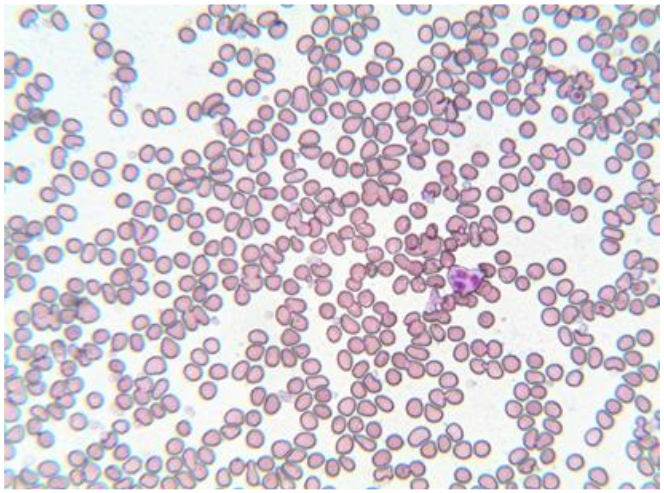

MEMORY T CELLS: COVID AND CANCER

August 2021

ABSTRACT

Memory T cells (MTC) resident in tissues such as the lung have shown the potential for long lasting immune response. These cells often outlast the antibodies generated via the B cells. We examine these cells and then consider them in the context of COVID and cancers.

Terrence McGarty
TTL 189

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tmcgarty@telmarc.com.

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1 INTRODUCTION

This is a brief note on tissue resident memory T cells (MTC)[1]. The understanding of the immune system is still and evolving process. Fifty years ago, we knew just a few basic fundamentals. Thirty years ago many of the basic building blocks were understood. Yet today we still get surprised by new and as of yet less well understood elements. The current corona virus pandemic has presented an interesting means to better understand the role of the memory T cells. At the same time these memory T cells have great potential in the control of many cancers. The cross fertilization between the corona virus and cancer immunotherapy is quite interesting and well worth following.

MTC are found resident in tissues that have been infected with some pathogen. They appear to be a direct response to the infection, the B cells, then the T cells in lymphoid tissue, via macrophages and dendritic cells, and then a return to the infected site allowing for long term protection. In concept, MTC are more effective that antibody (Ab) protection. However, the logic seems to infer that perhaps if a person is vaccinated then the localization engendered by a site infected is not present and then one may infer that localization of any MTC can be lost.

If the above logic has merit, then perhaps a conclusion is that in order to establish the long term protection from the MTC, one should be exposed to the virus, mitigating the extensive morbidity by having been vaccinated. This may then establish a localization for the MTCs. At present there does not seem to be any validation one way or the other for this hypothesis. We now attempt to establish a baseline for such an argument.

In contrast, in cancers, we have cellular involvement and localization signals. Thus the MTC in the case of cancers are established ex post facto.

1.1 INTENT

Our intent herein is to review the current understanding of the memory T cells and then do likewise for its import in the corona virus treatment and cancers.

As Farber has recently noted:

Early in the pandemic, my team spotted something surprising. When people were severely ill with COVID-19 and on a ventilator, the daily rinses of the plastic tubes in their windpipes contained immune cells from the airway. More surprisingly, what was in these airway samples was very different from what was found in the same patient's blood. The airway cells were producing high levels of cytokines — factors that recruit immune cells such as T cells to a tissue site and promote inflammation.

[1] It should be noted that there seems to be a multiplicity of abbreviations for these cells. We use MTC, but it is important to recognize that the cells we are focusing on are T cells, memory T cells, and tissue resident memory T cells. Generally, the meaning can be inferred by context.

By contrast, the corresponding blood samples were low in T cells, but high in other immune cells called monocytes, which were displaying unusual patterns of cell-surface receptors. Lung samples from patients who had died showed monocytes and a further type of immune cell (macrophages) clustered in the lung's tiny air sacs; this is associated with the damage that typifies severe COVID-19. The unusual receptors suggested to us that monocytes circulating in the blood had been both altered and summoned by the cytokines produced in the airway.

Had we not collected both airway and blood samples, we would not have put these pieces together. As this example shows, the pandemic has revealed major gaps in our understanding of the human immune system. One of the biggest is the reactions in tissues — at sites of infection and where disease manifests. Immune cells are often referred to as white blood cells.

But most, including more than 95% of T cells, reside and function in tissues, *"To fully grasp the immune system, researchers need to understand respiratory, gut and skin immunity." particularly lymphoid organs — such as bone marrow, spleen and lymph nodes — and in barrier surfaces, such as the skin, gut and mucous membranes. Although infection with the SARS-CoV-2 coronavirus leads to virus-specific CD4+ and CD8+ T cells that are detectable in the blood for months or longer3, it is unclear what their presence in circulating blood means for tissue-based immunity in the lungs — or elsewhere.*

Some immune cells are never found in blood. (Or rather, in many cases, we don't know if they fail to enter the circulation or whether they change their properties when they do.) Some, such as macrophages, derive directly from fetal progenitor cells to mature in tissues such as the lungs, liver and spleen. Others, such as memory T cells, develop from activated T cells that migrate to tissues following priming in lymph nodes during an infection.

