Chronic Inflammation in Oral Cancer - Adding Fuel to Fire

Dr Sanjay Chikkarasinakere Jogigowda, Dr Karthikeya Patil, Dr Shilpa Padar Shastry, Dr Tarun Vyas, Dr. Nagabhushana D, Dr Mahesh K P

1Reader, Department of Oral Medicine and Radiology, JSS Dental College and Hospital
JSS Academy of Higher Education and Research (JSSAHE&R), Mysuru
drsanjaycj_dch@jssuni.edu.in

2Professor and Head of the Department, Department of Oral Medicine and Radiology
JSS Dental College and Hospital, JSS Academy of Higher Education and Research (JSSAHE&R), Mysuru.
dr.karthikeyapatil@jssuni.edu.in

3Reader, Department of Oral Medicine and Radiology, Vydehi institute of Dental sciences and Research Centre,
No.82, EPIP area, Whitefield Bangalore, Karnataka- 560066
Shilpa.keshav@gmail.com

4Reader, Department of Oral Medicine and Radiology, RR Dental College And Hospital, Udaipur
tarunvyas14@gmail.com

5Reader, Department of Oral Medicine and Radiology, JSS Dental College and Hospital
JSS Academy of Higher Education and Research (JSSAHE&R), Mysuru.
drbhushan@rediffmail.com

ABSTRACT
The link between Inflammation and Cancer was first suggested by Rudolph Virchow in 1863, which attributed tumours formation to chronic irritation in the 19th century. The issue has been highly controversial as the infiltration of leukocytes in and around neoplastic tissue has been traditionally viewed as exerting an anti-tumour effect. The concept of antineoplastic immunosurveillance as an important component of the immune system is supported by several data including the presence of functionally active human tumour antigen-specific T cells in patients with cancer, the correlation of T-cell infiltration of several human tumors with disease outcome, the increased risk of certain malignancies in immunosuppressed individuals, and the recent development of immunotherapeutic modalities (cancer vaccine) for some malignancies (e.g., malignant melanoma). However, there is now substantial evidence to suggest the inflammatory cells and cytokines found in peritumoral stroma are more likely to contribute to tumours development, progression and metastasis than to mount an effective host anti-tumour response. In brief, if genetic damage is the “match that lights the fire” of cancer, some types of inflammation may provide the “fuel that feeds the flames.” It is interesting to note these concepts were originally proposed at the end of the 19th century, when the biological and molecular bases of cancer were almost completely unknown. Molecular medicine has now provided evidence that such a notion is indeed possible. This article highlights the role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of oral cancer and outlines some of the possible mechanisms involved.

Key words: Oral Cancer, Chronic Inflammation, Cytokines, Microenvironment

Introduction:
Rudolph Virchow in 1863, was the first person to suggest link between Inflammation and Cancer in which chronic irritation was attributed to tumor formation. The subject was found to be controversial as the infiltration of leukocytes in and around neoplastic tissue has been traditionally viewed as exerting an anti-tumor effect. The basic concept of antitumor immunosurveillance as an important component of the immune system is supported by several data including the presence of functionally active human tumor antigen-specific T cells in patients with cancer, the correlation of T-cell infiltration of several human tumors with disease outcome, the increased risk of certain malignancies in immunosuppressed individuals, and the recent development of immunotherapeutic modalities...
(cancer vaccine) for some malignancies (e.g., malignant melanoma). However, there is now substantial evidence to suggest the inflammatory cells and cytokines found in peritumoral stroma are more likely to contribute to tumor development, progression and metastasis than to mount an effective host anti-tumor response. In short, when genetic damage is the fire of cancer "this can be used to provide the fuel which feeds the flames. Certain kinds of inflammation." It is interesting to note these concepts were originally proposed at the end of the 19th century, when the biological and molecular bases of cancer were almost completely unknown. Molecular medicine has now provided evidence that such a notion is indeed possible. This review highlights the role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of oral cancer and outlines some of the possible mechanisms involved.

