The Science and Arts of Antibodies

Sudhir Gupta · Hans-Peter Schwarz

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Recently there have been major developments in understanding the basic mechanisms of actions of natural and adaptive antibodies, especially with regard to their anti-inflammatory and immunoregulatory properties, and the art of their clinical application in a variety of inflammatory and autoimmune disorders. This was the major theme of the International Forum of Immunoglobulin Research, held in Miami Florida on October 3–6, 2013.

There have been several recent developments in the understanding of the role of natural autoantibodies in immune homeostasis, tolerance, and antimicrobial activity. Natural IgM antibodies to apoptotic-cell membrane determinants represent a distinct subset of antibodies that potentiate the phagocytic clearance of apoptotic cells and apoptotic bodies. Furthermore, these autoantibodies display potent anti-inflammatory activities both *in vitro* and *in vivo*, which are mediated by MAPK phosphatase 1 signaling pathway (Caroline Gronwell, New York, USA). A role of natural IgM to be protective in human diseases comes from patients with systemic lupus erythematosus in which high levels of natural IgM to phosphocholine correlate with less active lupus disease, less organ damage, and lower risk of cardiovascular events. Srinivasa Kaveri (Paris, France) and associates have identified and characterized the natural autoantibodies of the IgG isotype directed against the human Fc receptors. They show that the F(ab')₂ of IVIG recognizes the FcγRIII (CD16) and FcγRII (CD32). Furthermore, the immunopurified anti-FcγIII and anti-FcγII antibodies present in IVIG recognized soluble and membrane-bound FcR. These observations provide further evidence of the existence of natural autoantibodies

towards a wide range of self-motifs, including those present in apoptotic bodies in immunoglobulin preparations that may participate in regulation of the immune response. Peter Lobo (Virginia, USA), using *in vitro* and murine knock out models, demonstrated an anti-inflammatory role of natural IgM anti-leucocyte antibodies (ALA). This anti-inflammatory effect of IgM is mediated, in part, by inhibiting TLR-4-induced translocation of NF-κB into the nucleus and inhibiting differentiation of naïve Th0 cells into Th1 and Th17 cells. He also showed that intra-peritoneal administration of IgM prevents NOD mice from developing autoimmune insulinitis, which also involves Th1 and Th17 cells. Therefore, anti-inflammatory and immunoregulatory properties of natural antibodies may justify the use of polyclonal IgM preparations and/or development of monoclonal IgM for the treatment of inflammatory and autoimmune disease.

Although a role of both 'natural' and 'immune' IgM antibodies in immune homeostasis, removal of self-antigen containing apoptotic cells and apoptotic bodies, and in protective immunity against pathogens is well-recognized, the mechanisms of IgM-mediated protection against microbes and immune-regulation; however, are not completely understood. Recent recognition and characterization of long since elusive Fc receptor for IgM (FcμR), in both humans and mice have shed light on some of these mechanisms. Hiromi Kubagawa (Alabama, USA) presented data on the cellular distribution of FcμR and potential functions. FcμR knock out mice are unable to secrete IgM; however, express surface IgM and other Ig isotypes on B cells, and secrete other immunoglobulins. Studies of these mice have reinforced the importance of both 'natural' and 'immune' IgM in microbial defense and in the regulation of autoimmunity.

The sugar moieties attached to the IgG constant fragment (Fc) are essential for IgG functionality such as binding to cellular Fc receptors and complement activation. Presence or absence of distinct residues such as fucose and sialic acid can

S. Gupta (✉)
University of California at Irvine, Irvine, CA, USA
e-mail: sgupta@uci.edu

H.-P. Schwarz
Baxter BioTherapeutics, Vienna, Austria

significantly change pro- and anti-inflammatory IgG activities. These changes in IgG molecules could be harnessed for immunotherapeutic uses of IgG. Jan Lunemann (Zurich, Switzerland) presented recent advances in understanding the role of the IgG-Fc glycan during immune responses and for immunotherapy with a focus on sialic acid and intravenous immunoglobulin (IVIG) treatment.

Recent advances in understanding the role FcγRIIIA in mediating anti-inflammatory and immunoregulatory actions of antibodies and a role of TRIM in activating immunity by intracellular antibody-pathogen complexes were discussed.

