Introduction

There has been an increase in the prevalence and the severity of bronchial asthma all over the world, this fact being true especially for the atopic type of asthma (20,24,38,41). According to the study by Vondra (57), such an increase does exist in the Czech Republic as well. The reasons for such an increase in atopic prevalence are not quite clear. Whilst hereditability forms the basis of the definition of atopy, it is clear that the environment must have had a major influence on phenotype (34,38,48,63). Longitudinal cohort studies will be required to establish which of the many possible factors are critical to the development of allergy and subsequent asthma. The ultimate aim shall be to identify avoidable factors and to establish prevention protocols (61,62).

SOME SERUM ACTIVITY MARKERS OF AIRWAYS INFLAMMATION IN DIFFICULT-TO-CONTROL ASTHMA PATIENTS

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Summary: The main aim of the present study was a search for a characteristic serum marker of inflammatory activity in the airways of asthmatics with difficult-to-control disease. Therefore, serum levels of interleukin-4 (IL-4), serum low-affinity Fc Epsilon Receptor II (sFceR II), Interferon-gamma (INF-gamma) Immunoglobulin-E (IgE), Interleukin-2 (IL-2), serum Interleukin Receptor 2 (sIL-2R) and Intercellular Adhesion Molecule-1 (sICAM-1) were measured in 2 groups of asthmatics: 1-26 patients with difficult-to-control asthma (DTCA), 2-22 asthmatics, minimally symptomatic (MSA).

Results: No significant difference in either measured parameters between the DTCA and MSA group in peripheral blood samples was found. Conclusion: The above mentioned serum markers of T- and B-cell activation as well as the serum ICAM-1 level are not sensitive enough to determine the type, activity and severity of the inflammatory process in the asthmatic airways.

Key words: Difficult-to-control asthma; Interleukin-4; Interleukin-2; Soluble Fc Epsilon Receptor II; Soluble Interleukin-2 Receptor; Intercellular Adhesion Molecule-1

Abbreviations used in the study

IL - Interleukin
INF - Interferon
TNF - Tumor necrosis factor
IgE - Immunoglobulin E
FcER - Fc epsilon receptor
LT - Leucotriens
PG - Prostaglandins
PAF - Platelet activating factor
Th - T-helper lymphocyte
APC - Antigen presenting cell
GM-CSF - Granulocyte-macrophage colony stimulating factor
ICAM - Intercellular adhesion molecule
VCAM - Vascular adhesion molecule
LFA-1 - Lymphocyte function associated antigen-1
VLA-4 - Very late antigen 4
BAL - Bronchoalveolar lavage
BHR - Bronchial hyperreactivity

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driving the inflammatory response with T-cells taking primacy, and B-cells (via IgE) providing a trigger stimulus involving common allergens. Molecular technology has allowed a myriad of mediators to be identified as contributing to both the acute and chronic aspects of the disease, resulting in bronchial hyperreactivity and smooth muscle contraction too much and too early in response to various "triggers" (specific - allergens, nonspecific - cold, exercise, smoke) (21).

The pathogenesis of the chronic inflammation of asthma is complex and still only partially understood. Once the inflammation has become established, it appears to be maintained by a tissue - driven response, (41). The aetiology of asthma is thought to be the development of a specific immune response in the airways. In allergic asthma, the allergen is captured and processed by antigen-presenting cells (APC) which are dendritic cells in the airways mucosa, other less efficient APCS in the airways include monocytes, epithelial cells, B - lymphocytes and fibroblasts. Following the capture of the antigen, the APC migrate to the regional lymph nodes in the airways, where they present the allergen, in association with the major histocompatibility antigen class II molecules, to lymphocytes.

A characteristic of allergic asthma is the selective development of CD 4+ lymphocytes of the Th 2 - type. This selective development of Th 2 - type lymphocytes in asthma is promoted by Interleukin - 4 (IL-4) and inhibited by IL-12. These allergen - specific lymphocytes enter the circulation and selectively home in to the submucosa and mucosa of the airways. Through the production of various cytokines, these lymphocytes direct the inflammatory reaction into the characteristic airways inflammation of asthma (27). Interleukin -4 appears to be an important cytokine in the development of allergic inflammation, not only because of its role in switching T-helper into Th2 lymphocytes, but because it promotes differentiation of B-lymphocytes to produce IgE (31,32).

