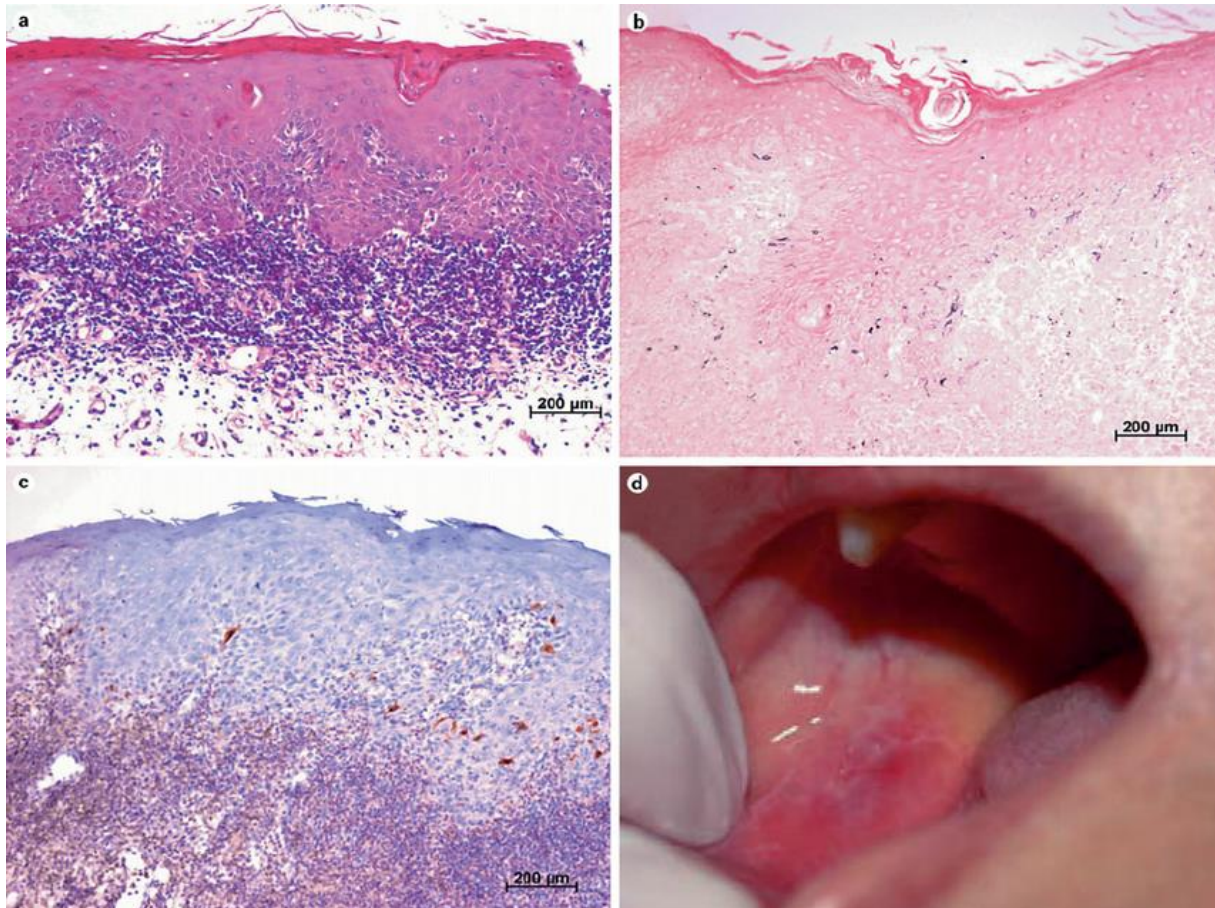# Cellular infiltrate in oral lichen planus, a review article

Source: Fricain JC, Sibaud V, Campana F, Lepreux S, Taïeb A. Mucosal pigmentation after oral lichen planus treatment with topical tacrolimus. Dermatology. 2005;210(3):229-32. PMID: 15785053.

Av: Vahid Shokoohinia

Veileder: Professor Karl Schenck ved Institutt for oral biologi, universitetet i Oslo

Table of contents

Cells of immune system

Hematopoiesis

The system contains more than 10 different blood cell types with various functions and originating from different ontogenic lineages. Leukocytes represent many specialized cell types involved in innate and acquired immunity. Erythrocytes provide O_2 and CO_2 transport, whereas megakaryocytes in the bone marrow generate platelets for blood clotting and wound healing. All blood cell types arise from hematopoietic stem cells (HSCs) that reside mainly in the bone marrow (BM), a major site of adult hematopoiesis.

In adult mammals, HSCs are at the apex of a hierarchy of numerous progenitor cell stages with increasingly restricted lineage potentials that give rise to all blood cell lineages. Of all blood cells, only HSCs fulfill the criteria for somatic stem cells, namely, long-term (and possibly lifelong) self-renewal and differentiation potential. HSCs differentiate into a cascade of progenitor cell stages with declining multilineage potential before unilineage commitment occurs. Recent results and new technologies have challenged the demarcations between stem and progenitor cell populations, the timing of cell-fate choices and the contribution of stem and multipotent progenitor cells to the maintenance of steady-state blood production.
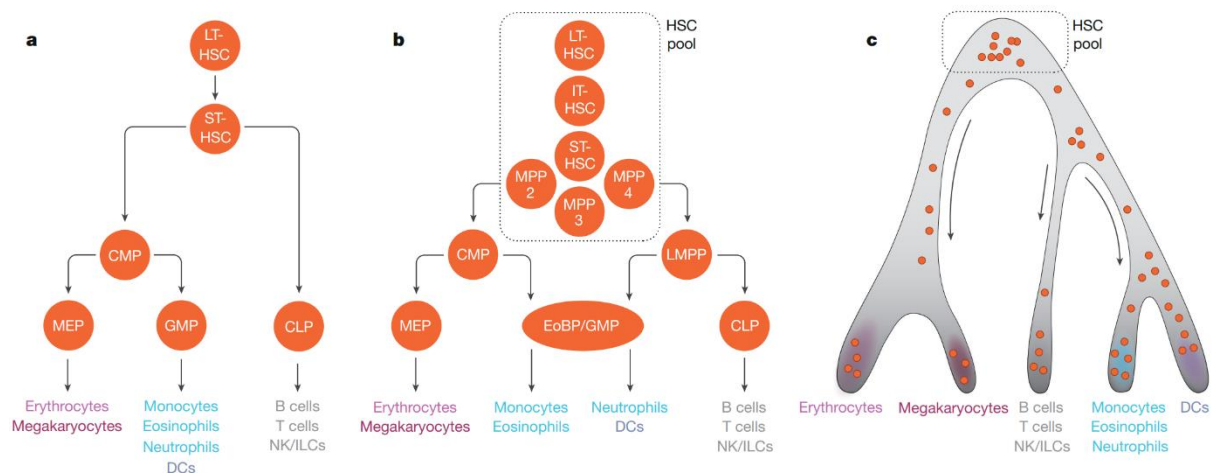
Source: Laurenti E et al. 2018

a, Visualization based on cutting-edge research around the year 2000:
b, During the years 2005–2015, this visualization incorporates new findings: the HSC pool is now accepted to be more heterogeneous both in terms of self-renewal (vertical axis) and differentiation properties (horizontal axis)
c, From 2016 onwards, single-cell transcriptomic snapshots indicate a continuum of differentiation.

HSC, hematopoietic stem cell; MPP, multipotent progenitor; LT-, long-term repopulating; IT-, intermediate-term repopulating; ST-, short-term repopulating; LMPP, lymphoid-primed MPP; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte–macrophage progenitor; MEP, megakaryocyte–erythrocyte progenitor; EoBP, eosinophil–basophil progenitor.

Neutrophils

Neutrophils are the most abundant circulating phagocytes in humans, and the first cells that are recruited into sites of infection and inflammation. In the circulation, mature neutrophils have an average diameter of 7–10 µm, their nucleus is segmented and their cytoplasm is enriched with granules and secretory vesicles.

Neutrophils are continuously generated from myeloid precursors in the bone marrow. The process is controlled by granulocyte colony stimulating factor (G-CSF). During maturation, the neutrophil goes through several stages, namely myeloblast, promyelocyte, myelocyte, metamyelocyte, band cell and, finally, polymorphonuclear (segmented) cell. Neutrophil granules are formed sequentially during maturation from the promyelocyte stage.

Neutrophils are kept in reservoirs in organs like bone marrow, spleen, liver and lung. These neutrophils are mature and called organ-marginated granulocytes. Organ-marginated granulocytes can be rapidly deployed to sites of inflammation or infection, and are also possibly constantly patrolling the aforementioned organs in search of tissue damage or microbial invasion.

Three types of neutrophil granules are formed consecutively during their maturation, and they are filled with pro-inflammatory proteins. These are azurophilic (primary) granules, which contain myeloperoxidase (MPO), specific (secondary) granules, which contain lactoferrin, and gelatinase (tertiary) granules, which contain matrix metalloproteinase 9 (MMP9; also known as gelatinase B).

Neutrophils have traditionally been thought of as simple foot soldiers of the innate immune system with a restricted set of pro-inflammatory functions, but recent evidence shows that they contribute to chronic inflammatory conditions and adaptive immune responses. For example, recent studies have described that neutrophils are involved in adaptive immunity by controlling the activation of T and B cells, and through the presentation of antigens to professional antigen-presenting cells in lymph nodes. (Mantovani, A., Cassatella, M. A., Costantini, C. & Jaillon, S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nature Rev. Immunol. 11, 519–531 (2011).)

