Regulation of IgE-synthesis and Proliferation: Stress Protein IgE as early warning signal for our body

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Emzymes: collagenase - EC 3.4.24.7 gelatinase - EC 3.4.24.-

Abbreviations: IL = interleukin, IFN = interferon, APMSF = (4-amidinophenyl-) methanesulfonylfluoride, NEM = N-ethylmaleimide, pCMB = p-chloromercuribenzoate, Diamide = azodicarboxylic acid bis (dimethylamide), DMPS = dimercaptopropan- sulfonate.

Abstract.

An "in vivo" test system on blood of atopic eczema patients has been developed which leads to the following regulatory scheme for IgE-synthesis and proliferation:
A dithiol/disulfide interchange mechanism is involved in IgE-synthesis. The associated redox state is sensitive to Hg^{2+}, Diamide, gIFN and Il-4. Il-4 reacts antagonistically to gIFN in blood samples and cell cultures, although in opposite directions.
Elements involved in the signal transduction pathway from gIFN or Il-4 to IgE were concluded to be their receptors, (a) cytosolic G protein(s), NADPH oxidase, a yet unknown electron transfer factor (etf), redox factor (ref), nuclear transcription factors and endonuclease. These elements are presumed to build-up an electron transfer chain, NADPH to DNA (IgE), which is coupled to ongoing mitochondrial energy formation.
The reduced ref involved stimulates DNA-binding and carries an essential sulfhydryl group, whereas the oxidized state, responsible for catalytic endonuclease activity, carries a catalytic disulfide: gIFN transduces redox signals during short time intervals (blood samples), whereas during longer intervals (e.g.cell cultures) the oxidized redox factor involved determines translational activities.
The involvement of Mg^{2+} - or Ca^{2+} - sensitive serine residues in the signal transduction to IgE synthesis is suggested by APMSF/ EDTA-titrations. An important role in modulating IgE concentrations is played by kinases, especially protein kinase C.
Toxic mercury or reactive oxygen species seem not to be responsible for the changes in patients IgE-levels, but for activation of metalloproteases and associated glucocorticoid-sensitive inflammations. Metalloproteases degrade gIFN. Particularly the course of leucocyte collagenase activity is conversely related to gIFN concentration.
The adaption of cells to stress conditions includes the gIFN and Il-4 controlled synthesis of IgE antibodies. Environmental pollutants, including formic aldehyde, sulfite/SO_2 , isocyanates and anhydrides, reacting irreversibly with the involved essential dithiol/ disulfide redox state or CO by binding to the NADPH oxidase, shift the redox state to the reduced form with increased probability of IgE synthesis (low for O_2^-). The oxidized form is not able to synthesize IgE, but instead O_2^- , and the risk of mitogen stimulated proliferations is extremely high. In addition, weakening of the immune system by dermal and intestinal dysbiosis (C.albicans), food (carbohydrates) as well as psychogenic stress (norepinephrine), leads to enhanced development of IgE antibodies.
The first expression of, for instance, food- or inhalative allergen-specific IgE may be purely

incidental and may relate to autoimmune diseases. The described pathogenesis of both atopic eczema and leukemia relates to the development of AIDS.

Key words: g-interferon, cytokines, electron transfer chain, redox potentials, metalloproteases, immune diseases.

Introduction

The most important triggering factors for atopic eczema are allergic in nature. The basic mechanism underlying the triggering events is at the level of IgE-synthesis (Godard et al, 1992). The sole inducer of IgE synthesis in cell cultures proved to be Il-4, and the antagonist for Il-4 in the same system is gIFN (Vercelli and Geha, 1989 a; Vercelli et al, 1989 b; Rousset et al, 1989). These results have not yet been confirmed in the IgE-levels of atopic eczema patients. However, this is above all due to weaknesses in the available assay systems for gIFN and Il-4. The systems presently in use for their detection in body fluids are either not sensitive enough, or are unable to differentiate between active and inactive cytokins. No significant circulating levels of Il-4 could be measured and no significant difference in the circulating levels of gIFN could be found (Rousset et al, 1989; Takahashi et al, 1992; personal communications). The reported successful treatment of 3 atopic eczema patients with recombinant gIFN without the knowledge of circulating gIFN or Il-4-concentrations (Reinhold et al, 1990) could not be repeated or resulted in hazardous side effects [unpublished]. We decided therefore to develop an "in vivo" test system for IgE and to investigate IgE-regulation in this new system, as well as to subsequently look at various factors which were possibly influencing gIFN concentration in vivo. Preliminary results leading to a complete model for the regulation of IgE-levels in blood plasma, the proliferation of blood cells, and the development of AIDS have been presented (Kiehl, 1994b; 1995c; 1996a).

Patients and Methods

38 patients with clinically proved atopic eczema (criteria of Hanifin and Rajka, 1980; Diepgen et al, 1991) (age range 18-52 years) and 19 healthy volunteers with no allergic history (age range 19-50 years) consented (1993 and 1994) to participate. All patients avoided any steroid or antihistaminic treatment for at least one month before admission.
Total IgE and specific IgE levels in serum or heparin plasma, white blood cells, T- and B-cells, CD4- and CD8-T-cells, the CD4/CD8-ratio and natural killer (NK) cells in peripheral blood were measured using standard enzyme immuno assays (Pasteur and Ciba Corning, FRG) and flow cytometry (Coulter, FRG). IgA, IgG and IgM values were obtained using a turbidometric assay (Baxter, FRG).
Mono and diamine-oxidase activities, histamine and other biogenic amine concentrations (Kiehl and Ionescu, 1993) were measured as described: Platelet-rich plasma (PRP) was obtained by centrifuging the stabilized (EDTA) blood at 53 x g for 10 min at 20^0C. An aliquot (0.6 ml) of peroxidase buffer (8.3 mg peroxidase in 100 ml 0.2 M sodium phosphate, pH 7.15), 0.2 ml PRP and 10µl10 % Triton X-100 were mixed. After 5 min 0.2 ml of 0.25 mM 2',7'-dichloro-fluorescein diacetate dissolved in 0.01 N NaOH and 10 µl 1 mM benzylamine (for monoamine oxidase) or 10 µl 50 mM putrescine (for diamine oxidase) were added and mixed. The absorbance at 502 nm was recorded at 15 to 25 min, at 20^0C, on a Shimadzu UV-160 spectrophotometer (Kiehl, 1991). Histamine was measured in EDTA plasma by the method of Shore (1971) using a Perkin-Elmer LS-2 filter fluorimeter. Dopamine, epinephrine and

norepinephrine concentrations in EDTA-plasma were determined by reverse phase HPLC with electrochemical detection (Weicker et al, 1984). For this, venous blood samples were taken on Na-EDTA in the supine position at 9 a.m. after 10 min of bed rest. Equipment, standardized method and reagents were supplied by Waters, Millipore, FRG.
The determinations for the concentrations of various fatty acids in plasma and red blood cells were performed by Scotia Pharmaceutical (Guildford, England) and Beiersdorf (Hamburg) (Manku et al, 1983; Hoving et al, 1988; Roemen et al, 1990; Kiehl et al, 1994). Microbiological investigations (Ionescu et al, 1990a, b,c, 1991): Oral, pharyngeal, nasal, vaginal and dermal microbiological samples were collected with sterile swabs using a standard technique. Gastric and duodenal intubations were performed in order to investigate possible contamination with pathogenic bacteria and yeast in the upper intestinal tract. Anaerobically yielded fecal samples were taken and serial dilutions were plated for quantitative investigation of large bowel microflora. All samples were cultured on appropriate growth-media for gram-positive, gram-negative, anaerobic and yeast strains.
Heavy metals in blood and urine with DMPS (Schiele, 1989) were detected by atomic absorption spectroscopy. The other clinical parameters were obtained using the Kodak EKTACHEM analyzer. If not stated otherwise, all collected samples were immediately used up for assay.

Collagenase, gelatinase and lactoferrin were quantitatively detected by ELISA (Bergmann et al, 1989). For this purpose, fresh venous blood samples had to be used. Sera were taken after a 15 minute coagulation time and together with heparin plasma, immediately frozen to -20^0C. The collected frozen samples were quickly transferred from the clinic to the university for assay and thawed just prior to assay. Blood samples of affected skin areas were taken as follows: The disinfected skin area (alcohol) was pricked with a lancet, the appearing blood collected with a heparinized capillary tube, the tube closed on one side and centrifuged. The part of the tube containing the plasma was cut off, the plasma transferred into a small reaction vessel and frozen to -20^0C.
Proteolytic degradation of gIFN was performed similarly to the described inactivation of α_1 -proteinase- or C1-inhibitor (Knäuper et at, 1990; 1991): Incubation of gIFN with collagenase and gelatinase (at a ratio of 100:1) was performed and measured at 0 h and 24 h.

The assays for "in vivo" (in vitro) IgE regulation were carried out as follows: patients with total IgE-values of about 1000 to 2000 U/ml at the beginning of their hospitalization gave their consent to participate in the study. Venous heparinized blood was taken at 9 a.m. and immediately processed. Samples of 1 ml were incubated and gently shaken (to prevent breaking of the cells) at 37^0C (see Results section), the reactions stopped by centrifugation, and the normally resulting yellow supernatant taken for detection of total IgE: Samples turning red during incubation may indicate breaking of cells (Triton experiments). A possible influence of the compounds/drugs used in the experiment on the assay system itself (at the minimal dilution of 1:12 = 84 μM Hg^{2+} or 84 μM EDTA) was not found. The results itself exclude methodological artefacts, which is confirmed on appropriate controls (standardized IgE-samples). At least two identical experiments were done per blood sample with a minimum of two different patients, and all measurements were performed in duplicate. The standard deviation was 2 to 5 % in all measurements performed; shown are mean values (Fig. 1 to 7).

The spectra of white blood cells were taken on a UV-3000 Shimadzu UV-VIS spectrophotometer. For this purpose, the most simple way was used: 0.5 ml of the blood sample was mixed with an equal volume of aqua dest to lyse erythrocytes (ca. 10 to 15 min.), and

appropriate amounts of the various reagents were then added. Controls for possibly interfering compounds (hemoglobin) were obtained after spinning the lysed samples. The supernatant was recorded to obtain the mixed spectrum of these interfering compounds.

Materials

Recombinant gamma-interferon (specific activity $2x10^7$ IU/mg) and recombinant interleukin-4 (specific activity $1x10^7$ IU/mg) were gifts from Bender (Vienna, Austria) and IC-Chemicals (Ismaning, FRG) respectively. APMSF was obtained from Boehringer (Mannheim, FRG); Cycloheximide, Diamide and NEM from Sigma (Deisenhofen, FRG), vacutainer$^{(R)}$ plus heparinized capillary tubes from Becton-Dickenson (Heidelberg, FRG) and Assistant (Sondheim, FRG) respectively. DMPS-HEYL ampullae were obtained from Heyl (Berlin, FRG). All other chemicals were commercially available and of the highest reagent grade quality.

Results

Patients' immune profiles (IgE-, IgG-, IgM-, IgA-levels, lymphocytic subpopulations and haematological parameters) showed the expected results (Kiehl et al, 1994): lymphocytes, T-, CD8-T- and B-cells, the CD8/CD4-T-cell-ratio, as well as eosinophiles and IgE, were significantly elevated. In contrast, CD4-T- and NK-cells were significantly lowered. Furthermore, histamine concentrations were elevated; concomitantly platelet histamine oxidase activities lowered (Kiehl and Ionescu, 1993).

Psychogenic stress (environmental agents, etc.) significantly elevates norepinephrine levels (Ionescu and Kiehl, 1988), but can be reduced by repeated autogenic training (relaxation therapy, Kiehl, 1992; Ionescu et al, 1992; Kiehl, 1993a,b,c). Investigations concerning the lipid profile in red blood cells or plasma demonstrate a low level of g-linolenic acid (Kiehl et al, 1994). The coupled low level of arachidonic acid can be raised by linoleic acid plus zinc supplementation (Kiehl et al, 1994). The patients showed intestinal and dermal dysbiosis, in most cases accompanied by significantly increased counts of pathogenic strains including Candida Albicans (Ionescu et al, 1990 a,b,c, 1991). Other routinely performed laboratory parameters were in the normal range, including Mg and Ca.

