

# Tissue-resident memory T cells

**Generation of tissue-resident memory T cells and the protection they provide against influenza and other infectious diseases**

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## **Abstract**

Vaccination to influenza does not provide long-term protection against influenza virus. The epitopes of influenza virus that are recognized by antibodies continuously change. Recognition of influenza virus should be based on epitopes that can be found on every influenza virus. T cells may be able to recognize these epitopes. Since influenza viruses enter via the lung, local protection would be logical. Local protection can be mediated by tissue-resident memory T (Trm) cells. Trm cells originate from circulating T cells that migrate to the site of inflammation and are retained inside the lung after infection. After primary infection, the Trm cells protect the body against reinfection. Not only are Trm cells able to clear the infection themselves, they can also activate both innate and adaptive immune responses. In conclusion, Trm cells seem to be important for protection against influenza virus and other infectious diseases.

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## Introduction

Influenza is one of the major human respiratory diseases. It is caused by influenza virus which infects people many times in life. Every year 3 to 5 million people become severely ill as a result of an influenza virus infection (<http://www.who.int/mediacentre/factsheets/fs211/en/>). Three types of influenza viruses are known to infect humans, which are influenza A, B and C (Taubenberger et al., 2008). Influenza A is the only influenza virus type known to be able to cause a pandemic (<http://www.who.int/mediacentre/factsheets/fs211/en/>). Influenza A viruses are divided into subtypes based on their types of hemagglutinin (HA) and neuraminidase (NA). Inside the envelope of the virus are 8 negative RNA-strands, which code for 11 viral proteins (Samji et al., 2009). Although not all variants are able to infect humans, often these variants can exchange RNA with variants that are able to infect humans.

The exchange of RNA fragments between different subtypes of influenza viruses can result in the acquisition of a different HA subtype, this process is called antigenic shift (Samiji et al., 2009). This is a way to evade the immune system which mainly recognizes HA and NA with the use of antibodies specific for a subtype of HA or NA. Another way to evade an immune response is by small mutations in the HA and NA. Small mutations prevent or at least limit the recognition of influenza virus by antibodies, which is called antigenic drift (Taubenberger et al., 2008). The pandemics of 1957 and 1968 were caused by antigenic shift, where human influenza viruses imported HA from avian influenza virus (Taubenberger et al., 2008).

Vaccination is an important method to prevent influenza. However, vaccination has to be repeated every year in the elderly and immune compromised to remain protective (Goodwin et al., 2006). Vaccination, however, only works optimally for several months and due to antigenic drift the original vaccine would not give sufficient protection after several years (Taubenberger et al., 2008).

The goal is to find an influenza vaccine that protects against many if not all influenza strains, which is called heterosubtypic protection. Universal or broadly protective vaccines need to target conserved epitopes that are present in all influenza virus strains. One means to provide long-term protection is to induce T cells that target these conserved epitopes. (Powell et al., 2007; Kreijtz et al., 2007).

So far the focus to attain long-term and broad protection against influenza virus has been on circulating effector memory T (Tem) cells that will be recruited to the lung upon reinfection.

Recently a new subset of T cells have been identified that reside at the site of previous infection, the tissue resident memory T (Trm) cells (Gebhardt et al., 2009; Iijima & Iwasaki, 2015). Local Trm cells may be crucial in the heterosubtypic protection against influenza virus, instead of Tem cells.

Trm cells stay inside the tissue, instead of circulating through the body via the blood vessels (Gebhardt et al., 2009). Just as the circulating T cells, Trm cells are also part of the CD4+ and CD8+ cell populations. So far, mainly CD8+ Trm cells were of interest to provide protection against virus infection. Lately CD4+ T cells have been given more emphasis as well.

This report aims to summarize current knowledge about the generation of Trm cells and the protection they provide against infectious diseases, specifically influenza.