Killing mechanism of neutrophils are
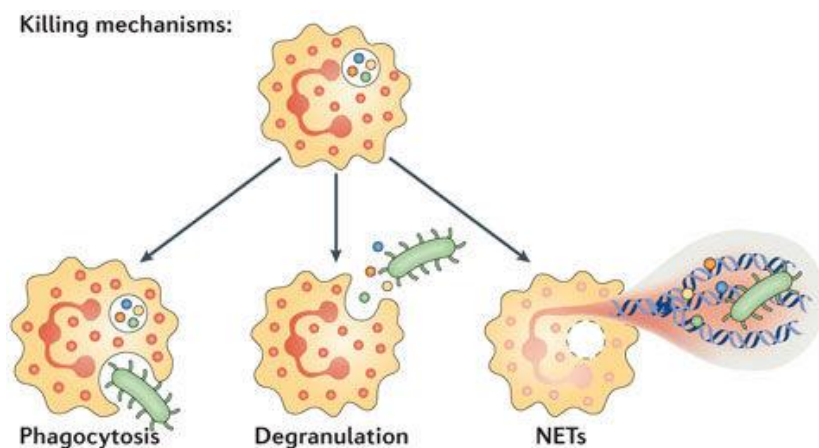
Source: Kolaczkowska et al. 2013

- Phagocytosis. After microorganisms are encapsulated in phagosomes, the cells kill the pathogens using NADPH oxygenase-dependent mechanisms (reactive oxygen species) or antibacterial proteins (cathepsins, defensins, lactoferrin and lysozyme).
- Degranulation. The antibacterial proteins, as mentioned above, are released from the neutrophil granules into the extracellular milieu.
- NETs. Highly activated neutrophils can eliminate extracellular microorganisms by releasing neutrophil extracellular traps (NETs). NETs are composed of a core DNA element to which histones, proteins (for example, lactoferrin and cathepsins) and enzymes (for example, MPO and neutrophil elastase) that are released from neutrophil granules are attached. NETs immobilize pathogens, thus preventing them from spreading but also facilitating subsequent phagocytosis of trapped microorganisms. They are also thought to directly kill pathogens by means of antimicrobial histones and proteases.

Eosinophils

Summarized from: Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. Nat Rev Immunol. 2013 Jan;13(1):9-22. & From: Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol. 2009; 101:81-121.

Eosinophils were first described in 1879 by Paul Ehrlich, who noted their unusual capacity to be stained by acidophilic dyes. Eosinophils are granulocytes that develop in the bone marrow from pluripotent progenitors of the myeloid lineage. They are released into the peripheral blood in a phenotypically mature state and spend only a brief time in the peripheral blood. Most eosinophils are localized in thymus, GI tract, uterus, respiratory tracts and mammary gland.

The recruitment of eosinophils is regulated by eotaxin-1 and interleukin-5 (IL-5).

Human eosinophil granules contain four major proteins: eosinophil peroxidase, major basic protein (MBP) and the ribonucleases eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). The granules also store numerous cytokines, enzymes and growth factors. Other prominent features of eosinophils include primary granules that contain Charcot–Leyden crystal protein (also known as galectin 10 and eosinophil lysophospholipase) and lipid bodies, which are the sites of synthesis of cysteinyl leukotrienes, thromboxane and prostaglandins.

Eosinophils contribute to immunity to helminths, play a role in fighting viral infections, and in pathology of some diseases including asthma. The pathology arises from dysregulated eosinophilia in the airways that causes collateral damage to the tissues.

Effector mechanism of eosinophils

- Degranulation, the release of granule contents into the extracellular space.
- Extracellular trap formation in which eosinophil granules are attached. These granules release their contents when stimulated, and they have a well-known role in combatting parasitic infection.
- Secretion of an array of proinflammatory cytokines which play a role in the pathogenesis of allergic diseases
- Interaction with other leukocytes
  - Eosinophils can initiate antigen-specific immune responses by acting as antigen-presenting cells (APCs) (Wang, H. B., Ghiran, I., Matthaei, K. & Weller, P. F. Airway eosinophils: allergic inflammation recruited professional antigen-presenting cells. J. Immunol. 179, 7585–7592 (2007))

Basophils

Basophils are the largest type of granulocyte and are stained by basic dyes. They have an indented or segmented nucleus and have granules. They represent about 0.5 to 1% of circulating white blood cells.

Basophils develop from common granulocyte-monocyte precursors in the BM and IL-3 can regulate this process.

Basophils can be activated by various signals including cytokines (IL-3, IL-18 and IL-33), antibodies (IgG, IgE and IgD) and nonspecifically by protease antigens (papain and Der p1).

Function of basophils

- Immunity against multicellular parasites, such as parasitic worms (helminths) or ticks
- Play central role in the development and maintenance of Th2 cytokine-dependent immunity. Basophils enter the lymph nodes and come in contact with naïve CD4^{+} T cells. They produce IL-4 that promotes the differentiation and maintenance of Th2 cells.

Mast cells

Mast cells are resident in tissues throughout the body but are most common at sites that are exposed to the external environment, such as the skin, the airways and the GI tract, including the oral cavity. Mast cells are a type of granulocyte derived from the myeloid stem cell, and have a similar morphology to basophils, but without indented nucleus.

Mast cells originate from hematopoietic cells. The committed mast-cell progenitors circulate in small numbers in the blood and are thought to migrate to tissues before undergoing the final stages of maturation. Interleukin-3 (IL-3) and stem-cell factor (also known as KIT ligand) are two main growth factors for mast cells. Tissue mast cells also have the ability to proliferate locally.

Mast cells are seen in close association with blood vessels and nerves at the sites that are most likely to be exposed to pathogens.

Mast cells produce three main classes of mediators: pre-formed granule-associated mediators like enzymes with tryptase-, chymase- or carboxypeptidase-like activities, vasoactive amines like histamine and proteoglycans like heparin; newly generated lipid mediators; and a wide variety of cytokines and chemokines.

Mast cells contribute to the innate immune response against a variety of bacteria by secreting vasoactive amines and cytokines. Mast cells and basophils also cooperate to protect the host against secondary infestation with ticks and contribute to immune responses against helminths. Resistance to infection can be conferred by FcR-mediated mast cell activation, and expression of FcεRI or of the low-affinity FcR for IgG by basophils. It remains to be established how mast cells and basophils communicate to confer resistance to ticks, however.

Mast cells and basophils are also major players during systemic and local allergic responses

Lymphocytes

Summarized from: Larosa DF, Orange JS. 1. Lymphocytes. J Allergy Clin Immunol. 2008 Feb;121(2 Suppl): S364-9. & From: Andersen MH, Schrama D, Thor Straten P, Becker JC. Cytotoxic T cells. J Invest Dermatol. 2006 Jan;126(1):32-41. & From: Walker JA, McKenzie ANJ. T(H)2 cell development and function. Nat Rev Immunol. 2018 Feb;18(2):121-133. PMID: 29082915 & From: LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008 Sep 1;112(5):1570-80.

Lymphocytes are white blood cells uniform in appearance, but varying in function and include T, B, and innate lymphoid cells including natural killer cells. These cells are responsible for antibody production, direct cell-mediated killing of virus-infected and tumor cells, and regulation of immune responses.

Lymphocytes originate from bone marrow–derived progenitors. Some progenitors develop into mature B-cells in the bone marrow while other progenitors migrate to the thymus and receive signals through the Notch receptor and are committed to the T-cell lineage.

T Lymphocytes

T lymphocyte progenitor cells arise in the bone marrow and migrate to the thymus for maturation. In the fetus and the juvenile, bone marrow (and the liver in early embryonal stages) is the source of large numbers of new lymphocyte progenitor cells, which migrate to populate the peripheral lymphoid tissues. In adult persons, development of new T cells in the thymus slows down and T-cell numbers are maintained through division of mature T cells outside of the central lymphoid organs. During their maturation process, T cells somatically rearrange gene segments, eventually leading to the expression of a unique antigen-binding molecule, the T-cell receptor (TCR). This antigen-binding molecule consists of two transmembrane molecules, either αβ or $\gamma\delta$, that are the result of rearrangement of first the γ, δ, β or α genes.

TCRs recognize a complex ligand comprising an antigenic peptide bound to a major histocompatibility complex (MHC) molecule and that, contrary to BCR, cannot recognize antigen alone. MHC molecules serve not only as ligands for the TCR, but also as non-antigen-specific ligands for the TCR co-receptors.
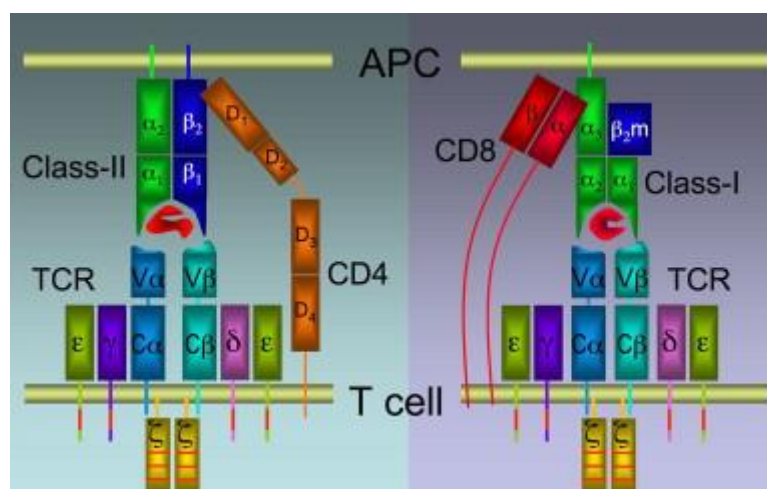
Source: Larosa et al. 2008

Two major forms of these polymorphic membrane-bound glycoproteins exist, MHC class I and class II molecules. Class I and class II molecules interact with different co-receptors on the T cells, that is,

CD8 and CD4, respectively. Whereas class I molecules are expressed by nearly all nucleated cells, class II molecules are constitutively expressed only by antigen-presenting cells (APCs); however, they can be induced in the majority of cells in the body, in particular by IFN-γ.

This initial antigen-specific activation and proliferation of naive T cells is referred to as priming. In this process T cells recognize their specific peptide-MHC ligand on APCs, and together with a second costimulatory signal, activation and proliferation of naive T cells takes place. Once primed, naive T cells differentiate into effector T cells that perform antigen-specific functions without the need for costimulation.

CD8 lymphocytes, cytotoxic lymphocytes, CTL

CD8+ T cells recognize antigen that is presented by MHC class I-derived molecules that sample peptides from protein degradation inside the cell and present these at the cell surface. CD8 T cells have ability to detect antigens both quantitatively and qualitatively.

The role of the CD8+ T cells is to monitor the cells of the body, detect and destroy any infected or tumor cell:

- CTLs kill cells that are infected with virus or intracellular bacteria, preventing them from being the hiding place for bacteria or being the source of more viral pathogen.
- CTLs are also thought to provide some degree of protection against spontaneous malignant tumors.

