Distribution and storage of inflammatory memory in barrier tissues

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Abstract | Memories of previous immune events enable barrier tissues to rapidly recall distinct environmental exposures. To effectively inform future responses, these past experiences can be stored in cell types that are long-term residents or essential constituents of tissues. There is an emerging understanding that, in addition to antigen-specific immune cells, diverse haematopoietic, stromal, parenchymal and neuronal cell types can store inflammatory memory. Here, we explore the impact of previous immune activity on various cell lineages with the goal of presenting a unified view of inflammatory memory to environmental exposures (such as allergens, antigens, noxious agents and microorganisms) at barrier tissues. We propose that inflammatory memory is distributed across diverse cell types and stored through shifts in cell states, and we provide a framework to guide future experiments. This distribution and storage may promote adaptation or maladaptation in homeostatic, maintenance and disease settings — especially if the distribution of memory favours cellular cooperation during storage or recall.

Barrier tissues
Epithelial tissues that interface directly with the external environment (that is, any surface directly and constantly exposed to the world outside the host), composed of a monolayer, pseudo-stratified or stratified epithelium, as well as an underlying stromal-derived component and other transient, resident or permanent resident cell lineages.

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The epithelial barriers of the skin, airways and intestines in mammals form essential interfaces that constantly sense and respond to external and internal signals[1–3]. Adaptation to environmental exposures is a conserved property of barrier tissues, and the cell types that compose these barriers must balance metabolic functions with host defence[4,5]. In simpler metazoans that lack specialized immune cells, this function falls solely on epithelial cells, whereas in more cellularly complex mammalian tissues, immune cells may mediate this process[5].

For a barrier tissue to optimally access information derived from a previous immune event to inform a present or future memory response, that information can be stored in locally accessible cell types that are residents or permanent residents and maintain appropriate qualitative features (FIG. 1). Deviations in memory storage or retrieval can predispose a tissue to pathological consequences: insufficient memory leads to increased infections; excessive memory retrieval drives chronic inflammation; and malignancy potentially arises from both insufficient and excessive memory[6,7]. The appropriately named adaptive immune system has key roles in promoting antigen-specific memory through encoding receptors — T cell receptors (TCRs) and B cell receptors (BCRs) — specific for microorganisms, environmental antigens and self-antigens in lymphocyte-bearing organisms[8,9].

However, antigen-specific adaptive immune memory is just one of the myriad ways in which tissues and organisms can adapt during immune events[10]. Indeed, the properties of memory are now being identified within other haematopoietic cells, such as innate immune cells, as well as more recently in the tissue parenchyma[10–16]. Here, we use the term memory to refer to a defined response to an initiating trigger that has an altered baseline, sensitivity, rapidity or maximum, and that persists until a secondary challenge[11] (FIG. 2a). Immunological, inflammatory and neuronal memory are all subject to independent environmental or host factors, including temperature, metabolism, hormones and circadian cycles, that may diminish or enhance a secondary response.

The division of the adaptive immune system into cell types (such as αβ and γδ T cells), cell subsets (such as $CD4^+$ and $CD8^+$ T cells) and cell state descriptors (such as type 1, type 2, type 17, circulating and resident-memory cells) has been invaluable in providing cellular mechanisms for the observed phenomena of tissue immunity[17,18]. Comprehensively identifying the sets of gene modules that define the types, subsets and states of the main parenchymal, stromal, neuronal and immune cell lineages in barrier tissues will afford an unprecedented view of tissue immunity[19]. To date, this has been technically challenging given the lack of

Immune event
Exposure to an environmental stimulus (such as allergens, antigens, noxious agents, diet, pathogens and microbial communities) or a host-derived stimulus (such as metastasis or sterile tissue damage) at a barrier tissue sensed by the host, triggering downstream transcription and/or epigenetic changes in cell state and/or cell composition in the tissue.

Memory
The properties of memory include an altered baseline, sensitivity, rapidity or maximum for a defined response upon secondary challenge to an initiating trigger.

Adaptive immune memory
Classically defined as a memory response by a cell that is considered part of the adaptive immune system (for example, T cells and B cells), based on the ability of its receptor to be formed through the recombination of genetic elements and stably inherited across cell divisions.

Cell types
Developmentally specified cell identity modules that are typically irreversible beyond enforced overexpression of lineage-overriding transcription factors.

Cell subsets
Typically developmentally stable cells, but their programming may be overridden based on niche availability or extreme environments.

Cell state
Characteristics that can be transiently acquired from tissue entry and/or an immune event, are distinct from cellular differentiation and are related to the quality (that is, type of inflammation) of an immune response.

