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Role of the thymus in the immune response to sheep erythrocytes in the lizard *Calotes versicolor**

RM. PITCHAPPAN AND VR. MUTHUKKARUPPAN

Department of Biological Sciences, Madurai University, Madurai 625021

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ABSTRACT

The role of the thymus in antibody response to sheep erythrocytes (SRBC) was investigated in the lizard, *Calotes versicolor*, by utilizing the experimental models of adult thymectomy and anti-thymocyte serum (ATS) treatment. When thymectomized lizards were treated with high dose regimen of ATS, plaque-forming cell (PFC) response to SRBC was abrogated; however, this response was not altered by the low dose. A definite recovery of anti-SRBC response after ATS treatment occurred in the presence of the thymus. On the other hand the PFC response to SRBC was enhanced one month after adult thymectomy and after low dose ATS treatment. Both low and high doses of normal rabbit serum suppressed the immune response to SRBC and it is suggested that this suppression might be due to antigenic competition.

These results indicate that (i) anti-SRBC response is thymus dependent and (ii) there are two kinds of thymus derived cells: one 'helper' collaborating in anti-SRBC response and another 'regulator' governing the magnitude of the response which is involved in antigenic competition. The phylogenetic status on the dual role of the thymus in immune functions has been discussed.

1. INTRODUCTION

EVER SINCE the discovery of the role of the thymus in immunogenesis, attempts have been made to elucidate the various aspects of its function in different groups of vertebrates.[1–4] Among poikilotherms, thymic dependence of the immune response to allografts and sheep erythrocytes (SRBC) has been demonstrated in fishes and amphibians.[1,4–6] However, it would be of great

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interest to delineate thymic functions and to understand the mechanism of immune dichotomy at the phylogenetic level of reptiles, which are considered to be originators of both birds and mammals. The very basis whether there exists functional dichotomy of lymphocytes into thymus-derived (T) and bone-marrow derived (B) cells among lower vertebrates is not yet clarified. Ruben[7] based on his findings in amphibians proposed that cellular co-operation may have been an early phylogenetic feature of immune responses.

The present experiments involving adult thymectomy and antithymocyte serum (ATS) treatment were, thus designed to investigate the role of the thymus in the immune response in a species of the lizard, *C. versicolor*. Our earlier studies on the development of immunity in reptiles provided information concerning the kinetics of the allograft and anti-SRBC response,[8,9] the development, lymphoid organization and immune functions of the spleen [10–12] and the thymus.[13–15] This paper presents evidence to demonstrate the thymic dependence as well as regulation of the immune response to SRBC in the lizard, *C. versicolor*.

2. MATERIALS AND METHODS

ANIMALS

Adult *Calotes* of both sexes, weighing 20–30 gm were used in the present study. They are about one year old[16] and sexually mature. The thymus in these lizards contain well defined cortex and medulla. The method of maintaining lizards in the laboratory has already been described.[17]

THYMECTOMY

Bilateral thymectomy was carried out surgically as described earlier.[1] Since the hatchling lizards are already immunologically mature [19,20] and since no inbred lines are available[19] neither thymectomy nor irradiation and reconstitution could be used as methods of eliminating T-cells. Therefore we resorted to the use of adult thymectomy coupled with ATS treatment

ANTI-THYMOCYTE SERUM

Anti-*Calotes* thymocyte serum was raised in rabbits as described in the previous paper.[21] Before injection into lizards ATS was inactivated at 57° C for 30 minutes and absorbed with *Calotes* erythrocytes.

