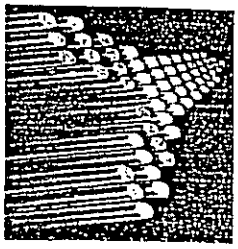
Friday, Oct. 28
2 pm - 6 pm
Chairmen:
Jacobsen (DK)
Stangl (D)
1994

International ALK - Ciba Corning Joint Symposium



Advances in "*in vitro* Allergy Diagnosis"

Preliminary Program

Stangl (D) Welcome

in vitro Allergy Diagnostics

Løwenstein (DK) Standardisation of allergen extracts for *in vitro* diagnostics

Du Buske (USA) Specific IgE determinations (Nominal and High Sensitivity)

Dreborg (S) Sensitivity and specificity in allergy diagnosis

Coffee break

Verification of allergy

Skrbic (CH) Diagnosis of insect venom allergy

Schou (DK) Different diagnostic tools in the diagnosis of mite allergy

Petran (D) Diagnosis of occupational allergy

Social program including dinner
8 pm

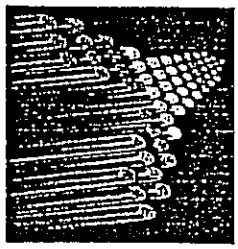
Saturday, Oct 29

8.30 am - 12 pm

Chairmen:

Jacobsen (DK)

Stangl (D)



Food allergy diagnosis

Høst (DK) Diagnosis and treatment of early infant allergy

Bindslev-Jensen (DK) Aspects of food allergy diagnosis

Jansen (A) Different mechanisms in food intolerance - biogenic amines

Status of diagnostic procedures

Jorde (D) Current status of diagnostic procedures

Grossman (D) IgE-complexes - clinical relevance

Kaehl (D) Total IgE as a monitoring/response tool to therapy

Jacobsen (DK) Importance of the specific allergy diagnosis

Stangl (D) Closing remarks

Lunch

TOTAL IGE AS A MONITORING/ RESPONSE TOOL TO THERAPY

R. Kiehl, Spezialklinik Neukirchen, 93453 Neukirchen, FRG

Introduction: The most important direct modulating factors for synthesis of IgE in culture systems proved to be γ-IFN and Il-4. However, this fact could not be confirmed in vivo on atopic eczema patients. We decided therefore to investigate IgE-regulation directly on the blood samples of these patients. Results obtained with the new developed "in vivo assay" system were presented.

Patients and Methods: The patients involved in this study were well characterized and avoided any steroid or antihistaminic treatment for at least one month before admission.
The assays to "in vivo" IgE-regulation were carried out the following way: patients at the beginning of their hospitalization and with total IgE-values of about 1000 to 2000 U/ml gave their consent to participate in this part of the study. Venous heparinized blood was taken at 9 a.m. and immediately processed. Samples of 1 ml were incubated at 37 °C (results parts), the reactions were stopped by centrifugation and the resulting supernatant was taken for detection of total IgE. The influence of the used compounds on the assay system itself has been checked on appropriate controls (standardized IgE-samples). At least 2 identical experiments were done with one blood sample by a minimum of two blood samples from different patients and all measurements were performed in duplicate. The standard deviation was 2 to 5 % in all the measurements.

Materials: Recombinant γ-IFN and Il-4 were gifts from Bender (Vienna, Austria) respectively IC-Chemicals (Ismaning, FRG).

Results: The overall concentrations/ activities of the leukocyte metallo proteases collagenase and gelatinase as well as the concentration of lactoferrin show no pathological changes in the patients. However, skin areas under acute inflammations demonstrate significantly elevated values and γ-IFN molecules were heavily degraded by the me-proteases under these conditions.
500 µM to 1 mM of the protease activator Hg^{2+} change the blood-IgE-levels. The degree of change varies from patient to patient. The measurements result in differences as big as 50 %. 10 mM of the me-protease inhibitor EDTA increase IgE-concentration. 40 µg/ml of the serine protease inhibitor APMSF are itself without effect on the IgE-concentrations but prevent nevertheless a rise of IgE-concentration normally induced by 10 mM EDTA.
10 to 100 µg/ml cycloheximide, an inhibitor of protein synthetic activity, lower the IgE-concentrations.
The results obtained with Hg^{2+} suggested the involvement of some kind of thiol redox state in the IgE-levels, we tested therefore further thiol group reagents: NEM is only slightly influencing, by contrast 1 mM Diamide reduces powerful the IgE-level even below the level obtained with 1 mM Hg^{2+}.
500 U/ml γ-IFN, a concentration of γ-IFN normally present in healthy controls (normal range, 50 to 500 U/ml) are almost without effect. Only 100 times higher concentrations, concentrations which are hazardous to the patients, reduce the IgE-levels significantly.