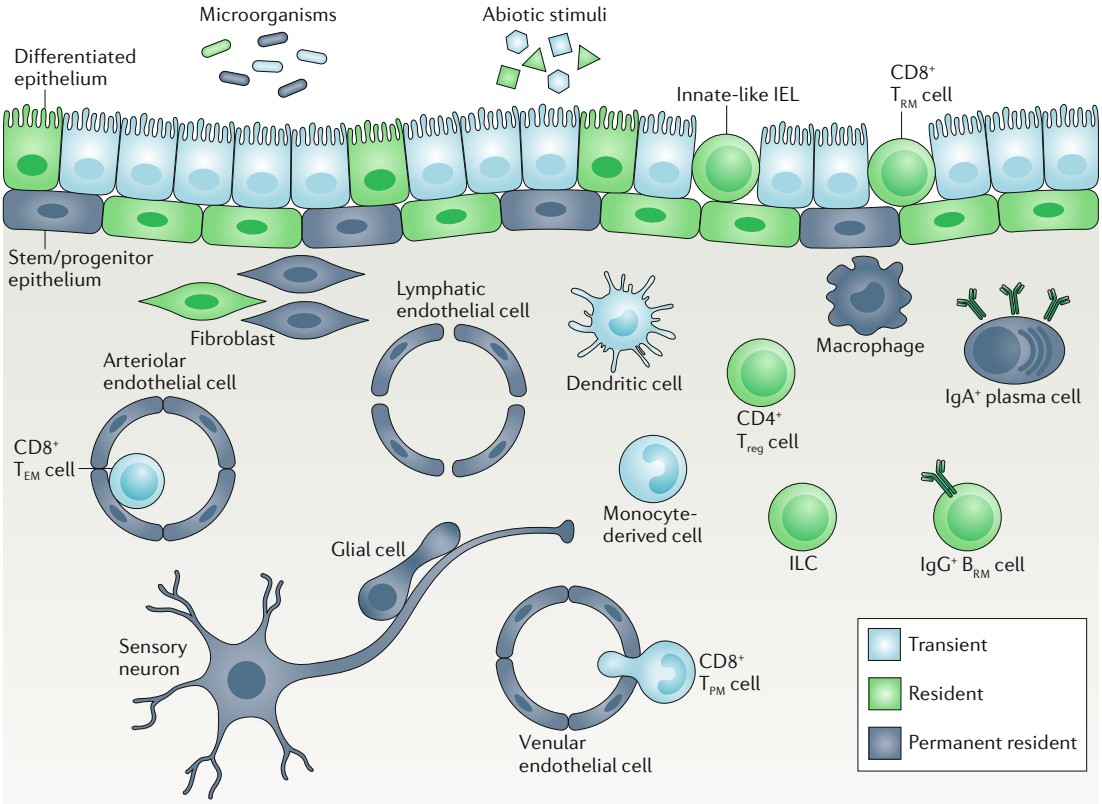

Fig. 1 | **Cell type residence and permanence in barrier tissues.** The cell types in a tissue at any given moment may be there short term (transient cells), have the capacity to reside long term (resident cells) or be essential constituents (permanent resident cells). The fundamental permanent resident unit of a barrier tissue consists of epithelial stem cells and stromal cells (fibroblasts and endothelial cells). Other permanent resident cells include macrophages and sensory neurons. We acknowledge that there are specific cases in which cell subsets that are typically transient (such as monocyte-derived mononuclear phagocytes) can acquire the characteristics of resident cells (such as Langerhans cells or macrophages) based on environmental perturbation and niche availability. Cell types that are non-essential to the fundamental tissue unit (such as plasma cells) can also exhibit characteristics of permanent residence. Moreover, microorganisms can be permanent residents, residents or transient visitors. Abiotic stimuli, such as nutritional components, can vary in 'residence' based on the frequency and duration of environmental exposure. B_{RM} cell, tissue-resident memory B cell; IEL, intraepithelial lymphocyte; ILC, innate lymphoid cell; T_{EM} cell, effector memory T cell; T_{PM} cell, peripheral memory T cell; T_{reg} cell, regulatory T cell; T_{RM} cell, tissue-resident memory T cell.

discrete markers for the prospective isolation of epithelial cells, stromal cells and some immune cells by subset and state, limiting our understanding to broad gene expression patterns within these subsets. However, recent advances in large-scale single-cell RNA sequencing (scRNA-seq) and epigenetic profiling are now enabling inquiries into the properties and adaptations of discrete cell subsets, as well as the relationships between them[20–22] (BOX 1).

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In this Review, we focus on the distribution and storage of inflammatory memory across the cell types and subsets that form barrier tissues, highlighting recent mechanistic insights, the potential functional consequences of encoding memory and how it may be reinforced. We propose a framework to guide future experiments aimed at understanding cooperative storage of memory across cell lineages and its impact on tissue adaptation and maladaptation. Elucidating the mechanisms of collective inflammatory memory may eventually allow for new approaches to programme and reprogramme memory for infectious and inflammatory disease (BOX 2).

Components of inflammatory memory

We use the term inflammatory memory to describe the broad responses that encompass protective immunological memory and account for protective, neutral and deleterious secondary responses, regardless of cell type. Our working definition expands on the latest thinking in the field, including the definitions of immunological memory

Gene modules
Sets of co-varying genes that may be co-regulated through the activity of one or more transcription factors, or a complex thereof, often associated with a specific cell attribute such as cell type (T cell) or cell state (forkhead box P3 (FOXP3)$^+$ regulatory T cell).

Inflammatory memory
A memory response by any cell lineage to an environmental or host-derived cue, typically acquired during an immune event.

Protective immunological memory
A functionally defensive memory response that enables the host to better respond to secondary challenge after an initial exposure. This function can comprise any of the potential mechanisms that may mediate protective recall, and these same mechanisms may concomitantly or separately mediate immunopathology.

Innate immune memory
Classically defined as a memory response by a cell that is considered part of the innate immune system (for example, macrophages and natural killer cells). However, we favour the use of innate immune memory for memory events triggered by germline-encoded receptors expressed by any cell lineage.

and distinctions between adaptation and memory provided in two recent perspective articles[10,13].

We formalize tissue inflammatory memory storage into five measurable discrete components that are connected through cooperativity (FIG. 2b). The first component of tissue inflammatory memory is specificity, which refers to the recognition of an initiating stimulus, and can range from unique receptor–ligand recognition, or recognition of context-specific cues, to complete promiscuity. The second component is quantity, which generally refers to an increase in the frequency of responding cells. The third is quality, which describes the polarization of responding cells towards a specified cell state dictated by one to several genes or gene modules, or the activity of their products. The fourth component is durability, which is a measure of the time period of increased quality or quantity of a response owing to a combination of cellular and epigenetic factors. The fifth component describes its distribution, which encompasses the cell lineages, types and subsets that show intrinsic alterations in the first four components in a tissue. Finally, these five components are linked by a sixth, cooperativity, which refers to the factors that operate and communicate between cells to promote collective memory retention and recall.

Specificity, quantity, quality and durability. Immunological memory is present across all kingdoms of life. Bacteria use CRISPR arrays to restrict viral infections, plants use sensitization or priming strategies to defend local and distal tissues, *Drosophila* fruit flies deploy RNA interference and mammals rely on antigen-specific T cells and B cells[12,23–25]. Much of the recent interest in immunological memory has focused not on its functional properties but, rather, on whether the cellular and molecular mechanisms used should be classified as immunological memory within and across species[25]. Yet the unifying principle of these mechanisms is that prior exposure to a specific environmental agent induces a change to a measurable parameter (such as specificity) of the host response that persists, often in the absence of the initiating trigger[11,13,25]. Protective immunity may result from complex cell state programmes[26], or even a single gene product being more robustly produced, as is the case for interferon-induced transmembrane protein 3 (IFITM3) and infection with influenza virus[27].

Two of the main routes to memory storage are through increasing the number of specific cells present (that is, quantity) or altering the response characteristics of these cells (that is, quality; such as polarization towards type 1 T helper cell, type 2 T helper cell or type 17 T helper cell states)[9] (FIG. 2). These shifts are related to clonal expansion and epigenetic alterations (BOX 3), respectively[12,24], and involve changes to how inputs are sensed in the tissue and to subsequent outputs. However, it is important to note that changes in quantity and quality are not mutually exclusive, and both are found in hallmark examples of adaptive and innate immune memory. Thinking beyond the concept that memory is stored within adaptive or innate immune cells is essential for us to more broadly consider the functional roles of inflammatory memory in tissue adaptation[25,28–30]. It is also important to consider that the time period (durability) over which memory is useful or detrimental, and whether it is an adaptation or memory, will vary based on the specific context (such as microbial or allergen exposure in daily, weekly, monthly or yearly intervals). Finally, it will be important to define whether the presence of the initiating trigger is required to maintain adaptation or whether memory persists in its absence[10].

