Ins and Outs of Antibodies

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In recent years, there have been major developments in the understanding of the mechanisms of assembly, intracellular transport, antibody secretion, and B cell differentiation into antibody-producing plasma cells. These, along with use of FcRs as targets for therapy for human diseases, were the main theme of the 4th International Forum on Immunology Research held at Berlin on October 7–10, 2015.

The endoplasmic reticulum (ER) plays a critical role in biogenesis of secretory and membrane proteins, in which Sec61 complex in the ER membrane represents a polypeptide-conducting channel. In the last decade, a number of human diseases have been linked to the components of the protein translocation machinery. These disorders have been grouped as Sec61-channelopathies. The mutations in the gene encoding Sec61 α -subunit are associated with a common variable immunodeficiency. Zimmerman [1] reviewed the chaperone network of immunoglobulin heavy chain binding protein (BiP) and its co-chaperones (ERjs or ERdjs) and nucleotide exchange factors and its role in correct folding and assembly of polypeptides that are delivered to their functional location in the cell or outside of the cell by vesicular transport. He discussed in detail the mechanisms of Sec61 mutation resulting in inefficient gating of Sec61 channel resulting in sustained leakage of ER Ca^{++} , resulting in apoptosis of plasma cells. It is also interesting to entertain a possibility of defect(s) in the assembly, folding, degradation, and transport out of the cells of secreted IgM or IgA in patients with selective IgM deficiency and selective IgA deficiency, respectively.

There has been major progress in understanding transcriptional regulation of terminal differentiation of mature naïve B cells to antibody-secreting B cells; however, only recently attention has been directed towards metabolic regulation of plasma cell differentiation. Aronov and Tirosh [2] reviewed a link between transcriptional and metabolic control of plasma cells. While mature naïve B cells express approximately equal amounts of secreted μ heavy chain (μs) and the transmembrane μ heavy chain (μm), B cells express membrane IgM but do not secrete IgM. μs molecules are continuously degraded by the proteasome-dependent ER-dependent associated degradation pathway (ERDA), whereas μm molecules assemble into BCR and are transported to the cell surface. During B cell differentiation to plasma cells, this process is reversed. Three major transcription factors, IRF4, Blimp1, and XBP1 regulate stepwise differentiation of plasma cells. These include three parallel signaling pathways, IRE1, PERK, and AFT6. Perhaps, there are additional signaling pathways. A role of each of these ER-resident sensors in protein folding, autophagy, and apoptosis of plasma cells is reviewed. Autophagy is a highly conserved intracellular digestive pathway that plays a role in the engulfment of unwanted substrates and directs them to the lysosome for degradation and recycling. Autophagy plays a role in the regulation of both innate and adaptive immune responses, and elimination of microorganisms. Autophagy can promote cell death but can also promote survival pathway.

Simon Sensi and his associates [3] reviewed the mechanisms of autophagy in immune functions and elimination of microbes. Furthermore, they reviewed a role of autophagy in the shaping of plasma cells for sustained antibody production.

Antibody drug conjugates have long been used in the treatment of a wide variety of tumors. However, these conjugates have been associated with systemic side effects. Heinrich Leonhardt and his associates [4] reviewed novel chemical

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and biotechnological strategies for the site-directed attachment of drugs to promote conjugate homogeneity and stability. These include enzymatic and co-enzymatic, as well as Tub-tag labeling approaches. It is likely that such conjugates may be used in microbial and autoimmune diseases.

