

Increased levels of soluble serum interleukin-2 receptor in extrinsic allergic alveolitis correlate with interleukin-2 receptor expression on alveolar macrophages

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In a group of 20 patients with extrinsic allergic alveolitis (10 with farmer's lung, 9 with bird-breeder's lung, and 1 with humidifier's lung), we observed increased levels of soluble serum interleukin-2 receptor (ssIL-2R) with an average of 75.2 pmol (control group average, 40.6 pmol). Analysis of bronchoalveolar lavage cells by immunocytochemistry with a CD25 antibody revealed only a slight increase to 4.2% IL-2R+ cells among alveolar lymphocytes but a pronounced rise to 21.2% IL-2R+ cells among alveolar macrophages. Furthermore, cytometry revealed a fourfold higher IL-2R expression on a per cell basis for alveolar macrophages as compared with alveolar lymphocytes. A clear-cut positive correlation ($p = 0.006$) was found for ssIL-2R and IL-2R+ alveolar macrophages, but only a borderline correlation was found for ssIL-2R and IL-2R+ alveolar lymphocytes ($p = 0.04$). The finding of a concomitant decrease of IL-2R+ alveolar macrophages and of ssIL-2R on allergen avoidance further supports the notion that alveolar macrophages may be the main source of the increased ssIL-2R in patients with extrinsic allergic alveolitis. (J ALLERGY CLIN IMMUNOL 1994;94:1057-64.)

Key words: Extrinsic allergic alveolitis, IL-2 receptor, alveolar macrophages, lymphocytes, ssIL-2 receptor

Extrinsic allergic alveolitis (EAA) or hypersensitivity pneumonitis is an interstitial lung disease, caused by inhalation of allergens derived, for instance, from birds or from moldy hay. Immune complex formation by precipitating antibodies was originally believed to initiate inflammation, but IgE-mediated mechanisms have been implicated

Abbreviations used

AL:	Alveolar lymphocytes
AM:	Alveolar macrophages
BAL:	Bronchoalveolar lavage
EAA:	Extrinsic allergic alveolitis
IL-2R:	Interleukin-2 receptor
mAb:	Monoclonal antibody
OD:	Optical density
ssIL-2R:	Soluble serum interleukin-2 receptor

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as well.^{1, 2} The histopathologic course is characterized by infiltration with mononuclear cells and by granuloma formation in the lung. This is reflected in the dramatically increased numbers of both lymphocytes and alveolar macrophages (AMs) in bronchoalveolar lavage (BAL). These cells show signs of activation in that there is, for instance, an increased expression of HLA Class II on AMs.^{3, 4}

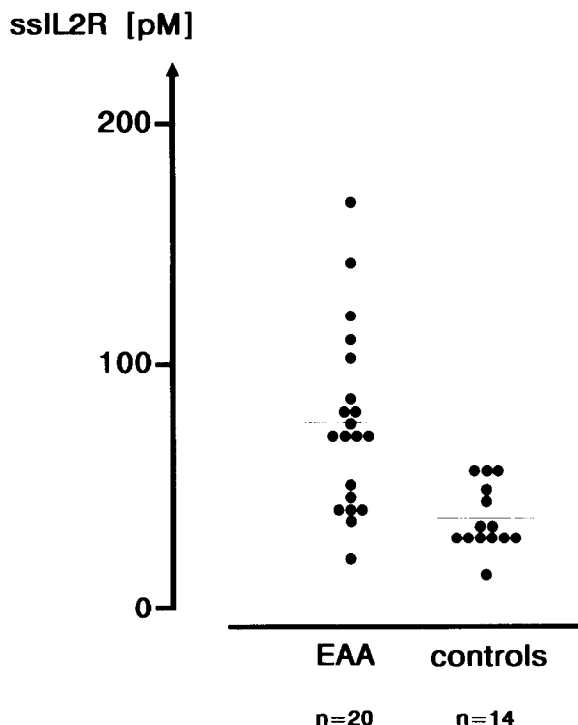


FIG. 1. Soluble serum IL-2R levels in patients with EAA and control subjects. Determination of soluble IL-2R α chain was done by ELISA with two mAbs. EAA group versus control group significant at $p < 0.001$.

