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Foreword

YL Lau
President, Hong Kong Society for Paediatric Immunology and Infectious Diseases

I warmly welcome you all to our First Annual Scientific Meeting of the Hong Kong Society for Paediatric Immunology and Infectious Diseases. Our Society is a young Society with a history of 2 years, yet has been very vibrant and forward looking, especially in promoting education and training in the 3 areas of infectious diseases, immunology and rheumatology. We are grateful to our Guest Speaker Professor Alain Fischer, who has contributed enormously to the field of Paediatric Immunology in the last 3 decades to come to honour our young Society. He is a most generous and warm scholar, always willing to help colleagues and the young generation of paediatricians. He will review for us the exciting topic on Haemophagocytic Lymphohistiocytosis.

Next, I would like to thank our Vice President Dr Leung Chi Wai sincerely in organising this Annual Scientific Meeting, as well as all my Council Members in their hard work to help grow our Society in the last 2 years.

Please enjoy our programme of science and learning, comprising 12 free papers and 2 lectures on this Saturday afternoon.

Haemophagocytic Lymphohistiocytosis – From Molecular Aspects To Diagnosis and Therapy

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Haemophagocytic lymphohistiocytosis (HLH) is an unique immunopathological entity characterised by polyclonal CD8 T lymphocyte activation and expansion associated with macrophage activation. In the absence of therapy, it can be fatal. Several genetic disorders can cause HLH.[1] They share a common immunological feature, i.e. a defective capacity of T and NK lymphocytes to kill target cells through the release of perforin and granzymes from lysosomal secretory granules. These conditions are the X-linked proliferative syndromes, the Hermansky-Pudlak syndrome type II, the Griscelli syndrome, the Chediak-Higashi syndrome and the various forms of familial lymphohistiocytosis. Molecular studies of these disorders have led to identify the role of key molecules, Rab27a, Munc13-4 and syntaxin 11 in the process of cytolytic granules exocytosis.[2-4] It led to propose a model accounting for the ability of cytotoxic T cells to act as a "serial killer" of viral infected cells.[5]

HLH clinical manifestations are most often, albeit not always, triggered by an infection especially a viral infection. It is thought that in the absence of efficient killing of infected cells, ongoing T cell stimulation leads to protracted CD8 T activation, cytokine release and therefore macrophage activation. Usage of chemotherapy consisting in VP-16 was first demonstrated to induce complete remission of HLH. It is still in use by many groups throughout the world.[6] It is however a fairly toxic treatment and not entirely logic because of the known immunological based pathophysiology of HLH. This is why we proposed a T-cell based therapy consisting of anti thymocyte globulins[7] that is indeed efficient without inducing myelosuppression. In all cases genetic forms of HLH require allogeneic stem cell transplantation as curative therapy. So far a 60% cure rate has been achieved[8,9] with a higher chance of success when HSCT is performed in patients who are in clinical remission of HLH. It is conceivable that in the future, more focused therapy, targeting the CD8 T cells or even pathological cytokines could be utilise to control HLH manifestations with a better safety index. There are appropriate animal murine models to test these strategies.[10]

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New Respiratory Viral Infections

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In the last 10 years, there are many new or newly identified respiratory viral pathogens described in human, including (1) SARS-coronavirus, (2) avian influenza viruses (H5N1, H9N2, H7N7, H7N3 and H10N7), (3) human metapneumovirus, (4) coronaviruses NL63 and HKU1, (5) bocavirus and (6) human rhinovirus C. I shall focus on those viruses that Hong Kong has contributed in either discovery or characterisation.

1. Severe acute respiratory syndrome (SARS) is caused by a previously unrecognised coronavirus (CoV) which jumped species and became adapted to be transmissible between humans in 2002.[1] Enormous scientific knowledge about this SARS-CoV has been accumulated and aspects of its pathogenesis defined.[2] However it is still unclear why some members of the same family or housing estate with SARS outbreak were more susceptible to SARS than others. Similarly, why children have a lower incidence of SARS with milder clinical course as compared to adults remains unknown.

   We have therefore studied the polymorphisms of 15 innate immune response genes in a case-control gene association study of over 1,000 SARS patients and controls, and identified several susceptibility genes, which may explain partly the genetic susceptibility to SARS. They are genes encoding for interferon-gamma,[3] p21, RANTES[4] and mannose binding lectin (MBL).[5] Of these, we also demonstrated MBL has direct biological activity against SARS-CoV.[5] This knowledge may also shed lights on therapeutics and prophylactics. We also compared the interferon and chemokine responses to SARS-CoV in adult and cord blood dendritic cells, and identified different developmental responses between neonates and adults as well as high induction of chemokines but low induction of interferons by SARS-CoV.[6] The dysregulation of chemokine and interferon responses in SARS may be mediated through the SARS-CoV non-structural protein 1 (nsp1).[7,8] Moreover, we did a risk-stratified seroprevalence study of SARS-CoV among children living in housing estates with SARS outbreak and in areas with no SARS.[9] We concluded subclinical SARS in children are rare. Hence the lower incidence of SARS in children is not due to subclinical infection, and children will not be ready sources of SARS infection.

