

Plenary session 3

Genes regulation, interferons and cancer

PL3-1

T-BET, A TRANSCRIPTION FACTOR THAT CONTROLS TYPE 1 IMMUNITY

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T-bet is the first Th1 specific transcription factor described that plays a central role in Th1 development. T-bet expression correlates with IFN- γ expression in Th1, NK and B cells. Ectopic expression of T-bet both transactivates the IFN- γ gene and induces endogenous IFN- γ production. Remarkably, retroviral gene transduction of T-bet into polarized Th2 and Tc2 primary T cells redirects them into Th1 and Tc1 cells, respectively, as evidenced by the simultaneous induction of IFN- γ and repression of IL-4, IL-10, TGF β and IL-5. Thus, T-bet initiates Th1 lineage development from naïve Thp cells both by activating Th1 genetic programs and by repressing the opposing Th2 programs.

We have recently published in vivo proof-of-principle studies that definitively demonstrate that T-bet controls the Th1/Th2 balance in vivo. Mice that overexpress T-bet (T-bet transgenic mice) have increased production of IFN- γ and decreased production of IL-4. Mice that are deficient in T-bet (T-bet ko mice), in contrast, have severely impaired production of IFN- γ and increased production of Th2 cytokines. T-bet controls IFN- γ production from CD4, NK, $\gamma\Omega$, CD8 and dendritic cells. T-bet ko mice are more susceptible to infection with *Leishmania Major*, *Salmonella*, *Listeria* and *M. Tuberculosis* but are protected from the development of autoimmune diseases such as inflammatory bowel disease. Inflammatory arthritis and systemic lupus erythematosus. T-bet deficient mice are, in contrast, highly susceptible to asthma as would be expected from their failure to mount Type I immunity which is protective in asthma and allergies. We have mapped the function of T-bet to multiple cell types in both the adaptive and innate immune system and have identified by genome wide location analysis the universe of T-bet target genes in both human and murine cells. Small molecules and biologics that can effectively and selectively inhibit T-bet activity represent an attractive approach to controlling autoimmune, infectious, neoplastic and allergic disease.

PL3-2

DYNAMIC MODULATION OF INTERCHROMOSOMAL INTERACTIONS DURING T CELL DIFFERENTIATION

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Association of regulatory elements on one chromosome can determine the transcriptional activation of genes on another chromosome, with implications for the functional regulation of cellular processes. We have previously shown that interchromosomal association of the TH1 IFN- γ locus and TH2 loci, which are mutually exclusively expressed by two subtypes of differentiated T helper cells, precedes differentiation and acts as a checkpoint for T cell fate determination. We now

report that the second TH1 locus, lymphotoxin (LT- α)/tumor necrosis factor (TNF- α) gene cluster on chromosome 17, physically interacts with regulatory elements on the TH2 locus on chromosome 11 in precursor CD4⁺ T cells. Upon TH1 cell differentiation, this interaction is terminated and replaced by interactions between the two expressed loci: namely, the LT α -TNF α locus on chromosome 17 and the IFN- γ locus on chromosome 10. Regulatory elements in the TH2 locus appear to play an active role in orchestrating interactions across these chromosomes. The interchromosomal interactions we describe appear to direct either activatory or silencing roles in gene transcription.

PL3-3

IRF FAMILY TRANSCRIPTION FACTORS IN THE REGULATION OF CYTOKINE GENE REGULATION AND IMMUNITY

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Prompt and regulated cellular responses is central to host defense and is coordinated by a genetic regulatory network in which a given transcription factor controls the diverse set of target genes depending on the cell type and/or the nature of cellular stimuli. Studies of the interferon regulatory factors (IRFs), originally discovered in the context of type I interferon (IFN) gene induction, have revealed their remarkable functional diversity in regulating the immune systems. The cellular IRF family in man and mouse consists of nine members, and the critical involvement of these members in the innate and adaptive immune systems has recently been gaining much attention. We showed that IRF-7 is essential for both the virus-activated cytosolic pathway (in cooperating with IRF-3) and the TLR-activated, MyD88-dependent pathway of IFN gene induction in plasmacytoid dendritic cells (pDCs). Evidence has also been provided that IRF-7 interacts with MyD88 and the endosomal persistence of MyD88-IRF-7 signaling critically determines the unique ability of pDCs to produce large amounts of IFNs. We found that IRF-5 also interacts with and activated by MyD88 and IRF-5 is critical for the TLR-dependent gene induction program (IL-12, IL-6, TNF- α and others), and that IRF-4 acts as a negative regulator of IRF-5. One of the emerging issues in the TLR field is the synergism between TLRs: We will present some of our recent data on this synergistic activation of the genes in relation to the activation of IRFs and other transcription factors. Finally, critical roles of IRFs in oncogenesis will be discussed. (Our work was done in collaboration with T. Mak, N. Yoshida, Ch. Taya, T. Matsuyama, and their colleagues).

