Plenary session 1

Signaling pathways and mechanisms

PL1-1

NEGATIVE REGULATORS OF CYTOKINE SIGNALING

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Cytokines are an integral component of the adaptive and innate immune responses. The signaling pathways triggered by the engagement of cytokines with their specific cell surface receptors have been extensively studied and have provided a profound understanding of the intracellular machinery that translates exposure of cells to cytokine to a coordinated biological response. It has also become clear that cells have evolved sophisticated mechanisms to prevent excessive responses to cytokines. The suppressors of cytokine signaling (SOCS) are a family of cytoplasmic proteins that complete a negative feedback loop to attenuate signal transduction from the hematopoietin class of cytokine receptors. SOCS proteins inhibit components of the cytokine signaling cascade via direct binding or by preventing access to the signaling complex. The SOCS proteins also appear to target signal transducers for proteasomal destruction. Analysis of genetically modified mice in which SOCS proteins are over-expressed or deleted have established that this family of negative regulators has indispensable roles in regulating cytokine responses in cells of the immune system as well as other tissues. Emerging evidence also suggests that disruption of SOCS expression or activity is associated with several immune and inflammatory diseases, raising the prospect that manipulation of SOCS activity may provide a novel future therapeutic strategy in the management of immunological disorders.

PL1-2

THE BIOLOGY OF γ_C FAMILY CYTOKINES AND THE IL-7-RELATED CYTOKINE TSLP

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Interleukins 2, 4, 7, 9, 15, and 21 are cytokines that signal through receptors that share the common cytokine receptor γ chain, γ_c. The gene encoding γ_c, *IL2RG*, is mutated in humans with X-linked severe combined immunodeficiency (XSCID). In XSCID, there is defective production of T and NK cells, whereas B cells are normal in number but non-functional. Defective signaling by IL-7 and IL-15, respectively, explains the defective T cell and NK cell development in XSCID and the related Jak3-deficient form of SCID, whereas the B cell defect is explained by defective signaling in response to IL-4 and IL-21. The genes encoding both IL-21 and IL-21R are dynamically regulated in response to TCR activation. *IL21R* gene induction is biphasic, with the late induction possibly reflecting IL-21-mediated re-induction of the gene. We find that IL-21 can be produced by both Th1 and Th2 cells and is produced in vivo in response to *Toxoplasma gondii*, an intracellular pathogen. It can also regulate T cell responses. Interestingly, IL-21 is pro-apoptotic for B cells and negatively regulates IgE production, but it is critical for normal immunoglobulin synthesis and drives the differentiation of B cells into post-switch cells and plasma cells. IL-21 is a potent inducer of Bcl-6 and Blimp-1, and we have identified an IL-21 response element in the Blimp-1 gene. IL-21 does not appear to be pro-apoptotic for T cells, and it acts in concert with IL-15 or IL-7 to drive synergistic expansion of effector $CD8^+$ T cells, an ability that results in a potent anti-tumor agent in murine models. Thus, IL-21 cooperates with distinct additional γ_c cytokines for its differential effects on T versus B cells. We have mutated all of the tyrosines in the IL-21R cytoplasmic domain in varying combinations and have identified a critical tyrosine that mediates the activation of Stat1 and Stat3 in response to IL-21 and which is critical for normal IL-21-mediated proliferation. We have found evidence for critical roles for both STAT-dependent and STAT-independent IL-21-mediated signaling. Another IL-7-related cytokine, thymic stromal lymphopoietin (TSLP) has a receptor that consists of IL-7Rα and TSLPR, the latter protein being the protein in the databases most similar to γ_c. In contrast to the effects of IL-21 on $CD8^+$ T cell development/expansion, TSLP is an amplifier of $CD4^+$ T cell expansion and plays a critical role in inflammation/asthma. Thus, lineage development and function depends in part on the complex interactions of an array of γ_c and related cytokines.

PL1-3

NOVEL UBIQUITIN BINDING PROTEINS IN NF-κB PATHWAY

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The attachment of ubiquitin (Ub) to a substrate serves as an important regulatory modification involved in numerous cellular functions such as proteolytic degradation, cell cycle, apoptosis, growth factor and cytokine signaling, endocytosis, transcription and DNA repair. The identification and characterization of numerous Ub-binding domains (UBDs) explains how Ub can regulate such distinct biological functions. We have recently cloned novel Ub-binding domains named UBM (Ub binding motif) and UBZ (Ub binding Zn finger), which are evolutionarily conserved in Y-family translesion polymerases (pols). These domains are required for binding of pols to Ub, their accumulation in replication factories and for in vivo translesion synthesis (TLS). Deregulation of this process results in the development of UV-induced tumour skin syndrome. Moreover, by using the yeast-two hybrid system screens several Ub-binding protein, involved in modulation of the NF-kB pathway, have been identified. The molecular basis of their role in NF-kB signaling will be discussed.