2. Avian influenza A virus H5N1 was first transmitted from chicken to human in Hong Kong in 1997.[10] Over 373 human H5N1 cases, associated with multiple recurrent H5N1 outbreaks in poultry, were reported since December 2003 in Asia, Europe and Africa, with mortality of 63%. The pathogenesis of H5N1 infection resulting in such high mortality is still far from clear, but is partly due to the host excessive inflammatory response.[11] We also studied the roles of death receptor ligands in H5N1 infection using human monocyte-derived macrophages (MDMs) as model. We found H5N1-infected MDMs could induce T cell apoptosis, mediated through TNF-related apoptosis-inducing ligand (TRAIL).[12] This may partially explain the severe lymphopenia in human H5N1 infection. Moreover we compared the chemokines and chemokines receptors expression between adult and cord blood MDMs infected with avian influenza viruses as chemokines are implicated in ARDS, and found avian influenza viruses induced higher chemokines and their receptors expression than human influenza viruses. Adult macrophages also had higher expression in CCL3, CCR1 and CCR5 than cord blood macrophages when infected by H5N1 virus.[13] We have preliminary data to suggest such cytokines and chemokines response may be virus strain specific.[14]

3. Human metapneumovirus (hMPV) was first described in 2001 in Netherlands[15] now documented to circulate and infect all children by 5-10 years old in Europe, America, Asia, Australia and S Africa,[16,17] including Hong Kong.[18] hMPV is responsible for URI and LRTI in infants, young children and the elderly. Co-infection of hMPV with other viruses such as SARS-CoV has been documented in Hong Kong and are not associated with more severe clinical course.[19] Interestingly, hMPV-associated LRTI can be reduced by conjugate pneumococcal vaccine, suggesting pneumococcal coinfection with hMPV is of clinical relevance.[20] Compared to respiratory syncytial virus (RSV), hMPV induced less cytokine and chemokine gene expression in alveolar epithelial cells, and could explain in parts the less severe clinical disease of hMPV.[21]

4. Human coronavirus (HCoV) NL63 was first described in a 7-month-old child with bronchiolitis and conjunctivitis in 2004 in Netherlands[22] and HKU1 in an elderly patient with pneumonia in 2005 in Hong Kong.[23] HCoV-NL63 has the same cellular receptor as SARS-CoV, i.e. ACE2 and is associated with URI, croup, asthma exacerbation in children.[24-26] HCoV-

HKU1 is associated with RTI in individuals of all ages and has been reported in Australia, Europe and America.[27,28] Acute enteric disease with HCoV-HKU1 detected in stool has been reported in France[29] but the overall significance of HCoV-HKU1 in gastroenteritis is still not clear.

5. Human bocavirus (HBoV) was first described in 2005 in Sweden in children with RTI,[30] now reported to circulate widely in the world. HBoV is related to parvovirus B19, and co-infection with other respiratory viruses is a frequent feature (>80%), raising issues on the significance of HBoV infection on its own. HBoV can be detected in serum of patients and such viremia suggests HBoV may cause diseases beyond the respiratory tract.[31,32] We have demonstrated HBoV can be detected in faecal specimens in children with acute gastroenteritis.[33] A single lineage of HBoV is associated with both respiratory tract and enteric infection.[33] Serodiagnosis of HBoV infection has been investigated, showing respiratory infections due to HBoV are systemic, and elicit B cell immune responses. Serological diagnosis correlate with high virus loads in nasopharynx and with viremia.[34]

6. A previously unidentified human rhinovirus (HRV) species, HRV-C, has been reported in Hong Kong, Australia and United States.[35] HRV-C is circulating worldwide and an important cause of febrile wheeze and asthmatic exacerbations in children.

