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Review article

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Assessing the generation of tissue resident memory T cells by vaccines

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Abstract

Vaccines have been a hugely successful public health intervention, virtually eliminating many once common diseases of childhood. However, they have had less success in controlling endemic pathogens including *Mycobacterium tuberculosis*, herpesviruses and HIV. A focus on vaccine-mediated generation of neutralizing antibodies, which has been a successful approach for some pathogens, has been complicated by the emergence of escape variants, which has been seen for pathogens such as influenza viruses and SARS-CoV-2, as well as for HIV-1. We discuss how vaccination strategies aimed at generating a broad and robust T cell response may offer superior protection against pathogens, particularly those that have been observed to mutate rapidly. In particular, we consider here how a focus on generating resident memory T cells may be uniquely effective for providing immunity to pathogens that typically infect (or become reactivated in) the skin, respiratory mucosa or other barrier tissues.

Sections

Introduction

T cells in tissue and T_{RM} cell biology

Vaccines: can they efficiently generate T_{RM} cells?

Challenges and future directions

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Introduction

Vaccines are one of the most effective and inexpensive public health interventions after clean water and hand hygiene[1,2]. They have been instrumental in the elimination of polio in the United States and in the eradication of smallpox worldwide[3]. Vaccine immunogenicity has historically been judged by antibody titres, which are often relied upon as a surrogate marker of protection[4]. This is based on their proven clinical association with protective immunity and the ease, speed and reproducibility of such assessments across laboratories. Antibody assays require small volumes of blood, remain stable over time with banked sera and are readily commercialized. Efforts to understand the role of T cell immunity elicited by vaccines have generally focused on T cells circulating in peripheral blood, and these studies are technically much more challenging and involve many more variables[5,6]. In the present Review, we focus on whether a recently described subset of memory T cells — namely tissue resident memory T cells (T_{RM} cells) — may also have a key role in vaccine-induced protective immunity.

Although sampling blood provides a useful approximation of systemic humoral immunity, the recent appreciation that most memory T cells reside in peripheral tissues highlights the need for better characterization of immune cells within tissues and organs[7]. There is a greater awareness of the subsets of memory T cells that do not recirculate, namely T_{RM} cells, and a growing appreciation of their role in immune homeostasis and protection[7–11]. T_{RM} cells and other T cells in tissues greatly outnumber circulating T cells; for example, each square centimetre of human skin is home to one million T cells, and phenotypical analyses suggest that more than 50% of these are T_{RM} cells, making skin T_{RM} cells twice as abundant as circulating memory T cells in the peripheral blood[10]. T_{RM} cells and other tissue T cells also provide rapid and potent recall responses in the skin and mucosal tissues[12]. However, obtaining useable numbers of T cells from tissue in patients is not straightforward and most methods are not available outside a handful of academic laboratories, posing challenges for studies in humans. Much of what we know about T_{RM} cells has come from mouse models, which for many reasons are only partially translatable to human biology[8,10,13,14]. In this Review, we first highlight some key features in the biology of T_{RM} cells. We then discuss how to enhance their role in vaccine-induced immune protection, and propose that a focus on tissue T cells, including T_{RM} cells, should be more routinely integrated into early-stage vaccine research and development.

T cells in tissue and T_{RM} cell biology

T_{RM} cells were initially described, somewhat provocatively, in the setting of systemic viral infection[15,16]. Although it was already known that effector memory T cells (T_{EM} cells) are found in peripheral tissues, T_{RM} cells are a distinct subset of memory T cells characterized by long-term residency in peripheral non-lymphoid tissues. T_{RM} cell populations from different tissue sites have been shown to share a core gene expression profile, as well as to have tissue-specific differences in gene expression[17–21]. Other memory T cells that are found in the extravascular space of tissue are heterogeneous; in human skin, T cells with relatively shorter and longer 'dwell' times in skin have been identified[22]. These non-T_{RM} cell populations eventually exit the skin and enter peripheral blood. This is in contrast to almost all T_{RM} cells, which remain as long-term residents within the tissue[23]. Both $CD4^+$ and $CD8^+$ T_{RM} cell populations have been described in skin, with $CD8^+$ T_{RM} cells associated with antiviral immunity and $CD4_+$ T_{RM} cells more closely linked with immunity to bacteria and fungi[24–26]. A comparison of the transcriptional profiles of resident and circulating T cells from multiple tissues demonstrated that T_{RM} cells from sites across the body (for example, lungs, skin and gut) have a core conserved transcriptional signature that distinguishes them from both T_{EM} cells and central memory T cells (T_{CM} cells), although the markers originally used to discriminate these cells from each other nearly 20 years ago have limitations[17,27,28]. The transcriptomes of T_{EM} cells and T_{CM} cells reveal that these cell types more closely resemble each other than they do T_{RM} cells[17,20,29]. Their anatomic location allows T_{RM} cells to act as immunological sentries, functioning as 'alarm' cells that are programmed to persist in tissues and elicit a rapid recall immune response upon antigen encounter[30,31] (Fig. 1). Fundamentally, they have been shown to provide enhanced immunity against re-infection and to accelerate pathogen clearance[32,33].

Identification of T_{RM} cells by surface phenotype has been debated for some time, and certain markers that are closely associated with T_{RM} cell biology (for example, CD69 and CD103) are neither universally nor continuously expressed by T_{RM} cells[34]. One controversy regarding T_{RM} cells as originally defined is the absence of recirculation once they have taken up tissue residence[8,11,35,36]. Although this can be measured in mouse models by parabiosis or treatment with trafficking inhibitors, it is considerably more difficult to assess in humans. More recently, this property of indefinite residence has been challenged; there are reports of (formerly) T_{RM} cells leaving tissue and entering the circulation[37,38]. Whether T_{RM} cells represent a terminally differentiated population that cannot leave tissue or whether there is built in plasticity to reverse some elements of the T_{RM} cell 'programme' is increasingly debated[39]. It could be argued that the original requirement that T_{RM} cells can 'never' migrate out of tissue does not allow for the potential for plasticity of biological systems. A better question is under what conditions certain T_{RM} cells can leave tissue, and what adaptive immune advantage is conferred. We will attempt to address this question below.

