Innate immune memory: a paradigm shift in understanding host defense

Mihai G Netea, Eicke Latz, Kingston H G Mills & Luke A J O'Neill

Researchers gathered at the Wellcome Trust Genome Campus in Hinxton, Cambridge, for the first Innate Immune Memory Conference dedicated to the adaptive characteristics of innate immunity, to further the understanding of this newly described immunological process that probably has a central role in host defense and inflammation.

Since the discovery of the clonal selection theory and the description of antigen recognition and presentation, immunologists have traditionally divided immune responses into innate and adaptive categories. The cells of the innate immune system, such as granulocytes, monocytes, macrophages or innate lymphoid cells (ILCs), including natural killer (NK) cells, are activated within minutes of an infection, and they are able to fight a broad range of pathogens efficiently. However, the consensus until recently was that they mount nonspecific responses and they are not able to confer immunological memory on their own. In contrast, the adaptive T cell– or B cell–dependent immune responses are antigen specific[1] and they often provide lifelong protection against re-infection[2].

The dogma that innate immunity is nonspecific has been challenged by the discovery of pattern-recognition receptors, which are responsible for the semi-specific recognition of pathogen-associated molecular patterns from different classes of microorganisms. In turn, this activates the production of proinflammatory mediators, phagocytosis and killing of pathogens[3]. In addition, the activation of innate immune responses has an essential role in stimulation of the acquired immune system[4]. An increasing body of evidence suggests that innate immunity can also display adaptive characteristics, a *de facto* innate immune memory[5,6] (**Fig. 1**). The key concept discussed at the Wellcome Trust Meeting on Innate Immune Memory held in Hinxton, Cambridge, 18–20 March 2015 was that the innate immune system can have memory, which could lead to a paradigm shift away from the view that it is simply an immediate mediator of host resistance and inflammation. The memory characteristics would involve a priming event whereby upon first exposure, cells of the innate immune system would be altered such that upon re-exposure to identical or heterologous stimuli, they would display a heightened response and boost host defense. Evidence was presented, possible mechanisms were discussed, and consequences for the development of vaccines or anti-inflammatory therapeutics were explored.

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Concepts and mechanisms

A major focus of research on the innate immune system has been its role in inflammation, exemplified by activation of the NLRP3 inflammasome and the interleukin 1 (IL-1) cytokine family, as described by Douglas Golenbock (University of Massachusetts Medical School) in his keynote opening lecture. Golenbock presented his work on NLRP3 as a driver of pathology in Alzheimer's disease. Chronic inflammation, possibly involving innate reprogramming of microglia and astrocytes such that plaque clearance is impaired, leading to enhanced activation of NLRP3, might be critical for disease pathogenesis. In the opening session, Mihai Netea (Radboud University) set the scene by describing how innate immune memory is a feature of organisms that have no adaptive immune response, such as plants and invertebrates[7–9]. In plants, epigenetic alterations lead to the priming of genes encoding host defense molecules to respond upon re-exposure—the so called 'systemic acquired resistance'[7]. Such a system makes evolutionary sense, since the 'training' of cells to respond more robustly upon re-exposure would provide a selective advantage. Netea went on to describe how similar events can occur in mammalian cells, in which epigenetic reprogramming also underlines innate immune memory, also called 'trained immunity' (**Fig. 2**). The key mechanisms that mediate the effects reported by Netea include epigenetic reprogramming induced by fungal structures such as β-glucan, accompanied by changes in positive histone regulatory marks such as H3K4me1, H3K4me3 and H3K27ac. This process is in turn responsible for the increased responses of the trained cell for genes encoding host defense molecules upon restimulation with either the same stimuli or different stimuli. A particularly notable set of genes that are modified encode glycolytic enzymes, which indicates that a shift in the cellular metabolism of glucose is critical for training of immunity, a theme that was explored by other speakers. Inhibition of glycolysis has been shown to limit training; this suggests

