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Antibody-dependent and -independent cytotoxic activity of spleen cells for *Plasmodium berghei* from susceptible and resistant rats

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SUMMARY

Antibody-dependent cell cytotoxicity (ADCC) mediated by spleen cells from 30- or 50-day old rats against ^{51}Cr-labelled rat erythrocytes parasitized by *Plasmodium berghei* in the presence of anti-*P. berghei* antibody showed only slight age differences. However, in the absence of specific antibody, the total cell-mediated cytotoxicy (CMC) per spleen was four times higher in the spleen cells from 50-day-old rats compared with those from 30-day-old rats. CMC accounted for about 50% of total cytotoxic activity in 50-day-old rat spleens. Spleen cells mediating ADCC and CMC are Thy-1.1 positive, and those mediating ADCC are nearly all non-adherent to Sephadex G-10 columns.

INTRODUCTION

More than one million children die each year from malarial infections. The most severe clinical aspects of the disease with highest mortality is seen in young children infected with *Plasmodium falciparum* between 6 months and 5 years of age (McGregor *et al.*, 1956). We have used an age-related rat model in order to investigate the nature of immunological defence mechanisms that may be lacking in young, susceptible rats. It is well known that while 30-day-old rats succumb to *P. berghei* infection, 50-day-old rats survive (Singer, Hadfield & Lakonen, 1955; Smalley, 1975). We have confirmed that 30-day-old Sprague Dawley rats were susceptible to infection with *P. berghei* and died 8–10 days post-infection. Death was preceded by fulminating parasitaemia, massive splenomegaly and severe anaemia. In contrast, 50-day-old rats survived *P. berghei* infection with low parasitaemia, reduced splenomegaly and less severe anaemia.

Immunity to *P. berghei* infection in rats can be passively transferred by immune serum and, more effectively, by lymphoid cells (Phillips, 1970). Thus, antibody-dependent cell cytotoxicity (ADCC) is an interesting candidate for providing immunity to *P. berghei* as it involves both cellular and humoral immune mechanisms. ADCC has previously been implicated in killing malaria parasites *in vitro* in man (Greenwood, Odulojo & Stratton, 1977; Brown & Smalley, 1980; Brown & Greenwood, 1985), rats (Fox & Solomon, 1981) and mice (Coleman *et al.*, 1975; McDonald & Phillips, 1978).

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Abbreviations: ADCC, antibody-dependent cell cytotoxicity; CMC, cell-mediated cytotoxicity; FBS, fetal bovine serum; PBS, phosphate-buffered saline.

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In the present work we have studied natural ADCC mediated by spleen cells from 30- and 50-day-old rats by measuring the release of ^{51}Cr from labelled rat erythrocyte target cells parasitized by *P. berghei* and coated with specific antibody. No significant age differences were found. However, when antibody was excluded from the assay, cell-mediated cytotoxicity (CMC) per spleen was found to have increased four-fold between 30 days and 50 days of age and might account for some of the age-related resistance to *P. berghei* that develops during this period. We have also found enhanced ADCC and CMC at 8 days after infection; elevated levels of ADCC following *P. berghei* infection have previously been reported by McDonald & Phillips (1978) in mice and by Fox & Solomon (1981) in young rats.

MATERIALS AND METHODS

Animals

Outbred Sprague Dawley rats originally obtained from Charles River Ltd (Margate, Kent) were taken from a colony maintained in our animal house. *Plasmodium berghei* was passaged weekly in 30-day-old rats. Six-week-old outbred female MF1 mice from our closed colony were used for a 6-day maintenance passage of *Plasmodium berghei.*

Plasmodium berghei (NK 65) was kindly supplied by Dr D. Walliker of the Institute of Animal Genetics, University of Edinburgh, Edinburgh.