Follicular helper CD4⁺ T (TFH) cells provide help to B cells to produce antibodies. They appear to play a critical role in providing help for germinal center (GC) formation, B cell differentiation into plasma cells and memory cells, and antibody production. Emerging data have shown that TFH cells may be involved in the pathogenesis of certain primary immunodeficiencies, autoimmune, and neuroautoimmune disorders. TFH cells have been identified by a combination of cell surface markers and transcription repressor Bcl-6. However, our knowledge about TFH cells has significantly increased. Hideki Uneo [5] discussed distinct subsets of human TFHs with unique phenotype and functions. Using a combination of CXCR3 and CCR6, three populations of TFH cells in the blood (TFH) are defined. CXCR3+CCR6-resembling Th1 cells (TFH1) express T-bet and produce IFN- γ , CXCR3-CCR6-resembling Th2 cells (TFH2) express GATA3 and secrete IL-4, IL-5, and IL-13, and CXCR3-CCR6+ cells resembling Th17 (TFH17) express ROR γ t, and produce IL-17, IL-17F, and IL-22. In addition, expression of ICOS and PD-1 further defines subpopulations within these subsets. Furthermore, TFH cells can be divided into two functionally distinct subsets: efficient helper (TFH2 and TFH17) and non-efficient helpers (TFH1). These subsets of TFH cells may provide important biomarkers for disease activity in autoimmune disease and antibody responses to vaccination.

Cindy Ma [6] used various monogenic primary immunodeficiency diseases to define the role of various signaling molecules and transcription factors in the development and generation of functional TFH cells. Using primary immunodeficiency diseases with mutations in *CD40L*, *ICOS*, *IL-12RB1*, *IFN- γ R1/2*, *NEMO*, *SAP*, *TYK2*, *STAT1* and *STAT3*, she demonstrated STAT-1 and TYK2 are redundant in TFH cell development, whereas IL-12-STAT3, CD40L, SAP, NEMO, ICOS, and IL-12RB1 are important in another subset of TFH cells and functional TFH cells.

Regulatory B cells (Breg) play an important role in regulating immune responses and disorders of Breg have been implicated in a number of autoimmune diseases in both humans and experimental animals. A number of markers have been used to identify Breg; however, no definitive marker to identify human Breg is available. IL-10 appears to be the major mechanism for Breg activity. Padriac Fallon and his colleagues [7] have provided an overview of complexity of surface markers to identify Breg in mice and humans. They discussed that a number of cytokines including IL-33, IL-35, IL-6, IL-1 β , and a low concentration of BAFF can induce Breg. They also

reviewed IL-10-independent mechanisms of immune suppression by Breg.

Primary immunodeficiencies are a heterogeneous group of disorders. Approximately 300 different primary immunodeficiencies, and more than 400 genes causing or associated with primary immunodeficiency diseases have been described. Several of immunodeficiencies are monogenic, whereas others are associated with mutations of several genes (e.g., common variable immunodeficiency). Since first described in 2009 [8], next generation sequencing (NGS) is increasingly used to identify genes and molecular diagnosis of a number of human diseases. Recently, NSG has been used for gene identification and molecular diagnosis of primary immunodeficiencies. Although NSG is a promising strategy for monogenic diseases, assigning the causal mutation is still a challenge. Lennart Hammarstrom and colleagues [9] have reviewed application of NGS to gene identifications in primary immunodeficiencies. They have summarized considerations for the interpretation of data and techniques for searching disease-related primary immunodeficiency genes. They suggested multi-omics approach, including epigenetics, transcriptomics, proteomics, and functional pathway testing are needed to supplement genetic data. Genes only load the gun, it is the environment and epigenetics that pull the trigger to induce damage, and the expression of disease. Epigenetic mechanisms modulate gene expression without altering DNA sequences. DNA methylation, and histone post-translational modifications are one of the most common epigenetic mechanisms influencing gene expression by changing the ability of different nuclear factors to interact with chromatin. Esteban Ballestar and colleagues [10] reviewed a role of epigenetic control during B cell activation, differentiation, and antibody production, and discussed the defects in DNA methylation in Wiskott-Aldrich syndrome and common variable immunodeficiency.

Although primary immunodeficiencies commonly present clinically with recurrent common and unusual infections, they also present with non-infections manifestations, including allergic diseases, autoimmune diseases, and malignancies. There are a number of mechanisms that are responsible for autoimmunity and autoimmune diseases in primary immunodeficiencies, including loss of central and peripheral tolerance [11]. Troy Torgerson and Allenspach [12] discussed difference mechanisms of autoimmunity, including the role of Aire, thymic Treg, peripheral Treg, clearance of apoptotic cells, and lack of apoptosis, and autoimmune disorders and primary immunodeficiencies based upon these mechanisms.

