RHINO-ORBITAL-CEREBRAL MYCOSIS AND CAVERNOUS THROMBOSIS

D.M. LI¹,², P.P. SHANG¹, L. ZHU³ and G.S. DE HOOG²

¹Department of Dermatology and Mycological Laboratory, Peking University Third Hospital, Beijing, China; ²Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ³Department Otorhinolaryngology, Peking University Third Hospital, Beijing, China

Received June 26, 2013 – Accepted January 13, 2014

Rhino-orbital-cerebral mycosis (ROCM) is a life-threatening fungal disease associated mostly with Mucoralean fungi. The infection presents as headache, vision loss, proptosis, ptosis, painful ophthalmoplegia, and peripheral face palsy, with a high mortality (>80% for infections that spread to the brain) and severe morbidity, such as eyeball exenteration and vision loss. In our hospital, a 61-year-old woman with diabetes was diagnosed with rhino-orbital-cerebral infection caused by Alternaria infectoria. Cavernous sinus thromboses (CST) were seen in surgery, pathology, and MRI. She did not respond to potent antifungal therapy until the adding of anti-thrombosis drugs. By analyzing our case, together with the ones that have been published, we realized that fungal thrombosis in the cavernous sinus is the main pathophysiological problem in ROCM that typically shows mass enhancement within the cavernous sinus in radiographic images, thrombosis with characteristics of ischemia and infarction in pathology. Anticoagulation/antithrombus therapy might be helpful in the management of ROCM if potent antifungal treatment does not have effect.
INNATE LYMPHOID CELLS: IMMUNOREGULATORY CELLS OF MUCOSAL INFLAMMATION

V. KUMAR

Department of Otolaryngology, Sunnybrook Health Science Center, University of Toronto, Toronto, Ontario, Canada

Received February 11, 2013 – Accepted February 14, 2014

Inflammation is a very complex immunopathological process occurring due to exaggerated activation of immune system in response to various inflammatory stimuli (i.e. bacterial, viral, fungal or parasitic antigens, xenobiotics, autoantigens and sterile inflammation of unknown cause (i.e. tumor associated inflammation), traumatic inflammation or allergic inflammation etc.). Innate lymphoid cells (ILCs) are particular newly discovered immune cells, which have characteristics of both innate and adaptive immune cells. These cells have shown very significant roles in the pathogenesis of inflammatory disorders at mucosal surfaces (i.e. respiratory tract, gastrointestinal tract and mucosal skin surfaces or barriers). The present review, explores their role in pathogenesis of inflammation at mucosal sites.
IgE GENERATION AND MAST CELL ACTIVATION

S.K. KRITAS¹, A. CARAFFA², P. ANTINOLFI³, A. SAGGINI³, A. PANTALONE⁴, G. NERI⁵, M. ROSATI⁶, M. TEI⁷, A. SPEZIALI⁷, R. SAGGINI⁸, F. PANDOLFI⁹, G. CERULLI⁷ and P. CONTI¹⁰

¹Department of Parasitology, Veterinary School, Aristotelian University, Thessaloniki, Greece; ²Orthopeadics Division, University of Perugia, Italy; ³Department of Dermatology, University of Rome Tor Vergata, Rome, Italy; ⁴Orthopeadics Division, University of Chieti-Pescara, Italy; ⁵Otorino Laryngology, University of Chieti, Italy; ⁶Gynecology Division, Pescara Hospital, Italy; ⁷Nicola’s Foundation, Arezzo, Italy; ⁸Riabilitation Division, University of Chieti-Pescara, Italy; ⁹Department of Internal Medicine, Catholic University of the Sacred Heart, Rome, Italy; ¹⁰Immunology Division, Medical School, University of Chieti-Pescara, Italy

Received December 16, 2013 – Accepted March 1, 2014

IgE is an important marker for allergy and plays a central role in the induction of allergic diseases through its binding of the high affinity receptor on mast cells. Mast cells can influence B cell survival, proliferation and differentiation into CD138+ cells. Among TH2 cytokines, interleukin (IL)-4 and IL-13 are responsible for class-switching in B cells which resolves in production of allergen-specific IgE antibodies that bind to specific receptor on mast cells. IgE synthesis by B cells is regulated by CD40 ligand, IL-4 and interferon-gamma, therefore inhibition of B cell antigen-specific IgE may prevent the cleavage of CD23 from B cells, having a therapeutic impact which also includes the removal of circulating free IgE, omalizumab, corticosteroids, mast cell stabilizers, leukotriene receptor antagonist, and others. B cell differentiation into IgE-producing cells requires two signals provided by TH2 cells and IL-4, however IL-4, IL-1 and IL-10 as well as several hormones are critical for the development of TH2 cells, while cytokines, such as interferon (IFN)-alpha, IFN-gamma, IL-12 and transforming growth factor (TGF)-beta play a negative role. However, the exact mechanism of this process has not yet been defined.
THE C-TERMINAL DOMAIN OF THROMBOMODULIN REGULATES MONOCYTE MIGRATION WITH INTERLEUKIN-6 STIMULATION