Enrichment of parasitized erythrocytes

Rat erythrocytes parasitized with *P. berghei* were enriched by Percoll gradient centrifugation as described by Wahlgren *et al.* (1983) for *P. falciparum.* Rat blood parasitized with *P. berghei* (about 20% parasitaemia) was washed twice in 0·01 M phosphate-buffered saline (PBS), pH 7·2 (Dulbecco A), and the erythrocytes suspended in PBS at a 10% haematocrit. Two

fractions were placed on top of 2·5 ml of 55% (v/v) Percoll (Pharmacia Ltd, Milton Keynes, Bucks). After centrifugation at 1500 *g* a distinct band of enriched parasitized erythrocytes was obtained. This was washed twice in PBS and suspended in a small volume of PBS. A four-fold enrichment was achieved, so that about 80% of erythrocytes were parasitized.

*Rat anti-*Plasmodium berghei *serum*

Rat anti-*P. berghei* hyperimmune serum was raised by a method originally described by Quinn & Wyler (1980) with slight modifications. Adult Sprague Dawley rats were given an intraperitoneal injection of 1×10^7 Percoll gradient-enriched *P. berghei*-infected rat erythrocytes in sterile PBS. When the parasites had disappeared from the blood, the rats were given an intraperitoneal injection of 1×10^8 Percoll gradient-enriched *P. berghei* every week for 6 weeks and were exsanguinated 2 weeks after the last injection. Pooled sera were absorbed three times with rat erythrocytes and heat-inactivated at 56° for 30 min. When tested for anti-rat erythrocyte antibodies by haemagglutination of rat erythrocytes, the titre was $< 1/2$.

Anti-*P. berghei* antibody was measured by means of a micro-ELISA technique. *Plasmodium berghei* antigen was prepared as described by Wahlgren *et al.* (1983) using the Percoll gradient method previously described. Enriched parasitized erythrocytes were sonicated for 2 min. The antigen was divided into aliquots and stored at −70°. The microplate method of enzyme-linked immunosorbent assay (ELISA) described by Voller *et al.* (1974) was used to measure anti-*P. berghei* antibody. Optimal dilution of the *P. berghei* antigen preparation was found to be 1/10. The absorbance at 405 nm of the contents of each well was determined in a Titertek multiscan spectrophotometer. All dilutions of antiserum were tested in duplicate. Maximum absorbance at 405 nm of a 1/10 dilution of a standard sera was allowed to develop to 1·7. At a 1/100 dilution absorbance at 405 nm was 1·04. This dilution was used in all ADCC tests.

Target cells

Parasitized rat erythrocytes (1×10^8, 20–25% parasitized with *P. berghei*) were injected intraperitoneally into 30-day-old rats. Seven days later (when the parasitaemia was about 20%) the rats were bled by cardiac puncture into PBS containing 100 units of heparin/ml and the cells centrifuged and washed twice with PBS. Four-hundred Ci of $Na_2{}^{51}CrO_4$ solution (Amersham International, Amersham, Bucks) were added to 0·1 ml packed parasitized erythrocytes and incubated for 2 hr at 37° with occasional shaking. The red cells were washed three times in RPMI medium (Flow Laboratories, Irvine, Ayrshire) with a 30-min interval between the second and third washes to allow diffusion of any loosely bound ^{51}Cr into the medium, and made up to 1×10^7 cells/ml RPMI medium.

Effector spleen cells

Spleen cells from Sprague Dawley rats of 30 or 50 days of age were prepared according to the method of Leiper & Solomon (1977). Spleens were macerated through a sieve, suspended in PBS and forced through 19- then 25-guage needles. Spleen cells were washed twice in PBS and the pellet suspended in RPMI medium.