The situation changes dramatically on addition of 1 mM Hg^{2+}: 500 U/ml γ-IFN are now sufficient to reduce significantly the IgE-concentrations. 1 mM Zn^{2+} fails to induce a similar behaviour as Hg^{2+} (Fig. 1 a). Our first effort in search for replacement of

toxic Hg^{2+} with 1 mM glutathione (reduced or oxidized) proved to be relatively ineffective. Titrations of patients blood samples with increasing amounts of Il-4 make clear, that this cytokine reduces effectively the IgE-levels at concentrations (1000 U/ml) where γ-IFN is relatively ineffective (Fig. 1b).

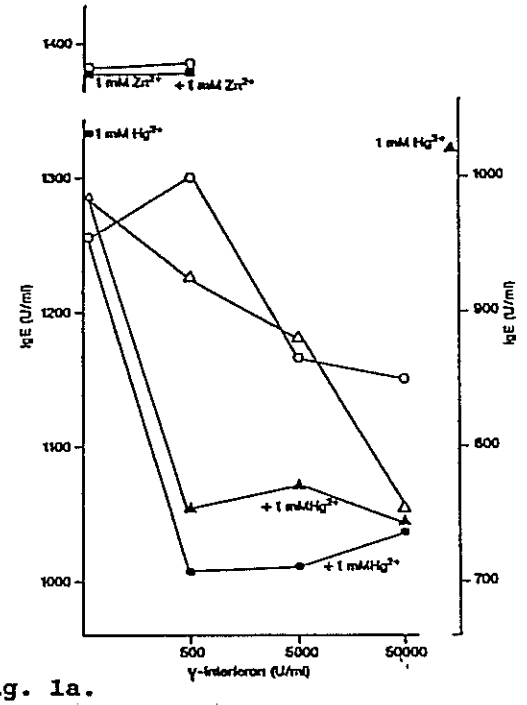


Fig. 1a.

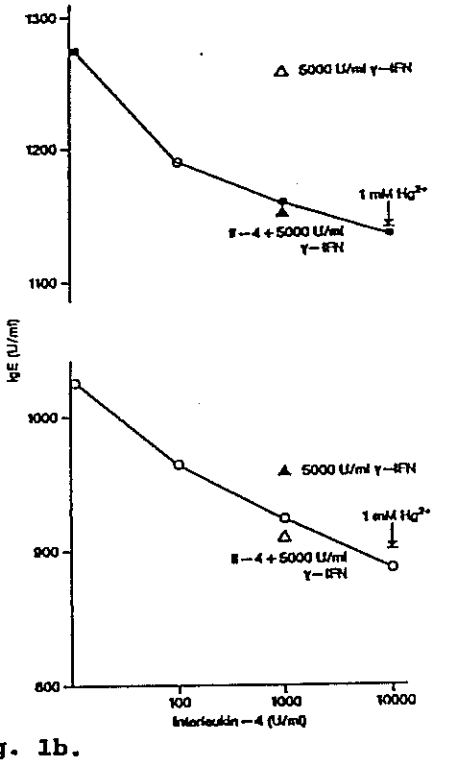


Fig. 1b.

Dual wavelength measurements on lysed blood samples of atopic eczema patients demonstrate complex spectra between the wavelength of 550 to 575 nm. The peakes of 560 and 568 nm seem to be sensitive towards O_2, NADPH and γ-IFN. Besides the cytochrome of the plasma membrane NADPH-oxidase the mitochondrial respiratory chain cytochromes are the most likely reacting components.