## Origin of Trm cells

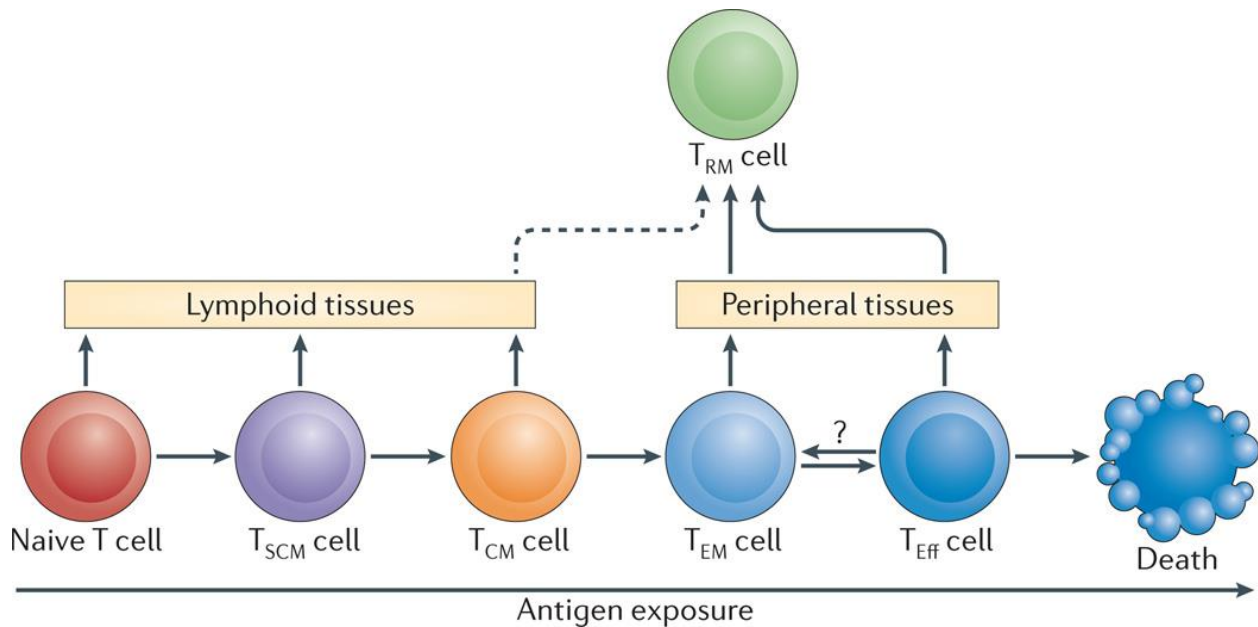


Figure 1. The progressive differentiation of naive T cells to other T cell subsets such as, stem cell memory T (T<sub>SCM</sub>) cells, central memory T (T<sub>CM</sub>) cells, effector memory T (T<sub>EM</sub>) cells, effector T (T<sub>EFF</sub>) cells and tissue-resident memory T (T<sub>RM</sub>) cells based on the extent of antigen exposure. T<sub>EFF</sub> cells are terminally differentiated. T<sub>RM</sub> cells originate from T<sub>EM</sub> and T<sub>EFF</sub> cells. T<sub>CM</sub> cells may become T<sub>RM</sub> cells as well. From Farber et al., 2014.

T cells can be divided in several subsets, which are able to differentiate in other subsets. First off, naive T cells are the cells that have not recognized any antigen. Naive T cells can differentiate in different subsets based on antigen exposure, which can be seen in Figure 1 (Farber et al., 2014).

Naive T cells can become stem cell memory T cells which are known to be stem cell-like and are known for their long life span and self-renewal. More common are central memory T (T<sub>CM</sub>) cell and effector memory T (T<sub>EM</sub>) cell. T<sub>CM</sub> are relatively slow and differentiate in T<sub>EM</sub> cell after antigen recognition, whereas T<sub>EM</sub> cells can become effector T (T<sub>EFF</sub>) cells rapidly after reinfection. A new subset that has drawn attention is the tissue resident memory T (T<sub>RM</sub>) cell, which are T cells that permanently reside within peripheral tissues and are unable to recirculate through the blood stream (Gebhardt et al., 2009; Mueller & Mackay, 2016).

T<sub>RM</sub> cells were first found in the dorsal ganglia and the skin after herpes simplex virus infection (Gebhardt et al., 2009). Before that it was unknown if the T cells that were found, were circulating T cells or tissue resident T cells. Circulating T cells can be mistaken for T<sub>RM</sub> cells, because at the moment of tissue staining not all T cells found in the tissue are tissue resident T cells. A portion of the cells are just circulating cells that temporarily reside within the tissue. These T<sub>RM</sub> cells do, however, originate from the same precursor as T<sub>EM</sub> and T<sub>CM</sub> cells (Gaide et al., 2015; Watanabe et al., 2015). Gaide et al. used high-throughput sequencing of the T cell receptor of T cells found in a distant lymph node, a draining lymph node and in the ear and tail

of mice. It was found that there were T cells present in all three tissues that expressed the same unique T cell receptor. This means that all these T cells belonging to different subsets have had a common precursor.

Watanabe et al. found that Trm cells originate from circulating T cells (Watanabe et al., 2015). A piece of human neonatal skin tissue that was almost devoid of Trm cells was used to prove this. This piece of skin was transplanted on a receiver, which was the NOD scid gamma (NSG) mouse. The NSG mouse strain is known to be an immunodeficient, lacking mature T cells, B cells and natural killer cells (Schultz et al., 2015). Dermatitis was induced by the intravenous infusion of mononuclear cells and T cells. A subset of the T cells that migrated into the grafted skin showed expression of Trm markers, which suggests the generation of Trm cells. Based on both observations it can be concluded that the circulating T cells have a common unique naïve T cell precursor with Trm cells that have been found in non-lymphoid tissue.