^{51}Cr-release assays

Aliquots (0·2 ml) of ^{51}Cr-labelled rat erythrocytes parasitized with *P. berghei* were pipetted into plastic LP3 tubes. Diluted (1/100) rat anti-*P. berghei* serum (0·02 ml) was added and the mixture kept at room temperature (20°) for 30 min for the specific antibody to bind to the parasitized target cells. Each tube was rocked on a Vortex shaker every 10 min to enhance coating of the antibody on target cells. Effector spleen cells were added in 0·2 ml RPMI medium. The optimal effector to target (E/T) cell ratio in the ^{51}Cr-release assay was determined over a range of E/T ratios from 20:1 to 1:1. E/T ratios of 10:1, 5:1 and 3:1 gave a three-point linear curve and these were adopted for all experiments. For each E/T ratio the tests were set up in triplicate. Maximum ^{51}Cr-release was determined by adding 0·4% (v/v) Triton X-100 to lyse all target cells. The negative control (spontaneous release) consisted of target cells and 0·2 ml of RPMI medium; both controls contained specific antibody. CMC was measured in parallel with ADCC; the tubes contained the same number of target cells but were not pretreated with the specific antibody. Tubes were stoppered and incubated for 18 hr at 37° in humidified air containing 5% CO_2. After incubation, 1 ml of RPMI medium was added to each tube. The supernatant was collected by centrifuging all tubes at 500 *g* for 10 min and the amount of ^{51}Cr release counted on a 2001 gamma counter (Wilj Electronics, Ashford, Kent). ^{51}Cr release due to antibody (ADCC) was calculated using the formula:

$$\%\,\text{ADCC} = \frac{\left(\begin{matrix}\text{test}\\ \text{release}\end{matrix} - \begin{matrix}\text{spontaneous}\\ \text{release}\end{matrix}\right) - \left(\begin{matrix}\text{release without}\\ \text{antibody}\end{matrix} - \begin{matrix}\text{spontaneous}\\ \text{release}\end{matrix}\right)}{(\text{maximum release} - \text{spontaneous release})}.$$

^{51}Cr release in the absence of antibody (CMC) was given by the formula:

$$\%\,\text{CMC} = \frac{\left(\begin{matrix}\text{release without}\\ \text{specific antibody}\end{matrix} - \begin{matrix}\text{spontaneous}\\ \text{release}\end{matrix}\right)}{(\text{maximum release} - \text{spontaneous release})}.$$

Total ADCC and total CMC are expressed as lytic units (LU) per spleen in Tables 3 and 4. One LU = 10% ADCC or 10% CMC. Total ADCC and CMC are calculated from the number of LU per number of recovered spleen cells per rat.

Splenomegaly

The degree of splenomegaly was calculated from a modification of weighted splenic index (Hackett, 1944). The splenic index for a normal rat of a given age was determined by the quotient:

$$\text{Splenic index} = \frac{\text{spleen weight of normal rat}}{\text{body weight of the same rat}} \quad (1)$$

and for an infected rat:

$$\text{Splenic index} = \frac{\text{spleen weight of the infected rat}}{\text{body weight of the same rat}} \quad (2)$$

The degree of splenomegaly was given by dividing Equation (2) by Equation (1).

Treatment of spleen cells with monoclonal anti-rat Thy-1.1 IgG plus complement

Anti-rat Thy-1.1 IgG plus complement treatment of normal rat

spleen cells and those from *P. berghei*-infected rats was performed as described by Herberman *et al.* (1975). Briefly, 2×10^8 spleen cells were preincubated with a 1:2·7 final dilution of the ascite, monoclonal-rat Thy-1.1 IgG (MRC OX-7) (Serotec Ltd, Oxford, Oxon) in RPMI medium fortified with 10% FBS for 30 min at 20°. The cells were washed in RPMI medium and resuspended in a 1:3 dilution of normal rabbit serum as a source of complement and incubated at 37° for 45 min. The cells were washed three times in RPMI medium, counted and assayed for ADCC and CMC. Controls consisted of spleen cells treated with complement alone and spleen cells treated with anti-Thy1.1 antibody alone.

Cell fractionation by Sephadex G-10 columns

The method described by Jerrells *et al.* (1980) was used with the following minor modifications for spleen cells. Spleen cells (1×10^8) in 1 ml RPMI medium were loaded on columns of Sephadex G-10 (Pharmacia, Hounslow, Middlesex) in 10-ml syringes. Both ends of the syringes were sealed and gently rotated so the Sephadex G-10 and spleen cells were mixed. The columns were incubated for 30–40 min at 37° and shaken every 10 min. Following incubation, the non-adherent cells were eluted with 20 ml of warm RPMI medium containing 20% (v/v) normal rat serum. Adherent cells were recovered by elution with 0·5% lidocaine (Sigma, Poole, Dorset) according to Jerrells *et al.* (1980).