In our study (Table 1) heavy metal ion concentrations in blood and urine of atopic eczema patients (before and after intravenous DMPS-treatment) were almost in the "normal" range as defined by Daunderer (1988), or described by Moyer (1996), but more importantly, identical to values found in healthy control persons. The upper value of the mercury concentrations in atopic eczema patients is slightly elevated in contrast to the values found in control persons, although only 7 controls could be checked. The number of dental amalgam fillings in these groups was identical.

The overall concentrations of the leukocyte metalloproteases, collagenase and gelatinase, in these patients showed no pathological changes compared to control persons. Also lactoferrin, an iron binding protein locally liberated during inflammation from granulocytes, has a normal overall level in atopic eczema patients vs. controls (Table 1).

However, the assay of a few capillary blood samples (sampling is difficult, therefore n = 3, Table 2) from skin areas under acute inflammation, but not from normal skin areas (not shown),

Table 1. Heavy metals in blood and urine, Me-proteases and lactoferrin in serum and plasma of atopic eczema patients and healthy control persons

	Heavy metals blood Hg, µg/l	Cu, mg/l	Zn, µg/dl	urine/DMPS[1] Hg, µg/l	Cu, µg/l	Zn, µg/l	dental amalgam fillings
Control persons (n)	0.8 ± 0.5 (19)	1.18 ± 0.3 (19)	88 ± 11 (19)	10.1 ± 3.1 (7)	232 ± 102 (7)	660 ± 286 (7)	7 ± 5 (19)
Atopic eczema patients (n)	1.2 ± 0.9 (26)	1.35 ± 0.4 (26)	101 ± 20 (26)	29.6 ± 28.1 (38)	550 ± 430 (38)	2250 ± 2170 (38)	6 ± 5 (26)
Normal value, Dzunderer Mayer	< 0.2 - 3.0 < 11	0.8 - 1.55	70 - 127	<50	<500	>2000	0
Significance Student t test	NS[2]	NS	NS	NS	NS	NS	NS

	collagenase, ng/ml serum	collagenase, ng/ml heparin-plasma	gelatinase, ng/ml serum	gelatinase, ng/ml heparin-plasma	lactoferrin, ng/ml serum	lactoferrin, ng/ml heparin-plasma
Control persons (n)	a) 46 ± 14 (7)	8 ± 7 (7)	1757 ± 761 (7)	204 ± 80 (7)	432 ± 123 (7)	100 ± 67 (7)
	b)[3] 46 ± 24 (13)	9.2 ± 8 (20)	1721 ± 813 (13)	234 ± 78 (20)	418 ± 232 (13)	84 ± 49 (20)
Atopic eczema patients (n)	46 ± 34 (6)	10.5 ± 9 (13)	1685 ± 865 (6)	264 ± 77 (13)	404 ± 341 (6)	68 ± 30 (13)
Significance Student t test	NS	NS	NS	NS	NS	NS

[1] DMPS = dimercaptopropansulfonate, [2] NS = not significant, [3] control persons plus atopic patients

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demonstrates that the leucocyte collagenase and gelatinase concentrations (heparin plasma) were significantly elevated. At the same time, the lactoferrin levels are greatly increased, which is in agreement with the acute inflammation conditions (Table 2 vs. Table 1).

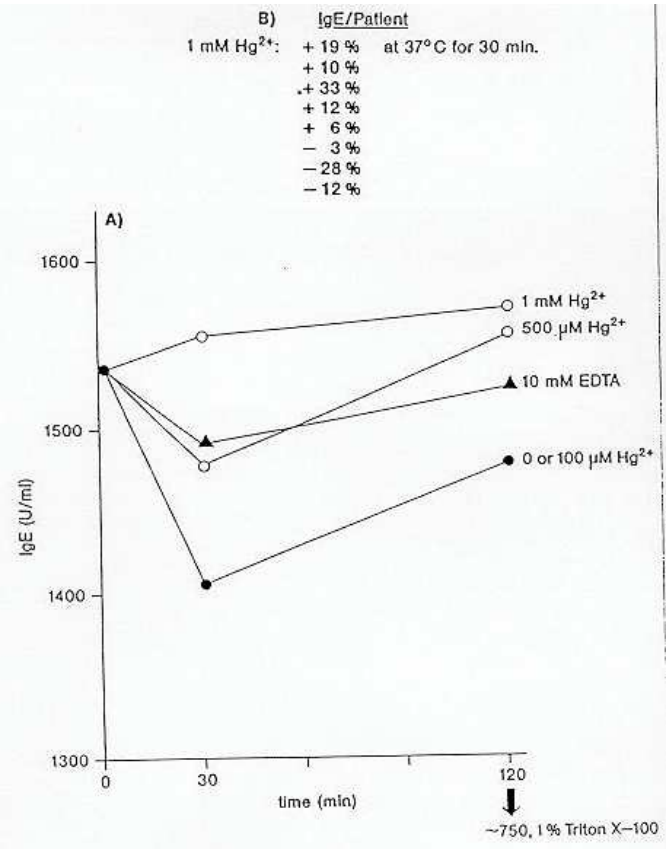
Table 2. Me-proteases and lactoferrin in heparin-plasma of circulating blood and affected skin areas of three atopic eczema patients

Atopic eczema patients		collagenase, ng/ ml	gelatinase, ng/ ml	lactoferrin, ng/ml
1.	circulating	44,1	323	74,9
	affected skin	130,7	2699	429,1
2.	circulating	48,9	486	137,1
	affected skin	140	1260	601,2
3.	circulating	8,2	288	85,9
	affected skin	65,3	653	287,3

Incubation of gIFN with leucocyte collagenase or gelatinase under in vitro conditions (substrate: enzyme = 100:1) was performed and degradation of gIFN to 20 and 0 %, respectively, was

observed. Comparison with in vivo conditions (normal value of gIFN in blood plasma = 2.5 to 25 ng/ml) shows a calculated variation in the gIFN concentrations as a function of the collagenase concentrations between 0 and 100 % during relative short time intervals: a ratio of 1:1 = ca. 1 ng gIFN:4 ng collagenase, 100:1 = 25 ng gIFN: 1 ng collagenase; 20 % degradation = 5 ng gIFN by 1 ng collagenase/24 h or 50-700 ng gIFN by 10-140 ng collagenase/24 h = ca. 2.5-30 ng gIFN/h, respectively.

The routinely used assay for total IgE-concentrations in atopic eczema patients has been extended and developed into a useful model system for the exploration of IgE-regulation (Methods, Fig.1 to 7). First, orientation measurements with old (~ 4 hrs) and fresh (0 hrs) blood samples made clear that only the assay with fresh blood samples at 37^0C resulted in significant and reproducible differences. The best IgE-range for the experiments proved to be between 1000 and 2000 U/ml. The differences for low IgE-values are too small and for high IgE-values the variances are too big.



We started with tests for the effects of protease activators and inhibitors on the blood-IgE-concentrations (Figs. 1 and 2). The normal course of IgE-concentrations in blood samples shows a decline during the first 30 minutes, followed by a slow rise. 100 µM of the metalloprotease activator Hg^{2+} (Bläser et al, 1991) are almost without effect, but 500 µM to 1 mM Hg^{2+} (conc. of Hg still below the applied conc. during Epicoutanous tests, for disinfektion or vaccination) significantly increase the IgE-values in the patients' blood samples (Fig. 1). 100 mM of the metalloprotease inhibitor EDTA (Bergmann et al, 1989) increase IgE-levels, as does Hg^{2+} (Fig. 1). The degree of change in blood-IgE-level caused by 1 mM Hg^{2+} varies from patient to patient. Our measurements result in differences as high as 50 % (+33 % to -28 %, Fig. 1B, inlet).
Therefore, it is dear that there is no representative patient and each patient has to

Fig. 1. A) Time dependent Hg^{2+}- and EDTA –sensitivity of the IgE-levels in patint blood samples. Blood samples were also used for the following experiments. Shown are mean values (Fig. 1 to 7) with a SD of 2 to 5 % (out of 4 x 2 assays**). Inlet B)** Standardized Hg^{2+} -sensitivity of various patients IgE-levels. Each value represents one patient.

be measured (and treated) on its own.
Under the given conditions, 40 µg/ml of the serine protease inhibitor APMSF (Laura et al, 1980) alone have no effect on IgE-concentrations, but prevent nevertheless an increase normally induced by 10 mM EDTA (Fig. 2). This effect is not obtained by the combination of Hg^{2+} and EDTA, but there is a slight increase in IgE-levels by combining 10 mM EDTA and 1 mM Hg^{2+}

compared to the compounds applied alone (Fig. 2).

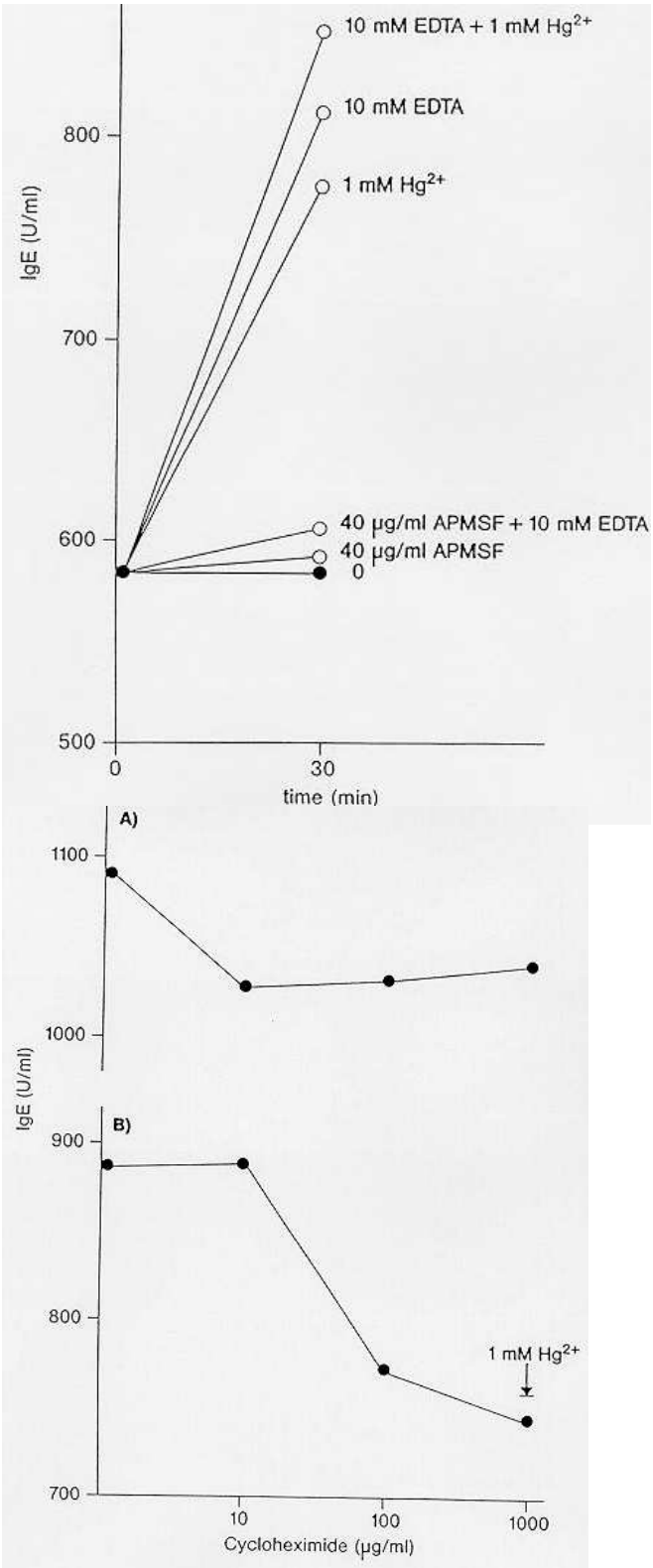
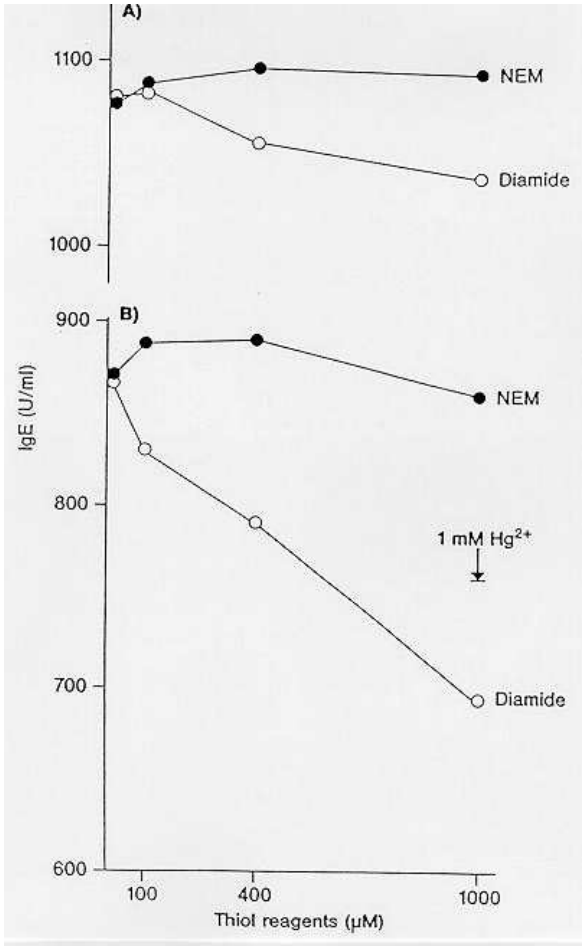


Fig. 3. A) and B) Concentration dependent inhibition of IgE-synthesis by Cycloheximide in blood samples of two different patients. The standardized Hg^{2+}-sensitivity (B) is given for comparison. The incubation times in the experiments shown in Fig. 3 to Fig. 7 were 30 minutes each.

