Interleukin 4 Receptor Expression on Human Lung Tumors and Normal Lung

Muhammad F. Tungekar, Helen Turley, Michael S. Dunnill, Kevin C. Gatter, Mary A. Ritter, and Adrian L. Harris

ABSTRACT

Interleukin 4 (IL-4) receptors were detected by a monoclonal antibody on tumor cells of 10 of 29 squamous cell carcinomas and 6 of 17 adenocarcinomas of the lung. None of the small cell carcinomas or carcinoid tumors stained. Parallel sections stained for epidermal growth factor receptors showed that all but 2 of the IL-4 receptor-positive tumors also expressed epidermal growth factor receptors. Positive labeling for IL-4 receptors was obtained on nonneoplastic bronchial epithelium and on lymphocytes and macrophages infiltrating the tumor stroma. The role of IL-4 and its receptor in normal human lung is unknown, but the expression of IL-4 receptors on particular subtypes of lung tumors suggests that they may have a role in differentiation or proliferation of squamous and adenocarcinomas.

INTRODUCTION

The discovery of peptide molecules that function as proliferation signals for a range of cell types has aroused tremendous interest in the field of tumor biology (1). Various growth factors and lymphokines such as interleukins and interferons belong to this family of peptide regulatory factors that exert their effects by binding to specific receptors on the surface of target cells (2). The interleukins were first identified as regulatory molecules of the immune system, although the pleiotropic nature of their effects over a wide range of other cell types is being increasingly acknowledged (3). The importance of unraveling possible tumor-modulating effects of lymphokines has been highlighted in recent years by novel therapeutic approaches utilizing interleukins and interferons as therapeutic agents (4). IL-4 (5) was originally described as a B-cell growth factor (5), but its receptors are present on cells of lymphoid and myeloid lineages and have been reported on two epithelial cell lines (6). It is known that IL-4 may either suppress or enhance the growth of hematopoietic progenitor cells depending on the lineage and differentiation of target cells (7) and it is therefore a candidate for lymphokine-based tumor therapy. IL-4 has been shown to have in vivo antitumor effects on cell lines derived from a variety of murine tumors on its own (8) and may potentiate similar effects of other lymphokines such as IL-1β and IL-2 (9). However, to date, there is no detailed information concerning the influence of IL-4 on tumor progression or data on the distribution of IL-4 receptors in any individual human organ system.

Expression of several oncogenes have been described in human lung cancer and are potentially involved in its development. One such is the protooncogene c-erbB2 (also known as neu), which shares sequence homology with the EGF receptor. c-erbB2 is normally expressed in bronchial mucosa but may be overexpressed in lung tumors without amplification or point mutation (10). Its presence in breast cancer has been well studied, although the prognostic significance of its amplification and overexpression is presently controversial (11-13). Peptide growth factor receptors, too, are widely distributed in tumors and normal tissues and show different levels of expression in tumors, compared with adjacent tissue. In particular EGF receptor has been demonstrated in many tumors of lung, breast, bladder, and brain (14-18) and, more importantly, a correlation with pathological stage and survival for breast and bladder carcinomas has been demonstrated (18, 19).

Recently, a preliminary study, in which a monoclonal antibody, MR6, recognizing the IL-4-R complex (19, 20) was used, showed that IL-4 receptors are present on a number of carcinomas of various types including one case of lung cancer (21). Because of the availability of this antibody the present study was undertaken to investigate the detailed expression of IL-4 receptors on human lung tumors. EGF receptor expression has been studied in parallel both as a control, since its distribution is already well documented in lung tumors, and in order to determine the extent of coexpression of EGF receptors with IL-4 receptors. This study shows that IL-4 receptors are coexpressed with EGF receptors on approximately one-third of cases of squamous cell carcinoma and adenocarcinoma. In contrast, both are absent from cases of small cell carcinoma and carcinoid tumor.

MATERIALS AND METHODS

Blocks from 63 surgically resected lung tumors were snap frozen and stored in liquid nitrogen. The tumor types, classified according to the World Health Organization classification (22), comprised 29 cases of squamous cell carcinoma, 17 cases of adenocarcinoma, 10 cases of small cell carcinoma, and 7 cases of carcinoid tumor. In 10 cases nonneoplastic lung tissue removed from the resection specimen was available for use as a control. The primary antibodies used are summarized in Table 1. In all cases, staining with Ki67, a monoclonal antibody reactive with a nuclear proliferation-associated antigen, was carried out to check the antigenic preservation in the tumor cells since the Ki67 antigen is known to be exquisitely sensitive to any form of degradation (23). Immunohistochemical staining was performed using the alkaline phosphatase:anti-alkaline phosphatase procedure as described previously (24).

Antibody MR6. The monoclonal antibody MR6 was raised against an extract of thymic tissue (19) and shown to react strongly with thymic cortical epithelial cells. By Western blotting MR6 detects a single polypeptide of Mr, 200,000 which is rapidly cleaved to Mr, 145,000 by proteolytic enzymes (20). This latter is known to be the approximate molecular weight of one of the four polypeptide chains associated with the IL-4 receptor. Flow cytometric and immunohistochemical studies showed the antigen to be present also on T- and B-lymphocytes, cells of myeloid lineage, and some renal epithelium (20). Recent experiments (22) strongly suggest that MR6 binds to the IL-4 receptor complex by demonstrating that MR6 inhibits IL-4-induced T-cell proliferation and completely abrogates the IL-4 production of specific antigen-induced IgE by B-cell populations. MR6 does not, however, block binding of IL-4 to its receptor, which indicates that it recognizes a molecule closely
associated with the three chains of the IL-4-R but which does not form
the ligand-binding component of the receptor.

