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Immunomodulatory phenomena during anti-influenza vaccination in the elderly

- **The aim** of the study was to evaluate immunomodulatory properties of anti-influenza vaccine and reasons of the poor response to anti-influenza vaccine in the elderly population.

- **Methods:** complete clinical assessment of the patients, flow cytometry (immunophenotyping, intracellular staining, apoptosis-related assays, cell cycle and proliferation, functional studies of cytotoxic lymphocytes - IFNγ and Tetramer assays), fluorescent and confocal microscopy, expression of cytokines (bioassays, ELISA, RT-PCR), cell cultures, LDH-based colorimetric cytotoxicity test, immunomagnetic cell separation, cell cryopreservation, serological assessment of immunoglobulins in sera (ELFA, hemagglutinin inhibition test).

Example of flow cytometry analysis of vaccine-induced apoptosis of lymphocytes confirmed by confocal image of the lymphocytes in different stages of apoptosis (matched with the appropriated quadrant of flow cytometry analysis).
Immunomodulatory phenomena during anti-influenza vaccination in the elderly

Results:

• Clinical status of the elderly patients analysed allowed to establish some clinical data which enhanced or silenced the immunoprotective effects of anti-influenza vaccine in the elderly (Pol Merkuriusz Lek. 2000;10:75-9; Brain Behav Immun 2003 - in press)

• The study confirmed necessity of annually repeated vaccination in the elderly - seroprotection as effective as in the earlier ages became after the second or third immunisation (J Clin Immunol 2003;23:214-22)

• We found that repetitive annual vaccination was able to decrease unprofitable proinflammatory profile in the elderly (Vaccine 2003; 21:3826-36)

• Evaluated profile of cytokines indicated the role of TH2 imbalance in the unresponsiveness to the vaccine in the elderly

• Disturbed expression of receptors inside immune synapse was associated with inadequate response to the vaccine (Mech Ageing Dev 2002; 123:1283-93)

• The study proved that successful anti-influenza vaccine recruits both innate and adoptive immune responses, namely cytotoxic NK and CD8+ T cells (Adv Exp Med Biol. 2001;495:311-4; Clin Exp Immunol 2003 - in press)

• We found that disturbed apoptosis (activation-induced cell death) and cell cycle arrest in the elderly correlated with poor response to the vaccine (Cell Biol Int. 2002;26:517-27)
Immunomodulatory activity of some muramylodipeptide (MDP) derivatives in co-operation with Department of Organic Chemistry, Gdańsk University of Technology

The aim of the study was to evaluate immunomodulatory properties of muramylodipeptide (MDP) derivatives conjugated with anti-bacterial tuftsin or anti-tumour batracilin in in vitro model with human immune cells.

Methods: flow cytometry (immunophenotyping, assessment of oxidative burst, apoptosis-related assays, cell cycle and proliferation, phagocytosis tests), DNA fragmentation assay, expression of cytokines (bioassays), cell cultures, MTT and LDH-based colorimetric cytotoxicity tests, cell separation.

Results
We confirmed immunomodulatory in vitro activity of the derivatives examined. At least two of the MDP-tufts in derivatives evaluated appeared to be useful adjuvants in applied experimental model of anti-bacterial therapy (J Med Chem 2003 - in press), while one of the MDP-batracilin derivatives was an efficient anti-tumour agent stimulating immune cells against some tumour cells (J Med Chem 2003; 46:978-86)
Susceptibility to apoptosis of Natural Killer (NK) cells in the elderly and young with low and high level of NK cytotoxic activity

NK cells are non-T and non-B large granular lymphocytes that participate in the initial stage of the immune defense. They are cytotoxic without prior sensitization or MHC restriction for transformed cells or cells infected with some viruses or bacteria. Our data indicate that level of NK activity correlates with a health status. Low NK cell function in elderly individuals is associated with increased incidence of severe infections. We propose that changes of NK activity correlates with disorders of apoptosis. In lymphocytes apoptosis plays an important role in maintaining T cell repertoire, deletion of autoreactive cells and cytotoxicity against target cells.

The aim of this study is to define the role of apoptosis in functional disorders of NK cells during the ageing process.
Susceptibility to apoptosis of Natural Killer (NK) cells in the elderly and young with low and high level of NK cytotoxic activity

Materials and methods

PBMC of young and elderly volunteers were isolated from the venous blood. The intensity of apoptosis was measured using the annexinV test, flow cytometric evaluation of DNA content (sub-G1 peak in DNA histograms), „ladder” by DNA gel electrophoresis, and fluorescence microscope.

Results:

Cultures of PBMC undergoing apoptosis - The 12-h stimulation with camptothecin and antigens of vaccine caused an increase in the percentage of sub-G1 peak in that population compared to the non-stimulated controls. The effect was observed in the young population. In the 24-h cultures the percentage of events in the sub-G1 peak did not differ between the young and elderly groups.

• Analysis of apoptosis of the cultured PBMC - In young group on increase in the ability to apoptosis concerns lymphocytes, however in the elderly it concerns mainly monocytes.

• The intensity of apoptosis is associated with the NK cytotoxic activity. High and medium NK activity correlates positively with a higher susceptibility to apoptosis. The effect was observed in both, the young and the elderly population.
Analysis of the replicative senescence process in human NK cells

• **Aim of the study**: to find out whether there is a correlation between differences in cytotoxic activity of NK cells and telomere length in the elderly and young population.

• **Methods**: flow FISH (Fluorescence In Situ Hybridization)

• **Results**: the preliminary studies on telomere length carried out in the elderly (over the age of 65) and in young population (under the age of 35) indicate that there are individuals with short as well as with long telomeres in both groups. These observation indicate that investigation on bigger experimental groups is necessary.
Role of corticotropin-releasing factor in the biology of keratinocyte
Błażej Zbytek, Andrzej Myśliwski

- **Hypothesis**: CRH is one of the elements of the cutaneous Stress Response System that is to serve as a local equivalent of hypothalamo-pituitary-adrenal axis.

- **Aim of the project**: Description of the model that will allow for elucidation of the phenotypic effects exerted by CRH on the main component of the epidermis: keratinocyte

- **Methods**: Cell culture, flow cytometry, real time RT-PCR, phospholipid biochemistry, ELISA, EMSA, proliferation assays

Role of corticortopin-releasing factor in the biology of keratinocyte

Results:

CRH inhibits proliferation of transformed and normal keratinocytes; stimulates early differentiation program in human keratinocytes; effects are dependent on PKC pathway, AP-1 and NF-KB
THE EFFECT OF AGEING AND FASTING/REFEEDING ON HORMONAL REGULATION OF MINERAL AND ENERGY HOMEOSTASIS IN RATS

Z. KMIEC, A. MYSLIWSKI, G. KOTLARZ, L. POKRYWKA

Objectives: Age-related changes become visible after stimulation such as fasting and refeeding.

Aims: To determine calcium, phosphate, calcitonin, parathormone ACTH, corticosterone, and leptin serum concentrations in young (4 month old) and old (24 month old) rats that were fasted for 48 h and refeed for the next 24 hours.

Methods: Hormone levels were determined by species-specific RIA.

Conclusions:

8. During fasting/refeeding, old rats effectively maintain calcium homeostasis through different mechanisms than young animals.

2. Although serum ACTH and leptin levels did not differ between both age groups, refeeding resulted in a prominent decrease of corticosterone levels in old rats suggesting altered regulation of hypophyseal-adrenal axis in ageing.