CTLs may kill target cells by one of at least three distinct pathways:

- Indirect killing of target cells by release of tumor necrosis factor-α and IFN-γ.
- Induction of apoptosis in target cells via death receptor triggering.
- Direct killing by release of granzyme B and perforin into the intercellular space between CTL and target cell.
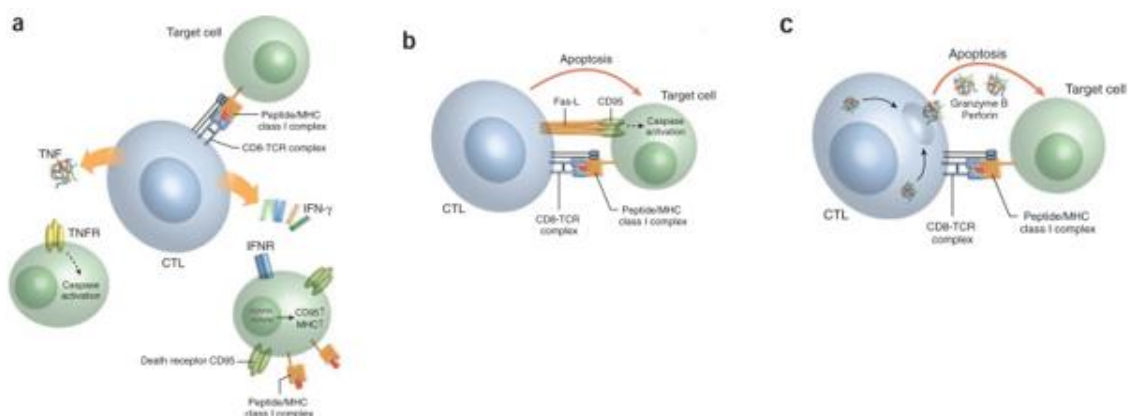
Source: Andersen et al. 2006.

Helper T Cells

Unlike CD8+ T cell priming, CD4+ T-cell priming results in the differentiation of various TH subsets distinguished by the production of particular cytokines and effector functions.

TH1 cells specialize in macrophage activation by IFN-γ production and contact-dependent stimulation by using a variety of cell surface costimulatory ligands, thus playing a major role in intracellular pathogen clearance and delayed-type hypersensitivity.

TH2 cells produce IL-4, IL-5, and IL-13. T helper 2 (TH2) cells orchestrate protective type 2 immune responses, such as those that target helminths and facilitate tissue repair, but also contribute to chronic inflammatory diseases, such as asthma and allergy.

TH17 cells are a subset of pro-inflammatory T helper cells defined by their production of interleukin 17 (IL-17). IL-17 is a potent inflammatory cytokine involved in the recruitment and proliferation of neutrophils. Th17 cells play an important role in maintaining mucosal barriers and contributing to pathogen clearance at mucosal surfaces, but they have also been implicated in autoimmune and inflammatory disorders.

Regulatory T cells (Treg, suppressor T cells) are a subpopulation of T cells that modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disease. Tregs are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells. Subclasses of Treg include:
- **Natural Tregs (nTregs)** develop in the thymus and constitutively express the high-affinity IL-2 receptor (CD25) in accordance with their high dependency on IL-2 for survival.
- **Induced Treg (iTreg)** are another population of Treg that may develop extrathymically from naive CD4+ T cells under the influence of TGF-β. These cells are functionally indistinguishable from nTregs.

Other T cells

Some T cells recognize antigen in the context of atypical MHC molecules. An atypical MHC is essentially any molecule other than MHC class I or II that can present antigen to a TCR. The best known T-cell subsets that use atypical MHC are NKT cells and γδ T cells.

Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and natural killer cells. Many of these cells recognize the non-polymorphic CD1d molecule, an antigen-presenting molecule that binds self and foreign lipids and glycolipids. They constitute only approximately 0.1% of all peripheral blood T cells.

γδ T cells use γ and δ TCR chains instead of α and β. These cells are present among peripheral blood lymphocytes but are found preferentially in epidermal and epithelial tissues. γδ T cells do not express CD4 or CD8 but recognize a variety of antigens, such as microbial and epithelial phosphoantigens, in a number of different non-classic MHC molecules.

Memory T cells

Memory T cells can be from the CD8+ or the CD4+ T cell lineage and are categorized into 2 subsets termed central memory (T_{CM}) and effector memory (T_{EM} T cells

- **T$_{CM}$** cells are sequestered in lymphoid tissue. They respond to antigen by dividing rapidly and can differentiate into effector cells, but have little or no effector function.
- **T$_{EM}$** cells circulate to the peripheral tissues and have limited proliferative capacity but more pronounced effector function.

Thus, T$_{EM}$ cells provide peripheral surveillance and rapid effector responses, whereas T$_{CM}$ cells quickly generate back-up effector cells when the immune response becomes more regional.

The features that typify memory T cells are the following:

- Persistence of an increased frequency of antigen-specific precursors (100-fold to 1000-fold more than naive host)
- Accelerated responsiveness and rapid effector molecule acquisition on encounter with specific antigen
- Antigen-independent steady-state maintenance which maintain a constant level of T cells (homeostatic proliferation)

B lymphocytes and plasma cells

B lymphocytes are named after bursal or bone marrow–derived lymphocytes and are a population of cells that express clonally diverse cell surface immunoglobulin (Ig) receptors recognizing specific antigenic epitopes.

B-cell development encompasses a continuum of stages that begin in primary lymphoid tissue (e.g. human fetal liver and fetal/adult marrow), with subsequent functional maturation in secondary lymphoid tissue, here bone marrow. The functional and protective end point is antibody production by terminally differentiated plasma cells. New B cells are continually produced from the bone marrow, even in adults.

Under development of B lymphocytes in primary lymphoid tissues, a combinatorial rearrangement of the V, D, and J gene segments occurs in the H chain locus and the V and J gene segments of the L chain loci. This results in a diverse repertoire of functional VDJ$_H$ and VJ$_L$ rearrangements encoding the pre-B-cell receptor (BCR).

Antigen-induced B-cell activation and differentiation in secondary lymphoid tissues are mediated by dynamic changes in gene expression that give rise to the germinal center (GC) reaction. The GC reaction is characterized by clonal expansion, class switch recombination (CSR) at the IgH locus, somatic hypermutation (SHM) of VH genes, and selection for increased affinity of a BCR for its unique antigenic epitope through affinity maturation.

B cells respond to T cell–independent type antigens such as lipopolysaccharides, and T cell–dependent foreign antigens. The maturation process results in production of high-affinity antibody-secreting plasma cell and memory B-cell generation.

Antibody class-switching is mostly determined by interaction with helper T cells for T cell-dependent antigens, but non-antigen dependent also occurs, e.g. in the intestinal milieu.

Innate lymphoid cells

Innate lymphoid cells (ILCs) are lymphocytes that do not express diversified antigen receptors as on T cells and B cells. ILCs are largely tissue-resident cells and are deeply integrated into the fabric of the tissues. ILCs are the innate counterparts of T lymphocytes, and each ILCs and its Th counterpart, react by providing positive and negative feedbacks and through immune regulatory and effector functions

ILCs develop from common lymphoid progenitor (CLP) that give rise to B cell, T cell precursors, and NK cell precursors (NKPs).

ILCs begin functioning during fetal development, as lymphoid tissue-inducer cells (LTi cells). These cells induce the development of most of the secondary lymphoid organs. They instruct mesenchymal stromal cells to produce the factors required to attract hematopoietic cells to the developing lymphoid structure and retain them there.

ILCs are classified into three groups:

Group 1 ILCs comprise NK cells and ILC1s. These cells produce interferon-gamma (IFN-γ). ILC1s are regarded as counterparts of Th1 cells, and react to intracellular pathogens, such as viruses, and to tumors, through the secretion of TGFb, IL-12 and IL-18 from activated monocytes.

Natural killer cells (NK) are large granular lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation

NK comprise a number of distinct populations with diverse characteristics. This diversity is generated by developmentally distinct NK cell subsets, killer cell immunoglobulin-like receptor (KIR) expression, NK cell differentiation, clonal-like expansion in response to pathogens and tissue distribution.

NK cells are present in most human tissues. A high frequency of NK cells can be found in peripheral blood, the lungs, the uterus and the liver, whereas NK cells are scarce in lymph nodes and tonsils, as well as some other peripheral organs.

Functions:

- NK cells are cytotoxic for virally infected cells and malignant cells; granules in their cytoplasm contain proteins such as perforin and proteases known as granzymes. Upon release in close proximity to a cell selected for killing, perforin forms pores in the cell membrane of the target cell, creating an aqueous channel through which the granzymes and associated molecules can enter, inducing either apoptosis or osmotic cell lysis. The mechanism of recognizing cells is:
  - Missing "self" (altered cell) hypothesis
    - NK cells possess two types of surface receptors: activating receptors and inhibitory receptors. These inhibitory receptors recognize MHC class I alleles, which could explain why NK cells preferentially kill cells that possess low

levels of MHC class I molecules. A common evolutionary adaptation to this is seen in both intracellular microbes and tumors: the chronic down-regulation of MHC I molecules, which makes affected cells invisible to T cells, allowing them to evade T cell-mediated immunity. NK cells apparently evolved as an evolutionary response to this adaptation

- o Antibody-dependent cell-mediated cytotoxicity
- NK cells work to control viral infections by secreting IFNγ and TNFα.

Group 2 ILCs contains a single subset, ILC2s, which produce type 2 cytokines, predominantly IL-5 and IL-13. They respond to the cytokines IL-25, TSLP, and IL-33. ILC2s act as counterpart of Th2 cells, respond to large extracellular parasites and allergens.

