FEBS Journal

Special Issue: FEBS EMBO 2014 Conference, Paris, France, 30 August-4 September 2014

September 2014

Volume 281, Issue Supplement s1

Pages 1–823

FEBS EMBO 2014 Conference, Paris, France, 30 August-4 September 2014

whole ICU stay. 4 of them did infected early (after 48 hours, but before day 7) and developed septic shock.
Results: Metabolomic analysis identified 7 lipid structures that have the potential to be used as early biomarkers of sepsis. These structures are: homoserine lactone, sorbaldehyde, succinic acid, methylmalonic acid, bromo-decanoic acid, 4-hydroxy-3-methyl-but-2-en-1-yl trihydrogendiphosphate and many others.
Conclusion: Lipid structures may regulate unexpected molecular pathways during sepsis. Their role in inflammation, immunity and infection must be investigated, as well as their potential to help direct clinicians in the treatment of this devastating disease.
Financial Support: FAPESP (#2009/17731-2).
Keywords: inflammation, Lipidomics, sepsis.

SUN-220
Lipopolysaccharide induces pro-inflammatory cytokines and MMP production via TLR4 in nasal polyp-derived fibroblast and organ culture

J.-S. Cho[1], J.-Y. Um[1], J. A. Kim[1], H.-M. Lee[1,2]
[1]Biomedical Science, [2]Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Korea University, Seoul, Korea

Nasal polyposis is characterized by persistent inflammation and remodeling in sinonasal mucosa. Toll-like receptors (TLRs) play a role in the innate immune response to microbes in the sinonasal cavity. The aim of this study was to evaluate whether nasal polyp-derived fibroblasts (NPDFs) and organ-cultured nasal polyps can synthesize pro-inflammatory cytokines and matrix metalloproteinases (MMPs) after exposure to lipopolysaccharide (LPS), a TLR4 agonist. NPDFs and organ-cultured nasal polyps were isolated from nasal polyps of 8 patients and exposed to LPS. The mRNA and protein expression levels of TLRs, cytokines, and MMPs were determined using a gene expression microarray, real-time RT-PCR, western blot analysis, enzyme-linked immunosorbent assay, and immunofluorescence staining. The enzymatic activities of MMPs were analyzed using collagen or gelatin zymography. The protein expression level of MMP-1 increased in nasal polyp tissues compared to inferior turbinate tissues. LPS induced mRNA expression of *TLR4*, *IL-6*, *IL-8*, and *MMP-1* and activated MAPK signaling in NPDFs. LPS promoted the release of interleukin (IL)-6 through extracellular signal-related kinase (ERK) and IL-8 through ERK and c-Jun N-terminal kinases (JNK). Production of IL-6 and IL-8 was induced by PI3K/Akt signaling in LPS-stimulated NPDFs. LPS increased the transcript and protein expression levels of MMP-1 and induced collagenase activity of MMP-1 via ERK and p38, but did not induce gelatinase activity of MMP-2 and MMP-9. LPS from *Rhodobacter sphaeroides* (LPS-RS) inhibited the stimulatory effects of LPS in NPDFs as well as in organ culture of nasal polyp. LPS triggers immune response via TLR 4 and activates MAPK and PI3K/Akt signaling pathway, which is involved in remodeling of nasal polyps.
Keywords: Cytokine, nasal polyp, toll-like receptor.

SUN-221
Liposomes for photodynamic therapy of vitiligo via pilosebaceous route

M. Bhargava[1], S. Bhargava[2], V. Bhargava[3]
[1]ICFAI University, [2]Signa Pharma, [3]KRV Hospitals Pvt. Ltd., Kanpur, India

Vitiligo is an acquired depigmentory skin disorder in which the pigment producing cells (melanocytes) are absent. It affects 0.1–4% of the population worldwide and is emotionally and socially devastating. Well established treatment modalities in today's therapeutic armamentrum have their own side effects and failure. Photodynamic therapy (PDT) an entirely new treatment modality which involves a photosensitizer and light. PUVA therapy used so far has marked failure in many of the clinical trials studies. Since no full therapeutic solution for vitiligo is available, PDT is the ray of hope. The aim of project was to develop and investigate the therapeutic efficacy of liposomes to produce immediate repigmentation (by melanin) along with correcting the cause simultaneously. Topical route has been chosen to directly target the diseased site and to minimize the systemic toxicity. Methoxsalen-melanin loaded liposomes were prepared by a lipid cast film method and were characterized *in-vitro* for their shape, size, percent antigen entrapment, Skin permeation and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* part of the study comprised of Induction of vitiligo by mushroom tyrosinase intradermally & photodynamic studies with different formulations. Cosmetic disfiguration and psychological sequel underlines the impact of vitiligo. Immunization with mushroom tyrosinase resulted in discoloration of the areas showing its effectiveness in inducing vitiligo. Sustained pigmentation resulted after application of formulation was suggestive of cure with fast repigmentation. Thus, pilosebaceous route is effective in targeting follicular melanocytes. Toxic manifestations of methoxsalen were also subsided when delivered in liposomes.
Keywords: liposomes, Methoxsalen, vitiligo.

