Generation of Monoclonal Antibodies Against Recombinant Capsular Antigen of *Burkholderia mallei* and Its Potential Application in Glanders Disease Diagnosis

D.T. Selvam*, U. Tuteja, H.V. Batra

Defence Research & Development Establishment, Gwalior, India

Glanders is primarily a disease of solipeds, most mammals have a certain degree of susceptibility and infection has been reported in goats, sheep, camels, cats, dogs and various other carnivores, human cases of glanders have rarely been reported. Glanders was eradicated in many countries through the use of the mallein test and also with the application of countermeasures like intensive blood testing, rigorous culling of positive animals and strict trading restrictions but sporadic cases still occur in Asia, Africa, the Middle East, and South America. The similarities that exist between *Burkholderia mallei* causative agent of Glanders and its closely related bacterial species *Burkholderia pseudomallei*, the causative agent of melioidosis are well documented. Due to its high homology and sharing of many antigens, serological and immunological tests developed for these agents are cross-reactive. In the present study, efforts were made for the development of specific monoclonal antibodies specific to a 14 kDa recombinant capsular protein of *B. mallei* that does not cross-react to *B. pseudomallei*. For this purpose genes of 14 kDa recombinant capsular protein of *B.mallei* was cloned and expressed in pQE expression system. After fusion 114 hybrids were screened for reactivity and 15 were found reactive and out of which 8 were stabilized and preserved. The specificity of the antibodies was checked with different bacterial strains and the isotypes of all the clones were determined. One clone was specifically reactive to *B. mallei* and 2 were reactive to *B. mallei* and *B. pseudomallei* and 5 clones were cross- reactive. These specific monoclonal antibodies will be helpful in the standardization of antigen detection system for Glanders.

doi:10.1016/j.ijid.2008.05.550

40.041

Determination of Minimal Inhibitory Concentration for Antibiotics against some *Mycoplasma mycoides subsp. mycoides* (LC type) Strains

Y. Singh[1,*], D.N. Garg[2]

[1] *CCS Haryana Agricultural University, Hisar, India*
[2] *CCSHAU, Hisar, Hisar, India*

Mycoplasmal reproductive disorders in bovines is gaining global significance for cattle industry because of economic implications inflicted due to lower reproductive efficiency of male and female bovines leading to temporary and permanent infertility. An indiscriminate use of antibiotics in treating the clinical cases result in development of resistance to these antimicrobial agents. During the present study, the minimal inhibitory concentration (MIC) for five antibiotics viz: sparfloxacin, norfloxacin, amoxicillin, lincomycin and spectinomycin against three strains of *Mycoplasma mycoides subsp. mycoides* (LC, Y-goat determined in standard mycoplasma broth having phenol red as an indicator. A fixed quantity of organisms (106 cfu/ml) was used as inoculum to each of the two fold dilution of antibiotic. Results were recorded after 24 hours and the final MIC was recorded after 7 days as μg/ml concentration of each antibiotic under test.

The MIC ranged from 0.02 to 2 μg/ml for sparfloxacin, 0.04 to 1 μg/ml for norfloxacin, 0.5 to 4 μg/ml for amoxicillin, 0.2 to 20 μg/ml for lincomycin and 0.5 to 25.6 μg/ml for spectinomycin with all the three test strains of *Mycoplasma mycoides subsp. Mycoides* (LC) of bovine genital origin. The MIC with sparfloxacin, norfloxacin and amoxicillin was found lower as compared to lincomycin and spectinomycin. The MIC for spectinomycin (25.6 μg/ml) with type strain of *M. mycoides subsp. Mycoides* (LC) and lincomycin (20 μg/ml) with MMLC from preputial swab (15 CPS/88) were highest which shows their complete resistance. The results of present study revealed that sparfloxacin, norfloxacin and amoxicillin were the effective antibiotics against bovine genital mycoplasmas (*Mycoplasma mycoides subsp. Mycoides*, LC type which can be utilized as potent therapeutic agent in the field.

doi:10.1016/j.ijid.2008.05.551

40.042

Cytological and Biochemical Characteristics of Cerebrospinal Fluid at Enteroviral Meningitis in 2005

D. Naunova Jovanovska*, S. Mihova, V. Kirova Urosevic, M. Cvetanovska, K. Grozdanovski, P. Kalamaras

Clinic for Infectious Diseases, Skopje, Former Yugoslav Republic of Macedonia, Clinic for Infectious Diseases, Skopje, Former Yugoslav Republic of Macedonia

At the Clinic of Infectious Diseases from April till October 2005, 93 patients were admitted with clinical signs of aseptic meningitis. The CSF samples obtained by lumbal puncture were cytological, biochemical, bacteriological and serological examined (analyzed). Enteroviral group antigen was detected (confirmed) i 8 CSF samples. The samples were bacteriological and slidex meningite kite test negative. All the patients were at the age of 1—25 years, with predominations of the male (62), and the females were presented with 31. WBC count in CSF ranged from 37 till 658, and the average was 201/mm^3. The lymphocyte predomination in CSF was detected in 82 patients (88%). Biochemical examination of CSF showed protein levels of 0,40g/L in 70 patients (76%), then in 23 patients (24,7%) from 0,40—0,74 g/L and the average value of protein levels was 0,54 g/L. The glucose level in CSF represented 40—60% of serum glucose levels. In 31 patient (33%) low values chlorides were detected. 62 patients (72%) had normal values od lactates in CSF (1,2—2,1 mmol/L). In 26 patients (28%) lactate levels were increased, but no further than 2,7 mmol/L.*Conclusion:* Cerebrospinal fluid in enteroviral meningitis showed an average value of white-blood cell count of 201/mm^3. In majority patients predominant cells in CSF were lymhpocytes, then protein levels were in referent values; glucose levels and the lactates in CSF were

normal. Only in one-quarter (25%) of analyzed CSF samples, the chemical examination showed deviation from normal values.

