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Interleukin-2 inhalation Therapy Temporarily Induces Asthma-like Airway Inflammation*

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Abstract
Background: Inhaled interleukin-2 (IL-2) is an effective and safe treatment in metastasing renal cell carcinoma (mRCC) but known to potentially elicit respiratory symptoms.
Objectives: The present study analyses the effects of IL-2 using a panel of measures including markers of airway inflammation.
Methods: Ten patients with mRCC (7m/3f; mean age, 63 yrs) were measured at baseline, 6-10 days after start of therapy (n = 5, inhaled IL-2 only; n = 5, inhaled IL-2 plus 1/11th of daily dose subcutaneously), and 16-29 days later under continuous combined (inhaled plus subcutaneous) therapy, including additional subcutaneous IFN-α in 8 patients.
Results: After start of therapy median FEV_1 declined from 108 to 85 to 90 % predicted and the provocative concentration of methacholine elicting a 20 % fall in FEV_1 ($PC_{20}FEV_1$) from 16 to 8 to 3 mg/mL, while the level of exhaled nitric oxide (FENO) rose from 27 to 79 to 60 ppb and the percentage of sputum eosinophils from 2 to 18 to 37 % ($p<0.01$, each), accompanied by cough and dyspnoea ($p<0.05$). One patient who stopped therapy, was back to baseline values when measured 2 months later. Cytokine production by blood or sputum T lymphocytes was not markedly altered by IL-2 inhalation.
Conclusions: IL-2 inhalation therapy in patients with metastasing renal cell carcinoma is capable of temporarily inducing symptomatic, functional and inflammatory alterations similar to those of bronchial asthma.

Keywords: immunotherapy, eosinophils, nitric oxide, bronchial hyperreactivity, lung function, induced sputum, lymphocytes, renal cell carcinoma

Introduction

Immunotherapy by intravenous or subcutaneous interleukin-2 (IL-2) is an established strategy in the treatment of advanced tumours, in particular metastatic renal cell carcinoma (mRCC) [1-4]. In patients with pulmonary metastases of mRCC, local IL-2 administration via inhalation is also an effective therapy [5-7]. It is associated with low toxicity and increased quality of life compared to systemic therapy. This has favoured its use especially in high risk patients, in whom a substantial benefit in survival is observed [8]. It is also possible to combine the standard systemic approach [4] with IL-2 inhalation [6, 8]. The rationale for the regional application is the exposure of tumour tissue and surrounding lymph nodes to as high as possible doses of IL-2, while reducing systemic toxicity as far as possible.

Several reports have characterised the anti-tumour responses to IL-2 in mRCC [7, 9, 10]. Some of them include data on the onset of respiratory symptoms [7,11] as well as eosinophilia in blood [12,13] and bronchoalveolar lavage fluid (BALF) [7, 10]. Eosinophilia, though per se non-specific, is known to be a key feature of bronchial asthma. This is believed to be a TH2-driven disease [14] but there is also impressive evidence against this [15]. The association of IL-2 inhalation therapy with eosinophilia plus respiratory symptoms, such as dose-limiting cough [7], suggests a closer inspection being worthwhile. Since non-invasive markers of airway inflammation, such as sputum composition [16] and the level of exhaled nitric oxide (FENO) [17], are considered particularly informative in asthma, it seems of interest to follow the response to IL-2 inhalation using these measures.

Based on this, we assessed respiratory symptoms, lung function, airway responsiveness to methacholine, the concentration of exhaled NO, the percentage of sputum eosinophils, and cytokine production by T cells of blood and sputum in patients undergoing IL-2 inhalation therapy, to compare the IL-2-induced alterations with those known for asthma.

Patients and Methods

Patients

Ten consecutive patients (3 f/7 m; mean ± SD age, 63 ± 11 yrs; FEV_1, 106 ± 6 %predicted [18]; Karnofsky index, 85 ± 7) with renal cell carcinoma and pulmonary metastases were studied (for baseline values see Table 1 and Fig. 1). Upon inclusion, none of the patients reported a history of respiratory diseases, ex-

*This manuscript is dedicated to Prof. Dr. med. Günther Gercken, formerly Department of Biochemistry and Molecular Biology, University of Hamburg, Hamburg, Germany.

Table 1. Time course of variables during IL-2 inhalation therapy.

