

RESEARCH COMMUNICATION

FLOW CYTOMETRIC ANALYSIS OF T CELL RESPONSE IN MICE INFECTED WITH *COWDRIA RUMINANTIIUM*

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ABSTRACT

DU PLESSIS, J. L., GRAY, C. & VAN STRIJP, M. F., 1992. Flow cytometric analysis of the T cell response in mice infected with *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 59, 337-338 (1992)

A 3-fold increase in the numbers of Lyt-2⁺ T cells in the circulating blood of mice infected and re-infected with the Welgevonden stock of *Cowdria ruminantium*, as determined by flow cytometry, is supportive evidence that immunity in heartwater is cell-mediated. The rise in Lyt-2⁺ cells only after re-infection of the mice is further evidence that the development of immunity in heartwater is dependent on the unhindered and adequate replication of *C. ruminantium*.

In an earlier study it was found that Lyt-2⁺ T cells obtained from the spleens of mice immune to *Cowdria ruminantium* conferred resistance to challenge to recipient mice injected with the cells (Du Plessis, Berche & Van Gas, 1991). Immune spleen cells depleted of Lyt-2⁺ T cells with monoclonal antibodies against this subset were unable to confer protection against challenge, whereas the depletion of L₃T₄⁺ T cells had no effect. To gain further evidence on the role of T cells in immunity to *Cowdria*, it was decided to assay by flow cytometry the numbers of both T cell subsets in mice before infection and after infection, re-infection and challenge.

Twenty specified pathogen-free, inbred, female, BALB/c mice, 6-8 weeks old, were infected as previously described (Du Plessis *et al.*, 1991) with the Welgevonden stock (Du Plessis, 1985) of *C. ruminantium* by inoculating them intravenously with 0,2 ml (400LD₅₀ doses) of a homogenate of tissues prepared from infected BALB/c mice. On Days 8 and 10 after infection (p.i.) the mice were treated with oxytetracycline¹ at a dosage rate of 30 µg/g body mass. On Day 23 p.i. the mice were re-infected and on Day 50 p.i. they were challenged with the homologous stock at the same dosage rate. No treatment was given after re-infection and challenge and all the mice survived the infection, re-infection and challenge.

Two days prior to infection and at intervals thereafter, purified samples of lymphocytes were prepared from the circulating blood of the mice according to the method described by Böyum (1968) appropriately modified. Approximately 0,5 ml of blood was drawn from the orbital sinus of each of 10 mice and the blood of 2 arbitrarily chosen mice was pooled into 1 ml of Eagle's medium containing 200 IU/ml heparin. The 5 samples were then carefully layered on 2 ml ficoll² (1,077 density) and centrifuged at 400 g for 30 min. Lymphocytes were collected from the interface and washed twice (washing

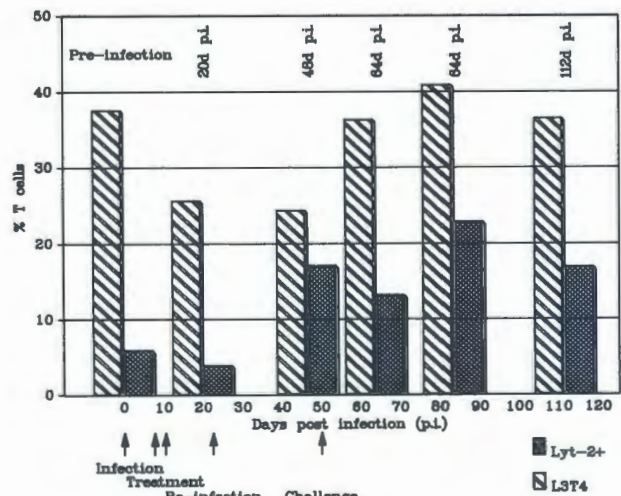


FIG. 1 Ratio of Lyt-2⁺ and L₃T₄ T cells over the course of 112 days p.i.

medium: 0,2 % bovine serum albumin and 0,01 % Na-azide in phosphate buffered saline) by centrifuging at 1000 r.p.m. for 10 min. The final pellet was resuspended in 1 ml of washing medium and the cells counted. 10⁶ cells in 200 µl washing medium were then allowed to react simultaneously for 30 min on ice with 10 µl monoclonal antibody (mAb) against mouse L₃T₄⁺ T cells conjugated to phycoerythrin³ and 10 µl mAb against Lyt-2⁺ T cells conjugated to fluorescein isothiocyanate³. The cells were then washed twice, suspended in 500 µl fixing medium (1,5 % formaldehyde and 1,5 % bovine serum albumin in PBS) and kept protected from light at 4 °C until they were examined by flow cytometry (EPICS Profile, Coulter Electronics).

