Memories in the snow: immune memory, persistent infection and chronic disease

Matthew E. Pipkin

Under the cover of snow in February 2011, immunologists convened in Banff, Alberta, Canada, for the Keystone symposium 'Immunologic Memory, Persisting Microbes and Chronic Disease'. These are wide-ranging topics that are typically addressed in separate experimental settings. However, a theme that emerged was the way in which these subjects are inextricably linked, and the importance of addressing them together and deciphering their molecular determinants.

The town of Banff is nestled in the Canadian Rockies at over 1,000m above sea level, and is an idyllic setting in which to congregate at a scientific conference. Moderate snow and foraging elk greeted members of the immunology community as they entered Banff National Park and convened at the Fairmont Banff Springs for what would be a challenging Keystone meeting, organized by E. John Wherry (U. Pennsylvania, USA), Yasmine Belkaid (NIAID, National Institutes of Health, USA) and Bali Pulendran (Emory U., USA). The work presented at the meeting was broad, ranging from host–pathogen interactions, immunity and homeostasis at mucosal surfaces, vaccines and antibody responses, to the regulation of T-cell differentiation by transcription factor networks and chromatin structure. Integrating these diverse topics was a challenge for the organizers, but it was handled aptly, and induced lasting memories.

Viromes set the mood

The immune system is an extraordinarily diverse compilation of cells that work in concert to establish a flexible and multi-layered defence system that spans the dimensions of space and time. Much of our understanding of immune responses is based on studies of healthy, inbred mice that are maintained in specific pathogen-free facilities. This is in contrast to human populations, which are outbred, live in diverse environments and consume varied diets. As infants, we are rapidly colonized by symbiotic flora and, as we age, we experience

bouts of acute infections acquired through diverse portals of entry, some of which are not cleared and go on to become chronic, life-long infections. The way in which these variables affect the immune system is largely unknown.

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Herbert (Skip) W. Virgin (Washington U. School of Medicine, USA) set the table for this symposium in his opening Keynote address by illustrating that persistent infections change the nature of the host, substantially altering immune function, immunological protection and predisposition to seemingly unrelated chronic diseases. Two key points delineated these concepts. First, the basal-level of immunity in a host, its immunophenotype, is shaped by its virome—that is, the collection of viruses that have established persistent infections—and can have positive or negative effects on the ability of the host to eradicate other unrelated infections. Second, persistent infections in the context of specific, inherited determinants in the host can combine with otherwise inconsequential environmental triggers to precipitate a diseased state. As evidence of this, the Virgin group has shown that the basal level of immunity is elevated by persistent γ -herpes virus infection, which confers protection against bacterial infections such as *Listeria monocytogenes*

and *Yersinia pestis* (the Plague). Conversely, a Crohn-disease-like disorder can develop in mice if they are chronically infected with murine norovirus and carry a hypomorphic *Atg16L1* mutation (a gene involved in autophagy), but only if they are also exposed to an otherwise-innocuous environmental stressor in the presence of symbiotic gut flora (Cadwell *et al*, 2010).

Despite these examples, little work has focused on elucidating the way in which chronic infections affect the development of cellular and humoral immunity on concurrent, unrelated infections or immunizations. This has implications for vaccination. For example, hosts with an immunophenotype that suppresses normal development of B-cell or T-cell memory might be bad candidates for receiving certain vaccines, and alternative strategies should therefore be considered. David Brooks (U. California Los Angeles, USA) provided evidence that the CD4⁺ follicular T-helper (T_{FH}) cell compartment is over-represented in mice chronically infected with the clone 13 strain of lymphocytic choriomeningitis virus (LCMV; Fahey *et al*, 2011). T_{FH} cells normally 'help' activated B cells in germinal centres, promoting their differentiation into memory B cells and antibody-secreting cells. Thus, skewing the abundance of T_{FH} could lead to sustained antiviral antibody production, as well as limiting excessive immunopathology. Furthermore, E. John Wherry and his group demonstrated that CD4 T cell gene expression is dysregulated during chronic LCMV infection. CD8 T cells from chronically infected mice are known to

develop an aberrant 'exhausted' phenotype; however, the Wherry group found that the CD4 T-cell phenotype is distinct from CD8 T-cell exhaustion, suggesting that distinct mechanisms are involved. In a separate study, they found that development of CD8 T-cell memory of a specific antigen is blunted in the context of unrelated, chronic viral or parasitic infections, in a mechanism that seems to involve bystander influences from inflammatory signals. These studies suggest that underlying chronic infection in the host probably profoundly influences concurrent immune responses, and that this is an essential parameter to consider regarding vaccination.

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This work indicates that deciphering the context defined by individual immunophenotypes is key, and this will depend on discovering and characterizing human viromes. Even among healthy people, the average person harbours almost a dozen chronic infections from a list of known viruses (Virgin *et al*, 2009). Next-generation sequencing platforms and advanced computational approaches now provide the sequencing depth and speed necessary to identify rare sequences in complex mixtures *de novo*. David Wang's group (Washington U. School of Medicine, USA) applied this technology to sequence nucleic acids in faecal samples from patients in an outbreak of acute gastroenteritis with unknown aetiology. They identified a new astrovirus as the potential pathogen underlying the outbreak; astrovirus family members are known to cause diarrhoea, but this member was previously unknown (Finkbeiner *et al*, 2009). A conceptually similar approach to characterizing the combinations of chronic infections comprising viromes will be an important step towards predicting immunophenotypes in human cohorts, especially when correlated with attendant, unrelated chronic disorders.

