Molecular Structures and High Resolution TEM

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Structure of C1-immune complexes revealed by cryo-electron tomography

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The complement system enables the mammalian host to recognize and clear bacteria, viruses, fungi and parasites from blood and interstitial fluids. The so-called classical pathway of complement activation may be initiated by antibodies [1]. Whereas the structural insights are advanced for antibodies recognizing antigens and binding Fc receptors, it is currently unclear how antibodies activate the complement system. By using dual axis cryo-EM tomography [2] and sub tomogram averaging [3] we reveal the three-dimensional arrangement of antibodies that binds and activates the first component, i.e. the C1 complex (790 kDa), of the classical pathway and thereby initiates the complement cascade that leads to clearance of the invading microbes.

For cryo-EM tomography we used functionally active antigen-antibody-C1 complexes formed on liposomes (Figure 1). Dual axis cryo electron tomography (FEI Titan Krios), followed by sub tomogram averaging and classification, resulted in a 4.5 nm resolution structure of the complex (Figure 2). This averaged EM map reveals the quaternary structure of the active C1-Ab complex on a biological membrane in a close to native state. Guided by biochemical experiments and atomic structures we were able to generate a pseudo-atomic model of the C1-antibody arrangement (Figure 3).

The model shows that six Ab bind to the antigen surface with Fab, while the remaining Fab and Fc form a platform through Fc:Fc interactions for C1 binding. Mutagenesis data and functional assays confirm that this interaction is mandatory for complement activation by all IgG Ab sub classes.

Requirement of hexameric Ab arrangement for classical complement activation on a haptenated surface forms a novel activation model which improves our understanding of (auto) immune diseases and might enable design of improved antibodies as vaccines or cancer therapeutics.

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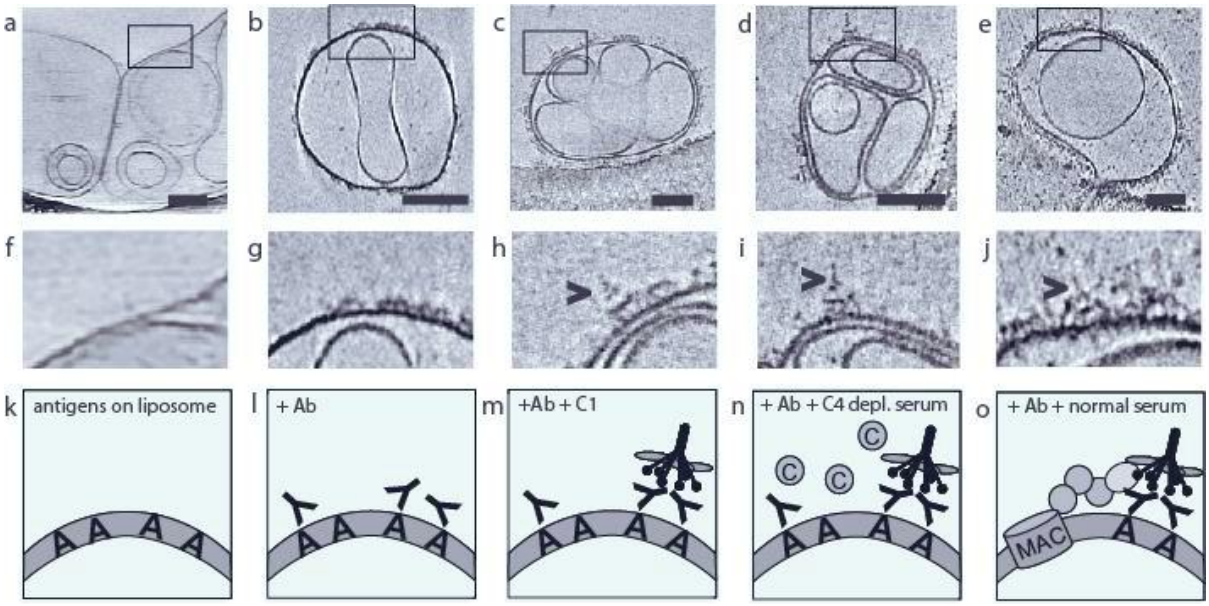

Figure 1. Cryo-ET imaging of complement-activation steps. a, Series of sections through tomograms of haptenated liposomes **b**, antibody opsonized liposomes **c,** followed by addition of C1 or **d**, addition of C4-depleted serum or **e,** normal serum leading to full complement activation. Black scale bars represent 100 nm. **f-j** enlargements from the areas indicated by a rectangle. Arrowheads point at C1-immune complexes. **k-o**: schematics.

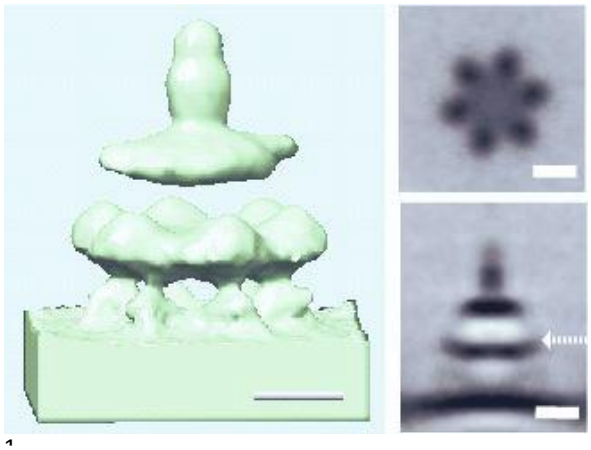

Figure 2. Sub tomogram average of C1-immune complexes. Isosurface (left) as well as horizontal and vertical sections (right). Scale bars represent 10 nm.

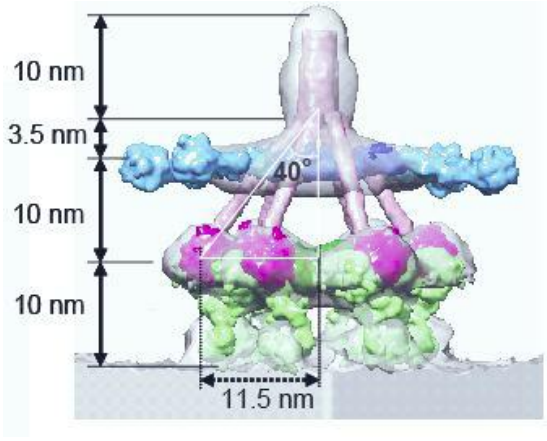

Figure 3. Atomic model if the C1-immune complex docked into cryo-ET map. Side view of the model with indication of overall dimensions.