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Defective regulation of erythrocyte autoantibodies in SJL mice

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Summary. When SJL mice are hyperimmunized with rat red blood cells (RBC), tolerance to self is readily broken and these animals develop a high autoantibody response to their own RBC. However, these mice also fail to generate those antigen-specific suppressor cells which normally regulate this induced autoantibody response.

INTRODUCTION

Mice of the SJL strain have been shown to be highly susceptible to the induction of experimental allergic encephalomyelitis (EAE), developing a relapsing form of the disease and making them an excellent animal model for multiple sclerosis (Brown & McFarlin, 1981). These animals are also high responders in terms of the induction of experimental thyroiditis and thyroglobulin autoantibodies (Vladutiu & Rose, 1971). Amagai & Cinader (1981) have demonstrated that this strain shows defects in tolerance induction and in the generation of suppressor cells. Since this may play a role in the development of autoimmunity we investigated in this strain both the induction of red cell autoantibodies and the generation of the antigen-specific suppressor cells which regulate this induced autoimmune state in normal strains. The results of this study are presented in this short communication.

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MATERIALS AND METHODS

Mice

Female SJL mice (8–12 weeks old) were purchased from OLAC 1976 Ltd, Shaws Farm, Blackthorn, Bicester, Oxon, U.K. All other mouse strains used were also obtained from this dealer.

Rat red cells

Rat blood cells (RBC) were obtained by cardiac puncture of Wistar rats maintained in our own colony. The rat RBC were washed three times before injecting 2×10^8 RBC intraperitoneally (i.p.) into mice weekly for 4 weeks to generate an autoantibody response as described previously (Playfair & Marshall-Clarke, 1973).

Generation of antigen-specific suppressor cells

Antigen-specific suppressor cells were generated as described previously (Cooke, Hutchings & Playfair, 1978). Briefly, mice received four weekly injections of 2×10^8 rat RBC i.p. Subsequently, their spleens were removed, and 50×10^6 splenocytes washed three times were transferred i.v. to histocompatible recipients as a source of suppressor cells.

Assay of autoantibody activity

Direct antiglobulin (Coombs') test (DCT). Mice were bled from the retro-orbital venous plexus and the red cells washed four times in isotonic saline before being tested. A single batch of rabbit anti-mouse immunoglobulin (shown by immunoelectrophoresis to react

against IgG and IgM) was stored in small aliquots at −20° and used at a standard final dilution of 1:80. Agglutination was scored microscopically on a scale ranging from positive, visible only under the microscope (1), to a massive agglutination involving all the cells (4), after 30 min incubation with antiserum at room temperature. Positive and negative controls were included in all determinations. This more rapid qualitative assay shows a complete correlation with a more quantitative assay based on titration of the developing antiserum. Low responder mouse strains typically have a score of 1 whereas high responders have a score of 3 or more.

Statistical analysis

Analysis of the differences between the DCT of different groups of mice was carried out using the Wilcoxon ranking test (Wilcoxon, 1978).

RESULTS AND DISCUSSION

The autoantibody response induced in different mouse strains in response to the injection with rat RBC appears to be independent of Ig allotype and H-2 type (Table 1). This is in contrast to the experimental induction of thyroiditis in mice where Vladutiu & Rose (1971) showed that induction of thyroiditis is linked to H-2. When female SJL mice were immunized with rat RBC they developed an autoantibody response characteristic of a high responder strain. Figure 1a shows the response of 10 female SJL mice to four weekly injections of 2×10^8 rat RBC. The serum agglutinin response to rat RBC determinants non-cross-reactive with mouse RBC was not elevated relative to other strains. The SJL is therefore not only a high responder for thyroglobulin autoantibody production but also for red cell autoantibodies. A surprising feature that was observed however was that during the actual course of immunisation with rat RBC, 30% of SJL mice died (Fig. 1b). The mice did not die of anaemia resulting from the high autoantibody response itself, and if the mice received only three injections of rat RBC none of the animals died. This pattern of mortality has never been observed in any of our studies in other mouse strains (more than 1000 mice).

Table 1. Autoantibody production is not linked to allotype or H-2

Strain	Response	Av DCT score*	Ig-1 allotype	H-2 halotype
BALB/c	Low	1	a	d
BIOT (6R)	Low	0	b	y2
C57 Bl/10	Low	1	b	b
C58	Low	0	a	k
A	Low	0·8	e	k
C3H-H-2^0	Low	1	a	02
ASW	High	3	a	s
NZB	High	3·5	e	d
C3H	High	3·5	a	k
CBA	High	3	a	k

* Scoring is described in 'Material and Methods'. At least five mice were in each group.

