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Editorial Overview: Molecular immunology: Targeting the immune system

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Marcus Groettrup is a professor for Immunology at the University of Konstanz. His interest is focussed on antigen processing by the proteasome. He has discovered the pre-T cell receptor and recently found a new function of the immunoproteasome in the pathogenesis of autoimmune diseases. Moreover, his laboratory is working on the function of the ubiquitin-like modifier FAT10 in targeting antigens to the 26S proteasome and in antigen presentation.

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Huib Ovaa is group leader at the Netherlands Cancer Institute (NKI) in Amsterdam and professor of chemical biology of post-translational modifications at Leiden University, the Netherlands. His interest goes to drug discovery and to ubiquitin signaling, ubiquitin-mediated proteolysis and antigen presentation. His lab has developed technologies for the total chemical synthesis of ubiquitin chains and ubiquitylated substrates and a wide range of activity-based probes.

It is only four decades ago that immunologists were nicknamed by their colleagues from biochemistry 'immunosophists' because they loved to discuss theories how the immune system may work at the chalkboard. This passion was probably nourished by the lack of mechanistic and molecular insights into exciting phenomena of the immune system. Important questions were: how are antigens recognized, how is the huge diversity of antigen receptors generated, how is autoimmunity avoided, how do immune cells know where to go, and why do we get immune to a pathogen? Immunology was studied by a small community in an ivory tower with little interest from chemists or pharmaceutical industry. This has dramatically changed in the meantime. The exponentially growing body of knowledge about molecular mechanisms in immunology has revealed very attractive targets for pharmacological modulation and has long caught the interest of pharmaceutical companies. Today's therapeutic success of small molecules and biologicals, especially monoclonal antibodies, in cancer therapy or the suppression of autoimmunity, allergy and transplant rejection is impressive. Nevertheless, there is ample room for improvement considering that ancient drugs with lots of adverse side effects like corticosteroids are still prescribed most frequently.

The Guest Editors of this first section on 'Molecular Immunology' in *Current Opinion in Chemical Biology* feel that this is a perfect occasion to initiate a separate section where chemical biology meets immunology. Unfortunately, the wealth of concepts, abbreviations and cell surface 'CD' receptor molecules pose a challenge to authors who want to explain to a chemist the immunological context of why an emerging target is so attractive and this in a review of only 2000 words. We think that the authors of this first Molecular Immunology section have done a splendid job on this. The topics which have been covered are certainly only a selection of the many targets to choose from but at least it is a start.

All roads lead to Rome, so we cannot miss out on Rome in this section. What is Rome? The obvious guess is the Nuclear Factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB). There is no signal transducer and transcription factor onto which so many different surface receptors of immune cells culminate. NF-κB is crucial for the initiation but also for the limitation of a pro-inflammatory immune response. Pathogen sensing toll-like receptors (TLRs), cytokine receptors, antigen receptors and many more trigger upon ligand stimulation the migration of the two subunits of NF-κB, p50 and p65, from the cytoplasm to the nucleus to activate genes. It is beyond the scope of a concise review to cover NF-κB activation by all of these receptors. This is why Wertz reviews the regulation of NF-κB

activation by one of these receptors, the tumor necrosis factor receptor-1 (TNFR1). At the end of the cascade an inhibitor protein of NF-κB (IκB), which keeps the p50/p65 heterodimer in the cytoplasm, is first phosphorylated and then polyubiquitylated in order to be sent for degradation to the 26S proteasome. To achieve this goal a complex interplay of phosphorylations/dephosphorylations and ubiquitylations/deubiquitylations occurs which decides on the extent of NF-κB activation. Linear head-to-tail linked ubiquitin chains and ubiquitin chains linked via K63 of ubiquitin serve as assembly scaffolds to bring together the right kinases and substrates. I. Wertz introduces the involved deubiquitylases as natural inhibitors of NF-κB and covers the biologicals and small molecules which have been designed mostly to inhibit TNFR1 mediated activation of NF-κB. On the way not from TNFR1 but from the antigen receptors of B and T cells to NF-κB is where the paracaspase MALT1 plays an important role. Hailfinger *et al.* review the central function of MALT1 in NF-κB activation but also in the development of some B cell lymphomas and the mucosa-associated lymphoid tissue (MALT) lymphoma, after which this protease was named. It is described how MALT1 is activated physiologically interestingly also by ubiquitylation but also pathologically by mutations in lymphomas. Assays for monitoring the protease activity of MALT1 are introduced in this review as well as recently developed small molecule inhibitors of MALT1 activity.

Deubiquitylating enzymes (DUBs) like A20, CYLD and OTULIN are pivotal for the regulation of NF-κB signaling, but these are only three out of approximately 80 DUBs in the human genome. The Ovaa laboratory focusses on the synthesis of active-site directed probes for DUBs which can be used to label and isolate them from cells and tissues. To generate these probes the C-terminus of ubiquitin or a ubiquitin-like modifier is equipped with a chemical warhead like vinylmethylester, alkylbromide, or more recently propargyl which bind to active site cysteine residues which most DUBs bear in their catalytic center. These activity-based probes can be generated from recombinant ubiqutin-intein fusion proteins or they can be derived via complete chemical synthesis. In their review, Ekkebus *et al.* also review how ubiquitin chain specific probes can be generated by native chemical ligation. Ubiquitin chains can be linked via any of the seven lysines of ubiquitin and DUBs can discriminate between them based on which lysine is used for isopeptide bond formation. This implicates that linkage specific DUBs can be specifically labeled and identified with the help of ubiquitin chain specific probes.

