行政院國家科學委員會補助專題研究計畫

成果報告

（計畫名稱）

參與登革出血熱的遺傳因子(3/3)

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中華民國 97 年 5 月 29 日
中文摘要
登革病毒感染造成的疾病可以從輕微的登革熱至嚴重的登革出血熱，若沒有即時的支持性治療，登革出血熱的死亡率高達 10-45%。國際衛生組織估計全世界每年有 9,000,000 登革熱及 500,000 登革出血熱，它對人類帶來的衝擊可比擬瘧疾、肺結核或肝炎。台灣也不能免於此疾病的威脅。大高雄地區在民國 91 年爆發登革病毒感染疫情，確定個案總共有 5278 例，其中 240 例為登革出血熱，造成二十一例死亡個案，登革出血熱病例的死亡率高達 8.8%，也比東南亞的 1%高出很多。登革熱感染的防疫與控制已成為臺灣重要的健康問題，疾管局一向把登革熱的防治列為最重要的工作目標。登革病毒四型台灣都有，出現登革出血熱／登革休克症候群（DHF/DSS）的機會越來越大，登革病毒感染引起的登革熱是輕微的疾病，但 DHF/DSS 如果沒有早期適當的輔助治療會是致命的。登革病毒感染的致病機轉不是很清楚，早期的抗体依靠增強反應假說只說明流行病學上第二次再感染時，如果是不同型，則易引起嚴重的 DHF/DSS，但對發生過程機轉的說明力有未逮，完全沒有任何解釋。我們提出了一個登革出血熱是一個急性類似噬血症候群疾病的假說：登革病毒感染會引發免疫反應的不正常活化，登革病毒的 prM, NS-1 和血小板及內皮細胞間有構造上的分子模擬，引起自體免疫疾病，產生抗血小板及內皮細胞自体抗体，同時產生過多的細胞激素，尤其是干擾素-γ，造成巨噬細胞的活化，去吞噬抗體結合的血小板及，內皮細胞，因而造成血小板減少及血管壁滲透增加，引發凝血和溶血的不平衡，形成登革出血熱及嚴重的登革休克症候群。利用 2002 年發生於大高雄地區的登革熱大流行，其中有 DHF 的高死亡率（8.8%），分析其危險因子和下列有關：(1) 年齡大於 55 歲，(2) 有潛在性的疾病，(3) 患者發病時有不正常的血液及生化指標，(4) 過多的細胞激素，(5) 二次登革感染，(6) 過高的自體抗體，(7) 過多的 ferritin。以急性類似噬血症候群的免疫致病機轉能解釋登革病毒感染引起登革出血熱特有的臨床症狀，病理的變化，以及過去眾多的流行病學的觀察。

英文摘要
Dengue virus infection causes dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), but its pathogenesis is not clearly demonstrated. A new hypothesis of immunopathogenesis is proposed for the development of DHF/DSS. An aberrant immune over-activation is induced post dengue virus infection, which not only impairs the immune response to clear the virus, but also results in overproduction of cytokines that affect monocytes, endothelial cells, and hepatocytes, as well as the production of cross-reactive anti-platelet and anti-endothelial cell autoantibodies. A molecular mimicry occurs between platelet, endothelial cells and dengue virus antigens. Platelets and endothelial cells are bound by the cross-reactive anti-dengue virus antibodies such as anti-E, anti-NS1 or anti-prM antibodies. The IFN-γ activated macrophage would then phagocytosize the opsonized targets. Dengue virus-induced vasculopathy and coagulopathy are involved in the pathogenesis of hemorrhage, and the imbalance between coagulation and fibrinolysis activation increases the likelihood of severe hemorrhage in DHF/DSS. This theory of transient hemophagocytic activity in the immunopathogenesis of DHF/DSS can account for specific characteristics of clinical, pathologic, and epidemiological observations in dengue virus infection. Using the data of the 2002 year Dengue big outbreak in Kaohsiung, we found that high fatality from dengue infection was associated with the following patient conditions: (1) age above 55 years, (2) underlying diseases with hypertension, chronic renal insufficiency, or diabetes, (3) abnormal thrombocytopenia, APTT and PT prolongation,
hemoconcentration and leukocytosis, (4) abnormal elevation of AST, ALT and BUN, (5) higher serum IFN-γ, IL-6 and IL-10, (6) secondary dengue infection, (7) higher anti-platelet autoantibody level, and (8) higher ferritin level. A transient macrophage activation might participate in the inflammatory disease process of DHF/DSS and contribute to the high fatality of elderly dengue infected patients with other underlying disease.
Introduction

