6. Mucida, D. *et al. Nat. Immunol.* **14**, 281–289 (2013).
7. Reis, B.S. *et al. Nat. Immunol.* **14**, 271–280 (2013).
8. Honda, K. & Littman, D.R. *Annu. Rev. Immunol.* **30**, 759–795 (2012).
9. Egawa, T. & Littman, D.R. *Nat. Immunol.* **9**, 1131–1139 (2008).
10. Muroi, S. *et al. Nat. Immunol.* **9**, 1113–1121 (2008).
11. Steinke, F.C. *et al. Nat. Immunol.* **15**, 646–656 (2014).
12. Luckey, M.A. *et al. Nat. Immunol.* **15**, 638–645 (2014).

Neutrophils worm their way into macrophage long-term memory

John R Grainger & Richard K Grencis

Neutrophil function is perhaps best studied in bacterial infection, during which they are directly involved in pathogen killing. After helminth invasion, however, neutrophils acquire an alternative transcriptional profile that allows them to 'train' macrophages to acquire long-term protective features.

Helminth infections affect almost 2 billion people worldwide, particularly in the developing world, and are a major cause of morbidity. Their high prevalence results in part from poor development of protective immunity despite ongoing exposure to these infections. Better understanding of the mechanisms that contribute to long-term protection is crucial for establishing more targeted approaches to deal with disease and support vaccine development. Although rodent models of hookworm infection have been used to study secondary protection since the early 1900s[1], understanding of the cell types that contribute to this protection and the tissue-specific mechanisms involved are still poorly understood. The protective ability of granulocyte populations, including basophils, eosinophils and mast cells, has been well studied in infection with various helminths[2]. Whether neutrophils, another granulocyte population that is rapidly recruited to almost all sites of infection and injury[3], also have a role in protection has been poorly explored. In this issue of *Nature Immunology*, Chen *et al.* describe a previously unknown and unexpected role for neutrophils in 'training' macrophages to acquire a long-term protective function against helminth larvae as they transit through the lungs[4].

Neutrophil function during infection has been investigated most extensively in response to bacteria; during such infection, they have been long thought of as terminally differentiated cells with a direct protective role[3]. Protection is mediated by their phagocytic ability, along with their production of a plethora of bactericidal factors, including reactive oxygen species, elastase and neutrophil extracellular traps (NETs)[3]. The recruitment of neutrophils is not limited to bacterial infection, however, as they swiftly transit to the site of pathogen entry in response to various types of invader, including helminths[2,5,6]. Although they may serve a direct role in killing larvae, one possibility is that here too their dominant function is protection against bacteria, as helminths can carry bacteria into tissues as a result of barrier breach during invasion[2,6].

A less-well-explored aspect of neutrophil function is their ability to influence the function of cell populations with which they are closely localized during infection, such as monocytes-macrophages. Perhaps the best described example of neutrophils' modulating macrophage function is the uptake of apoptotic neutrophils (efferocytosis), which leads to monocytes-macrophages' taking on a proresolution activity[7]. During infection with *Staphylococcus aureus*, however, neutrophils can secrete factors that are able to differentiate macrophages toward an alternatively activated (M2) state[8]; this suggests that there are multiple mechanisms by which neutrophils can influence their innate cell neighbors during infection.

Chen *et al.* demonstrate that during primary infection with *Nippostrongylus brasiliensis* (a rodent helminth with a lifecycle similar to that of the human hookworm), neutrophils can 'instruct' lung macrophages to take on altered functions that confers on them the ability to rapidly kill larval stages of the parasite during a secondary challenge[4] (**Fig. 1**). This macrophage 'memory' is still evident at 45 days after infection and around 30 days after clearance of the helminth. Enhanced killing is dependent on signaling via the α-chain of the receptor for interleukin 4 (IL-4) and the production of arginase-1, which have been described as protective during infection with various helminths[2]. In addition, expression of integrin α_M (CD11b) is increased on lung macrophages following primary infection with *N. brasiliensis* and, using an *in vitro* larval killing assay, the authors show that this integrin is important in augmenting macrophage-mediated killing[4]. Unexpectedly, upregulation of the expression of arginase-1 and CD11b on lung macrophages, and hence secondary protection against the helminth, is critically dependent on the presence of neutrophils during primary infection. In agreement with those findings, sorted lung macrophages from mice infected with *N. brasiliensis* but depleted of neutrophils are unable to mediate protection against helminth challenge following intravenous transfer into naive hosts, unlike macrophages from control mice infected similarly but treated with isotype-matched control antibody.