## Migration and retention of Trm cells

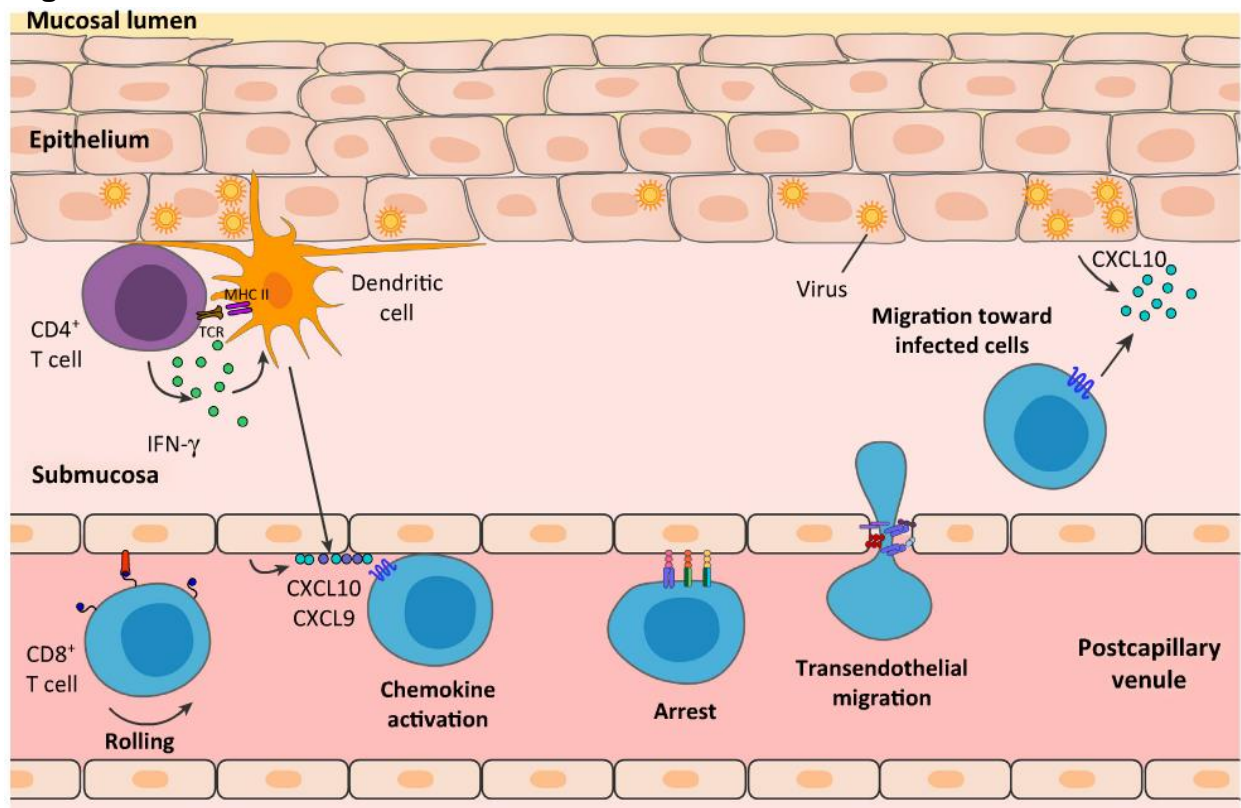


Figure 2. Schematic overview of the migration of CD8+ T cells towards infection. From Iijima & Iwasaki, 2015.

Migration is the initial step in the generation of Trm cells. As discussed above, Trm cells originate from circulating T cells. These T cells have to migrate into peripheral tissue before they can establish a Trm population. Migration as seen in Figure 2 mainly occurs during inflammation and can be characterized by distinct phases (Iijima & Iwasaki, 2014). First of all, T cells begin to roll over the activated endothelium when the blood flow is slow enough. This rolling is mediated by cell-adhesion molecules, known as selectins. E-selectin and P-selectin are expressed by the endothelium and T cells can bind to these selectins by expressing corresponding ligands. Endothelial cells will then display chemokines to T cells to induce cell-cell binding by integrins which results in arrest. After arrest it is possible for the T cells to migrate through the endothelial cell layer which is called transendothelial migration.

The CD4+ T cells that have already entered the tissue produce high amounts of IFN-gamma. In turn dendritic cells produce CXCL9 and CXCL10 which are presented by endothelial cells to the CD8+ T cells (Nakajima et al., 2002). CXCL9 and CXCL10 are recognized by CXCR3 which is expressed in T cells that are released from T cell receptor signals, which is the case in circulating T cells (Iijima & Iwasaki, 2015). However, it should be noted that the chemokines and T cell receptor needed to migrate into the tissue, is different between organs. In absence of CD4+ T cells, CD8+ T cells are hindered in their ability to migrate to the inflamed tissue. To establish a Trm population it is important that T cells are retained within the tissue and do not recirculate. After successful retention, a true Trm cell population is formed.

