

Short communication

Differential modulation of T cell functions by soluble IL-4R (sIL-4R) in two cases of severe atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with elevated levels of serum IgE (1). The production of IgE is controlled by CD4⁺ T-cells (2). The T-cell product interleukin (IL)-4 has been shown to play an important role as a potent inducer of IgE. In contrast, the T-cell product IFN- γ was shown to inhibit IL-4 induced IgE production. Mechanisms resulting in increased IgE synthesis in AD may be related to either decreased IFN- and/or increased IL-4 production. In fact, several studies have shown that lymphocytes from AD patients exhibited such a complex pattern of cytokine dysregulation (3) that is associated by an increased frequency of IL-4 producing T-cells in both peripheral blood and local inflammatory tissue of the skin (4). Therefore, direct or indirect modulation of T-cell functions may be the target of new therapeutic approaches for allergic diseases. Recent studies in human and mice demonstrated that many of the IL-4 dependent T-cell functions, including the production of total and allergen-specific IgE, could be inhibited not only by IFN- γ (5), but also by a recombinant soluble (s)IL-4R (6, 7). sIL-4R represents a

naturally produced soluble receptor molecule that is secreted by T-cells upon stimulation and activation (8), and it binds IL-4 with a similar affinity as the membrane bound IL-4R.

To obtain preliminary data about possible immuno-modulatory effects of sIL-4R we examined whether such a drug may modulate allergen-specific and allergen-induced lymphocyte functions. For this purpose two patients with most severe AD (according to the criteria by Hanifin et al [9]) were analyzed, one of them (P.H.) was resistant to conventional and experimental therapeutic approaches including systemic IFN- γ and local cyclosporin A treatment. Peripheral blood mononuclear cells (PBMC) from these patients were subjected to several in-vitro assays to study the effects of sIL-4R under cell culture conditions.

Case reports

Patient 1

ML, a 10-year-old boy, suffered from severe AD (COSTA-score (10) = 82; ranging from 0 to 100 points with 100 points assigned for maximal affection) with general erythroderma and frequent bacterial superinfections since early childhood and a positive family history for atopy. Bronchial asthma persisted since early infancy with exacerbations es-

Abbreviations: AD: atopic dermatitis, *Derp*: dermatophagoides pteronyssinus, Tet: tetanus toxoid, PBMC: peripheral blood mononuclear cells, rhIFN-: recombinant human interferon-gamma, sIL-4R: soluble interleukin 4 receptor

pecially during physical exercise. He suffered from food allergies to fish, soy, egg, and others. Total serum IgE was at 87,400 ng/ml and specific IgE antibodies against all tested food and inhalant allergens were detected. Anti-inflammatory therapy consisted of topical steroids and antihistamines only.

Patient 2

PH was a 5-year-old girl with a negative family history for atopy. Since her 6th month of life she had severe AD (COSTA-score = 68) with ubiquitous xerosis, general erythema, multiple excoriations. Her serum IgE was 126,000 ng/ml. She suffered from multiple food allergies against nearly all main protein sources including fish, egg, cow's milk, wheat, and others. Food allergy was diagnosed by double blind placebo controlled food challenge (soy), open food challenge (corn, rice, egg), and selective elimination diets. In addition, clinical signs of bronchial obstructions were noted (wheezing; shortness of breath). Therapy consisted of a very strict diet (weight: between 25th–50th percentile; height: between 10.–25. percentile). She received sodium cromoglycate orally and ointment containing 0.03% betamethasone 2–3 times per day. Bronchial asthma was managed with sodium cromoglycate and beta-2-adrenergic agonists.

Cell preparation – PBMC were prepared from heparinized blood by lymphoprep gradient centrifugation (Gibco/BRL, Gaithersburg, MD, USA) for 20 min at 2000 rpm at room temperature (RT). Cells from the interface were washed three times (10 min, 3000 rpm, RT) in RPMI 1640 (Biochrom, Berlin, FRG).

