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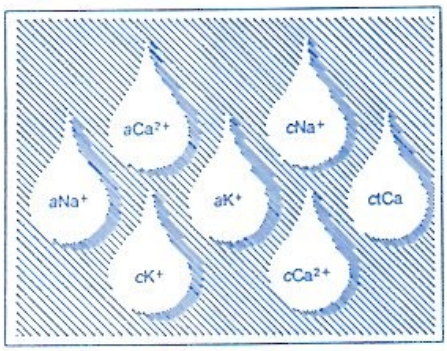
The Confluence of Critical Care Analysis and Near Patient Testing

Proceedings of the 17th International Symposium



Société Française de Biologie Clinique

American Association for Clinical Chemistry
Electrolyte/Blood Gas Division

Japan Society of Clinical Chemistry
Committee for Blood Gases and Electrolytes

Meridien Hotel , Nice, France, June 4-7, 1998

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Organized and Jointly Sponsored by:
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Proceedings Editors:
Paul D'Orazio, Chiron Diagnostics, Medfield, MA USA
Niels Fogh-Andersen, Herlev Hospital, Herlev, Denmark
Lasse Larsson, Linkoping University, Linkoping, Sweden

Printed by:
OMNIPRESS, Madison, WI USA
1998

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Molecular regulation of IgE synthesis and proliferation: stress protein IgE as early warning signal for our body

Reinhold Kiehl, Laboratory and Research for Mol. Medicine/Biology (RKI),
Saliterweg 1, D-93437 Furth im Wald, Germany
Phone: 0049(0)9973/801056 Fax: 0049(0)9973/801057

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.

I hope the consequences out of the described molecular pathways lead into new diagnostic tools and succesful ways for treatment of these diseases and the suppression of associated (chronic) inflammations.

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

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

JFCC, Proc. of the 17th Int. Sym. Nice, 1998

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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 et al, 1989; Rousset et al, 1989) These results have not yet been confirmed in the IgE-levels of atopic eczeme 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 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 (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 IgE concentration in vivo and their influence on inflammation (Kiehl, 1994-1998).

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 perpheral blood were measured using standard enzyme immuno assays (Pharmacia, 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° C. 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 10 µl 10% 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 1mM 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°C, 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 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 α_1-proteinase- or Cl-inhibitor (Knäuper et al, 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°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 (Fig. 1 to 4).
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 ® 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 (Kiehl et al, 1994) . 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, 1993b, 1993c). 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,

1990a, 1990b, 1990c). 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 (1988), or described by Moyer (1996), 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.

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.

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+} (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. 100 mM of the metalloprotease inhibitor EDTA (Bergmann et al, 1989) 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 ist 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. 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.

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. 1). 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. 1). 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, Horowitz, 1986), are almost without effect (Fig. 2). Only concentrations 100 times higher, a level which is hazardous to the patients, reduce the IgE-levels significantly (Fig. 2). The situation changes dramatically on addition of 1mM Hg^{2+}: 500 U/ml gIFN are now sufficient to significantly reduce IgE-concentrations (Fig. 2).

Fig. 1

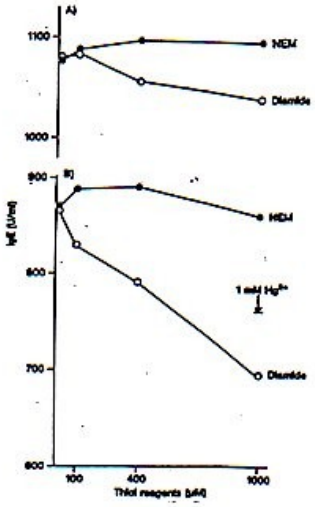
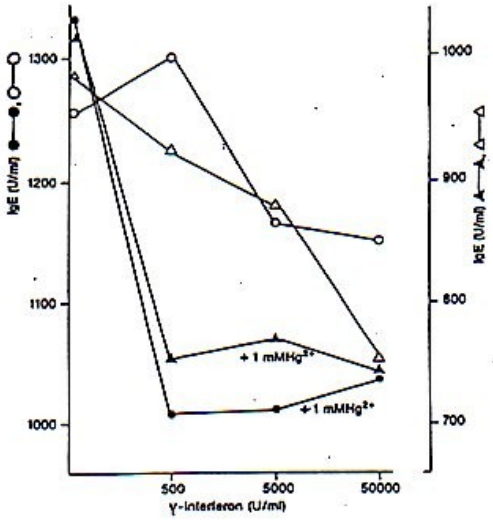


Fig. 2



1mM Zn^{2+}, however, fails to induce such an effect. Fig. 3 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.
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. 4).