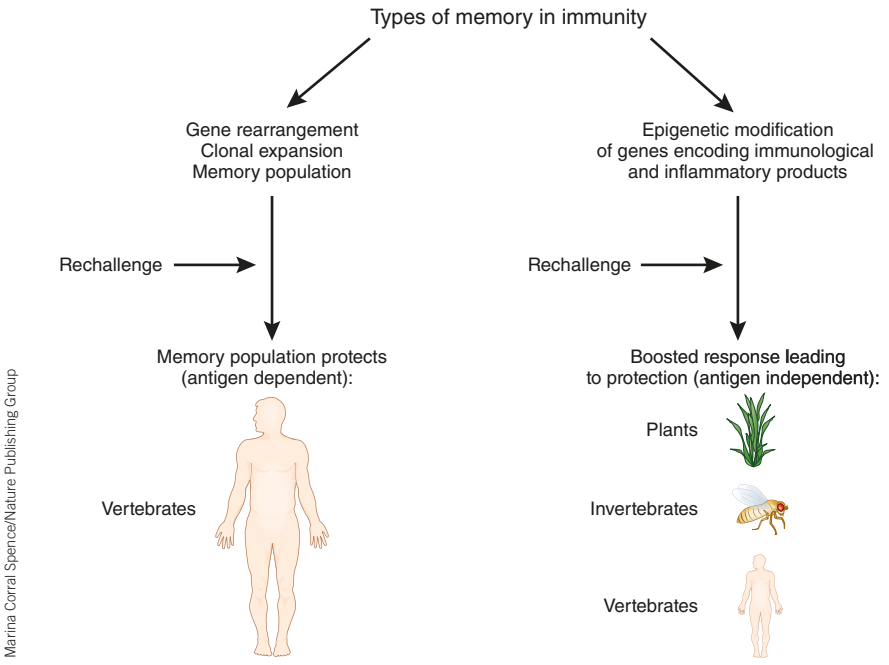

Figure 1 Conventional adaptive immune memory is present only in vertebrates, whereas innate immune memory is an ancient property of host defense present in plants, invertebrates and vertebrates.

that these metabolic alterations lie at the heart of the underlying process, which probably involves histone- and DNA-modifying enzymes that depend on co-factors produced by metabolic pathways.

Gioacchino Natoli (Istituto Europeo di Oncologia) continued the theme of epigenetic modification during innate immune responses, describing the crosstalk between interferon-γ (IFN-γ) and IL-4 in the stimulation of macrophages. This work explored one possible mechanism whereby parasitic infections interfere with anti-microbial responses. Natoli discussed transcriptional and chromatin-mediated mechanisms involved in this negative crosstalk, placing them in the context of the current knowledge on transcriptional and epigenetic control of macrophage function. Using a genomic approach, he showed that a large fraction of the transcriptional and epigenomic changes induced by stimulation with IFN-γ are reduced or suppressed by IL-4. These inhibitory effects of IL-4 *in vitro* are retained after the cytokine is washed out and cells are then treated with IFN-γ. Detailed mechanistic analysis was consistent with the proposal of indirect crosstalk mediated by transcription factors downstream of the main STAT transcription factors activated by IL-4 and IFN-γ. Macrophages can therefore carry an 'epigenomic memory' of prior exposure to IL-4 (as an indicator of parasitic infection), such that there will be a more limited response to IFN-γ during a bacterial infection. The effect of the epigenetic profile for determining the function of cells of the immune system was also emphasized in the presentation of John O'Shea (US National Institutes of Health), who showed that the identity and function of such cells depend on the enhancer landscape at the level of chromatin. In addition to the chromatin modifications that accompany trained immunity, another possible mechanism of innate immune memory might involve long-term regulation of long non-coding RNA (lncRNA). Indeed, as demonstrated by Kate Fitzgerald (University of Massachusetts Medical School), numerous lncRNAs are transcriptionally induced upon signaling via sensors of the innate immune system. Since lncRNAs can mediate both the activation of various classes of immunological genes and their repression, the pattern and timing of the induction of lncRNA could profoundly affect the type of immune responses to secondary stimulations. The power of genotype-phenotype investigation as a methodology with which to identify new properties of innate immune responses, and thus possible innate immune memory, was revealed by Ramnik Xavier (Harvard University). Screening the genes encoding products that regulate signaling via the nitric oxide synthase (NOS2) complex and reactive oxygen species (ROS) identified both positive and negative regulators of the process of ROS production. Notably, ubiquitination of the $p22^{phox}$ and $gp91^{phox}$ subunits of the phagocyte NADPH oxidase regulates signaling via the NADPH oxidase Nox2, and therefore this can be used as a therapeutic target in inflammatory disorders characterized by dysregulated release of ROS.

