

# Preface

High amounts of specific antibodies are produced upon antigen stimulation during adaptive immune responses. Antibody production can rapidly resume, long after a primary response, and in higher amounts, during memory responses. Altogether, antibodies of every individual recognize a wide repertoire of antigens encountered during life, including autoantigens. As a result, several grams of antibodies with multiple specificities per liter of plasma circulate throughout the body via the bloodstream. Antibodies can reach remote organs in the periphery within minutes. They are involved in a variety of biological responses in health and disease. They can both protect from infections and induce allergic, autoimmune, or other inflammatory diseases. Genetically engineered monoclonal antibodies are increasingly used in passive immunotherapy, mostly, but not exclusively in cancer. Immunoglobulins pooled from the plasma of thousands of normal donors are injected intravenously (IVIg) as an anti-inflammatory treatment in an increasing number of autoimmune diseases. Actively produced specific antibodies account for the therapeutic effects of the overwhelming majority of protective vaccines, whether prophylactic or therapeutic. How antibodies work, however, is far from being fully understood and appreciated.

Antibodies bind to specific antigens by their Fab portions with a wide range of affinities. Binding is necessary for antibodies to act on antigens. Binding, however, is not sufficient. Antibodies indeed exert little or no effect when binding to antigen only. They have no biological activities per se. Antibodies, however, *mediate* many biological activities. They are mediators rather than effectors of adaptive immunity. Biological activities mediated by antibodies require their Fc portion. The Fc portion of immunoglobulins consists of the C-terminal constant domains of the two heavy chains that are characteristic of antibody classes and subclasses. Antibody-mediated biological activities indeed depend on the class of antibodies. The reason is that the Fc portion of antibodies of different classes differentially interacts with other molecules that can induce a variety of effector functions. These molecules are of two types: soluble molecules such as components of complement, and Fc Receptors (FcRs) expressed on the membrane of various cells.

For long, the existence of FcRs has been inferred from the observed biological effects of so-called “cytophilic” antibodies. In spite of the classical opposition between cell-mediated immunity and humoral immunity, some biological properties of antibodies were indeed found to depend on cells. When binding to

antigens, antibodies called “opsonins”—literally, which prepare the food to be ingested—enabled phagocytes to internalize particulate antigen-antibody complexes. Anaphylaxis and antibody-dependent cell-mediated cytotoxicity were found to result from the release of vasoactive and cytotoxic mediators, respectively, stored in the granules of different cell types. There were “homocytotropic” antibodies, which triggered responses in homologous tissues, and “heterocytotropic” antibodies, which triggered responses in heterologous tissues; there were antibodies whose cytophilic properties were heat-labile and antibodies which were heat-stable; there were washing-resistant and washing-sensitive cell-sensitizing antibodies, all of which could trigger similar responses but under different conditions. There were also enhancing and regulatory antibodies of different IgG subclasses. Although the concept of receptors for the Fc portion of cytophilic antibodies was proposed to account for the enhanced internalization of opsonized antigens by macrophages in the 1960s (Berken and Benacerraf 1966), the term Fc Receptors was not coined until 1972 by Frixos Paraskevas to describe IgG receptors on B lymphocytes (Paraskevas et al. 1972). By being given a name, FcRs gained a material existence. They could be identified on cell membranes and they became susceptible to molecular analysis.

FcRs for the various classes of immunoglobulins were indeed identified using several means to visualize cell-bound antibodies. FcRs with a high affinity were first found on a limited number of cells by assessing the binding of radiolabeled immunoglobulins. Using this approach, IgG and IgE receptors were found on macrophages and mast cells, respectively. Homogeneous cell lines made it possible to assess FcR numbers on single cells, to measure association and dissociation constants, and thus to calculate affinity constants. These were between  $10^8$  (Unkeless and Eisen 1975) and  $10^{10} \text{ M}^{-1}$  (Kulczycki and Metzger 1974).

Many more receptors for the same and for other immunoglobulin isotypes were subsequently identified by assessing the binding of red cells sensitized with antibodies under the microscope. These receptors had no measurable affinity for radiolabeled monomeric immunoglobulins, but they could bind multivalent immune complexes with high avidity. Using this “rosetting” procedure, all myeloid cells and some lymphoid cells expressed FcRs, and FcRs for all five immunoglobulin classes were recognized. These findings led to the distinction of high-affinity receptors referred to as FcRI, which bind antibodies as monomers, and of low-affinity receptors referred to as FcRII, which bind antigen-antibody complexes only. FcRs were also found on parasites (Torpier et al. 1979; Vincendeau and Daëron 1989), bacteria (Langone 1982), and even virus-encoded FcRs were described on infected cells (McTaggart et al. 1978; Litwin et al. 1990; Litwin and Grose 1992).