**The new model of Neoplasia and the “Seed and Soil” theory.**
Cancer and the surrounding inflammatory infiltrate can be “friends” rather than “foes.” Neoplasia can be considered the expression of a “pathological imbalance of tissue-cell societies” where the tumor cells interact with the surrounding microenvironment. This microenvironment consists of an insoluble extracellular matrix, a stroma composed of fibroblasts, vascular and local immune cells, and a milieu of cytokines and growth factors, and is now known to influence many cell processes of tumor onset and progression, such as gene expression, growth, death, differentiation, migration, and invasion. This concept of cellular function being influenced by local surroundings applies to the process of metastasization. In 1889 by Paget published the seminal “seed and soil” hypothesis explaining the non-random pattern of metastasis. He suggested that certain tumors (equated to the 'seed') had a special affinity for certain bodies (equated to the 'soil') and that metastases had only been formed when the soil and seed were compatible. Endothelial cells in the vasculature of different organs express different cell-surface receptors and growth factors that influence the phenotype of metastases that develop locally. The outcome of metastasis depends on multiple interactions (“cross-talk”) of metastasizing cells with homeostatic mechanisms, including specific binding to endothelial cells and responses to local growth factors. These findings support the role of inflammatory peritumoral microenvironment, stromal factors, and cellular cross-talking in being important elements of neoplasia pathogenesis and development.

**Chronic Inflammation**
Chronic swelling may develop from acute inflammation if the addictive agent persists, but the reaction is chronic from the beginning more often than not. Chronic inflammation is marked by the infiltration by mononuclear cells, such as macrophages, lymphocytes and plasma cells, and tissue killing as well as repairing attempts, as opposed to the mostly acute vascular change of inflammation. The macrophage is the key to the chronic inflammatory reaction. Because it releases a large number of bioactive products. These brokers are part of the organ's strong defense against invasion and injury. The downside, however, is that persistent or pathological macrophage activation can result in continued tissue damage.

**Inflammation and Neoplastic Progression**
Peyton Rous was the first to recognize that cancers are caused by viral or chemical carcinogens which induce somatic changes from 'neoplastic sub-thresholds.' These countries, now known as "initiation," involve DNA changes, are irreversible and can remain permanently in otherwise normal tissue until a second type of stimulation occurs (now known as "promotion"). Promotion can be caused by exposure of initiated cells to chemicals such as phorbol esters, injury factors, partial organ resection, hormones or chronic irritation. Many promoters induct cell proliferation, recruit inflammatory cells, boost the production of reactive oxygen species leading to oxidative DNA damage and reduce the repair of DNA, whether directly or indirectly. Subversion of cell death and/or chronically swollen tissue repair programs leads to replication of DNA and the spread of cells losing normal growth control. Normal inflammation is automatic, since anti-inflammatory cytokines are produced closely following pro-inflammatory cytokines. However, the persistence of initiating factors or a failure to resolve the inflammatory response seem to be due to chronic inflammation. Why do tumours continue to react inflammatory? Because chronic inflammation, tissue damage, and chronic infection may stimulate cytokines and chemokines that contribute to development of malignant disease.

<table>
<thead>
<tr>
<th>Table 1. Actions of cytokines and chemokines which may facilitate cancer growth, invasion and metastasis</th>
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<tr>
<td>DNA damage via reactive oxygen</td>
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<td>Inhibition of DNA repair via reactive oxygen</td>
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Functional inactivation of tumour suppressor genes
Autocrine/paracrine growth and survival factors for malignant cells
Induction of vascular permeability and extravasation of fibrin/fibronectin
Tissue remodelling via induction/activation of matrix
Metalloproteinases
Control of tumour-cell migration, direct and indirect
Control of leucocyte infiltrate
Modulation of cell:cell adhesion molecules
Subversion of host immune responses
Stimulation of angiogenesis and angiogenic factor production
Resistance to cytotoxic drugs
Loss of androgen responsiveness

**INFLAMMATION IN TUMOUR MICROENVIRONMENT**

- NF-κB Activity
- Tumor-promoting machine, as it provides a mechanistic link between inflammatory and neoplastic processes.
- Resist apoptosis-based tumor surveillance mechanisms.
- MMPs & Proteases
  - Modulates ECM making tumor cells to invade
  - • Stimulate the growth of inflammatory cells
  - • Proteins such as Bcl which inhibits apoptosis and promotes immortalization
- CSF-1 & COX-2

Fig 1: Inflammation in Tumor Microenvironment.