Y.W. LIN1,2, C.Y. HUANG1,3,4, C.M. SHIH1,3,4, W.L. CHANG2, S.K. SHYUE5, Y.T. TSAI2, C.Y. LIN2, C.Y. LEE2, Y.J. CHANG6, N.C. CHANG1,3,4, F.Y. LIN1,3,4 and C.S. TSAI2,4

1Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei; 2Division of Cardiovascular Surgery, National Defense Medical Center, Taipei; 3Division of Cardiology, Department of Internal Medicine and 4Cardiovascular Research Center, Taipei Medical University Hospital, Taipei; 5Institute of Biomedical Sciences, Academia Sinica, Taipei; 6Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan

Received October 26, 2012 – Accepted March 21, 2013

Thrombomodulin (TM) is expressed on the surface of monocyte, which is important in the regulation of cell migration, proliferation, and inflammatory responses. In a previous study, we demonstrated that TM on monocyte is negatively associated with cell migration. However, the mechanisms involved in this process are unclear, therefore, we explored the mechanisms in this study. Chemotactic assays and immunofluorescence showed that TM siRNA increased the chemotaxis of the IL-6-activated THP-1, and aggravated actin assembly relative to the IL-6-treated control. In contrast, cells overexpressing plasmids containing full-length or domain 5 of TM followed by IL-6 treatment displayed lower chemotaxis and less actin assembly. Western blot analysis showed that TM knockdown markedly increased cytoskeleton components cofilin and LIMK1 phosphorylation in IL-6-treated THP-1, whereas, transfected cells with HA-TM FL or HA-TM D5, but not HA-TM D1-3 plasmids, reversed the effects. Activation of ERK1/2 and JNK/SAPK, upstream regulators of cytoskeleton components, were also inhibited in overexpressed group. Immunoprecipitation assay demonstrated that actin interacts with TM and intersectin1 in THP-1. Decreased interaction between intersectin1 and actin in TM knockdowns suggested that the interaction is mediated by TM. Our findings indicate that TM domain 5 is a negative regulator and seems to have the ability to inhibit paxillin, cofilin, LIMK1, and actin activation. The mechanisms for the repression effect of domain 5 may be mediated by inhibition of the ERK1/2 and JNK/SAPK activation. Expression of domain 5 of TM may represent a promising approach for controlling monocyte migration, and TM may have potential applications in treatment of inflammatory diseases.
Adult skeletal muscle regeneration involves serial steps among which inflammation in the wounded area is critical for the healing process. However, accelerated tissue regeneration and the inhibition of excessive inflammation are always the targets of tissue engineering, because excessive inflammation in the early stage can impede the regeneration in the following step. In this study, a feasible ibuprofen-loaded poly (L-lactide) (PLLA) fibrous scaffold was designed to evaluate the ability of preventing excessive inflammatory response and promoting regeneration using 35 Sprague-Dawley (SD) rats. The cytotoxicity assay of PLLA and ibuprofen-loaded PLLA fibrous scaffolds (IBU/PLLA) showed that there were no significant cell cytotoxicity on L6 cells. The histological results showed that the IBU/PLLA group had slighter inflammation than PLLA and control groups during the whole process. In the later stage, the regeneration process of the IBU/PLLA group took place on the 7th day, which was almost more than one week earlier than the PLLA and control groups. qRT-PCR analysis further displayed that the IBU/PLLA group had a lower level of inflammatory factors and higher expression of repair factors than the PLLA and control groups, especially from the 7th day, and lasted until the 21st day. Furthermore, there were no statistical differences between the PLLA group and the control group from histological results and qRT-PCR analysis. Taken together, through the muscle wound healing process, the results demonstrated that the ibuprofen-loaded PLLA fibrous scaffolds had better control of excessive inflammation and faster process of healing than non-ibuprofen-loaded groups.
HIF-1α PROVOKES DELAYED NEUTROPHIL APOPTOSIS BY DECREASING 24p3 EXPRESSION AND INTRACELLULAR IRON CONTENT

A. PÉREZ-LADAGA¹, M.A. MUÑOZ¹, C. MASTORA¹ and A. SOLA¹,²

¹Department of Experimental Pathology, Instituto de Investigaciones Biomédicas (IIIB-CSIC, IDIBAPS), Barcelona, Spain; ²CIBER-BBN, Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain

Received February 12, 2013 – Accepted August 1, 2013

Neutrophil apoptosis is delayed in medical conditions associated to anoxia or hypoxia, prolonging tissue destruction and fostering the inflammation. Hypoxia Inducible Factor-1α (HIF-1α), is a main regulator of delayed neutrophil apoptosis but the mechanism of action is poorly characterized. Neutrophil gelatinase-associated lipocalin (24p3) participates actively in iron metabolism and the regulation of iron-responsive genes. Recently, a connection has been described between HIF-1α and 24p3. The purpose of the present study was to determine whether constitutive apoptosis in neutrophils requires 24p3 and whether HIF-1α represses 24p3 affecting cell death iron intracellular levels. To this end we used in vivo ischemic models and anoxic approaches based on the reactivation of the delayed apoptosis. We found that the stabilization of HIF-α during anoxic periods provoked a delay in neutrophil apoptosis through decrease of 24p3 expression and intracellular iron content. The ischemia drastically inhibited the synthesis of 24p3 in circulating neutrophils, increasing the tissue damage. Reactivation of neutrophil apoptosis with opsonized E.coli induced increases in intracellular levels of iron and 24p3. In conclusion, contrary to other cell types, constitutive apoptosis in neutrophils requires 24p3. During hypoxia or ischemia, HIF-1α stabilization represses 24p3 expression, consequently iron levels are depleted and neutrophil apoptosis is delayed.
ENHANCED ANTI-DIABETOGENIC EFFECT OF INTRAVENOUS IMMUNE GLOBULIN MODIFIED BY FERROUS ION EXPOSURE

S. PAVLOVIC¹, N. ZDRAVKOVIC¹, N. PEJNOVIC¹, I.K. DJOUMERSKA-ALEKSIEVA², N. ARSENIJEVIC¹, T.L. VASSILEV² and M.L. LUKIC¹

¹Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; ²Department of Immunology, Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Received February 2, 2013 – Accepted November 8, 2013

The aim of this study was to investigate the immunomodulatory capacity of native and Fe(II)-exposed intravenous immune globulin (IVIg) in multiple low dose streptozotocin-induced diabetes and to delineate the mechanisms of their influence on immune cell functions. Optimal doses (200-600mg/kg) of IVIg prevented the development of hyperglycemia, glycosuria and attenuated mononuclear cell infiltration in pancreatic islets. Fe(II) exposure of IVIg decreased their optimal therapeutic dose to 100mg/kg which significantly decreased the serum levels of proinflammatory cytokines compared to the same dose of native IVIg. This was accompanied by lower numbers of TNF-α, IFN-γ and IL-17 producing CD4⁺ T cells and increased frequencies of CD4⁺IL-10⁺ and CD4⁺IL-4⁺ T cells in the pancreatic lymph nodes and islets on day 16 after diabetes induction. Ferrous ion-exposed IVIg enhanced the bias towards Th2 response while the regulatory Foxp3⁺ T cells were not affected.
A newly created hybrid enzyme (COX-2-10aa-mPGES-1), which mimics the specific biosynthesis of the inflammatory PGE$_2$ through COX-2’s coupling to mPGES-1, was stably expressed in HEK293 cells. The stable cell line, which consistently expresses the superior triple catalytic (Trip-Cat) activities from COX-2 and mPGES-1, was able to directly convert arachidonic acid into the pathogenic PGE$_2$ and distinguish it from other PGE$_2$ synthesizing pathways, as confirmed by enzyme immunoassay, LC/MS analysis and a specific $^{14}$C-AA (arachidonic acid) metabolite analysis approach. A competitive assay confirmed that the endogenous cPGES and mPGES-2 in the HEK293 cells had little involvement in the presence of the expressed COX-2-10aa-mPGES-1 for the synthesis of pathogenic PGE$_2$. Furthermore, subcutaneous injection of the stable cell lines into nu/nu mice revealed 100% (10 out 10) occurrence of tumor mass formation beginning on Day 7 and a continuous progression of the masses to the maximal size which required sacrificing the mice. In contrast, only 10% occurrence of tumor masses, though smaller and with slower growth rates, were observed for the group of vector-transfected HEK293 control cells expressing only endogenous cPGES and/or mPGES-2. The PGE$_2$ produced from multiple pathways by the HEK293 cells co-expressing the individual wild type COX-2 and mPGES-1, and in the presence of endogenous cPGES and mPGES-2, showed also a significantly increased tumor occurrence rate to 30%, which confirmed that the sole coupling of COX-2 to mPGES-1 is a powerful tumor-advancing factor. This result implies that the engineered COX-2-10aa-mPGES-1 could be a promising molecule as a drug developing target against the pathway of COX-2 coupled to mPGES-1 to treat inflammatory diseases and cancers.
KMUP-1 increases nitric oxide (NO) via endothelium nitric-oxide synthase (eNOS). Deficiency of eNOS and peroxisome proliferator-activated receptor-γ (PPARγ) is the pathogenesis of diabetic nephropathy (DN). This study aims to investigate whether KMUP-1 inhibits streptozotocin (STZ)-induced proinflammation in early DN. In experiments, STZ was used to induce diabetes in Wistar rats. Twenty-four male rats were randomly divided into four groups, including control, STZ (65 mg/kg, i.p.), STZ+KMUP-1 (1 mg/kg) and STZ+KMUP-1 (2.5 mg/kg). KMUP-1 HCl was dissolved in distilled water for oral administration. The morphology of renal tissues was evaluated by periodic acid-schiff (PAS) staining and immunohistochemistry of eNOS. The expressions of matrix metalloproteinase-2/-9 (MMP-2/-9), eNOS, B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax) and PPARγ of renal tissues were examined by Western blotting technique. NO production was evaluated by Griess reagent. Oxidative stress was evaluated by measuring reactive oxygen species (ROS). Results indicated that STZ-induced diabetic mellitus (DM) and subsequent DN, including excessive deposition of extracellular matrix (ECM) accompanied by enhanced MMP-2/-9, raised ROS production, increased Bcl-2/Bax ratio and decreased eNOS/PPARγ over a period of 4 weeks. KMUP-1 inhibited STZ-induced hyperglycemia, BUN, MMP-2/MMP-9, and restored eNOS-PPARγ expression in renal tissues. Immunohistochemistry (IHC) of eNOS in glomeruli of renal cortical tissue sections indicated that KMUP-1 restored the eNOS caused by STZ. PAS staining of glomeruli indicated that KMUP-1 could not significantly reduce STZ-induced ECM expansion. Moreover, KMUP-1 increased Bcl-2/Bax and decreased ROS. In summary, KMUP-1 inhibits STZ-induced proinflammation in early DN by restoring PPARγ/eNOS and inhibiting MMP-9.
RELEASE OF HIGH MOBILITY PROTEIN BOX-1 IS GREATLY REGULATED BY NUCLEAR FACTOR OF ACTIVATED T CELL-2 IN HUMAN MONOCYTES