Prospective studies of infants with acute respiratory diseases published recently have demonstrated the role of these new respiratory viruses in addition to that of RSV.[36,37] Together HRV, HCoV, hMPV and HBoV accounted for 60% of viruses recovered from these infants.[36]

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Cellular Signaling Events in Cytokines and Sulphamethoxasole Interactions

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Introduction: The use of sulphonamides as antimicrobial agents has been practiced for decades. It is frequently used with another antimicrobial agent trimethoprim to provide a more effective antimicrobial spectrum in treating bacterial and protozoan infections in immunocompromised patients. During bacterial infection, proinflammatory cytokines including tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-12 as well as anti-inflammatory cytokines including IL-4 and IL-10 are produced by macrophages. The complexity of cytokine interactions provides a favorable environment for the host immune system to fight against pathogens. In previous studies, the metabolites of sulphamethoxazole have been demonstrated to have a role in the antimicrobial effects but without the details on molecular mechanisms. In the present study, we delineated the mechanisms and effects of sulphamethoxazole metabolites on cytokine production.

Methods: With the use of primary human differentiated blood macrophages as our model, the cells were treated for 30 minutes with the drug metabolites and followed by the addition of bacterial endotoxin (lipopolysaccharide LPS) for 15 minutes to 3 hours. Cytokine mRNA and protein productions were measured by Quantitative Real-time PCR and ELISA, respectively. For the investigation of signaling events including the role of kinases and transcription factors, cellular and nuclear protein fractions were collected and analysed by specific Western blot assays.

Results: The results showed that the LPS-induced cytokines including TNF-α, IL-6 and IL-10 were downregulated by the sulphamethoxazole metabolites both at the transcription and translation levels. Since the expression of cytokines is mediated by the action of signaling kinases such as mitogen-activated protein kinases (MAPK) and transcription factors, we measured the activity status of MAPK in the sulfphamethoxazole metabolites-treated macrophages. The results showed that sulphamethoxazole metabolites abrogated the LPS-induced MAPK phosphorylation, concomitant with their effects on cytokine downregulation. Furthermore, the activation of nuclear factor-κB (NF-κB) induced by LPS was also suppressed by the metabolites.

Conclusion: In conclusion, our data elucidated that in addition to their antimicrobial effects, sulphamethoxazole and its metabolites may play a role in limiting the propagation of uncontrolled inflammation, via the suppression of MAPK and NF-κB activities, in microbial infections.

Post-chemotherapy Booster Diphtheria-Tetanus-Pertussis Vaccination in Children with Haematological Malignancy

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Introduction: The role of post-chemotherapy booster vaccination in children who have completed treatment for haematological malignancy remains to be established. We evaluated the effects of chemotherapy on humoral immunity to vaccine-preventable diseases and the booster immune responses to diphtheria-tetanus-pertussis (DTP) revaccination in children with haematological malignancy.

Methods: Children aged 1-18 years old with haematological malignancies with chemotherapy terminated for 6 months (baseline) were eligible. Subjects were randomised into vaccine and control group. In the former, one dose of DTP vaccine (Aventis Pasteur Inc., USA) was administered. IgG antibody titers against diphtheria, tetanus, pertussis and hepatitis B were measured by enzyme immunoassay at baseline and 2 months after vaccination. Protective antibody levels against diphtheria, tetanus, pertussis and hepatitis B were set at 0.1 IU/ml, 0.1 IU/ml, 24 U/ml and 10 mIU/l, respectively.

Results: Twenty-nine children (15 vaccinees and 14 controls) were recruited, with similar (p=0.85) mean (SD) ages being 8.2 (3.56) and 8.4 (3.83) years. Twenty-four children had acute lymphoblastic leukaemia and 4 children had acute myeloid leukaemia. Protective antibody levels against diphtheria, tetanus, pertussis, and hepatitis B were found at baseline in 97%, 83%, 86% and 17% of them. After one dose of DTP, all vaccinees demonstrated protective specific antibody levels with significant surges (median [interquartile range]) in antibody levels against diphtheria (139 [37-154] IU/ml; p<0.001), tetanus (470 [34-805] IU/ml; p<0.001) and pertussis (631 [90-906] U/ml; p=0.002). There were no significant changes in antibody levels against diphtheria (p=0.45), tetanus (p=0.36) and pertussis (p=0.12) in the control group, or against hepatitis B (p=0.27) in both groups.

Conclusion: Majority of children with haematological malignancy demonstrated good IgG responses to one booster dose of DTP vaccine at 6 months after cessation of chemotherapy.

Funding support: Hong Kong Paediatric Bone Marrow Transplant Fund Research Grant.

Conflict of Interest Statements: Nil to declare.

Post-chemotherapy Booster DTP Vaccination in Children with Solid Tumours

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Introduction: We evaluated the effects of chemotherapy on humoral immunity to vaccine-preventable diseases and the booster immune responses to diphtheria-tetanus-pertussis (DTP) revaccination in children with solid tumours.

