Stress protein IgE

Reinhold Kiehl, Laboratory and Research for Mol. Medicine/Biology (RKI-Institute)
Saliterweg 1, D-93437 Furth im Wald, kiehl@rki-i.com, www.rki-i.com

Abstract.

The molecular regulation of IgE synthesis by various factors, including gIFN and cytokines, is a highly complex event and their consequences on inflammation were until now difficult to imagine. Earlier results concerning the sulfur chemistry in the living cell are now able to lead into a more open and detailed picture of the molecular events connected with alterations in the IgE-levels of living matters and their influence on inflammation. Then, ancient stress protein IgE is concluded to be an early warning signal for our body. It is connected to some wide spread immune diseases like atopic eczema/allergy, leukemia and AIDS as well as with thrombosis.

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

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[1]. 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[2,3] .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[3,4] (and personal communications). The reported successful treatment of 3 atopic eczema patients with recombinant gIFN without the knowledge of circulating gIFN or Il-4-concentrations[5] could not be repeated or resulted in hazardous side effects [unpublished].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 IgE concentration in vivo and their influence on inflammation [6-10].

Patients and Methods.

38 patients with clinically proved atopic eczema [11,12] (age range 18-52 years)(Fig.1) 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.

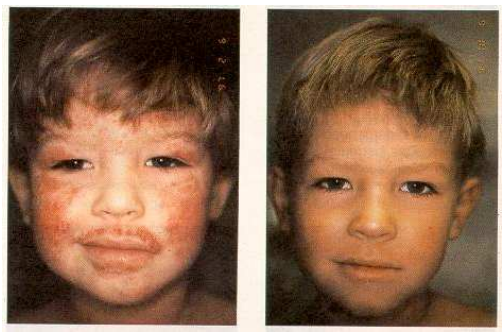


Fig. 1. Patient with allergic manifestation of atopic eczema.

Collagenase, gelatinase and lactoferrin were quantitatively detected by ELISA[13].For this purpose, fresh venous blood samples had to be used. Sera were taken after a 15 min coagulation time and together with heparin plasma, immediately frozen to –20°C. The collected frozen samples were quickly transfered 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° C.

Proteolytic degradation of gIFN was performed similarly to the described inactivation of a_1-proteinase- or Cl-inhibitor[14,15]. 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°C (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.

Other methods used are described elsewhere[6-10,16].

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 ® plus heparinized capillary tubes from Becton-Dickinson (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 regent grade quality.

Results

Patients` immune profiles showed the expected results[17] . Furthermore, histamine concentrations were elevated; concomitantly platelet histamine oxidase activities lowered[16.] Psychogenic stress (environmental agents, etc.) significantly elevates norepinephrine levels[18] but can be reduced by repeated autogenic training (relaxation therapy)[19-23] .Investigations concerning the lipid profile in red blood cells or plasma demonstrate a low level of g-linolenic acid[17]. The coupled low level of arachidonic acid can be raised by linoleic acid plus zinc supplementation[17]. The patients showed intestinal and dermal dysbiosis, in most cases accompanied by significantly increased counts of pathogenic strains including Candida Albicans[24-26]. Other routinely performed laboratory parameters were in the normal range, including Mg and Ca.

In our study 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[27], or described by Moyer[28], but more importantly, identical to values found in healthy control persons. 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 normal overall level in atopic eczema patients vs. controls.

However, the assay of a few capillary blood samples from skin areas under acute inflammation, but not from normal skin areas, 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 1).

Table 1. 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
1. affected skin	130,7	2699	429,1
2. circulating	48,9	486	137,1
2. affected skin	140	1260	601,2
3. circulating	8,2	288	85,9
3. 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(Table 2).

Table 2. Proteolytic degradation of γ-IFN by leucocytes collagenase or gelatinase

γ-IFN : collagenase / ng : ng		incubation time / hrs	degradation / %	
25	1	24	20	in vitro
2,5-25 normal range	1-140 atop. ecz. patients	min to hrs.	0-100	in vivo

γ-IFN : gelatinase / ng : ng		incubation time / hrs	degradation / %	
25	1	24	(~ 0)	in vitro
2,5-25 normal range	120-2700 (Table 2,3) atop. ecz. patients	min to hrs.	(0-100)	in vivo

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. First, orientation measurements with old (ca. 4 hrs) and fresh (0 hrs) blood samples made clear that only the assay with fresh blood samples at 37°C resulted in significant and reproducible differences.

We started with tests for the effects of protease activators and inhibitors on the blood-IgE-concentrations. 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+},[29] 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. 100 mM of the metalloprotease inhibitor EDTA [13] increase IgE-levels, as does Hg^{2+}. 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%). Therefore, it is clear that there is no representative patient and each patient has to be measured (and treated) on its own.

Under the given conditions, 40 µg/ml of the serine protease inhibitor APMSF [30] alone have no effect on IgE-concentrations, but prevent nevertheless an increase normally induced by 10 mM EDTA. This effect is not obtained by the combination of Hg^{2+} and EDTA.

The IgE-level decrease drastically (ca. 50%) during incubation of the samples with 1% Triton X-100 for two hours. 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. In the case of incubation with cycloheximide the supernatant remains yellow.

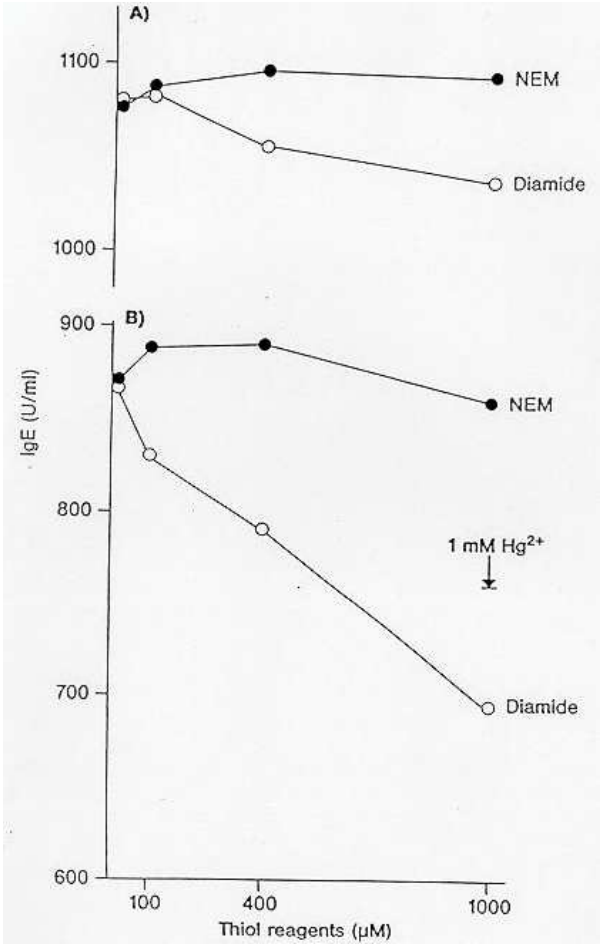


Fig. 2a.A) and B) Concentration dependent inhibition of IgE-synthesis by Diamide (NEM) in blood samples of two different patients. The standardized Hg^{2+} - sensitivity (B) is given for comparison.