			Visit 1 (baseline)	Visit 2	Visit 3
Cough *		Score	0 (0-1)	1 (1-1)	1 (1-1)
Sputum production		Score	0 (0-0)	0 (0-0)	0 (0-1)
Dyspnoea *		Score	0 (0-0)	0.5 (0-1)	1 (1-1)
FEV_1#		L	3.07 (2.40-3.89)	2.23 (1.90-2.62)	2.36 (2.07-3.31)
FVC		L	3.52 (2.72-4.62)	3.05 (2.20-3.74)	3.24 (2.50-4.59)
$PC_{20}FEV_1$ Mch #		mg/mL	16.0 (13.3-16.0)	8.0 (2.0-16.0)	2.7 (0.8-10.7)
FENO at 50 mL/s #		ppb	27.0 (20.8-30.7)	78.7 (31.1-109.7)	59.8 (54.5-122.3)
Sputum	eosinophils #	%	2.1 (2.0-3.4)	18.1 (11.4-23.4)	37.0 (20.9-42.9)
	neutrophils *	%	44.2 (34.0-64.4)	50.1 (27.4-57.6)	24.6 (17.4- 32.9)
	lymphocytes	%	6.9 (5.2-11.7)	7.0 (5.4-9.2)	7.1 (5.9-10.6)
	histamine *	ng/mL	4.5 (0.7 -13.7)	11.4 (6.0-43.3)	12.0 (5.8-23.4)
Blood	eosinophils #	%	3.8 (2.4-4.7)	10.1 (7.0-11.1)	14.9 (11.4-24.9)
	lymphocytes	%	26.7 (21.4-29.6)	26.8 (16.5-35.4)	20.5 (12.7-31.9)
	serum IgE	kU/L	57.8 (23.0-168.0)	61.7 (32.6-184.0)	52.6 (21.9-119.0)

The table shows median values and interquartile ranges (in parentheses).
Significant change over visits by Friedman's ANOVA: *$p<0.05$, #$p<0.01$ (for details see Results)

cept patient #9 who showed a, though undocumented, history of asthma with normal airway responsiveness to methacholine. Two patients (#1,10) showed a positive skin prick test to at least one common allergen. One was a current smoker and 6 ex-smokers, all having stopped smoking ≥20 years ago, after, on average, 10 pack years. Patients were consecutive patients and not selected for a history of respiratory diseases. All of them had undergone nephrectomy before IL-2 therapy and gave their written informed consent after approval of the study by the local Ethics Committee. Owing to limitations in the patients' ability to cooperate, a few functional values were missing at some visits (Fig. 1).

Study Protocol

Measurements were performed at baseline, 8.5 (6-10) days (median, range) after start of therapy, and 21 (16-29) days later. The total dose of recombinant IL-2 (Proleukin®, Chiron, Emeryville, CA) was 36 MIU/d in 9 patients and was reduced to 18 MIU/d in two of them (#2,4) after the second visit. One patient received 18 MIU/d throughout (#10). Five patients started with inhalation only, administering $1/11^{th}$ of their daily dose subcutaneously after the second visit, while all others included the subcutaneous IL-2 from the beginning on. Eight patients received additional subcutaneous IFN-α (3x6 MIU per week; Roferon®, Hoffman-La Roche Inc., Nutley, NJ) after the second visit, as usual with such protocols [19]. Patients inhaled 5x per day, with one day per week off [19], using breath-triggered nebulisers that minimized dead space deposition (Jetair d20, Salvia Lifetec, Kronberg, Germany).

On each visit, clinical state and symptoms were assessed, blood samples taken, FENO and lung function measured, and methacholine challenge and sputum induction performed, in that order. Salbutamol (200 μg) was inhaled after administration of the last methacholine dose.

Functional Assessments

An electronic spirometer (Viasys, Würzburg, Germany) was used to measure the forced expiratory volume in one second, FEV_1, and forced vital capacity, FVC, following established criteria [18]. On each visit patients were asked for symptoms, comprising cough, sputum production, and dyspnoea, and the presence of any of these was coded as 0 or 1. Airway responsiveness to inhaled methacholine was expressed as the concentration of methacholine, $PC_{20}FEV_1$, eliciting a 20 % fall in FEV_1. The protocol covered concentrations up to 16 mg/mL, hyperresponsiveness being assumed when $PC_{20}FEV_1<8$ mg/mL [20].

Sputum induction followed a standard protocol [21]. Prior to induction patients inhaled 200 μg salbutamol and 15 min later 3 % saline for 10 min from an ultrasonic nebuliser. Samples were separated from saliva, homogenised by Sputolysin®, and differential counts derived from ≥400 nonsquamous cells on May-Grünwald-Giemsa-stained cytospins by two independent investigators, the average being taken for analysis. Histamine in supernatants was quantified by ELISA (IBL, Hamburg, Germany).