CULTURE TECHNIQUE

Millipore filter-well organ culture method of Auerbach[22] was followed for culturing *Calotes* spleen fragments.[16] The culture medium was prepared

by mixing L–15 Lebovitz medium (Grand Island Biological Co., and tripple distilled water in the ratio of 3 : 1 and supplemented with 5% chick embryo extract, 5% heat inactivated fetal calf serum (Difco) and antibiotics (50 units, each of penicillin and streptomycin per ml of medium). After 40–60 hours of priming with 0·1 ml of 25% SRBC, spleens were dissected out from the lizards and cultured in the presence of 0·02 ml of 0·1% SRBC. The cultures were incubated at 37° C for five days in a humid atmosphere of 50% oxygen in air. Medium was changed on alternate days. At the termination of cultures the number of plaque-forming cells (PFC) was determined by the Cunningham liquid monolayer technique as previously described.[9]

3. RESULT

EFFECT OF ADULT THYMECTOMY AND ATS TREATMENT

Since prior thymectomy was shown to potentiate the effects of the anti-lymphocyte sera (ALS),[23] adult thymectomy was coupled with ATS treatment in the present study. Thymectomized lizards were injected intraperitoneally (i.p.) with either 0·18 ml (low dose) or 0·25 ml (high dose) of ATS for five consecutive days because of the effectiveness of this route in reducing the splenic PFC to SRBC[24] and the dose dependency of ALS induced suppression.[25] Since the capacity of ALS to suppress the primary immune response was not overcome by increasing the antigen dose in mice,[26] a dose of 0·1 ml of 25% SRBC was used in the present study as standardized earlier.[16] Further, the lizards were immunized after the completion of serum treatment.[27] Control cultures were derived from thymectomized and normal rabbit serum (NRS)/saline treated lizards (figure 1).

While the low dose ATS group showed a PFC response similar to the saline controls, there was significant suppression of this response in high dose ATS group ($P < 0·005$) (figure 2). Another interesting observation was the suppression of PFC response in NRS treated lizards, in contrast to the low dose ATS group ($P < 0·05$).

EFFECT OF ADULT THYMECTOMY

While observing the histopathology of adult thymectomized lizards one month after operation, the periarteriolar region of the spleen was found to be specifically depleted.[28] The immunocompetence of such a depleted spleen has been studied by immunizing them with 0·1 ml of 25% SRBC and culturing as described earlier. The PFC response of thymectomized lizards was more than that of sham operated controls ($P < 0·05$) suggesting an augmentation of this response in the absence of the thymus (figure 3).

ROLE OF THE THYMUS IN RECOVERY AFTER ATS TREATMENT

Lizards were either thymectomized or sham-operated and injected with 0·25 ml of ATS for five consecutive days. Twenty-one days after the completion of serum treatment, animals were immunized with SRBC and the PFC response was determined. There was a definite recovery of anti-SRBC response after ATS treatment in sham-thymectomized lizards, in contrast to the thymectomized group ($P < 0·05$) (figure 3).

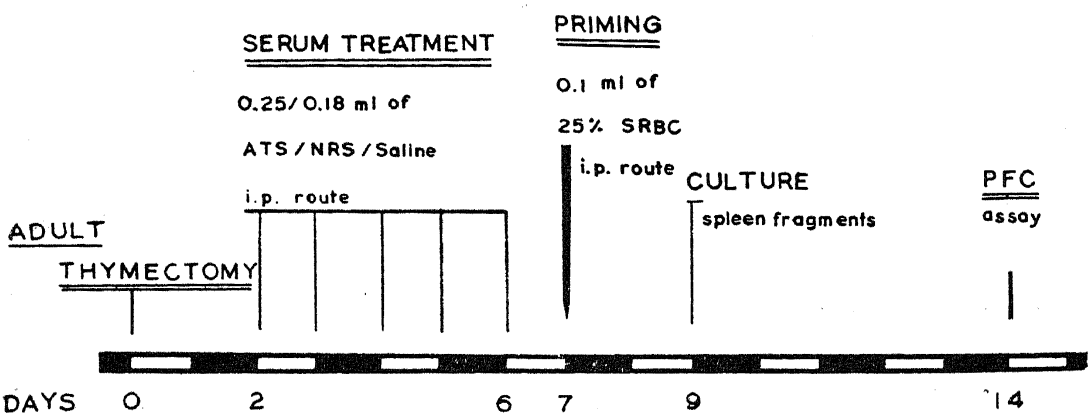

Figure 1. Experimental protocol followed to study the effect of antithymocyte serum (ATS) treatment on anti-sheep erythrocyte response (details in text).

