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Cellular expression of interleukin 2 (IL-2) and its receptor (IL-2R, CD25) in lung tumours

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In view of the unclear prognostic and diagnostic role of interleukin 2 (IL-2) and its receptor in human tumours, we examined the cellular expression of IL-2 and of the subunit α of its receptor (IL-2Rα, CD25) in relation to the proliferative activity of various subtypes of lung tumours. The immunocytochemical ABC technique was applied to archival tissue material of neuroendocrine lung tumours: lung carcinoids, including typical carcinoids (TC), atypical carcinoids (AC) and small-cell lung cancers (SCLC) and squamous cell lung cancers (non-small cell lung cancers, NSCLC). Expression of IL-2 was detected in all types of lung tumours. The highest frequency of IL-2 expression (93%) was noted and the most pronounced semi-quantitatively evaluated expression of IL-2 was detected in AC tumour cells. The expression was more pronounced as compared to neoplastic SCLC ($p = 0.01$) and NSCLC cells ($p = 0.005$). The results suggest a negative correlation between IL-2 expression and the proliferative activity of tumour cells (evaluated by expression of Ki-67) in AC. The frequency of detection of IL-2 receptor (IL-Rα, CD25) was the highest in NSCLC (94%). Semi-quantitative expression of IL-2R, like that of IL-2, also dominated in the group of atypical lung carcinoids but manifested a significant difference only as compared to typical carcinoids ($p = 0.014$). Within the groups of tumours studied no correlation could be detected between cellular expressions of IL-2 and IL-2R. Our results demonstrate variable expression of IL-2 and its receptor in various types of lung tumours, but no simple relationship could be detected between tissue expression of the markers and proliferative activity. Appraisal of the diagnostic and/or prognostic significance of the results requires further study.

Key words: lung cancers, proliferating marker Ki-67, IL-2, IL-2 receptor

INTRODUCTION

Primary lung cancer is at present the most frequently diagnosed type of tumour and one of the principal causes of human death worldwide. Incidence and mortality due to lung cancer manifest rising tendencies, particularly in the developing countries [20].

The interest of investigators in interleukin 2 (IL-2) and its receptor (IL-2R) is linked, first of all, to tumour immunotherapy and syndromes associated with immune defects (*e.g.* AIDS) [10, 14]. Modified human IL-2 obtained by genetic engineering acts in a similar way to human endogenous IL-2. It strongly stimulates immune responses (lymphocytes T),

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augments the proliferation and activity of cytotoxic NK cells, and increases the release of other cytokines (*e.g.* TNF, IL-1) by monocytes and macrophages [18, 19, 27]. Nevertheless, the final results of anti-neoplastic therapy with IL-2 remain far from satisfactory. Most studies have demonstrated production of IL-2 by immunocompetent cells isolated from the blood of tumour-bearing patients. The results have frequently been incoherent or contradictory. Few of the investigators have focused their attention on the tissue expression of IL-2 and its receptor and their reciprocal relationship. It also remains unclear why IL-2 inhibits growth of certain cells of human tumours, while the proliferation of others continues in parallel with similarly pronounced expression of IL-2Rβ in their cell membranes. It has been suggested that the endogenous IL-2 has, like other growth factors and hormones, been involved in growth control in tumour cells [12]. Opinions have also been expressed that endogenous IL-2 stimulates tumour cell proliferation (in squamous cell carcinomas of the head and neck) and that inhibition of tumour growth can be obtained using anti-sense IL-2 [23].

Studies on the role of the receptor for IL-2, and the soluble form of its subunit α (sIL-2R) in particular, have mainly involved measurements of their serum levels in patients [13–15]. Recent studies have focused on the determination of correlations between IL-2Rα expression and the growth of tumour cells *in vivo* [10]. The results of these studies have shown that over-expression of IL-2Rα is responsible for stimulated proliferation of tumour cells [26, 28]. Numerous investigators have demonstrated over-expression of IL-2Rα in tumours, including cancers of the lung, skin, prostate and oesophagus, and in leukaemias [10, 11]. One of the mechanisms of growth promotion in tumour cells by IL-2Rα is supposed to involve inhibition of apoptosis [22, 25].