Discussion: It is clear, that the regulation of IgE is a highly complex event with the involvement of many factors, the most important ones in cell culture systems proved to be γ-IFN and Il-4.
The effects of Hg^{2+} on blood IgE-levels were seen at a concentration range of 0,5 to 1 mM. These concentrations are by a factor of about 10^5 higher as the normal ranges measured in blood of control- or atopic eczema patients. Mobilisation of Hg^{2+} by DMPS results in 10^2 to 10^3 times higher values in these persons, which is still about 10^2 to 10^3 times below the effective concentrations on IgE-level. Toxic Hg^{2+} seems then not to be responsible for changes in the IgE-levels of our patients. **However, mercury mobilisation may lead in metallo protease** activation and glucocorticoid sensitive inflammations. Nevertheless, present γ-IFN-molecules get significantly degraded in blood samples of infected skin, most probably by ROS-activated me-proteases.
The involvement of a Mg^{2+}- or Ca^{2+} sensitive serine residue in the signal transduction pathway leading to elevation of IgE-level is suggested by the APMSF/EDTA-titrations.
Reduction of the IgE-level by cycloheximide shows that ongoing IgE production in B-cells is blocked and indicates that de novo

IgE synthesis is measured.
The involvement of a dithiol/disulfide interchange mechanism/ redox state in the regulation of IgE-synthesis should be proved by the titrations with Hg^{2+}, Diamide, γ-IFN (± Hg^{2+}) and Il-4. Important: Il-4 reacts antagonistically to γ-IFN in blood samples and cell cultures, although to opposite directions. The interaction of conformation with redox state at the already existent γ-IFN-concentration in the patients determines the highly varying IgE-values in the blood samples of these patients on addition of Hg^{2+}. The ineffective glutathione titrations demonstrate that the involved thiol groups were located inside the involved B-cells.— We used "in vivo" conditions for our experiments, in contrast cell cultures were grown in artificial systems under the use of mitogen stimulated B cell proliferation - a fact which may explain the different results obtained in these systems.
Elements involved in the signal transduction pathway from γ-IFN or Il-4 to IgE are most probably their receptors, (a) cytosolic G protein(s), NADPH-oxidase/electron transfer factor, redox factor, nuclear transcription factors and endonuclease (Fig. 2a, 2b). The full reduced electron transfer chain, NADPH to IgE (DNA), is in favor of a high synthese rate for IgE (low for O_2^-), in contrast a totaly oxidizied one is not able to synthesize IgE (but O_2^-) and the risk for mitogen stimulated proliferations is extremely high.

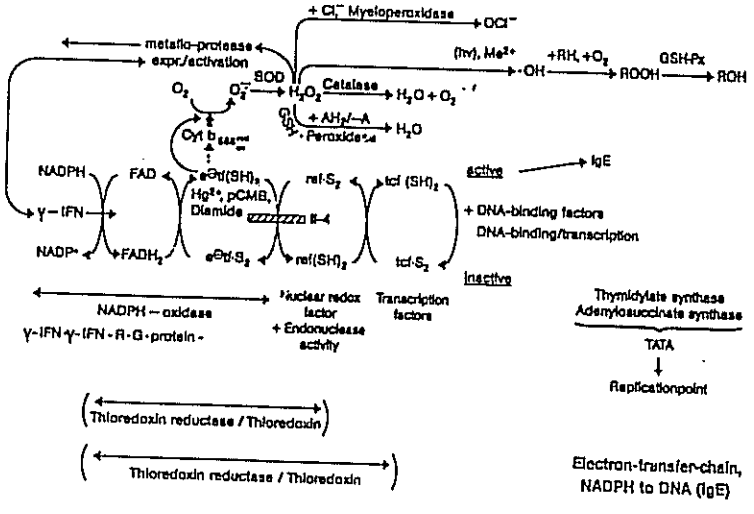


Fig. 2a.