The ability of neutrophils to 'train' lung macrophages is associated with their acquisition of a distinct global transcriptional profile early after infection, compared with that of lipopolysaccharide-elicited neutrophils at the same site. In particular, they have augmented expression of genes associated with type 2 immunity, including *Retnla*, *Chi3l3* and *Il13*. Furthermore, coculture experiments reveal that production of the cytokine IL-13 by neutrophils obtained from mice after primary infection with *N. brasiliensis* is critical for favoring the enhancement of factors associated with the alternative activation of lung macrophages and upregulation of CD11b expression. Thus, in a manner analogous to M2 macrophages, neutrophils are described in this setting as taking on an alternatively activated ('N2') phenotype. One reason for the importance of these N2 neutrophils in long-term 'training' may be their large numbers and close proximity to monocytes-macrophages during larva-driven inflammation.

Acquisition of N2 features by neutrophils has been described before during infection with *S. aureus*[8]. Here, as in infection with *N. brasiliensis*, neutrophils are able to induce M2 polarization of macrophages, although

John R. Grainger is with the Manchester Collaborative Centre for Inflammation Research and Faculty of Life Sciences of The University of Manchester, Manchester, UK. Richard K. Grencis is with the Faculty of Life Sciences of The University of Manchester, Manchester, UK.
e-mail: john.grainger-2@manchester.ac.uk or richard.k.grencis@manchester.ac.uk

this was attributed to IL-10 and the chemokine CCL2 (the monocyte chemotactic protein MCP-1). How neutrophils take on an N2 phenotype rather than classically activated (N1) phenotype was not investigated during infection with *S. aureus*[8] and is not fully described during infection with *N. brasiliensis* in the current study[4]. It is likely that type 2 cytokines produced early by innate lymphoid cells (such as ILC2 cells) or factors derived from antigen-presenting cells mediate this switch. Alternatively, products secreted directly from helminths can result in altered function of innate cells[9]. Further exploration will be needed to establish how neutrophils enter into this state and whether N2 neutrophils are a common feature of various infections.

Perhaps one of the most surprising observations by Chen *et al.* is that acquired immunity to the larval stage of the parasite trafficking through the lungs does not require the adaptive immune system[4]. It is intriguing that appropriate 'training' of the innate immune system alone is entirely responsible for the protection observed in this model. Such 'training' of the innate immune system can provide protection against *Candida albicans* in the absence of an adaptive immune response[10], and lung macrophages can be maintained in an immunologically altered state following viral infection[11]. The longevity of specific populations of macrophages residing in the lungs may provide an explanation for the lack of necessity for adaptive immune responses to provide secondary protection at this site. Although it is unclear from this study[4] which population of macrophages is targeted by neutrophils, the lung alveolar macrophage population represents a likely candidate. Alveolar macrophages are an extremely long-lived population that can expand locally after infection[12]. It is well established that epigenetic regulation is critical for the M2 polarization of macrophages[13]. Therefore, long-term epigenetic reprogramming of these cells may underlie their modified protective state.

Whereas the data in the present study underscore the importance of 'training' lung macrophages in acquired immunity, other tissue sites are also critical in protecting against helminths. During infection with *N. brasiliensis*, the parasite first invades through the skin, then transits through the bloodstream to the lungs and ultimately travels to the gut, where the adult worm produces eggs for transmission to new hosts (**Fig. 1**). Following a primary infection, the majority of the protection against the helminth is mediated in the gut, in which secretions initiated by cells of the immune system, especially goblet cells, and peristalsis (so-called 'weep and sweep' responses)