The immunoglobulin isotype switch to IgE is dependent on three cytokines: IL-4, INF-gamma and IL-10. IgE secretion by B-lymphocytes requires an additional T-cell derived signal that is provided by a cognate interaction between B-cells and a membrane structure on the B-lymphocytes termed CD 40 (7,43,45,46).

The second cytokine that is critically important in the regulation of IgE synthesis is INF-gamma (1). Interferon gamma functions as an inhibitor of allergic responses through its capacity to inhibit the effects of IL-4 on B-cells. IgE production in atopy represents a combination of excess IL-4 production occurring in the relative absence of Interferon - gamma (28,39).

Interleukin-10 is produced by IL-4 producing T-cells and inhibits the synthesis of INF-gamma by other T-lymphocytes.

In contrast to the Th2-subtype of lymphocyte, Th 1-cells differentiate in the presence of a different range of antigens associated with the delayed type hypersensitivity response. Th 1 cells generate predominantly Interferon-gamma (INF-gamma), IL-2 and TNF-beta (12,13,15,16).

The production of cytokines by Th-lymphocyte subtypes is shown in Table 1.

A summary of cytokine actions in allergies is presented in Table 2.

Table 1: Different types of cytokines produced by Th1 and Th2 - cells are described (according to Borish, 1992).

| cytokine | activity |
| --- | --- |
| IgE regulation | IL-4 | IgE isotype switch |
| | IL-2, IL-5, IL-6 | synergize with IL-4 |
| interferon gamma | inhibits IL-4 |
| IL-10 | inhibits interferon-gamma |
| Eosinophilia | IL-3, IL-5, GM-CSF | eosinophils |
| Mast cell development and activation | IL-3, IL-9, IL-10 | mast cell growth factors |
| | hematopoietic stem cell factor | |
| | connective tissue activating peptide (CTAP) | |
| Inflammation | INF-gamma, GM-CSF, TNFs, IL-1, IL-4, IL-6, IL-8 | neutrophil-activating factors |
| | GM-CSF, TNFs, IL-1, IL-3, IL-5 | eosinophil-activating factors |
| | INF-gamma, GM-CSF, macrophage-CSF, TNFs, IL-1, IL-2, IL-3, IL-4 | macrophage-activating factors |

Either locally produced or circulating IgE antibodies bind to high-affinity IgE receptors (FcER1) on mast cells and circulating basophils, and to low-affinity IgE receptors (FcERII) on eosinophils, monocytes, macrophages, lymphocytes, dendritic cells and platelets (Fig. 1) Cross-linking of the IgE receptors by allergen on the surface of these cells leads to their activation, and the release of various preformed and newly formed mediators in the intercellular spaces.
of the airways. The attraction, activation and prolonged survival of eosinophils in the airway submucosa and mucosa are caused by the production and secretion of IL-3, Granulocyte-Macrophage Colony-Stimulating factor (GM-CSF), IL-5, RANTES and Tumour Necrosis Factor - alpha (TNF-alpha) (15,16,17,18,19,55).

**Figure 1**: Illustrates schematic pathophysiologic patterns of allergic mucosal inflammation and its consequences in the airways of asthmatics (see text for further details).

The movement of circulating eosinophils and other inflammatory cells into the airways is controlled by „priming“ of the cells by cytokines, local production of chemokinetic and chemotactic factors, and expression of adhesion molecules on inflammatory cells, endothelium and extracellular matrix (ICAMs, VCAMs, LFA-I, VLA-4) (7,9,23,29,54,56).

The relative roles of the different adhesion molecules in the development of airway inflammation are not known. Monoclonal antibodies to Intercellular Adhesion Molecule -1 (ICAM-1), Lymphocyte Function Antigen -1 (LFA-1) and Very Late Activation -4 (VLA-4) molecules have been shown to inhibit the influx of eosinophils in the airways following allergen challenge (37,58).

An important characteristic of the chronic airway inflammation of asthma is that constitutional and secondary inflammatory cells may be involved in the maintenance of the chronic inflammation. It appears that the production of pro-inflammatory cytokines by sensitized lymphocytes, but also the expanded involvement of mast cells, eosinophils, constitutive cells, such as epithelial and endothelial cells, and fibroblasts, all apparently play a role in the maintenance of chronic inflammation that characterizes asthma (41,44).

