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Trained immunity — basic concepts and contributions to immunopathology

Jordi Ochando [1,2✉], Willem J. M. Mulder [3,4], Joren C. Madsen [5,6], Mihai G. Netea [7,8] and Raphaël Duivenvoorden[4,9✉]

Abstract | Trained immunity is a functional state of the innate immune response and is characterized by long-term epigenetic reprogramming of innate immune cells. This concept originated in the field of infectious diseases — training of innate immune cells, such as monocytes, macrophages and/or natural killer cells, by infection or vaccination enhances immune responses against microbial pathogens after restimulation. Although initially reported in circulating monocytes and tissue macrophages (termed peripheral trained immunity), subsequent findings indicate that immune progenitor cells in the bone marrow can also be trained (that is, central trained immunity), which explains the long-term innate immunity-mediated protective effects of vaccination against heterologous infections. Although trained immunity is beneficial against infections, its inappropriate induction by endogenous stimuli can also lead to aberrant inflammation. For example, in systemic lupus erythematosus and systemic sclerosis, trained immunity might contribute to inflammatory activity, which promotes disease progression. In organ transplantation, trained immunity has been associated with acute rejection and suppression of trained immunity prolonged allograft survival. This novel concept provides a better understanding of the involvement of the innate immune response in different pathological conditions, and provides a new framework for the development of therapies and treatment strategies that target epigenetic and metabolic pathways of the innate immune system.

Innate immune defence against infection is largely based on the detection of molecular patterns that are present in infectious agents, such as bacteria, fungi or viruses, and are recognized as danger signals. Recognition of these pathogen signals leads to the activation of different types of immune cells, including T and B lymphocytes, natural killer (NK) cells, monocytes, macrophages, neutrophils and dendritic cells (DCs)[1]. This multicellular response to infection is coordinated into two distinct phases: the innate and the adaptive immune response. The innate immune response represents the first phase of the immune response and is mediated by physical, chemical and cellular defences in distinct types of myeloid cells (such as monocytes, macrophages and DCs) or lymphoid cells (NK cells and innate lymphoid cells). The second phase of the immune response is mediated by the adaptive response, which is mediated by T and B lymphocytes[2].

The innate immune response is evolutionarily conserved and has traditionally been associated with a rapid and non-specific inflammatory response. Innate immune cells recognize pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) expressed on their surface and cytoplasm[3]. This ancient mechanism of immunological defence can also

be triggered by self-derived damage-associated molecular patterns (DAMPs) released in the context of sterile inflammation[4]. Activation of PRRs in innate immune cells induced by PAMPs and DAMPs rapidly induces the secretion of pro-inflammatory cytokines, such as IL-6, IL-1β and tumour necrosis factor (TNF)[5]. These pro-inflammatory cytokines subsequently stimulate the generation and presentation of antigenic epitopes that activate the adaptive immune response[6]. Notably, the adaptive immune response is only present in jawed vertebrates and, although slower than the innate response, generates an antigen-specific response after an initial encounter with a pathogen. This high antigenic specificity is enabled by the recombination of variable (V), diversity (D) and joining (J) genes segments during the T and B cell receptor rearrangement phase of T and B cell development, which results in the generation of a T cell and B cell receptor repertoire with an immense breadth of specificities. Following an encounter with a pathogen, the lymphocytes that can recognize antigens present on that pathogen expand and some of those cells differentiate into long-lived memory T and B cells that can be readily reactivated upon subsequent encounters with the same pathogen[7].

✉e-mail:
Jordi.Ochando@mssm.edu;
Raphael.Duivenvoorden@
radboudumc.nl

Key points

- Trained immunity is a functional state of the innate immune system that is characterized by long-term epigenetic and metabolic reprogramming of cells associated with potent immune responses.

- Experimental and clinical studies have demonstrated that exogenous pathogen-associated molecular patterns and endogenous danger-associated molecular patterns induce trained immunity.

- Trained immunity is a functional adaptation of the innate immune system against secondary infections, but can lead to aberrant inflammatory activity in conditions such as autoimmunity.

- Sterile inflammation owing to ischaemia–reperfusion injury and organ transplantation induces trained immunity and precipitates allograft rejection.

- Therapeutic inhibition of trained immunity (for example, in autoimmunity or transplantation) or its induction (for example, in infections or cancer) are promising strategies for treating immunity-related diseases.

The absence of immunological memory historically distinguished innate from adaptive immunity but this concept is now challenged. Although memory T and B lymphocytes undoubtedly provide long-term protection against reinfection, higher vertebrates represent only 5% of all animal species on Earth, including mammals, reptiles, birds, fish and amphibians[8]. The absence of T and B lymphocytes in the remaining 95% of organisms does not necessarily mean the absence of immune memory. Non-vertebrates have evolved to respond to pathogenic infectious agents and in 2003 one study showed that copepods, which are minute crustaceans that rely solely on an innate immune system, had immunological memory and could prevent reinfection with a parasitic tapeworm[9]. This innate immune memory phenomenon was subsequently observed in other invertebrate and vertebrate species, including humans, indicating that it is ubiquitous and evolutionarily conserved[10].

The term 'trained immunity' was first introduced in 2011 and describes the immunological memory response that innate immune cells can mount in response to past insults[11]. Although cells return to an inactivated state after an initial stimulus, epigenetic changes enable a faster and stronger response upon antigen re-encounter[12]. PAMPs and DAMPs are classic

Author addresses

[1]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[2]Transplant Immunology Unit, National Center of Microbiology, Instituto de Salud Carlos III, Madrid, Spain.

[3]Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands.

[4]Biomedical Engineering and Imaging Institute, Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
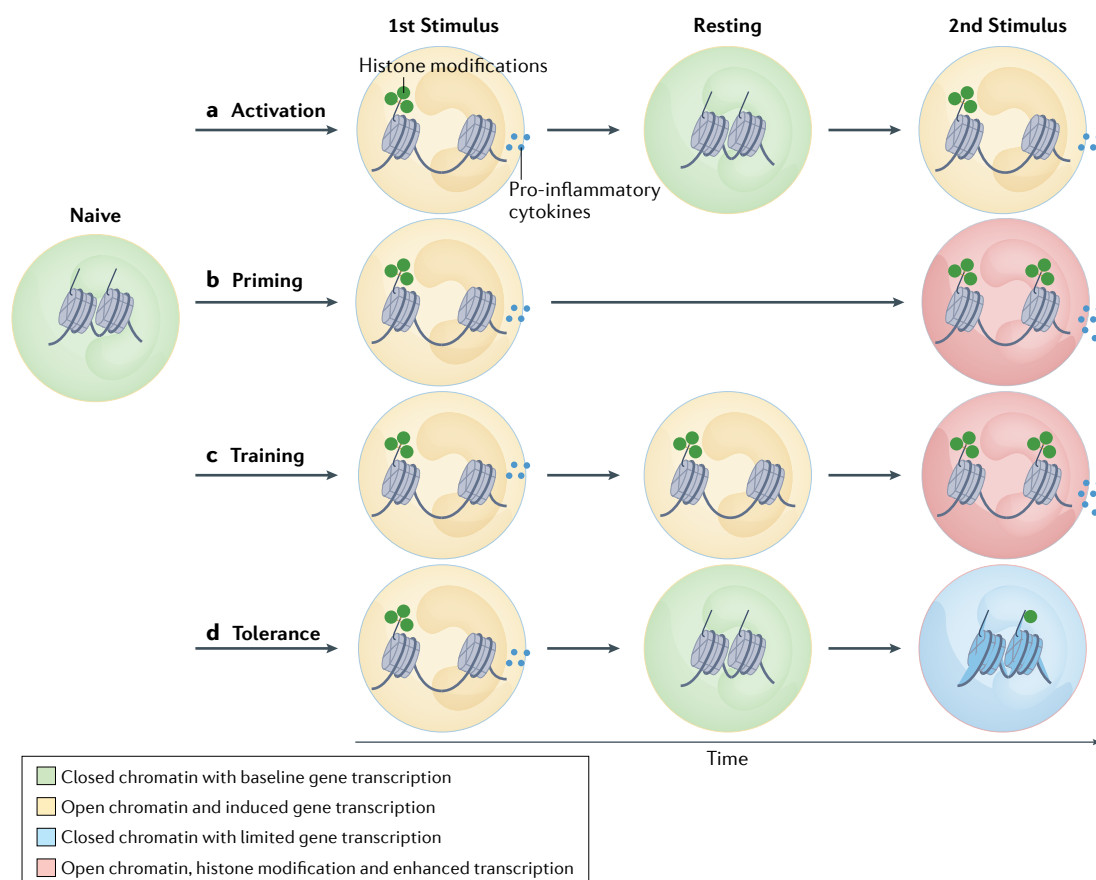
[5]Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, Boston, MA, USA.

