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Salivary Epidermal Growth Factor in Mice Modulates the Differentiation of CD4CELLS in Thymus.

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Abstract: ***The CD4 helper T cells are the key players of cell mediated immunity. Upon antigenic interaction they show variable subsets depending upon specific cytokine profile. The development, maturation and differentiation of CD4 cells with other subtypes take place in well-coordinated manner. Such developmental processes influenced by thymic epithelial cells. Many scientists have suggested that the extirpation of salivary gland causes adverse effects on thymus gland, as this salivary gland considered as richest source of growth factors especially EGF(Epidermal Growth Factor). But the exact effect of this EGF on differentiation of thymocytes is unknown. So the present research work was carried out to find out the effect of salivary EGF on development, maturation and differentiation of CD4 cells. For this we have sialoadenectomised (removal of submandibular gland) and salivariadenectomised (removal of submandibular and sublingual glands) the 20th day old male albino mice and maintained under normal conditions in the animal house along with control up to the age of ten weeks. There after the mice from both the groups were sacrificed; thymus was dissected out and subjected for immunofluorescence study of CD4 cell andCD4 cell count by flow cytometry. It has been observed that in sialoadenectomy and salivariadenectomy there was reduction in number of CD4 cells and degeneration of collagenous content of thymic epithelial network whiCh lead to disturbance in thymocytes maturation and selection, in particular CD4 helper T cells.***
Keywords: CD4; Thymus; EGF; sialoadenectomy; salivariadenectomy; Immunofluorescence study

I. INTRODUCTION

The thymus gland is like a supreme commander of the body defence system. The whole control and regulation of the defence against constant bombardment of pathogens lies in the immune system. T cell development begins with the arrival of small number of lymphoid precursors migrating from the blood into the thymus, where they proliferate, differentiate, and undergoes selection process that results in development of mature T cells. Until recently it was believed that the intact architecture of the thymus was a necessary condition for this developmental process to occur. But in 2002, it was demonstrated that T cell could be induced to develop in absence of thymic fragments when bone marrow stem cells were cultured on a stromal cell line [1].

T cell development begins with the arrival the common lymphoid progenitor cell from bone marrow into thymus via circulation. As shown in Fig. 1, Here this cell proliferation, differeciation and selection process which leads to production of mature thymocytes. The progenitor-T cell (Pro T cell) do not express signature surface markers such as the T cell receptor (TCR), CD3, co-receptors CD4 and CD8, proteins such as RAG1 and RAG2 (required for the rearrangement of TCR gene). This progenitor T- cell is Double negative- DN (CD4- and CD8-) which is subdivided into four stages: DN1(c-Kit+, CD44 high, and CD25−.), DN2 (c-Kit+, CD44 high, and CD25−), DN3- Pre T cell (During the critical DN2 stage of development, rearrangement of genes for TCR-γ, δ and β chain begins; with Pre-T α chain), DN4 (Pairing between β chain and Pre-T α chain and produces Pre-TCR- CD3 complex). The formation of Pre TCR-CD3 complex leads to survival, proliferation of T cell with β chain loci rearrangement and up-regulation and expression of CD4 and CD8, now the cells are termed double positive (DP) cells. Cells that do not undergo beta-selection die by apoptosis. In the thymus immature double positive (CD4+ CD8+ CD3low) thymocyte undergoes two selection processes give single positive (SP) mature thymocytes. In the positive selection thymocytes bearing receptors capable of binding self-MHC molecules are selected, that results in MHC restriction. In negative selection, elimination of thymocytes bearing high affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC is carried out, those results into self-tolerance. Cells that fail in selection are eliminated by apoptosis. For the selection process the interaction of DP cell with thymic epithelial cell (with dendritic cell and macrophages also) at cortex and medulla (cTEC and mTEC) region is most important. The mature T cells while in

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circulation get activated by interaction of TCR-CD3 complex with a processed antigenic peptide bound to either a class I (CD8 cell) or Class II (CD4 cell) MHC molecule on the surface of antigen presenting cell (APC). This resulting into initiation of cascade of biochemical events that induces the resting cell enter into cell cycle, proliferating and differencing into effector or memory cells. The CD4 functional T helper cell is further distinguished by their cytokine profiles into Th1, Th2, Th17, Treg (regulatory) and Tf (follicular) produces number of products which are capable of influencing a variety of immune cells, and the T cell response generated can be essential for a successful outcome from infection (Kindt et al., 2006).