The above-mentioned findings of chronic inflammatory changes in the airway mucosa even in mild forms of asthmatic patients, has led to a change in therapeutic strategy (3). The inhibitory effects of corticosteroids on cytokine production, on cellular responses to cytokines, such as expression of adhesion molecules, increased survival of inflammatory cells and enhanced release of mast cell mediators, now provide a scientific basis for the early use of corticosteroids as a first-line measure in the treatment of asthma (2,4). Furthermore, the effects of corticosteroids on the synthesis of arachidonic acid metabolites are well-known. With this change in the management strategy, a decline in morbidity and mortality associated with asthma has been proven (20,24,41). Despite these fundamental changes in the management of asthma, some asthmatics still remain „difficult - to - control“ (DTCA) (25).

Multiple reasons for the failure of modern treatment strategy in these patients can be identified: 1) non-compliance with treatment, 2) failure to adhere to indicated preventive measures (smoking, exposure to allergens), 3) concomitant disorders (sinusitis, nasal polyps gastro-oesophageal reflux, bronchitis, left ventricular failure, psychogenic factors), 4) inherently severe asthma from the beginning of the disease.

The investigation of the latest category of severe asthmatics represents the main topic of the present study.

**Aims of the study**

1) To measure the serum level of IL-4, INF-gamma, the soluble form of the low-affinity receptor on B-lymphocytes (sFcER II/CD-23) and of IgE in patients with difficult - to - control asthma (DTCA), as well as in a group of asthma patients with minimal symptomatology (MSA).
2) To examine activation markers of the T and B-lymphocytes by measuring IL-2, soluble receptor of IL-2 (sIL-2R/CD-25), IL-4 and sFcER II (CD-23) in the peripheral blood of both asthmatic groups.
3) To investigate the serum concentration of intercellular adhesion molecule-1 (ICAM-1) as a marker of airway inflammatory activity, in both asthmatic groups.

**Study groups**

1) **Group of difficult - to - control asthma patients (DTCA)**

The group was composed of 26 asthmatics in whom daily symptoms were present despite a complex anti-inflammatory and bronchodilatory therapy according to the International Treatment Strategy being instituted. There were 14 women and 12 men in the group, their mean age being 52.1 years (range 24-72 years) and the
mean disease duration was 19.8 years (range 2-60 years). Atopic asthma was diagnosed in 14 (53.8%) patients, 5 of them suffering from asthma since childhood. Sustained systemic corticoid treatment was necessary in 16 (61.5%) patients.

2) Group of minimally symptomatic asthma patients (MSA)
This group consisted of 22 asthmatic subjects, 13 women and 9 men, whose mean age was 50.9 years (range 22-73 years), and who had an average diagnosis of asthma of 15.1 years (range 3 to 55 years). The atopic form of asthma was found in 15 (68.1%) of subjects, it being present since childhood in 3 of them.

Two patients had no sustained therapy (only occasionally was the rescue bronchodilatory drug necessary). Twenty subjects were on inhaled corticosteroids with a daily dose of 0.2-0.8 mg.

The symptom score was classified into 4 grades according to the international consensus: grade 1 being no dyspnoea, no cough, grade 4 representing dyspnoea and cough every day and night for the last year. Similarly, the average daily use of rescue β-2 inhalations for the last year, was estimated. The best value of the forced expiratory volume in 1 second (FEV\textsubscript{1}-1%) and of the forced vital capacity (FVC %) was measured following two inhalations of fenoterol (0.4 mg) from a dosier-aerosol device.

**Methods**

After obtaining an informed consent, the patients were referred to the immunological laboratory for blood sampling. The patients were asked to abstain from all corticosteroid drugs for a period lasting at least 36 hours. Blood sampling for immunological examination took place in a period of 1 to 2 weeks following clinical and functional investigation.

1. Blood sampling

   Peripheral blood was obtained by venepuncture of cubital vein. Blood sample was left at room temperature for 1.5 hours. After centrifugation serum samples were stored at -70°C until measurement. Samples were thawed only once.

2. Determination of the serum level of IgE, cytokines and soluble membrane molecules

   Determination was performed by standard immunochemical method using commercially available diagnostic kits according to the instructions of manufacturers. Absorbance was measured by Titertek, Flow, UK spectrophotometer.

Total IgE: Immunotech, Marseille, France
IL-2 level: Quantikinine, RD Systems, Minneapolis, MN, USA
IL-4 level: Quantikinine, RD Systems, Minneapolis, MN, USA
Interferon gamma: Endogen, Inc., Cambridge, MA, USA
sIL-2R: Immunotech, Marseille, France
sICAM-1: Quantikinine, RD Systems, Minneapolis, MN, USA
sCD23: Immunotech, Marseille, France

**Statistical evaluation**

An unpaired T-test for the evaluation of differences between groups was used. In some parameters, the variability analysis with the Bonferroni method was applied. Statistical evaluation was performed using the software package BMDP.