Most of the studies on IL-2 in lung tumours have pertained to non-small cell lung cancers (NSCLC) studied either *in vitro* or *in vivo* [4, 12, 14, 29]. However, production of the cytokine was studied mainly in blood immunocompetent cells (lymphocytes, monocytes). In general, a suppression of the secretion of selected cytokines, which is dependent upon the clinical advancement of the lung tumour, has been noted [5]. Moreover, decreased IL-2 production has been demonstrated to correlate with the duration of patient survival and it has been suggested that decreased IL-2 production at the moment of SCLC diagnosis provides an important independent prognostic factor.

In general, in studies on the receptor for IL-2 (IL-2R) in lung tumours elevated serum levels of sIL-2R have been noted. This finding has correlated with the progression of the tumour and with the aggressiveness of the disease and, in some studies, with decreased levels of IL-2 itself [4, 13, 16]. On the other hand, attempts to establish a relationship between serum sIL-2R levels and respective levels of IL-2Rα (Tac molecule, CD25) in lung tumour tissue have had divergent results. The roles of IL-2 and its receptor, produced by the tumour cells themselves, have remained poorly defined.

In view of the incomplete clarification of the reasons for the ineffective therapy of lung tumours and the irreproducibility of data on cytokine expression by tumour cells, we decided to examine tissue expression of IL-2 and its receptor (IL-2Rα, CD25) in histologically different types of lung tumour.

MATERIAL AND METHODS

The material for the studies included buffered formalin-fixed paraffin-embedded histologically different subtypes of lung tumours: typical (TC) (n = 14) and atypical lung carcinoids (AC) (n = 8), small-cell (SCLC) (n = 24), and squamous cell lung cancers (the NSCLC group of tumours) (n = 17). The material was histopathologically evaluated by two independent pathomorphologists. The positive control material involved reactive lymph nodes from patients free of neoplastic disease (n = 15). In studies on the tissue material routine staining techniques for histopathological examination were used, including haematoxylin and eosin, silver impregnation and immunocytochemistry. The avidin-biotin peroxidase complex (ABC) technique was employed [8] with the use of monoclonal mouse antibodies (MAbs) to human IL-2 at the dilution of 1:100 (R&D Systems) and to human CD25 (IL-2Rα) at the same dilution (NOVOCASTRA), as well as MAbs to human Ki-67 antigen (clone MIB-1) at the dilution of 1:2 (DAKO). The colour reaction was evoked with the horseradish peroxidase substrate, 0.05% DAB in 0.05 M Tris-HCl buffer, pH 7.6, supplemented with 0.001% H_20_2. A positive reaction was manifested in at least three sequential sections as a dark brown or black precipitate in the cell membranes, cell nucleus and/or cytoplasm. Control reactions employed control sera of the respective species in 0.05 M Tris-HCl, pH 7.6, supplemented with 0.1% BSA and 15 mM sodium azide (negative control).

Table 1. Evaluation of IL-2 and IL-2R (CD25) expression in the studied preparations according to Remmele and Stegner [24]. The final score represents a product of the positive cell score (**A**) and the score reflecting the intensity of the colour reaction (**B**)

A
0: no cells with positive reaction
1: to 10% cells with positive reaction
2: 11 to 50% cells with positive reaction
3: 51 to 80% cells with positive reaction
4: > 80% cells with positive reaction
B
0: no colour reaction
1: low intensity of colour reaction
2: average intensity of colour reaction
3: intense colour reaction

No reaction: score 0 points; weak reaction: score 1 to 2 points; average intensity of the reaction: score 3 to 4 points; intense reaction: score 6 to 12 points

