

ALKALINE PHOSPHATASE ACTIVITY AND DNA CONTENT IN THYMUS GLAND OF SIALOADENECTOMISED MALE MICE**Madhuri V. Walvekar*, Shilpa P. Khairmode and Nitin T. Potphode**

Department of Zoology, Shivaji University, Kolhapur, 416004, Maharashtra, India.

(*E-mail: madhuri_walvekar@rediffmail.com)

ABSTRACT

To study the effect of biologically active polypeptides secreted by submandibular gland on the development and differentiation of thymus and thymocytes, we have sialoadenectomised the male albino mice at the age of 20 days and maintained under normal condition in the animal house up to the age of ten weeks. Thereafter the sialoadenectomised mice were scarified; thymus was dissected out and subjected for estimation of alkaline phosphatase activity and DNA content. The body weight, gland weight, alkaline phosphatase activity and DNA content all these parameters was decreased significantly as compared to normal. These results reveal that due to absence of submandibular gland secreted growth factors there was decrease in the weight of thymus gland. Decrease in the DNA content indicates reduction in the number of thymocytes and decrease in the alkaline phosphatase activity indicates decrease in functioning of thymus.

KEY WORDS: alkaline phosphatase, Sialoadenectomy, submandibular gland, thymus.**INTRODUCTION**

By both morphological and functional analyses, the relationships between the submandibular glands and the immune system have been investigated and have indicated marked effects of submandibular glands on the T-cell system (Gruber and Ledney, 1980a, b; Kemp *et al.*, 1985, 1986). Furthermore, atrophy of the thymus and the depletion of lymphocytes from the thymus-dependent areas in the spleen and lymph nodes have been reported after the extirpation of the submandibular glands from mice and rats (Martinez-Hernandez *et al.*, 1973; Letic-Gavrilovic and Jankovic, 1983). Considerable evidence has accumulated to indicate the existence of a lymphoid-thymotrophic factor (Barka, 1980) or factors (Naughton *et al.*, 1969, 1972; Kemp *et al.*, 1985, 1986) in the epithelial cells of the submandibular glands affecting the immune system.

In contrast to this some authors have reported hyperplasia of the thymus and spleen after sialoadenectomy (Takeda and Grollman, 1968; Hiramatsu *et al.*, 1979). Although several authors were not able to confirm the relationships between the submandibular or sublingual gland and the thymus or spleen (Dean and Hiramoto, 1983), other authors have shown that the suppression of allograft rejection (Kongshavn and Bliss, 1970) and development of delayed-type hypersensitivity (Roberts *et al.*, 1976; Hiramatsu *et al.*, 1979) could be caused by the submandibular gland extracts. Recent papers suggest that particular types of cells - even some T cell subpopulations and some progenitor or accessory cells of the immune system - are influenced by factors from submandibular glands (Koch and Rowe, 1976; Hoffman and Burnett, 1974; Gruber and Ledney, 1983). These facts led us to investigate the effects of sialoadenectomy on the alkaline phosphatase of thymus gland. The differentiation of the thymus and thymocyte was studied using alkaline phosphatase (AP) as a marker of the thymocytes. To study atrophy of thymus DNA content was studied

MATERIALS AND METHODS

Male Swiss albino mice [*Mus musculus* Linn] were used for present investigation. The mice were bred and reared in departmental animal house (CPCSEA/233) in separate cages under proper conditions of light, temperature and humidity. They were supplied with Amrut mice feed (Pranav Agro Industries) and water *ad Libitum*. For these study 20 male mice of 20 days old, weighing 9-15 gm were used. They were grouped into two groups- sham operated mice and sialoadenectomised mice. Bilateral sialoadenectomy (removal of submandibular gland) and sham operations was carried out on 20 animals. Sham-operated animals were subjected to identical procedures, except that the glands were not removed. The animals were equally distributed into the sialoadenectomised and the sham-operated groups. Operated mice were maintained in animal house with proper care up to the age of 10 weeks. The mice from all the above groups were sacrificed by cervical dislocation and the thymus gland was dissected out and used for following study. Following parameters were studied-

1. Estimation of alkaline phosphatase activity by Linhardt and Walter method (1965)

The thymus was cleaned, weighed and homogenized in chilled distilled water. The concentration of homogenate was 1mg/ml for alkaline phosphatase estimation. The homogenates were centrifuged at 10°C at 5000 rpm for 10 minutes. The supernatants were used for estimation of alkaline phosphatase activity by using P-nitro phenyl phosphate as substrate.

