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Cytokine Release from Cultured Peripheral Blood Mononuclear Cells of Patients with Severe Atopic Dermatitis

UWE REINHOLD,[1] GRAHAM PAWELEC,[2] WOLFGANG WEHRMANN,[1] SYLVIA KUKEL,[1] PETER OEHR[3] and HANS-WILHELM KREYSEL[1]

[1]*Department of Dermatology, University of Bonn,* [2]*Immunology Laboratory, Medizinische Klinik, University of Tübingen, Tübingen, and* [3]*Institute of Nuclear Medicine, University of Bonn, Bonn, FRG*

Peripheral blood mononuclear cells (PBMC) from 14 patients with severe atopic dermatitis (AD) and 11 healthy donors were tested for their capacity to produce tumour necrosis factor-alpha (TNF-α) after PHA stimulation and compared with their in vitro production of interferon-gamma (IFN-γ). The mean TNF-α production in AD patients did not differ vis-à-vis controls. However, a significant portion of patients with AD which was defective in generating IFN-γ in vitro showed in addition significantly a decreased production of TNF-α. No correlation could be found between TNF-α and neopterin production in either group, whereas there was a close overall correlation between the amount of TNF-α and IFN-γ detectable in culture supernatants of patients and controls. Furthermore, a significant correlation was found between TNF-α and IFN-γ generation in vitro and serum IgE concentration in AD. Based on cytokine production in vitro and IgE concentration in vivo, patients with severe AD could be divided into two groups. Furthermore, 3 AD patients with normal IFN-γ generation and low serum IgE concentration but suffering from eczema herpeticum formed a subgroup which showed an increased TNF-α production in vitro. The data suggest alterations in cytokine production in a subgroup of patients with AD which bear a reciprocal relationship to abnormal IgE regulation.

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U. Reinhold, Department of Dermatology, University of Bonn, D-5300 Bonn 1, FRG.

Atopic dermatitis (AD) is a chronic, pruritic, inflammatory skin disorder, associated with several immunologic abnormalities. Some of the changes include increased elevated serum IgE concentrations in 43–80% of the patients, a low proliferative responsiveness to mitogens and antigens, and a decreased natural killer cell (NK) activity (1). IgE has been reported to be involved in the pathogenesis of the disorder and serum IgE levels have been shown to be positively correlated with the severity of the disease, but many authors have failed to demonstrate a significant association (2, 3, 4). It has been described that a subgroup of patients may have normal IgE levels (4) despite severe AD.

Our recent data indicate that a significant proportion of patients with severe AD have an impaired capacity to secrete interferon (IFN)-γ in vitro, which was negatively correlated with IgE and IgG_4 levels in vivo (5). In addition, defective IFN-γ production has been found in patients with primary immunodeficiencies (e.g. Wiskott-Aldrich syndrome, Hyper-IgE syndrome, ataxia teleangiectasia) which are commonly associated with AD-like eruptions and increased serum IgE concentration (6). A low IFN-γ generation suggests a major role in the pathogenesis of increased IgE production in AD, since IFN-γ inhibits the IgE- and IgG_1-enhancing effects of IL 4 on B lymphocytes (7).

In addition to its regulatory effects on B and T cells, IFN-γ activates monocytes and stimulates IL 1 production. Activated monocytes produce numerous factors, including tumour necrosis factor (TNF-α) and neopterin (8). The different activities of TNF-α include induction of fever and cachexia, induction of several cell-surface antigens, regulation of B cell proliferation and immunoglobulin (Ig) production (9, 10). Moreover, TNF-α enhances antigen- and mitogen-induced human T lymphocyte proliferation responses. Recent data indicate synergistic effects of human TNF-α and IFN-γ (11). On the basis of the observed impaired IFN-γ production in AD and its possible consequences for the activation and cytokine generation of monocytes/macrophages, we have studied the production of TNF-α, IFN-γ and neopterin, estimated by specific immunoassays following mitogenic stimulation in a selective group of patients with severe AD.