Semi-quantitative evaluation of the results and statistics

The intensity of expression of IL-2 and IL-2R (CD25) proteins in lung cancers obtained using ABC was estimated by the use of the semi-quantitative IRS scale according to Remmele and Stegner [24], taking into account the intensity of the colour reaction and the number of positive cells. The final score represented the product of the scores representing the two variables and ranged from 0 to 12 points. In each preparation 10 visual fields were appraised at a total magnification of 400x, and mean scores were calculated (Table 1). Expression of Ki-67 proliferative antigen (nuclear reaction) was calculated, taking into account the mean percentage of immunopositive cells in 10 visual fields of a light microscope. The expression was defined in line with the modified semi-quantitative scale of Gatter et al. [7], where a score of 1 denoted up to 10% of cells with a positive reaction and scores of 2, 3 and 4 corresponded to, respectively, 11–25%, 26–50% and ≥ 51% cells with a positive reaction. All the preparations were examined with an OLYMPUS B-2 light microscope.

In order to determine the statistical significance of variations in the intensity of expression of the investigated proteins and the Ki-67 antigen we first calculated the mean values of the staining scores in each group of lung cancers. The means were compared using the Mann-Whitney U test for non-parametric independent data. Correlations between data rows were determined employing Spearman's rank correlation index. A p value below 0.05 was assumed to indicate significant difference.

RESULTS

Cellular expression of Ki-67 antigen in lung tumours

Expression of Ki-67 proliferation antigen involved exclusively cell nuclei of neoplastic cells (Fig. 1A, B). The number of immunopositive cell nuclei varied in individual types of lung tumour (Table 2). The significantly lowest proliferative activity was demonstrated by typical lung carcinoids as compared to the remaining groups of tumours; AC, SCLC and NSCLC did not differ significantly in the expression of Ki-67 antigen (Fig. 4). In the control material (non-neoplastic lesions) individually labelled cell nuclei were observed in germinal centres of the lymphoid follicles.

Cellular expression of interleukin-2 in lung tumours

Cellular expression of IL-2 was demonstrated in all four types of lung tumour studied. It was documented in 13/14 (93%) typical carcinoids, in 7/8 (88%) atypical lung carcinoids, in 21/24 (88%) small cell and in 13/17 (76%) squamous cell lung carcinomas. Marked individual differences were observed in IL-2 expression in individual patients (Table 2). IL-2 was detected in the cytoplasm of tumour cells and the most evident cytoplasmic reaction was detected in the cells of typical lung carcinoids (Fig. 2A, B). IL-2 expression was also visible in the cells of inflammatory infiltrates which accompanied lung tumours, but such cells were not subjected to semi-quantitative evaluation in the present study. Semi-quantitatively, the most pronounced expression of IL-2 was detected in tumour cells of atypical carcinoids and it was significantly higher than the expression noted in SCLC ($p = 0.01$) or NSCLC ($p = 0.005$). Moreover, a tendency was observed for expression of IL-2 in cells of TC to be augmented in comparison with those in NSCLC ($p = 0.058$) (Fig. 5).

In the control material (non-neoplastic lesions) expression of IL-2 was seen mainly in lymphocytes T and macrophages/monocytes within inflammatory lesions in the lymph nodes.

Cellular expression of the subunit α of the IL-2 receptor (IL-2Rα, CD25) in lung tumours

Immunocytochemical studies have demonstrated expression of IL-2Rα (CD25) in 5/14 (36%) cases