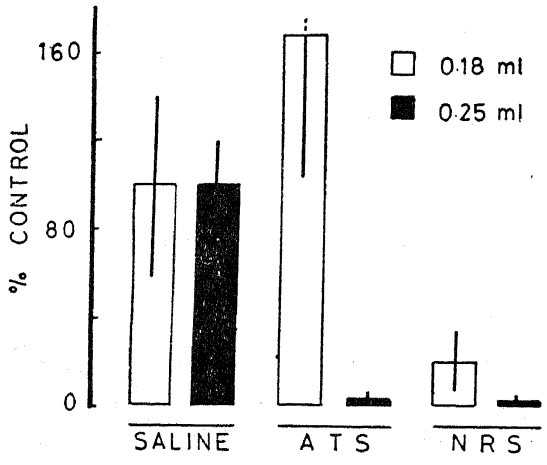

Figure 2. Effect of ATS on anti-SRBC response. Lizards were thymectomized and injected with either ATS, NRS (normal rabbit serum) or Saline (low dose: 0·18 ml or high dose: 0·25 ml each day) for five consecutive days. One day after the completion of serum treatment they were primed with SRBC and the PFC response was determined. PFC is expressed as % control

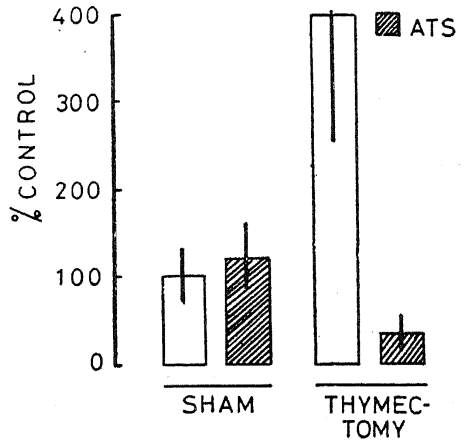

Figure 3. Role of thymus in maintaining the immune system. Sham-thymectomized and thymectomized lizards were either treated with ATS (0·25 ml × 5 days) or untreated and their capacity to elicit anti-sheep erythrocyte responses were assessed three weeks later. PFC is expressed as % control

4. DISCUSSION

Three important findings emerging from these experiments are:

(1) abrogation of the anti-SRBC PFC response as a result of prior treatment of thymectomized lizards with ATS (high dose) or NRS,

(2) the augmentation of this response one month after adult thymectomy or after low dose ATS treatment and

(3) the recovery of this response from ATS induced immuno-suppression in the presence of the thymus.

THYMIC DEPENDENCE OF ANTI-SRBC RESPONSE

The present study revealed that there was an enhanced PFC production in low dose ATS group in contrast to NRS group, whereas this response was abrogated after treatment with high dose of ATS (figure 2). The persistance of the response at low dose and its suppression at high dose suggests that the amount of cytotoxic activity present in the ATS is the deciding factor. Further it has been shown that the ATS used in the present study is fairly specific for thymocytes.[21] Therefore, it may be inferred that the anti-SRBC response is thymus dependent in *C. versicolor*.

This kind of suppression of humoral antibody response to a variety of antigen by ALS/ATS in mammals has been reported.[29] It was shown that anti-theta serum treated spleen cells did not respond to SRBC[30] and adult

thymectomy coupled with ALS treatment suppressed the serum antibody response to heterologous erythrocytes.[31] Thymic dependency of anti-SRBC response in the lizard was further evidenced by the recovery of this response after high dose treatment of ATS in the presence of the thymus (figure 3).

TWO POPULATIONS OF T-CELLS

From the result of the present investigation, two kinds of T-cells have been proposed. One population is a helper which collaborates in anti-SRBC response, relatively less sensitive to ATS (eliminated from the system in high dose regimen) and long lived (present even one month after adult thymectomy). Another population, regulator exerts a regulatory role in anti-SRBC response and is involved in antigenic competition. It is this population which is eliminated from the system in low dose regimen of ATS or by adult thymectomy within a month of operation, thus culminating in the enhanced responsiveness.

