Pflügers Archiv
E J P

Belgian Society of Fundamental and Clinical Physiology and Pharmacology

Abstracts of the Autumn Meeting
22 November 2003, Liège (Université de Liège), Belgium

(Abstracts for Pharmacology are published in "Fundamental & Clinical Pharmacology")

Springer
Pflugers Arch – Eur J Physiol (2004) 447: R1–R12

25

CERAMIDES PLAY A CRITICAL ROLE IN SPONTANEOUS NEUTROPHIL APOPTOSIS

G. Seumois*, M. Fillet, L. Gillet, A.Vanderplasschen , P. Lekeux and F. Bureau

Neutrophils (polymorphonuclear leukocytes) are short-lived terminally differentiated blood cells that play a vital role in inflammatory responses. Circulating neutrophils are physiologically cleared from the circulation by spontaneous apoptosis. A negative modulation in this process contributes to the development of inflammation. Ceramides are nowadays regarded as potential mediators of apoptosis. In various cell types, generation of ceramides at the cell membrane occurs early during apoptotic processes. The aim of this study was to examine whether ceramides may be involved in spontaneous neutrophil apoptosis. Blood neutrophils spontaneously die within 12-18 hours in culture. During culture, by mass spectrometry analysis, 6 forms of ceramides were detected (C14, C16, C18, C20, C22, C24) among which the C16 and C24 forms were the most abundant. The levels of C16 and C24 increased progressively from 6h hours of culture. The death is so preceded by an accumulation of two types of ceramides (C16 and C24). DL-PPMP is a specific inhibitor of glucosylceramide synthetase and D-erythro-MAPP a specific inhibitor of alkaline ceramidase. Both inhibitors raised endogenous levels of ceramide after 3 hours of incubation. Treatment of neutrophils with DL-PPMP (20 µM) or D-erythro-MAPP (100 µM) enhance the apoptosis rate as 50% of cells were annexin-V-FITC positive less then 9 hours of incubation. This suggest that an accumulation of endogeneous ceramide enhances the rate of spontaneous neutrophil apoptosis. GM-CSF (125 u/ml), an inhibitor of neutrophil apoptosis, reduces the apoptosis rate of blood neutrophils as 50% of the cells were annexin-V-FITC negative after 24 h of culture. Levels of ceramide C16 and C24 were although highly reduced after 30 minutes incubation. This indicate that delay of spontaneous neutrophils apoptosis occurred by GM-CSF, is preceded by a decrease of ceramide C16 and C24 levels. In conclusion, these results suggest that ceramides, especially C16 and C24, are key regulators of spontaneous neutrophil apoptosis.

*Present address: Department of Physiology, Faculty of Veterinary Medicine, University of Liege, Bât. B42, B-4000 Liege, Belgium.

26

COMPARISON OF PULMONARY DYSFUNCTIONS CAUSED BY SENDAI VIRUS IN BALB/C AND DBA MICE.

P. Faisca, D. Bui Tran Anh, D. Desmecht*

The present study aimed at detecting different susceptibilities of inbred mouse strains (Balb/c and DBA/2) to Sendai virus infection. Double-chamber plethysmography was the technique implemented to detect the putative resistant / sensitive phenotypes. Twenty female mice of 13 weeks old of each strain were inoculated with 50µl of a viral suspension containing 1000 PFU. Body weight and pulmonary function values (PFV) were monitored daily for 7 days, and lung lesions were examined by histopathology in groups of five mice of each strain at 5, 6 and 7 days after inoculation. DBA/2 presented alterations in their PFV since the third day of infection, that reach their peak at the fourth and fifth day and slightly decreased in the seventh day, even though remaining always different compared to baseline. Peak flows, minute volume, breathing frequency (Fr) and specific airway resistance have all significantly increased by the third day while tidal volume and inspiratory (Ti) and expiratory time have all decreased. In contrast Balb/c only presented significant changes in Ti, Fr, specific minute volume and %Ti. Those alterations were only observed at the fourth and fifth day, and returned to normal in the following days. The alterations in PFV appeared sooner than variation in body weight and were correlated with the observations of lung lesions by histopathology.
The data collected suggest that Balb/c and DBA/2 have different susceptibilities against the Sendai virus, Balb/c being more resistant to infection, and that double-chamber plethysmography is a trustful and sensitive technique to evaluate the severity of respiratory diseases.