Group 3 ILCs include natural cytotoxicity receptor (NCR)– ILC3s, NCR+ ILC3s, and LTi cells, and can produce IL-17 and/or IL-22. ILC3s act as counterpart of Th17 cells, and combat extracellular microbes, such as bacteria and fungi. They respond to CCL20 and IL-23 from epithelial cells and DCs and IL-1b from macrophages.

| Stimuli		Mediators	Immune function
Tumors, intracellular microbes (Virus, bacteria, parasites)	NK ILC1	IFN-γ Granzymes Perforin	Type 1 immunity (Macrophage activation, cytotoxicity)
Large extracellular parasites and allergens	ILC2	IL-4 IL-5 IL-13 IL-9 AREG	Type 2 immunity (Alternative macrophage activation)
Mesenchymal organizer cells (Retinoic acid, CXCL13, RANK-L)	LTi	RANK Lymphotoxin TNF IL-17 IL-22	Formation of secondary lymphoid structures
Extracellular microbes (Bacteria, fungi)	ILC3	IL-22 IL-17 GM-CSF Lymphotoxin	Type 3 immunity (Phagocytosis, antimicrobial peptides)

Source: Vivier et al. 2018

Monocytes and macrophages

Summarized from: Rees AJ. Monocyte and macrophage biology: an overview. Semin Nephrol. 2010 May;30(3):216-33. PMID: 20620668. & From: Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008 Dec;8(12): PMID: 19029990;

Monocytes develop from long-term hematopoietic stem cells (LT-HSCs) in the bone marrow under the influence of CSF-1 and the transcription factor PU.1. Monocytes are released into the circulation, and after localization to the tissues develop into macrophages or monocyte-derived DCs (MoDCs), especially in the context of inflammation. Monocytes circulate in the bloodstream for about one to three days. The immediate demand in acute injury requires reservoirs buffered by mobilizing monocytes for infiltration of damaged organs from reservoirs in the bone marrow and the spleen.

Macrophages are large white blood cells. The majority of macrophages are stationed in tissues and form the mononuclear phagocytosis system (aka reticuloendothelial system). Tissue macrophages

have the ability of self-renewal and take various forms with various names, throughout the body (e.g., histiocytes, Kupffer cells, alveolar macrophages, microglia, and others).

Macrophages can undergo polarization (activation), a process by which a macrophage expresses different functional programs in response to microenvironmental signals. As the result of macrophage polarization, two different type of macrophages can develop *in vitro*:
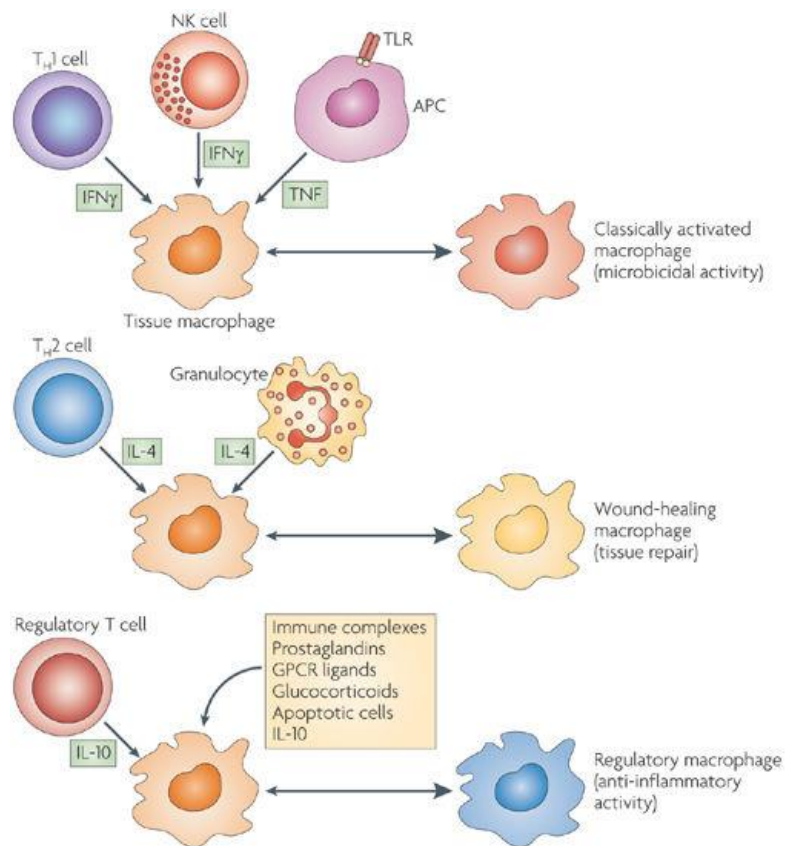
Nature Reviews | Immunology

Source: Mosser et al. 2008

- **M1 macrophages** are *classically activated macrophages*. Classically activated macrophages refer to effector macrophages that are produced during cell-mediated immune responses by IFN-γ and tumour-necrosis factor (TNF). IFN-γ is secreted by TH1, NK and the M1 itself. Under classical activation, macrophages are primed to secrete pro-inflammatory cytokines, produce increased amounts of superoxide anions and oxygen and nitrogen radicals to increase their killing ability. The role of classically activated macrophages are host defense and most importantly to intracellular pathogens. Classically activated macrophages must be tightly controlled because they can be key mediators of the immunopathology that occurs during several autoimmune diseases, including rheumatoid arthritis and inflammatory bowel diseases.
- **M2 macrophages** *(wound healing macrophages)* are alternatively activated macrophages and can develop in response to innate or adaptive signals. One of the first innate signals to be released during tissue injury is thought to be IL-4. Basophils and mast cells are important

early sources of innate IL-4 production, although other granulocytes also might contribute. IL-4 production rapidly converts resident macrophages into a population of cells that are programmed to promote wound healing; IL-4 stimulates arginase activity in macrophages, allowing them to convert arginine to ornithine, a precursor of polyamines and collagen, thereby contributing to the production of extracellular matrix. Wound-healing macrophages can also be detrimental to the host when their matrix-enhancing activity is dysregulated, similarly to the dysregulated activity of classically activated macrophages in autoimmunity. The tissue fibrosis that occurs during chronic schistosomiasis has been attributed to the uncontrolled activation of wound-healing macrophages.

- **Regulatory macrophages** can arise following innate or adaptive immune responses. Although stress responses are not typically considered part of innate immunity, glucocorticoids can inhibit macrophage-mediated host defense and inflammatory functions by inhibiting the transcription of pro-inflammatory cytokine genes and decreasing mRNA stability, giving rise to a population of regulatory macrophages. Production of IL-10 is the most important characteristic of regulatory macrophages. In addition, these regulatory macrophages also downregulate IL-12 production.
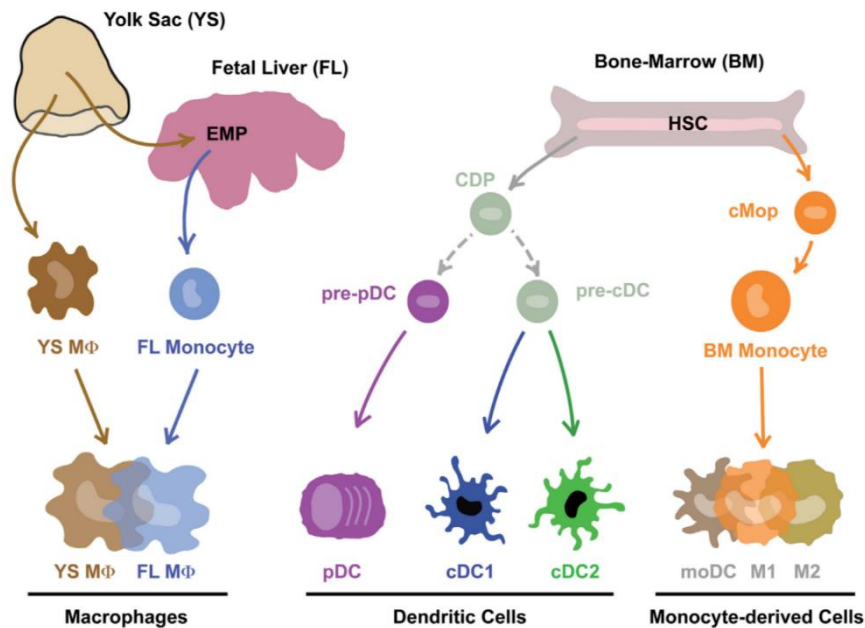
Dendritic cells (DCs)

Dendritic cells (DC) are a class of bone marrow derived cells arising from myeloid hematopoiesis and form an essential interface between the innate sensing of pathogens and the activation of adaptive immunity. The initiation and control of immune responses depends upon dendritic cells (DC), a class of bone marrow-derived cells found in blood, tissues and lymphoid organs. The function of DC is to present antigens to T cells.

Dendritic cells are present in tissues under epithelia that are in contact with the external environment.

Types of dendritic cells

- **Conventional dendritic cell (myeloid dendritic cell, cDC)** originate from myeloid line in hematopoiesis and mature from common DC progenitors (CDPs). They migrate to periphery and capture antigens and then migrate to the lymphoid organs to initiate immunity. They are further divided into cDC1 and cDC2 subclasses.
- **Plasmacytoid dendritic cell (pDC)** originate from myeloid line in hematopoiesis. They are found in the thymic medulla and lymph node T cell areas and can induce tolerance (pDC-mediated induction of T-cell tolerance). pDC are specialized to sense and respond to viral infection through several mechanisms by the rapid production of high quantities of type I and type III interferons and secretion of cytokines. Ligation of surface receptors modulates activation or tolerance through the regulation of the IRF7 and nuclear factor-κB pathways.
- **Monocyte-derived dendritic cells (moDC)** arise from monocytes under stimulation by interleukin 4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF).

Source: Guilliams et al. 2015

Follicular dendritic cells (FDCs) are non-migratory cells of the immune system found in primary and secondary lymph follicles of the B cell areas of the lymphoid tissue. They are derived from mesenchymal cells. Their functions are organizing lymphoid microarchitecture, antigen capturing for memory B-cell support, debridal removal and regulating immune tolerance. These cells must not be confused as part of hematopoietic derived DCs.

Langerhans cells

Summarized from: Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol. 2008 Dec;8(12):935-47.

Langerhans cells (LCs) are DCs in the epidermis/epithelium, whereas dermal/lamina propria DCs belong to a broader subset of interstitial DCs. Through their extended dendrites, LCs form a continuous cellular network that surveys the epidermis and the epithelium for foreign antigens, providing the first immunological barrier to the external environment.

LCs are named after Paul Langerhans, who was the first to report the presence of dendritic, non-pigmented cells in the epidermis. These cells are now recognized as a type of leukocytes that are derived from the bone marrow and are a dendritic cell population found in the epidermis and epithelium. Epidermal and epithelial LCs account for 3–5% of all nucleated cells in the epidermis of mice and humans and are arranged in a network that occupies the interstices between neighboring keratinocytes.