**Wound healing versus Invasive Tumor Growth**

The tissue of normality is highly organized and segregated. Epithelial cells sit on the top of the membrane separated from the vascularized chamber. Platelets are enhanced during wounds or tissue attack and form a haemostatic jacket where vasoactive mediators regulating vascular permeability, serum fibrinogen influx and fibrin clot formation are released. Chemotactic factors such as growth factor-β transforming and platelet-derived factor growth derived from activated plates, initiate granulation tissue formation, fibroblast activation and proteolytic activation and enzymes induced and activated necessary to restructure the extracellular matrix (for example, matrix metalloproteinase and urokinase-type plasminogen activator). The combined recruitment and re-epitulization of granulocytes, monocytes and fibroblasts throughout the wound is achieved by the venous network. In a conversational signaling dialogue, epithelial and stromal cell types facilitate healing. The reciprocal signaling decreases once the wound is healed.

Less organized are invasive carcinomas. An angiogenesis and lymphangiogenesis associated with neoplasia generates a chaotic blood vessel and lymphatic organization where neoplastic cells interact with other cell types (mesenchymal, haematopoeia and lymphoid), together with a remodeled extracelled matrix. Although during neoplastic progression, the vascular network is not disrupted the same as during wounding, numerous parallel interactions occur. Neoplastic cells produce a range of cytokines and chemokines for granulocyte, mast, monocyte/macrophage, fibroblast and endothelial cells, which are mitogenic and/or chemical attractants. Activated fibroblasts and inflammatory cells...
infiltrate proteolytic, cytokine or chemokine enzymes that are mitogenic to neoplastic cellulose, as well as neoangiogenic and lymphangiogenic endothelial cells. These factors can promote tumor growth, stimulate angiogenesis, cause migration and maturation of the fibroblast, and enhance metastatic spread through interaction with venous or lymph networks.

**Cytokine and Chemokine balances regulate neoplastic outcome**

The balance of cytokines (Fig 2) in any given tumor is critical for regulating the type and extent of inflammatory infiltrate that forms. Tumors producing little or no cytokines or an over-abundance of anti-inflammatory cytokines induce restricted vascular and inflammatory reactions, resulting in restricted tumor growth. The development of an abundance of pro-inflammatory cytokines, on the other hand, can lead to an inflammation that potentiates angiogenesis and thus promotes neoplastic growth. Otherwise, high levels of monocytes and/or neutrophil infiltration may be associated with cytotoxicity, angiostasis and tumor regression in response to an altering balance of pro-compared anti-inflammatory cytokines. Interleukin-10 is usually a tumor product and a tumor-related macrophage.

![Diagram of cytokine and chemokine balance regulating neoplastic outcome](image)

**Fig 2: Balance in Cytokines and Chemokines regulate Neoplastic outcome**

**Local inflammation and systemic anti-inflammation: a paradox**

Neoplastic disorders are paradoxical with regard to inflammatory reactions. Tumors produce and infiltrate leukocytes with inflammatory cytokines or chemokines. However, neoplastic disorders are associated with a defective ability to cause inflammatory reactions in sites other than the tumor and cancer patients' circulating monocytes are defaulting when responding to chemoattractants. Various factors originating in the tumor microenvironment may contribute to the systemic antiinflammation associated with cancer. Chemokines leaking into the systemic circulation are likely to desensitize circulating leucocytes; increased concentrations of TNF receptors and the type II decoy IL-1 receptor may buffer inflammatory cytokines; and tumors also produce anti-inflammatory cytokines. Thus a defective capacity to mount a systemic inflammatory response in cancer patients could coexist with continuous leucocyte recruitment at the tumor site.