H. LIU¹, Q. ZHAO², Q. SONG¹, F-H. ZHOU¹, H-J. KANG¹, L. PAN¹ and Y-M. YAO³

¹Critical Care Medicine Department, Chinese PLA General Hospital, Beijing, People’s Republic of China; ²Gastroenterology Department of Nanlou, Chinese PLA General Hospital, Beijing, People’s Republic of China; ³Department of Microbiology and Immunology, Burns Institute, First Hospital Affiliated to the Chinese PLA General Hospital (formerly 304th Hospital), Beijing, People’s Republic of China

Received April 23, 2013 – Accepted January 13, 2014

Close talk between inflammatory mediators and immunological cytokines has been discovered and reported. In this study, the role of nuclear factor of activated T cell-2 (NFAT2) in regulation of high mobility group box-1 (HMGB1) release was investigated. THP-1 cell and HEK293T cell were incubated and stimulated by lipopolysaccharide (LPS). Firstly, binding site between HMGB1 and NFAT2 was identified by co-immunoprecipitation (IP). Box A, Box B and CT domain of HMGB1 were constructed, as well as Rel-homology-domain (RHD), pre-RHD and pro-RHD of NFAT2. THP-1 cell was harvested, cell lysate and culture medium were collected at appointed times. Binding between HMGB1 and NFAT2 was measured, HMGB1 protein level in culture medium was analyzed at the same time. Secondly, the role of NFAT2 in regulating HMGB1 release was investigated. When THP-1 cell was cultured for 24 h, HMGB1 protein level was measured at appointed times with or without siRNA to inhibit NFAT2 expression. Our data show that HMGB1 bound to NFAT2 in THP-1 cell cytoplasm. Further experiments showed that box B domain of HMGB1 could bind to pre-RHD of NFAT2. After stimulation by LPS, interaction between HMGB1 and NFAT2 was discovered decreasing gradually. However, HMGB1 protein level increased in culture medium at the same time. Furthermore, HMGB1 release could be enhanced by NFAT2 inhibition. Taken together, release of HMGB1 could be regulated by NFAT2 in human monocytes.
Chronic inflammation may be one of the factors that contribute to the development of diabetic nephropathy (DN). However, erythropoiesis, erythrocyte circulatory half-life and erythrocyte deformability may be influenced by inflammation. Thus, red blood cell distribution width (RDW) levels increase in inflammatory conditions. We investigated the RDW values and related factors in patients with uncomplicated type 2 diabetes mellitus (DM) and diabetic patients with DN. We carried out a retrospective study on patients with type 2 DM admitted to our hospital. Subjects were divided into three groups. Group 1 consisted of healthy subjects. Group 2 consisted of patients with uncomplicated type 2 DM. Patients with various stages of DN were included in Group 3. The RDW values in group 1 subjects were significantly lower than those in group 2 and 3 patients (p<0.05). The RDW values of group 3 patients were higher than those in the other two groups (p<0.05). While the RDW values had positive correlation with blood pressure, serum creatinine, HbA1c, body mass index, proteinuria, platelet (PLT), triglyceride, low density lipoprotein (LDL), total cholesterol (TC), and fasting blood glucose (r values: 0.95, 0.72, 0.56, 0.86, 0.82, 0.76, 0.88, 0.84, 0.88, 0.86, respectively) (p<0.05 for all), there was negative correlation between estimated glomerular filtration rate (eGFR), albumin, high density lipoprotein (HDL) and RDW levels (r values: -0.92, -0.88, -0.78, respectively) (p value < 0.05 for all).
CASPASE-1-DEFICIENT MICE ARE MORE SUSCEPTIBLE THAN WILD-TYPE MICE TO PNEUMONIA INDUCED BY INFLUENZA A (H1N1)