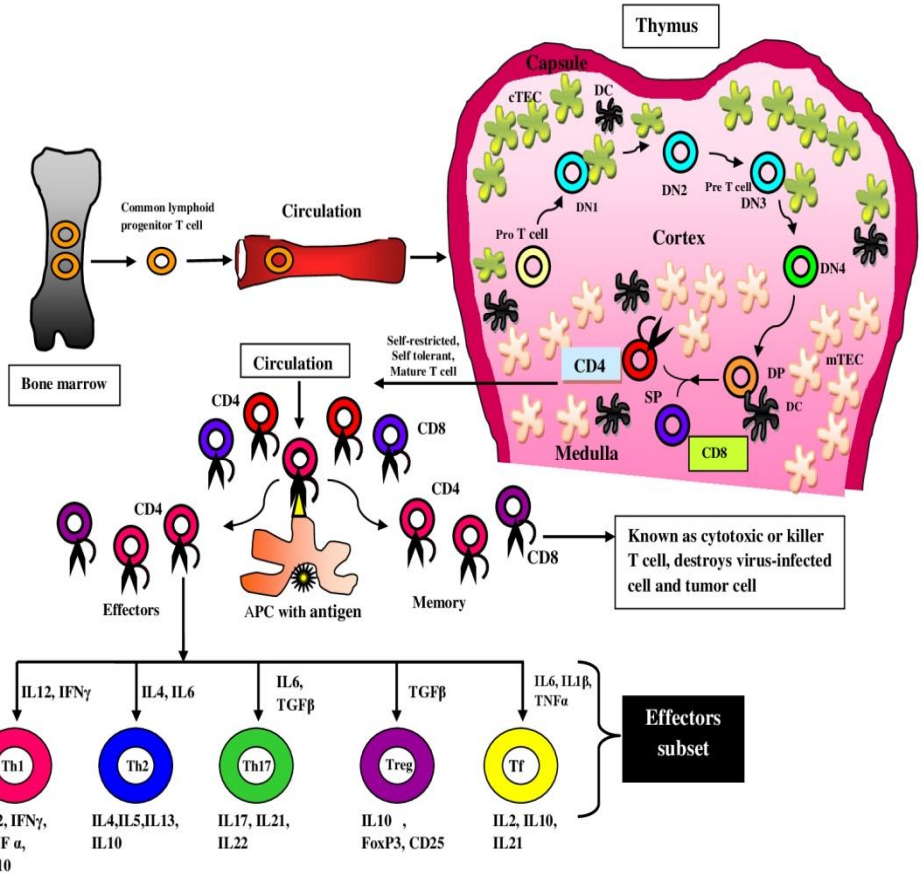


Fig.1The Overall scheme of the development, differeciation, maturation, activation of thymocytes in the thymus gland with its subsets.

Our body is the unique bio signature of coordination system. It's not a single but all cells, tissues and organs work together with the specific interrelation and communication. The morphological and functional studies on submandibular gland and immune system indicate relationship between these two system[3] [4] [5] [6]. The mouse submandibular gland secrets number of biologically active polypeptides and it is rich source of EGF which is a potent growth factor for various types of cells [7]. EGF is the best characterized and most extensively studied single polypeptide chain of 53 amino acid residues (Mr 6045) [8] [9] [10]. The level of EGF in the salivary gland is androgen-dependent [11] [12] [13] [14]. Previously little work was done on thymus gland after sialoadenectomy and all workers have observed that there was decrease in body and thymus gland weight with reduction in thymocytes [15] [16] [17] [18] [19] [20] [21] [22]. But research regarding status of particular type of thymocytes in absence of salivary glands is still unknown. The present research work was done to study effect of sialoadenectomy and salivariadenectomy on CD4 cells in thymus gland of mice.

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II. MATERIALS AND METHODS

A. *Animals*

For the present research work male albino mice (Mus musculus) were used. They were breed and reared in departmental animal house (1825/PO/EReBi/S/15/CPCSEA) in groups of three to four per cage. The mice were supplied with Amrut mice feed (Pranav agro industry) and water ad libitum. All experimental procedures were performed between 8 am to 10 am and care was taken to avoid all stressful conditions. The protocols which were used for the present study were approved by Institutional Animal Ethics Committee (IAEC).

B. *Selection of animals*

Healthy male offspring of Swiss albino mice, *(*Mus musculus Linn) of 20 days old weighing 8 to 10 g were selected for the present study. They were divided into three groups as follows:

1) *Control:* The offspring were sham operated i.e. the mice were preceded through the similar surgical procedure except the submandibular and sublingual glands were not removed.
2) *Sialoadenectomy:* The male offspring were subjected to sialoadenectomy i.e. submandibular glands was removed.
3) *Salivariadenectomy:* The male were sialoadenectomised i.e. submandibular and sublingual glands were removed. The whole surgical procedure during sham operations, sialoadenectomy and salivariadenectomy was carried out under mild ether anaesthesia. The mice were operated early morning in between 8 to 10 am.

C. *Study of cd4 Cells By Immunofluorescences Staining Technique:*

1) *Sample collection and preparation:* Operated mice were maintained in animal house with proper care up to the age of ten weeks. The mice from all the above groups were sacrificed by cervical dislocation and the thymus gland was dissected out and fixed in 10% NBF for a day. Paraffin embedded tissue sectioned at 2-3µ then deparaffinised followed by hydration.
2) *Immunofluorescences Staining Protocol: (Fig. 2):* The sections were treated with Antigen retrieval solution with citrate buffer (pH 6) in pressure cooker or microwave oven (15 minutes at high flame and 30 minutes at the low flame). Sections were washed with0.05 % Tween20 and then marked. Then sections were treated with activated 10% goat serum blocker for 30 minutes. Then immediately the slides were incubated with primary antibody (CD4 antibody-GK 1.5, Thermo scientific) (Primary antibodies are dissolved with 980 µl BSA) at 1:50 dilution overnight at 4^{o}C. (Negative control was prepared i.e. a section without primary antibody). Then they were washed with 1X PBS-Tween20. The slides were incubated with secondary antibody (Goat Anti-RatIgG Dylight 488, Thermo scientific) for 1hour, again washed in 1X PBS-Tween20. Then sections were stained by DAPI for 1 minute in dark. Observations were made by using fluorescence microscope.