For measuring FENO we used a Sievers NOA 280 analyser whose calibration was checked in each test. Following current recommendations, patients exhaled against a resistance, achieving plateau values at 50 mL/s [22]. To correct for potential deviations from target flow, concentrations were assessed over different flows and the final value derived by interpo-

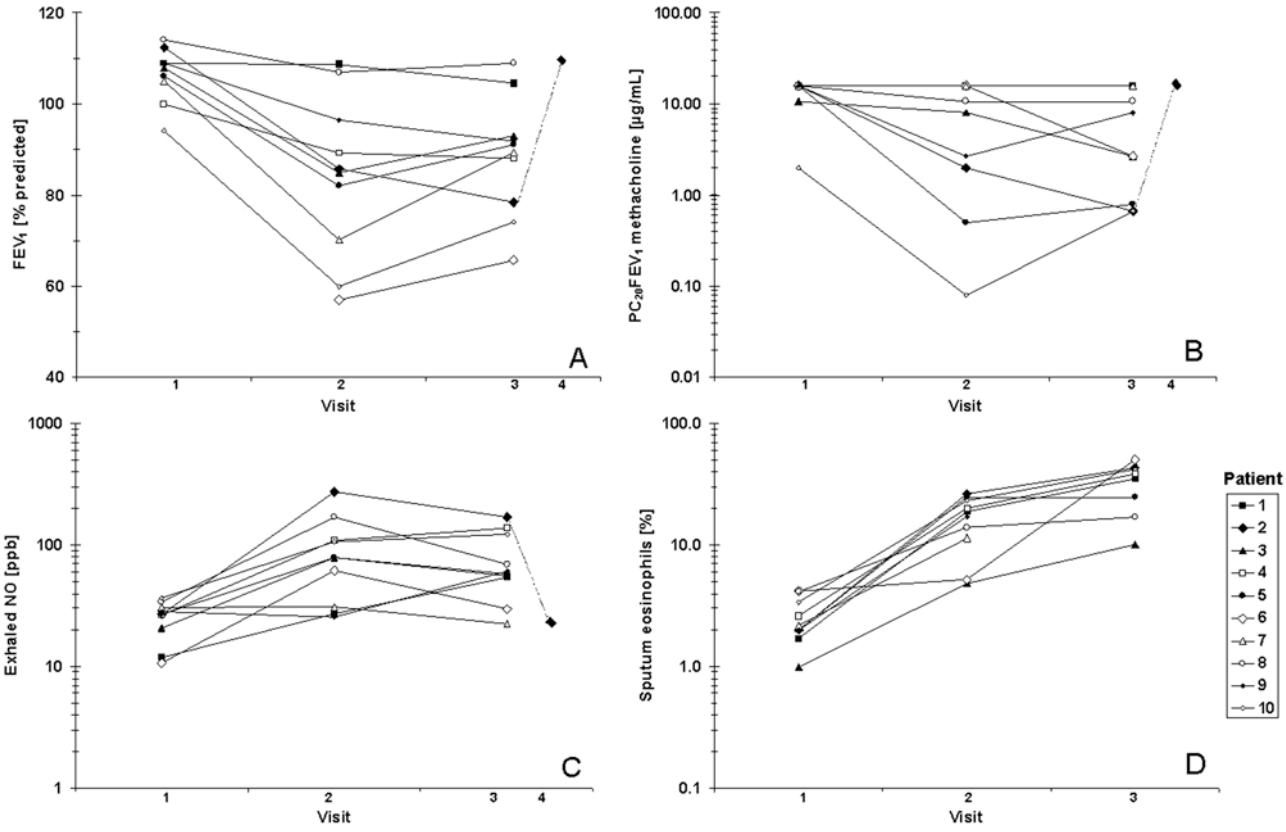

Fig. 1. Individual values of FEV_1 (panel A), $PC_{20}FEV_1$ (B), exhaled NO (FENO) (C), and the percentage of sputum eosinophils (D) on the three study visits. Please note the log scales in B, C, and D. Visit 1 refers to baseline before start of IL-2 therapy, visits 2 and 3 to the measurements after initiation of therapy (for time intervals see text). The additional visit 4 refers to patient no. 2, who was re-measured two months after discontinuation of therapy. To enable a comparison between the slightly different treatment regimes, open symbols denote patients who received only inhaled IL-2 between visits 1 and 2, and closed symbols patients who received $1/11^{th}$ of their daily IL-2 dose subcutaneously from the beginning on (for doses see text). After visit 2, all patients administered $1/11^{th}$ of their daily IL-2 dose subcutaneously. Furthermore, after that visit additional subcutaneous IFN-α was given to all patients except no. 2 and 3.

lation.