MODE OF NRS INDUCED SUPPRESSION

In all NRS groups which were maintained as controls for antiserum treatment, suppression of anti-SRBC response was observed (figure 2). Such suppressive effect of NRS has been reported by many investigators and explained on the basis of antigenic competition.[21,31,32] In this phenomenon, the response to the second antigen is abrogated by prior treatment with another non-cross reacting antigen[33,34] and the degree of immuno suppression is dependent on the amount of first antigen and the interval between the two antigens.[24,33] Thus, the massive doses of NRS followed by the immunizing dose of SRBC seemed to have induced antigenic competition in the lizard, resulting in the abrogation of the response to the second antigen, SRBC. In our recent studies, lizards have been shown to be capable of eliciting antigenic competition, when two non-crossreacting heterologous erythrocytes were used for immunization.[14] Further, one month after thymectomy antigenic competition was eliminated, thereby establishing this phenomenon to be thymus dependent in lizards.[14] This is in conformity with the thymic dependency of this phenomenon in mammalian system.[33] However, more critical work is required to verify whether the NRS induced suppression in the lizard is a result of antigenic competition.

THYMIC REGULATION OF ANTI-SRBC RESPONSE

It has been shown that mice given progressively increasing amounts of ATS had exponential decrease in the number of splenic anti-SRBC PFC.[24] However, the results obtained in the present investigation showed an increased antibody production instead of ' partial suppression ' at the low dose

regimen used (figure 2). This may mean the abolition of certain thymic control exerted over antibody synthesis in the low dose ATS treatment. This was further confirmed by long term thymectomy experiments (figure 3). One month after thymectomy, the response in thymectomized group was more than in sham controls. This kind of augmentation has been reported widely in recent years and the procedures like adult thymectomy[31,35] or the injection of ALS[31,35,36] have been shown to eliminate one type of T-cell, culminating in augmentation of antibody response.

Enhanced responsiveness may also be explained on the basis of increased metabolic space available in the lymphoid system for B-cells as a result of thymectomy or ATS treatment.[31] Though there was an enhanced response to SRBC in low dose ATS group and one month after adult thymectomy the inability of expressing antigenic competition one month after adult hymectomy[14] forms a crucial evidence to substantiate the concept of suppressor/regulater T-cells.

PHYLOGENY OF THYMIC FUNCTIONS

While considering the phylogeny of immune system, it is well understood that the appearance and establishment of small lymphocytes, specific recognition of foreigness and adaptive immunologic functions are related to the origin and evolution of the thymus.[37] Among the primitive living vertebrates, the hag fish has an accumulation of lymphocytes in the pharyngeal velar muscle complex—the thymic precursor.[38] In the teleost *Tilapia mossambica* thymectomy prolonged the allograft survival and abrogated the anti-SRBC response. Further, the immunological maturation of these responses coincides with the lymphoid differentiation of the thymus; and the time of maturation of cell mediated immune response precedes that of humoral immunity.[1]

In amphibians, thymic dependency of cell mediated and humoral immune responses has been described by many investigators.[4] During ontogeny the establishment of transplantation immunity precedes that of humoral response and the ontogeny of alloimmune response is correlated with the lymphoid maturation of the thymus.[4–6,39] Following thymectomy IgG as well as IgM antibody response against SRBC has been inhibited. This is quite different from the findings in mammal where IgM antibody is not affected by thymectomy.[40] Thymus has also been shown to exert a regulatory influence on the level of immunoglobulins.[41] Further, anti-haemocyanin titer was high in thymectomized larvae.[42]

Among reptiles, information concerning the immunoglobulins and transplantation reactions are available.[43–45] Other studies in the lizard immune system from this laboratory relating the ontogeny of the thymus[15] and spleen and their role in humoral and cell-mediated immune responses[10,12,46] and the present paper describing the thymic dependence and the regulation of the immune response to SRBC attributes a mandatory role to the thymus in the immune system of the lizard.

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