

Laboratory for Clinical Research on Infectious Diseases

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Research Projects

The research activities in our department aim to elucidate the immune mechanisms that can control and eliminate acute respiratory infections (ARIs), with the aim of developing effective vaccines against these microorganisms. We also seek to understand the mechanisms by which dengue virus infects the human host and how these infections can be treated. In addition, our group is registered as a member of the World Health Organization (WHO)/Global Outbreak Alert & Response Network (GOARN) and will join the outbreak response team in the global effort to control emerging and re-emerging infectious diseases.

A) Research related to ARIs

ARIs, primarily pneumonia, are associated with high morbidity in children and adults and are the leading cause of death in children. The two main bacterial pathogens responsible for pneumonia are *Streptococcus pneumoniae* and *Haemophilus influenzae*. Since there is an increase in the prevalence of drug-resistant respiratory pathogens, it is strongly recommended that antimicrobial agents are appropriately used in both children and adults. In addition, it is desirable not only to increase the use of the presently available pneumococcal polysaccharide vaccine but also to develop new pneumococcal vaccines.

1. Study of ARIs in Thailand

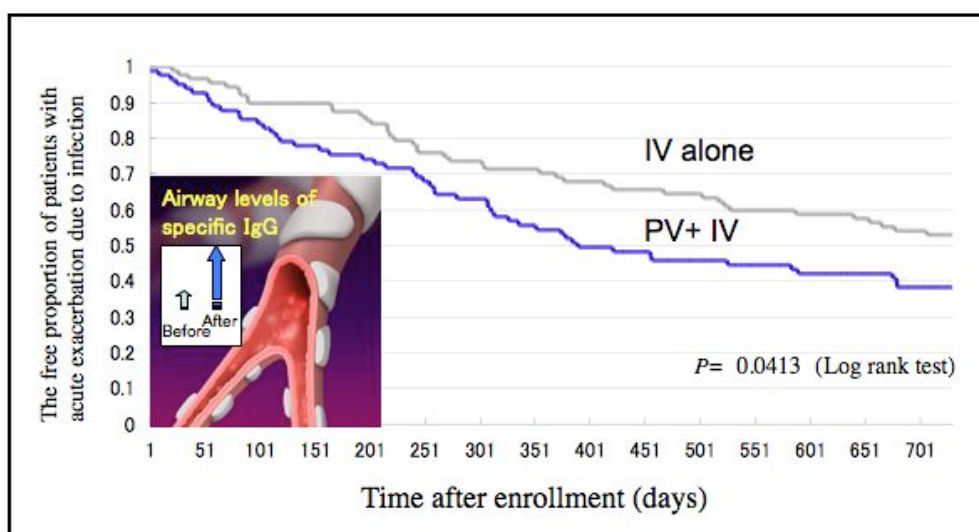
A new research proposal entitled “Surveillance of Emerging Respiratory Infections and Analysis of Mechanism of Secondary Bacterial Pneumonia in Thailand,” which will be undertaken in collaboration with Dr. Kawakami, Tohoku University and the Formation Program of the Research Center for Emerging and Reemerging Infectious Diseases, is the most important project of our laboratory. This study involves both an epidemiological component and a pathophysiological component. The former involves the identification of pathogenic viruses and bacteria-associated ARIs and will serve as a surveillance system for emerging viral infections in Thailand. The pathophysiological component of the study involves the analysis of how virus-host-bacteria interactions promote secondary bacterial infections, through up-regulation of bacterial receptors that increase bacterial adherence and growth on airway epithelial cells. Moreover, we are seeking to identify microbial or host factors that promote the occurrence, severity and poor prognosis of community-acquired pneumonia. This study involves the Thailand–Japan Research Collaboration Center on Emerging and Re-emerging Infections and three hospitals in Bangkok.

2. Clinical applications of the pneumococcal polysaccharide vaccine and the development of new pneumococcal vaccines

a) Clinical application of a 23-valent capsular polysaccharide vaccine (PV)

We recently carried out a prospective study to determine the effect of PV in combination with influenza vaccine (IV) in patients with chronic lung diseases (CLD). We found that the combined vaccination program reduced the incidence of acute exacerbation in patients with chronic obstructive pulmonary diseases (COPD). While PV had an additive effect in combination with IV in terms of acute exacerbation due to infection during the first year after vaccination, this was not observed during the second year. Since immune response to PV was primarily found during the first year after vaccination, the clinical effect of PV seemed to be associated with a serotype-specific immune response. We also started a project in 2008 that examines the effects of PV in combination with IV on long-term-care residents. Our goal is an implementation of nationwide routine vaccination of the elderly in Japan.

Figure 1. Comparison of the frequency of patients who showed acute exacerbation of COPD after being vaccinated with PV+ IV or IV alone. (PV: pneumococcal vaccine, IV: influenza vaccine)



b) Application of pneumococcal conjugate vaccine in adults

HIV-infected people are susceptible to pneumococcal infections such as pneumonia but the efficacy of PV in these people is limited. We therefore examined whether HIV-infected adults in Uganda would mount an immunological response to a pneumococcal conjugate vaccine (CV) if it was administered before the introduction of antiretroviral therapy (ART). While HIV-infected subjects with peripheral CD4 lymphocyte counts of 200–500/ μ l exhibited decreased serum opsonic activity before vaccination, CV vaccination significantly elevated opsonic activity and serotype-specific serum IgG levels. A single dose of CV could afford protective immunity in HIV-infected African adults before the introduction of ART.