[6]Division of Cardiac Surgery, Department of Surgery, Massachusetts General Hospital, Boston, MA, USA.

[7]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands.

[8]Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany.

[9]Department of Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands.

inducers of such training — they can generate trained immunity in the circulation and tissues (that is, peripheral trained immunity), and in HPSCs in the bone marrow (that is, central trained immunity)[13]. Importantly, although trained immunity has evolved under continuous evolutionary pressure to provide enhanced protection against pathogens, maladaptive innate immune responses might contribute to chronic inflammatory diseases and autoimmunity[14].

In this Review, we examine the concept of trained immunity and describe technical approaches that are currently available to study its underlying molecular mechanisms. Furthermore, we explore the implications of trained immunity in the field of nephrology and discuss potential therapeutic approaches for the manipulation of trained immunity in the context of kidney diseases.

Basic concepts of trained immunity

Trained immunity describes the immunological process by which innate immune cells acquire immunological memory. After exposure to certain stimuli, innate immune cells can adjust their response to subsequent insults, resulting in an enhanced response to previously encountered infectious agents[11]. This challenging concept was fully demonstrated in 2012, when non-specific protective effects of the Bacillus Calmette–Guérin (BCG) vaccine were reported[15]. In severe combined immunodeficient mice, which lack T and B lymphocytes and therefore cannot mount an adaptive immune response, BCG vaccination against *Mycobacterium tuberculosis* conferred cross-protection and reduced mortality caused by a lethal *Candida albicans* infection. In addition to pathogenic stimuli, self-derived molecules, such as DAMPs (for example, vimentin) and cytokines (for example, granulocyte–macrophage colony-stimulating factor), can also induce trained immunity[16]. Mechanistically, PAMPs and DAMPs induce trained immunity by eliciting long-term epigenetic reprogramming at the promoters of inflammatory genes and causing metabolic rewiring in memory macrophages[15].

Importantly, monocyte activation, priming and training refer to different processes[12] (FIG. 1). Resting monocyte and macrophages can be activated through Toll-like receptor (TLR) signalling, which induces metabolic rewiring and epigenetic changes that are associated with the production of inflammatory cytokines; these changes are transient and dynamic over the course of an inflammatory event[17]. Of note, macrophages might be re-stimulated before the gene transcription induced by the initial stimulus subsides, which will enhance their inflammatory response; these pre-stimulated cells are considered to be primed. For example, priming studies in vivo demonstrated that mice co-infected with *Legionella pneumophila* 3 days after influenza virus infection results had significantly higher broncho-alveolar lavage fluid concentrations of IL-1β than single-infected mice[18]. However, all mice infected with influenza virus and then co-infected with *L. pneumophila* died within 1 week of priming; signs of morbidity included decreased body weight and hypothermia, despite ongoing inflammation. In contrast to innate immune priming, the

Fig. 1 | **Differences between innate immune cell activation, priming, training and tolerance. a** | Activation of naive macrophages by a primary stimulus is accompanied by histone modifications that lead to an enrichment of open-chromatin in regions containing inflammatory genes and their regulatory elements, which facilitates the transcription of cytokines. Once the activating stimulus is removed, activated macrophages return to a naive epigenetic state characterized by closed chromatin without histone marks. **b** | However, if restimulation occurs before the gene transcription events induced by the initial stimulus subside, the inflammatory response of these primed cells is enhanced and this effect is also associated with histone modifications. **c** | By contrast, if the primary stimulus results in persistent epigenetic changes, even after the cell has returned to baseline, and restimulation of that innate immune cell results in an enhanced cytokine response, the cell is considered to have undergone trained immunity. **d** | Innate immune tolerance is another potential outcome following a primary stimulus. These macrophages might display functional defects associated with non-permanent epigenetic changes for weeks after resolution of the primary challenge, which result in loss of function following restimulation.

induction of trained immunity contains a component of immune memory. In models of trained immunity, the initial gene transcription induced by the primary stimulus ceases before the secondary stimulation and the cells are thus considered to have returned to homeostasis. If restimulation of these innate immune cells at a later time point results in enhanced responsiveness compared with the initial stimulation, the cells are considered to have undergone trained immunity. Training studies in vivo demonstrated that 1 month after influenza virus infection (that is, following elimination of the virus), infection with *S. pneumoniae* resulted in increased production of IL-6 by monocyte-derived alveolar macrophages compared with animals infected with *S. pneumoniae* but without a prior influenza challenge[19]. This enhanced cytokine production was associated with epigenetic changes and conferred a survival advantage, given that IL-6 deficiency abrogated protection. Therefore, although primed monocytes display increased gene expression upon secondary stimulation, trained monocytes maintain long-term

epigenetic changes at specific gene promoters after the immune activation status returns to baseline. These epigenetic changes are associated with immune protection after restimulation.

Crucially, the opposite of priming and trained immunity can also occur, resulting in blunted innate immune function (FIG. 1). This process, which is termed innate immune tolerance, might be associated with loss of function after restimulation. Supporting this concept, monocytes and macrophages display functional defects for weeks after the resolution of sepsis, independently of metabolic exhaustion or unresponsiveness to endotoxin[20]. Innate immune tolerance studies in vivo showed that histone H3 Lys27 acetylation (H3K27ac) peak heights correlated significantly with functional paralysis in alveolar macrophages weeks after the resolution of primary *E. coli*-mediated pneumonia, indicating that this type of pneumonia elicits tolerogenic training of alveolar macrophages[21]. Immune tolerance data from animal models is consistent with the severe

metabolic defects observed in patients with sepsis, which are characterized by multiple deficiencies in glycolytic and oxidative phosphorylation pathways that lead to immunometabolic paralysis[20]. Interestingly, induction of trained immunity can reverse innate immune tolerance. One study showed that LPS-exposed monocytes in vitro treated with the *C. albicans* cell wall component β-glucan retain the ability to produce pro-inflammatory cytokines after LPS re-exposure compared with non-β-glucan-treated cells[22]. In addition, this study demonstrated that ex vivo β-glucan also increases the release of TNF and IL-6 in monocytes from healthy volunteers injected with LPS before LPS re-exposure in vitro, which suggests that it might be possible to modulate immune responses through the regulation of epigenetic histone reprogramming at specific genes and of metabolic rewiring.

Epigenetic reprogramming

Epigenetic control of histone modifications leading to long-term opening of chromatin is the basis of trained immunity[17]. For example, following a primary stimulus, certain epigenetic modifications enable faster expression of relevant effector genes in response to a second stimulus. Specifically, non-permanent histone modifications associated with gene activation, including H3K4 monomethylation (H3K4me) and trimethylation (H3K4me3), and H3K27ac have been observed in trained macrophages[23]. These epigenetic marks, which can be detected through several approaches (FIG. 2 and BOX 1), result in the opening of chromatin at promoters of genes encoding pro-inflammatory cytokines such as IL-6, IL-1β and TNF and are associated with protection against re-infection[24].

Metabolic rewiring

Under steady-state conditions, immune cells have low biosynthetic activity and their energy requirements are predominantly met through oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO). Upon activation, the energy demand of innate immune cells increases and aerobic glycolysis, glutaminolysis, cholesterol metabolism and fatty acid synthesis can be used to meet those additional needs. Metabolic intermediates, such as acetyl-CoA, fumarate, succinate, nicotinamide adenine dinucleotide (NAD⁺) and mevalonate, which are produced as a result of this activation-induced metabolic rewiring, regulate the epigenetic landscape[25]. Epigenetic rewiring is associated with a metabolic shift in trained macrophages, which includes an increase in aerobic glycolysis compared with untrained macrophages that is dependent on the activation of the mammalian target of rapamycin (mTOR) via the dectin-1–AKT–hypoxia-inducible factor 1α (HIF-1α) pathway[26]. mTOR detects cellular nutrient, oxygen and energy levels via various upstream inputs and then activates transcriptional regulators (for example, HIF1α, transcriptional repressor protein YY1 and peroxisome proliferator-activated receptor-γ coactivator 1-α (PCG1α)) that stimulate glycolysis and mitochondrial oxidative metabolism[27]. Inhibition of the mTOR pathway or glycolysis with rapamycin, 2-deoxyglucose (2-DG) or metformin during

training interferes with pro-inflammatory cytokine production in macrophages and results in loss of protection against infection. The upregulation of glycolysis in trained monocytes is associated with both mitochondrial respiration and accumulation of lactate[13]. Of note, in BCG-induced trained macrophages, the basal and maximal extracellular acidification rate, which is an indicator of glycolysis, and the oxygen consumption rate, which reflects oxidative phosphorylation, increased before LPS re-stimulation compared with untrained macrophages. These findings indicate upregulation of both aerobic glycolysis and oxidative phosphorylation in trained macrophages[28] (FIG. 2).

