行政院國家科學委員會專題研究計畫　成果報告

利用單株抗體與 anti-idiotype antibody 等生物技術找尋血小板自體抗原並研發血小板缺乏紫癜症之專一性療法

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計畫主持人：張懿欣

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Identification of natural platelet autoantigens and development of specific therapy for idiopathic thrombocytopenic purpura using monoclonal and anti-idiotypic antibody techniques (2/3)

計劃編號：NSC 90-2314-B-040-016
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一、中文摘要

血小板缺乏性紫癜症（idiopathic thrombocytopenia purpura，ITP）是一種常見的血小板疾病，病人體內之抗血小板自體抗體會破壞血小板而使血小板數目減少。尋找人體中自然存在之血小板自體抗原的 epitopes 並了解自體抗體對血小板功能所產生的影響，為設計研發嶄新之 ITP 治療方式不可或缺的工作。我們希望研發 ITP 之專一性療法，解決現有療法之缺點。本計劃為全程執行期限 3 年之第 2 年計劃。我們在第 1 年計劃中以人類血小板注射小白鼠，利用活體內之自然免疫與抗原呈現作用製造單株抗體(mAb)來篩選抗原性較強之血小板抗原。我們已經成功的篩選到一些可以分泌單株抗體的融合瘤細胞株。並成功地確認這些抗體的特性。此外，我們也發現上述單株抗體的確可以和臨床上 ITP 患者的血清互相競爭與血小板抗原結合。目前我們正在確認這些單株抗體的 isotypes 與所認識的抗原種類。希望透過此方式在未來可以找尋自然存在於人體之血小板自體抗原，並於活體中分析其 mAb 對血小板破壞速率的影響。而最終之目的則是製造與血小板 epitopes 結構相似之 anti-idiotype antibody (AIAbs)，抑制 ITP 病人自體抗體與血小板結合，以研發 ITP 之專一性療法。

關鍵詞：血小板缺乏性紫癜症、單株抗體、anti-idiotypic antibody

Abstract

ITP is a common disorder that autoantibodies against platelets can result in platelets destruction and ultimately thrombocytopenia. Patients receiving current ITP interventions must confront to the disadvantages, such as side effects caused by non-specific intervention, expensive, and relapse. Accordingly, it should be a great interest to develop an ITP treatment that exhibits the advantages of specificity, convenience, cost-effectiveness and free of major side effects. In this project, we hope to develop a specific intervention for ITP to tackle the existed difficulties in clinical treatment. The present study is a 3-year study project. In the first year of project, we

had successfully obtained several hybridomas that secreted high titer of anti-human platelet antibodies. Besides, these isolated monoclonal antibodies can compete the binding of platelet antigens with sera from ITP patients. The characteristics of these clones, isotypes of these monoclonal antibodies as well as the antigens recognized by these antibodies are now under investigating. Ultimate purposes of this 3-year study project are to identify new platelet autoantigen(s), study the kinetics of *in vivo* platelet destruction and develop an ITP-specific treatment taking the advantages of mAb and AIAb techniques.

二、緣由與目的

ITP is a common disorder of immune regulation (reviewed in ref. 1). Autoantibody is produced against platelets and leading to the phagocytic destruction of these cells. The resultant thrombocytopenia induces purpura and hemorrhage if the platelet count reaches a critical level (usually <30,000/ml).

Contemporary treatment for ITP is nonspecific and palliative rather than specific and curative. Followings are the summery of contemporary ITP interventions and the related disadvantages (reviewed in ref. 1):

1. **Corticosteroids**

   Treatment with corticosteroids prevents sequestration of antibody-coated platelets by the spleen. An effective response (platelet count >100,000/ml) occurs in only 36-44% of all patients treated. However, the often slow platelet response and the potentially severe adverse effects of corticosteroid therapy are frequently a deterrent.

2. **Splenectomy**

   Splenectomy removes both the potential site of destruction of damaged platelets (2) and a significant source of anti-platelet antibody production (3). About 10% of patients will relapse after an initially successful splenectomy; relapse is usually within the first year but it can happen as long as 5 years later.

3. **IVIg**

   IVIg transiently induces acceptable platelet counts in about 75% of patients. IVIg usually leads to a rapid rise in platelet count; however, IVIg is a non-specific therapy for ITP since it is composed of pooled human gammaglobulin. Besides, IVIg is very expensive and adverse effects associated with its infusion are common and sometimes troublesome.

4. **Intravenous infusion of anti-D**

The role of anti-D in acute ITP is still evolving. Side effects include mild hemolysis with a fall in hemoglobin lasting 1-2 weeks. However, Anti-D was less

effective than IVIg (4). The use of anti-D for individuals with Rh-positive status and ITP has been the focus of many trials to determine its success and cost-effectiveness relative to other treatments (5-7).

SPECIFIC AIMS

Although several measures have been used in the ITP intervention, it is still lack of an ITP treatment that simultaneously exhibits the advantages of specificity, convenience, cost-effectiveness and free of major side effects. We design to uncover new platelet autoantigen(s) and develop a specific intervention for ITP to tackle the existed difficulties in clinical treatment. Ultimate purposes of this 3-year study project are to identify new platelet autoantigen(s), study the kinetics of *in vivo* platelet destruction and develop an ITP-specific treatment taking the advantages of mAb and anti-idiotype antibody AIAb techniques.

In the second year of study, mAbs that can specifically against human platelet autoantigen and compete the binding of platelet antigens with sera from ITP patients were selected. Besides, the isotypes of these mAbs and specific antigens recognized these mAbs were identified. The establishment of AIAb against these mAbs are now undergoing.

三、結果與討論

We had successfully induced anti-human platelet antibodies in mice immunized by human whole platelets and completed the fusion process for hybridomas. Several cell clones which can secrete high titer of anti-platelet antibodies were obtained. Followings are the achievements of our studies in the second project year:

1. **Selection and purification of mAb-Fab that share the same epitope recognition with human anti-platelet antibodies in ITP serum**.

Thirteen clones of hybridoma that can secrete mAb against platelet antigens were obtained in the first project year. The ability of these 13 mAbs to compete binding to platelet antigens with sera from 15 ITP patients were subsequently analyzed in this project year. By using competitive ELISA, 6 clones which can secrete mAbs with high competitive capacity to bind the platelet antigens were selected. Three clones among these 6 hybridomas have strong competitive ability to bind to platelet antigens. Therefore, further experiments were conducted to characterize the nature of these 3 clones.

2. Characterization of the isotypes and antigens recognized by the selected mAbs.

According to the above results, isotypes of the mAbs that were highly competitive to bind to platelet antigens were analyzed by ELISA. One and 2 mAbs secreted by these 3 clones were IgG1 and IgG2a, respectively. The antigens recognized by these clones and the purification of the Fab fragments of these highly competitive mAbs are now undertaking.

四、計劃成果自評

In summery, we have successfully identified 3 clones that secrete high titer of murine anti-human platelet mAbs which harbored strong competitive capacity to bind to platelet antigens. One and 2 mAbs secreted by these 3 clones were IgG1 and IgG2a, respectively. The antigens recognized by these clones and the purification of the Fab fragments of these highly competitive mAbs are now undertaking. The progress of this project is somewhat delayed due to certain experimental problems and difficulties during these 2 years, however, we believe that accomplishment of this project should be able to add some new clues to the pathogenesis of ITP and contribute to the development of new therapeutic strategy of IPT treatment.

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