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WBC & THEIR ROLE IN TUMOR MICROENVIRONMENT (TME) OF ORAL SQUAMOUS CELL CARCINOMA-A REVIEW

Health Science

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ABSTRACT

Oral premalignant lesions (OPLs) that affect approximately 4.5% of the world's population usually precede the occurrence of Oral squamous cell carcinoma. These lesions are now included in oral potentially malignant disorders (OPMD). Majority of OPLs regress, yet, up to 30% of them ultimately progress through increasingly grades of dysplasia &culminate as oral cancer. Therefore, OPLs represent an intermediate phase during the evolution of normal mucosa into malignant tumor, owing to their acquisition of a subset of the genomic alterations from those necessary to develop into Oral Squamous cell Carcinoma(OSCC).(1) In India OSCC is responsible for more than 20% of new malignancies diagnosed every year, being the most prevalent malignancy in the nation. There are several prognostic factors which help to evaluate the risk associated with the OSCC and serve as subsequent treatment guidelines. Increasing evidence has, so far, suggested that inflammation may be linked to pathogenesis of oral cancer. Also, the tumor microenvironment is considered a crucial component in the understanding of the biologic behavior of a neoplasm. Leukocytosis is common in patients with progressive oral squamous cell carcinoma, is related with T-classification, lympho-vascular permeation, and recurrence or metastasis, &, therefore could decrease survival. Tumor-related leukocytosis results from hematopoietic colony-stimulating factors and inflammatory cytokines from solid tumors. (2,4) One of the new most promising histopathological factor in prognostic evaluation of OSCC is, the density of tumour infiltrating lymphocytes (TILs). Different subsets of lymphocytes have different or even opposing functions in the tumor microenvironment. (2,6,7) Neutrophils contribute to cancer progression or regression via multiple mechanisms, including the suppression of cytotoxic as well as helper T cell responses and the stimulation of tumor angiogenesis.(8) B cells also act as antigen-presenting cells, promote differentiation of Th1 cells and Tcyt cells, and directly kill cancer cells through release of Granzyme B, thus, having a tumor suppressive role. (9) Infiltrating eosinophils in the tumor microenvironment (TME), supply direct and indirect mitogenic growth mediators that stimulate proliferation of neoplastic cells, as well as educate other stromal cell types to induce paracrine and juxtacrine mitogenic signaling molecules to support neoplastic growth which also appears true for oral squamous cell carcinomas (OSCCs). (10) The plasmacytoid dendritic cells (pDC), are specifically important in cancer immunity as, these cells have been identified in many solid malignant tumors, including those of head and neck & may play a key role in tumor occurrence and development.(9,12) Mast cells have a long life and form a heterogeneous population of cells that seem to have both a positive and negative regulatory effect on the immune system. MCs accumulate into tumor microenvironment by the help of tumor cell-released chemoattractants such as SCF or CCL15 and actively recruit cells of the innate immune system mainly neutrophils, macrophages, and eosinophils and cells of the acquired immune system (B and T cells) to orchestrate antitumor immune responses (13) In cancer tissues, the infiltration of macrophages is significantly increased. Macrophages are recruited to this edge by tumor-derived chemotactic agents and are a major infiltrating cell type in the leading edge of a carcinoma.(16) OSCC are highly immunogenic tumors that are often characterized by abundant infiltration of immune cells, however, their function & prognostic value vary. (19, 20)

KEYWORDS

Tumor micro-environment (TME), Oral squamous cell Carcinoma(OSCC), Tumor infiltrating Lymphocytes(TILs), Dendritic cells, Tumour-Associated Tissue Eosinophilia (TATE)

INTRODUCTION

Oral premalignant lesions (OPLs) that affect approximately 4.5% of the world's population usually precede the occurrence of Oral squamous cell carcinoma. These lesions are now included in oral potentially malignant disorders (OPMD). OPLs include distinct pathological entities like: leukoplakia, Oral submucous fibrosis, and lichen planus & remain a diagnostic dilemma without a clearcut carcinomatous transitional alteration recognition and with limited preventative and therapeutic options. Majority of OPLs regress, yet, up to 30% of them ultimately progress through increasingly grades of dysplasia &culminate as oral cancer. The overall annual risk of transformation of of OPLs is variable region-wise. However, OPLs represent an intermediate phase during the evolution of normal mucosa into malignant tumor, as, OPLs have acquired only a subset of the genomic alterations necessary to develop into OSCC alongwith an accompanying mileu of an oral inflammatory environment caused by exogenous factors such tobacco and/or alcohol use, poor dentition and by endogenous factors including auto-immune diseases. In all probability, the genomic alterations cooperate and co-regulate the immune microenvironment in OPLs to facilitate the progression of OPLs to invasive cancer. (1)