*Present address: Department of Pathology, Faculty of Veterinary Medicine B43, B-4000 Liège, Belgium

27

COMPARISON OF PULMONARY DYSFUNCTION CAUSED BY THE MOUSE PNEUMOVIRUS IN BALB/C AND DBA MICE.

D. Bui Tran Anh, P. Faisca, D. Desmecht*

Pneumonia virus of mice (PVM) belongs to the genus *Pneumovirus* which is classified among the Paramyxoviridae. This virus has been demonstrated to mimick the natural infection of human beings with respiratory syncytial virus (RSV) far more closely than mouse models of RSV pneumonia developed so far. This study aimed at determining whether the genetic background might influence the pattern of pneumonia caused by pneumoviruses. Ten female mice of 13 weeks old of BALB/c and DBA strains were inoculated intranasally with 50µl of a viral suspension containing 1000 PFU of the J3666 strain of PVM. Body weight, clinical signs and double chamber plethysmographic pulmonary function values (PFV) were monitored daily for 7 days after inoculation. Clinical signs were more precocious and more marked in DBA. Weight loss was more severe in DBA compared to BALB/c: -9.7%±4.6 vs. -4.1±2.5 five days after inoculation ($p<0.05$) and -17.8%±3.7 vs. -11.7±3.2 seven days after inoculation ($p<0.05$) respectively. There was no mortality among BALB/c, whereas 40% of DBA had died by 6 days after inoculation. Similarly, pulmonary function values were altered earlier and more deeply in DBA than in BALB/c. It is concluded that BALB/c and DBA strains do not respond in a similar way to PVM, DBA being more susceptible. It is therefore suggested that the genetic background critically alters the severity of pneumonias attributable to pneumoviruses. The study also enlightens that, in the context of mass screening of resistance phenotypes among inbred mouse strains, double chamber plethysmography offers an easy, sensitive and reliable method.

*Present address: Department of Pathology, Faculty of Veterinary Medicine, B-4000 Liège, Belgium

28

ACTIVATION OF CIRCULATING POLYMORPHONUCLEAR NEUTROPHILS DURING EXERCISE-INDUCED MUSCLE DAMAGE

G. Camus*, J.-L. Croisier, J.-P. Chapelle, G. Deby-Dupont, C. Deby, J.-M.Crielaard, M. Lamy

To address the question of whether exercise-induced activation of circulating polymorphonuclear neutrophils (PMN) as assessed by plasma myeloperoxidase (MPO) concentration could be related to exercise-induced muscle damage, five moderately active male subjects underwent two isokinetic exercise sessions in the eccentric mode. Each session consisted of 3 stages of 30 maximal contractions (at 60°/s angular velocity) of the knee extensor and flexor muscle groups of both legs with 1 min rest phases between each stage. These exercise sessions were separated by a period of 3 weeks during which the volunteers were submitted to 5 training periods consisting of five stages of 10 submaximal contractions at 75 % peak torque of the knee extensor and flexor muscle groups of both legs according to the above exercise protocol.
The first isokinetic session was followed by severe muscle pain (delayed onset muscle soreness ; DOMS as assessed by visual analogue scale) in previously active muscles and significant increases in blood levels of MPO ($P < 0.05$), serum creatine kinase (CK) and myoglobin (Mb) ($P < 0.001$). After the training period, the mean values of DOMS, CK and Mb were significantly decreased compared to pre-training session values, and remained practically unchanged at 30 min, 48 and 72 h recovery after the isokinetic session. Mean plasma MPO concentrations were also significantly lowered compared to pre-training values at the 30 min, 48 and 72 h recovery time points.
From these results, we concluded that training lowered exercise-induced muscle damage, which were at least partly involved in the activation of circulating PMN.

*Present address : Center for the Biochemistry of Oxygen, Research and Development
Institute of Chemistry (B6a), 4000 Liège Belgium