**Results**

The clinical characteristics of both investigated groups are depicted in Table 3. No significant difference of age and of the asthma duration between both groups was found, whereas the symptom score, and the number of rescue β-2 inhalations was significantly higher in the DTCA patients.

A significant difference in FVC and FEV\textsubscript{1} values between the study groups was estimated, both values being much lower in the DTCA patients cohort (Table 4).

**Table 3:** Clinical characteristics of two asthmatic patient groups under investigation are represented.

| Group                      | N   | Age (years) | Asthma duration (years) | Symptom score (1-4) | Rescue β-2 inhalations (N) |
|---------------------------|-----|-------------|-------------------------|---------------------|--------------------------|
| Difficult-to-control asthma | 26  | 52.1±14.0   | 19.8±14.8               | 3.17±0.50           | 7.8±2.9                  |
| Minimal symptomatic asthma | 22  | 50.9±13.9   | 15.1±12.6               | 1.15±0.32           | 0.9±1.1                  |

Statistical significance: n.s., p<0.001, p<0.001

**Table 4:** This Table describes functional characteristics of both asthmatic groups under study.

| Group                      | N   | Forced vital capacity (% predicted) | Forced expiratory volume in 1 second (% predicted) |
|---------------------------|-----|------------------------------------|--------------------------------------------------|
| Difficult-to-control asthma | 26  | 79.2±16.7                          | 59.3±18.8                                       |
| Minimal symptomatic asthma | 22  | 92.4±13.6                          | 72.5±21.6                                       |

Statistical significance: p<0.005, p<0.02

Table 5 demonstrates the serum concentrations of IL-4, INF-gamma, sFcER II and IgE in both asthmatic groups, as
well as in 20 healthy subjects (blood donors). No significant changes in IL-4 values between both asthmatic groups, as well as between asthmatics and control subjects, was found.

While no significant difference between DTCA and MSA asthmatics in INF-gamma was apparent, its value was significantly higher in both asthmatic cohorts in comparison with healthy people.

The same result is true for the sFcERII serum concentrations.

The average serum IgE level is much higher in the DTCA asthma patient group than in the MSA and control subjects, but because of a very high standard deviation in the DTCA group, statistical significance could not be reached. This high standard deviation was caused by an normally high IgE value in one very severe atopic patient, being 7,104 IU/ml.

Table 5: Serum concentrations of interleukin-4 (IL-4), interferon gamma (INF-gamma), serum Fc epsilon receptor II (sFcERII) and immunoglobulin E (IgE) in examined asthmatic groups are described.

| Group       | N  | IL-4 (pg/ml) | INF-g (pg/ml) | sFcERII (pg/ml) | IgE (IU/ml) |
|-------------|----|--------------|---------------|-----------------|-------------|
| DTCA 1      | 26 | 14.4±2.3     | 4.45±1.05     | 4.78±7.15       | 47.39±137.4 |
| MSA 2       | 22 | 15.8±4.1     | 3.13±1.10     | 3.25±1.57       | 129.5±165.2 |
| Controls 3  | 20 | 16.4±4.7     | 3.76±0.53     | 1.98±1.10       | 122.4±105.2 |
| Statistical significance | n.s. | 1-2 n.s. | 1-3 p<0.01 | 2-3 p<0.05 | 1-2 n.s. | 1-3 p<0.01 | 2-3 p<0.05 | 2-3 n.s. |

DTCA - difficult-to-control asthma; MSA - minimal symptomatic asthma

Table 6 summarizes the results of serum IL-2, sIL-2R and of ICAM-1 concentrations in three groups under study. No significant differences in IL-2 were noticed between DTCA and MSA patients, as well as in sIL-2R between DTCA, MSA compared with control subjects.