Cell proliferation assay – To assess the proliferative response of lymphocytes to allergens and antigens 4×10^5 PBMC/well were incubated in round bottom microtiter plates (Greiner, Nürtingen, FRG) in the presence and absence of 5 µg/ml recombinant human sIL4R (Behringwerke, Marburg, FRG) that was prepared as described (11). Cells were incubated for 5 days in culture medium alone or with either 500 SQU/ml *Dermatophagoides pteronyssinus* (Der p) (Scherax, Hamburg, FRG), 50 mg/ml ovalbumin (OVA) or caseine (Sigma, Taufkirchen, FRG), or 5 µg/ml tetanus toxoid (Behringwerke). The cultures were performed in triplicates and pulsed with 1 mCi ^3H [TdR]/well (Amersham, Braunschweig, FRG) 24 hours prior to cell harvest. Due to limited availability of lymphocytes from both children detailed dose response experiment could not be performed for all reagents. The dose of 5 µg/ml sIL-4R was chosen based on previously published results using murine sIL-4R (13).

Table 1: Differential effects of sIL-4R on allergen and antigen stimulated Ig-production

	P.H.		M.L.	
	IgE (ng/ml)	IgG (ng/ml)	IgE (ng/ml)	IgG (ng/ml)
Medium	40 ± 12	612 ± 48	12 ± 4	846 ± 90
Medium + sIL-4R	32 ± 6	312 ± 25	8 ± 2	240 ± 30
Der p	115 ± 17	3240 ± 241	30 ± 4	5050 ± 240
Der p + sIL-4R	82 ± 7	1240 ± 140	11 ± 2	850 ± 75
Tetanus	93 ± 6	1630 ± 80	21 ± 3	1550 ± 200
Tetanus + sIL-4R	89 ± 5	5280 ± 410	27 ± 2	4820 ± 360

2×10^6 PBMC/well were incubated with Der p extract (500 SQU/ml) or tetanus toxoid (5 µg/ml) and sIL-4R (5 Iµg/ml) for 14 days. Cell free culture supernatants were harvested and analyzed for total IgE and IgG production by FEIA and ELISA, respectively, as described in Material and Methods; shown are mean SD ± from one of two experiments.

Determination of total and allergen-specific Ig production in culture supernatants – For immunoglobulin production 2×10^6 PBMC/well were incubated for 14 days in flat bottom 24-well-plates (Greiner) and stimulated as described. Cell free culture supernatants were harvested and assayed for total and allergen-specific IgE by FEIA (Pharmacia CAP System) (Pharmacia, Uppsala, Schweden). Analysis was performed according to the instructions of the manufacturer. Detection limit was 0.7 ng IgE/ml. IgG was analyzed by ELISA as previously described (11).

Results and discussion

Differential effects of sIL-4R on antigen and allergen induced Ig production

To evaluate the effects of sIL-4R on immunoglobulin (Ig) production, PBMC were cultured for 14 days and the concentrations of Ig were measured by ELISA or FEIA. PBMC from both patients showed high spontaneous cell proliferation and Ig-production in cell culture. This phenomenon may be explained by the presence of already preactivated lymphocytes as reflected by enhanced expression of T cell activation markers including HLA-DR,

Table 2: Inhibition of allergen-specific IgE by human recombinant sIL-4R

	anti-Der p IgE (ng/ml)	
	P.H.	M.L.
Medium	< 0.7	1.6 ± 0.1
Medium + sIL-4R	< 0.7	< 0.7
Der p	2.5 ± 0.2	4.5 ± 0.2
Der p + sIL-4R	< 0.7	< 0.7

2×10^6 PBMC were cultured with or without Der p extract (500 SQU/ml) and sIL-4R (5 Iµg/ml) for 14 days. Cell free culture supernatants were analyzed for anti-Der p specific IgE by FEIA as described in Material and methods. Shown are mean ± SD from one out of two similar experiments.

Table 3: Modulation of T-cell proliferation by sIL-4R and IFN- γ in a patient with therapy-resistant AD

Conditions	cpm
Medium	3,778 $\pm$ 290
Medium + IL-4	5,304 $\pm$ 305
Medium + sIL-4R	4,340 $\pm$ 195
Medium + IFN- γ	3,407 $\pm$ 250
Medium + sIL-4R + IFN- γ	3,120 $\pm$ 165
Der p	7,885 $\pm$ 496
Der p + IL-4	10,122 $\pm$ 628
Der p + sIL-4R	5,182 $\pm$ 300
Der p + IFN- γ	6,555 $\pm$ 4,90
Der p + sIL-4R + IFN- γ	4,084 $\pm$ 287

0.4 $\times$ 10⁶ PBMC were cultured in 96-well round-bottom tissue culture plates under described conditions for 5 days. 3H[ThR] was added 24 hour prior to cell harvest (1 elCi/well). Results represent mean $\pm$ SD from quadruplicates of one of two similar experiments.

