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Development and Activation of T cell subsets– An Overview from a Periodontal Perspective

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***Abstract**: Innate immunity and adaptive immunity form the two main components of human immune system. Unlike the innate immunity, adaptive immune response is specific and more complex which may be cell-mediated or humoral-mediated. T lymphocytes, the effectors of cell-mediated immunity (delayed hypersensitivity) are immune cells derived from bone marrow and develop in the thymus. The diversity of the T cell receptor enables recognition of wide variety of specific antigens expressed by antigen presenting cells (APC) in the Major Histocompatibility (MHC) context. Following complete activation, T cells differentiate into any of the subtypes depending on the cytokine milieu. Plasticity among T helper subsets have also been noted. Knowledge of how the immune-inflammatory mechanisms are regulated is essential for understanding the pathogenesis of inflammatory diseases like periodontitis where the complex interplay between microbial flora and the host immune mechanisms govern the balance between tissue homeostasis and disease progression. This review highlights on the processes of development, subsequent activation of T cells, and differentiation into various subtypes, briefly summarizing their role in periodontal disease.*

Keywords: T cells, lymphocytes, T cell development, T cell activation, adaptive immunity, T cell subsets, periodontal disease.

Introduction:

The immune system of the human body is built by a nexus of cells, tissues, and organs that release cytokines that modulate the innate and adaptive immune responses, which work in

synchronicity to protect the body against infectious organisms. Innate immunity refers to the natural non-specific defence mechanisms which include a wide range of mechanical, chemical, and cellular elements that are activated immediately or within hours of an antigen exposure in the body, to effectively neutralize the antigen [1]. Adaptive immunity refers to the acquired specific immune responses against antigens. Unlike innate immune response it is more complex and takes days or even weeks to become established. It is marked by antigenic specificity, immunologic memory, and potential to distinguish self antigens from non-self. Adaptive immunity might be humoral or cell-mediated. The latter is modulated by cytokines that are released by immunogenic cells such as T lymphocytes and macrophages. Humoral immunity as mediated by B lymphocytes produce antigen-specific antibodies. Components of adaptive immune response include T and B lymphocytes, natural killer cells, and APCs.

Variations to the onset and regulation of immune and inflammatory mechanisms, determine the patient susceptibility trait for a variety of complex diseases like periodontitis which is a multifactorial chronic inflammatory condition of the periodontium that is initiated and perpetuated by tooth associated polymicrobial biofilm resulting in progressive attachment loss and bone destruction [2]. While periodontitis is primarily a polymicrobial disease, it is the variation of the interplay between the microbial flora and the host immune mechanisms that determines the level of offset in the intricate balance between tissue homeostasis and disease progression.

The adaptive immune response has a hand in periodontal disease when the initial inflammation fails to resolve, through the activation of the innate immune reaction. With the emergence of the adaptive immune response, there is potential to orchestrate a wide variety of cytokines and signalling molecules which can modulate the immuno-inflammatory process. When this immune-inflammatory process is unable to quickly resolve the cause of the inflammatory reaction, there is significant potential for deleterious effects to the inflamed tissues, due to its persistent activation. It has for a long time been observed that in periodontal disease progression, the Early Stage as described by Seymour is similar to the delayed-type hypersensitivity reaction, while the Stable disease activity is a T cell-mediated response, whereas the progressive Established state is predominated by B cells [3].

The indispensable role of T cells in adaptive immunity came into light following the study by Ivanyi and Lehner in 1970 [4]. The T lymphocytes stem from the multipotent progenitors of bone marrow `and develop in the thymus in a segmented sequential manner. T cell activation

necessitates the identification of antigenic peptide by its receptor followed by co-stimulation with the APCs like dendritic cells, Langerhans cells, and macrophages along with appropriate cytokines in the local environment. The differentiation and clonal accumulation of the T cells can manifest into many forms, and hence various T cell types have been described [5]. This review seeks to highlight the extent of our knowledge in the development and activation of T cells, as well as the underlying signalling cascades that effect the various T cell subsets, so as to provide a better understanding on their potential role in the outbreak and progress of periodontal disease.

Basic morphology of T cells:

T lymphocytes are small rounded cells measuring about 8-10 microns in diameter, with dense chromatin laid large nucleus and cytoplasmic border which contains few ribosomes, mitochondria, and lysosomes. Following activation, the cells enlarge in size, with more number of organelles. They have cell surface receptors (TCR) for antigen recognition with different specificities.