Fc γ Rs play an important role in mediating effector functions of antibodies, and their impaired functions and polymorphisms are associated with autoimmune diseases. There are six Fc γ Rs in humans including Fc γ RI, Fc γ RIIA, Fc γ RIIB, Fc γ RIIC, Fc γ RIIIA, and Fc γ RIIIB. All Fc γ Rs with the

exception of FcγRIIB are activation receptors. FcγRIIB contains a cytoplasmic immunotyrosine inhibitory motif, binds to monomeric IgG, and mediate inhibitory signal. FcγRIIB are expressed on B cells and monocyte and macrophages. FcγRIIB has been most extensively studied as regards its role in immune regulation, and association with autoimmune disease. Decreased expression of FcγRIIB and mutations in FcγRIIB are associated with autoimmune diseases including systemic lupus erythematosus (SLE) and chronic demyelinating polyneuropathy (CIDP). FcγRIIB has also been used as a target for therapy for autoimmune disease and cancer. Falk Nimmerjahn [13] reviewed FcγRIIB biology translating into novel therapeutic approaches. He discussed changes in N-glycan of the Fc portion of antibodies to increase the interaction with FcγRIIB to deliver stronger inhibitory signal to the advantage to suppress autoimmune response. Gestur Vidarsson and colleagues [14] also reviewed functional FcγR polymorphism for lower or increased binding affinity to IgG that may affect induction of autoimmunity and susceptibility to infections. They also discussed genetic and environmental factors that may contribute to the level of glycosylation of the IgGFc, which in turn may influence their effector functions and clinical significance. In relation to alloimmune thrombocytopenia of pregnancy, they discussed a role of CRP and oxidative stress in increased clearance of platelets. Mark Cragg and his associates [15] discussed the biology of FcγRIIB and how that could be used to modify monoclonal antibodies in order to improve efficacy of monoclonal antibodies in the treatment of autoimmune diseases and cancer; in the case of autoimmune diseases, by delivering inhibitory signals and inducing apoptosis in autoantibody-producing B cells, and mediating apoptotic signal in malignant B cells. They discussed their work on antigenic modulation of CD20:IgG complexes. FcγRIIB is involved in the internalization of rituximab (anti-CD20) from the surface of both autoimmune B cells and malignant lymphoma B cells resulting in the resistance to treatment of these diseases. *Cis* interaction of the Fc portion of anti-CD20 with FcγRIIB on the same cell was responsible for internalization of anti-CD20 (antibody bipolar bridging). This may lead to decreased half-life of antibodies. In this regard, anti-FcγRIIB antibodies in humans have been agonistic or antagonistic. Anti-FcγRIIB antibody that blocks the *cis*-binding of rituximab and therefore reduces its internalization, appears to increase the efficacy of rituximab. Peter Sondermann [16] discussed two opposite strategies for the treatment of autoimmune disease targeted at IgG-FcγRs interactions. In one approach, soluble FcγRIIB (SM101) competes with the interaction of IgG (autoantibody) with FcγRs. Since there are no significant allelic variations in the extracellular domain of FcγRIIB, SM101 sequences are unlikely to be immunogenic. In early studies in humans, SM101 appears to be safe and effective in idiopathic thrombocytopenia (ITP) and SLE. Sondermann also discussed

its half-life, fate of IgG-SM101 immune complexes (IC), and lack of its degradation by FcRn. In the second approach, anti-FcγRIIB monoclonal antibody (SM201) does not block IgG or immune complexes. However, in the presence of IC, SM201 recruits additional FcγRIIB molecules into signaling complex leading to amplification of inhibitory signal. These approaches are intended to be more efficacious with fewer side effects. SM101 is already in clinical trial.

In summary, molecular mechanisms of the ins and outs of antibodies and diverse role of FcRs reviewed here should contribute to better understanding of several immunodeficiency and autoimmune diseases, and in designing better targeted therapies of immune-mediated diseases.

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