Meeting report

Gene regulation and signal transduction in the immune system

Tiffany Horng, Shalini Oberdoerffer and Anjana Rao

Address: Department of Pathology, Harvard Medical School, Immune Disease Institute, Boston, Massachusetts 02115, USA.

Correspondence: Tiffany Horng. Email: horng@idi.harvard.edu

Published: 10 July 2008

Genome ***Biology*** 2008, **9**:315 (doi:10.1186/gb-2008-9-7-315)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/7/315

A report of the meeting 'Gene Expression and Signaling in the Immune System', Cold Spring Harbor, USA, 22-26 April 2008.

Major themes at this year's Cold Spring Harbor meeting on gene expression and signaling in the immune system included transcriptional control of leukocyte development and differentiation, antigen receptor gene assembly and modification, signal transduction by antigen receptors in control of lymphocyte biology, signal transduction in regulation of inflammatory gene expression and the analysis of chromatin structure and other epigenetic mechanisms in control of gene expression.

Metabolic regulation in lymphocyte activation

The link between metabolism and regulation of lymphocyte activity was addressed by Doreen Cantrell (University of Dundee, UK), who presented data regarding the role of phosphatidylinositol-3-OH kinase (PI3K) signaling in regulating T-cell biology. She has used an *in vitro* model of murine CD8 T-cell differentiation in which the strength of PI3K signaling is varied, by culture with either of the cytokines interleukin (IL)-2 or IL-15, or with pharmacological inhibitors of the PI3K pathway. She showed that high levels of PI3K signaling (as in the IL-2 cultures) produced large cells with an effector phenotype, while low levels (as with IL-15) resulted in small, memory-like cells. This reflected regulation of two major functional programs: metabolism, through control of protein synthesis, and trafficking, through regulated expression of the chemokine receptor CCR7 and the selectin molecule CD62L. Cantrell suggested that this enables the PI3K pathway to match cellular metabolic demands with migration *in vivo*, such that high levels of PI3K signaling would support energy-demanding effector T-cell functions in the periphery, whereas low levels would direct T cells back to the nutrient-rich environment of the lymph node.

The intracellular signaling protein Slp2 is also involved in regulating energy metabolism in lymphocytes. Joaquin Madrenas (University of Western Ontario, London, Ontario) suggested that induction of Slp2 in activated mouse T cells and B cells is necessary to meet the increased metabolic demands associated with the transition from the quiescent, G0-arrested state to actively proliferating and differentiating lymphocytes. He showed that induction of Slp2 led to an increase in the amount of mitochondrial membrane and the number of mitochondria, and consistent with this, an increase in ATP stores. Conversely, Slp2 downregulation inhibited T-cell activation, as assessed by IL-2 production. Ectopic expression of Slp2 also protected T cells from apoptosis triggered by the cell-autonomous, mitochondria-dependent pathway. Therefore, while its exact role in mitochondrial regulation is not clear, Slp2 may be critical for diverse aspects of lymphocyte activation, including energy metabolism and survival.

Signal transduction in pro-inflammatory gene expression

The NF-κB family of transcription factors critically regulates pro-inflammatory gene expression in response to a range of stimuli. Alexander Hoffmann (University of California, San Diego, USA) reported that one member, NF-κB2/p100, can function as a novel noncanonical inhibitor in the IκB family, by mediating retention in the cytoplasm of the NF-κB heterodimer RelA/p50. In contrast to inflammatory signaling, which activates RelA/50 by degrading canonical IκBs (α, β, and ε), p100-bound heterodimers are liberated by developmental stimuli such as stimulation via the lymphotoxin-β receptor (LTβR). Because canonical and noncanonical IκBs also differ in their stimulus-dependent resynthesis and half-lives, IκB homeostasis changes depending on the cellular stimulus. Hoffmann suggested that integration of NF-κB activation by inflammatory signals and developmental signals such as LTβR is essential for specifying physiologically relevant transcriptional programs; dysregulation of this system, for instance by perturbed IκB homeostasis, may contribute to cancer or chronic inflammation.

In this context, Sankar Ghosh (Yale University School of Medicine, New Haven, USA) demonstrated a novel function for a familiar friend, IκBβ. Surprisingly, mice lacking this NF-κB inhibitor were more resistant to septic shock, and consistent with this, produced less tumor necrosis factor α (TNFα). This suggests a role for IκBβ in potentiating inflammatory gene expression in some contexts. Indeed, newly synthesized IκBβ (unlike the constitutive pool that inhibits NF-κB activity) seems to function as a NF-κB coactivator at the TNFα promoter.