**Inflammation and Oral Cancer (OSCC)**

**Infections of oral cavity and OSCC**

OSCC is a multifactorial disease that has not identified a single clearly identifiable cause factor. The oral cavity is currently being studied for inflammation or infection-related carcinogenesis. In view the oral cavity, which includes a variety of surfaces, including over750 distinct bacterial taxa, with huge diversity of microorganisms, one or more of these microbes will be involved in their carcinogenesis. In OSCC development, Table 2 provides a summary of

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infectious agents and carcinogenic mechanisms.

Table 2. Infectious agents and attributed carcinogenic mechanisms in oral carcinogenesis

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<tr>
<th>Risk factor</th>
<th>Potential Carcinogenic Mechanism</th>
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<tr>
<td>Oral biofilm (Dental plaque)</td>
<td>Induction of cellular proliferation, inhibition of apoptosis, interference with cellular signalling mechanisms, Mutagenic interaction with saliva</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>Microbial action on oncogenic inflammatory reactions and proto-oncogenes, Providing opportunty to initiate HPV infection and serve resevoir for latent virus</td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td>Interference with cellular signalling mechanism, Converting ethanol to acetaldehyde</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Dysplastic changes in oral leukoplakia, Converting ethanol to acetaldehyde</td>
</tr>
<tr>
<td>Human papilloma virus</td>
<td>Epithelial cell immortalization, Activation of proto-oncogenes inactivation of p53 tumor supressor gene</td>
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<tr>
<td>Herpes simplex virus</td>
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Helicobacter pylori, a gastric adenocarcinoma associated, is the first species of bacteria to be considered a definitive cause of cancer in humans. Many other possibilities were explored following this discovery. Salmonella typhi, cervical chlamydia carcinoma trachomatis, chlamydia pneumonia lung cancer and streptococcus bovis bowel cancer were associated with gallbladder carcinoma. There was no such direct OSCC connection. The organisms of tumor specimens are crucial in the OSCC studies. Exiguobacterium oxidotolerans, Prevotella melaninogenica, Staphylococcus aureus and Veillonella parvula were specific bacteria found in tumor specimens. Out of 40 samples, three bacteria, namely Capnocytophaga gingivalis, Prevotella meninogenica, and Streptococcus miti, were found to be raised in OSCC in another study, using salivary samples. It has been suggested that specific oral bacteria play a part in carcinogenesis, either through induction of chronic inflammation or by interference, either directly or indirectly, with eukaryotic cell cycle and signalling pathways, or by metabolism of potentially carcinogenic substances like acetaldehyde causing mutagenesis.

There are also a number of yeasts sharing the same environment with the bacteria. The most common yeast found in the human oral mucosa and generally regarded as commensals is a species of Candida. Besides being opportunistic, it has been shown that leukoplakia with candidal infection (formerly known as candidal leukoplakia) has a higher rate of malignant transformation than non-infected leukoplakia, and the estimated rate is up to 10%. C. albicans may have a direct or indirect role in oral carcinogenesis. Candida might induce OSCC by directly producing carcinogenic compounds (e.g. nitrosamines). From the molecular perspective, mucosal bacterial infections may influence carcinogenesis by inducing chronic inflammation in the adjacent connective tissue leading to upregulation of cytokines and growth factors. Similarly, C. albicans has been found to induce IL-8 secretion of endothelial cells by stimulating the cells to produce TNF-α. The transcript factor NF-κB, a key coordinator of innate immunity and inflammation, is also an important tumor promoter. Candidal infection may activate particular toll-like receptors (TLRs), which are known to be activated after tissue damage and microbial infection. They can also communicate with the tumor promoter NF-κB. NF-κB is involved in carcinogenesis, especially where cancer-related inflammation is evident. The association between C. albicans, TLR and NF-κB, and the production of cytokines and enzymes in the prostoglandin synthesis pathway, such as COX-2, is another potential mechanism that shows how C. albicans might influence the development of OSCC.

