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Review

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Review

Tissue-Resident Memory T Cell: Ontogenetic Cellular Mechanism and Clinical Translation

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Abstract

Mounting evidence has indicated the essential role of tissue-resident memory T (T_{RM}) cells for frontline protection against viral infection and for cancer immune surveillance (Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defense. Nat Rev Immunol 2016, 16, 79–89. doi:10.1038/nri.2015.3.). T_{RM} cells are transcriptionally, phenotypically, and functionally distinct from circulating memory T (T_{cirm}) cells. It is necessary to understand the unique ontogenetic mechanism, migratory regulation, and biological function of T_{RM} cells. In this review, we discuss recent insights into cellular mechanisms and discrete responsiveness in different tissue microenvironments underlying T_{RM} cell development. We also emphasize the translational potential of T_{RM} cells by focusing on their establishment in association with improved protection in mucosal tissues against various types of diseases and effective strategies for eliciting T_{RM} cells in both pre-clinical and clinical studies.

Keywords: tissue-resident memory T cell, mucosal immunity, T-cell differentiation, vaccine, immunotherapy

Abbreviations: APC: antigen-presenting cell; ATAC: assay for transposase-accessible chromatin; BAL: bronchial alveolar lavage; ChIP: chromatin immunoprecipitation; COVID-19: coronavirus disease 2019; CTL: cytotoxic T lymphocytes; DC: dendritic cell; HPV: human papillomavirus; ICB: immune checkpoint blockade; IEL: intraepithelial lymphocytes; IFN-γ: interferon gamma; IgA: immunoglobulin A; IgG: immunoglobulin G; IL-17A: interleukin-17A; IL-2: interleukin-2; KO: knocked-out; LAIV: live attenuated influenza virus; LAP: latency-associated peptide; MAV: melanoma-associated vitiligo; mDC: migratory dendritic cell; MHC: major histocompatibility complex; RAMDs: repair-associated memory depots; RBD: Receptor binding domain; RSV: respiratory syncytial virus; S1P: sphingosine-1-phosphate; S1PR1: sphingosine-1-phosphate receptor 1; S1PR1: sphingosine-1-phosphate receptor 1; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SLO: secondary lymphoid organ; T_{cirm}: circulating memory T cell; T_{CM}: central memory T cell; T_{eff}: effector T cell; T_{EM}: effector memory T cell; TGF-β: transforming growth factor beta; T_{LLEC}: long-lived effector T cell; TME: tumor microenvironment; TNF-α: tumor necrosis factor alpha; TPCK: ntosyl-L-phenylalanine chloromethyl ketone; T_{RM}: tissue-resident memory T cell; T_{SCM}: T memory stem cell; tSNE: t-distributed stochastic neighborhood embedding; WT: wild type.

Introduction

Host cellular immunity against infection and tumor involves heterogeneous types of T cells especially within the tissue compartments affected. Effector $CD8^+$ T cell (T_{eff}) is a subset of highly activated cytotoxic T lymphocytes (CTL), which can eliminate infected or cancer cells through direct contact killing and secreting cytotoxic cytokines. Adaptive T-cell immune response is indicated by three major phases including expansion, contraction, and memory. Antigen-specific T_{eff} cells clonally expand from naïve T cells during the first phase after antigen presentation, followed by the contraction phase, and then the memory phase with the formation of memory T-cell subsets, providing immune surveillance against reinfection or tumor recurrence in circulation and tissues. Memory T cells consist of circulating memory T cells (T_{cirm}) and tissue-resident memory T cells (T_{RM}). T_{cirm} cells can migrate through peripheral blood, tissues, and secondary lymphoid organs, whereas T_{RM} cells are non-recirculating and persist long-term in epithelial, mucosal, and other tissues [1]. Based on recent studies, T_{cirm} cells can be further classified into central memory T cell (T_{CM}), effector memory T cell (T_{EM}), T memory stem cell (T_{SCM}), and long-lived effector T cell (T_{LLEC}) [2–5]. The intrinsic gene expression profile determines the differentiation and proliferation capacity of various memory T-cell subsets after reactivation. T_{CM} and T_{SCM} display a naïve-like differentiation potential with high CCR7 expression and increased interleukin-2 (IL-2) secretion for lymphoid organ homing and enhanced proliferation capacity. T_{CM} and T_{SCM} can repopulate into different types of memory T cells in various tissues and secondary lymphoid organs [2, 3]. On the

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other hand, T_{EM} and T_{LLEC} can secrete effector molecules such as *GzmA*, *Ifng*, *Zeb2*, and *GzmB* swiftly but with reduced migration capacity due to the lack of CCR7 expression and terminally differentiated characteristics [4, 5]. T_{RM} also displays a potent and frontline cytotoxic effect upon pathogen re-challenge and tumor progression. Notably, recent research confirmed that T_{RM} can form different memory T-cell populations in non-lymphoid tissues or lymphoid organs after restimulation [6–8]. Since molecular pathways involved in T_{RM} development have been previously reviewed [9–12], we discuss recent advances in cellular interaction networks and the impact of tissue microenvironment on T_{RM} early induction. We also discuss the function of T_{RM} in cancer and infectious disease control together with preclinical and clinical studies on T_{RM} induction and activation.

