The Midwinter Conference of Immunologists is a popular annual fixture for immunologists to discuss the hot topics in immunology while enjoying the mild weather of the West Coast.

Immunology at Asilomar: from molecules to mice

Christopher B. Wilson[1], Christel H. Uittenbogaart[2] and Diane J. Mathis[3]

[1]University of Washington, Department of Immunology, Box 357650, Seattle, WA 98195, USA. [2]University of California, Los Angeles School of Medicine, Departments of Pediatrics, Microbiology, Immunology and Molecular Genetics, Los Angeles, CA 90095, USA. [3]Joslin Diabetes Center, Harvard Medical School, 1 Joslin Place, Boston, MA 02115, USA.

The 42nd Midwinter Conference of Immunologists, held at the Asilomar Conference Center in Monterey, California in January 2003, addressed the development and function of the immune system. Below, the topics discussed at Asilomar are briefly described.

Determining lymphoid cell fate epigenetically

The development and differentiation of lymphoid cells into distinct lineages with specialized functions is an ordered process of sequential cell-fate choices, which requires cells to extinguish the expression of some genes and to enable the expression of others. These changes in gene expression programs are controlled epigenetically. In mammals, epigenetic information is encoded by differential methylation of DNA on cytosine, post-translational modifications of histones and higher-order chromatin structure[1,2]; these, in turn, modulate the accessibility of genes to transcription factor binding and, in concert with differences in transcription factor abundance, determine the probability of gene expression. Epigenetic codes are potentially heritable even in the absence of the factors that induced them, thereby providing a mechanism for permanent changes in gene expression necessary for lineage specification. However, they are also potentially reversible.

L. Dempsey

Scientists basking in the beautiful winter sunshine of Asilomar.

CD4 and CD8 expression begins when immature double-negative thymocytes mature into double-positive thymocytes, after which CD4 or CD8 expression must be silenced as double-positive thymocytes mature into single-positive cells. The CD4 silencer and one of several CD8 enhancers primarily control these developmental switches[3]. Gerald Crabtree (Stanford, CA) showed that SWI/SNF-like BAF chromatin remodeling complexes help to control these developmental transitions. Mice with mutations affecting the Brg or BAF57 subunits of BAF complexes have defects in activation of the CD4 silencer and CD8 enhancers, which result in premature and persistent expression of CD4 in normally double-negative thymocytes and a defect in the expression of CD8 (ref. 4). Brg binds to the CD4 silencer, and the loss of CD4 silencing in BAF mutants resembles that seen in mice in which the CD4 silencer or the CD4 silencer–binding transcription factor Runx1 have been knocked-out[5]. Similarly, the gene encoding TdT (*Dntt*) is turned on in double-negative thymocytes and then irreversibly silenced after positive selection[2]. Stephen Smale (Los Angeles, CA) showed that the silencing of TdT is associated with deacetylation and subsequent methylation of histone H3 on lysine 9, which begins in the TdT promoter and then spreads outward bidirectionally. Although methylation of H3 of lysine 9 has a causal role in irreversible gene silencing and repositioning to pericentromeric heterochromatin in other systems (Matthias Merkenschlager, London), to date it has not been shown that silencing of TdT is causally related to progressive H3 lysine 9 methylation in thymocytes.

T cell effector functions also seem to be enforced epigenetically. DNA methylation and binding of the methylated-CpG binding protein MBD2 in the IL-4 and IL-13 locus seem to be essential for silencing IL-4 expression in T_H1 and $CD8^+$ T cells. $CD8^+$ T cells deficient in DNA methylation owing to a conditional knockout of DNA methyltransferase I (Chris Wilson, Seattle, WA) and T_H1 CD4 T cells deficient in MBD2 (Steven Reiner, Philadephia, PA) inappropriately express IL-4 and IL-13 or IL-4, respectively, in high amounts. Data from these groups also support the hypothesis that the repressive effects of DNA methylation and MBD2 binding in the IL-4 and IL-13 locus are in genetic competition with the agonistic effects of GATA-3, the master T_H2 transcriptional regulator[6]. Reiner's studies suggest that this may be mediated in part by GATA-3 interfering with MBD-2 binding in the locus. An additional constraint on inappropriate T_H2 cytokine expression may be provided by two GATA-3 inhibitors—FOG (friend of GATA-1) and ROG (repressor of GATA) (Kenneth Murphy, St. Louis, MO). This indicates that T_H2-polarizing conditions or ectopic GATA-3 expression may induce T_H2 T cell differentiation in part by increasing the ratio of GATA-3 to these antagonists. Further study will be needed to evaluate epistasis between epigenetic modifications in the T_H2 cytokine loci and the abundance of GATA-3, and its antagonists, in the control of T cell effector fate specification.