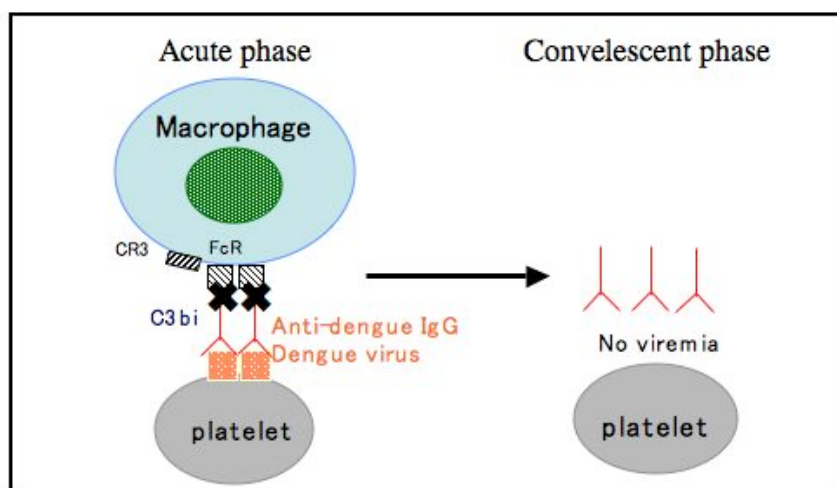
c) Development of a nasal mucosal pneumococcal surface protein A (PspA) vaccine

PspA is a cross-reactive, choline-binding protein that is expressed by all pneumococci and is known to elicit protective antibodies in animals. To develop a cost-effective pneumococcal vaccine that can be used with infants and the elderly, who are at high risk for pneumococcal diseases, we are currently investigating possible mucosal adjuvant candidates (such as Flt 3 ligand DNA and TLR ligands) that can be used in combination with the PspA antigen.

B) Mechanisms with which dengue virus infections lead to thrombocytopenia.

We examined patients with acute-phase secondary dengue virus infections in a prospective hospital-based study in the Philippines to determine the relationship between platelet-associated IgG (PAIgG) and IgM (PAIgM) levels and thrombocytopenia. An inverse correlation between platelet counts and PAIgG or PAIgM levels was found in these patients, and anti-dengue virus IgG and IgM activity was found in the platelet eluates. These data suggest that platelet-associated immunoglobulins with anti-dengue virus activity play a pivotal role in the induction of thrombocytopenia in secondary dengue virus infections (Figure 2). Notably, we recently found, in an *ex vivo* setting, that patients with thrombocytopenia during acute phase secondary dengue virus infections showed increased phagocytosis of platelets (unpublished data). When we performed a randomized, controlled study of patients with secondary dengue virus infections who were treated both with and without a high dose of IVIG, we found that IVIG treatment did not significantly hasten the recovery from thrombocytopenia. This suggests that Fcγ receptor-mediated platelet clearance by macrophages does not play a major role in the mechanisms that lead to thrombocytopenia in patients with secondary dengue virus infections.

Figure 2. The thrombocytopenia resulting from secondary dengue virus infections may result from a platelet-associated IgG (PAIgG)-mediated mechanism.



C) Response to emerging and re-emerging infectious diseases

GOARN was organized to serve as a surveillance network system that would rapidly detect the outbreak of infectious diseases, identify their causes, and improve clinical management. The objective of GOARN is to combat the international spread of such outbreaks by ensuring that appropriate technical assistance rapidly reaches affected areas and by promoting long-term epidemic preparedness. Our group is registered as a member of GOARN and participated in a regional training course on the International Outbreak Response in November, 2007. Our team will join the WHO-organized response team when there is an outbreak of infectious diseases in developing countries.

Recent publications

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2. Kurita, S., Koyama, J., Onizuka, S., Motomura, K., Watanabe, H., Watanabe, K., Senba, M., Apicella, M.A., Murphy, T.F., Matsushima, K., Nagatake, T., and Oishi, K. (2006). Dynamics of dendritic cell migration and the subsequent induction of protective immunity in the lung after repeated airway challenges by nontypeable *Haemophilus influenzae* outer membrane protein. *Vaccine*. **24**, 5896-5903.
3. Chen, M., Hisatomi, Y., Furumoto, A., Kawakami, K., Masaki, H., Nagatake, T., Sueyoshi, Y., Iwanaga, T., Aizawa, M., and Oishi, K. (2007). Comparative immune response of patients with chronic pulmonary diseases during the 2-year period after pneumococcal vaccination. *Clin. Vac. Immunol*. **14**, 139-145.
4. Dimaano, E., Saito, M., Honda, S., Miranda, E.A., Alonzo, M.T., Valerio, M.D., Mapua, C.D., Inoue, S., Kumatori, A., Matias, R., Natividad, F. F., and Oishi, K. (2007). Lack of efficacy of high dose intravenous immunoglobulin treatment of severe thrombocytopenia in patients with secondary dengue virus infection. *Am. J. Trop. Med. Hyg.* **77**, 1135-1138.
5. Furumoto, A., Ohkusa, Y., Chen, M., Kawakami, K., Masaki, H., Sueyasu, Y., Iwanaga, T., Aizawa, H., Nagatake, T., and Oishi, K. (2008). Additive effect of pneumococcal vaccine and influenza vaccine on acute exacerbation in patients with chronic lung disease. *Vaccine* (in press).