Although the inhibitory FcγRIIB plays an important role in anti-inflammatory and immunoregulatory action of IVIG, signaling via FcγRIIIA has recently been identified as another major anti-inflammatory mechanism. Renato Monterio (Paris, France) presented evidence for a novel mechanisms of action of IVIG in which IVIG functionally interacts with FcγRIIIA inducing ITAMi signaling that can prevent development of autoimmunity and inflammation, independent of FcγRIIB. An interaction of FcγRIIIA with uncomplexed IgG1 or IVIG, or with bivalent anti-FcγRIII F(ab')₂ results in dampening of calcium responses, ROS production, endocytosis and phagocytosis. This inhibitory action requires the inhibitory configuration of the ITAM motif present within the FcγRIII-associated FcRγ subunit. This allowed SHP-1 recruitment and formation of intracellular inhibisome clusters containing FcγRIII and the targeted activating receptor. Once bound to extracellular microbes, antibodies induce a broad range of effector mechanisms, including phagocytosis and complement activation that are well characterized. However, intracellular pathogens like viruses and certain bacteria may evade the effect of antibodies upon infection of their target cells. More recently, it has been shown that antibodies that are bound to the surface of non-envelop viruses or bacteria are carried into the cell, where these pathogen-attached antibodies are recognized by a highly conserved high affinity cytosolic antibody receptor, TRIM21 (Leo James, UK). TRIM21 initiates both sensor and effector responses that reduce viral replication and induce an antiviral state. An important role of TRIM21 in antiviral immunity is evident in a murine model of infection in which removal of TRIM21 results in uncontrolled viremia and death of the animal.

Schwann cells are the myelinating glial cells of the peripheral nervous system that exhibit a high degree of differentiation plasticity as they were shown to re- and dedifferentiate upon injury and disease as well as to actively participate in regenerative and inflammatory processes. By virtue of expression of MHC antigens, Schwann cells may serve as accessory antigen presenting cells and present antigens to cells of the adaptive immunity. Patrick Kury (Dusseldorf, Germany) reviewed data on Schwann cell's role in antigen-presentation, on interactions with the complement system, and Schwann cell surface molecules/receptors and on secreted

factors involved in immune cell interactions or para-/autocrine signaling events, functional evidences of molecules, regulators and factors involved in this process.

Neuroinflammation in relation to various neurological pathologies was discussed, particularly in relation to spinal cord injury, MMN, and Alzheimer's disease (AD).

Th17 have shown to play a role in inflammation and autoimmunity. However, recent studies have provided evidence for the presence of pathogenic and non-pathogenic Th17 cells. These Th17 subsets have distinct and divergent roles in inducing tissue inflammation. Comparative genomic sequence analyses between the pathogenic and non-pathogenic Th17 cells have delineated extensive population heterogeneity within the Th17 subset. Vijay Kuchroo (Boston, USA) reviewed some of the unexpected factors that may drive pathogenic divergence, and the functional consequences of Th17 cell diversity that may allow for the selection of more precise targets for intervention in autoimmune and inflammatory diseases.

Multifocal motor neuropathy (MMN), a rare inflammatory neuropathy with persistent conduction blockade as the electrophysiological hallmark. The Node of Ranvier and its surrounding structures are likely to play a role in the pathogenesis of conduction block because of the high density of sodium channels essential in the generation of action potentials and impulse present within the node of Ranvier. Damage to voltage-gated Na channels possibly due the presence of anti-GM1 antibodies (GM1 is expressed in the nodal axolemma) may result in conduction block, and suggest immune-mediated pathogenesis. Hassel Fransenn (Utrecht, The Netherlands) discussed the physiology and pathology of the Node of Ranvier in MMN. Although the GM1-specific antibody appears to play a role in the pathogenesis of MMN, other factors may also be involved. Leonard van den Berg, (Utrecht, The Netherlands) reviewed and presented data to support a role of, and the mechanisms by which anti-GM1 IgM antibodies, which may involve complement activation. Complement activation appears to be an important player in conduction blockade and the clinical manifestations of MMN. In experimental animal models, binding of anti-GM1 antibodies to GM1 in the axolemma causes blocking and dysruption of sodium channels; sodium channels are critical for nerve conduction. Once again complement activation may play a role. Unlike other neuropathies like chronic demyelinating polyneuropathy (CIDP) and Gullian-Barre syndrome (GBS), MMN does not respond effectively to plasma exchange and corticosteroids. The rapid response to IVIG in MMN may suggest its effect on sodium channels rather than on remyelination. It is likely that beneficial effects of IVIG may also be due to attenuation of complement activation, including C3 deposition in the nerve. Carol Koski (New Mexico, USA) presented data on various clinical trials in MMN.