Dengue virus infection just like other infectious diseases has an iceberg characteristic. Most cases are symptomless, followed, in increasing rarity, by undifferentiated fever, dengue fever (DF), and life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). DF is an acute febrile illness with symptoms of headache, retro-orbital pain, myalgia, arthralgia, rash, leukopenia, and mild thrombocytopenia. Although symptoms usually resolve after 5 to 7 days, there is a possibility of progression to DHF, an acute vascular permeability syndrome accompanied by abnormalities in hemostasis. DF/DHF is the most important arboviral disease of humans, occurring in tropical countries of the world where over 2.5 billion people are at risk of infection. WHO has estimated that between 50 and 100 million cases of DF and several hundred thousand cases of DHF occur each year, depending on the epidemic activity. DHF is the leading cause of hospitalization for children in Southeastern Asia. The dengue virus infection-induced dengue disease, however, has its own unique features, and are distinct from other viral diseases. Currently, there is no effective treatment except supportive care, and no approved vaccines are available. The reasons for these deficiency are due to the lack of understanding of the pathogenesis of dengue disease and the difficulty of developing an effective dengue vaccine. Dengue virus infection causes two major clinical characteristics: thrombocytopenia and plasma leakage that are unique and distinct from other virus-caused diseases. At defervescence, the stage when the patient’s condition becomes unfebrile and temperature falls, there is a turning point. In the case of DF, this point marks the beginning of recovery, while in the case of DHF, this point can mark the start of a rapid progress to shock, if patients do not receive timely intravascular fluid resuscitation. Once the shock is prolonged to an irreversible stage, it can be fatal. When appropriate intravenous fluid replacement is provided, the DHF/DSS patients will recover in one or two days with no disease sequelae. The severe DHF/DSS mostly occurs in secondary infection, especially when the second infection is by serotypes different from the previous one. This is an exception to the dogma that an established immune response can offer protection from subsequent infection. On the contrary, the previous immunity would enhance the disease process. The antibody-dependent enhancement (ADE) hypothesis has been proposed to explain this phenomenon. The heterologous anti-dengue antibodies, either from a previous infection or maternally derived, will not neutralize the virus, but would instead enhance the virus entrance into target cells via the Fc receptor after binding the dengue virion, thus leading to a high virus burden and more severe disease development [5].

2. Clinical and pathologic manifestation of dengue virus infection

DF is an acute febrile illness with headache, retro-orbital pain, myalgia, arthralgia, rash, leukopenia, and mild thrombocytopenia. Biphasic fever and rash are the most characteristic features of classic dengue fever. Symptoms resolve after 2 to 7 days. DHF is an acute vascular permeability syndrome accompanied by abnormalities in hemostasis. The clinical features include plasma leakage, bleeding tendency, and liver involvement. Liver involvement is common in dengue virus infection with mild elevation of serum transaminases. Capillary leakage develops rapidly over a period of hours, near or at the end of the febrile period when the symptoms of classic DF resolve. Pleural effusion, ascites, and hemoconcentration are indicative of intravascular volume loss. The condition can quickly progress to shock if patients do not receive
intravascular fluid resuscitation. The hemorrhagic manifestations range from a positive tourniquet test to spontaneous bleeding from the nose or the gastrointestinal tract. Plasma leakage with consequences of hemoconcentration and marked thrombocytopenia are the two major characteristic features of DHF/DSS. There are ample studies to show that dengue virus can infect a variety of human primary cells including monocytes/macrophages, dendritic cells, B cells, hepatocytes, Kupffer cells and cell lines of endothelial and epithelial origin. Although these \textit{in vitro} infections are modulated by the cell type and viral strain, it is not clear what are the true \textit{in vivo} targets (except the immature dendritic cells on the skin) for dengue virus, and how the damage or dysfunction of these cells or organs induced by dengue virus infection, either directly or indirectly, is responsible for the progressive development of DHF/DSS. Any pathogenic theory has to explain how the two specific cells (platelet and endothelial cells) are involved in the development of dengue disease.