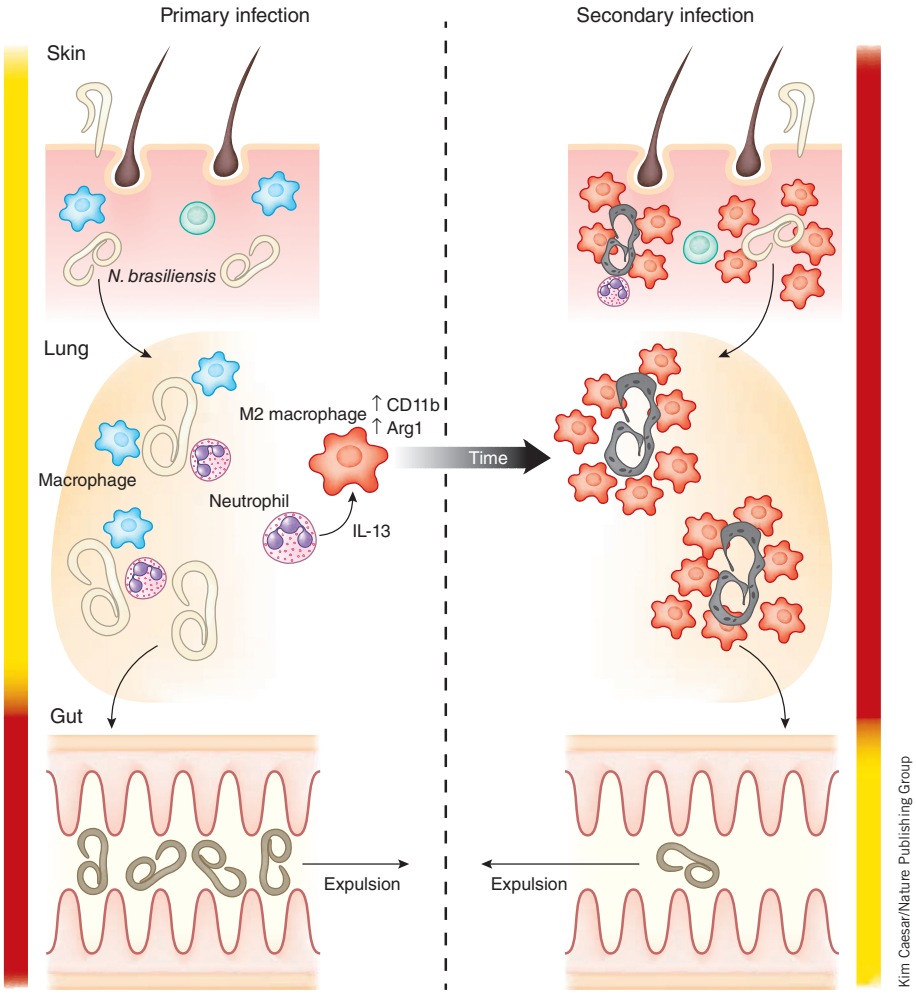

Figure 1 Neutrophils 'train' lung macrophages to take on features that protect against helminth larvae during secondary infection. In a primary infection (left), following entry through the skin, *N. brasiliensis* larvae are poorly impeded by the immune system and transit to the lungs, where neutrophils are rapidly recruited. At this site, neutrophils take on an N2 phenotype that is associated with production of IL-13, as well as other factors, that 'train' macrophage function to support the killing of parasites (blue, before training; red, after acquisition of the M2 phenotype). However, larvae are not killed at this stage, and they transit to the gut, where during primary infection worms are completely expelled, in large part by enhancement of goblet-cell secretion and gut peristalsis. The macrophages in the lungs remain in a 'trained' state for at least 45 days, until there is a subsequent invasion of *N. brasiliensis*, at which time they kill the parasite in a manner dependent on CD11b and arginase-1 (Arg1). Although in this setting much protection is mediated in the skin (in an antigen-specific manner), these reprogrammed lung M2 macrophages provide a second level of protection that is independent of the adaptive immune system. In contrast to primary infection, during secondary challenge (right) the gut is not a key mediator of protection, as small numbers of worms survive to reach this site. Vertical bars (right and left margins) indicate minor (yellow) or major (red) sites of protection.

