Infections, rheumatisms and autoimmunity

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Autoimmune chronic inflammatory diseases are thought to have a prevalence of one in 30 people in the Western world. Understanding how environmental factors can eventually lead to such a burden is of crucial importance. In a recent meeting held in Milan, Italy, the cellular and molecular basis of autoimmunity arising following infection, and protection against autoimmunity induced by infection, were discussed by more than 100 scientists from around the world.

Innate immune response

In the first session of the meeting, a comprehensive update regarding how infectious agents interact with cells of the immune system was made. A Cook (University of Cambridge, UK), MH Claesson (University of Copenhagen, Denmark), T Avcin (University of Ljubljana, Slovenia) and HJ Girshick (University of Wuerzburg, Germany) discussed small, structurally conserved molecules (pathogen-associated molecular patterns), which are recognized to play a fundamental role in the early recognition of several infectious agents (e.g., Gram-positive and Gram-negative bacteria and RNA or DNA viruses) by the immune system. These molecules (bacterial cell-surface lipopolysaccharides, lipoproteins, lipopeptides, lipoarabinomannan, proteins such as flagellin from bacterial flagella, viral dsRNA, the unmethylated CpG islands of bacterial and viral DNA, and certain other RNAs and DNAs) are sensed by Toll-like receptors (TLRs) [1,2], a type of pattern-recognition receptor. They belong to the same receptor superfamily as the IL-1 receptors (IL-1 TLR superfamily), which all have the so-called Toll–IL-1 receptor (TIR) domain. There are three TIR-domain subgroups: group 1 are receptors of interleukins and have extracellular immunoglobulin domains; group 2 are TLRs and directly or indirectly bind pathogen-associated molecular patterns; and group 3 are cytosolic adaptor proteins whose role is to transmit signals from proteins of groups 1 and 2. Some TLRs seem to rely mainly on MyD88 to produce cytokines, such as IL-6 and TNF-α, while others rely on adaptor molecules and transcription factors, such as TIR-domain-containing adapter-inducing IFN-β, TNF receptor-associated factor 6, IL-1 receptor-associated kinase-1, and IFN regulatory factor-7.

H Amital (Tel Aviv University, Israel) and AL Zignego (University of Florence, Italy) focused on the issue of infectious agents (cytomegalovirus, *Helicobacter pylori*, hepatitis B virus [HBV] and hepatitis C virus) and innate immune response.

At least 11 TLRs are recognized in humans, and among these, TLR3, TLR7, TLR8 and TLR9 are expressed intracellularly within one or more endosomal compartments. TLR3 shows specificity for polyinosinic:polycytidylic acid compounds and dsRNA, TLR7 and TLR8 recognize imidazoquinilones and ssRNA, and TLR9 binds dsDNA. In a normal immune response, these TLRs should induce an antipathogen immune response while avoiding the induction of autoimmune diseases. This means that the key functional outcome of TLR ligation is the production of an inflammatory response through transcription factors, such as NF-κB. Of the TLRs, the DNA- and RNA-binding members have been shown to lead to the production of large amounts of IFN-α, mostly derived from plasmacytoid dendritic cells. This cytokine is of special interest to investigators studying autoimmune systemic lupus erythematosus (SLE) or Sjogren's syndrome because its expression has been correlated with disease severity in some studies. In summary, the inflammatory response downstream of TLR ligation is mainly characterized by an inflammatory milieu comprising IL-6, TNF-α and IFN-α, which represents an optimal combination for inducing immune cells to clear an infectious agent, and also the most appropriate setting to favor autoreactive B-cell clonal expansion. In addition, several studies have shown that endogenous mammalian ligands of TLR7, such as small ribonucleoproteins, are capable of stimulating B cells and plasmacytoid dendritic cells. Interestingly, it was recently reported that TLR7 ligands induce higher IFN-α production in females.

Models of innate immune response & autoimmune diseases

L Guillevin (University of Paris Descartes, France) and CGM Kallemberg (University of Groningen, The Netherlands) focused their discussion on the role of infectious agents in autoimmune vasculitic diseases. In animal models of lupus nephritis, some TLRs (TLR3 and TLR9) are specifically immunolocalized in the kidneys, suggesting a possible pathogenetic role in the manifestation of the disease [3]. Experimental evidence shows that MRL/lpr mice that spontaneously develop proliferative glomerulonephritis in the context of an SLE-like