3. Hypotheses on the pathogenesis of dengue hemorrhagic fever

Several hypotheses for the pathogenesis of dengue hemorrhagic fever have been proposed. Among them, the theory of the antibody-dependent enhancement (ADE) of infection plays a central role. The ADE hypothesis was formulated to explain the finding that severe manifestations of DHF/DSS occur in children experiencing a second dengue virus infection that has a different serotype from the previous one. There are indeed preexisting antibodies to previous dengue virus that cannot neutralize but rather enhance dengue virus infection \textit{in vitro}. Sera obtained before infection from children who later developed DHF/DSS were much more likely to demonstrate ADE \textit{in vitro} than those who had only DF. Newborn babies less than 1 year old who have acquired maternal anti-dengue IgG antibody are also susceptible to developing DHF/DSS post primary infection. Epidemiological studies support the association of DHF/DSS with secondary dengue virus infection. However, the association of DHF/DSS with prior immunity to other dengue serotypes by itself explains neither the pathogenetic basis of the association nor the molecular mechanism of DHF/DSS clinical manifestations. It is not clear how augmentation of dengue virus infection by enhancing antibodies leads to DHF/DSS, in addition to increasing the viral mass.

Virus virulence, the capacity of a virus to produce disease in a host, is an alternative hypothesis for the pathogenesis of DHF/DSS. The different manifestations of DF, DHF, and DSS may be caused by variants of dengue virus with different degree of virulence. The risk of DHF/DSS is higher in secondary infections with dengue virus of serotype 2 compared to other serotypes. Structural differences have also been found among various isolates of DF and DHF patients. Furthermore, it was reported that a high dengue viremia titer was associated with increased disease severity. Peak viral titers were 100- to 1000-fold higher in patients with DSS than those with DF in dengue-infected Thai children. Viral load is a contributing factor in the development of DHF/DSS. However, the virus load is highly dynamic, varying from individual to individual, or even from day to day post infection. It declines quickly on the day fever subsides. How the viral load is reflective of its virulence, the high growth rate \textit{in vivo}, or the consequence of the host immune response, needs further differential investigation. However, persons infected with the same dengue virus will have different clinical manifestations, suggesting that host factors must play important roles in the development of the dengue disease.
Immunopathogenesis in dengue hemorrhagic fever has been proposed. Serotype cross-reactive antibodies from the previous infection bind to virions without neutralization and enhance the entry of virus into monocytes. The number of virus-infected monocytes increases. As a result, the level of dengue virus-specific T cell activation is markedly enhanced. The T cells, especially the cross-reactive T cells, produce cytokines such as IFN-γ, IL-2, and TNFα, and lyse dengue virus-infected monocytes. TNFα is also produced by activated monocytes. The complement cascade is activated by a virus-antibody complex as well as by several cytokines to release C3a and C5a which also have direct effects on vascular permeability. The synergistic effects of IFN-γ, TNFα, and activated complement proteins trigger plasma leakage of endothelial cells in secondary dengue virus infection. However, several issues remain unclear under this theory. Not all DHF/DSS cases are secondary infections. Although most of the DHF/DSS in children are secondary infection, the DHF/DSS in infants are primary infection, and some of the adult DHF/DSS are primary infection. Complement activation may be the result of severe disease, not the cause of DHF/DSS. DHF develops rapidly, usually over a period of hours, and resolves within 1 to 2 days in patients who receive appropriate fluid resuscitation. It is also not clear why both endothelial cells and platelets are the major targets affected in dengue disease. No discernible sequelae are usually found. This scenario is not easily reconciled with the known tissue-destructive effects of inflammatory cytokines.