Table 6: This Table presents the results of serum levels of interleukin-2 (IL-2), interleukin-2 receptor (IL-2R) and intercellular adhesion molecule-1 (ICAM-1) in different patient groups under study.

| Group       | N  | IL-2 (pg/ml) | IL-2 R (pg/ml) | ICAM-1 (ng/ml) |
|-------------|----|--------------|----------------|----------------|
| DTCA 1      | 26 | 9.91±0.70    | 1130.8±350.9   | 437.8±128.3    |
| MSA 2       | 22 | 9.84±0.91    | 1260.9±564.0   | 457.2±190.4    |
| Controls 3  | 20 | 9.53±1.11    | -              | 189.0±42.3     |
| Statistical significance | n.s. | 1-2 n.s. | 1-3 n.s. | 2-3 n.s. | 1-2 n.s. | 1-3 p<0.001 | 2-3 p<0.001 |

DTCA - Difficult-to-control asthma; MSA - Minimal symptomatic asthma

As far as ICAM-1 concentrations are concerned, a highly significant increase in both asthmatic groups in comparison with healthy subjects was estimated.

When our asthmatic patients were divided into atopic and nonatopic groups without regard to their disease severity, no statistical difference in serum levels of all measured parameters between both groups could be found (Table 7 and 8).

Table 7: Serum levels of IL-4, INF-gamma, sFcERII and IgE in atopic, non-atopic asthmatics and healthy people are illustrated in this Table.

| Group       | N  | IL-4 (pg/ml) | INF-gamma (pg/ml) | sFcERII (pg/ml) | IgE (IU/ml) |
|-------------|----|--------------|-------------------|-----------------|-------------|
| Atopics 1   | 29 | 15.1±4.2     | 4.14±0.8          | 3.79±2.9        | 464.6±1398.7 |
| Non-atopics 2 | 19 | 15.0±1.9     | 4.60±1.0          | 4.70±8.4        | 125.4±201.4  |
| Controls 2  | 20 | 16.4±4.7     | 3.76±0.5          | 1.98±1.1        | 122.4±105.1  |
| Statistical Significance | 1-2 n.s. | 1-3 n.s. | 1-3 p<0.05 | 2-3 p<0.01 | 1-2 n.s. | 1-3 n.s. | 1-3 p<0.001 | 2-3 p<0.001 |

Table 8: This Table demonstrates the differences of serum IL-2, IL-2 R and ICAM-1 between atopic, non-atopic asthmatics and healthy blood donors.

| Group       | N  | IL-2 (pg/ml) | IL-2 R (pg/ml) | ICAM-1 (ng/ml) |
|-------------|----|--------------|----------------|----------------|
| Atopics 1   | 29 | 10.0±0.9     | 1215.4±510.4   | 448.9±148.1    |
| Non-atopics 2 | 19 | 9.8±0.7      | 1171.5±386.2   | 446.5±185.6    |
| Control 3   | 20 | 9.5±1.1      | -              | 189.0±42.3     |
| Statistical Significance | 1-2 n.s. | 1-3 n.s. | 2-3 n.s. | 1-2 n.s. | 1-3 p<0.001 | 2-3 p<0.001 |

Discussion

It is quite clear, that application of fiberoptic bronchoscopy with endobronchial (eventually transbronchial) biopsy has allowed a direct appraisal and investigation of mucosal cellular events of Airways inflammation in asthmatic patients (5,22,26). However this investigation, being invasive, cannot be repeatedly used in routine clinical practice. Nearly the same is true for the investigation of samples, taken from bronchoalveolar lavage (BAL) fluid. These methods are mainly applied in research studies.

Recently, a method of induced sputum cell counts, using hypertonic saline solution inhalation, has been pro-
posed as a non invasive alternative to BAL in clinical practice for investigation of various cells, coming from the periphery of the airways, the results being comparable to that of bronchoalveolar lavage (49). Despite its noninvasive, technical equipment requirement and long duration (45-60 minutes), makes this method less convenient for daily clinical use.

Because of the difficulty in routinely obtaining samples from the lung, there has been considerable interest in potential blood-born markers of airways inflammation. Some changes have been shown in T-lymphocyte activation markers and cytokines in peripheral blood, particularly in more severe asthma. However it is not quite clear what is their relationship to events in the airways (2,36). Bearing this in mind, the evaluation of our measurements will be discussed in the following paragraphs.

Interleukin - 4 (IL - 4)

Interleukin-4 is not only responsible for B-cells isotype switching in favour of IgE production, but it also may account, at least in part, for selective eosinophil recruitment at sites of allergic tissue reactions. Dr. de Vries (12) had been unable to find circulating IL-4 in patients with very high IgE serum levels, probably because the IL-4 is released locally and acts by cell-to-cell contacts in a paracrine or autocrine way. However, Matsumoto measured higher IL-4 serum levels in atopics asthma in comparison with nonatopic subjects, but no significant correlations with IgE or sFcERII (CD23) levels were found (35).