Adenosine is one of the cellular key building blocks. It acts in itself as a signaling molecule, it is a constituent of RNA, and as a phosphotriester (ATP) it provides the energy that drives many cellular processes. It can also be found as part of post-translational protein modifications. Proteins can be modified through ADP ribosylation and AMPylation. The study of these modifications needs a good set of chemical tools. Westcott and Hang provide an overview of the reagents described in the literature that help to study these modifications and they discuss the type of reagents that remain to be developed. In doing so the authors focus their review on the importance of ADP-ribosylation and AMPylation in infection at the host pathogen interface.

The proteasome is an abundant proteolytic cellular machine, which is responsible for the turn-over of ubiquitylated proteins. The proteasome contains six catalytic sites (3 different active site subunits in duplo) and various proteasome species exist such as the immunoproteasome that contain different active site subunits. The proteasome is also a *bona fide* drug target: the broad spectrum proteasome inhibitors bortezomib and carfilzomib are for example used in the treatment of multiple myeloma. Despite this success much is to be learned from inhibition of distinct catalytic sites. Kisselev and Groettrup review the current state-of-the-art in the design of subunit selective inhibitors and explain what medical needs may be met with these inhibitors such as the treatment of autoimmune diseases with selective immunoproteasome inhibitors.

Proteases as potential drug targets are also involved in the processing of protein antigens to peptidic T cell epitopes presented on major histocompatibility complex (MHC) class I and class II molecules for the stimulation of cytotoxic T lymphocytes (CTL) and T helper cells (Th), respectively. Their inhibition may allow the modulation of T cell responses. Antigen processing for the MHC class I pathway is initiated by the proteasome followed by processing of the released polypeptides mostly by aminopeptidases in the cytoplasm and endoplasmic reticulum (ER) to trim them for a perfect fit into the peptide binding groove of class I molecules. Stratikos reviews in his article this pathway and how aminopeptidases can be inhibited with a focus on the ER aminopeptidases (ERAP)1 and 2. Knock out mice lacking ERAP1 have shown that its activity markedly affects the peptide repertoire presented on class I molecules. In his review E. Stratikos explains how phosphinic pseudopeptide transition analogs can be used to inhibit ERAPs and to enhance CTL responses to cancer cells. Another challenging task is to achieve immunomodulation through the selective inhibition of over a dozen of different cathepsins. Cathepsins are centrally involved in antigen processing in the endolysosome for presentation of peptides on MHC class II molecules. Van Kasteren and Overkleeft outline in their review how the different cathepsins operate during processing of the MHC class II invariant chain which accompanies MHC class II molecules on their way from the ER to the endosome.

The invariant chain blocks the peptide binding groove of class II molecules and needs to be degraded in a stepwise fashion to allow peptide antigens to bind to class II. The delicate balance between peptide epitope production and destruction in the class II loading compartment is explained and how inhibitors of the different cathepsins can contribute to the preservation of antigen and enhancement of antigen presentation. That cathepsin inhibition can also affect the functional maturation of endosomal toll like receptors through cathepsin K mediated limited proteolysis is considered as well.

Although peptide antigen presentation by MHC class I and class II complexes has been studied for many decades, the presentation of lipid antigens has been studied to a lesser extent. A review has been contributed to this issue by Layre *et al.* where the presentation of non-peptide antigens to T-cells is discussed in great detail. CD1 and MR1 proteins can present lipids and other non-peptidic small molecules to lipid- and small molecule reactive T-cells. The development of specific CD1 and MR1 tetramers has now also enabled the study of this subset of T-cells opening a new field where many discoveries are expected to be made in the near future.

A review with a clear focus on cancer immunotherapy is contributed to this issue by Boyman and colleagues. They outline how interleukin(IL)-2 has been used in the past to treat the 'immunogenic' cancers melanoma and renal cell carcinoma. The side effects of systemic IL-2 administration, however, limit the therapeutic success. The attempts to avoid this dilemma include the use of mutated IL-2 molecules, the IL-2 muteins or superkines, the use of IL-2 fusion proteins, as well as the use of IL-2 antibody complexes. These reagents bind selectively to the three chains of the IL-2 receptor which differentially affects immunosuppressive regulatory T cells and effector T cells. T cells reactive against cancer tissues can therefore be activated and expanded. Rosalia *et al.* explain and summarize in helpful tables which IL-2 and IL-15 variants bind which IL-2R chains and how far their preclinical and clinical evaluation has proceeded to date.

Sundberg *et al.* discuss novel opportunities for the treatment of immune disorders through small-molecule mediated control of cytokine function. Although protein-based drugs have been proven effective in the manipulation of cytokine function and the treatment of autoimmune and autoinflammatory diseases, such drugs cannot easily modulate intracellular targets. The authors specifically discuss the importance of the development of small molecule modulators of cytokine function and they provide an excellent overview of current small molecules that do so together with their targets. The authors also discuss emerging targets and how innovations in small molecule science such as DNA-encoded synthesis will help to lift traditional drug discovery approaches to the next level.

At the end of this Editorial Overview, the Guest Editors of this first section on 'Molecular Immunology', Marcus Groettrup and Huib Ovaa, would like to thank all contributors for their excellent reviews and smooth cooperation. Impressive progress has been made in recent years in the areas reviewed in this issue. We are aware that the ten reviews included in this section by far do not cover all the important areas of 'Molecular Immunology' where interesting new concepts, drug targets, and therapeutic avenues emerge. This nourishes our hope that this issue will be the first of a long and prosperous series of issues on this exciting topic.