Fig. 3

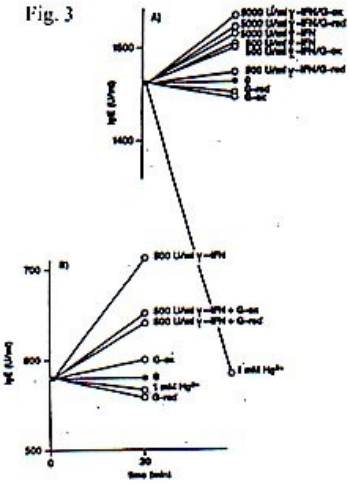
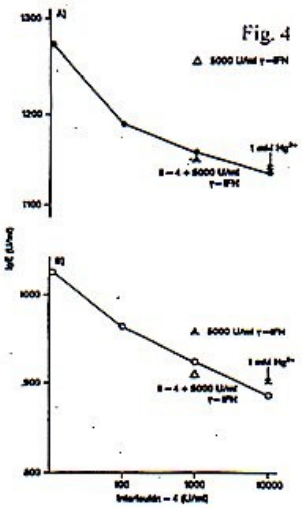


Fig. 4



Dual wavelength measurements on lysed blood samples of atopic eczema patients 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 respiratory burst oxidase (Foroozan et al, 1992), 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. 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 (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(Bannasch and Schleicher, 1991), 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 (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 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) (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 serince-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). 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 (Kiehl and Ionescu, 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 (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, (Wenzel et al, 1990) etc; contact allergy). Platelets aggregation results presumably in a changed energy metabolism in these particles with build-up of vitamin 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 (Kiehl, 1997). ROS may also be produced by prolonged exposure of skin cells to UV-light and responsible for development of skin carcinomas (Kiehl, 1996-1998). Nitirc 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 (Allister and Vallance P., 1996) (Fig. 5).

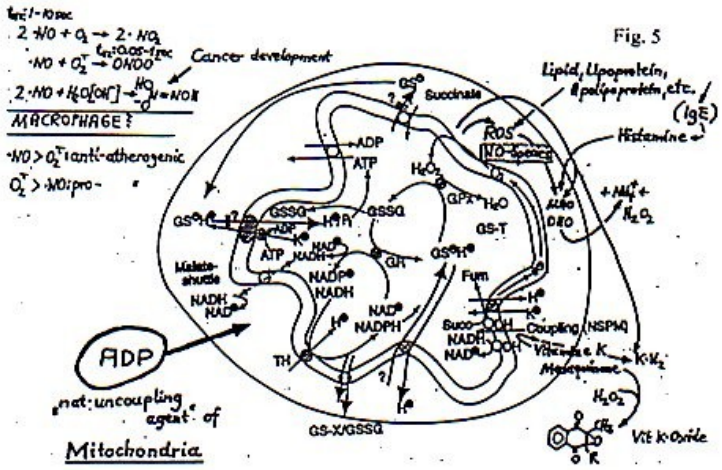


Fig. 5

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 melcules 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; Knäuper et al, 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, 1994-1998).

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 (Kiehl, 1980). 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 (Wenzel et al, 1990). 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 (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, 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.

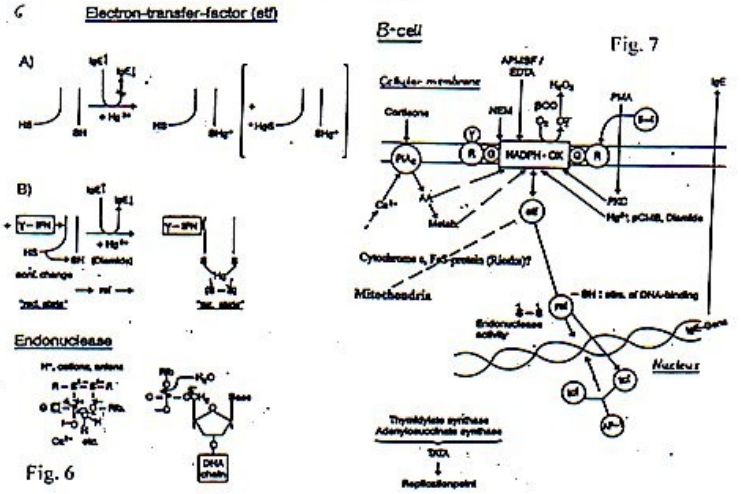
Furthermore, and even more importantly, the results obtained with Hg^{2+} indicate the involvement of a redox reaction in the regulation of IgE-synthesis (Kiehl, 1994-1998)

