STUDIES ON MEGAKARYOCYTOPOIESIS IN ADULT CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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We have previously demonstrated that based on enzyme linked immune staining with antibody against thrombospondin and monoclonal antibody (MoAb) against GPIIb, 24% (7/29) of ACITP didn't display maturation impairment of megakaryocytes. Afterwards, we studied megakaryocytopoiesis in 70 new ACITP patients with 17 males and 53 females, the mean age was 35 years old. The following parameters were examined: morphology of megakaryocytes (MK) on bone marrow smear; image analysis of megakaryocytes in bone marrow biopsy immunochemically stained with MoAb of GPIIIa and ABC kit according to the method of Fox using VIDAS system; expression of oncogenes c-myc and c-cis in MK by in situ hybridization using synthetic OS1 c-myc and OS1 PDGF-B/c-cis as probes and digoxigenin end labelled oligooxynucleotide method introduced by Farquharson and in vitro culture of CFU-MK with plasma clot method.

The results showed that three types of megakaryocytopoiesis could be distinguished: (1) Type I (n=38) characterized by increase in number of MK accompanied by retardation of maturation on bone marrow smear, normal number of CFU-MK, normal c-cis but increased c-myc expression; (2) Type II (n=24), normal number of MK, slight decrease of CFU-MK, decreased c-cis and c-myc expression; and (3) Type III (n=8), decrease of MK number without maturation impairment, marked decline of CFU-MK formation as well as c-cis and c-myc expression.

Treatment with corticosteroid was more effective for type I (65.8%) and type II (25%) than for type III (12.5%) (p < 0.05~0.01). 21 cases resistant to the steroid treatment were further treated with interferon α-2b, the response rate was 55.6% (5/9) and 36.4 (4/11) for types I and II respectively, while no response was observed in one case with type III.

It could be concluded that in ACITP, there are a certain number of cases in whom the thrombocytopenia is not due to impairment of megakaryocytopoiesis, they respond poorly to steroid or interferon treatment. The mechanism needs further elucidation.

Hemophilia in Taiwan

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Hemophilia (A and B) is the most commonly encountered hereditary coagulation disorder. It results from a deficiency of factor VIII or IX coagulant activity (VIII:C or IX:C) and is inherited by means of sex-linked recessive transmission, being confined almost entirely to males. It causes not only a direct threat to the individual life, but also a serious family, social and economic problem. We are going to present the clinical, laboratory and family studies of cases of hemophilia in Taiwan, the status of treatment and complications as well as molecular biological studies of genetic defects. The first Taiwan hemophilia treatment center was established in 1984 in our university hospital.

Three serious problems were found, delayed diagnosis, frequent serious musculoskeletal damage and crippling and inadequate treatment. Patients with hemophilia A receive about 40,000 units of factor VIII per person per year, and hemophilia B, about 20,000 units of factor IX per person per year. Of 151 cases of hemophilia A and 41 cases of hemophilia B, 16 (10.6%) and one (2.4%) have developed inhibitors respectively. Of 238 cases, 101 (42.2%) had liver function abnormality. Of 232 cases, 42 (18.1%) were positive for anti-HIV. Of 236 cases, 201 (85.0%) were positive for anti-HBV, 35 (14.8%) were HBV carriers. Of 201 cases, 171 (85.6%) were positive for anti-HCV. Of 17 cases of hemophilia A and 1 case of hemophilia B were found, the data revealed that they are probably caused by extreme lyonization in a heterozygote. Of 102 cases of hemophilia A from 87 families studied by means of the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) and/or dideoxy fingerprinting (ddF) and then direct sequencing, a single base substitution was found in 21 cases, small deletions in 12 cases and small insertions, in 4 cases. Of 17 cases of hemophilia B also studied by PCR-SSCP and direct sequencing, 16 had single point mutations and one had a gross gene deletion. These data indicate that there are no essential differences between China and other nations in many aspects of hemophilia.
The molecular cloning of coagulation factor VIII and the analysis of factor VIII gene mutations in the Chinese hemophilia A patients

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Hemophilia A is a X-linked hereditary disease which is caused by the deficiency of coagulation factor VIII. The molecular cloning of the gene encoding human FVIII constitute the basis for production of the recombinant FVIII and the gene therapy of hemophilia A. By using the retrotranscriptase/polymerase chain reaction (RT/PCR) methods, we have succeeded in the construction of two types of FVIII cDNA: one of 7828bp long containing the total open reading frame (ORF) of FVIII; another is of 5828bp in which the major part of the B domain and a 36bp stretch of N-terminal of light chain were deleted. At the moment we are carrying out the FVIII cDNA expression in the insect virus/insect cell system. The preliminary results show that the expression products from FVIII cDNA without B-domain has the biological activity of FVIII.