We proposed an alternative immunopathogenesis for dengue virus infection in 2001. Dengue virus infection causes aberrant immune responses, which not only impair the immune response to clear the virus, resulting in overproduction of cytokines, but also leading to abnormal production of autoantibodies. Platelets are destroyed by cross-reactive anti-platelet autoantibodies. Dengue-virus-induced vasculopathy and coagulopathy must be involved in the pathogenesis of hemorrhage, and the imbalance between coagulation and fibrinolysis activation increases the likelihood of severe hemorrhage in DHF/DSS. Hemostasis is maintained unless the dysregulation of coagulation and fibrinolysis persists. The overproduced IL-6 might play a crucial role in the enhanced production of anti-platelet or anti-endothelial cell autoantibodies, elevated levels of tPA, as well as a deficiency in coagulation. Capillary leakage is triggered by the dengue virus or by antibodies to its antigens. In this chapter, we will illustrate the issue of aberrant immune activation after dengue virus infection and its role in the manifestation of dengue disease. Coagulopathy and anti-NS1 antibody cross-reactivity to platelet and endothelial cells have been addressed in previous chapters of this book.

4. Dengue virus-induced aberrant immune activation.

We have reported finding an abnormal immune status in dengue patients during a dengue serotype 3 Taiwan outbreak in 1998. The immunophenotypes of consecutive blood samples from 29 dengue patients, of whom 21 had DF and 8 had DHF/DSS, showed that a transient reverse in the CD4/CD8 ratio occurred at day 6 to 14 post fever onset. The CD4/CD8 ratio inversion was manifested in 10 out of 29 dengue patients, and it was more frequently encountered in DHF/DSS than in DF patients. Analysis of the clinical blood cell count of these 10 cases showed that increment of immature neutrophils developed at fever day 5-6, CD4\textsuperscript{dim} or CD8\textsuperscript{dim} monocytosis at day 6-7, and atypical lymphocytosis at day 8-10 post fever onset. The PHA-stimulated T cell response was also depressed.
High levels of T-cell activation markers such as the soluble IL-2 receptor, soluble CD4, soluble CD8, IL-2, and IFN-γ, as well as monokines, (e.g., TNFα, IFN-β, and GM-CSF), were detected in dengue-infected children, and these markers were higher in DHF/DSS patients than in DF patients. High serum levels of inhibitory cytokines such as IL-10 or the soluble receptors of sTNFRI and sTNFRII were also found in DHF. We also reported a mixed Th1/Th2 cytokine profile in primary infection of infant DHF and secondary infection of children DHF. Both proinflammatory cytokines (IFN-γ, TNF-α) and anti-inflammatory cytokines (IL-10, IL-6) were elevated in the serum of infants and children with DHF/DSS. However, the concentrations of IFN-γ and IL-10 showed no difference between children with secondary dengue infections and infants with primary dengue infections. No increases of IL-4 and IL-2 were found. This is not compatible with Rothman’s viewpoint that cross-reactive T cells in response to secondary infection of different serotype can produce more cytokines and cause more severe DHF [17]. It seems that enhancing antibodies, from either a maternal source (primary infection in infants) or a previous infection (secondary infection in children), will promote infection of FcγR-bearing cells, and thus resulting in a large infected cell mass. The host responds to dengue virus infection via activation of T cells and generation of inflammatory cytokines. The T cell and cytokine responses are proportional to the infected cell mass. The inflammatory cytokines of IFN-γ and anti-inflammatory cytokines of IL-10 are present simultaneously in DHF/DSS patients. The inhibitory cytokines were induced to counteract the overt inflammation. Cytokines can cause cell activation synergistically or antagonistically; the net outcome will depend on the balance between various cytokine actions.

Dengue virus can infect immature dendritic cells, monocytes, as well as B cells. Monocytes are supposed to be the major target for dengue virus infection. Although the immature dendritic cells were reported to be 10-times more permissive for dengue virus infection than monocytes or macrophages, no antibody enhancement of infection was observed even though they express FcγR receptors. The infection of dendritic cells stimulates their maturation and cytokine production of TNFα and IFNα, but not of IL-6 and IL-12. The levels of IL-12 are higher in DF than DHF patients. No IL-12 could be detected in patients with DHF grades III and IV.