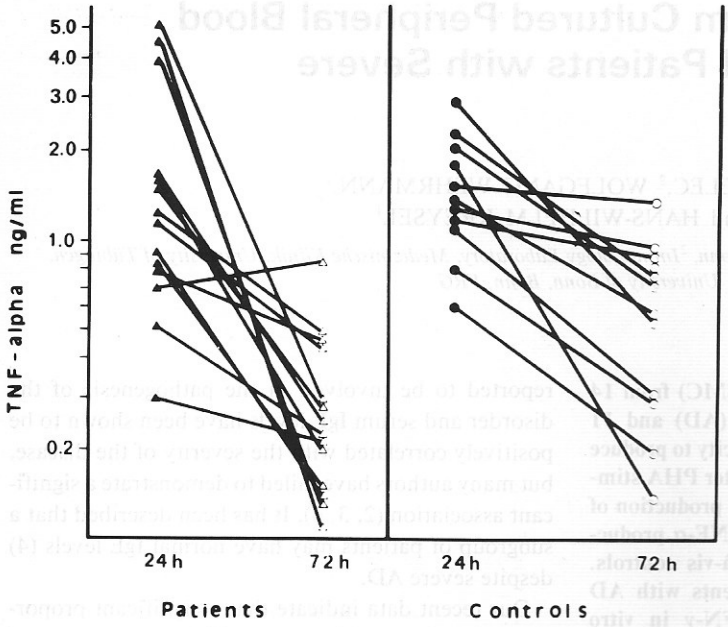

Fig. 1. In vitro TNF-α production at 24 and 72 h in patients with severe AD.

PATIENTS AND METHODS

Subjects

Peripheral blood mononuclear cells (PBMC) of 14 patients with severe AD were studied. The patients were selected from the Department of Dermatology, University of Bonn, FRG. AD was diagnosed according to the criteria of Hanifin & Rajka (12). All patients suffered from an exacerbated severe form of AD involving the face, limbs and trunk. All patients had severity score factor > 7 (i.e. severe disease as defined by Rajka (13)). None of the patients was receiving systemic steroids or acyclovir at the time the studies were conducted. PBMC from 11 normal subjects without symptoms and history of atopy were also studied; their serum IgE was <100 IU/ml.

Cell cultures

Heparinized blood (10 IU/ml) was collected from different donors, diluted with phosphate-buffered saline (PBS) and PBMC were isolated using standard Ficoll-Isopaque gradient centrifugation. Cells were washed three times with PBS and finally suspended in RPMI 1640 + 25 mM HEPES + 2 mM *l*-glutamine (Gibco, Karlsruhe, FRG), gentamicin (100 µg/ml, Serva, Heidelberg, FRG) and 10% inactivated pooled human serum. Cells were cultured for subsequent analysis of TNF-α, IFN-γ and neopterin production in the presence of phytohemagglutinin (PHA) at a dose of 1% stock solution (unpurified, batch 13N1076, Gibco, Karlsruhe). Cultures were distributed in 2 ml aliquots into 24-well Costar culture plates (Tecnomara, Fernwald, FRG) at 1×10^6/ml for a period of 5 days at 37°C, 5% CO_2. Daily production of TNF-α, IFN-γ and neopterin was measured by removing the supernatant of activated PBMC and then resuspending the cells in fresh medium, thus avoiding the accumulation of secreted factors. Supernatants were stored at −70°C until they were assayed for their content of IFN-γ and neopterin.

TNF-α, IFN-γ and neopterin assays

The TNF-α content of culture supernatants ascertained after stimulating PBMC for 24 and 72 h with PHA was measured by a radio-immunoassay (IRE-Medgenix, Fleurus, Belgium). IFN-γ was assayed at 24, 48 and 72 h by using an immunoradiometric assay (IRMA) from Celltech (Slough, Bucks, England). The neopterin content of activated PBMC supernatants was quantified by using a radio-immunoassay method (Henning, Berlin).

Serum IgE quantification

Serum IgE concentrations were determined by a solid-phase radio-immunoassay (Phadebas IgE Prist, Pharmacia, Freiburg, FRG).

Data analysis

The differences between datasets were assessed using Mann-Whitney test (U-test). Data are expressed as means ± SD. For correlation analysis, the Spearman correlation coefficient was used.

RESULTS

TNF-α production

PHA-stimulated TNF-α generation by PBMC from 14 patients with severe AD and 11 healthy volunteers is shown in Fig. 1. All supernatants contained measurable titres of TNF-α. TNF-α production reached a maximum at 24 h, falling to low levels at 72 h in both groups. The mean TNF-α production (24 h) in patients with AD (1.7 ± 0.7 ng/ml) did not differ vis-à-vis controls (mean 1.5 ± 0.4 ng/ml). The patient group

Table I. *Two distinct subgroups of patients with severe AD, based on their serum IgE concentration and cytokine production in vitro*