A.A. LIEW¹, T. NARASARAJU², M.C. PHOON¹, S. WANG³, K.B. TAN³ and V.T. CHOW¹

¹Host and Pathogen Interactivity Laboratory, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Kent Ridge, Singapore; ²Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma, USA; ³Department of Pathology, National University Health System, Singapore

Received June 21, 2013 – Accepted January 7, 2014

Although inflammasome-mediated caspase-1 activity and subsequent maturation of IL-1β are critical in host immune response against influenza, their roles in lung pathogenesis are not completely understood. Enhanced susceptibility of caspase-1-deficient mice to influenza has been attributed to decreased inflammation and augmented epithelial damage, while increased IL-1β release has been shown to exacerbate lung pathology. We challenged caspase-1-knockout and wild-type mice with high doses of influenza A (H1N1) virus (500 pfu). Only 10% mortality occurred in wild-type mice, in contrast to 40% mortality in caspase-1-knockout mice which exhibited severe pathologic manifestations as a result of overwhelming inflammatory and cytokine/chemokine responses. Infected caspase-1 knockout mice revealed neutrophil-dominant recruitment and elevated apoptotic inflammatory cells in the lungs, while 20% of these animals displayed focal lymphocytic infiltrates in the brains suggestive of mild focal encephalitis. In infected caspase-1 knockout mice, cytokine/chemokine levels were reduced at 3 days post-infection, but robust increase in the levels of TNF-α, IL-6, MIP-1α, MCP-1, MIP-1β and RANTES were observed at 6 days post-infection, coinciding with exaggerated neutrophil influx. These results support our previous findings implicating excessive neutrophil infiltration in pathologic complications during influenza pneumonia. In addition, we observed an induction of IL-1β release in caspase-1 knock-out mice at 6 days post-infection, indicating the involvement of caspase-1-independent activation of IL-1β. Microarray analyses of the lungs of infected caspase-1 knockout mice revealed upregulation of immune and inflammatory genes including CCL8, CCL4, IFN-γ, ICOS, PRF-1, KLRA4, KLRA7, KLRA18, NKG7, LTB4R and PTX3. Collectively, our data suggest that caspase-1-mediated effects are critical against severe influenza infection which can also trigger robust caspase-1-independent inflammatory responses, thus alluding to the complexity of innate immune regulation.
VIRAL ANTI-INFLAMMATORY PROTEINS TARGET DIVERGING IMMUNE PATHWAYS WITH CONVERGING EFFECTS ON ARTERIAL DILATATION, PLAQUE AND APOPTOSIS

J.A. DAVIDS1,2, E. DAI1,3, H. CHEN1, M.Y. BARTEE1,2, L. LIU1,3, A. FORTUNEL1, R. MOYER2, G. MCFADDEN2 and A.R. LUCAS1,2,3

1Divisions of Cardiology and Rheumatology, Department of Medicine and 2Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL, U.S.A.; 3Robarts’ Research Institute, Department of Microbiology and Immunology, and Division of Cardiology, Department of Medicine, University of Western Ontario, London, ON, Canada

