Protection of mice against *Plasmodium berghei* infection by a tuftsin derivative

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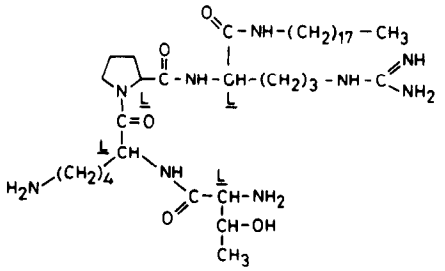
In *Plasmodium berghei* infections, the mortality rate and parasitaemias were significantly reduced and the mean survival time was considerably enhanced by pretreating the animals with a tuftsin derivative, Thr-Lys-Pro-Arg-NH-$(CH_2)_2$-NHCO$C_{15}H_{31}$. This effect of the modified tuftsin was further increased upon its incorporation in the liposome bilayer. These results indicate that tuftsin and its derivatives may prove useful in enhancing nonspecific host resistance against protozoan infections.

Protozoan infection *Immunomodulation* *Tuftsin* *Liposome* *Macrophage*

1. INTRODUCTION

The tetrapeptide tuftsin (Thr-Lys-Pro-Arg) resembles an integral component of IgG and is released physiologically as the free peptide fragment after enzymatic cleavage [1]. Several studies have shown that this peptide is a potent natural killer activator of phagocytic cells including macrophages [2–6]. Since activated macrophages have been demonstrated to kill intraerythrocytic malarial parasites in vitro [7], we considered it of interest to examine the effect of tuftsin and its derivatives on *Plasmodium berghei* infection in mice. Our present data clearly show that pretreatment with the tuftsin derivative II (fig.1) significantly enhances the mean survival time and reduces the parasitaemias in infected animals.



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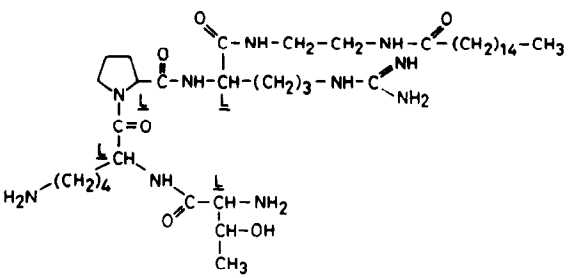

(II)

Fig.1. Hydrophobic derivatives of the tetrapeptide Thr-Lys-Pro-Arg (tuftsin).

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2. MATERIALS AND METHODS

2.1. *Animals*

Randomly bred male Swiss mice weighing 20 ± 1 g were used in all experiments reported here. The animals had access to food (pellet diet, Hindustan Lever, Bombay) and water ad libitum.

2.2. *Parasite*

P. berghei parasite was obtained from the National Institute of Communicable Diseases, New Delhi, and maintained in Swiss mice through serial blood passage.

2.3. *Tuftsin and its derivatives*

Tuftsin and its derivatives (fig.1, I and II) were synthesized and characterized as in [8].

2.4. *Liposomes*

Liposomes were prepared from equimolar amounts of egg phosphatidylcholine (PC) and cholesterol with or without II (8% by egg PC wt) in Tris-buffered saline (10 mM Tris containing 150 mM NaCl, pH 7.4) by probe sonication [8]. The sonicated preparation was centrifuged at 15000 × *g* for 30 min (10°C) to sediment undispersed lipids and titanium particles. The amount of II incorporated in the liposome bilayer was estimated as in [8].

2.5. *Animal experiments*

Tuftsin or one of its derivatives in Tris-buffered saline (0.5 ml) was administered intravenously to mice prior to or after the infection. The animals were challenged with 1×10^6 parasitized erythrocytes/mouse intraperitoneally, and kept under observation to record parasitaemias and mortality. Parasitaemia was determined by counting 10^4 red cells in a thin blood smear stained with Giemsa, and expressed as the number of parasitized cells/100 erythrocytes. The animals that were given only Tris-buffered saline (0.5 ml) or egg PC-cholesterol liposomes were used as controls. The day of challenge was referred to as day zero while other days were referred to as minus or plus (before and after challenge, respectively) to indicate the day of treatment with tuftsin and its derivatives.