Tissue-Resident Memory T-Cell Ontogeny

Naïve T-cell preconditioning and priming

Naïve T cells circulate constantly throughout the blood, second lymphoid organs (SLO), and lymph system. Naïve T-cell activation requires antigen stimulation by different types of antigen-presenting cells (APCs), including dendritic cells (DCs), macrophages, monocytes, and B cells [13]. The fate of naïve T-cell terminal differentiation is decided in the early division of activated T cells, which is associated with cytokine or indirect interactions with APCs. Using single-cell RNA sequencing, Yeo and colleagues have revealed the transcriptional divergence in the first division of activated naïve $CD8^+$ T cells [14]. $CD8^+$ T cells in the first division display two distinct clusters in the unsupervised tSNE (t-distributed stochastic neighborhood embedding) analysis. One subcluster was likely the terminal effector cell while the other cluster was the naïve and T_{CM} populations, which were segregated into "pre-memory" and "pre-effector" T-cell clusters accordingly [14]. However, whether T_{RM}-related genetic features are determined at the time of the first cell division has not been well described. Apparently, DNGR-1^+ DCs were required for optimal T_{RM} priming by promoting T-bet induction and providing key molecules including IL-12, IL-15, and CD24 [15]. Defective antigen cross-presentation mediated by DNGR-1 and Batf3-dependent DCs illustrated a reduced number of $CD103^+$ T cells in the skin as early as day 14 post-local infection. Kok and colleagues utilized a single-cell barcode label system to trace single naïve T-cell progeny into different memory T-cell pools [10]. They found that core T_{RM} signature genes had already been highly expressed in a group of peripheral effector T cells at day 6 post-activation, indicating that a subset of circulating effector T cells consisted of the T_{RM} precursors. The fate of T_{RM}, therefore, was likely decided in the early effector phase. Consistently, core T_{RM} regulators including *Runx3*, *Hobit*, and Blimp1 were highly expressed at the early effector phase in T_{RM} precursors [16–20]. In the first division of activated cells, Ezh2 was upregulated in the "pre-effector" T cells [14]. The Ezh2 gene encodes the catalytic subunit of the polycomb complex PRC2 that can trigger the trimethylation of histone H3 at Lys27 to induce gene repression. Ezh2 mediates several memory-associated gene repressors including *Tcf7*, *Eomes* and *Klf2*, which are also downregulated in the T_{RM} formation process [17, 21, 22]. This intrinsic feature implies a possibility that T_{RM} fate decision events occur in the first cell division, prior to the effector phase, but further research is still needed to fully address this hypothesis.

Some studies suggested that T-cell differentiation fate is decided after T-cell activation. This notion, however, has been challenged by a study on T_{RM} differentiation. Migratory DC (mDC) localized in skin-draining lymph nodes could activate latent TGF-β into its activated form. Activated TGF-β subsequently preconditioned naïve T cells to acquire the propensity to differentiate into T_{RM}. This preconditioning process happens during the immune homeostasis phase, which is prior to the T-cell activation (Figure 1A) [23]. Apart from the role of DC in T-cell priming, several studies focus on the function of monocytes and macrophages in T-cell preconditioning. One study indicated that monocytes may use an autocrine pattern to prime T_{RM} differentiation (Figure 1A). During the process, systemic monocyte-secreted IL-10 induces inflammation-recruited monocytes to release TGF-β that in turn upregulates CD103, the key marker of mucosal-associated T_{RM}, on primed T cells [24]. Another study emphasized that monocytes can be recruited into inflamed tissues in a CCR2-dependent manner to drive lung T_{RM} differentiation [25]. It remains unknown whether this monocyte-induced T_{RM} differentiation happens prior to or after the T cell activation. Another type of APC, $CCR2^+$ macrophages, may play a critical role in intestinal and cervical vaginal T_{RM} formation [26, 27]. Different from its parental circulating $CCR2^+$ monocytes, this cluster of macrophages may illustrate tissue residency. They can secrete IFN-β and IL-12 for intestinal $CD8^+$ T_{RM} persistence and CCL5 for $CD4^+$ T_{RM} maintenance in the vagina. IFN-β, IL-12, and CCL5 are not restricted to be expressed within the inflamed peripheral tissue because they can also be found in the spleen, where T_{RM} may share a similar mechanism to T_{cirm} for long-term maintenance. Notably, T_{RM}-related circulating APCs mentioned above are unlikely able to promote T cell differentiation into T_{RM} in circulation, only after being recruited into the peripheral inflammation environment [25]. Future studies, therefore, are needed to investigate what kind of tissue-specific microenvironment can preferentially recruit circulation-derived APCs to generate tissue-specific signals for differentiation and retention of T_{RM}.

