Soluble Interleukin 2 Receptor in Lung Cancer*
An Indirect Marker of Tumor Activity?

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Circulating levels of the soluble interleukin 2 receptor (sIL-2R) could provide an in vivo measure of the immunologic response to human tumors. We performed a total of 326 sIL-2R serum assays in 126 patients with lung cancer (67 at diagnosis, 59 during and after treatment), 112 patients with pulmonary benign diseases, and 63 voluntary healthy subjects. Patients with lung cancer had a median value of sIL-2R of 791 U/ml, which was superior to that of both controls (398 U/ml, p<0.001) and patients with noninflammatory benign diseases (583 U/ml, p<0.02). However, infectious pulmonary disorders, such as tuberculosis and pneumonia, were associated with the highest values of the substance (median, 1150 U/ml; p<0.001). At the diagnosis of lung cancer, sIL-2R correlated neither with the stage of disease nor with the cell type. On the contrary, posttreatment levels of the receptor were significantly related to disease status (RO = .41, p<0.002), particularly in the subgroup of nonsurgical patients (RO = .48, p<0.001). Patients with abnormal sIL-2R levels had a nearly significant reduction in survival as compared with patients with normal values (p<0.1). Measurements of sIL-2R could be useful in monitoring patients under treatment for bronchogenic carcinoma, as well as in prognostication. In this setting, sIL-2R might open a new class of biologic markers, providing information that is complementary to those of the more classic tumor-derived markers. (Chest 1991; 99:1433-37)

Interleukin 2 (IL-2) is a well-characterized cytokine with various immunologic functions, the most important being the capacity to initiate the proliferation of activated T cells.1 This property has stimulated the recent renewal of interest in immunotherapy of cancer.2 IL-2 acts with a specific surface receptor (IL-2R), absent on resting T cells but appearing within hours of activation.3 Activated lymphocytes produce and release into the circulation a soluble form of the same receptor (sIL-2R)4 that retains the capability of binding the lymphokine.4 In humans, the serum concentration of sIL-2R can be easily measured by means of an enzyme-linked immunosorbent assay (ELISA), according to the method described by Rubin et al.5 Empiric observations, performed in both healthy subjects and patients suffering from diverse pathologic conditions, have shown that serum levels of sIL-2R can be abnormally elevated in patients with virus infections,6,7 sarcoidosis,8 Graves' disease,9 organ transplants,10,11 lymphoproliferative disorders,12-14 and solid tumors.15,16

Lung cancer is the most common and lethal malignant neoplasm in the Western world and is becoming one of the major health problems in undeveloped countries as well.17 Its devastating incidence and clinical seriousness have stimulated innumerable researches directed toward any possible approach. One of these approaches was the quest for biologic markers capable of helping clinicians in their processes of diagnosis, staging, disease monitoring, and prognostication. So far, a large number of tumor markers have been proposed,18 with some distinguished results.19,20 However, none is completely satisfactory and obviates the need for additional investigations.

Recently, we have reported increased levels of sIL-2R in the serum samples of patients with untreated lung cancer.21 In diverse human malignancies, the existence of a correlation between sIL-2R and some clinical parameters, such as tumor burden and treatment response, has been observed.15,16 In this report, we analyze the results of 326 additional sIL-2R serum assays performed in both voluntary healthy subjects (HS) and patients with a variety of pulmonary disorders. The aim of the study was to confirm the sensitivity of the assay in diagnosing lung cancer and to explore its specificity; a second purpose was to evaluate its potential usefulness as a biologic marker of the disease.

METHODS
From October 1989 to April 1990, 67 new patients, with a cytologically or histologically confirmed bronchogenic carcinoma, were seen at the two cooperating Institutions: the A. Carle Hospital...
of Chest Diseases, Cuneo, and the S. Paolo University Hospital, Milan, Italy. Male patients constituted 79 percent (53/67) of the cohort studied. Pathologic diagnoses comprised 29 cases of squamous cell carcinoma, 10 cases of small cell carcinoma, 14 cases of adenocarcinoma (including alveolar cell types), and 2 cases of large cell anaplastic carcinoma. Twelve unclassified carcinomas, including mixed histologies, were grouped separately. All patients were tested for sIL-2R serum levels, before starting their anticancer treatment. Six of them, presenting with a superimposed bacterial infection (five postobstructive pneumonias and one lung abscess) had their assay postponed until there was a complete clinical and radiologic recovery. Other routine staging procedures consisted of physical examination, blood chemistry, chest roentgenograms and tomograms, bronchoscopy, and respiratory function tests. Computed tomography of the thorax, abdomen, and brain were performed in all non–small cell lung cancer patients assessable for surgery or irradiation. Selected surgical candidates underwent mediastinoscopy. Bone radionuclide scannings and bone marrow biopsies were added to the baseline evaluation of patients with small cell lung cancer. Other diagnostic procedures were carried out as clinically indicated. All patients were classified according to the 1987 staging system of the International Union Against Cancer (UICC).12 Twelve patients were classified into stage I disease, 11 in stage II, 19 in stage IIIA and IIIB, and 25 in stage IV. Eventually, 14 patients underwent surgical resection, three had radiotherapy as a primary treatment, and 33 were treated with chemotherapy (MACC regimen12 plus lomustine [Doridomine R, Angelini, Italy] 150 mg orally three times a day). The remaining seven patients with non–small cell cancer received only supportive care, symptomatic irradiation, or individualized chemotherapy. Nearly all the patients with a small cell type tumor were treated with a previously described scheme of combination chemotherapy.14 Survivals were recorded from the time of the first pretreatment assay to death or to the last date of follow-up.

