Isotypes: Diving Deeper into Immunoprofiling

The immune system is a library cataloging an individual's unique experiences with pathogens and self-antigens. Immunoprofiling is the quantification of these experiences by measuring the unique characteristics of an individual's immune system such as cell type and activation status, cytokines, and antibody expression. Altogether, the data can indicate disease, inflammatory status, and immune fitness.

The information can guide precision medicine by improving diagnostic accuracy, identifying disease early, assessing treatment effectiveness, and stratifying patients into effective treatment protocols. Further, immunoprofiling provides unique research opportunities, enabling researchers to study disease subpopulations, or endotypes, with the future goal of improving therapies. While any measurement of immune function is helpful for immunoprofiling, antibodies are among the most economical and informative.

Antibodies are easily accessible through a simple blood draw and contain detailed information about an individual's disease status because of their specificity to unique antigens. IgG antibodies in particular are strong indicators of an immune response within the body. They make ideal biomarkers because they circulate in the blood and are direct manifestations of disease that can often appear before physical symptoms. Functional protein microarrays can identify the unique antibody repertoire from a patient against thousands of potential antigens. Historically, researchers have focused on just IgG antibodies. However, IgG antibodies are only one of many antibody isotypes. Profiling multiple isotypes provides greater patient and disease detail than single isotype analysis because each isotype contributes discrete information, creating a fuller picture of what the immune system senses.

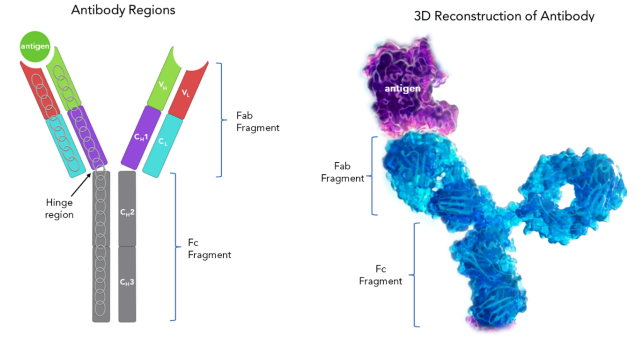

Figure 1 The Basic Structure of an Antibody. Left, General overview of antibody structure highlighting location of Fab and Fc regions. Right, 3D reconstruction illustrating antibody shape.

Antibodies are 10nm, Y-shaped proteins produced by B lymphocytes (Figure 1). There are five distinct antibody isotypes, IgG, IgM, IgA, IgD and IgE with four additional subclasses of IgG and two additional subclasses of IgA. Some isotypes can multimerize; IgM can form pentamers and IgA dimers. Each antibody is composed of two heavy chains and two light chains, held together by disulfide bonds. The stem of the antibody, the Fc region, is made up of two heavy chains with two to three constant domains. The Fc region of each isotype is unique. This region is involved in anchoring the antibody in the B cell membrane, transducing antigen stimulated signaling, and acts as ligand to Fc receptors to regulate other immune cells. The branches on top of the antibody, called the Fab fragment, consist of heavy chains attached to a pair of smaller light chains. At the amino terminal end are three hypervariable domains that are produced through somatic mutations and random recombination of gene segments. This is where antigen binding occurs. Unlike the Fc domain, hypervariable regions are created with enormous diversity, resulting in billions of binding possibilities. Each individual B-cell and its progeny are dedicated to producing the same antibody (i.e., monoclonal). The two regions, Fc and Fab, are both involved in immune system function in different ways.

During B cell development, immature bone marrow B cells initially express membrane bound IgM, forming part of the B-cell receptor (BCR) with the Fc region bound to the cell membrane and the Fab region exposed extracellularly to bind with circulating antigen. A signal transduction region (CD79) couples with the IgM to complete the complex. BCRs with strong affinity for self-antigens will usually undergo clonal deletion via apoptosis. Surviving B cells further mature in the spleen or lymph nodes where they can express IgD or IgM transmembrane antibodies. If stimulated by foreign antigen, class switching, and clonal expansion occur. The B cells form germinal centers and express high levels of secreted antibody specific to the stimulating antigen (LeBien & Tedder, 2008; Mårtensson et al., 2010; Melamed et al., 1998; Tonegawa, 1983). The isotype expressed is a function of the antigen and its location within the body. For

example, B cells activated in the intestinal lumen produce circulating IgA, an antibody capable of functioning under low pH and high peptidase activity. IgA also obstructs pathogen entry into the intestine and is found in mucosal membranes. IgG is the most abundant isotype and is most often produced in response to bacteria and viruses and circulates in the lymph and blood. IgE activates mast cells and eosinophils, is involved in allergic reactions, and protects against larger microbes and parasites. The function of IgD is not well known. It is expressed in very low numbers and plays a role in B cell activation and class switching. Lastly, IgM is involved in complement signaling and mediating cellular immunity (Table 1). Antibodies are not only highly specific to a given antigen, but also have different effector functions mediated by their Fc regions. The Fc regions can act as ligands to Fc receptors and can also react with complement to regulate the activity of other immune cells like neutrophils, natural killer cells, macrophages and microglial cells leading to their recruitment and activation. This is a regular function of IgM antibodies to activate other immune cells of the innate immune system. As shown below, antibody isotype can play a key role in immunotherapies.

