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# Immunological Phenomena in Human Oral Carcinoma in India

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## INTRODUCTION

ORAL CANCER is one of the 10 most common cancers in the world, and in countries like India, Pakistan, Bangladesh and Sri Lanka it is the most common cancer. Hospital-based registries in India reveal that oral cancer accounts for about 30–40% of all malignancies in the country [1]. The prevalence of oral premalignant lesions, such as leukoplakia and submucous fibrosis, has also been found to be high in this country. The aetiology of these high incidences of oral premalignant and malignant lesions is not fully understood. Chewing and smoking of tobacco, and consumption of alcohol have been reported to be associated with oral premalignant and malignant lesions [2–4]. Studies have also revealed an association of the herpes group of viruses with oral cancer [5–9]. Despite marked improvements in various therapeutic modalities, the rate of 5-year survival among oral cancer patients is still low. Throughout the world extensive investigations have been undertaken during the past two decades, on the various aspects of oral cancer. Immunology of oral cancer has been a subject of active research. This article aims to review the major immunological studies undertaken so far in India.

## CELL MEDIATED IMMUNITY

It is now generally accepted that there are immunological derangements in cancer patients, and alterations in the cell-mediated immunity (CMI) in malignancy have become apparent in several studies [10, 11]. The assessment of CMI is, therefore, believed to be useful in the diagnosis and/or prognosis, as well as in the treatment of cancer. The failure of immune surveillance has been reported to be an important factor in the development and progression of cancer [12]. Tumour specific antigens may be present both on the surface as well as in the interior of tumour cells. Specific antibodies to the neoantigens may bind to the surface antigens leading to complement fixation and subsequent lysis of the tumour cells. Immunologically specific cytotoxic T-cells may bind to surface antigens and destroy the tumour cells or inhibit their growth. Studies on animal models have revealed the blocking of antigen-recognition sites on lymphocytes by circulating tumour specific antigens. Reports on such blocking factors in human cancers are rather rare [13, 14].

### *Lymphocytes*

Alteration of the surface characteristics of lymphocytes can lead to changes in their circulation pattern inside the body. Changes in the distribution pattern of lymphocytes subjected to alterations of surface characters by neuraminidase treatment [15] or by anti-lymphocyte serum treatment [16] have been demonstrated.

Vijayakumar [17] undertook a study to evaluate the alterations in the surface characteristics of lymphocytes from oral cancer patients in India, by noting the homing pattern of the lymphocytes in mice. Lymphocytes from oral cancer patients and normal subjects were labelled with radioactive chromium and injected into the experimental animals. The radioactivity in the liver and spleen of the mice was measured subsequently to understand the homing pattern of the lymphocytes. Compared to normal, the lymphocytes from oral cancer patients showed a drastic change in homing pattern. This study suggested that the surface characteristics of the lymphocytes in oral cancer patients are altered possibly due to the presence of blocking factors (antigen, antibody or antigen–antibody complexes). Incubation of the lymphocytes prior to injection into mice resulted in regaining of the normal homing pattern, possibly due to the removal of blocking factors.

Balaram and Vasudevan [18] quantitated total lymphocytes, B-cells and the T-lymphocyte subsets, $T_G$ cells (IgG Fc receptor bearing T-cells) and $T_M$ cells (IgM Fc receptor bearing T-cells), in the peripheral blood of oral cancer patients and normal subjects. A significant increase in the number and proportion of $T_G$ cells and a significant reduction in the $T_M$ cells were noted in the cancer patients. The increase in $T_G$ subset was obvious in the early stages of the disease, whereas the reduction in $T_M$ subset was evident only in the advanced stages.

Vijayakumar and Vasudevan [19] used enumeration of T lymphocytes, by rosette formation with sheep erythrocytes (SRBC), as a parameter to assess CMI in oral cancer patients. In the study, total rosette forming cells (TRFC-T cells forming rosettes with SRBC at low temperature on prolonged incubation) and high affinity rosette forming cells (HARFC-T-cells forming rosettes at 29°C with fewer SRBC) were enumerated. No changes were observed in TRFC in the patients. On the other hand, HARFC were found to be decreased in the cancer patients, and this decrease became more pronounced with progression of clinical stage. Further, normal HARFC levels were observed in the patients with total tumour remission, while it remained depressed in patients with residual tumour after treatment. It was, therefore, concluded that the enumeration of HARFC may be a simple, reliable and a relatively less expensive method to assess CMI in cancer patients. In a later study, Rajendran *et al.* [20] reported depression of HARFC in patients with oral submucous fibrosis.