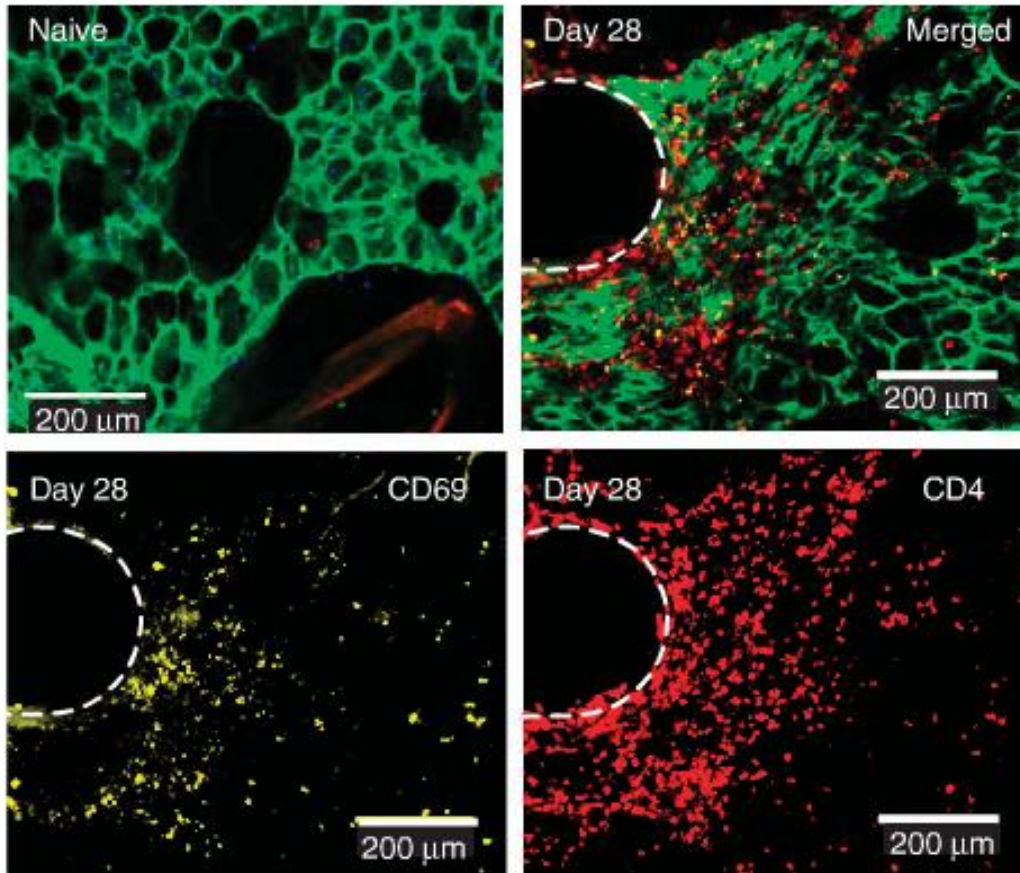


Figure 2. CD69-expression on CD4 T cells in niches inside the lung after influenza virus infection. CD69 expression is shown in yellow, cells separated from the circulation are shown in red staining represents the cells that were separated from the circulation and collagen is shown in green. From Turner et al., 2014.

Migration alone is not enough for T cells to become fully differentiated Trm cells, as it also happens during a normal immune response by the circulating T cells. It is important to keep the previously circulating T cells on its place within the lung tissue. This is mediated mainly by two surface molecules, CD69 and CD103 (Iijima & Iwasaki, 2015). The location of CD69 and CD103 expressing cells in the lung can be seen in Figure 3.

CD69 is found in both CD4+ and CD8+ Trm cells (Turner et al., 2014). CD69 is the antagonist of S1P1 for cell-surface expression (Skon et al., 2013). S1P1 is an egress receptor that promotes recirculation. CD69 is able to promote the degradation of S1P1 and can therefore prevent migration back into the bloodstream.

CD103 is responsible for attaching Trm cells to other cells in their environment by binding to E-cadherin that is expressed by those other cells. CD103+ has been seen on CD8+ Trm cells but not on CD4+ Trm cells. Also, CD103 is less common than CD69 on CD8+ Trm cells (Iijima & Iwasaki, 2015). Without CD103, however, the Trm population may be less stable than it would be otherwise.

CD103 expression is stimulated by TGF-beta, because it reduces the expression of a CD103 inhibitory transcription factor called T-bet (Laidlaw et al., 2014; Iijima & Iwasaki, 2015). Again CD4+ T cells are important for CD8+ Trm cell generation, because they produce TGF-beta. In the absence of CD4+ T cells within the tissue, causes CD8+ T cells to have higher expression levels of T-bet. Which inhibits the retention of CD8+ Trm.

Trm cells can be recognized based on that they cannot be labeled via the bloodstream, because they are dissociated from the bloodstream (Turner et al., 2014). Labeling can be done *in vivo* with antibodies specific for a CD4 or CD8. The portion of T cells that are accessible for the antibody are being labeled, the T cell that are not accessible are not labeled.

The other way to recognize Trm cells is based on their expression of cell surface markers such as CD69 and CD103.