These tissue-homing T cells take up long-term residence in tissues and can develop properties that are distinct in each.

Thus, the progression of our understanding of the details of the immune system often take interesting side steps. With COVID, the increased understanding of memory T cells resident in the infected or exposed cells lends to a better understanding of another arm of long term immunity. In this report we attempt to lay out the construct of the memory T cell and its application in COVID, and one can assume many other viral infections, as well as cancer, an area which has established the importance of memory T cells.

The collection of immune cells and other hematopoietic cells are found in multiple locations. We demonstrate this complexity below:

Bone

- Stem Cells
- Progenitor Cells

Immune Organs (Nodes, spleen, thymus)

- B cells
- T cells

Blood

- Granulocytes
- Leukocytes
- Erythrocytes
- Platelets

Tissues

- Macrophages
- Mast Cells
- Dendrites
- T cells
- Neutrophils

1.2 T Cells and Complexity

The focus of this report is the memory T cells. Now T cells are generally the attack agents of the adaptive immune system. The B cells generate Ab and as such "light up" the antigen on the invading organism and the T cells go in for the "kill". However, over time more and more has been learned regarding the multiplicity of T cells. We list some of them below.

T Cell Type	*Characteristic*
T helper[2]	TH1 cells Subset of CD4+ helper T cells that secrete a particular set of cytokines, including IFN-γ, and whose principal function is to stimulate phagocyte-mediated defense against infections, especially with intracellular microbes. TH2 cells Functional subset of CD4+ helper T cells that secrete a particular set of cytokines, including IL-4, IL-5, and IL-3 and whose principal function is to stimulate IgE and eosinophil/mast cell–mediated immune reactions. Th17 cells: A subset of CD4+ helper T cells that secrete a particular set of inflammatory cytokines, including IL-17 and IL-22, that are protective against bacterial and fungal infections and also mediate inflammatory reactions in autoimmune and other inflammatory diseases.
T naive[3]	The naive T cell pool is generally considered to be a fairly quiescent, homogeneous pool of antigen-inexperienced cells. However, recent studies have revealed important differences between naive T cells in terms of phenotype, dynamics, differentiation status, location and function. This heterogeneity may be influenced by factors such as age, thymic function and total numbers of T cells and tends to be overlooked in most immunological studies, even though it affects the performance of the immune system. These insights call for a revised view of the naive T cell pool.
T effector	In the adaptive immune system, this function of killing cells harboring viruses is mediated by cytotoxic T lymphocytes (CTLs), the **effector cells of the CD8+ lineage**. The same mechanism is used to eliminate phagocytes containing ingested bacteria that escape from phagosomes into the cytosol and are no longer susceptible to the killing activity of the phagocytes. In innate immune reactions, the same function of killing infected cells is mediated by natural killer (NK) cells