Phenotype switch of circulating memory T cell during reactivation

T_{cirm} cells including T_{EM} and T_{CM} cells distributed throughout the body. After re-encountering the antigen, memory T cells can be rapidly reactivated, performing the cytokine-secreting function and differentiating into various T-cell phenotypes [28]. Although the intrinsic characteristics of T_{cirms} are significantly different from that of T_{RM}, several studies demonstrated that both T_{EM} and T_{CM} have the potential to differentiate into local T_{RM}. Using the P14 memory T-cell adoptive transfer model, Slütter and colleagues demonstrated that the recruitment of T_{EM} is the major source of lung T_{RM} [29]. Splenic P14 T_{EM} and T_{CM} were adoptively transferred into recipient mice intranasally infected with the PR8 influenza virus, respectively. The T-cell phenotype in the lung was evaluated 21 days after the transfer. They found that only $CD62L^-$ T_{EM} can differentiate into T_{RM} in an antigen-independent manner (Figure 1B). The lung harbors many T_{EM} and T_{RM} cells but in distinct niches: T_{EM} cells were found in the alveoli and respiratory bronchioles, whereas T_{RM} was largely detected in the peribronchiolar foci generated during viral infection, inflammation, and recovery [30]. Another study refined the lung T_{RM} replenishment process, indicating that the majority of $CD8^+$ T_{RM} cells were maintained within repair-associated

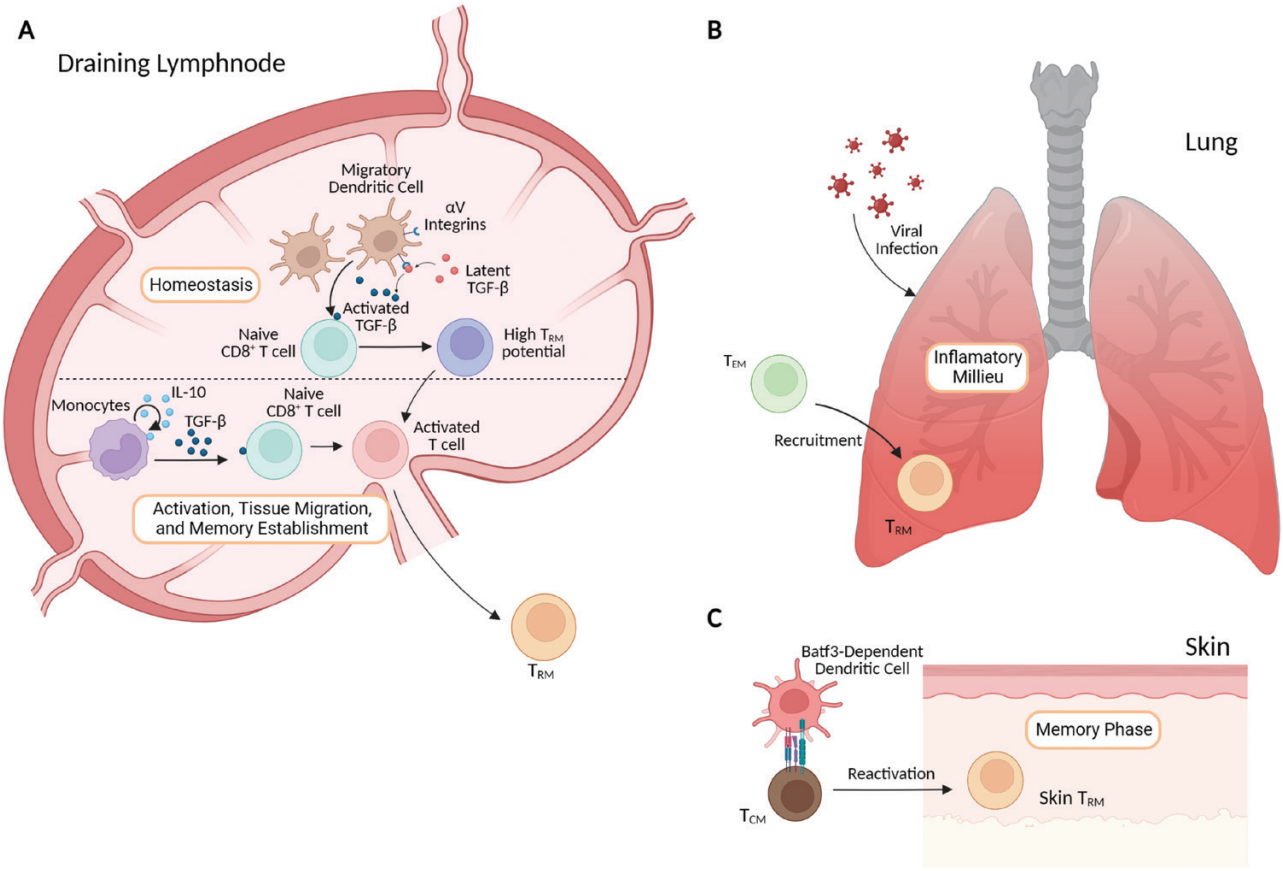

Figure 1. Tissue-resident memory T cells developed from naïve T cells and T_{cirm}. (A) Naïve T cell preconditioning and priming. During homeostasis, αV Integrin expressing migratory DC could activate latent TGF-β, which subsequently interacts with naïve $CD8^+$ T cells. Preconditioned naïve T cells will preferentially develop into T_{RM} after immune activation. After intravenously vaccinating adjuvanted peptide antigen, IL-10 released by blood monocytes acts in an autocrine mechanism to induce TGF-β production that in turn promotes T_{RM} generation. (B) Circulating memory T-cell phenotypic switch. Circulating T_{EM} will be recruited into inflamed lungs infected with the PR8 influenza virus and acquire the ability to develop into T_{RM} during the memory phase. Batf3-dependent DCs could reactivate T_{CM}, and the preconditioned reactivated cells will be biased to differentiate into skin T_{RM}. Created with Biorender.com.

