LYMPHOKINE PATTERNS IN CARCINOMA LARYNX USING SKIN WINDOW TECHNIQUE

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Tumour antigen induced lymphokine response was studied in patients with laryngeal carcinoma and benign tumours. Release of factors were studied in relation to the stage of disease. Malignant tumours show significant production of MIF (Macrophage Inhibitory Factor), Leucocyte Inhibitory Factor (LIF) and Neutrophil Chemotactic Factor (NCF). NCF showed increasing production with the stage of disease and lymph node metastasis showing direct relationship but MIF production diminished with increasing stage and lymph node metastasis showing inverse relationship. The study shows possible relation of tumour dissemination and failure to produce MIF response.

Immune response is known to develop against malignant tumours. Malignant tumour cells express newer antigen on their cell membranes which elicit both T cell and B-cell mediated response. There are definite in vitro evidences that T-Cell sensitization develops in patients bearing malignancies. The effector T-cells eliminate tumour after releasing short range mediators lymphokines like migration inhibitory factor, chemotactic factor etc. These migration inhibitory factors suppress cell migration from the site of the inflammation. In early part of inflammation neutrophil migrates followed by lymphocyte and monocytes-macrophage cells. The lymphokines effect locomotion of neutrophils and mononuclear cells thus resulting in immune modulation of inflammatory process.

Skin window technique used in the present study appears to be an in vitro correlation of delayed cutaneous hypersensitivity. Tumour antigen applied on the skin abrasion appeared to sensitize the host T-cells and effects cell migration. The production of lymphokines has been studied in relation to malignant tumour and their stages.

Material and Methods

A. Patients and Control Population:

Fourteen patients with laryngeal carcinoma and four with benign tumours and amyloidosis have been studied.

Commonest site of primary tumour was supraglottis (72%) followed by glottis (28%). Eight of the 14 patients also had regional lymph node metastasis. Two cases had stage II disease, 4 stage III and 8 patients had advanced (stage IV) disease (Shaw, 1979).

B. Immunological:

Biopsy piece from each case was taken and crushed in mortar and pestle and soluble antigen was prepared. Total protein content was measured by Biuret method (King and Wootton, 1975) and protein content was adjusted to 2 mg%. These antigenic preparations were found to be nontoxic.

The method of skin abrasion was modified from the one described earlier by Dizon et al, 1963. Briefly 6 abrasions each of 2 cm diameter were
created on the volar aspect of the forearm using sand paper (No. 120) until capillaries appeared on the exposed dermis. Three abraded areas were covered with naked cover slips (22 × 22 cm) and fixed with leucoplast while on three other coverslips one drop (01 ml) of tumour antigen was layered before covering the abraded skin. The coverslips were removed after 6 hours and replaced by another set of coverslips. The coverslips were removed after 36 hours and stained with Leishman method.

Differential leucocyte counts were done at 5 different sites on each coverslip and only those sites were considered for study where at least 10 cells were counted and mean value was determined. Difference of 15% in differential cell counts on naked and autologous tumour antigen containing coverslips was considered to be a significant change (Table) and this determined the lymphokine response. (Microphotograph no. 1 & 2.

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Mean ± SD% of cell in patients with Antigen</th>
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<tbody>
<tr>
<td></td>
<td>Stage II n=2</td>
</tr>
<tr>
<td>A. Neutrophil</td>
<td>710 ± 56</td>
</tr>
<tr>
<td>B. Lymphocytes</td>
<td>1842 ± 32</td>
</tr>
<tr>
<td>C. Macrophages</td>
<td>765 ± 23</td>
</tr>
<tr>
<td>D. Eosinophils</td>
<td>350 ± 2</td>
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Delayed hypersensitivity (DH) was tested using 01 ml of autologous tumour antigen. Antigen was injected intradermally on the volar aspect of forearm. After 72 hours size of induration and erythema was noted (Bates et al, 1979).

**Results**

Skin window test revealed neutrophilic chemotactic factor production (NCF) in 12 out of 14 cases (85.7%), macrophage migration inhibitory factor (MIF) in 2 out of 14 cases (14.3%), while neutrophilic inhibitory (NIF) factor production was seen in 85.7% of cases. Four cases each showed basophilic hypersensitivity and delayed cutaneous hypersensitivity to tumour antigen in patients bearing malignant tumours (Fig. 1).

![Photomicrograph 1. Shows skin window response after 36th hour of naked coverslip.](image1)

![Photomicrograph 2. Shows skin window response after 36th hour coverslip containing autologous tumour antigen.](image2)

![Graphical representation of skin window and dermal responses according to type of lesion.](image3)

Fig. 1. Graphical representation of skin window and dermal responses according to type of lesion.

Production of these factors in patients of benign tumours and patients having amyloidosis of larynx was insignificant.

In patients of stage IV tumours (n = 14) there were seven cases showing NCF and 7 showed NIF. In one case each BMH and DH to tumour...