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[31] (Fig. 2a).

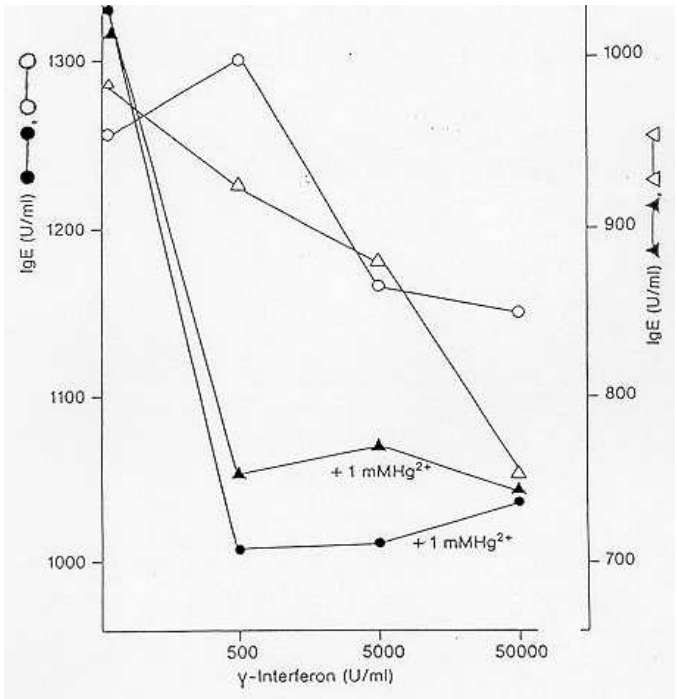
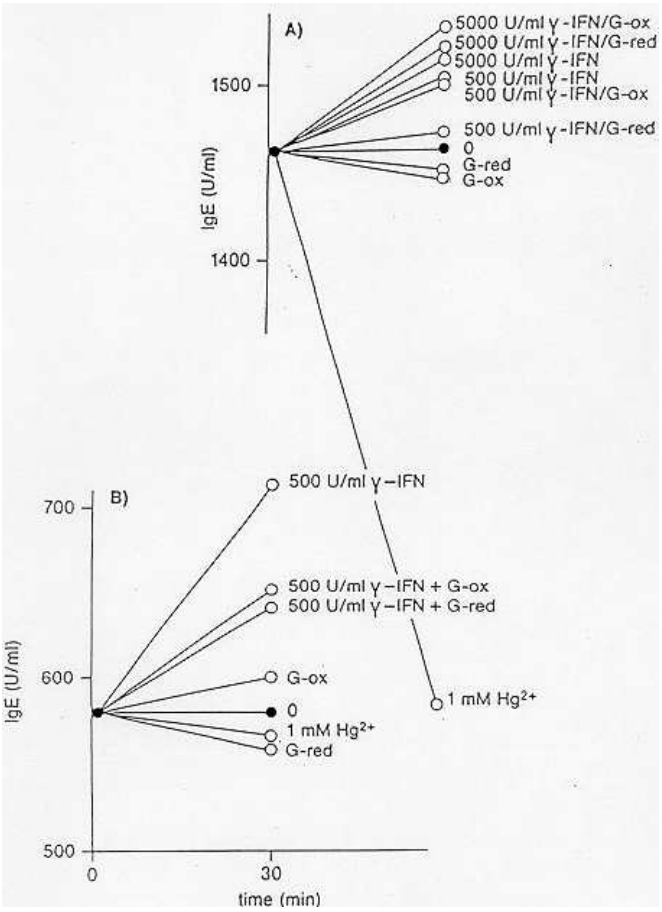


Fig. 2b. Concentration dependent variation of IgE-synthesis by γ.-IFN in the absence (o---o, Δ---Δ) or presence (•---•,♠---♠) of Hg^{2+} in blood samples of two different patients.

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. 2a). Tritrations 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)[32]are almost without effect (Fig. 2b). Only concentrations 100 times higher, a level which is hazardous to the patients, reduce the IgE-levels significantly (Fig. 2b). The situation changes dramatically on addition of 1mM Hg^{2+}: 500 U/ml gIFN are now sufficient to significantly reduce IgE-concentrations (Fig. 2b).



Tritrations 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. 3b).

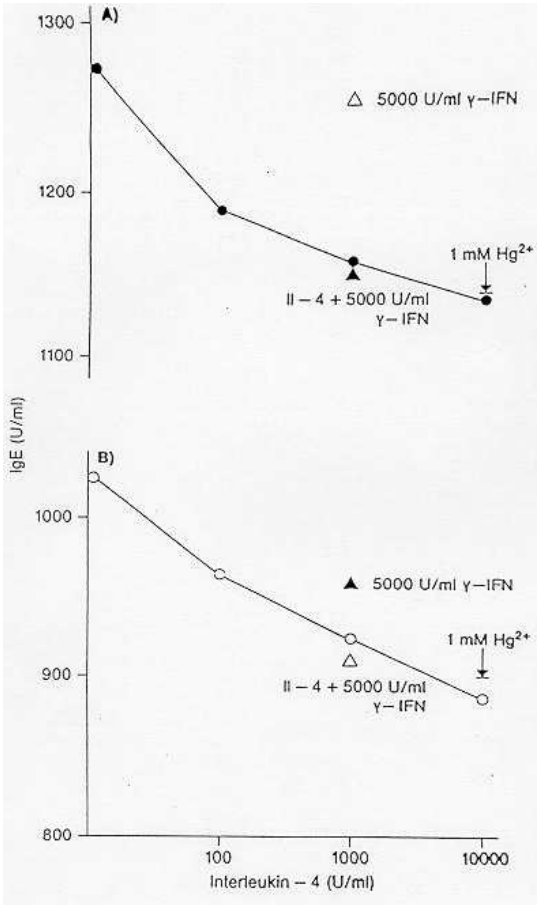
Dual wavelength measurements on lysed blood samples of atopic eczema patients[6-10] demonstrate complex spectra

between the wavelength 550 to 575 nm. Peaks are visible at 558, 560 and around 568 nm. 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 inervals (a few minutes). Besides the cytochrome of the plasma membrane

1mM Zn^{2+}, however, fails to induce such an effect. Fig. 3a demonstrates our first effort in the search for a replacement for toxic Hg^{2+} . As can be deduced from this figure , 1mM glutathione (reduced or oxidized) proved to be relatively ineffective for this purpose.