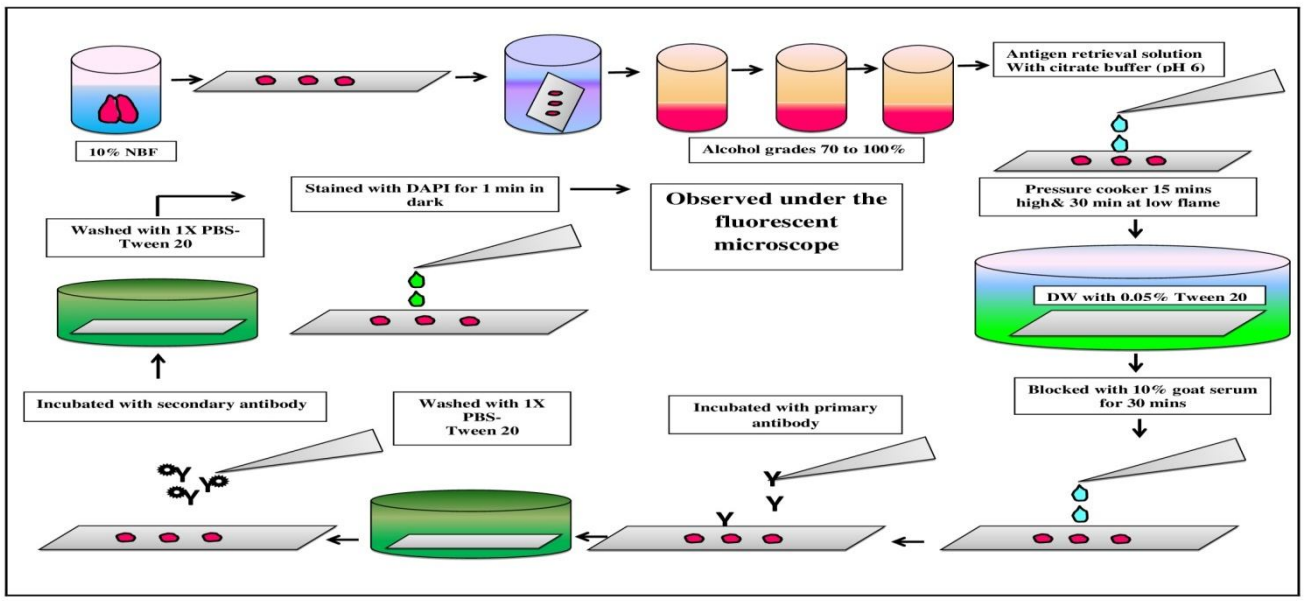


Fig. 2 Diagrammatic view for the CD4 cells staining protocol by immunofluorescence technique.

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D. Three Colour Direct Flow Cytometric Analysis Of cd4 cells

1) *Collection of Sample:* The blood sample was collected aseptically by venipuncture at tail region of mice from all groups, and stored into sterile K3 EDTA BD Vacutainer blood collection tubes (Lavender top) mixed well. The anticoagulated blood samples were stored at room temperature 20-25^{0}C.
2) *Preparation of working solution::*The analysis of CD4 cells performed with a standard BD FACSCalibur system (Becton Dickinson, U.S. based) which is provided with 15- mW, 488-nm, argon-ion laser and detectors for three fluorescent parameters. The FL4 option provides a second 635-nm, red-diode laser and an additional detector. Laser light is focused onto the flow cell by a focusing lens. BD CellQuest Pro TM software (Becton Dickinson) was used for the flow cytometric data acquisition, data analysis, and report generation in three colour flow cytometric experiments and lymphocyte subset analysis. The labelled verified BD Trocount tubes were rinsed with sheath fluid. 20 µl of BD Tritest CD4 FITC/CD8PE/CD3 PerCP reagent was pipette out into the bottom of the BD Trocount tubes, just above the stainless steel retainer without touching the pellet. Then 50 µl of anticoagulated whole blood samples was mixed with BD Tritest CD4 FITC/CD8PE/CD3 PerCP reagent and mixed well with the vortex mixer. The samples were incubated for 15 minutes in the dark at room temperature (20-25^{0}C). Then 2ml of BD FACS Lysing solution was added, kept in the dark at room temperature (20-25^{0}C) for 10 minutes. After that the tubes were centrifuged at 3000rpm, for 7 to 8 minutes. The supernatant was discarded; tubes were blotted. Afterwards 2 ml of sheath fluid was added to tubes, again centrifuged at 3000rpm, for 7 to 8 minutes, supernatant was discarded; tubes were blotted. Subsequently 500 µl of sheath fluid was added. This sample is known as working solution.