In addition to monocytes or dendritic cells, human B cells can also support dengue virus replication in vitro. The dengue virion production, as well as the enhancing antibody phenomenon on dengue virion and TNF-α/IL-6 cytokine release were similar in both B cells and monocytes. King et al., have reported that B cells were the principal circulating mononuclear cells infected by dengue virus. The majority of the virus was recovered from CD20+ B cells of dengue patients. The effect of dengue infection on B cells, together with the overproduction of B-cell growth factor IL-6, might trigger the maturation of plasma cells and the subsequent generation of autoantibodies.

Dengue-infected patients are usually leukopenic for several days during the acute infection, characterized by a decrease in the absolute number of neutrophils and monocytes. However, a transient neutrophilia and monocytosis occurs before leukopenic. Dengue virus stimulation induces the activation of neutrophils to release chemokines such as IL-8, MIP-1α, and MIP-1β, as well as neutrophil granular enzyme, MPO and to express CD11b/CD18 and Toll-like receptor 4. The neutrophil or monocyte activation will also contribute to the damage of target organs in DHF/DSS. All these immune deviations not only delay virus clearance, but also trigger cytokine
overproduction and auto-anti-platelet antibodies that will initiate or participate in the subsequent pathogenesis of dengue virus infection.

5. The anti-platelet and anti-endothelial cell autoantibodies in dengue disease

Thrombocytopenia is common in dengue fever and is always found in DHF/DSS. The pathogenesis of thrombocytopenia is poorly understood. Although dengue virus-induced bone marrow suppression was reported to depress the platelet synthesis and result in thrombocytopenia [29], this interpretation is not so likely because the platelet count spontaneously rebounds later. The decreased numbers of circulating platelets can instead be explained as a result of the destruction or consumption of the platelets. Parvovirus infection is reported to be associated with childhood idiopathic thrombocytopenic purpura. In dengue disease, dengue-2 virus can also bind to human platelets in the presence of virus-specific antibodies, and an immune-mediated clearance of platelets has been proposed to be involved in the pathogenesis of thrombocytopenia in DHF/DSS. We also reported IgM anti-platelet autoantibodies in dengue patients or in dengue virus-infected mice. The titer of IgM anti-platelet antibodies is higher in DHF/DSS than in DF patients. In vitro assay, the anti-platelet antibodies can cause platelet lysis with the help of complement, and this also inhibits ADP-induced platelet aggregation. The anti-platelet antibody is unique for dengue disease, and is not found for other viruses such as enterovirus-infected patient sera. However, the auto anti-platelet antibody alone is not sufficient to cause platelet destruction because convalescent phase dengue patient serum also contains the anti-platelet antibody, but the platelet count has rebounded to a normal level. There must therefore be other factors to precipitate the effect of anti-platelet antibodies.

Anti-endothelial cells antibodies have also been detected by flow cytometric analysis, with higher anti-endothelial cell antibodies in DHF/DSS than in DF patient sera. Both IgM and IgG anti-endothelial cell antibodies were found. The levels of anti-endothelial cell antibodies showed no difference between non-shock DHF and DSS in infants or children. No relationship between the levels of anti-endothelial cell autoantibodies and the increase in hematocrit, an evidence of plasma leakage, was observed. The anti-platelet and anti-endothelial cell IgM or IgG levels showed no difference between primary infection of infant and secondary infection of children. Again, the auto anti-endothelial cell antibody alone is not sufficient to cause endothelial cell damage or dysfunction because the convalescent phase dengue patient serum also contained the anti-endothelial cell antibody. The plasma leakage is a transient event, occurring primarily at defervescence, followed by recovery after 1-2 days. A molecular mimicry between the dengue virus and endogenous self-proteins has been proposed to be one of the mechanisms for the induction of autoimmunity during dengue virus infection.