When monoclonal antibodies were raised against FcRs, cell population analysis by flow cytometry confirmed the distinction between high- and low-affinity FcRs and their differential tissue distribution. It also revealed a further heterogeneity amongst low-affinity receptors for IgG expressed by different cell types (Unkeless et al. 1988). Low-affinity FcRs were therefore subdivided into Fc $\gamma$ RII and Fc $\gamma$ RIII. More recently another high-affinity receptor for IgG found in mice but not in

humans, was named Fc $\gamma$ RIV (Nimmerjahn et al. 2005). Human FcRs identified by referenced monoclonal antibodies were given CD numbers and used as phenotypic markers of cell populations: CD16 corresponds to Fc $\gamma$ RIII, CD32 to Fc $\gamma$ RII, CD64 to Fc $\gamma$ RI, CD23 to Fc $\epsilon$ RII and CD89 to Fc $\alpha$ RI. Fc $\epsilon$ RI have no CD number.

As FcRs were increasing in numbers, biochemical analysis disclosed their molecular heterogeneity. High-affinity IgE receptors first (Holowka et al. 1980), then IgG (Ernst et al. 1993) and IgA receptors (Pfefferkorn and Yeaman 1994) were found to contain several polypeptides. Most are composed of 2–3-extracellular-domain immunoglobulin-binding FcR $\alpha$  subunit noncovalently associated with a widely expressed, highly conserved homodimeric common subunit named FcR $\gamma$  (Orloff et al. 1990) and, when expressed in mast cells or basophils, with a 4-transmembrane subunit named FcR $\beta$  whose expression is restricted to these cells (Kurosaki et al. 1992). cDNAs encoding the various FcR subunits having been cloned and expressed in different cells, their functional roles could be analyzed. FcR $\gamma$  and FcR $\beta$  were found to control both the membrane expression of FcR $\alpha$  (Takai et al. 1994; Kinet 1999) and the ability of membrane FcRs to generate activation signals when engaged by antigen–antibody complexes. FcR $\gamma$  and FcR $\beta$  were indeed shown to contain Immunoreceptor Tyrosine-based activation Motifs (ITAMs) (Reth 1989). Two single-chain low-affinity IgG receptors expressed in humans only also contained one ITAM, whereas another single-chain low-affinity IgG receptor expressed in mice and humans was found to contain an Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) (Daëron et al. 1995). Other receptors triggered neither activation nor inhibition signals, but permitted a strictly controlled internalization of antibodies. The 5 extracellular domain-containing polyIg receptor enables pentameric IgM and dimeric IgA to transcytose through polarized cells (Brandtzaeg 1983), whereas the  $\beta$ 2-microglobulin-associated MHC-I-like FcRn not only mediates the intestinal absorption of maternal IgG through the fetal gut epithelium, but protects IgG from degradation in adults (Raghavan et al. 1993; Roopenian et al. 2003).

When FcR genes were cloned, their phylogenetic relationship was established (Qiu et al. 1990) and their heterogeneity was further enriched. Capital letters were added to FcR names to designate human genes and their murine orthologs. More recently, a novel family of FcR-like (FCRL) molecules was disclosed in mice and humans, which dramatically expanded the FcR field (Ehrhardt et al. 2007). Many FCRLs still have no known ligand. Some can bind immunoglobulins. They have similar structures, similar signaling properties and similar genetic organizations as classical FcRs, but also marked differences. Genetic polymorphisms were unraveled in classical human FcRs, some of which were associated with disease, mostly autoimmune diseases, and/or with a differential efficacy of therapeutic antibodies. FcR knockout, knockin, and transgenic mice were genetically engineered that proved to be invaluable analytical tools to assess FcR function *in vivo*. Special efforts have been made to generate humanized mice in which murine FcRs have been more or less extensively replaced by human FcRs, sometimes with the same tissue distribution as in humans. These unique mice should be a major advance to analyze the contribution of the various FcRs to the protective and the pathogenic roles of antibodies in mouse models of human diseases. They should be also of

interest to tailor and to assess the efficacy of novel therapeutic antibodies for specific purposes. Indeed, nonhuman primates that are often viewed as the best animal models for preclinical vaccine trials, seem not to have the same FcRs as humans (Trist et al. 2014).

As knowledge on FcRs progressed, the complexity of the mechanisms by which antibodies work increased dramatically. The difficulty to understand their biological effects in health and disease and to use them as therapeutic tools increased in parallel. As a consequence, it became difficult for most scientists, including immunologists, to embrace the multifaceted and often antagonistic properties of antibodies. The aim of this issue of *Current Topics in Microbiology and Immunology* on FcRs was to gather in a single volume the contributions of internationally recognized FcR experts on essential novel aspects of FcR biology in physiology and pathology. To our knowledge, such a book has not been published for many years. This volume is divided into five parts, which, we believe, cover the main aspects of current knowledge on FcRs.

The Part I titled *Old and New FcRs* contains three chapters. It provides novel information on old receptors and information on novel FcRs. Hiromi Kubagawa et al. report their recent findings on human and murine Fc $\mu$ R. The existence of this long suspected receptor for IgM now lies on solid grounds. It also has unexpected properties that other FcRs do not have. Randall Davis et al. provide a state-of-the-art overview of the FCRL family with their known ligands, and they discuss their potential functions. Finally, Leo James describes TRIM21. This intriguing intracellular receptor with an extraordinarily high affinity for IgG and IgM has unique structural and functional properties that endow it with major protective properties, especially against viral infection.