doi:10.1016/j.ijid.2008.05.552

40.043

Burkholderia pseudomallei Down-Regulates Host Defense Gene Expression in Non-phagocytic A549 Cells

S.J. Chai[1,*], R. Mohamed[2], S. Nathan[2]

[1] *Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia*
[2] *Malaysia Genome Institute, Heliks Emas Block, UKM-MTDC Smart Technology Centre, Universiti Kebangsaan Malaysia, Bangi, Malaysia*

Melioidosis is an endemic disease found in many tropical countries of South East Asia and Northern Australia. This disease is caused by *Burkholderia pseudomallei*, a Gram-negative soil saprophytic bacterium known to invade, survive and multiply in both phagocytic and non-phagocytic cells. Currently, limited information is available regarding the mechanism of host response against this bacterium. In this study, we have utilized the microarray technology to understand the complex interplay between *B. pseudomallei* and its host. A549 human lung epithelia cells were exposed to *B. pseudomallei* clinical isolate R15/05 as well as a less virulent animal isolate Sheep 4523/98 with a M.O.I of 10:1 for 3 and 18 hours. Using the Illumina Sentrix Human-8v2 Expression BeadChip, we monitored changes in gene expression in host cells after exposure to *B. pseudomallei*. Analysis of microarray data showed 39 genes differentially expressed in both the clinical and animal isolate, 3 and 18 hours post infection. Among these 39 genes, many of those involved in the host defense response were down-regulated. These included several cytokines, chemokines as well as genes involved in the NFkB and STAT signaling pathways. The down-regulation of these defense genes might be attributed to the host's response to prevent inflammation in order to survive the pathogen invasion. In addition, this phenomenon might also reflect the ability of *B. pseudomallei* to suppress host cell's defense response, either by manipulating the host innate defense system or interfering with associated signaling pathways. Analysis of the microarray data has helped to shed new light on the *B. pseudomallei* infection process and survival strategy inside its host cell.

doi:10.1016/j.ijid.2008.05.553

40.044

Relationship Between IgeE Antibodies to the *Staphylococcus aureus* Enterotoxin B (SEB) with the Severity of Atopic Dermatitis in Children

S. Kamali Sabzevari*, Z. Pourpak, R. Shokouhi Shoormasti, T. Salehi, M. Moin

Immunology, Asthma and Allergy Research Institute, Medical Sciences/University of Tehran, Tehran, Iran (Islamic Republic of)

Background: The skin of patients with atopic dermatitis (AD) exhibits a striking susceptibility to colonization and infection with *Staphylococcus aureus*. In this context it has been previously shown that *S. aureus*-derived superantigens could function as classic allergens, inducing production of functionally relevant specific IgE antibodies. The aim of this study was to determine the relationship between IgE antibodies to the *Staphylococcus aureus* enterotoxin B (SEB) with the severity of Atopic Dermatitis in children.

Methods: In a cross-sectional study of 30 children with atopic dermatitis, AD is diagnosed based on standard criteria Hanifin and Rajka, clinical severity of AD was determined by the SCORAD index. Specimen for *S. aureus* culture was isolated from 3 different areas of the skin. Total serum IgE and IgE specific to SEB was measured by ImmunoCAP system.

Results: Thirty children, 19 male and 11 female, aged from 3 months to 8 years with AD entered this study. Five of 30 children were sensitized to SEB. The degree of disease severity correlated to a higher extent with the presence of SEB-specific antibodies. Among patients 13.7% were colonized with *S. aureus* producing staphylococcal enterotoxins B. Children colonized with toxigenic S. aureus strains had higher disease severity [SCORAD index of 65.1 ± 18.5 in positive SEB versus 23.9 ± 16.9 in negative SEB group ($P = 0.005$)].

Conclusion: Our results demonstrate a relationship between severity of disease in AD patients and IgE antibodies to SEB. Sensitization to *S. aureus*-derived superantigens may be involved in disease exacerbation. The presence of SEB-specific antibodies had additional explanatory value for disease severity and therefore may be helpful in the characterization of children with severe atopic dermatitis. It is recommended that all Atopic Dermatitis patients be considered for *Staphyloccocus aureus* culture, *Staphyloccocus aureus* Antibody evaluation and prophylactic antistaphylococcal treatment especially in severe cases.

doi:10.1016/j.ijid.2008.05.554

40.045

Pre- and Intra-Operative Risk Factors Which Influence Early Outcome in Infective Native and Prosthetic Aortic Valve Endocarditis. A Single Center Study

P.M. Dohmen[1,*], D. Gabbieri[1], J. Linneweber[1], H. Grubitzsch[1], C. von Heymann[2], K. Neumann[3], W.F. Konertz[1]

[1] *Department of Cardiovascular Surgery, Charite Hospital, Berlin, Germany*
[2] *Department of Anesthesiology, Charite Hospital, Berlin, Germany*
[3] *Department of Biometry and Clinical Epidemiology, Charite Hospital, Berlin, Germany*

Background: In-hospital mortality in patients suffering for infective native and prosthetic aortic valve endocarditis is still high. Purpose of this study was to identify pre-operative and intra-operative predictors of early outcome.

Methods: Between January 2004 and December 2006, 75 patients, mean age 61.6 ± 14.1 years (range 19-85 years), underwent surgical treatment for infective native or prosthetic aortic valve endocarditis. Patients were identified after the modified Duke and Renzulli criteria for infective endocarditis. Pre- and intra-operative variables were analy-