Immunoreceptors: evolution, structure and therapeutic applications

Jeanette H. W. Leusen

The 'Immunoreceptors' meeting took place in July 2012 in beautiful Snowmass Village in Colorado, USA. At an altitude of more than two kilometres, researchers and clinicians discussed the molecular aspects of immunoreceptors, ranging from B- and T-cell receptors, to complement and Fc receptors.

Flying in from Denver to Aspen in a small aeroplane with beautiful views of the Rocky Mountains, against a backdrop of lightning storms, had already raised high expectations for this meeting on 'Immunoreceptors'—the eleventh in the series—organized by Jenny Woof (U. Dundee, UK) and Peter Sun (NIH, USA). The relatively small number of participants—around 100—made the meeting a great place to interact, and young investigators could often engage with established researchers. With approximately 50 oral presentations, including short presentations selected from abstracts, and four poster sessions in five days, the meeting was the perfect place both to learn more about the dynamics of the immune system and to discuss the new insights and developments with fellow scientists. In this report, I focus on the highlights of the meeting, roughly divided into four topics.

Evolution of immunoreceptors

Approximately 500 million years ago, the adaptive immune system was divided into two groups: the antigen receptors that depend for diversity on the rearrangement of V(D)J gene segments and somatic hypermutation, and the variable lymphocyte receptors (VLRs) that are generated through recombinatorial use of a large panel of highly diverse leucine-rich-repeat (LRR) sequences. The keynote speaker, Max Cooper (Emory U., USA), has studied the adaptive immune system in the surviving jawless vertebrates—lampreys and hagfish—considered by many to be living fossils. The unique structural characteristics and specificities of lamprey antibodies

indicate their usefulness as diagnostic reagents for infections and malignancies. Cooper's keynote focused on the parallels and differences between the two arms of the adaptive immune system in jawless vertebrates and the T- and B-cell lineages in jawed vertebrates.

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Erin Adams (U. Chicago, USA) discussed $\gamma\delta$ T cells, a distinct lineage of T cells in which the T-cell receptor (TCR) is composed of γ and δ chains instead of the usual α and β glycoproteins. The evolutionary studies from her group have revealed that the γ locus seems to be evolving rapidly within the primate lineage. CD1d-specific human $\gamma\delta$ TCRs recognize their ligands differently compared with murine T22-reactive $\gamma\delta$ T cells [1]. Moreover, as certain $\gamma\delta$ T cells are also well equipped to recognize tumour-enriched ligands, the question is how can we translate all this new knowledge into therapeutic applications.

Randall Davis (U. Alabama at Birmingham, USA) is well known for his work on Fc receptor-like molecules (FcRLs), an ancient multigene family of transmembrane proteins expressed by B cells. Most of these molecules harbour both inhibitory (ITIM) and activating (ITAM-like) motifs in their cytoplasmic tails. Immunoreceptor tyrosine-based activation motifs (ITAM) are important for signal transduction in immune cells. Hence, they are found in the tails of important cell-signalling molecules such

as the CD3 and ζ -chains of the TCR complex, the CD79- α and CD79- β chains of the B-cell receptor (BCR) complex and within Fc receptors. The inhibitory counterpart ITIM was first identified in the intracytoplasmic domain of Fc γ RIIB. FcRLs are unique in that they simultaneously express both contradictory motifs, and it is intriguing to unravel the signalling pathways. For FcRL5 in mice, Randall disclosed that the molecule serves as a binary regulator, the function of which correlates directly with Lyn kinase and src homology phosphatase 1 (SHP1) phosphatase activity in innate-like B cells, suggesting that these immunoreceptors have dual biological properties.

Structure of immunoreceptors

Jenny Woof has looked at other species for more insights into IgE and Fc ϵ R interaction. Although the investigation of human and rodent systems has provided detailed insights into immunoglobulin (Ig) function, these examples represent only a fraction of the structural insights possible in Ig-Fc receptor interaction. Horses have IgE-mediated allergic diseases, and Woof has investigated equine IgE-Fc ϵ RI interaction by using a panel of IgE-IgG1 domain swap-and-loop exchange mutants. She revealed that the C ϵ 3 domain contains the major determinants for Fc ϵ RI binding, but that optimal binding and effector function also requires the C ϵ 2 and C ϵ 4 domains. Given the results of Brian Sutton's work (King's College, London, UK), horses might be more similar to humans than expected. Sutton focused on the bend between human C ϵ 2 and C ϵ 3, as he noticed that this angle becomes more acute on Fc ϵ RI binding, which he confirmed

by using FRET labelling. Interestingly, the therapeutic IgE antibody omalizumab that inhibits FcεRI binding causes an ‘unbending’ of the IgE: this result suggests that an allosteric inhibitory mechanism might be involved [2].