Distribution and cooperativity. Quantitatively and/or qualitatively enhanced responses may be distributed across multiple cell types in the relevant tissue for rapid inflammatory memory[14,31,32]. Work over the past decade has elucidated discrete lymphocyte subsets that can 'remember' specific immune events and persist long term in tissues[18,33–36]. This lymphocyte storage of memory occurs in the context of the basic mammalian barrier

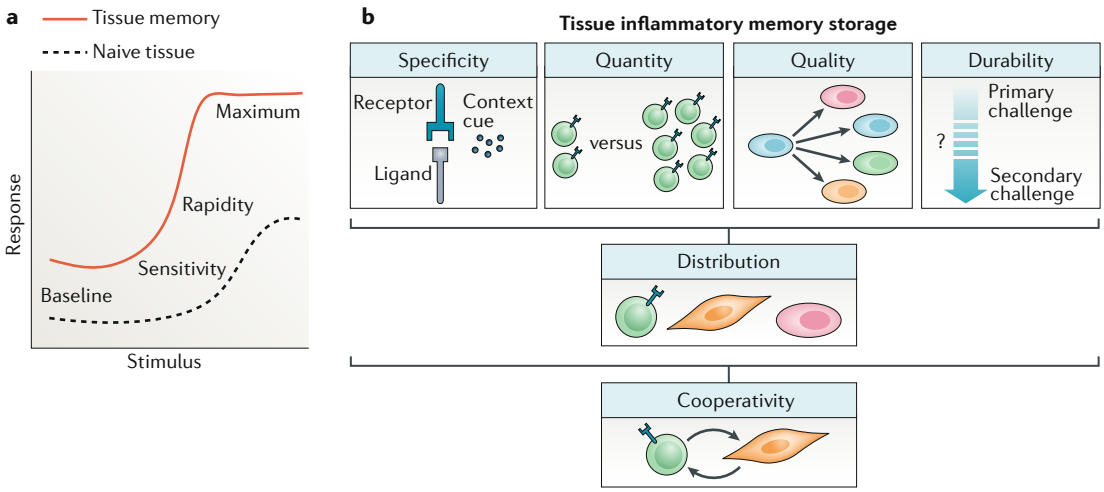

Fig. 2 | **Properties of tissue inflammatory memory storage. a** | The properties of memory in response to a stimulus include increases in the baseline, sensitivity, rapidity or maximum. **b** | The discrete components of tissue inflammatory memory storage include specificity, quantity, quality (that is, cell state), durability and distribution among various cell types. Cooperativity may exist across similar or distinct cell types, with each cell type being defined through its own discrete components. Part **a** adapted with permission from REF.[11], Elsevier.

Box 1 | **Techniques for measuring inflammatory memory**

Achieving a comprehensive understanding of the cell types and states that comprise a barrier tissue, and shifts thereto, has been technically challenging given a lack of discrete cell markers for prospective isolation. Recent advances in large-scale single-cell RNA sequencing[20–22] now enable directed inquiries into human epithelial cell adaptation, as well as deep characterization of the cellular composition of barrier tissues. Coupling these methods with validated antibody panels[173,174] will facilitate interpretation of gene expression in the context of established protein lineage markers. Furthermore, deploying recently developed methods for assessing single-cell epigenetic features, such as chromatin accessibility, methylation state, chromatin marks[175] and three-dimensional genome architecture[176], will help to elucidate underlying epigenetic mechanisms of memory, especially when coupled with judicious sample selection strategies[15] and transcriptional measurements from the same single cells[175,177]. To help position identified cell types and states within their proper tissue contexts, high-content spatial profiling methods for RNA or protein can be applied[178–182]. These end-point measures can be further coupled with live cell imaging modalities and lineage tracing[183] to elucidate the dynamic attributes of the various forms of memory, as well as perturbation methods to screen and functionally test putative memory mechanisms[184–186].

tissue unit of epithelial cells (the parenchyma), supported by stromal cells (fibroblast and endothelial cells), neurons and haematopoietic cells (macrophages)[5,14,37] (FIG. 1).

Cell types other than lymphocytes can also remember immune events. Specific examples include how macrophages can become 'trained' to adapt to inflammatory challenges[38], how fibroblasts and endothelial cells can be primed by inflammatory cytokines[39–41] and, most recently, how inflammation shapes epithelial barriers through its actions on the stem cells that give rise to them[15,16]. But how do parenchymal, stromal, neuronal and haematopoietic cells participate in the distribution and storage of tissue inflammatory memory, both independently and cooperatively[10,15,16,28,38–40,42]? In an attempt to integrate key concepts of inflammatory memory distribution and storage, we describe select examples for each cell type, beginning with those cell subsets for which there is more robust experimental evidence and ending with emergent findings on the distribution of memory. We conclude with more speculative views on the cooperative storage of memories and highlight the need for experimental approaches to test the physiological relevance of inflammatory memory cell states in tissues.

Tissue leukocytes in memory storage

Evidence for the existence and functional roles of tissue-resident memory T cells revitalized the study of tissue-resident immune cells, a field originally pioneered through the study of intraepithelial lymphocytes[17,33,35,43–45]. The initial work on tissue-resident memory cells helped to identify functional markers, such as CD69 and CD103, that are enriched within tissue-resident CD8$^+$ T cell subsets[36,46,47]. These markers have prompted more recent work characterizing the transcriptional networks that establish tissue-residency programmes and their functional contributions to tissue immunity[18,36,46]. Efforts continue to extend this paradigm to other lymphocyte subsets[36,48].

B cells and plasma cells. The production of antigen-specific antibodies by memory B cells and terminally differentiated plasma cells serves to protect epithelia from bacterial colonization and subsequent invasion[26,49]. If an antibody can neutralize an environmental agent before epithelial colonization, it may completely restrict the need to call in the next layer of lymphocytes and molecular mechanisms of defence[26] (FIG. 3). These antibodies may be synthesized locally and/or in lymphoid organs[50].

Recent work identified discrete subsets of tissue-resident antibody-producing cells. After pulmonary infection of mice with influenza virus, a subset of lung memory B cells expressing CD69 and CXCR3 could persist long term and promote faster viral clearance upon reinfection by producing IgA and IgG[51]. Furthermore, the process of selecting B cells that are adept at neutralizing viral escape preferentially occurs in long-lived germinal centres within the lungs[52], and antigen re-encounter, rather than non-specific inflammation, was shown to be important for establishing resident memory B cells[53]. Together, these studies provide evidence that the presence of memory B cells in the lung parenchyma leads to enhanced quantity, quality and rapidity of responses, resulting in enhanced protective immunological memory. However, some tissues, such as the lower female genital tract, may rely on rapidly recruited CXCR3$^+$ memory B cells, rather than resident plasma cells, for protective immunological memory[54]. Although the duration of resident memory B cell responses remains to be determined, solid-organ transplant studies in humans support the potential for plasma cells to preserve lifelong memory in the intestine[55].

Whether antibody-mediated B cell memory is productive or detrimental varies according to context. Recent theory and studies suggest that antibody-mediated protection strategies may actually support niche establishment of microorganisms that express certain antigenic determinants by selecting for preferential retention[56,57]. Paradoxically, the presence of pathogen-specific memory B cells in the lungs may even allow for influenza A virus to infect cell types other than epithelial cells, illustrating how the virus may exploit host defence strategies[58]. The presumed antimicrobial functions of specific induced antibody mechanisms require careful consideration in light of host–microorganism co-evolution[59].