Methods: Children aged 1-18 years old with solid tumours and with chemotherapy terminated for 6 months (baseline) were eligible. Subjects were randomised into vaccine and control group. In the former, one dose of DTP vaccine (Aventis Pasteur Inc., USA) was administered. IgG antibody titers against diphtheria, tetanus, pertussis and hepatitis B were measured by enzyme immunoassay at baseline and 2 months after vaccination. Protective levels against diphtheria, tetanus, pertussis and hepatitis B were set at 0.1 IU/ml, 0.1 IU/ml, 24 U/ml and 10 mIU/l respectively.

Results: Twenty-seven (13 vaccinees and 14 controls) children were recruited, with similar (p=0.38) mean (SD) age being 9.4 (3.8) and 8.0 (4.2) years. Eight children had osteosacroma, 5 had brain tumour, 5 had lymphoma, 2 had rhabdomyosarcoma and 7 had Wilm's tumour, clear cell sarcoma, neuroblastoma, germ cell tumour and nasopharyngeal carcinoma. Protective antibody levels against diphtheria, tetanus, pertussis and hepatitis B were found at baseline in 100%, 100%, 86%, and 45% of them. All vaccinees demonstrated protective specific antibody levels with significant surge (median [interquartile range]) in antibody levels against diphtheria (36 [12.0-72.5] IU/ml; p=0.03), tetanus (434 [172.0-620.0] IU/ml; p<0.001) and pertussis (484 [100.5-3503.5] U/ml; p=0.002). There were no significant changes in antibody levels against diphtheria (p=0.36); tetanus (p=0.51) and pertussis (p=0.59) in control group, or against hepatitis B (p=0.56) in both groups.

Conclusion: Children with solid tumours demonstrated good IgG responses to one booster dose of DTP vaccine at 6 months after cessation of chemotherapy.

Funding support: Hong Kong Paediatric Bone Marrow Transplant Fund Research Grant.

Conflict of Interest Statements: Nil to declare.

HIV-1 Tat Plays a Role in Dysregulating Lipopolysaccharide-induced Cytokine Expression: Implications for Immune Defects in AIDS

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Introduction: During bacterial infection, macrophages/monocytes are activated by lipopolysaccharides (LPS), the bacterial cell wall-associated endotoxins, to trigger innate and adaptive immune responses. However, these responses are impaired in HIV-infected patients especially in children, and such immune defects may contribute to the higher incidence of secondary bacterial infections and rapid progression of AIDS. The mechanisms on how HIV impairs these immune responses are not fully understood. Previous reports including ours indicated that Tat, the transactivator for transcriptional activation of the HIV genome, is partly responsible for mediating the retrovirus-induced subversion of immunity and enhancement of HIV replication. Therefore, we hypothesise that Tat plays a role in the dysregulation of the LPS-induced immune responses, thereby contributing to the pathogenesis of AIDS.

Methods: Primary human blood monocytes were pretreated with recombinant Tat protein prior to LPS addition. Expression levels of specific cytokines were assayed by Q-RTPCR and ELISA. Levels of signalling kinases and nuclear factors were examined by Western analysis.

Results: Our results demonstrated that Tat differentially suppresses the LPS-induction of IFN-β but enhances the induction of IL-6. On the contrary, Tat was shown to have a slight enhancing effect on the LPS-induction of TNF-α. To investigate the underlying mechanisms of Tat in cytokine dysregulation, we showed that the HIV protein inhibits LPS-induced activation of ERK1/2 but not p38 MAP kinase. We also demonstrated that Tat suppresses LPS-induced degradation of IκBα, resulting in the release and activation of NFκB for subsequent transcription of downstream cytokines and targeted genes.

Conclusion: Taken together, these results imply that Tat may suppress the host anti-viral responses due to IFN-β suppression and yet promote HIV replication via the enhancement of IL-6 expression. Hence, during Gram-negative bacterial infection in AIDS patients, Tat may play a role in dysregulating the immune responses induced by the bacteria for providing a favourable environment for HIV survival and replication.

Conflict of Interest Statements: There is no conflict of interest. Parts of the results were presented in Annual Scientific Meeting 2008, Hong Kong Society for Immunology. (Supported in part by HK Research Grants Council, HKU7408/04M and HKU7594/06M)

Modified Clinical Manifestations of Measles in Young Infants <7 Months Old

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Introduction: Measles still causes significant morbidity and mortality and the diagnosis relies on the early recognition of clinical manifestations. We identified a group of young infants who presented with modified clinical courses after contracting measles, but there is scarce literature information concerning this area.