E. Flow Cytometry Of cd4 Thymocytes

The working solution was preceded further through flow cytometer, BD FACS Calibur. Each tube was installed onto the sample injection port. The sample was carried to flow cell through stainless steel sample injection tubes surrounded by a droplet containment sleeve. The sleeve worked in conjunction with a vacuum pump to eliminate droplet as a fluid backflow from sample injector tubes. The pressurized sample was run through flow cell in BD FACS Calibur. The acquisition and analysis of data was carried out with the help of BD Cell Quest Pro software). Fluorescent measurements were collected by using a logarithmic amplifier and forward scatter and side scatter by using a linear amplifier. Generally 10.000 events were recorded. Thymocytes were gated according to forward and side scatter patterns of light in the form of dot plots. Data is acquired on a PowerMacG5 workstation running OSX and BD CellQuest Pro TM software. The observations were recorded.

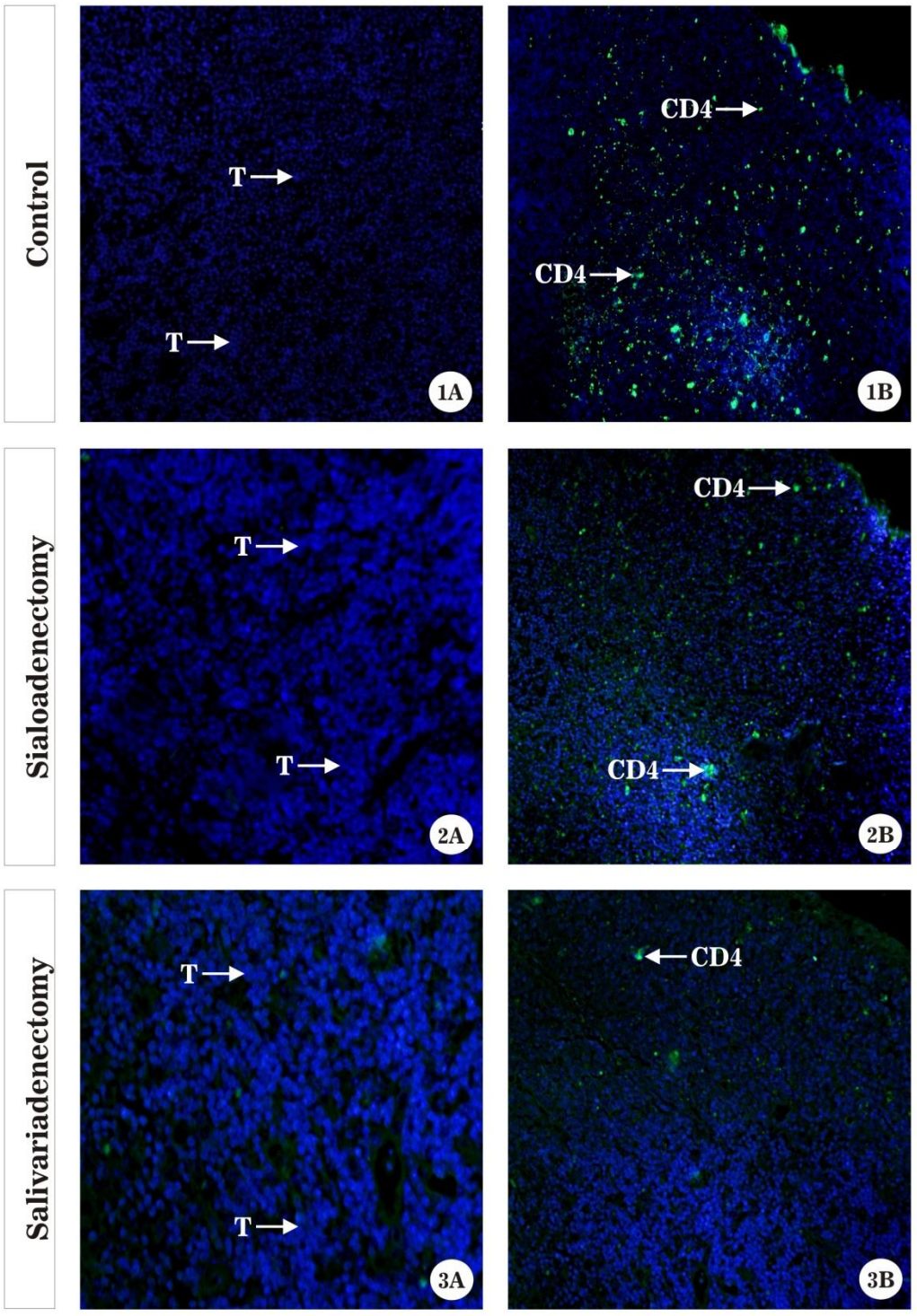
III. RESULTS

The plate numbers I and II, showed immunofluorescence staining of CD4 cells of thymus gland in control, sialoadenectomy and salivariadenectomy group at 200X and 400 X magnifications respectively. Fig. 1A, 2A and 3A from plate no. I and II showed the negative control sections which were treated with only secondary antibody (Goat Anti-RatIgG Dylight 488, Thermo scientific) and visualised with DAPI stain. The brilliant blue stained cells were observed with well differentiated medulla and cortex region with corticomedullary junction. The thymus is well populated with thymocytes. The cellularity was somewhat reduced in sialoadenectomised and salivariadenectomised group as compared to control.The plate numbers I and II with Fig. 1B, 2B, 3B showed the positive control sections of thymus gland. The positive control sections were treated with Anti CD4 (Primary antibody-CD4antibody-GK 1.5, Thermo scientific; secondary antibody -Goat Anti-RatIgG Dylight 488, Thermo scientific) visualised with DAPI. Thymocyte showed immunore activity towards Anti CD4 (Primary antibody- CD4 antibody-GK 1.5, Thermo scientific). There was bright green staining of CD4 cells in thymus of in all the groups. Here there was a reduction in cortex and medulla region with the reduction in the number ofCD4 cells in the sialoadenectomised and salivariadenectomised group as compared to that of control.