Statistics

Standard errors (SE) of means were calculated for all values and statistical significance between means calculated by Students' *t*-test.

RESULTS

Haematological status of 30- and 50-day-old rats before and after infection with *P. berghei*

When 30- and 50-day-old rats were infected with *P. berghei* (2×10^6 parasitized erythrocytes/50 g body weight) no deaths occurred in the older age group, but 17% of the younger rats had died by 8 days post-infection and all were dead after 13 days. At the onset of infection, reticulocyte counts were higher (12% total erythrocytes) in the younger rats than in the older group (2%). Severe anaemia occurred in the younger group from 8 days post-infection and the proportion of reticulocytes increased to 37% total erythrocytes. In contrast, there was only a slight compensatory reticulocytosis in the 50-day-old rats. There was little difference in the percentage of reticulocytes parasitized between the two age groups (Fig. 1). After virtually all the reticulocytes had become parasitized in the younger rats, an increasing proportion of mature erythrocytes became parasitized. However, because of the higher numbers of reticulocytes relative to mature erythrocytes in young rats (particularly by 8 days post-infection), the parasite burdens were nearly five times greater in young rats (Fig. 2). Parasite burdens started to decrease in the older, resistant rats as the infection was controlled.

ADCC and CMC in spleens of 30- and 50-day-old rats

In spleen cells from normal rats, antibody increases cytotoxicity much more in 30-day-old than 50-day-old rats (Table 1). This is

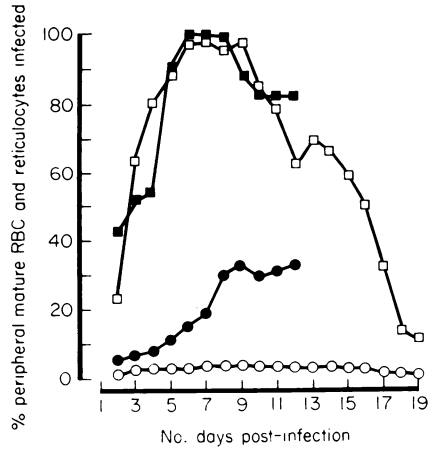

Figure 1. Percentage parasitaemia of reticulocytes and mature erythrocytes from 30- and 50-day-old rats infected with *P. berghei*: (■) 30-day-old rat reticulocytes; (□) 50-day-old rat reticulocytes; (●) 30-day-old rat erythrocytes; (○) 50-day-old rat erythrocytes.

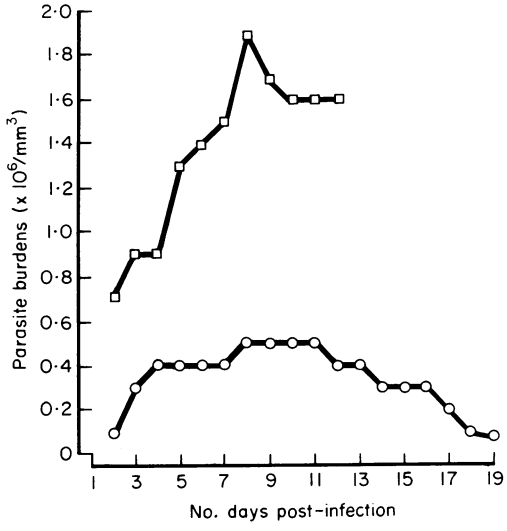

Figure 2. Total parasite burdens in blood cells of 30-day-old (□) and 50-day-old (○) rats infected with *P. berghei*.