Posttreatment sIL-2R measurements were performed, mostly on an outpatient basis, in 59 patients during the same 7-month period. Patients had to show no clinically evident signs of infection at the time of the test. Those receiving chemotherapy had their venous blood samples drawn just before the next cytotoxic drug administration. A uniform time was not picked for the assessment of the disease status; rather, intervals between medical examinations ranged from three to four weeks during chemotherapy and every one to three months in the case of surgery, irradiation, or no active therapy. In addition to the sIL-2R assay, biochemical, hematologic, and radiologic routine tests, along with the reassessment of tumor activity, were carried out. Computed tomographies and scanning studies were repeated, if clinically indicated. Standard criteria for objective response were used.16 Minor regression was defined as any unequivocal tumor volume reduction that did not fulfill the criteria of at least partial remission.

As a control, we assayed 112 patients with various benign pulmonary disorders, grouped into noncancerous patients with nonphlogistic illnesses and patients with various types of lung and bronchial inflammations. In the first group, we included bronchial asthma, chronic respiratory failure and cor pulmonale, pneumocnosis, old inactive tuberculous lesions, and pulmonary embolism. In the second group, we placed acute pneumonia, active pulmonary tuberculosis, pleurisy, exacerbation of chronic obstructive pulmonary disease, lung abscess, sarcoidosis, and other granulomatous disorders.

A further group of healthy individuals provided our normal reference. They were also used to estimate the normal approximation of the IL-2R distribution.

sIL-2Rs were measured using monoclonal antibodies (anti-TAC and 7G7/B6) directed against distinct epitopes of the human IL-2R, as previously described.17 In practice, serum samples, after centrifugation and storage at −20°C, were assayed using the commercial ELISA kit (provided by T Cell Science, Inc, Cambridge, MA, USA), and following the manufacturers’ instructions. sIL-2R concentrations were expressed in units per milliliter. The sensitivity of the test was 50 U/ml. Intra-assay and interassay coefficients of variation were 4 percent and 11 percent, respectively. Our reference value for sIL-2R was up to 700 U/ml (93rd percentile of HS values). A preliminary statistical analysis revealed the nonnormality of the distribution of sIL-2R (moments skew:2.53, kurtosis:7.27). Consequently, nonparametric statistics were adopted.18 The rank sum test, the Kruskall Wallis nonparametric analysis of variance, or the Spearman rank correlation were used, as appropriate. Survivals were investigated by the log-rank test and curves were generated by the life-table technique.19 Statistical significance level was 5 percent, two-sided tests. Data, other than survivals, were computed.
RESULTS

A total of 326 sIL-2R serum assays were evaluated. Sixty-seven were performed on patients with untreated lung cancer, 84 were assayed on the serum samples of 59 subjects seen for a clinical reassessment of the same disease. One-hundred twelve control patients (42 with noninflammatory and 70 with inflammatory pulmonary diseases) were studied, as well. The number of HS tested for sIL-2R was 63. Figure 1 gives the distribution of sIL-2R in the five subgroups. For each group, values above the reference are easily recognizable. In particular, 42 of 67 lung cancer patients had pretreatment sIL-2R levels exceeding 700 U/L (63 percent of sensitivity), with a median value equal to 791 U/ml. By comparison, the HS and the patients with noninflammatory benign disorders had median values of 398 (p<0.001) and 583 U/ml (p<0.02), respectively. At the other end of the spectrum, 56 of the 70 patients with inflammatory diseases of the lung had abnormal values (80 percent sensitivity), with a median concentration of 1150 U/ml (p<0.01, vs the untreated lung cancer group). Therefore, no differential diagnosis between inflammatory and cancerous lung radiopacities is possible on the basis of a high value of sIL-2R. Rather, very elevated levels are more likely to originate from an infection or a tumor-associated infection.

Within the HS group, values of sIL-2R did not vary among sexes (median levels of the 27 female and of the 36 male patients were 452 and 477, respectively; p = NS), but increased in parallel with age (RO = .28, p<0.05). As far as patients with noncancerous lesions are concerned, the highest values of the test were observed in mixed bacterial cavitating infections or in active pulmonary tuberculosis, followed by acute pneumonitis and pleural effusions (median values were 3,346, 1,931, 1,095, and 990 U/ml, respectively); in contrast, the lowest levels were those of patients with silicosis and other pneumoconiosis (median value, 453 U/ml). Since the differential diagnosis of lung cancer is often made with abscesses, pneumonitis, and tuberculosis, these data underline further the uselessness of elevated sIL-2R in this setting.

