Role of complement factors on nciph

Role of complement factors, ADAMTS13 and Von Willebrand factor in pathogenesis of non cirrhotic intrahepatic portal hypertension (NCIPH) in humans

Introduction

(You need to start with background on gut-liver axis, the fact that bacterial infections in gut can induce inflammatory mediators which come to liver through mesenteric-portal vein). The complement system is a fundamental element of the innate immune system as well as the adaptive immune responses. Improper complement activation leads adverse effects, for instance in diverse renal diseases, (expand) TTP, thrombotic microangiopathies, portal hypertensionand transplant rejection. (expand). (You need to have more background on the various complement factors, their function, the 3 different pathways of activation etc) NCIPH is a liver disorder of vascular origin defined by a portal venous pressure exceeding 5mm Hg between portal vein and inferior vena cava (sanyal 2008), characterized by occlusion of the 3rd /4th order branches of the hepatic portal vein (madhu 2008). It is suspected that this occlusion is due to formation of microthrombi in the venules of the portal vein. Our clinical studies indicate that in 30-40% of patients with cryptogenic chronic liver disease, liver biopsy confirms the diagnosis as NCIPH (Madhu, Avinash 2009, Goel, madhu 2013). The focus of this proposal is to uncover the cause for thrombosis that leads to NCIPH.

Thrombotic micro angiopathy (TMA) is characterized by a sequence of events, including disruption of endothelial cell integrity, intravascular activation of platelets, formation of platelet–fibrin thrombi, and obstruction of the microcirculation followed by hemolytic anemia with consumption of

erythrocytes and platelets (1). The factors causing TMA include pathogenic infections, immunosuppressive drugs and blood group incompatibility (7) however, the mechanisms of toxicity remain indistinct. The Von Willebrand protein is a glycoprotein which predominantly functions in hemostasis and is mainly synthesized in endothelial cells and megakaryocytes

You need to add a lot more background on VWF, explain what is UL-vWF, the various domains etc) In normal conditions, hepatic portal vein endothelial cells that are injured/activated secrete vast quantity UL-vWF, which would usually been cleaved by ADAMTS13 (A disentegrin and metalloprotease with eight thrombospondin 1 – like domanins) to maintain homeostasis.

The function of vWF is is facilitated by shear stress caused by the blood flow in the venous circulation. Under static condition the ULvWF is not linear and may be coiled, hiding the ADAMTS13 cleavage sites, but under the blood flow, the shear stress induces unfolding of ULvWF and exposure as a linear multimer of vWF. ADAMTS13 attaches to monomeric subunit strings of ULvWF through its CUB domain and cleaves the molecule at 842Tyr-843Met peptide bond in VWF A2 domain. Thus the ULVWF fragmented and the monomeric sub units will no longer induce adhesion and aggregation of platelets in the circulation. Prevention of cleavage by ADAMTS13 will induce adhesion of ULvWF and accumulation of platelets, leading to microangiopathy as in (expand) TTP, which is a disease caused as a consequence of ADAMTS13 deficiency and lack of cleavage of ULVWF.

Lack of cleavage of vWF multimers by ADAMTS13 leads to platelet aggregation and occlusion of the vessel resulting in microangiopathy.

Alternatively, over secretion of vWF from the endothelial cell will lead to the same. The vWF synthesis in endothelial cells may be activated by bacterial toxins, inflammatory cytokines, calcium ionophores or phosphodiesterase inhibitors (ref). The synthesized ULvWF may be anchored on the surface of endothelial cells by P-selectin which is produced concurrently with VFW from the Wieble-palade bodies of endothelial cells(padilla 2004).

Thus, pathogenesis in NCIPH patients could be either defective Vwf multimeric processing by ADAMTS13, excess expression of multimeric vWF which overwhelms the cleavage capacity of ADAMTS13 or any other factor which interferes with this system. Preliminary data indicates that NCIPH patients have an imbalance in vWF and ADAMTS13 but the reason driving this disparity is not clear. In this proposal it is hypothesized that activation of vWF secretion and down regulation of ADAMTS13 may be provoked by inflammatory cytokines induced by an inflammatory response in NCIPH cases. It is possible that inflammatory cytokines secreted during acute inflammation or infection can inhibit ADAMTS13 biosynthesis and result in thrombotic microangiopathy in the hepatic venules, that leads to portal hypertension (You need some ref which show that cytokines can inhibit ADAMTS13-those must be quoted before this sentence). Other than toxins, ULvWF synthesis can be activated by histamine, TNFα, and interleukins such as IL8, IL6 and IL10 (Aubrey Bernardo, 2004) which are mainly activated by the complement pathway (ref for activation by complement pathway). More over nitric oxide has also been shown to induce microthrombi in circulation (ref), and the role of this in NCIPH cases also needs investigation.