particularly noticeable at an E/T ratio of 10:1 when 7·3% (CMC) was increased to 29·7% (ADCC). Also, in spleens from infected 30-day-old rats at the same time E/T ratio, CMC of 6·8% was increased to 57·8% (ADCC). There were no comparable increases at this E/T ratio in spleen cells from 50-day-old normal or infected rats. The percentage CMC of spleen cells from 50-day-old rats was significantly higher ($P < 0{\cdot}001$) than those from 30-day-old rats at all three E/T ratios. Eight days after infection, the percentage ADCC had increased by 1·5- to two-fold ($P < 0{\cdot}001$) at all E/T ratios in both age groups. There was only slight evidence for increased percentage CMC after infection, except in 50-day-old rat spleens when an E/T ratio of 10:1 was used. At this E/T ratio the percentage ADCC was at virtually the same level as the percentage CMC.

Relationship of parasitaemia and splenomegaly

Eight days after infection with *P. berghei* the percentage

Table 1. ADCC and CMC against parasitized erythrocytes mediated by spleen cells from normal and *P. berghei*-infected rats

Age (days) (no. rats)	Effector/target ratio	% ADCC ± SE: Normal spleen	% ADCC ± SE: Infected spleen*	% CMC ± SE: Normal spleen	% CMC ± SE: Infected spleen*
30	3:1	10·7 ± 0·5	15·9 ± 1·0	0	1·6 ± 0·7
(29)	5:1	18·9 ± 2·5	29·9 ± 1·6	0·2 ± 0·2	3·2 ± 0·4
	10:1	29·7 ± 0·8	57·8 ± 0·7	7·3 ± 0·4	6·8 ± 0·8
50	3:1	4·8 ± 0·9	9·8 ± 0·4	4·1 ± 0·1	5·2 ± 0·1
(32)	5:1	13·8 ± 1·6	26·1 ± 0·8	6·0 ± 0·4	7·6 ± 1·0
	10:1	21·0 ± 1·2	35·7 ± 0·8	18·3 ± 0·9	35·0 ± 1·8

* Eight days post-infection.

Table 2. Mean percentage parasitaemia, splenomegaly and increased number of spleen cells in 30- and 50-day-old rats 8 days after infection with *P. berghei*

	Rat age (days) 30	Rat age (days) 50
No. rats	43	46
Parasitaemia (%)	26·3 ± 1·1	3·5 ± 0·9
Splenomegaly measured by wet wt (normal = 1·0)	7·7 ± 0·8*	2·9 ± 1·0
Splenomegaly measured by number of recovered spleen cells per 2×10^8 cells (normal = 1·0)	13·0 ± 4·1*	3·1 ± 0·6

* Spleens of 30-day-old rats greater than those from 50-day-old rats ($P < 0{\cdot}001$).

parasitaemia in rats infected at 30 days of age was much higher than in those infected at 50 days (Table 2). Based on wet weight measurements, splenomegaly was 2·5 times greater in young rats compared to the older group ($P < 0{\cdot}001$). This was due to an increase in the number of cells as the cell count was four-fold greater ($P < 0{\cdot}001$) (Table 2).

Total splenic ADCC and CMC

When total ADCC and CMC activities per spleen were calculated at E/T = 10:1 for normal rats (Table 3) it was seen that while total ADCC was the same in both age groups, the total CMC was four-fold higher in the older rats. The increased ADCC and CMC activities eight days after infection are considerably greater in the younger age group due to the higher splenomegaly (Table 2).

Production of anti-*P. berghei* antibody

Sera taken from six 30- and 50-day-old rats at 8 days post-infection showed evidence of anti-*P. berghei* antibody by the ELISA technique. Whereas standard hyperimmune serum gave an absorbance at 405 nm of 1·7 at 1/10 dilution, the value for pooled sera from 50-day-old rats was 0·79 and from 30-day-old rats 0·66 (background absorbance was 0·54). No specific

Table 3. Total ADCC and CMC in spleens of normal 30- and 50-day-old rats and in rats 8 days after infection with *P. berghei*

Age (days)	Total ADCC (Lu*/spleen†): Normal	Total ADCC (Lu*/spleen†): Infected	Increase (normal = 1·0)	Total CMC (Lu/spleen†): Normal	Total CMC (Lu/spleen†): Infected	Increase (normal = 1·0)
30	128·0 ± 3·5	4280·2 ± 52·5	33·4	31·3 ± 1·7	503·2 ± 59·2	16·1
50	148·3 ± 8·1	848·8 ± 19·7	5·7	129·0 ± 6·3	831·7 ± 43·0	6·4

* One lytic unit (LU) = 10% ADCC or 10% CMC.