T cells. If a microbial pathogen evades antibody detection and successfully infiltrates an epithelial barrier, conventional T cell-mediated effector programmes provide the next layer of specific control. After education in secondary lymphoid organs, conventional T cells gain the ability to migrate to their target tissue and extravasate from the vascular bed into the parenchyma to establish tissue residency[31,32,60–62]. These steps of tissue homing are required for circulating cells to become tissue residents and are therefore distinct from the developmental determination of permanent resident cell types, such as parenchymal, stromal and neuronal cells. The literature on tissue-resident memory lymphocytes has been extensively covered in recent reviews[36,46,47].

Experiments using mouse models for infection-independent transplantation of activated CD8$^+$ T cells directly into tissues, and the depletion of vaccination-induced circulating memory cells, have highlighted the

functions of resident memory cells in enhancing tissue-restricted infection control[36]. Pioneering studies demonstrated that antigen-specific CD8$^+$ T cells transferred directly into the skin markedly suppressed replication of herpes simplex virus, and, in a parabiotic mouse model of vaccinia virus skin infection, resident memory T cells provided 300-fold better protection than central memory T cells[33,35]. Transfer of lung memory CD4$^+$ T cells into naive recipient mice also afforded complete protection from influenza virus-induced mortality[34]. Collectively, these findings illustrate that enhancing the quantity of antigen-specific, tissue-resident CD8$^+$ or CD4$^+$ T cells may mediate more effective host protection.

Some fundamental questions that arise from these observations include what are the qualitative effector mechanisms used by these cells; how many cells are required for immunity; and how are they retained long term? The answers to these questions may be interdependent, as distinct effector mechanisms can act directly on specific target cells (such as through cytotoxicity) or affect a tissue more broadly (such as through interferon release)[17,45], and this may explain how antigen-specific stimulation can lead to heterologous immunity to unrelated pathogens[63,64]. Tonic expression of several co-inhibitory receptors may restrain the functional activation of expressed cytotoxic and cytokine gene modules, as suggested by scRNA-seq of human colonic intraepithelial CD8$^+$ T cells in healthy individuals and in patients with ulcerative colitis[65]. How effector capacity is intrinsically and/or extrinsically modulated in tissue-resident CD8$^+$ T cells will be of interest to explore further[46,47].

To understand what constitutes effective protective immunological memory, it is crucial to define the relative number of tissue-resident memory T cells required to effectively survey permanent resident cell types (such as epithelia, fibroblasts and neurons)[66,67]. Quantitative microscopy illustrated that a lymphocytic choriomeningitis virus (LCMV)-induced T cell in the small intestine may only need to scan six target cells, relative to the 80 targets it was 'responsible' for by previous estimates and that, at least after LCMV infection, 90% of memory CD8$^+$ T cells in barrier tissues are resident memory T cells[32]. The use of quantitative models to determine contact-dependent and contact-independent mechanisms across distinct lymphocyte subsets and environmental challenges will be essential to more accurately quantify surveillance capacity[67].

It is currently difficult to generalize about the requirements for the best-known key extrinsic 'residence' factors for T cells — namely, antigen, transforming growth factor-β (TGFβ) and IL-15 — as even seemingly related tissues, such as the upper respiratory tract and the lungs, appear to have different requirements[68]. Local antigen is not required for resident memory T cell programming, but it can amplify numbers of tissue-resident memory T cells by approximately 50-fold (REFS[53,69–71]). As TGFβ and IL-15 are *trans*-presented growth factors that maintain tissue-resident memory T cells[62,72], understanding the relative contributions of distinct cell sources of these cytokines will be key[73,74] (see Memory niches). Furthermore, the eliciting type of inflammation and/or antigen strength may play a role in the capacity to form CD8$^+$ and/or CD4$^+$ resident memory cells in the lungs[75,76]. It will also be important to determine how metabolism intersects with these cues, as recent work shows the importance of lipid metabolism and extracellular ATP in shaping fitness and persistence of tissue-resident memory T cells[77–79]. Other work has explored residency in human tissues, leveraging unique diseases and treatments to clarify specific mechanisms of tissue-resident memory T cells[80,81].

CD4$^+$ T cells in tissues and CD8$^+$ tissue-resident memory T cell subsets follow different rules. We speculate that non-migratory tissue-residency programmes may help to confine cytotoxic programmes to sites of previous inflammatory exposure, whereas when inflammation subsides, CD4$^+$ T helper cells may patrol new sites[33,82–86]. However, forkhead box P3 (FOXP3)$^+$CD4$^+$ regulatory T cells have been shown to exploit mechanisms for long-term tissue retention yet 'memory-less' suppressor function[87–89]. Future work is needed to elucidate how spatio-temporal aspects of tissue memory circuits relate to organismal memory[90].

These studies on tissue-resident memory T cells illustrate how the presence of newly recruited cell subsets can fundamentally invert the typical circuits within a naive tissue from the innate immune system to the adaptive immune system (FIG. 3). Recent work on CD8$^+$ T cells resident in the uterus and skin suggests that these cells autonomously dominate the secondary recall

Box 2 | **Therapeutic implications of inflammatory memory**

'Programming' memories through vaccine-induced immunological memory provided by B cells and plasma cells, and in some cases T cells, has been transformative in reducing the infectious disease burden in modern society[187]. Furthermore, the concept of trained immunity was inspired by the heterologous immunity to pathogens beyond tuberculosis afforded by Bacillus Calmette–Guérin vaccination, presumably through monocytes and macrophages[12]. Understanding how to prophylactically rebalance myeloid cell subsets in viral and bacterial diseases could yield future dividends, although it is still early in our understanding of this phenomenon[188]. Furthermore, leveraging the broader principle of tissue inflammatory memory for therapeutic aims in barrier tissues will require a better understanding of how memory is distributed and stored in parenchymal, stromal and neuronal cells to provide effective immunological memory.

Whereas acute inflammation and inflammatory memory are necessary for protective barrier tissue adaptation, chronic activation or reactivation can lead to disease pathology[6,29]. Significant deviations in the composition of cell types and cell subsets, and the emergence of unique cell states, are often seen in diseases such as psoriasis, eczema, chronic rhinosinusitis, asthma, Crohn's disease and ulcerative colitis[15,65,189]. These diseases are defined by their tissue-restricted presentation, unique genetic predispositions[190], microbial dysbioses[56,167] and context-dependent triggers, yet are unified through fundamental epithelial barrier remodelling and dysfunction that has been appreciated for decades.

For each of these diseases, the contribution of both innate and adaptive immune cells is well appreciated[49,191,192], yet therapies targeting cytokines and leukocytes are successful in only some patients[193,194], with many becoming treatment refractory, suggesting that alternative mechanisms beyond adaptive immunological memory are involved[13,31,62]. Therapies that effectively modulate resident memory leukocytes, in addition to the recruitment of transient leukocytes, will be essential for treating established disease. Furthermore, reprogramming of the distributed components of inflammatory memory in parenchymal, stromal and neuronal cell types may serve as important adjunctive therapies to restore tissue health[15]. Taken together, these approaches may also open opportunities for refining therapeutic strategies towards specific diseases in barrier tissues, rather than suppressing broad types of inflammation or recruitment to many barrier tissues simultaneously.