Received August 8, 2013 – Accepted January 20, 2014

Abdominal aortic aneurysms are often fatal due to atherosclerosis, thromboembolism, rupture, and hemorrhage, however, treatment is limited to expectant monitoring and surgical intervention. Inflammation is detected in aneurysms and in plaque with associated increased apoptosis, chemokines, cytokines, hemorrhage, and thrombosis. We compared treatment with three different myxomavirus-derived anti-inflammatory proteins targeting apoptosis, thrombosis, and chemokine pathways. The effect of each protein on aortic dilatation and plaque growth was assessed after angioplasty in Apolipoprotein E null mice. Four myxomavirus-derived proteins were studied; Serp-1 a serine protease inhibitor (serpin) targeting thrombotic and thrombolytic proteases, Serp-2 a cross-class serpin inhibiting granzyme B and caspases 1 and 8, M-T7 a broad spectrum C, CC, and CXC chemokine inhibitor, and R171E, an inactive M-T7 mutant. Cell invasion, elastin breaks, plaque progression, and aortic dilatation were significantly reduced by Serp-1, Serp-2, or M-T7 protein treatment, but not by R171E. PCR array analysis detected altered expression of a group of shared 40 apoptotic genes in monocytes after treatment with each active protein, but not R171E. Interference with inflammatory cell responses, through highly divergent inflammatory response pathways, produces similar reductions in monocyte invasion, arterial dilatation, and plaque growth potentially through modified expression of apoptotic genes.
OSTEOCLAST GENERATION AND CYTOKINE PROFILE AT PROSTHETIC INTERFACES: A STUDY ON TISSUE OF PATIENTS WITH ASEPTIC LOOSENING OR IMPLANT-ASSOCIATED INFECTIONS

U. DAPUNT¹, T. GIESE², F. LASITSCHKA³, B. LEHNER¹, V. EWERBECK¹ and G.M. HÄNSCH²

¹Department of Orthopaedics and Trauma Surgery, Heidelberg University, Heidelberg, Germany; ²Institute for Immunology, Heidelberg University, Heidelberg, Germany; ³Institute for Pathology, Heidelberg University, Heidelberg, Germany

Received October 28, 2013 – Accepted February 13, 2014

Aseptic loosening of implants or loosening due to persistent bacterial infection remains a severe complication in orthopaedic surgery. To investigate underlying cellular and molecular mechanisms, particularly with regard to bone loss, tissue samples of patients requiring surgery were examined. By histological methods and by quantitative RT-PCR, respectively, infiltration of leukocytes, expression of osteoclast-typical genes and of proinflammatory cytokines was determined. Samples were taken directly from osteolytic sites and for comparison from adjacent sites, distant sites and from muscle. At osteolytic sites, cathepsin K and the metalloproteinases MMP1 and MMP9 were found, as was expression of inflammation-related cytokines, particularly of interleukin (IL)-1ß, CXCL8, S100A9 and a very moderate expression of receptor activator of NfκB ligand (RANKL) and tumour necrosis factor (TNF) α. Of note, expression of these parameters gradually decreased from sites of osteolysis to adjacent tissue, to distant tissue to muscle. In patients with infection and osteolysis, expression of cytokines, notably of CXCL8, was markedly enhanced, especially in adjacent and distant tissues, where expression was 10- to 20-fold higher compared to tissue of aseptic patients. A possible source of CXCL8 could be infiltrated cells, particularly neutrophils, because they were found in infected tissue only. Histological examination of the biopsies revealed an additional CXCL8 source, namely endothelial cells of small blood vessels. In conclusion, aseptic loosening and implant-associated infection are associated with osteoclast generation and a local inflammatory response. The proinflammatory environment could promote the differentiation of precursor cells to osteoclasts, thereby linking inflammation to bone resorption. The higher expression of cytokines, particularly of CXCL8 in tissue of patients with bacterial infection, could explain the accelerated time course of bone resorption as it occurs in infection compared to aseptic loosening.
The aim of this study was to investigate the relationship between pentraxin-3 and other biochemical parameters in women with polycystic ovary syndrome (PCOS). We compared 58 women with PCOS to 34 body mass index- and age-matched normally menstruating healthy controls. Women with PCOS had significantly higher DHEA-S, free testosterone, LH and FAI, but lower pentraxin-3 levels when compared to healthy controls (0.86±0.21 and 0.91±0.14 respectively, p=0.014). Levels of CRP and lipoprotein-a were higher in the PCOS group. Overweight PCOS had significantly higher insulin, HOMA-IR, FAI, free testosterone and CRP and statistically significantly lower HDL and SHBG levels when compared to controls. Pentraxin-3 levels of obese and normal weight PCOS were similar. CRP and pentraxin-3 might contribute reciprocally to metabolic events and chronic low-grade inflammation in women with PCOS.
Probiotic bacteria have been shown to have health benefits in various situations (inflammation, allergy, infection). We previously showed that a bacteria-free fermentation product of *Bifidobacterium breve* C50 (BbC50sn) induced high IL-10 secretion by human dendritic cells. As IL-10 is a regulatory cytokine, the aim of the present study was to examine whether DCs cultured in the presence of BbC50sn could induce regulatory T cells in an allogeneic context. Purified CD4^+^CD25^-^ human T cells were co-cultured with allogeneic BbC50sn-treated dendritic cells for 4 weeks. The T cell population (BbC50sn-T) was analysed both at phenotypical and functional [ability to inhibit a mixed lymphocyte reaction (MLR)] levels. We showed that T lymphocytes acquired phenotype characteristics of regulatory T cells after 4 weeks of co-culture with BbC50sn-DCs, and inhibited *in vitro* T lymphocyte proliferation and IFN-γ production in an MLR. Transwell experiments demonstrated that this suppressive activity was not T cell contact-dependent but probably mediated by a soluble factor. Although BbC50sn-T cells secreted significant amounts of IL-10 and TGF-β, their suppressive effect is most likely not mediated through these cytokines. This is, to our knowledge, the first demonstration of *in vitro* regulatory T cell induction by a bacteria-free fermentation product in an allogeneic context.
Intravesical hyaluronic acid (HA) and chondroitin sulphate (CS) instillation are effective for urinary tract infections (UTIs) and bladder pain syndrome. This study aimed to evaluate the tolerability, safety and efficacy of intravesical HA and CS instillation in patients with late radiation tissue cystitis (LRTC). In this pilot study, tolerability was reported as discontinuation or deviation of the protocol, safety as general or local side effects, efficacy as improvement of bladder capacity and frequency, quality of life (QoL) through the European Quality of Life 5-Dimensions (EQ-5D) (details at http://www.controlled-trials.com/ISRCTN37534393). Thirty-two patients with LRTC were enrolled. Twenty-seven patients (84.8%) received a mean of 12.2±0.3 months of instillation therapy. Only two patients (6.2%) developed a urinary tract infection from instillation, which required antibiotic treatment, nevertheless not compromising the therapy schedule. No male patient developed a urethral stricture. Intravesical instillation was associated with a significant increase (>50%) of bladder capacity from baseline (66.9ml) both at 3 months (101.9 ml; \( p<0.001 \)) and 12 months (174.4 ml; \( p<0.001 \)). EQ-5D index significantly increased from baseline to both 3 and 12 months (0.26, 0.69 and 0.96, respectively; \( p<0.001 \)). Intravesical co-administration of HA and CS improved bladder function, symptoms and QoL in patients with LRTC.
LETTER TO THE EDITOR