The IgE-level decreaes drastically (ca. 50 %) during incubation of the samples with 1 % Triton X-100 for two hours (Fig. 1). The supernatant after Triton incubation is dark red. 10 to 100 µg/ml cycloheximide, an inhibitor of protein synthetic activity, similarly lower IgE-levels, though not as drastically (Fig.3). In the case of incubation with cycloheximide the supernatant remains yellow.

Due to the unexpected results obtained with Hg^{2+} which suggested involvement of some kind of thiol redox state in the IgE-levels, we tested the thiol reagent NEM and the dithiol oxidizing compound Diamide (Kosower et al, 1969) (Fig. 4). NEM shows only a slight influence, while 1 mM Diamide strongly reduces the IgE-level, even below the level obtained with 1 mM Hg^{2+} (Fig. 4).

Fig. 2. Standardized APMSF/EDTA-titrations of patients blood samples. One patient as example.



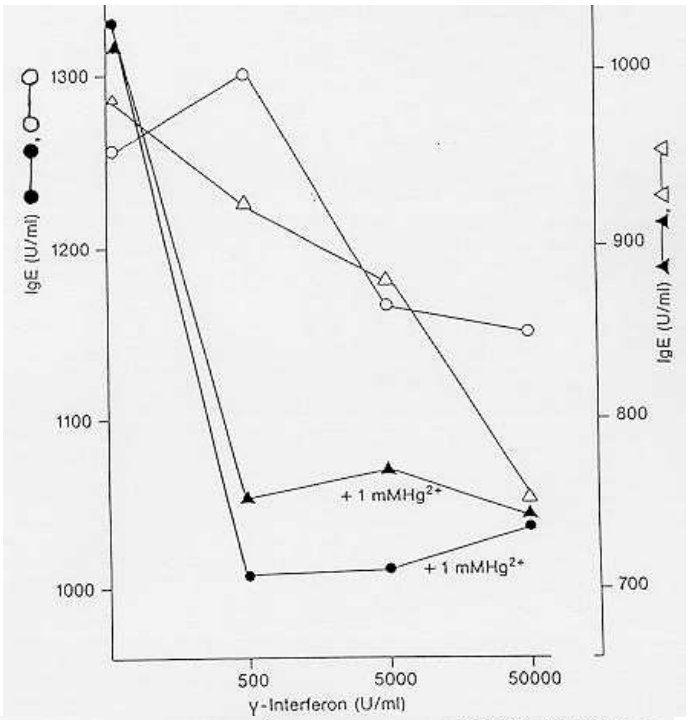


Fig. 4. A) and B) Concentration dependent inhibition of IgE-synthesis by Diamide (NEM) in blood samples of two different patients. The standardized Hg^{2+}-sensitivity (B) is given for comparison.

Fig. 5. Concentration dependent variation of IgE-synthesis by γ-IFN in the absence (O--O,Δ--Δ) or presence (●--●,♠--♠) of Hg^{2+} in blood samples of two different patients. The standardized Hg^{2+}-sensitivity is given for comparison.

Titrations of patients' blood samples with gIFN demonstrates the relative ineffectiveness of this compound in lowering IgE-levels: 500 U/ml, the concentration normally present in healthy controls (normal range, 50 to 500 U/ml, Horowitz, 1986), are almost without effect (Fig. 5). Only concentrations 100 times higher, a level which is hazardous to the patients, reduce the IgE-levels significantly (Fig. 5). The situation changes dramatically on addition of 1 mM Hg^{2+}: 500 U/ml gIFN are now sufficient to significantly reduce IgE-concentrations (Fig. 5). 1 mM Zn^{2+}, however, fails to induce such an effect.

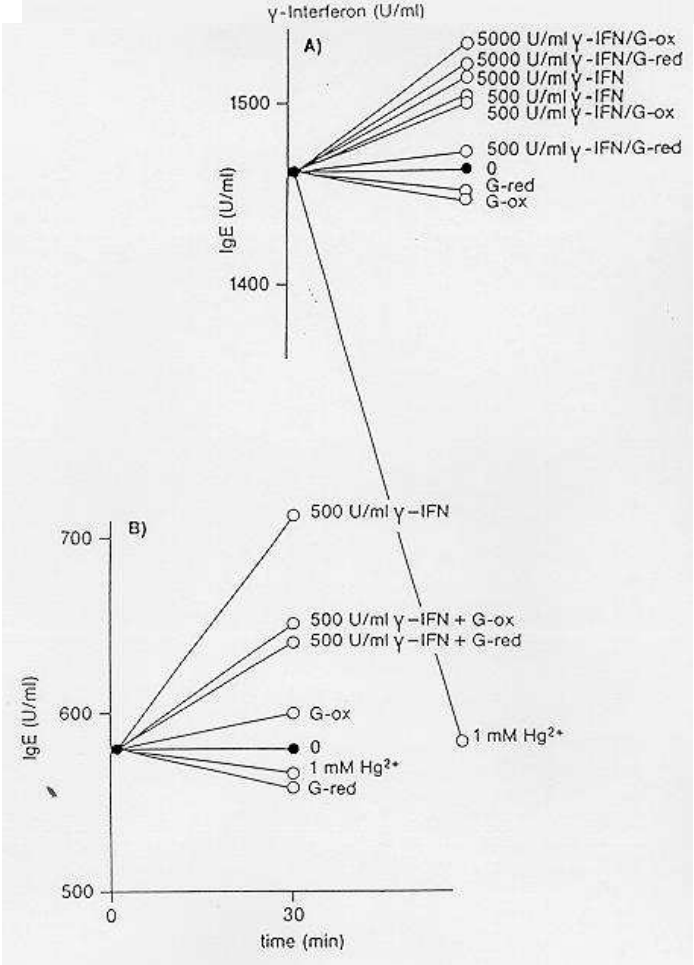


Fig. 6. A) and B) concentration dependent γ-IFN-sensitivity of the IgE-levels in blood samples of two different patients in the presence or absence of 1mM reduced or oxidized glutathione, respectively. G = glutathione, red = reduced, ox = oxidized. The standardized Hg^{2+}-sensitivity is given for comparison.

Fig. 6 demonstrates our first effort in the search for a replacement for toxic Hg^{2+}. As can be deduced from this figure, 1 mM glutathione (reduced or oxidized) proved to be relatively ineffective for this purpose.
Titrations of patients' blood samples with increasing amounts of Il-4 indicate that this cytokine effectively reduces the IgE-levels at concentrations (1000 U/ml) where gIFN is relatively ineffective (Fig. 7).
Dual wavelength measurements on lysed blood samples of atopic eczema patients demonstrate complex spectra between the wavelengths 550 to 575 nm. Peaks are visible at 558, 560 and around 568 nm (Fig. 8). Control measurements on isolated hemoglobin samples make clear that these peaks are not due to the blood hemoglobin content. Using cell counts, a rough calculation of the content of cytochrome b_{558}, a characteristic respiratory burst oxidase component, we obtained OD-values and results comparable to the estimated values on

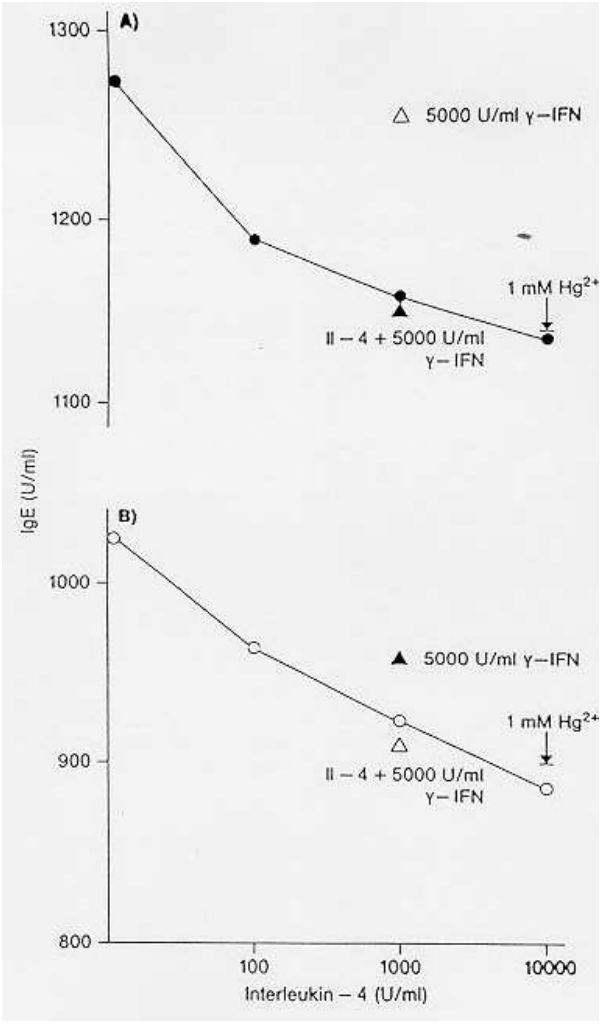


Fig. 7. A) and B) concentration dependent inhibition of the IgE-synthesis by Interleukin-4 in blood samples of two different patients (o--o,•--•). The standardized Hg^{2+} -sensitivity, as well as additional γ-IFN values are given for comparison.

neutrophils or neutrophil membranes obtained by Foroozan et al (1992). Although the few assays performed thus far could not be standardized, the peaks at 560 and 568 nm (but not at 558 nm) seem to be sensitive to O_2, NADPH and gIFN, which is indicated by changes in their OD in relative short time intervals (a few minutes). Besides the cytochrome of the plasma membrane respiratory burst oxidase, the mitochondrial respiratory chain cytochromes around 560 nm (especially in ubiquinol-cytochrome c oxidoreductase) (Hatefi, 1985) are the most abundant and most likely reacting components. We should emphasize, however, that the measurements could not be performed on fresh, but on 3 to 4 hrs old blood samples, and the absolute background absorption during the measurements of 2.5 resulted in standardization problems. Compounds like cytochrome c could not be detected.

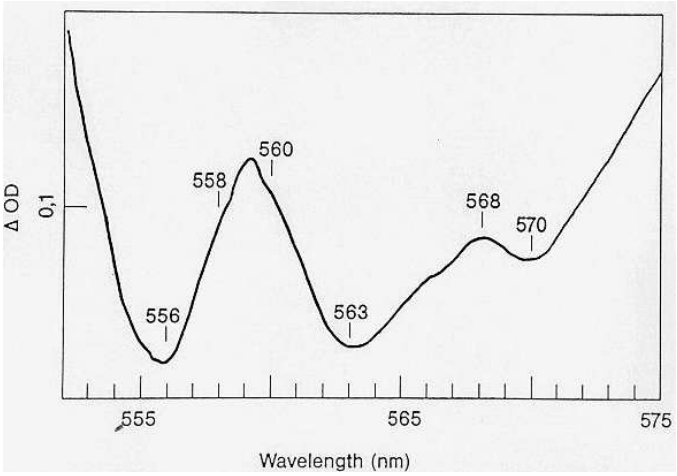


Fig. 8. Dual wavelength spectrum of whole lysed blood. Conditions as described in "Patients and Methods".