Understanding of the epigenetic reprogramming induced following infection or vaccination might provide evidence of the molecular mechanism of trained immunity. There is emerging evidence of a central role for cellular energy metabolism in immunoregulation (**Fig. 3**). The importance of changes in energy metabolism for the activation of innate immunity was emphasized by Luke O'Neill (Trinity College, Dublin). O'Neill detailed the role served by the enzyme PKM2 for induction of the Warburg effects in activated macrophages and of the metabolite succinate for the stimulation of IL-1β synthesis[10]. In addition, the direction of the electron-transport chain in the mitochondria is crucial for the balance between production of the proinflammatory cytokine IL-1β and that of the anti-inflammatory cytokine IL-10. A normally functioning electron-transport chain is needed for IL-10 production, reflective of homeostatic balance. However, under conditions of inflammatory activation, reverse electron flow promotes IL-1β synthesis. For long-term changes in the macrophage phenotype, the effect of Krebs cycle metabolites such as succinate and α-ketoglutarate for inhibition or induction of histone demethylases such as JMJD3 is an example of the close interaction of cellular metabolism and epigenetic reprogramming. Another important example of the immunological-metabolic interplay was provided by the work of Charles McCall (Wake Forest University) demonstrating the effect of histone deacetylases of the sirtuin family for the process of lipopolysaccharide-induced tolerance. NAD^+-dependent sirtuin proteins reprogram cellular energy metabolism, with sirtuin-1 blocking stimulation of inflammation dependent on the transcription factor NF-κB subunit RelA. Likewise, sirtuin-6 disrupts increases in glycolysis dependent on the transcription factor HIF-1α[11]. The importance of these processes during severe infections was demonstrated by the reduced survival in the acute phase of sepsis, when sirtuin-1 was inhibited, while a restoration of energy homeostasis and survival was observed when the inhibition was exerted during the late adaptation phase.

The cell populations involved in training immunity are beginning to be understood. Helen Goodridge (Cedars-Sinai Medical Center) discussed microbial programming of myelopoiesis. During infection, myeloid cells are mobilized from the bone marrow to supply neutrophils and inflammatory monocytes to handle the infection. Microbial products can promote the differentiation of hematopoietic stem cells and progenitor cells and program the function of the macrophages they produce[12]. Bacterial lipopeptides promote the production of macrophages that are phagocytic but

