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3D protein structure modeling:

A tool to provide insight into the platelet alloimmune response

Fetal / Neonatal alloimmune thrombocytopenia (FNAIT) is a severe bleeding syndrome in which fetal / neonatal platelet destruction is mediated by maternal antibodies directed to specific antigens (or alloantigens) inherited from the father[1]. These antigens depend on polymorphisms of genes coding for several membrane glycoproteins (GPIb-IX-V, GPIIbIIIa, and GPIaIIa) or lipo-protein (CD109) receptors expressed at the platelet surface. These polymorphisms are classified in the Human Platelet Alloantigen, or HPA, nomenclature[2]. αIIbβ3 (or GPIIbIIIa) carries the majority of the HPA systems described to date (HPA-1, 3, 4, 6, 7, 8, 9, 10, 11, 14, 16, 17, 19, 20 and 21). This complex is highly immunogenic and is responsible for most FNAIT. αIIbβ3 belongs to the large family of the integrins that is composed of heterodimeric membrane receptors involved in cell-cell or cell-matrix interactions[3]. It mediates platelet aggregation as a receptor for fibrinogen, a major plasmatic adhesion molecule[4]. Resting platelets express on their surface about 50 000 copies of αIIbβ3 and 30 000 additional copies when activated.

Protein 3D structures (or structure models) help understand relationships between the protein dynamics and their biological functions. They provide new insight into atomic mechanisms of macromolecular recognition and conformational changes. Integrin structures are available from the Protein Data Bank (PDB). We have used a 3D structure of αIIbβ3 (PDB code 3FCS) to propose an explanation for the structure effect of the β3 Lys253Met substitution indentified in a Glanzmann patient, a mutation impairing αIIbβ3 expression[5]. Immune response relies on both immunogenicity and antigenicity. Antigenicity can depend on the 3D molecular structure surrounding the polymorphic site. We have used a 3D structure of αIIbβ3 and modeling experiments to study the impact of HPA polymorphisms on the complex structure, and their role in antigenicity. Different HPA allelic forms of αIIb and β3 were modeled from the structures 3FCS or 3IJE respectively and resulting structure characteristics of residue accessibility, mobility, and electrostatic change were analyzed.

Four polymorphisms of βIb identified in a context of FNAIT were studied, HPA-20w6, Cab27, Cab3 and Lec (table 1). The other αIIb polymorphisms described, HPA-3, -9 and Ak, could not be studied because they locate on an unresolved part of the αIIb crystal structure. As an example, figure 1 reports the 3D structures of the αIIb Cab2a- and Cab2a+ allelic forms. The corresponding computed electrostatic map has been projected on the molecular surface of each allelic form of αIIb. Red color corresponds to a negative charge whereas blue is positive and white neutral. The Ser472Asn substitution does not really affect either the local positive electrostatic charge or the residue mobility or its accessibility. Structure features of all αIIb polymorphisms studied (summarized in Table 1) suggest that alloantibody presence depends on residue accessibility (accessibility ranging between 50 and 90%) but not really on mobility or electrostatic characteristics.

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3D structure of the β3 HPA polymorphisms 1a, 1b, 4a, 4b, 6a, 7a, 10a, 11a, 14a, 16a, 17a, 19a and 21a have also been studied. Figure 2 shows a model of the β3 backbone structure (green ribbon) and the molecular surface (grey). HPA polymorphic amino acids are represented as magenta spheres. Analysis of the structure features of the β3 HPA residues studied confirmed the observation made for the αIIb HPA polymorphisms. Alloantibodies rely on the residue presence at the surface of the structure (accessibility) but do not tightly depend on its mobility or its electrostatic charge (not shown).

Finally, a comparative study of a selection of published primary sequences of the β subunits of the integrins revealed that residues involved in β3 HPA polymorphisms are not evolutionarily conserved. These results were also reported by Landau et al8 who used a similar approach. Mutations affecting evolutionarily conserved amino acids generally result in defective expression or function of αIIbβ3 that impair platelet aggregation (Glanzmann Thrombasthenia syndrome).[8]

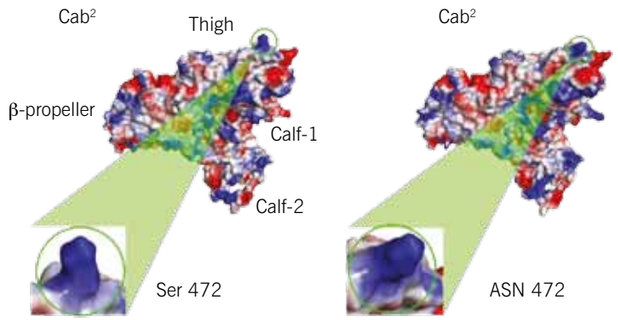

Figure 1:
Modeling of the Cab2a+ and Cab2a- forms of the αIIb structure. The Ser472 (Cab2a-) and the Asn472 (Cab2+) αIIb forms were modeled from the PDB structure 3FCS. The computed electrostatic maps were projected on the molecular surfaces of each structure, blue, red and white colors respectively corresponding to positive, negative and neutral electrostatic charges. Detailed pictures present the structure features of the polymorphic site for each allele. It appears that the Ser472Asn substitution impairs neither the accessibility nor the electrostatic charge nor the mobility of the side chain (respectively assessed by computing the solvent surface accessible surface, the electrostatic charge and the B-factor).

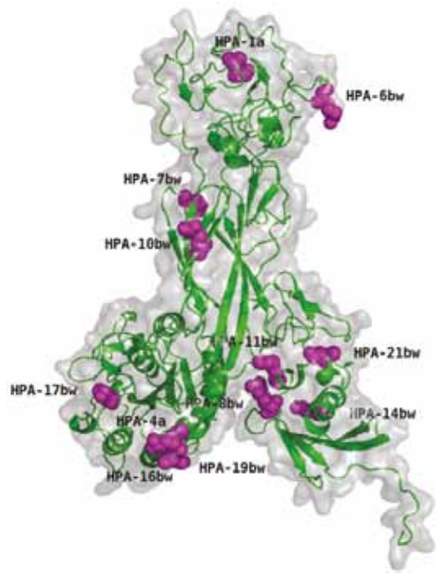

Figure 2:
Location of the HPA polymorphisms of the β3 structure. 3D model of the β3 obtained from the PDB structure 3IJE. Shown are the peptidic backbone (green), the molecular surface (grey) and the atoms of the HPA polymorphic residues (magenta spheres). HPA codes are indicated. This representation was made by using PyMOL (http://www.pymol.org)