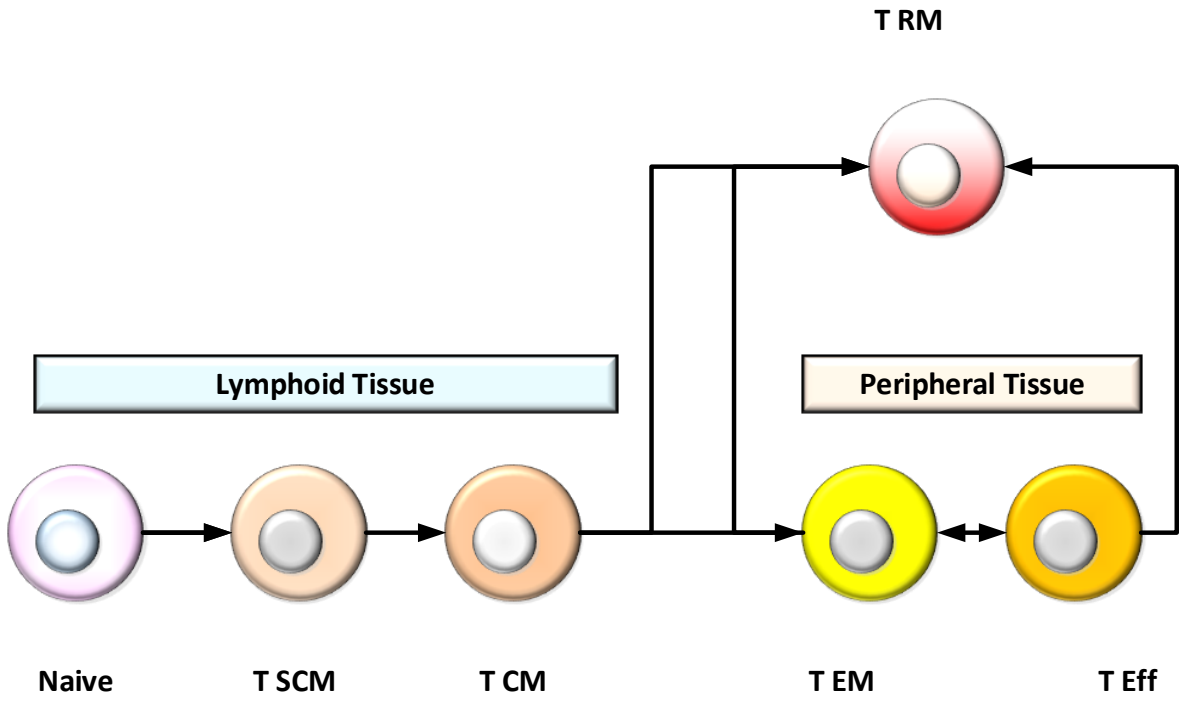
[2] See Abbas et al

[3] See van den Broek et al

T Cell Type	*Characteristic*
T effector memory[4]	T cells are subdivided into CD45RA−CCR7+ central memory T (TCM) cells, which traffic to lymphoid tissues, and CD45RACCR7− effector memory T (TEM) cells, which can migrate to multiple peripheral tissue sites. They further showed that TCM cells produced more interleukin-2 (IL-2) than TEM cells, which produced more effector cytokines, and they proposed a differentiation model with TCM cells being an intermediate stage in development of naive T cells into TEM cells in peripheral tissue sites. The existence of TCM and TEM subsets in lymphoid and peripheral tissue sites was confirmed in mouse models
T central memory	See above
T resident memory[5]	Tissue-resident memory T (Trm) cells constitute a recently identified lymphocyte lineage that occupies tissues without recirculating. They provide a first response against infections reencountered at body surfaces, where they accelerate pathogen clearance. Because Trm cells are not present within peripheral blood, they have not yet been well characterized, but are transcriptionally, phenotypically, and functionally distinct from recirculating central and effector memory T cells.
T memory stem[6]	T memory stem (TSCM) cells are a rare subset of memory lymphocytes endowed with the stem cell–like ability to self-renew and the multipotent capacity to reconstitute the entire spectrum of memory and effector T cell subsets. Cumulative evidence in mice, nonhuman primates and humans indicates that TSCM cells are minimally differentiated cells at the apex of the hierarchical system of memory T lymphocytes. Here we describe emerging findings demonstrating that TSCM cells, owing to their extreme longevity and robust potential for immune reconstitution, are central players in many physiological and pathological human processes. We also discuss how TSCM cell stemness could be leveraged therapeutically to enhance the efficacy of vaccines and adoptive T cell therapies for cancer and infectious diseases or, conversely, how it could be disrupted to treat TSCM cell driven and sustained diseases, such as autoimmunity, adult T cell leukemia and HIV-1.

[4] See Farber et al

Understanding T cells appears to be an evolving process. We show some of the progression below. The key issue is that T cells can come in a variety of forms and progress from lymphoid tissue, through the blood stream and eventually into peripheral tissues. The focus herein will be on the T RM or resident memory T cells, the long lasting cells generated in the local tissue where the initial infection had occurred.



We know how the T cells are produced and activated. The migration of the activated T cells is also somewhat understood. The focus here is in understanding the long lasting T memory cells which are resident in tissues susceptible to a second infection. This applies to virial infections of the type in the current corona virus pandemic as well as "infections" of the type we see with many cancers.

1.3 OVERVIEW

In this report we address several issues:

1. Present the basic fundamental of memory T cells especially those which are tissue resident. A set of key issues here are: (i) how are these formed, (ii) how do they enter the targeted cells, (iii) what are their life span, and (iv) how do they compare to other immune response such as classic antibody (Ab) responses.

[5] See Schenkel and Masopust

[6] See Gattinoni et al

2. Examine the significance of memory T cells in the context of COVID-19. The most significant works seems to have focused on classic Ab and their long term durability. However memory T cells (MTC) appear to have longer durability and efficacy. However studying these effects is complex due to the cell residence which makes study more involved.

3. Prior to COVID the MTC studies focused on cancer and the development of MTCs in malignant tissues. This has often been focused on malignancies of epithelial tissues. Again the same set of questions arise as above. We examine the literature here and compare the MTC responses.

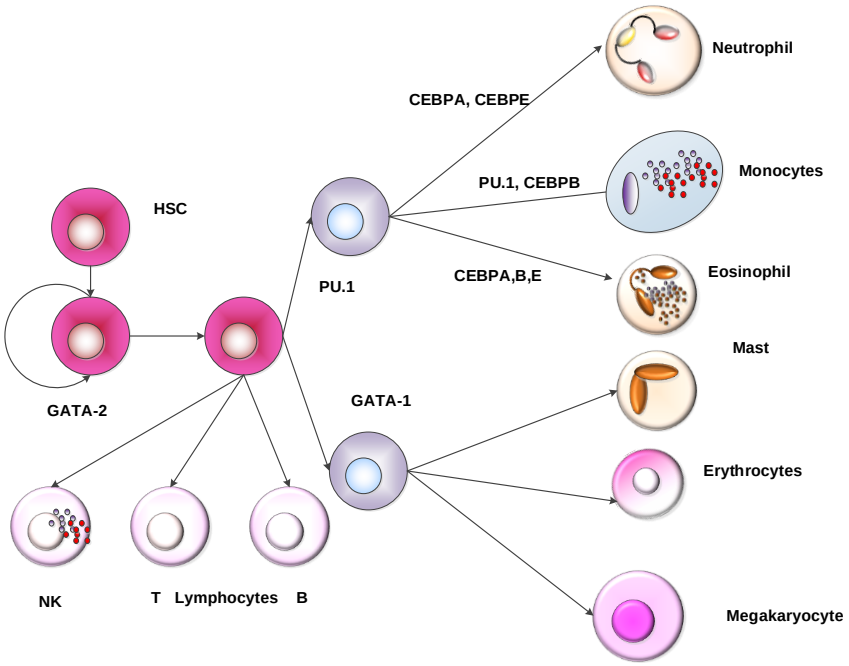
4. A key question that does not seem to have been addressed is; can MTCs be activated and established by vaccination rather than infection?