Box 3 | **Epigenetic mechanisms of inflammatory memory**

We note that the term epigenetic mechanisms can encompass many distinct layers of regulation: from the activity of transcription factors and non-coding RNAs to the accessibility of enhancers, the modification of histone tails and overall three-dimensional genome architecture. We favour the definition of epigenetics as the perpetuation of a transient event or signal within a cell and its progeny[195]. The sequence-specific activity of transcription factors acting on a cell's chromatin landscape can promote the stable or transient acquisition of a cell state programme[104,195,196], and this is key to specifying and maintaining examples of cellular memory through influence over chromatin accessibility and histone modifications[105,106,114,197–200].

This cellular memory fundamentally underlies the stable inheritance of developmental cell type information, tissue-specific adaptations, and the immunological and inflammatory memory considered here[10]. Cell type, cell subset and cell state gene modules, informed by this epigenetic landscape, have different capacity and propensity to be retained as memories depending on the nature of the mechanisms that enforce them. It will be of interest to further identify the conserved and unique aspects of inflammatory memory modules used by each cell lineage and the cooperativity of these transcription factors with pioneer cell identity factors and ubiquitous chromatin modifiers[46,101,104,114,201–203].

response relative to recruited effector T cells[91]. In summary, after establishment of inflammatory memory, the key sensor of an experienced tissue becomes the TCR, instead of the Toll-like receptor (TLR), and activates antiviral immunity via the output of B cells, dendritic cells, natural killer cells, keratinocytes[3,63,64] and, potentially, other resident cell subsets.

Innate lymphoid cells and innate-like T cells. The qualitative components of resident memory T cells, such as the production of cytokines in the absence of lymph node (re-)education, are often properties of the anticipatory developmental programmes of innate lymphoid cells (ILCs) and innate-like lymphocytes[48,92,93]. Furthermore, antigen-specific resident memory T cells, similarly to ILCs, can activate a generalized state of tissue alarm or alertness through the production of interferon-γ (IFNγ) acting on other resident cell subsets[63,64,91,92]. What, then, is the role of antigen-specific memory in tissues, when the qualities appear similar to those developmentally programmed into resident lymphocytes?

The distribution of memory into antigen-specific cell subsets may serve to allow for wider sensing of environmental exposures through TCRs and BCRs, together with more tailored cell state outputs than those by innate lymphocytes and/or innate-like lymphocytes[4,94]. Consistent with this idea, *Tcrd*$^{-/-}$ and *Tcra*$^{-/-}$ mice have a hyper-inflammatory phenotype in the intestinal tract, perhaps from the chronic compensatory activation of ILCs[17,94]. The extent to which antigen-specific tissue-resident memory cells can replace or extend the homeostatic roles of ILCs is an exciting area of investigation[92,93,95,96]. The progressive loss, or increased specialization towards different subsets, of ILCs and innate-like T cells with increasing immunological events in tissues may replace their generalized tissue maintenance and repair functions in favour of host defence[92,97–99].

Macrophages and dendritic cells. Work over the past few years has shown that macrophage and dendritic cell subsets share core developmental cell-type identity programmes as well as tissue-specific cell state adaptations that are driven by their local environment[5,100,101]. At the same time, investigators have developed the concept of trained immunity, whereby a primary exposure can functionally reprogramme myeloid cells to a secondary challenge with a similar or distinct pathogen-associated molecular pattern or microorganism[12,38,102]. The unifying aspect of tissue-specific identity and trained immunity is that epigenetic transcriptional response modules integrate with cell type and subset gene regulatory modules to drive the emergence of specific states that are adapted to that tissue and/or microbial challenge[10,103–106] (BOX 3). Although the importance of fetal-derived macrophage subsets relative to recruited monocytes in retaining tissue-resident memory remains to be determined, foundational work in this area highlights that myeloid progenitors in the bone marrow also become functionally reprogrammed[107–110].

The concept of trained immunity was largely inspired by two parallel, but related, experimental approaches. Original experiments with *Candida albicans* infections in mice suggested that a non-lethal dose of *C. albicans* primes organisms and protects against a future, otherwise lethal, infection in a cytokine-dependent and macrophage-dependent manner[28]. However, priming with a pathogen or a pathogen-associated molecular pattern does not always lead to an enhanced response, as is the case for lipopolysaccharide (LPS) tolerance[42]. Studying the transcriptional and epigenetic response of LPS tolerance has highlighted that, whereas some pro-inflammatory mediators are suppressed, antimicrobial effector molecules are primed, which illustrates the layered control of specific effector transcriptional modules in macrophages[42]. Intriguingly, exposure to β-glucan derived from *C. albicans* can reverse some aspects of LPS tolerance[111], suggesting contextual control over the formation and erasure of memory.

Early studies on the role of trained immunity in tissues suggest that IFNγ from T cells can directly prime alveolar macrophages, which correlate with enhanced bacterial protection after viral exposure[107]. Current work is also examining how environmentally responsive transcription factors (such as PPARγ) and cytokine-responsive transcription factors (such as STAT6) cooperatively modify macrophage cell states[112]. It will be of interest to determine how macrophage-mediated trained immunity operates within the broader context of tissue immunity.

Non-leukocytes in memory storage

Whereas tissue residency is an acquired cell state of an effector lymphocyte or monocyte, it is a developmental property of epithelial, stromal and neuronal cell types[46,113,114]. Across all four cell lineages, if a cell (or its progeny) can persist between the initial immunological event and subsequent recall, then it may have the capacity to meaningfully contribute to collective inflammatory memory. Of course, there may also be instances of bystander effects whereby alterations occur in a cell lineage without impacting subsequent recall. Here, we consider recent insights into epithelia-retained inflammatory memory, which poses an intriguing problem regarding how long-term storage of information could

Lipopolysaccharide (LPS) tolerance
Macrophages exposed to sustained stimulation with LPS or high-dose LPS acquire a hypo-responsive state in which sets of inflammatory genes are blunted in their secondary response to LPS or other inflammatory cytokines.