Researchers have speculated that LCs are part of the peripheral nervous system, and that they are related to melanocytes or that they were of ectodermal origin. Recent findings show, however, that epidermal LCs have unique origins in the steady state and are maintained either through self-renewal or by a local pool of proliferating host hematopoietic precursor cells that reside in the skin. The exact nature of the circulating precursor cells that give rise to dermal langerin+ DCs has not yet been identified. It will be interesting to test the contribution of several potential DC precursor cells,

including circulating monocytes and the recently described common DC precursor (CDP) and macrophage DC precursor (MDP) cell. However, during inflammation, circulating monocytes have been shown to replenish epidermal LC populations.

Immunological Methods

Immunohistology (immunohistochemistry and immuno-fluorescence)

Immunohistology involves the process of selectively imaging antigens in cells of a tissue section by exploiting the principle of antibodies that bind specifically to antigens in biological tissues.

Visualizing an antibody-antigen interaction can be accomplished by:
- Enzyme reaction staining: an antibody is conjugated to an enzyme, such as peroxidase, that can catalyze a color-producing reaction like immunoperoxidase staining
- Immunofluorescence: the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine

Antibodies used for specific detection can be polyclonal or monoclonal.
- **Polyclonal antibodies** are made by injecting animals with the protein of interest, or a peptide fragment and, after a secondary immune response is stimulated, isolating antibodies from whole serum. Polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes.
- **Monoclonal antibodies** are made by immunizing an animal, collect B cells from the spleen and testing them for antigen-specificity. Specific B cells are then fused with cancer B cells (plasmacytoma) to create hybridomas that are immortalized to create antibodies. The antibodies show specificity for a single epitope only.

The staining procedure can be
- **Direct method** is a one-step staining method and involves a labeled antibody reacting directly with the antigen in tissue sections.
- **Indirect method** involves an unlabeled primary antibody that binds to the target antigen in the tissue and a labeled secondary antibody. This method is more sensitive than direct detection strategies because of signal amplification due to the binding of several secondary antibodies to each primary antibody
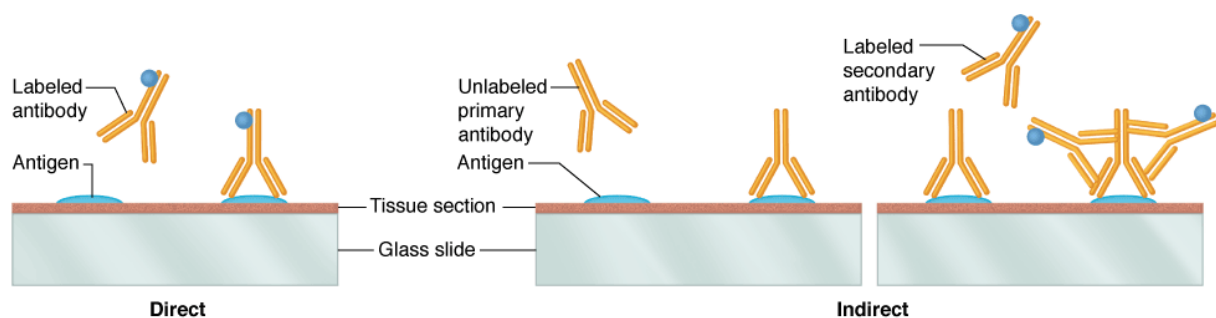

Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas,*
12th Edition: http://www.accessmedicine.com
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In situ hybridization (ISH)

In situ hybridization is a technique that uses a labeled complementary DNA, RNA or modified nucleic acid strand (i.e. probe) to localize a specific DNA or RNA sequence in a portion or section of tissue. This is distinct from immunohistochemistry, which usually localizes proteins in tissue sections.

DNA ISH (DNA in situ hybridization) can be used to determine the structure of chromosomes. RNA ISH (RNA in situ hybridization) is used to measure and localize RNAs (mRNAs, lncRNAs, and miRNAs) within tissue sections.

Labeling methods can be radioactive labeling, fluorescent-labeling or antigen-labeling, which can be visualized either autoradiography, fluorescence microscopy, or immunohistochemistry, respectively. Using radioactive or fluorescent labeling is called for direct labeling, and using antigen labeling called indirect labeling.
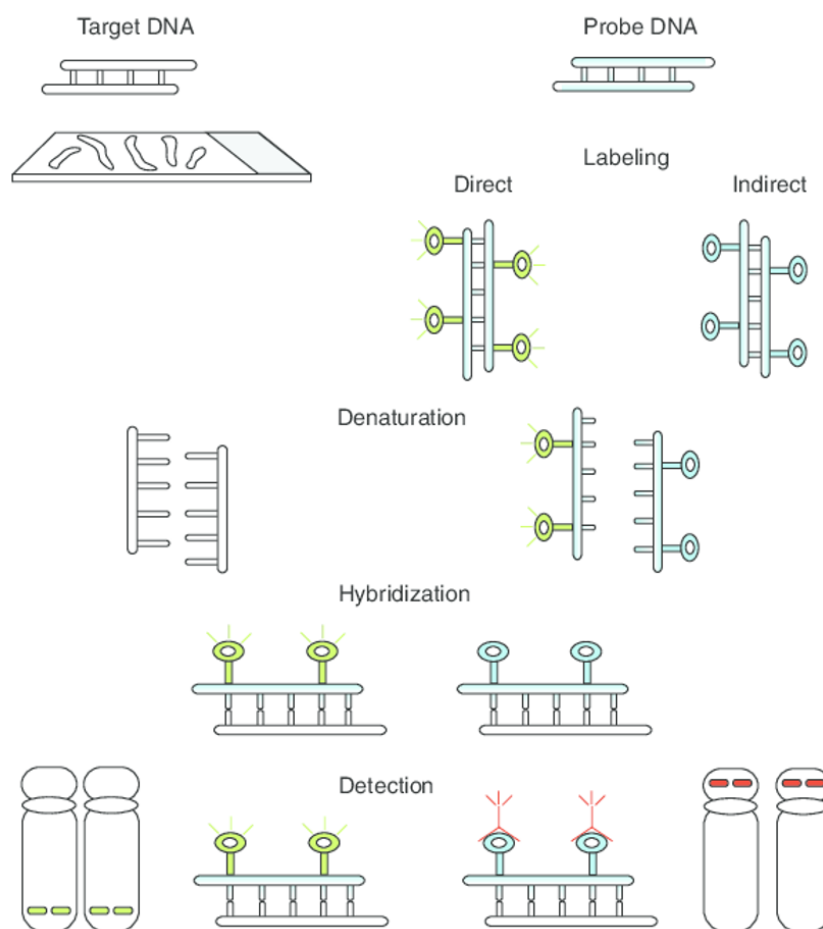
Source: Dorritie K, Montagna C, Difilippantonio MJ, Ried T. Advanced molecular cytogenetics in human and mouse. Expert Rev Mol Diagn. 2004 Sep;4(5):663-76. Review. PMID: 15347260;

Flow cytometry

Flow cytometry is a laser- or impedance-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering. Cells are suspended in a stream of fluid and pass through an electronic detection apparatus. A flow cytometer allows simultaneous

multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

A flow cytometer has five main components: a flow cell, a measuring system, a detector, an amplification system, and a computer for analysis of the signals.

The measuring system commonly use measurement of impedance (or conductivity) and optical systems lamps and laser beams.

Labeling of cells can be obtained by different methods like fluorescent-antibody, isotope labeling.

Source: https://benchling.com/pub/tabor-flowcal
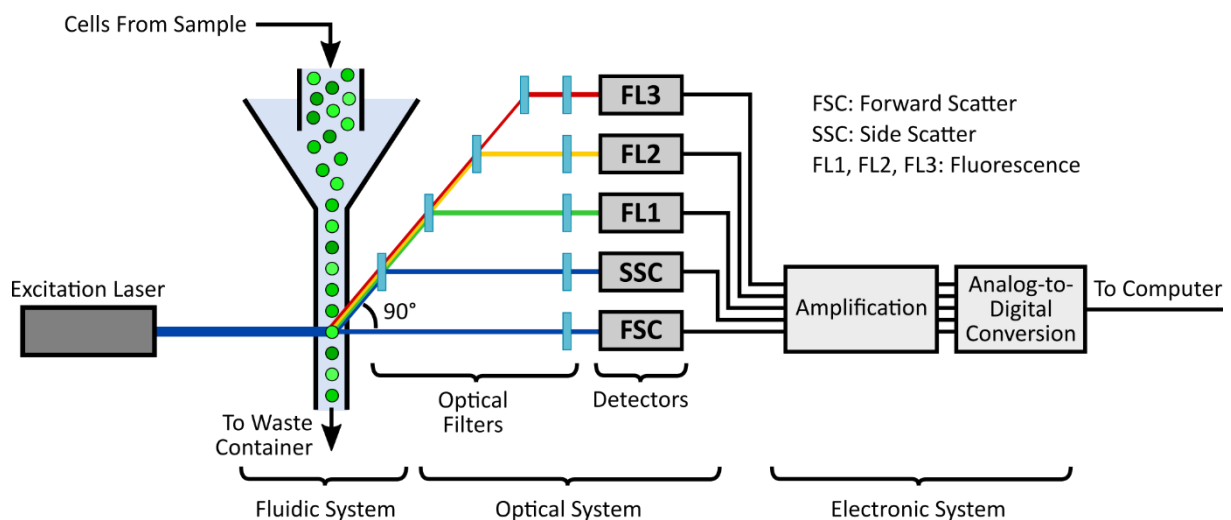
Real time reverse transcription polymerase chain reaction

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR), commonly used in molecular biology to detect RNA expression.

RT-PCR is used to qualitatively detect gene expression through the creation of complementary DNA (cDNA) transcripts from RNA. In RT-PCR, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR. RT-PCR can be achieved as either a one-step or a two-step reaction. The difference between the two approaches lies in the number of tubes used when performing the procedure. In the one-step approach, the entire reaction from cDNA synthesis to PCR amplification occurs in a single tube. On the other hand, the two-step reaction requires that the reverse transcriptase reaction and PCR amplification be performed in separate tubes.

The quantitative real-time RT polymerase chain reaction (qRT-PCR), is another laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR, and not at its end as in conventional PCR. Real-time PCR relies on the detection and quantification of a fluorescent reporter that accumulates during the course of the PCR reaction in a directly proportional manner to amplicon generation.

Two common methods for the detection of PCR products in real-time PCR are

- non-specific qPCR: A DNA-binding dye binds to all double-stranded (ds) DNA in PCR, causing fluorescence of the dye. An increase in DNA product during PCR therefore leads to an increase in fluorescence intensity measured at each cycle.
- sequence-specific qPCR: DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter permits detection only after hybridization of the probe with its complementary sequence.