SUN-222
Loading and release efficiencies of Bortezomib on chitosan coated magnetic nanoparticles

G. Unsoy[1], S. Yalcin[2], R. Khodadust[1], P. Mutlu[3], N. Taghavi Pourianazar[1], U. Gunduz[1]
[1]Biotechnology, METU, Ankara, [2]Food Engineering, Ahi Evran University, Kirsehir, [3]Central Laboratory Molecular Biology and Biotechnology R&D, METU, Ankara, Turkey

Conventional chemotherapeutics are unspecifically distributed all over the body and they affect both cancerous and normal cells. This treatment results in excessive toxicities. Targeted drug delivery has emerged to overcome the lack of specificity of conventional chemotherapeutics. Nanotechnology opened a new door to the development of particles with nano sizes that can be fabricated from a multitude of materials in a variety of compositions, including quantum dots, polymeric magnetic nanoparticles, and

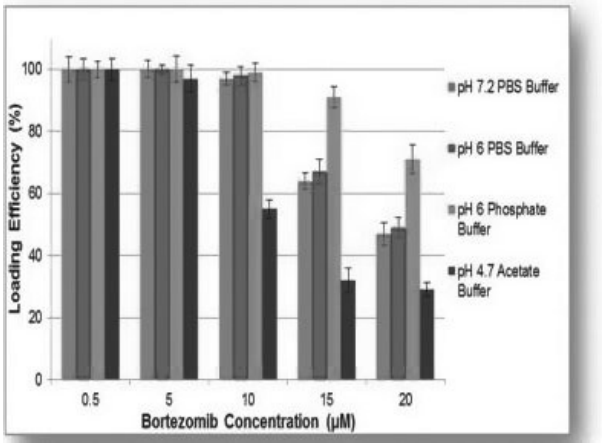

Fig. 1. Effect of initial Bortezomib concentration on loading efficiency in acetate buffer (pH 4.7), phosphate buffer (pH 6.0), phosphate buffered saline (pH 6.0 and pH 7.2).

dendrimeric. Nanoparticles are promising to circumvent these challenges, by enabling high amounts of drugs to be loaded and targeted to the tumor site. Delivery of drugs via nanoparticles increases the half life and reduces toxic side effects of drugs, by improving their pharmacokinetic profile and therapeutic efficacy.

Chitosan magnetic nanoparticles (CS MNPs) were generated for targeting of tumor cells in the presence of magnetic field (Unsoy *et al*., 2012) and loaded with the anti-cancer drug Bortezomib (Velcade®). Bortezomib loading efficiency of CS MNPs was optimized (Figure 2). Highest loading capacity and Drug release characteristics of CS MNPs were identified. Stability of Bortezomib loaded CS MNPs were determined. Bortezomib, a highly potent proteasome inhibitor, was successfully loaded on CS MNPs and their release behavior was pH dependent. Bortezomib loaded pH sensitive CSMNPs were perfectly stable at physiological pH.

As a result, synthesized and Bortezomib loaded CS MNPs can be effectively used in magnetic targeted drug delivery as nanocarrier for the pH dependent release of Bortezomib in cervical cancer cells.

Keywords: Bortezomib, cervical cancer, nanoparticle, targeted drug delivery.

SUN-223
Local inflammatory response induced by CcH1, a P-I metalloproteinase isolated from *Cerastes cerastes* venom

H. Boukhalfa-Abib, F. Laraba-Djebari
Department Cellular and Molecular Biology, USTHB, Faculty of Biological Sciences, Algiers, Algeria

Envenomings induced by snakebite are characterized by local tissue damage, including, necrosis of skeletal muscle, edema, hemorrhage and inflammatory response, as well as by systemic alterations such as hemorrhage, cardiovascular shock, acute renal failure and coagulopathies. Local and systemic effects caused by snake venoms have been associated with diverse components of the venom, such as phospholipases A_2 and metalloproteinases. Snake Venom Metalloproteinases (SVMPs) play a relevant role in the complex multifactorial inflammatory response induced by snakebite. CcH1, an P-I class SVMP isolated from *Cerastes cerastes* snake venom, is a weak hemorrhagic protein, able to induce myonecrosis and to degrade fibrinogen, fibrin, type IV collagen and laminin. In this study, we analyzed the inflammatory reaction induced by CcH1 in gastrocnemius muscle, aiming to identify the cellular components involved in muscular cytokine release.