IL-4 serum concentrations in our asthmatic patients were not increased either in severe or mild degrees of the disease in comparison with healthy control subjects. It appears that the measurement of IL-4 in peripheral blood cannot be a useful marker of inflammatory reaction in the asthmatic airways. Interestingly enough, IL-4 serum concentrations were not of discriminatory potency between atopic and nonatopic subjects as could be expected.

Soluble low-affinity FcER II (CD-23)

Soluble Fc epsilon receptor II is a fragment of FcE RII expressed mainly on B-cells, its expression being poor on normal B-cells. Interleukin-4 enhances this expression while INF-gamma inhibits the effect of IL-4 on FcE RII expression. IgE production and FcE RII expression on B-cells are two closely related events 35. FcE RII is both an autocrine and a paracrine B-cell stimulatory factor and soluble FcE RII (sFcE RII) may thus possibly be a parameter of B-cell activation.

An increase in sFcE RII in 77 asthmatics was found by Matsumoto, but no difference between atopic and nonatopic subjects was present and no significant relationship to IL-4 and total IgE was shown (35). In Billery’s work on 48 atopic subjects an elevation in percentage and absolute values of B-cells expressing FcE RII by flow cytometry was measured. Allergics to pollens had an elevation only during the season (6). There was no correlation between sFcE RII and IgE levels, eosinophilic cationic protein (ECP) or symptom score. According to their view, the upregulation of FcE RII in atopic reflects allergen exposure and cannot serve as a diagnostic marker of atopy out of the exposition period. The evaluation of CD23 isn’t useful in monitoring allergic patients because it lacks correlation with clinical symptoms. Similarly, de Amici in 19 adults with allergic asthma bronchiale could not find any correlation between sFcE RII and asthma score (11).

An increase of sFcE RII in 55 atopic asthmatics in comparison with healthy people was found by Rogala, but no relationship to total IgE, to allergen specific IgE or to IgG4 could be estimated (47).

The results of our measurements of sFcE RII are very similar to the above mentioned data. Moreover, in atopic subjects a significant correlation with IgE was found (Table 9). An increase in sFcE RII may by a marker of immunopathologic events in the airways of asthma patients but the lack of relationship to the disease severity indicates that its measurement is of no importance for the practicing clinician.

Interferon - gamma (INF-gamma)

Interferon-gamma was discovered in 1957 by Issacs as an antiviral protein, produced by cells infected by viruses (31). Since then, other mechanisms of its action (antiproliferative, immunomodulatory) have been demonstrated. Interferon-gamma is known to antagonize the effect of IL-4 on IgE synthesis in the presence of CD-4 T-cells. The administration of recombinant INF-gamma to patients with hyper-IgE syndrome was followed by a 50% decrease of the IgE blood level, without influencing other immunoglobulin types (7). Peripheral blood monocytes of atopic asthmatics were shown to produce less INF-gamma, following phytohemagglutinin stimulation. When recombinant INF-gamma was added, the INF-gamma was increased to normal values (33,39,64).

INF-gamma serum levels in asthmatics have shown different results. While in Soliman’s work on atopic asthmatics no significant changes of INF-gamma compared to controls were found (52), Kimura was able to demonstrate a significant increase of INF-gamma in severe forms of asthma, in comparison with mild asthmatics (28).

A significant increase of the INF-gamma serum level in both groups of our asthma patients could be evaluated as a T-cell activation marker in the inflammatory process of their airways wall. On the other hand, no difference in its concentration between mild and severe asthmatics makes this finding of no useful purpose for treatment strategy changes in difficult-to-control asthmatics (e.g. recombinant INF-gamma application).
As shown in table 9, a significant positive correlation between INF-gamma and IL-4 in all asthmatic groups was calculated. The importance of this finding is not clear to us, and no data explaining this relationship in the available literature were found.

