Condensation of a lecture given at IPRAC, April 1 to 4, 2001, Praque, Czech Republic, Allergie, Abstrakta, Rocnik 3, Supplementum 1, 2001.

Atopy/Contact Dermatitis/Asthma:Metal Ions/Cd;Hg;Ni;Pt;Pd

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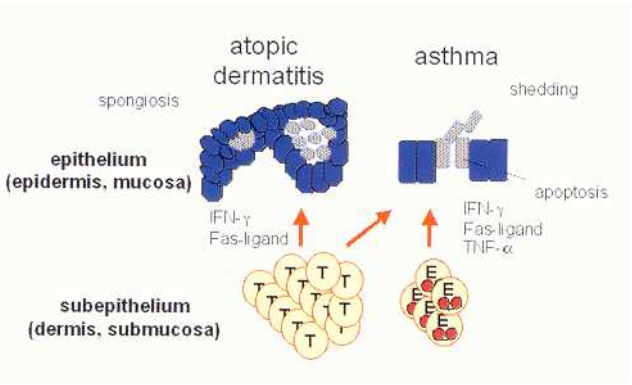
Key words: g-interferon, cytokines, electron transfer chain, redox potentials, metalloproteases, immune diseases.
Abbreviations:IL=interleukin,IFN=interferon,APMSF=(4-amidinophenyl)-methanesulfonylfluoride,NEM=N-ethylmaleimide,pCMB=p-chloromercuribenzoate, Diamide= azodicarboxylic acid bis (dimethylamide), DMPS = dimercaptopropan-sulfonate.

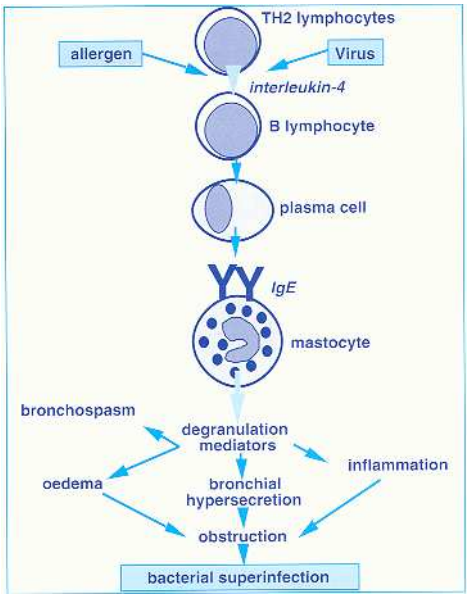
Mercury, metalloproteases, IgE-Ievel, inflammation and allergic manifestations



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 immune system.
The effect of mercury on IgE-levels was seen at concentration ranges of 0,5 to 1 mM; concentrations which are about 1mio times higher than the normal range in blood of control or atopic eczema persons. Mobilization of mercury by DMPS results in 10^2 to $l0^3$ times higher values in these persons, which is still about 10^2 to 10^3 times lower than our measured effective concentrations of Hg on IgE-levels. Nevertheless, someone describes 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 high dosages of mercuric-chloride *(50* µg/100 g body weight) were repeatedly injected into rats, which corresponds to about *5* mg/1 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) 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 or α^2 -macroglobulin. The signal