Thus it appears that EAA may represent a cell-mediated immune reaction, whereas precipitating antibodies are merely markers of exposure.

Activation of both lymphocytes and monocytes or macrophages can be exerted by the lymphokine interleukin-2 (IL-2), which acts through the inducible p55, p75 IL-2 receptor (IL-2R).^{5, 6} In this report we can in fact demonstrate a pronounced expression of the IL-2R p55 α chain on AMs in EAA, and this accompanies an increase in the serum level of the IL-2R α chain. These data suggest that increased levels of soluble serum IL-2R (ssIL-2R) in EAA may be a useful indicator of local inflammation and, more specifically, may reflect the degree of activation of AMs.

METHODS

Study population

Twenty patients with EAA (10 women and 10 men), between the ages of 20 and 63 years, were investigated. The diagnosis was based on a history of antigen exposure, typical clinical features (dyspnea, cough, chills, weight loss, fever), diffuse opacities on chest x-ray films,

and the functional pattern of interstitial lung disease. Pulmonary function tests included dynamic spirometry, flow volume spirometry, blood gas analysis, and single-breath diffusing capacity. All patients had precipitating antibodies against the relevant antigens as determined by Ouchterlony assay, kindly performed by Dr. de-Haller, Geneva, Switzerland. As a control group, five apparently healthy volunteers (nonsmokers) were examined. Clinical evaluation showed no pulmonary abnormalities in these subjects. The study was cleared by the Ethics Committee of the Medical Faculty, University of Munich.

Assay for ssIL-2R

The assay for the ssIL-2R is an ELISA that uses two monoclonal antibodies (mAbs) directed against different epitopes of the IL-2R α chain (Immunotech, Marseille, France). The first antibody is coated on the surface of wells of a microtiter plate. The samples to be measured are incubated in these wells, a second mAb conjugated with alkaline phosphatase after antibody titration was added. p-Nitrophenyl phosphate was used as a substrate. The standard curve was determined by six standards. Serum samples were obtained from all persons who underwent the study, frozen at -20°C , and analyzed.

BAL

After informed consent was obtained, all patients underwent fiberoptic bronchoscopy and BAL. Lavage was performed by instilling 160 ml of 0.9% saline solution in 20 ml aliquots into the lingula or middle lobe and withdrawing the fluid immediately. Total cell counts were determined, and cytocentrifuge smears were prepared for cytologic and immunocytochemical analysis. Differential counts of 100 cells were made (Wright-Giemsa staining).

Immunocytochemistry

For immunocytochemical staining, mAbs were used in conjunction with the alkaline phosphatase antialkaline phosphatase technique.⁷ For identification of the IL-2R on AMs and lymphocytes, the CD25 mAb ACT-1 was used. To control for nonspecific binding, a separate slide was stained in parallel with a CD8 antibody, which produces no antigen-specific signal with macrophages. T-cell subsets were identified with CD4 and CD8 monoclonal antibodies. All mAbs were obtained from Dakopatts (Hamburg, Germany). Alveolar lymphocytes (ALs) always produce strong staining for these markers, which is clearly discernible from background staining. Hence, for the enumeration of positive lymphocytes, the slides were analyzed visually by light microscopy, and a total of 200 cells were counted by two independent investigators. In selected samples, IL-2R+ lymphocytes were also evaluated by cytometry (see below).

TABLE 1. BAL characteristics of patients with EAA and healthy control donors

Group	Age (yr)	Sex (M/F)	S/NS	BAL				
				Recovery (cells $\times 10^6$)	AM (%)	AL (%)	AGr (%)	CD4/CD8 ratio
EAA ($n = 20$)	51 ± 13	10/10	1/19	$30.5 \pm 26.7^*$	$42 \pm 19^*$	$44 \pm 25^*$	4 ± 2	0.9 ± 1.2
Control ($n = 5$)	31 ± 5	3/2	0/5	4.3 ± 1.4	90 ± 3	7 ± 3	2 ± 1	—

S/NS, Smoker/nonsmoker; AGr, alveolar granulocytes.

