anti-idiotype antibody

(1/3)

Identification of natural platelet autoantigens and development of specific therapy for idiopathic thrombocytopenic purpura using monoclonal and anti-idiotypic antibody techniques (1/3)

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anti-idiotypic antibody

idiopathic thrombocytopenia purpura ITP

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 have successfully obtained several hybridomas that secreted high titer of anti-human platelet antibodies. The characteristics of these clones are now under investigating. Ultimate purposes of this 3-year study project are to identify new platelet

epitopes

ITP

ITP

3 1

1

(mAb)

mAb

epitopes anti-idiotype antibody (AIAbs) ITP

ITP

autoantigen(s), study the kinetics of *in vivo* platelet destruction and develop an ITP-specific treatment taking the advantages of mAb and AIAb techniques.

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 first year of study, mAbs that specifically against human platelet autoantigen with stronger antigenicity were raised by injecting mice with whole human platelets through natural immunization and *in vivo* antigen presentation process.

We have successfully induced anti-human platelet antibodies in mice immunized by human whole platelets and completed the fusion process for hybridomas. In addition, we have obtained several cell clones which can secrete high titer of anti-platelet antibodies screened by ELISA. The characteristics of the antibodies secreted by these clones are now under investigating. Followings are the achievements of our studies in the first project year:

1. **Characterization of the minimum number of human platelets required for the activation of murine immune response.**

Four BALB/C mice were intraperitoneally immunized with $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ human whole platelets respectively to induce anti-human platelet antibodies. Mouse injected with PBS was used as control. The mice were boosted with equal amount of human whole platelets or PBS 3 times at 2, 4, and 6 weeks after first immunization. Peripheral blood from tail vein was collected and the titer of anti-human platelet antibodies was analyzed by whole platelet ELISA and immunoblotting.

The results showed that the number of injected human platelets must be as least more than $1 x10^7$ to effectively induce anti-human platelet antibodies, therefore, anti-human antibodies were successfully induced in mouse injected with $1x10^7$ or $1x10^8$ platelets.

According to Western blotting analysis, antibodies in each mouse recognized differential platelet antigens. The major antigen recognized were 52 and 81 kD respectively, however, sera from one mouse recognized multiple platelet antigens with high molecular weights.

2. **Fusion, culture and screening of hybridomas that can secrete anti-human antibodies.**

According to the above results, at least $1x10^8$ human platelets were used to boost mice in all the following immunization process. On the forth day of the forth boost, the mice with highest

titers of anti-human platelet antibodies analyzed by ELISA, as described detail in Methods, was sacrificed. Spleen of this mice was fused with myeloma cells and an introduced to 24 well tissue culture plate for further growth. We have successfully selected, screened and obtained several hybridomas that secreted high titer of anti-human platelet antibodies. The characteristics of these clones are now under investigating.

In summery, we have characterized the minimal number of human platelets that are required to activate murine response and successfully identified several clones that secrete high titer of murine anti-human platelet monoclonal antibdoies. The characteristics of these clones, including type of light chain and antigen recognized by these mAbs are now under investigating. Sera from ITP patients are now collecting to identify if these mAbs secreted by these clones can compete the binding ability of platelet autoantibdoies in ITP sera.

1. Karpatkin S. Autoimmune (idiopathic) thrombocytopenic purpura. *Lancet* 349: 1531-1536, 1997.
2. Shulman NR, Weinrach RS, Libre Ep, et al. The role of the reticuloendothelial system in thee pathogensis of idiopathic thrombocytopenic purpura. *Trans Assoc Am Physns* 78: 374-391, 1965.
3. Karpatkin S, Strick N, Karpatkin M, Siskind G. Cumulative experience on the detection of anti-platelet antibody in 234 pateints with idiopathic thrombocytopenic purpura, systemic lupus erythmatosus and other disorders. *Am J Med* 52: 776-785, 1972
4. Blanchette V, Imbach P, Andrew M, et al. Randomized trial of intravenous immunoglobulin G, intravenous anti-D, and oral prednisone in childhood acute immune thrombocytopenic purpura. *Lancet* 344: 703-707, 1994.
1. Ware R, Zimmerman S. Anti-D: mechanism of action *Semin Hematol* 35(suppl): 14-23, 1998.
2. Scaradavou A, Woo B, et al. Intravenous anti-D treatment of immune thrombocytopenic purpura experience in 272 patients. *Blood* 89: 2689-2700, 1997.
5. Bussel J. Recent advances in the treatment of idiopathic thrombocytopenic purpura. *Semin Hematol* 35(suppl 1): 1-4, 1998.