Methods: We conducted a retrospective study in a tertiary referral centre of Hong Kong including 131 infants <1 year old with measles confirmed by serological or virological methods over a period of 8 years. The study population (N=114) was divided into 2 groups: 25 infants are <7 months and 89 infants 7-12 months of age. Their clinical manifestations were compared and analysed, which include the onset of skin rash in relation to fever, fever duration, maximum temperature, presence of coryzal symptoms, cough, conjunctivitis, Koplik's spots, staining of convalescent rash, and other associated symptoms and complications.

Results: The duration of fever was significantly shorter and the onset of skin rash earlier in the younger age group ($p<0.001$ and $p=0.026$ respectively). Besides, there were significantly less conjunctivitis ($p=0.003$), coryzal symptoms ($p<0.001$) and staining of skin rash during convalescence ($p=0.014$) in infants <7 months old. There were no significant differences for the presence of Koplik's spots ($p=0.08$), cough ($p=0.61$), pneumonia ($p=0.68$) and the use of anti-microbial agents ($p=0.87$).

Conclusion: Our study revealed the milder manifestations of measles in young infants <7 months of age. We should maintain a high index of suspicion for measles in young infants presenting with fever and rash. Early use of rapid diagnostic test should be considered in such situation in order to facilitate early diagnosis and appropriate isolation measures to interrupt transmission and prevent potential outbreak.

Conflict of Interest Statements: None.

The Pattern and Progression of Multiple-Tree Nut Allergy in Peanut Allergic Children

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Background:

- Tree nuts, sesame seeds and peanuts are not taxonomically related
- However, tree nut and sesame allergy are commonly associated with peanut allergy
- Avoidance of tree nuts is a common precaution in peanut allergic children, irrespective of tree nut sensitisation status
- Delaying the introduction of peanuts and tree nuts beyond 2-3 years of age has been recommended in both the US and UK Ewan *Lancet 1998, American Academy of Pediatrics 2004*
- This strategy has now come into question due to a lack of evidence of impact in reducing the incidence of nut allergy *Greer et al. Pediatrics 2008, Lack NNWSPP 2007*

Methods:

- Consecutive referrals of infants <2 yo with presumed peanut allergy based on:
  1) Unequivocal history of immediate reaction, and/or
  2) >95th positive predictive value for clinical reaction using skin prick test (SPT) wheal diameter ≥4 mm *Hill et al, Pediatr Allergy Immunol 2004;15(5):435-41*
- Baseline SPT to peanuts, cashew, hazelnut, and sesame, as well as peanut-specific serum IgE antibody levels, were documented. All patients were advised to strictly avoid all peanut, tree nuts, and sesame seeds and were followed up 1-2 yearly with SPT and assessment for up to 10 years
- At 4 years of age [median], a SPT panel of 7 additional tree nuts (walnut, pecan, Brazil nut, almond, macadamia, pine nut and pistachio), together with peanut, cashew, hazel nut and sesame, was conducted.
- These tests were completed 1-2 yearly until the patient was clinically tolerant or had passed a formal food challenge for each food.

Results:

- 267 consecutive children were diagnosed with peanut allergy during the 5 year study period.
- At initial assessment (mean age 14 months), 121 (45%) of patients were sensitised to 1 or more tree nuts and 67 (25%) to sesame.
- By age 4, 66% (147/223) were sensitised (SPT ≥3 mm), and 40% (89/223) were clinically allergic to at least 1 tree nut despite advice regarding strict dietary avoidance of all nuts.
- 30% (66/223) were sensitised to ≥3 tree nuts.

Conclusion:

- The prevalence of tree nut sensitisation in this cohort of peanut allergic children increased despite education to avoid all nuts, including tree nuts.
- The mechanisms of de novo sensitisation to tree nuts in peanut allergic children who are avoiding these nuts is unknown.

Clinical Implications:

Peanut allergic children should be monitored for the development of tree nut and sesame sensitisation and allergy

None to declare conflict of interest.

Grant:

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Human Rotavirus Vaccine RIX4414 (*Rotarix*™) is Highly Efficacious in Hong Kong During the First Two Years of Life

Hong Kong Rota-029 Study Group

Introduction: The oral live attenuated human rotavirus vaccine RIX4414 (*Rotarix*™) has been shown to be highly effective for the prevention of rotavirus (RV) gastroenteritis (GE) in Europe and Latin America. Infants participating in a phase III, double-blind, randomised, placebo-controlled and multi-centre trial conducted in Singapore, Hong Kong and Taiwan (e-track107070, 107072,107076/ NCT444563/ 028/029/030) were followed up to approximately two years of age to assess protection against severe RVGE. The individual results for Hong Kong are presented here.