* $p < 0.005$.

Cytometry

Staining of AMs for CD25 results in a broad distribution of staining intensities with the difficulty of discriminating weakly positive and negative cells by eye. Therefore these cells were evaluated by cytometry. For these analyses, 100 cells or more per specimen were studied, by using a microscope (Zeiss UEM, objective magnification $\times 25$, num. ap. 0.55, optovar 1.6, filter OG 455 nm; Carl Zeiss Inc., Thornwood, N.Y.) and CCD-TV camera (Hamamatsu Corp., Bridgewater, N.J.). Each cell was segmented semiautomatically or interactively and was measured with a SAMBA image analysis system (Dynatech Laboratories, Inc., Chantilly, Va.) and the program MINT 5.⁸ This program performs a standardized measurement for optical density (OD), including shading correction. The OD readings were corrected by subtracting the background of the respective slides. The selected images were digitized into 512×512 pixels, resulting in pixel distances of $0.273 \mu\text{m}$. Transmission was performed pixelwise into extinction and digitized into 256 channels (8 bit). Mean specific staining intensity was determined by subtraction of the mean OD for the control (CD8) staining from the mean OD for the specific (CD25) staining.

Statistics

For comparison of patients and control subjects, Student's *t* test was used, and correlation analysis was done with the Kendall test.

RESULTS

Soluble serum IL-2 receptors

In 13 of 20 patients with EAA, values for ssIL-2R were above the control range (>50 pmol), and the average value for all patients was 75.2 pmol/L, whereas the average value for control donors was 40.6 pmol (Fig. 1, Table I). Levels of ssIL-2R did not correlate with age, sex, or type of allergen (data not shown).

Cell surface IL-2 receptors on lavage cells

To identify the source of ssIL-2R, we studied the cell surface expression of IL-2R on BAL cells by using immunocytochemistry. In control donors no

or only very few IL-2R+ cells can be observed. In patients with EAA, however, the CD25 antibody clearly stained a large proportion of AMs (Fig. 2, A), whereas the CD8 control antibody produced only a background signal on these cells (arrows in Fig. 2, B). In addition, a few lymphoid cells (small arrow in Fig. 2, A) were positive for CD25.

Enumeration of positive cells by cytometry demonstrated an average of $1.5\% \pm 2.0\%$ of AMs and $0.4\% \pm 0.8\%$ of ALs positive for IL-2R in control donors. In patients with EAA 21.2% of AMs were positive for IL-2R. This includes three patients with less than 10% CD25-positive AMs and one patient with more than 50% positive AMs (Fig. 3). In contrast, lymphoid cells of patients with EAA exhibited less staining, with an average of 4.2% positive cells, and the highest value of 10% IL-2R+ lymphocytes was observed in only 2 of 20 patients. Thus when AMs and ALs are compared, it is evident that the majority of IL-2R-positive cells are AMs. In addition, although the small lymphoid cells have a high cell surface density, the amount of IL-2R on a per cell basis is higher on AMs. This can be demonstrated by cytometric evaluation of specific OD, which is 385 ± 161 channels for AMs compared with 86 ± 28 channels for ALs ($n = 3$). Thus on a per cell basis, the IL-2R-positive ALs exhibit a fourfold higher expression of this receptor molecule.

The high expression of IL-2R on AMs in EAA suggests that AMs are a likely source of ssIL-2R. Correlation analysis revealed a higher significant correlation of ssIL-2R and CD25+ AMs ($p = 0.006$, $r = 0.4$) (Fig. 4), whereas only a borderline correlation was observed with CD25+ ALs ($p = 0.04$). These data suggest that ssIL-2R may be derived from AMs.

