# Immune complexes on protein chips: measurement of antibody function using antigen microarrays

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#### Antigen microarrays, protein chips

- Protein arrays are ordered arrangements of several (upto thousands) of proteins, allowing the measurement of such high numbers of molecular interactions
- Interactions between antigens and antibodies can also be monitored on microarrays

 Identification of the targets of circulating antibodies out of a library of antigens generates an immunologic fingerprint: the antibody profile



Colored proteins printed on a nitrocellulose-covered glass slide

### Antibody profiling



fluorochrome labeled detection antibody



serum antibody

antigen printed on the array



Serum antibodies bound to the antigen array are detected by secondary labeled antibodies Result: quantity of antigen specific antibodies in serum

#### Antibodies...

come in different flavours, called

classes :IgD, IgM, IgG,IgA,IgEsubclasses :IgG1, IgG2, IgG3, IgG4IgA1, IgA2



# The role of antibodies in the serum is to

- neutralize targets
- activate complement
- enhance phagocytosis
- activate leukocytes

Antibodies belonging to different classes and subclasses possess different effector functions

### Traditional antibody profiling

 measures the binding of certain antibody classes or subclasses only – out of the many isotypes that potentially bind

 provides no information about the effector functions Functional antibody profiling by AbC-array technology

 measures the overall effect of the binding of antibodies of different isotypes on the complement system.

## The complement system



Erdei&Gergely, Immunobiology 2000

• is a group of proteins with diverse biological functions

• is mostly inactive in the blood until danger signals trigger its activation

• is capable of molecularly tagging the site of activation and recruit leukocytes to this site



Janssen et al., Nature 2006 Nov 9

#### Functional antibody profiling



fluorochrome labeled detection antibody



C36

serum antibody

Serum complement protein





Serum antibodies and complement proteins bound to the antigen array are detected by secondary antibodies Result: quantity and quality of antigen specific antibodies in serum

### **Fluorescent detection**

helps the parallel measurement of antibody and complement binding information which can be merged and interpreted afterwards



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