3D structure of human myeloma IgG subclasses.

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Human immunoglobulins G (hIgG) are divided into four subclasses, hIgG1-4. In all IgG subclasses, Fc and Fab subunits are nearly identical. However, the connector between subunits (hinge) differs. Fabs are responsible for recognition of antigen, while Fc is mainly responsible for effector functions such as activating the complement. After 40+ years of research, the relationship between structure and function in hIgG subclasses remains unclear. Why, for instance, does hIgG1 activate complement very well and hIgG4 does not? In 1989 we proposed that the difference in effector functions of hIgG subclasses may be explained in terms of orientation of subunits within the molecule and subunits ability to move (flexibility) [1-5]. Since many IgG are flexible by nature, classical structural approaches, such as X-ray crystallography, do not work. In fact, even now, just a few whole IgG structures are available. To address the challenge of working with IgGs we employed a complex of methods including electron microscopy (EM). We used negative staining and freeze-drying with high-resolution shadowing. Our work resulted in a series of publications on human myeloma hIgG1-4 structure [2-4]. We found that four hIgG subclasses have a different shape, and subunits (of each subclass) have different mobility. We also described the typical shape of the IgG molecule as tripod-like, unlike the Y-shape popular in textbooks. Table 1 summarizes our findings.

Interestingly, EM of most intriguing hIgG2 and hIgG4 originally did not produce meaningful results and was placed "on hold" for 25 years. These data were recently re-visited and a new EM approach was imposed on old data. Using single particle 3D reconstruction we were able to reconstruct the 3D structure of hIgG2 [4] and hIgG4 [5]. The 3D models showed fine details of the structure, which had never been seen before. The structure of subunits served as an internal control. In particular, hIgG2 has a unique structure when one Fab is in tight contact with Fc. hIgG4 is a rigid asymmetric molecule with one Fab subunit closer to Fc than other. The 3D structures of hIgG2 and hIgG4 are presented on Fig. 1.

Understanding how structure of IgG relates to function is critical for antibody-based drug development. For instance, even normal IgG effector function(s) can cause devastating negative effects by activating complement cascade. An improperly selected IgG candidate for drug can cause allergy and other complications. Regardless of our extensive knowledge about IgGs, some drug-companies are choosing hIgG1, which is a less promising candidate due to its high potential to activate C1q. Isolation and purification of IgG drug candidates is another problem: in many cases, drug-companies utilize methods that can alter the 3D structure of IgG and therefore affect its effector function. From this perspective, EM is a simple, quick and reliable method to estimate the properties of IgG and facilitate the best choice in drug-developing pipelines.

References:

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3. Ryazantsev et al., 1990, Eur. J. Biochem. 190: 393−399.
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Table 1. Human myeloma IgG subclasses summary.

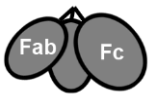
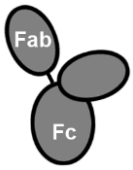
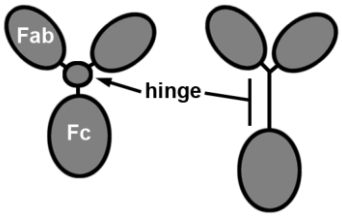
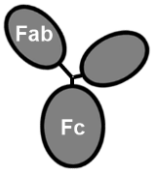
IgG1	IgG2	IgG3	IgG4
			
C1q binding +++	+/-	++++	-/+
Flexibility +++	+/-	++++	-/+

Figure 1. Comparison of 3D structure of hIgG2 and hIgG4.