Long-lasting effects of trained immunity

Evidence for the long-lasting protective effects of the BCG vaccine that are mediated by innate immune cells support the existence of long-term trained immunity, which has been investigated in longitudinal population-based cohort studies[29,30]. The long-lasting effect of trained immunity was also observed in a randomized controlled trial in which older patients hospitalized for a new infection were randomly assigned to receive the BCG vaccine or placebo[31]. These patients were followed for 1 year after discharge to determine the rate of reinfections. Interestingly, the rate of infections was 79% lower in the BCG group, primarily owing to fewer viral pulmonary infections. The role of trained immunity in this protection is supported by epigenetic analyses that revealed an enrichment of H3K4me3 at the promoters for *IL6* and *TNF* compared with the placebo group, which was associated with pro-inflammatory cytokine secretion in macrophages. These analyses also demonstrated that BCG had a cross-protective effect against *C. albicans* and *Staphylococcus aureus* infections for at least 3 months after vaccination. A 6-month follow-up phase III placebo-controlled randomized clinical trial of BCG vaccination against SARS-CoV-2 demonstrated a 68% relative reduction in the risk of developing COVID-19 in older individuals 6 months after vaccination compared with those in the placebo group[32]. Another study demonstrated that BCG induces an increase in the peripheral blood mononuclear cell production of TNF and IL-1β after LPS stimulation in vitro 1 year after vaccination compared with pre-vaccination levels, which supports the sustained training effects of BCG vaccination[33].

The longevity of trained immunity can be explained by its effects in the bone marrow. One study showed that intravenous BCG injection induced the expansion of HSPCs and promoted their differentiation towards myelopoiesis[13]. Moreover, macrophages derived from the bone marrow of BCG-vaccinated mice (collected 1 or 5 months post-infection) were more resistant to *M. tuberculosis* in vitro than those derived from unvaccinated mice; this difference was associated with distinct gene expression signatures. Importantly, vaccination induced epigenetic modifications in myeloid precursor cells and transfer of bone marrow from vaccinated to naive mice enhanced the protection provided by myeloid cells against pulmonary infection by *M. tuberculosis*[13]. These data suggest that epigenetic modifications induced on bone marrow myeloid precursors are
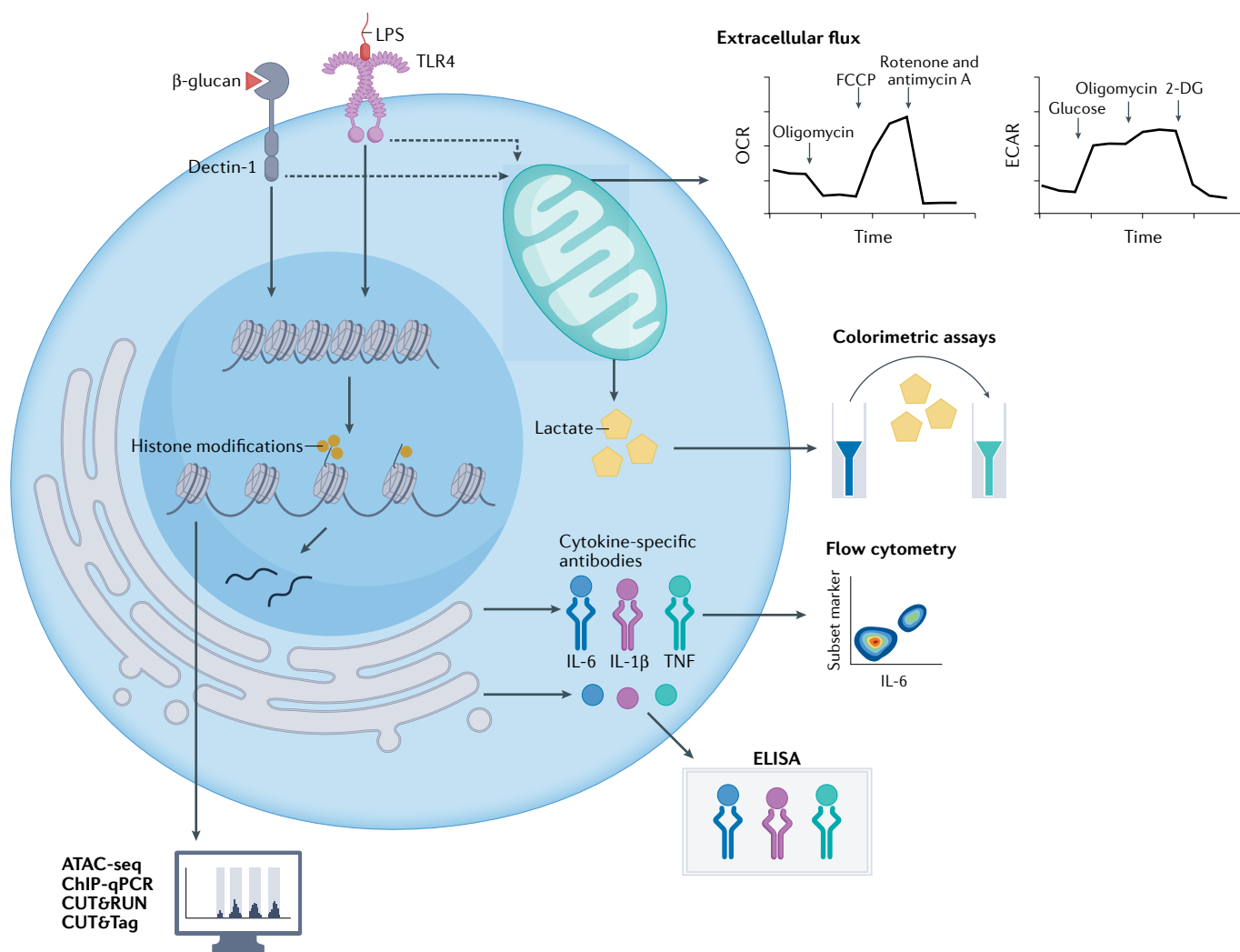
Fig. 2 | **Cellular, molecular, metabolic and epigenetic approaches to studying trained immunity.** Different methodologies can be used to assess trained immunity in innate immune cells. Flow cytometry can be used to quantify the production of pro-inflammatory cytokines[167], such as tumour necrosis factor (TNF) and IL-6, in fixed and permeabilized innate immune cells after restimulation with lipopolysaccharide (LPS). Several studies have also used ELISA to measure the pro-inflammatory cytokine response (IL-6, TNF and IL-1β) of trained monocytes in vitro and in vivo[168,169]. In vitro, trained innate immune cells have higher basal and maximal mitochondrial activity, which can be assessed using colorimetric or fluorometric assays[170] to provide insights into trained immunity. For example, cultured trained innate immune cells produce protons via the lactate pathway and the acidification of the culture medium can be assessed by measuring the extracellular acidification rate (ECAR) as an indicator of glycolysis, whereas the oxygen consumption rate (OCR) can be used as a measure of oxidative phosphorylation[171,172]. Seahorse XF analysers can be used to obtain an in-depth analysis of a variety of mitochondrial functions in single cells in vitro with relatively high throughput through the use of compounds that perturb the cellular bioenergetic profile[173]. Specifically, the sequential addition of oligomycin (ATP synthase inhibitor), FCCP (mitochondrial uncoupler), and a combination of the complex I inhibitor (rotenone) and complex III inhibitor (antimycin A) provides information on three key parameters of mitochondrial activation — ATP turnover, proton leak and maximal respiration[174,175]. Finally, induction of trained immunity can also be investigated by evaluating chromatin remodelling, using tools such as chromatin immunoprecipitation (ChIP), assay for transposase-accessible chromatin using sequencing (ATAC-seq), cleavage under targets and release using nuclease (CUT&RUN) and cleavage under targets and tagmentation (CUT&Tag).

retained throughout development and are maintained once the cells migrate out of the bone marrow and infiltrate inflamed tissue in response to certain infections. Supporting this hypothesis, another study demonstrated that BCG leads to imprinting of persistent transcriptomic signatures on human HSPCs that skew them towards myeloid development. These changes in transcription were associated with specific epigenetic modifications and both alterations could still be detected in peripheral monocytes 3 months after vaccination[34]. In addition to BCG, other stimuli can induce trained immunity, including β-glucan, which induces trained immunity via its receptor, C-type lectin domain family 7 member A (also known as dectin-1). β-glucan-mediated induction of trained immunity occurs via modulation of HSPCs in the bone marrow. Specifically, β-glucan enhanced myelopoiesis by increasing the numbers and frequency of haematopoietic progenitors, such as Lin−Sca1+c-Kit+ (LSK) cells, and inducing metabolic and transcriptomic alterations associated with trained immunity[35]. For example, β-glucan binding to dectin-1 activated the mTOR pathway and calcium-dependent nuclear factor of activated T cells (NF-AT) signalling, resulting in epigenetic reprogramming of immune gene promoters[25,36].