Origin and Development of T cells:

The fact that T cells develop in the thymus was discovered by Jacques Miller in 1961. The T cells originate from the hematopoietic stem cells (HSC) in foetal liver during the embryonic stage and in bone marrow later. A part of these omnipotent HSC progenitors trigger the transcription of recombination activating gene 1 and 2 (RAG 1 and RAG2) and become lymphoid-primed multipotent progenitors and then common lymphoid progenitors (CLP) [6]. The CLP cells travel to the thymus to become early thymic progenitors (ETP).

The ETP cells can evolve into T, B lymphocytes, myeloid cells, dendritic and NK cells. In the substance of thymus, the ETP confronts various growth factors and ligands, which triggers the differentiation and proliferation of these cells. Expression of Notch-1 receptors and interaction with delta-like ligands is critical for commitment of the cells towards T-cell lineage [6].

In the cortex of thymus, ETP becomes double negative (DN) cells that neither express CD4 nor CD8 ($CD4^-CD8^-$) which later transforms into DN2 cells when it obtains the $CD25^+$ and $CD44^+$ receptors. These cells lose the B potential during this stage and starts expressing

proteins such as, RAG1, RAG2, signalling proteins CD3 chains, kinases, phosphatases ZAP-70, LCK and LAT complexes for TCR gene rearrangement [6].

DN2 cells now migrate into the sub capsular zone where they become DN3 cells which can choose either of the two different pathways of differentiation. They can either express the αβ chains of the TCR and follow the process of selection to generate $CD4^+$ or $CD8^+$ T Cells or express the γδ chains to generate a subpopulation of γδ T lymphocytes with special functional characteristics [7].

β Selection:

The DN3 cells committed to form αβ TCR undergo a process of β selection where they form functional pre-TCR complex with an invariant α chain and functional β chain. This functional β chain allows to exhibit CD4 and CD8 molecules thereby, converting them to double positive (DP) cells.

Positive Selection:

These DP cells enter the thymic cortex for the stage of positive selection where, the T cells are checked for their arrangement of TCR α locus and whether these cells are capable of recognising antigen peptide-MHC complexes. The epithelial cells present in thymic cortex (cTEC) evaluate DP cells with their self-peptides in the MHC-Class I and II context. About 1-5% of the DP cells interact with these molecules and receive a vital survival signal while other DP cells undergo apoptosis. This stage of selection allows the thymocytes to differentiate into single positive (SP) cells. Those SP cells which recognise MHC-Class I turn out to be $CD4^-CD8^+$ cells, and those recognising MHC-Class II become $CD4^+CD8^-$ cell phenotypes [8].

Negative Selection:

Subsequently, the SP cells infiltrate the thymic medulla where, negative selection takes place to obliterate the T lymphocytes that attach too firmly with self-peptides expressed on MHC. The positively selected SP cells are subjected to a wide variety of own-antigens disclosed by epithelial cells of thymic medulla (mTEC) and dendritic cells. The cells with high affinity undergo apoptosis, thus persuading the knock down of potentially autoreactive T cells. Only the SP cells which successfully survive the negative selection process mature to become naïve T cells [9]. It allows for self-tolerance in immunity.

The typical mature naïve T lymphocytes which undergoes a chain of these processes, and leave the thymus through cortico-medullary junction are self-tolerant, single positive, and self-restricted. Later, they migrate to secondary lymphoid organs like lymph nodes where they are primed and mature into effector cells.

T cell receptor complex:

TCR is the cell surface receptor which the T cells conceive during early development in the primary lymphoid organ (thymus) and helps in antigen recognition. The TCR complex was discovered by Tak Wah Mak in 1984. It is a complex of various proteins that act together for activation of T cells.

The two separate polypeptide chains α and β, or γ and δ chains produced by independent genes comprise the TCR. Those with αβ chains make up 95% of all T cells and are the most abundant while, T cells with γδ-TCR are mainly found in gut epithelium and make up 1-5% of all peripheral T cells which are essential for barrier membrane homeostasis. CD3 group proteins consisting of heterodimers CD3εγ, CD3εδ and homodimer CD3ζ form the accessory proteins of TCR complex.

Although TCR is cell surface bound receptor, it cannot mediate signal transduction on its own when it engages with antigenic peptide as, it has a short cytoplasmic tail thereby requiring co-receptors, accessory proteins, associated enzymes, adaptor proteins, and activated transcription factors to carry out and mediate signal transduction [10].

T cell receptor diversity:

The wide range of antigen diversity in T lymphocytes is brought about by variations in the structure of TCR created during the DN2 and DN3 phases, through somatic recombination of selected gene-encoded segments.