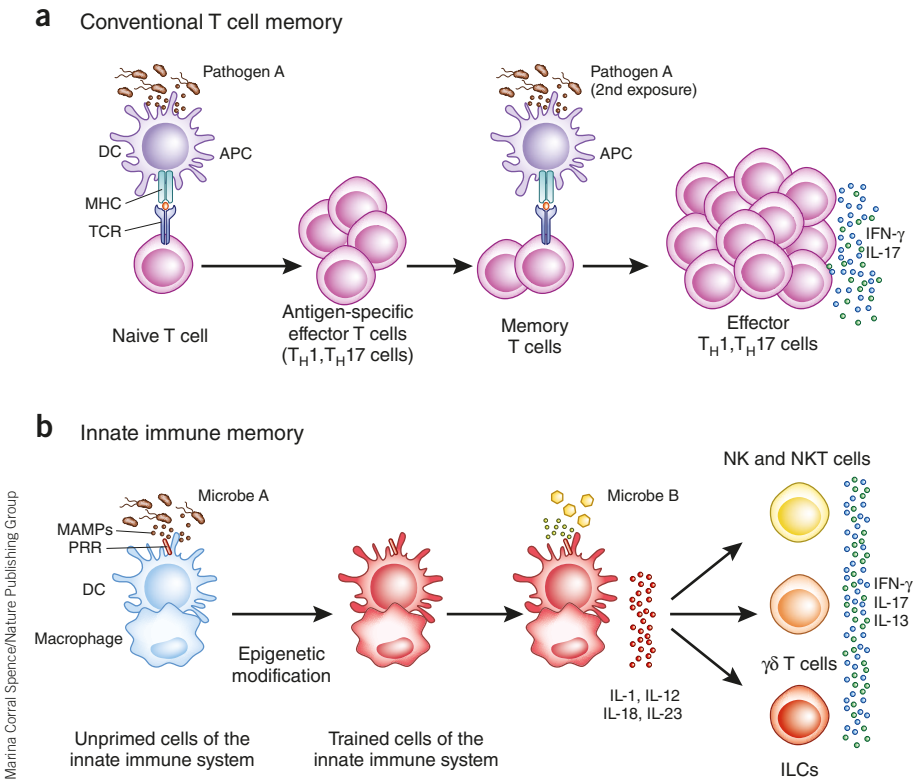

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Figure 2 Immune memory responses in vertebrates comprise both conventional T cell memory responses (**a**) and innate immune memory dependent on myeloid cells, NK cells and innate lymphocyte populations such as NKT cells, γδ T cells and ILCs (**b**). DC, dendritic cell; MHC, major histocompatibility complex; TCR, T cell antigen receptor; MAMP, microbe-associated molecular pattern; PRR, pattern-recognition receptor; APC, antigen-presenting cell.

less inflammatory, and soluble factors released by the progenitor cells have paracrine effects in this system. Again, epigenetic changes probably have a role in differentiation events. The supply of myeloid cells with specific functions based on the earlier effect of particular microbial products thus represents a form of innate immune memory. Kristin Bieber (University of Tübingen) explored this theme further, examining 'emergency monopoiesis' during infection with various Gram-positive and Gram-negative bacteria. This was associated with an increase in monocyte progenitors in the bone marrow and mature monocytes in the spleen. There was a diversion into the monocytic lineage and away from dendritic cells. The increase in monocytes probably occurred to enable pathogen control, but depletion of dendritic cells might also contribute to immunosuppression, as occurs in bacterial sepsis.

In addition to myeloid cells, NK cells also display memory characteristics. Published studies have shown that infection of mice with cytomegalovirus leads to NK cell proliferation that can mediate protection against reinfection with cytomegalovirus in a T cell–independent manner. These 'memory' NK cells can rapidly degranulate and produce cytokines upon reactivation and confer protective immunity in an adoptive-transfer model[13]. Similarly, after hapten sensitization, long-lived NK cells are responsible for contact-hypersensitivity responses, independently of B lymphocytes and T lymphocytes[14]. Hongwei Ren (Cambridge University) reported that vaccinia virus expressing defective virulence factor K7 can induce protection against lethal infection with this pathogen, and this protection can be transferred by NK cells. A cytokine 'cocktail' of IL-12, IL-15 and IL-18 is also able to induce long-term activation of NK cells for cytokine production and lysis of primary leukemia tumor cells, and in an *in vivo* mouse model these NK cells are able to inhibit K562 human myelogenous leukemia cells, as shown by Maximillian Rosario (Washington University School of Medicine). Two studies have independently reported that the innate immune memory properties of NK cells are mediated by epigenetic changes[15,16].

Given the burden of evidence of the role of epigenetic modulation as a source of long-term changes in the immunological phenotype, it can thus be proposed that the epigenetic reprogramming of cellular function is at the core of long-term training of immunity for both myeloid cells and NK cells. A key question, however, is how long-lived these cells are and whether the altered epigenetic landscape is retained after proliferation.

Colonization and infection

Functional reprogramming of innate immunity during an encounter with a colonizing or invading microorganism was a central feature of several presentations. Regulation of the innate immune system at the level of mucosal surfaces by colonizing microorganisms was the focus of the work presented by David Artis (Weill Cornell Medical College). He highlighted emerging knowledge of the roles of ILCs in regulating immunity to infection and promoting inflammation and tissue repair. As the field develops, a key challenge will be to define how ILCs integrate with other cells of the innate and adaptive immune systems to control primary and memory responses in different tissues[17]. In addition to being modulated by colonizing microorganisms, the function of ILCs at the level of the mucosa is also modulated by nutrients. In this context, Christoph Wilhelm (US National Institutes of Health) reported that vitamin A deficiency increases the ILC2 population while decreasing ILC3 cells and thus results in a greater resistance to helminths. Colonizing microorganisms can also induce long-term trained immunity, as described by Lisa Rizzetto (Fondazione Edmund Mach). The fungus *Saccharomyces cerevisiae* induces training of monocytes and macrophages, an effect that differs for various strains, with those isolated from human subjects being the most potent. Moreover, the authors have demonstrated that the induction of trained immunity and improved microbial killing by monocytes trained with *S. cerevisiae* is dependent on differences in cell-wall composition.