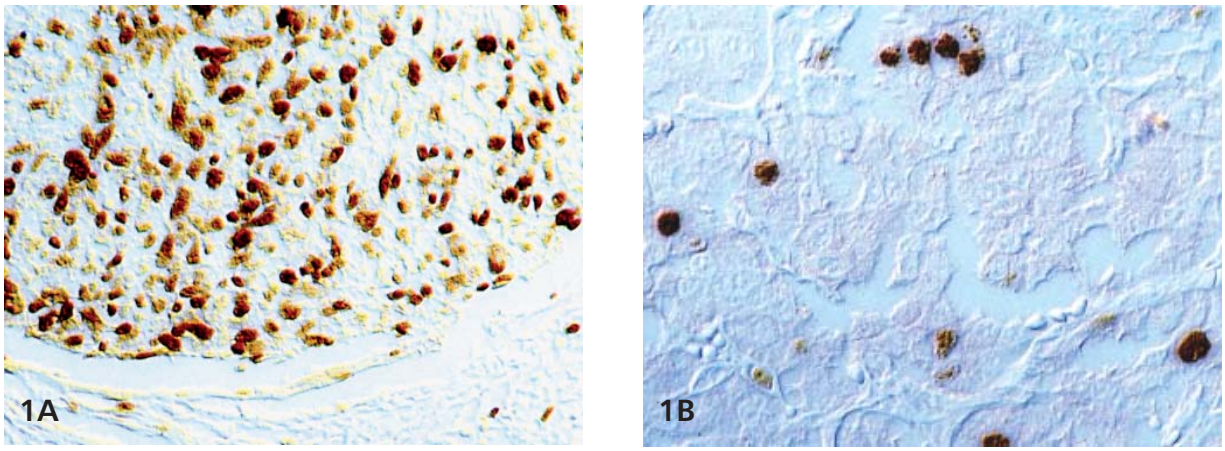

Figure 1. A. Small-cell lung carcinoma. Numerous MIB-1-positive (Ki-67 antigen) in cell nuclei. Patient no. 4; **B.** Immunocytochemical localisation of Ki-67 in typical lung carcinoid. Patient no. 10. ABC technique. All bar markers represent 20 μm.

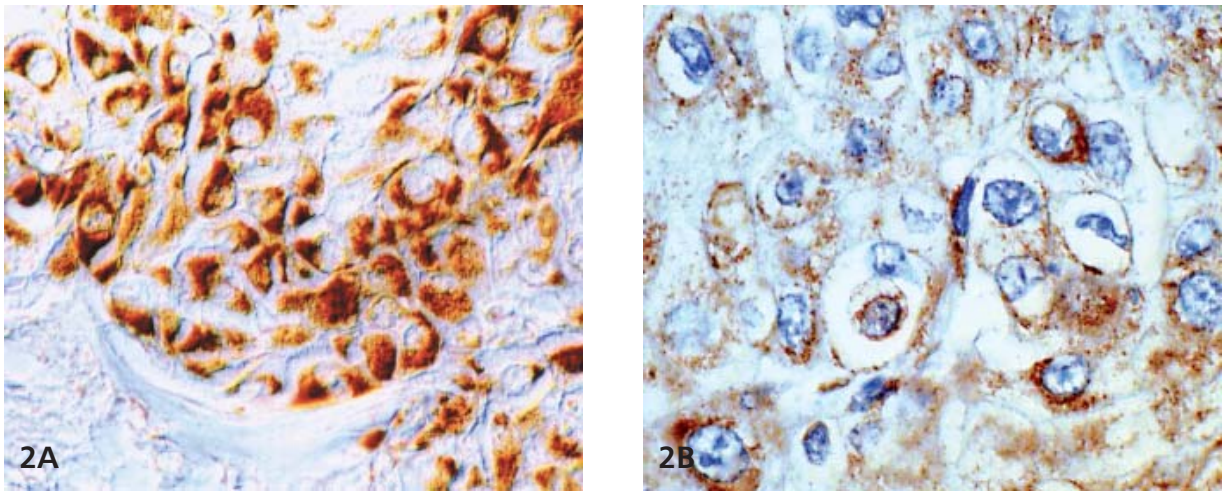

Figure 2. A. Immunocytochemical localisation of IL-2 in typical lung carcinoid. Patient no. 2. ABC technique; **B.** Immunocytochemical localisation of IL-2 in squamous cell lung carcinoma. Patient no. 9. ABC technique. Counterstained with haematoxylin (B). All bar markers represent 20 μm.