Methods: Three thousand and twenty-five healthy infants (28.2% of total enrolled for this study), 6-12 weeks of age at Dose 1 were enrolled in Hong Kong and randomised into two groups (1:1) to receive 2 doses of RIX4414 vaccine or placebo at a 0,2 month schedule. Routine childhood vaccinations were given concomitantly. Vaccine efficacy (VE) was calculated from 2-weeks post-Dose 2 until approximately 24 months of age. Severity of RVGE was assessed using the 20-point Vesikari scale

(severe RVGE ≥11 on the Vesikari scale). A GE episode was defined as occurrence of diarrhoea (three or more looser than normal stools, within a day) with or without vomiting. Diarrhoeal stool samples were analyzed for RV by ELISA and typed by RT-PCR based method followed by a reverse hybridisation assay. Safety data was collected throughout the study.

Results: RIX4414 and placebo group had a similar demographic profile. During the efficacy follow-up period (mean duration of 18 months), 1 (0.1%) severe RVGE was reported in the RIX4414 group and 23 (1.5%) in the placebo group (p-value <0.001). The dominant G-types observed during this surveillance period were G1, G3 and G9. The VE against severe RVGE due to circulating wild-type RV was 95.6% (95% CI: 73.1%; 99.9%). In the placebo group there was a 1 in 65 risk of hospitalisation due to RVGE and a 1 in 15 risk of hospitalisation due to all cause GE. An overall reduction of 36.8% (95%CI:12.5;54.6) in hospitalisation due to all cause GE was noted for the vaccine group. There was no evidence of difference between the RIX4414 and placebo group for non-GE related SAEs reported during the study.

Conclusion: These results demonstrate that in Hong Kong two oral doses of RIX4414 (*Rotarix*™) offer high and sustained protection against severe RVGE during the first two years of life when the disease burden is highest. These data are in line with efficacy results obtained in Singapore and Taiwan as well for a multicountry study in Europe.

Mycobacterial Evasion of Immunity via Cytokine Dysregulation: A Potential Role for Mitogen-Activated Protein Kinase Phosphatase-1

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Introduction: *Mycobacterium tuberculosis* (MTB) is a major cause of morbidity and mortality in the world. To combat against this pathogen, immune cells release cytokines including tumour necrosis factor (TNF)-α, which plays a pivotal role in the development of protective granulomas consisting of macrophages and other immune cells to contain the mycobacteria. Our previous results showed that Bacillus Calmette Guerin (BCG), a mycobacterial model used to investigate the immune response against MTB, stimulates the induction of TNF-α via a double-stranded RNA-dependent protein kinase (PKR) and mitogen-activated protein kinase (MAPK) in human blood monocytes. Since MAPK is regulated by MAPK phosphatase-1 (MKP-1) in response to lipopolysaccharide (LPS), the involvement of MKP-1 in BCG-induced MAPK activation and its consequent cytokine expression was examined.

Methods: Primary human blood monocytes were treated with BCG and assayed for MKP-1 expression by quantitative RT-PCR and Western analysis.

Results: Our results demonstrated that following exposure to BCG, there was an increase in the expression of MKP-1. Additionally, there was a significant abrogation of BCG-induced MKP-1 expression in the presence of the p38 MAPK and ERK1/2 inhibitors. The results suggested that the induction of MKP-1 was regulated by p38 MAPK and ERK1/2 in response to BCG activation. Next, the roles of MKP-1 in BCG-induced MAPK activation and TNF-α expression were elucidated with the use of MKP-1 siRNA for gene-specific knock-down. Surprisingly, when MKP-1 was blocked by MKP-1 siRNA, there was a significant decrease in the levels of phospho-MAPK and TNF-α inducible by BCG, indicating the phosphatase plays a pivotal role in cellular defense against mycobacterial infection.

Conclusion: Taken together, MKP-1 plays a critical role in the regulation of TNF induction in response to mycobacterial infection, and its induction may suggest an effective host defense mechanism to combat the pathogen invasion.

Conflict of Interest Statements: There is no conflict of interest (supported in part by grants to ASL from RFCID and RGC-CERG).