Regulatory receptors, allergy and autoimmunity

Several genetic loci seem to affect T_H2 cytokine production and predisposition to allergic diseases. One such locus, on human chromosome 5q23–25, contains the *TIM* genes. Rosemary DeKruyff (Stanford, CA) reviewed data indicating that these genes are polymorphic between mouse strains that differ in their predisposition to T_H2 responses and airway hyperreactivity[7]. *In silico* analysis indicates that the human locus contains a total of three *TIM* genes, whereas the syntenic region of mouse chromosome 11B1.1 seems to contain eight *Tim* genes. These genes are differentially expressed by T_H1 and T_H2 T cells and may modulate their function. Studies by Raif Geha (Boston, MA) indicate that products of the *C3* and *C3ar1* receptors and *Il10* genes modulate allergic skin disease. The relative contribution of these and other genes to T_H2 responses and allergy, and the mechanisms involved, are areas of ongoing investigation.

The TIM receptor family is only one of a growing list of activating and inhibitory receptors on leukocytes. The expanding B7 superfamily consists of ligands for costimulatory receptors and for receptors that negatively regulate the T cell response. The multiplicity of these receptors and ligands gives rise to questions about redundancy, uniqueness to compartment or cell types, timing and stage of the immune response, and functional hierarchy. Arlene Sharpe's (Cambridge, MA) results show that the CD28-B7-1 and CD28-B7-2 interactions play a role not only in the induction of autoreactive T cells in experimental autoimmune encephalomyelitis (EAE), but also in the effector phase of the response. In contrast to B7-1 and B7-2, PD-L1 and PD-L2 are expressed on nonhematopoietic and hematopoietic cells and bind to the PD-1 receptor on activated T cells[8]. She also showed that PD-L1 negatively regulates T cell activation and cytokine production even in the presence of CD28-B7 stimulation and may play a role in T cell tolerance. Kenneth Murphy reported the identification of another new member of this family, BTLA, which is an inhibitory receptor expressed on T_H1 T cells. BTLA knockout mice have increased susceptibility to EAE. Juan Lafaille (New York, NY) showed that the IL-2 receptor-α chain (CD25) also plays a critical role in protection from EAE. Mice with a monoclonal population of $CD4^+$ T cells expressing a receptor for myelin basic protein invariably develop spontaneous EAE, which can be prevented by the transfer of small numbers of wild-type or IL-2 knockout, but not CD25 knockout, polyclonal $CD4^+$ T cells[9]. Similarly, regulatory T cells block the hyper-IgE response of mice with monoclonal $CD4^+$ T cells and B cells after immunization with antigen in alum, apparently by inhibiting the generation of T_H2 T cells and the germinal-center B cell response.

Inhibitory and activating receptors also regulate innate immunity. Lewis Lanier (San Francisco, CA) showed that cytomegalovirus (CMV) encodes ligands for the Ly49 family of receptors, and differences in Ly49 receptors, explain the genetic resistance of some mouse strains to CMV infection. Two activating receptors, Ly49H and NKG2D, cooperate in the recognition and lysis of CMV-infected cells by NK cells from resistant C57Bl/6 mice. Although CMV m152 blocks the expression of NKG2D ligands (Rae-1 proteins), Ly49H directly recognizes CMV m157. In contrast to recent publications[10,11], Lanier's experiments also suggest that NK cell activation *via* NKG2D is largely intact in DAP-12 or syk Zap70 double knockout mice. Further study will be needed to reconcile the findings of these three groups. Marco Colonna (St. Louis, MO) showed that TREM-2, which signals *via* DAP-12, is important for the proper development of osteoclasts, dendritic cells (DCs) and microglial cells, as indicated by studies in TREM-2–deficient humans with Nasu-Hakola disease.