Pillai *et al.* [21] found reduced total lymphocyte counts in oral cancer patients. This study also revealed a significant increase in the proportion and count of B-lymphocytes in premalignant lesions. Reduction in the absolute number and

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proportion of T-cells was observed both in the malignant and premalignant lesions. The number of circulating $T_G$ cells was considerably increased and that of $T_M$ cells was decreased in both group of patients. Sasidharan *et al.* [22] reported a decrease in the absolute count of peripheral lymphocytes and normal B-cell counts in oral cancer patients. It was also shown that there is an impairment in delayed hypersensitivity in cancer patients, especially in those with metastases. They also attempted to evaluate tumour directed cellular immune responses in oral cancer patients by leucocyte migration inhibition assay [23]. A significant depression in mean migratory index was observed in cancer patients. The depression was more pronounced in advanced stages of the disease.

Jamkar *et al.* [24] studied the natural killer (NK) cell activity in oral cancer patients and attempted to correlate the results with tumour size and metastasis in regional lymph nodes. NK cell activity was found to decrease with an increase in the tumour size. Increased involvement of regional lymph nodes however, was accompanied by an increase in NK cell activity. Circulatory levels of interferon (IFN) producing ability of T-cells after treatment of oral cancer patients with staphylococcal enterotoxin were studied by them [25]. Circulatory IFN was below the detectable level in stage I patients, and it occurred at the highest levels in stages II and IV. It was found that IFN failed to enhance NK cell activity *in vitro* in some cases, and it was concluded that these patients were not suitable candidates for IFN therapy.

Das *et al.* [26] have studied non-specific cellular immune response in oral cancer patients and in age-matched normal subjects, using delayed type hypersensitivity (DTH) responses to purified protein derivative (PPD), dinitro-chlorobenzene (DNCB) and *Candida albicans* extract, absolute T-cell counts, absolute lymphocyte counts and blastogenesis index of phytohaemagglutinin (PHA) induced lymphocytes as parameters. Impairment of both *in vivo* and *in vitro* parameters was observed in the patients, and this was more pronounced in advanced clinical stages with or without lymph node involvement. DTH response to DNCB, PPD and *Candida* extract was found to correlate with tumour differentiation and lymphoreticular response. Reduction in T-cell population was observed in the primary stage of the disease, and a considerable impairment of PHA-induced lymphocyte blastogenesis was evident when the tumour was well-established and disseminated. DTH response to DNCB, T-cell counts and lymphocyte blastogenic indices returned to normal in the treated patients without recurrences. Similar observations have been reported in two studies carried out by Rao *et al.* [27] and Saranath *et al.* [28] on oral cancer patients. Studies conducted outside India have also noted altered lymphocyte counts in oral cancer [29, 30].

Mukhopadhyaya *et al.* [31] carried out studies on the immunoreactivity of the lymphocytes from peripheral blood, draining lymph nodes and tumours from oral cancer patients. T- and B-lymphocytes and T-cell subsets and blastogenesis response of lymphocytes to PHA, NK and K-cell cytotoxicity and modulation of NK-cell activity by recombinant interferon-$\alpha$ (rIFN$\alpha$) were studied. Impairment of T-cell function was evident in the peripheral blood lymphocytes (PBL) and tumour infiltrating lymphocytes (TIL). T-cell activity was found to be normal in lymph node lymphocytes (LNL). NK cell and antibody dependent cellular cytotoxicity (ADCC) functions were intact in PBL, but were severely depressed in LNL and TIL. Moreover, the NK activity of the LNL and TIL was not responsive to rIFN$\alpha$.

*Lymphokines*

Murali *et al.* [32, 33] investigated the role of interleukin-2 (IL-2) in T-cell response and the ability of macrophages to secrete IL-1 and to exhibit tumoricidal activity in oral cancer. Augmentation of PHA response by IL-2 and expression of IL-2 receptors (CD25-Tac antigen) and production of IL-2 by PBL, TIL and LNL upon activation with mitogen were studied. An augmentation of PHA response by IL-2 was observed, but the augmented levels in PBL and TIL were found to be lower than those in the PBL from normal subjects. CD25 antigen expression was found to be depressed in the PBL from oral leukoplakia patients, treated oral cancer patients and in the TIL from oral cancer patients. IL-2 production in the PBL, TIL and LNL from oral cancer patients was normal, but was reduced in the PBL from leukoplakia patients. These observations revealed that there is no correlation between the IL-2 mediated events and the proliferative responses of mitogen stimulated T-cells from different lymphoid sources in cancer patients.

