

## **THE REGULATION OF THE AUTOIMMUNE RESPONSE IN ACTIVE HEYMANN'S NEPHRITIS BY T CELL SUBSETS IN LEWIS RATS**

**E de Heer, M R Daha, L A van Es**

*University Hospital, Leiden, The Netherlands*

### **Summary**

The cellular regulation of autoantibody production was investigated in rats with Heymann's nephritis. In these rats we showed that only the draining lymph nodes contained autoreactive B-cells. Resistance to the induction of Heymann's nephritis was achieved by pre-treatment with Cyclosporin A (CyA) or by induction of high dose tolerance. Tolerance could only be transferred by antigen specific, OX8<sup>+</sup> splenic T cells from high dose tolerance rats, but not with lymphoid cells from CyA treated rats. Enhancement could be transferred with OX8<sup>-</sup> T cells. This study shows that the autoimmune response in rats with Heymann's nephritis is under the regulatory influence of T cell subsets.

### **Introduction**

Heymann's nephritis can be induced in Lewis rats by one footpad immunization with a brush border preparation called Fx1A [1,2]. Using enzyme-linked assays [3,4] we localized the site of antibody production by autoreactive B cells during the induction of Heymann's nephritis and we quantitated levels of circulating autoantibodies. By transfers of lymphoid cells from high dose tolerance rats or CyA-treated donors into subsequently challenged recipients we investigated whether an increased resistance to Heymann's nephritis is maintained by lymphoid cells and where these cells reside. The nature of these suppressor cells was identified further by the same transfer system using affinity column fractionated lymphocytes.

### **Materials and methods**

Fx1A and purified autoantigen (RTE-Gp) were prepared as described [3] with minor modifications. Enzyme-linked assays to quantify antibody production by B cells and measurement of circulating autoantibodies by ELISA have been

described [3,4]. Inbred Lewis rats were immunized in the front footpads with Wistar Fx1A, FCA and B pertussis as described [3]. High dose tolerance was induced according to Litwin et al [5]. Pre-treatment with CyA (Sandoz) was performed by daily intramuscular injections of 15mg/kg for 14 days. At day one and day seven 10mg Wistar Fx1A was administered subcutaneously in FIA.

Cell separations were performed according to Duarte et al [6] with minor modifications. Suppression was calculated from immunized controls that received no cells (set at 0% suppression).

## Results

*Site of autoreactive B cells* Single cell suspensions were prepared from various lymphoid organs obtained from Lewis rats that had been immunized with Fx1A. Two to 15 weeks after immunization these lymphoid cells were assayed in vitro for their capability to produce autoantibodies against purified autoantigen. As shown in Figure 1 only the lymphoid cells obtained from the draining lymph nodes produced in vitro autoantibodies while all other lymphoid cells tested were unable to produce detectable amounts of specific autoantibodies. Furthermore removal of the draining lymph nodes at four weeks after immunization postponed the development of abnormal proteinuria, while the site of autoantibody production had shifted to the cervical and parathyroid lymph nodes (data not shown).