Chromatin remodelling is the key mechanism underlying trained immunity, thus epigenetic methods represent a valid approach to investigating trained immunity (FIG. 2). If monocytes are trained by certain stimuli, their chromatin is remodelled to facilitate the expression of inflammatory genes. Useful techniques for the detection of epigenomic traits include chromatin immunoprecipitation (ChIP), assay for transposase-accessible chromatin using sequencing (ATAC-seq), cleavage under targets and release using nuclease (CUT&RUN), and cleavage under targets and tagmentation (CUT&Tag). ChIP is the most traditional technique used for epigenome assessment[176] and involves the immunoprecipitation of nucleosomes with distinct histone modifications, coupled with subsequent purification of cross-linked DNA. When training is induced, nucleosomes in the promoters of inflammatory genes, such as tumour necrosis factor (TNF) and IL-6, are enriched in trimethylated histone H3 Lys4 (H3K4me3) and acetylated H3K27 (H3K27ac), which are associated with open chromatin. After ChIP is completed, polymerase chain reaction (PCR) can be performed on ChIP-DNA to quantify the presence of H3K4me3 or H3K27ac at inflammatory gene promoters (ChIP-PCR). Alternatively, ChIP-DNA can be prepared for next-generation sequencing to study the entire epigenome (ChIP-seq).

ATAC-seq, CUT&RUN and CUT&Tag are more recent approaches than ChIP and their main advantage is a low cell number requirement. ATAC-seq assesses chromatin accessibility at a genome-wide level[177] and revealed an enrichment of open chromatin in inflammatory gene regions in trained monocytes. CUT&RUN and CUT&Tag are antibody-based immunotethering technologies[178,179] that rely on antibodies that bind to the protein of interest (for example, H3K4me3, H3K27ac or transcription factors). In addition, Protein A or Protein G fused to either micrococcal nuclease (MNase; CUT&RUN protocol) or Tn5 Transposase (CUT&Tag protocol) are tethered to the antibody to enable targeted digestion and the creation of a library of open chromatin fragments. Data obtained through CUT&RUN and CUT&Tag confirmed that trained monocytes had an enrichment for histone modifications associated with gene activation (for example, H3K4me3 and H3K27ac) in inflammatory gene regions.

These alterations were protective in mice challenged with a lethal secondary cytotoxic stress (that is, lethal *C. albicans* infection). β-glucan-induced trained immunity can also reprogram granulopoiesis and neutrophil development through transcriptomic and epigenetic modulation to promote an antitumour phenotype[37]. This protective effect against tumour development was dependent on reactive oxygen species (ROS) and type I interferon signalling, and could still be observed in mice lacking B and T cells. Of note, although initially described in monocytes and macrophages, trained immunity can also be induced in neutrophils[38], NK cells[39], innate lymphoid cells[40] and non-immune cells[41], including endothelial cells[42], epithelial cells[43] and haematopoietic precursors[13]. For example, BCG vaccination of healthy humans also induced long-lasting enhancement of neutrophil function, which was associated with genome-wide epigenetic modifications in H3K4me3 in neutrophils[38]. Here, we focus mainly on the potential role of trained monocytes and macrophages (BOX 2).

Trained immunity and immunopathology

Many kidney diseases are associated with immune pathological conditions — several immunological disorders (for example, anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis or systemic lupus erythematosus (SLE)) can cause damage to the kidney and lead to chronic kidney disease (CKD). Moreover, patients with CKD often have impaired immunity owing to the effects of uraemic toxicity, as well as treatment-associated immune alterations, especially in patients with kidney failure who require kidney replacement therapy (KRT) such as dialysis or kidney transplantation. These immune alterations associated with kidney disease and KRT include chronic inflammation and premature immune ageing, and patients with advanced CKD are more susceptible to infections, have a diminished response to vaccination and display profound innate immune system alteration compared with the general population[44]. Below, we discuss the molecular regulation of trained immunity in the context of pathological conditions that involve the kidney.

Autoimmune diseases

Several systemic autoimmune diseases, such as SLE, are complicated by kidney involvement. Although SLE can affect any organ of the body, the kidneys are involved in ~50% of patients[45]. The pathogenesis of SLE is not fully understood and the mechanisms underlying the observed loss of self-tolerance in this disease are unclear. SLE is characterized by an imbalance between the induction of apoptosis and the removal of apoptotic cells. Apoptotic material can originate from any cell type, including neutrophils, of which approximately one billion undergo apoptosis every day[46]. Moreover, neutrophils in patients with SLE have an increased propensity to form neutrophil extracellular traps[46]. Apoptotic cellular debris and neutrophil extracellular traps contain nucleic antigens that can trigger an inflammatory response by activating cytosolic nucleic acid sensors and TLRs in innate immune cells. Their activation induces type I interferon production, which can stimulate the adaptive immune system, and therefore has the potential to activate self-reactive T and B cells[45,46]. Engagement of self-reactive lymphocytes drives the production of autoantibodies against nuclear components, such as double-stranded DNA, RNA, histones and small nuclear ribonucleoproteins. Binding of nuclear antigens to the subendothelial space of the glomerular capillaries might lead to the formation and deposition of immune complexes in the kidneys, leading to the activation of complement and recruitment of leukocytes that characterize lupus nephritis[45].

Historically, SLE research has focused largely on the adaptive immune system. However, monocytes and macrophages are increasingly recognized to have a key role in this disease[47]. For example, phagocytosis of apoptotic cells is reduced in macrophages from patients with SLE compared with healthy individuals, and their ability to clear immune complexes is also impaired[48,49]; these macrophages also show signs of enhanced activation[50]. Of note, circulating cytokine levels, including IL-6, TNF and IL-1β, are higher in patients with SLE compared with healthy individuals and blood levels of these cytokines correlate positively with disease activity and autoantibody levels[51,52]. In lupus nephritis, macrophage infiltration is prognostic for disease progression[53].

Several studies have begun to elucidate the molecular mechanisms that underlie the increased activation of macrophages in SLE, including immunometabolic and epigenetic reprogramming. In a mouse model of lupus nephritis (MRL-lpr), kidney myeloid cells were isolated for single-cell RNA sequencing, which revealed a marked enrichment of glycolysis-related gene expression

in kidney-derived macrophages[54]. The study demonstrated, through transcriptional and metabolic assays, that Fc-gamma receptor (FcγR) cross-linking induces a switch to aerobic glycolysis in macrophages that is regulated through mTOR–HIF1α signalling. This work also demonstrated that metabolic reprogramming is required to induce IL-1β production, and that inhibition of this glycolytic switch with 2-Deoxy-ᴅ-glucose (2-DG) suppresses IL-1β production in primary human kidney macrophages[54]. In addition, in MRL-lpr mice infused with IgG immune complexes, treatment with 2-DG decreased expression of IL-6, TNF and IL-1β in kidney tissue and decreased kidney neutrophil infiltration, demonstrating that these immunometabolic circuits, which are known to be involved in trained immunity, participate in SLE[54].