Fig. 3a. 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. 3b. 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 -IFNvalues are given for comparison.



respiratory burst oxidase[41] ,the mitochondrial respiratory chain cytochromes around 560 nm (especially in ubiquinol-cytochrome c oxidoreductase)[42] are the most abundant and most likely reacting components. Compounds like cytochrome c could not be detected.

Discussion

Mercury, metalloproteases, IgE-level; inflammation and allergic manifestations

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 immun system[35].

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[36] which is still about 10^2 to 10^3 times lower than our measured effective concentrations of Hg on IgE-levels. Nevertheless, Bannasch and Schleicher[36] 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[37] high dosages of mercuric-chloride (50 µg/100 g body weight) were repeatedly injected into rats, which corresponds to about 5 mg/l blood (a concentration near our described effective concentrations), which 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)[38-40] 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 serince-proteinases, or with different mercurial compounds, or reactive oxygen species (ROS). They are also inhibited by their specific inhibitor TIMP[41] or α^2-macroglobulin[13]. The signal for upregulation of their secretion is suppressed by immunosuppressive drugs, like glucocorticoids [42,43] .

Activation of isolated metalloproteases requires µM concentrations of mercurials[29]: 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[42,43]. 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). 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. 90ng/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.
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 therapy should not work.
We could show[16] 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 (thrombosis). 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[44], etc; contact allergy). Platelets aggregation results presumably in a changed energy metabolism in these particles with build-up of vitamin

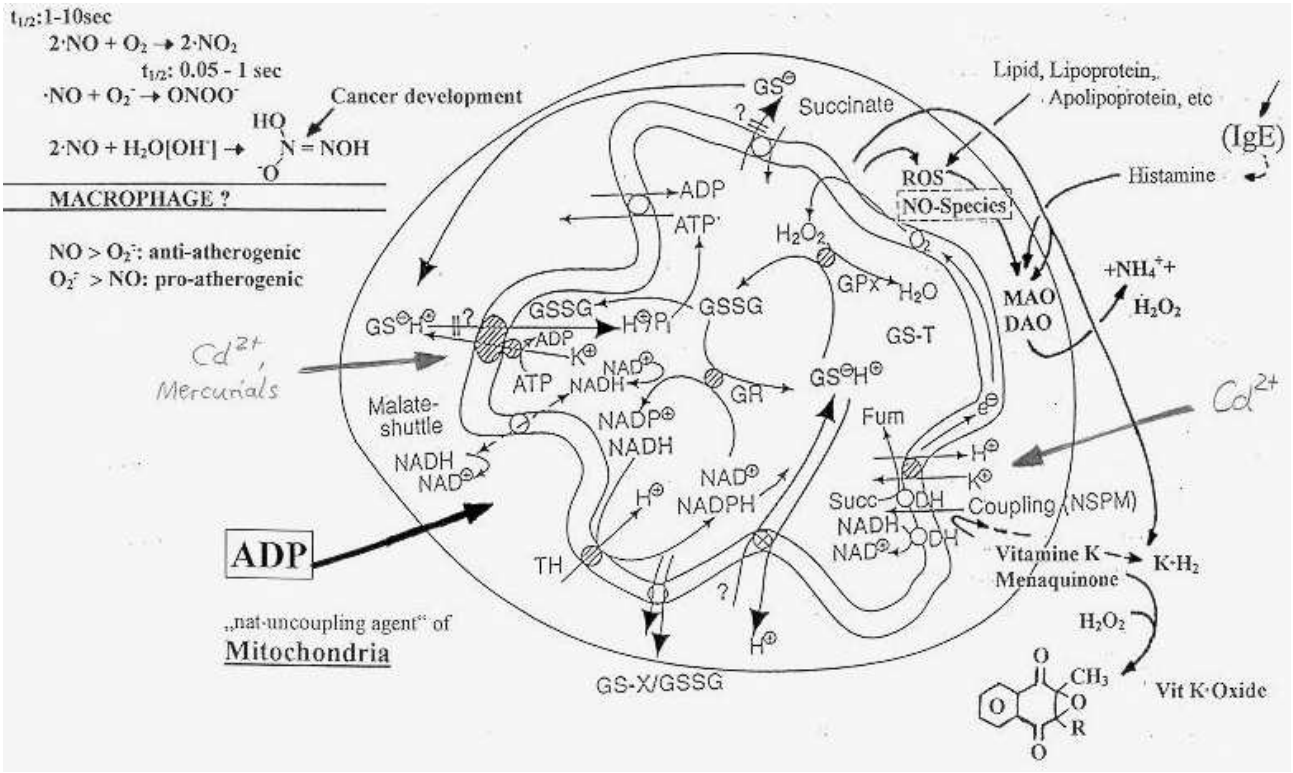


Fig. 4. Interplay of IgE (histamine)/RO- and NO-species on the metabolism of platelets mitochondria (development of thrombosis).

KH_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 [8-10].ROS may also be produced by prolonged exposure of skin cells to UV-light and responsible for development of skin carcinomas[8-10]. Nitric acid (NO) seems not to be a physiologic regulator of the cardiovascular system. However, abnormalities of the L-arginine: NO pathway could contribute to the pathophysiology of diseases like thrombosis[45] (Fig. 4).

gIFN-molecules were significantly degraded by metalloproteases (at least by activated leucocyte collagenase) unter 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[14,15] .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[6-10].

The 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[46]. 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 form their storage compartments[44]. 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, although during days of growing, has been obtained in cell culture systems[3].