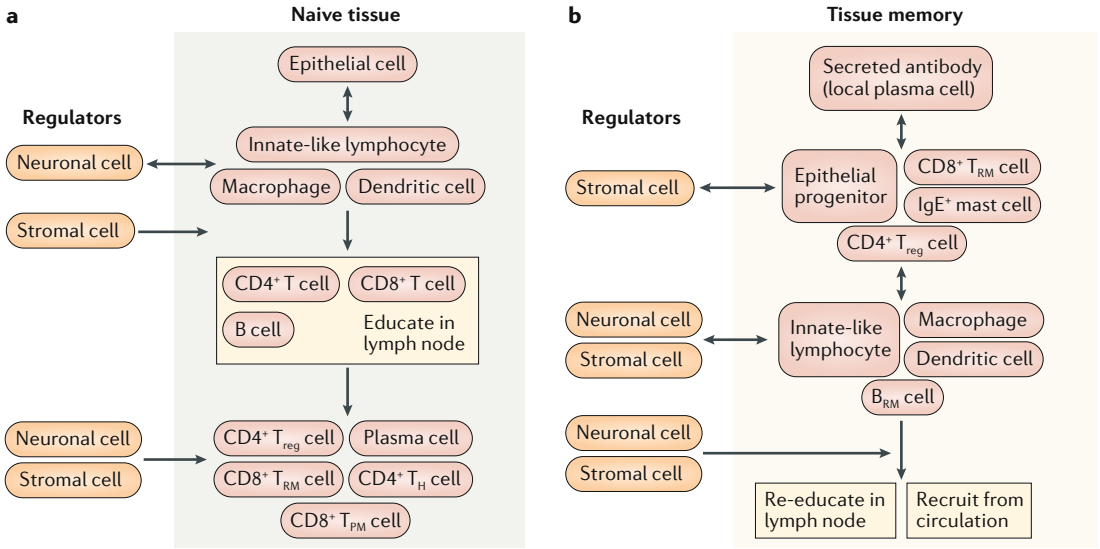

Fig. 3 | **Inflammatory memory alters circuits in barrier tissues.** The flow of information through a naive tissue (part **a**) or a hypothetical experienced tissue (part **b**), in which memory has been established, with key regulators at each step highlighted on the side of the vertical flow. Arrows indicate the predominant direction of information flow. By establishing memory in specific cell subsets, such as antibody-producing plasma cells, resident memory $CD8^+$ T cells and epithelial progenitors, the typical flow of information can be 'inverted' in a tissue that has inflammatory memory. B_{RM} cell, tissue-resident memory B cell; T_H cell, T helper cell; T_{PM} cell, peripheral memory T cell; T_{reg} cell, regulatory T cell; T_{RM} cell, tissue-resident memory T cell.

be achieved within a rapidly regenerating compartment, as well as recent insights into the contribution of stromal and neuronal cells to inflammatory memory.

Epithelial cells. The architecture of stratified (skin), pseudo-stratified (airways) and monolayer (lungs and gut) epithelia are distinct, yet all are composed of specialized epithelial cell subsets that collectively perform tissue-essential functions. The production of these cell types is regulated at the level of multipotent epithelial progenitor and stem cells[113,115–117]. This design is crucial for the epithelial development, turnover (in the order of weeks to months) and response to induced demands[5–7,118]. If immunological events are to be remembered by epithelia directly, the progenitor compartment would be the prime candidate for storage, as memory assigned to terminally differentiated cells could be rapidly lost.

Groundbreaking insights came from the analysis of dietary regulation of progenitor cell function in the intestine. In this study, mice given a high-fat diet showed increased stemness of both intestinal stem cells and progenitor cells, such that the stem cells became independent of their normal requirement for a Paneth cell niche in organoid-forming assays[119]. Furthermore, fatty acid-sensing transcription factor PPARδ was seen to drive elements of the WNT pathway and enhance stemness and tumorigenicity of intestinal progenitors. The enhanced stemness of organoids grown from the epithelia of mice given a high-fat diet persisted across multiple passages, suggesting differences in the output of mature epithelial cells and a potential cellular memory component of stemness that was retained ex vivo[119].

Another pioneering study in this area highlighted that a brief period of psoriasis-like inflammation could fundamentally alter skin epithelial stem cells to more rapidly repair a subsequent wound at that site[14,16]. The contributions of skin-resident macrophages or T cells were formally excluded from this process and, instead, the AIM2–caspase 1–IL-1β axis seemed to be essential for implanting and recalling this memory at a later date[16]. The inflammatory memory state in progenitor cells persisted for at least 180 days, showing durability of the response.

Identifying non-immune cellular mechanisms that might sustain human inflammatory disease at barrier tissues, another recent study uncovered striking changes in epithelial cellular diversity and mature functional cell types by performing scRNA-seq of samples from patients with chronic rhinosinusitis[15]. Intriguingly, this study found that alterations in mature cells were driven by the type 2 cytokines IL-4 and IL-13 acting directly on basal airway epithelial progenitor cells, shifting their cell state and restraining their differentiation capacity. Some of these inferred type 2 cytokine signatures determined in vivo could be propagated through long-term in vitro culture and expansion of sorted human basal progenitor cells, suggesting that these cells could serve as repositories for allergic inflammatory memory[15].

Taken together, these studies indicate that memory of immune events can be integrated by epithelial progenitors through diverse sensors, altering their recall responses and functional outcomes across distinct barrier tissues and species[15,16,119] (FIG. 3). These studies have yet to address, however, how memory may be preferentially retained within heterogeneous progenitor cell subsets[120]. Furthermore, it remains to be determined how inflammatory memory retained by epithelial cells affects stress-induced ligands, which interact with innate-like T cells, or chemokines that recruit conventional T cells,

and potentiate cooperation[45,47,121]. Notably, these studies of inflammatory memory by epithelial stem cells reveal putative transcription factors on the basis of increases in the accessibility of specific chromatin regions and draw parallels with the mechanisms of transcriptional maintenance observed in myeloid and lymphoid immunological memory[12,28,47,103,106,122,123]. It is tempting to speculate that genetically encoded susceptibility to disease may modify the epigenetic landscape within disease-relevant cell states at baseline to alter predisposition[65].

Stromal cells. Stromal cells include fibroblasts and vascular and lymphatic endothelial cells. They are essential constituents of barrier tissues that provide structure, conduits for cell migration and developmental cues to surrounding cell types[31,124]. Furthermore, stromal cells have key immunological functions, are pathognomonic in various settings and can lead to the establishment of fibrotic disease[125]. Of note, experiments performed using type I interferons provided the initial evidence illustrating cytokine-induced priming of subsequent responses, and the underlying mechanisms were further investigated, in large part, with fibroblasts[126,127].

A recent review[39] elegantly unifies recent experimental evidence of how stromal cells can also exhibit characteristics of inflammatory memory, and we distil select elements here. Most studies have investigated synovial fibroblasts, isolated from inflamed or non-inflamed joints, and their enhanced responsiveness after priming to stimuli such as tumour necrosis factor and LPS[40,128,129]. Intriguingly, synovial fibroblasts, unlike macrophages or endothelial cells, did not show LPS tolerance, illustrating how distinct cell types may differentially regulate identical genes to facilitate a future stereotyped or learned response[40,128–130]. Extending preliminary insights obtained from scRNA-seq studies to determine whether specific human disease-associated fibroblast subsets preferentially retain more complex memory cell states will be of great interest[65,131].

Neurons. Although the cell bodies of neurons do not reside within epithelial barrier tissues, their processes densely innervate them[2,132]. Recent work showed that autonomic and peripheral sensory neurons can exert substantial regulation of immunological processes at barrier tissues through directly interfacing with specific immune cell subsets[2,133,134] (FIG. 3).