Each TCR polypeptide chain contains two extracellular portions – a constant (C) region and a variable (V) region connected by a joining segment (J). Each variable region has three hypervariable regions called complementarity-determining regions (CDR1, 2, and 3) which are capable of generating a number of different combinations to create different TCR specific for antigens. There is another hypervariable region in the β chain, CDR4 which does not participate in antigen recognition but interacts with super antigens. CDR3 is known to be the chief CDR for Ag recognition while, CDR1 of α and β chains are known to associate with N-

and C-terminal parts of the peptide antigen respectively. The CDR2 is responsible for recognising the MHC molecule.

TCR α and γ chains are produced by recombination of V, J, and C segments whereas, β and δ chains by recombination of V, D, J, and C segments together with P- and N- terminals that account for great diversity in TCRs. Also, TCR can be re-edited called as TCR revision and alteration of its antigen specificity is possible during its development in later stages [11, 12].

Naïve T cells activation:

T cell activation and differentiation requires three signals –

i) Interaction between TCR and antigenic peptide expressed by MHC molecules

ii) Co-stimulation

iii) Cytokines activity that induce clonal embellishment [13]

Further, the cytokine microenvironment accompanying T cell activation decides the nature of immune response generated subsequently.

First signal – Antigen recognition:

There is regular flow of T lymphocytes from the blood into lymph nodes/ aggregated lymphatic follicles. The naïve T lymphocytes within the secondary lymphoids essentially express L-selectin, which helps in binding to the specialized endothelial cells that display the addressins PNAd (peripheral node addressin) or MAdCAM-1 (mucosal addressin cell adhesion molecule-1) in the post-capillary veins. The T cells can bind to other endothelial cells only when the TCR is stimulated by inflammatory mediators [14].

Within the lymph nodes, T lymphocytes migrate at a speed of 11-14 μm/ minute whereas, DCs migrate at speeds of 3-6 μm per minute, which enable them to establish new contacts with T cells constantly. T lymphocytes develop short-term contact with numerous DCs but halts and binds to only those expressing a compatible antigen specific to their TCR and the duration of interaction might be transient (3-11 minutes) or steady (several hours) which is dependent on the affinity for that particular antigen. Stable contacts are established by manifestation of adhesion factors like ICAM-1, mature DCs, and highly antigenic ligands [15]. The CD4 and CD8 T cells capture antigens exhibited by MHC Class II and I molecules respectively while the γδ T cells are not MHC restricted and recognize a wide variety of antigens.

Following antigen recognition by TCR, several "TCR micro clusters" are formed which assists the rearrangement and induction of signalling molecules around the contact zone with DC. It unites the DC and T lymphocyte and is called an "immunological synapse" comprised of well-organized dynamic molecular complex with three concentric zones.

- Central zone comprises of TCR complex, co-stimulatory and co-inhibitory molecules, and co-receptors - primary and secondary activation signals.
- Peripheral zone consisting adhesion molecules LFA-1-ICAM-1 and CD2-LFA-3 that, stabilize the bond between the cells due to the affinity.
- Distal supramolecular activation clusters are made of phosphatase CD45 and F-actin [16].

After antigen recognition, a nexus of signalling pathway is induced on the inner aspect of the membrane and cytoplasm ending in stimulation of the main transcription elements: NF-κB, NFAT, and AP-1.

Signalling Pathways: Depicted in figure 1.

Second signal – Co-stimulation:

A surface molecule that cannot stimulate T cell activation on its own but can potentially amplify or decrease the signalling initiated by TCR complex is called a co-stimulatory molecule. Co-stimulation is the second signal of T cell activation which licences the T lymphocyte to react to an antigen. It potentiates the production of IL-2 and further interactions between these molecules induce anti-apoptotic signals that can extend the life span of T lymphocyte, stimulate activity of adhesion compounds and increase production of cytokines and growth factors which promote T cell proliferation and differentiation.

- Co-stimulatory receptors on T cells are CD28, CD27 and HVEM
- Co-stimulatory molecules which are inducible and expressed only after activation are SLAM, ICOS [17, 18].

POSITIVE SIGNAL = Receptor on T cell + Corresponding ligands on APCs

Mostly, these co-stimulatory molecules belong to CD28/B7 family or TNF/TNFR family [17, 18]. Without co-stimulation, the process of T lymphocyte activation is not complete, and becomes anergic. Through this mechanism, inappropriate self-responses are prevented. Markers of T cell activation are CD69, CD71, CD25, and HLA-DR. Expression of CTLA-4 is also enhanced on activated T lymphocytes, which outcompetes CD28 for binding to B7 proteins. This acts as a barricade to prevent T cell over activation [19].