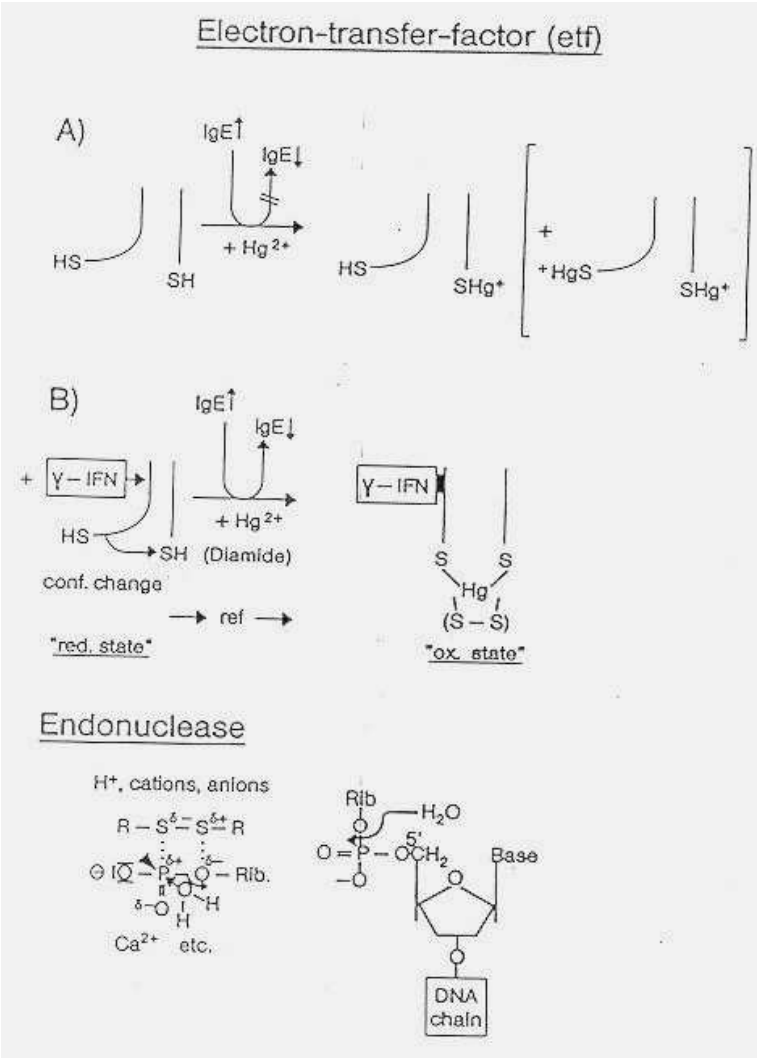


Fig. 5a. Reduced and oxidized state of the electron transfer factor (etf.) Possible mechanism of DNA cleavage by endonuclease. red.state = reduced state, ox.state = oxidized state, ref = nuclear redox factor.

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, during relative short time intervals (=minutes in harmoy with the O_2-build-up in neutrophils) 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 [6-10].

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

This dithiol/disulfide redox state is sensitive to Hg^{2+}, Diamide, gIFN and I1-4, but not to Zn^{2+},[6-8]. gIFN probably directly or indirectly changes the conformation of the involved protein, etf [46] 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 1owers the effective concentration of gIFN by a factor of 10^2 to 10^3 or more (Fig. 5a). I1-4 reacts antagonistically to gIFN in blood samples and in cell cultures, although in opposite directions and at different time scales (minutes vs days)[3]. 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+}. The described mechanism relates to the origin of BSE, Creutzfeld-Jakob and similar diseases [8-10].

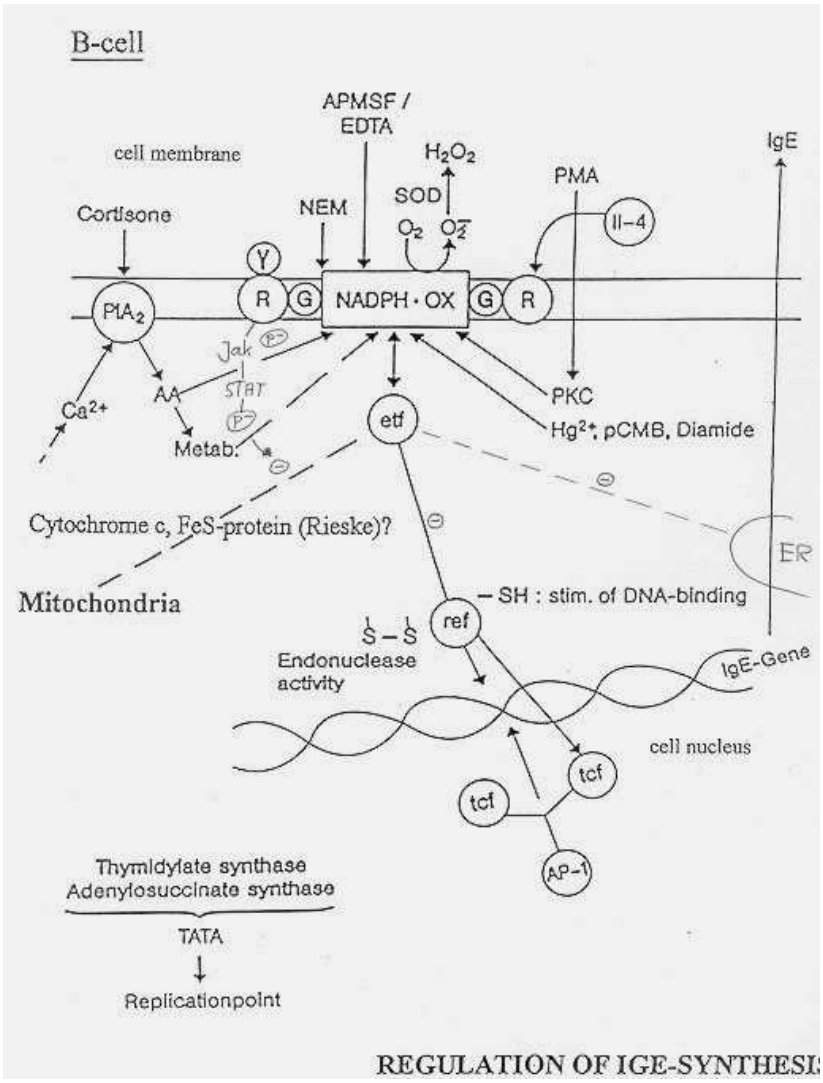


Fig. 5b. Regulation of IgE-synthesis. γ = γ -IFN. R= receptor, G = G-protein, OX = oxidase, SOD = superoxid dismutase, PMA = phorbol myristate acetate, AA = arachidonic acid, etf = electron transfer factor, stim. = stimulation.

Our ineffective titrations of the redox state, indicated by the various IgE-1evels, with extremely high concentrations of glutathione (ox. or red.)[21-23] 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[21-23], 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)[21-23] to be involved in the Hg^{2+} or diamide-induced elimination of IgE-synthesis. lt is concluded, that etf is a FeS-protein (Fig. 5b)[6-10]. 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.

Elektron transfer chain, NADPH to DNA

The redox 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[47] and is starting point of the e-transfer chain, NADPH to DNA (IgE)