Development of T_{RM} cells

T_{RM} cell development continues to be studied extensively. Two general models of T_{RM} cell development from naive T cell precursors have emerged, which for simplicity we will term the 'local divergence' and the 'systemic divergence' models (Fig. 2). The local divergence model proposes that pluripotent effector T cells enter tissues and are influenced by local signals to differentiate into T_{RM} cells and establish long-term tissue residence[22,27,29,40]. In contrast, the systemic divergence model proposes that there is a subpopulation of circulating effector T cells in blood that are already poised to enter the tissue and differentiate into T_{RM} cells. In this model, a subset of T cells are preconditioned to have greater capacity to migrate into inflamed tissue and to respond to the environmental signals within the tissue that drive T_{RM} cell differentiation[27,39,41–44]. Upon reflection, these two models need not be mutually exclusive. Evidence supporting the notion that precursors of T_{RM} cells undergo their maturation after antigen encounter and, in addition, differentiate further in peripheral tissues includes reports on the common clonal origin of T_{CM} cells and T_{RM} cells (bearing the same CDR3 sequence as assessed by high throughput sequencing) following skin immunization[42]. It remains unknown exactly which factors prime some incompletely differentiated effector T cells to acquire a T_{RM} cell identify when exposed to tissue microenvironments, although transforming growth factor-β (TGFβ) appears to be a dominant signal[19]. It is also not clear why other effector T cells do not respond to these tissue factors and, instead, develop into mature circulating T_{EM} cells and T_{CM} cells or else undergo apoptosis in the tissue. It is unlikely that this differentiation process is simply stochastic. A recent study using single-cell RNA sequencing analysis of gut T_{RM} cells over time was unable to identify

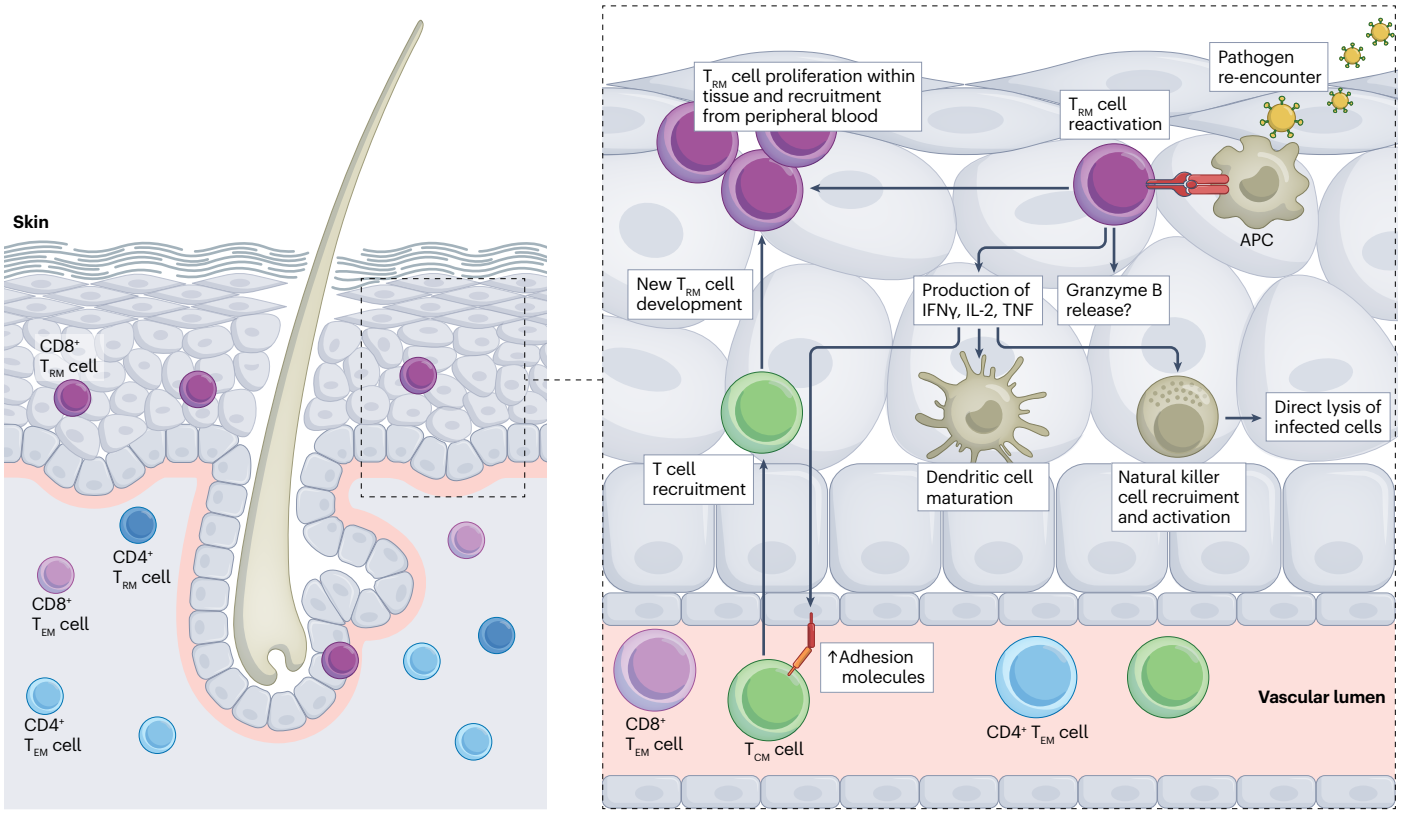

Fig. 1 | CD8$^+$ T_{RM} cell reactivation upon secondary pathogen encounter. Tissue resident memory T cells (T_{RM} cells) are uniquely positioned within tissues to respond rapidly to pathogen re-encounter, and this response is multifaceted. Upon recognition of cognate antigen, which is presented to them by epithelial cells or antigen-presenting cells (APCs), CD8$^+$ T_{RM} cells rapidly secrete inflammatory cytokines (for example, IFNγ and TNF). The downstream effects of these cytokines include upregulation of adhesion molecules on endothelial cells (such as VCAM1, ICAM1 and E-selectin) and expression of chemokines, which in turn facilitates recruitment of circulating lymphocytes (B cells and T cells), dendritic cell maturation, and the recruitment and activation of natural killer cells. In addition, some T_{RM} cells express high levels of granzyme B at baseline, leading to direct lysis of infected host cells. Reactivation leads to T_{RM} cell proliferation within tissues as well as recruitment of peripheral lymphocytes that become T_{RM} cells. T_{CM} cell, central memory T cell; T_{EM} cell, effector memory T cell.

circulating precursors of T_{RM} cells before the effector T cells entered the tissue[45], supporting the concept of local divergence. In favour of the systemic divergence model, a subset of phenotypically distinct circulating mature T cells have been identified; these are presumed to be dedicated T_{RM} precursor cells as they share more than 90% of the transcriptional profile of authentic T_{RM} cells in tissue[39,46]. This subset is made up of T cells that differ from each other transcriptionally in accordance with the tissue to which they home[27,39,46,47]. It is proposed that these T_{RM} cell precursors in peripheral blood have been imprinted with tissue-specific homing molecules shortly after antigen encounter in the lymph node[48]. A model that is a compromise between these two extremes proposes that in skin (for example), activated dendritic cells of the classical DC1 subset that express TGFβ migrate to draining lymph nodes and cross-present antigens to activate naive T cells[47,48]; these activated T cells divide asymmetrically, with some progeny being directed towards a T_{RM} cell programme[19] whereas others retain plasticity and become T_{CM} cells[42], including some T_{CM} cells expressing skin-homing markers[49]. Once resident in tissue, T_{RM} cells are poised to participate in host defence. It is increasingly appreciated, however, that some T_{RM} cells may develop the capacity to respond to antigenically related autoantigens, contributing to autoimmune disease in joints and skin, either spontaneously or after therapy with immune checkpoint inhibitors[8–11,50–52].