Bartonella Henselae Infection Presented as a Tender Groin Swelling

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An 8-year-old boy who enjoyed good past health presented acutely with a painful left groin swelling. There was no other site of involvement. The pain was so severe that the initial diagnosis was an acute surgical emergency, an incarcerated inguinal hernia. Urgent operation was performed and multiple enlarged inguinal lymph nodes were identified intraoperatively. The lymph nodes were matted together with perinodal inflammation and fibrosis. Histologically, there were aggregates of histiocytes with

central prominent necrosis in which variable neutrophilic infiltration and karyorrhexis were seen. Other areas showed marked lymphoid follicular hyperplasia and scattered epithelioid histiocytes. Caseous necrosis and Langhan's giant cells were absent. There was no evidence of malignancy. Special stains including Ziehl-Neelsen, PAS and Warthin-Starry stains were all negative. The overall features were consistent with suppurative granulomatous lymphadenitis. Immunological staining for *Bartonella henselae* demonstrated many short bacilli in the centres of the suppurative granulomas. Serological test for *Bartonella* antibody was positive. The diagnosis was confirmed to be cat-scratch disease.

To exclude the possibility that other lymph nodes or organs were involved, CT thorax and abdomen was performed and findings were normal. Azithromycin was administered for 2 weeks. There was no recurrence of the disease during subsequent follow-up.

On detailed questioning, the parents revealed a history that the family kept a cat at home but the boy could not recall whether he had been scratched by the cat or not. *Bartonella henselae* infection was rare in Hong Kong. It generally presents as regional lymphadenopathy, which suppurates in 30% of cases. Cat is a common reservoir of the bacteria. Up to 90% of infected individuals have a history of contact with apparently healthy cats or kittens. Histological examination of the affected lymph node commonly showed the presence of epithelioid granulomas. Diagnosis required the demonstration of the organism in the histological sample or a positive serum antibody to the bacteria.

Conflict of Interest Statements: This is to declare that there is no conflict of interest.

An Adolescent Girl with Persistent Headache

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Introduction: *Cryptococcus neoformans* is a ubiquitous encapsulated yeast which can infect immunocompromised and, less commonly, immunocompetent patients. However, this fungal infection is infrequently reported in the paediatric population. We report a case of cryptococcal meningitis in a previously healthy adolescent girl who presented with persistent headache. She recovered without neurological sequelae.

Case Report: A healthy 14-year-old Chinese girl was admitted to Princess Margaret Hospital with complaints of fever, headache and weight loss for eight weeks. She had stayed in a rural area of China (Fujian) for two months before traveling back to Hong Kong for medical advice. There was no known contact with birds or bird droppings. Physical examination revealed a thin and tired girl with high fever. Neck stiffness and bilateral optic disc swelling were noted on physical examination. The rest of the physical examination was unremarkable. Complete blood count showed normal white cell count of 7 x 10^9/L, and a neutrophil count of 4.9 x 10^9/L. Her haemoglobin level was 12.3 g/dL. Erythrocyte sedimentation rate was elevated to 44 mm/hour but C-reactive protein was less than 1 mg/L. Urgent contrast CT scan of the brain showed meningeal enhancement with no gross cerebral oedema. Lumbar puncture revealed cerebrospinal fluid (CSF) leucocytosis of 98 cells per cubic mm, high protein level of 1.08 g/L and low glucose level of 0.9 mmol/L. Cerebrospinal fluid cryptococcal antigen titre was 512. *Cryptococcus neoformans* was also detected by Indian ink staining and later confirmed by culture. Her chest radiograph was normal and tuberculin skin test was negative. HIV antibody test was negative as well.

She was commenced on a six-week course of intravenous amphotericin B and oral flucytosine. Treatment was well tolerated apart from initial mild renal impairment which resolved with adjustment of drug dosage. She had gradual resolution of fever and headache one week after admission. Follow-up Magnetic Resonance Imaging (MRI) examination of the brain one week later showed meningoencephalitis without any evidence of cerebral infarction or hydrocephalus. Lumbar puncture was repeated two weeks and four weeks after treatment. The cryptococcal antigen titre dropped from 512 to 8. Fungal culture was negative. Immune function tests including lymphocyte subset profile, lymphocyte proliferation assay, immunoglobulin levels and complement activity were normal.

Discussion: Cryptococcal meningitis is an uncommon but potentially fatal disease which may affect immunocompetent children. Early diagnosis with appropriate anti-fungal treatment is essential. Clinical suspicion of the disease should be raised in children who present with indolent or atypical meningitis, with or without history of contact with birds or bird droppings. Many questions still remain unanswered regarding the optimal management and prognostic factors of cryptococcal meningitis in children. Further studies are required to improve our understanding of this rare infection in children.

Clinical, Immunological and Molecular Characteristics of 67 Chinese patients with X-linked Agammaglobulinaemia

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Background: X-linked agammaglobulinaemia (XLA) is caused by an arrest of B cell development arising from Bruton's tyrosine kinase (*Btk*) gene mutations. To date over 1000 patients were registered in the international *Btk* mutation database, but there is lack of systematic data on XLA in the Chinese population.