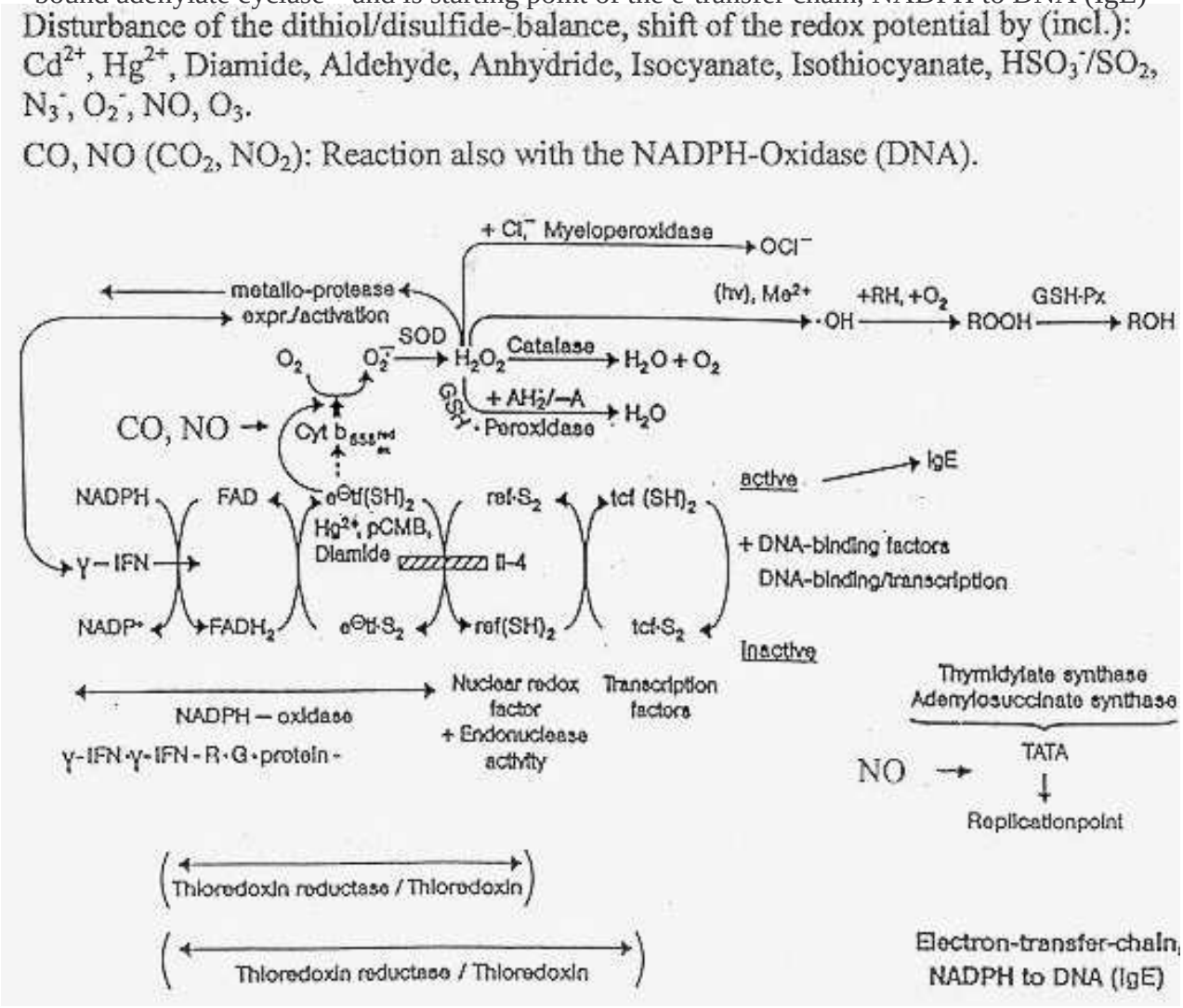


Fig. 6. Electron-transfer-chain, NADPH to DNA (IgE).expr. = expression, SOD = superoxid dismutase, GSH Px (GSH Peroxidase) = glutathione peroxidase, Cyt b = cytochrome b, γ -IFN-r = γ -IFN-receptor.

(Figs. 5b and 6)[6-8] .The defect in NADPH to DNA (IgE) at atopic eczema patients lies at the level of etf/ref [6-9]. 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 I1-4/ receptor is rather obscure[48]. The described down regulation of gIFN mRNA and gIFN production in mitogen activated T-culture cells by I1-4[3] takes days and is therefore related to the late responses of gIFN on endonuclease (Figs. 5a,b and 6) and its anti-proliferative effects[6-9,48]. During short time intervals, I1-4 transduces opposite to gIFN redox signals. Coupling of the I1-4 receptors to the electron transfer chain at the level of etf/ref (via G-protein?) may be responsible for this behavior (Fig. 5b). The down regulation of IgE level, the production of several cytokines (I1-1, TNF incl.), as well as gIFN[49], prostaglandin E_2[17] and

superoxide production[50] by I1-4 implies the possibility that I1-4 may play a role as an antiinflammatory cytokine[51,52].

Extremely high serum IgE levels exist in patients with the socalled hyper-IgE syndrome. In this case, regulation by I1-4 or gIFN is almost impossible[3] 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 desribed) under oxidative stress conditions[50].

<u>The redox potential is responsible for stress protein IgE or O_2-synthesis and proliferation</u>

The adaption of cells to oxidative stress[50], to heat shock, to environmental stress, etc. is nothing other than their natural defense mechanism for protection against injury (Fig.7a,b)[6-10]; see to the thesis also the classification of „maligne lymphome“ by Höffkes[53].