DRESS SYNDROME INDUCED BY SULPHASALAZINE

K. PAŁGAN and Z. BARTUZI

Department of Allergology, Clinical Immunology and Internal Diseases, Nicolaus Copernicus University in Toruń, Collegium Medicum of L. Rydygier in Bydgoszcz, Poland

Received January 3, 2014 – Accepted February 17, 2014

DRESS syndrome (Drug rash with eosinophilia and systemic symptoms) is a severe drug-induced hypersensitivity syndrome characterized by diffuse skin rash, fever, eosinophilia, atypical lymphocytes and organ involvement. We report a case of drug reaction with eosinophilia and systemic symptoms (DRESS) to sulphasalazine. A 54-year-old woman developed a widespread papulovesicular rash after treatment with sulphasalazine (1000 mg daily). She was successfully treated with systemic corticosteroids.
LETTER TO THE EDITOR

PATHS TO UNDERSTAND AND TO LIMIT INFLAMMATION IN TRANSFUSIONS

O. GARRAUD¹,², H. HAMZEH-COGNASSE¹, S. LARADI² and F. COGNASSE²

¹EA3064, Faculty of Medicine, University of Lyon, Saint-Etienne, France; ²Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France

Received August 22, 2013 – Accepted January 28, 2014

Transfusion is generally very safe. However, in a small number of cases, it leads to mild and even serious - life-threatening - hazards (most often after platelet component transfusion). In cases where no preventable accident happens (that exclude infection, chain error, malpractice, antigen-antibody conflict), the symptomatology resembles inflammation. The present essay reports on the causes of inflammation in transfusion and in which way one may intervene on the different steps of the process - from donor to patient - to limit such incidents/accidents.
MACROPHAGE ACTIVATION AND PATTERNS OF INFLAMMATION IN OBESE AND NON-OBESE WOMEN WITH BREAST CARCINOMA

M. RENNE¹, F. CONFORTI², C. Camastra², A. DONATO² and G. DONATO²

¹“Tommaso Campanella” Foundation, Department of Oncologic Surgery, Catanzaro, Italy;
²Chair of Pathology, Department of Health Science, School of Medicine and Surgery, University “Magna Graecia” of Catanzaro, Catanzaro, Italy