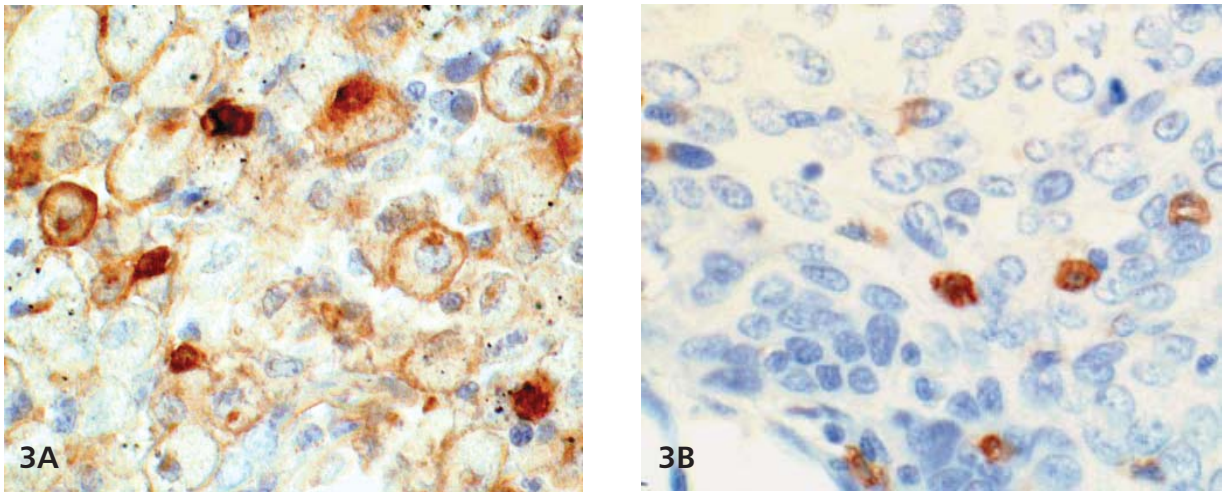

Figure 3. A. Immunocytochemical localisation of IL-2Rα (CD25) in squamous cell lung carcinoma. Note the membranous localisation of CD25. Patient no. 10; **B.** Cytoplasmic and membranous localisation of IL-2Rα (CD25) in squamous cell lung carcinoma. Patient no. 6. ABC technique. Counterstained with haematoxylin. All bar markers represent 20 μm.

Table 2. Semi-quantitative evaluation of Ki-67 antigen, IL-2 and IL-2R expression in patients with different types of lung carcinoma according to Remmele and Stegner [24]

Patient (no.)	Typical carcinoid			Atypical carcinoid			SCLC			NSCLC		
	Ki-67	IL-2	IL-R	Ki-67	IL-2	IL-R	Ki-67	IL-2	IL-R	Ki-67	IL-2	IL-R
1	1	6	3	2	12	10	4	0	0	3	3	3
2	2	12	0	1	9	0	4	2	0	4	3	3
3	2	3	0	4	12	6	2	2	0	2	6	0
4	2	4	0	3	8	3	4	2	3	3	3	3
5	1	12	12	4	0	3	1	4	0	3	3	6
6	1	12	0	4	6	4	3	3	0	4	6	3
7	1	3	4	4	6	6	4	6	6	4	0	6
8	1	3	0	4	6	10	4	0	6	4	6	8
9	2	6	0				3	3	4	3	6	3
10	1	1	0				3	6	3	4	3	4
11	1	2	0				3	4	6	4	3	4
12	1	6	0				1	6	4	3	0	3
13	2	0	3				4	2	2	3	0	6
14	1	8	3				3	6	6	3	2	3
15							4	3	2	3	0	3
16							1	3	3	4	3	6
17							4	0	4	4	2	2
18							4	6	0			
19							3	6	6			
20							4	6	0			
21							4	6	0			
22							4	8	10			
23							4	3	6			
24							4	6	0			