disease express *TLR3* mRNA in the kidneys at levels comparable with expression in the spleen, while all other TLRs are expressed at low levels in the kidney. Immunostaining for TLR3, TLR7 and TLR9 revealed their expression by F4/80-positive infiltrating macrophages in 20-week-old nephritic MRL/lpr mice. In addition, TLR3 localized to glomerular mesangial cells. Stimulation of both cell types with ligands induced IL-6 production, and TNF-α and IFN-γ enhanced ligand-induced IL-6 production. In the NZB/NZW mouse model of lupus nephritis, on the other hand, TLR9 expression (which links unmethylated CpG-DNA, the bacterial DNA rich in CpG islands, and the natural ligand) is present in the tubular cells, and is correlated with proteinuria, while in human lupus nephritis, TLR9 has been shown to be induced by DNA–immune complexes formed with specific autoantibodies. In human SLE, tubular cells of nephritic kidney obtained from patients express TLR9, which is also expressed in B cells and plasmacytoid dendritic cells – and exposure to CpG-DNA ligands or to immune-complexed self RNA triggers activation of autoreactive B cells and plasmacytoid dendritic cells [4]. Surprisingly, experiments with chromatin-containing immune complexes in SLE sera are capable of inducing proliferation of rheumatoid factor-specific B cells (AM14-transgenic B cells) and DNA-specific B cells (3H9-transgenic B cells). This proliferation was sensitive to treatment with DNAse and required unmethylated CpG sequences. Data suggest that DNA isolated from serum immune complexes in SLE contains disproportionately more guanosine/cytosine nucleotides than adenine/thymine residues, and as such can simultaneously stimulate B-cell receptor and TLR9 activation. In rheumatoid synovitis, an increased expression of TLR3 and TLR7 was shown to accompany an increased synthesis of proinflammatory cytokines by dendritic cells [5].

Innate immunity & activation of T & B cells

Given that TLRs can be found in target tissues of SLE, clearcut evidence that B cells can be activated through TLR ligation would be of pivotal relevance.

The first important observation is that signals that target the B-cell or cytokine receptors may control the level of TLR7 and TLR9 expression in human naive B cells and mouse follicular B cells, thus overcoming this important checkpoint and increasing responsiveness to their ligands.

The second relevant point is that TLR9 activation exerts a powerful synergistic effect along with B-cell receptor ligation, and T cells help in determining class-switch recombination and maturation of naive B cells up to the final step of antibody-secreting cells. As class-switch recombination, occurring after B-cell-specific activation, is a key event in the autoimmune response, the involvement of TLR ligation in a possible autoimmune cascade appears plausibile.

ME Gershwin (University of California, Davis, CA, USA) and D Vergani (King's College London, UK) discussed liver diseases and infectious agents. In primary biliary cirrhosis (PBC), it has been shown, *in vivo*, that most patients with PBC have both reverse transcriptase-PCR and immunohistochemical evidence of human betaretrovirus infection in the lymph nodes. Moreover, the viral proteins colocalize to cells exhibiting aberrant autoantigen expression [6]. *In vitro* data showed that lymph node homogenates from patients with PBC induced autoantigen expression in normal biliary epithelial cells in coculture. Even in PBC, CpG induced secretion of antimitochondrial antibodies in peripheral blood mononuclear cells and also upregulated B-cell expression of TLR9, CD86 and KCa3.1. In addition, K^+ channel blockers suppress secretion of antimitochondrial antibodies without a reduction of CpG-B-cell-enhanced IgM production. Therefore, data suggest that the hyper-responsiveness of B cells in PBC accelerates B-cell-mediated autoimmunity [7].

In chronic HBV infections, supernatants from TLR3- and TLR4-stimulated Kupffer cells and TLR3-stimulated sinusoidal endothelial cells from wild-type mice were able to potently suppress HBV replication. Using neutralizing antibodies, we demonstrated that the TLR3-mediated, but not the TLR4-mediated, effect is exerted exclusively through IFN-β. This could have implications for the development of TLR-based therapeutic approaches against HBV [8].

Conclusion

All these data have led investigators to believe that understanding the molecular events leading to T-cell, B-cell, monocyte/macrophage and dendritic cell activation will help to devise new therapeutic strategies to control the inflammatory events that occur in autoimmune diseases and develop new therapies that target key molecules or signals involved in the different phases of the diseases [10]. Of particular interest is the counter-regulation that occurs during simultaneous stimulation of TLR7 and TLR9 on human plasmacytoid dendritic cells and B cells. Interestingly, it has been observed that the capacity for potent IFN-α induction by TLR9 ligands such as CpG-C and CpG-A is markedly reduced by concurrent small-molecule TLR7 stimulation [9].

Future perspective

Ligands of TLR7, and other modalities to control innate immune activation, could be employed to downregulate TLR9 activation in B cells and autoantibody synthesis.

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Executive summary

- More and more evidence of a strict coupling between innate immunity and development of autoimmunity has been uncovered by recent investigations and by *in vivo* studies in humans.
- A full molecular understanding of the various signals occurring in the different cell populations of the immune system will allow investigators to devise new methods to control innate immune activation and autoimmunity occurring downstream of innate immunity activation.
- Innate immunity-related receptors and signaling molecules could become therapeutic targets to stop autoimmunity.

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