References

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PL3-4**VISUALIZING REAL TIME ACTION OF IRF-8 IN MACROPHAGES AND DENDRITIC CELLS****K Ozato¹, L Laricchia-Robbio¹, T Tamura, P Taylor¹, J McNally², T Karpova²**¹Laboratory of Molecular Growth Regulation, NICHD; ²National Cancer Institute, National Institutes of Health, Bethesda MD 20892, USA

IRF-4 and IRF-8 are immune system specific members of the IRF family. We previously showed that (i) IRF-8 promotes differentiation of macrophages from myeloid progenitor cells and (ii) IRF-4 and IRF-8 differentially regulate dendritic cell (DC) subset development. IRF-8 is required for generation of plasmacytoid DC and CD8 α + DC, important for type I IFN and IL-12p40 production. To study the mechanism by which IRF-8 regulates transcription, we visualized real time movement of IRF-8 in macrophages by using a photobleaching technique. Fluorescence recovery after photobleaching was employed for a system where wild type (WT) IRF-8-GFP induce macrophage differentiation in IRF-8^{-/-} progenitors. WT IRF-8, but not mutants moved very rapidly within the nucleus with fast and slow kinetics. The fast moving component bound to chromatin for less than 0.1 second, while the slow moving compartment bound for > 25 seconds but less than 1 min. Results are consistent with photobleaching performed for other nuclear regulatory proteins. We found that an interaction with partner proteins, PU.1 and IRF-1 decreased the mobility of IRF-8, indicating that formation of continuously changeable multi-protein complexes affects chromatin binding property. Furthermore, IFN- γ and LPS dramatically altered the mobility of WT IRF-8, but not the mutants. Thus the macrophage activating signals change IRF-8's global chromatin binding properties. Together, photobleaching approaches provide new understanding on the real time behavior of a transcription factor.

References

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PL3-5**TRANSCRIPTION REGULATION OF IFN-STIMULATED GENES. MECHANISTIC INSIGHT INTO ACUTELY INDUCIBLE GENE TRANSCRIPTION****D Levy, H Chang H**

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IFN-stimulated gene (ISG) expression represents a highly regulated genetic switch, in which essentially silent genes can be acutely induced to very high levels of transcriptional in a rapid response to IFN stimulation. Transcription is mediated by the ISGF3 transcription factor complex, composed of tyrosine phosphorylated STAT1 and STAT2, in conjunction with the DNA binding protein, IRF9. While STAT2 has been shown to contain the majority of the transactivation potential of ISGF3, the mechanisms of target gene activation are incompletely defined. We have documented some unique biochemical aspects of this regulatory switch. ISG transcription is triggered by a novel initiation complex, containing a subset of standard TAF components, allowing robust ISG transcription to continue in the face of viral inhibitory mechanisms. We have also investigated a requirement for histone deacetylase (HDAC) activity for transcriptional regulation of ISGs and establishment of an antiviral state. Our results show that HDAC activity is provides a positive signal for gene activation and is required at a step subsequent to Pol II recruitment but prior to the transition from transcriptional initiation to committed elongation. Modeling STAT2-dependent transcription in yeast suggests that class I HDAC enzymes target histone H4 acetylation during ISG activation. These biochemical and epigenetic regulatory features may be general attributes of acutely activated transcription units.

PL3-6**THE INTERFERONS AND CANCER IMMUNOEDITING****RD Schreiber**

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We have shown that mice lacking an intact immune system are more susceptible than their wild type counterparts to formation of spontaneous and chemically induced tumors. We also found that tumors from immunodeficient mice are more immunogenic than those from immunocompetent recipients. Based on these observations, we formulated the name "cancer immunoeediting" to describe this process because it stresses the dual host-protective and tumor-sculpting actions of immunity on developing tumors. Over the last three years we have refined our thinking about cancer immunoeediting such that we now envisage it as a process comprised of three phases each beginning with the letter **E**: **E**limination (the host-protective phase of the process comparable to cancer immunosurveillance), **E**quilibrium (the tumor-sculpting phase comparable to tumor dormancy) and **E**scape (the phase where tumor growth occurs in a manner unrestrained by the immune system). Recent work from our lab and other labs has focused on defining the molecular and cellular events that underlie each of the three phases of cancer immunoeediting. We have placed our specific efforts on (1) identifying the components of innate and adaptive immunity that participate in the host-protective, elimination phase of the process; (2) defining the molecular targets of cancer immunoeediting and identifying the cellular editors responsible for the tumor sculpting that occurs in the equilibrium phase of the process; and (3) elucidating the critical alterations that a developing tumor must undergo to escape immunologic control. The presentation will summarize some of our most significant new findings stemming from these efforts with special emphasis placed on the different roles of IFN- γ , IFN- α/β and STAT1 in the process.

PL3-7**LYMPHANGIOGENESIS IN DEVELOPMENT AND HUMAN DISEASE****Kari Alitalo and collaborators**

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Angiogenesis and permeability of blood vessels are regulated by vascular endothelial growth factor (VEGF) via its two receptors VEGFR-1 and VEGFR-2. The VEGFR-3 receptor does not bind VEGF and its expression becomes restricted mainly to lymphatic endothelia during development. We have found that homozygous VEGFR-3 targeted mice die around midgestation due to failure of cardiovascular development. We have also purified and cloned the VEGFR-3 ligand, VEGF-C. Transgenic mice expressing VEGF-C show evidence of lymphangiogenesis and VEGF-C knockout mice have defective lymphatic vessels. The proteolytically processed form of VEGF-C binds also to VEGFR-2 and is angiogenic. VEGF-D is closely related to VEGF-C, similarly processed and binds to the same receptors. Thus VEGF-C and VEGF-D appear to be both angiogenic and lymphangiogenic growth factors. VEGF-C overexpression led to lymphangiogenesis and growth of the draining lymphatic vessels, intralymphatic tumor growth and lymph node metastasis in several tumor models. Furthermore, soluble VEGFR-3, which blocked embryonic lymphangiogenesis, also blocked lymphatic metastasis in breast and lung cancer models. These results together with recent clinical cancer studies suggest that paracrine signal transduction between tumor cells and the lymphatic endothelium may be involved in lymphatic metastasis of human cancers.

References

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