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O18

On-chip complement activation for functional profiling of serum antibodies on antigen microarrays

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The development of high-density protein arrays makes it possible to simultaneously measure hundreds or even thousands of antigen–antibody interactions. Profiling of serum antibodies with such arrays helps us in understanding the complexity of responses of the adaptive immune system against microbes, tumors and autoantigens. Complement components are also able to recognize and interact with both foreign substances and self-molecules, including antibodies, and initiate a cascade of proteolytic cleavages, resulting – along with other effects – in the covalent attachment of complement components to molecules in nanometer proximity.

By adapting the conditions of antibody profiling on antigen arrays we made use of this molecular tagging to identify antigens that activate the complement system. Antigen arrays were incubated with serum under conditions that favor complement activation and the deposited complement C3 fragments were detected by fluorescently labeled antibodies. We used genetically C3 deficient mice or inhibition of the complement cascade to prove that the technique requires complement activation for the binding of C3 to features of the array. Antigens on the array can initiate complement activation both by antibody dependent or independent ways. Using two-color detection, antibody and complement binding to the relevant spots was measured simultaneously. Currently we are assessing the potential added diagnostic value of the technique in autoimmune conditions.

We propose that measurement of complement deposition on antigen microarrays supplements information from antibody binding measurements and provides an extra, immune-function related fingerprint of the tested serum.

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O19

Small glycoproteins of the extracellular matrix interact with complement to varying degrees

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The extracellular matrix consists both of large macromolecules and smaller proteins with regulatory functions. An important group of the latter class of molecules is the family of small leucine rich repeat (SLRP) glycoproteins. We have previously shown that fibromodulin, a member of this family, binds directly to C1q of complement and thereby activates the classical pathway of complement.

In the present study we expand the knowledge regarding interactions between SLRPs and complement and are able to show specific interactions between several extracellular matrix components and complement. Similar to fibromodulin, some SLRPs bind C1q and activate the classical pathway of complement avidly. To some extent the alternative pathway is activated but the lectin pathway is not triggered. We have also detected specific and strong interactions between some of these proteins and complement regulator factor H. This interaction may be responsible for the relative weakening of SLRP-induced complement activation in the terminal pathway.

Furthermore, we confirm published data stating that biglycan and decorin bind C1q but do not activate complement. For some of the analysed proteins a similar pattern is seen, although the interactions are weaker. We also investigated whether the SLRPs bind the head or stalk domains of C1q and performed inhibition experiments to identify overlapping binding sites on C1q.

We propose a role for SLRPs in regulation of complement activation in the extracellular matrix. This mechanism would be particularly relevant in diseases characterised by chronic inflammation, such as rheumatoid arthritis.

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O20

C3c as a fluid phase marker for inflammation—generation and characterization of monoclonal antibodies against neopeptides on activated or inactivated split products of C3

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There is a need for markers for systemic inflammation in acute or chronic diseases, and factors of the complement system are obvious targets. Available methods are of low sensitivity and are both elaborate and time consuming; they include