Discussion

Regulation of IgE synthesis by gIFN and cytokines is a highly complex event.

IgE synthesis results from a complex interaction between T cells, B cells and monocytes, under the control of T cell- and monocyte-derived lymphokines (Il-4, Il-5 and Il-6). In addition to their direct effects on IgE synthesis, these lymphokines have other functions, many of which are related to IgE. Thus, Il-4 is not only a crucial signal for the induction of IgE synthesis, but also a growth factor for murine mast cells, together with Il-3, a product of Th2 clones. Furthermore, Il-4 is a potent inducer of FcεR2b expression on both B cells and monocytes. On the other hand, Il-4 secreting T cells also produce Il-5, which promotes B-cell maturation and induces differentiation of eosinophil precussors. Eosinophils, in turn, express CD 23 when activated.

Thus, the activation of the IgE system leads to increased IgE synthesis and to enhanced expression of FcεR1 (on mast cells) and FcεR2 (on B cells, monocytes and eosinophils). This in

turn results in increased receptor-ligand interactions, with release of different chemical mediators involved in the pathogenesis of allergic reactions. The above summary was reported in 1989 with the results of research groups on cell culture systems (Vercelli and Geha, 1989 a). Similar conclusions were reached by Maggi et al (1989), altough they also implicated the necessity of Il-2 in the synthesis of IgE.

gIFN (100 to 250 U/ml) blocked Il-4- induced IgE-synthesis (at saturating concentrations of 200 to 300 U/ml). The synthesis of IgG, IgM and IgA production in the presence or absence of Il-4 was not affected by gIFN or α-IFN. Moreover, it could be shown that ongoing IgE production is blocked by cycloheximide (50 μg/ml), indicating that de novo IgE-synthesis was measured. Results obtained during a 2-9 day cell culture study concluded that enhanced production of Il-4, together with reduced production of gIFN, contributes to the high levels of IgE (Rousset et al, 1989). Attempts to demonstrate then the presence of Il-4 in the cell cultures, as well as in the blood serum of the atopic eczema patients, failed even at very high IgE-levels. Interesting in this aspect, however, is the resulting down-regulation of gIFN mRNA levels and gIFN production by Il-4 in mitogen activated culture cells (Rousset et al, 1989).

The physiological involvement of gIFN in the expression of IgE has been questioned (Zhang et al, 1991). Data obtained on cultured cells suggests that the signals delivered for IgE production by gIFN independent Il-4 and CD40 stimulation may mimic the pathway for IgE production seen in vivo. Nevertheless, besides involvement in allergic diseases, the interferons (especially gIFN) seem to be involved in various diseases, including progression to the full syndrome of AIDS (Kirkpatrick, 1989).

It is clear that the regulation of IgE is a highly complex event involving many factors. The most important direct modulating factors for synthesis of IgE in culture systems proved to be gIFN and Il-4. However, as outlined in the Introduction, this could not be confirmed in vivo in atopic eczema patients. The degradation of IgE in turn requires proteases, which themselves require gIFN (down regulation) and Il-1 (up regulation) for expression in cell cultures (Anemori et al, 1991). gIFN, Il-1 and Il-4, on the other hand, are possible substrates of these proteases.

<u>Mercury, metalloproteases, IgE-level; inflammations and allergic manifestations</u>
In search for an assay system more closely related to the in vivo conditions of atopic eczema patients, we decided to directly investigate the blood samples of these patients.

During our first attempt we titrated the blood samples with activators and inhibitors of proteases, since some of these compounds were thought to be involved in triggering atopic eczema. Particularly the metalloprotease activator mercury should have been able, in our opinion, to influence gIFN-levels by activation of metalloproteases for degradation of this important regulatory factor. Mercury has been suspected for decades now of triggering allergic manifestations via the immune system (Enwonwu, 1987).

The effect of mercury on IgE-levels was seen at concentration ranges of 0,5 to 1 mM; concentrations which are about 10^5 times higher than the normal range in blood of control or atopic eczema persons. Mobilization of mercury by DMPS results in 10^2 to 10^3 times higher values in these persons (see also Bannasch and Schleicher, 1991), which is still about 10^2 to 10^3 times lower than our measured effective concentrations on IgE-levels. Nevertheless, Bannasch and Schleicher (1991) describe immune changes (in the lymphocyte-subpopulations) induced in

their opinion by mercury mobilization. However, these changes, especially in patients with allergic diseases, were not verified. In another study (Murdoch and Pepys, 1986) high dosages of mercuric-chloride (50 µg/100 g body weigth) were repeatedly injected into rats, which corresponds to about 5 mg/l blood (a concentration near our described effective concentrations), with enhancement of antibody production. Thus, low toxic mercury concentrations seem not to be responsible for the changes in IgE-levels in our patients.

Matrix metalloproteinases (collagenase, gelatinase, stromelysin) (Emonard and Grimaud, 1990; Parmgren et al, 1992; Tschesche et al, 1992) are highly glycosylated enzymes, active at neutral pH, which require intrinsic Zn^{2+} and extrinsic Ca^{2+} for full activity, and are therefore inhibited by chelating agents (like EDTA) and have the ability to degrade, for example, the extracellular matrix. They are secreted from the connective tissue cells such as fibroblasts and from neutrophils as inactive proenzymes, and can be activated by treatment either with proteinases such as serine-proteinases, or with different mercurial compounds, or reactive oxygen species (ROS). They are also inhibited by their specific inhibitor TIMP (Osthues et al,1992) or α_2 -macroglobulin (Bergmann et al, 1989). The signal for upregulation of their secretion is suppressed by immunosuppressive drugs, like glucocorticoids (Hempelmann et al, 1991; Shapiro et al, 1991).

Activation of isolated metalloproteases requires µM concentrations of mercurials (Bläser et al, 1991): 10 µM $HgCl_2$, for instance, activates about 40 % of the proteases (collagenase) within approximately 4 hrs. These conditions were obtained in our patients after mercury mobilization and may therefore be responsible for glucocorticoid-sensitive inflammations (Hempelmann et al, 1991; Shapiro et al, 1991). However, under normal conditions the circulating protease and lactoferrin concentrations in the patients were found to be normal. The collagenase and gelatinase assays have been done by ELISA. ELISA measures only protein concentrations. In blood samples of healthy donors, metalloproteases are inhibited by TIMP, protected by α_2 -macroglobulin and the anticoagulant heparin from reaction with substrate or binding to antibodies (for instance during ELISA), which leads to the lowest concentrations (and activities) (Table 1).

In EDTA plasma, α_2 -macroglobulin is inactive and residual heparin and/or TIMP protect and/or inactivate(s) only part of the present latent proteases resulting in moderate concentrations (collagenase ca. 90 ng/ml, gelatinase ca. 600 ng/ml, and lactoferrin ca. 300 ng/ml at healthy donors, Tschesche, personal communication) and activities. In the sera (coagulated blood), α_2 -macroglobulin is inactive, heparin missing and therefore almost all the metalloproteases are activated by oxidation (below). As to expect, the highest concentrations (and activities) of the proteases (and of lactoferrin) were then obtained in the sera (Table 1).

The few measurements with capillary blood samples (collected under heparin protection) of affected skin areas (areas under acute inflammation) demonstrate that at these areas activation processes exist. The few heparin molecules, possibly in here available, may not be able to block the high concentrations of free latent and/or activated metalloproteases for binding to antibodies during ELISA (competition). On this ground, a heparin-therapie should not work.

We could show (Kiehl and Ionsecu, 1993) that circulating immune complexes and IgE in the patients blood activates the coagulation system with elevation of platelet aggregation and histamine release with further enhancement of aggregation. This process could be related to

significantly lowered diamine-oxidase activities of platelets. We now conclude that this process starts with rising IgE concentrations in the circulating blood or affected skin areas (activation of the contact system by surfactants, Wenzel et al, 1989, etc; contact allergy). Platelets aggregation results presumably in a changed energy metabolism in these particles with build-up of vitamin $K \cdot H_2$ and H_2O_2 /ROS, inhibition of diamine-oxidase by ROS (H_2O_2) with elevation of histamine, inactivation of α_2-macroglobulin and activation of metalloproteases by ROS/H_2O_2. ROS may also be produced by prolonged exposure of skin cells to UV-light.

gIFN-molecules were significantly degraded by metalloproteases (at least by activated leucocyte collagenase) under in vivo conditions, although our in vitro assay showed no such behavior. However, one should keep in mind that the concentrations of the circulating gIFN molecules are very small and in the concentration ranges of most hormones. Degradation of two plasma components, namely Cl-inhibitor and α_1-proteinase inhibitor, by metalloproteases has already been demonstrated (Knäuper et al, 1990; 1991). The implication for metalloprotease regulation is evident, and the impact of the changing active gIFN-concentrations on the IgE-levels of atopic eczema patients will be discussed below. On this point, we compared total IgE measurements using samples of circulating blood with skin Prick-tests and skin Epicutanous-tests. As expected, the measurements do not match either (Kiehl, 1994b; 1995c; 1996a).

A standardized titration of blood samples from different patients (IgE ca. 1000 U/ml) with 1 mM $HgCl_2$ resulted in unexpected positive and negative variations (> 50 %) of their IgE-values, suggesting involvement of a redox reaction in the Hg-IgE-interaction: Hg^{2+} is, like Cd^{2+}, able to react as a dithiol reagent (Stiggall et al, 1979). The metalloprotease inhibitor EDTA elevates IgE-levels (at least in the experiments where Hg^{2+} induces positive variations). The EDTA results may be interpreted in favor of a direct influence of the metalloprotease on IgE concentrations, however the results of the Hg^{2+}-titrations are in direct contradiction to such an interpretation.

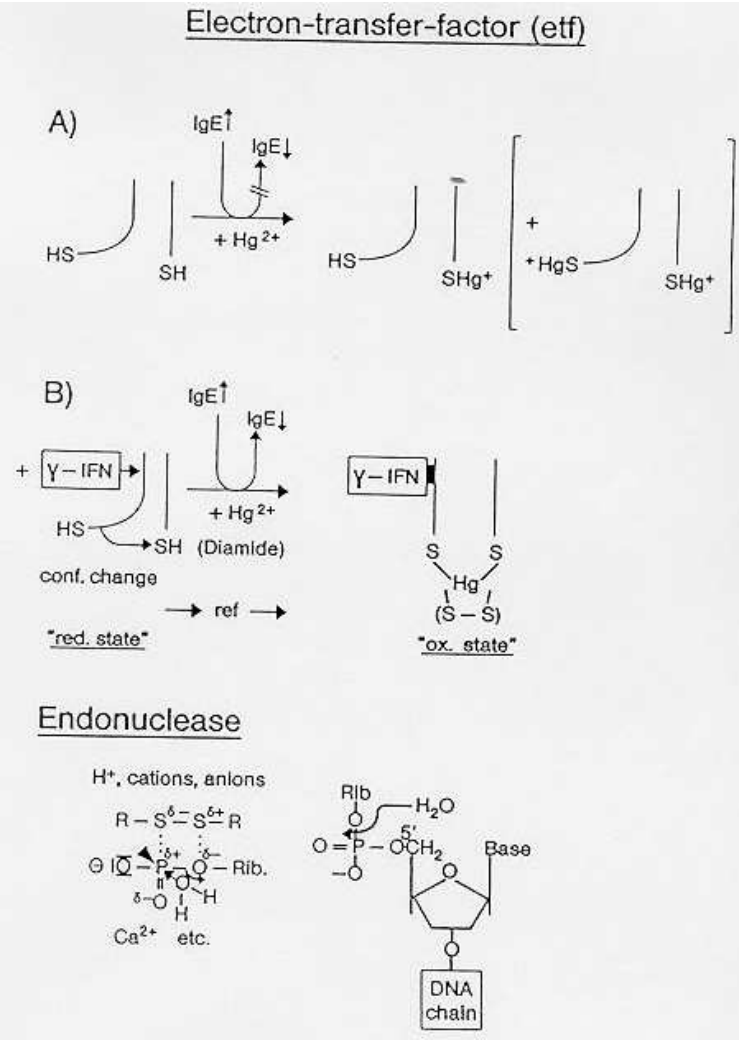
The serine protease inhibitor APMSF itself has no effect on IgE-level, which means that this protease is not involved in IgE-regulation either directly or indirectly, and serine proteases are not involved in our measured metalloprotease activities. APMSF probably interacts with serine residues after they are liberated by EDTA treatment, and thus prevents upregulation of the IgE-level due to EDTA. This suggests the involvement of external Mg^{2+} - or Ca^{2+} - sensitive serine residues in the signal tranduction pathway leading to elevation of IgE-levels.