Effect of allergen avoidance after challenge

Two patients were studied directly after allergen exposure and several weeks after strictly avoiding allergen contact. Lavage cells directly after expo-

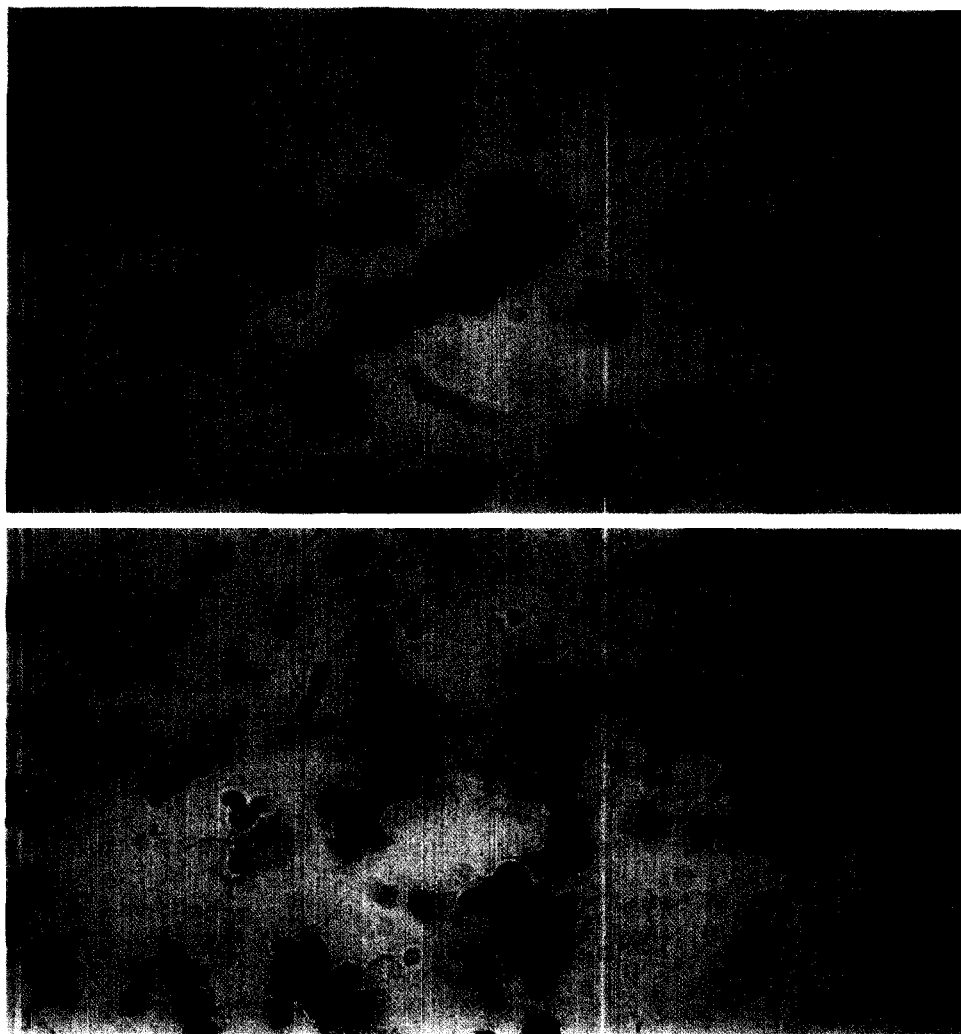


FIG. 2. Expression of IL-2 receptors on AMs in EAA. Cytospin preparations of lavage cells were stained by the alkaline phosphatase anti-alkaline phosphatase technique with a CD25 mAb (**A**) or with a CD8 control antibody (**B**). Arrow in **panel A** indicates an IL-2R+ lymphocyte. Arrows in **panel B** indicate negative AMs.

sure showed high percentages of CD25+ AMs with 27% and 19% (Fig. 5), whereas percentages of CD25+ ALs were low (10% and 7%). After allergen avoidance, expression on AMs decreased by a factor of about 1.5, whereas there was no obvious change in percentage of CD25+ ALs. Levels of ssIL-2R were high immediately after allergen challenge (110 and 120 pmol) and decreased by a factor of 1.5 after allergen avoidance. Thus the concomitant decrease of IL-2R+ AMs and of ssIL-2R provides further support to the notion that ssIL-2R may be derived from AMs.