are involved in mediating complete expulsion of the parasite[2]. In a secondary infection, on the other hand, the skin has been described as a major (if not complete) bulwark of immunity[5]. At this tissue site, the adaptive immune system is critical in providing antigen-specific protection, as basophils armed with immunoglobulin E are required in favoring the M2 polarization of macrophages. Regardless of the setting, it appears critical that macrophages take on the M2 phenotype and, in particular, produce arginase[5]. A hypothesis for the differences between the skin and lungs in their requirement for the adaptive immune system is that the macrophages in the skin turn over much more rapidly than those in the lungs and thus long-term 'training' of skin macrophages is not possible. Even in secondary infection, any parasites that do survive transit through both the skin and lungs are expelled by the gut (**Fig. 1**).

Overall, this study[4] highlights the importance of neutrophils beyond their well-described role as a pathogen-killing population during bacterial infection. Future research based on this study by Chen *et al.*[4] will no doubt aim to elucidate whether the N2 differentiation and

function of neutrophils can be manipulated to support long-term protection against helminth infection. What remains to be explored is whether the 'training' of macrophages by neutrophils is a common feature of lung inflammation and if targeting of this interaction could provide a novel therapeutic avenue for some of the vast array of respiratory diseases afflicting the world today, such as chronic obstructive pulmonary disease or asthma.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Africa, C.M. *J. Parasitol.* **18**, 1–13 (1931).
2. Allen, J.E. & Maizels, R.M. *Nat. Rev. Immunol.* **11**, 375–388 (2011).
3. Amulic, B. *et al. Annu. Rev. Immunol.* **30**, 459–489 (2012).
4. Chen F. *et al. Nat. Immunol.* **15**, 938–946 (2014).
5. Obata-Ninomiya, K. *et al. J. Exp. Med.* **210**, 2583–2595 (2013).
6. Pesce, J.T. *et al. J. Immunol.* **180**, 464–474 (2008).
7. Serhan, C.N. & Savill, J. *Nat. Immunol.* **6**, 1191–1197 (2005).
8. Tsuda, Y. *et al. Immunity* **21**, 215–226 (2004).
9. Hewitson, J.P., Grainger, J.R. & Maizels, R.M. *Mol. Biochem. Parasitol.* **167**, 1–11 (2009).
10. Quintin, J. *et al. Cell Host Microbe* **12**, 223–232 (2012).
11. Didierlaurent, A. *et al. J. Exp. Med.* **205**, 323–329 (2008).
12. Hashimoto, D. *et al. Immunity* **38**, 792–804 (2013).
13. Satoh, T. *et al. Nat. Immunol.* **11**, 936–944 (2010).

Bcl-6 gets T cells off the sugar

Kevin Man & Axel Kallies

The transcriptional regulator Bcl-6 represses aerobic glycolysis in CD8$^+$ and CD4$^+$ type 1 helper T cells.

During the course of an adaptive immune response, T cell metabolism is dynamically regulated and is subject to differentiation stage–specific regulation. In particular, antigen-triggered activation of T cells is characterized by the transition from the catabolic state of naive or quiescent cells to the anabolic state of rapidly proliferating effector cells[1]. During this process, the cells 'rewire' their metabolic profile and switch from oxidative phosphorylation and fatty acid oxidation to aerobic glycolysis. The transcription factors that mediate this metabolic transition in T cells remain poorly defined. In this issue of *Nature Immunology*, Weinmann and colleagues reveal that the BTB–zinc-finger transcription factor Bcl-6 is a critical regulator of the metabolic changes that parallel the transition from effector T cell to memory T cell[2]. Specifically, Bcl-6 acts as a repressor of genes encoding molecules involved in aerobic glycolysis that are upregulated by high environmental concentrations of interleukin 2 (IL-2) during the effector phase of the immune response. Thus, this study offers important insight into how the formation of long-term memory cells is linked to the transcriptional regulation of distinct metabolic pathways.