Table 9: In this Table, calculated significant correlations between various measured serum parameters in different asthmatic groups are shown.

| Significant correlations between different measured serum parameters |
|---------------------------------------------------------------|
| **Group**          | IL-4 x INF-gamma | IL-4 x INF-gamma | IL-4 x INF-gamma | IL-4 x INF-gamma |
|                   | r=0.900 p<0.05   | r=0.871 p<0.01   | r=0.919 p<0.01   | r=0.948 p<0.001  |
| Atopics            | IL-4 x INF-gamma | sFcERII x IgE    | IL-2 x ICAM-1    | IL-2 x ICAM-1    |
|                   | r=0.535 p<0.05   | r=0.95 p<0.05    | r=0.495 p<0.05   |
| Non-atopics        | IL-4 x INF-gamma |                    |                    |
|                   | r=0.861 p<0.001  |                    |                    |

**Immunoglobulin-E (IgE)**

Immunoglobulin-E was first isolated from serum in 1966 by Ischizaka (8). Its highest concentrations are found in atopic people, who are characterized by a persistent IgE-mediated response to common environmental allergens (1,7). Atopy, which contributes to diseases such as asthma, eczema and allergic rhinitis, is defined as a disorder of the IgE response to common allergens, such as pollen, animal dander, house dust mites and fungi. These diseases are frequently detected by a raised total IgE serum level, and positive skin tests to common Aeroallergens. Despite the high prevalence of atopy in population studies (about 30-40%), the incidence of manifested allergic disease (asthma bronchiale, rhinitis, eczema) is present in only 5-10% of them (8,16,17). A number of variables have been shown to affect both IgE serum levels and bronchial hyperresponsiveness. Smoking has been shown to lead to an increase in total IgE serum levels. The effect of age, environmental factors undoubtedly was proven to influence the basal values of IgE (17,18).

The IgE plasma levels in nonatopics are usually normal or only slightly elevated.

The above mentioned findings are in accordance with our results. IgE serum concentrations were highest in the group of severe asthmatics and in patients with atopy. The absence of statistical significance was due to one excessive IgE value in one patient with severe atopy. This extreme concentration was not false result because repeated measurements were in a similar range. The IgE serum level positively correlated with sFcE RII concentration, while the correlation of IgE with sICAM-1 was found in a significantly negative value (r= -0.535 P< 0.05). No similar estimation for this correlation in the available literature was found.

One of our primary aims for studying these serum parameters in severe asthma was the identification of patients characterized by high IL-4, high IgE, high sFcERII and low INF-gamma. The intention was, to treat these patients by administering recombinant INF-gamma to block the influence of IL-4 on IgE switching in B-cell. Unfortunately, not one patient with this laboratory phenotype was estimated. In between, further progress in the therapy of atopic asthmatics has been demonstrated, e.g. by using specific anti-IL-4 or anti-IgE antibodies (12,14).

**Interleukin-2 (IL-2) and s IL-2 R**

Interleukin-2 is an essential growth factor for T-cells (28). IL-2 acts in an autocrine fashion to stimulate T-cell proliferation and also serves to regulate immunoglobulin production and the growth of cytotoxic T-lymphocytes and Natural Killer Cells (1,16). Upon activation of the T-lymphocytes by antibodies, an increase of the HLA-DR antigen, of the alpha subunit of the IL-2 receptor (CD-25) and of VLA-1 are found. IL-2 R on the T-cell surface is appearing 24 hours after antigen challenge and is present there for several days. Interleukin-2 provides a necessary signal for the transition of the activated T-cells from the G 1 to the s-phase of the cell cycle. During activation, the lymphocytes secrete glycoproteins related to their surface proteins, including the sIL-2 R (40,50).

While the determination of activated T-lymphocytes in peripheral blood provides information only about the immunological situation in this compartment, the determination of sIL-2 R gives an overview of the general situation of the allergic situation of the allergic inflammation in specific organs (50).

Increased plasmatic concentration of sIL-2 R were found in acute asthma attack and its decline correlated with an increase in FEV-1 and a decrease of symptoms following treatment by corticosteroids (1,2,10,29). A segmental allergen provocation test in asthmatics is followed by an enormous increase of sIL-R in BAL fluid (55,56). The concentrations of IL-2 and sIL-2 R were higher in the BAL fluid of symptomatic patients with bronchial asthma than in those of asymptomatic patients (40). Serum sIL-2 R was elevated even in 77 minimally symptomatic adult asthmatics in comparison with 75 control subjects (35). No difference in sIL-2 R levels between atopic and nonatopic subjects could be demonstrated (35). Quite different results were described by Walker (59). In his work, the sIL-2 was significantly elevated only in non-atopic asthmatics.

According to Kips and Midander, no uniform relationship exists between s IL-2R and allergic Airways inflammati-
on as well as between s IL-2R and severity of asthmatic symptoms (29,36).