The detergent Triton X-100 (and probably other detergents too) drastically lowers IgE-levels, probably by liberating IgE-degrading proteases from their storage compartments (Wenzel et al, 1989). The IgE-level was also reduced by cycloheximide, a protein synthesis inhibitor, which shows that ongoing IgE production is blocked and indicates that de novo IgE synthesis is measured. A similar result has been obtained in cell culture systems (Rousset et al, 1989).

Patients` IgE-level in the circulating blood system is regulated by degradation and (re)synthesis (secretion seems not to be a rate-limiting step), and these two processes are regulated by various factors, including interleukins and gIFN. We were able to demonstrate this well-known fact in our simple assay system, although the background level of IgE was very high (70 to 90 %). It was then possible to calculate degradation, as well as synthesis rates of the patients' steady state IgE-level, by doing a few assays. Furthermore, and even more importantly, the results obtained with Hg^{2+} indicate the involvement of a redox reaction in the regulation of IgE-synthesis.

Involvement of a redox reaction/thiol-disulfide interchange mechanism in the regulation of IgE synthesis

This reaction has further been substantiated by titrations with the dithiol oxidizing reagent Diamide and the thiol reagent NEM. Diamide affects the IgE-level similarly to the dithiol reagent Hg^{2+}, but, in contrast, NEM and zinc were relatively ineffective. These data prove the involvement of a dithiol/disulfide interchange mechanism in the regulation of IgE-synthesis. The obvious question raised involves the effects of gIFN or Il-4 on this mechanism. In contrast to the assays on cell cultures (Rousset et al, 1989), gIFN is relatively ineffective, or has even the opposite effect in our in "vivo" assay system. The addition of Hg^{2+} plus gIFN to blood samples (normally with almost no response to either one of these compounds), results in down-regulation of IgE in a similar way as seen in cell culture systems by gIFN alone (Rousset et al, 1989). Low concentrations of Il-4 suppress the IgE-levels in our blood samples in contrast to gIFN. This suppressive effect is directly opposite to the Il-4-dependent up-regulation of IgE in cell cultures (Rousset et al, 1989).



To summarize, the described dithiol/disulfide redox state is sensitive to Hg^{2+} (Diamide), gIFN and Il-4. gIFN probably directly or indirectly changes the conformation of an involved protein (see also Kiehl, 1980) in such a way that two associated thiols become vicinal and able to react with Hg^{2+}. Hg^{2+} itself keeps this conformation and thus lowers the effective concentration of gIFN by a factor of 10^2 to 10^3 or more (Fig. 9). Il-4 reacts antagonistically to gIFN in blood samples and in cell cultures, although in opposite directions (Rousset et al, 1989). The interaction of conformation with redox state at the existing gIFN-concentrations in our patients explains the highly varying IgE values in the blood samples of these patients on addition of Hg^{2+}.

Fig. 9. Reduced and oxidized state of the electron transfer factor (etf). Possible mechanism of DNA cleavage by endonuclease. red.state = reduced state, ox.state = oxidized state, ref = nuclear redox factor.

Such results raise the following questions: Where is the described dithiol/disulfide redox interchange reaction taking place? In plasma, membrane, or cytosol? Which natural oxidation factor normally replaces Hg^{2+} or Diamide in this dithiol/disulfide interchange? What leads to the differences between patients blood samples and the cell cultures raised from their blood cells?

Our ineffective titrations of the redox state, indicated by the various IgE-levels, with extremely high concentrations of glutathione (ox. or red.) (Kiehl, 1993 a,b,c) for plasma, demonstrate clearly that the thiol groups involved were located inside the involved B cells: glutathione cannot cross cell membranes. Another point is that externally delivered glutathione is then, of course, not able to replace Hg^{2+} or Diamide in the described dithiol/disulfide interchange mechanism. Hg^{2+} and Diamide, effective at high concentrations, react inside the cells (Kiehl, 1993 a,b,c), most likely with a dithiol-containing protein localized, at least for some time, on the inner cell membrane. Cytosolic glutathione should not be involved: the results and the described mechanism require the involvement of a membrane-bound protein. NEM is ineffective although reacting normally with reduced cellular glutathione and the cellular glutathione concentrations were too high (up to 10 mM, Kiehl, 1993 a,b,c) to be involved in the Hg^{2+} - or Diamide-induced elimination of IgE-synthesis. The different results obtained when using blood samples or cell cultures may be explained by the conditions in which the cells live. We used "in vivo" conditions for our experiments, in contrast to cell cultures which were grown in artificial systems using mitogen-stimulated B cell proliferation.

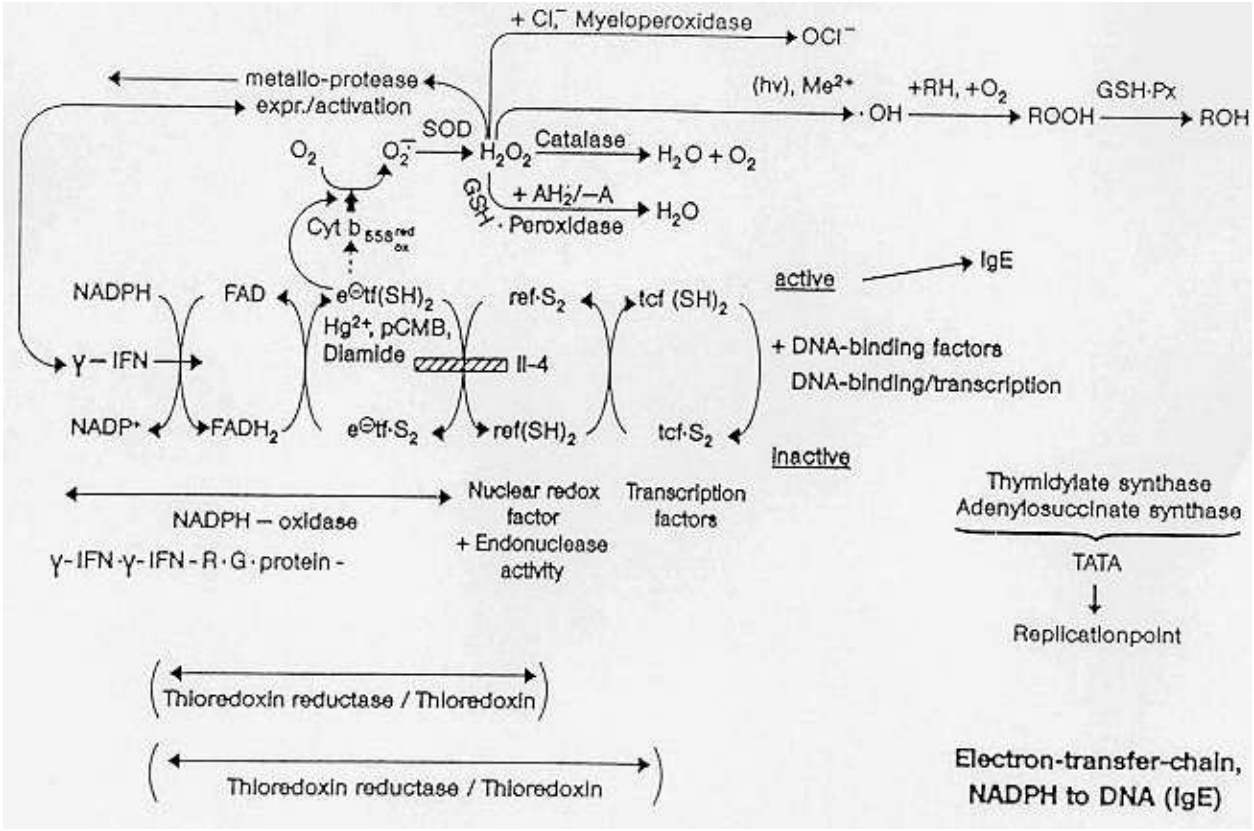


Fig. 10. Electron-transfer-chain, NADPH to DNA (IgE). expr. = expression, SOD = superoxide dismutase, GSH•Px (GSH Peroxidase) = glutathione peroxidase, Cyt b = cytochrome b, γ-INF-r = γ-IFN-receptor.

The most likely receptors involved in the signal transduction pathway from gIFN or Il-4 to IgE are cytosolic G protein(s), NADPH-oxidase/oxidation factor, redox factor, nuclear transcription factors and endonuclease [Fig. 10]. This conclusion will be substantiated in the further discussion section.

Electron transfer chain, NADPH to DNA

The plasma membrane NADPH oxidase [Fig.10 and 11] is the superoxide forming enzyme of phagocytes and B-lymphocytes.The production of oxygen-free radicals is important to their

antibacterial defense, but overproduction can contribute to tissue injury due to inflammation and risk of cancer development (Maly et al, 1989). It is a rather complicated electron transfer system, catalyzing the transfer of reducing equivalents from NADPH to O_2 and, in so doing, sharing some similarity with the mitochondrial respiratory chain (Hatefi, 1985; Segal, 1989; Babior, 1992; Park et al, 1992; Kiehl, 1993 a,b,c; Kiehl, 1996c). Serine (or threonine) phosphorylation has been found as a result of activation of the cells (for instance through PMA, Volkmann et al, 1984) [Fig. 11]. The activation of NADPH oxidase is absolutely dependent on Rac 2 (a G-protein, Knaus et al, 1992), GTP (Mg), GTPase activity and Ca^{2+}, and is then likely to be subject to regulatory cofactors, like SDS or arachidonic acid (Kiehl and Ionescu, 1993), in vivo (Park and Babior, 1993) and it should be associated with a phosphatase in order to be transformed to the resting state. Somewhat surprising, however, the process of activation is coupled to ongoing mitochondrial ATP formation and blocked by 1 mM NEM (Cohen and Chovaniec, 1978). But most importantly, the reduction of cyt b_{558} (Parkos et al, 1988) does not occur simultaneously with O_2^--build-up (Foroozan et al, 1992), and cyt b_{558}-deficient systems are also able to generate O_2^- (Babior, 1992) [Fig. 10].

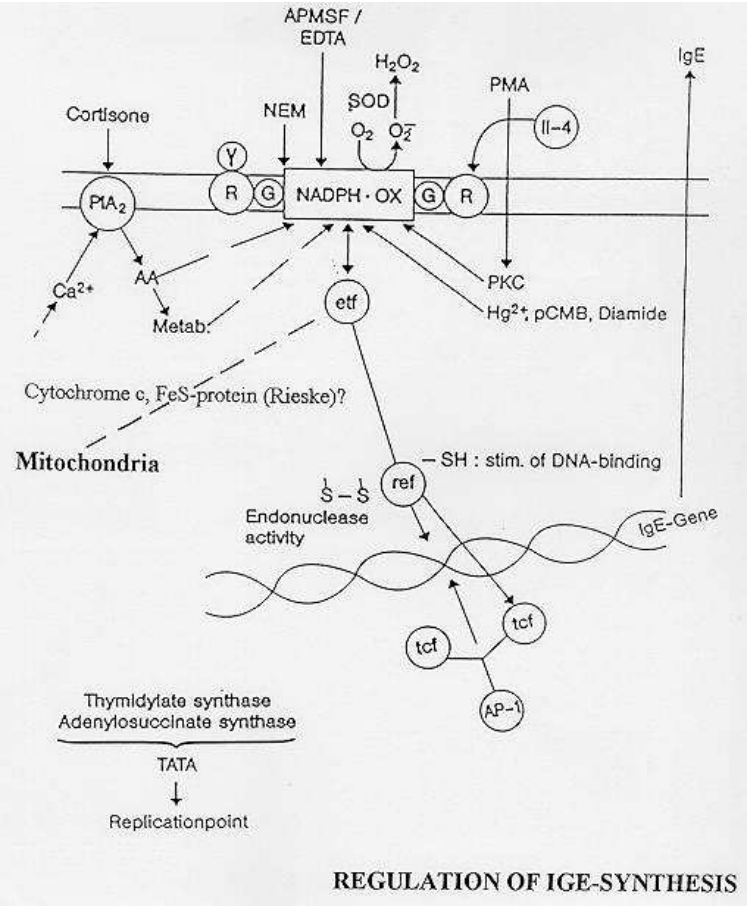


Fig. 11. Regulation of IgE-synthesis. γ = γ-IFN. R = receptor, G = G-protein, OX = oxidase, SOD = superoxide dismutase, PMA = phorbol myristate acetate, AA = arachidonic acid, etf = electron transfer factor, stim. = stimulation.