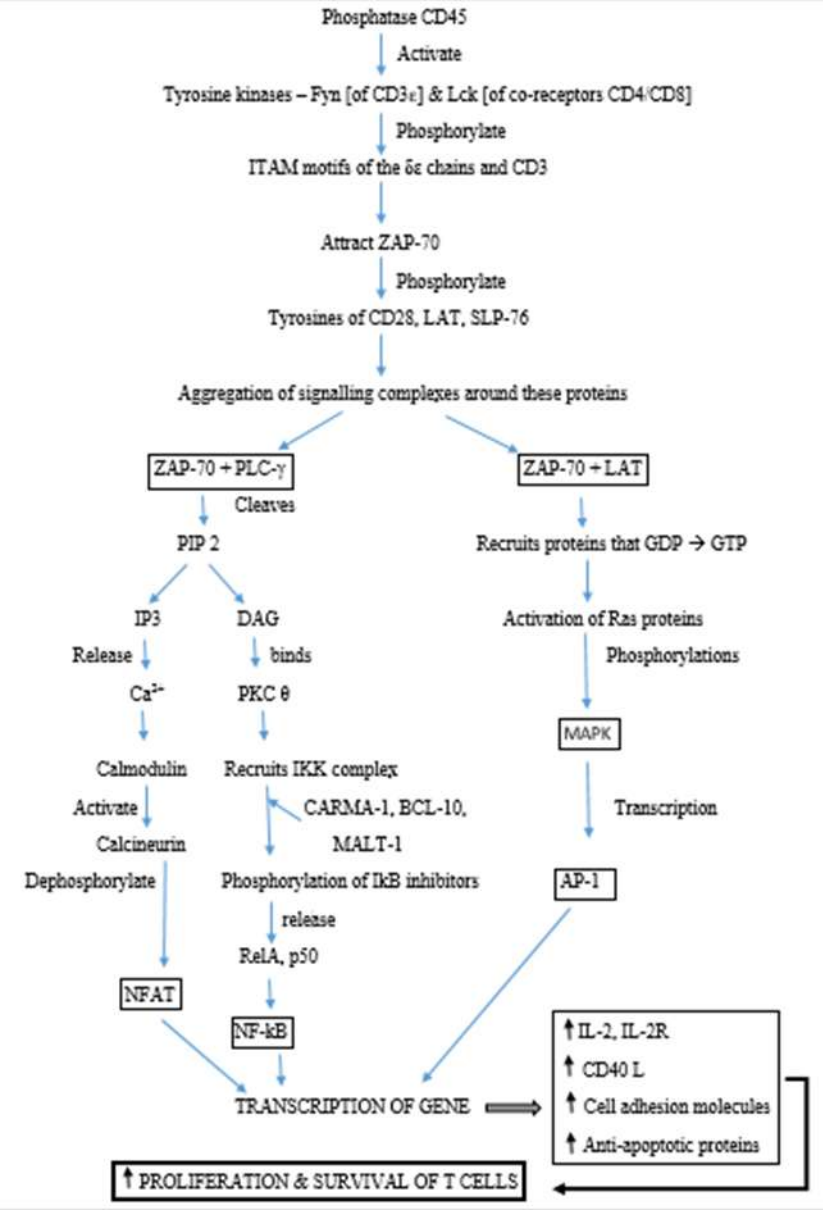

Figure 1: Signalling Pathways in T lymphocyte activation [Illustrated by the authors of this article]

LAT= Linker of Activated T cells; DAG= Diacylglycerol; IP3= Inositol triphosphate; PLC-γ= Phosphoinositide phospholipase C; SLP-76= Lymphocyte cytosolic protein; PKCθ= Protein kinase C; RAS= family of GTP

binding proteins; LCK= lymphocyte specific protein tyrosine kinase; FYN= member of Src tyrosine kinase; ZAP-70= ζ chain associated protein kinase of 70 kDa.

Third signal – Clonal expansion:

Following Ag recognition and co-stimulation, T lymphocytes stimulate the production of IL-2 and exhibit IL2Rα/CD25 which is their high affinity receptor temporarily. CD25 unites with other parts of the IL2R like the β chain (CD122) and γ chain (CD132). Although, it abstains from partaking in the signalling, it amplifies IL-2 affinity up to 100 times. IL-2 serves as an autocrine and also paracrine growth factor. Clonal expansion is activated by IL-2 which results in multiplication of T cells with TCR similar to the native one, which can only recognize the antigen that stimulated its activation. The other molecules participating in this process are IL-15 and IL-21 [20]. A death phase follows activation and establishment of T cell types, where 90% of the effectors undergo apoptosis. The mechanisms initiating the contraction phase are, interactions among Fas-FasL, TNF, and TNFR I and II, as well as CD40-CD40L. Additionally, products like IFN- γ, perforins, and IL-2 modulate this phase [21].