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+} (Kiehl, 1994-1996). gIFN probably directly or indirectly changes the conformation of the involved protein, etf (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. 6). 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) (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+}. The described mechanism relates to the origin of BSE, Creutzfeld-Jakob and similar diseases (Kiehl, 1996-1998).

Our ineffective titrations of the redox state, indicated by the various IgE-levels, with extremely high concentrations of glutathione (ox. or red.) (Kiehl, 1993a, 1993b, 1993c) 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

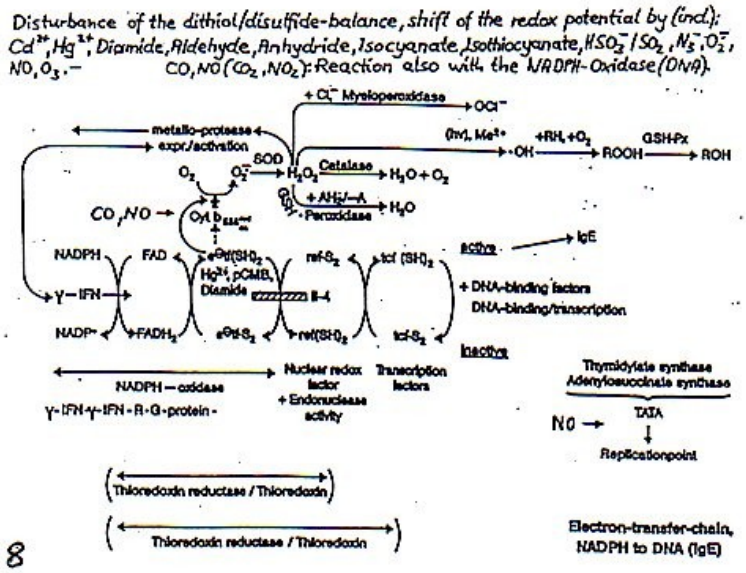
is then, of course, not able to replace Hg^{2+} or Diamide in the described dithiol/disulfide interchange mechanism. Hg^{2+} and Diamide, effective at high concentrations, react inside the cells (Kiehl, 1993a, 1993b, 1993c), most likely with a dithiol-containing protein localized, at least for some time, 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, 1993a, 1993b, 1993c) to be involved in the Hg^{2+} or diamide-induced elimination of IgE-synthesis. It is concluded, that etf is a FeS-protein (Fig. 7) (Kiehl, 1994-1998). 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 ot 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) and is starting point of the e-transfer chain, NADPH to DNA (IgE) (Figs. 7 and 8) (Kiehl, 1994-1996) . The defect in NADPH to DNA (IgE) at atopic eczema patients lies at the level of etf/ref (Kiehl, 1994-1997). 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 (Meager, 1990). The described down regulation of gIFN mRNA and gIFN production in mitogen activated T-culture cells by I1-4 (Rousset et al, 1989) takes days and is therefore related to the late responses of gIFN on endonuclease (Fig 6-8) and its anti-proliferative effects (Kiehl, 1994-1997; Meager, 1990). 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. 7). The down regulation of IgE level, the production of several cytokines (I1-1, TNF incl.), as well as gIFN (Anemori et al, 1991), prostaglandin E_2 (Kiehl et al, 1994) and superoxide production (Sies, 1993) by I1-4 implies the possibility that I1-4 may play a role as an antiinflammatory cytokine (Hart et al, 1989; Reynaud et al, 1989).



8

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 (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 desribed) under oxidative stress conditions (Sies, 1993).

The redox potential is responsible for stress protein IgE or O_2-synthesis and proliferation

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 (Fig. 9), (Kiehl, 1994-1998); see to the thesis also the classification of „maligne lymphome" by Höffkes, 1997).

In the summation and the interplay of cyclic processes we are dealing with coupled settings of individual oscillating threshold values.