SCLC — small-cell lung carcinoma; NSCLC — non-small cell lung carcinoma (squamous cell lung carcinoma)

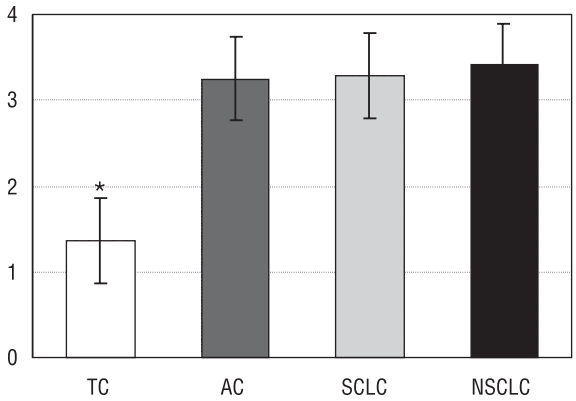

Figure 4. Average expression of Ki-67 antigen in studied groups of lung tumours on the four-point scale of Gatter et al. [7]; TC — typical lung carcinoid; AC — atypical lung carcinoid; SCLC — small-cell lung carcinoma; NSCLC — non-small cell lung carcinoma (squamous cell lung carcinoma); *p = 0.001 for TC *vs.* AC; p = 0.00001 for TC *vs.* SCLC and p = 0.000002 for TC *vs.* NSCLC.

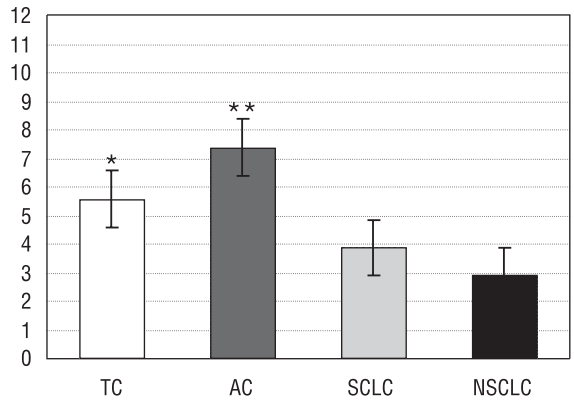

Figure 5. Expression of IL-2 on the semi-quantitative 12-point IRS scale [24] as related to histological type of lung tumour; TC — typical lung carcinoid; AC — atypical lung carcinoid; SCLC — small-cell lung carcinoma; NSCLC — non-small cell lung carcinoma (squamous cell lung carcinoma); *p = 0.058 for TC *vs.* NSCLC; **p = 0.01 for AC *vs.* SCLC; p = 0.005 for AC *vs.* NSCLC.

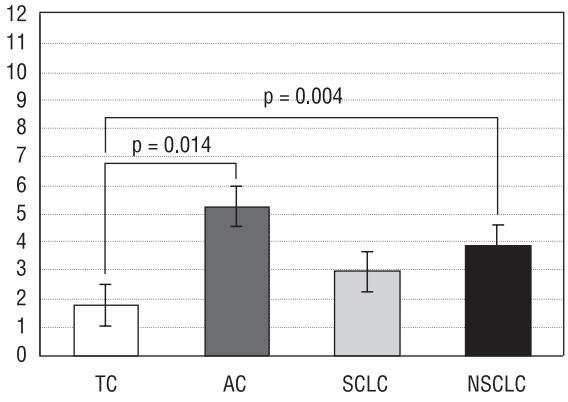

Figure 6. Expression of IL-2 receptor (IL-2R) on the semi-quantitative 12-point IRS scale [24] as related to histological type of lung tumour; TC — typical lung carcinoid; AC — atypical lung carcinoid; SCLC — small-cell lung carcinoma; NSCLC — non-small cell lung carcinoma (squamous cell lung carcinoma).