It was shown that the generation of lung Trm cells specifically depends on dendritic antigen presentation and TGF-beta signaling (Wakim et al., 2015). It was stated that TGF-beta signaling may be sufficient to induce the generation of Trm cells, but that the lung has very low levels of TGF-beta and therefore CD8+ Trm cells require dendritic cells. These dendritic cells induce CD103+ expression in Trm cells by displaying membrane-bound TGF-beta1. CD103+ dendritic cells may be the most capable of doing this. Not only are CD103+ dendritic cells able to produce TGF-beta1, but they can also cross-present antigens to T cells.

Most studies regarding Trm cells have been done in mice. Not many articles focus directly on studying humans. Turner et al used human lung biopsies to consolidate their findings in mice (Turner et al., 2014). Their findings show that CD69+ CD8+ T cells specific for influenza virus were found in clusters inside the lung, just as what was found in mice. For comparison cytomegalovirus (CMV) was used. CMV-specific CD8 T cells were both found in the lung and spleen in comparable ratios. However, relative amount of influenza virus-specific CD8 T cells in the lung was much higher than what was found in the spleen. Different pathogens can have different effects on the generation of Trm cells. A site specific pathogen causes relative higher amounts of Trm cells on a specific part of the body. Influenza virus, which is a lung specific virus, causes the generation of a relatively high number of Trm cells in the lung.



## Lifespan of Trm cell populations

The Trm cell population in the lung is not in contact with the circulation and is able to maintain itself for at least 120 days (Turner et al., 2014). This means that the lung Trm cell population does not need replenishment from the lymphoid tissues. It was suggested that the retention of Trm cells in the lung on specific locations may prevent the loss of virus-specific T cells, because otherwise newly formed T cells may compete with other T cell populations (Turner et al., 2014). A self-sustaining T cell population that does not suffer from competition from other populations is ideal.

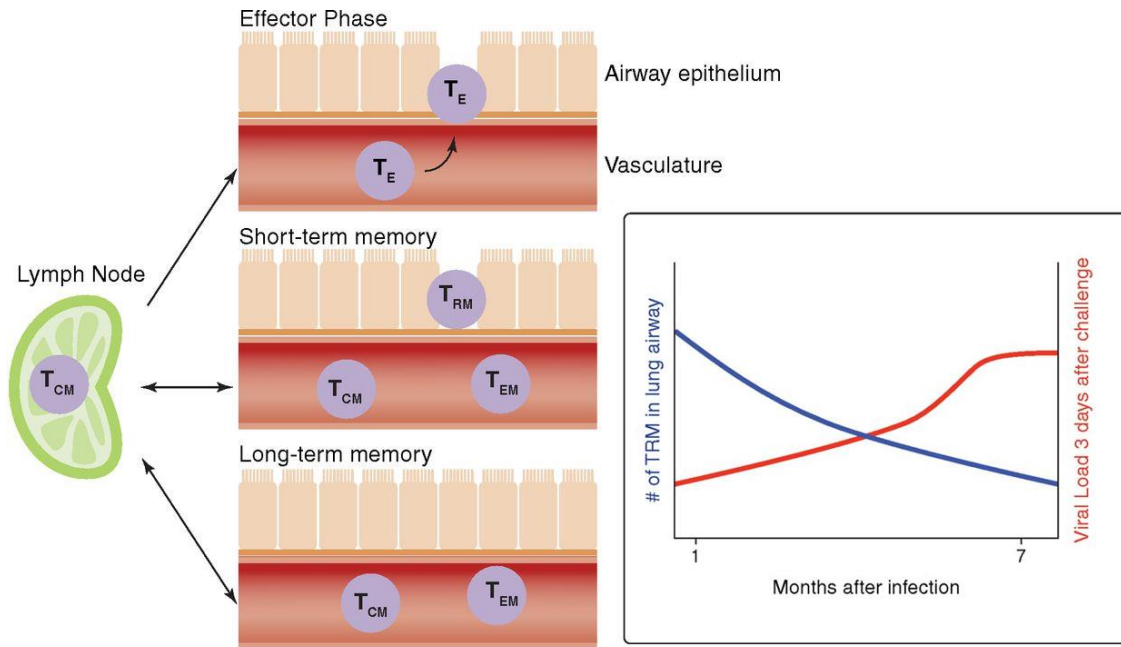


Figure 3. Influenza virus infection causes T<sub>eff</sub> to enter the lung tissue. Shortly after infection Trm cells arise from the T<sub>eff</sub> cells, however these Trm cells disappear after several months. Adapted from Anderson et al., 2014.

However, Wu et al. shows that the initial Trm population wanes after 7 months as can be seen in Figure 3 (Wu et al., 2014; Anderson et al., 2014). Figure 3 shows that T<sub>eff</sub> cells migrate towards the inflammation and become Trm cells. These Trm cells remain on the site of the previous inflammation for a relatively short amount of time, but disappear after an extended period of time. Also viral load is negatively correlated with the number of Trm cells in the lung, which indicates the importance of Trm cells for viral control. Thus, Trm cell populations are self-sustainable but only for a limited time.