memory depots (RAMDs) after the initial induction, yet T_{EM} was excluded in this niche [31]. It was suggested that circulating T_{EM} differentiated into a small fraction of T_{RM} that was maintained in the lung interstitium [31]. Osborn and others then demonstrated that T_{CM}, rather than T_{EM}, was capable of differentiating into T_{RM} in the skin (Figure 1C) and in the liver [32, 33]. Both studies also indicated that naïve T cells could differentiate more efficiently into CD103-expressing T_{RM} cells than T_{CM}, which was corroborated by a subsequent paper [34]. The differences observed in these studies were probably related to the time point of T-cell adoptive transfer because the induction and development of T_{RM} could be affected by different experimental conditions. Slütter and colleagues established an antigen-nonspecific inflammatory environment in the lung prior to the T_{cirm} adoptive transfer. The local inflammatory environment might consistently recruit T_{EM} from circulation to replenish the T_{RM} pool. Osborn and colleagues transferred T_{cirm} before the local antigen administration and memory T-cell reactivation. T_{CM} could be swiftly reactivated and preferentially differentiated into T_{RM} in the memory phase after local infection or antigen presentation in the mucosa. Detailed mechanisms behind these environmental conditions remain to be investigated. For example, although APCs play an active role in the reactivation of memory T cells [15, 35, 36], whether APCs affect memory T-cell differentiation into T_{RM} remains elusive under different environmental conditions.

Discrete tissue microenvironment elicits diversified T_{RM} characteristics

T_{RM} cells in different tissues possess distinct gene expression profiles, which may indicate unique epigenesis processes and adaption to specific tissue microenvironments. CD69 and CD103 upregulation may jointly inhibit lymphocyte egression from tissue and draining lymph nodes. The interaction between sphingosine-1-phosphate (S1P) and sphingosine-1-phosphate receptor 1 (S1PR1) was found to be critical in lymphatic trafficking [37, 38]. Increased surface expression of CD69 on activated T lymphocytes promotes S1PR1 degradation, which may restrain T-cell egression from the secondary lymphoid organ. E-cadherin is an adherent junction protein that is expressed on mucosal epithelial cells. CD103 expressed on the surface of T_{RM} can bind to E-cadherin and thus promotes the tissue residency of lymphocytes [39, 40]. T_{RM} cells in the lung, intestine, skin, and salivary glands (SG) display a substantial expression of both CD103 and CD69, but T_{RM} cells in the liver, fat, and kidney rarely express CD103 [6, 41]. TGF-β is the key regulator of CD103 expression, as it mediates the phosphorylation and nuclear translocation of Smad3. Subsequently, Smad3 can bind to the promoter

and enhancer of CD103-coding gene *ITGAE*, regulating the CD103 expression [21]. Moreover, *in vitro* culture of liver-derived $CD69^+CD8^+$ T cells with TGF-β increases CD103 surface expression. These results demonstrated that the tissue cytokine milieu might instruct the transdifferentiating approach of heterologous T_{RM}.

Besides the tissue cytokine milieu, the requirement of local antigen presentation during T_{RM} induction is discrepant in different tissues. The route of vaccination may affect the requirement for eliciting T_{RM} effectively in different organs (Table 1) by imprinting local T-cell tropism as described previously [28, 53, 54]. Delivering immunogen to the target tissue is required for generating specific T_{RM} in that compartment. Moreover, it is also feasible to utilize a nonspecific inflammatory signal or chemokine to "pull" prime immunization-generated circulating memory T cells into the target peripheral tissue to induce T_{RM} differentiation. This kind of vaccination strategy is referred as "prime-and-pull" immunization [44, 51]. Caminschi and colleagues utilized zymosan as a vaccine adjuvant to bypass the local antigen presentation to induce T_{RM} in the lungs. During the experiment, naïve mice were transferred with *in vitro* activated $CD8^+$ T cells. Subsequently, pulmonary inoculation of zymosan could recruit seeded T cells into the lungs, resulting in an increase of T_{RM} at the memory phase [45]. The molecular and cellular mechanism underlying "prime-and-pull" immunization, however, remains to be investigated.

