia rodhaini in mice is further investigated by selective immunosuppressive treatment with corticosteroids and anti-thymocyte serum.

MATERIALS AND METHODS

Experimental animals and protozoa

All mice were of the Balb/c strain, bred at the Department of

Tropical Veterinary Medicine and Protozoology, Utrecht. Only female animals were used, and were aged 6-8 weeks when the experiments started. The mice were housed and fed as described previously (Zivkovic et al., 1984a). The Antwerp strain of Babesia rodhaini, obtained from Professor Rodhain in 1955, was used as the source of stabilate and fresh parasitized blood as described before (Zivkovic et al., 1984a). The stabilate caused lethal infections in all naive mice injected intraperitoneally (i.p.) with 0.1 ml diluted 1:1000.

Estimation of parasitaemia

The course of parasitaemia was monitored every second day by examining Giemsa-stained thin blood smears. The smears were considered negative when no parasites were found in 2 minutes of microscopic examination. Low parasitaemias were estimated by counting the parasites in at least 2000 red blood cells. When more than half of the erythrocytes were infected the parasites were counted in 300 cells. Parasitaemias were expressed as the percentage of erythrocytes infected and no allowance was made for multiple organisms in a single cell.

Immunization procedure

Balb/c mice were immunized against Babesia rodhaini by a method described previously (Zivkovic et al., 1984a). In brief: mice were infected with stabilate diluted 1:10. On the third day of patency they were treated with amicarbalide*, 12 mg per kg bodyweight, and challenged with a large number of Babesia parasites two weeks afterwards. After another two weeks the animals were treated with amicarbalide, 24 mg/kg, i.p.

Mice immunized in this way did not develop parasitaemia after splenectomy and were protected against a high challenge dose for over 8 months.

Animals that did not receive the second, sterilizing injection of amicarbalide at 24 mg/kg were found to be carriers of Babesia rodhaini.

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Corticosteroid treatment

Hydrocortisone acetate. Groups of seven immune and 7 non-immune mice were treated with hydrocortisone acetate* at a dose rate of 100 mg/kg bodyweight i.p. One day after the first injection had been given, both groups were inoculated with 0.1 ml of stabilate diluted 1:10. Treatment with hydrocortisone acetate was repeated on days 3, 7, 11, 15 and 19 after stabilate inoculation. A group of 7 naive mice was treated in the same way with hydrocortisone acetate without being infected with Babesia rodhaini.

Dexamethason. Groups of eight immune and 8 non-immune mice were treated with dexamethasone sodium phosphate** administered in the drinking water at a level of 10 mg/l or 20 mg/l. Treatment was started 2 days before challenge inoculation with 0.1 ml fresh parasitized blood (parasitaemia 2%) diluted 1:10, and was continued for 24 days. The immune animals consisted of Babesia carriers and of mice that had received a second amicarbalide treatment (24 mg/kg) and were supposed to be non-carriers. The carrier animals did not receive the challenge inoculation.

Anti-thymocyte serum (ATS) treatment

Groups of immune and non-immune mice were treated with ATS which was kindly provided by Dr. Kreeftenberg, National Institute of Health, Bilthoven, The Netherlands. The serum was produced in rabbits by immunization with mouse thymus cells. It was administered subcutaneously after absorption with 10% (v/v) normal mouse erythrocytes for 30 minutes at 37°C in order to minimize haemagglutinating activity. Normal rabbit serum, absorbed with mouse erythrocytes, was administered in the same way to control animals.

To determine dosages and frequency of ATS administration a titration in mice was performed, using the relative decrease of the circulating small lymphocytes as an indication of the ATS activity. As a result the treatment was started with 0.5 ml ATS per animal followed by 5 supportive injections of 0.25 ml every third day. The mice were challenged with 0.1 ml B.rodhaini stabilate diluted 1:10, two days after the first ATS injection.

* Hydro-adreson, Organon NV, Holland.
** Oradexon, Organon NV, Holland.

Three control groups of immune mice were included: a group treated with ATS but not challenged; a group treated with normal rabbit serum (NRS) instead of ATS and challenged; and a group challenged but not serum treated. Two naive control groups were also included (Table III).

Statistical analysis

Data were analysed using Student's t-test.