Case no.	TNF-α (ng/ml)	IFN-γ U/ml	Neopterin nmol/l	IgE IU/ml	*E.herpeticum*
High serum IgE					
3	0.8	18	6.4	2 800	–
5	0.9	35	5.5	2 260	–
7	1.2	86	2.3	1 610	–
8	0.7	13	3.7	20 000	–
9	0.3	10	9.4	16 500	–
10	1.4	140	4.6	1 300	–
12	0.5	17	4.3	1 560	–
14	0.8	32	4.4	1 200	–
Mean ± SD	0.8±0.4*	44±46*	5.1±2.1	5 904±7 695	
Low serum IgE					
1	4.5	340	7.8	244	+
2	5.1	220	5.9	110	+
4	1.7	120	3.6	228	–
6	1.6	210	6.8	86	–
11	1.7	140	2.7	98	–
13	3.8	70	4.5	250	+
Mean ± SD	3.1±1.6	183±95	5.2±2.0	169±79	
Controls (n=11)	1.5±0.4	193±46	6.8±2.1	<100	–

*$p<0.005$ vs. controls.

was heterogeneous in that, in 3 patients, TNF-α production was increased, while others generated very low levels in response to PHA (Fig. 1, Table I). Interestingly, all 3 patients generating high TNF-α levels had eczema herpeticum. The concentrations of TNF-α detected at 72 h reached low values in both groups, with a non-significant tendency to be lower in the patients (controls: mean 0.7±0.4 ng/ml; patients: mean 0.3±0.2 ng/ml).

Relationship between TNF-α production and in vitro generation of neopterin and IFN-γ

We observed a close correlation between the amounts of TNF-α and IFN-γ detectable in culture supernatants of patients (r=0.84, $p<0.001$) and controls (r=0.92, $p<0.001$) (Fig. 2). In the presence of 1% PHA, IFN-γ production reached a maximum at 24 h, remained unchanged up to 48 h, and decreasing thereafter to low levels (data not shown). Patients producing less than 50 U/ml IFN-γ also showed TNF-α values of less than 1 ng/ml. No significant correlation could be found in either investigated group between TNF-α and neopterin production or in IFN-γ vs. neopterin production. The maximum of neopterin generation at 72 h was 5.2±2.1 nmol/l in patients and 6.8±2.1 nmol/l in controls, which was not statistically different.

Relation of TNF-α production in vitro to serum IgE concentration

Comparison of TNF-α results in supernatants from AD patients with their IgE concentration in vivo revealed a negative correlation ($r=-0.43$) which was not statistically significant. As a result of the detected correlation between TNF-α and IFN-γ concentration, a concordant negative correlation ($r=-0.47$) between IFN-γ and serum IgE concentration was established. As is shown in Table I, patients with severe AD can be subdivided into two groups, based on their serum IgE concentration and TNF-α and IFN-γ production in vitro. Patients with a marked increase in serum IgE (1 000–20 000 IU/ml) were defective in generating TNF-α and IFN-γ in vitro, whereas patients showing a mild elevation (<300 IU/ml) of serum IgE concentration were comparable to controls regarding their in vitro TNF-α and IFN-γ production. As all patients were suffering from the severe form of AD (severity score factor >7) no correlation between

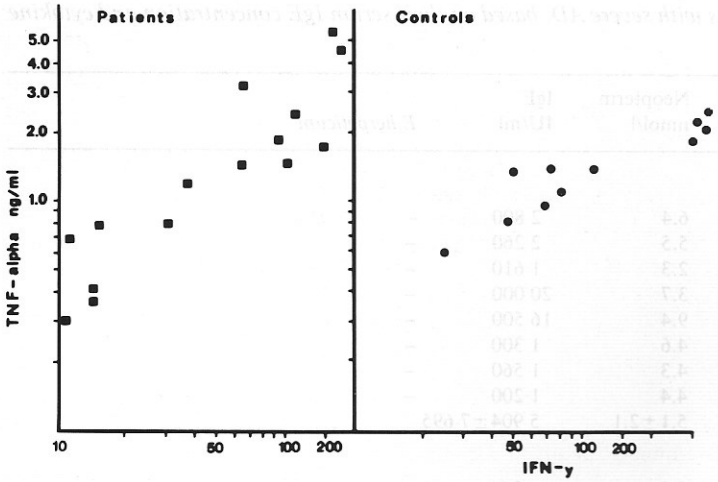

Fig. 2. Significant correlation between TNF-α and IFN-γ generation in vitro in patients ($r=0.84$, $p<0.001$) and controls ($r=0.92$, $p<0.001$).

TNF-α production and clinical severity could be analysed in this study.