Lifespan of these Trm cell populations may depend on programmed cell death protein 1 (PD-1), which is expressed in low levels in Trm cells after viral pulmonary inoculation. PD-1 is a protein that is often expressed at low levels on CD8 T cells that are chronically stimulated with an antigen. High levels of PD-1 are correlated with reduced capacity of T cells to proliferate and produce cytokines (Trautmann et al., 2006). This can cause immune dysfunction. PD-1 expression profiles in Trm cell populations may give insights into the lifespan of these Trm cells.

## Trm cells and infectious diseases

Trm cells play a key role in the rapid immune response to foreign antigens and pathogens (Ariotti et al., 2014, Gaide et al., 2015). Tcm are able to help the Trm cells in the clearance of the antigen after infection, but are slower in their reaction time (Ariotti et al., 2014; Gaide et al., 2015). Trm cells are important for the first line of defense against a pathogen.

Wu et al. goes a step further and found out that the number of virus-specific Trm was crucial for protection and that Teff cells had little effect on the protection against an influenza viral infection (Wu et al., 2014). Teff cells did activate after the infection, but they had no substantial role in the protection. This was mainly caused by again slow reaction of Teff cells, contrast to fast reacting Trm cells. Teff cells did proliferate in the local tissues after infection, but this was seen after viral titers were already declining. This indicated that Trm cells already reacted to the infection.

Among others it has been shown that Trm cells protect against infection of influenza virus, herpes simplex virus (HSV), vaccinia virus (VACV) and *Leishmania Major*. The latter is not a virus but a parasite (Gebhardt et al., 2009; Ariotti et al., 2014, Schenkel et al., 2014; Iijima & Iwasaki, 2014, Glennie et al., 2015; Reilly et al., 2016; Zens et al., 2016; McMaster et al., 2015; Jiang et al., 2012).

HSV is a virus that causes skin infections. After the skin infection HSV can lie dormant in the ganglia close to the site of infection. After some time HSV can then reactivate. Gebhardt et al. found CD8+ T cells present in the ganglia after HSV infection (Gebhardt et al., 2009). Not only are these CD8+ T cells found in the ganglia, but also in the skin where HSV infects the body. When a HSV infected ganglia was transplanted in a donor, reactivation of the virus induced an immune response from CD8+ Trm cells inside the ganglia against HSV.

The study of Gebhardt et al. also found that skin CD8+ Trm cells expressed CD103 and CD69. Furthermore, the Trm cells were dissociated from the circulation and had a lower proliferation rate than their circulating counterparts. The most interesting part of the study involves the protection the skin Trm cell population offers when the skin is infected again. Without antibodies, the previously HSV-infected skin showed a 100-fold better control of HSV after reinfection compared to the control skin.

Another example of a virus that can infect the skin is VACV. This virus also generated CD8+ Trm cells were also generated after infection of the skin (Jian et al., 2012). Just as in HSV, this population of CD8+ Trm cells protected against a subsequent VACV infection and do this very rapidly. CD8+ Trm cells are more effective at protecting against another infection with VACV than Tcm cells. Trm cells spread through the whole skin as a result of tissue-wide distribution of Tem cells that become those Trm cells.

CD8+ Trm cells have more functions than simply killing infected cells, they also secrete cytokines that are able to induce both innate and adaptive immune responses (Schenkel et al., 2014). Among others CD8+ Trm cells have shown to be able to induce interferon-induced transmembrane protein 3 (IFITM3) in skin cells by secreting IFN-gamma (Ariotti et al., 2014). IFITM3 is a protein that has antiviral activity.

In addition to CD8+ Trm also preexisting CD4+ Trm cells are important in the protection against re-infection of among others influenza virus, HSV-2 and *L. major* (Zens et al., 2016; Iijima & Iwasaki, 2014; Glennie et al., 2016).

During secondary HSV-2 infection, CD4+ Trm cells managed to quickly suppress viral replication before it could spread to the nervous system (Iijima & Iwasaki, 2014). These CD4+ Trm cells were found inside memory lymphocyte clusters as a result of chemokine secretion of macrophages. Also, CD4+ Trm cells were more effective at providing protection than the circulating CD4+ Tem cells.

However, a study by Glennie et al. found that CD4+ Trm cells are dependent on circulating CD4+ T cells in their protection against a secondary infection with *L. major* (Glennie et al., 2015). Without circulating CD4+ T cells, the number of parasites did not decrease. Circulating CD4+ T cells alone did decrease the number of parasites, but in combination with CD4+ Trm cells the number was even further decreased. CD4+ Trm cells may be involved in the recruitment of circulating CD4+ T cells in order to control the infection. Recruitment of CD4+ T<sub>H</sub>17 cells was promoted by their expression of CXCR3. Thus, both CD4+ and CD8+ Trm play their role in the protection against reinfection.

## **Trm cells and influenza**

As described above, Trm cells that are found in the lung after infection with influenza provide protection against influenza (Wu et al., 2014). Only inoculation via the lung generated a local Trm cell population.