In India OSCC is responsible for more than 20% of new malignancies diagnosed every year, being, the most prevalent malignancy in the nation. This is due to the fact that, in India its incidence is associated with the ubiquitous habit of chewing betel quid, composed of betel leaves with areca nut, often admixed with smokeless tobacco in most cases besides use of various forms of smoked tobacco. There are several prognostic factors which help to evaluate the risk associated with the OSCC and serve as subsequent treatment guidelines. Those widely accepted are: tumor size, nodal status and distal metastasis, the backbone of the TNM system, while several others, commonly described when evaluating the OSCC specimen, are still in dispute: perineural and lymphovascular invasion, tumour thickness/depth of invasion, bone invasion, surgical margin status and tumor differentiation grade.(2)

In 1863 Rudolf Virchow postulated induction hypothesis stating that cancer originates at site of inflammation because he observed the presence of leukocytes in non-neoplastic tissue. Increasing evidence of a high WBC count in tumor stroma has, so far, suggested that inflammation may be linked to pathogenesis of oral cancer. Also, according to many researchers, inflammatory cells and their cytokine production seems to correlate with tumor severity. There is growing evidence suggesting that, cellular proliferation in an environment rich in inflammatory cells, growth factors and activated stroma is associated with DNA damage potentiate the growth of cancer cells. (3) Also, the tumor microenvironment is considered a crucial component in the understanding of the biologic behavior of a neoplasm. Tumor-host interaction is an essential but, complex phenomenon inherent to tumor progression and is an increasingly important target for anticancer strategies.

Inflammation is a recognized hallmark of tumor progression, and infection, and inflammation has been involved in various steps of oncogenesis. The tumor microenvironment (TME), partially composed of inflammatory cells, orchestrates the neoplastic process, promoting tumor proliferation, survival and migration. A significant rise in granulocyte count has been observed both in the peripheral blood and in tumor tissues of patients with different types of solid malignancies. Numerous functional in vitro and in vivo studies have also proven that tumor mass stimulated neutrophil density increase in the local micro-environment which, in turn, promoted angiogenesis, immunosuppression, invasion, migration and metastasis of the tumor cells. Leukocytosis is common in patients with progressive oral squamous cell carcinoma, is related with T-classification, lympho-vascular permeation, and recurrence or metastasis, &, therefore could decrease survival. Tumor-related leukocytosis is a consequence of release of hematopoietic colony-stimulating factors and inflammatory cytokines from solid tumors. Production of cytokines, chemokines and granule proteins promotes, which promotes tumor growth, angiogenesis, and increase its metastatic potential. Additionally, Leukocytosis, reflecting elevated neutrophil and monocyte counts, might independently predict Overall Survival and Progression Free Survival from these parameters according to some researchers. (4)

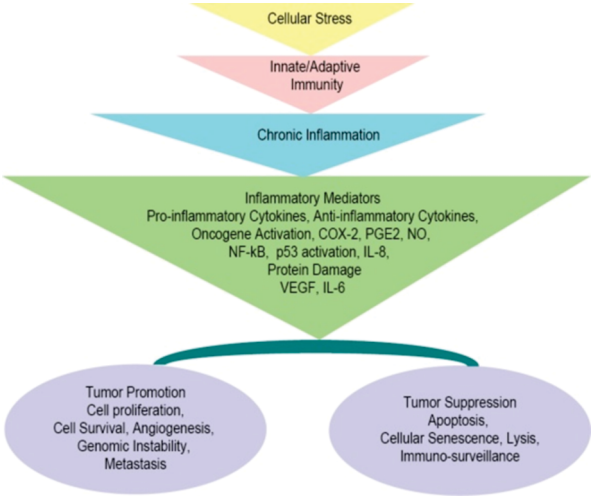

Fig-1: Chronic inflammation alters the cellular levels of inflammatory mediators & aid inflammatory associated molecular signature for early detection of oral dysplasia and squamous cell carcinoma.