6. Vasculopathy in dengue disease

The most characteristic feature of DHF/DSS and the best indicator of disease severity is plasma leakage. Plasma leakage is caused by a diffuse increase in capillary permeability and manifests as any combination of hemoconcentration, pleural effusion, or ascites. It usually becomes evident on days 3-7 of the illness, during which time dengue fever resolves (defervescence). Plasma leakage occurs systemically, progressing quickly, but will resolve within 1 to 2 days in patients who receive appropriate fluid resuscitation. No subsequent tissue or organ
dysfunction is observed. The perivascular edema is obvious, but, no obvious destruction of vascular endothelial cells has been reported. The plasma leakage can be due to altered vascular permeability or the structural destruction of endothelial cells. Although the by-standard effects of cytokine or mediator release in dengue infection were possible for the functional alteration of endothelial cells, it is difficult to reconcile the specific endothelial cells damage in dengue disease when similar cytokines or bio-active mediators are generated post any other viral infection. Dengue virus has been reported to infect endothelial cells in vitro, leading to apoptosis, production of cytokines and chemokines such as IL-6, IL-8, and RANTES as well as complement activation and ICAM-1 expression, but no infection on human DHF/DSS biopsy has been demonstrated. Therefore, the dengue virus direct effect on endothelial cells remains questionable. The effect of anti-NS1 antibodies that cross-react with non-infected endothelial cells to cause apoptosis and its signaling pathway will be discussed in another chapter of this book [37-39].

Other anti-dengue virus antibodies also cross-react with the endothelial cells, for example, the anti-prM antibody can bind to endothelial cells and one of the antigens is heat shock protein (HSK) 60.

Serum levels of thrombomodulin (TM), a marker of endothelial cell damage, found in infants and children with DHF/DSS were higher than those of controls, but their levels were not correlated with the severity of DHF in infants and children. Although there was no correlation between the serum levels of TM and the levels of anti-endothelial cell autoantibodies or the increase in hematocrit in infants and children with DHF/DSS, this still indicates that endothelial cell structural damage occurred in vivo under certain conditions. It seems that immune mediated damage by leukocyte recruitment can cause structural injury to endothelial cells. This vascular leakage can be induced during dengue virus infection. Moreover, although the endothelial cell surface molecules that are recognized by these autoreactive antibodies need to be identified, a cross-reactivity exists between endothelial cells and dengue virus antigens due to molecular mimicry. The endothelial cells do not need to be infected by dengue virus to be targeted. The cross-reactive anti-dengue antibodies such as anti-E, anti-NS1 or anti-prM can bind to the un-infected endothelial cells to cause damage. Because the endothelium plays a crucial role in maintaining hemostasis, damage of endothelial cells during dengue virus infection may skew the procoagulant/anticoagulant balance of endothelium and increase the bleeding tendency. The sequestration of platelets by activated endothelial cells might also contribute to the development of thrombocytopenia.

7. Immunopathogenesis of DHF

The characteristic features of DHF/DSS include capillary leakage, thrombocytopenia, and coagulopathy. We propose a modification of the original immunopathogenesis of dengue virus infection-caused disease, taking into consideration of several key observations: 1. Dengue virus infection induces transient immune aberrant activation of CD4/CD8 ratio inversion and cytokine overproduction. 2. Dengue virus infection induces autoimmunity because of molecular mimicry. 3. Anti-E, anti-NS1 or anti-prM antibodies can cross-react with platelets and endothelial cells. The binding to platelets causes platelet lysis in the presence of complement, whereas the anti-NS1 antibody binding to endothelial cells induces the NO-mediated apoptosis. 4. Monocytes are active by dengue virus infection or by cytokine such as IFN-γ. 5. Macrophage activation was
found in vivo with the increase of serum ferritin level.