One biological activity of gIFN is to prime cells to mount a respiratory burst in response to various stimuli acting at the cell surface (e.g. the mitogen LPS) to induce tumoricidal activity. The increased activity is obtained with 500 to 1000 U gIFN/ml after 3 days in vivo and up to 15 days in vitro by expression of the genes and tissue levels of cyt b_{558}. gIFN markedly increased the mRNA transcript for the large ß-subunit of cyt b. Nevertheless, gIFN is able to induce a respiratory burst despite a deficiency or complete lack of b_{558} (Babior, 1992; Ezekowitz and Newburger, 1986), probably by improving the connection between the NADPH-binding subunit and the remaining subunits (activation process) (see also Segal, 1989). It should be emphasized that atopic eczema patients express normal cytochrome concentrations in contrast to chronic granulomatous disease (CGD) patients. The modulation of their IgE concentrations ($\pm$ Hg^{2+}) by gIFN requires minutes in vivo (blood samples) similar to the O_2^--build-up in neutrophils, but days in vitro (mitogen stimulated culture cells).

To summarize, the signal of gIFN for O_2^- or IgE production (activation process) is probably mediated by its receptor to the NADPH oxidase, most likely at first to the NADPH-binding

subunit via G-protein (Rac-2). This system, thus, very much resembles the receptor-linked membrane-bound adenylate cyclase (Hall, 1990).

NEM is able to block the activation process but not the activated oxidase. A free thiol group probably becomes reversibly masked during activation. There is a low rate of reduction of cytochrome b by NADPH in anaerobiosis, which does not account for the high rate of O_2^- production and NADPH oxidation. The reduced cytochrome b is rapidly oxidized by air. This behavior very much resembles cytochrome oxidase (Gibson et al, 1965), although separation of cytochrome reduction and O_2^- -generation (as described) resembles more ubiquinol-cytochrome c oxidoreductase (Bechmann et al, 1992) and its iron-sulfer (FeS)-protein.

The thiol group reagent pCMB abolishes O_2^- -forming activity and raises the E_m of the cytochrome from -245 mV to -175 mV, supporting the notion (Bellavite et al, 1983) that one or more SH groups of a yet unknown electron transfer factor (etf, e.g. FeS-protein), able to reduce O_2, may be coupled to cytochrome b (Fig. 10) (see also Kiehl, 1993 a,b,c). Our spectra on whole lysed blood samples suggest that NADPH oxidase may be more complex than published to date (including Park et al, 1994). The pCMB-sensitive thiol group(s) most likely participate(s) in the described Hg^{2+}- or Diamide-sensitive thiol/disulfide redox state responsible for IgE-regulation. In addition to these essential thiol groups directly involved in regulation of O_2^- generation or IgE synthesis, there is probably a Ca (Mg)-sensitive serine residue indirectly linked to former activities.

gIFN plus cytokines stimulate cell proliferation (leukocytes) and activate the defense mechanism. The pathway for signals delivered by these molecules includes the nuclear transcription factors (the oncogenes = jun, fos, myc, myb, etc.), AP-1-binding factors (mediating the activity of the transcription factors) and an ubiquitous redox factor (ref. 1) (Meager, 1990; Abate et al, 1990; Xanthoudakis et al, 1992). High concentrations of c-myc and c-fos in gIFN/cytokine or mitogen stimulated B-lymphocytes, for instance, correlate with elevated antibody production, in contrast to the late response of B-lymphocytes (cell cultures) to the gIFN/cytokines which results in inhibition of antibody production (Meager, 1990) as reported by others (Rousset et al, 1989, own).

The proto-oncogenes c-fos and c-jun function cooperatively as inducible transcription factors, and their activity is probably mediated via AP-1-binding factors by a redox mechanism. Thioredoxin plus Thioredoxin reductase/NADPH or high concentrations of DTT were able to stimulate the activity, suggesting the involvement of (an) essential thiol-group(s) in DNA-association and a natural reduction factor for regulation of DNA-association (Abate et al, 1990).

An ubiquitous nuclear factor (Ref-1) stimulating several transcription factors (e.g. fos, jun, AP-1-proteins, NFxB, myb) has thus been isolated (Xanthoudakis et al, 1992). This factor is similar to the transcriptional factors stimulated by Thioredoxin plus Thioredoxin reductase/NADPH or high concentrations of DTT, and carries therefore essential sulfhydryl groups. The Ref-1-reduced form of the regulatory cysteine residue in the transcription factors is a sulfhydryl. Following the suggestions for =xyR protein in E.coli (Storz et al, 1990), it has been proposed that the oxidized cysteine residue in fos and jun is in the form of a reversible sulfenic acid derivative. However, the main argument for this suggestion is incorrect: One of the two cysteine residues probably involved may be sufficient in the mutant form of OxyR to show wild type induction of OxyR regulated genes in vivo, but may not be sufficient for regulation of these genes. The reduced state

of wild type Ref-1 is able to be oxidized reversibly by peroxides (HO_2^-) and similar compounds [Fig. 10].

The primary C-terminus of Ref-1 seems to be required not only for redox function, but also for a Ref-1 associated endonuclease activity (Xanthoudakis, 1992). Only the reduced Ref-1 is capable of stimulating DNA-binding activity, whereas it is not yet clear whether the reduced or oxidized Ref-1 functions as an endonuclease. Studies performed by us on transport and ATPsynthesis in mitochondria (Kiehl, 1976; Bäuerlein and Kiehl, 1976; Kiehl and Bäuerlein, 1976; Stiggall et al, 1979; Kiehl, 1993 a,b,c; Kiehl, 1994 a,b; Kiehl, 1995 a,b,c; Kiehl, 1996 a) lead us to believe that activated disulfides were able to perform phosphoryl transfer or transport activities.

The subsequent conclusion with respect to Ref-1 would then be that the oxidized state of ref-1 is a disulfide and responsible for catalytic endonuclease activity (Fig. 9). As already mentioned, gIFN has a bifunctional effect on protein synthesis. C-myc (antibody) synthesis, for instance, will be stimulated or inhibited depending on fresh blood sample or culture cell incubation time respectively. The antiproliferative effects of gIFN demonstrate that translation inhibition pathways were activated by gIFN treatment of the cells (Meager, 1990). Thereby, inactive endonucleases were turned into active enzymes presumably carrying a catalytic disulfide. gIFN should then transduce redox signals during short time intervals, whereas during longer intervals (e.g. cell cultures) the oxidized redox factor determines translational activities.

The results and conclusions described thus far are summarized in Figs. 10 and 11. The transcription factors c-myc and c-fos are involved in antibody production, nevertheless the clinical parameters of atopic eczema patients, especially their immunparameters, imply IgE-specific transcription factors. The redox factor (= oxidation factor) has to be linked to the electron transfer or transcription factors respectively.

<u>The redox potential is responsible for IgE or O_2^- -synthesis and proliferation</u>
The full reduced electron transfer chain, NADPH to DNA (IgE), favors a high synthesis rate for IgE (low for O_2^-). In contrast, a totally oxidized chain is not able to synthesize IgE (but O_2^-) (Fig. 10) and the risk for mitogen stimulated proliferations is extremely high (relation to skin carcinomas induced by UV-light). We are on the replication point (TATA-Box), where thymidylate synthase (Shishieh et al, 1992) and adenylosuccinate synthase (Kiehl, 1993 a,b,c; Kiehl, 1994 a,b; Kiehl and Ionescu, 1992; Gallert et al, 1994) with sulfenic acid/sulfenylphoshite residues (Kiehl, 1994 a,b) (Fig. 12) play the most important role. As with prokaryotes, the replication should proceed at this point in two directions. High redox potentials (red. state of the e^- -transfer chain) favor the synthesis of IgE in one direction (activation of the defense mechanism). Low redox potentials (ox. state of the e^--transfer chain) favor the synthesis of O_2^-. Any elevation in the IgE-level should be a warning signal for our body, for instance against foreign mitogens of invading microbes, and is a precurser to the production of defending free O_2^- radicals. Overproduction of O_2^- (oxidative stress) leads to an irreversible break of the disulfide bonds involved (most probably build up of $–SO_2$- radicals and $–S^-$) (Fig.12) and uncontrolled (hyper-) proliferations in the other direction. A controlled proliferation would imply that the synthesis of the disulfide carrying proteins (factors) involved runs faster than their chemical modification by O_2^-. An overview of the complexity of the transcription apparatus itself may be obtained from the literature (Gill and Tjian, 1992; Tjian and Maniatis, 1994).

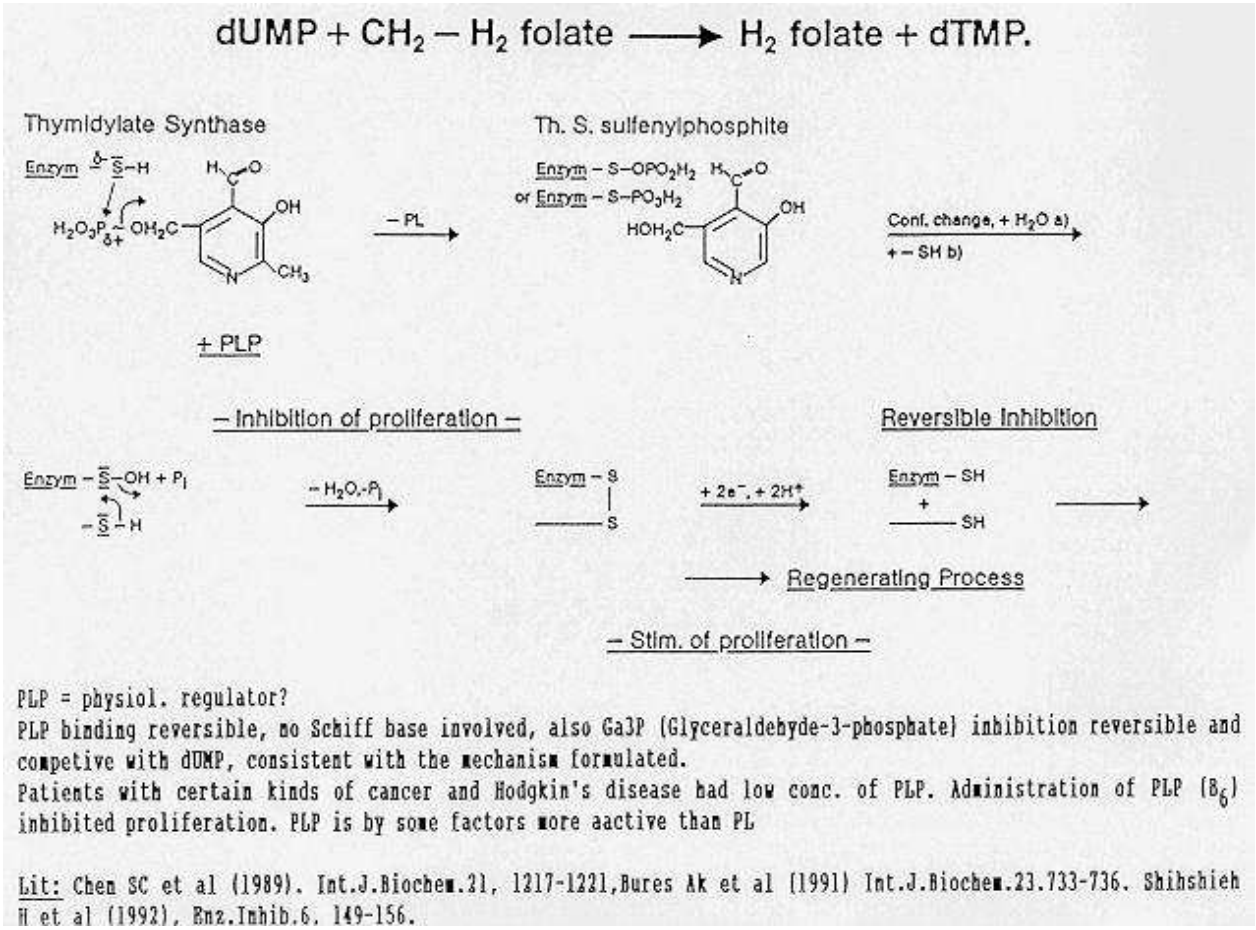


Fig. 12. Regulation of the thymidylate synthesis activity by PLP (pyridoxal-phosphate) and disulfide/dithiol interchange (redox potential). PL = pyridoxal.