A combined technique described as quantitative reverse transcription polymerase chain reaction or real-time RT-PCR (RT-qPCR) can be utilized for quantification of RNA, by combining real time PCR techniques in RT-PCR. Compared to other RNA quantification methods, such as northern blot, qRT-PCR is considered to be the most powerful, sensitive, and quantitative assay for the detection of RNA levels. It is frequently used in the expression analysis of single or multiple genes, and expression patterns for identifying infections and diseases.
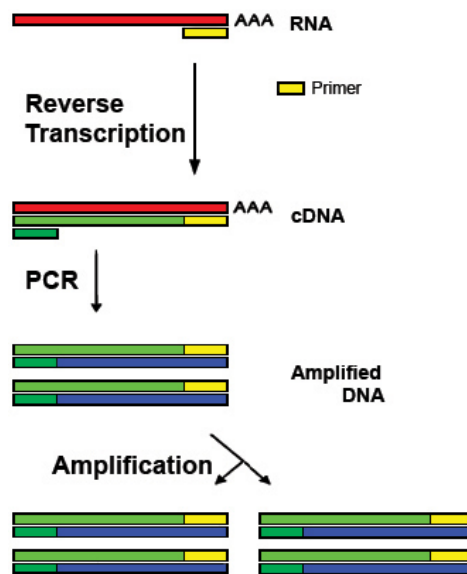
Source: http://www.differencebetween.net/science/difference-between-rt-pcr-and-qpcr/

Oral Lichen Planus

Lichen Planus (LP) is a chronic inflammatory disorder of the epidermis and the epithelia. Lichen planus commonly involves the mucosa of the oral cavity (oral lichen planus, OLP), but can involve other sites, namely the skin, the vulvar and vaginal mucosa, the glans penis, the scalp (resulting in alopecia), and the nails. Oral LP may occur alone or in conjunction with other forms of LP.

Epidemiology

The disease prevalence rates between less than 1 percent and 3 percent. OLP most commonly occurs in middle-aged adults with an average age between 50 to 60 years. Women may be more likely to develop oral LP than men.

Pathogenesis

LP is considered to be a T-cell mediated chronic inflammatory reaction that results in a cytotoxic reaction against epithelial basal cells. A potential pathway for CD8+ T cell-mediated cytotoxicity in oral LP is

- CD8+ T cells are activated by antigens presented on MHC I molecules on keratinocytes or by encounters with activated CD4+ helper T cells or cytokines produced by activated CD4+ helper T cells
- Activated CD8+ T cells induce keratinocyte apoptosis through mechanisms such as secretion of tumor necrosis factor (TNF)-alpha, secretion of granzyme B, or Fas-Fas ligand interactions
- Activated CD8+ T cells produce chemokines that attract additional inflammatory cells, thereby promoting continued inflammation

Upregulation of matrix metalloproteinases is considered as another important mechanism in OLP pathogenesis. MMPs disrupt the epithelial basement membrane zone and allow entry of immune cells into the epidermis.

Clinical manifestation in oral cavity

Mucosal lesions are usually multiple and almost always have a bilateral, symmetrical distribution. OLP lesions have a variety of manifestations and different forms may merge or coexist in the same patient.

OLP lesions has the following subtypes based on their manifestation:

- **Plaque-like OLP** may be difficult to distinguish from leukoplakia.
- **Reticular OLP** characterized by the presence of white lines called as "Wickham's striae", in a reticulated or lacy pattern on the oral mucosa and interspersed with papules or rings. Large, painful, hyperkeratotic plaques on the tongue are a less common manifestation. This form is considered as the classic manifestation of OLP.
- **Erythematous or atrophic OLP** typically occurs in conjunction with reticular lesions. Areas of mucosal atrophy, appearing as red patches among the white papules, patches, or plaques of reticular LP are usually present

- **Erosive or ulcerative OLP** is characterized by erosions or frank ulcers. Rarely, bullae that easily rupture occur. Lesions consistent with erosive OLP are usually accompanied by both reticular and erythematous lesions

Sign and symptoms

- The manifestations of oral LP are usually seen bilaterally and in a symmetric distribution.
- Unlike reticular LP, which is often asymptomatic, the erythematous and erosive forms of oral LP are typically accompanied by symptoms. Patients may note pain, a burning sensation, swelling, or irritation, and may experience mucosal bleeding in response to mild trauma, such as toothbrushing.
- Oral LP may occur in isolation or in conjunction with other clinical manifestations of LP, including cutaneous LP, genital LP, nail LP, and lichen planopilaris (scalp LP).
    - **Cutaneous LP** is typically characterized by the appearance of polygonal, violaceous, pruritic papules on the trunk or extremities. Oral LP is common among individuals with cutaneous LP. It is estimated that more than 50 percent of individuals with cutaneous LP also have oral lesions.
    - **Lichen planopilaris** is a form of scarring alopecia characterized by atrophic plaques and subtle follicular hyperkeratosis.
    - **Nail LP**, Nail involvement in LP may manifest as grooves or ridges in the nail plate
    - **Genital (vulvar, vaginal, or penile) LP** with clinical findings like erythema, erosions, white reticulated plaques, and scarring. Lesions may be asymptomatic, pruritic, or painful, and may be accompanied by dysuria or dyspareunia. Annular lesions are a common manifestation of penile LP
    - Rarely, LP involves the ocular, nasal, laryngeal, esophageal, anal, or ear mucosa.

Diagnosis

A diagnosis of oral lichen planus (LP) is confirmed through review of the patient history, physical examination, and histologic findings.

The clinical evaluation should include a patient history that assesses the following:

- History of LP involving other body sites or other skin disorders that may present with similar findings
- Presence of associated symptoms (e.g. pain, burning)
- Presence of symptoms suggestive of other sites of mucosal involvement (e.g. dysphagia, hoarseness, stridor, ocular irritation, dysuria, dyspareunia, hematuria)
- Medication list to evaluate for the possibility of an oral lichenoid drug eruption (may occur weeks to months after drug initiation)
- History of dental restorations, use of dental appliances, or oral exposure to substances that may cause oral lichenoid contact eruptions

Tissue biopsies of oral LP help to confirm the diagnosis and are particularly of value for erythematous and erosive LP, which share features with multiple other mucosal disorders, including oral malignancy. Thus, biopsies are taken from all patients who exhibit features of these subtypes.

Biopsies to confirm oral LP are less essential in patients who present with classic reticular LP, particularly in patients in whom a diagnosis of LP has already been confirmed through biopsy of an extraoral manifestation of this disorder.

Differential diagnosis

- **Leukoedema**, a common, benign finding in the oral cavity that presents as white-gray, somewhat translucent plaques on the mucosa
- **Oropharyngeal candidiasis**
- **Leukoplakia**, a manifestation of squamous epithelial hyperplasia that may be a precursor to oral squamous cell carcinoma
- **Oral squamous cell carcinoma** can present as erythematous or white patches, ulcers, or exophytic
- **Oral lichenoid drug reaction** may be caused by a variety of systemic medications and share clinical features with oral LP. Histologic findings of a deep mixed infiltrate with lymphocytes, plasma cells, and neutrophils (with or without eosinophils) and perivascular inflammation favor this diagnosis
- **Oral lichenoid contact reaction (allergic contact mucositis)** may be caused by a variety of substances. Mercury-containing amalgam used for dental restoration is the most common cause. The clinical and histologic features of oral lichenoid contact reactions are similar to oral LP but are unilateral and nearby the dental material. Patch testing and recognition of the proximity of an offending substance to the eruption can aid with diagnosis.
- **Autoimmune blistering diseases** like Mucous membrane pemphigoid and other autoimmune blistering diseases may present with oral erosions and desquamative gingivitis similar to that seen in erosive LP. Biopsies for routine histologic examination and direct immunofluorescence are useful for distinguishing these disorders from oral LP.
- **Graft-versus-host disease** lacy, reticulated plaques or erosions that resemble oral LP may occur in chronic graft-versus-host disease (GVHD). The histologic findings of these disorders are also similar. The patient history is useful for differentiating chronic GVHD from oral LP. Oral involvement in acute GVHD is less well characterized than chronic GVHD, but has been associated with erythematous, erosive, ulcerative, or lichenoid oral lesions.

Histopathological features of OLP

Summarized from: Cheng YS, Gould A, Kurago Z, Fantasia J, Muller S. Diagnosis of oral lichen planus: a position paper of the American Academy of Oral and Maxillofacial Pathology. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Sep;122:332-54.

Microscopic features of OLP include hyperparakeratosis, hyperorthokeratosis, and combinations of the two; cytoid (Civatte) bodies; basal cell hydropic change in epidermal and epithelial tissue; and a band-like, chiefly lymphocytic, infiltrate in the lamina propria. Additional findings include saw-tooth rete ridges, atrophy, acanthosis, a homogeneous eosinophilic deposit at the epithelium-connective tissue junction, and ulceration. Compared to cutaneous disease, oral lesions less often exhibit saw-tooth rete ridges and more frequently exhibit atrophy.
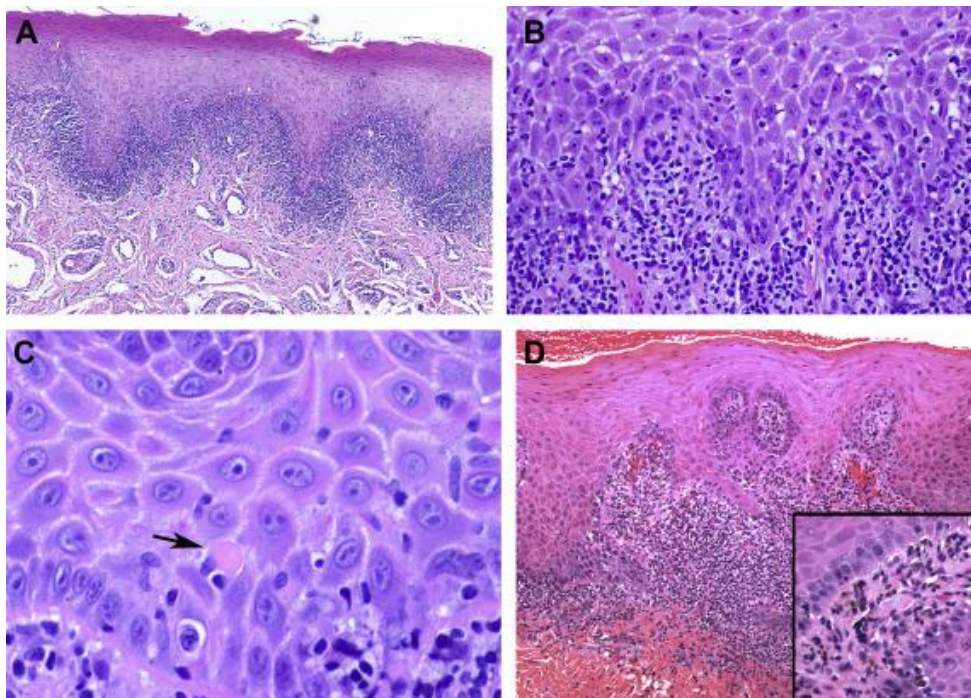
Source: Cheng et al. 2016