One study highlighted that a specific subset of heat-sensing neurons in the skin drives IL-23 production from dermal dendritic cells, leading to IL-17 production from dermal γδ T cells, and promoting psoriasis-like inflammation[132]. Intriguingly, this pathway can be triggered solely by activating neurons optogenetically and is beneficial for host defence during *C. albicans* skin infection[135]. Further work on understanding painful bacterial infections emphasized important roles for pain-sensing neurons in directly detecting bacterial toxins and regulating the inflammatory response to intradermal infections[136,137]. There has also been particular interest in studying ILCs, where investigators have identified several distinct means of neuroimmune interaction, effectively placing a new sensory and regulatory layer above the typical innate-like functions of ILCs at barrier tissues[133,138].

Is there a memory of painful exposures that can be recalled to inform future immunological encounters? Although the aforementioned circuits have not been explicitly tested for memory formation, recent evidence suggests the possibility that activating cell bodies or peripheral axons of neurons can influence both avoidance behaviour and anticipatory peripheral immune responses, even in the absence of other inflammatory cues[135,139,140].

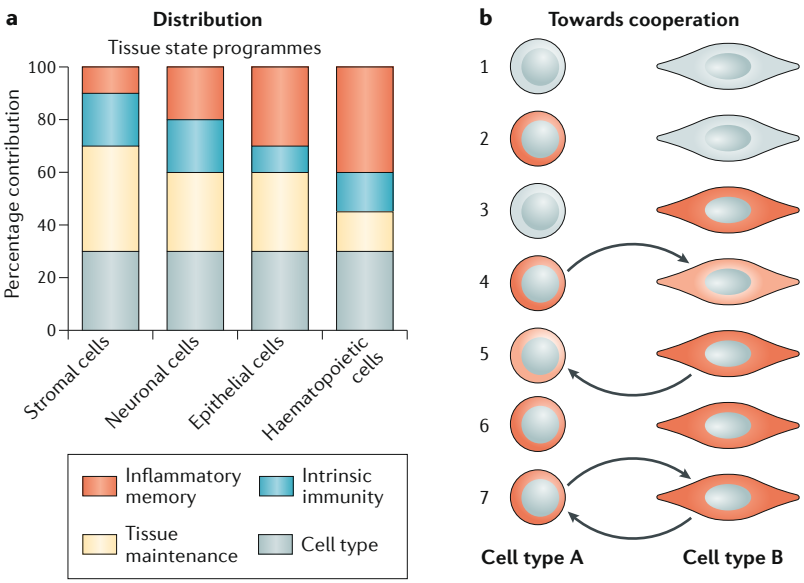

Fig. 4 | **Distribution and cooperation in tissue memory. a** | A schematic illustrating that stromal, neuronal, epithelial and haematopoietic cells can contain distinct combinations of cell state modules that define their cell type, typical role in tissue maintenance, intrinsic immune capacity and inflammatory memory capacity. The presented values reflect hypothetical relative distributions. **b** | Potential distributed inflammatory memory states for a system comprising two cell types: (line 1) no memory; (lines 2, 3) one cell type, or the other, has inflammatory memory; (lines 4, 5) one cell type, or the other, has inflammatory memory that partially influences the other cell type; (line 6) both cell types collaborate in forming inflammatory memory; or (line 7) both cell types, through cooperation, can mutually reinforce one another.

Distribution towards cooperative memory

Cooperativity is a common phenomenon in biology, acting as a crucial regulator of interactions at the molecular, cellular and species levels. Importantly, cooperation can lead to the emergence of higher-order functions that would not have been possible for the individual component[4,5,56,141]. This specialization is well appreciated as a facilitator of cell type and tissue evolution in metazoans. However, our study of immunological memory has largely focused on the individual components, rather than on their collaborative actions (multiple cell subsets working independently) or cooperative actions (multiple cell subsets working together). Here, based on recent evidence suggesting that multiple cell types distribute memory storage, we propose that inflammatory memory can be retained in cooperative cell circuits (FIG. 4). These circuits may help to reinforce cell states and/or promote cell residence in tissues.

Intercellular circuits. Looking beyond the intrinsic functions of each cell's state, we ask how distinct lineages might collaborate or cooperate towards the larger goal of barrier tissue adaptation (FIG. 4). Epithelial cells have long been appreciated as producers of cytokines and chemokines during barrier tissue challenges[9,49]. In particular, during type 2 immunity, epithelial cells can release the instructive cytokines thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 (REF.[49]). Importantly, IL-33 expression by basal cells isolated from human donors with chronic obstructive pulmonary disease can be sustained long term[142]. Together, this work has highlighted that information flow occurs from the epithelium to lymphocytes and myeloid cells.

Only recently has it been appreciated that immune effector cytokines can act directly on permanent residents (that is, stem cells) of the epithelial barrier[14]. In pioneering work, ILC3-derived IL-22 was shown to influence tissue stem cells in the intestine and promote tissue regeneration[143]. More recent work in human ulcerative colitis links $CD4^+$ T cell-derived IL-22 with enterocyte fate[65]. Although IL-22 is generally beneficial owing to its pro-regenerative activity, it may occasionally be detrimental, depending on its cell source and inflammatory context[94,143,144], shifting epithelial cell states away from normal metabolic function and towards tumorigenesis[94,145].

Direct interactions also facilitate information flow. Such interactions have been revealed in greater detail, for example, through the application of scRNA-seq to generate an atlas of the mouse small intestine, highlighting which specific subsets of stem cells and differentiated cells express MHC class II molecules[116,120,146]. Expression of MHC class II and antigen presentation machinery was increased in cycling intestinal stem cells, reduced in enterocytes and absent in secretory cell subsets[120]. By testing lineage-defining cytokines from T helper cell subsets, the authors found that IL-17 was associated with an increase in transit amplifying cells, IL-13 with increased tuft cells and IL-10 with increased stem cell self-renewal[120].

Recent work also identified discrete circuits between epithelial instructive cytokines and lymphocyte effector cytokines in type 2 immunity[147–149]. This work showed that specialized secretory epithelial cells known as tuft cells are responsible for the production of IL-25. Helminth infection enhances tuft cell IL-25 production, leading to an increased abundance of both $IL\text{-}25^+$ tuft cells and $IL\text{-}13^+$ ILC2s[147]. Follow-up work clarified that dietary-derived or parasite-derived succinate can also activate this circuit[148,149]. It will be interesting to determine the points at which homeostatic and induced circuits exhibit long-term adaptation or memory responses in one, or both, of these cell types.

Memory niches. Although we know some of the cytokines, chemokines, growth factors and adhesion molecules that are important for the maintenance of resident memory T cells and B cells, the cellular sources of these factors remain incompletely defined. Furthermore, the way in which inflammation shapes their expression by distinct cell lineages has not been fully explored. We highlight some vignettes into how niches are formed in barrier tissues and speculate about how memory in niche cells of any lineage may alter the size or type of memory that is retained.