for upregulation of their secretion is suppressed by immunosuppressive drugs, like glucocorticoids .
Activation of isolated metalloproteases requires µM concentrations of mercurials: 10 µm HgCl2, 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 . 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 rnolecules, 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 (1) 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, etc; contact allergy). Platelets aggregation results presumably in a changed energy metabolism in these particles with build-up of vitamin K2 and H202/ROS, inhibition of diamine-oxidase by ROS (H202) with elevation of histamine, inactivation of α^2-macroglobulin and activation of metalloproteases by ROS/ H202 (2). ROS may also be produced by prolonged exposure of skin cells to UV-light and responsible for development of skin carcinomas.. 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 .
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 α1-proteinase inhibitor, by metalloproteases has already been demonstrated. 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 (2).
The standardized titration of blood samples from different patients (IgE ca. 1000 U/ml) with 1 mM HgCl2 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 (3). 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 . 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 .
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 harmony with the 02-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 (2).
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 IL- 4, hut not to Zn^{2+} (2). gIFN probably directly or indirectly changes the conformation of the involved protein, etf (7) 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 . IL-4 reacts antagonistically to gIFN in blood samples and in cell cultures, although in opposite directions and at different time scales (minutes vs days) . 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 (2).
Our ineffective titrations of the redox state, indicated by the various IgE levels, with extremely high concentrations of glutathione (ox. or red.) (2) 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 , 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) to be involved in the Hg or diamide-induced elimination of IgE-synthesis. It is concluded, that etf is a FeS-protein. 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 O2 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 and is starting point of the e-transfer chain, NADPH to DNA (IgE) (2). The defect in NADPH to DNA (IgE) at atopic eczema patients lies at the level of etf/ref . 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. The described down regulation of gIFNmRNA and gIFN production in mitogen activated T-culture cells by IL-4 takes days and is therefore related to the late responses of gIFN on endonuclease and its antiproliferative effects (2). 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 . The down regulation of IgE level, the production of several cytokines (IL-1, TNF incl.), as well as gIFN), prostaglandin E2 and superoxide production by IL-4 implies the possibility that IL-4 may play a role as an antiinflammatory cytokine .

Extremely high serum IgE levels exist in patients with the so called hyper IgE syndrome. in this case, regulation by IL-4 or gIFN is almost impossible 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 .

The redox potential is responsible for stress protein IgE or 02-synthesis and proliferation

The adaption of cells to oxidative stress , 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 (nonphagocytes), TNF and IL-1 control collagenase, and gIFN and IL-4 control IgE. In most (or all?) cases, the activation of NADPH oxidase (O2 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 were able to react irreversibly with the involved essential dithiol / disulfide redox state. The pollutants include formic aldehyde, sulfide /S02, isocyanates, anhydrides, etc. These compounds keep the electron transfer chain in the reduced form (low or no O2 production) and, under activating (defence) conditions, the IgE concentrations rise to pathological ranges. The oxidized form is not able to synthesize IgE but instead O2, and the risk of mitogen stimulated proliferations (leukemia, carcinomas and CGD) ist extemely high. Another compound, CO (and NO), binds to the NADPH oxidase , preventing the reduction of O2 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 .

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 . Thus, it is not surprising that the process of NADPH oxidase activation (IgE synthesis) and regulation is coupled to ongoing mitochondrial energy formation . All the compounds influencing mitochondrial energy formation (2) then also influence IgE and O2-level and connects to psoriasis vulgaris (4) and AIDS (2). Dermal and intestinal dysbiosis, food, as well as psychogenic stress (2*)* 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 . Carbohydrate for instance, delivered by food, is a growth factor for these fungi and weakens immune response by changing the energy metabolism of lymphocytes .
Psychogenic stress elevates norepinephrine levels, lowers dependent cellular cAMP concentrations (2,5) and weakens thereby immune response (arachidonic acid, prostaglandin, leukotriene, cytokine concentration, etc.) 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 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 (2).
Ni, Pt; Pd: Contact dermatitis/ Asthma
Skin diseases, diseases of the respiratory tract and above all **asthma**, genetic defects as well as cancer are rapidly increasing in the western conurbations with use of cat cars - since 1990 very heavely in the east-Bloc states/ fall of the wall and exchange of old cars without cat against cat cars (6,7).
The most commen occupational as well as public contact allergen Ni (8) and the most significant **atmospheric asthmatic pollution Pt(Pd)** (9) (automobile exhaust) are responsible for this fact (6,7,9): In the air finest distributed Pt at a metropolis like Munich results in about 300 to 400 g/ year plus haevy pollutions of aliphatic and aromatic hydrocarbons (10), root particles (10-12) and a direct inhallation by pedestrians/ infants and pregnant woman. The **mechanisms** acting: Ni does not bind to sulfur but to N. Colloidal Pt(Pd) and Ni for instance have a preference for C, alkene, alkine; alkylate, catalyse additive reactions, oxidations, hydrogenations. Pt (Cis-Pt) inhibits/ stimulates proliferations/ IgE-synthesis.
A summation of the causes for multifactorial diseases, e.g. allergies, respiratory tract diseases and asthma is described by us (13).

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