The immune cell component of tumor microenvironment plays an important role in carcinogenesis and has prognostic potential in many types of cancer. One of the new & most promising histopathological factor in prognostic evaluation of OSCC is, the density of tumor infiltrating lymphocytes (TILs). The term TILs was first coined by Clark and his colleagues approximately 50 years ago, and since then the TILs have been extensively studied as the prognostic and predictive factor in many solid malignancies. It is important to know, the prognostic value of TILs as the group of mononuclear inflammatory cells, in oral squamous cell carcinoma. (2)

Innate and adaptive immunity which are controlled by a heterogeneous family of TILs, play important roles in immunosurveillance of tumor mileu and therefore, enhance tumor destruction capacity of the stroma. Both types of effector responses are regulated by a heterogeneous family of TILs. TILs are present in the earlier stages of head and neck SCC and are known to be a critical component of anticancer immunity in the form of adaptive immune response. A low lymphocyte count might thus, indicate an insufficient host immune response by lymphocytes to cancer cells. The risk of cancer development and progression might increase in such immune-compromised, lymphopenia elicting population. (5)

Different subsets of lymphocytes have different or even opposing functions in the tumor microenvironment. Cytotoxic T-lymphocytes, characterized by the expression of CD8, have the ability to directly target and destroy tumor cells through binding to MHC class I molecules, & their presence in the tumor microenvironment has therefore, been associated with a better prognosis in many types of cancers. Regulatory T-cells (Tregs) are involved in maintaining immunological tolerance to host tissues and are therefore considered to be suppressors of the anti-tumor immune response. The prognostic value of Tregs varies significantly among different types of cancer & hence, they are the commonly evaluated biomarker using immunohistochemical staining for FoxP3, a marker which is the most specific Treg marker. The role of CD4+ helper T-cells is unclear, because a wide range of CD4+ cell subsets with different functions exists. CD4+ Th1 cells promote the anti-tumor immune response by stimulating CD8+ cytotoxic T-cells and CD4+ Th2 cells are also related to anti-tumor immunity. On the other hand, CD4+ regulatory T-cells are thought to inhibit an effective anti-tumor immune response.(6)

Among T-lymphocytes, NK cells act as immune effectors independent of sensitization, in an HLA-free fashion. As a consequence, tumor cell clearance is promoted in a nonspecific manner, through NK cell degranulation, cytokine release and cytotoxicity. The signaling mechanisms for NK cell tumor infiltration function by, the so-called phenomenon of "missing self". Such phenomenon is often seen in cells undergoing malignant transformation, as they exhibit a phenotype lacking MHC class I molecules that engage the NK cells & thus, promoting tumor cell clearance. The DNA damage accumulation that occurs in many environmentally-induced cancers, including nicotine and UV radiation-associated OSCC, activates NK cells by means of upregulated stress ligands. Thus, these effective nonspecific cytotoxic effects of NK cells against malignant cells, indicate their positive prognostic role in solid cancers and, were detected in OSCC in peripheral blood and tumor tissue, with the help of increased expression of suppressive cytokines interleukine-10 (IL-10) and tumor growth factor-β (TGF-β) and decreased expression of activating receptor NKp46. These findings may open new perspectives in targeting the inhibitory signaling pathway, with consequent NK cell activation. (7)

Neutrophils are important players in tumor immunology and a thorough investigation of these cells has helped to throw light on aspects of tumor immune escape mechanisms as well as in enabling to establish more suitable biomarkers for cancer diagnostics and therapy. Neutrophils are known to contribute to cancer progression or regression via multiple mechanisms, including the suppression of cytotoxic as well as helper T cell responses and the stimulation of tumor angiogenesis. Moreover, neutrophils participate in cancer metastasis via formation of premetastatic niche in target organs or via NET-mediated trapping of circulating cancer cells. Clinical studies identified blood neutrophil-to-lymphocyte ratio and the number of tumor-infiltrating neutrophils to be negative prognostic factors in a variety of different cancers, including HNC. (8)

Theoretical backgrounds for cancer-related inflammation have gradually emerged despite lack of explanation of the mechanisms of interaction between systemic inflammation and cancer. Neutrophils exhibit both anti- & pro-tumor responses concurrently in the tumor microenvironment. In an anti-tumor response, these cells, limit tumor growth through direct, antibody-dependent cytotoxic effects and activation of immune cells. However, cancer cells also function to induce the systemic activation of neutrophil extracellular matrix traps, & thereby lead to increased adhesion, destruction of basement membrane, invasion of cancer cells and metastasis. The cytokines from neutrophils, including vascular endothelial growth factor, fibroblast growth factor 2, oncostatin M, matrix metalloproteinase 9 and elastase are involved in chronic inflammation and cancer progression. In addition, neutrophils have been found to be related with suppression of cell-mediated immunity for cancer surveillance. Rao et al. in 2004 reported that elastase from neutrophils can also inhibit recruitment of T lymphocyte into the inflammation sites. (5)