RESULTS

Hydrocortisone acetate treatment

In Balb/c mice immunized against B.rodhaini only a slight and transient parasitaemia was observed after challenge with stabilate diluted 1:10 (Table I, group 2). Treatment of these immune mice with repeated doses of hydrocortisone acetate, starting one day before challenge, resulted in a fluctuating parasitaemia with peaks after each hydrocortisone injection.
Only after several peaks did these mice become highly parasitaemic (> 30%) and die (Table I, group 1). Three weeks after challenge only one out of seven mice had survived; it showed a parasitaemia never exceeding 8%, and which cleared completely after withdrawal of the hydrocortisone treatment.

In naive mice hydrocortisone treatment did not affect the course of B.rodhaini infection. The non-immune treated and control mice showed the same pattern of infection as demonstrated by the similar prepatent period, peak parasitaemia, mortality and survival time.

Dexamethasone treatment

Dexamethasone was applied via the drinking water to obtain a constant high blood level of corticosteroids. The protective immunity was completely removed by treatment of immune mice with 20 mg dexamethasone per liter drinking water starting 2 days before challenge inoculation.
All mice died after demonstrating moderate to high parasitaemia (Table II, group 1). This treatment also provoked high parasitaemia and mortality in carrier animals (Table II, group 3). In groups of immune mice receiving 10 mg dexamethasone per liter, all animals developed parasitaemia ranging from 1 up to 100% and

TABLE I

Effect of hydrocortisone on protective immunity to Babesia rodhaini in Balb/c mice*.

Group	Hydrocortisone acetate	Challenge inoculation	Prepatent period (days)	Peak parasitaemia (%)	Mortality	Survival time (days)
		IMMUNE MICE				
1	+	+	5.7[1] ± 1.3	39.9 ± 20.5	6/7	24.2[2] ± 6.3
2	-	+	4.0 ± 0	3.1 ± 2.7	0/4	
		NON-IMMUNE MICE				
3	+	+	3.7 ± 1.4	40.9 ± 26.6	7/7	10.1[2] ± 0.9
4	-	+	4.1 ± 0.2	54.9 ± 7.8	4/4	9.4 ± 0.5
5	+	-			1/7	21

* Mice were challenged with Babesia rodhaini stabilate diluted 1:10.

1) Significant difference with other groups $P < 0.01$

2) Significant difference $P < 0.001$

Parameters of infection are expressed as mean values ± S.D.

TABLE II

Effect of dexamethasone-sodium-phosphate in drinking water on protective immunity to Babesia rodhaini in Balb/c mice*

Group	Dexamethasone	Challenge inoculation	Prepatent period (days)	Parasitaemia rate	Parasitaemia mean peak (%)	Mortality	Survival time (days)
		IMMUNE MICE					
1. non-carriers	20 mg/l	+	3.5 ± 1.6	8/8	59 ± 27	8/8	18.6 ± 4.4
2. non-carriers	10 mg/l	+	4.8 ± 3.9	8/8	27 ± 24	5/8	19.6 ± 3.8
3. carriers	20 mg/l	-	8.8 ± 3.3	7/8	38 ± 30	8/8	18.0 ± 1.7
4. carriers	10 mg/l	-	12.4 ± 6.6	8/8	40 ± 39	4/8	19.5 ± 5.7
		NON-IMMUNE MICE					
5. -	-	+	3.8 ± 1.7	8/8	70 ± 10	8/8	8.5 ± 2.9

* Mice were challenged with fresh, parasitized blood diluted 1:10
Parameters of infection are expressed as mean values ± S.D.

TABLE III

Effect of anti-thymocyte serum (ATS) on protective immunity to Babesia rodhaini in Balb/c mice[a].

Group	Treatment	Challenge inoculation	Prepatent period (days)	Peak parasitaemia (%)	Mortality	Survival time (days)
			IMMUNE MICE			
1	ATS	+	3.3 ± 1.1 [2]	10.2 ± 14.4 [3]	7/9	18.9 ± 2.3 [4]
2	ATS	-	6.8 ± 4.9 [1]	0.12 ± 0.05	1/6	23
3	NRS[b]	+	4.1 ± 1.9	1.1 ± 1.3	0/10	-
4	--	+	3.8 ± 1.0	1.5 ± 1.2	0/6	-
			NON-IMMUNE MICE			
5	-	+	2.7 ± 0.8 [2]	51.7 ± 13.7 [3]	6/6	10.7 ± 1.5 [4]
6	ATS	-	-	-	0/4	-

a) Mice were challenged with Babesia rodhaini stabilate diluted 1:10. Parameters of infection are expressed as mean values ± S.D.

b) NRS = Normal Rabbit Serum.