DISCUSSION

IL-2 is a cytokine produced by activated T cells, and although it has recently been demonstrated

by targeted disruption of the IL-2 gene in transgenic mice that absence of IL-2 is compatible with life,⁹ it is still clear that IL-2 is essential for a normal immune response.^{9, 10} IL-2 acts through binding to a specific cell surface receptor, initially discovered on T lymphocytes.¹¹ Although resting T cells express an intermediate affinity IL-2 receptor β chain (p75),^{12, 13} activation by appropriate stimulation including IL-2, will induce the p55 α chain (CD25 Tac), resulting in the high affinity α, β heterodimer.^{10, 14} This high affinity IL-2R can also be induced on B cells,¹⁵ monocytes,¹⁶⁻¹⁸ and macrophages.¹⁹ Binding of IL-2 to this unique heterodimeric receptor will induce proliferation and cytotoxicity in T cells,^{20, 21} differentiation and immunoglobulin production in B cells,^{19, 22} and

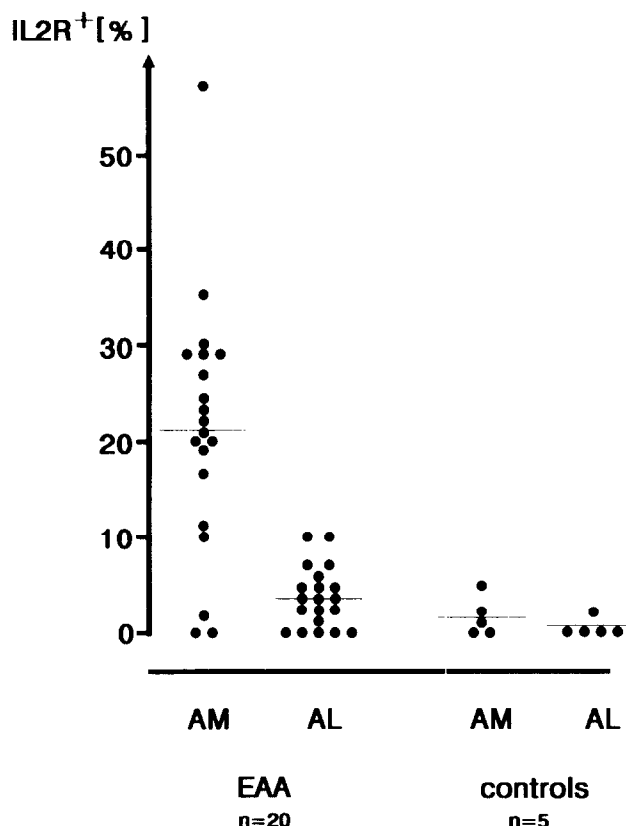


FIG. 3. Expression of IL-2R on BAL cells in patients with EAA and control subjects. Cytospin preparations were stained with antibody or CD8 isotype control. Staining of large ($> 14 \mu\text{m}$) AMs was evaluated by cytometry, and percent positive cells was calculated by subtracting CD8 control staining from specific CD25 staining. AL staining was counted visually. Differences for both AMs and ALs were significant at $p < 0.005$.

cytotoxicity and cytokine production in monocytes.^{16, 23} Hence, it is obvious that IL-2, by acting through its specific receptor, may induce a plethora of effects contributing to inflammation.

In inflammatory disease, such as rheumatoid arthritis and Crohn's disease, elevated levels of IL-2 have been detected in serum.^{24, 25} In lung disease elevated levels of spontaneously produced IL-2 were detected in supernatants of lavage cells from patients with sarcoidosis.²⁶ Furthermore, expression of IL-2 receptors on lymphocytes^{3, 27} and macrophages¹⁹ was noted in these patients. In contrast, only one study on EAA addressed that IL-2/IL-2R system, and in this study there was no clear-cut increase of CD25+ T cells (2% in the control group compared with 4% in the EAA group).²⁸ In this report we observed a similar low level expression of IL-2R on EAA lung lymphocytes (average, 4%). The notable finding in our report is, however, the expression of CD25+ on

AMs in EAA. AMs and ALs, each account for approximately 40% of the lavage cells in EAA. Considering that five times more of the AMs than ALs are positive for CD25 (20% vs 4%) and that on a per cell basis CD25 was found to be fourfold higher on AMs compared with ALs, it is clear that an EAA IL-2R expression on AMs by far exceeds expression on ALs (approximately 20-fold). Assuming that IL-2 is produced locally when sensitized individuals are exposed to allergen, we may speculate that IL-2 activates ALs and AMs for production and release of other inflammatory mediators, thereby initiating and perpetuating the inflammatory response.