Secondly the extent of complement activation and its participation in development of TMA will be assessed in NCIPH patients. Complements like C3, C4, and C3b are involved in the alternative pathway, and the activation of these complement factors are regulated by the complement factor H (CFH). Hence, any problem in any of these factors may leads to thrombosis (nancy 2013). Though the focus is mainly on liver thrombotic microangiopathy, recognition of the causatives for thrombosis in NCIPH may provide insight on pathogenesis of other thrombotic diseases.

Aims

Aim 1: Evaluate hemeostatic mediators in circulation of patients with NCIPH: In this aim, the level of circulating ADAMTS13, ULvWF, nitrate levels, inflammatory cytokines and complement factors in NCIPH patients will be measured. Studies on TMA implicate elevation of (mention specific cytokines) these cytokines in thrombosis (ref) and hence the role of these factors in NCIPH will be evaluated. Inflammatory cytokines such as TNFα, IL6, IL8, and IL10 in plasma of NCIPH cases and controls will be determined using ELISA. It is believed that changes in the levels of these components may give a clearer understanding in outlining the cascade of thrombotic origin in NCIPH. Details of cases such as the age, time point (not yet discussed with Dr. Ashish)

Aim2: Examine the role of ADAMTS13 deficieny on endothelial cell adhered VWF in the context of NCIPH: In this aim, the functional relationship of ADAMTS13 and proteolytic cleavage of UL-VWF on endothelial cells in NCIPH will be evaluated in an in vitro system. Induction of VWF on human umbilical vein endothelial cells (HUVECs) in response to levels of inflammatory

cytokines seen in NCIPH patient circulation will be examined initially. Subsequently, normal and NCIPH patient plasma will be diluted in different ratios (from 0 to 5) in buffer (20 mM HEPES, pH 7. 4, 150 mM NaCl and 5 mM CaCl2) and incubated with HUVECs for 5 to 30 minutes, followed by testing for surface bound UL-vWF polymers. These experiments will also be done in the presence of shear stress by culturing cells on microslides with precise channels (IBIDI) to replicate flow within both venous and arterial circulation. The continuous flow of Media (specification-mention details) is supplied to the slides to mimic the physiological system of shear stress. Laminar shear as well as turbulent shear at junctions where the venous and arterial circulation meets within the liver will be replicated in this system.

Aim3: Explore the role of ADAMTS13-VWF balance on complement binding to endothelial cells in the context of NCIPH: Here, the effect of ADAMTS13 deficiency on complement activation and adhesion to VWF on endothelial cells will be studied. In vitro experiments using HUVECs (human umbilical vein endothelial cells) in culture will be carried out to recreate the proposed intrahepatic vascular milieu in NCIPH, where absence of ADAMTS13 cleavage results in accumulation of ULWF (ultralarge von Willebrand factor) on endothelial cells. Towards this, HUVECs grown in culture will be stimulated with histamine for 2 minutes to induce secretion of ULVWF, which will be immuno-stained prior to their cleavage by plasma derived ADAMTS13. These cells will then be treated with serum from controls and NCIPH patients under normal as well as orbital shear conditions. After 15 minutes incubation, cells will be washed and immunostained for complement proteins. In the second set of experiments it tested for platelet adhesion to these cell lines which

purified from the peripheral blood of the patients and control individuals. It is anticipated that plasma from NCIPH patients will show an increased complement and Platelet binding to ULVWF as illustrated in figure. For platelet adhesion experiments on invitro cell lines platelet rich plasma will be incubated for 15 minutes, washed and imaged for platelet adhesion.

ADAMTS13 is a disintegrin and metalloprotease with eight thrombospondin-1-like domains composed of an amino-terminal reprolysin-type metalloprotease domain followed by a disintegrin domain; a thrombospondin-1-like domain; a cysteine-rich domain containing an arginine-glycine-aspartate (RGD) sequence and an adjacent spacer portion; 7 additional thrombospondin-1-like domains; and 2 similar CUB domains at the carboxyl-terminal end of the molecule. CUB domains, found only in ADAMTS13 among the ADAMTS enzymes, contain peptide sequences present in Complement subcomponents C1r/C1s; embryonic sea Urchin