A) Oral mucosal stratified squamous epithelium exhibits a thickened surface layer of parakeratin, saw-tooth rete ridge morphology
B) A dense predominantly lymphocytic infiltrate is situated in the lamina propria
C) Lymphocyte-mediated injury of oral mucosal stratified squamous epithelium, with keratinocyte apoptosis represented as a colloid (Civatte) body (arrow)
D) Melanosis and melanin incontinence with associated melanophages can sometimes be found, especially in biopsies from individuals with dark complexions

T cells

In OLP lesions, the cell infiltrate is dominated by T cells. A dense T cell (CD4+ and CD8+) infiltrate is always seen at the epithelial-connective tissue interface, scattered within the epithelium, lamina

propria and in the deeper connective tissue (Javvadi et al. 2016). There are significantly more CD4+ and CD8+ cells in the epithelial-connective tissue interface region than in deeper part of lamina propria (Javvadi er al. 2016). Most intraepithelial mononuclear cells are CD8+ and in some cases intraepithelial CD8+ cells are adjacent to degenerating keratinocytes (Khan et al. 2003). CD4+ cells are observed mainly in the deep lamina propria and few exist near the epithelium (Khan et al 2003). Whether the T cells have receptors that react with specific antigens or whether they constitute a pool of random specificities that are attracted by T cell chemokines released from the lesion remains unknown. As epithelial cells show damage and degradation, epithelial components can be candidate antigens. As basal epithelial cells in OLP lesions lose normal keratin expression (Schreurs et al. unpublished), keratins might constitute such antigens. Recently, Murota et al. (2001) found increased levels of circulating anti-keratin antibodies (K8, K18 and K19) and peptide-antibody complexes (K8 and K18) in patients with autoimmune hepatitis, together with partial loss of K8 and K18 and aberrant expression of K19 in the liver, as detected by immunohistochemistry. Furthermore, soluble complexes of HLA-I and K8 peptide were also found in plasma of patients with coronary artery disease (Mihailovic et al. 2019). Stimulation of patients' PBMCs with a peptide corresponding to aa248-279 of K8 resulted in an increase of both CD8^{+} T effector and CD4^{+} T effector memory responses compared to controls, while mRNA for PD-1, which promotes self-tolerance, was upregulated in controls only. It is therefore also possible that K8 and K18 play a role as autoantigens in OLP.

There is no significant difference between the % mean (±SD) of CD4+ and CD8+ cells either at the epithelial-connective tissue interface or in the deeper part of lamina propria (Javvadi er al. 2016). Other studies have, however, reported that the numbers of CD4+ T cells are significantly higher in cutaneous LP as compared with OLP, while the fraction of CD8+ cells was significantly higher in OLP (Weber et al. 2017, Villarroel Dorrego et al. 2002). These differences in CD4/CD8 ratios might be due to different degrees of disease activity. Early lesions may show higher proportions of CD4+ cells, which promote the influx of CD8+ cells seen in older lesions (Villarroel Dorrego et al. 2002).

CD4+ T cell subsets

CD4+ T lymphocytes can differentiate into distinct Th cell types such as Th1, Th2, Treg, Th17, Th22, Th9, and Tfh within the context of certain cytokines environment (Wang et al. 2015). In OLP lesions, CD4+ Th subsets are the major lymphocyte subset in the subepithelium and the lamina propria. Deeper down, the cells cluster and form the typical lymphocyte-rich band that may have a prominent role in the pathogenesis of OLP (Wang H et al. 2015). The Th1 subset, characterized by mainly producing INF-γ, IL-2, and TNF-α, can promote the activation and cytokine production of macrophages and cytotoxic T lymphocytes. The Th2 subset secretes the cytokines IL-4, IL-5, and IL-10. It has been suggested that a Th1/Th2 imbalance in the cytokine network may determine OLP immunopathology (Wang et al. 2015, Roopashree 2010). Kalogerakou F (2008) and Rhodus NL (2006) also indicate that a Th1/Th2 imbalance may determine the character of immune responses in OLP pathogenesis.

Th17 cells play critical roles in the development of autoimmunity, allergic reactions and host defense by producing the powerful pro-inflammatory cytokine IL-17 and, to a lesser extent, tumor necrosis factor-α and IL6 (Javvadi et al. 2016, Bettelli et al. 2006). In OLP, the majority of Th17 cells are located within and deep into the main band of inflammatory cells and very few are observed at the epithelial-connective tissue interface in OLP (Javvadi LR et al. 2016). Levels of Th17 mRNA are

increased in the lesions of erosive OLP patients (Wang et al. 2017, Javvadi et al. 2016). Another study showed that the percentage of Th17 cells is significantly higher in erosive lesions (35%) compared with tissue controls (4%) (P = 0.001). By contrast, the percentage of Th2 T-cell clones (TCCs) is significantly higher in reticular OLP (35%) compared with tissue controls (20%) (P = 0.02), whereas the percentage of Th2 TCCs is significantly decreased in erosive OLP (24%) compared with their tissue controls (70%) (P = 0.001) (Piccinni MP et al. 2014). Th17 cells can have a predominant role in erosive OLP while Th2 cells may predominate in reticular OLP (Wang et al. 2017, Piccinni et al. 2014). In erosive OLP, Th17 cytokines (which have a role in inflammation) may be responsible for the more evident oral mucosal damage, whereas the increased numbers of Th2 cells (which perhaps are not pathogenic) might explain the less evident epithelial damage associated with reticular OLP (Piccinni et al. 2014).

Th9 cells can be seen in reticular OLP, indicating that Th9 cells may play a role in reticular disease (Wang et al. 2017). In the presence of TGF-β and IL-4, naive Th cells can develop into potent Th9 cells, and Th2 and Th9 may be synergistically acting to maintain immune balance in OLP immune microenvironments (Wang et al. 2017).

Tregs express the transcription factor forkhead box P3 (FoxP3) and are believed to have a pivotal role in the regulatory networks that control the immune response and peripheral tolerance to self and non-self. (Nishikawa H et al.2014,). FoxP3+CD4+ T cells can be divided into at least three subpopulations with different properties, including naïve/resting (nTregs; Fraction I), effector/activated (eTregs; Fraction II), and non-suppressive FoxP3+CD4+ T cells (Fraction III) (Miyara M 2009, Schreurs O et al. 2016). In OLP, Most of the Tregs belong to fraction III (Schreurs et al. 2016). Within OLP lesions, FoxP3+ cells are almost evenly distributed in the inflammatory infiltrate at the epithelial-connective tissue interface and in the superficial connective tissue, while there are significantly more CD4+ and CD8+ cells in the epithelial-connective tissue interface region than the superficial connective tissue infiltrate region in OLP and significantly more IL17A+ cells are present in the deeper infiltrate (Javvadi LR et al. 2016). Numbers of subepithelial FoxP3+ T cells in OLP differ, however, according to disease state. The infiltrate in atrophic OLP shows a larger population of FoxP3+ cells throughout the whole infiltrate as compared to reticular and ulcerative OLP (Schreurs O et al. 2016).

The interplay between FoxP3+ cells and IL17A+ cells must be in a proper balance to ensure an efficient immune response rather than pathological damage (Javvadi LR et al. 2016). There are significantly more FoxP3+ cells than IL17A+ cells in OLP lesions, and significantly more IL17A+ cells are present in the deeper infiltrate than at the epithelial-connective tissue interface (Javvadi er al. 2016, Schreurs et al. 2016). This shows that the balance between Tregs and IL-17+ cells is altered in OLP, thus supporting the proposition that disturbance in local immune regulation is important in the pathogenesis of OLP (Firth FA et al. 2015). Alternatively, too few actively suppressing Tregs are present in the OLP lesion (Schreurs et al. 2016) to balance for the effects of cells with pro-inflammatory capacity.

CD8+ T cells

In OLP forms where the basal membrane is continuous, CD8+ cells are concentrated in the lamina propria with only occasional intraepithelial CD8+ cells. In contrast, clusters of CD8+ cells are seen in the basal epithelial layer in regions of BM disruption (Zhou et al. 2002). The number of CD8+ T cells in the epithelium of OLP lesions in regions of BM disruption is significantly greater than in regions of BM

continuity (Zhou et al. 2002). Both the CD8+ and CD4+ lymphocyte infiltrates are observed in the epithelial layer in addition to the subepithelial "band-like" lymphoid cell infiltrated zone (Enomoto et al. 2008). High-grade intraepithelial CD8+ lymphocyte infiltration has been found to be associated with a higher remission rate (Enomoto et al. 2008).

Chemokine signaling via CCR5 and CXCR3, which are known to be selectively expressed by T cells, are predominantly involved in T-cell infiltration of oral lichen planus (Iijima et al. 2003). Furthermore, the presence of CCL5 and CXCL10 in the cytolytic granules of tissue-infiltrating CD8+ T cells expressing CCR5 and CXCR3 reveals a potential self-recruiting mechanism involving activated effector cytotoxic T cells (Iijima et al. 2003).

CD4+ T helper cells can activate the cytotoxic CD8+ T lymphocytes by secreting Th1 type cytokines, such as IFN-γ, IL-2, and TNF-α. Furthermore, activated CD8+ cytotoxic T lymphocytes may then trigger basal keratinocyte apoptosis in OLP lesions (Wang et al. 2015).