Other studies focused on the epigenome of monocytes and macrophages, and found substantial alterations in histone acetylation and methylation in the context of SLE. H4 acetylation, which increases chromatin accessibility for gene transcription, was increased in the *TNF* locus in circulating monocytes of patients with SLE compared with healthy individuals[55]. Whole-genome chromatin immunoprecipitation showed that H4 acetylation is higher overall in patients with SLE than in controls, and pathway analyses revealed that H4 acetylation was enriched at the promoter regions of genes that drive inflammatory pathways[56,57]. Interestingly, >60% of genes with increased H4 acetylation were potentially regulated by the interferon regulatory factor 1 (IRF1) transcription factor[58]. Subsequent studies demonstrated that IRF1 interacts directly with histone acetyltransferases, which promotes H4 acetylation[56,59]; overexpression of IRF1 results in H4 hyperacetylation. Of note, innate immune memory could be induced in macrophages through

exposure to IFNγ, which induced changes in the histone chromatin marks of interferon-stimulated genes[60].

Although the study of trained immunity in autoimmune diseases is currently largely unexplored, the involvement of training pathways suggests a potential role for trained immunity in SLE (FIG. 3). Further functional, metabolic and epigenetic studies on myeloid cells in different anatomical sites might provide additional mechanistic insights into the potential role of trained immunity in SLE. For example, one study investigated the transcriptional regulation of bone marrow progenitors in a murine SLE model (NZBW/F1) and in patients with SLE, and demonstrated alterations in haematopoiesis, with a skewing towards myelopoiesis in both mice and humans[61]. Interestingly, these changes were associated with a 'training' LSK signature, consistent with data on the effects of β-glucan training on LSK progenitors and granulopoiesis[35,37]. Induction of trained immunity in the bone marrow of patients with SLE might therefore promote the development of pro-inflammatory granulocytes that home to peripheral tissues, including the kidneys[62,63]. Future studies are needed to confirm the presence of histone modifications in the bone marrow progenitors and circulating monocytes of patients to confirm this hypothesis. Furthermore, longitudinal studies are needed to assess peripheral and central trained immunity at different stages of the disease, and to investigate how trained immunity might relate to disease outcomes. Interestingly, therapeutic interventions that target metabolic and epigenetic processes can ameliorate SLE and inhibition of trained immunity might underlie some of this protection[54,64]. For example, a 2020 study reported that hydroxychloroquine, which is commonly used to prevent SLE flares, inhibited trained immunity by suppressing H3K27ac and H3K4me3 of inflammation-related genes[65]. Trained immunity was also reported to modulate inflammation-induced fibrosis in systemic sclerosis, suggesting that it might be involved in a broad range of systemic autoimmune diseases[66].
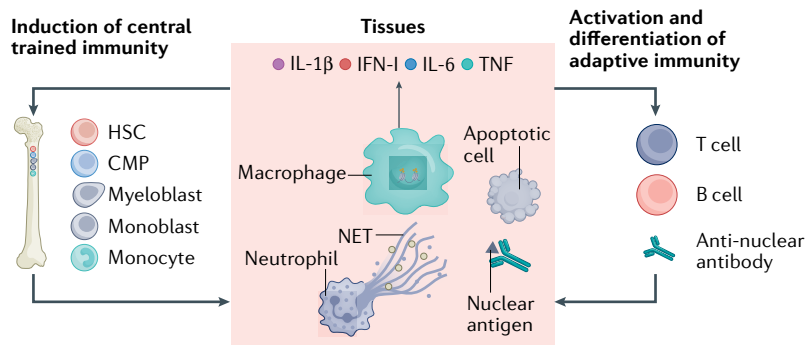
CKD and dialysis

The uraemic state in patients with CKD, including those receiving dialysis, can have a profound effect on the immune system[67]. This CKD-associated immune dysregulation, which also compromises responses to vaccination[68], was highlighted by the COVID-19 pandemic[69] — patients with CKD and organ transplant recipients were at one of the highest risks of SARS-CoV-2-associated morbidity and mortality[70,71]. In addition, patients with advanced CKD are more susceptible to other immunity-mediated diseases, including virus-associated cancers, periodontitis and atherosclerosis, compared with the general population[72–75].

The exact mechanisms underlying immune dysregulation in patients with kidney disease are not completely defined, but oxidative stress induced by the retention of uraemic toxins and decreased clearance of inflammatory cytokines seem to have an important role[76]. Oxidative stress results in the production of advanced glycation end-products (AGEs), which are recognized as DAMPs through the receptor for AGE (RAGE) and can trigger innate immune activation. The oxidation of low-density

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Box 2 | Isolation and phenotyping of monocytes

Purification of mononuclear cells, including monocyte populations, from whole blood or buffy coats can be achieved by gradient centrifugation[180,181]. Monocytes can also be enriched using magnetic beads[182]; this approach is fast and very gentle on myeloid cells but does not isolate monocyte populations with the same degree of purity as flow cytometry approaches such as fluorescence-activated cell sorting (FACS). Compared with FACS, microfluidics-based isolation methods are even gentler on myeloid cells and therefore less prone to induce non-specific cell activation, which would have a confounding effect when measuring the cellular response to subsequent challenges with an activating stimulus such as lipopolysaccharide (LPS)[183]. Flow cytometry not only enables the detection of human monocyte populations using CD11b and CD64[184,185] as lineage markers, but can also be used to detect cell-specific expression of pro-inflammatory cytokines through intracellular cytokine staining[167]. Trained monocytes produce more inflammatory cytokines than cells exposed to a primary stimulus and staining of LPS-restimulated monocytes with anti-cytokine antibodies, after cell fixing and permeabilization, allows the simultaneous measurement of production of different cytokines, such as tumour necrosis factor (TNF) and IL-6. Flow cytometry can also be used to measure mitochondrial activity[186] through the use of fluorescent cell permeable dyes that enable the quantification of total mitochondrial mass (with dyes that accumulate in mitochondria, regardless of their membrane potential), and the assessment of mitochondrial membrane potential (with positively-charged dyes that preferentially accumulate in polarized mitochondria) or of mitochondrial superoxide production (with mitochondria-specific dyes that become fluorescent in the presence of superoxide)[187]. For example, metabolic reprogramming in macrophages induced by LPS in vitro was studied using a combination of MitoTracker Red and MitoTracker Green to analyse glycolytic flux and mitochondrial respiration[188], whereas the production of ROS associated with oxidative phosphorylation could be analysed with MitoSox[189].

Fig. 3 | Proposed role of trained immunity in SLE. Nucleic antigens from apoptotic cells and neutrophil extracellular traps (NETs) induce inflammation and the production of type I interferon (IFN-I), IL-1β, IL-6 and tumour necrosis factor (TNF). IFN-I and IL-1β cause metabolic and epigenetic reprogramming of myeloid and granulopoietic progenitors in the bone marrow. This reprogramming creates trained monocytes and neutrophils with an enhanced inflammatory phenotype and increased capacity for cytokine production after stimulation by nucleic antigens, autoantibodies and inflammatory cytokines. This overproduction of cytokines also stimulates the adaptive immune system, contributing to the activation of autoreactive T and B cells, and the formation of anti-nuclear antibodies. Collectively, these processes generate an inflammatory cycle that leads to tissue destruction. CMP, common myeloid progenitor; HSC, haematopoietic stem cell.

lipoprotein (oxLDL) is another well-described consequence of oxidative stress, and patients with CKD have significantly higher serum levels of oxLDL than healthy individuals[77,78]. Accumulation of oxLDL in the intima of arterial walls leads to aberrant inflammation owing to inflammasome activation in innate immune cells. These processes eventually lead to plaque rupture, which manifests as acute myocardial infarction or stroke[79]. In addition, oxLDL promotes the production of pro-inflammatory myeloid cells in the bone marrow, contributing to a systemic pro-inflammatory state[80]. Patients with advanced CKD can also have elevated serum uric acid levels, which not only induce acute inflammation in the context of gout, but are also associated with chronic systemic inflammation[81].

CKD is associated with increased production of CD14++CD16+ pro-inflammatory monocytes in the bone marrow[82]. This activated profile is accompanied by elevated circulating levels of pro-inflammatory cytokines, such as IL-6, IL-1β and TNF[83]. Interestingly, this enhanced inflammatory activity observed in CKD is retained after patients receive a kidney transplant and uraemia is resolved[84,85]. Although not investigated as such, this sustained propensity for increased production of pro-inflammatory cytokines could be mediated by trained immunity. Notably, uraemia is associated with epigenetic changes, such as changes in DNA methylation, in leukocytes[86]. Moreover, maladaptive training of myelopoiesis underlies the development of inflammatory comorbidities[87], which might have a role in the strong susceptibility of patients with advanced kidney disease to other immune-mediated pathological conditions, including periodontitis and atherosclerosis.