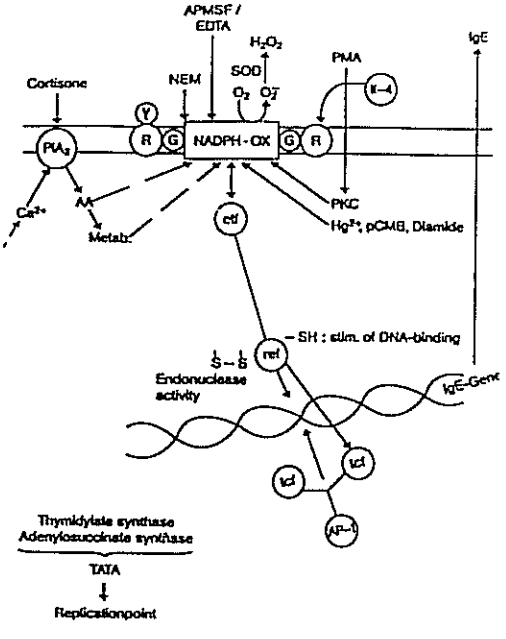
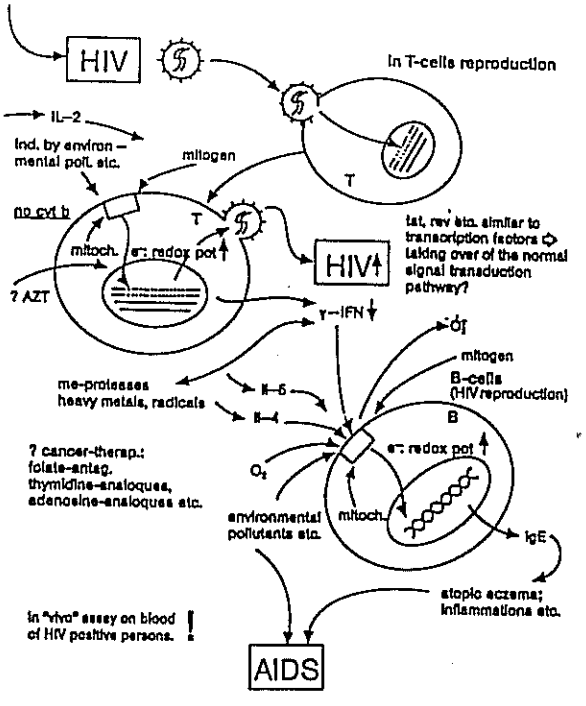


Fig. 2b.

IGE-REGULATION

The oxidized state of ref-1 is a disulfide and responsible for catalytic endonuclease activity. γ-IFN has a bifunctional effect on protein synthesis. IgE synthesis will be stimulated or inhibited depending on fresh blood samples or culture cells respectively incubation time. Translation inhibition pathways were activated by γ-IFN treatment of the cells. Thereby, inactive endonucleases were turned into active enzymes presumably carrying a catalytic disulfide. γ-IFN should then normally transduce redox signals during short time intervals (fresh blood samples) whereas during longer intervals (cell culture) the oxidized redox factor determines translational activities.

The adaption of cells to stress conditions includes the γ-IFN and Il-4 controlled synthesis of IgE antibodies. Environmental pollutants (formic aldehyde, sulfite/SO_2, isocyanates, anhydrides), assumed to induce atopic eczema, were able to react irreversibly with the involved essential dithiol/disulfide redox state. CO_2 by binding to the NADPH oxidase, prevents reduction of O_2, thereby shifting the electron transfer chain to the reduced state with enhanced probability for IgE-synthesis.
B-cells have an immense need for energy. Their fuel is meanly glutamine and the process of activation and regulation is coupled to ongoing mitochondrial energy formation. All compounds influencing mitochondrial energy formation are then also influencing IgE-level. Dermal and intestinal dysbiosis, food as well as psychogenic stress are the mean triggering factors of allergic manifestations.
It should be stressed, the total (unspecific plus specific) IgE concentrations were normally 10^2 to 10^3 times higher than the measured specific ones. The first expression of specific IgE antibodies may purely be incidental and recalls to autoimmune diseases. The pathogenese of atopic eczema and of cancer (proliferation) relates to development of AIDS (Fig. 3).



The most probable mechanism for the pathogenesis of AIDS.

Fig. 3.

References: Kiehl, R. (1994) 13. Vortragstagung der Fachgruppe Biochemie in der GDCh, 03/16th to 18th at Darmstadt, Abstract P 2.6; Analytica Conference, 04/19th to 21st at München, Abstract-book, p. 195-196; Biol.Chem. H.-S. 375, S. 61; BIOTEC 94, 11/16th to 19th at Düsseldorf, in press, and Eur. J. Clin. Chem. and Clin. Biochem., subm.

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