Turner et al. found that the CD4+ T cells moved to other places in the lung after influenza virus infection. Clusters of CD4+ Trm cells found were close to the site of infection and were

If Trm cells can be generated in the lung depends on the expression of cell-adhesion molecules, such as selectins, on the endothelium (Iijima & Iwasaki, 2015). CD4+ Teff cells can always migrate into the lung. Inflammation is not a requirement for the migration of CD4+ Teff cells, but inflammation is important for the establishment of a large population of CD4+ Trm cells. CD8+ Teff cells depend on inflammation in order to only be able to migrate. The lung only expresses cell-adhesion molecules needed for CD8+ Teff migration during inflammation.

The mechanisms about how Trm cells specific for influenza virus provide protection against reinfection is largely unknown. Reilly et al. found that a Trm cell population was generated in the lungs after influenza virus challenge (Reilly et al., 2016). This Trm cell population protected the mouse from a usually lethal dose of heterosubtypic influenza virus. Important is the fact that this Trm cell population produced IFN-gamma, which is important for the protection against viruses (McMaster et al., 2015)

Just as in some other infectious diseases, Trm cells generated after influenza virus show to be most effective with the help of other immune responses. Trm cells function optimally when in conjunction with virus-specific antibodies (Wu et al., 2014).

The protection against influenza reinfection by Trm cells has to be as efficient as possible with low collateral damage to the lung. A study that completely focused on Trm cells present in the human lung found that CD8+ Trm cells in the lung were balancing between controlling infections and avoiding damage to the surrounding tissue (Hombrink et al., 2016). CD8+ Trm cells had a gene-expression program that was associated with inhibition of T cell activation, including inhibitory receptors, suppressive transcription factors and a T cell receptor signaling inhibitor.

## **Vaccination and the generation of Trm cells**

That Trm cells are generated after infection and provide protection against secondary infections is rather clear. Most studies about the generation of Trm cells use functional pathogens. It would be of interest to know if Trm cells are also generated after vaccination. Instead of protection against reinfection, Trm cells generated after vaccination may protect against primary infection. It is important to know what is needed to generate a large Trm cell population after vaccination.

Morabito et al. and Zens et al. showed indeed that vaccination generates a Trm cell population that does protect against a viral infection. Respiratory syncytial virus (RSV) generated a CD8+ Trm cell population in the lung after intranasal administration (Morabito et al., 2016). Important to note is that when the vaccination against RSV was given intraperitoneal, virus control was a lot lower. Lower viral control was attributed to the effect of the CD8+ Trm cell population that is induced after intranasal vaccination, but not after intraperitoneal vaccination.

That the route of vaccination is important for the generation of a Trm cell population and its protection against a virus was confirmed by Zens et al. Intranasal administration of two seasonal live-attenuated influenza virus (LAIV) vaccines generated a stable Trm cell population consisting of CD4+ and CD8+ Trm cells that were able to provide heterosubtypic protection (Zens et al., 2016). The generation of Trm cells was only achieved when vaccines were administered intranasal. Inactivated influenza virus (IIV) vaccination administered subcutaneous or intraperitoneal, was not able to generate Trm cell populations and provide heterosubtypic protection. IIV only provided strain-specific humoral immunity.

The duration that Trm cells were studied was 6 weeks, a lot shorter than 7 months by Wu et al. (Wu et al., 2014). Thus, the actual duration for which Trm cells provide protection against influenza after LAIV vaccination remains unclear.

## Discussion

Trm cells have gained more interest in their capability to protect against infections. As of yet, most functions of Trm cells are unknown and research is mainly based on detecting Trm cells. The presence of Trm cells is usually determined in two ways. The first way this can be done is by looking at the properties of Trm cells. Trm cells are hard to reach via the circulation and do not reenter the circulation or migrate via the circulation (Gebhardt et al., 2009; Turner et al., 2014). The other option is to look at the expression of cell surface markers of these T cells residing in the lung. Trm cells are known to express CD69 which is a protein that prevents the cell from migrating and recirculation by antagonizing S1P1, an egress receptor. While CD69 is commonly seen on all cells of the Trm cell population, CD103 is mainly seen on the CD8+ Trm cells. CD103 mediates attachment to the environment by attaching to E-cadherin which is expressed by neighboring cells.

Unfortunately, the lifespan of Trm cell populations seems to be limited. Trm cell population against influenza virus lasted for >120 days, >6 weeks and 7 months (Turner et al., 2014; Zens et al., 2016; Wu et al., 2014). The large differences found in Trm cell lifespan does not give a clear answer to the actual lifespan. The variation may be explained in part by that there is usually a limit to how long a study can last. A study that studied Trm cells for a longer period of time by Wu et al. showed that Trm cells disappeared after approximately 7 months after virus infection. Trm populations that could maintain itself without replenishment from the circulation have been found, but this maintenance was only followed for 120 days and not 7 months (Turner et al., 2014).