Tissue-Resident Memory T Cells and Disease Control

Anti-viral infection

Before the introduction of the T_{RM} concept, a population of T cells, capable of migrating into non-lymphoid tissue and then developing into local memory T cells, had been identified in a *Listeria* and vesicular stomatitis virus (VSV) infection model [52]. After T_{RM} was defined as T cells expressing CD69, CD103, and $CD49a^+$ in different tissues [6, 55, 56], the potential of T_{RM} in controlling viral infection has been rapidly explored [57]. For example, lung $CD8^+$ T_{RM} induced by influenza A virus infection can provide protection against cross-subtype influenza virus re-infection [58]. Influenza virus-specific $CD8^+$ T_{RM} was also found to be persistently embedded in the nasal tissue after primary infection. While the protective efficacy of lung T_{RM} might reduce gradually due to the time-dependent apoptosis [29], the frequency of nasal T_{RM} did not significantly decay over time, providing longer-term protection against influenza virus re-infection [47]. In humans infected with the influenza virus, antigen-specific $CD8^+$ T_{RM} was identified in the lungs, and the high expression of CD69 and CD103 aligned with the phenotypic characteristics of lung T_{RM} observed in animal studies [59, 60]. Interferon-γ (IFN-γ) and interleukin-17A (IL-17A) released by tissue-resident memory $CD4^+$ T cells might mediate protection against respiratory syncytial virus (RSV) infection [61]. Meanwhile, the frequency of human RSV-specific airway $CD8^+$ T_{RM} cells was negatively correlated with the disease severity during the RSV primary infection [62, 63]. Airway $CD8^+$ T_{RM} cells were also potentially involved in the recovery process [64]. Long-lasting vaginal T_{RM} induced by human papillomavirus (HPV)-vectored vaccine protected mice against recombinant vaccinia virus challenge in an intravaginal prime-boost immunization regimen [65]. Skin T_{RM} generated by acute vaccinia virus infection provided global immunity against re-infection [66]. These results demonstrated consistently a protective role of T_{RM} cells against various viral infections.

After the outbreak of the coronavirus disease 2019 (COVID-19), the importance of T_{RM} has been highlighted especially in the field of vaccine research. Besides T_{CM} and T_{EM} induced by intramuscular vaccination, we and others reported that a large number of cytokine-secreting T_{RM} elicited by intranasal vaccination provided superior protection against SARS-CoV-2 in the lungs [67–69]. Due to the lack of sufficient sterile immunity induced by mRNA or inactivated vaccines, the COVID-19 pandemic remains a continuous public threat [69]. Individuals who received two-dose intramuscular vaccination, either by BNT162b2 or ChAdOx1 nCoV-19 (also known as AZD1222), exhibited similar or slightly decreased peak viral load during breakthrough infection compared to infected individuals with no vaccination history [70, 71]. A recent cohort study during the Omicron wave further suggested that intramuscular vaccination, single or two doses prior to infection reduced the risk of transmitting infection [72]. However, the two-dose intramuscular vaccination failed to fully prevent the acquisition and transmission of the virus. To prevent SARS-CoV-2 nasal infection, we have reported a heterologous COVID-19 vaccination regimen using a PD1-enhanced DNA vaccine (ICCOV) intramuscular prime followed by a live attenuated influenza-vectored vaccine (LAIV-RBD) boost [69, 73]. This regimen induced the highest frequency of $CD8^+$ T_{RM} in the lungs of vaccinated animals compared with the individual vaccine (e.g ICCOV, LAIV-RBD or BioNTech) tested alone or with either BioNTech or Sinovac as the intramuscular prime. Since this regimen prevented live SARS-CoV-2 nasal infection effectively, our findings strengthened the importance of vaccine-induced mucosal $CD8^+$ T_{RM} in the respiratory system for protection [69]. Consistently, 60.7% of $CD8^+$ T cells in nasal samples derived from convalescent patients (36–70 days after viral clearance) exhibited a T_{RM} phenotype, suggesting that the enrichment of T_{RM} in nasal mucosa is likely essential for SARS-CoV-2 prevention [74].

Although T-cell responses elicited by spike-based intramuscular vaccine maintain cross-reactivity to multiple SARS-CoV-2 variants [75], intranasal vaccination could further enhance cross-protection [76–78]. Previous research reported the significantly reduced neutralization capacity of vaccine-elicited mAb against highly-mutated Omicron variants, but conserved T cell immunity, especially vaccine-induced

Table 1. Induction route and antigen presentation required by TRM development

Tissue	Local antigen presentation Required?	Induction route
Lung	Yes [42–44]/or bypassed by adjuvant [45]	Intranasal/intravenous [46]
URT	No [47]	Intranasal
Skin	No [48]	Intradermal/intraepithelial
Gut	N/A	Intraperitoneal/oral [49]
Liver	Yes [50]	Intraperitoneal
Vagina	No [51]	Intravaginal [52]

URT: upperrespiratorytract.

$CD8^+$ T cell responses, illustrated durable and potent cross-reactivity [79, 80]. Since only intranasal vaccination could establish lung T_{RM} cells, rather than intramuscular immunization, it is rational to infer that vaccine-elicited lung T_{RM} cells also confer cross-reactivity against various SARS-CoV-2 variants. However, consistent with the findings in lung T_{RM} cell-mediated protection against influenza viral infection [29], vaccine-elicited cellular immunity against SARS-CoV-2 infection underwent a gradual decline [81, 82]. A similar numerical decay can possibly be found in vaccine-elicited SARS-CoV-2 specific lung T_{RM} cells. Therefore, vaccine regimens that could induce a high frequency of durable lung T_{RM} cells at the time of vaccination might be advantageous for prolonged protection.