The Part II deals with *FcR Signaling*. It also contains three chapters. Denis Thieffry et al. present their novel bioinformatic approach of FcR signaling, using high-affinity IgE receptors in mast cells as a model. They show how computational modeling can help to integrate the complexity of signaling pathways. Pierre Launay et al. focus their review on calcium channels that have long been known to be critical in FcR signaling. They discuss the role of novel channels that control intracellular calcium and how these channels are tightly regulated. Finally, Michael Huber et al. address the mechanisms by which the lipid phosphatase SHIP1 negatively regulates Fc $\epsilon$ RI signaling and how both the expression and function of SHIP1 are controlled. This hematopoietic cell-specific phosphatase is a major regulator of many signaling pathways, particularly but not only, in mast cells.

The Part III entitled *FcR Biology* deals with various FcR functions, mostly, but not exclusively under physiological conditions. It contains six chapters. Marc Daëron first discusses how FcRs function as adaptive immunoreceptors (with an adaptive specificity, structure and signaling) that trigger adaptive biological responses with an extensive combinatorial functional diversity, depending on the functional repertoire of FcR-expressing cells selected by antibodies. Pauline Rudd et al. provide a comprehensive overview of the role that glycosylation plays in FcR functions. The glycosylation of antibodies is well known to determine their

binding to FcRs. That of FcRs is much less known. The interplay between carbohydrate–carbohydrate and carbohydrate–protein interactions, in ligands and receptors, opens a novel field of investigation. Birgitta Heyman discusses how, depending on the antigen and on the antibody class, antigen–antibody complexes can exert potent adjuvant effects or, on the contrary, suppress antibody responses. Understanding the mechanisms behind these versatile effects is essential for antibody-based immunotherapy. Renato Monteiro et al. review the anti-inflammatory properties of IgA and IgA receptors. IgA indeed uses several receptors and several mechanisms to regulate inflammatory processes generated during immune responses and the resulting tissue damage observed in autoimmune and inflammatory diseases. Jeffrey Ravetch et al. discuss how humanized mice can be used to assess the role of IgG–Fc $\gamma$ R interactions in the in vivo effects of therapeutic antibodies used in the clinic. They focus on such a mouse in which all the Fc $\gamma$ Rs have been deleted and replaced by human Fc $\gamma$ Rs with a human tissue distribution. Finally, Sally Ward et al. review the mechanisms used by FcRn to protect IgG from lysosomal degradation through recycling and transcytosis, to deliver antibodies across cellular barriers to sites of pathogen encounter, to maintain and regulate renal filtration and to present antigen. These multifaceted functions open new FcRn-targeted therapies.

The Part IV specifically deals with *FcRs and Disease*. It contains three chapters. Robert Kimberly et al. address the issue of FcR polymorphism in human diseases. They review the single nucleotide polymorphisms, as well as the copy number variations in classical FcRs, including FcRn, but also in FCRLs, and they discuss their roles in infectious and inflammatory diseases associated with these genetic variations. René Toes et al. focus on the roles of autoantibody–FcR interactions in rheumatoid arthritis. Specifically, they show how anti-citrullinated protein antibodies determine joint damage through the interplay between activating and inhibitory receptors. Finally, Mark Hogarth et al. provide a comparative analysis of human and non-human primate Fc $\gamma$ Rs, in viral infection. They focus on the polymorphism of macaque Fc $\gamma$ Rs and HIV infection and discuss how faithful the macaque model is for designing safe and efficient HIV vaccine strategies.

The Part V bears on *FcRs and Therapeutic Antibodies*. It contains three chapters. Mark Cragg et al. first discuss how ITIM-containing inhibitory Fc $\gamma$ RIIB, that were shown to decrease the efficacy of therapeutic antibodies such as Rituximab or Trastuzumab can, on the contrary, enhance that of antibodies against members of the TNF Receptor superfamily such as anti-CD40 antibodies. Jantine Bakema and Marjolein van Egmond review the mechanisms involved in FcR-dependent passive immunotherapy of cancer. They specifically focus on therapeutic antibodies of the IgA class, instead of the anti-tumor IgG antibodies that are commonly used. Finally, Falk Nimmerjahn et al. review the anti-inflammatory activity of normal IgG and, specifically, the role of IgG glycosylation in this property. They discuss how IgG sialylation critically determines the therapeutic effects of IVIg in several models of autoimmune diseases by affecting both innate and adaptive immune responses through several receptors and mechanisms.

We hope this volume will interest scientists and clinicians, immunologists and non-immunologists, who are willing to know more about FcRs and to master better antibodies for therapeutic purposes. We also wish they will share with us the pleasure we had to put these chapters together when they read them.

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