Neuroinflammation appears to play a role in exacerbating the primary trauma and therefore worsen recovery in spinal cord injury (SCI). It is believed that leukocyte activation and recruitment in the acute phase of injury results in the production of cytotoxic molecules that are harmful to the nervous tissue. Therefore, suppression of excessive inflammation in the spinal cord could serve as a therapeutic strategy to attenuate tissue damage. Since IVIG has anti-inflammatory and immunomodulatory effects, its effect in SCI was examined in an experimental model of SCI. Michael Gehling (Toronto, Canada) discussed their data that demonstrate that IVIG attenuates the immune response and result in improved neurobehavioral recovery following cervical SCI in rats through a mechanism that involves the attenuation of neutrophil recruitment and reduction in the levels of cytokines and cytotoxic enzymes. These observations require well-designed controlled clinical trials in humans.

Alzheimer's disease is a complex and multifactorial disease in which neuroinflammation appears to play an important role. Sanjay Pimplikar (Cleveland, USA) discussed both Genome-wide Association Studies (GWAS) and system biology approaches to identify a number of genes of the innate immune pathways including TEM2, CR1, CD33 and others, which are risk factors for AD. He also discussed the role of abnormal mitochondrial functions and decreased energy metabolism in the pathogenesis of AD.

Scott Counts (Grand Rapids, USA) presented data in primary human fetal brain cultures and in the 3× transgenic models of AD to demonstrate the mechanisms of the neuroprotective role IVIG, primarily its effect on oxidative stress. Based upon these studies, he proposed that if dosage and treatment conditions are adequately optimized, IVIG might be effective in preventing and/or delaying the progression of AD.

Preclinical data in transgenic mouse models of Alzheimer's disease (Sam Gandy, New York, USA), and in vitro studies have demonstrated potential effectiveness of IVIG treatment

in AD, which appeared to be due to naturally occurring anti-amyloid antibodies and immunomodulatory properties of IVIG. Furthermore, early small clinical trials of IVIG reported encouraging results of decreases in cognitive decline and increased brain glucose metabolism. Normal Relkin (New York, USA) reviewed data from several previous Phase II clinical trials of IVIG in AD, and presented data of more recent Phase III placebo-controlled double-blinded Gammaglobulin Alzheimer' Partnership (GAP) study sponsored by the National Institute of Aging, and Baxter Healthcare. Unfortunately, this study of 390 patients failed to demonstrate the primary outcome of slowing cognition and functional decline after 18 months of therapy with IVIG. However, positive cognitive signals were observed in pre-specified moderate AD and APOE-ε4 carrier subgroup. IVIG treatment was safe and generally well tolerated. Biomarker analysis demonstrated a dose-dependent increase in plasma and CSF IgG and decrease in β amyloid-42 levels. This would suggest that additional studies of different dosage of IVIG in selected subgroup of AD are warranted.

In AD, two amyloid molecules that appear to induce neuroinflammation and are considered pathogenic. However, amyloid proteins may be anti-inflammatory as well. In the brains of patients with multiple sclerosis (MS), transcripts of aB crystalline (cryab) are present in abundance. Lawrence Steinman (Stanford, USA) presented data for anti-inflammatory properties of cryab in a number of animal models of human diseases, including experimental autoimmune encephalomyelitis (EAE). He discussed the structure of the core hexapeptide common to many amyloid proteins, and demonstrated reversal of paralysis in EAE, which was associated with lowering of plasma proinflammatory cytokine IL-6 and IL-2. The hexapeptides binds to several proinflammatory mediators and complement components. Therefore, beneficial properties of amyloid forming hexapeptides might provide a potential new therapeutic potentials.