Objective: To describe the spectrum of clinical presentations and *Btk* gene mutations in a large Chinese cohort, and to predict the functional consequences of mutations based on structure information and analysis of sequence conservation during evolutionary courses.

Methods: From 1988-2007, 16 children with XLA were evaluated in our unit. One patient from Singapore and 50 patients originating from 11 provinces in Mainland China were referred for molecular diagnosis. Demographic and clinical data were collected. *Btk* mutation analysis was performed on genomic DNA by direct sequencing.

Results: All patients diagnosed to have XLA had history of recurrent bacterial infections, panhypogammaglobulinaemia, and markedly reduced numbers of B-lymphocytes in the peripheral circulation (all <2%). The mean age of symptom onset was 1.95±2.06 years while the mean age at diagnosis was 7.1±4.06 years. Recurrent respiratory tract infections (97.6%), otitis media (39.2%) and sinusitis (21.6%) were the most common infections, and we observed a high percentage of patients having arthritis (33.3%). Fifty-five patients were confirmed to have *Btk* mutations, in which 6 were de novo mutations. Among the 50 mutations, missense mutations were the most common (n=20, 40%) followed by frameshift mutations (n=13, 26%) and splice site mutations (n=8, 16%). Patients with frameshift mutations involving variant sites and missense mutations involving residues which are not conserved, or located outside secondary structural elements were considered to be 'mild' mutations while nonsense, frameshift, gross exon deletions and missense mutations at conserved residues or important binding sites were considered as 'severe' mutations. The mean age of onset of the two groups (3.0±2.5 years and 1.7±1.8 years, respectively) approached statistical significance (p=0.072).

Conclusion: This report summarised the diverse spectrum of mutations and clinical heterogeneity observed in Chinese XLA patients. Analysis of the mutations and predicted alteration in protein function revealed a possible genotype-phenotype correlation. The characterisation of disease pattern and mutation spectrum of XLA in Chinese is useful in raising the awareness this rare disorder in the Chinese population.

Epidemiology of Community-Acquired Urinary Tract Infection Due to Extended-Spectrum β-Lactamase Producing *E. Coli* and *Klebsiella Species* in Paediatric Population: A Case-Control Study

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Introduction: Community-acquired extended-spectrum Beta-lactamase producing *E. Coli* and *Klebsiella* (ESBL-EK) infection had been increasingly recognised from adult studies, however, relatively little data is available from the paediatric perspective. This study aimed to determine the incidence, outcome and risk factors associated with paediatrics community-acquired ESBL-EK urinary tract infection (UTI).

Methods: *E.Coli* and *Klebsiella spp.* from urinary isolates of paediatric patients admitted for UTI from 1st January 2000 to 31st December 2006 were identified via microbiology database of the study hospital. Clinical and demographic data was obtained from medical record. Case-control study was performed on subgroup of community-acquired UTI, with ESBL-EK UTI as cases and UTI due to non-ESBL producing strains as control. Potential risk factors, outcome and resistant pattern were recorded.

Results: Five hundred and twenty-eight episodes of *E.Coli* or *Klebsiella spp.* UTI were identified, of which 467 were community-acquired. Thirty-five episodes of ESBL-EK UTI were identified, 26 episodes being community-acquired (74%). Incidence of ESBL-EK UTI increased from 5.7% in 2000 to a peak of 17.9% in 2004 and remained high thereafter. All ESBL-EK were sensitive to imipenum and amikacin, but significant resistance was noted to trimethoprim-sulfamethoxazole (68%), gentamicin (58%) and ticarcillin/clavulanate (54%). Risk factors associated with community-acquired ESBL-EK UTI from the univariate analysis included:

history of hospitalisation within the past 1 year (p= 0.01); use of any antibiotics 30 days prior to the onset of UTI (p<0.01); use of second-generation cephalosporin (p<0.01); trimethoprim/sulfamethoxazole (TMP/SMX) (p=0.01) and ampicillin/amoxicillin (p=0.05). From the multivariate logistic regression model, only prior use of second-generation cephalosporin was significantly associated with ESBL-EK UTI. No significant difference was recognised in all the outcome measurements.

Conclusion: Definite increase of community-acquired ESBL-EK UTI was observed in paediatric population. Recent exposure to second-generation cephalosporin was identified as a significant independent risk factor, thus more judicious use of antibiotics is a key factor in limiting the increment of ESBL-EK caseload. Early identification of at risk children and prompt initiation of appropriate empirical antibiotics is also vital.

Conflict of Interest Statements: Nil.