What defines a T_{RM} cell?

Retention of T_{RM} cells within tissues is a precondition for residency and is mediated by a combination of variables. Some of the first changes are downregulation of the transcription factor Kruppel-like factor 2 (KLF2) and upregulation of CD69 on T cells within the destination tissue[20]. Both KLF2 and CD69 mediate tissue retention of developing T_{RM} cells via their actions on the sphingosine-1-phosphate receptor 1 (S1PR1), which under normal conditions promotes cell egress from tissues via efferent lymph[8,53]. More recently, a related receptor S1PR5 has also been implicated in enforcing tissue residence[54]. In addition to CD69, many T_{RM} cells are positive for CD103 (also known as αE integrin)[27]. In skin, most CD8$^+$ T_{RM} cells are CD103$^+$; CD103$^-$ T_{RM} cells are fewer in number and more motile[9,27,36]. However, as noted earlier, T_{RM} cell surface markers are not uniformly expressed in all tissues. CD103 expression may be limited to certain tissues and cell subsets as CD103$^-$ T_{RM} cells have been found in tissues such as the brain[55,56]. In addition, co-expression of CD69 and CD103 does not guarantee tissue residency, highlighting the importance of parabiosis and intravascular labelling and other

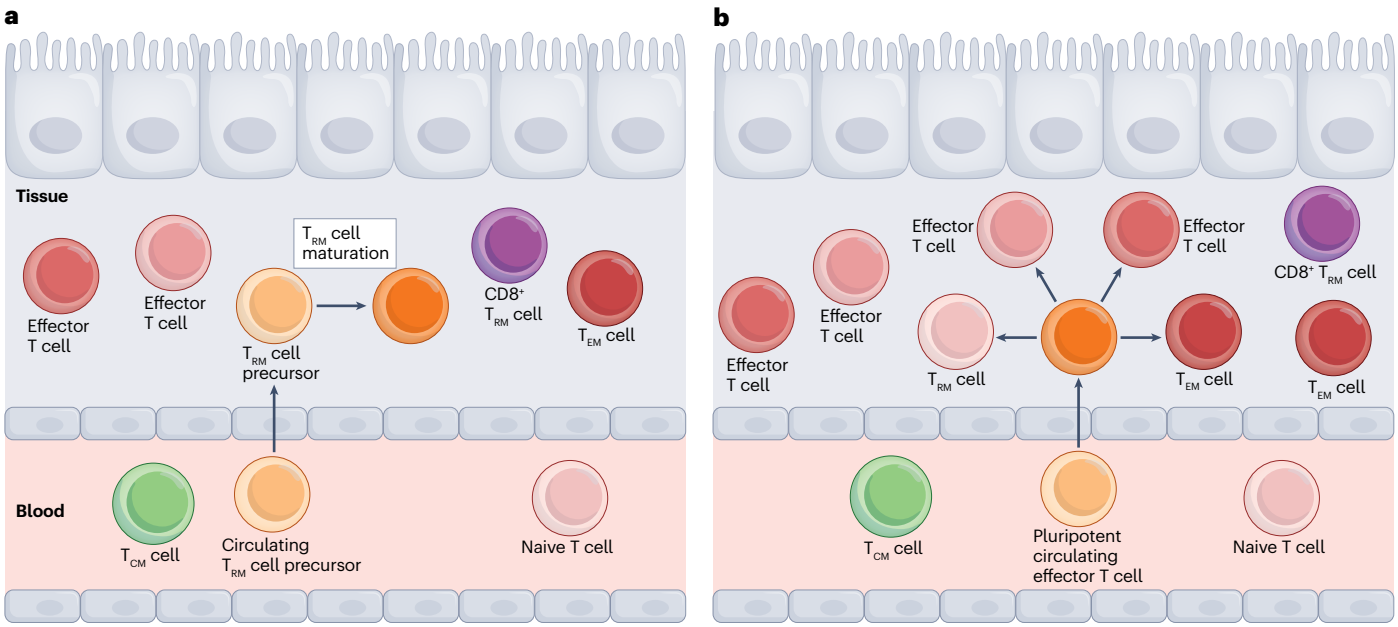

Fig. 2 | Two models of T_{RM} cell divergence. a, Recent studies propose there is a population of 'circulating T_{RM} cell precursors' that are phenotypically distinct from central memory T cells (T_{CM} cells), effector memory T cells (T_{EM} cells) and tissue resident memory T cells (T_{RM} cells). The transcriptional profiles of these putative circulating T_{RM} cell precursors resemble those of T_{RM} cells, and the cells are thought to be destined to become T_{RM} cells. This is in line with the model of systemic divergence. **b**, Other data support the theory of local divergence of T_{RM} cells, in which T_{RM} cells differentiate within tissues from multipotent or pluripotent effector T cells in the early stages of the immune response. These two theories should be viewed as potentially complementary, and that there may, in fact, be significant overlap between these theories in T_{RM} cell development in vivo.

functional techniques in determining the true nature of residency[26,57]. A recent study examining T_{RM} cell development over time with single-cell RNA sequencing found that genes associated with T cell receptor (TCR) activation (for example, *Nr4a2*) and AP1 dimerization partners (for example, *Junb*, *Fosl*) were associated with development of gut T_{RM} cells in mice after infection, even at very late time points, but it is unknown whether this is generalizable to other tissues[45].

The respective tissue microenvironment almost certainly plays a significant role in T_{RM} cell differentiation and maintenance, and transcriptional analyses indicate heterogeneity in gene expression signatures across T_{RM} cells in different tissues[8]. It is tempting to speculate that different tissue microenvironments act via distinct signalling pathways to drive T_{RM} cell differentiation and maintenance, thus leading to the generation of different T_{RM} cell populations throughout the body[18,19,58,59]. It remains unclear whether these populations contribute to pathogen control in different ways, or whether different pathogens in the same tissue generate different T_{RM} cell populations. The tissue architecture of the microenvironment may also influence the location of T_{RM} cells within a tissue. In the skin, for example, $CD4^+$ T_{RM} cells reside predominantly in the dermis, whereas $CD8^+$ T_{RM} cells localize to the epidermis. CD103 staining is far more intense in the $CD8^+$ T_{RM} cell population[8,60]. In the epidermis, $CD8^+$ herpes simplex virus (HSV)-reactive $CD103^+$ T_{RM} cells display a crawling dendritic cell-like migration pattern, probing keratinocyte junctions in a manner that suggests active surveillance for infected keratinocytes[61]. In the lungs, the relative abundance of extracellular matrix components may influence T_{RM} cell localization. It has been proposed that $CD4^+$ T_{RM} cells and $CD8^+$ T_{RM} cells, respectively, gravitate towards areas rich in distinct collagens[8,9]. Thus, T_{RM} cell subtypes may have evolved mechanisms to localize within mucosal and epithelial tissues in ways that enhance the likelihood of a rapid coordinated response to local infection[40,62,63].