Dengue virus infection causes intense immune activation. Aberrant immune responses such as cytokine overproduction and generation of autoantibody acting against platelets and endothelial cells occur after dengue virus infection. A molecular mimicry between platelets or endothelial cells with the E, NS-1 or prM of dengue virus would explain the cross-reactivity of anti-E, anti-NS1 or anti-prM antibody to the host cells, and participation in the attack of platelet and endothelial cells during the disease development. A high serum ferritin level, a macrophage activation marker in vivo, was found to be highly elevated in dengue patients, suggesting that it is a common phenomenon in dengue patients. Monocytes or macrophages were activated by cytokines such as IFN-γ during the dengue disease process. The activated macrophages would then phagocytize the autoantibody-coated platelet, and thus contribute to the development of thrombocytopenia in DHF/DSS. Dengue virus could cause a severe hemophagocytic syndrome (Figure 1). The anti-E, anti-NS1 and anti-prM cross-reactive antibody to platelets and endothelial cells provide an explanation for the target specificity and unique feature of thrombocytopenia and plasma leakage in the development of DHF/DSS. The macrophage activation might also be responsible for the sustained disease process, with a high fatality rate, as observed in the elderly dengue virus-infected patients.

Figure 1. Autoantibody-associated immunopathogenesis of dengue disease.
Publications

Papers published in American Journal of Infectious Disease, 2008 January thematic issue


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<td>（中文）抗登革病毒抗体在登病疾病的致病角色</td>
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報告內容應包括下列各項：
一、參加會議經過

二、與會心得

三、考察參觀活動 (無是項活動者省略)

四、建議

五、攜回資料名稱及內容

六、其他

一、參加會議經過

美國實驗生物(Experimental Biology)年會每年舉行一次，由七個學會 American Association of Anatomists, The American Association of Immunologists (AAI), The American Physiological Society, American Society for Biochemistry and Molecular Biology, American Society for Investigative Pathology, American Society for Nutrition, American Society for
Pharmacology and Experimental Therapeutics 共同聯合舉行，今年在美國加州聖地牙哥市，於 97 年 4 月 5 日至 4 月 9 日共五天的會議，我和我的博士學生張志鵬一起參加，主要是 AAI 的年會，AAI 的大會除了有七個 Major Symposium: Genetics of Autoimmunity, Microenvironmental Influence on Immune Function, TNF Family members in Inflammation, Autoimmunity and Cancer, Organ-Specific Regulation of Innate Immunity, managing B Cell Lifespan and Lifestyle with BARR Family Interactions, Control of Effector and Membory T Cell Fate, Can Basic Advances in Immunology Generate Effective Cancer Therapies 外，還有 AAI President’s Program, AAI Distinguished Lectures, AAI special Events，以及和各學會合辦的跨領域的各種研討會，及壁報展示。

我的 poster 安排在 4 月 7 日，並於 12:30-13:30 於壁報旁供人詢問。主題是抗登革病毒抗体在登病疾病的致病角色，抗登革病毒抗体的角色有二個，一是 antibody-dependent enhancement of dengue virus infection 可以由 anti-E Ab and anti-prM Ab 來擔任，它對於帶有 FcR 的細胞，是透過增加 dengue virion 結合到細胞上，而增加 dengue virus entry，不僅增加 dengue virus-infected cell mass, 同時也增加 dengue virus replication, 因為 dengue virus-infected cell percentage 及 dengue virus protein mean fluorescent intensity 都增加。這些 enhancing antibody 的作用是 concentration-dependent，在高濃度時是抑制(neutralization)，低濃度時是增強(enhancement)。這種增強作更特別的是 anti-prM Ab 可以增強沒有 FcR 的細胞如 BHK 或 A549 cell lines，它利用可以同時結合 BHK 或 A549 cells 表面的蛋白質和登革病毒顆粒的雙重結合性，將登革病毒顆粒拉進及細胞，再經由登革病毒受體而感染細胞，這種 dual specificities 是很特別的，BHK 或 A549 細胞表面上被鑑定出來的一個蛋白質是熱休克蛋白(heat shock protein 60)，這種雙重作用在登革病毒疾病上也是特有的。抗登革病毒抗体的另一個角色是致病性的角色，我們提出登革出血熱是一個急性類似噬血徵候群疾病的假說：登革病毒感染會引發免疫反應的不正常活化，產生抗血小板及內皮細胞自體抗體，同時產生過多的細胞激素，尤其是干擾素-γ，造成巨噬細胞的活化，去吞噬抗體結合的血小板及，內皮細胞，因而造成血小板減少及血管壁滲透增加，引發凝血和溶血的不平衡，形成登革出血熱及嚴重的登革休克症候群。這急性類似噬血徵候群的免疫致病機轉能解釋登革病毒感染引起登革出血熱特有的臨床症狀，病理的變化，以及過去眾多的流行病學的觀察。