For instance: ... Collagenase, γIFN, Il-4, IgE, O_2^-...

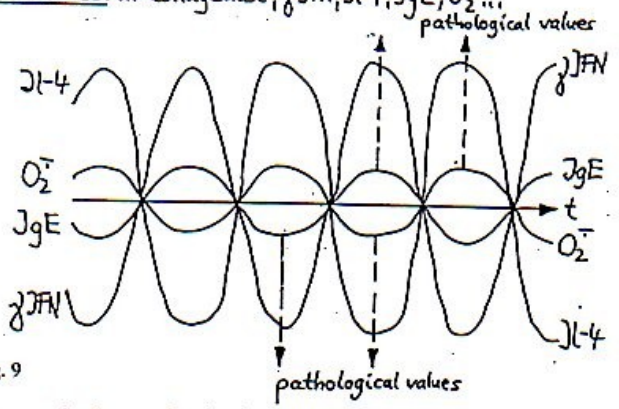


Fig. 9

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) an 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 (Behrend, 1989) 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 (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).

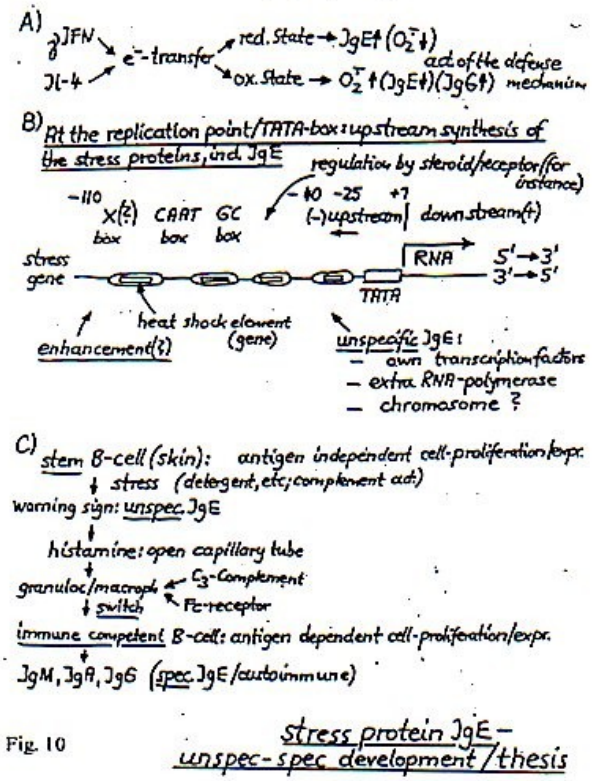


Fig. 10 stress protein IgE – unspec-spec development/thesis

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 (Ardawi and Newsholme, 1985). Thus, it is not surprising that the process of NADPH oxidase activation (IgE synthesis) (Foroozan et al, 1992) and regulation is coupled to ongoing mitochondrial energy formation (Cohen and Chonvaniec, 1978). All the compounds influencing mitochondrial energy

formation (Kiehl, 1994; Kiehl, 1993a, 1993b, 1993c; Kiehl, 1976; Bäuerlein and Kiehl, 1976; Kiehl and Bäuerlein, 1976; Stiggall et al, 1979; Kiehl and Hanstein, 1984; Kiehl, 1994b) then also influence IgE and O_2^- -level and connects to psoriasis vulgaris (Kiehl and Ionescu, 1992) and AIDS (Kiehl, 1996, 1997). Dermal and intestinal dysbiosis, food, as well as psychogenic stress (Kiehl 1994,1995, Ionescu et al, 1990a, 1990b, 1990c, 1991; Ionescu and Kiehl, 1988; Kiehl, 1992; Ionescu et al, 1992; Kiehl, 1993a, 1993b, 1993c) 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).

Psychogenic stress elevates norepinephrine levels, lowers dependent cellular cAMP concentrations (Kiehl, 1992; Ionescu et al, 1992; Kiehl 1993a, 1993b, 1993c) and weakens thereby immune response (arachidonic acid, prostaglandin, leukotriene, cytokine concentration, etc.) (Kiehl, 1996b), and elevates IgE concentration. The greatest number ot specific IgE-antibodies are devoloped 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 I1-4 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 (Kiehl, 1994-1997).

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) (Kiehl, 1996, 1997).

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.

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