PL1-4

GENETIC ANALYSIS OF THE WNT AND PI3K SIGNALING PATHWAYS

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Glycogen synthase kinase 3 (GSK3) comprises two protein-serine/threonine kinases that are implicated in several human diseases including inflammation, bipolar disorder, cancer, type-2 diabetes and neurodegeneration. GSK3 lies downstream of both the phosphatidylinositol 3' kinase (PI3K) pathway and the Wnt pathway but is independently and selectively regulated by each.
We have approached functional dissection of these pathways by genetic analysis of GSK3 alleles in mice and cells. Mammalian genomes contain two, distinct genes for GSK3, encoding related protein kinases, GSK3α and β, which display similar catalytic properties. Mice engineered to lack GSK3β revealed a phenotype similar to disruption of components of the NF-κB transcription factor pathway. By contrast, mice lacking GSK-3α are viable but show abnormalities in cytokine signaling. We have knocked out both alleles of GSK3α or GSK3β in mouse embryonic stem (ES) cells and have also generated fibroblast cells lacking both alleles of both isoforms. Inactivation of all four alleles of GSK-3 in ES cells confers a profound block to differentiation and these cells retain full pluripotentiality regardless of inducing signals. We have exploited these engineered cells to probe the regulation of GSK-3 and to identify targets and functions of the kinase. For example, Wnt stimulation induces GSK3-dependent phosphorylation of LRP5 which causes recruitment of Axin to the LRP/Frizzled proteins at the membrane and blocks GSK-3 phosphorylation of □-catenin. In parallel with the ES cell analysis, we have generated strains of mice that contain floxed alleles of either or both GSK-3α and β allowing selective disruption of the genes in various tissues such as liver, muscle and T cells. Both the mice and ES cells are shedding light on mechanisms of cell fate determination, hormone and cytokine responsiveness and provide insight into the therapeutic utility of GSK-3 inhibitors.

PL1-5

ACTIVATION OF PROINFLAMMATORY CASPASES

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The proinflammatory caspase-1 and caspase-5 are activated in a complex called inflammasome. Two types of NALP inflammasomes exist. The NALP1 inflammasome composed of NALP1, the adaptor protein ASC, caspase-1 and caspase-5, and the NALP2/3 inflammasome that contains, in addition to NALP2 or NALP3, CARDINAL, ASC and caspase-1 but not caspase-5. In addition to the bacterial component peptidoglycan, inflammasomes are activated by 'danger signals' such as gout-associated uric acids crystals (MSU). We will present data that show that MSU engage the caspase-1-activating NALP3 inflammasome, resulting in the production of active interleukin (IL)-1beta and IL-18. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC and NALP3 are defective in crystal-induced IL-1beta activation. Moreover, an impaired neutrophil influx is found in an in vivo model of crystal-induced peritonitis in inflammasome-deficient mice or mice deficient in the IL-1beta receptor (IL-1R). These findings further support a pivotal role of the inflammasome in several autoinflammatory diseases.

PL1-6

INTERLEUKIN-6: FROM BSF-2 TO TOCILIZUMAB

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In the late 1960s, the essential role of T cells in antibody production had been reported. In search of molecules mediating T cell helper function, I discovered activities in the culture supernatant of T cells that induced proliferation and differentiation of B cells. This led to my life's work: studying one of those factors, interleukin-6 (IL-6). To my surprise, IL-6 turned out to play additional roles, including myeloma growth factor and hepatocyte-stimulating factor activities. More importantly, it was involved in a number of diseases, such as Rheumatoid arthritis, Juvenile idiopathic arthritis (JIA), Multiple myeloma, and Castleman's disease. I feel exceptionally fortunate that my work not only revealed the framework of cytokine signaling, including identification of the IL-6 receptor, gp130, NF-IL6, STAT3, and SOCS-1, but also led to the development of a new therapy for chronic inflammatory diseases. Blocking IL-6 signals by a humanized antibody against IL-6 receptor (Tocilizumab) could show significant therapeutic effect on those chronic inflammatory diseases. In Japan, clinical trials have been completed and in 42 countries all over the world the final trials will conclude in 2007.
Interestingly, long-term administrations of the antibody normalize the serum IL-6 levels, indicating that the antibody might fundamentally restore the immune disorders. Recently, it has been shown that IL-6 together with TGF-β is essential for the development of a new T cell subset Th17 which causes autoimmune diseases. If this is the case, our clinical observations could be explained.
We begin "from clinic to basic" by analyzing cell, protein and gene profiles before and after the treatment. This could tell us what causes autoimmune diseases.

PL1-7

TRIGGERING ANTIVIRAL RESPONSES AND HOST REGULATION BY THE CYTOPLASMIC RNA HELICASE RIG-I

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Recent studies show the involvement of cytoplasmic RNA helicase family, RIG-I, MDA5 and LGP2 in antiviral innate immune responses. RIG-I consists of N-terminal CAspase Recruitment Domain (CARD) and a domain with signatures of DExD/H box helicase (helicase domain). In the absence of viral infection, RIG-I does not transduce signals. The helicase domain detects viral replicating RNA, then CARD triggers the activation of downstream signaling cascade: IPS-1 (MAVS, VISA or Cardif), TBK-1/IKK-I and IRF-3/-7. RIG-I binds to double stranded (ds)RNA, however it does not simply function as a binding receptor for dsRNA, since RIG-I with disrupted ATP binding site, which binds to dsRNA indistinguishably as wt, is incapable of signaling. Furthermore, our in vitro assay demonstrated that RIG-I dissociates dsRNA into single stranded RNA in the presence of ATP. Thus, we propose a model that in the absence of dsRNA, RIG-I forms "closed" conformation and upon binding to dsRNA, it conforms into "open" structure exposing CARD. ATP hydrolysis is required to induce the drastic conformational change.
RIG-I signaling results in the activation of transcription factors such as IRF-3, -7 and NF-kB affecting various cellular functions. Most well established effect is induction of cytokine secretion including type I IFNs. These soluble factors are critical for rapid expansion of antiviral responses. In addition to these indirect effects, we found that RIG-I directly suppresses host cell replication. Physiological significance of this biological activity will be discussed.