Meanwhile, we are performing the molecular analysis of the genetic defect of hemophilia A in Chinese population. The Southern analysis using FVIII cDNA probes covering the entire gene showed no large deletion among 60 DNA samples from severe or moderate hemophilia A. Only one out of 60 cases exhibited a modified Taq I fragment, probably resulting from a point mutation at the CpG site. It is thus possible that the molecular defects of most Chinese hemophilia A patients are not caused by gene deletion while most point mutations should be located outside the CpG site contained in Taq I sites. Experiments using PCR-SSCP for partial ORF of FVIII are now well under way in our laboratory.

Plasminogen Activation System in Disseminated Intravascular Coagulation

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Disseminated intravascular coagulation (DIC) syndrome is characterized by extensive coagulation and secondary fibrinolysis. Activation of the fibrinolytic system results in breakdown of fibrin by plasmin. Plasmin is formed from proteolytic cleavages of plasminogen by plasminogen activators. Tissue-type plasminogen activator (t-PA) has been shown increased in some cases with DIC, and the increase of type 1-plasminogen activator inhibitor (PAI-1) was suggested to indicate poor prognosis in patients with septic shock, although the results have not been consistent in the literature.

We studied serial changes of the plasma levels of t-PA and PAI-1 in DIC induced by various causes including Tsutsugamushi disease, hemorrhagic fever with renal syndrome, infections in acute leukemia and snake bites, and found that increases of these major endothelial cell regulators of the plasminogen activation system are a universal finding in DIC. The differences, however, in the degree and the sequence of the changes suggested that the response of endothelial cells triggered by the direct cause of DIC might be different from that associated with common pathologic consequences of DIC.

To elucidate the pathophysiologic mechanism responsible for the increase of t-PA and/or PAI-1 in DIC, we determined the effect of in vitro R. tsutsugamushi infection of cultured human umbilical vein endothelial cells on the secretion of t-PA and PAI-1 since R. tsutsugamushi had been demonstrated to invade endothelial cells and might play a role in development of DIC associated with this illness. Although both t-PA and PAI-1 were increased in plasma of the patients with DIC due to R. tsutsugamushi infection, only PAI-1, but not t-PA, was actively synthesized and secreted from the cultured human umbilical vein endothelial cells infected with R. tsutsugamushi in vitro. Plasma t-PA observed in high levels during R. tsutsugamushi infection might have resulted as a common pathologic consequence of DIC. Increased PAI-1 levels, however, may at least partly be attributed to active secretion of PAI-1 from the endothelial cells invaded by R. tsutsugamushi.
Antithrombotic peptides from snake venoms

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Thrombin-like enzymes (TLEs) have been utilized for the prevention of venous thromboembolism because of its defibrinogenating effect in vivo. TLE selectively cleaves fibrinopeptide A from fibrinogen resulting in the formation of a non-cross linked fibrin. However, we recently confirmed that TLE (from Calloselasma rhodostoma) exhibited antiplatelet activity when it was intravenously infused in experimental rabbits, suggesting that sustained defibrinogenation is the primary cause leading to "reversible" impairment of platelet function although the production of fibrin degradation products at the initial stage may also be causative. In addition, TLE caused a marked elevation of PGI2 level in human umbilical vein endothelial cells indirectly through the formation of non-cross linked fibrin. In vivo, TLE inhibited platelet plug formation in mesenteric microvessels irradiated by filtered light in fluorescein sodium pretreated mice. In this model, PGI2 showed only a brief antithrombotic effect whereas heparin showed no effect. Furthermore, TLE also reduces the viscosity of whole blood in experimental animals. In conclusion, TLE therapy may be considered for patients afflicted with either venous thrombosis or arterial thrombosis.