Thus IL-2 and IL-2R expression may be an informative tool with which to evaluate activity of disease in EAA. There is, however, no ready access to AL cells in these patients. Still, the local expression of cellular IL-2 receptors may be reflected in increased levels of soluble IL-2 receptors. Such soluble IL-2 receptors, representing the α chain have

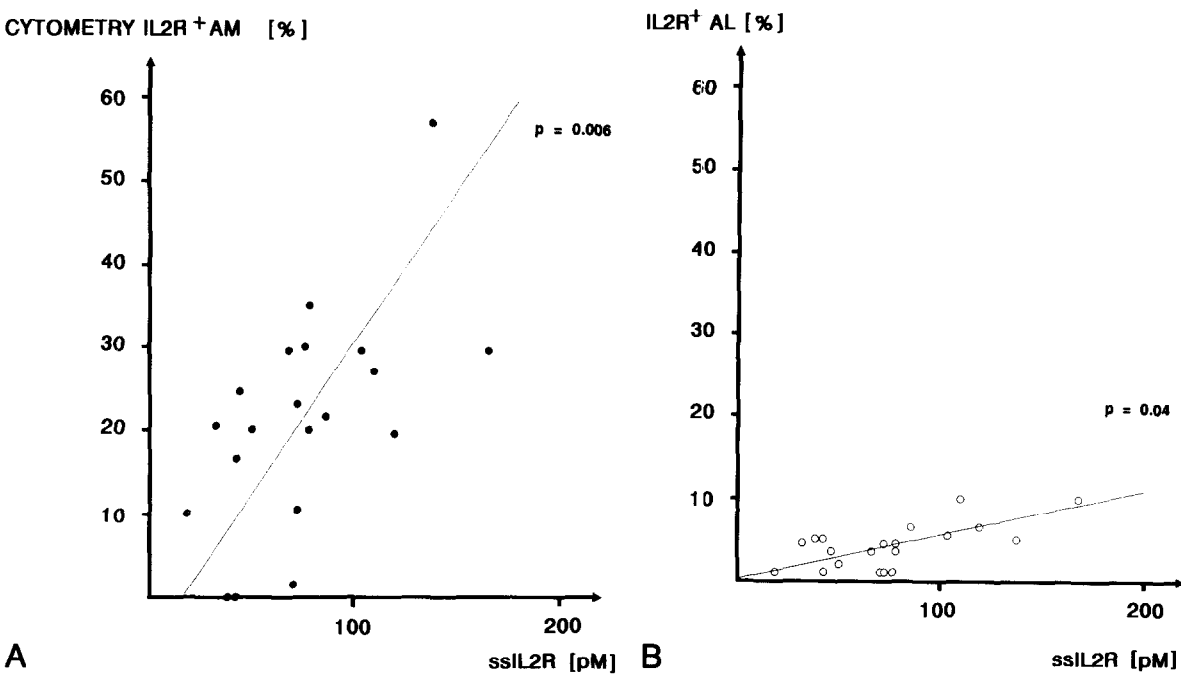


FIG. 4. Correlation of ssIL-2R and IL-2R expression on alveolar cells. **Left panel**, AMs. **Right panel**, ALs.