B cells are another major player in the adaptive immune response, more specifically in the humoral response. When an antigen compatible with their specific receptor binds to these cells alongwith simultaneous release of cytokines from Th cells, B cells can differentiate to plasma cells and produce antibodies specific to the antigen that triggered their differentiation. Besides this, B cells also act as antigen-presenting cells, promote differentiation of Th1 cells and Tcyt cells, and directly kill cancer cells through release of Granzyme B. These functions indicate a tumour suppressive role of B cells. In a similar fashion as, T cells, immunesuppressive subtypes of B cells have been detected, termed B-regulatory (Breg) cells. Similar to Treg cells, these cells produce TGFβ and IL-10, promoting the same down-

stream effects as described for Treg cells.(9)

Well-recognized checkpoints mediated that immune system puts in position indicate that, the immune mechanism recognizes malignant cells as foreign agents. Bone marrow-derived myeloid cells such as macrophages, neutrophils, eosinophils, mast cells, and dendritic cells usually infiltrate malignant tumors in large numbers. Eosinophils are bone marrow-derived minority circulating (1%–5%) multifunctional granulocytes. Eosinophils are, destructive cells owing to their cytotoxic activities attributable to their granules,which contain cationic proteins like- the major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and the eosinophil peroxidase. Infiltrating immune cells, such as, eosinophils, in the tumor microenvironment (TME) supply direct and indirect mitogenic growth mediators that stimulate proliferation of neoplastic cells, and simultaneously, educate other stromal cell types to induce paracrine and juxtacrine mitogenic signaling molecules ,which in turn, supports neoplastic growth as also seen in oral squamous cell carcinomas (OSCCs). (10)

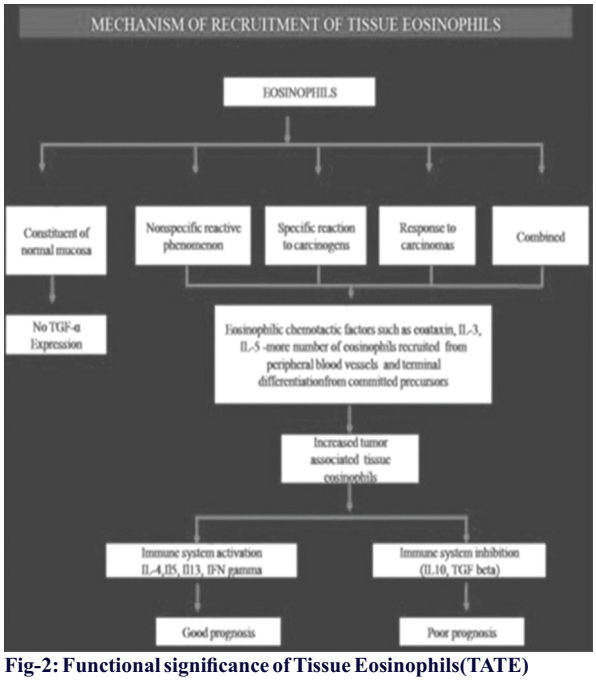

Fig-2: Functional significance of Tissue Eosinophils(TATE)

Eosinophils are mainly involved in initiation and propagation of diverse inflammatory responses including infections, tissue injury and allergic diseases and are known modulators of innate and adaptive immunity. The phenomenon of eosinophilic infiltration of tumor stroma which is not associated with either tumor necrosis or ulceration was first described in1896 by Przewoski in carcinoma of cervix and is referred to as Tumour-Associated Tissue Eosinophilia (TATE). It is characterized by the presence of eosinophils as a component of peritumoral and intratumoral inflammatory infiltrate. Under the impact of appropriate stimuli (e.g., infections, tumors, etc.), the eosinophils release several mediators such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL- 10, IL-12, IL-13, IL-18, interferon (INF)-γ, transforming growth factor (TGF)- α, TGF- β, Eosinophil Cationic Protein (ECP), Major Basic Protein (MBP), Eosinophil Peroxidase (EPO), Eosinophil-Derived Neurotoxin (EDN), Tumor Necrosis Factor (TNF)- α, Chemokines (RANTES, endotaxin-1), Platelet-Activating Factor (PAF), Leukotriene C4 (LTC4), Neuromediators and Indoleamine 2,3-dioxygenase (IDO). These molecules when released contribute to tumor regulation and lead not only to cell death but also to initiation of inflammatory symptoms.

