Increased susceptibility of *Trypanosoma lewisi* infected, or decomplemented rats to *Salmonella typhimurium*[1]

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Summary. Rats infected with *Trypanosoma lewisi* or decomplemented by injection of cobra venom factor or complement activating factor of trypanosomes were found to be more susceptible to infection with *Salmonella typhimurium*. Decomplemented rats subsequently infected with *T. lewisi* developed higher blood parasitemia than did normal *T. lewisi* infected rats.

We have previously reported that *Trypanosoma lewisi* contains materials which are capable of activating complement in vitro[2,3]. This finding has also been reported in vivo for rats infected with *T. lewisi*[4] as well as in cattle infected with *T. congolense*[5] and in Gambian sleeping sickness in man[6,7]. The significance of decreased complement levels in trypanosome infected hosts is not understood, but may be of importance in the depression of the immune response observed in such animals[8,9] or in the susceptibility to secondary infections[10] and tumors[11].

Thus 250-g male Wistar rats were infected with 10^9 *T. lewisi* obtained from rat blood 7 days after injection. 24 h previous to *T. lewisi* infection, groups of rats were decomplemented by i.p. injection of 80 units of cobra venom factor[12] or 1.0 mg of complement activating factor from *T. lewisi*[3]. 7 days later, the rats were challenged by i.p. injection of 10^9 *Salmonella typhimurium* (this number was established to be $^1/_2$ of an LD_{50} dose). Blood samples were taken from all animals prior to injection of *S. typhimurium* for parasite counts and for determination of hemolytic complement levels. In addition, blood samples were taken from surviving rats 48 h after injection of *S. typhimurium* for hemolytic complement determination. The overall experimental design is outlined in table 1.

Blood parasite counts were established using a hemacytometer. The level of hemolytic complement contained in each serum sample was determined by the microtiter dilution method using sheep erythrocytes optimally sensitized with rabbit hemolysin. The last well in which all the sensitized erythrocytes were lysed after incubation at 37 °C for 1 h was considered to be a measure of the complement levels of the animals.

As is apparent from table 2, the animals of groups 1 and 10 (decomplemented with CoF or CAF-T respectively and then infected with *T. lewisi*) had approximately 4 times as high a parasitemia as groups 2 and 3 (infected at the same time with *T. lewisi*). It is also evident that as expected, the decomplemented animals had low or no hemolytic titers as compared to the nontreated controls (group 7) and that the *T. lewisi* infected animals (groups 2 and 3) had been partially depleted of complement as well. In addition, rats which had been deprived of a functional complement cascade or infected with *T. lewisi* were much more susceptible to subsequent infection with *S. typhimurium*. In fact, the rats of groups 2 and 10 (infected with *T. lewisi* and decomplemented with CAF-T) began to die 4–5 h after challenge with the freshly cultured *S. typhimurium* while the control group (6) and the CoF complement depleted group (4) did not commence to die until 18 h after challenge. No deaths were observed in the untreated group (6) or in the complement depleted control groups (5 and 8). At the termination of the experiment, 48 h after injection of *S. typhimurium* and 9 days after infection with *T. lewisi*, all the remaining animals appeared healthy.

While the adverse effects of secondary infections on hosts parasitized with trypanosomes are well established (reviewed by Losos[10] the relationship between such secondary infections and complement depletion by trypanosomes has not been explored. It stands to reason that interference with the host defense mechanisms, such as complement, would allow invading pathogens nearly unhindered growth, especially if the complement cascade was interfered with at the C3 level (for example with CoF) as C3 is of considerable significance in protecting the host against infection[13].

Table 1. Injection schedule of each of group of rats

Group	No. of animals	*T. lewisi*	CoF*	CAF-T**	*S. typhimurium*
1	10	10^9	80 units		
2	10	10^9			10^9
3	10	10^9			
4	10		80 units		10^9
5	10		80 units		
6	10				10^9
7	10				
8	6			1 mg	
9	6			1 mg	10^9
10	6	10^9		1 mg	

* Cobra venom factor, injected in 3 equal amounts 24 h prior to *T. lewisi* or *S. typhimurium* injection. ** Complement activating factor of *T. lewisi*, injected in 3 equal amounts prior to injection with *T. lewisi* or *S. typhimurium*.