What maintains T_{RM} cells in tissue?

If vaccines are to be designed to generate T_{RM} cells, then understanding the survival dynamics of T_{RM} cells becomes important. A recent human study showed T_{RM} cell clones surviving in the skin for up to 10 years following bone marrow transplantation[34]. One variable mediating T_{RM} cell survival in this study was a stem cell-like profile adopted by T_{RM} cells, which promoted superior survival of these cells when exposed to myeloablative radio-chemotherapy. Upon stimulation with cognate antigen, these T_{RM} cells became active, changing their metabolism and undergoing clonal expansion. Other important mediators thought to influence T_{RM} cell survival include IL-15 and ICOS1 (refs. 27,64). Fatty acid internalization and oxidative metabolism appear to be an additional mechanism for enhancing T_{RM} cell survival, and there is evidence that there is variation on this theme in different tissues. For example, whereas fatty acid-binding protein 4 (FABP4) and FABP5 appear to be important for fatty acid metabolism in skin T_{RM} cells, FABP1 seems to be critical for liver T_{RM} cell metabolism and persistence[29,65]. However, our overall understanding of T_{RM} cell maintenance remains limited and is a key area for future research.

Impact of infection and inflammation on T_{RM} cell biology

In humans, T_{RM} cells are thought to accumulate in tissues over time in response to repeated infections and to provide protective immunity against previously encountered pathogens[33,66]. T_{RM} cells express

anti-apoptotic factors, including BCL2, which may support their maintenance in tissues[8,27]. However, the duration of T_{RM} cell survival differs between tissues. For example, T_{RM} cells in the lung do not persist for as long as those in the skin and other tissues; the reasons for this are still unclear but are important when considering vaccines to pulmonary pathogens. A recent review discussed that lung T_{RM} cells may, in fact, migrate into the mediastinal lymph nodes after some time of residence within the lungs, rather than undergoing cell death[24,67]. It was recently shown that pools of T_{RM} cells in the skin can be replenished by both the replication of existing T_{RM} cells and the recruitment of circulating precursors[50]. Upon re-infection or antigen encounter following vaccination, there is local proliferation of T_{RM} cells as well as egress of some T_{RM} cells to lymphoid tissue, where they may again take up residence[38,39,68]. T_{RM} cells that egress from tissues have been shown in one report to re-enter the circulation, where they have a high propensity to home back to their tissue of origin[37]. In a skin xenograft model, a subset of $CD4^+$ T_{RM} cells were identified that had the ability to leave the skin to join the circulating pool of T cells as $CD103^+$ T cells and to later re-enter the skin at distant tissue sites to form skin T_{RM} cells after local infection[39,69]. This general observation is consistent with multifocal skin diseases known to involve T_{RM} cells, such as psoriasis and mycosis fungoides[36,70]. The development of inflammatory patches and plaques at new sites in the skin remote from the original inflamed sites is likely to involve this mechanism; however, whether this is truly dedifferentiation of T_{RM} cells or migration and maturation of what have been termed 'migratory memory' T cells is unknown[10]. The study of epigenetic changes that occur with T_{RM} cell differentiation is in its infancy, and whether these changes are reversed in 'former' T_{RM} cells will be important to document. Much remains undiscovered regarding T_{RM} cell longevity, replenishment and regeneration. Many of the factors that may drive egress of T_{RM} cells from tissue into the circulation also remain obscure at this time.

T_{RM} cell generation following tissue infection has been studied in both humans and mice, and several factors have been identified that contribute to the induction of T_{RM} cells. In the lungs, it is now generally accepted that tissue microenvironmental niches receptive for T_{RM} cells are generated during and after clearance of viral infection, and T_{RM} cells are spatially compartmentalized and maintained near sites of pathogen entry and accumulation[71,72]. It may be that longer lived T_{RM} cells specific for respiratory pathogens reside in the oropharyngeal mucosa, which may be an alternative site to target for T_{RM} cells. In another study, T_{RM} cells accumulated at sites of tissue injury and regeneration[73]. Both observations suggest local tissue regulation of T_{RM} cell maintenance. Antigen processing also contributes to the generation of all T cell memory, including T_{RM} cells. In the case of $CD8^+$ T_{RM} cell generation, classical DC1s have the ability to cross-present antigen to naive $CD8^+$ T cells in lymph nodes, which appears to be critical for antiviral $CD8^+$ T_{RM} cell development[46,47,58]. Much of what is known about gut T_{RM} cells derives from studies of lymphocytic choriomeningitis (LCMV) infection, which generates effector T cells from splenic naive T cells after intravenous challenge. However, LCMV delivered intraperitoneally enters the mediastinal node[74] and leads to the seeding of all lymphoid and non-lymphoid tissues with effector T cells that mature into T_{RM} cells. Do such cells educated in the spleen versus the lymph node differ in their behaviour or homing properties? And do LCMV-specific effector T cells that enter intestinal tissue encounter virally infected cells and perform effector functions, or simply passively differentiate in the gut microenvironment? There may be fundamental differences between the T_{RM} cells that arise in the setting of acute tissue inflammation, TCR activation and immune protection (for example, during infections with influenza A virus (IAV) in the lungs, or with vaccinia virus (VACV) or HSV in the skin) and those that are seeded into non-inflamed tissues by systemic infection (for example, in infection with a non-cytopathic virus such as LCMV in multiple tissues), although there are some clear commonalities.

Vaccines: can they efficiently generate T_{RM} cells?

Above, we have provided an overview of the current understanding of the factors that influence T_{RM} cell generation and their maintenance. In this section we will more specifically concentrate on relevant research related to vaccines and T_{RM} cells, primarily highlighting findings in animal models, as well as the substantial gaps in our knowledge. Naturally acquired infection in peripheral tissues generally elicits a long-lived T cell memory response, including the induction of T_{RM} cells. However, relatively little is known about the generation of T_{RM} cells by vaccines, and the contribution of other tissue-dwelling T cells, including T_{RM} cells, to vaccine-induced protection[33,75]. Newly available research tools and approaches could be more widely applied to parse out the roles of T_{RM} cells, circulating T cells and antibodies in vaccine-mediated protection against infection. Overall awareness of and interest in T_{RM} cells in vaccine-elicited immunity should be an element of early-stage vaccine research and development efforts. Although there are practical limitations to such research in humans, much can be learned at a fundamental level in small animal and non-human primate models. Finally, we comment below on the factors that may prove important in generating T_{RM} cells, including, first, the route of antigen administration; second, the mode of antigen delivery; and last, the role of vaccine adjuvants.