Both indirect & direct tumoricidal activity has been attributed to eosinophils. Direct activity is done by the release of cytotoxic proteins in TME and indirect activity involves the enhancement of their permeability into tumor cells facilitating penetration of tumor-killing cytokines. Additionally, eosinophils are likely to promote tumor angiogenesis also by, the production of several angiogenic factors. However, the eosinophils are like a "Double-edged sword in tumor battle field" that support cell-mediated tumor immunity in early stages while inhibiting the same in advanced stages via IDO and cytokines and promoting tumour angiogenesis. Thus, TATE can be a "Boon or a Bane" depending on the stage of the tumor. It can be used as independent surrogate marker for determination of prognosis in OSCC. (11)

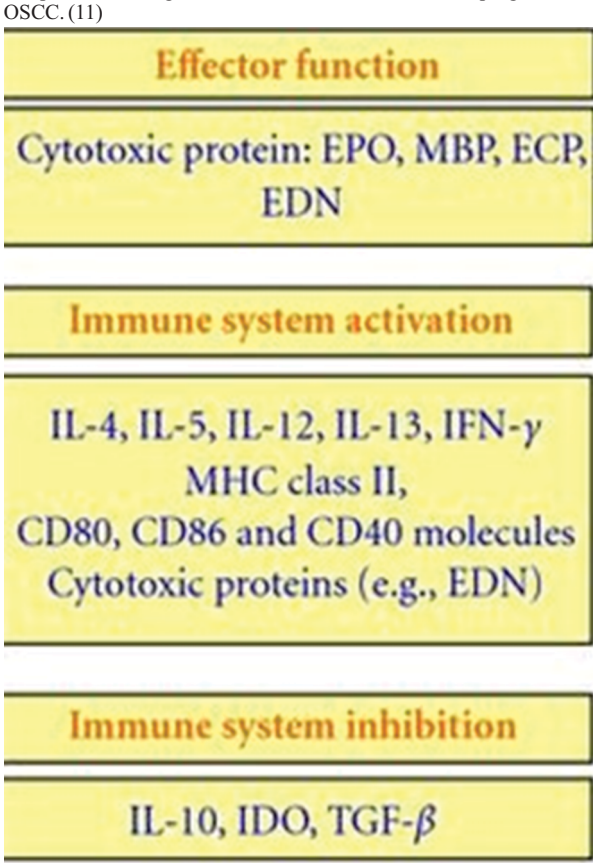

Fig-3: Eosinophil function in immune response giving good &/or poor prognosis

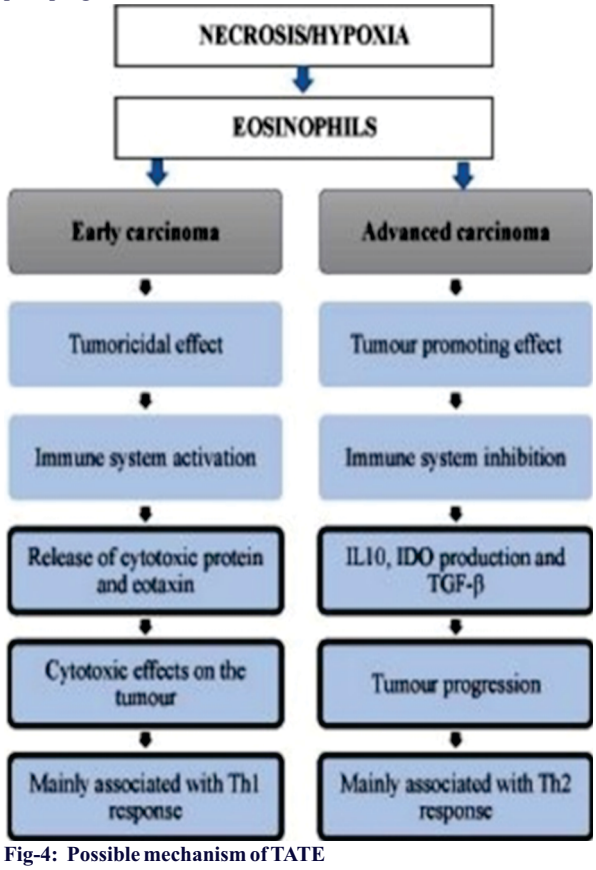

Fig-4: Possible mechanism of TATE

Clinical correlative studies of patients with either blood or tissue eosinophilia, or both in, other human malignancies, has led to the conclusion that, tissue eosinophilia at the tumor sites tends to be "protective," whereas blood eosinophilia tends to be an indicator of tumor metastasis. However, due to the ambiguous evidence of either of the two in oral cancer pathogenesis, the ratio of TATE/TABE in OSCC could be decisive of the pathology. (10)

Dendritic cells are the antigen presenting cells in tissues and are of two main types: myeloid (mDC) & plasmacytoid (pDC). Conventional (c) DC derived from myeloid progenitor cells, are of two main classes - cDC1 and cDC2. In addition to DC originating from lymphoid and myeloid progenitor cells, DC can also be derived from monocytes under inflammatory conditions. (12)

Recent studies suggest that pDC, specifically, are rather important in cancer immunity as these cells have been identified in many solid malignant tumors, including those of head and neck.Several researchers suggest that the immune cells, including plasmacytoid dendritic cells (pDC), may play a key role in tumor occurrence and development. Plasmacytoid dendritic cells are one of two principal subsets of human dendritic cells (DCs) which are derived from lymphoid progenitor cells and have an important role in combating viral infections. At steady state, pDCs circulate in the blood and are absent from peripheral tissues. Plasmacytoid dendritic cells (pDC) are a unique population of bone-marrow-derived immune cells accounting for only 0.3%-0.5% of peripheral blood cells. From among all dendritic