Anti-tumor Effect

For virus-associated cancers, U.S. Food and Drug Administration (FDA) has approved two vaccines, the human papillomavirus (HPV) vaccine and the Hepatitis B (HBV) vaccine. The HPV vaccine has been used for the prevention of cervical cancer, vaginal cancer, vulvar cancer, penile cancer, anal cancer, and oropharyngeal cancer [83, 84]. The HBV vaccine has been proven to efficiently reduce the incidence of hepatocellular carcinoma (HCC) [85]. Although the underlying mechanism of vaccine-elicited immune responses against relevant cancer has not been well understood, $CD8^+$ T_{RM} illustrated a prominent role in patients. The high frequency of $CD39^+$ $CD103^+$ $CD8^+$ T_{RM} like tumor-infiltrating lymphocytes has been correlated with a better survival rate in head and neck squamous cell carcinoma (HNSCC) [86]. Similarly, a subset of $CD69^+$ $CD103^+$ $CXCR6^+$ $CXCR3^+$ $CD8^+$ T cells expressing liver retention markers were highly expanded in some controlled HBV patients [87]. These results suggested that the induction of a substantial number of $CD8^+$ T_{RM} cells might be crucial for future optimization of cancer immunotherapies. For non-virus-associated cancers, vaccine-induced $CD8^+$ T cell immunity might play a prominent role in eliminating tumor cells compared with the $CD4^+$ T cells and antibody responses [88, 89]. Notably, vaccine-induced anti-tumor systemic and resident T-cell responses often depend on the types of vaccine regimens engaged [90]. In recent years, the heterologous prime-boost immunization strategy has been widely applied to amplify anti-tumor T cell responses [91–95]. The potential of T_{RM}-mediated tumor killing *in situ* has been documented in several intradermal vaccination-subcutaneous tumor planting models [90, 96–98]. T_{RM} could further improve T_{cirm}-mediated immunity against subcutaneous tumor progression [90, 99–101]. Interestingly, T_{RM} cells may improve anti-tumor immunity in distant tissues [96]. Researchers utilized an intranasal DC-targeting peptide vaccine (STxB-E7) to establish substantial lung T_{RM} immunity. TC1 tumor cells were subsequently grafted into the tongue of recipient mice after intranasal vaccination. Vaccine-elicited lung T_{RM} cells were capable of eliminating tumor cells in the head and neck of vaccinated mice [96]. As for T cell immunity against lung metastasis, some studies provided effective vaccine regimens to control lung metastasis, yet the role of T_{RM} has not been well demonstrated. For example, an albumin-chaperoned peptide-based cancer vaccine illustrated that T_{RM} showed a trend toward better anti-lung metastasis effect compared with T_{cirm} despite of lack of statical significance [102]. Apart from the cancer vaccine, researchers utilized melanoma-associated vitiligo (MAV) to induce T_{RM}. While T_{cirm} provided protection against melanoma lung metastasis, T_{RM} mediated long-term protection against melanoma within lymph nodes and skin [100]. Although the high responsiveness of $PD\text{-}1^+$ $CD103^+$ lung T_{RM} has been associated with improved clinical outcomes in lung cancer patients [103], vaccine combinations tested (e.g. MAV model) did not induce potent T_{RM} immunity to improve the efficacy against lung metastasis. The possible reason for failure might be related to the sub-immunodominant antigen selection during vaccine design, limited potency of chaperoned vaccine adjuvant, and improper vaccination route [104]. Vaccine research should strengthen the role of T_{RM} cells against lung metastasis.

Clinical Approaches for Enhancing T_{RM} Induction and Activation

Cancer Immunotherapy

Immune checkpoint blockade (ICB) and chimeric antigen receptor (CAR) T-cell therapy have been widely applied in cancer treatment. Due to the demonstrated advantage of T_{RM} in protection, clinical trials may attempt to maximize the effect of T_{RM} by enhancing its activation and frequency. Although several cytokines and ligand-receptor pairs have been identified to be critical for T_{RM} activation, few have been applied to clinical settings. Currently, PD-1 has been considered one of the surface markers of $CD8^+$ T_{RM} infiltrating human non–small cell lung carcinoma (NSCLC). Anti-PD-1 and anti-PD-L1 treatments could rescue intra-tumoral $CD8^+$ T_{RM} and increase the target oncolysis [105, 106]. Meanwhile, the frequency of T_{RM} was positively correlated with better clinical outcomes in lung cancer patients. A subset of $PD\text{-}1^+$ T_{RM} was also illustrated as the major responder to secret a large amount of cytokine during the ICB treatment [103].