此次的另一個目的是參加美國實驗生物醫學雜誌（Experimental Biology & Medicine,
EMB）編輯會議及美國實驗生物醫學協會（Society of Experimental Biology and Medicine, SEBM）的相關活動，SEBM 也是 Experimental Biology 的 Guest Society，EBM 亞洲編輯辦公室已於 97 年 1 月設立於成大醫學院，由我擔任亞洲主編，負責篩選亞洲地區的學者上網投稿資料之初審，協助英文用法的改進。此外也將以成功大學為亞洲 SEBM 發展基地，結合相關人力及資源，致力於增加亞洲的 SEBM 會員數、增加亞洲地區圖書館的 EBM 訂閱數、增加亞洲 EBM 投稿數以及提高亞洲 EBM 投稿的成功率。在 4 月 6 日 11:00 的編輯會議也做了亞洲編輯辦公室的一月至三月的工作報告。

二、與會心得

美國實驗生物年會是一個由各學會聯合舉辦的年會，參加的人數上萬，雖然因為會場太大，趕場多，容易迷失，但因主題多，尤其是各學會聯合的跨領域的研討會，可以得到不同觀點的看法，對於整個學術研究的發展有很大的提昇，這是單一學會舉辦所不能獲得的效果。台灣的每年三月也由生物醫學聯合年會，但規模及效果都不如，主要是參加的研究人員報告不踴躍，學術氣氛較差。

三、建議

台灣在登革熱的研究已建立具國際競爭力的實力，我們有全世界密度最高的登革熱研究團隊，在未來應好好利用這優勢，持續在特定領域領先，國外登革疫苗的研發將會有 dengue virus infection induce autoimmunity 的顧慮，面臨長久安全性的挑戰。我們在免疫致病機轉及抗体依賴性的增強作用上的著力，使我們掌握學術上的關鍵，如果能瞭解中和性抗体和增強性抗体的差異，即有機會能發展出能誘導中和性抗体，而不是增強性抗体的登革疫苗。亞洲地區以臨床治療為重心。台灣有登革疫情但不嚴重，不過有很特別的地方。我們有很多的基礎研究人員，這幾年來的研究成果已建立國際登革學術研究的聲譽。所以如果能持續投入大量的研究資源，我們是有機會在登革研究領域發展出世界一流的研究中心。

四、攜回資料名稱及內容

攜回大會手冊及摘要各一份。
Abstract

The role of anti-dengue antibodies in the pathogenesis of Dengue disease

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Dengue disease includes from mild dengue fever to severe dengue hemorrhagic fever (DHF), which is an important health problem in tropical or sub-tropical areas. The immunopathogenesis of DHF is initiated by aberrant immune activation and autoantibody production caused by virus infection. There is a molecular mimicry between dengue antigens and self-proteins. The anti-dengue antibody cross-react with platelet and endothelial cell, and would trigger the subsequent dysfunction of endothelial cells and hemorrhage during the acute infection. The antibody-dependent enhancement theory plays a central role in the dengue disease. The enhancing antibody is dependent on the types of the target cells and the specificity of the enhancing antibody. The enhancing antibody can be either anti-prM or anti-E antibody. For anti-E Ab-mediated enhancement on monocytic cells, it can be concentration-dependent: enhancing at sub-neutralization level or enhance regardless of concentration by Fc and FcR interaction. For anti-prM Ab-mediated enhancement, it enhanced the dengue virion binding on both FcR or non-FcR bearing cells with dual specificity. The anti-dengue antibodies seem to be either enhancing antibody or the pathogenic antibody, and play a major role in the DHF pathogenesis.