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PLATE NO. I : IMMUNOFLUORESCENCE STUDY OF CD4 CELLS



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A. Captions

1) *PLATE NO.:* IEffect of sialoadenectomy and salivariadenectomy on thymus gland of male mice stained with staining of CD4 cell by immunofluorescence technique.

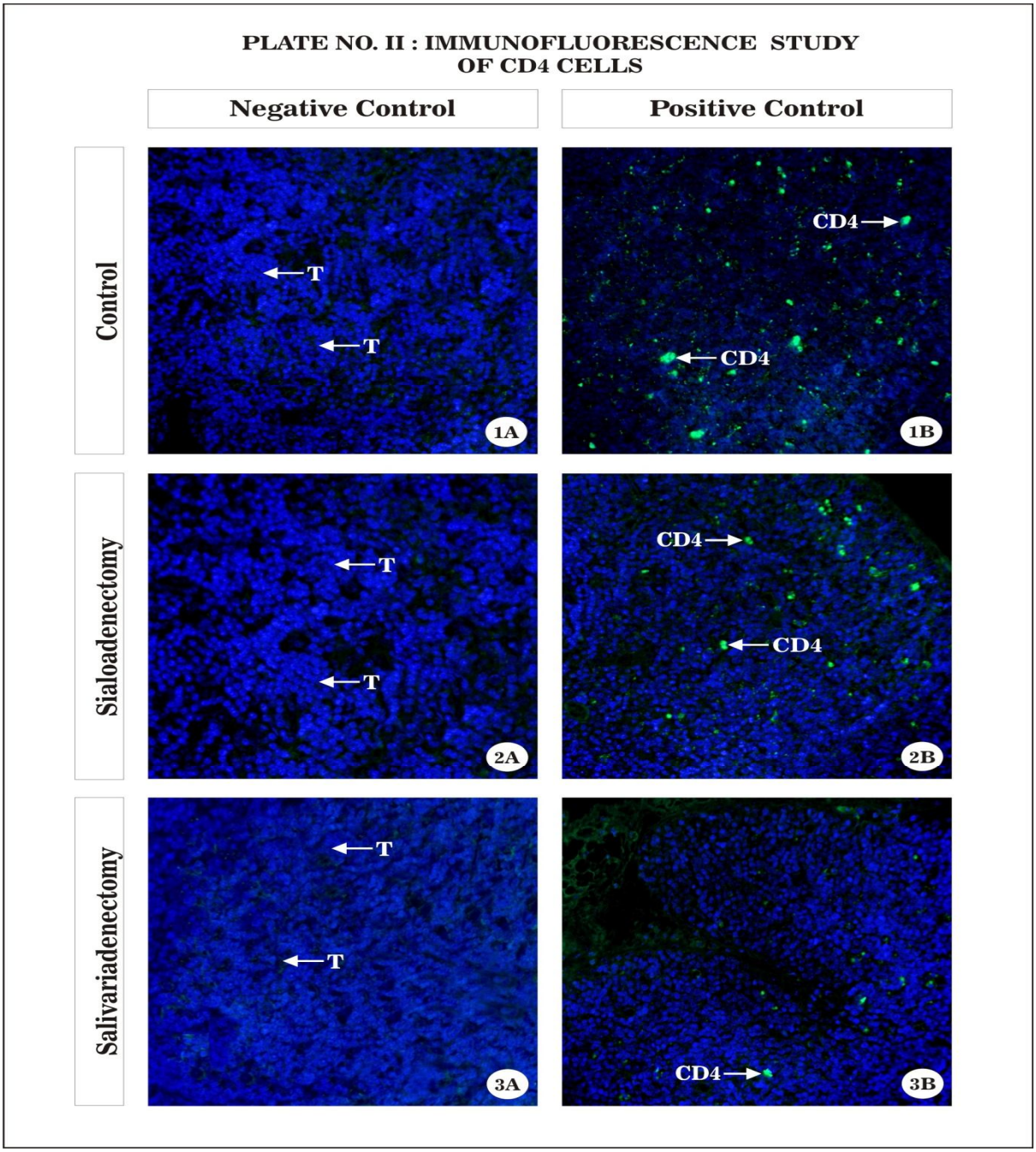
2) Figure no. 1A and 1B shows negative and positive control of T.S. of thymus gland of control group at 200X magnification respectively.

3) Figure no. 2A and 2B shows negative and positive control of T.S. of thymus gland of sialoadenectomised group at 200X magnification respectively

4) Figure no. 3A and 3B shows negative and positive control of T.S. of thymus gland of salivariadenectomised group at 200X magnification respectively

B. Captions in figures:

1) *Cortex, M: Medulla, T:* Thymocyte, CP: Capsule, CD4: CD4 T cells



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C. Plate No. II

Effect of sialoadenectomy and salivariadenectomy on thymus gland of male mice stained with staining of CD4 cell by immunofluorescence technique.