2 MEMORY T CELLS

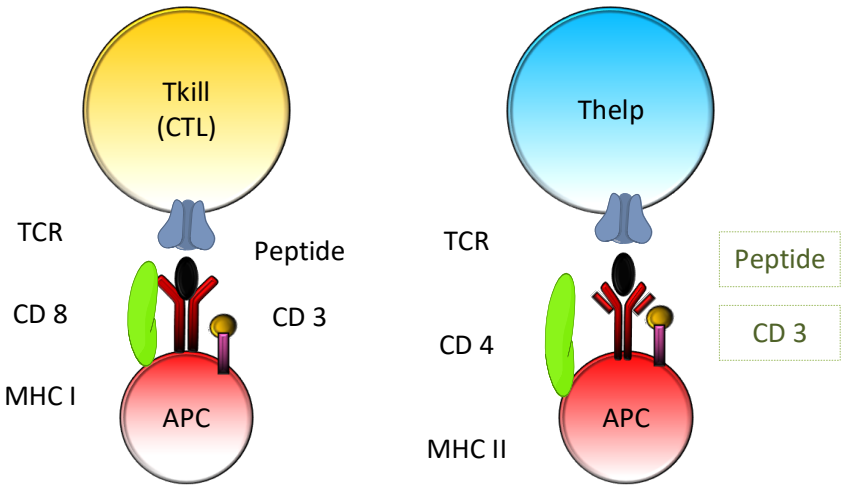
We first present a brief summary of the immune system in order to provide a simple structure for understanding the memory T cells.

2.1 BASIC T CELLS

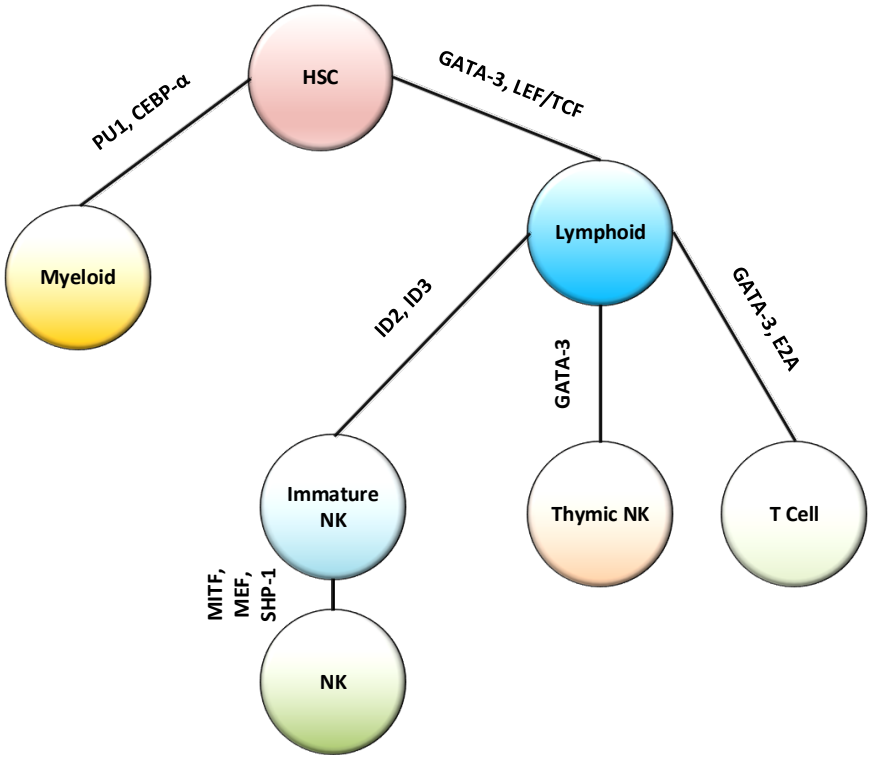
The following graphic is a simplified layout of the development and structure of many of the key immune system cells. Fundamentally the immune system is an amalgam of the innate and adaptive system. The innate is a powerful tool in the initial battle against invaders. In many ways it is the sledge hammer approach but can be fine-tuned. The adaptive is a bit of a scalpel approach, targeting specific invaders.