Table 1

Effect of tuftsin and its derivatives on mean survival time of mice infected with *P. berghei*

Animal group	Total number of animals	Animals surviving on day +12	Mean survival time (± SD)
Control	20	0	8.7 ± 1.6
Tuftsin-treated	10	3	10.4 ± 2.9
I-treated	10	0	9.2 ± 2.5
II-treated	10	5	14.2 ± 6.8

Tuftsin or one of its derivatives was given on days −3 to 0. The dose of these peptides/animal per day was 50 μg

3. RESULTS AND DISCUSSION

Mice that were pretreated with tuftsin or one of its derivatives were challenged with *P. berghei*, and their mortality and parasitaemias were recorded up to day +21. The animals in both the control and treated groups started dying from day +7 and all the animals in control and I-treated groups succumbed to infection by day +12 (table 1). However, under identical conditions, about 30% animals from the tuftsin-treated group and 50% animals from the II-treated group survived on day +12. Moreover, the mean survival time of the animals that received pretreatment with II was significantly ($p < 0.001$) greater than that of the control group. Further, although the rate of increase of parasitaemia in II-treated animals was similar to that observed in control mice up to day +7, the parasitaemia levels in II-treated animals did not increase further at least up to day +17. These results clearly demonstrate that mice pretreated with II are partially protected against lethal *P. berghei* infection. A pretreatment dose of 50 μg/animal per day of II for more than 1 day seems to be necessary to obtain this partial protection, since the mortality rate in animals pretreated with a single dose (200 μg) on day −3 or with lower doses (10 or 25 μg) on days 0 to −3 was very similar to that observed in the saline-treated animals.

Further experiments were done to ascertain whether enhanced uptake of II by phagocytic cells would increase its effect on *P. berghei* infection. As liposomes are known to localize primarily in

tissues rich in these cells [9], we speculated that uptake of II by the cells may further increase by incorporating it in the liposome bilayer. To examine this possibility liposomes containing varying amounts of II in their bilayer were administered to mice before and after challenging them with *P. berghei* and mortality as well as parasitaemias recorded. Fig.2 shows that both the mortality rate and parasitaemias in the animals that received pretreatment with egg PC-cholesterol-II liposomes were significantly reduced, as compared with those

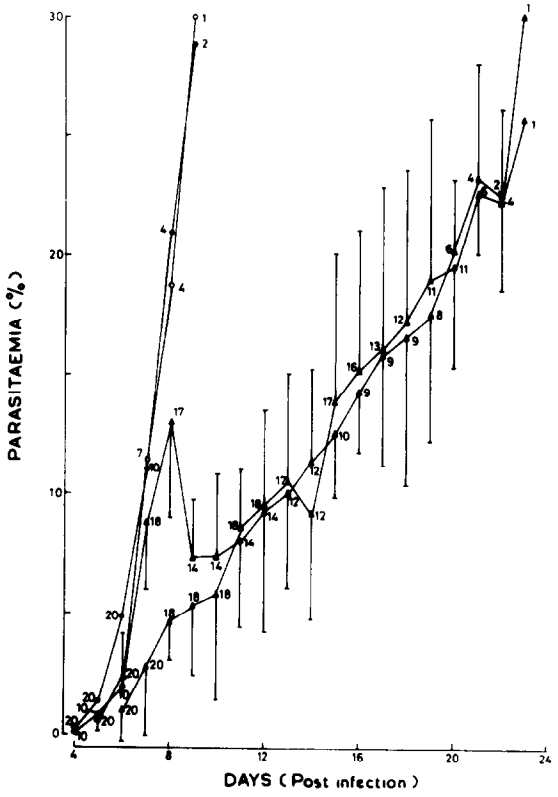

Fig.2. Parasitaemias in *P. berghei*-infected mice that received pretreatment on days −3 to 0 with egg PC-cholesterol-II liposomes. The doses of liposomised II were 50 μg/animal per day (▲) and 100 μg/animal per day (△). The animals that were given Tris-buffered saline (●) and egg PC-cholesterol liposomes (○) were used as controls. Values shown are means ± SD. The figures shown on each point represent the number of mice surviving on that day. No mortality in any of these four groups was observed up to day +6. For the sake of clarity only the mean values of parasitaemias are shown in the control groups.

pretreated with saline or egg PC-cholesterol liposomes. However, similar treatment on days 0 to +3 did not confer much protection (fig.3). Besides, a better response was observed if the pretreatment dose of II was about 100 μg/animal per day instead of 50 μg/animal per day. This dose was optimal, as increasing the dose to 200 μg resulted in lower protection (fig.4). These results clearly show that II upon incorporation into the liposome bilayer still retains its antiparasitic activity. The mean survival times of mice pretreated with 50 and 100 μg doses of II in liposomes were about 16 and 19 days respectively (fig.4), which were greater than that observed with II in free form (table 1).

