

## SPECIFIC IMMUNITY IN MICE TO HEARTWATER

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### ABSTRACT

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Mice develop a specific immune response following infection with the mice strains of heartwater. In the case of the Küm strain the agent can persist in some tissues for up to 365 days. Transfer of spleen cells from immune mice confers protection against homologous challenge in recipient mice showing that cell mediated immunity is important. A comparison with immune mechanisms occurring in other *Rickettsia* is discussed.

Mice are able to develop a specific immunity to the various mice strains of heartwater as shown by homologous challenge (Du Plessis, 1982; MacKenzie & Van Rooyen, 1981). The duration of this immunity in the case of the Küm strain is at least 18 months (Du Plessis, 1982). Whether this is related to the persistence of the organisms in the tissues is not known, but Du Plessis (1982) was able to recover the organism from the liver, spleen and myocardium of mice 365 days after infection. The myocardium and the lung appeared to have the highest infectivity whereas peritoneal macrophages were only infective for up to 30 days following primary infections.

In immune mice, infective material could not be demonstrated in peritoneal macrophages following re-infection which demonstrates the fact that at least the Küm strain does not replicate in peritoneal macrophages of re-infected immune mice but does replicate for a short time in macrophages of susceptible mice. Immune sheep serum has no effect on the infection whether given 6 h prior to infection, or repeated 4 days later (Du Plessis, 1982).

Mc Hardy & MacKenzie (1981) showed that Balb/C mice were more susceptible to the Kwanyanga strain of heartwater than either Balb/B or Balb/K mice. That these strains of mice differ only at the H<sub>2</sub> locus, provides evidence that the H<sub>2</sub> locus is associated with susceptibility to heartwater.

The protective immune response which develops in mice appears to be primarily cell-mediated in nature. Du Plessis (1982) infected mice with the Küm strain by blocking the infection with gloxazone (Dithiosemicarbazone) 8 days after infection to prevent mortality and then re-challenged 14 days later to ensure they were fully immune. Three days prior to the harvesting of their spleens they were treated with gloxazone (60 µg/g) in order to sterilize any persistent infection. Tissue homogenates were prepared from immune mice and inoculated into susceptible mice to show that the gloxazone had sterilized the infection. Spleen suspensions were made and macrophages removed by allowing them to attach to glass for 30 min. The non-attached cells were inoculated intraperitoneally into susceptible mice so that  $4 \times 10^7$  cells were inoculated into each mouse. When challenged 30 and 60 days later, the mice were immune to challenge, whereas mice treated in a similar manner with non-immune lymphocytes were fully susceptible.

Du Plessis (1982) showed that, if  $1 \times 10^7$  spleen cells from immune mice are incubated with 600LD<sub>50</sub> of the Küm strain for 20 min. before inoculation into susceptible mice, then these mice do not die from the infection. If the experiment is repeated using non-immune spleen cells, the mice die from the infection. Likewise, if immune spleen cells are mixed with the Küm strain

and inoculated directly into susceptible mice, the mice die from infection (Stewart, laboratory observation, 1985). This would suggest that *in vitro* incubation of immune spleen cells containing both memory lymphocytes and macrophages results in the destruction of the infective agent within 20 min. *In vitro* incubation is necessary for this reaction to occur, probably to allow close contact between immune cells and the infective agent. Once inoculated into mice, the immune cells are diluted with non-immune cells, thus allowing the parasite to localize and multiply. The fact that the Küm strain has been shown to enter macrophages for a short period and can be destroyed *in vitro* by immune spleen cells, but not by non-immune cells, suggests that the memory cells cause destruction of the agent, possibly by the release of lymphokines and the activation of macrophages, which are then able to destroy the agent.

The fact that the Küm strain enters macrophages may explain why it is possible successfully to infect mice by the intraperitoneal route, whereas other mice strains must be given by the intravenous route. Whether the other strains of heartwater also enter macrophages remains to be seen.

Stewart (laboratory observations, 1984) showed that nonspecific cytotoxicity does not develop in mice infected with either the Küm or Kwanyanga strain of heartwater. P815 cells were used as target cells, and spleen cells from infected mice as effector cells in a chromium release assay.

### Comparison with other rickettsial infections

A comparison of the various immune mechanisms which have been shown to occur in heartwater with other *Rickettsia* is given in Table 1. This subject has recently been reviewed by Tringali, Montenegro, Walker & Mansueto (1983). The role of humoral antibodies has been shown to play a role in the case of *R. prowazekii*, and *R. mooseri* (Zinsser, Castaneda & Hager, 1985) and *R. rickettsii* (Ricketts & Gomez, 1908). However, humoral antibodies do not appear to play a role in infection with the Küm strain of heartwater (Du Plessis, 1982). Adoptive transfer of spleen cells collected from immune animals to susceptible animals appears to be an important finding in all *Rickettsia*, including heartwater. However, only in the case of *R. mooseri* and *R. tsutsugamushi* (Shirai, Cotanzaro, Phillips & Osterman, 1976) and *R. conorii* have the transferred cells actually been shown to be T lymphocytes. The increased susceptibility to infection following the use of antilymphocyte serum or infection of athymic mice, however, shows the importance of cell-mediated immunity in *R. rickettsii* (Walker & Henderson, 1978), *R. akari* (Kenyon & Pedersen, 1980) and *R. conorii* (Kokorin, Kabanova, Shirokova, Abrosimova, Rybkina & Pushkareva, 1982) infection. The stimulation of peripheral blood lymphocytes from immune humans by specific antigen of *R. mooseri* and *R. prowazekii* in an *in vitro* blast transformation response further

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TABLE 1 Comparison of the immune mechanism between *Cowdria ruminantium* and other *Rickettsia*

	Specific antibody protective	Positive transformation	Transferred lymphocytes protect	Protective cells shown to be T cells	A.L.S. <sup>(1)</sup> treated or nude mice more susceptible
<i>R. prowazekii</i>	Yes	Yes	Yes		
<i>R. mooseri</i>	Yes	Yes	Yes	Yes	
<i>R. tsutsugamushi</i>			Yes	Yes	
<i>R. rickettsii</i>	Yes				Yes
<i>R. akari</i>			Yes		Yes
<i>R. conorii</i>			Yes	Yes	Yes
<i>Cowdria</i>	No	NI <sup>(2)</sup>	Yes	NI	NI

<sup>(1)</sup> A.L.S. = Antilymphocyte serum

<sup>(2)</sup> NI = No information available

confirms the importance of cell-mediated immunity in rickettsial infections (Bourgeois, Dasch & Strong, 1980).

Macrophages appear to be the most important final common pathway for rickettsial clearance (Tringali *et al.*, 1983). Little is known about the role of macrophages in heartwater infection; however, the absence of large numbers of mononuclear cells from histopathological lesions of heartwater in farm animals suggests that other mechanisms may be involved (Pienaar, Basson & Van der Merwe, 1966). On the other hand, the fact that immune spleen cells can kill the Kumm strain *in vitro* suggests that macrophages in combination with lymphocytes do play a role, at least *in vitro*.

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