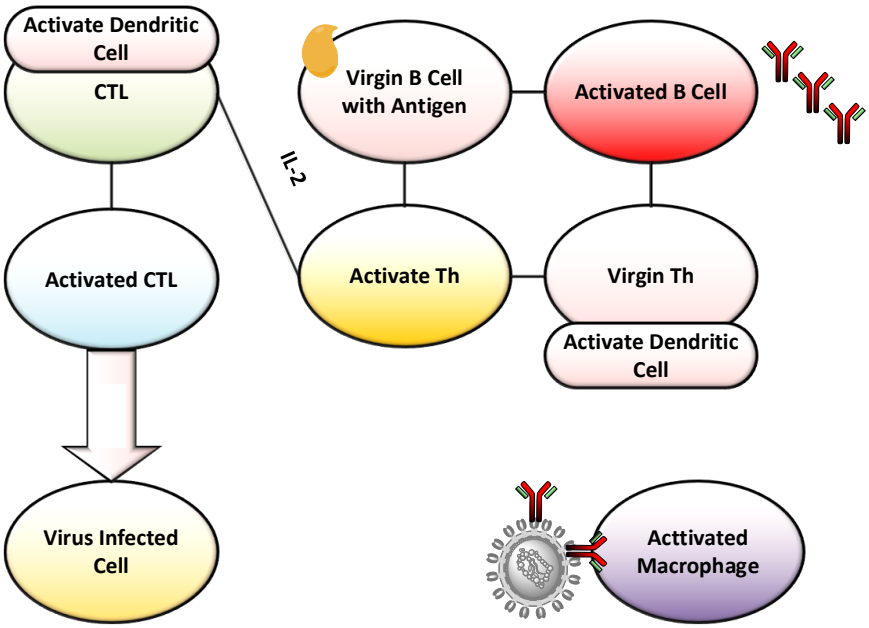
The above is a simplified version of what we currently understand to be the primary cells in the immune system including the red cells. The T cells are generally thought to be divided into two sets, helper and killer. The killer has an MHC I ligand and the helper an MHC II.



The relationship between the cells addressing invasions is shown below where the T cells are but one path.



Finally the overall dynamics of the adaptive system is shown below in a simplified manner.



It should be noted that none of the T cells mentioned above are tissue resident. In contrast the memory T cell is totally tissue resident and retains the ability to attack the invader it was primed for as noted above.

As Hunter et al note:

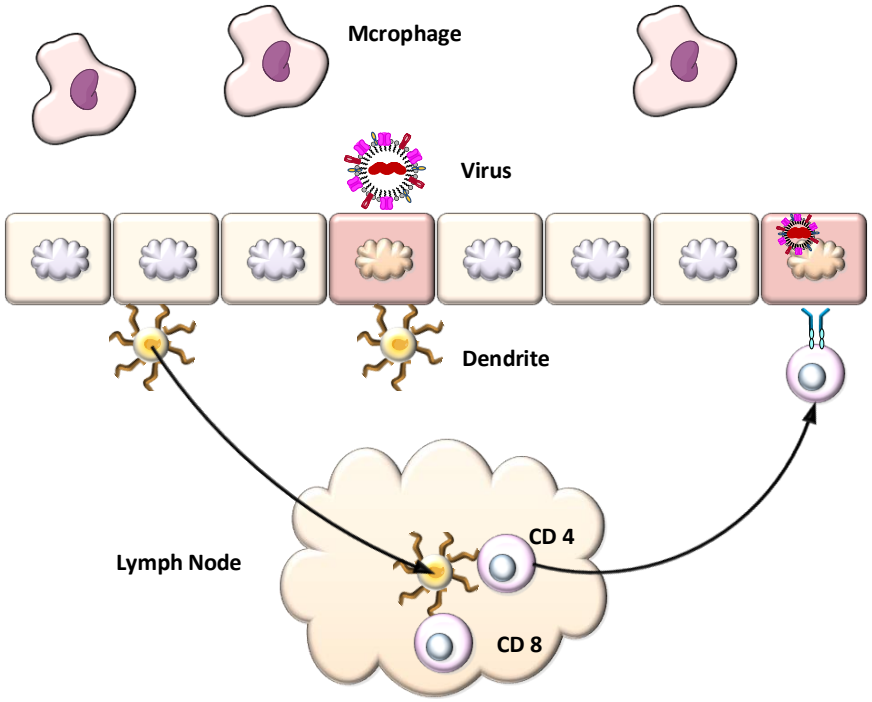
T cell migration within and between peripheral tissues and secondary lymphoid organs is essential for proper functioning of adaptive immunity. While active T cell migration within a tissue is fairly slow, blood vessels and lymphatic vessels (LVs) serve as speedy highways that enable T cells to travel rapidly over long distances. The molecular and cellular mechanisms of T cell migration out of blood vessels have been intensively studied over the past 30 years. By contrast, less is known about T cell trafficking through the lymphatic vasculature. This migratory process occurs in one manner within lymph nodes (LNs), where recirculating T cells continuously exit into efferent lymphatics to return to the blood circulation.