Apart from bacteria and yeasts there is also evidence that viruses take part in oral carcinogenesis. The role of the human papilloma viruses (HPV) and herpes simplex viruses (HSV) has been investigated in a number of studies. Studies indicated that HPV-16 and -18 were the most common types detected in individuals with OSCC. Generally, there is no clinic lesion or sign of inflammation in HPV (+) OSCC patients, but there is a relation...
proposed by Tezal et al that chronic inflammation in periodontal pockets may give an opportunity to initiate HPV infection and its persistency. In this study the base of tongue in squamous cell carcinoma patients were found to be 70% positive for HPV-16 and HPV (+) tumors, and had significantly higher rates of alveolar bone loss, which is indicative of chronic periodontitis. Infections in the oral cavity are likely to play a role in oral carcinogenesis. Since there are numerous factors that cannot yet be distinguished, further studies with larger sample sizes are warranted.\textsuperscript{30} Infections in the oral cavity are likely to play a role in oral carcinogenesis. Since there are numerous factors that cannot yet be distinguished, further studies with larger sample sizes are warranted.

**Non-Infectious chronic inflammation and OSCC**

Chronic inflammatory diseases such as ulcerative colitis, atrophic gastritis and Barret’s esophagus have been causally associated with cancer development.\textsuperscript{31} Within the oral cavity, the best example of chronic inflammation are periodontal disease (as mentioned before) and oral lichen planus (OLP), which is regarded as having a malignant potential in a wide range of 0-12.5%.\textsuperscript{30, 31, 32} OLP has been suggested as a unique disease model to study non-infectious and chronic inflammation. In the tissue microenvironment of OLP it is expected to find cytokines/chemokines directly associated with oral carcinogenesis, and suggested that OLP related OSCC is very likely to develop from another pathway than non-OLP OSCC.\textsuperscript{32} Its been hypothesized that inflammatory mediators such as cytokines and chemokines released from infiltrating T lymphocytes induce fundamental Protein changes in oral epithelial cells leading to OLP development into oral squamous cell carcinoma (OSCC).\textsuperscript{32} These modified proteins can play a key role in the local microenvironnement and OSCC development. Intensive lymphocyte and mutagenic effects are associated with OLP basal cells, but there is noticeably little apoptotic phenomenon in this cell compartments. The concept that the scarcity of epithelial apoptotic phenomena can be a result of inflammatory infiltrate stimuli is becoming increasingly apparent. The macrophagic migration inhibitor and RANTES chemokines that are releases through infiltrate, have been shown to have antiapoptotic effects on epithelial cells. The proliferation rate for basal epithelial cells has significantly improved with studies of cell proliferation in OLP, which can be induced by inflammatory infiltrate stimuli. This is probably a significant event in OLP cancer development. Conflicting results have emerged from the analysis of the p53 protein expression and the interpretation of its function in OLP. In the affected OLP epithelium, p53 was found to be significantly over expressed against ordinary oral mucosa.