† Number of spleen cells recovered from normal 30-day-old rats = $8{\cdot}6 \times 10^8$, from 50-day-old rats = $1{\cdot}4 \times 10^9$, from infected 30-day-old rats = $1{\cdot}48 \times 10^{10}$, and from infected 50-day-old rats = $4{\cdot}75 \times 10^9$.

Table 4. Sephadex G-10 column fractionation of spleen cells from 50-day-old rats mediating ADCC

	Normal cells				Infected cells*			
		ADCC				ADCC		
	No. viable cells recovered ($\times 10^8$)	LU/10^8 cells	LU/ fraction	% recovery	No. viable cells recovered ($\times 10^8$)	LU/10^8 cells	LU/ fraction	% recovery
Unfractionated input	18	21·0±1·2	378	—	40	35·7±0·8	1428	—
Non-adherent	13	21·7±0·4	282	74·6	32	51·2+1·1	1638	114·7
Adherent, eluted	2	1·6±0·2	3	0·8	4	5·8±0·4	23	1·6

* Eight days post-infection with *P. berghei*.

Table 5. Treatment of spleen cells from normal and infected 30- and 50-day-old rats with monoclonal anti Thy-1.1 IgG plus complement

		% ADCC±SE		% CMC±SE	
Rat age (days)		Untreated spleen cells	Treated spleen cells	Untreated spleen cells	Treated spleen cells
Normal*	30	33·9±0·9	0	8·7±0·6	0
	50	11·3±0·9	0	18·3±0·2	0
Infected†	30	75·2±1·2	0	13·2±0·6	0
	50	23·4±0·	0	43·6±1·6	0

* Ten rats were used in each age group, E/T = 10:1.
† Eight days after infection with *P. berghei*.

antibody was detected in the supernatants of overnight cultures of spleens from such infected rats under conditions for CMC.

Sephadex G-10 column fractionation

In both normal and infected rat spleens Sephadex G-10 adherent cells comprised about 10% of the total cells and accounted for less than 2% of total ADCC (Table 4).

Treatment of spleen cells with anti-Thy-1.1 IgG

When spleen cells from normal 30- or 50-day-old rats, or from rats of both ages 8 days after infection with *P. berghei*, were treated with monoclonal anti-rat Thy-1.1 IgG and complement, all ADCC and CMC activity was destroyed, even at an E/T ratio of 10:1 (Table 5). Treatment of Sephadex G-10 non-adherent spleen cells from normal and infected 50-day-old rats with anti-Thy-1.1 IgG and complement showed that at a dilution of 1/100 or less, all ADCC activity was destroyed. At a dilution of 1/500 about 50% ADCC was destroyed, but there was no effect on ADCC activity at a dilution of 1/2000.

DISCUSSION

Greenwood *et al.* (1977) detected increased numbers of killer cells (ADCC to chicken erythrocyte targets) in the blood lymphocytes of children suffering from acute *P. falciparum* infection. Brown & Smalley (1980) observed that blood mononuclear cells (95% lymphocytes and 5% monocytes and neutrophils) from children (aged between 3 months and 6 years) and adult Gambians infected with *P. falciparum* were capable of killing *P. falciparum in vitro* in the presence of specific antibody. Later, Khusmith & Druilhe (1983) observed that purified normal human monocytes added to cultures of *P. falciparum* inhibited parasite growth and this was much more marked in the presence of specific IgG antibody. Recently, Brown & Greenwood (1985) have shown that the combination of blood mononuclear cells and autologous serum from patients in Gambia was more effective at inhibiting growth of *P. falciparum in vitro* than serum alone. They suggested that mononuclear cells are an important defence mechanism during early stages of malaria infection.