Transcriptional control of leukocyte development and differentiation

The transcription factor Foxp3 has a critical role in specifying the gene expression program of regulatory T cells (T_{reg}). Alexander Rudensky (University of Washington, Seattle, USA) reported that a sub-module of this Foxp3-dependent program is co-regulated by IRF4, a transcription factor necessary for differentiation of the Th2 subclass of effector T cells. While specific deletion of IRF4 in T_{reg} did not alter their suppressor activity *in vitro*, these cells had lower levels of expression than many of the genes associated with Th2 differentiation or function (for example, ICOS, cmaf), and mice lacking IRF4 in T_{reg} succumbed to a Th2-skewed lymphoproliferative disease. Because IRF4 is a direct target of Foxp3 and is directly associated with Foxp3, Rudensky suggested that Foxp3 co-opts IRF4 for regulation of a Th2-specific submodule of the T_{reg} transcriptional program.

Two talks addressed early events in lineage commitment in hematopoiesis. Using a multi-lineage progenitor assay, Hiroshi Kawamoto (RIKEN Research Center for Allergy and Immunology, Yokohama, Japan) showed that during thymic differentiation murine T-cell precursors lose B-cell potential before myeloid potential. Furthermore, he estimated that 30% of thymic macrophages are derived from T-cell progenitors *in vivo*. These data fit a model wherein T and B lymphocytes are derived from common myelo-lymphoid progenitors (CMLPs), as opposed to common lymphoid progenitors (CLPs). In ending his talk, Kawamoto presented the provocative idea that B and T lineages had diverged before the evolutionary emergence of adaptive immunity.

Although this caused a stir in the audience, Harinder Singh (University of Chicago, USA) hinted that he might have an explanation of Kawamoto's findings. Singh presented data on the role of the transcription factor Ikaros in B-cell fate determination in mice. He showed that Ikaros has dual functions, promoting B-cell development by restraining the expression of pro-myeloid factors (such as Gfi1), and acting directly to induce the recombinase (*Rag*) genes and thereby recombination at the immunoglobulin heavy-chain locus. Interestingly, Ikaros was found to bind at pericentromeric satellite DNA and could therefore play a role in the silencing of genes encoding key developmental regulators such as Gfi1, PU.1 and Egr1. Singh presented the intriguing concept that Ikaros may have played a primordial role in repressing the *Rag* genes. Later on, an Ikaros variant evolved that could refine activation of the *Rag* genes, thereby allowing for the development of modern B and T cells.

Global analysis of chromatin structure

Covalent modifications to histones are essential for dynamic regulation of gene expression. A plethora of studies in the past few years, and several talks at this meeting, mapped histone modifications genome-wide. Keji Zhao (National Heart, Lung, and Blood Institute, NIH, Bethesda, USA) used chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) to interrogate 38 histone modifications at the promoters of all genes in human resting T cells. This analysis identified 4,300 different patterns, of which 3,100 were unique (that is, found only at one gene). In contrast, some patterns were found at multiple genes; more than 3,000 genes, for instance, were marked by a 'modification backbone' consisting of 17 modifications. By assaying single nucleosomes, Zhao determined that 14 of these 17 modifications co-localize to the same nucleosome. To address histone modifications at enhancers, he assayed 41,000 DNase hypersensitive sites (which are putative enhancers), and discovered 13,000 distinct patterns, of which 1,100 were unique. Zhao's study underscored the diversity of histone modification patterns at gene regulatory elements, but also showed that a limited number of such patterns exist.

Matthias Merkenschlager (Imperial College School of Medicine, London, UK) presented provocative work describing a noncanonical function of cohesin in gene regulation (independent of its function in chromosome segregation), which seems to require the insulator-binding protein CTCF. These results suggest a model whereby, in mammalian cells, CTCF recruits cohesin. Chromatin immunoprecipitation followed by DNA microarray analysis (ChIP-chip) showed that CTCF and cohesin co-localize to a subset of conserved noncoding sequences. These results suggest a model whereby CTCF recruits cohesin to these sites to mediate an insulating function (and perhaps other activities). Interestingly, Merkenschlager noted that because cohesin does not co-localize with CTCF in *Drosophila*, this mechanism of gene regulation by cohesin is vertebrate specific.

Epigenetic regulation of inflammatory gene expression

Two talks addressed the role of epigenetic mechanisms in regulation of inflammatory gene expression. In particular, they addressed the induction of primary response genes (direct transcriptional targets) and secondary response genes (indirect transcriptional targets that require *de novo* protein synthesis for their induction) following Toll-like

receptor signaling. Steve Smale (University of California, Los Angeles, USA) showed in mouse cells that secondary response genes require signal-dependent chromatin remodeling by the BAF complex for their induction; in contrast, the promoters of many primary response genes are marked by CpG islands, which have low histone density and do not assemble into stable nucleosomes, and these promoters are induced independently of chromatin remodeling. Ruslan Medzhitov (Yale University School of Medicine, New Haven, USA) extended this analysis of mouse macrophages to show that in their basal state, promoters of primary response genes are marked by trimethylation on lysine 4 of histone H3 (H3K4), acetylation on lysine 9 of H3 (H3K9), and engaged RNA polymerase II, whereas promoters of secondary response genes acquired these features in a signal-dependent manner. Medzhitov proposed a model whereby arrest of transcription elongation at primary response genes is derepressed by inducible recruitment of pTEFβ, the essential elongation factor, via a 'histone code' for transcription elongation.