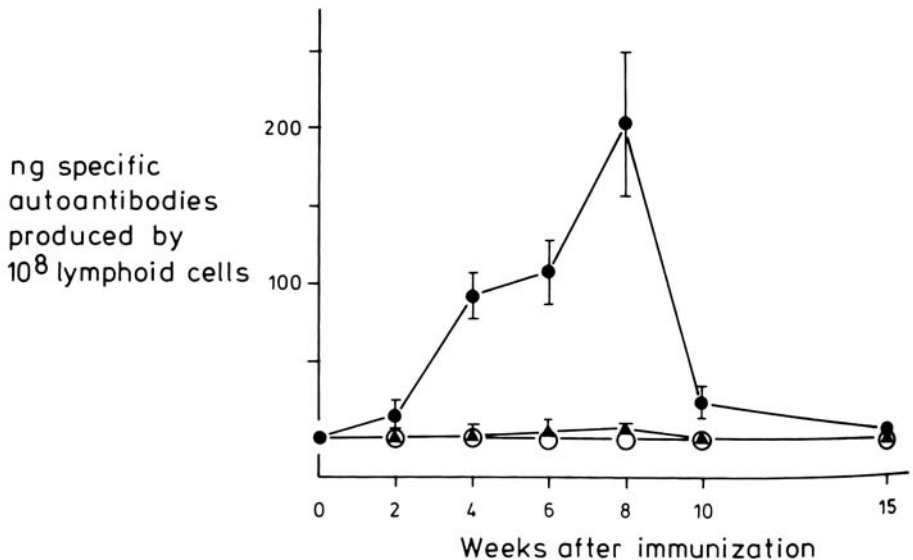


Figure 1. In vitro production of autoantibodies against RTE-Gp by  $1 \times 10^8$  lymphoid cells harvested at different time periods from the draining lymph nodes (●), bone marrow (▲) and spleen (○) of rats that were immunized with 5mg Fx1A in FCA combined with administration of B pertussis vaccine

*Establishment of tolerance* Rats were rendered high dose tolerant with high doses of Fx1A in FIA by eight subcutaneous injections. Pre-treatment with CyA was performed as described in materials and methods.

A subsequent nephritogenic challenge with Fx1A in FCA resulted in absence of the development of proteinuria eight weeks after the challenge.

The autoimmune response in high dose tolerant rats was suppressed. Both the capability of the draining lymph nodes to produce *in vitro* autoantibodies and the level of circulating autoantibodies were reduced to less than 10 per cent of the response measured in non-tolerant controls.

*Site and nature of suppressor cells* Lymphoid cells obtained from various lymphoid organs of high dose tolerance donors or CyA treated donors were injected intravenously into naive recipients that subsequently received a nephritogenic footpad challenge.

As shown in Table I only cells obtained from the thymus or spleen of high dose tolerance rats were able to transfer tolerance. By no means could tolerance be transferred from CyA pre-treated rats.

TABLE I. Demonstration of regulatory T cells in high dose tolerance (HDT) rats and absence of regulatory cells in CyA treated rats by adoptive transfer of lymphoid cells into subsequently challenged recipients

Donor	Organ or cell-fraction	No. of cells transferred per rat x 10 <sup>6</sup>	Circulating antibodies at 4 weeks µg/ml	ng autoantibodies produced by the DLN <i>in vitro</i>	Percent suppression
HDT	thymus	100	ND	17±14*	96±5*
	spleen	80	ND	119±60*	73±14*
	lymph nodes	100	ND	390±64	11±17
Normal	—	—	—	—	—
CyA	thymus	87	ND	399±85	9±19
+	spleen	70	ND	366±141	17±32
Fx1A	lymph nodes	84	ND	456±137	-4±36
HDT	OX8 <sup>+</sup> T cells	7	0.45±0.04*	115±50*	56±7*
(spleen)	OX8 <sup>-</sup> T cells	80	1.75±0.36	380±77*	-46±29*

\*p<0.001. DLN=draining lymph nodes; ND=not determined

Since high dose tolerance seemed to be mediated by a cell of thymic origin we separated the splenic T cells from high dose tolerance rats into a subset that was largely depleted of OX8<sup>+</sup> cells and a fraction that was enriched for OX8<sup>+</sup> T cells.

As shown in Table I transfer of OX8-enriched T cells into subsequently challenged recipients suppressed their response by 56 per cent, while transfer of OX8 depleted T cells enhanced their response by 46 per cent. This indicated that in tolerant rats T cells with both suppressor and helper function had developed.

## Discussion

The results presented in this paper clearly show that the amount of antibodies produced in vitro by the draining lymph nodes correlated with the level of circulating antibodies. Abrogation of autoimmune tolerance seemed to have occurred only locally, but had been maintained in the other compartments of the immune system.

Both high dose tolerance and CyA treatment were effective in increasing tolerance to the induction of Heymann's nephritis. However, only high dose tolerance could be transferred by antigen specific OX8<sup>+</sup> T cells. This indicates that tolerance induction by CyA seemed to be based on the prevention or elimination of helper T cells.

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