With most vaccines, generation of neutralizing antibody is associated with protection against a specific pathogen and remains a goal of vaccine development. Less attention has been paid to the generation of memory T cells, which is also associated with protection but is less likely to be measured in vaccine studies. It is assumed that for the generation of T_{RM} cells, the immune challenge (whether through natural infection or immunization) ought to occur in a peripheral tissue site that is destined to recruit these cells. Current intramuscular vaccines do not elicit T_{RM} cells, at least whenever this has been studied[76,77]. In animal models, parsing out whether protection from infectious challenge is mediated predominantly by T cells or B cells is now achievable[75]. Examples include the use of B cell-depleting or antibody-depleting strategies prior to infection, which helps focus attention on the role of T cells[75]. In addition, T cell-depleting antibodies (which typically deplete T cells from the blood but not from tissues) or agents that limit T cell egress from lymph nodes or from the circulation can more directly discriminate between the roles of circulating T cells and T_{RM} cells[77,78]. For example, after inoculation with a vaccine that generates a T_{RM} cell, followed by challenge infection, pathogen clearance is unaffected by blocking the circulating T cell response (via treatment with FTY720, an S1P1R agonist), pointing to the central role of T_{RM} cells in these immune responses[14,75,79,80]. Parabiosis models can also pinpoint the relative contributions of circulating T cells and T_{RM} cells[57]. New technology promises to answer many open questions; for example, T_{RM} cells may now be quantified and the spatial and functional relationships of T_{RM} cells in the overall tissue architecture may be characterized through in situ high-resolution multiplex imaging as well as through deep immune profiling and repertoire analysis (for instance, using CODEX, TCR sequencing, single-cell RNA sequencing and related technologies).

Vaccine administration: impact of delivery route on T_{RM} cells

Intramuscular injection into the deltoid or gluteus maximus muscle is the most common route of vaccine administration. It is generally well tolerated in children and adults and is convenient and accessible. The musculature is well vascularized and drained by lymphatics, enabling recruitment of immune cells from blood and rapid delivery of vaccine antigens to the draining lymph nodes, which is clearly sufficient for the induction of humoral immunity. Despite these practical clinical advantages, there are substantial disadvantages of this approach for T cell immunity[77]. Unlike skeletal muscle, skin and other epithelial tissues are consistently exposed to the external environment and have been evolutionarily shaped accordingly[11]. In other studies, intratracheal, intranasal or intravenous[81] delivery of experimental vaccines for *Mycobacterium tuberculosis* and respiratory syncytial virus (RSV) induced a T_{RM} cell response that was more robust and more protective against subsequent challenge as compared with intramuscular or intraperitoneal delivery[51,82,83]. It could be argued that skeletal muscle has not faced any evolutionary pressure from infectious pathogens (Fig. 3). As such, muscle has relatively few dendritic cells, and intramuscular immunization generates weak $CD8^+$ T cell and T_{RM} cell responses in mice as compared with mucosal and epidermal immunization[75]. Adjuvant-mediated recruitment of dendritic cells to muscle presumably involves their transit from blood.

Vaccination against IAV has historically involved immunization with whole virus, either inactivated or attenuated, with the primary intent of generating neutralizing antibodies. Intramuscular injection of inactivated IAV has been shown to elicit a particularly poor $CD8^+$ T cell response[84]. In a comparison of a live attenuated and an inactivated influenza vaccine, intranasal delivery of the live attenuated vaccine generated both neutralizing antibodies and lung T_{RM} cells[80]. Intraperitoneal or subcutaneous delivery of the inactivated vaccine generated neutralizing antibodies, but not lung T_{RM} cells. Neither systemic (intraperitoneal) administration of the live attenuated vaccine nor intranasal delivery of the inactivated IAV vaccine elicited a measurable T_{RM} cell response, suggesting a requirement for both the route of vaccine administration and a live attenuated formulation to generate a robust T_{RM} cell response. Mucosal vaccines have been recently reviewed (see refs. 85,86). Briefly, emerging data suggest that mucosal vaccination against respiratory pathogens can elicit tissue immunity and prevent or eliminate infection at the site of entry. This is in comparison with current intramuscular SARS-CoV-2 vaccines, which prevent severe illness and mortality but lack substantial efficacy in preventing upper respiratory infection and viral transmission.

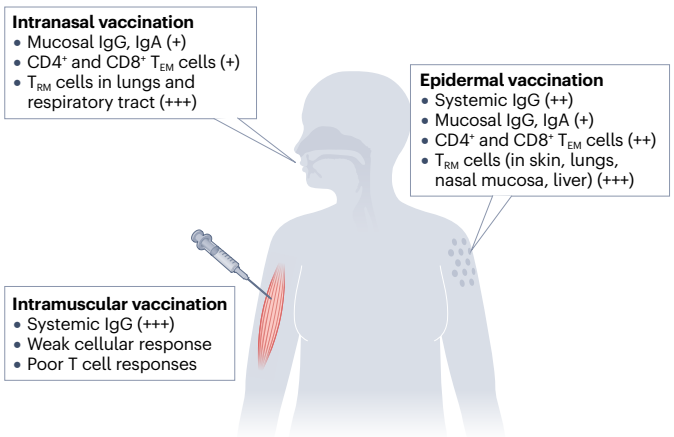

Fig. 3 | Host immune responses to different routes of vaccine administration. Both vaccine formulation and route of administration shape the immune response generated by the host immune system. Intranasal vaccination leads to the production of mucosal IgA and the generation of $CD4^+$ and $CD8^+$ effector memory T cells (T_{EM} cells), and a tissue resident memory T cell (T_{RM} cell) response in the respiratory tract. Intramuscular injection leads to a humoral immune response that produces systemic IgG, but this route generates relatively weak effector and memory T cell responses, most likely because there are few dendritic cells in muscle. Epidermal vaccine administration results in strong humoral and cell-mediated immune responses. This route generates systemic production of IgG, as well as mucosal IgG and IgA. It also elicits a strong cellular response with the generation and proliferation of both T_{EM} cells and T_{RM} cells in skin and also in distant tissues, including the lungs, nasal mucosa and liver. Adding adjuvants to vaccine formulations can potentially enhance and shift the immune response. For instance, adjuvanted intramuscular vaccinations have greater immunogenicity and may, in some cases, generate mucosal antibodies in addition to a systemic antibody response.