Classification of T cells:

T lymphocytes can broadly be categorized into helper, cytotoxic, regulatory and memory T cells which are further divided into various subsets. An overview of various types of T cells, their effector functions and their role in periodontal disease is presented below. (Table-1&2)

TABLE 1: T helper cells

T cell subsets	***Common character***	***Types***	***Inducing cytokine***	***Transcription factor***	***Effector cytokine***	***Subtypes***	***Actions***	***Role in Periodontal disease***
T helper cells	Express CD4 surface molecule	Th1	IL-12, INF-γ/ IFN-α, IL-18	T-bet	INF-γ	-	Strong cell-mediated immune response against intra-cellular pathogens, delayed hypersensitivity type of reaction	Recruits RANKL cells → osteoclastogenesis

	& activated following Ag presentation by MHC-Class II molecule	Th2	IL-4, IL-33, IL-11, IL-25	GATA-3	IL-4, IL-10, IL-13, IL-5, IL-9, IL-25	-	Anti-inflammatory, Suppression of Th 1 response, and effective against extra-cellular pathogens.	Inhibit MMP, RANKL; Associated with B cell function
		Th17	IL-6 and/or IL-1 with TGF-β	RORγt	IL-17A,IL-17F, IL-21, IL-22	-	Recruitment of PMNs, Protection against extra-cellular pathogens, has both pro-inflammatory and anti-inflammatory roles	Induce production of IL-6, IL-8, PGE2, RANKL
		Treg	TGF-β, IL-2	FoxP3	TGF-β, IL-10, IL-35	Natural [nTreg] and Inducible [iTregs →Tr1, Th3, iTr35]	Helps in resolution of inflammation, Immuno-suppressive action, Tolerogenic responses (especially Tr1), and prevents autoimmunity	Helps in suppression of pro-inflammatory environment.
		Th9	TGF-β, IL-4	PU.1 (?)	IL-9, IL-10	-	Mast cell differentiation and secretion of IL-1β, IL-13, IL-6 and TGF-β	Not yet recognized
		Th22	IL-6, TNF-α	AHR	IL-22, IL-17, IFN-γ	-	Found in epithelium and produce defensins	Yet to be identified
		Tfh	IL-6, IL-21, IL-12	BCL-6	IL-21, IL-4,	-	Germinal centre formation, conversion of B	Associated with B cell

					IL-10		Cells to plasma cells, production of Ab's	functions

TABLE 2: Other T cell subsets

T cell subsets	***Common character***	***Types***	***Actions***	***Role in Periodontal disease***
Cytotoxic T cells	Express CD8 surface molecule & activated following Ag presentation by MHC-class I molecule	Tc	Fights against virus-infected or tumour cells directly	Fights against virus-infected or tumour cells directly by producing TNF-α, IFN- γ
		CD8+ Treg	Produce regulatory mechanism by secretion of cytokines, direct cell-cell contact with target cells, and inducing anergy in APCs	Helps in suppressing pro-inflammatory environment
Memory T cells	Long-lived cells capable of mounting quick & strong immune response against their cognate Ag on re-exposure	T_{CM}	Found in circulation, Lymph nodes & express CD45RO, CCR7, L-selectin, high levels of CD44	Immunologic memory
		T_{EM}	Found in circulation, tissues, express CD45RO and high levels of CD44	
		T_{RM}	Found within tissues, express Integrin αeβ7, CD45RO	
		T_{VM}	Abundant in circulation, express CD45RO	
Natural Killer T cells	Expression of invariant TCR α chain Vα24-JαQ	--	Recognize glycolipid antigens exhibited by CD1d molecules which are MHC-like molecules	Regulatory role, combats autoimmune responses, Secretes IL-4, IFN-γ

Mucosal Associated Invariant T cells [MAIT]	Adaptive immune cells with innate-like effector function	--	Recognize antigens exhibited by MHC Class I-like protein called MR1 and produce pro-inflammatory cytokines	Largely unknown
Gamma delta T cells [γδ T]	γδ TCR rather than αβ TCR	--	Not MHC-restricted and can recognize whole antigens, phosphoantigens	Barrier surveillance

Conclusion:

The development of T cells is a segmented process and consists of an irreversible progression of distinct stages. It is evident that various T cell subsets play a crucial role in the immunopathogenesis of periodontal diseases resulting in tissue destruction but the mechanisms of immune regulation which underpin the disease progression is highly complicated. Understanding of the T cell activation, signalling pathways and how these interactions regulate T cell activities might be beneficial for the development of targeted therapeutic interventions in the near future.

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