The genes that regulate host cell resistance and susceptibility to viral infection have not been completely characterized, but are probably good therapeutic targets for limiting infections and perhaps controlling viromes. Sara Cherry's group (U. Pennsylvania, USA)

has used both RNA interference (RNAi) and cDNA complementation to screen for genes that regulate viral infection of cells. Importantly, this approach facilitates the screening of cells from different organisms for which a given virus has tropism, and thus identifies distinct and overlapping pathways that are used by a virus in its reservoirs, vectors and terminal hosts. The Cherry lab has identified hundreds of genes that regulate infectivity with this strategy and, interestingly, has discerned that common sets of host genes are required for infection with different viruses of the same group; for example, when two different (+)-stranded RNA viruses are compared. Conversely, different sets of genes are essential for infection when two viruses of distinct groups are compared; for example when a (+) and a (–)-stranded RNA virus is compared. Finally, establishing new models of viral infection that can be probed in simplified *in vitro* systems is an important avenue for uncovering conserved mechanisms of viral pathogenesis. Wang's group has identified viruses that infect *Caenorhabditis* nematodes (Felix *et al*, 2011), opening the door to the genetic dissection of host–virus interactions in worms—a system that is highly tractable with RNAi.

Ambience matters to T cells

Concepts of effector-cell and memory-cell development are mainly generalized from models of systemic infection and immunization. Less is known about the way in which their development is regulated by different tissue contexts during localized infections. Francis Carbone's group (U. Melbourne, Australia) has shown that skin infection induces effector memory CD4 T cells to develop and localize to the skin, where they exhibit high mobility in the dermis and efficiently exchange between the blood and skin. Conversely, although the responding CD8 T cell counterparts also localize to the skin, they become stationary and remain stably anchored in the epidermis (Gebhardt *et al*, 2009). Notably, immunization through the skin provides protection from subsequent infection at distal cutaneous sites, whereas systemic inoculation does not induce localization to the skin or protection. The skin-homing phenotype of CD4 T cells correlates with expression of E- and P-Selectin ligands, whereas epidermal persistence of CD8 T cells is linked to expression of CD103, an integrin protein involved in binding adhesion molecules expressed by epithelial cells in the skin and gut. Thus,

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clarifying what regulates CD103 expression could help to elucidate what controls the development of protective, tissue-resident memory cytotoxic T cells.

In an analogous setting, Leo Lefrançois (U. Connecticut Health Center, USA) presented data to show that oral, as opposed to systemic, *L. monocytogenes* infection favours development of tissue-resident memory T cells in the gut, and that this correlates with CD103 expression. He showed that CD103 is not expressed on effector cells that express KLRG1—a key marker of 'terminal' effector cells—and that TGF- β RII is expressed on developing memory T cells, rather than KLRG1⁺ effector T cells. Therefore, signals that induce CD103 expression might also promote memory-cell formation, and TGF- β signals could have a role. Interestingly, earlier studies had noted that dendritic cells expressing CD103 could induce gut tropism on T cells through their capacity to produce retinoic acid (Iwata *et al*, 2004). At the meeting, Yasmine Belkaid linked retinoic acid and its receptor on T cells—RAR α —to fundamental aspects of early T-cell activation, and showed that it is necessary for priming pro-inflammatory T cells in response to mucosal vaccination and oral infection (Hall *et al*, 2011). Previously, RAR α was thought to be important mainly for induction of peripherally induced regulatory-type CD4 T cells, rather than conventional effector T cells. These studies emphasize that distinct tissue contexts use specific signals in unique ways to shape T-cell differentiation in order to balance immunity and tolerance, and further work should focus on these contexts.

Memory T-cell basics: introspection

Activation of naive T cells leads to their differentiation into effector and memory T cells. The molecules and mechanisms that promote memory T-cell differentiation, especially those that are intrinsic to the cell, are not as well understood as those that promote effector T cell development. In the CD8 T cell response, inflammatory cytokines—such as type I interferons IL-12 and IL-2—induced by infection promote the development of

