Plenary session 2

Innate and adaptive immunity

PL2-1

VIRUS SIGNALING AND CONTROL OF INTERFERON AND CYTOKINE PRODUCTION: LESSONS FROM RNA

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Virus infection of mammalian cells triggers the effector actions of interferon regulatory factors (IRFs) in part through dsRNA responsive signaling pathways that induce interferon alpha/beta (IFN) production and host defenses that limit viral replication. This "host response" represents a first line immune defense against infection. Hepatitis C virus (HCV) is an RNA virus and major human pathogen that mediates persistent infection in millions of people. Our studies are focused on understanding HCV-IFN interactions and the molecular basis of HCV persistence. Cell-based studies have shown that HCV RNA is a potent inducer of the host response, that viral replication is sensitive to the action of IRF-3 target genes, and that HCV replication fitness and persistence are linked to viral control of IRF-3 and IFN actions. HCV triggers host defenses in part through the cellular CARD domain protein and helicase, RIG-I, which recognizes and binds to structured motifs of the genomic viral RNA. This initiates CARD signaling events to IPS-1, which is also a CARD protein and an essential adaptor molecule for virus signaling of host defense. The resulting activation of IRF-3 drives IFN production in hepatocytes. Biochemical studies have revealed that HCV targets and cleaves IPS-1 through the actions of the essential viral protease, termed NS3/4A. This work has identified novel therapeutic applications of HCV protease inhibitors as small molecules that can restore the host response to HCV infection. In conclusions, our studies define RIG-I as a cytoplasmic sensor or viral dsRNA, and show that HCV targets and disables this pathway to support persistent infection.

PL2-2

INTERFERONS AT THE INTERFACE OF INNATE AND ADAPTIVE IMMUNITY

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Type I Interferons (IFN-Is) are important regulators of the interface between innate and adaptive immunity. Yet, despite being one of the first cytokines cloned, a molecular understanding of how IFN-Is direct the activation of intracellular signals and mediate their potent biological responses has not been fully elucidated. To this end Stat2 knockout mice were generated. Although Stat2-/- fibroblasts are largely defective in their response to IFN-Is, Stat2-/- leukocytes are still able to mediate a subset of Stat1 dependent responses. Unexpectedly, Stat2-/- macrophages have acquired the ability to induce MHC-II in response to IFN-I stimulation. A molecular model of this surprising observation will be discussed.
Additional efforts to understand how IFN-Is direct the activation of intracellular signals have entailed the generation of IFN-a receptor chain 1 (IFNAR1) and chain 2 (IFNAR2) point mutants. Ectopic expression of these mutant receptors in tissues harvested from IFNAR1 and IFNAR2 knockout mice should provide an opportunity to identify receptor residues that are critical in mediating the biological response to IFN-Is.

PL2-3

INNATE RECOGNITION PATHWAYS LEADING TO CYTOKINE PRODUCTION BY DENDRITIC CELLS

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Direct pathogen recognition is a major trigger of dendritic cells (DC) activation leading to adaptive immunity. We have been studying three distinct pattern-recognition pathways that mediate murine DC activation by potential pathogens. One pathway involves DC recognition of mimics of viral genomes such as CpG-containing DNA, double stranded RNA or single stranded RNA. These nucleic acids can be recognised by toll-like receptors (TLRs) 9, 7/8, or 3 and, depending on the DC subset in question, promote synthesis of high levels of IFNα and/or IL-12/IL-6/TNFα. A related but distinct pathway involves cytosolic recognition of viral double-stranded RNA and can induce secretion of high levels of interferons by all DC subsets upon direct viral infection. Finally, a third pathway involves recognition of yeasts by a C-type lectin, dectin-1, which signals via Syk kinase and elicits a distinct pattern of cytokine production by DC, characterised by high levels of IL-2 and IL-10. We hope that these and further studies will help build a global picture of the receptors and signalling pathways that regulate DC activation by pathogens, thereby improving the use of DC as tools for immunotherapy in cancer and infectious disease.