In another manner, T cell trafficking through lymphatics also occurs in peripheral tissues, where T cells exit the tissue by means of afferent lymphatics, to migrate to draining LNs and back into blood. In this review, we highlight how the anatomy of the lymphatic vasculature supports T cell trafficking and review current knowledge regarding the molecular and cellular requirements of T cell migration through LVs. Finally, we summarize and discuss recent insights regarding the presumed relevance of T cell trafficking through afferent lymphatics. …

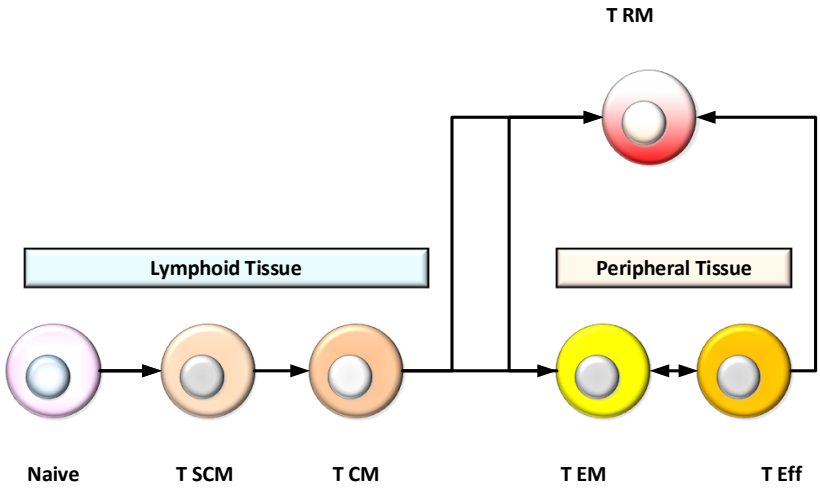
In an antigen-inexperienced host, the frequency of naïve T cells specific for any given antigen is extremely low, several thousand at most. Given that the diversity of possible antigens is almost countless and that T cell activation requires direct contact with antigen, naïve T cells constantly circulate through secondary lymphoid organs (SLOs) in pursuit of antigen.

Upon encountering antigen in SLOs, antigen-specific naïve T cells proliferate and become activated effector T cells (Teff) that egress from SLOs and enter peripheral tissue at sites of inflammation. Most Teff die after antigen is cleared but a few antigen-experienced T cells remain for longterm protection and either develop into tissue-resident memory T cells (TRM), into central memory T cells (TCM) that recirculate between SLOs and blood, or into effector-memory T cells (TEM) that circulate through blood and home to inflamed tissue. In addition to the abovementioned antigen-experienced cell types, regulatory T cells (Tregs) also circulate between blood, tissue, and SLOs

A typical viral attack and response is shown below based upon Spitaels et al:



Farber has presented a proposed detail on MTC. Graphically it is below followed by commentary:



A schematic model for the differentiation of circulating and tissue-resident memory T (TRM) cell subsets is shown. The progressive differentiation of the three major circulating subsets — stem cell memory T (TSCM) cells, central memory T (TCM) cells and effector memory T (TEM) cells — from activated naive T cells is shown relative to the extent of antigen exposure.

Effector T (TEff) cells represent terminally differentiated cells, and death is one outcome of increased antigen exposure and proliferation. Naive, TSCM and TCM cells circulate and migrate to lymphoid tissue, whereas T EM and TEff cells are the subsets of T cells that have the capacity to traffic to peripheral tissues. TRM cells in peripheral tissue sites may derive from either TEM or T Ef cells that migrate to these sites through tissue-specific factors.

It is possible that T CM cells could develop into TRM cells in lymphoid sites (dashed arrow). TRM cells in the peripheral compartments are probably terminally differentiated as they do not circulate or convert to other memory T cell subsets.

2.2 BASIC MEMORY T CELLS

We now commence with a discussion of basic MTCs.

From Farber et al the heterogeneity of T cells and their distinguishing markers is shown below. If as discussed above, we assume that MTC, also noted a T RM, are sequenced in some direct manner from previous versions as noted then we have a changing set of levels of expression of specific markers as we progress across the cell types.

	Circulating Memory Cells			Resident Memory Cells	
	T SCM	T CM	T EM	T RM	T RM CD103
CD45RA[7]	+[8]	-	-	-	-
CCR7[9]	+	+	-	-	-
CD69[10]	-	-	-	+	+
CD103[11]	-	-	-	-	+
IL-2[12]	+++	+++	+ +	+/-	+/-
IFNγ[13]	+	+ +	+ + +	+++	+ + +
TNF[14]	+	+ +	+ + +	+++	+ + +

Let us commence with understanding the memory T cell. As Beura et al had noted:

Immunosurveillance of ***secondary lymphoid organs (SLO)*** *is performed* ***by central memory T cells that recirculate through blood.***

Resident memory T (Trm)cells remain parked in nonlymphoid tissues and often stably express CD69.

We recently identified Trm cells within SLO, but the origin and phenotype of these cells remains unclear. Using parabiosis of ''dirty'' mice, we found that ***CD69 expression is insufficient to infer stable residence of SLO Trm cells****.*

Restimulation of nonlymphoid memory CD8+ T cells within the skin or mucosa resulted in a substantial increase in bona fide Trm cells specifically within draining lymph nodes. SLO Trm cells derived from emigrants from nonlymphoid tissues and shared some transcriptional and phenotypic signatures associated with nonlymphoid Trm cells.