IL-2Ra (CD25), and transferrin receptor (CD71) (data not shown). When sIL-4R was added to Der p stimulated cell cultures, reduced production of total IgE and IgG was detected in both patients (Table 1). In contrast to only minimal inhibitory effects on total IgE production, sIL-4R suppressed total IgG production by about 75%. Similar inhibitory effects were also detected on spontaneous IgG production. These results indicate that ongoing IgG production was more sensitive to sIL-4R than total IgE production. Since sIL-4R acts via binding of IL-4, these data also suggest that IgG production is more sensitive to IL-4 than IgE. A different sensitivity of IgE and IgG class-switch to IL-4 has been described (12). It was reported, that a switch to IgE may require higher amounts of IL-4 than the switch to IgG1 (mouse) (13) and IgG4 (human). Such differences may provide an explanation why sIL-4R was a potent inhibitor of IgG production, but not of ongoing IgE production.

In contrast to suppressive effects of sIL-4R on allergen-dependent immune functions, a different pattern was observed on tetanus toxoid induced Ig production. It was a surprising finding that addition of sIL-4R to tetanus-stimulated lymphocytes resulted in a marked increase of IgG production. It is known that IL-4 inhibits many immune functions of type-1 T cells that are responsible for a normal antigen-specific immune response (14). Therefore, sIL-4R was not only a potent inhibitor of allergen-induced, IL-4 dependent, immune functions, but simultaneously favored the development of an antigen-specific lymphocyte response. An explanation for this shift in Ig production could be that inhibition of IL-4 functions by sIL-4R favored the production of the IL-4 independent IgG 1 and IgG 3 subclasses and suppressed IL-4 dependent IgE/IgG 4 production.

sIL-4R suppressed allergen-specific IgE-production – In both patients sIL-4R suppressed allergen-specific IgE production below the detection limit (0.7 ng/ml) of the assay system (Table 2). The inhibition of allergen specific IgE production paralleled the suppression of allergen-induced lymphocyte proliferation (Table 3). Differences in the inhibition of total and allergen-specific IgE production by sIL-4R may be explained by differences in the state of B-cell differentiation. As shown previously, B-cells that had already differentiated into IgE secreting plasma cells were relatively resistant to immuno-modulatory agents aimed to suppress IgE production. Previously performed kinetic experiments on murine lymphocytes indicated that sIL-4R (and IFN- γ) had to be present at early stages of B-cell activation and proliferation in order to prevent IgE production (13). Addition at later time points was less effective to suppress ongoing IgE production. Therefore, ongoing total IgE production was less sensitive to sIL-4R as compared to newly induced allergen specific IgE in the cell culture system.

Effect of sIL-4R on lymphocyte proliferation – One of the patients (PH) exhibited a disease pattern that was not only resistant to conventional therapy, but also did not improve following experimental therapy with immuno-modulating drugs including high-dose IVIG, topical application of cyclosporin A and systemic treatment with recombinant human (rh) IFN- γ . Based on these clinical observations it was examined whether sIL-4R would have any effects on lymphocyte proliferation (Table 3). Addition of IL-4 enhanced spontaneous and Der p induced cell proliferation. This response was sensitive to addition of sIL4R as indicated by a reduction of cell proliferation. The strongest degree in inhibition of lymphocyte proliferation was found when both sIL-4R and IFN- γ were added to the culture at the same time.

These data indicate that sIL-4R represents a potent agent that modulates many allergen-dependent immune functions in-vitro, even in lymphocytes from patients with most severe AD and resistant to rhIFN- γ therapy. Furthermore, the soluble receptor favored development of antigen-specific T/B-cell functions. These results point to a bidirectional activity of sIL-4R that counteracts the dysregulation of several immune functions in these patients. Clinical studies are now required to assess whether sIL-4R is able to alter IgE production and allergic inflammation under *in vivo* conditions.

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