Imaging of the immune system

Recent years have seen phenomenal advances in the imaging of biological processes at all levels. Immunologists have been quick to apply these technological novelties. This set of presentations showed that imaging does not just provide pretty pictures—though it does do that—but also affords new insights.

In mice whose endogenous gene encoding I–Aβ was replaced with an EGFP-tagged Aβ molecule, Hidde Ploegh (Boston, MA) examined the trafficking of major histocompatibility complex (MHC) class II molecules in live antigen-presenting cells (APCs) *in vivo* or *ex vivo* in real time. For DCs, tubular endosomes extended intracellularly and polarized towards an inciting T cell, resulting in stunning projections. This occurred only when fruitful interactions took place between MHC-peptide complexes on the DCs and T cell receptors (TCRs) on the T cells. David Parker (Portland, OR) also showed beautiful images of antigen presenting cells (APCs) at work, obtained from live fibroblasts that expressed MHC class II molecules fused to antigenic peptide or to GFP. He found that in APCs, as in T cells, an immunological synapse is formed as the result of effective TCR-MHC-peptide engagements. Events occurred rapidly: clusters of MHC molecules formed in the contact zone within 1 min of APC contact with $CD4^+$ T cells, then coalesced into synapses within 3–20 min. The APC synapse was peptide specific and at least partially dependent on CD80-CD28 and ICAM-1–LFA-1 interactions. Anergic T cells also formed synapses, but these contained molecules not found in the synapses of activated T cells. For example, c-Cbl (a negative regulator of T cell signaling) accumulated in the former but not the latter.

Ellen Robey (Berkeley, CA) used two-photon laser scanning microscopy to study thymic differentiation and T cell–DC interactions in lymph nodes. Double-positive thymocytes were highly motile within reaggregation thymus organ cultures, interacting preferentially with stromal cells that displayed positively selecting MHC-peptide complexes. Both short- and long-lived interactions could be seen, sometimes on the same stromal cell, and it was not clear what factors favored one or the other. $CD8^+$ T cells in excised lymph nodes were also very motile; DCs moved around as well, but much more slowly. It was calculated that a single DC within the lymph node was contacted on average 500 times per hour by a T cell, a value significantly different from what was seen in the thymus, probably reflecting the need to scan for rare peptides or rare cell-types. Cognate antigen inhibited the free crawling of T cells in lymph nodes and halted the T cells on DCs in long-lasting contacts.

Lymphocyte dynamics

For individuals of the same species, sex and age, the size of any given tissue falls within a rather narrow distribution. This reflects controls on total numbers of cells and the contributions of diverse cell populations. Numbers are controlled by four major processes: import, proliferation, death and export. The immune system adheres to these same general principles, although it does have several particular features that add 'spice' to the dynamics of its cell populations. First, lymphocytes constantly circulate through blood and lymph vessels and sometimes through tissues. Second, to ensure effective surveillance, there exists an impressive array of compartments for specific lymphocyte types and activity states. Third, specific components of these populations are subject to constant flux in response to pathogen challenge. How these particularities are taken into account in controlling lymphocyte dynamics was addressed during a lively session.