Studies have been conducted on the FcR and HLA-DR expression and IL-1 production by LPS and rIFN$\alpha$ stimulated peripheral blood monocytes from oral cancer patients, patients with oral premalignant lesions and from normal healthy subjects and by similarly stimulated macrophages from draining lymph nodes of oral cancer patients [31]. The LPS and rIFN$\alpha$ stimulated peripheral blood monocytes from untreated oral cancer patients showed a significant reduction in the percentage of FcR expressing cells when compared to normal subjects and patients with premalignant lesions. The number of oral cancer patients who showed IL-1 production in response to LPS/rIFN$\alpha$ stimulation was lower in comparison to normal subjects and patients with premalignant lesions. It was further observed that the production of IL-1 in response to LPS stimulation of macrophages from metastatic lymph nodes was lower than that by the macrophages from non-metastatic lymph nodes.

Cytotoxic effector functions of the peripheral blood macrophages from normal healthy subjects and from oral leukoplakia and oral cancer patients, subjected to treatment with LPS and rIFN$\alpha$, IL-2 alone or in combination at sub-optimal concentrations have been investigated [34]. The cytotoxicity of unstimulated monocytes, as assessed by the neutral red dye uptake by T-24 bladder carcinoma target cells, was considerably higher in untreated oral cancer patients and in the treated patients with tumour recurrence. This observation revealed that the monocytes from the subjects with tumour load were in a preactivated condition. After activation, the levels of cytotoxicity and the number of subjects exhibiting significant cytotoxic activity were lower among cancer patients when compared to healthy subjects. IFN$\alpha$ was found to be the most potent modulator in comparison to LPS and IL-2; a synergistic effect was apparent when two modulating agents were combined.

Gangal *et al.* [35] and Takate *et al.* [36] investigated the generation of lymphokine activated killer (LAK) cells in the three populations of lymphocytes, PBL, LNL and TIL, from oral cancer patients. The IL-2 treated TIL were found to have a high LAK activity, especially on autologous targets, but reduced NK/ADCC activity. In this study, PBL showed good NK/ADCC activities and an LAK activity that is better than

by LNL. LNL were found to have low NK/ADCC activity, but a considerable LAK activity, though lesser than those of TIL and PBL. IL-2 activated LNL showed an increased proportion of $CD8^+$ cells and a suppression effect on the generation of LAK cells in autologous PBL. Further, the frequency of IL-2 reactive T-cells was high in TIL and PBL, but was low in LNL. The reduced LAK activity of LNL was attributed to the existence of suppressor cells and a relatively fewer number of IL-2 reactive T-cells, the LAK progenitors, in this population of lymphocytes.

## HUMORAL IMMUNITY

*Immunoglobulins*

Several studies have revealed the impairment of humoral immunity in oral cancer patients. Pathological processes in the tissues and organs usually produce tissue damage with concomitant release of proteins in the circulation. The immunoglobulins (Igs) play an important role in the inactivation and neutralisation of the antigens associated with the tumour. Serum Ig levels serve as useful parameters to assess the status of humoral immunity in cancer patients. Khanna *et al.* [37] observed a significant rise in serum IgM and IgA levels in oral cancer patients. The rise was more pronounced in advanced clinical stages, but showed no correlation to tumour size, differentiation and treatment modalities. Dissemination of tumour mass was found to be a possible significant factor in the rise of Ig levels.

Vijayakumar *et al.* [38] estimated the levels of IgG, IgM, IgA, IgD and IgE in patients with oral cancer and in normal subjects. IgA, IgD and IgE levels were found to be elevated in the patients and were found to increase with progression of clinical stage. IgA and IgD levels returned to normal levels in patients with clinical cure, whereas IgE remained slightly elevated. The immunoglobulin levels remained high in patients who had residual tumours. Based on these observations, the authors suggested that estimation of IgA, IgD and IgE levels could be useful in diagnosis and prognosis of oral cancer. Rajendran *et al.* [19] observed elevated levels of IgA, IgD and IgE in oral submucous fibrosis. Scully *et al.* [39] from the U.K. have reported elevated levels of IgG, IgA, IgD and IgE in oral cancer patients.

Tissue Ig levels have been evaluated in oral cancer by Pillai *et al.* [40]. IgG and IgA were found in the saline extract of oral cancer tissues, but not in normal oral mucosal tissues. Ravindran *et al.* [41] observed elevated levels of IgG and IgA in the saline extracts of oral cancer and oral submucous fibrosis tissues, the elevation being more pronounced in oral cancer. IgG occurred in trace amounts in the extracts of normal and leukoplakia tissues, while IgA in trace amounts was found only in leukoplakia, not in normal tissues. A trace of IgM occurred only in oral submucous fibrosis. In these two studies, Ig were found to be present only in saline extracts, not in 3 mol/L KCl extracts of the tissues, indicating that Ig were present either in the soluble cytoplasmic component or on the surface of cells, loosely attached to other membrane proteins. An association of herpes simplex virus type I and oral cancer has been reported [5-9], and if the virus is an aetiological factor in oral cancer, viral antigens could be expected on the tumour cells. This might explain the presence of Ig in oral cancer tissue extracts. Another possibility is the presence of oral cancer-associated antigen(s) [53] on the cell surface which would induce the production of specific antibodies.