of typical lung carcinoids. In atypical lung carcinoids expression of IL-2R was shown in the same proportion of patients as demonstrated expression of IL-2 (88%). In SCLC expression of IL-2R pertained to 15/24 (62%) cases. The most frequent tissue expression of IL-2R was detected in squamous cell lung cancers (94%) (Table 2). The product of the immunocytochemical reaction was observed in diffusely spread or focally arranged cells within their cell membranes and cytoplasm (Fig. 3A, B). In semi-quantitative comparisons of IL-2R expression using the IRS scale, as with the IL-2 expression, it was most pronounced in the atypical lung carcinoid group but differed significantly only when compared with the typical lung carcinoids ($p = 0.014$) (Fig. 6). A significantly lower expression of IL-2R was found in typical lung carcinoids, in comparison both with AC ($p = 0.014$) and NSCLC ($p = 0.004$).

In the control material (non-neoplastic lesions), IL-2Rα was detected in scattered or grouped cells of mainly T lymphocytes.

Correlations between reciprocal expression of IL-2 and IL-2R and between the expression of both markers and Ki-67

Spearman's correlation could not be detected between expressions of IL-2 and IL-2R in any of the types of lung tumour. In addition, no significant relationships could be detected between expression of IL-2 and its receptor and the cellular expression of the Ki-67 proliferation antigen in the groups of tumours studied. Only in the AC group was a tendency noted for a negative correlation between the expression of IL-2 and that of Ki-67 antigen ($r = -0.590$), although this relationship was statistically insignificant ($p = 0.123$).

DISCUSSION

Interleukin-2 participates in graft rejection and its action is probably local in character. After the injection of cells with transfected IL-2 an exclusively local inhibition of tumour cell growth was noted at very low serum levels of IL-2 [17]. IL-2 induces antitumour activity by increasing numbers of NK and MHC-restricted tumour-specific cytotoxic cells [10]. Moreover, a plethora of secondary cytokines is induced in the response to IL-2 *in vivo* [18]. Some authors have reported that direct effects of IL-2 on tumour cells could not be noted [21]. In advanced stages of several malignant tumours decreased production of IL-2 by lymphocytes and a decreased expression of receptors for IL-2 on cytotoxic cells have been noted [14]. Data on the secretion of IL-2 and its receptor by tumours cells are incomplete. Most such studies were performed on NSCLC [4, 7, 9, 12].

Our studies, comprising four subtypes of lung tumour, demonstrated differences in the cellular expression of IL-2 depending on the histological subtype of lung tumour. The exclusively cytoplasmic expression of IL-2 was noted in tumour cells. The semi-quantitatively most pronounced expression was detected in atypical lung carcinoids, neuroendocrine lung tumours of worse prognosis in relation to survival than typical lung carcinoids [31]. No significant relationship was established between IL-2 expression and the intensity of proliferative activity (expression of Ki-67 antigen) and a tendency was noted for a negative correlation between the two parameters only in atypical lung carcinoids ($r = -0.591$; $p > 0.05$). The low number of cases in the group ($n = 8$) made the result insignificant. It remains difficult to comment on the observations of some investigators regarding whether expression of endogenous IL-2 in squamous cell cancers of the neck and head is required for the proliferation of tumour cells [23]. The least pronounced expression of IL-2 was detected in pulmonary squamous cell cancers, which could indicate that production of the cytokine is suppressed in this type of tumour and may provide an ominous prognostic factor. Selective suppression of cytokine release was reported in lung cancers (IL-2, IFN-α and IFN-γ), depending upon tumour size and variably expressed in individual patients with SCLC and NSCLC [5]. In our studies no significant differences were noted in the tissue expression of IL-2 between SCLC and NSCLC. Some studies have pointed to a complete

absence of IL-2 and IFN-γ secretion in NSCLC both *in vitro*, and *in vivo* [9]. Some authors have observed a correlation between shorter survival in advanced pulmonary cancers and a decreased IL-2 level in serum [14]. Not having access to full data on the patients, we cannot comment on the results.