When naive T cells encounter foreign antigen in conjunction with other immunomodulatory signals, such as costimulation and IL-2, they undergo rapid clonal expansion and differentiate into effector or memory precursor T cells. During this process, they reprogram their mitochondrial metabolism to aerobic glycolysis (the 'Warburg effect')[3]. In aerobic glycolysis, glucose-derived pyruvate in the cytosol is converted to lactate, and small amounts of pyruvate enter the mitochondria to be oxidized to carbon dioxide in the tricarboxylic acid cycle. In contrast to activated T cells, resting T cells use oxidative phosphorylation and fatty acid oxidation to fulfill their metabolic requirements and generate chemical energy in the form of ATP from the breakdown of pyruvate in the tricarboxylic acid cycle. Although aerobic glycolysis, used by activated cells, is a relatively inefficient process, it produces intermediary metabolites that fuel anabolic biosynthetic pathways that support the rapid proliferation and function of effector T cells. This includes the synthesis of nucleotides, amino acids and lipids needed to generate cellular progeny[3]. Upon clearance of the antigen, most effector T cells undergo apoptosis, whereas a proportion of memory precursor cells give rise to long-lived memory T cells. Although the metabolic profile of memory T cells is similar to that of naive T cells, they are unique in that they have larger mitochondria and actively transcribe genes encoding molecules involved in mitochondrial biogenesis and the uptake of fatty acids, which is stimulated by the memory-promoting cytokine IL-15 (ref. 4). Therefore, during memory formation, activated T cells revert back to catabolic metabolic pathways, reengaging in oxidative phosphorylation and fatty acid oxidation for energy production[5] (**Fig. 1**).

Gene-regulatory networks that guide the transition from mitochondrial oxidative phosphorylation to aerobic glycolysis are only beginning to be elucidated, and even less is known about the transcription factors that regulate the reversion from aerobic glycolysis to oxidative phosphorylation and fatty acid oxidation during memory development. T cell activation induces early transcriptional regulators, including c-Myc[6] and ERRα[7], that are important for inducing the expression of genes encoding molecules involved in glycolysis and other intermediary metabolic pathways. Another transcription factor dependent on the T cell antigen receptor, IRF4, is required for sustained glycolysis in effector CD8$^+$ T cells. Genetic ablation of IRF4 results in profound impairment in clonal expansion and loss of antigen-specific effector T cells during viral or bacterial infection[8]. Furthermore, IRF4 is required for the efficient expression of HIF-1α, an oxygen-sensitive key transcriptional regulator of metabolic processes that mediates the transition from oxidative phosphorylation to aerobic glycolysis and allows metabolic adaptation to hypoxic microenvironments. HIF-1α can also be induced by signaling via Toll-like receptors and activity of the metabolic checkpoint kinase complex mTORC1 even under conditions of normal oxygen tension[9]. Maintenance of HIF-1α expression in CD8$^+$ T cells requires sustained IL-2 signaling, and it is partly through this mechanism that IL-2 promotes effector differentiation and glycolysis[10].

In their study, Weinmann and colleagues show that HIF-1α and Bcl-6 reciprocally regulate genes encoding molecules that control glucose metabolism in an IL-2-sensitive manner[2]. Using a variety of *in vitro* approaches in CD4$^+$ type 1 helper T cells (T_H1 cells) and CD8$^+$ T cells cultured in low and high concentrations of IL-2, the authors confirm published results[11], showing that genes encoding molecules involved in glucose metabolism are upregulated by high concentrations of IL-2. These results correlate with increased occupancy of c-Myc and HIF-1α at the promoters of genes encoding molecules involved in glycolysis. Conversely, low concentrations of IL-2 favor Bcl-6 expression and DNA-binding activity, which allows Bcl-6-dependent repression of the same genes. The authors further demonstrate that the T-box transcription factor T-bet, which is critical for the differentiation of T_H1 cells and CD8$^+$ effector T cells, is also required for the IL-2-dependent increase in the expression of genes encoding glycolysis-related molecules[2]. T-bet antagonizes Bcl-6-dependent repression by interacting with Bcl-6 and masking its

Kevin Man and Axel Kallies are with the Molecular Immunology Division at the Walter and Eliza Hall Institute of Medical Research, Parkville, Australia.
e-mail: kallies@wehi.edu.au.