McDonald & Phillips (1978) found an increase in 'non-specific ADCC' (chicken erythrocyte targets) during 6–15 days post-infection of C57BL mice with *P. chabaudi*. When rat erythrocytes were used as targets an increase in splenic ADCC at 8 days following infection of young rats with *P. berghei* was also observed (Fox & Solomon, 1981). Small increases in splenic ADCC to erythrocyte target cells parasitized with *P. berghei* have been observed in infected mice (Coleman *et al.*, 1975). In our work, at 8 days post-infection with *P. berghei* both age groups of rats showed similar 1·5–2·0-fold increases in the percentage ADCC but only 50-day-old rats showed a significant increase in the percentage CMC at E/T = 10:1. The greater splenomegaly in 30-day-old *P. berghei*-infected rats means they have a very high total ADCC and greatly increased total CMC by 8 days post-infection (Table 3). Possibly, the higher parasite burden (Fig. 2) in the circulation of the spleen of the younger rats had stimulated a greater multiplication of the effector cells.

'Splenic-mediated cytotoxicity' against ^{51}Cr-labelled *P. berghei*-parasitized erythrocytes has been studied in mice by Coleman *et al.* (1975). Most of the ^{51}Cr release was due to cell-mediated cytotoxicity, and the presence of 'immune serum' generally produced only small increases in ^{51}Cr release, i.e. ADCC was a minor component. Spleen cells from mice taken at the time of peak parasitaemia after transfusion with packed erythrocytes (termed 'immune spleen cells') showed slightly greater CMC but the ADCC component was still minor. This difference may be due to the use of more potent antibody in our work.

High parasitaemias in malarial infections can be correlated with increased numbers of spleen cells in the enlarged spleen (Zuckerman, 1977; Aikawa, Susuki & Gutierrez, 1980). This finding is borne out in our rat model. It is of interest that anaemia and enhanced phagocytosis as well as splenomegaly are all thymus-dependent responses in mice (Roberts & Weidanz, 1979). Splenomegaly seems to be an abortive attempt by young rats to increase the number of killer cells in order to control the infection. However, the cells mediating ADCC or CMC (which may be subsets of natural killer cells) appear only to be important in the early stages of a malarial infection.

There is suggestive evidence to indicate that some immunity to *P. berghei* is provided by T cells as Spira, Silverman & Gaines (1970) found that adult rats succumbed to *P. berghei* after treatment with anti-lymphocyte serum and Smalley (1975) observed higher parasitaemias in adult rats treated with anti-thymocyte serum. Also, neonatal thymectomy of adult rats abolished resistance to primary infection with *P. berghei* (Brown, Allison & Taylor, 1968). These earlier observations become more interesting in the light of our finding that all cytotoxic cells mediating ADCC and CMC against *P. berghei*-infected target cells express Thy-1.1 antigen. As most cells are non-adherent to Sephadex G-10 columns, this tends to preclude a macrophage or monocyte as the effector cell.

We have found that the total CMC per spleen is four times higher in 50-day-old rats compared to 30-day-old rats. This increase in antibody-independent cytotoxicity with age is in accord with the finding that 50-day-old rat spleen cells are four times more efficient at killing *P. berghei in vitro* than those from 30-day-old rats (Solomon, 1986). Additionally, we have found that 50-day-old rats produce slightly more anti-*P. berghei* antibody at 8 days post-infection than 30-day-old rats. This suggests that ADCC may operate *in vivo* at this stage of infection in both age groups.

This age relationship lends credence to the use of young rats as a model for the study of childhood malaria. While specific antibody will not be present in the very early stages of infection of adults when only CMC or natural cytotoxicity could provide natural immunity, the presence of specific anti-malarial antibody of maternal origin in the circulation of very young children may increase cytotoxicity via ADCC in the early stages of infection.

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