Cytokine Production by T Cells

Intracellular cytokine production was assessed in lymphocytes of blood and sputum, whereby IL-2 and IFN-γ represented T_H1-, and IL-4 and IL-5 T_H2-cytokines. Cells were diluted in RPMI 1640 and one aliquot stimulated for 4 h with PMA (20 ng/mL) plus ionomycin (1 µg/mL), while secretion was blocked (monensin, 5 µM). After permeabilization (Fix&Perm, Caltag, Burlingame, CA), cells were incubated with antibodies against CD3, CD8 and the selected cytokines (BD Biosciences, Franklin Lakes, NJ). As PMA interacts with CD4 [23], helper cells (T_H) were identified as CD3-positive and CD8-negative cells. Results of flow cytometry (BD FacsCalibur) were expressed as percent T cells positive for a cytokine, whilst negative (T_H) or positive ($T_{C/S}$) for CD8.

Statistical Analysis

Data were expressed as median values and interquartile ranges. The three visits were compared by Friedman's nonparametric ANOVA. P-values are given explicitely and were assumed significant when <0.05.

Results

Functional Assessments

When comparing data of the three visits, patients reported an increase in cough ($p = 0.012$) and dyspnoea ($p = 0.034$) over time (Table 1). IL-2 therapy also induced significant ($p = 0.0011$) changes in FEV_1 (Table 1, Fig. 1A), while those in FVC did not reach significance ($p = 0.17$). $PC_{20}FEV_1$ decreased over visits ($p = 0.0088$; Fig. 1B), whereas the level of exhaled NO (FENO) increased ($p = 0.0075$; Fig. 1C), as well as the percentage of sputum eosinophils ($p = 0.00034$; Fig. 1D). Percentages of sputum neutrophils were also affected by IL-2 ($p = 0.030$), but not those of macrophages ($p = 0.093$) and lymphocytes ($p = 0.88$). Supernatants exhibited a rise in histamine concentration ($p = 0.016$). The percentage of peripheral blood eosinophils increased greatly ($p = 0.00030$), without significant changes in blood lymphocytes ($p = 0.17$) or serum IgE ($p = 0.46$). None of the variables showed apparent differences between groups in whom therapy was started with or without subcutaneous IL-2 (Fig. 1).

We additionally measured one patient (no. 2) two months after discontinuation of therapy. This patient had shown marked responses to IL-2 in terms of FEV_1, $PC_{20}FEV_1$, FENO, sputum histamine and eosinophils, which were abolished after the end of therapy (sputum eosinophils could not be evaluated on the additional visit) (Fig. 1).

Cytokine Production in T Lymphocytes

Intracellular cytokine production in lymphocytes of induced sputum or peripheral blood was altered only after stimulation (Table 2). In blood, changes were statistically significant regarding IFN-γ, IL-4 (both T_H) and IL-5 ($T_{C/S}$), and in sputum regarding IFN-γ ($T_{C/S}$).

Table 2. Cytokine production in T lymphocytes during IL-2 inhalation therapy.

		Visit 1 (baseline)	Visit 2	Visit 3
Blood				
IL-2	T_H	0 → 52	0 → 47	0 → 48
	$T_{C/S}$	0 → 33	0 → 27	0 → 25
IFN-γ	T_H	0 → 12	0 → 9	0 → 8 *
	$T_{C/S}$	0 → 39	0 → 35	0 → 28
IL-4	T_H	0 → 9	0 → 8	0 → 5 *
	$T_{C/S}$	0 → 19	0 → 19	0 → 13
IL-5	T_H	2 → 20	3 → 26	2 → 21
	$T_{C/S}$	2 → 14	3 → 28	2 → 13 *
Sputum				
IL-2	T_H	1 → 14	1 → 7	2 → 8
	$T_{C/S}$	1 → 8	2 → 8	0 → 10
IFN-γ	T_H	2 → 21	0 → 20	1 → 14
	$T_{C/S}$	1 → 21	1 → 45	1 → 37 *
IL-4	T_H	6 → 13	2 → 16	2 → 8
	$T_{C/S}$	2 → 16	1 → 26	2 → 13
IL-5	T_H	12 → 23	11 → 13	11 → 11
	$T_{C/S}$	10 → 17	5 → 12	6 → 10

Median values of the percentage of T cells positive for the respective cytokine, either without or with (→) stimulation. T_H cells were identified as CD8-negative, $T_{C/S}$ cells as CD8-positive T cells.
* Significant ($p<0.05$) changes over visits were observed only after stimulation.

Discussion

The present data demonstrate that high-dose IL-2 inhalation therapy is capable of temporarily inducing alterations resembling those of mild asthma, as judged from symptoms, airway obstruction, hyperresponsiveness and markers of airway inflammation.

