Anti-anti-tissue Factor: X-ray Structure and the Immune Network Hypothesis

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Introduction

Vascular damage that permits blood to leave the vasculature will trigger the generation of clotted blood and thereby slow or stop blood loss. The critical need to form clots in response to vascular injury is addressed by a highly sensitive and fast-acting collection of many molecular parts called the coagulation cascade. However, this high sensitivity can lead to a deleterious initiation of the cascade within the vasculature. For instance, the rupture of an atherosclerotic plaque can initiate coagulation, and the resulting blood clot (thrombus) can become life-threatening. As a counterbalance to its high sensitivity, the cascade operates under tight control provided by cofactors required by the various coagulation proteases.

The extrinsic coagulation cascade refers to reactions among a collection of trypsin-like serine protease enzymes and their cofactors, which are initiated when FVIIa combines with its cell-surface bound cofactor, tissue factor (TF). The TF/FVIIa complex performs a proteolytic cleavage of factor X (FX) to form the activated protease FXa. In association with its cofactor factor Va (FVa), FXa performs a proteolytic cleavage of prothrombin to form thrombin. Thrombin cleaves fibrinogen to fibrin, promoting formation of a fibrin clot, and it activates platelets for inclusion in the clot. Thrombin also starts to turn off the coagulation process when, in combination with thrombomodulin, it activates Protein C, which, in turn, degrades key cascade components. The TF/FVIIa complex can also perform activating cleavages on FIX and FVII.

Among strategies being pursued to limit activation of the coagulation cascade is an anti-TF monoclonal antibody, humanized D3 (D3h44). This antibody acts by binding to TF in an epitope that overlaps strongly with a substrate binding region. The structures of both D3h44 alone and in complex with TF are known [1]. We have generated a mouse monoclonal antibody (6A6) that binds to D3h44 with high affinity and high specificity and that blocks the binding of D3h44 to TF. The immune network hypothesis predicts that a secondary antibody raised against the antigen-recognition region of a primary antibody will mimic the primary antigen. In the present case, the primary antigen is TF, the primary antibody is D3h44, and the secondary antibody is 6A6. Thus, the hypothesis draws a parallel between TF and 6A6. To investigate the validity of the hypothesis in this system, we have determined the x-ray structure of a complex between Fab fragments of D3h44 and 6A6.

Material and Methods

The Fab fragment of D3h44 was produced in E. coli. The Fab fragment of murine 6A6 was produced via papain cleavage of the intact IgG. After combination and purification by size exclusion chromatography, crystals were grown from 20% PEG 3350 at pH 7.0 by the hanging drop method. Data were collected at beamline 19-ID at the Structural Biology Center (SBC) of the APS. The data extend to a resolution of 2.5 Å in space group P1 with cell parameters a = 78.9 Å, b = 85.8 Å, c = 92.4 Å, α = 77.5º, β = 75.9º, and γ = 63.3º. The structure was solved by molecular replacement by using models based on prior D3h44 structures and from the murine Fab structure in Protein Data Bank entry 1GIG. The current agreement factors are R = 25.2% and Rfree = 30.2%.

Results

On the basis of the faint sequence homology we had identified between complementarity-determining regions (CDRs) L1 and H2 of 6A6 and the known D3h44 epitope of TF, we had determined that the approximate distance separating CDRs L1 and H2 was close to the distance between the two homologous sequences of TF. Once the 6A6/D3h44 complex structure was sufficiently solved and refined, we were able to judge the accuracy of the rough structural alignment. The overall structural correspondence is poor. There are few, if any, strict analogs between the interactions of TF/D3h44 and 6A6/D3h44. Of course, on the basis of the biochemical characterization, we expected strong overlap in the regions of D3h44 contact by each of the binding partners, and this is the case. But even at a gross level, there are significant differences. For instance, the heavy chain of D3h44 is far more important than the light chain in the TF/D3h44 complex, but the situation is reversed in the 6A6/D3h44 complex. Thus, the immune network hypothesis is demonstrated here in only a very limited, functional way.

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Reference

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