Our results of serum IL-2 and s IL-2R levels are in accordance with the above cited findings, these parameters not being able either to discriminate between the different severity of asthmatic symptoms or to differentiate atopic and nonatopic asthmatics. Therefore, we don’t recommend the measurement of these parameters in the serum of asthmatic patients as an activity marker of allergic airways inflammation.

**Intercellular adhesion molecule-1 (ICAM-1)**

The recruitment of leucocytes to sites of inflammation involves a well co-ordinated sequence of events, in which several cell adhesion molecules and chemotactic cytokines play an active role (21,30). Initial low affinity selectin-dependent vascular rolling is followed by leucocyte activation by endothelium-derived chemoattractants (e.g. IL-8, monocyte chemoattractant peptide-1-MCP-1) and a transition to beta-integrin-dependent high affinity leucocyte adherence cytokine-mediated upregulation of the Ig superfamily of adhesion proteins, ICAMs and vascular cell adhesion molecules (VCAMs), leading ultimately to transendothelial migration.

ICAM-1 is a 80-115 kD glycoprotein with a large cell distribution. Its increased secretion is stimulated by various cytokines (e.g. IL-1, TNF-alfa, INF-gamma). ICAM-1 serves as a ligand for beta-2 leucocyte integrins LFA-1 and MAC-1 and enhances adhesion of leucocytes provided with these receptors to the tissue expressing ICAM-1 (30).

Within 6 hours of segmental allergen challenge of the sensitized asthmatic airways, a marked endothelial upregulation of E-selectin and ICAM-1 occurs accompanied by an influx of leucocyte function associated antigen-1 (LFA-1) leucocytes comprising neutrophils and eosinophils (37).

Epithelial cell expression of ICAM-1 in asthmatics has been compared with controls. While Montefort found no difference in ICAM-1 expression between asthmatic and control epithelium (37), Vignola demonstrated that ICAM-1 expression was increased in asthmatics and that it correlated with clinical score and lung function (44,54). A soluble form of ICAM-1 (sICAM-1) has been detected in the sera of patients with bronchial asthma (42,51). Its increased concentrations were found both in atopic as well as in non atopic asthmatics. Serum concentrations of sICAM-1 were higher during asthma attacks than in the same patients during remission (51). Serum sICAM-1 levels were also lower during treatment with oral prednison (42,51).

On the other hand, biopsies from patients with severe „steroid resistant" asthma have revealed a marked upregulation of ICAM-1 and VCAM-1 in the absence of allergen exposure and while taking high doses of corticosteroids. According to Holgate, the examination of ICAMs may offer a possible biomarker of asthma activity (21). It has been shown that pretreatment of monkeys with a monoclonal antibody specific for ICAM-1 attenuates the eosinophil infiltration and airway responsiveness to inhaled methacholine (58). These studies suggest that ICAM-1 may by pivotal in the pathogenesis of airway hyperresponsiveness and asthma.

Based on the potential importance of cell adhesion molecules in allergic and inflammatory reactions, various approaches are being developed to develop specific antagonists (60). While inhibition of adhesion molecules is an attractive new approach to the treatment of inflammatory disease, there may be potential dangers in inhibiting immune responses, leading to increased infections and increased risk of neoplasia (3).

As far as our measurements of sICAM-1 are concerned, significantly higher levels in both asthmatic groups compared to controls, may be a marker of permanent allergic inflammation in the airways of these patients without relationship to clinical asthma severity. The upregulation of ICAM-1 in our patients was independent of their atopic profile. The significant negative correlation to IL-2 and IgE concentrations is hard to explain.

Because of the lack of differentiation between mild and severe asthmatics, we consider the estimation of sICAM-1 in peripheral blood as a non-sensitive marker of airway inflammation.

**Conclusions**

1) The group of difficult-to-control asthmatics cannot be differentiated by a specific cytokine profile in peripheral blood samples from minimally symptomatic patients.
2) Some increased levels of T-and B-cell activity markers (INF-gamma, sFcER II) and of sICAM-1 even in minimally symptomatic asthma patients, point to permanent chronic inflammatory process in their airways.
3) No patient of the difficult-to-control asthma cohort with a laboratory phenotype of high IgE, high sFcER II, low INF-gamma and high IL-4 serum concentration could be found, so as to allow the causative therapy by recombinant interferon-gamma.
4) Despite some interesting results observed in investigations of the above mentioned parameters, we do not consider their estimation in peripheral blood samples of severe asthmatics helpful in therapeutic strategy changes.

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