In mouse and hamster studies, a CpG-adjuvanted, recombinant SARS-CoV-2 vaccine targeting the Omicron Spike protein was administered via the intramuscular and intranasal routes[87]. Intranasal vaccination was superior in producing cross-neutralizing antibodies and promoted more rapid clearance of virus following Omicron challenge. The intranasal route elicited a T helper 1 cell (T_H1 cell)-biased Spike-specific $CD4^+$ and $CD8^+$ splenic T cell response that was cross-reactive (to pre-Omicron variants) and likely contributed to vaccine-mediated protection.

Humoral and cellular responses were assessed in a mouse model comparing intramuscular and intranasal administration of a chimpanzee adenoviral vector vaccine targeting the SARS-CoV-2 Spike protein[88]. Although both routes of administration resulted in comparable serum-neutralizing antibody titres, a single intranasal dose generated superior immune protection against SARS-CoV-2 challenge compared with a two-dose regimen given intramuscularly. Furthermore, a single intranasal immunization prevented both upper and lower respiratory tract infection by SARS-CoV-2. Following intranasal, but not intramuscular, immunization, $CD103^+CD69^+$ T cells were present in the lung, likely representing T_{RM} cells. The authors suggested that the superior protection conferred by the intranasal route was due to the mucosal immune response generated following intranasal vaccination and pointed to the potential value of strategies to discern which elements of the immune response contributed to protection (for example, B cell and T cell depletion, passive transfer or parabiosis experiments).

A series of elegant studies dissected mechanisms of vaccine-elicited B cell and T cell protection and directly compared intranasal and parenteral (subcutaneous or intramuscular) routes of administration. The authors developed IAV and SARS-CoV-2 protein subunit vaccines formulated in a carbomer-based nanoemulsion adjuvant system (Adjuplex) including either the Toll-like receptor 9 (TLR9) agonist CpG or the TLR4 agonist glucopyranosyl lipid A (GLA). The Adjuplex adjuvant system is a strong promoter of dendritic cell antigen cross-presentation and a potent inducer of $CD8^+$ T cell responses to protein subunit vaccines. Immunization by either the intranasal or subcutaneous route led to effective control of SARS-CoV-2 Beta variant infection in mice with intact antibody responses, but only the intranasal route induced protection against the virus in the absence of antibody-mediated neutralization[89–91]. Protection in response to intranasal vaccination was associated with durable T cell-mediated immunity and with T cells in the lung parenchyma that expressed

Glossary

Central memory T cells
(T_{CM} cells). A subset of long-lived memory T cells that express CCR7 and CD62L, allowing them to home to and patrol secondary lymphoid organs for known pathogens to respond more quickly to a subsequent infection.

Effector memory T cells
(T_{EM} cells). A subset of memory T cells that express integrins and chemokine receptors, allowing them to localize to inflamed tissues and mediate a rapid and potent immune response following repeat encounter with a known antigen.

Tissue resident memory T cells
(T_{RM} cells). A subset of memory T cells that are distinct in that they take up long-term residence within a peripheral tissue. Here, they can quickly mount a robust immune response upon pathogen encounter in non-lymphoid tissue.

CD103, CD69 and CD49a. In contrast, subcutaneous administration of the SARS-CoV-2 vaccine was associated with abundant splenic and lung $CD8^+$ T cells, but these were localized predominantly to the vasculature, and lacked cell surface markers of T_{RM} cells. Thus, a growing body of work points to the possibility of fine-tuning mucosal immunization with a series of adjuvants to generate tissue-specific immunity mediated by T_{RM} cells.

One immunization strategy to elicit a tissue-targeted T_{RM} cell response is the 'prime and pull' approach that has been used in animal models[92,93]. The 'prime' step involves conventional parenteral immunization, followed by the 'pull' step which relies on topical application of an innate immune inflammatory stimulus (for instance, chemokine, adjuvant or another inflammatory mediator) to recruit activated T cells into particular tissues[21,94,95]. This method successfully recruited and retained effector T cells in local tissue environments and generated T_{RM} cells for at least 1 year in some models[21]. This suggests that uncommitted effector T cells generated by parenteral inflammation can be altered by recruitment into inflamed peripheral tissues, suggesting that for these cells, T_{RM} cell programming occurs after tissue entry. However, it is not clear how this method can be applied to an entire tissue, as opposed to a specific site within a tissue. Additionally, it is unclear which T cells are best at forming T_{RM} cells when 'pulled' into a tissue, and whether current or recent TCR activation is required. At the very least, these experiments clearly demonstrate that persistent antigen stimulation is not required for the establishment of tissue residence. T_{RM} cell precursors 'pulled' into tissue by inflammation clearly can establish residence, but whether they differ in fundamental ways from T_{RM} cells that experience TCR activation in their destination tissue requires further study.

The route of administration as well as the T_{RM} cell response elicited by VACV may have contributed to the success of the smallpox vaccine; subsequent human studies were able to confirm this, and show that the appearance of a skin 'pox' lesion conferred better immunity, even when the mode of intended vaccination was subcutaneous or intramuscular[96,97]. The cellular requirements for the evolution of a pox lesion are undefined, but it appears to involve epidermal infection by live VACV. Studies of recombinant VACV in mice demonstrated that epidermal disruption (skin scarification) was best at eliciting a T cell response (including T_{RM} cells and T_{CM} cells), which itself is sufficient to provide protection against subsequent cutaneous and lethal respiratory challenges; this protection did not require neutralizing antibodies[75]. Modified vaccinia virus Ankara (MVA) is a highly attenuated VACV licensed as a third-generation smallpox and monkeypox vaccine and has been widely used as an investigational vaccine vector[98]. Because it is replication-incompetent and lacks approximately 10% of the VACV genome, immunization via epidermal disruption with MVA avoids many of the undesirable side effects of VACV[99–101], including the formation of a florid pox lesion that heals with a noticeable scar. MVA is licensed for intramuscular or subcutaneous administration, whereas only VACV is licensed for human administration by skin scarification/epidermal disruption. Epidermal disruption (in distinction from intradermal immunization) may be important in recruiting and activating dendritic cells at the immunization site[47], through the release of cytokines or by the generation of 'danger' signals. Immunogenicity of an ovalbumin (OVA) peptide-expressing MVA construct (MVA_{OVA}) was recently assessed in mice. MVA_{OVA} was administered via epidermal disruption or via intradermal, intratracheal, subcutaneous or intramuscular routes. Epidermal disruption generated a more robust T cell response that was transcriptionally unique from the other routes tested[77]. Others have suggested that vaccines designed to recruit T_{RM} cells to the respiratory tract should be administered intranasally[102]; however, recent experiments of poxvirus vector vaccines have generated a measurable T_{RM} cell response via epidermal disruption[77,103]. Poxvirus vector vaccines are uniquely suited for skin, and in this study, skin scarification elicited a superior lung T_{RM} cell response compared with all but the intratracheal route and was superior in protecting mice against lethal $VACV_{OVA}$ challenge. Importantly, these data showed a dose-sparing effect on T_{RM} cell generation by vaccination via epidermal disruption compared with intramuscular, subcutaneous and intraperitoneal routes, with intradermal being dose sparing but at a lower level. Previous studies have also shown a dose-sparing effect of MVA vaccine in eliciting neutralizing antibodies when delivered intradermally versus subcutaneously[104]. Current concerns surrounding limited vaccine stockpiles raise interesting prospects for future research[105]. Specifically, the Jynneos vaccine against smallpox and monkeypox was recently granted emergency use authorization by the US Food and Drug Administration (FDA) and a clinical trial is currently recruiting patients to evaluate vaccine immunogenicity when administered intradermally at 20% of the conventional dose (NCT05512949). Further studies comparing this or other MVA vaccines by various routes of administration, including epidermal disruption, could be informative. Combined, such studies could highlight the potential value of further dose de-escalation via intradermal administration and development and testing of monkeypox vaccine products suitable for administration by skin scarification or similarly needle-free techniques.