Received August 23, 2013 – Accepted December 5, 2013

Tumor-associated macrophages (TAMs) with M2 phenotype provide an immunosuppressive microenvironment for tumor growth. In contrast, a great deal of evidence indicates that macrophages in obese patients’ adipose tissue undergo a phenotypic change from M2 to M1 polarization accelerating adipose tissue inflammation. Interestingly, obesity is considered a major risk factor for breast cancer. Here, we report a case-control study comparing normal-weight and obese women with breast cancer. Activation states of macrophages and patterns of inflammation associated to breast invasive ductal carcinoma were analyzed by immunohistochemistry. iNOS positive macrophages were counted as a M1 polarized population. Our study demonstrates that classically activated macrophages in obese patients outnumber classically activated macrophages in the breast of non-obese patients with infiltrating ductal carcinoma. However, cancer cell linked factors may strongly antagonize M1 macrophages’ positive effects by both stimulating their switching to M2 TAMs and inhibiting their effects.
LETTER TO THE EDITOR

RHEUMATOID ARTHRITIS-LIKE DISORDER MIMICKING SERUM SICKNESS: DO NOT FORGET ACUTE B HEPATITIS!

G. FAMULARO¹, G. MINISOLA² and I. GASBARRONE¹

¹Internal Medicine, ²Rheumatology, San Camillo Hospital, Rome, Italy

Received May 4, 2013 – Accepted December 3, 2013

We report on a patient who presented with a severe symmetric polyarthritis that was found to be part of the prodromal stage of acute hepatitis B. This syndrome is seen in a minority of cases, resembles serum sickness and parallels the duration and level of hepatitis B viremia. It is a type III immune complex reaction with complement consumption and activation which ultimately triggers inflammation and may also occur in the context of other infections, vaccines, and use of antibiotics or sera. We emphasize the need to search for acute B hepatitis in all patients with polyarthritis or other symptoms of serum sickness.
This study compared the analgesic and anti-inflammatory efficacy of intra-alveolar administration of a thermosetting gel containing Doxycycline Hyclate 3% + Ketorolac Tromethamine 0.5% (Tg-DHKT) (Thermosetting gel, Monteresearch, Bollate, Italy) on patient discomfort after third molar surgery. This study was a single-blind, randomized clinical trial, including two study groups of 39 and 41 patients each, who required surgical removal of a single mandibular impacted third molar. After the extraction the test group received an intra-alveolar injection with Tg-DHKT and the second group a thermosetting gel containing only Doxycycline Hyclate 3% (Tg-DH). Each patient’s symptoms (pain, swelling, reddening, bleeding and body temperature) was assessed with a follow-up questionnaire (PoSSe scale). Nimesulide 100 mg, a painkiller, every 8 hours was prescribed to the Control and Test groups if necessary (maximum 3 doses); if they needed to assume it they were asked to mark it on the questionnaire. Results showed that on the second day after surgery pain, oedema and reddening decreased faster in the Control group (Tg-DH). There was no difference between the two groups when postoperative bleeding was evaluated. In both groups bleeding decreased in the same way, probably due to the mechanical characteristics of the gel itself. 46% of patients of the Test group did not require to take any painkiller at home. Our data demonstrate that the use of Tg-DHKT is less effective in the prevention of postoperative symptoms after third molar extraction compared to Tg-DH. However, almost half of patients in the Test group did not need to take more pain medication at home, suggesting that a single postoperative local administration of Tg-DHKT is a safe and effective concept for controlling pain, oedema and inflammation after third molar extraction.
KERATOCYSTIC ODONTOGENIC TUMOR SURGICAL MANAGEMENT: RETROSPECTIVE ANALYSIS ON 77 PATIENTS


Department of Oral and Maxillo-Facial Sciences, “Sapienza” University of Rome, Italy

Received September 19, 2012 – Accepted July 3, 2013

In 2005 the WHO introduced the former odontogenic keratocyst to the category of benign odontogenic tumours. The change in terminology was based on the observation that the odontogenic keratocyst behaves as a neoplasm and not like a benign cystic lesion. The present paper is a retrospective analysis on the management of keratocystic odontogenic tumor over a period of 11 years (2001–2012) in the Department of Maxillo-Facial Surgery at the University of Rome “Sapienza”, with particular focus on the surgical choices and the relative rates of relapse. The patient population consisted of 34 females and 43 males. Administered treatment modalities consisted of enucleation in 55 cases and radical resection in 22 cases. Nineteen percent of patients who underwent enucleation suffered KCOT relapse. No relapse was observed in the radical resection group with follow-up of 3-7 years. The goals of the treatment include elimination of the pathology and decrease of potential recurrence while minimizing harm to the patient. In the Authors’ experience, conservative treatment still encompasses a high rate of recurrence; otherwise, resection provides the lowest recurrence rate, yet causes the most suffering to the patient. The issue surgeons encounter is whether to choose a conservative approach, reducing the morbidity to the patient, knowing that several operations may be required to eliminate recurrence; or being more aggressive and potentially more destructive, at the same time ensuring the best condition to avoid recurrence. Other studies are needed in order to find definitive guidelines for this challenging pathology.