Some researchers believe, with sparse apoptosis and increased cell proliferation, that an epithelial cell response to an intensive T lymphocytic attack in PLO is designed to preserve the epithelial structure. They assumed that the inflammatory infiltration generates anti-apoptotic and proliferative stimuli to defend against this possibilities, activating a high proportion cellular DNA repair system associated with p53.\textsuperscript{32} OLP inflammatory cells may contribute to an excess of nitric oxide \textit{via} expression of inducible nitric oxide synthetize, which finally induces the formation of both 8-oxo-7, 8- dihydro-2′-doxyguanosine (8-oxodG) and 8-nitroguanine in the nucleus of epithelial cells. 8-oxodG formation is known to promote carcinogenesis, as a cause of G-T transfusion. Furthermore, mutations in OLP could be caused by the effect of cyclooxygenase-2, also generated by inflammatory cells. Although the premalignant nature of OLP seems to support existing molecular studies, OLP’s malignant potential remains controversial, and different research groups have suggested distinct approaches. But the role of the inflammatory microenvironment is crucial in the initiation and promotion of malignant keratinocyte transformation, as can be demonstrated in other chronic inflammatory disorders related to cancer growth. In this respect, OLP could serve to study the role of chronic inflammation in oral carcinogenesis as a unique disease model.\textsuperscript{32} There are many hypotheses for the possible association between periodontal disease and cancer, total and by site. But inflammation does play a role in both periodontal disease and cancer. The chronic inflammation induced by the periodontal pathogens may serve to promote initiated cells and may lead to breakdown of normal growth and possible carcinogenesis. The chronic inflammation also may be a sign of a lack of surveillance of tumor growth by the body. Lastly, the formation of endogenous nitrosamines by the bacteria is promoted by inadequate oral hygiene and periodontal disease.

Chronic traumas\textsuperscript{33} in the oral cavity were also associated with oral carcinogenesis in some recent studies and case reports. A study on the etiological factors of tongue carcinoma was conducted Chronic traumas were observed in 44.7% of the patients and 17% of the control group (p=0.004).

**Implications for Prevention and Treatment**

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TNF blockade: 33, 34 Besides the TNF data and growth and spread of cancer, some tests indicate that TNF has a role to play in developing cachexia, and this could also be another advantage for TNF antagonistic therapy. Thalidomide inhibits TNF (and VEGF) mRNA processing, and continuous low-dose thalidomide has shown activity in progressive myeloma patients 59. Several trials are underway with etanercept for the purpose of assessing the role of anti-TNF therapy as a single agent, or in combination with other malignant therapies. Also assessed is the role of etanercept in the improvement of the negative consequences of other cancer therapies. Infliximab is also available in ongoing, planned clinical trials. Like other “biological” cancer treatment approaches, anti-TNF therapy in an adjuvant setting with minimal illness can be optimal. In patients receiving TNF-antagonists for inflammatory disease, a careful record of the incidence of malignant diseases may be indicative of these chemo-processing agents’ potential.

Chemokine antagonism: 35, 36 Chemokinetic-driven tumors and tumors involving chemokines in metastases (e.g. lymph nodes) can be a suitable target for chemokine anti-chemokine addicts currently under development. Data from mouse experiments support this approach.

Nonsteroidal anti-inflammatory agents: Cyclooxygenase enzymes and angiogenesis are inhibited by NSAIDs. Inducing cytokines and being expressed in both inflammatory and cancer diseases, cyclooxygenase-2 is induced 37

Conclusion:
Recent and ongoing molecular studies are rapidly changing our view of neoplastic processes. One of the most important novel findings has been the understanding of the real role of the peritumoral inflammatory microenvironment which is now known to actively participate in the induction, selection and expansion of the neoplastic cells. This has significantly contributed to the institution of novel, so-called “stromal therapy” in cancer prevention and therapy. Furthermore, it has led to better understand the relationship between several chronic inflammatory disorders and cancer development. As a consequence, there is now enough evidence that the increased risk for malignant transformation in these disorders is related to inflammation-associated damage to DNA (such as oxidative damage) and disruption of tissue architecture and function via the “activation” of stromal cells and components able to influence cell survival, growth, proliferation, differentiation and movement.

Inflammation is a recently defined contributor of oral carcinogenesis. In this multi-step process, inflammation might have a role in initiation as well as progression. Important components of this association are cytokines and chemokines produced by activated innate immune cells, which stimulate tumor growth and progression. Moreover, genetic susceptibility and gene/environment interactions are becoming more important in the attempt to eliminate the burden of cancer. The evidence found so far is sending out signals that OSCC may cease to exist in the future, and the referral will only be for a group of diseases that manifests symptoms of a similar sort. Further studies with larger sample groups in premalignant diseases of oral mucosa as well as OSCC are required to confirm these findings.

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