The present study shows that in *P. berghei* infection parasitaemias and mortality rates are

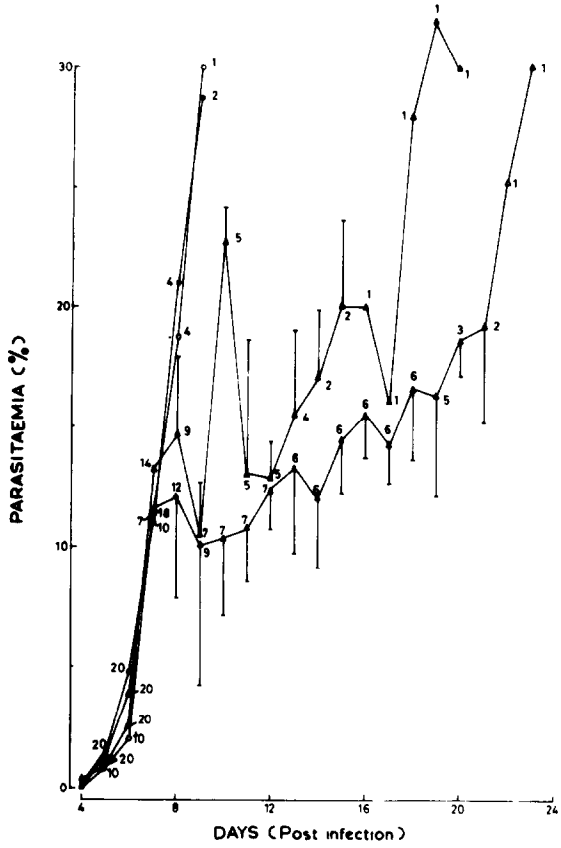

Fig.3. Parasitaemias in *P. berghei*-infected mice that were treated on days 0 to +3 with egg PC-cholesterol-II liposomes. For other details see fig.2.

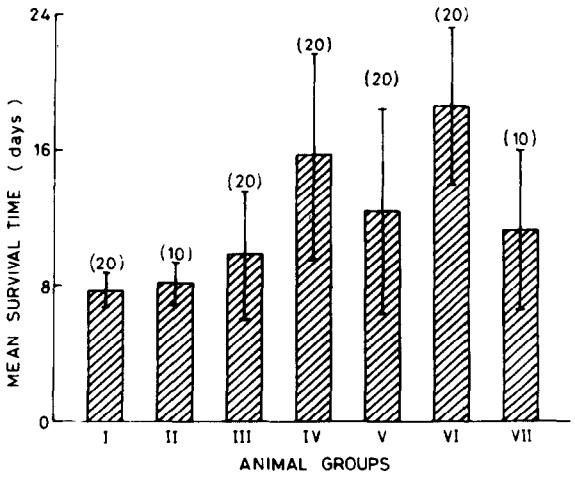

Fig.4. Survival time of mice that received treatment with egg PC-cholesterol-II liposomes, before or after the *P. berghei* infection. I, mice treated with saline; II, mice treated with egg PC-cholesterol liposomes; III, mice treated with 50 μg/animal per day dose of liposomised II on days 0 to +3; IV, mice treated with 50 μg/animal per day dose of liposomised II on days −3 to 0; V, mice treated with 100 μg/animal per day dose of liposomised II on days 0 to +3; VI, mice treated with 100 μg/animal per day dose of liposomised II on days −3 to 0; VII, mice treated with 200 μg/animal per day dose of liposomised II on days −3 to 0. Values shown are means ± SD. Figures shown in parentheses represent the total number of animals taken in each group.

significantly reduced in II-treated animals as compared with untreated infected controls. Also, the survival time of the infected mice is considerably ($p < 0.001$) enhanced by their pretreatment with the tuftsin derivative. This protective effect of II against *P. berghei* infection should arise probably from II-induced stimulation of macrophages and other related cells [2–6].

Suppression of host immune response seems to be a general feature in almost all parasitic diseases [10]. Agents that can bring the immune status back to normal should therefore find an important application in controlling the disease. In this context, several molecules of natural and synthetic origin, which possess strong immunomodulatory activity, have been examined for their antiparasitic action [11], but none of them appears to induce as marked a resistance against protozoan infections as observed in the present study. We, therefore, conclude that tuftsin and its derivatives could prove useful in enhancing nonspecific resistance against protozoan and other parasitic infections.

ACKNOWLEDGEMENTS

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