Of note, the effector T cells elicited by MVA_{OVA} skin scarification or by the intratracheal route had overlapping transcriptional profiles[77]. A fuller appreciation of the immune mechanisms by which skin scarification elicits lung T_{RM} cells (in addition to cutaneous T_{RM} cells) and allows for dose-sparing vaccination will require further exploration. Similarly, it may be instructive to compare various modes of cutaneous immunization, including epidermal disruption and epidermal injection with a particular focus on the generation of T_{RM} cells.

Vaccine platforms

Few vaccine platforms have been rationally designed or rigorously evaluated to assess their role in the generation of T_{RM} cells and other tissue-dwelling T cells. One group designed a peptide vaccine targeting wild-type and in silico optimized HLA-A*0201-restricted $CD8^+$ T cell epitopes derived from 11 structural, non-structural and accessory proteins of SARS-CoV-2. Following a single subcutaneous injection,

mice generated abundant draining lymph node and splenic $CD8^+$ T cells with surface markers and a phenotype suggestive of the potential for maturation into lung and mucosal barrier T_{RM} cells, although this was not validated by sampling those sites[106]. Another approach uses a pH-dependent antigen-delivery system allowing for release of the vaccine's 'cargo' antigen at a favourable pH, and enhancing antigen processing by the MHC class I pathway. This strategy prolonged antigen presentation leading to a more robust $CD8^+$ T cell response[40]. Thus, the platform harnessed two factors known to promote $CD8^+$ T_{RM} cells in natural infection — namely, enhancing antigen processing by the MHC class I pathway and extending the duration of antigen presentation.

Adenoviral vector vaccines, such as VACV vectors, mimic viral infection to elicit an immune response, although the normal target tissue of the parent virus is different[107]. Recently developed mRNA-based vaccines employ a unique mechanism that relies on host cell machinery to synthesize and present antigen to the immune system. In this way, they simulate certain features of natural infection, eliciting a combined cellular and humoral response. Several leading SARS-CoV-2 vaccines rely on adenoviral vectors (AstraZeneca, Janssen/JNJ and Sputnik-5) or on mRNA platforms (Pfizer/BioNTech and Moderna)[108–111]. Early results suggest that the adenoviral-vectored and mRNA SARS-CoV-2 vaccines generally elicit robust $CD4^+$ T cell and, to a lesser extent, $CD8^+$ T cell responses, although one study of an adenoviral-vectored SARS-CoV-2 vaccine demonstrated comparable $CD4^+$ and $CD8^+$ T cell responses to immunization[112]. The published clinical results describe circulating T cells, so any extension to T_{RM} cells is hypothetical. Preclinical studies of intranasally delivered adenoviral-vectored vaccines have demonstrated a strong, focused immune response against the receptor binding domain of SARS-CoV-2 Spike protein through induction of mucosal IgA in addition to serum-neutralizing antibodies, $CD4^+$ and $CD8^+$ T cells and a population of $CD8^+$ T_{RM} cells[103,113]. In a humanized mouse model utilizing a heat-shock protein chaperone that promotes vaccine antigen cross-presentation, a SARS-CoV-2 Spike protein vaccine elicited T_{RM} cells in the lungs and airways[114]. A comprehensive analysis by systems vaccinology of the Pfizer/BioNTech COVID vaccine suggests that the mRNA platform does not elicit lung T_{RM} cells when administered via the intramuscular route in mice, despite measurable $CD8^+$ T cell and natural killer cell responses[115]. A recent mouse model study of an mRNA SARS-CoV-2 vaccine compared different strategies of vaccination, including contralateral versus ipsilateral prime–boost doses, as well as intravenous and intranasal administration[116]. Recent studies of mRNA SARS-CoV-2 and influenza vaccines in humans have tested the intradermal route of administration. These vaccines have been shown to be safe and effective via this route when compared with intramuscular administration[117,118]. In one study, the intradermal route led to a less robust cellular immune response when compared with the intramuscular route, although it was delivered intradermally at only 20% of the dose used intramuscularly[117]. This study showed the greatest generation of a circulating T cell and lung T_{RM} cell response occurred when combining intramuscular injection with a subsequent intranasal booster dose.

Current concerns about vaccine-induced antibody waning and the emergence of SARS-CoV-2 antibody-escape variants underscore the potential importance of $CD8^+$ T_{RM} cells in long-term protection against COVID-19. Antibodies to either the Pfizer or Moderna vaccine (targeting the original Spike protein) cross-react poorly, if at all, with the Spike from the Omicron BA.1, BA.2 or BA.5 viral variants[119], suggesting that the correlation between the number of vaccine doses and the reduction in serious illness and/or hospitalizations is likely, at least in part, to be T cell-mediated[120]. Indeed, mRNA vaccine-elicited T cell responses have been implicated in protection against recent variants of concern[121,122]. Newer mRNA constructs may address this protective antibody deficiency, at least temporarily. However, it is likely that the selective pressure on the virus induced by the newer vaccines will drive the emergence of additional escape variants.

As noted above, pulmonary T_{RM} cells generally do not persist long term and the immunity they confer may be relatively transient. SARS-CoV-2 breakthrough infections in fully vaccinated individuals are characterized by asymptomatic or mildly symptomatic upper airway involvement with transient but high levels of viral replication in nasopharyngeal mucosa[123]. Circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells have been found in a high proportion of convalescent individuals with COVID-19. These T cells target a wide range of SARS-CoV-2 proteins in addition to the immunodominant Spike. SARS-CoV-2-reactive $CD4^+$ T cells were also found in ~50% of unexposed individuals, consistent with such cells being generated in response to prior exposure to common cold coronaviruses, highlighting a potential role for these cells in cross-protective immunity[124,125]. The presence of SARS-CoV-2-specific T cells in bronchoalveolar fluid and biopsies from patients with COVID-19 and the presence of T_{RM} cells in lung biopsies taken from patients up to 10 months following infection with SARS-CoV-2 point to their potential role in immune protection[126,127]. In addition, such T_{RM} cells could be isolated from cadaveric tissues of patients who had survived infection with COVID-19 and died of other causes. Again, these findings point to the importance of tissue-specific immunity and studies to explore these issues following SARS-CoV-2 immunization are practical in small and large animal models. Furthermore, rational, pan-coronavirus vaccine design should incorporate conserved $CD4^+$ and $CD8^+$ T cell epitopes along with novel vaccination strategies to promote long-term tissue residence[126].