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It is not only immunoglobulins that bind to Fc receptors. Terry Du Clos (U. New Mexico, USA) and Peter Sun have shown that acute phase proteins, such as C-reactive protein (CRP) and pentraxin, can interact with FcγRI and FcαR, but not FcεR. These interactions have been defined by structural work, and the binding residues of CRP and CD89 are known [3]. Functionally, CRP crosslinking activates CD89-transfected cell lines, and CRP enhances neutrophil surface expression of CD89, phagocytosis and cytokine secretion *in vitro*. At the moment, *in vivo* studies are underway. Continuing with Fc receptors, Marije Overdijk (Genmab BV, the Netherlands), gave a short presentation about how human IgG isotypes interact with mouse FcγR. Mouse models are still the most used platform to test new therapeutic antibodies, and questions consistently arise about whether this is really comparable with the human system. Overdijk presented clear evidence that human IgG1 is the most potent isotype in mice, suggesting these concerns are unfounded, and that it interacts similarly with murine and human effector cells [4]. At the end of the conference, she received the award for best short presentation.

Immunoreceptor dynamics

Michael Dustin (New York U. School of Medicine, USA) has long studied the immunological synapse between a T cell and an antigen-presenting cell. The synapse can be divided into two radially symmetrical zones: the TCR-rich and F-actin-poor central supramolecular activation cluster (cSMAC), and the adhesion molecule and F-actin-rich ring defined as a peripheral supramolecular activation cluster (pSMAC). TCR microclusters have been defined as a third compartment important for sustained signalling. Microcluster formation is F-actin-dependent, but large microclusters formed by high-avidity ligands become

F-actin-independent as they progress toward the centre of the immunological synapse (reviewed in [5]). Dustin presented evidence that the endosomal sorting complexes required for transport (ESCRT) protein TSG101 is required for cSMAC formation. Gillian Griffiths (U. Cambridge, UK) gave an excellent presentation on synapses, cilia and secretion. She presented beautiful images that made clear that the centrosome has a crucial role in cytotoxic T lymphocytes, docking at the plasma membrane in response to TCR signalling and directing lytic granules along microtubules for secretion within the immunological synapse. Griffiths revealed observations that there are striking similarities between the immunological synapse and cilia, with signalling, endocytosis and exocytosis all focused at the point of centrosome docking. These findings suggest that the immunological synapse is actually a frustrated cilium [6].

Many studies on the dynamics of immunoreceptors are driven by sophisticated microscopic technologies. Facundo Batista (London Research Institute, UK) presented new super-resolution images of the BCR showing that it is pre-clustered in the B-cell plasma membrane. On the basis on this new microscopy and genetic analysis, Batista showed that during BCR signalling, cytoskeleton reorganizations release receptor nanoclusters that can interact with co-receptor CD19 held in place by the tetraspanin network. These results suggest that receptor compartmentalization is important in the stimulation of the BCR, and further implies a potential role for CD19 in mediating ligand-independent BCR tonic signalling. Diane Lidke (U. New Mexico, USA) used a new hyperspectral microscope with the ability to acquire spectral images—128 spectral channels over 500–800 nm—at a rate of 30 frames per second. She has used this technique to track mobility and aggregation of quantum dot-labelled FcεRI during signalling. Sally Ward used a combination of total internal reflection fluorescence microscopy (TIRFM) and wide-field imaging of live cells to study intracellular IgG and FcRn transport. All these techniques resulted in beautiful images and movies, which provided further insights into receptor dynamics.

Immunoreceptors and therapeutics

Tanya Mayadas (Harvard U., USA) presented work by using elegant combinations of transgenic and knockout mice to study systemic lupus erythematosus (SLE). SLE is

a chronic, multi-organ inflammatory autoimmune disorder associated with high levels of autoantibodies and immune complexes. However, autoantibodies alone are not sufficient for the development of lupus, as FcγRIIA, FcγRIIIB and the leukocyte integrin Mac1 have also been genetically linked to the disease. Mayadas's work uses mice carrying human FcγRIIA and FcγRIIIB transgenes crossed with FcγR-knockout mice. These are sometimes crossed with Mac1 knockout mice. Mayadas has developed a model for SLE nephritis that is induced by the passive transfer of human SLE serum into these mice. Unexpectedly, disease could only be triggered in human FcγR-positive mice that additionally lacked Mac1, indicating that Mac1 protected against FcγR-mediated development of SLE [7]. Follow-up studies exploiting intravital microscopy suggest that Mac1 attenuates FcγRIIA mediated neutrophil recruitment.