effector T cells that control acute infection. Consequently, many of these cells lose their ability to form memory T cells, which normally persist long-term and protect against secondary infection. Susan Kaech (Yale U., USA) presented data to support the concept that signal transducer and activator of transcription 3 (STAT3), which is activated by the cytokines IL-10 and IL-21, promotes and sustains memory-cell potential in virus-specific CD8 T cells. STAT3 activation is needed for mature, protective memory CD8 T cells to develop after infection, and to sustain the expression of two transcription factors characteristic of memory cells: BCL6 and Eomesodermin. STAT3 is also needed to upregulate suppressor of cytokine signalling 3 (SOCS3), a protein that can temper the activation of other STAT factors, such as STAT1 and STAT4, which are normally activated by inflammatory cytokines. Thus, Kaech proposed a model in which the combination of IL-10/IL-21 signalling promotes memory CD8 T cell development by both activating STAT3-dependent genes and titrating inflammatory signals perceived by activated CD8 T cells during infection. Hai-Hui (Howard) Xue (U. Iowa, USA) demonstrated that TCF1, an effector transcription factor of the Wnt signalling pathway, is required for central memory T-cell development and maintenance and that part of this regulation occurs by directly promoting expression of eomesodermin, a transcription factor that is important for memory T-cell differentiation. Although the Wnt ligands responsible for this are unknown, this study provides a molecular link between memory CD8 T-cell development and Wnt/ β -catenin signalling. Collectively, these studies are among the first to identify signals and molecules that induce memory as opposed to effector T-cell development.

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The molecular mechanisms that crystallize the gene expression programmes that initiate and maintain the differentiation of effector and memory T cells are unknown. They are, however, probably determined at the level of chromatin structure and dependent on specific regimes of transcription

factors. Nucleosomes are the fundamental repeating subunit of chromatin and are also the scaffold that recruits transcriptional regulatory complexes to control gene activity. Matthew Pipkin (La Jolla Institute for Allergy and Immunology, USA) presented a new approach to map nucleosome positions and occupancy in mammalian cells at unprecedented resolution, by combining genomic selection and next-generation sequencing and then overlaying these maps with the *in vivo* binding patterns of the transcription factor RUNX3, which is required for cytotoxic T-lymphocyte (CTL) maturation. In differentiated CTLs, nucleosomes are depleted over key *cis*-regulatory regions, and this reciprocally corresponds with RUNX3 occupancy at those sites. Thus, Pipkin proposed a model in which RUNX3 factors compete directly with nucleosomes for DNA binding to regulate the 'accessibility' of key *cis*-regulatory regions that mediate transcription in CTLs.

Related to this topic, Nan-ping Weng's group (National Institutes of Health, USA) used gene expression profiling and analysis of histone-tail modifications in nucleosomes by chromatin immunoprecipitation, followed by next-generation sequencing, to provide some of the first evidence for why effector genes in memory CD8 T cells manifest transcription so much faster than in naive cells. Rapidly induced genes in memory CTLs were found to harbour histone modifications characteristic of active genes, or those that are 'poised' to be transcribed, whereas in naive CD8 T cells they have modifications indicating chromatin remodelling is necessary for them to become transcriptionally competent (Araki *et al*, 2009).

Finally, Nicholas Haining's group (Dana Farber Cancer Institute, USA) applied a systems approach to identify genes that underpin 'altered' T-cell differentiation states associated with chronic viral infection. A narrow band of genes that might comprise a signature specific to dysregulated T cells was identified by superimposing T-cell gene-expression profiles from patients with human immunodeficiency virus (HIV) that effectively control the virus, and those that contain the virus inefficiently, with the gene-expression profile of exhausted murine CD8 T cells from mice chronically infected with LCMV. Further overlaying these orthogonal data sets with gene-expression profiles resulting from experimental ligation of PD1—an inhibitory receptor expressed on exhausted

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T cells—revealed that the transcription factor BATF regulates the exhausted T-cell phenotype (Quigley *et al*, 2010). Collectively, these presentations described new approaches aimed at discovering the cell-intrinsic regulatory networks and mechanisms that determine T-cell differentiation in healthy and diseased states.

In summary, we hope that this snapshot conveys several important themes from the symposium. First, host–pathogen interactions are complex, regulate immune memory and underlie seemingly unrelated chronic diseases, and most of the genes that control these interactions are unknown. Second, clarifying what controls the immune response as it develops *in situ* is crucially important. Third, elucidating the basic mechanisms of gene expression programmes is essential for understanding cell differentiation in health and disease. Fourth, deciphering the biology of these processes and the way in which they can be controlled by vaccination or immunotherapy will rely on rapidly advancing technologies that enable systems-level approaches to acquire and synthesize large and orthogonal data sets.

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REFERENCES

- Araki Y *et al* (2009) *Immunity* **30**: 912–925
- Cadwell K *et al* (2010) *Cell* **141**: 1135–1145
- Fahey LM *et al* (2011) *J Exp Med* **208**: 987–999
- Felix MA *et al* (2011) *PLoS Biol* **9**: e1000586
- Finkbeiner SR *et al* (2009) *J Virol* **83**: 10836–10839
- Gebhardt T *et al* (2009) *Nat Immunol* **10**: 524–530
- Hall JA *et al* (2011) *Immunity* **34**: 435–447
- Iwata M *et al* (2004) *Immunity* **21**: 527–538
- Quigley M *et al* (2010) *Nat Med* **16**: 1147–1151
- Virgin HW, Wherry EJ, Ahmed R (2009) *Cell* **138**: 30–50

Matthew E. Pipkin is at the La Jolla Institute for Allergy and Immunology, Division of Signalling and Gene Expression, La Jolla, California, USA.

E-mail: mpipkin@liai.org

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