Figure no. 1A and 1B shows negative and positive control of T.S. of thymus gland of control group at 400X magnification respectively.

Figure no. 2A and 2B shows negative and positive control of T.S. of thymus gland of sialoadenectomised group at 400X magnification respectively

Figure no. 3A and 3B shows negative and positive control of T.S. of thymus gland of salivariadenectomised group at 400X magnification respectively

Captions in figures:C: Cortex, M: Medulla, T: Thymocyte, CP: Capsule, CD4: CD4 T cells

Table 1: Effect of sialoadenectomy and salivariadenectomy on CD4 cells in the thymus gland of male mice studied by flow cytometry. Values are Mean ± SD. Number in parenthesis denotes the number of animals. $P < 0.05$= almost significant, $P < 0.01$= significant, $P < 0.001$= highly significant, $P > 0.5$=Non Significant (NS).

Sr. No.	Parameter	Control	Sialoadenectomy	Salivariadenectomy
1	CD4%	20.396 ± 1.0242	3.83 ± 1.138	1.526 ± 0.391
	Statistical significance	1:2, $P < 0.01$	2:3, $P < 0.01$	1:3, $P < 0.05$
2	Absolute CD4 count	966.4 ± 132.7754	35.6 ± 2.7019	28.6 ± 2.3022
	Statistical significance	1:2, $P < 0.01$	2:3, $P < 0.01$	1:3, $P < 0.01$

The table 1 showed the data for flow cytometric analysis of blood samples for control, sialoadenectomised and salivariadenectomised group respectively. The CD4 % in control group was 20.396 ± 1.0242. There was significant reduction in CD4 % i. e. 3.83 ± 1.138 in sialoadenectomised mice as compared to the control (1:2, $P < 0.01$). The CD4 % again reduced almost significantly in salivariadenectomised group 1.526 ± 0.391 as compared to the control (1:3, $P < 0.05$). While reduction of CD4 % from sialoadenectomised to salivariadenectomised group was significant (2:3, $P < 0.01$).The absolute CD4 count was decreased significantly from control with 966.4 ± 132.7754 to 35.6 ± 2.7019 in sialoadenectomised mice (1:2, $P < 0.01$), then up to 28.6 ± 2.3022 in salivariadenectomised group as compared to control (1:3, $P > 0.01$). The reduction in absolute CD4 count in sialoadenectomised to salivariadenectomised was also significant (2:3, $P < 0.01$).

IV. DISCUSSION

EGF is capable of activating EGF receptor (EGFR or ErbB1) tyrosine kinase, which in turn activates intracellular signal transduction, enhances transcription of growth-related genes, and usually promotes cell growth and differentiation [23]. According to many workers, after sialoadenectomy the plasma EGF decreases rapidly and was undetectable by 3 weeks, indicating that the submandibular gland is a major source of circulating EGF [24] [25] [26] .After the sialoadenectomy and salivariadenectomy there was atrophy of thymus gland. The early atrophy is associated with the reduction in cortico medullary region and cellular density including decrease in CD4 thymocytes. The aging-associated thymus atrophy is related both to the restriction of thymus growth due to a reduction in the provision of growth factors as well as to a decrease in the proliferation of thymic epithelial progenitor cells (TEPCs). Here in present study the absence of salivary EGF may be the cause of atrophic signs in thymus. The immunofluorescence activity of T cells towards Anti CD4 primary and secondary antibodies showed intense bright bluish staining of CD4 cells in thymus gland of control, sialoadenectomised and salivariadenectomised mice. The number of stained CD4 cells was decreased in thymus gland of sialoadenectomised and salivariadenectomised group as compared to control, this indicates occurrence of pathological conditions due to absence of salivary glands secreted growth factors specially EGF.

To confirm the effect of salivary glands secreted growth factors on differentiation, activation and cellularity of T cell at peripheral region the flow cytometric analysis of CD4 cell from the blood sample was done. CD4+ T helper lymphocytes play a central role in regulation of immune response: 1) they have capacity to help B cells for generating antibodies, to recruit and activate macrophages, to recruit neutrophils, eosinophils and basophils to sites of infection and inflammation. 2) Low CD4+ T lymphocyte counts (CD4 counts) are associated with a variety of conditions, including many viral infections, bacterial infections, parasitic infections, sepsis, tuberculosis, coccidioidomycosis, burns, trauma, and intravenous injections of foreign proteins, malnutrition, over-exercising,