[7] https://www.ncbi.nlm.nih.gov/gene/5788

[8] The inserts indicate relative expression. From Faber et al they note: +, low expression levels; ++, medium expression levels; +++, high expression levels.

[9] https://www.ncbi.nlm.nih.gov/gene/1236

[10] https://www.ncbi.nlm.nih.gov/gene/969

[11] https://www.ncbi.nlm.nih.gov/gene/3682

[12] https://www.ncbi.nlm.nih.gov/gene/3558

[13] https://www.ncbi.nlm.nih.gov/gene/3458

[14] https://www.ncbi.nlm.nih.gov/gene/7124

These data indicate that nonlymphoid cells can give rise to SLO Trm cells and suggest vaccination strategies by which memory CD8+ T cell immunosurveillance can be regionalized to specific lymph nodes.

From Spitael et al we have specifics on the two types of memory cells:

*1. **CD4+ memory T cells** have long been studied less intensely than CD8+ memory T cells. The main reason for this is that CD4+ memory T cells do not expand as exuberantly as CD8+ memory T cells, and consequently are not present in large numbers after re-exposure to antigen. For respiratory viruses, CD4+ Trm cells seem to be important for optimal protection against reinfection. For influenza virus, it has been shown that CD4+ T cell epitopes are conserved within different subtypes of influenza virus. Interestingly, in people infected with seasonal influenza virus, virus-specific CD4+ T cells have been isolated which cross-react with emerging reassortant strains like H5N1*

*2. **Memory CD8+ T cells**, like CD4+ memory T cells, have the ability to rapidly generate effector functions. They also produce a burst of secondary CTLs that can rapidly contain secondary infections. Repetitive reactivation of the memory CD8+ T cells, either through booster vaccinations or successive infections, augments the effector-like properties of memory CD8+ T cells and the frequency of Tem cells in the resulting memory T cell pool. The importance of memory CD8+ T cells has already been illustrated in humans.*

People without detectable pre-existing antibodies to the 2009 pandemic H1N1 strain were monitored following the global spread of this virus. From this it was evident that people that showed no or minor disease symptoms had higher levels of pre-existing IAV-specific CD8+ Tem cells. This study also showed no clear correlation between disease severity and pre-existing memory CD4+ T cells. This is rather striking because Wilkinson and colleagues noticed an inverse correlation between the presence of pre-existing CD4+ memory T cells and disease severity following a controlled challenge. The reason for these different observations is currently unclear.

Viral outbreaks are quite common. However, the mechanism for spreading them oftentimes delimits the exposure such as that in Ebola. The current corona virus pandemic however has a transmission mechanism which is quite extensive and frankly is still not well understood.

2.2.1 Memory Cell Actions

The above is an opening salvo discussing the memory T cell. Now from Abbas et al we have an expanded discussion:

T cell–mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen, which may persist for years, even a lifetime.

Memory cells provide effective defense against pathogens that are prevalent in the environment and may be repeatedly encountered. Despite the importance of immunologic memory, many

fundamental questions about the generation and maintenance of memory cells have still not been answered.

Memory cells may develop from effector cells along a linear pathway, or effector and memory populations follow divergent differentiation and are two alternative fates of lymphocytes activated by antigen and other stimuli. ***The mechanisms that determine whether an individual antigen-stimulated T cell will become a short-lived effector cell or enter the long-lived memory cell pool are not fully established.***

The last statement is a critical observation. The driving question is: can MTCs, which we know exist and are effective, be generated in a classic manner or do they require local infection to assure localization of the MTC in the target cell space? They continue:

However, as effector T cells contract, a small pool of memory precursor effector cells, often called MPECs, develops from which memory populations are mainly generated. ***The signals that drive the development of memory cells are also not fully understood.*** *These signals may include the strength of TCR stimulation, the level of costimulation, the cytokine environment, and others. No single transcription factor determines whether an antigen-stimulated T cell will become a terminal effector cell or a memory cell; rather, this choice may be controlled by quantitative differences in numerous transcription factors and epigenetic reprogramming.*

The clear conclusion is that there is still a great deal unknown about MTC.

2.2.2 Defining Properties

We continue with Abbas et al in discussing the defining properties.

The defining properties of memory cells are their ability to survive for prolonged periods after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells. Several features of memory cells account for these properties.

(i) ***Memory cells express increased levels of anti-apoptotic proteins, which may be responsible for their prolonged survival.*** *Whereas naive T cells live for weeks or months and are replaced by mature cells that develop in the thymus, memory T cells may survive for years. Thus, as humans age in an environment in which they are constantly exposed and responding to infectious agents, the proportion of memory cells induced by these microbes compared with naive cells progressively increases****. In individuals older than 50 years of age, half or more of circulating T cells may be memory cells.*** *The anti-apoptotic proteins that promote memory cell survival include BCL-2 and BCL-XL, which block apoptosis induced by a deficiency of survival signals. The presence of these proteins allows memory cells to survive even after antigen is eliminated and innate immune responses have subsided, when the stimuli for effector T cell survival and proliferation are no longer present.*

(ii) ***Memory cells respond more rapidly to antigen stimulation than do naive cells specific for the same antigen.*** *For example, studies in* ***mice have shown that naive T cells differentiate into effector cells in response to antigen in 5 to 7 days, but memory cells acquire effector functions***

within 1 to 3 days*. This is one reason why secondary responses to antigen exposure are more rapid than primary responses. A possible explanation for this accelerated response is that the* ***gene loci for cytokines and other effector molecules are fixed in an accessible chromatin state in memory cells****, in part because of changes in methylation and acetylation of histones. These epigenetically modified genes are poised to respond rapidly to antigen challenge.*

This response issue is a critical issue. The time factor is driven by the priming of the immune system as well as the genetic facilitation allowed in the MTC.