Although the concept of trained immunity has not been fully investigated in the context of CKD and dialysis, several studies suggest that it might have a direct role in the immune alterations observed in affected patients. For example, monocytes stimulated with oxLDL have an enhanced capacity for production of IL-6 and TNF in response to TLR 2 and 4 agonists, compared with untreated cells. This effect is accompanied by upregulation of H3K4me3 on the promoters of the genes encoding these proteins and was completely abolished when monocytes were pre-incubated with the methyltransferase inhibitor methylthioadenosine[88]. Uric acid-stimulated monocytes also produced higher levels of IL-1 and IL-6 than untreated cells upon stimulation with TLR 2 and 4 agonists; the enhanced capacity for cytokine production could be reversed by pharmacological inhibition of histone methyl transferases, suggesting a trained immunity process[89]. Consequently, therapies that inhibit trained immunity through histone methyltransferase inhibition, have been proposed to prevent chronic innate immune activation in hyperuricaemia[90], which is common in patients with CKD[91]. Future research will need to explore the role of trained immunity in patients with advanced CKD further, including its contribution to inflammatory comorbidities and therapeutic targeting potential[92].

Organ transplantation
Several factors that contribute to allograft rejection, including ischaemia–reperfusion injury (IRI) and the presence of infection, involve immune mechanisms that have been implicated in trained immunity (FIG. 4).

Ischaemia–reperfusion injury. In transplantation, the donor kidney is subjected to ischaemia during organ retrieval, which leads to pathophysiological events such as hypoxia and tissue injury owing to limited blood supply. Following transplantation, the return of the blood supply to the transplanted organ induces reperfusion injury, which is mediated by the production of ROS, alterations in intracellular calcium, endothelial cell dysfunction, complement activation and cell death[93]. Multiple stimuli generated during this process of IRI can activate the innate immune system and trigger potent inflammatory responses[94]. IRI-derived DAMPs released into the extracellular space, such as mitochondrial ATP, which is released from damaged cells, induce metabolic changes in innate immune cells[95,96]. Extracellular ATP activates the macrophage cell surface purinergic receptor P2X7 and induces the production of pro-inflammatory cytokines, such as TNF and IL-1β, which are associated with allograft rejection[97,98]. Interestingly, P2X7 blockade abrogated the T helper 1 (T$_H$1) and T$_H$17 T cell immune response, thereby reducing the number of effector T cells, and promoted long-term transplant survival in mice[99]. Thrombospondin (TSP1), which is a glycoprotein that regulates nitric oxide and has an important role in cellular metabolism[100], is also rapidly upregulated during IRI and mediates acute kidney injury[101]. TSP1 binds to TLR4 and triggers multiple pathways of inflammation, including the production of IL-6, TNF and IL-1β in monocytes and macrophages[102–104]. Of note, oxidative stress-triggered IL-6 production in mouse myeloid cells enhanced their capacity to activate allogeneic T cells in a mixed lymphocyte reaction[105]. Moreover, targeting IL-6 reduced inflammation following myocardial ischaemia–reperfusion[106,107], and early blockade of IL-6
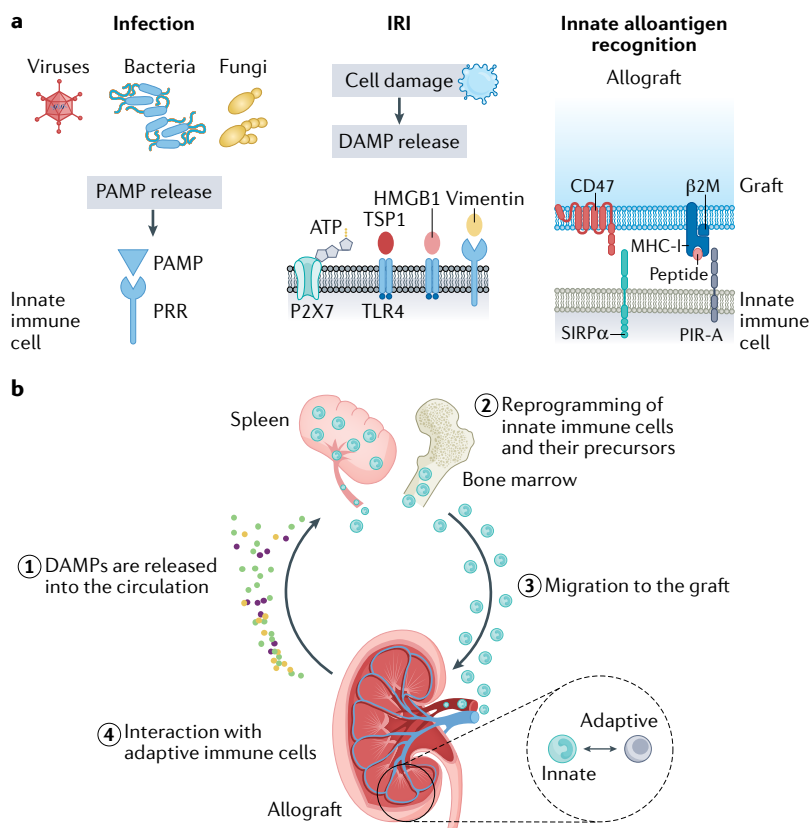
in the cardiac post-transplant setting reduces infiltration of adaptive allogeneic leukocytes and extends graft survival in mice[108]. Overall, these data indicate that IRI, which is associated with poor graft survival[109], upregulates innate immune pathways that are known to be associated with trained immune memory[110]. Preconditioning and postconditioning of donor organs through pharmaceuticals and novel strategies such as RNA interference favours innate immune tolerance[94]. In addition to kidney transplantation, IRI also occurs in thrombotic diseases, sepsis and trauma, all of which can affect the kidney[111]. Consequently, the potential role of trained immunity in these IRI outcomes has broad therapeutic implications.

Fig. 4 | **Trained immunity in kidney transplantation. a** | Events that activate innate immunity in organ transplantation include ischaemia–reperfusion injury (IRI) and the release of damage-associated molecular patters (DAMPs), such as high-mobility group box 1 (HMGB1) or advanced glycation end products (AGEs), which are produced under conditions of oxidative stress and the presence of viral, bacterial and fungal infections. In the context of infection, microbial pathogen-associated molecular patterns (PAMPs) can reprogram macrophages into pro-inflammatory cells that respond to and can kill a broad range of infectious pathogens, and offer protection to susceptible transplant recipients. However, these trained macrophages produce high levels of pro-inflammatory cytokines that can also amplify inflammation and lead to acute rejection. Innate immune cells can also develop a memory response to specific alloantigens, including CD47, which is recognized by signal-regulatory protein-α (SIRPα), and MHC class I (MHC-I) detected via Ig-like receptor A (PIR-A). **b** | Early after transplantation, DAMPs are released from the donor kidney into the circulation (1). Trained immunity-inducing DAMPs reach the bone marrow and reprogram innate immune cell precursors (2). Reprogrammed innate immune cells migrate to the graft where they encounter additional training signals (3). Once in the transplanted organ, reprogrammed innate immune cells interact with adaptive immune cells and contribute to organ rejection (4). β2M, β2-microglobulin; PRR, pattern-recognition receptor; TLR4, Toll-like receptor 4; TSP1, thrombospondin.

Innate allorecognition. Although kidney transplantation is a life-saving procedure for patients with kidney failure, it requires the lifelong use of immunosuppressive drugs. These therapies currently used in the clinic to prevent allograft rejection primarily target the adaptive immune system because B and T lymphocytes are directly responsible for antibody- and cell-mediated immunity against the graft[112]. However, increasing evidence indicates that innate immune cells also have an important role[113]. Of note, the long-term viability of kidney transplants remains suboptimal despite a notable improvement in high-risk groups[114] and targeting the innate immune system could act synergistically with immunosuppressive drugs that target adaptive immunity to improve patient outcomes.