RESULTS

Staining for IL-4 receptors using monoclonal antibody MR6
was seen in tumor cells of 10 of 29 squamous cell carcinomas
and 6 of 17 adenocarcinomas (Table 2). All of the small cell
carcinomas and carcinoid tumors were unlabeled. In all samples
lymphocytes and macrophages were also positively labeled. In
the nonneoplastic lung tissue and also in many tumor samples
strong staining of bronchial epithelium was also seen. Staining
was seen both on the cell membranes and in the cytoplasm. All
of the tumors showed homogeneous staining apart from one
adenocarcinoma in which it was distributed focally.

Of the 2 monoclonal antibodies reactive with the EGF recep-

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>MR6</td>
<td>M, 45,000, IL-4 receptor</td>
<td>M. A. Ritter (18–21)</td>
</tr>
<tr>
<td>EGFR1</td>
<td>Epidermal growth factor receptor</td>
<td>ICRF (10)</td>
</tr>
<tr>
<td>G15</td>
<td>Epidermal growth factor receptor</td>
<td>Dr. Gregoriou, Molecular Biophysics, Oxford, England</td>
</tr>
<tr>
<td>Ki67</td>
<td>Proliferation-associated antigen</td>
<td>Dakopatts (16)</td>
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Table 2 Details of tumor types and antibody staining results

<table>
<thead>
<tr>
<th>Histological type</th>
<th>IL-4-R+/EGFR-</th>
<th>IL-4-R+/-EGFR+</th>
<th>IL-4-R-/EGFR+</th>
<th>IL-4-R-/EGFR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma (n = 29)</td>
<td>0</td>
<td>10</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Adenocarcinoma (n = 17)</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Small cell carcinoma (n = 10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Carcinoid (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Fig. 1. Squamous cell carcinoma expressing IL-4 receptors (A; detail at higher power in C). This is contrasted with the stronger staining of the same tumor for epidermal growth factor receptors (B) and a different squamous cell carcinoma unlabeled by the anti-IL-4 receptor antibody (D). Both IL-4 receptors and epidermal growth factor receptors are present on normal bronchial epithelium (arrows in A and B). Note that in both C and D surrounding reactive lymphoid cells are strongly labeled for IL-4 receptors (*).
tor, EGFR1 labeled 26 of 29 squamous cell carcinomas and 11 of 17 adenocarcinomas, giving a higher positivity rate than G15 which stained 18 of 27 and 9 of 17 of these tumors. Since all of the G15 cases were also EGFR1 positive, the results in Table 2 are given under the single heading of EGFR. All samples of small cell carcinoma and carcinoid tumor tested were negative for both antibodies. All of the IL-4 receptor-positive squamous cell carcinomas also expressed EGF receptors as did 4 of the 6 IL-4 receptor-positive adenocarcinomas (Fig. 1). In all cases such coexpression was seen in the same areas and appeared to be involving the same cells.

In the nonneoplastic lung, more intense reactivity for EGF receptors was seen in basal compared to luminal bronchial epithelial cells, whereas MR6 uniformly stained the bronchial mucosa: this was consistently seen regardless of the reactivity of tumor itself for these markers. MR6 also stained lymphocytes and macrophages infiltrating the stroma.

**DISCUSSION**

This study has demonstrated that IL-4 receptors are frequently expressed by squamous cell carcinomas and adenocarcinomas of the lung, whereas small cell carcinomas and carcinoid tumors consistently lack them. This selective distribution of IL-4 receptors among lung tumor types is strikingly similar to that of EGF receptors as observed by us and others (26). These observations may relate to mechanisms of development of different types of lung tumors. Although many studies have indicated that all lung tumors arise from a common stem cell type (27) and are frequently of mixed morphological type (28), the clinical profile of small cell carcinoma is quite distinct from that of the other tumor types and requires different clinical management. The absence of both EGF receptors and IL-4 receptors in small cell carcinoma and their presence in other types of lung cancer imply a different set of regulatory mechanisms affecting squamous cell carcinomas and adenocarcinomas, presumably due to differences in the routes of differentiation followed by the putative tumor stem cells.

The present study has shown that both EGF and IL-4 receptors are expressed in normal bronchial mucosa, although there appears to be down-regulation of expression of EGF receptors in the more differentiated luminal epithelial cells. IL-4 receptor activation induces expression of class I and II major histocompatibility complex molecules on cells of monocytic lineage (29). If this occurs in normal and malignant epithelium also, it may be important in modulating the local immune response. It will be of interest to assess coexpression of IL-4 receptors with these regulatory molecules.

In the case of squamous cell carcinomas and adenocarcinomas the present findings may be of therapeutic value. Since both chemotherapy and radiotherapy are relatively ineffective in their management, it may be possible in the future to influence the growth of these tumors by utilizing the presence of EGF receptors and IL-4 receptors for therapeutic manipulations.

**REFERENCES**

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