P791 COMMON VARIANTS IN COMPLEMENT PROTEINS, C3 AND CR1, ENHANCE COMPLEMENT ATTACK ON PNH ERYTHROCYTES AND INCREASE RISK FOR EXTRAVASCULAR HAEMOLYSIS

Topic: 11. Bone marrow failure syndromes incl. PNH - Biology & Translational Research

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Background:

In paroxysmal nocturnal haemoglobinuria (PNH), absence of glycosylphosphatidylinositol (GPI) anchors leads to loss of the complement inhibitor, CD59, on PNH erythrocytes (E) causing complement-mediated intravascular haemolysis. Treatment with anti-C5 antibody (eculizumab or ravulizumab) rescues PNH-E but loss of another GPI-anchored regulator, CD55, leads to accumulation of C3 fragments on E and extravascular haemolysis (EVH) due to C3-driven erythrophagocytosis. Therefore, approximately 30% of patients still require blood transfusion. Deposition of C3b and generation of downstream inactivation fragments such as iC3b and C3dg is controlled by binding of complement receptor type 1 (CR1) to C3b with factor I (FI), forming a trimolecular complex (TMC). We hypothesise that polymorphisms in C3 and CR1 influence the efficacy of this inactivation process and dictate propensity for EVH.

Aims:

To determine the combined effect of C3-S/F and CR1 density polymorphisms in susceptibility to EVH by the inactivation of C3b/iC3b on PNH-E.

Methods:

Forty-two eculizumab-treated PNH patients were genotyped for their single nucleotide polymorphism in C3 (rs2230199;S/F) and CR1 (rs11118133;H/L). C3 loading on PNH-E of patients were measured by flow cytometry as part of the routine analysis at the Leeds Haematological Malignancy Diagnostic Service. Lactate dehydrogenase, haemoglobin levels and reticulocyte count were also recorded. Soluble CR1 (sCR1) constructs were generated using recombinant technology and C3 variants were purified from human plasma using classical chromatography techniques. Interactions between C3b and CR1 and formation of the alternative pathway regulatory TMC (C3b:CR1:FI) were analysed using surface plasmon resonance (SPR).

Healthy donors were genotyped for their CR1 density polymorphism and relative CR1 expression on E was quantitated by flow cytometry. Impact of CR1 density polymorphism on C3b inactivation was studied using C3b-coated streptavidin beads and solubilised E from CR1 H/H or H/L donors and C3b breakdown to iC3b/C3dg was measured using flow cytometry.

Results:

There was a trend for higher mean percent of C3 loading in patients who have the C3-S/S allele (n=28, 30%) compared to patients with the C3-S/F allele (n=12, 18%) and a patient who had the C3-F/F allele (7%). Patients with high (H/H) CR1 expression (n=25, 20%) showed a trend for lower mean percent of C3 loading on PNH-E than patients with intermediate (H/L) CR1 expression (n=17, 32%). In patients with the C3-S/S polymorphism, haemoglobin levels were significantly lower (p=0.0132) and higher numbers of patients (20 of 29) had at least one...
transfusion event.

Using SPR, we demonstrated that CR1 bound C3b-F with a higher affinity than C3b-S. This led to higher levels of TMC formation with FI and more effective decay of C3-F convertase (C3b-F:Bb) by CR1. Solubilised E from a CR1-H/H donor converted iC3b more effectively to C3dg compared to E from CR1-H/L donors.

**Summary/Conclusion:**

These data indicate that both C3-S/F and CR1 density polymorphisms may influence C3b loading on PNH-E, with C3 fragments influencing EVH risk. Weaker binding of C3b-S to CR1 led to a decreased regulatory TMC formation with FI and slower decay of C3-S convertase (C3b-S:Bb). These data also indicate that E from individuals with lower CR1 expression convert the iC3b to C3dg more slowly. Overall, these data demonstrate mechanisms that enhance erythrophagocytosis in susceptible PNH patients by increased C3 loading on PNH-E and a decreased in removal of phagocytic opsonin, iC3b.