PL2-4

CELLULAR RESPONSES TO DOUBLE-STRANDED RNA: DISTINGUISHING SELF FROM NON-SELF

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Non-specific activation of the interferon system by short interfering (si) RNAs can complicate the use of RNA interference to specifically downregulate gene expression. To uncover the basis of these nonspecific activities, we analyzed the effect of chemically synthesized siRNAs on mammalian double-stranded RNA (dsRNA)-activated signaling pathways. Interestingly, siRNAs ranging from 21 to 27 nucleotides (nt) in length activated the interferon system when they lacked 2-nt 3' overhangs, a characteristic of Dicer products. We found that the recognition of siRNAs is mediated by the RNA helicase RIG-I and that the presence of 3' overhangs impairs its ability to unwind the dsRNA substrate and activate downstream signaling to the transcription factor IRF-3. These results suggest a structural basis for the discrimination between microRNAs that are endogenous Dicer products, and nonself dsRNAs such as by-products of viral replication. These findings will enable the rational design of siRNAs that avoid non-specifc effects or alternatively that induce bystander effects to potentially increase the efficacy of siRNA-based treatments of virus infections or cancer.

PL2-5

INTRAVIRAL MICROSCOPY OF T CELL INTERACTIONS WITH ANTIGEN-PRESENTING CELLS

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Cell migration and coordinated cell-cell interactions are hallmarks features of the immune system. Recent advances in real-time in vivo imaging technology have added a new dimension to our efforts to understand the dynamics and complex interplay of the key cellular players in the steady state and during ongoing immune responses. In particular, multiphoton intravital microscopy (MP-IVM) allows prolonged three-dimensional observations of highly dynamic events that occur hundreds of micrometers below the surface of solid tissues in living animals. Using a newly developed MP-IVM model in mouse popliteal lymph nodes, we have analyzed how CD8 T cells are activated and how they interact with tumor antigen-presenting target cells in the presence and absence of CD4+CD25+ regulatory T cells (Treg). We observed that the inital activation (priming) of naive T cells occurs in three distinct interactive phases characterized by a several hours-lasting period of short serial contacts followed by a phase of tight, sustained clustering, which after the first day converts to a prolonged period during which the activated T cells are highly motile, engage in short contacts with antigen-presenting cells and proliferate rapidly. Several days later, the activated T cells differentiate into full-fledged cytotoxic T lymphocytes (CTL), whose cytotoxic activity could be studied at the single-cell level using MP-IVM in popliteal lymph nodes. We found that CTL without Treg killed their antigen-presenting targets at a much faster rate than regulated CTL. To be susceptible to Treg-mediated suppression the CTL had to be responsive to TGF-beta, and suppression was rapidly reversed when Treg were depleted. Treg did not intefere with CTL proliferation or differentiation, but selectively disabled antigen-triggered CTL degranulation. Together, our experiments show that Treg create a local milieu in lymph nodes that permits CTL to acquire effector potential, but withholds the license to kill.

PL2-6

THE IMPACT OF CHEMOKINE AND CYTOKINE NETWORKS ON T CELL PRIMING

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Upon activation in peripheral tissues dendritic cells (DCs) migrate to draining lymph nodes where they prime naïve T cells and initiate adaptive immune responses. We have been studying how DC migration influences T cell priming in a vaccination setting where bone marrow-derived mature DCs are administered subcutaneously and the response of specific T cells is monitored in the draining peripheral lymph nodes. We reported that inflammation in peripheral tissues increases the efficiency of DC migration to lymph nodes and consequently the magnitude and quality of $CD4^+$ T cell responses. We now show that $CD4^+$ and $CD8^+$ effector memory T (T_{EM}) cells, which are normally excluded from lymph nodes, are efficiently recruited to lymph nodes that have been immune stimulated either by migrating mature DCs or adjuvants. In stimulated lymph nodes antigen-activated T_{EM} cells produce cytokines and, in the case of $CD8^+$ CTL, kill antigen-presenting DCs. We also show that P-selectin and CXCL9 are up-regulated on high endothelial venules of DC-draining lymph nodes and that *in vivo* administration of P-selectin, but not E-selectin, antibodies inhibits migration of effector $CD4^+$ T cells while migration of $CD8^+$ T_{EM} cells is impaired if cells are generated from CXCR3-deficient mice. Activated $CD8^+$ T_{EM} cells suppress proliferation or enhance differentiation of naïve $CD4^+$ T cells depending on whether MHC class I and class II epitopes are displayed on the same or on different DCs, respectively. Taken together these results indicate that in some conditions T_{EM} cells can be recruited to lymph nodes and modulate secondary immune responses.