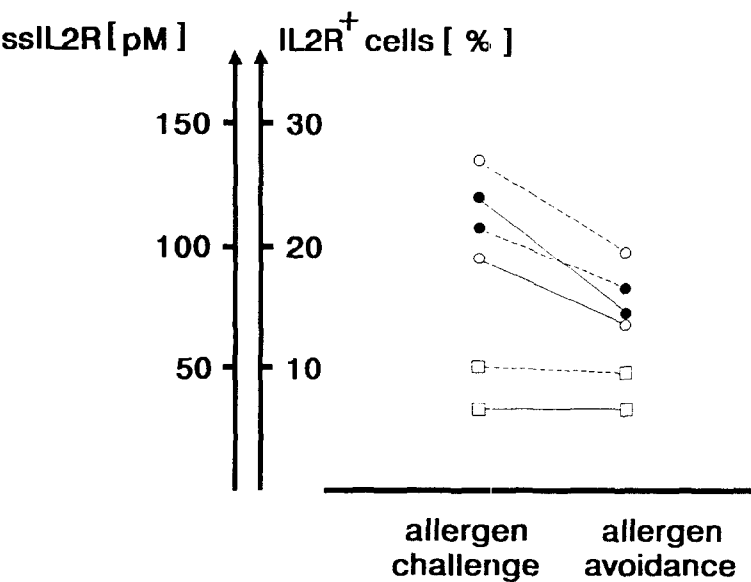


FIG. 5. Effect of allergen avoidance on ssIL-2R and cell-associated IL-2R. Patients were studied before and 4 to 6 weeks after allergen avoidance. ● = ssIL-2R; ○ = IL-2R+ AMs; □ = IL-2R+ ALs; broken line, patient 1; solid line, patient 2.

been detected in the supernatants of lymphocytes⁵ and in body fluids, and ssIL-2R may be increased by several times in various diseases, which involve active immunologic processes.⁶ In sarcoidosis an

average fourfold increase of ssIL-2R was noted in active disease, but not in inactive disease or after steroid treatment,^{29, 30} suggesting that ssIL-2R may be useful for disease monitoring.

For EAA, we report here for the first time, elevated levels of ssIL-2R, with some patients exhibiting normal values of ssIL-2R, but at the same time they showed increased IL-2R expression on AMs. This discrepancy could not be explained by any clinical characteristic. We could not detect any CD25+ mononuclear cells in patient blood (not shown), whereas at the site of local allergen exposure in the lung, a strong up-regulation is noted, suggesting that ssIL-2R is derived from the lung. It can, however, not be excluded that circulating CD25+ cells in the lung compartment may contribute to ssIL-2R levels. Furthermore, the higher expression of CD25 on AMs suggests these cells to be a likely source of ssIL-2R. This contention is also supported by the allergen avoidance studies, which documented a concomitant decrease of ssIL-2R and of CD25+ AMs, whereas the low percentage of lymphocytes remained stable. Thus in contrast to the widely accepted assumption that ssIL-2R is derived from T cells, our data suggest that ssIL-2R in EAA may be derived from AMs. These data are obtained from patients in different stages of EAA, but it appears that ssIL-2R may be a useful parameter for monitoring disease activity and allergen exposure in patients with EAA.