As already outlined, the defect in NADPH to DNA (IgE) in atopic eczema patients lies at the level of etf/ref. The gIFN effects in relation to IgE synthesis are described, but the question about the Il-4 interference in the described redox regulation of IgE synthesis is difficult to answer. The mechanism of signal transduction by the Il-4/ receptor is rather obscure (Meager, 1990). The described down regulation of gIFN mRNA and gIFN production in mitogen activated T-culture cells by Il-4 (Rousset et al, 1989) takes days and is therefore related to the late responses of gIFN. During short time intervals, Il-4 transduces opposite to gIFN redox signals. Coupling of the Il-4 receptors to the electron transfer chain at the level of etf/ref (via G-protein?) may be responsible for this behavior (Fig. 10). The down regulation of IgE level, the production of several cytokines (Il-1, TNF incl.), as well as gIFN (Anemori et al, 1991), prostaglandin E_2 (Kiehl et al, 1994) and superoxide production (Sies, 1993) by Il-4 implies the possibility that Il-4 may play a role as an antiinflammatory cytokine (Hart et al, 1989; Reynaud et al, 1989).

Extremely high serum IgE levels exist in patients with the socalled hyper-IgE syndrome. In this case, regulation by Il-4 or gIFN is almost impossible (Rousset et al, 1989) and the electron transfer chain should be in the full reduced form. The defect in NADPH to IgE for electron-transfer is most probably located at the level of etf/ref as described for the normal atopic eczema patients. All the factors regulating NADPH oxidase also, of course, influence the IgE level. An important role in modulating IgE concentrations then is also played by phosphorylation and

dephosphorylation of the involved proteins by kinases (e.g. PkC) and phosphatases. An indirect influence on the IgE level exists (as described) under oxidative stress conditions (Sies, 1993).

The natural defense mechanism of cells

The adaption of cells to oxidative stress (Sies, 1993), to heat shock, to environmental stress, etc. is nothing other than their natural defense mechanism for protection against injury. The general scheme of activation of this defense mechanism seems to be the use of stimulatory or inhibitory cytokines/hormones including, for instance, tumor necrosis factor (TNF) and Il-1 control NADPH oxidase (non-phagocytes), TNF and Il-1 control Mn-superoxide dismutase (SOD), gIFN and Il-1 control collagenase, and gIFN and Il-4 control IgE. In most (or all?) cases, the activation of NADPH oxidase (O_2^- production) occurs simultaneously to the expression of former enzymes.

In the case of IgE synthesis (and probably also in the expression of some other compounds), environmental pollutants assumed to induce atopic eczema (Behrendt, 1989) were able to react irreversibly with the involved essential dithiol/ disulfide redox state. The pollutants include formic aldehyde, sulfite/ SO_2, isocyanates and anhydrides. These compounds keep the electron transfer chain in the reduced form and, under activating (defense) conditions, the IgE concentrations rise to pathological ranges. Another compound, CO, binds to the NADPH oxidase (Segal, 1989), preventing the reduction of O_2 and thereby shifting the electron transfer chain to the reduced state, which is accompanied by the enhanced probability of IgE synthesis. Depending on concentration, most compounds have proven to prime cell proliferation in an animal model and in human studies (Hayes, 1992).

Mitochondrial oxidative phosphorylation serves as sole producer of energy

B-cells have a considerable need for energy. Their proliferation, synthesis and excretion of immunoglobulins require this energy in the form of nucleotide-triphosphates. In contrast to brain cells, lymphocytes (B-cells) use glutamine instead of glucose as fuel. Mitogenic stimulation increases rate of glutamine utilization and glutamine is essential for cell division of lymphocytes in culture. Other amino acids are not able to be substituted for glutamine (Ardawi and Newsholme, 1985). Under these conditions, mitochondrial oxidative phosphorylation serves as the sole producer of energy in the form of nucleotide triphosphates and NADPH. The NADPH oxidase (IgE synthesis) requires at least NADPH/ATP (kinases)/GTP (G-proteins) to function. Thus, it is not surprising that the process of their activation (Foroozan et al, 1992) and regulation is coupled to ongoing mitochondrial energy formation. All the compounds influencing mitochondrial energy formation (Kiehl, 1976; Bäuerlein and Kiehl, 1976; Kiehl and Bäuerlein, 1976; Stiggall et al, 1979; Kiehl and Hanstein, 1984; Kiehl, 1993 a,b,c; Kiehl, 1994 a,b;) then also influence IgE-level.

Dermal and intestinal dysbiosis, food, as well as psychogenic stress (Kiehl and Ionescu, 1993; Kiehl and Ionescu, 1994; Ionescu et al, 1990 a,b,c; Ionescu and Kiehl, 1988; Kiehl, 1992; Ionescu et al, 1992; Kiehl, 1993 a,b,c) are the main triggering factors of allergic manifestations. Polysaccharidic, as well as protein antigens of C. albicans, play a definite role in inducing allergic reactions in patients (Gumonski et al, 1991). Carbohydrate for instance, delivered by food, is a growth factor for these fungi and weakens immune response by changing the energy metabolism of lymphocytes (Ardawi and Newsholme, 1985).

The pathogenesis of atopic eczema and leukemia relates to the development of AIDS

Psychogenic stress elevates norepinephrine levels, lowers dependent cellular cAMP

concentrations (Kiehl, 1992; Ionescu et al, 1992; Kiehl, 1996 a,b,c) and weakens thereby immune response (arachidonic acid, prostaglandin, leukotriene, cytokine concentration, etc.) (Kiehl, 1996 b), and elevates IgE concentration. The greatest number of specific IgE-antibodies are developed against food- or inhalative allergens. It should be stressed that the total (unspecific plus specific) IgE concentrations were normally 10^2 to 10^3 times higher than the measured specific ones. Perhaps the gIFN independent IgE production by cultured cells on Il-4 and CD 40 stimulation (Foroozan et al, 1992) is related to this fact. The first expression of specific IgE antibodies may be purely incidental and resembles autoimmune diseases. The described pathogenesis of atopic eczema and leukemia (proliferation) relates to the development of AIDS: 1.) the normal signal transduction pathway may be overtaken by the HIV-virus and 2.) at the replication point the inserted genetic material of the virus is expressed instead of the cellular DNA. The most likely mechanism for pathogenesis of HIV-infection has been presented (Kiehl, 1994 a, b; 1995 c; 1996 a) [Fig. 13].

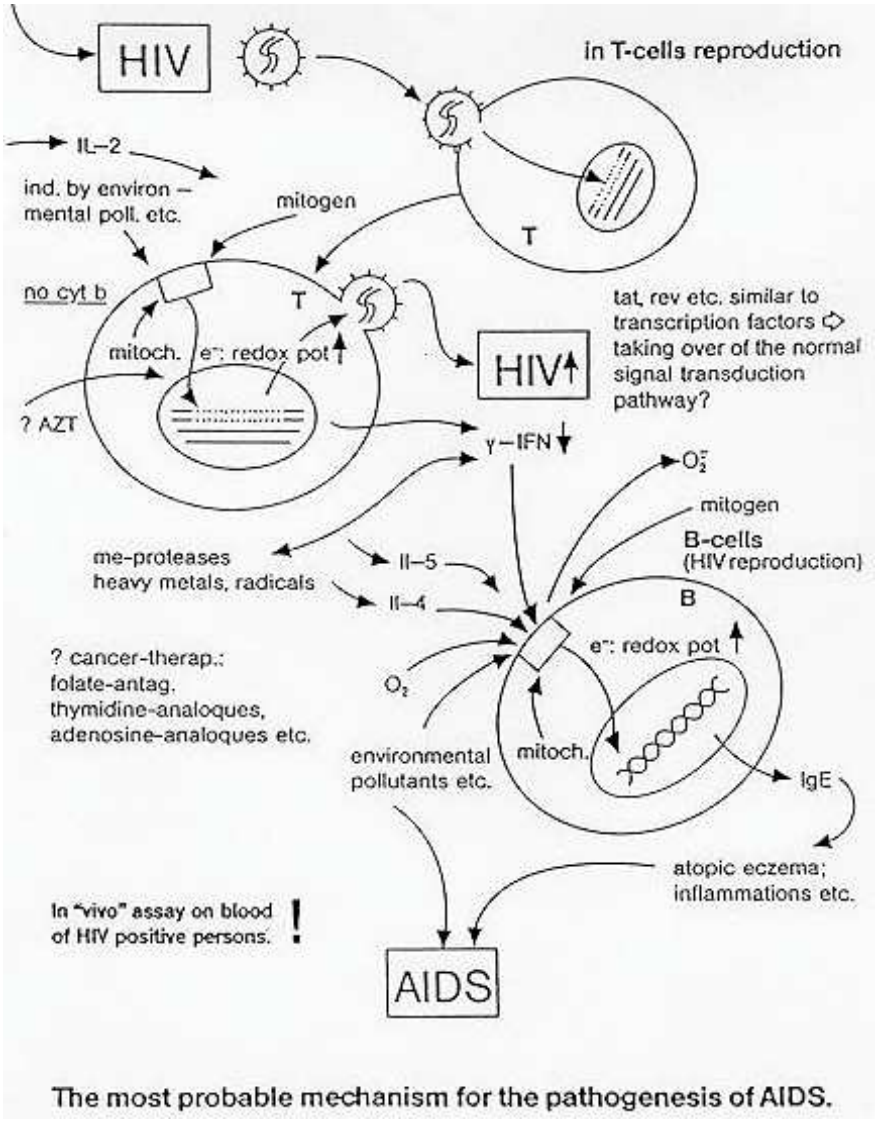


Fig. 13. The most probable mechanism for the pathogenesis of AIDS. AZT = 3`-Azido-3`-desoxythymidine, cyt = cytochrome, ↑ = high, rising, ↓ = low, falling.

<u>A system for "in vivo" experiments without animals</u>

Altough our regulatory in vivo system, as presented, is able to summarize most data by us and others, a few important elements still have to be proven. However, the point is that it is a beneficial system which not only allows investigation of causes, but also leads to treatment of some wide-spread immune diseases, such as leukemia and AIDS. Another important point to be emphasized is that our developed assay system can be used for detection of almost every blood parameter. The assay is fast and comparable to "in vivo" conditions, and cell culture systems with artifical „in vivo“ conditions can be spared. This fact is most important for research on AIDS-patients where robots or automated devices have to be used for analyzing HIV-containing samples. Pharmacologists would be able to do "in vivo" experiments without the use of experimental animals, and the system is reliable for the evaluation of the toxic

limits of chemicals as well (Kiehl, 1994 b, 1995 c, 1996 a). Results are obtained in a very short time and the pharmacological screening for the biological profile of a substance is therefore easy to perform.

Conclusion: The strategy for diagnosis and therapy of atopic eczema, leukemia and AIDS should be 1.) in "vivo" titration of the redox state involved with Hg^{2+}, gIFN and Il-4; 2.)measurement of the active gIFN and cytokine (Il-4, Il-2, etc.) concentrations in plasma with appropriate assays (which have to be developed); 3.) supplementation with the individual right amount of active gIFN and cytokines (Il-4, Il-2, etc.) and 4.) avoidance of environmental pollutants. If not possible, modification of the involved redox state with appropriate compounds (which have to be developed) (Kiehl, 1996 c).