Eosinophilic inflammation [14-16] and elevated levels of exhaled NO [17] are generally considered hallmarks of asthma, as they are much less found in other diseases such as chronic obstructive pulmonary disease (COPD) or interstitial lung disease. The mechanisms causing asthma in human subjects are only partially known, despite a large number of studies on the role of environmental exposures, genetic disposition and animal models [15]. Not even the link between asthma and atopy is undisputable, as the intrinsic asthma demonstrates.

Our findings on airway inflammation were consistent in showing parallel changes in eosinophils and NO, as known after allergen exposure or, conversely, anti-inflammatory treatment. Obviously, these changes also occurred with a non-allergic stimulus such as IL-2. The change in eosinophil numbers was accompanied by a change in airway responsiveness. It seems noteworthy that such an association was also observed in patients with asthma showing a short duration of their disease but not in those with a long duration [24].

Previous attempts of experimentally inducing an asthma-like state in human subjects showed limited success. Possibly respiratory tract infections by viruses bear the closest resemblance to what is seen in asthma, as they can temporarily induce airway eosinophilia [25], a rise in FENO [26] and hyperresponsiveness [27] even in healthy subjects. It has been reported previously that intravenous IL-2 can affect lung function, in terms of a reduction in FEV_1 and FVC, as a "not clinically relevant, interstitial lung defect" [11]. After IL-2 inhalation we found a reduction in FEV_1 and FEV_1/FVC, with much smaller changes in FVC (table 1), particularly on visit 3, as well as airway hyperresponsiveness. These changes were more indicative of asthma than of interstitial lung disease. Noteworthy enough, salbutamol has been reported to be useful for symptom relief after IL-2 inhalation [28].

One of the patients reported on signs of asthma upon inclusion but none had chronic obstructive bronchitis (COPD). We cannot exclude that the daily inhalations per se promoted the changes that arose within a few days, as we could not treat a control group with sham inhalations. Clinical experience with conventional inhalation therapy by nebulisers of similar characteristics as utilised here seems not to support this assumption. It is unlikely that the mannitol (50 mg per day) which was contained in the IL-2 solution was responsible for the effects, as inhalation of doses as high as 635 mg mannitol does not elicit significant effects on the respiratory system in healthy subjects [29]. Regarding their extrapulmonary status, patients were stable during the study, rendering immunological alterations from the tumour disease unlikely. The onset of systemic IL-2 administration, which involved only $1/11^{th}$ of the total daily dose, and of IFN-α application did not seem to affect responses (Fig. 1).

Our data built upon systemic IL-2 therapy which is known to result in increased numbers of blood eosinophils and percentages of hypodense eosinophils [12], changes that disappear after discontinuation of therapy and are not elicited by IFN-γ or IFN-β. One patient whom we were able to measure after termination of inhalation therapy, showed his baseline values restored, despite the intermediate induction of eosinophilia, airway hyperresponsiveness and obstruction (Fig.1). Furthermore, we were able to obtain peak expiratory flow (PEF) data in one additional patient,

who did not complete the protocol, but stopped therapy for about one month, started it again for about 3 weeks and stopped again. This patient showed median a.m. and p.m. values of 307 and 370 L/min during the first period of therapy, 462 and 466 L/min after discontinuation, 417 and 432 L/min when starting therapy again, and 507 and 517 L/min when stopping again. Thus there seemed to be a PEF fall and variation in relation to the intermittent therapy. The observation that changes were reversible does not necessarily speak against the hypothesis that high-dose IL-2 inhalation mimicked asthma in some aspects, as it is increasingly recognised that asthma might occur as a temporary condition and disappear even in the absence of therapeutic interventions.

Besides eosinophilia, anaemia, thrombocytopenia, and lymphocytopenia can occur with systemic IL-2 therapy. Some data also suggest infectious complications, but the well-known flu-like syndromes of systemic application may be confounding. Such effects we did not observe, probably because the major dose of IL-2 was administered locally via inhalation.

Most data on the effects of IL-2 on airway function arose from animal studies. In rats subcutaneous IL-2 led to increased numbers of eosinophils in BALF, as well as an increase in airway responsiveness related to inflammation but not to edema or the vascular infiltrate [30]. Similar results were obtained in guinea pigs. IL-2 can greatly enhance early and late phase responses in ovalbumin-sensitized animals and increase numbers of eosinophils, lymphocytes and mast cells around airways, without affecting IgE levels [31]. Only two patients studied by us showed signs of atopy, and median levels of serum IgE were neither markedly elevated nor did they change during the study. Those of histamine in sputum, however, increased, pointing towards mast cell activation.