cell subsets , pDCs are unique owing to their morphology, surface markers, their migration in vivo and the ability to produce high levels of type I IFNs upon activation. According to previous research, impaired tumor-infiltrating pDC are a strong predictor for LN metastasis in oral cancer because, increased tumor-infiltrating pDC through TNF-α-mediated NF-κB activation, contribute to upregulation of CXCR-4 expression, and, ultimately cause tumor cell proliferation and migration toward LN via the CXCR4/SDF-1 chemoattraction axis. (9, 12)

Mast cells are an important component of the immune system in host defence that, reside in all the vascularised tissues, especially in all the connective tissues and mucosal surfaces of the human body, but mature mast cells are not present in the circulation . These cells have many granules, are motile & are derived from haemopoietic progenitor cells. They are also known as mastocyte or as labrocyte, derived from the Latin word mastocytus , mast meaning "well fed" or "fattening" in German. Mast cells were first described by Paul Ehrlich in 1878 as cells belonging to the connective tissue staining purple to blue with aniline blue dye due to the presence of an abundant number of granules. In different tissues, mast cells tend to have differing morphology and cytogenicity and hence this property is useful in differentiating them by staining them for their contents. However, their most critical role is in mediating type 1 hypersensitivity reaction and acting as effector cells in IgE-mediated host immune responses. They are activated by diverse mechanisms &, are found to be significantly associated with increased mitotic activity, extracellular matrix degradation, angiogenesis, intensification of microvascular hyper permeability, and recruitment of inflammatory cells including macrophages. The mast cells cannot be identified in circulation but can be seen in tissues. The major component of the granules of mast cells is heparin. They are divided into two types based on the substances present in their granules: 1. connective tissue mast cells and 2. mucosal mast cells. The chief function of the mast cells is that they participate in innate immunity through secretion of effector molecules. Another important aspect of mast cells recently discovered is their ability to change their phenotype according the site and the duration of stimuli to which they are exposed. This is termed 'mast cell plasticity'. Mast cells have a long life and form a heterogeneous population of cells that seem to have both a positive and negative regulatory effect on the immune system. (13)