2. Isolation and estimation of DNA (Jay Raman-1988)

The Isolated DNA from thymus was used for estimation of DNA content using diphenylamine reagent. The optical density was measured at 595 nm. (Jay Raman-1988).

STATISTICAL ANALYSIS

All data is reported as mean \pm SD. The significance of difference between means was assessed by one way analysis of variance for independent or correlated samples ANOVA followed by Tukey's post hoc test.

RESULTS AND DISCUSSION

Effect of sialoadenectomy on different parameters on thymus gland of male mice is shown in table 1. Body weight and the weight of the thymus were significantly reduced in sialoadenectomised mice, three and four weeks after sialoadenectomy, as shown in table (P< 0.0001). In sialoadenectomised mice the alkaline phosphatase activity and DNA content was also reduced as compared to control and the decrease was highly significant (P< 0.0001).

Table 1. Effect of Sialoadenectomy on different parameters of thymus gland of male mice. Values are mean \pm SD.

Parameters	Control	Sialoadenectomised	Statistical significance
Body weight (g)	45.4 \pm 0.5477	35.6 \pm 0.8944	P < 0.0001
Thymus gland weight (mg/g body weight)	51.6 \pm 2.701	36.00 \pm 2.345	P < 0.0001
DNA content (μg/mg tissue)	2.24 \pm 0.0543	2.01 \pm 0.0183	P < 0.0001
Alkaline phosphatase activity (units of alkaline phosphatase activity /mg protein)	32.06 \pm1.471	27.22 \pm0.766	P < 0.0001

We have demonstrated that after removal of submandibular gland there was reduction in the body weight [consistent with several reports (Ossario and Kraemer, 1965; Takeda *et al.*, 1967; Menendez-Patterson *et al.*, 1985)]. Severe atrophy of the thymus was caused by depletion of thymocytes. Decrease in the DNA content after sialoadenectomy indicates that there is decrease in number of thymocytes. But our these results i.e. decrease in number of thymocytes after sialoadenectomy are in agreement with Letic- Gavrilovic and Jankovic (1983,1986,1989), Letic- Gavrilovic *et al.*, demonstrated that, three weeks after extirpation of the submandibular salivary glands, there was a reduction of body weight [consistent with several reports (Ossario and Kraemer, 1965; Takeda *et al.*, 1967; Menendez-Patterson *et al.*, 1985)], severe atrophy of the thymus caused by the depletion of thymocytes, and significant atrophy of the spleen. But these results are in contrast to some of the reports, which showed hyperplasia of the thymus after removal of submandibular gland (Takeda and Grollman, 1968; Hiramatsu *et al.*, 1979). Thus there is controversy regarding effect of submandibular gland secretion on the thymus whether submandibular gland secreted growth factors causes atrophy of thymus by the depletion of thymocytes or not.

Although several authors were not able to confirm the relationships between the submandibular or sublingual gland and the thymus or spleen (Dean and Hiramoto, 1983), other authors have shown that the suppression of allograft rejection (Kongshavn and Bliss, 1970) and development of delayed-type hypersensitivity (Roberts *et al.*, 1976; Hiramatsu *et al.*, 1979) could be caused by the submandibular gland extracts. Recent papers suggest that particular types of cells - even some T cell subpopulations and some progenitor or accessory cells of the immune system - are influenced by factors from submandibular glands (Koch and Rowe, 1976; Hoffman and Burnett, 1974; Gruber and Ledney, 1983). The thymus and T-cell system are composed of different. To solve this discrepancy whether sialoadenectomy increases or decreases the number of thymocytes we have done the sialoadenectomy on 20th day of the mice and at the age of ten weeks we have found that there is decrease in the alkaline phosphatase activity indicating something pathological conditions and decrease in DNA content indicating reduction in the number of thymocytes.

The salivary gland factors responsible for these effects have not been identified, although an esteroprotease (enzyme with esterase and protease activity) which induces transformation of thymic lymphocytes in vivo and in vitro has been isolated (Naughton *et al.*, 1972). A salivary gland component which could be involved in modifying immune responsiveness is epidermal growth factor (EGF), a protein capable of modifying development, which is found in high concentrations in mouse submandibular glands (Cohen, 1962; Byyny *et al.*, 1974; Roberts, 1974). Specific receptors of EGF have been detected in a variety of tissues, including the membranes derived from the mouse thymus (O'Keefe, Hollenberg and Cuatrecasas, 1974).

Thus our results clearly indicate that in absence of submandibular gland secreted growth factors there is decreases in the weight of thymus gland and causes reduction in the size. Decrease in the DNA content also supports that there is reduction in the number of thymocytes and decrease in alkaline phosphatase activity indicates something pathological conditions in the thymus gland.

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