IL-2 is known as a key regulator of cell activation and proliferation in the immune system, and its antitumour action is thought to involve the induction of cytokines with tumoricidal potency, such as IFN-γ, and expansion of lymphokine-acitivated killer (LAK) cells. There are also links between IL-2 and eosinophil maturation and activation [32]. Furthermore, eosinophils can synthesize, store and release IL-2 [33] and IL-2 mRNA is present in cells of BALF and bronchial mucosa [34]. BALF concentrations of IL-2 and its soluble receptor sCD25 were increased in asthma, although serum sCD25 did not seem a suitable marker compared to FENO [35]. We did not measure sCD25 in sputum owing to methodological obstacles [36].

Administration of IL-2 can lead to elevated plasma levels of IL-5 [13] and enhanced mRNA for IL-5 in T [37] and mononuclear cells, in close association with eosinophilia. Also, LAK cells showed an increase in IL-5 production upon stimulation with IL-2 [38], without a consistent pattern in the plasma levels of IL-4 and IFN-γ [13]. Prior to eosinophil numbers the concentration of major basic protein increased, indicating degranulation and activation [13]. In our patients, IL-2-induced eosinophil recruitment did not appear to depend on pre-existing eosinophilia, as upon baseline only one patient showed eosinophil counts in blood ≥6 % and 3 patients ≥3 % in sputum. Furthermore, the response was also not prevented by the administration of IFN-α which inhibits the antigen-induced eosinophil influx in mice [39].

It is not clear whether the eosinophilic response plays a role in the anti-tumour effects of IL-2 or represents a useful marker, as suggested for its local effect in the bladder [40]. Eosinophils are capable of exerting cytotoxicity against tumour cells *ex vivo* after activation by IL-5, or indirectly through IL-2 *in vivo* [41].

The analysis of cytokine production by sputum lymphocytes was performed on an exploratory basis and rendered difficult by the low number of sputum cells. All of the data (Table 2) have to be viewed with certain care, due to the multiple tests involved. There seemed to be a transient increase in the stimulated IL-5 production by $T_{C/S}$ and possibly T_H blood cells, in accordance with the acute eosinophilia, accompanied by phase-lagged minor changes in stimulated IL-4 and IFN-γ production. In sputum only a rise in stimulated IFN-γ production by $T_{C/S}$ lymphocytes was obvious and possibly a transient increase in IL-4 production. IL-2 (plus IFN-α) did not markedly affect IL-2 production, either in spite or because of the elicited T_H2-like inflammatory response. Owing to the limited amount of sputum material we could not assess other potential shifts in cell activation by additional markers.

The relationship between IL-2 and asthma is rather intricate. There have been doubts whether the dichotomy between T_H1 and T_H2 responses, as proposed in mice, is appropriate in human subjects [15]. Currently one can only speculate on an impact of our findings for the understanding of asthma but the asthma-like response after IL-2 inhalation seems striking. Interestingly enough, IL-2 is capable of shifting the polarisation of naive T cells into the T_H2 phenotype [42]. It might also be worth mentioning that, e.g., complement activation is one of the factors related to IL-2-induced cytokine production [43] and that, conversely, complement 3a and 5, as parts of the innate immune system, seem to be involved in asthma [44,45], in addition to acquired immune responses.

To avoid unwarranted interpretations, it should be emphasized that IL-2 inhalation was well accepted. Different from systemic therapy, patients on aerosol therapy were still capable of fulfilling their social role and side effects seemed to be limited to local toxicity, such as dose-dependent cough. Corticosteroids were not given, as they are known to impair the anti-tumour response. IL-2 inhalation therapy seems to be advantageous particularly in high-risk patients with co-morbidities, and a direct comparison of systemic vs predominantly inhalational IL-2 therapy suggested a significant survival benefit in the inhalation group [8]. More detailed studies are to be expected on the basis of a recent positive opinion of the Committee for Orphan Medicinal Products of the European Agency for the Evaluation of Medicinal Products (EMEA). The finding that changes were reversible, additionally underlined that the inhalation therapy did not force the patients to pay an, at least partial, control of lung metastases by acquiring a chronic, irreversible airway disease.

In conclusion, the present data suggest that asthma-like symptoms, functional changes and airway inflammation can be elicited through high-dose interleukin-2

inhalation. This supports the assumption that bronchial asthma is to be put into a wider framework than T_H2 responses.