Figure 2 shows the distribution of sIL-2R values within the population with an untreated lung cancer, according to the stage of disease (stage IIIa and IIIb were merged into one), and to the cell type. Despite a modest tendency toward higher sIL-2R values in metastatic diseases, the difference was statistically insignificant, considering either all the four stages of disease (RO = 0.11, p = NS), or stage IV vs stages I, II, and III grouped together. There was no significant relationship with the cell type observed.
Figure 3 refers to posttreatment sIL-2R assays. Individual values, obtained at each follow-up time, are grouped together according to the category of treatment response. In this type of analysis, the correlation between sIL-2R and disease status was highly significant (RO = 0.41, p < 0.002). It was even more significant (RO = 0.48, p < 0.001) when the postsurgical patients were removed from the calculation (in all, 14 patients were disease free after operation); in this case, median values of sIL-2R for complete remission, partial remission, minor regression, no change, and progressive disease were 452, 564, 759, 785, and 1,197 U/ml, respectively.

The evaluation of the survival probability is rather premature at the time of writing, but nevertheless, the preliminary actuarial analysis shows curves separating very clearly and differences approaching the statistical significance (p < 0.1). Patients with levels of sIL-2R higher than 700 U/ml seem to be unfavored (median survival of 16 weeks vs a median still unreached at 28 weeks).

**Discussion**

This study has shown that increased levels of the soluble IL-2 receptors can be found in serum of patients with lung cancer and no evidence of infection. Higher levels can be observed in any type of infectious pulmonary diseases and lung inflammations by other causes; whereas, lower levels, often within the normal range, may be observed in nonphlogistic pulmonary disorders. We have demonstrated a large overlapping of the sIL-2R values deriving from different diseases and no cut-off levels. Hence, as already pointed out, the demonstration of an abnormally elevated concentration of serum sIL-2R is of little or no value in the suspected lung neoplasm.

In the past, abnormal values of sIL-2R have been reported in various benign disorders, such as viral infections and immunologic reactions. To the already long list, we now add tuberculosis and other infections of the lung, especially if provoked by bacteria and accompanied by suppuration and cavitation. Moderately elevated levels of sIL-2R have been observed in patients with diverse solid tumors, such as sarcomas, breast, lung, and gastrointestinal cancers. Some conflicting data have also been reported. For example, only 40 patients with breast cancer were tested in a positive study, whereas a second study, specifically addressed to breast cancer, was unable to detect any difference between controls and diseased patients. In lung cancer, however, current findings confirm both our preliminary data and the evidence deriving from 66 additional patients.

There is an aspect of potential practical interest in this study. We have found a surprisingly high correlation between sIL-2R values and disease status, particularly when postsurgical patients were omitted from the analysis and this latter was limited to patients receiving chemoradiotherapy or palliative care. This finding could suggest a use of the test for disease monitoring. To our knowledge, there are no previous reports that can help to validate or criticize these data, with one exception. In a very recent article that summarizes all the relevant experience of the authors, Lissoni and coworkers reported that in seven chemotherapy responders (of whom four had a carcinoma of the lung), the mean serum concentration of sIL-2R fell from 857 to 503 U/ml and rose from 739 to 1,313 U/ml in the other 13 patients (five with lung cancer) who progressed despite the treatment.

We have also found—but the survival analysis is very preliminary and formally nonsignificant—that patients with abnormal levels of sIL-2R had a worse prognosis, and this could be useful in prognostication. On the contrary, we were unable to show significant differences among either metastatic and limited diseases or histotypes. However, the capability of detecting at the significance level small but real differences, with the test we have applied to our relatively small sample is not great. Hence, our nonsignificant results do not necessarily mean negative results. This appears particularly true, taking into consideration the fact that the same correlation was found to be significant in another series.

But why should abnormal values of sIL-2R be associated with a progressive disease and shortened survival? We cannot really answer the question, but it is possible that the release of this receptor might serve an important immunoregulatory role by competing for IL-2 with cell-surface receptors and thus by decreasing the local or regional immune response. In fact, there are several indications that increased sIL-2R levels could be associated with a defect in the immune response. Aged individuals have depressed cell-mediated immunity and aged donor cells produce less IL-2 than do those from young donors; in aged normal subjects, however, we and others have measured increased concentration of sIL-2R. In cancer patients, high levels of sIL-2R are associated with signs of immune dysfunction, such as a reduced CD4/CD8 ratio and a lower IL-2 serum concentration. In animal models, the most elevated sIL-2R serum levels are associated with the more aggressive implanted tumor. And finally, the ability of sIL-2R to inhibit the IL-2 dependent clone expansion has been demonstrated by in vitro studies.

In conclusion, the circulating levels of the soluble receptor for IL-2 could be confirmed as an indirect marker of tumor activity. Of the complex host-tumor relationship, it explores the first component of the binomial. Potentially, it may provide complementary information to those deriving from the more classic
tumor markers. Hopefully, it could help to evaluate clinically our lung cancer patients and contribute to their treatment.

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