Antibody	Subclasses	Primary Function	Secreted
IgA	IgA_{1-2}	Pathogen neutralization, anti-inflammatory, mucosal	Yes
IgG	IgG_{1-4}	Pathogen detection & removal	Yes
IgD	None	Immune tolerance	No
IgE	None	Mast cell activation, anti-parasitic, tumor surveillance & mediation of type I hypersensitivity	Yes
IgM	None	Complement activation, cell to cell signaling, BCR	Yes

Table 1. Antibody Isotypes

Many complex diseases including cancer, neurodegenerative diseases, and autoimmune diseases, involve numerous organ systems, and as a result may induce expression of multiple isotypes and subclasses of antibodies, some of which may be present before the target organ is affected. For example, autoimmune diseases not only exhibit autoantibodies with antigen specificity, but also isotype specificity. IgM and IgG are the most abundant in nearly all autoimmune diseases; however, IgA autoantibodies are prominent in antiphospholipid syndrome of the vascular system while IgE is prevalent in SLE (Suurmond & Diamond, 2015). Consequently, isotype identification in addition to antibody specificity can add greater clarity to disease detection and pathology. In rheumatoid arthritis, combining quantification of antibody isotypes IgG, IgM, and IgA with antigen specificities (RF, ACPA and RA33) improved identification of rheumatoid arthritis by up to 30% (Sieghart et al., 2018).

The appreciation of subclasses can help identify pathogens, disease status and drive therapeutic development. For example, the CNS infecting yeast C. neoformans stimulates IgG2a antibodies over all other antibodies, and Mycobacterium tuberculosis induces more IgG3 than any other antibody (Suurmond & Diamond, 2015). Consequently, the antibody isotype may help clarify among difficult to diagnose diseases. In the case of SARS-CoV-2, researchers at University of Cape Town have demonstrated, with the use of a dual color IgA and IgG protein microarray, a deficit in anti-Spike Protein IgA production in HIV patients who also become infected with SARS-CoV-2 (Smith et al., 2023). This information demonstrates how previous immunological experiences can alter patients' immunoprofiles and further supports the need for precision medicine to direct treatment.

The antibody isotype may be an important choice in designing immunotherapies. Antibody Fc receptor stimulated microglial cells increase uptake and degradation of tau and -synuclein particles in vitro, an encouraging finding leading to research of monoclonal antibody therapies to treat neurodegenerative diseases involving protein aggregates (Parkinson's, Alzheimer's, Huntington's). However, IgG1 antibodies have a greater chance of eliciting neuroinflammation and adverse events than IgG4 antibodies that do not react with complement (Katsinelos et al., 2019). In this case, immunoprofiling may help determine the best choice of antibody subtype for safety and efficacy.

Isotype screening has traditionally been done with immunochemistry using ELISA technology and isotype specific secondary antibodies. However, ELISA lacks the throughput and sensitivity of more modern protein microarrays. Functional protein microarrays are an excellent, high throughput means of quickly profiling the myriad of antibodies present in an individual's serum. Until recently, array assays detected IgG, the most common antibody in serum. Sengenics offers a validated, high-quality dual color detection analysis assay capable of simultaneous identification of specific IgG and IgA antibodies from a small patient sample. Using the patented KREX technology, only correctly folded proteins with their discontinuous epitopes intact are present on the array, resulting in highly specific antibody-antigen binding, a necessity for dual isotype profiling where non-specific binding could confound the results. The technology helped profile individuals exposed to SARS-CoV-2, indicating that healthy vaccinated individuals produced both IgG and IgA anti-spike antibodies, and as mentioned above, HIV patients did not produce anti-spike IgA (Smith et al., 2023). This information can help direct HIV patient care.

Accounting for antibody isotypes in addition to specificity adds unique data to antibody immunoprofiling that have the potential to uncover novel, high resolution biomarker signatures that could easily be missed by focusing solely on a single isotype. A dual color detection assay can greatly enhance diagnostic potential, provide greater depth of endotyping, improve predictions of adverse outcomes, and uncover new disease related pathways.

References

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