The surgical procedure required for kidney transplantation inevitably causes vascular tissue damage, which promotes the recruitment and extravasation of inflammatory monocytes into the allograft[115]. Once in the transplanted kidney, circulating monocytes can differentiate into different subsets of macrophages following recognition of DAMPs and non-self alloantigens, and their phenotype is also influenced by the systemic presence of immunosuppressive drugs. One study reported that the innate alloresponse worsened if recipient mice had been previously primed with donor cells[116]. After initial priming of lymphocyte deficient (RAG$^{-/-}$) mice with allogeneic splenocytes or skin grafts, monocytes from the recipient mice identify and eliminate allogeneic spleen cells injected subcutaneously 4 weeks later[116], demonstrating that monocytes and macrophages develop a memory response against alloantigens. Further evaluation of the mechanisms that mediate non-self-recognition by monocytes demonstrated that polymorphic differences in signal-regulatory protein-α (SIRPα) between donor and recipient mice, which were recognized by CD47 expressed on host monocytes, drives activation of the innate immune response against the allograft[117]. Moreover, a subsequent study found that paired Ig-like receptor A (PIR-A) expressed in recipient innate immune cells recognizes donor allogeneic MHC class I antigens, which leads to the development of innate immune memory to foreign tissues[118].

We also found evidence that innate immune memory is involved in rejection in a vascularized heart transplant model in mice[119]. When trained immunity was inhibited with myeloid cell-specific mTOR-inhibiting nanobiologics, chromatin immunoprecipitation (ChIP) of allograft monocytes showed reduced H3K4me3 of inflammation-related genes, including TNF and IL-6, which was associated with reduced secretion of these cytokines. In this setting, vimentin and high-mobility group box 1 (HMGB1) were the inflammatory mediators that induced trained macrophages in the transplanted heart. Vimentin is an endogenous intermediate filament protein that activates the dectin-1 receptor, which is highly expressed on endothelial cells, whereas extracellular HMGB1 is a DAMP that binds to TLR4 and induces a robust TNF-mediated immune response in vivo[120,121]. Both of these inflammatory mediators are upregulated in organ transplantation[122,123] and they both bind cell surface receptors known to induce trained immunity (that

is, dectin-1 and TLR4) — we confirmed that sequential treatment with vimentin and HMGB1 induces trained immunity in the context of organ transplantation[119]. Importantly, we found that inhibition of trained immunity using a myeloid cell-specific mTOR-inhibiting nanobiologic promoted a tolerogenic milieu by enhancing the number of regulatory T cells in the allograft, and induced indefinite graft survival without the need for continuous immunosuppression. In another study of cardiac allografts in mice[123], both allogeneic and syngeneic transplantation induced upregulation of HMGB1. However, whereas this upregulation decreased in syngeneic grafts over the first week post-transplant, high HMGB1 levels were sustained in allogeneic grafts, which was accompanied by interstitial infiltration and acute allograft rejection. Overall, these findings suggest that induction of trained innate immunity, through activation of PRRs during ischaemia–reperfusion injury, contributes to allograft rejection and indicates that the endogenous DAMPs that induce innate immune memory affect graft survival.

Infection. Organ transplant recipients are more susceptible to infections than the general population owing to their lifelong immunosuppressive treatment[124]. In these patients, macrophages can contribute to acute and chronic allograft immunopathology via antigen processing and presentation, co-stimulation, pro-inflammatory cytokine production and tissue injury[125,126]. However, these innate immune cells also have important protective roles in the context of infection, including the intracellular killing of pathogens. Importantly, infection can lead to kidney transplant complications and graft loss owing to the production of inflammatory cytokines by innate immune cells. For example, bacterial infection with *Staphylococcus aureus* at the time of skin transplantation prevents the induction of organ transplant acceptance in mice because of excessive IL-6 production[127]. Therefore, although macrophages can induce potent IL-6 cytokine responses that confer protection against *S. aureus* infection[128], this response represents a potential risk in the context of kidney transplantation because an excessive inflammatory immune response can lead to graft loss. Notably, immunosuppressive therapy with either cyclosporine or sirolimus could not prevent graft loss driven by the response to *S. aureus* infection in mice[127], which suggests that common immunosuppressive drugs fail to regulate inflammatory cytokine production by macrophages in response to a bacterial infection. Similar results were obtained using the bacteria *Listeria monocytogenes*, which prevented transplantation tolerance in mice despite therapeutic treatment with co-stimulation blockade[129,130]. These findings are consistent with data indicating that IL-6 produced by macrophages mediates costimulatory blockade-resistant graft rejection in murine models[131]. Given that trained macrophages produce high levels of IL-6 following re-stimulation, these studies suggest that macrophages trained by bacterial infections might represent a risk to kidney transplanted patients.

Viral infections have a similar effect on macrophage-mediated inflammatory cytokine production. Although latent cytomegalovirus (CMV) infection is present in the majority of the general immunocompetent population, CMV is an important cause of morbidity and is mainly observed in immunosuppressed hosts, including kidney transplant recipients[132]. CMV induces expression of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), which had been previously shown to mediate epigenetic reprogramming of innate immune cells and trained immunity[15,133]. CMV is present in the myeloid lineage of mice with a latent infection[134] and induces the production of pro-inflammatory IL-6 in CMV-infected macrophages[135]. Of note, IL-6 induces reactivation of CMV-infected monocytes that produce CMV virions at higher rates than monocytes treated with other cytokines[136]. Accordingly, circulating CMV-infected monocytes can extravasate to tissues and significantly increase their production of IL-6 upon restimulation with bacterial products, such as LPS[137]. Unbiased bulk and single-cell transcriptomics paired with functional assays (fungal killing) of cells from patients infected with CMV demonstrated that human CMV-infected monocytes do not effectively phagocytose opportunistic fungal pathogens, and that this functional impairment occurs in the context of decreased expression of fungal PRRs[138]. Moreover, CMV-infected monocytes upregulate the expression of phagocytic receptors, pro-inflammatory chemokines, activate the inflammasome and induce the expression of innate immune transcripts associated with allograft rejection[138]. Notably, latent infection with CMV prevents the induction of prolonged allograft survival following transplantation and therefore represents a major barrier in kidney transplantation[139]. Considering that latent CMV is prevalent worldwide — estimated seroprevalence of 83% in the general population[140] — and is reactivated by inflammation, CMV infection is a relevant clinical pathology associated with decreased long-term graft function[132].

Therapeutic modulation of trained immunity
Therapeutically targeting trained immunity represents a promising approach to regulating innate immunity and, consequently, the adaptive immune responses that are triggered and modulated by innate immune cell surface molecules and soluble mediators. Trained immunity can be manipulated to either enhance immune response against infections and malignancies or to inhibit the responses that drive autoimmune diseases and allograft rejection. Trained immunity is regulated at multiple levels, including ligand–receptor interaction, as well as metabolic and epigenetic regulation. These different regulatory levels present several targets for therapeutic intervention (FIG. 5).