We and others have shown that spleen cells or T cells transferred from rat RBC-primed normal mice to naive syngeneic recipients induce suppressor effector cells which significantly prevent the subsequent induction of the autoantibody component of the response to rat RBC (Fig. 2: CBA mice at week 2, $P=0{\cdot}01$; (CBA × BALB/c)F_1 mice at week 4, $P=0{\cdot}02$) without affecting the serum agglutinin response or the splenic plaque-forming cell response to rat RBC (Naysmith & Elson 1977; Cooke *et al.*, 1978; Gare & Cox, 1978). It has been shown that the observed suppression can be mediated by primed T cells and is not attributable to suppressor effector cells but to cells which are capable of inducing suppressor activity in the recipient. (Hutchings & Cooke, 1981; Naysmith, Ortega-Pierres & Elson, 1981). The ability of SJL mice to develop antigen-specific suppressor cells was tested by transferring the spleen cells from the surviving rat RBC primed SJL mice into naive SJL recipients which were then challenged with rat RBC. It can be seen from Fig. 1a that suppressor cells were not developed in SJL mice immunized with rat RBC. This experiment has been repeated three times and significant suppression has never been observed.

The failure of the SJL mouse to generate suppression despite the high autoantibody response argues against suppression being attributed to primed B cells suppressing via antibody itself. Our previous work had suggested that antibody or B cells might play a role in triggering the emergence of T cells capable of inducing the suppressor circuit (Cooke, Hutchings & Marshall-Clarke, 1980). Since the experiments described in this paper are within a wholly syngeneic system it is impossible to distinguish between a lack of suppression due to a deficit of cells in the donor capable of inducing suppression, and a deficit of cells in the recipient inducible to become suppressor effectors.

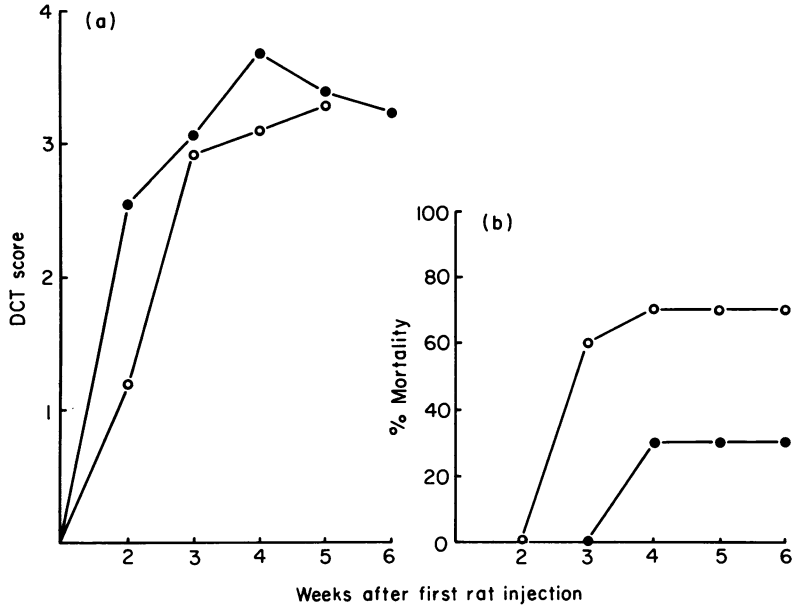

Figure 1. Autoantibody production and mortality induced in female SJL mice by rat RBC: (●) rat RBC only; (○) rat RBC primed spleen cells and rat RBC.

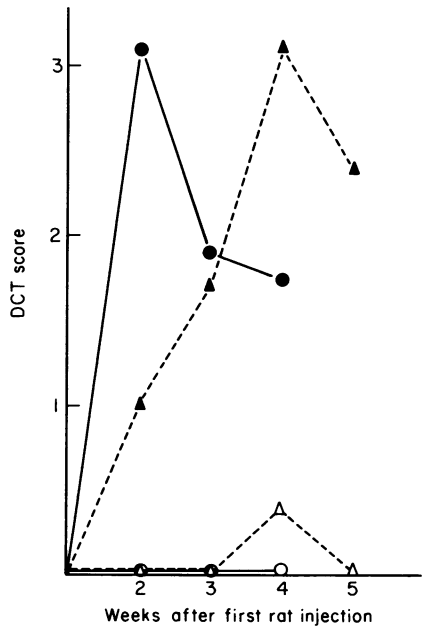

Figure 2. Autoantibody production and its regulation in normal mice. (●) CBA mice, rat RBC only; (○) CBA mice, rat RBC-primed CBA spleen cells and rat RBC; (▲) (CBA × BALB/c)F_1 mice, rat RBC only; (△) (CBA × BALB/c)F_1 mice, rat RBC-primed (CBA × BALB/c)F_1 spleen cells and rat RBC.

From Fig. 1b it can be seen that recipients of primed spleen cells also showed a greater propensity to be killed by the schedule of immunization with rat RBC. This may reflect the presence of rat RBC-primed T and B cells in the transferred spleen populations which would lead to a more rapid antibody response. The exact cause of death is unknown but it had many of the features characteristic of anaphylactic shock.

In conclusion, we have shown that SJL mice are high responders in terms of autoantibody production to rat RBC and also that this strain has a defect in the ability to generate suppressor cells capable of regulating an induced autoantibody response which is presumably responsible for the prolongation of the autoantibody response. This is comparable with the studies of other workers showing the SJL mouse to be a 'good' responder in terms of induced autoimmunity and also supports the observation of Amagai & Cinader (1981) that these mice are defective in tolerance induction and do not generate antigen-specific suppressor cells.

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