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The many functions of the, not so, neonatal Fc receptor FcRn were studied by Rick Blumberg (Harvard U., USA). FcRn is well known for its transfer of IgG across the placenta, but it also regulates the concentration of IgG and albumin in the body. Furthermore, it is an important co-receptor for phagocytosis and, as the Blumberg lab has demonstrated, it is an important mediator of both MHC class II and MHC class I restricted presentation and cross-presentation, respectively. This process involves cooperation with classical Fcγ receptors and is operative *in vivo*, as inflammatory (CD11b⁺) dendritic cells are highly active in these FcRn-dependent functions in a variety of inflammatory models.

John Cambier (U. Colorado and National Jewish Health, Denver) has a long track record of studying B-cell anergy. Anergy is a way of silencing newly produced B cells that express autoreactive antigen receptors. Cambier explored the role of anergic B cells in the development of type 1 diabetes. Insulin-specific B cells (IBCs) were detected in the normal repertoire. In humans, IBCs disappeared from the anergic (BND) compartment before development of diabetes, and

before insulin autoantibodies are detectable. Further analysis demonstrated a correlated loss of all BND anergic B cells regardless of autoantigen specificity. Cambier suggests that this might reflect activation of anergic B cells consequent to injury or infection, and participation in disease development. In NOD mice, IBCs are important for the development of diabetes, and Cambier showed that a proportion of these cells become activated long before disease onset.

The BCR is also under investigation by Jürgen Wienands (U. Göttingen, Germany), whose interest lies specifically in the signalling of this receptor. It is well known that the ITAM-containing Ig α /Ig β heterodimer signals through the tyrosine kinase Syk and its substrate SLP65. Wienands has added a new component to this mix: the Cbl-interacting protein of 85 kDa or CIN85. Loss of CIN85 expression in B cells severely compromises B-cell activation and subsequent antibody responses [8]. Wienands also showed data from an interesting series of experiments addressing the basis of enhanced signalling by membrane (m)IgG. He showed that the mIgG tail is tyrosine phosphorylated and engages the Grb2 adaptor protein.

The therapeutic application of all this immunoreceptor knowledge was the topic of the last session of the meeting. Menno van Lookeren Campagne (Genentech Inc., USA) studies the complement receptor of the Ig superfamily CR1g, which has anti-inflammatory and immune-suppressive properties. Newly developed proteins that resemble soluble versions of this receptor have been found to act as potent complement inhibitors and have therapeutic efficacy in rodent models of rheumatoid arthritis. Therapeutic antibodies were used in the research behind many of the talks at the meeting—Overdijk used CD20 antibodies to

treat lymphomas and autoimmune diseases, and Ward used Abdegs to treat IgG-mediated arthritis. But all therapeutic antibodies are still of the IgG isotype. Jeanette Leusen (U. Medical Center Utrecht, the Netherlands) and Thomas Valerius (U. Kiel, Germany) promoted the use of IgA as an effective molecule against solid tumours. Human IgA antibodies against the epidermal growth factor receptor are more capable of killing tumour cells *in vitro* than their IgG counterparts. Leusen presented work by using three mouse models, transgenic for human Fc α R, to show that IgA is also effective *in vivo*. Surprisingly, it was not neutrophils that were responsible for the tumour death *in vivo*, but macrophages.

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The best clinical results were presented by Carl June (U. Pennsylvania, USA), who presented the use of chimeric antigen receptors (CARs). CARs are fusion proteins comprised of single-chain variable fragments (scFv) from monoclonal antibodies and intracellular signalling domains such as the CD3 ζ -chain cytoplasmic tail. Fifteen years ago, this was done retrovirally in HIV patients, but B-cell malignancies are now treated with adoptive T-cell transfer. The therapy is very promising, and three chronic lymphocytic leukaemia patients treated with CD19 CAR T cells have already gone into full remission. Many other molecules and malignancies can be targeted, such as CD28 or 4.1 BB, as well as melanoma targets (for more discussion, see [9]).

In summary, this meeting on immunoreceptors covered a broad range of topics from molecular biology, signalling and technology-driven imaging to therapeutic applications. From a therapeutic viewpoint, there is much to be gained from a detailed understanding of the biology of effector molecules such as complement and Fc receptors. This knowledge can be translated to improve clinical strategies that enhance complement or Fc receptor binding through glyco- or protein engineering. Therefore, structural and dynamic insights remain pivotal, and I certainly look forward to the next meeting, to be held in 2014.

CONFLICT OF INTEREST

The author declares that she has no conflict of interest.

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