Blocking ligand–receptor interactions
Different receptors can mediate the induction of trained immunity. One of the best described is dectin-1, which is expressed mainly by DCs, monocytes and macrophages[141], and the dectin-1 signalling cascade triggered by binding to β-glucan can be blocked with receptor-blocking antibodies or laminarin[142,143]. Another well-described pathway that induces trained immunity is mediated by the intracellular PRR NOD-2, which
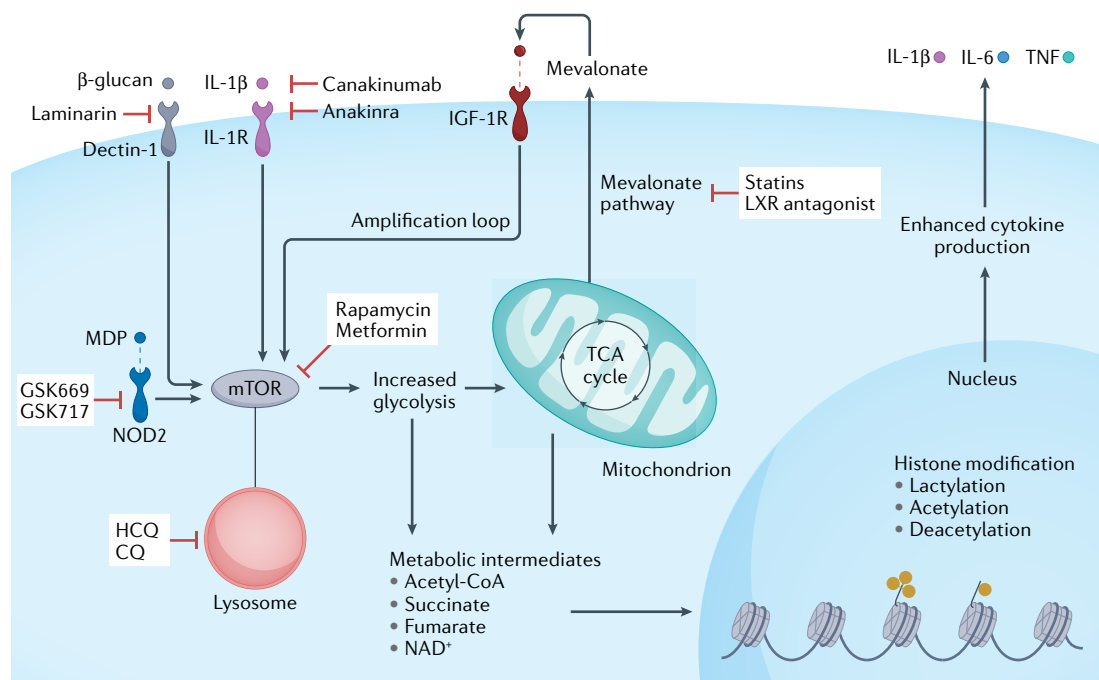
Fig. 5 | Therapeutic targeting of trained immunity. Trained immunity depends on histone modifications that enhance the production of pro-inflammatory cytokines and can be induced through different receptors, including the dectin-1 receptor, the IL-1 receptor (IL-1R), and the nucleotide-binding oligomerization domain-containing protein 2 (NOD2). Dectin-1 can be activated by β-glucan (component of fungal cell walls) and blocked by receptor-blocking antibodies or laminarin. IL-1R can be activated by IL-1α and IL-1β, and can be blocked by anakinra, whereas canakinumab can be used to prevent IL-1β binding to IL-1R. The intracellular pattern recognition receptor NOD-2 recognizes peptidoglycans containing muramyl dipeptide (MDP; component of bacterial cell walls) and can be blocked by the small-molecule inhibitors GSK669 and GSK717. Engagement of the aforementioned receptors by their ligands triggers activation of mechanistic target of rapamycin (mTOR), which is a key regulator of cell metabolism, and stimulates glycolysis and mitochondrial oxidative metabolism. The metabolic intermediates derived from these metabolic processes (acetyl-CoA, fumarate, succinate, nicotinamide adenine dinucleotide (NAD$^+$) and mevalonate), are directly involved in mediating the epigenetic changes that underpin trained immunity. Mevalonate-induced training is mediated through increased function of insulin-like growth factor-1 receptor (IGF-1R). Trained immunity can also be suppressed with mTOR inhibitors (for example, rapamycin and metformin). Moreover, mTOR is activated at the surface of lysosomes, which can be prevented with chloroquine (CQ) and hydroxychloroquine (HCQ), which interfere with lysosomal function. Lipid metabolism also has an important role in activating trained immunity. Statins and nuclear liver receptor (LXR) blockers can inhibit trained immunity by blocking mevalonate production.

recognizes peptidoglycans containing muramyl dipeptide (MDP)[15]; MDP is a cell wall component in both Gram-positive and Gram-negative bacteria[144,145]. NOD-2 receptor activation to induce trained immunity can be achieved with a synthetic small peptide conjugate comprising *N*-acetyl muramic acid and the short amino acid chain of ʟ-alanine ᴅ-isoglutamine dipeptide[146,147]. By contrast, the small-molecule inhibitors GSK669 and GSK717 can inhibit NOD-2 receptor activation[148].

The NOD-2 receptor is also activated by BCG, which is widely delivered through intravesical instillation as immunotherapy for high-risk non-muscle-invasive bladder cancer[149]. The induction of trained immunity has an important role in the BCG-mediated antitumour effects[150]. The working hypothesis is that inducing trained immunity can counter the immunosuppressive tumour microenvironment, thereby allowing the immune system to recognize and eliminate tumour cells. Of note, repeated administration of intravesical BCG increases the concentration of pro-inflammatory cytokines[151]. Accordingly, a myeloid-specific nanobiologic could

induce trained immunity through NOD-2 activation and mediated potent suppression of tumour growth in a mouse model of melanoma[152]. Treatment with BCG is also used to prevent recurrence of superficial bladder tumours[153], which improves overall patient survival[154]. Moreover, BCG vaccination might have a broad effect against infection as it can enhance protection against a variety of viruses, including herpes simplex virus and human papilloma virus[155].

IL-1 is another receptor signalling pathway with a fundamental role in the induction and modulation of innate immune responses that is associated with trained immunity. The receptors of this family signal through a Toll/IL-1 receptor (TIR) domain that leads to nuclear factor-κB (NF-κB) activation[156]. The IL-1 pathway can be activated (for example, through IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ), or antagonized (for example, via IL-1Ra, IL-36Ra, IL-38) depending on the cytokines and receptors that are triggered. Trained immunity induced by β-glucan depends on IL-1β production, and blocking antibodies against IL-1R (for example, anakinra)

and anti-IL-1β antibodies (for example, canakinumab) prevented the development of peripheral trained macrophages[157]. Another study showed that anakinra prevented central trained immunity through inhibition of cell-cycle progression and increased glycolysis in bone marrow progenitors[35]. These compounds are effective in the treatment of a wide range of conditions, including rheumatoid arthritis, familial Mediterranean fever, macrophage activation syndrome, gout and atherosclerosis[157,158] and their beneficial effects might be partly mediated through regulation of trained immunity. Of note, IL-1 also has an important role in the systemic inflammation observed in patients receiving haemodialysis[159]. A randomized, placebo-controlled study showed that, in patients treated with haemodialysis, 4-week treatment with anakinra markedly reduced serum C-reactive protein (CRP) and IL-6 levels[160]. Moreover, in patients with stage 3–4 CKD, treatment with the IL-1 inhibitor rilonacept for 12 weeks reduced serum CRP concentrations and improved vascular function measured by flow-mediated vasodilation[161]. These effects might also be partly mediated through effects on immune training, although blocking IL-1 has immunomodulating effects beyond trained immunity[157].

Immunometabolic targeting

Cellular metabolic pathways provide another opportunity to intervene and control the epigenetic reprogramming that underlies trained immunity. Trained immunity can be potently suppressed by inhibiting mTOR with rapamycin or metformin[162]. Moreover, mTOR is activated at the lysosomal surface and lysosomes have a pivotal role in coordinating immunometabolism[163]. Interestingly, the trained immunity phenotype is characterized by activation of key regulators of lysosome genes[22], and chloroquine and hydroxychloroquine, which are weak bases that diffuse passively to the lysosome where they interfere with its function, are potent inhibitors of trained immunity[65]. Compounds that affect cellular lipid metabolism might also modulate trained immunity. Statins and nuclear liver receptor (LXR) blockers inhibit the cholesterol synthesis pathway and thereby the production of mevalonate, which is a mediator of trained immunity via mTOR[164,165]. By contrast, the synthesis of fatty acid induced by aldosterone can induce trained immunity and this effect can be inhibited by aldosterone receptor blockers[166].

Conclusions

In the past decade, trained immunity, which is characterized by the induction of epigenetic memory in innate immune cells, has emerged as a new concept in immunology, providing new insights into the immune response to infections and the pathophysiology of immunity-mediated diseases. Although originally described as an evolutionary adaptation to protect organisms from reinfection, new evidence shows that this immunological concept extends to other areas of pathology. In the context of kidney diseases, the research field of trained immunity is still in its infancy. Currently, little is known about the role of trained immunity in patients with kidney failure receiving KRT. In autoimmune diseases, evidence of trained immunity has been reported in SLE and systemic sclerosis, although further investigations are required to determine the mechanisms by which trained immunity mediates disease activity, and whether inhibition of trained immunity can prevent disease flares. In other autoimmune disorders such as ANCA vasculitis, which frequently leads to kidney failure, the role of trained immunity is largely unknown, although the involvement of pathways associated with innate immune training suggest a potential contribution to the pathological development of the disease. In organ transplantation, animal studies have demonstrated the role of trained immunity in graft rejection, although human data are still lacking. Important questions remain about the effect of IRI on innate immune training and how trained immunity relates to short-term outcomes, such as delayed graft function, and long-term outcomes, such as formation of donor-specific antibodies and graft survival. Understanding the mechanisms underlying trained immunity in these clinical scenarios has important therapeutic implications. For example, emerging experimental and clinical studies suggest that activation of receptors such as NOD2 might be an effective strategy to induce trained immunity, whereas ligand–receptor blockade or intervention at the metabolic level might help to suppress the pathological effects of trained immune cells. Future research should clarify whether targeting the development of innate immune memory and modulating the trained immune response represents an innovative and effective therapeutic approach in nephrology.

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Competing interests
J.O., W.J.M.M., and M.G.N. declare that they are scientific founders of Trained Therapeutics Discovery. The other authors declare no competing interests.

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