Vaccine adjuvants

As summarized above, T_{RM} cell generation following vaccination is influenced by both the route of administration (favoured by mucosal or epidermal administration) as well as the vaccine formulation (with live attenuated vaccines being most effective). Adjuvants offer the possibility of either enhancing the T_{RM} cell response generated by these methods or, possibly, circumventing these requirements[14]. The focus of adjuvant research has long been on enhancement of the antibody response. This follows in that alum, the only adjuvant in FDA-licensed vaccines for many decades, is primarily a driver of T_H2-type humoral responses and not a strong promotor of cell-mediated immunity. Newer adjuvants or combination adjuvants, especially those that promote T_H1 cell and T_H17 cell immunity, also appear to be good promotors of $CD4^+$ and $CD8^+$ T cells[14]. Deploying rationally designed adjuvants may ultimately prove helpful in enabling parenteral vaccines to overcome an apparent requirement for mucosal or epidermal delivery to optimally elicit T_{RM} cells. A good example of this is the development of the carbomer-based nanoemulsion adjuvant system (Adjuplex) incorporating either GLA or CpG, as discussed previously.

Shingles presents an interesting opportunity to consider T_{RM} cell-targeted vaccines over antibody-targeted vaccines. This is because varicella zoster virus (VZV) and other herpesviruses are reactivated in the skin, where VZV-specific and HSV-specific T_{RM} cells have been shown to reside. Therefore, vaccination strategies aimed at boosting skin T_{RM} cells may elicit faster and stronger immune responses upon primary pathogen encounter and viral reactivation. One group found that Zostavax, a live attenuated, subcutaneous, endogenously

adjuvanted VZV vaccine, had no effect on the proportion of VZV-specific T_{RM} cells within the skin[128]. In clinical trials, Zostavax had 61.1% efficacy in adults aged 60 years and older[129]. By contrast, Shingrix is a recombinant, AS01B-adjuvanted vaccine that is highly protective against VZV, exhibiting 97.2% overall efficacy in adults aged 50 years and older[130]. The Shingrix vaccine relies on the glycoprotein E antigen — the most abundant glycoprotein expressed on VZV-infected cells and a target of both neutralizing antibodies and T cells. Unadjuvanted glycoprotein E does not elicit a strong immune response. However, when glycoprotein E is combined with the AS01B adjuvant system it generates robust glycoprotein E-specific antibody and $CD4^+$ and $CD8^+$ T cell responses[131]. Thus, Shingrix relies on glycoprotein E to direct the immune response and AS01B to shape and enhance the response. No tissue samples were collected as part of this study by Heineman et al.[131]; however, an ongoing clinical trial plans to identify and characterize cutaneous T_{RM} cells following Shingrix vaccination[132].

In general, vaccines that generate T cell responses have important potential advantages over vaccines geared primarily towards antibody production. Certain vaccines that induce a robust T cell (and T_{RM} cell) response target highly conserved, mostly internal and/or non-structural, viral proteins[133]. Antibody-mediated neutralization, on the other hand, relies primarily on recognition of surface conformational epitopes, which are known to be less well conserved across viral strains and subject to evasive mutation. By engineering vaccines to elicit a significant T cell response, we can circumvent evasion of the humoral immune response through viral recombination and antigenic drift. T cell vaccine responses are therefore capable of broad heterosubtypic protection across viral strains[80], and T cell vaccines are thus good candidates for universal influenza or pan-coronavirus vaccines.

Challenges and future directions

There remain many challenges to achieving a more complete understanding of the roles of circulating T cells and tissue resident T cells in vaccine-mediated protective immunity. Defining the subsets of circulating T cells that are destined to become T_{RM} cells[49], what prompts their egress from blood to repopulate local T_{RM} cell pools and what stimulates their regeneration within tissues are promising areas for study. Emerging and future insights into these issues would identify questions that could be addressed in vaccine clinical trials. The mRNA vaccine platform proved very successful with SARS-CoV-2 vaccines in the COVID-19 pandemic and may become a leading strategy for future vaccine development. Next to nothing is known regarding the effects of mRNA vaccines on the generation of T_{RM} cells, although a recent study showed that the levels of T_{RM} cells in nasopharyngeal samples increased after each of the two doses of the Pfizer BioNTech mRNA COVID-19 vaccine[134]. However, longitudinal sampling could be informative in light of the previously noted decline in nasopharyngeal immunity seen in SARS-CoV-2 breakthrough infections. Gaining a better understanding of the T cell and T_{RM} cell responses to mRNA vaccines and devising strategies to enhance those responses in rodent and non-human primate models could be of immediate value.

The major barrier to studying T cell-targeted vaccines is obtaining human tissue samples required to study tissue resident T cells[7]. The development and use of tissue banks to address the dearth of human tissue samples available for research has been invaluable in other research settings. The Network for Pancreatic Organ Donors with Diabetes (nPOD) is one such tissue bank that collects and distributes cadaveric pancreatic and other tissue samples from individuals with recent onset of type 1 diabetes or those who are at increased risk of developing type 1 diabetes. A similar effort to facilitate access to relevant tissues and enable studies of T cell vaccine would be valuable in furthering our understanding of these vaccines. Dr Donna Farber has been uniquely successful in studying human T_{RM} cell biology in multiple tissues. The success of the Farber laboratory in leveraging the organ donor process in New York can serve as a model for the study of human tissue T cells, including T_{RM} cells. Lungs that are not suitable for transplantation and nasopharyngeal, gastrointestinal or urogenital tissues could be banked and distributed to study vaccine-elicited protection at these mucosal sites. Indeed, the expansion of such tissue banks for use in vaccine studies has recently been proposed[7].

One promising area of research may be the identification of better cutaneous immunization platforms, in addition to epidermal disruption or skin scarification, that generate skin and lung T_{RM} cells. Whether classical epidermal disruption can be refined with other approaches remains unexplored. A challenge to skin-directed vaccines is the potential for reactogenicity and local inflammatory responses, thus demonstrating safety and tolerability of such vaccines will be important. Newer, more potent and well-tolerated adjuvants may alleviate such concerns. More data are needed to elucidate and deconvolute the respective roles of antibodies, circulating T cells and T_{RM} cells in immune responses, defining how they differ and where they overlap.

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