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REFERENCES

1. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, et al.: A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 1987;316:889-897.
2. Atzpodien J, Körfer A, Franks CR, Poliwoda H, Kirchner H: Home therapy with recombinant interleukin-2 and interferon alpha 2 b in advanced human malignancies. Lancet 1990;335:1509-1512.
3. Rosenberg SA, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE: Treatment of 283 consecutive patients with metastatic melanoma or renal-cell carcinoma using high-dose bolus interleukin-2. JAMA 1994;271:907-913.
4. Motzer RJ, Mazumdar M, Bacik J, Russo P, Berg WJ, Metz EM: Effect of cytokine therapy on survival for patients with advanced renal cell carcinoma. J Clin Oncol 2000;18:1928-1935.
5. Heinzer H, Mir TS, Huland E, Huland H: Subjective and objective prospective, long-term analysis of quality of life during inhaled interleukin-2 immunotherapy. J Clin Oncol 1999;17:3612-3620.
6. Huland E, Heinzer H, Huland H, Yung R: Overview of interleukin-2 inhalation therapy. Cancer J Sci Am 2000;6 (suppl 1):S104-112.
7. Lorenz J, Wilhelm K, Kessler M, Peschel C, Schwulera U, Lissner R, Struff WG, Huland E, Huber C, Aulitzky WE: Phase I trial of inhaled natural interleukin-2 for treatment of pulmonary malignancy: toxicity, pharmacokinetics and biological effects. Clin Cancer Res 1996;2:1115-1122.
8. Huland E, Heinzer H, Huland H: High risk patients with with pulmonary metastases of renal cell carcinoma: safety and efficacy of inhaled interleukin-2 compared to systemic IL-2 [Abstract]. Eur Urol 2003;1(Suppl. 2): 98A.
9. Sondel PM, Kohler PC, Hank JA, Moore KH, Rosenthal NS, Sosman JA, Bechhofer R, Storer B: Clinical and immunological effects of recombinant interleukin 2 given by repetitive weekly cycles to patients with cancer. Cancer Res 1988;48:2561-2567.
10. Zissel G, Aulitzky WE, Lorenz J, Huber C, Müller-Quernheim J: Induction of accessory cell function of human alveolar macrophages by inhalation of human natural interleukin-2. Cancer Immunol Immunother 1996;42:122-126.
11. Ardizzoni A, Bonavia M, Viale M, Baldini E, Mereu C, Verna A, Ferrini S, Cinquegrana A, Molinari S, Mariani GL, et al.: Biologic and clinical effects of continuous infusion interleukin-2 in patients with non-small cell lung cancer. Cancer 1994;73:1353-1360.
12. Sedgwick JB, Frick WE, Sondel PM, Hank JA, Borden E, Busse WW: The appearance of hypodense eosinophils during interleukin-2 treatment. J Allergy Clin Immunol 1990;85:557-566.
13. van Haelst Pisani C, Kovach JS, Kita H, Leiferman KM, Gleich GJ, Silver JE, Dennin R, Abrams JS: Administration of interleukin-2 (IL-2) results in increased plasma concentrations of IL-5 and eosinophilia in patients with cancer. Blood 1991; 78: 1538-1544.
14. Durham SR, Till SJ, Corrigan CJ: T lymphocytes in asthma: bronchial versus peripheral responses. J Allergy Clin Immunol 2000;106:S221-226.
15. Salvi SS, Babu KS, Holgate ST: Is asthma really due to a polarized T cell response toward a helper T cell type 2 phenotype? Am J Respir Crit Care Med 2001;164:1343-1346.
16. Jayaram, Parameswaran K, Sears MR, Hargreave FE: Induced sputum cell counts: their usefulness in clinical practice. Eur Respir J 2000;16:150-158.
17. Kharitonov SA, Barnes PJ: Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163: 1693-1722.
18. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC: Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European Community for Steel and Coal. Official statement of the European Respiratory Society. Eur Respir J 1993;16(suppl):5-40.
19. Huland E, Heinzer H, Mir TS, Huland H: Inhaled interleukin-2 therapy in pulmonary metastatic renal cell carcinoma: six years of experience. Cancer J Sci Am 1997;3:S98-105.
20. Jörres RA, Nowak D, Kirsten D, Grönke L, Magnussen H. A short protocol for methacholine provocation testing adapted to the Rosenthal-Chai dosimeter technique. Chest 1997;111:866-869.
21. Holz O, Jörres RA, Koschyk S, Speckin P, Welcker L, Magnussen H. Changes in sputum composition during sputum induction in healthy and asthmatic subjects. Clin Exp Allergy 1998;28:284-292.
22. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 200515;171:912-930.
23. Picker LJ, Singh MK, Zdraveski Z, Treer JR, Waldrop SL, Bergstresser PR, Maino VC: Direct demonstration of cytokine synthesis heterogeneity among human memory/effector T cells by flow cytometry. Blood 1995;86:1408-1409.
24. Grönke L, Kanniess F, Holz O, Jörres RA, Magnussen H: The relationship between airway hyperresponsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. Clin Exp Allergy 2002;165:57-63.
25. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST: Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995;151:879-886.
26. Kharitonov SA, Yates D, Barnes PJ: Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 1995;8:295-297.
27. Laitinen LA, Elkin RB, Empey DW, Jacobs L, Mills J, Nadel JA: Bronchial hyperresponsiveness in normal subjects during attenuated influenza virus infection. Am Rev Respir Dis 1991;143:358-361. Comment in: Am Rev Respir Dis 1991;144:1422-1423.
28. Barzrca S, Meydan N, Barklak A: Prevention of interleukin-2-induced severe bronchospasm with salbutamol. J Aerosol Med 2003;16:183-184.
29. Holzer K, Anderson SD, Chan HK, Douglass J: Mannitol as a challenge test to identify exercise-induced bronchoconstriction in elite athletes. Am J Respir Crit Care Med 2003;167:534-537.
30. Renzi PM, Sapienza S, Du T, Wang NS, Martin JG: Lymphokine-induced airway hyperresponsiveness in the rat. Am Rev Respir Dis 1991;143:375-379.

