

Protective Immunity against *Plasmodium berghei* malaria after administration of Interleukin-12

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Abstract

Interleukin-12 (IL-12) has been shown to induce protection in mice against *Plasmodium cyanomolgi* and in rhesus monkey against *Plasmodium yeolii*. This study is to investigate whether recombinant IL-12 can induce protection in BALB/c mice against *Plasmodium berghei*. Five mice were given intraperitoneal injection of 7.5 µg/kg body weight recombinant mouse IL-12 two days prior to challenge with 5×10^4 of *P.berghei*, while mice in the control group were injected with 0.5 ml of normal saline prior to challenge. In both groups, the parasitaemia appeared on the fourth day after the infection. There was a slight reduction in the parasite burden in mice given IL-12 and the mice also survived longer compared to controls. Statistical significance of the difference could not be determined due to the small sample size. Nevertheless, the results of the study suggested that IL-12 may be able to protect mice against *P.berghei* infection.

Key words: Interleukin-12, *Plasmodium berghei*, protection.

INTRODUCTION

Malaria continues to be a serious public health problem in many parts of the world. Estimates of the global incidence of malaria range from 300 to 500 million cases per year, with 2 to 3 million deaths.¹ Despite many interventional efforts, morbidity and mortality estimates continue to rise. Chemotherapy and vector control programs have been largely ineffective due to the emergence and spread of insecticide-resistant mosquito vectors and drug-resistant *Plasmodia*. The search for an effective vaccine against human malaria has not been successful despite the progress made in antigen identification over the last 15 years.

Interleukin-12 (IL-12) is a recently described cytokine that has been increasingly recognized for its critical role in host defence against infections. IL-12 is a heterodimeric protein consisting of two subunits (p35 and p40) that promote Th1 type immune response.² The administration of IL-12 systemically to mice has been shown to increase host resistance to several intracellular pathogens including *Leishmania major*, *Toxoplasma gondii*, *Listeria monocytogenes*, *Mycobacterium tuberculosis* and *Plasmodium chabaudi*.^{3,4} The present study is to investigate whether there is induction of protective response by administration of IL-12 against

Plasmodium berghei in BALB/c mice.

MATERIALS AND METHODS

Ten 4-week-old male BALB/c mice were used in the study. They were divided into two groups which consisted of 5 mice per group. Two days prior to challenge with about 5×10^4 *P.berghei* through intraperitoneal injection, the control mice were given 0.5 ml of normal saline, while the test mice were given recombinant mouse IL-12 (7.5 µg/kg body weight) intraperitoneally. Blood films of each mouse were monitored daily for the presence of malaria parasites after being infected. Collection of blood was by the tail vein. The blood films were stained with Giemsa and the parasite density was calculated based on the number of counted red blood cells.

RESULTS AND DISCUSSION

The daily parasite density and mean parasitaemia in control and test mice are shown in Table 1. The peak parasite densities for the test mice were observed on the seventh day post infection, except for mouse E4 where the peak was observed on the eighth day post infection. Mouse E5 died the earliest i.e on the ninth post infection day. Mice E1, E3 and E4 died on the tenth post infection day. All four mice died when the

TABLE 1: Daily parasite density ($\times 10^5/\text{mm}^3$) and Mean of parasitaemia in Control Balb/c mice and Test mice (given Interleukin 12) after infection with 5×10^4 *Plasmodium berghei*.

Day post infection	Test Group ($\times 10^5/\text{mm}^3$)					Control Group ($\times 10^5/\text{mm}^3$)					Daily mean of parasitaemia		
	E1	E2	E3	E4	E5	C1	C2	C3	C4	C5	Test Group ($\times 10^5/\text{mm}^3$)	Control Group ($\times 10^5/\text{mm}^3$)	% decrease in the test group
Day 1	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 4	1.2	1.5	0.6	0.4	0.9	1.07	2.13	2.9	1.2	1.7	0.9	1.8	50.0
Day 5	6.8	20.0	7.7	3.2	7.3	6.9	12.3	25.6	9.5	13.2	9.0	13.5	33.3
Day 6	23.5	34.9	17.1	22.4	30.7	23.5	55.3	45.9	16.0	22.8	25.7	32.7	21.4
Day 7	77.8	58.5	83.7	69.3	87.1	58.6	122.4	142.6	39.3	68.0	75.3	86.0	12.6
Day 8	65.9	38.5	73.3	73.2	60.3	55.4	116.9	Dead	41.7	Dead	62.2	71.3	12.8
Day 9	48.6	48.6	31.7	67.9	Dead	Dead	Dead	Dead	Dead	Dead	49.2	Dead	N/A
Day 10	Dead	27.4	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	27.4	Dead	N/A
Day 11	Dead	23.9	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	23.9	Dead	N/A
Day 12	Dead	34.6	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	34.6	Dead	N/A
Day 13	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	N/A

N/A : Not Applicable

parasitaemia was observed to decline. Mouse E2 survived the longest until the twelfth post infection day. In this mouse, the parasite density was observed to decline after its peak at the seventh post infection day. However, on the twelfth post infection day, the parasite density started to rise again before the animal died.

For all the control mice, parasitaemia was first detected on the fourth post infection day. In all control mice except C4, the peak parasite density was observed on the seventh post infection day with the highest parasite density ($142.64 \times 10^5/\text{mm}^3$) observed in mouse C3. Two mice (C3 and C5) died on the seventh post infection day, while the other three died on the eighth post infection day. Three of the control mice died at the peak of parasitaemia while the other two died when the parasitaemia was observed to start declining. The percentage of the difference in daily mean parasite densities between the test and the control group was taken as the percentage of reduction in parasite burden. Fifty percent reduction was observed in the test group at day four post infection. The percentage of reduction subsequently reduced to 33.3% and finally to 12.8%. Due to the small sample size, the significance of the difference between the two groups could not be determined statistically.

Malaria parasites have developed resistance to many antimalarial drugs. Therefore, newer methods for prevention of the disease is required. Studies have shown that administration of recombinant IL-12 to mice and monkeys prior to challenge with *Plasmodium yoelii* and *Plasmodium cynomolgi* respectively can induce protection against the parasites.^{5,6} In this study, mice given a single dose of recombinant IL-12 were observed to have daily parasite density lower than the control mice. The percentage of parasite reduction as indicated by the percentage of the difference in the parasite densities (50%) was observed to be highest on the fourth post infection day and the percentage dropped subsequently. Sedegah *et al*, (1994) reported induction of 100% protection by intraperitoneal injection of IL-12 against *P.yoelii* sporozoites in mice. In this study, recombinant IL-12 has been shown to reduce parasite burden but not achieve total prevention in mice challenged with the erythrocytic stage of *P.berghei*. In addition to a reduction of parasite burden, mice given IL-12 survived longer compared to the controls. All control mice died at the peak of parasitaemia, while all mice given IL-12 survived the peak parasitaemia period. The finding suggests a possible protective role

played by IL-12 in those mice. The mice may probably survive longer or greater protection would have been induced if a higher dose or multiple doses of IL-12 were given. Nevertheless, the finding of this study agrees with other studies in mice that support assessment of recombinant IL-12 for immunoprophylaxis of malaria infection.

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