Not only colonization but also systemic infections can induce epigenetic changes in cells of the host immune system. Melanie Hamon (Pasteur Institute) presented data showing that infection with *Listeria monocytogenes* induces specific histone modifications in cells of the host immune system, such as deacetylation of H3K18 through a sirtuin-2-dependent pathway. In turn, *Sirt2*$^{-/-}$ mice have a lower bacterial load than that of wild-type control mice[18]. Furthermore, the *Listeria* toxin listeriolysin O dampens the DNA-damage response, as shown by an increase in phosphorylation of the histone variant γ-H2AX. These data provide a glimpse into the true 'epigenetic arms race' between the host and the pathogen in controlling the induction and modulation of the immune response[19]. Similar long-term effects on the immune system by infections have now been reported by

William Gause (Rutgers New Jersey Medical School), who showed that infection with the helminth parasite *Nippostrongylus brasiliensis* primes macrophages for direct damage of the larvae by the host and induces a long-lived macrophage phenotype that upon reinfection can damage the parasite and protect the host independently of memory T cells and B cells[20]. Intriguingly, helminth-induced alternatively activated (N2) neutrophils, which produce IL-13 and probably other factors, are required for the development of this anti-helminth macrophage population. Whether the long-lived nature of the macrophage phenotype requires the sustained immunological milieu of the inflamed lung remains untested.

Autoinflammatory diseases

The induction of a stronger innate immune response may not always have beneficial effects. As emphasized by Brigitta Stockinger (Medical Research Council National Institute for Medical Research), the pathogenesis of autoimmune diseases is driven by a combination of inflammatory mediators released by myeloid cells and over-reactive T cell populations, such as the T_H17 subset of helper T cells. New innate lymphocyte populations such as γδ T cells and $V_\gamma4^+$ cells that express markers of conventional T cells are also an important source of the T_H17 cytokines IL-17 and IL-21, as discussed by Kingston Mills (Trinity College, Dublin). $V_\gamma4^+$ T cells serve a critical pathogenic role in mouse models of autoimmune diseases through their early production of IL-17 but are also involved in an amplification loop for T_H17 cells. Most interestingly, these cells can respond to IL-1 and IL-23, without engagement of the T cell antigen receptor, but also display memory characteristics associated with cells of the adaptive immune system.

Eicke Latz (University of Bonn) spoke on inflammation induced by macrophage reprogramming in the context of atherosclerosis. Cholesterol crystals deposited in the atherosclerotic plaque activate the NLRP3 inflammasome in macrophages and drive IL-1β and IL-18, both of which contribute to the pathogenesis of atherosclerosis. For the prevention of this process, Latz described pharmacological approaches that lead to higher solubility of cholesterol, which results in increased oxysterol production. This in turn promotes increased clearance of cholesterol and atheroprotective functions. In this process, macrophages become reprogrammed to promote reverse cholesterol transport. Reprogramming macrophages in this way could provide a novel therapeutic approach to atherosclerosis. In a related study, Anette Christ (University of Massachusetts Medical School) assessed