*Complement system*

The complement system plays an important role in the normal immune response, and is reported to be the primary humoral mediator of antigen antibody reaction [42]. In many pathological conditions, some of the components of the complement system elicit primary or secondary responses [43]. Vijayakumar *et al.* [44] studied the changes in the levels of total complement activity and C3 and C4 fractions in oral cancer patients at different clinical stages. The total complement activity and C3 and C4 levels were elevated and this elevation was in accordance with the clinical stage of the disease. In the patients with a clinical cure, the levels returned to normal values. Elevated levels of total complement activity and C3 and C4 have also been reported in oral submucous fibrosis [45]. Results of these studies suggest the possibility that the complement system tries to maintain immunological homeostasis by compensating for the weakened cellular immune responses.

*Circulating immune complexes*

The neoantigens expressed by tumour cells may be released into the extracellular environments and hence, be found in free form and/or as antigen–antibody complexes or circulating immune complexes (CIC) in the serum or in other body fluids. The soluble CIC can serve as major blocking factors in circulation and modulate the effector mechanisms against tumour cells. Attempts have been undertaken to study CIC in oral cancer patients and in patients with oral premalignant lesions. Vijayakumar *et al.* [46] reported significantly elevated levels of CIC in the serum samples from oral cancer patients. The CIC levels were found to be increasing with progression of clinical stage. The patients who had a clinical cure were found to have considerably decreased levels of CIC, whereas it remained elevated in patients who had residual lesions. Analysis of Ig content of CIC revealed significantly elevated levels of IgG and IgM in the CIC from patients. IgD was found only in the CIC from patients, and the incidence of IgA was remarkably high in patients. The authors stressed the usefulness of the evaluation of CIC levels in the diagnosis and prognosis of the cancer, and also, in monitoring the tumour growth. Mukhopadhyay *et al.* [47], Raghunath *et al.* [48, 49] and Abraham and Balaram [50] have also reported elevated levels of CIC in oral cancer patients. Scully *et al.* [39] had previously reported increased CIC levels in the cancer patients from abroad.

Remani *et al.* [51] estimated CIC and their Ig contents in the serum samples from patients with oral leukoplakia, oral submucous fibrosis and oral cancer. The CIC and their Ig contents were significantly elevated in oral submucous fibrosis and oral cancer. The authors noted the possible usefulness of the estimation of CIC and their Ig content in monitoring the malignant transformation of oral submucous fibrosis.

## TUMOUR ASSOCIATED ANTIGENS

Identification and characterisation of tumour associated antigens (TAA) in CIC can lead to the assessment of their potential use as diagnostic and/or prognostic markers and may provide insight into the aetiology and host interaction with a developing tumour [52]. Recently Shanavas *et al.* [53] demonstrated an oral cancer associated antigen in the CIC and cancer tissue from oral cancer patients. Antiserum raised against the CIC from oral cancer patients (OCIC), after thorough absorption with normal serum constituents, reacted

with the serum samples and CIC from oral cancer patients and with a saline extract of oral cancer tissue. The antiserum failed to react with normal serum samples and extract of normal oral tissue. An antiserum raised against the saline extract of oral cancer tissue (crude oral cancer antigen—COCA) and adsorbed with normal tissue extract reacted with OCIC and COCA. The TAA isolated from COCA by gel-filtration on Sephadex G-200, was found to have an immunological reactivity similar to that of the antigen obtained from OCIC. Attempts are being made to characterise this antigen and to prepare monoclonal antibodies against it.

## CONCLUSION

The primary role of the normally functioning immune system is to provide defence against malignant cells which are constantly arising throughout the body. Therefore, the failure of immune surveillance has been reported to be an important factor in the development of cancer. Only a limited number of studies have been undertaken so far on the immunology of oral cancer in other countries apart from India [9, 10, 29, 30, 39]. These studies have noted impairment of cell-mediated and humoral immunity in the patients. The studies so far conducted in India show that there is alteration both in humoral and cellular immune responses in oral cancer patients and the assessment of immunological status may be of use not only in the diagnosis and/or prognosis of oral cancer, but also for the effective management of the disease. In depth studies on oral cancer are being undertaken in India to find the possible aetiological factors responsible for the disease, to discover a biological marker to predict the malignant transformation of premalignant lesions and to develop monoclonal antibodies for diagnosis and treatment.


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