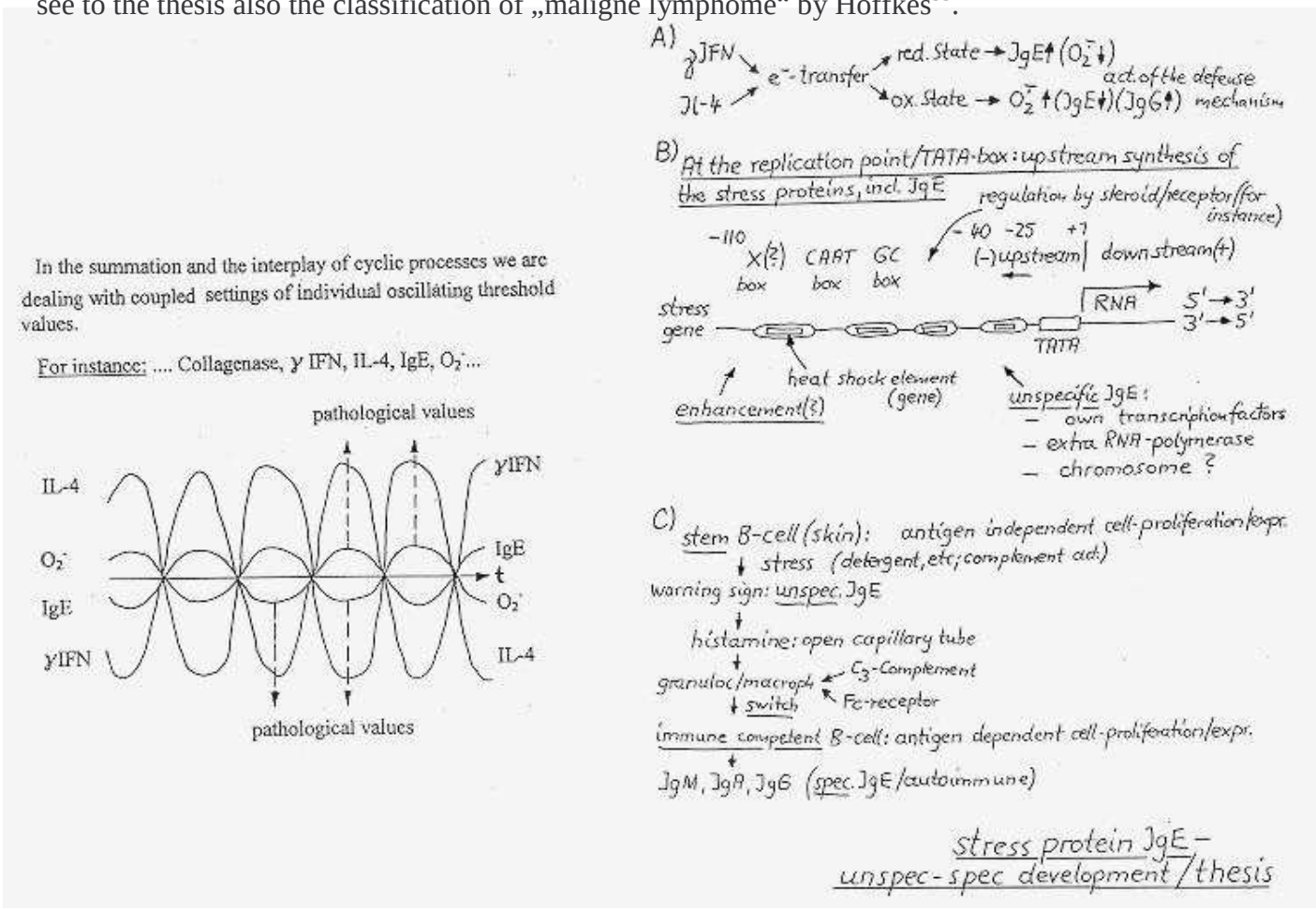


Fig. 7a,b. Stress protein IgE – unspecific/specific development/ thesis.

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 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[54] were able to react irreveribly with the involved essential dithiol/ disulfide redox state. The pollutants include formic

aldehyde, sulfide /$S0_2$, isocyanates and anhydrides. These compounds keep the electron transfer chain in the reduced form (low or no O_2^- production) and, under activating (defence) conditions, the IgE concentrations rise to pathological ranges. The oxidized form is not able to synthesize IgE, but instead O_2^-, and the risk of mitogen stimulated proliferations (leukemia, carcinomas and CGD) ist extemely high. Another compound, CO (and NO), binds to the NADPH oxidase[55], 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[56] (Fig. 6).

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 and their fuel is glutamine instead of glucose[57]. Thus, it is not surprising that the process of NADPH oxidase activation (IgE synthesis)[33] and regulation is coupled to ongoing mitochondrial energy formation[58]. All the compounds influencing mitochondrial energy formation[6,21-23,59-64] then also influence IgE and O_2^- -level and connects to psoriasis vulgaris[65] and AIDS[8-10]. Dermal and intestinal dysbiosis, food, as well as psychogenic stress [6,7,24-26,18-23] 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[66]. Carbohydrate for instance, delivered by food, is a growth factor for these fungi and weakens immune response by changing the energy metabolism of lymphocytes[57].

Psychogenic stress elevates norepinephrine levels, lowers dependent cellular cAMP concentrations[19-23] and weakens thereby immune response (arachidonic acid, prostaglandin, leukotriene, cytokine concentration, etc.)[67], and elevates IgE concentration. The greatest number ot specific IgE-antibodies are devoloped against food- or inhalative allergens. lt 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 I1-4 and CD 40 stimulation[33] 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 [6-9].

Conclusion: The strategy for diagnosis and therapy of atopic eczema, leukemia, AIDS and suppression of inflammation should be 1) in ,,vivo" titration of the redox state involved with Hg^{2+}, gIFN and I1-4; 2.) measurement ot the active gIFN and cytokine (I1-4, 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 (I1-4, I1-2, etc.) and 4.) avoidance of environmental pollutants. If not possible, modification of the involved redox state with appropriate compounds (which have to be developed)[8-10].

Acknowledgement

We thank Prof. H. Tschesche, University of Bielefeld, Prof. E. Siess, University of Munich, Laboratory Drs. Hofmeister,Weiden, Laboratory L+S, Bad Bocklet, Laboratory Dr. Bayer,Stuttgart, Mrs. C. Cavanna, Mrs. E. Dirscherl and Mrs. J. Sperlich

References

1. Godard Ph, Bousquet J, Michel FB, eds. Advances in allergology and clinical immunology. The Parthenon Publishing Group 1992; 3-137.
2. Vercelli D, Jabava HH and Geha RS. A two signal model for the induction of IgE synthesis in humans. In: Pharmacia Allergy Research Foundation. Award Book, Johansson S.G.O., ed. 1989; 13-17.