MCs accumulate into tumor microenvironment by the help of tumor cell-released chemoattractants such as SCF or CCL15 and actively recruit cells of the innate immune system mainly neutrophils, macrophages, and eosinophils and cells of the acquired immune system (B and T cells) to orchestrate antitumor immune responses .Simultaneously, Mast cells also promote tumor vascularization by facilitating increase of microvessel densities in squamous cell carcinoma by its role in up-regulation of angiogenesis/ neovascularization in oral squamous cell carcinoma. This is ,because, the perilesional and intra tumoural area of oral squamous cell carcinoma expresses CD105, VEGF, VEGFR1, and VEGFR2 and show positive correlation with angiogenic activity of the tumour. MCs are also involved in lymphangiogenesis by releasing VEGF-C and VEGF-D .Mast cell heparin , induces , vessel proliferation & increases the half-life of basic FGF, which is a strong angiogenic substance. This promotes tumor angiogenesis & facilitates the local invasion. Mast cells are a rich source of proteases especially, tryptase & chymase, which directly degrade the extracellular matrix(ECM) through their proteolytic activity & indirectly stimulate angiogenesis, thereby facilitating invasion & metastasis through ECM remodeling. MMPs from mast cells contribute to ECM degradation. The mast cells possess both pro- and antitumorigenic properties and have a variable impact both on the development of primary tumor and on tumor progression & metastasis in OSCC.(13,14)

The interaction between components of TME and tumor cells decides the fate and advancement of OSCC. It is often seen that, the WBC present in TME tend to have anatagonistic functions in oral oncogenesis. For eg.: a class of macrophages, myeloid-derived suppressor cells, regulatory T cells and CD4+ T helper type 2 (Th2) cells all share pro-tumor functions, whereas another class of macrophages, dendritic cells, natural killer cells, CD8+ T cells and CD4+ Th1 cells tend to show anti-tumor actions. Therefore, historically, solid tumors are immunologically classified into "cold" and "hot," depending on the non-inflamed or inflamed TME milieu, respectively. It has been observed by researchers that, inflamed tumors respond favorably to immune checkpoint blockade (ICB) therapy owing to the presence of an abundance of tumor infiltrated lymphocytes (TILs) that have enhanced :

1) interferon-γ (IFN-γ)-expressing CD8+ T cells,
2) expression of checkpoint markers including programmed death-1 ligand 1 (PD-L1) and
3) high mutational burden.

In contrast, non-inflamed tumors which are characterized by an immunosuppressive milieu are poorly infiltrated by immune cells and rarely express PD-L1, while characterized by an immunosuppressive milieu, and additionally, they might also have high genomic instability. Thus, the spectrum of tumor immunity ranges from immunologically ignorant tumors, characterized by genomic stability, highly proliferative tumor cells and low infiltration of T cells, as well as low expression of antigen-presentation machinery markers including MHCI to those tumors with an inflamed tumor milieu.(15)

In comparison to, normal oral mucosa, the proportion of macrophages in the stroma of TME region of hyperplasia and cancer tissues, the infiltration of macrophages is significantly increased. The activation of macrophages is the main event in the pathogenesis of multiple diseases. Tumor cells present at the leading edge of tumor and undergoing EMT, shoulder the responsibility of establishing a microenvironment suitable for tumor progression after undergoing EMT. Macrophages play a role in the tumor progression by being brought in owing to tumor-derived chemotactic agents and then, they serve as the major infiltrating cell type of the leading edge. After reaching the leading edge of tumor progression, tumor-associated macrophages (TAMs) can promote tumor cells motility and hydrolytic remodeling of the extracellular matrix (ECM) by secreting epidermal growth factors (EGFs) and other pro-migration factors and regulating the production of collagen fibers to enhance tumor cells invasion.(16)
Tumor-associated macrophages (TAMs) are both most abundant as well as necessary immune cell types of tumor edge and are derived in adults from a particular group of inflammatory monocytes ($CD64^{+}/CD16^{-}$ $CCR2^{+}$ $Ly6C^{+}$). The exposure of the inflammatory monocyte cells to microenvironmental stimuli results in complex phenotypic modifications in a time- and location-dependent manner. Various macrophage subtypes are derived as a result of activation of different regulatory mechanisms and transcription pathways. However, M1 and M2 subtypes of macrophages represent the extreme polarization phenotypes of this cell.