It is unknown if Trm cells actually are not present or that they simply cannot be found in the tissue anymore. Reinfection may cause a small number of surviving Trm cells present in the tissue to proliferate again and provide protection. Thus, conclusions cannot be drawn about the maintenance of Trm populations over an extended period of time.

Even though the lifespan of Trm cells is mostly unknown, they have been shown to provide protection during the period they can be found in the tissue. Trm cells were important for the protection against reinfection of the skin, vaginal wall and lung (Gebhardt et al., 2009; Jiang et al., 2012; Morabito et al., 2016; Glennie et al., 2015; Iijima & Iwasaki, 2014; Turner et al., 2014). Earlier research mainly found CD8+ Trm cells after infection, but lately CD4+ Trm have also been found. Both Trm cell types protect against a secondary infection.

It was shown that virus titers already started to decrease before Treg cells began proliferating, which indicates that Trm cells were responsible for the decrease in virus titers (Wu et al., 2014). Trm cells were able to provide general protection against viruses via IFITM3 in skin cells (Ariotti et al., 2014). Besides the general protection, Trm cells also activated and recruited both the innate and adaptive immune system. This indicates that although Trm cells seem to play an important role in the protection against a secondary infection, they do this optimally with the help of the innate and adaptive immune system (Wu et al., 2014; Schenkel et al., 2014). It is possible that Trm cell function is mainly based on activating the immune response, instead of clearing the pathogen completely by themselves.

Important to generate a Trm cell population is the route of inoculation or vaccination. In the lung it was shown that local inoculation or vaccination is a prerequisite for Trm cell generation (Wu et al., 2014; Zens et al., 2016).

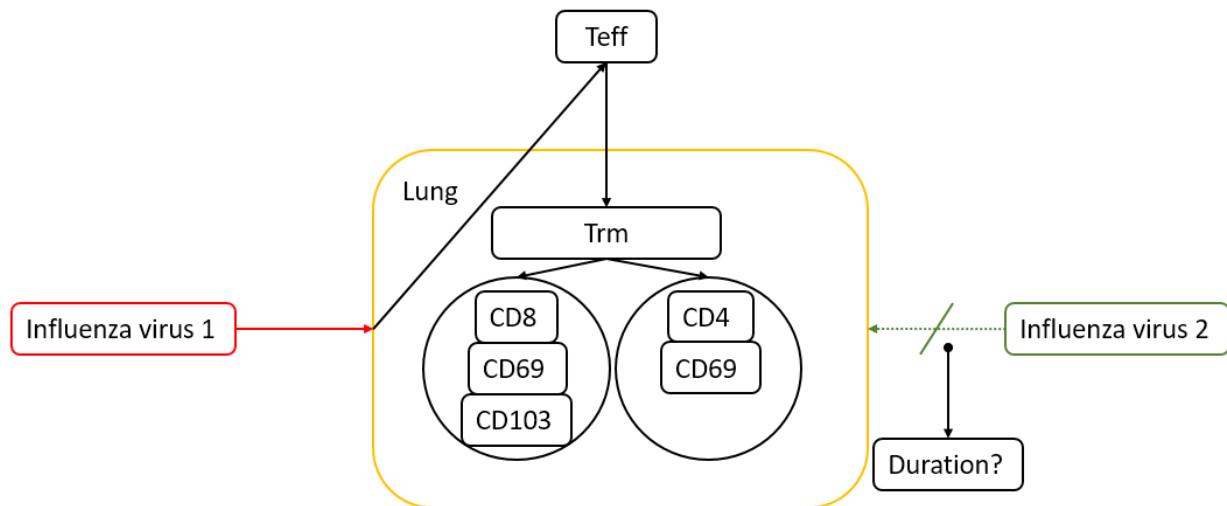


Figure 4. Schematic overview of the protection of Trm cells to influenza virus. Influenza virus 1 manages to infect and as a result Teff cells migrate to the lung to clear the pathogen. Some Teff remain in the lung and become Trm cells. Trm cells can be CD8+ or CD4+. Both express CD69 which keeps them from recirculating. Only CD8+ Trm expressed CD103 mediates attachment to the environment. The lung is protected against a new influenza virus infection. Unfortunately Trm cells seem to disappear after some time.

In conclusion, CD4+ and CD8+ Trm cells both seem to be important for longer-lived protection against influenza. Figure 4 shows a short summary about the generation of Trm cells in reaction to influenza virus infection.

It is important to focus on the mechanisms that Trm cells use to provide protection against other infectious diseases to understand the protection that Trm cells provide against influenza.

Studies that determine the duration of protection against influenza are also very important. Preferably this will be done in animals that have a longer lifespan than mice. Human studies may also provide information about the lifespan of Trm cells. Not only that, human studies are also crucial to confirm what has been found in other animals. However, obtaining human lung tissue is problematic as it raises ethical issues. Combined results may lead to the uncovering of the mechanisms involved in the Trm cell generation and the protection they provide against influenza.

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