Samples were collected in a similar manner 20 days p.i., 25 days after re-infection and 14, 34 and 62 days after challenge.

T cells were analyzed for their surface immunofluorescence by excitation with a 200 mW argon ion

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¹ Liquamycin, Pfizer

² Pharmacia

³ Coulter Immunology

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TABLE 1 Percentage $L_3T_4^+$ and $Lyt-2^+$ T cells per group of 2 mice infected with *C. ruminantium*

Sampling intervals	T cell	% T cells per mouse group					Average %
		1	2	3	4	5	
Pre-infection	$L_3T_4^+$	36,6	37,5	45,4	20,5	48,1	37,6
	$Lyt-2^+$	2,9	8,1	3,1	3,1	12,2	
20 days post-infection	$L_3T_4^+$	20,4	22,6	26,8	24	34	25,6
	$Lyt-2^+$	2,1	4	5,6	1,7	6,1	
25 days post re-infection	$L_3T_4^+$	25,2	21,2	30,1	20,2	25,5	24,4
	$Lyt-2^+$	18	16,9	20	15,2	15,2	
14 days post-challenge	$L_3T_4^+$	31,6	40,2	36,4	41,1	32,4	36,3
	$Lyt-2^+$	14,3	16,6	10,5	14,2	10,5	
34 days post-challenge	$L_3T_4^+$	37,6	44,1				40,9
	$Lyt-2^+$	24,1	21,7				
62 days post-challenge	$L_3T_4^+$	36,5					36,5
	$Lyt-2^+$	16,9					

laser at a power of 15 mW and a wavelength of 488 nm. Logarithmic green and red fluorescence were simultaneously detected and observed through a 525 nm band pass filter and a 590 nm long pass filter, respectively. Instrument calibration and standardization were performed using Immunocheck⁴ and Standardbrite⁴ fluorescent beads. Colour compensation was adjusted during analysis. For each stained sample 10 000 events were analyzed for fluorescence intensity after gating on the lymphocyte population on a forward angle versus a logarithmic 90° light scatter histogram. The percentage of positively stained cells was obtained by placing analysis cursors according to the position of the autofluorescence displayed by control unstained cells. Results were displayed as both univariate and bivariate fluorescence histograms.

The percentages $L_3T_4^+$ and $Lyt-2^+$ T cells per 10⁶ cells from each group of 2 mice collected before infection and on 5 occasions thereafter are shown in Table 1. The ratio of $Lyt-2^+$ and L_3T_4 T cells over

the course of 112 days p.i. is graphically represented in Fig. 1. It can be seen that at 25 days after re-infection there was an approximate 3-fold increase of $Lyt-2^+$ cells above the averages of 5,9 and 3,9 % recorded before and 20 days after infection, respectively. There was a slight drop in the number of $Lyt-2^+$ cells at 14 days after challenge followed once more by a rise 34 days after challenge. Increased numbers of $Lyt-2^+$ cells persisted until the last sampling 62 days after challenge. There was no significant variation in the numbers of $L_3T_4^+$ T cells between 24,4 and 40,9 % over the course of the sampling period.

The increased numbers of $Lyt-2^+$ T cells in the circulating blood of mice immune to *C. ruminantium* concurs with an earlier finding that immunity in heartwater is mediated by this particular subset of T cells (Du Plessis *et al.*, 1991). Furthermore, the finding that the first distinct rise in circulating $Lyt-2^+$ cells occurred only after the mice were re-infected and not treated again, is consistent with the observation that the transfer of immune spleen cells collected from donor mice after they had been re-infected rendered the recipient mice resistant to challenge, whereas cells collected from donor mice before they were re-infected failed to do so (Du Plessis *et al.*, 1991). This strongly suggests that the treatment of donor mice during the course of the infection inhibits the production of immunologically committed $Lyt-2^+$ T cells and that it is only after the uninterrupted course of the re-infection that $Lyt-2^+$ cells increase in numbers and that lymphocytes acquire the ability to confer protection to recipient mice. Unhindered, adequate replication of the heartwater agent must therefore take place before immunity develops.

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⁴ Coulter Electronics, Hialeah, Florida