Cancer cells secrete inflammatory chemokines to recruit monocytes/macrophages to the inflamed TME and activate residential macrophages in tissues, thus generating a TAM population. TAMs then secrete several cytokines, chemokines, growth factors, and inflammatory mediators that lead to anti-inflammatory and tumor-supportive effects due to their cytotoxic and tumoricidal activities.(15)

Table-1: Types of Macrophages & their role in OSCC (17)

M1 Macrophages in OSCC	M2 Macrophages in OSCC

1. Differentiation is induced with lipopolysaccharide (LPS) and/or interferon-γ.	1. The differentiation of M2 macrophage is a result of induction by IL-4 and/or IL-13
2. It is characterized by increased expression of specific proinflammatory cytokines, for instance, TNF-α, IL-6, IL-1β, and IL-12, and is responsible for eliminating pathogens and tumor cells	2. It is characterized by anti-inflammatory and protumor effects mediated by the inhibition of the immune response.
3. M1 macrophages can be identified by the expression of CD80 or CD86	3. CD163 or CD206 are explicitly expressed by M2 macrophages.
4. The role of M1 macrophages in cancers remains controversial. Although the accumulating evidence shows its anticancer potential,as well as, reported effect of promoting cancer cell metastasis and proliferation.	4. M2 macrophages contribute to tumor progression and metastasis via immunosuppressive, pro-angiogenic, and cell-invasive function.
5. The M1 polarization state depends on microbial stimulus and a T helper type 1 (TH1) cytokine profile (classical activation pathway).	5. M2 polarization depends on a T helper type 2 (TH2) cytokine profile (alternative activation pathway)

Cd163 is a member of the scavenger receptor cysteine-rich family class B receptor family and is mainly expressed on mature tissue macrophages. CD163 operates on macrophages by binding to the hemoglobin–haptoglobin complex and thereby such macrophages are able to infiltrate into inflammatory tissues subsequently leading to the resolution of inflammation.

The macrophage mannose receptor, CD206 which is a C-type lectin is also expressed by tissue macrophages, dendritic cells, and specific lymphatic or endothelial cells. Similar to CD 163, CD206 is also vital to immune homeostasis in TME, and is significantly expressed in the TME.

From the available research data, it is understood that macrophages have a bipolar role in tumor development. So, they also tend to display an immunosuppressive M2-like phenotype during disease progression. Therefore, current macrophage-based immuno-therapeutic approaches aim to alter the macrophage subtype balance in the TME, in favor of the M1. A very recent study, stratifying the chimeric antigen receptor (CAR) technology from T cells, showed that human monocyte-derived CAR engineered macrophages (CAR-M), demonstrated antigen-specific phagocytosis in vitro, while a single systemic injection of CAR-M in mice models, significantly reduced tumor burden and improved the overall survival. Furthermore, CAR-M demonstrated tumor specific localization in various tumors models and longevity in mice. Intriguingly, they also showed outstanding indirect anti-tumor properties including, secretion of pro-inflammatory molecules, resistance to immunosuppressive cytokines (M1 phenotype persistence), conversion of M2 macrophages to M1, as well as recruitment of- and presentation of antigens to- T cells.(18)

The microenvironment of Oral premalignant diseases & OSCC contains activated cytokines and chemokines, prostaglandins, reactive oxygen species, and various transcription factors. Some of these mediators have the ability to induce proliferation, epithelial-to-mesenchymal transition (EMT), and invasion of genetically predisposed lesions (with mutations in tumor-suppressor genes and/or oncogenes), thereby promoting tumor development. In established OSCC, chronic inflammation is also a common feature, being involved in tumor progression, invasion, and metastasis. Despite a dense inflammatory infiltrate often seen in OSCC tumor tissue, efficient antitumor response is, not present, and rather represent an aberrant host inflammatory response. OSCC are highly immunogenic tumors that are often characterized by abundant infiltration of immune cells, however, the function & prognostic value of immune cells may depend on the anatomical location of the tumor within the oral cavity & how the immune cells are organized in the tumor microenvironment. Understanding the characteristics of the inflammatory infiltrate in oral cancer is, therefore, essential to determine pathogenesis and new strategies to predict outcomes and control the disease. OSCC patients also show an increase in immune cells in the tumor microenvironment with a markedly pro-inflammatory immune environment and a unique salivary cytokine profile. (19, 20)

Future Pathways

Balkwill et al proposed the following aspects that will help us to develop an effective cancer therapy and someday even prevention:

(1) Recognition of tumor-promoting inflammation and tumor-suppressive immunity in tumor development, including stages of tumor initiation, promotion, malignant conversion, invasion and metastasis
(2) To identify which cell type performs tumor promoting inflammation and which cell type performs tumor suppressive immunity in tumor development
(3) Isolation of signal transduction pathways which mediate the cell type specific tumor-promoting inflammation or tumor suppressive immunity
(4) To put together the dynamic functional interaction map entailed in innate immune cells, adaptive immune cells, stromal and cancer cells.(21)

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