Over-expression of IL-2Rα has been demonstrated in several human tumours, including cancers of the lungs, skin, prostate and oesophagus and in leukaemias [10, 11]. It is assumed that IL-2Rα-positive cells proliferate more rapidly and manifest chromosomal instability, which may promote enhanced aggressiveness of the tumour, more pronounced resistance to drugs and worsen the prognosis [6, 10, 11].

Our results demonstrated the presence of the IL-2α chain (CD25, Tac molecule) in all subtypes of lung tumour, although to a variable extent with respect to frequency and the intensity of its expression. CD25 was located exclusively in the cell membranes of tumour cells. The most frequent presence of IL-2R was demonstrated in the group of squamous cell lung cancers and the least frequent in typical lung carcinoids. In semi-quantitative appraisal, the highest expression of both IL-2R and IL-2 proteins was detectable in the atypical lung carcinoids. Data in the literature provide evidence for the presence of functional IL-2R in the cultured cells of human squamous cell cancer of the head and neck [30]. However, immunofluorescence studies of cultured cells of SCLC did not show expression of IL-2Rα chain on their surface [17]. Analysis of serum levels of the so-called "soluble" form of IL-2R (sIL-2R), particularly in non-small cell lung tumours, demonstrated elevated levels of this as compared to the control. This correlated with the progression of the tumour and aggressiveness of the disease and, in some studies, also with decreased levels of IL-2 itself [4, 13, 16]. However, findings on a correlation between sIL-2R and its equivalent in tumour tissue (i.e. IL-2Rα) are incoherent. Some authors have demonstrated positive [16] and others a negative correlation between concentrations of sIL-2R and Tac-positive cells [4, 13]. In our own study no significant relationships could be detected between the expression of IL-2 and the expression of its receptor at the tissue level in any of the tumour types studied.

Evaluation of tumour proliferative activity is thought to predict the clinical course of the tumour and may serve as an additional diagnostic index. Several approaches can be used for the evaluation of a tumour cell proliferative index [2]. One of these involves the immunocytochemical expression of the Ki-67 antigen using monoclonal antibodies [2, 7]. The Ki-67 antigen is present at any stage of the cell cycle (except G0) and in histological preparations the staining pertains to dividing cell nuclei [7]. Co-localisation of the antigen has been demonstrated within peri-nucleolar chromatin and the role of the antigen is suggested in the higher-order organisation of this [3]. In the present study, as expected, the most numerous dividing cells, evaluated by their anti-Ki-67 staining, were detected in SCLC and squamous cell lung cancers, and also in atypical pulmonary carcinoids. In agreement with the literature data, the lowest number of dividing cells was found in typical lung carcinoids [1]. The same authors observed a negative correlation between proliferative activity and the duration of survival [1]. In our study we used the detection of Ki-67 antigen for the examination of a potential relationship between IL-2Rα and the cell proliferation index in various types of lung tumour. However, in none of the studied tumour types was such a correlation demonstrable.

CONCLUSION

In summary, in the present studies we found expression of IL-2 and the α chain of its receptor (IL-2Rα) both in neuroendocrine lung tumours (typical and atypical pulmonary carcinoids and small-cell lung cancer) and in non-small cell lung cancers (squamous cell lung cancers). The most frequent detection of IL-2 was observed in typical lung carcinoids (93%), and IL-2R expression was most frequent in squamous cell lung cancers (94%). However, the expression of IL-2 and its receptor were not correlated. Moreover, in most cases the occurrence of IL-2 and IL-2R also failed to show any relationship with the expression of Ki-67, used in this study as an index of cell proliferation. Semi-quantitative analysis demonstrated the most pronounced expression of IL-2 in atypical pulmonary carcinoids. Additional studies are necessary to confirm the negative correlation between IL-2 expression and the proliferation index of the neoplastic cells reported by other authors [5]. The most pronounced semi-quantitatively evaluated expression of IL-2R was also documented for atypical carcinoids, although this was not correlated with a tumour's proliferative activity.

The diagnostic and prognostic significance of our results requires further study.

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