Another preclinical study combined the MER proto-oncogene tyrosine kinase inhibition (anti-MerTK) and anti-PD-1 with radiotherapy for NSCLC treatment, which improved the survival rate in bilateral lung adenocarcinoma by augmenting the percentage of intra-tumoral T_{RM} [107]. Compared with recently infiltrating T lymphocytes, preexisting intra-tumoral T_{RM} cells demonstrated survival advantage because of their resistance to high-dose ionizing radiation [107]. Besides the survival advantage of T_{RM}, a clinical study indicated that the combination of ICB and high-dose radiation could induce interferon beta (IFN-β) production and subsequently could upregulate MHC-I molecules on the surface of tumor cells for improved stimulation of anti-tumor T cell responses [108, 109]. More clinical trials are ongoing for the improvement of combined therapies.

Vaccine is another approach to cancer immunotherapy. More than a thousand clinical trials are ongoing and over half of the candidates are therapeutic vaccines [110]. The therapeutic vaccine is inoculated after the cancer establishment and thus may counter intractable challenges: 1) immune evasion of the vaccine-covered antigen, 2) effective delivery of the vaccine to target sites, 3) overcoming immunosuppressive TME, and 4) rescue of exhausted T cells [110–113]. Existing vaccine candidates mainly focus on eliciting immediate effector T-cell responses, broadening tumor antigen coverage, and achieving improved tumor clearance and memory establishment. Developing vaccine strategies by enhancing T_{RM} responsiveness might be another promising area for future therapeutic anti-tumor vaccine research and development.

Clinical Vaccine Candidates for Enhancing T_{RM}

Multiple intranasal vaccine candidates elicit potent mucosal immunity with improved protection against respiratory infection in animal studies. Common intranasal vaccine vectors include adenovirus (Adv), adeno-associated virus (AAV), recombinant vaccinia virus (rVV), modified vaccinia virus Ankara (MVA), and live-attenuated influenza A virus (LAIV) due to their live-attenuated safety profiles in humans. To date, FluMist is the only FDA-approved intranasal vaccine for people excluding ages older than 50, pregnant women, and immunocompromised populations [54]. After being reformulated into a quadrivalent vaccine in 2018, the efficacy of FluMist is comparable to that of the conventional flu vaccine. It is encouraging that a single intranasal inoculation could provide protection against cross-subtype influenza virus for the year [114]. Clinical data released, however, did not show correlations of humoral and cellular immune responses with protection in vaccinees. Kyra and colleagues, however, evaluated T_{RM}-mediated protection between quadrivalent intranasal and intramuscular vaccines in mice [115]. Intranasal vaccination elicited significantly lower neutralizing antibody responses in sera but a significantly higher frequency of influenza-specific T_{RM} in the lungs compared with intramuscular injection. Meanwhile, vaccine-induced T_{RM} provided protection against the lethal dose viral challenge. Although this study did not deplete B cells to exclude the influence of mucosal IgA and tissue-resident memory B cell (B_{RM}), the implication that T_{RM} may play a pivotal role in decreasing death and severe symptoms is consistent with previous findings [51, 116, 117].

To induce potent T_{RM} cells in the lung, respiratory antigen presentation is critical. The dose of intranasal inoculation is a critical factor for clinical application. Nowadays, clinical trials commonly utilize the AccuSpray Device to deliver 200–500μL liquid in a single inoculation dose [118]. The vaccine squirted from the nozzle as liquid particles can easily adhere to the upper respiratory mucosa, but the deposition efficacy to lung tissue is relatively limited (Figure 2A). In the mouse model, intranasal inoculation volume varies from 20–80μL. A dose higher than 20μL is sufficient for the vaccine component to reach the lung. To increase the exposure area of both upper and lower respiratory tracts to the vaccine component for better lung T_{RM} induction, a recombinant adenovirus type-5-vectored (Ad5) COVID-19 vaccine adopted the Aerogen Ultra Device for delivering vaccine particles. 2×10^9 viral particles were nebulized for each administration. Determined through laser diffraction, the average diameter of the aerosolized vaccine particle was 5.4μm. Vaccines were administered by 30 to 60s nebulization inhalation (Figure 2B). As measured on day 14 post-vaccination, single dose aerosol vaccine elicited a similar level of antigen-specific T cell response in blood compared to intramuscular vaccination. Additionally, the group receiving an intramuscular prime vaccination followed by an aerosolized booster at 28-day intervals displayed the highest sera IgG, IgA, neutralizing antibody, and spike-specific T cell response in blood on day 14 after the immunization regimen [73]. However, the metrological control for each recipient might not be as accurate as intramuscular vaccination. The liquid leakage during inhalation or misuse of devices might cause vaccine waste and unwarranted environmental contamination. Notably, 12 intranasal vaccine candidates that successfully established potent respiratory T_{RM} against SARS-CoV-2 infection in preclinical research have entered phase I/II clinical trials. Probably due to the complicated operation