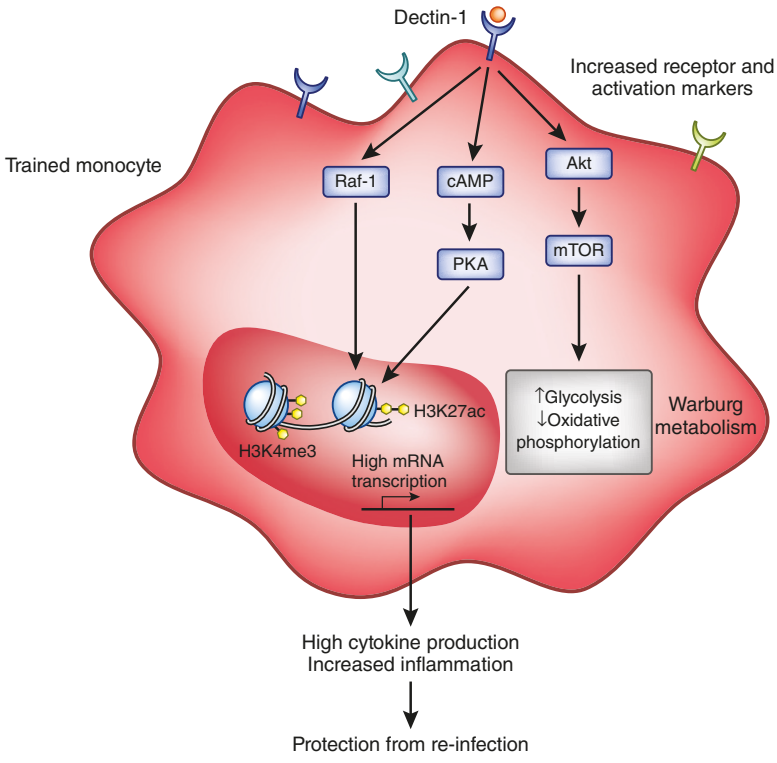

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Figure 3 Integration of immunological and metabolic pathways in trained monocytes, which lead to a shift toward aerobic glycolysis (Warburg effects), as well as epigenetic changes at the level of histone methylation and acetylation. This functional reprogramming leads to increased activity of the cell upon secondary stimulation and protection against reinfection from related or unrelated pathogens. Raf-1, Akt, PKA and mTOR are kinases.

trained immunity in cell populations of the innate immune system and hematopoietic progenitors in mouse models of hypercholesterolemia. This involved examination of Toll-like receptor responses in bone marrow and splenic cells from mice fed a high-fat, high-cholesterol diet or in mice 'reset' from the high-fat diet to a low-fat diet. In both cases, there was an increase in responsiveness, which would indicate that the initial high-fat, high-cholesterol diet had caused a persistent alteration in the cells that was not corrected by returning the mice to a regular diet. Hypercholesterolemia, therefore, seems to reprogram macrophages to be more inflammatory, even when mice lose weight. In contrast, Larisa Labzin (University of Bonn) showed that high-density lipoproteins can reprogram the inflammatory profile of macrophages by inducing expression of the trans-repressive transcription factor ATF3. Induction of ATF3 by the so-called 'good cholesterol' HDL *in vivo* or *in vitro* broadly downregulates the inflammatory response to various Toll-like receptor stimulants, which would explain the mechanism by which HDL exerts anti-inflammatory effects on macrophages[21].

Long-term reprogramming of macrophages has a role not only in atherosclerosis but also in inflammatory neurological diseases. Bart Eggen (University Medical Center Groningen) presented data demonstrating long-term inhibition of the proinflammatory profile of microglia by a single exposure to lipopolysaccharide. These effects were accompanied by induction of suppressive epigenetic marks such as methylation of H3K9 and decreased cytokine production[22]. Similarly, Jonas Neher (Hertie Institute and German Center for Neurodegenerative Diseases) showed that short-term peripheral immunological stimulation in 3-month-old mice by a 'training' stimulus or a 'tolerizing' stimulus exerted differential effects in a mouse model of Alzheimer's disease (APP23) 6 months later. The authors proposed that innate immune memory in the brain is a non-genetic modifier of neurological disease.

Vaccination and immunotherapy

The field of vaccination has provided probably some of the best evidence of innate immune memory in humans. Evidence of nonspecific protective effects of vaccines in humans is supported by an increasing number of epidemiological studies, as reported by Christine Stabell-Benn (University of South Denmark). She presented data showing that

immunization with live attenuated vaccines, such as bacillus Calmette-Guérin (BCG) and measles virus, reduce mortality from non-target diseases[23]. Emerging immunological studies suggest that the vaccines mediate these nonspecific effects via trained innate immunity[24]. Further evidence that innate immune responses induced by exposure to one pathogen or vaccine can affect the immune response to another was provided by Matthijs Kox (Radboud University). He reported that in healthy volunteers, previous vaccination with BCG enhanced functional antibody responses following immunization with a vaccine against pandemic H1N1 influenza virus. Furthermore, *ex vivo* cytokine responses induced by immunization with the vaccine against influenza virus were enhanced in people previously immunized with BCG. An additional mechanism of BCG-induced protective immunity against *Mycobacterium tuberculosis* was provided by Joanna Kirman (University of Otago), who demonstrated that induction of the T_H1 subset of helper T cells does not correlate with protective efficacy against tuberculosis but that ILC1 and ILC3 cells in the lungs instead control early mycobacterial infection. Collectively, these findings further support the concept of training effects on cells of the innate immune system and support the hypothesis that vaccination history affects immunological status in a clinically relevant manner.