Antigen receptor gene assembly and diversification

In regard to mechanisms that target the VDJ recombinase to the antigen receptor loci, Marjorie Oettinger (Harvard Medical School, Boston, USA) showed that the PHD domain of the recombinase subunit Rag2 binds to histone peptides that are trimethylated at H3K4 and symmetrically dimethylated at H3 arginine 2, in both mouse and human systems. While dispensable for *in vitro* VDJ recombination, the PHD finger was essential *in vivo* for recruiting Rag2 to its target genes. Moreover, recombination was reduced by PHD mutations that abolish histone binding, or by altering global levels of H3K4 trimethylation. These results suggest an essential role for the PHD finger in targeting Rag2 to antigen receptor genes and, therefore, in VDJ recombination.

David Schatz (Yale University School of Medicine, New Haven, USA) also addressed the issue of *in vivo* targeting of the Rag1 and Rag2 proteins. He used an elegant system of mouse strains that harbor catalytically inactive Rag1 mutants, so that Rag1 and Rag2 binding could be captured in the absence of ongoing VDJ recombination. Schatz established that the Rag proteins are recruited to 'recombination centers' - small, focused regions in the antigen receptor loci - to initiate VDJ recombination. Moreover, the two Rag proteins can be recruited independently to these loci; for Rag1, recruitment may be mediated by recombination signal sequences with 'open', accessible chromatin, whereas Rag2 binding mirrored H3K4 trimethylation patterns, consistent with the results of Oettinger.

The role of the enzyme activation-induced cytidine deaminase (AID) in antibody diversification was discussed by Michael Neuberger (MRC Laboratory of Molecular Biology, Cambridge, UK). Somatic hypermutation and class switch recombination are initiated by AID-mediated deoxycytidine deamination, resulting in a U:G mismatch and uracil excision or mismatch recognition and repair. Whereas AID targeting to a rearranged immunoglobulin gene variable region results in somatic hypermutation, AID mediates class switching at S regions in the immunoglobulin gene constant region. In an effort to understand the molecular basis of this differential activity, Neuberger used a yeast two-hybrid screen and identified CTNNBL1 (beta catenin-like protein 1) as an AID-interacting protein. Interestingly, he found that an AID mutant deficient in CTNNBL1 binding retains deamination activity but is impaired in class switching, suggesting that CTNNBL1 may specifically regulate AID-dependent class switching.

Cell-fate determination in the immune system

A few talks focused on the role of asymmetric division in cell-fate determination, and provided compelling evidence suggesting that the cell fate determinant Numb is critical in determining lineage commitment in the immune system. Tannishtha Reya (Duke University Medical Center, Durham, USA) used a reporter system that marks Notch expression to study stem-cell commitment, and showed that all of the following are potential outcomes of stem cell division: symmetric renewal; symmetric commitment; and asymmetric division. Interestingly, she found that the relative ratios of the outcomes could be regulated in a context-dependent manner. For instance, she found a normal balance between asymmetric and symmetric division in chronic myeloid leukemia, but increased symmetric renewal in acute myeloid leukemia (AML). Importantly, enforced expression of the Notch signaling antagonist Numb corrected the imbalance in AML, providing a basis for potential future therapies.

Steve Reiner (University of Pennsylvania, Philadelphia, USA) and Sarah Russell (University of Melbourne, Australia) also described asymmetric cell division, but in the context of an immune response. Reiner showed that following antigen triggering, T cells become polarized with respect to the immunological synapse they make with the antigen-presenting cell. Cell-fate determinants, such as Numb and the transcription factor T-bet, become localized to the immunological synapse (proximal) side of the cell, whereas protein kinase C-ζ becomes partitioned to the distal side of the cell. Following the first division, factors asymmetrically segregate into the daughter cells, such that the proximal daughter gives rise to effector cells and the distal daughter develops into memory cells. Reiner further speculated that T-bet is responsible for commitment to an effector lineage, whereas the transcirption factor EOMES promotes memory cells. Similarly, Russell showed that the polarity determinants Par complex and Scribble complex antagonize each other to polarize T cells during an immune response. She showed that, whereas cell-surface proteins such as CD8

and LFA1 are not polarized during cell division, Scribble remains proximal and Numb remains distal throughout, thus providing a basis for formation of asymmetric daughter cells.

The immune system has been a rich model for addressing how signal transduction and gene regulation control diverse and fundamental biological processes. This was underscored at the 2008 meeting. We look forward to the next meeting in 2010, and anticipate follow-up to the work reported here as well as new stories on signaling and transcription in the immune system.