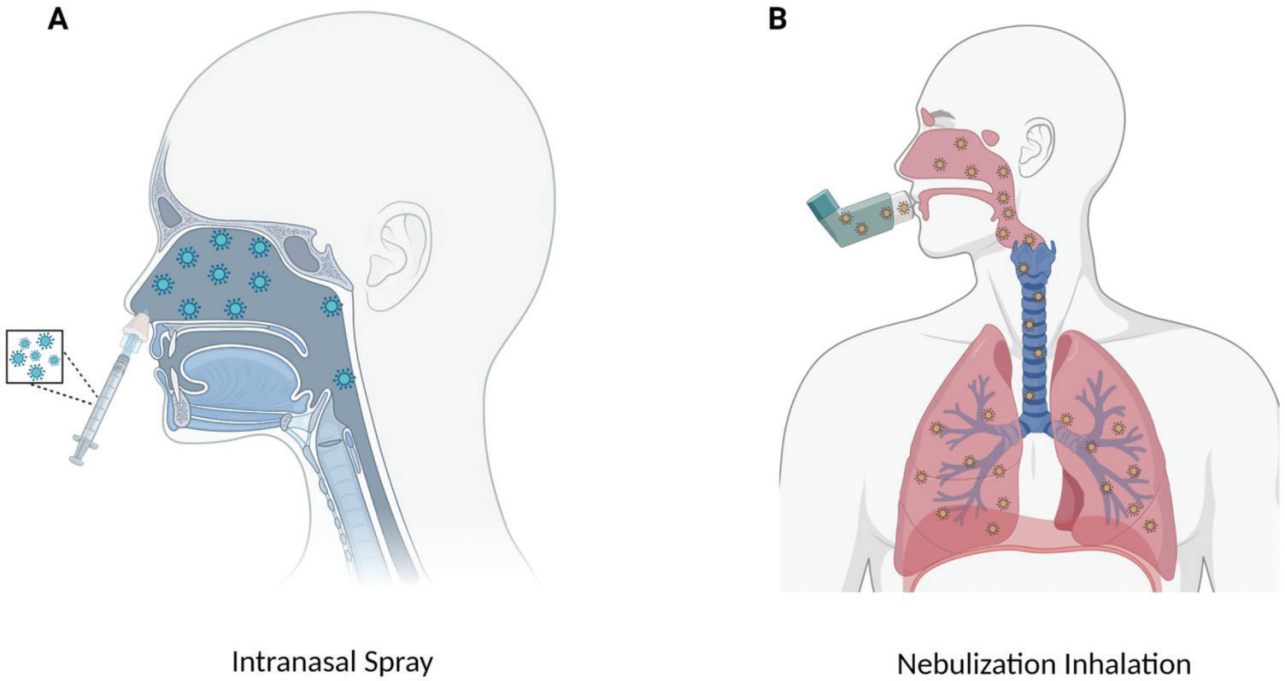

Figure 2. Clinical devices for inhaled vaccine. (A) FluMist and some ongoing clinical trials adopt Aerogen Ultra Device to deliver the intranasal vaccine. 200–500μL liquid containing vaccine component is nebulized via nasal inoculation. The issue is that large vaccine droplets easily attach to the upper respiratory mucosa and hardly reach to lungs. (B) An Ad5 COVID-19 vaccine utilizes Aerogen Ultra Device for vaccine inoculation. This administration procedure will last for 30–60 s and includes rounds of respiration which increases the exposure to vaccine particles in both upper respiratory mucosa and lung. Created with Biorender.com.

and vaccinees' reluctance for mucosal tissue and bronchial alveolar lavage (BAL) sampling, the functionality of T_{RM} cells elicited by vaccine candidates was poorly demonstrated in current clinical data. Further clinical data is required to prove the induction of functional and durable T_{RM} in humans.

Conclusion and Future Directions

Given the evidence that T_{RM} may provide prolonged protection to curb disease progression in tissue compartments, the knowledge of cellular ontogeny would be beneficial for future vaccine strategies and immunotherapy improvement. The evolving approaches may include the following areas. To optimize the T_{RM} induction, how to enlarge the size of the T_{RM} precursor pool and how to create the local milieu for biased T_{RM} development remains to be investigated comprehensively. Previous studies have tested the strategy of targeting antigens to DCs using anti-DEC205-, anti-CTLA4-, or soluble PD-1-based fusion vaccines, which could significantly increase DC activation and antigen cross-presentation for better T cell activation [119–121]. An alternative approach is to develop a strategy to enhance T_{RM} preconditioning activity between T_{RM} precursor and tissue cells. To this end, a better understanding of the cell-cell interaction between T_{RM} precursor and tissue cells during homeostasis or the early activation phase is necessary. Exploit key cytokines and regulating molecules in the T_{RM} development process into vaccine adjuvant may be another approach to improve T_{RM} induction. On the other hand, to enhance T_{RM} activation, like the anti-PD-1 immunotherapy in cancer treatment that promotes T_{RM} activation, engaging ligand-receptor interaction which specifically activates T_{RM} without inducing immune hyperactivation will be of great clinical application value. Besides, the exploration of new vaccination methods should combine with the optimization of delivery routes and devices. Reducing vaccine leakage, accurately controlling inoculation dose, and delivering vaccine components including useful adjuvants efficiently to the target site are decisive factors to be considered in future vaccine development.

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Conflicts of interest

We declare no competing interests.

Data availability

Not applicable.

Author contributions

H.X. and Z.C. wrote the manuscript. R.Z. contributed to the data and revision of the manuscript.

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Clinical trial registration

Not applicable.

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