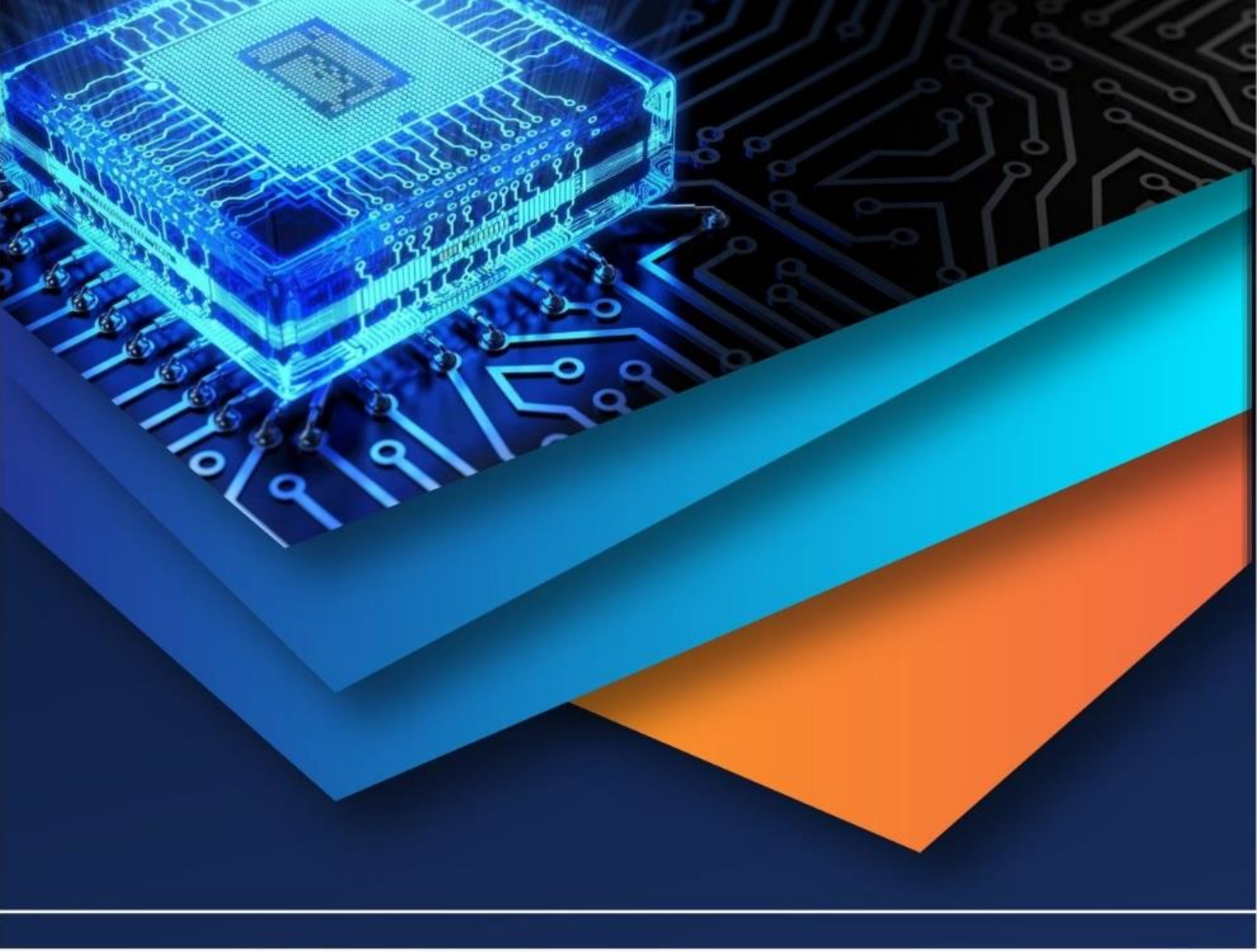
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pregnancy, corticosteroid use, normal daily variation, psychological stress, and social isolation. There are also a number of people who are completely healthy and who have low CD4 counts for no apparent reason [27].For the differentiation and maturation of T cell subset of CD4 helper cells the thymic epithelial network play precious role. In our previous finding we clearly observed the degeneration of epithelial network with the destruction of capsule and septal region through histological studies. With this there was a decrease in collagenous material confirmed with M.T. staining technique [28]. The collagen contributes to the entrapment, local storage and delivery of growth factors and cytokines. It may be possible that due to reduced collagen content the EGF was not provided properly. The CD4 thymocyte and its subset show special cytokine profile, but in absence of EGF the development and differeciation of CD4 may be disturbed. So the number of fluorescently stained CD4 thymocytes reduced after sialoadenectomy and salivariadenectomy. In a conclusion the present findings and previous supportive evidences are seems to be two side of same coins. It is possible that there is alliance or relationship between the whole development thymus and EGF that exert effect on each other. But it is confirmed that salivary gland secreted EGF definitely affect the immune system especially CD4 cells of thymus gland.