Not all the effects of exposure to attenuated or killed bacteria are associated with enhancing innate immune responses. Camille Locht (Inserm–Institut Pasteur de Lille) reported that a live attenuated candidate vaccine against *Bordetella pertussis*, while effective at preventing infection with virulent *B. pertussis,* had surprising anti-inflammatory effects. Nasal administration of live attenuated *B. pertussis* BPZE1 protected mice against mortality induced by highly pathogenic influenza virus by dampening the virus-induced cytokine storm in an antigen-nonspecific manner, without affecting the viral load.

A presentation by Bali Pulendran (Emory University) provided evidence that early molecular signatures, identified by systems-biology approaches in humans soon after vaccination, can be used to predict the immunogenicity of vaccines against yellow fever and influenza virus[25] and are beginning to provide new insights into the influence of innate immunity on adaptive immune responses. Among these insights are the demonstration of a role for the gut microbiome in enhancing antibody responses to vaccination and the role of the integrated stress response in programming dendritic cells to induce $CD8^+$ T cells. Pulendran suggested that through these mechanisms, the microbiome can function as an 'endogenous adjuvant' that would greatly influence responsiveness to vaccination.

There is complementary evidence, from the cancer field, of nonspecific immunostimulatory effects of BCG. Intravesical immunotherapy with BCG is a first-line therapy for bladder cancer and is the only agent for the management of bladder cancer with carcinoma *in situ* lesions that has been approved by the US Food and Drug Administration. Trained immunity has been reported to mediate these effects, at least in part[26]. Data presented by Angus Dalgleish (St. George's, University of London) suggested that this might be a general feature of mycobacteria and was not necessarily dependent on administration of replicating bacteria, as anti-tumor effects were observed with heat-killed *Mycobacterium vaccae* or *Mycobacterium obuense*. The results of a randomized clinical trial of advanced pancreatic cancer showed a highly significant survival advantage for patients treated with heat-killed whole-cell preparation of *M. obuense* in combination with gemcitabine relative to that of patients treated with gemcitabine alone. Although the mechanism was not clear, there is evidence that the killed mycobacteria can nonspecifically stimulate NK cells and γδ T cells, as well as T_H1 cells.

Future perspectives

After the 3 days of the conference, a picture emerged that innate immune memory is a fundamental characteristic of host defense with an important effect on the understanding of human diseases. Although the classical adaptive immune memory is specific, antigen dependent and mediated by gene rearrangement, innate immune memory is nonspecific, antigen independent and mediated through epigenetic reprogramming. Emerging data suggest that innate immune memory has a shorter duration than that of T cell– and B cell–dependent adaptive immunity.

Much remains to be learned in this exciting new field of immunology in the coming years. One direction in which efforts should focus is the molecular mechanisms that mediate trained immunity, with more in-depth understanding of the epigenetic substrate, the integration between cellular metabolism and the epigenetic changes, and the cell populations able to mount characteristics of trained immunity, including the precursors of cells of the innate immune system in the bone marrow and tissue macrophage populations. Other intriguing questions include mechanisms whereby innate immune memory might persist and for how long, and whether innate immune memory might be transmitted epigenetically in the germline, which is the case in plants[27] and may even be the case for metabolic traits in mammals[28]. Another crucial direction for research is to explore the breadth of human diseases, both infectious and inflammatory, in which innate immune memory is probably very important, as well as the role of some microorganisms with which humans are chronically infected (for example, herpes virus or cytomegalovirus). Finally, an increasing number of studies on the therapeutic applications of the concept of innate immune memory are expected to emerge, not only toward the design of an improved generation of vaccines to combine adaptive and innate immune memory but also toward modulation of the potentially deleterious consequences of inflammatory diseases. Much will be learned in the coming years in this new field of immunological research, and a follow-up Wellcome Conference on Innate Immune Memory is planned for the spring of 2017.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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