The inflammatory reaction induced by CcH1 in gastrocnemius muscle was assessed by tissue analysis and the release of pro-inflammatory mediators. Indeed, upon intramuscular injection, CcH1 induces formation of blisters and leukocyte infiltration into dermis, indicating an important pro-inflammatory effect of CcH1. Moreover, the injection of CcH1 in the gastrocnemius muscle, revealed a marked elevation of muscular levels of pro-inflammatory cytokines (TNF-α and IL-6), 50% higher than controls. The muscular concentrations of these cytokines returned to normal levels after 24 h of injection. Muscle injected with CcH1 resulted also the production of pro-inflammatory gelatinases, observed by zymography analysis. Two gelatinolytic bands were detected an apparent molecular weight of approximately 60 kDa and 100 kDa corresponding to latent forms of MMP-2 and MMP-9, respectively. These results suggest that the early production of cytokines induced by CcH1 may stimulate accumulation of leukocytes that will produce MMPs, which will enhance the levels of inflammatory mediators thus potentiating the local response and the tissue damage.

In conclusion, these results indicate that CcH1 is able to induce an inflammatory response characterized by a marked leukocyte infiltrate, MMPs and cytokines production in muscle tissue, indicating that multiple pathways may be involved in muscle inflammatory reaction. The characterization of the cell types and mediators involved with tissue damage and inflammatory response induced by metalloproteinases in snakebite accidents may contribute to the improvement of current therapies, adding to the currently available therapy with antivenoms.

Keywords: Inflammation, Metalloproteinase, Snake venom.

SUN-224
Macrophages engulfing apoptotic cells produce non-classical retinoids to enhance their phagocytic capacity

G. Joós, Z. Sarang, É. Garabuczi, R. Rühl, Z. Szondy
Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary

Previous work in our laboratory has shown that transglutaminase 2 (TG2) acting as a coreceptor for integrin β3 is required for proper phagocytosis of apoptotic cells. In the absence of TG2 SLE like autoimmunity develops in mice, similarly to other mice characterized by a deficiency in the clearance of apoptotic cells. In the present study we demonstrate that increasing TG2 expression alone in wild-type macrophages is not sufficient to enhance engulfment. However, during engulfment the lipid content of the apoptotic cells triggers the lipid sensing receptor liver X receptor (LXR), which in response upregulates the expression of the phagocytic receptor Mertk and the phagocytosis related ABCA1, and that of retinaldehyde dehydrogenases leading the synthesis of a non-classical retinoid. Based on our retinoid analysis this compound might be a dihydro-retinoic acid derivative. The novel retinoid then contributes to the upregulation of further phagocytic receptors including TG2 by ligating retinoic acid receptors. Inhibition of retinoid synthesis prevents the enhanced phagocytic uptake induced by LXR ligation. Our data indicate that stimulation of LXR enhances the engulfment of apoptotic cells via regulating directly and indirectly the expression of a range of phagocytosis-related molecules, and its signaling pathway involves the synthesis of a nonclassical retinoid. We propose that retinoids could be used for enhancing the phagocytic capacity of macrophages in diseases such as systemic lupus erythematosus, where impaired phagocytosis of apoptotic cells plays a role in the pathomechanism of the disease.

Keywords: efferocytosis, Macrophages, phagocytosis.

SUN-225
Maresin improves protective role of EPA in A549 cell line treated with benzo(a)pyrene

J. Gdula-Argasinska[1], A. Garbacik[2], J. Czepiel[3], A. Jurczyszyn[4]
[1]*Department of Radioligands, Faculty of Pharmacy, Medical College, Jagiellonian Univeristy,* [2]*Laboratory of Forensic Chemistry, Faculty of Chemistry, Jagiellonian University,* [3]*Department of Infectious Diseases, Faculty of Medicine, Medical College, Jagiellonian University,* [4]*Department of Hematology, University Hospital, Krakow, Poland*

Fatty acid metabolites – eicosanoids are important signalling molecules and regulate a variety of physiological and pathophysiological processes including inflammation.

The aim of our study was to assess the effect of eicosapentaenoic acid (EPA) supplementation with added Maresin and/or