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Table 2. *T. lewisi* parasitemia (organisms per ml of whole blood) and hemolytic complement levels in each group of rats

Group	No. of parasites/ml (mean ± SD)	Complement levels before *S. typhimurium* infection (mean ± 1 SD)	Complement levels 48 h after *S. typhimurium* infection (mean ± 1 SD)
1	$6.01 \times 10^9 \pm 2.54 \times 10^9$ (10)**	1.0 ± 1.41 (10)	0.6 ± 0.97 (10)
2	$1.39 \times 10^9 \pm 0.76 \times 10^9$ (10)	7.0 ± 4.35 (10)	ND*
3	$1.38 \times 10^9 \pm 0.60 \times 10^9$ (10)	7.2 ± 2.53 (10	8.0 ± 1.00 (10)
4	–	1.2 ± 2.14 (10)	1.33 ± 1.15 (3)
5	–	1.2 ± 2.14 (10)	0.4 ± 0.84 (10)
6	–	20.0 ± 6.47 (10)	22.4 ± 7.93 (9)
7	–	24.0 ± 11.31 (10)	23.6 ± 11.18 (10)
8	–	0 (6)	0 (6)
9	–	0 (6)	ND
10	$5.83 \times 10^9 \pm 3.41 \times 10^9$ (6)	0 (6)	0 (6)

* ND, not done due to 100% mortality within group. ** Number in bracket denotes number of animals in the group.

From the experiments reported in this communication, it is quite clear that deprivation of C3 by CoF, followed by infection with *S. typhimurium* increased the susceptibility of the rats to this bacteria. However, interference at the C1 level of the complement sequence by injection of CAF-T or on *T. lewisi* infected rats had much more severe consequences after infection with *S. typhimurium*. Thus all the rats in the latter griups died with classical symptoms of Salmonella enteritis while only 70% of the CoF-treated rats succumbed to the infection. In addition, all death occurred in the *T. lewisi* infected group and the CAF-T treated group 5–12 h after *S. typhimurium* infection while CoF treated rats also injected with *S. typhimurium* did not begin to die until 18 h after infection. It is realized that *T. lewisi* infection may contribute to increased pathogenicity of *S. typhimurium* in other ways than by lowering hemolytic complement levels, however, no such influences should be the case in animal treated with CAF-T, as the control group (8) showed no other adverse effects within the duration of the experiment.

It was also noted that CoF and CAF-T treatment of rats previous to infection with *T. lewisi* (groups 1 and 10 respectively) increased the blood parasitemia of both groups. This again would indicate that interference with the nonspecific host defense mechanisms allow infections to proceed relatively unhindered. This phenomenon may be the result of several mechanisms, for example, initial and continued depletion of complement would not allow normal immune clearance of the parasite thus allowing it to circulate within the body in much greater numbers.
A great deal more work is required to establish mechanisms by which trypanosomes interfere with host defenses especially in view of their ability to localize in microenvironments and produce a variety of soluble substances for instance, hemolytic factors[14], complement activating factors[2] and mitogenic factors[15].

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The effect of epinephrine on granulocyte adhesion

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Summary. Preincubation of blood from normal human volunteers with epinephrine significantly decreased the granulocytes ability to adhere to nylon fibres. Possible significance for the in vivo correlation is discussed.

Granulocytes must adhere to the endothelial walls of capillaries and then diapedesis through the vessel wall in order to become localized in an area of infection. Kinetic studies have revealed that over half of the granulocytes within the vasculature normally are adherent to the endothelial walls[2]. These marginated granulocytes are in equilibrium with freely circulating ones.
Defects in granulocytes' ability to adhere to endothelial linings might be expected to be associated with increased incidence of infection. The granulocyte adhesiveness to nylon fibres or glass in vitro has been used as a measure of adhesion in vivo but whether the in vitro testing system parallels what occurs in vivo remains to be proven. Since epinephrine has been shown to decrease granulocyte adhesion in vivo, it would be expected to cause a decreased adhesion in vitro as well. The purpose of this study was to determine if epinephrine inhibits granulocyte adhesion in vitro.
Methods and materials. The adhesion of granulocytes to nylon fibres was measured using a modification of a previously described method[3]. Nylon fibre (3 denier, 4 cm, Type 200, Fenwall Laboratories, Morton Grove, Illinois) was weighed and 70 ± 0.5 mg was packed into a Pasteur

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