DISCUSSION

We have shown previously that in vitro generation of IFN-γ after PHA stimulation of PBMC is defective in a significant proportion of patients with severe AD and is negatively correlated with their IgG_4 and IgE concentrations in vivo (5). In this study we were able to show that the mean TNF-α production in AD patients did not differ from that of controls, but that in addition to IFN-γ, the synthesis of TNF-α in vitro was defective in a subgroup of AD patients. When comparing the results of both TNF-α and IFN-γ produced in response to PHA, we observed a close correlation between the amounts of both cytokines detectable in culture supernatants of both controls and patients, with the exception of the 3 eczema herpeticum patients. Although monocytes/macrophages have been thought to be the major source of TNF-α, recent studies establish that T cells, NK cells as well as certain non-hematopoietic cells, are capable of producing TNF-α and a significant positive correlation between IFN-γ and TNF-α production by T cell clones has been noted (14, 15, 16). In agreement with these results and confirming our previous data, AD patients generating low IFN-γ concentrations in vitro were also defective in producing TNF-α. These data suggest multiple defects in cytokine production in a subgroup of patients with AD.

The mechanism of reduced TNF-α and IFN-γ production in AD is not clear and was not investigated here. It may be due to defective cellular interactions, active suppression, or an intrinsic defect of monocyte/macrophage and/or T cell activation. However, defective cytokine production was only observed in a distinct group of patients who concomitantly showed a marked increase in serum IgE concentrations. This further suggests that defective TNF-α and IFN-γ production is associated with altered IgE regulation. Recent data have shown that human IgE synthesis is regulated by various factors produced by activated T lymphocytes, including IL 4 and IFN-γ and it has been suggested that IFN-γ suppresses IgE production by its ability to inhibit the action of IL 4 (7, 17). Moreover, IFN-γ is involved in the activation of monocytes which are known to be a major source of TNF-α (18). Therefore it seems possible that defective TNF-α production by monocytes is a consequence of defective IFN-γ production by T lymphocytes, or is an intrinsic defect of the AD monocytes themselves. This is consistent with recent data showing defective IL 1 production by monocytes/macrophages from AD patients (19). In contrast, we did not find any deviation in neopterin production in vitro by AD PBMC, compared with controls, and did not observe any correlation between neopterin generation and TNF-α/IFN-γ production. This contrasts with the studies by Woloszczuk et al. (8) which have revealed that IFN-γ is a highly potent inducer of neopterin derived from monocytes/macrophages. However, maximal activation of the key enzyme of pteridin biosynthesis in monocytes/macrophages was already achieved by very small doses of IFN-γ which were comparable to those concentrations found in patients generating low levels of IFN-γ in vitro. As far as we know, correlation studies of TNF-α and neopterin production by acti-

vated monocytes/macrophages have not yet been performed.

It has been shown that IL 4 plays an important role in the modulation of the IgE system by upregulating not only the synthesis of IgE but also the expression of its receptors (7). We have shown that the frequency of lymphocytes bearing low-affinity receptors for the Fc portion of human IgE ($Fc_\varepsilon R_L$/CD23) is increased in AD patients and negatively correlated with serum IgE concentration in vivo and IFN-γ production in vitro (5). Furthermore, the frequency of monocytes bearing $Fc_\varepsilon R_L$/CD23 is increased in AD patients, particularly in the presence of high serum IgE. Very recent data have shown that IL 4 can strongly induce the expression of $Fc_\varepsilon R_L$/CD23 on normal human monocytes (20). Therefore, it seems possible that the presence of a large population of $Fc_\varepsilon R_L/CD23^+$ monocytes and a high concentration of serum IgE combined with low IFN-γ concentration may modulate receptor–ligand interactions on the monocyte membrane and may lead to modulated release of monocyte-derived mediators such as TNF-α and IL 1. Interestingly, our study revealed significantly enhanced TNF-α production in 3 patients with severe AD and the simultaneous presence of eczema herpeticum. These patients showed in vitro IFN-γ production comparable to the control group and low serum IgE concentrations. Several authors have reported increased TNF-α production in association with fever and infection in a variety of diseases, including endotoxin shock (21), cancer (22), malaria and Leishmaniasis (23), meningococcal septicemia (24) and acute renal allograft rejection (25). Our results indicate that eczema herpeticum which is a generalized virus infection of the skin frequently associated with fever and severe illness is another clinical state with concomitantly increased TNF-alpha production. Whether this is the case only in AD patients with low IgE serum concentration and normal IFN-γ generation in vitro remains to be elucidated in further studies.

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