REFERENCES

[1] M. Michie, J.C. Zuniga-Pflucker, “Regulation of thymocytes differentiation: pre-TCR signals and beta-selection,”Semin. Immunol.,vol.14, pp. 311-323, 2002.
[2] T.J. Kindt, R. A. Goldsby and B.A. Osborne, Kuby Immunology, 6th ed, Revised. W.H. Freeman and Co. Ltd. New York. U. S. 2006.
[3] D. F. Gruber and O. D. Ledney, “Lymphomyelopoietic activity of Submandibular Gland-conditioned Medium,”Immunol. Lett.,vol. 1, pp. 227-231,1980.
[4] D. F. Gruber and G.D. Ledney, “Submandibular Gland-conditioned Medium Enhancement of Bone Marrow Colony-forming Cells in Tumor-bearing Murine Recipients,”Experientia., vol. 39, pp. 101-103, 1983
[5] A.Kemp, L. Mellow and E. Sabbadini, “Suppression and Enhancement of in vitro Lymphocyte Reactivity by Factors in Rat Submandibular Gland Extracts,” Immunology., vol.56, pp. 261-267, 1985.
[6] A. Kemp, L. Mellow and E. Sabbadini, “Inhibition of Interleukin 1 Activity by a Factor in Submandibular Glands of Rats,”J. Immunol., vol. 137, pp. 2245-225, 1986.
[7] T. Barka, “Biologically Active Polypeptides in Submandibular Glands”. Histochem.Cytochem.,vol. 28, pp. 836-859, 1980
[8] S. Cohen, “Isolation of a mouse submaxillary gland protein Accelerating incisor eruption and eyelid opening in the new-born animal”. J. biol. Chem., vol. 237, pp. 1555, 1962.
[9] C. R. Savage, T. Inagami and S. Cohen, “The primary structure of epidermal growth factor”,Biochem.,vol. 247, pp. 7612, 1972.
[10] G.Carpenter, “The regulation of cell proliferation: advances in the biology and mechanism of action of epidermal growth”. Inves.Dermatol.,vol.71, pp. 283, 1978.
[11] R. LByyny,D. N. Orth andS. Cohen, “Radioimmunoassay of epidermal growth factor,”Endocrinol.,vol. 90, pp. 1261-1266, 1972.
[12] M. L. Roberts, “Testosterone induced accumulation of epidermal growth factor in the submandibular salivary glands of mice assessed by radioimmunoassay”. Biochem.Pharmacol.,vol. 23,pp. 3305, 1974.
[13] L. Frati, G. Cenci, G. Sbaraglia, D.V. TetiandI. Covelli, “Levels of epidermal growth factor in mice tissues measured by a specific radioreceptor assay”,Life. Sci., vol. 18, pp. 905, 1976.
[14] Y. Hirata and D.N. Orth, “Concentrations of epidermal growth factor, nerve growth factor and submandibular gland renin in male and female mouse tissue and fluids,”Endocrinology.,vol.105, pp.1382-1387, 1979.
[15] J. A. Ossario, and A. Kraemer, “Simulative Effect of Parotin on the Body Weight of Sialoadenectomised Rats,”Rev. Brasileria.Biol., vol. 25, pp. 233-237, 1965
[16] T. Takeda, Y. Yamasaki, H. Yamabe, Y. Suzuki, H. Haebara, T. Irino and A. Grollman, “Atrophy of the lymphoid tissues of mice induced byextracts of the submaxillary gland,”Proc. Soc. Exp. Biol. Med.,vol. 16, pp. 212-216, 1967
[17] A. Martinez-Hernandez, P.K. Nakane, and G. B. Pierce, “Relationship between the submaxillary gland and the thymus,”Lab. Invest., vol. 29, pp. 266-271,1973.
[18] G. L. De Lange, “The effect of salivary gland extracts on the histology of lymphoid organs and salivary glands in mice,”Arch. Oral. Biol.,vol. 20,pp.515-520, 1975
[19] D. H. Dean and R. N. Hiramoto, “Effects of Submandibularectomy and Castration on Thymus and Spleen Weights in Male Mice,”Experientia.,vol.39, pp.1351-1352, 1983.
[20] A. Menendez-Patterson, J. Suarez, S. Cornejoand B. Marin, “Sex Difference in the Effect of Extirpation of the Submandibular Salivary Glands in Rats,”‘Arch. Oral.Biol., vol. 30, pp. 243-248, 1985
[21] A. Lectic-Gavrilovic and B.D. Jankovic, “Immunological reactions in rats treated with different extracts of submandibular salivary gland,” Periodicum.Biologorum(Supp-1)., vol. 88, pp. 154-155,1986
[22] A. Letic-Gavrilovic, C. RadojicicandK. Abe, “Effects of Submandibularectomy on the Thymus and T-cell System of Mice.”J. Dent. Res.,vol. 68, pp.1252, 1989
[23] T. Zhang, Z. She, Z. Huang, J. Li, X. Luo and D. Yihui, “Application of sialic acid/ polysialic acid in the drug delivery systems,”As. J.Pharma’. Sci.,vol. 9, pp.75-81, 2014
[24] S. Arancibia, I. Assenmacher, “Submaxillary glands in an endocrine context”,J. Biol. Buccale., vol.13(3), pp.185-203, 1985
[25] Y. Oka and D. Orth, “Human plasma epidermal growth factor/ Burgosterone is associated with blood platelets,”J. Clin. Invest.,vol. 72, pp. 249-259, 1983
[26] R. M. Gubits, andP. A. Shaw, “Epidermal growth factor gene expression is regulated differently in mouse kidney and submandibular gland,”Endocrinol., vol. 119, pp. 1382-1387, 1983.
[27] M. Irwin, “Low CD4 Counts: A variety of causes and their implications to a multi-factorial model of AIDS,”British medical journal.,Online, 2003
[28] M. V.Walvekar, S. P. Khairmode and S. R. Desai, “Effect of salivary gland secreted growth factors on histology of thymus gland in mice,”Int.J.Curr.Biotechnol., vol. 2(4), pp.1-9, 2014.

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