1) Prepatent period was arbitrarily considered to start at the time when other groups were challenged.

2) Significant difference $P < 0.05$.

3) Significant difference $P < 0.01$.

4) Significant difference $P < 0.001$.

resulting in mortality of about 50% (Table II, groups 2 and 4).

Anti-thymocyte serum treatment

The ATS treatment of immune mice increased the susceptibility to challenge inoculations. The peak parasitaemias in these animals were significantly higher than in the non-ATS treated immune mice or in mice treated with normal rabbit serum, but remained far below those observed in non-immune mice challenged with B.rodhaini. ATS treatment of challenged immune mice ultimately resulted in death of 7 out of 9 animals. The time of death was significantly delayed compared with naive controls (Table III).

DISCUSSION

Treatment with a synthetic corticosteroid, hydrocortisone acetate, is known to remove a non-specifically induced protection against Babesia microti in mice (Clark et al., 1977b).

In our experiments hydrocortisone acetate abolished specifically induced immunity to Babesia rodhaini in mice, as shown by high mortality. Six out of seven animals died with high parasitaemia.

Dexamethasone given in the drinking water had a similar suppressive effect of the protection against B.rodhaini in immune mice.

The high doses of both hydrocortisone acetate and dexamethasone administered might exert a toxic action. The toxic effect of the drugs could have been the reason why one out of seven animals treated only with hydrocortisone acetate died, and why, of the dexamethasone-treated mice, one animal died without showing parasites in its blood. The possibility of a non-identified secondary infection due to impairment of the immune response can also not be excluded.

Sensitivity of mononuclear phagocytes to corticosteroid in vivo and in vitro has been reported (Hibbs, 1974; Clark et al., 1977b). Scott (1975) showed that non-specific anti-humor activity of Corynebacterium parvum-activated macrophages was sensitive to in vivo treatment with cortisone. Masur et al. (1982) showed that non-activated macrophages of mice were sensitive to in vitro treatment with hydrocortisone and unable to respond to Toxoplasma gondii. Lymphokine-activated macrophages were not sensitive. The immunosuppressive effect of the corticosteroid bethamethasone on protection of rodents against Babesia spp. has been demonstrated by Young and Cox (1971).

The immunosuppressive action of corticosteroid on mononuclear phagocytes is thought to be the main reason for abolishment of immunity in our experiments. However, the high dosages of the drugs needed for this purpose, the significantly prolonged prepatent period in the hydrocortisone-treated mice and the significantly delayed time of death of immune animals when compared to non-immune infected animals indicate that the immunity breakdown is not complete. The reason for this could be that only non-activated macrophages are corticosteroid sensitive while already activated macrophages are not affected (Masur et al., 1982). It is also possible that T-lymphocytes still maintain this low level protection.

Immunosuppressive effects of ATS on T-lymphocyte populations in vivo and in vitro have been described by Ruebush and Hanson (1980) and by Bach (1975). In the present study ATS used in vivo only partly abolished immunity to Babesia rodhaini. Although seven out of nine animals died, only one of them had a high parasitaemia, while all the others died with low parasitaemia. As these animals did not differ from the two survivors in their infection pattern, the level of parasitaemia did not seem to correlate with the outcome of the infection. This is in contrast to corticosteroid treatment where death is preceded by high parasitaemia. The long period of time between peak parasitaemia and the moment of death, as well as very low parasitaemia suggest a cause of death other than Babesia rodhaini alone.

In our experiments on the effect of ATS on an already established immunity to Babesia rodhaini, very high dosages were administered when compared to those used by Eling (1979) during the induction of immunity to Plasmodium berghei in mice. These high ATS levels may have caused toxic effects other than T-lymphocyte suppression only (Bach, 1975). This is supported by death of one out of six immune challenged animals, following ATS treatment.

The very pronounced abolishment of immunity after corticosteroid treatment and only partial abolishment after ATS treatment, as well as previously described resistance of this immunity to an irradiation dose of 875 rad (Zivkovic et al., 1984a), support the idea that mononuclear phagocytes are the most important cell population in the effector phase of specific immunity to Babesia rodhaini. The importance of macrophages in non-specifically induced protection against B.microti in mice was shown by Clark et

al. (1977b), who removed BCG-induced protection with hydrocortisone acetate injection.

These findings argue for the same effector mechanism in specifically and non-specifically induced immunity to Babesia parasites.

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