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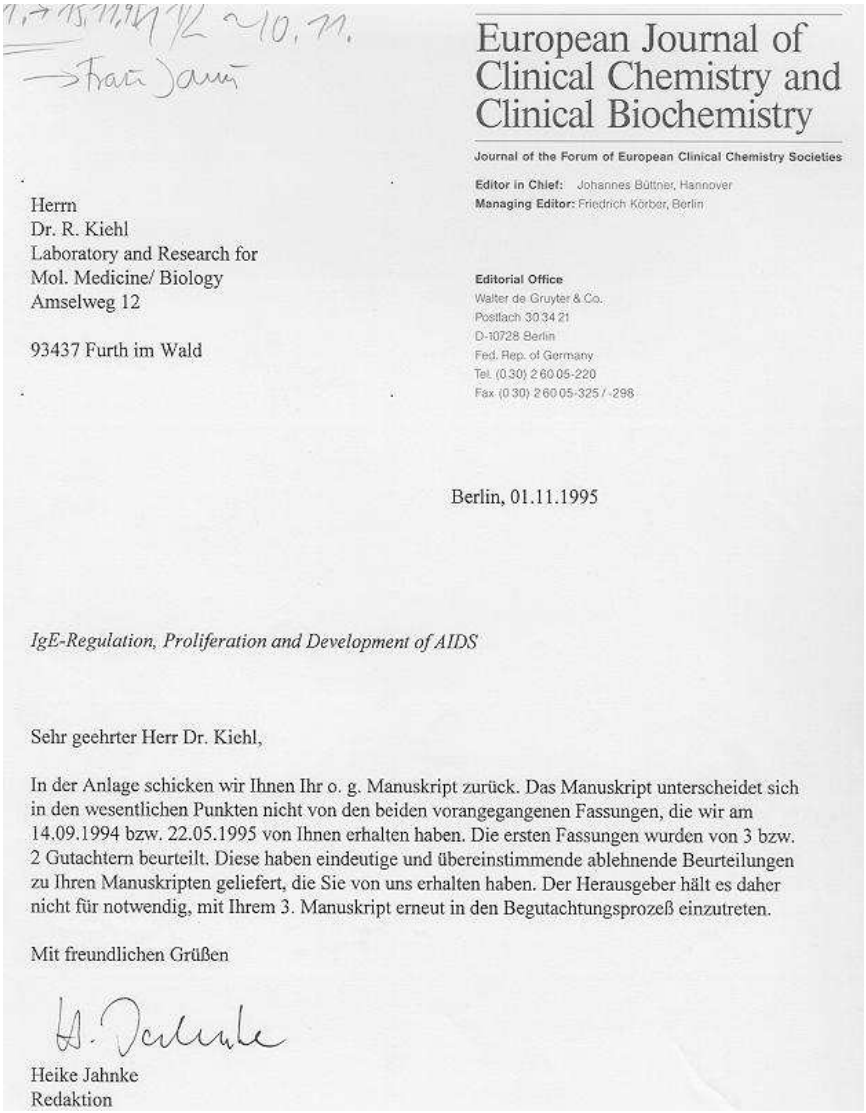

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Herrn
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Berlin, 01.11.1995

IgE-Regulation, Proliferation and Development of AIDS

Sehr geehrter Herr Dr. Kiehl,

In der Anlage schicken wir Ihnen Ihr o. g. Manuskript zurück. Das Manuskript unterscheidet sich in den wesentlichen Punkten nicht von den beiden vorangegangenen Fassungen, die wir am 14.09.1994 bzw. 22.05.1995 von Ihnen erhalten haben. Die ersten Fassungen wurden von 3 bzw. 2 Gutachtern beurteilt. Diese haben eindeutige und übereinstimmende ablehnende Beurteilungen zu Ihren Manuskripten geliefert, die Sie von uns erhalten haben. Der Herausgeber hält es daher nicht für notwendig, mit Ihrem 3. Manuskript erneut in den Begutachtungsprozeß einzutreten.

Mit freundlichen Grüßen

Heike Jahnke
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Referee 1 :
Dr Kiehl

His/her report on
Reg.No./Author(s)/Title
no. 144/94
R. Kiehl

IgE Regulation by Dithiol/Disulfide Interchange: In vivo Study on Blood of Atopic Eczema Patients Superior to Cell Culture Systems

Tables and Figures: o no comment o Table No. o Figure No. o can be omitted

Summary:
Innovative value of results:

Practical significance:

Accuracy of methods:

Statistical evaluation, quality control:

Style of documentation:

Recommendation:
o accept in present form

o accept with slight changes

o return to author for major alterations

X reject in its present form

X reject for the following reasons

Report (use separate sheet if necessary):

The avalanche of data in this manuscript does not allow to pinpoint a clear message, Most likely all these results can easily be explained in that the antibody system used to measure IgE is influenced by the great variety of drugs that are added to the system. Besides the lack of a clear cut message this manuscript also shines by a complete lack of appropriate controls.

This paper should be rejected for the following reasons:

Title: The title is incorrect since this is not an *in vivo* but an *in vitro* study and no experimental comparison to "cell culture systems" has been performed

Summary: It is impossible to dissect between established facts, hypothesis, data and conclusions. This is not a concise abstract.

Material and Methods: The description of the "*in vivo* assay" is so vague that it is impossible to understand what has been done. The last part of the respective paragraph on page 6 is completely incomprehensible. As a minor point, the sources of most materials are not given.

Results: Again, this section can neither be understood nor does it present data in an acceptable way. The figures lack error bars and reasonable legends. There is no explanation for the symbols used or for additions like the little table on top of Figure 1. There is no information on how many subjects have been tested and what has been tested. Based on the poor presentation of the data the reasoning for or the significance of the single experiments remains obscure.

Discussion: The discussion is far too long and diffuse. Liked in the summary, it is not clear what the own results are and what they mean in the light of established knowledge.

References: Several references do not comply with the rules of the Journal.

In general, this paper is of poor quality and the science does not appear sound. The presentation is further hampered by a very clumsy English.

Reg.No./Author(s)/Title

no. 144/94

R. Kiehl

IgE Regulation by Dithiol/Disulfide Interchange: In vivo Study on Blood of Atopic Eczema Patients Superior to Cell Culture Systems

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o reject in its present form

X reject for the following reasons

Report (use separate sheet if necessary):

The paper by Reinhold Kiehl is badly written. The paper is far too long, there is no clear hypothesis and the data are very difficult to understand. Especially the description of a common pathogenic pathway of atopic eczema and cancer and AIDS is difficult to follow.
The tables are difficult to read, as already stated the paper is too long, the discussion is going to kill any reader of this article.
For these reasons, I can not recommend the paper for publication in the "European Journal of Clinical Chemistry and Clinical Biochemistry".

Practical significance: o accept with slight changes

Accuracy of methods: o return to author for major alterations

Statistical evaluation, quality control: o reject in its present form

Style of documentation: o reject for the following reasons

Report (use separate sheet if necessary):

Ich habe die Arbeit begutachten wollen, habe aber sogleich realisiert, dass es eine Arbeit ist, die ich bereits einmal begutachtet habe.

Ich bin nicht bereit, diese Arbeit nochmals zu begutachten, da sie im Prinzip immer noch genau so wirr, abstrus und unmöglich ist, wie zur Zeit der Ersteinsendung.

Ich habe damals ein paar sehr persönliche Fragen zum Autor gehabt, auf die ich jetzt nicht noch einmal zurückkommen möchte. Ich bin der Meinung, dass Sie sich dieser Arbeit möglichst rasch und schmerzlos entledigen sollten.

In diese Begutachtung wurde mit viel Mühe in den ersten Satz eine „Ausparung“ (weis,umrandet) reingeschnitten: Dieser „Götz“ gilt uneingeschränkt für den Gutachter...! R.Kiehl

Archiv der Pharmazie
An international Journal
Pharmaceutical and Medicinal Chemistry
Prof. Dr. R. W. Hartmann, Editor
FR 12.1 Pharmazeutische Chemie
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MS NO: R 002

AUTHOR: R. Kiehl

TITLE: „IgE regulation, proliferation, development of AIDS"

PLEASE FAX SIGNED REVIEW OR MAIL SIGNED ORIGINAL BY: JULY 24, 1995

RECOMMENDATIONS AND COMMENTS: ☐ no change ☐ minor revision ☐ major revision ☒ publish elsewhere ☐ do not publish

The paper of Kiehl is a very interesting piece of work. However, it is not within the scope of the journal, which deals with drug research, especially structure related scientific work. I recommend a biochemical or a biochemically orientated medical journal.

(please use additional sheet if necessary)

Summary Rating:	Excel.	Good	Fair	Poor
Originality	☐	☐	☐	☐
Technical quality	☐	☐	☐	☐
Clarity of presentation	☐	☐	☐	☐
Importance to field	☐	☐	☐	☐

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Dr. Reinhold KIEHL
Laboratory and Research for
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Amselweg 12
93437 Furth im Wald

Date: August 23, 1995 ha-ri

RE: **R 002** „IgE regulation, proliferation, development of AIDS"

Dear Dr. Kiehl,

Thank you for your letter and for the above mentioned manuscript. We are unfortunately unable to accept your manuscript for publication in *Archiv der Pharmazie - Pharmaceutical and Medidinal Chemistry*. The reason for this decision is the referees' opinion that the paper is not within the scope of the journal (see Instructions for Authors 1/95). We are sorry to have to disappoint you, but trust that you will understand our decision.

Sincerely yours

(Prof. Dr. R. W. Hartmann)

enclosure: referees' comments

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Frankfurt, den 29.05.1996

Herrn
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Labor- und Forschungs-GmbH
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Sehr geehrter Herr Dr. Kiehl,

Ihren Brief vom 22.05.1996, enthaltend einen Leserbrief an das BIOspektrum und einen Manuskriptentwurf für einen Übersichtsartikel, sowie Kopien weiterer Korrespondenz, habe ich erhalten und gründlich durchgesehen. Ich nehme dazu wie folgt Stellung:

- Der Leserbrief behandelt ein hochaktuelles Thema, dem auch die Tagespresse sowie Funk und Fernsehen breiten Raum einräumen. Es wäre meines Erachtens verfehlt, die darin enthaltenen Informationen nur den Lesern des BIOspektrums, also fast nur Mikro- und Molekularbiologen und Biochemikern anzubieten. Für das BIOspektrum ist er auch viel zu lang. Ich empfehle Ihnen, den Leserbrief sehr zu kürzen und an die Tagespresse wie Süddeutsche Zeitung, FAZ, Welt, Zeit, Stern, Spiegel zu senden. Zu Ihrer eigenen Sicherheit sollten Sie allerdings darauf achten, persönliche Angriffe gegen Herrn Ionescu zu entfernen.

- Bezüglich des Übersichtsartikels kann ich mich nur dem anschließen, was Ihnen Herr Mietzsch von der Redaktion bereits geschrieben hat. Das BIOspektrum publiziert keine Originalarbeiten, sondern nur Übersichtsartikel zu aktuellen Themen - und das nur auf Aufforderung durch das Kuratorium oder den wissenschaftlichen Beirat. Ihr Artikel ist meiner Ansicht nach für das BIOspektrum nicht geeignet. Ich kann nur hoffen, daß Ihre Originalarbeit von Chemical and Diagnostic Virology angenommen wird. Ich habe da meine Zweifel, weil die Arbeit von der Thematik her dort nicht hinpaßt, und weil eine Reihe von Behauptungen in Ihrer Arbeit zum jetzigen Zeitpunkt noch als spekulativ erscheinen muß. Hier gibt es für Sie noch viel zu tun, bevor Sie die Fachwelt überzeugen können.

FREIE UNIVERSITÄT BERLIN
Fachbereich Chemie
Institut für Biochemie
Arbeitsgruppe Neurochemie

FU BERLIN

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Datum
10.8..95

Lieber Herr Gabrielczyk,

zu Ihrer Anfrage vom 25.7.:

Das Thema des Artikels von Herrn Kiehl ist hochinteressant und für das BIOspektrum geeignet. Der Artikel ist jedoch nicht wirklich als Übersichtsbeitrag sondern eher als Originalarbeit über eine eigene Studie geschrieben ("Ergebnis klinischer Studien" heisst es im Untertitel), mit den hierfür charakteristischen Abschnitten METHODEN, MATERIALIEN, und mit vielen Literaturzitaten. Ich glaube nicht, dass wir unsere Zeitschrift für Originalpublikationen öffnen sollten, zumindest nicht ohne Fachgutachter, die die Qualität der beschriebenen Experimente bewerten können. Vielleicht sollte man Herrn Kiehl bitten, über die Signalübertragung durch Gammainterferon einen kurzen Übersichtsartikel zu schreiben, in den er als einen Unteraspekt die klinischen Studien einfügen kann).

Ich gehe davon aus, dass ich für Heft 6/95 sowohl ein Highlight von einem Kollegen aus der Neurochemie als auch ein Editorial von mir vorbereite. Lassen Sie mich kurz (FAX) wissen, bis wann beides spätestens bei Ihnen sein soll.

Anbei ein Methoden-Artikel über die neue Massenspektrometrie in der Proteinchemie. Mittelfristig bereite ich einen Methodenartikel über "PTIR-Spektroskopie in der Biochemie" vor.

Mit besten Grüssen

Ihr

F. Hucho

F. Hucho