3. Rousset F, Pene J, Chretien I, et al. The role of 11-4, IFN-γ. and IFN-α, in the regulation of IgE synthesis by peripheral blood lymphocytes of atopic eczema and allergic children in vitro.In: Pharmacia Allergy Research Foundation, Award Book, Johansson, S.G.O.. ed.1989; 25-33.
4. Takahashi T, Sasaki Y, Hama K, et al. Production of I1-4, Il-2, IFN-γ. and TNF-α, by peripheral blood mononuclear cells of patients with atopic dermatitis. J Dermatol Sci 1992;3: 172-180.
5. Reinhold U, Wehrmann W, Kukel S, et al. Recombinant interferon-γ . in severe atopic dermatitis. Lancet 1990;335: 1282.
6. Kiehl R. IgE-Regulation by Dithiol/Disulfide Interchange: In ,,vivo" study on blood of atopic eczema patients superior to cell culture systems. 13. Vortragstagung der Fachgruppe Biochemie in der GDCh, 03/l6-l8, Darmstadt, P 2.6; Analytica Conference, 04/l9-21, München, p. 195-196; Total IgE as a monitoring/ response tool to therapy. Int. Alk-Ciba Corning Joint Symposium, l0/28-29, Benzheim; In ,,vivo" study on blood of humans reliable for pharmacologists and other applicants: spare of animals and cell cultures. BIOTEC MEDICA 94, ll/l6-l9, Düsseldorf, P 846; 1994.
7. Kiehl R. IgE-Regulation, proliferation and development of AIDS. Int.Conf.on New Applications of Emerging Molecular Diagnostics for Infectious Diseases, 6/26-27, London, P 11; Glutathione: The essential factor for live functions. 4th Int. Congress on Mnino Acids, 8/7-ll, Vienna, Amino Acids 9(l),20; IgE-Regulation, proliferation and development of AIDS A) Total IgE as monitoring/response tool to therapy. WASOG Joint meeting, 10/15-20, London, Procedings p.54, P1119; 1995.
8. Kiehl R. IgE-Regulation, proliferation and development of AIDS: gIFN, interleukins, reactive oxygen species and total IgE as monitoring/response tools to therapy. Analytica Conference, 04/23-26, München, p 498, P 144 and BSACI-Meeting, Uni Manchester, 09/4-6, p.25, P 55; Molekulare Medizin (Pathobiochemie, Biochemie), Habilthesis, LMU Munich, Med. Fakultät, 1996.
9. Kiehl, R.: Kopplungsfaktor Glutathion-Einführung in die Elektrophysiology und Thermodynamik von Mitochondrien. 16.Vortragstagung der GDCh, Fachgruppe Biochemie, Kassel, 03/12-14, p.3; Coupling factor glutathione: introduction into the eletrophysiology and thermodynamics of mitochondria. 5 th Int. Congr. On Amino Acids, Chalkidiki, 08/25-29, Amino Acids 13, p.50; Regulation of stress protein IgE-synthesis/proliferation: gInterferon, interleukins, reactive oxygen species and total IgE as monitoring/response tools to therapy, Molecular Diagnostics, Joint Congress DGKC and DGLM, Münster, 09/30-10/2, p.105, P 112; Stress protein IgE, circulating immune complexes and thrombosis. MEDLAB, 12 th JFCC, Basel, 08/17-22, p.105, A54 and 1. Joint Meeting der DGfl und der DGZ/GBM, Langen, 11/20-22, P16; Regulation der IgE-Syntheses, Proliferation und Entwicklung von AIDS, BIOforum 12, GIT Verlag, p.686-690, 1997.
10. Kiehl, R.: Molecular regulation of IgE synthesis and proliferation: stress protein IgE as early warning signal for our body. Proc. of the 17 th Int. Symposium of the Electrolyte/ Blood Gas Intercontinental Working Group on the Confluence of Crit. Care Analysis and Near Patient Testing, 06/4-7, Nice; Allergy 43,53; 1998.
11. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Dermat Venerol (Stockh.) 1980 (Suppl); 92: 44-47
12. Diepgen TL, Fartasch M, Hornstein OP. Kriterien zur Beurteilung der atopischen Hautdiathese. Dermatosen 1991; 39: 79-83.
13. Bergmann U, Michaelis J, Oberhoff R, et al. Enzyme linked immunosorbent assays (ELISA) for the quantitative determination of human leukocyte collagenase and gelatinase. J Clin Chem Biochem 1989; 27: 351-359.
14. Knäuper V, Reinke H, Tschesche H. Inactivation of human plasma α_1-proteinase inhibitor by human PMN leucocyte collagenase. FEBS Letters 1990; 263: 355-357.
15. Knäuper V, Triebel S, Reinke H, et al. Inactivation of human plasma Cl-inhibitor by human PMN leucocyte matrix metalloproteinases. FEBS Letters 1991; 290: 99-102.
16. Kiehl R, Ionescu G. Pathological changes in platelet histamine oxidases in atopic eczema. Med of Inflammations 1993; 2: 403-406.
17. Kiehl R, Ionescu G, Manuel Ph, et al. Klinische, immun- und lipidmodulatorische Effekte einer Behandlung mit ungesättigten Fettsäuren bei atopischer Dermatitis. H~G Zeitschrift für Hautkrankheiten 1994; 69 (1): 42-48.
18. Ionescu G, Kiehl R. Plasma catecholamine levels in severe atopic exzema. Allergy 1988; 43: 614-616
19. Kiehl R. Einsichten in den Wirkmechanismus gefunden, Grundlagen der Fumarsäuretherapie. Psoriasis Magazin (Deutscher Psoriasis Bund e.V.) 1992; 4: 8-9.
20. Ionescu G, Kiehl R, Müller-Steinwachs J. Autogenic training and norepinephrine levels in atopic eczema, allergic asthma and psoriasis. Allergy 1992; (Suppl. 47): 59, PS. 5 Cl. 388.
21. Kiehl R. Evidence for mitochondrial F_0F_1-ATPase being a K^+ -pump. GBCh joint meeting with the BS (London), Düsseldorf, September 12-l5th. Biol Chem H-S 1993a; 374: 742.

