115

Gliotoxin Mediated Apoptotic Cell Death in Jurkat cells

Cecilie Andersen[1,2], Rolf Pettersen[1], Dina Aresvik[1], Marianne Wright[1], Tore Abrahamsen[3,4]. *[1]Department of Paediatric Research and Institute of Microbiology, Rikshospitalet University Hospital, Oslo, Norway; [2]Institute of Microbiology, Rikshospitalet University Hospital, Oslo, Norway; [3]Division of Paediatrics, Rikshospitalet University Hospital, Oslo and University of Oslo, Norway; [4]University of Oslo, Oslo, Norway*

Introduction: In the immunocompromised host invasive aspergillosis (IA) is an increasing problem with high mortality despite new and improved antifungals. The mycotoxin gliotoxin is secreted during hyphal growth of Aspergillus fumigatus. The toxin is detected in the serum of patients with proven IA. Gliotoxin is immunosuppressive and mediates proliferation, apoptosis and necrosis of different cell-types. Its exact role in the pathogenesis of IA is still debated.
Objectives: We wanted to further investigate the effect of gliotoxin on T-cells by studying death responses in the T cell line Jurkat E6 induced by this toxin.
Methods: Jurkat E6 cells were incubated with or without 50-2000ng/ml gliotoxin for 1 to 24 hours. Apoptotic cells death was determined using Annexin-V-FLUOS and propidium iodide (PI) staining. Analysis of active caspase 3, -8 and -9 were performed using selective antibodies, loss of mitochondrial membrane potential by JC-1 staining, and ROS production using hydroethidine staining after incubation with gliotoxin 250ng/ml for 4 hours. All assessments were performed by flow cytometry. Staurosporine (1μM for 2 hours) was used as positive control.
Results: We observed time- and concentration dependent cell death in response to gliotoxin. Annexin V Fluos and PI staining suggested apoptotic cell death with a maximal response after 4 to 6 hours of incubation with 500ng/ml gliotoxin. Furthermore, cell death was almost completely blocked by the pan-caspase inhibitor Z-VAD-FMK (100μM). After 4 hour incubation with gliotoxin, we found caspase-3 activation (27-48% positive cells), caspase-9 activation (21-54% positive cells) and cells with loss of mitochondrial membrane potential (49-78%) suggesting apoptosis and mitochondrial involvement. We also found activation of caspase-8 (20-52% positive cells) suggesting an involvement of death receptor pathway as well. ROS generation was detected in less than 10% of the cells after treatment.
Conclusions: Our data show a caspase dependent apoptotic cell death of Jurkat T cells in presence of gliotoxin. This T cell apoptosis may contribute to reduced immunity to the fungus.

116

Prevalence of Virulence Factors and Antimicrobial Resistance in Uropathogenic *Escherichia coli* (UPEC) Isolates from Immunocompromised and Immunocompetent Hosts

Monica Merçon[1], Adriana Hamond Regua-Mangia[2], Kinue Irino[3], Lúcia Martins Teixeira[4], Guilherme Santoro Lopes[1]. *[1]Departamento de Medicina Preventiva, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Departamento de Ciências Biológicas,Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [3]Seção de Bacteriologia, Instituto Adolfo Lutz, São Paulo, Brazil; [4]Instituto de Microbiologia Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

Background: *E. coli* is the most frequent etiologic agent of urinary tract infection (UTI). There are limited data comparing the prevalence of virulence factors in UPEC isolates from immunocompetent and immunocompromised hosts.
Objectives: To compare the prevalence of virulence factors and antimicrobial resistance in UPEC strains isolated from immunocompromised and immunocompetent subjects.
Materials and Methods: UPEC isolates were characterized according to serotype, hemolytic and hemagglutination properties, virulence determinant genes (pap1/pap2, sfa1/sfa2, afa1/afa2 hly1/hly2, aer1/aer2,cnf1/cnf2) and antimicrobial susceptibility. Clinical presentation and patients' characteristics were abstracted from charts.
Results: UPEC isolates from 36 immunocompromised patients (26 renal and 1 liver transplant recipients, 1 AIDS patient and other 8 subjects receiving immunossupressive therapy) and 27 immunocompetent subjects with UTI were studied. Secondary bacteremia occurred only in immunocompromised hosts (7 cases, p=0.02). Resistance to quinolones tended to be less frequent among these patients (OR1.70; 95% IC=0.91-3.14, p=0.096). There was no significant difference between groups in the prevalence of resistance to other antimicrobial drugs. There was no significant difference between groups in the distribution of O-H serotypes, mannose resistant or sensitive hemagglutination, and virulence determinant genes. Quinolone resistance was associated with a lower prevalence of alfa-hemolysis (p=0.01), O serotypes classically associated with pyelonephritis (p=0.017), and sfa1/sfa2 gene (p=0.003).
Conclusions: Despite the higher frequency of secondary bacteremia among immunocompromised patients there was no significant difference in the prevalence of virulence factors in UPEC isolates from these patients as compared with isolates from immunocompetent controls. Quinolone resistance was associated with a lower prevalence of virulence factors.

117

Hyperinfection Syndrome in an Immunocompromised Host

Aggelos Toskas, Aikaterini Masgala, Vasiliki Liosi, Mina Psychogiou, Ioanna Deladetsima, Georgios Bamias, Kiriaki Dimarogona, Georgios Petrikkos, Georgios Daikos. *"Laikon" hospital, 1st Department of Propaedeutic Medecine, University of Athens, Athens, Greece*

Background: Strongyloidiasis has worldwide distribution and is one of the most important enteric elminthic infections. In patients with impaired cell mediated immunity, Strongyloides stercoralis may disseminate to multiple organs and cause a life-threatening complication called hyperinfection syndrome. Herein we present the clinical course of an immunocompromised patient with Strongyloides hyperinfection syndrome.
Materials, Methods and Results: A 62 year old farmer was admitted to our department because of fever and severe headache for the last 48 hours. He was on high dose of corticosteroids because of retroperitoneal fibrosis. On admission, he was lethargic with nuchal rigidity. Cerebrospinal fluid (CSF) examination revealed 4000 leukocytes/uL with neutrophil predominance (82%) and CSF cultures grew *Enterococcus feacalis*. Blood and urine cultures yielded no organism and the patient was treated with Ampicilline and Gentamicin. Transesophageal echocardiogram and computed tomography of abdomen and thorax, were negative. Two days after initiation of treatment the patient was improved, but on the 10th day he started vomiting. An upper endoscopy was performed and jejunum biopsies revealed *Strongyloides stercoralis*. On stool examination filariform larvae were seen. Bronchoalveolar lavage as well as CSF sediment examination revealed filariform larvae, so the diagnosis of hyperinfection syndrome was established. The patient was treated with albendazole 400 mg BID, while corticosteroids were being tapered. Although the patient was on treatment, the stool remained positive until the 10th week. The total duration of treatment was 12 weeks. During a 4 months follow up, no relapse was noted.