B cells and plasma cells

Few studies have examined the prevalence of plasma cells in OLP lesions and none have addressed this specifically. A figure in Raybaud et al. (2018) shows, however, that there are significant numbers of CD138+ plasma cells and CD20+ B cells in OLP lesions. The authors found that many are infected with EBV and speculate that this be a relevant factor in the pathogenesis of OLP. Others have shown the presence og CD19 B cells in both red and white lesions.

In a study focusing on the association between EBV infection and OLP, EBV was commonly found in OLP (74%), with significantly higher frequency (83%) in the erosive form (RL) than in the reticular/keratinized type mild form (58%) (Raybaud et al. 2018). Nearly all EBV+ cells detected in OLP lesions were CD138+ plasma cells (PCs). These findings add a further puzzling element to OLP pathogenesis, given that PCs are now considered to be major regulatory immune cells involved in several autoimmune diseases including OLP (Raybaud et al. 2018).

Mast cells

The numbers of mast cells were significantly higher in oral lichen planus as compared with oral lichenoid reactions and normal oral mucosal tissues (Ramalingam et al. 2018, Reddy et al. 2012). Others maintain that the difference between numbers of mast cells in OLP and in OLR is questionable (Sharma R et al. 2011). Mast cells are found in the superficial, middle and deeper layers of the lamina propria in OLP, but the greatest degranulated mast cell density is located in the most superficial layers of the connective tissue (Zhao ZZ et al. 1997, Jose et al. 2001). This can be explained by the fact that mast cells that migrate from blood vessels in the deeper connective tissue to the extravascular compartment subsequently move toward the subepithelial zone, where they exert their biologic effect on blood vessels and help in recruitment of inflammatory cells to the lesional area (Sharma et al. 2011).

Spatial associations of mast cells with nerves are found in OLP, especially in the superficial layers of the connective tissue, and there is a moderate correlation between the overall mast cell density and the frequency of mast cell-nerve interactions in OLP (Zhao ZZ et al. 1997).

There is a growing awareness that oral mucosal T lymphocytes and mast cells interact in a bidirectional fashion in maintaining the chronicity and the pathogenesis of OLP. In the development of the lesion, mast cell-derived TNF-α can activate T cells which further secrete RANTES and matrix metalloproteinases (MMP). RANTES causes continued degranulation of mast cells, while MMPs prepare the endothelium and the surrounding connective tissue matrix for the migration of T cells (Zhao ZZ et al. 2002, Sharma R et al. 2011)

In addition, degranulating mast cells release chymase and tryptase that damage the epithelial basement membrane directly or indirectly via activation of matrix metalloprotein-9 secreted by OLP lesional T cells (Jontell M et al. 1986, Sharma R et al. 2011).

Taken together, mast cells may play an important role in the pathogenesis of OLP.

Langerhans cells

CD1a cells are located in both the suprabasal part of the epithelium and in the connective tissue of OLP lesions and their numbers are significantly higher as compared with healthy mucosa (Gustafson et al. 2008). CD1a-positive LCs in OLP have in general have a dendritic appearance. CD1a-, and Langerin expressing cells are more abundant in the epithelium of OLP lesions than in connective tissue (Santoro et al. 2005). Moreover, in comparison with normal oral epithelium there is an increase in mean numbers of CD1a-expressing LCs per high power field in OLP epithelium (Kulkarni et al. 2016). Langerin-positive cells, which sometimes, but not always, exhibited a dendritic morphology, occur also with significantly higher numbers as compared with healthy mucosa, in both the suprabasal part of the epithelium and in the connective tissue of OLP lesions. Some CD83-positive cells also exhibit a dendritic morphology and are mainly found in lymphocyte-rich areas of the inflammatory cell infiltrate of OLP. CD1a-, Langerin-, and CD83- positive cells are sometimes located in clusters of cells with lymphocyte morphology (Gustafson et al. 2008)

LC have an important role in antigen-processing activity and subsequent presentation to T cells and have higher numbers in both OLP and lichenoid reaction, which suggests that they may play a significant role in the adaptive immune response of lichenoid conditions (Gueiros et al. 2012, Kulkarni et al. 2016).

Dendritic cells

OLP is characterized by the recruitment of different subsets of DCs, such as stromal DC-SIGN+ DCs (mDC), and pDCs, associated with the development of a mature phenotype by a significant number of DCs (Santoro et al. 2005). DCs shows significant increase in expression of TLR7/TLR8/TLR9, which may be correlated with antigens or their mimics as possible molecules initiating the autoimmune response in OLP (Wang Y). Higher densities of CD1a+ DCs and Langerin+ Dcs are also observed in OLP as compared with NOM in the region adjacent to the epithelium (Santoro et al. 2005, Souto et al. 2016).

pDCs are detected in the vast majority of the OLP cases, whereas they are virtually absent from the normal mucosa (Santoro et al. 2005). This DC population is principally observed inside the stromal lymphoid infiltrate, arranged as clusters around the small vessels or as single cells; they are also

detected in the epithelium as single cells and occasionally as small aggregates in approximately half of OLP cases. pDCs express CCR7, a chemokine receptor-mediating migration that is upregulated by PDCs upon maturation, and may be responsible for CCR7+ effector T-cell recruitment in OLP (Santoro et al. 2005)

Macrophages and monocytes

In oral lichen planus, macrophages are seen in close proximity to the epithelial basal cells, where cell damage occurs (Matthews JB et al. 1985). They may therefore play a role in the pathogenesis of this condition. Some macrophages act as scavengers since they contain phagocytosed material such as melanin and degenerate epithelial material. (El-Labban et al. 1972)

The interplay between macrophages and T cells emphasizes the importance of macrophages in the progression of the disease. Infiltrating monocytes recruited into the lesion can develop into a pro-inflammatory M1 phenotype due to the high levels of GM-CSF, TNF-α, and IFN-γ produced at the site. M1 macrophages can participate in the progression of the disease by three main mechanisms: initiation of inflammation, activation and priming of T cells, and direct destruction of the basal membrane. Macrophages can be located close to the damaged basal layer and therefore contribute to the destruction of the basal membrane. There seems to be a vicious cycle of perpetuating inflammation and damage to the basal membrane, as this destruction can further initiate inflammation through the release of danger associated molecular patterns (DAMPs) (Merry et al. 2012). In addition, T-cell receptor (TCR) ligation and activation, triggers the secretion of chemokines that draw other damaging cells to the lesion, such as tumour necrosis factor (TNF)-producing macrophages (Sato et al. 2006). If the macrophage phenotype could be manipulated from M1 to M2, there could be the potential to correct a range of oral inflammatory disorders such as lichen planus (Merry et al. 2012).

Granulocytes

In both OLP and lichenoid reactions, eosinophils are scattered predominantly within the superficial connective tissue, or within blood vessels rather than having perivascular distribution (Firth NA et al. 1990). Eosinophil numbers are higher in oral lichenoid mucositis than in oral lichen planus and this could be used as an adjunct histologic criterion in the diagnosis of oral lichenoid mucositis (Reddy et al. 2012).

Neutrophilic granulocytes are present in OLP lesions but constitute a minor fraction of the cells in the infiltrate (Mravak-Stipetić et al. 2014).

Salivary PMNs present functional features distinct from those in peripheral blood. Some phagocytic functions of sPMNs are enhanced in OLP, which might mean that they either have a role in the pathogenesis or reflect clinical changes in these conditions (Lukac et al. 2003). There are few articles that have investigated neutrophils in OLP as they are not thought to play a decisive role in the pathogenesis of OLP.

Natural killer cells

Histology and immunohistochemistry of biopsy specimens from both red and white lesions in OLP show scattered presence of CD56 lymphocytes under the basal membrane in red lesions and an almost undetectable CD56 lymphocytes infiltrate in white lesions (Lorenzini et al. 2013). On the other hand, lower functional activity of NK cells in peripheral blood is detectable in erosive LP patients (also red lesion) compared to those with non-erosive LP of the oral mucosa or healthy controls (Simon et al. 1989, Biocina-Lukenda et al. 1998).

Conclusion

CD4+ and CD8+ cells are located both in the epithelial-connective tissue interface region and the superficial part of lamina propria. The exact etiology of OLP is unclear but the high numbers of T cells indicate that they are pivotal in disease development. The T cell infiltrate may be initiated by specific antigens or it may be established by antigen-unspecific mechanisms. The latter may be caused by a form of immune dysregulation, mediated by the release of cytokines by activated CD4+ T-cells and failing function of Tregs which are mostly of a non-suppressive type, leading to the attraction of other inflammatory cells and finally to the destruction of basal keratinocytes by cell mediated cytotoxicity via activated CD8+ cytotoxic T-cells. The subtype of CD4+ cells may be important in defining the clinical and pathological picture of OLP. Th9 and Th2 cells may play a role in reticular OLP, while Th17 cells may play a role in erosive OLP. Tregs are located at the epithelial-connective tissue interface and in the superficial connective tissue and are mainly of the non-suppressive subtype. In addition, there can be a failing balance between Treg and IL17 lymphocytes functions which might play an important role in the regulation of the immune responses.

The prevalence of B cells and plasma cells in the infiltrate of OLP is not well mapped. The few observations available indicate that there are significant numbers of these cells and this should be studied more systematically. EBV infection of B cells and plasma cells in OLP lesions seems to be frequent and this may constitute a pathological factor.

Mast cells infiltrates can be seen in all layers of lamina propria, but when they are located more superficially to the epithelium, they often are degranulated. Degranulation of mast cells can activate T cells, nerve endings and contribute to damage of the basal membrane.

LCs play roles in antigen capture and presentation to T-cells in OLP, and their quantitative increase shows that they can be involved in the pathogenesis of OLP.

OLP shows higher number of DCs in the cellular infiltrate, among them mDC1 and pDC. DCs undergo maturation in OLP tissues and can activate pathogenic T cells locally or in the regional lymph nodes.

Macrophages are mainly located near the basal membrane and the superficial part of lamina propria. The main phenotype of macrophages infiltrate is M1 which with its interaction with T cells exacerbates inflammation in OLP. Macrophages also act as scavengers and phagocyte debris in inflamed tissue of OLP.

Neutrophilic granulocyte infiltration in OLP lesions is limited. Salivary neutrophils show enhanced phagocytic function. There are more eosinophils in the infiltrate of lichenoid reactions as compare with OLP, which can be explained by the hypersensitivity character of lichenoid reaction.

Natural killer cells can be seen mostly in relation to basal membrane degeneration in red lesions of OLP.

Further studies are still needed to elucidate the obviously complex pathogenesis of the disease.

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