T lymphocytes exist in naive, effector or memory states, each with distinct requirements for survival and activity[12]. Naive T cells depend critically on TCR-MHC-peptide and cytokine receptor–cytokine interactions for survival. When confronted with a lymphopenic environment, these same cells proliferate slowly and convert to a memory-like phenotype. This 'homeostasis-driven proliferation' (HDP) also depends on TCR-MHC-peptide and interleukin 7 (IL-7) receptor–IL-7 engagement. How do naive T cells read these cues in the two contexts, and why do they respond to them differently? Ananda Goldrath (Boston, MA) exploited gene expression profiling of purified cell populations to show that survival cues perceived through the TCR and IL-7R elicit some common transcriptional changes, as well as others that are distinct. T cells undergoing HDP show a muted version of transcriptional programs exhibited by effector and memory cells. They also display a unique program that includes induced expression of genes encoding heat-shock proteins and stress-activated protein kinases. Steve Jameson (Minneapolis, MN) compared the influence of 'true' *versus* 'perceived' space in inciting naive lymphocytes to proliferate. It is known that acquired (by irradiation) and congenital lymphopenia (arising from *Rag* or *SCID* mutations) provokes HDP. Cross-competition experiments with monoclonal $CD4^+$ T cell populations established that HDP can be provoked by a dearth not only of T cell numbers but also of T cell specificities. Mike McCune (San Francisco, CA) addressed some of the same questions in humans with HIV infection-induced lymphopenia. Both naive $CD4^+$ and $CD8^+$ T cells are lost after infection, as are certain hematolymphoid environments, such as the thymus. $CD4^+$ cells disappear as a result of two processes: loss of mature cells owing to accelerated destruction of end-stage effectors, and decreased production of cells from thymic and/or bone marrow progenitors. Both IL-7 and growth hormone seem to positively regulate T cell production in humans, offering some therapeutic promise.

B lymphocyte dynamics follow most of the same rules as those of T cells. Antonio Freitas (Institut Pasteur, Paris, France) showed, in mixed bone-marrow chimera and parabiotic systems, that B cells are produced in large excess over what is needed to fill or maintain peripheral compartments, implying competition at some level. Experiments placing B cells from B cell receptor (BCR) transgenic mice in competition with polyclonal B cells resulted in lower-than-expected representation of the transgenic cells and a change in their life span, illustrating the role of antigen specificity in the competition process. For which resources are the cells competing? Antigen is the obvious possibility, probably through complex influences on the ability of B cells to gain access to limiting microenvironments required for their sustenance.

The rules governing NK cell dynamics are just beginning to be established (Steve Jameson). These cells also undergo IL-15–dependent HDP after transfer into an NK cell–deficient environment. Unlike naive T cells, however, they show little change in their phenotype or activity as far as it is known.

Finally, it is becoming increasingly evident that the population dynamics of APCs, and in particular DCs, need to be considered when assessing immune responses. What factors control their influx into or efflux from particular peripheral lymphoid organs or nonlymphoid tissues? What molecules control their survival rates, provoke proliferation or trigger death? Yong-Won Choi (Philadelphia, PA) discussed TRAF-6, a common adaptor for signals emanating from receptors of the IL-1R–TLR and TNFR superfamilies. On the basis of studies with DCs from mice lacking TRAF-6, it seems that these receptors are required for proper differentiation, maturation and activation of DCs.

1. Turner, B.M. *Cell* **111**, 285–291 (2002).
2. Smale, S.T. & Fisher, A.G. *Annu. Rev. Immunol.* **20**, 427–462 (2002).
3. Ellmeier, W., Sawada, S. & Littman, D.R. *Annu. Rev. Immunol.* **17**, 523–554 (1999).
4. Chi, T.H. *et al. Nature* **418**, 195–199 (2002).
5. Taniuchi, I. *et al. Cell* **111**, 621–633 (2002).
6. Murphy, K.M. & Reiner, S.L. *Nat. Rev. Immunol.* **2**, 933–944 (2002).
7. McIntire, J.J. *et al. Nat. Immunol.* **2**, 1109–1116 (2001).
8. Greenwald, R.J., Latchman, Y.E. & Sharpe, A.H. Negative co-receptors on lymphocytes. *Curr. Opin. Immunol.* **14**, 391–396 (2002).
9. Furtado, G.C., Curotto de Lafaille, M.A., Kutchukhidze, N. & Lafaille, J.J. *J. Exp. Med.* **196**, 851–857 (2002).
10. Gilfillan, S., Ho, E.L., Cella, M., Yokoyama, W.M. & Colonna, M. *Nat. Immunol.* **3**, 1150–1155 (2002).
11. Diefenbach, A. *et al. Nat. Immunol.* **3**, 1142–1149 (2002).
12. Prlic, M., Lefrancois, L. & Jameson, S.C. *J. Exp. Med.* **195**, F49–F52 (2002).