31. Renzi PM, Sapienza S, Waserman S, Du T, Olivenstein R, Wang NS, Martin JG: Effect of interleukin-2 on the airway response to antigen in the rat. Am Rev Respir Dis 1992;146:163-169.
32. Tompkins MB, Novotney C, Grindem CB, Page R, English R, Nelson P, Tompkins WA: Human recombinant interleukin-2 induces maturation and activation signals for feline eosinophils in vivo. J Leukoc Biol 1990;48:531-540.
33. Levi-Schäffer F, Barkans J, Newman TM et al.: Identification of interleukin-2 in human peripheral blood eosinophils. Immunology 1996;87:155-161.
34. Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB: Phenotype of cells expressing mRNA for T_H2-type (interleukin 4 and interleukin 5) and T_H1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. Am J Respir Cell Mol Biol 1995;12:477-487.
35. Lanz MJ, Leung DY, McCormick DR, Harbeck R, Szefler SJ, White CW: Comparison of exhaled nitric oxide, serum eosinophilic cationic protein, and soluble interleukin-2 receptor in exacerbations of pediatric asthma. Pediatr Pulmonol 1997;24:305-311.
36. Motojima S, Ogata H, Tateishi K, Kushima A, Yamai T, Fukuda T, Makino S: Soluble interleukin-2 receptor in sputum from patients with bronchial asthma. Arerugi 1993;42:162-166.
37. Enokihara H, Furusawa S, Nakakubo H, Kajitani H, Nagashima S, Saito K, Shishido H, Hitoshi Y, Takatsu K, Noma T, et al.: T cells from eosinophilic patients produce interleukin-5 with interleukin-2 stimulation. Blood 1989;73:1809-1813.
38. Ishimitsu T, Torisu M: An eosinophil chemotactic lymphokine in the supernatant of lymphokine-activated killer (LAK) cell culture: relationship to interleukin-5. J Surg Res 1993;55:307-313.
39. Nakajima H, Nakao A, Watanabe Y, Yoshida S, Iwamoto I: IFN-alpha inhibits antigen-induced eosinophil and CD4+ T cell recruitment into tissue. J Immunol 1994; 153:1264-1270.
40. Huland E, Huland H: Tumor-associated eosinophilia in interleukin-2-treated patients: evidence of toxic eosinophil degranulation on bladder cancer cells. J Cancer Res Clin Oncol 1992;118:463-467.
41. Rivoltini L, Viggiano V, Spinazze S, Santoro A, Colombo MP, Takatsu K, Parmiani G: In vitro anti-tumor activity of eosinophils from cancer patients treated with subcutaneous administration of interleukin 2. Role of interleukin 5. Int J Cancer 1993;54:8-15. Erratum in: Int J Cancer 1993;54:887.
42. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, Zhu J, Paul WE: Interleukin 2 plays a central role in Th2 differentiation. PNAS 2004;101:3880-3885.
43. Thijs LG, Hack CE, Strack van Schijndel RJ, Nuijens JH, Wolbink BJ, Eerenberg-Belmer AJ, Van der Vall H, Wagstaff J: Activation of the complement system during immunotherapy with recombinant IL-2. Relation to the development of side effects. J Immunol 1990;144:2419-2424.
44. Humbles AA, Lu B, Nilsson CA, Lilly C, Israel E, Fujiwara Y, Gerard NP, Gerard C: A role for the C3a anaphylatoxin receptor in the effector phase of asthma. Nature 2000;406:998-1001.
45. Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Kohl J, Wahl L, Kuperman D, Germer S, Aud D, Peltz G, Wills-Karp M: Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. Nat Immunol 2000;1:221-226. Comment in: Nat Immunol 2000;1:190-192.

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