22. Kiehl R. Psoriasis Vulgaris. Dear Colleague, New in dermatology (A service of Leo Pharmaceutical Products) 1993b; 2(2):4-5
23. Kiehl R. Glutathione, the essential factor for mitochondrial energy-linked functions. 3rd Int. congress on amino acids, vienna, august 23rd-27th. l993c
24. Ionescu G, Kiehl R, Ona L, et al. Abnormal fecal microflora and malabsorption phenomena in atopic eczema patients. J Adv Med 1990a; 3 (2): 71-91.
25. Ionescu G, Kiehl R, Wichmann-Kunz F, et al. Immunobiological significance of fungal and bacterial infections in atopic eczema. J Adv Med 1990b; 3 (1): 47-58.
26. Ionescu G, Kiehl R, Wichmann-Kunz F, et al. Chronische Infektionen bei atopischer Dermatitis. Ortho Molekular 1990c; 3: 128-133.
27. Daunderer M. Metallvergiftungen, Diagnostik und Therapie, Kompendium der Klinischen Toxikologie, Teil 3, Band 9. Daunderer M. eds. ecomed Verlagsgesellschaft mbH and Bayer laboratory, Stuttgart, Germany: experience values. 1988.
28. Moyer TP. Heavy metalls - the forgotten toxins. AACC: TheDrug Mon and Clin Tox Div, Newsletter 1996; 11: 1-5.
29. Bläser J, Knäuper V, Osthnes A, et al. Mercurial activation of human polymorphonuclear leukocyte procollagenase. Eur J Biochem 1991; 202: 1223-1230.
30. Laura R, Robinson DJ, Bing DH. (p-Amindinophenyl) methane-sulfonyl Fluoride, an irreversible inhibitor of serine proteases. Biochemistry 1980; 19: 4859-4864.
31. Kosower NS, Kosower EM, Wertheim B. Diamide, a new reagent for the intracellular oxidation of glutathione to the disulfide. Biochem Biophys Res Commun 1969; 37: 593-598.
32. Horowitz 0. Interferon. In: Methods in Enzymatic Analysis, Vol. 9, Proteins and Peptides. Bergmeyer H U, ed. VCH Verlagsgesellschaft, Weinheim 1986; 239-247.
33. Foroozan R, Ruedi JM, Babior BM: The reduction of cytochrome b 558 and the activity of the respiratory burst oxidase from human neutrophils. J Biol Chem 1992; 267: 24400-24407.
34. Hatefi Y. The mitochondrial electron transport and oxidative phosphorylation system. Ann Rev Biochem 1985; 54: 1015-1069.
35. Enwonwu CO. Potential health hazard of use of Mercury in dentistry: critical review of the literature. Environm Research 1987; 42: 257-274.
36. Bannasch L, Schleicher P. Immunstatus vor und nach Quecksilbermobilisation. Natur- und GanzheitsMedizin 1991; 4: 53-56.
37. Murdoch RD, Pepys J. Enhancement of antibody production by Mercury and Platinium group metal halide salts. Jnt Arch of Allergy and Appl Immunol 1986; 80: 405-411.
38. Emonard H, Grimaud JA. Matrix metallo proteinase. Cellular and Molecular Biology 1990; 36: 131-153.
39. Parmgren MS, de Shazo RD, Carter RM, et al. Mechanisms of neutrophil damage to human alveolar extracellular matrix: The role of serine and metallo proteases. J Allergy clin Immunol 1992; 89: 905-915.
40. Tschesche H, Knäuper V, Krämer S, et al. Latent collagenase and gelatinase from human neutrophils and their activation. MATRIX 1992; 1(Suppl): 245-255.
41. Osthues A, Knäuper V, Oberhoff R, et al. Isolation and characterization of tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) from human rheumatoid synovial fluid. FEBS Letters 1992; 296: 16-20.
42. Hempelmann U, Haag-Weber M, Hörl WH, et al. Effect of immunosuppressive drugs on the release of metalloproteinases from human plymorphonuclear leukocytes. Transplant Int 1991; 4: 26-30.
43. Shapiro SD, Campbell EJ, Kobayashi DK, et al. Dexamethasone selectively modulates basal and lipopolysaccharide-induced metallo proteinase and tissue inhibitor of metallo proteinase production by human alveolar macrophages. J of Immunology 1991; 146: 2724-2729.
44. Wenzel H, Feldmann A, Engelbrecht S, et al. Activation of the human leukocyte proteinases elastase and cathepsin G by various surfactants. Biol Chem H-S 1990; 371: 721-724.
45. Allister RJ, Vallance P. The L-arginine: nitric oxide pathway in the human cardiovascular system, JIFCC 1996; 8: 152-158.
46. Kiehl R. Is there a nudeotide carrier present in OS-ATPases? FEBS Letters 1980; 109: 280-282.
47. Hall A. The cellular functions of small GTP-binding proteins. Science 1990; 249: 635-640.
48. Meager A. Cytokines. Meager A., ed. Open University Press, Milton Keynes 1990.
49. Anemori EN, Bair MJ, Bauer EA, Amento EP. Stromelysin expression regulates collagenase activation in human fibroblasts. J Biol Chem 1991; 266: 23477-23482.
50. Sies H. Strategies of antioxidant defense. Eur J Biochem 1993; 215: 213-219.
51. Hart PH, Vitti GF, Burgers DR, et al. Potential anti-inflammatory effects of interleukin 4: Suppression of human monocytic tumor necrosis factor a, interleukin 1, and prostaglandin E2. Proc Natl Acad Sci USA 1989; 86: 3803-3809.
52. Reynaud C, Dayer JM, Galve de Rochemonteix B, et al. Interleukin 4 (11-4) inhibits the respiratory burst

and secretory functions of human alveolar macrophages (AM). Eur Resp J 1989; 2 (55): 320-324.
53. Höffkes HG. Maligne Lymphome, Uppenkamp M, ed. Springer Verlag 1997.
54. Behrendt H. Grundlagen der Allergie und mögliche Angriffspunkte für Umweltchemikalien. Allergologie 1989; 12: 95-99.
55. Segal AW. The elecron transport chain of the microbicidal oxidase of phagocytic cells and its involvement in the molecular pathology of Chronic Granulomatous Disease. J Clin Invest 1989; 83: 1785-1793.
56. Hayes AW Ed. Comments on Toxicology 1992; 4(4).
57. Ardawi MSM, Newsholme EA. Metabolism in lymphocytes and its importance in the immune response. Essays in Biochem 1985; 21: 1-44.
58. Cohen HJ, Chovaniec ME. Superoxide production by digitonin-stimulated Guinea pig granulocytes. The effects of N-ethyl maleimide, divalent cations and glycolytic and mitochondrial inhibitors of the activation of the superoxide generating system. J Clin Invest 1978; 61: 1088-1096.
59. Kiehl R. Wirkung von Thiol- und Sulfensäurereagentien wachsender Alkylkettenlänge auf die mitochondriale Energieübertragung. Thesis. Universität Heidelberg and Max-Planck-Institut für Med. Forschung, Heidelberg 1976.
60. Bäuerlein E, Kiehl R. Lipophilic Thiourea and Thiouracil as inhibitors of oxidative phosphorylations. FEBS Letters1976; 61: 68-71.
61. Kiehl R, Bäuerlein E. The Action of lipophilic maleimides in mitochondrial energy transduction. FEBS Letters 1976; 72: 24-28.
62. Stiggall DL, Galante IM, Kiehl R, et al. Involvement of a dithiol-containing protein in mitochondrial energy-linked functions and its relation to coupling factor B and F2. Biochim Biophys Acta 1979; 196: 638-644.
63. Kiehl R, Hanstein WG. Oxonol response and energy transduction in an ATP-Pi-exchange complex. Biochim Biophys Acta 1984; 766: 375-385.
64. Kiehl R. Sulfer associated redox reactions, involvement in signal transduction and phosphate transfer-relevance for pathogenesis of diseases. Biol Chem H-S 1996; 375: S61.
65. Kiehl R, Ionescu G. A defective purine nudeotide synthesis pathway in psoriatic patients. Acta Dermato Venereologica 1992; 72: 253-255.
66. Gumonski Pl, Dunoyer-Geindre S, Latge JP. Candida cell wall antigens inducing allergic symptoms. NATO ASI, Series H 1991; 53: 383-398.
67. Kiehl R. Anwendung der Durchf1ußzytometrie in der klinischen Zelldiagnostik. X.Arbeitstagung 03/21-22, Regensburg, Abstract book and clin. Lab. 1996; 7+8: 655.

Epilog:

Sehr geehrter Herr Körber,

anbei übersende ich Ihnen das Manuskript (Nr.144/94), welches im **European Journal of Clinical Chemistry and Clinical Biochemistry** eingereicht war, zurück. Zu einem differenzierten Gutachten fühle ich mich aus Zeitgründen einfach nicht in der Lage. Ich würde jedoch warnen, diese Arbeit zu akzeptieren, da sie bezüglich ihrer klinischen Relevanz mir überaus fragwürdig zu sein scheint, darüber hinaus ist der Autor in einer sehr paramedizinisch orientierten „Spezialklinik“ beschäftigt, wo abenteuerliche diagnostische und therapeutische Verfahren zur Anwendung gelangen und jede wissenschaftliche Publikation zum Nachweis der Bonität dieser Alternativklinik verwendet wird. Vorsicht scheint mir angezeigt zu sein.

Ein **„Gutachter“** von Eur.J.Clin.Chem.Clin.Biochem.