IL-15, which is a key maintenance factor for intraepithelial lymphocytes, seems to be synthesized by specific subsets of hair follicle-proximal keratinocytes[150–152]. IL-15 maintains both innate-like T cells and subsequent waves of recruited resident memory $CD8^+$ T cells[46,62,72]. This creates another interesting problem: how are niches reallocated to accommodate newly recruited T cells? Early work in this area highlighted how, upon inflammation, the network pattern of dendritic epidermal T cells and Langerhans cells present in mouse epidermis at steady state becomes altered and more complex through their replacement by resident memory $CD8^+$ T cells[153]. Similarly, a recent study into coeliac disease illustrated how chronic inflammation can reshape the epithelial niche for innate-like γδ T cells, which are usually sustained by butyrophilin-like 3 and butyrophilin-like 8 molecules in homeostatic conditions, leading to the entry of gluten-reactive, IFNγ-producing γδ T cells[154].

The total storage capacity of tissues for distinct antigen-specific resident memory T cell populations has not been formally explored, but early studies suggest that the niche may be capable of accommodating several waves of recruitment without displacement[155]. Moreover, researchers are starting to consider how inflammatory cues during subsequent waves of tissue damage either help to retain and reinforce or displace and reprogramme existing memory niches. Indeed, extracellular ATP was shown to preferentially deplete bystander resident memory T cells in the tissue to free up niches for new infection-relevant specificities[79]. Stromal cells will likely play a central role in niche formation[156]. Exaggerated niche structures known as ectopic lymphoid follicles can form in the lungs via type I interferons, leading to CXCL13-mediated support of germinal centre reactions[157]. Studies exploring how cellular circuits regulate relative cell numbers during homeostasis and disease will help to inform exploration of this important topic in tissues, with single-cell approaches enabling this line of inquiry[37,65].

It will be interesting to determine whether ILCs and innate-like T cells serve as essential early settlers in a tissue to preserve future niches for adaptive lymphocytes, in addition to their sentinel role[92]. Competition for niches between embryonic or adult-derived macrophages may also be relevant in trained immunity. Whereas, in some cases, cells of the same type may compete for niches, distinct cell types can also form cooperative arrangements to maintain memory[108]. In some barrier tissues, myeloid cells appear to be instrumental for the preservation of memory within the sub-epithelial compartment, for example, through production of the chemokine CCL5, which retains clusters of resident memory $CD4^+$ T cells in the genital mucosa[158]. There is also evidence for cooperation between closely related cell subsets, whereby the presence of IFNγ-producing $CD4^+$ T cells helps $CD8^+$ T cells enter the epithelium[159]. Even the earliest work on resident memory $CD8^+$ T cells illustrated the potential

Tuft cells
Rare chemosensory epithelial cells with a 'tuft-like' brush of microvilli present in epithelial (primarily mucosal) tissues of mammals, characterized by expression of taste receptors and production of instructive allergic inflammatory cytokines.

Dendritic epidermal T cells
γδ T cell receptor-expressing cells selectively localized in the epidermis that have been identified in rodents and cattle, but not in humans. In mice, essentially all dendritic epidermal T cells express the same T cell receptor constituting a prototypical innate-like T cell.

for cooperativity between CD4$^+$ and CD8$^+$ T cells in local immunity[33].

Beyond internal cues, tissue-resident cells may also integrate cues from the external environment into their intrinsic cell state programmes[160]. Some cell subsets even require an environmentally derived ligand for persistence. For example, aryl hydrocarbon receptor ligands, such as those derived from cruciferous vegetables in the intestine, are essential to maintain both intraepithelial innate-like T cells and some conventional CD8$^+$ T cell subsets[161]. Similarly, microorganism-specific T cells adapt their residency programme to balance effector and immunoregulatory functions in response to their microbial environment[93,162,163]. Comparing wild or 'pet store' mice with specific pathogen-free mice will be essential to determine how environmental factors are stored as transient adaptations or long-term memory[164]. Using TCR-transgenic systems and swapping antigens across bacterial species illustrated that microbial specificity, rather than antigen, dictates the resultant T cell effector state[165,166]. More broadly, abundant products of colonic microbial fermentation — that is, short-chain fatty acids — have been shown to promote the production of peripheral regulatory T cells[167] and also the antimicrobial function of macrophages[168]. How diet, leukocytes and microorganisms intersect to impact the quantity, quality and distribution of memory will be of interest to explore further[169].

Challenges and future perspectives

The concept of innate and adaptive immunity has been, and will continue to be, essential to establish a framework for how immune responses are initiated, maintained and remembered[9,170]. Here, we extend recent theory and data to suggest that most, if not all, cells in tissues can adapt to, and potentially remember, immunological events[12,25,28,30]. The process of inflammatory memory is fundamentally concerned with promoting tissue adaptation to environmental exposures during homeostasis, maintenance and disease settings. This process draws from any of the cell types, subsets and states that may be available at the time of exposure, and the specific molecular mechanisms that each individual cell has within its repertoire. Even molecular structures associated with classical adaptive responses, such as the TCR and the BCR, have hard-wired 'innate' functions that are deployed in advance of, or in parallel with, their 'adaptive' properties[171,172].

One additional area for further investigation relates to the durability of tissue memory, and this will require rigorous definitions of what constitutes short-term and long-term adaptation relative to memory responses[10]. In simple terms, durability reflects a combination of factors, including the stability of inherited memory within a cell, the lifespan of that cell and the ability of that cell to propagate its memory to progeny within a barrier tissue. We highlight that, by necessity, the two longest-lived cell types in a barrier tissue must be its adult epithelial stem cells and underlying stroma. Furthermore, the dense innervation of barrier tissues, in conjunction with the long-lived nature of sensory and autonomic neurons, raises the possibility that neuron-encoded memory might be able to influence subsequent immune responses in tissues[2]. Determining the mechanisms of inflammatory memory will allow for experimental systems to rigorously test cell states for their potential relevance to tissue adaptation and maladaptation. In particular, it will be of interest to determine the duration, distribution and interaction of these mechanisms across cell lineages, relative contributions to specific responses and how they are shaped by host and environmental factors[15,29]. Improving the throughput and cost-effectiveness of techniques to map tissues will provide new opportunities to programme and reprogramme inflammatory memory formation prophylactically and during disease.

Does a particular type of inflammation preferentially allow residence and maintenance for the same polarized T cell type? It remains to be seen how the diversity of immunological experience in a tissue influences the specificity, quantity, quality, durability and plasticity of memory storage. This will likely be dictated by whether memory is truly cell intrinsic or stored in cell-level or tissue-level cooperative circuits, as well as the overall capacity of the tissue for information flow. Furthermore, how the increased specificity of responses to immune events following memory formation intersects with the repair and regenerative capacity of barrier tissues remains to be systematically explored. Although there is an overt emphasis here on local immunity, it is also important to remember that redistributing memory through migration to new sites within the same tissue, or between tissues, is essential for overall organismal health.

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