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REFERENCES

- Pepys J, Jenkins PA. Precipitin (F.L.H.) test in farmer's lung. *Thorax* 1965;20:21-35.
- Pforte A, Breyer G, Prinz J, et al. Expression of the Fc receptor for IgE/FcεRII, CD23) on alveolar macrophages in extrinsic allergic alveolitis. *J Exp Med* 1990;171:1163-9.
- Agostini C, Trentin L, Zambello R, et al. Pulmonary alveolar macrophages in patients with sarcoidosis and hypersensitivity pneumonitis: characterization by monoclonal antibodies. *J Clin Immunol* 1987;7:64-70.
- Haslam PL, Parker D, Townsend PJ. Increases in HLA-DQ, DP, DR and transferrin receptors on alveolar macrophages in sarcoidosis and allergic alveolitis. *Chest* 1990;97:651-61.
- Rubin LA, Kurman CC, Fritz ME, et al. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J Immunol* 1985;135:3172-7.
- Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med* 1990;113:619-26.
- Erber WN, Pinching AJ, Mason DJ. Immunocytochemical detection of T- and B-cell populations in routine blood smears. *Lancet* 1984;1:1042-6.
- Gais P, Rodenacker K. Requirements of a multipurpose image analysis system. In: Burger G, Ploem JS, Goerttler K, eds. *Clinical cytometry and histometry*. London: Academic Press, 1987:36-8.
- Schorle H, Holtschke T, Hünig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* 1991;352:621-4.
- Smith KA. Interleukin-2: inception, impact, and implications. *Science* 1988;240:1169-76.
- Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. *J Immunol* 1981;126:1393-8.
- Sharon M, Klausner RD, Cullen BR, Chizzonite R, Leonard WJ. Novel interleukin-2 receptor subunit detected by cross-linking under high-affinity conditions. *Science* 1986;234:859-63.
- Dukovich M, Wano Y, Thuy LB, et al. A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors. *Nature* 1987;327:518-22.
- Robb RJ, Greene WC. Direct demonstration of the identity of T cell growth factor binding protein and the Tac antigen. *J Exp Med* 1983;158:1332-7.
- Loughnan MS, Nossal GJV. Interleukins 4 and 5 control expression of IL-2 receptor on murine B cells through independent induction of its two chains. *Nature* 1989;340:76-9.
- Herrmann F, Cannistra SA, Lindemann A, et al. Functional consequences of monocyte IL-2 receptor expression. Induction of IL-1β secretion of IFN-γ and IL-2. *J Immunol* 1989;142:139-43.
- Holter W, Grunow R, Stockinger H, Knapp W. Recombinant interferon-γ induces interleukin 2 receptors in human peripheral blood monocytes. *J Immunol* 1986;136:2171-5.
- Rambaldi A, Young DC, Herrmann F, Cannistra SA, Griffin DJ. Interferon-γ induces expression of the interleukin 2 receptor gene in human monocytes. *Eur J Immunol* 1987;17:153-6.
- Hancock WW, Muller WA, Cotran RS. Interleukin 2 receptors are expressed by alveolar macrophages during pulmonary sarcoidosis and are inducible by lymphokine treatment of normal human lung macrophages, blood monocytes, and monocyte cell lines. *J Immunol* 1987;138:185-91.
- Morgan DA, Ruscetti FW. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 1976;193:1007-8.
- Gillis S, Smith KA. Long term culture of tumour-specific cytotoxic T cells. *Nature* 1977;268:154-6.
- Zubler RH, Lowenthal JW, Erard F, Hashimoto N, Devos R, MacDonald HR. Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. *J Exp Med* 1984;160:1170-83.
- Malkovsky M, Loveland B, North M, et al. Recombinant interleukin-2 directly augments the cytotoxicity of human monocytes. *Nature* 1987;325:262-3.
- Tebib JG, Boughaba H, Letroublon MC, et al. Serum IL-2 level in rheumatoid arthritis: correlation with joint destruction and disease progression. *Eur Cytokine Netw* 1991;2:239-43.
- Brynskov J, Tvede N. Plasma interleukin-2 and a soluble/shed interleukin-2 receptor in serum of patients with

- Crohn's disease (CD). Effect of cyclosporin. *Gut* 1990;31:795-9.
26. Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *New Engl J Med* 1983;308:793-800.
27. Müller-Quernheim J, Krönke M, Strausz J, Schykowski M, Ferlinz R. Interleukin-2 receptor gene expression by bronchoalveolar lavage lymphocytes in pulmonary sarcoidosis. *Am Rev Respir Dis* 1989;140:82-8.
28. Semenzato G, Agostini C, Zambello R, et al. Lung T cells in hypersensitivity pneumonitis: phenotypic and functional analyses. *J Immunol* 1986;137:1164-72.
29. Lawrence EC, Brousseau KP, Berger MB, Kurman CC, Marcon L, Nelson DL. Elevated concentrations of soluble interleukin-2 receptors in serum samples and bronchoalveolar lavage fluids in active sarcoidosis. *Am Rev Respir Dis* 1988;137:759-64.
30. Keicho N, Kitamura K, Takaku F, Yotsumoto H. Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis. *Chest* 1990;98:1125-9.