(iii) ***The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.*** *As we discussed earlier,* ***proliferation leads to a large clonal expansion in all adaptive immune responses, and the memory cells that remain from the expanded clone are typically 10- to 100-fold more numerous than the pool of naive cells before antigen encounter.*** *The increased clone size is one* ***reason that antigen challenge in a previously immunized individual induces a larger response than the first immunization in a naive individual.***

MTC have a significant presence. The assertion at the end of the above relates to Ag challenges in immunized persons but it is at best an analogy not a dispository observation.

(iv) ***Memory cells are able to migrate to peripheral tissues and respond to antigens at these sites. … naive T cells migrate preferentially to secondary lymphoid organs where they respond to antigens for the first time, but memory cells can migrate to virtually any tissue****. These differences are related to differences in the expression of adhesion molecules and chemokine receptors. In addition, memory T cells are less dependent on costimulation than are naive cells, allowing memory cells to respond to antigens presented by a wide range of APCs in peripheral tissues; in contrast, as we have discussed earlier, naive T cells are dependent on antigen presentation by mature DCs in secondary lymphoid organs.*

This localization factor is critical.

(v) ***Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool****. The cycling of these cells may be driven by cytokines. Because of the capacity for self-renewal, memory cells have been likened to stem cells. Although they survive for long periods, memory cells are functionally inactive and have to be restimulated by antigen to become functional effector cells.*

(vi) ***The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.*** *The most important cytokine for the maintenance of memory CD4+ and CD8+ T cells is IL-7, which also plays a key role in early lymphocyte development (see Chapter 8) and in the survival of naive T cells (see Chapter 2). Predictably, high expression of the IL-7 receptor (CD127) is characteristic of memory T cells. Memory CD8+ T cells also depend on the related cytokine IL-15 for their survival. IL-7 and IL-15 induce the expression of anti-apoptotic proteins and stimulate low-level proliferation, both of which maintain populations of memory T cells for long periods. The ability of memory cells to survive without antigen recognition has been best demonstrated by experiments in mice in which antigen receptors are genetically deleted after*

mature lymphocytes have developed. In these mice, the number of naive lymphocytes drops rapidly, but memory cells are maintained.

The most reliable phenotypic markers for memory T cells appear to be the surface expression of the IL-7 receptor and a protein of unknown function called CD27 and the absence of markers of naive and recently activated T cells. In humans, most naive T cells express the 200-kD isoform of the surface molecule CD45 called CD45RA (for "restricted A"), and most memory T cells express a 180-kD isoform of CD45 called CD45RO).

Both CD4+ and CD8+ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions. Three major subsets of memory T cells are known.

(i) Central memory T cells (TCM) express the chemokine receptor CCR7 and the adhesion molecule L-selectin and home mainly to lymph nodes. They have a limited capacity to perform effector functions when they encounter antigen, but they undergo brisk proliferative responses and generate many effector cells on antigen challenge. They provide a pool of memory cells that can respond to antigen challenge and develop into effector cells.

(ii) Effector memory T cells (TEM), on the other hand, do not express CCR7 or L-selectin, and they home to peripheral sites, especially mucosal tissues. On stimulation by antigen, TEM cells rapidly produce effector cytokines such as IFN-γ or become cytotoxic, but they do not proliferate much. This effector subset, therefore, is poised for a rapid response to exposure to a microbe, but complete eradication of infection may also require large numbers of effectors generated from the pool of central memory T cells. A subset of TEM cells in humans expresses the CD45RA isoform, which is characteristic of naive T cells. This population is called TEMRA cells (T effector memory RA+); whether it has unique functional properties is not known.

(iii) Tissue-resident memory T cells (TRM) are present in various nonlymphoid tissues, do not circulate in the blood, and may provide rapid defense against microbes in the tissues. Most of these cells express high levels of CD69, the molecule that reduces expression of S1PR1 (see Chapter 3). As a result, these cells do not respond to the high concentrations of S1P in the lymph and blood, facilitating their retention in tissues. A fourth population of memory T cells, called peripheral memory T cells (TPM), has been described. These cells make important contributions to secondary responses in tissues and, unlike TRM cells, are capable of moving between the circulation and tissues.

Memory T cells are also heterogeneous in terms of cytokine profiles. For example, some CD4+ memory T cells may be derived from activated T cells that are not committed to the Th1, Th2, or Th17 phenotype (described in Chapter 10), and when reactivated by exposure to antigen and cytokines, they can differentiate into any of these subsets. Other memory T cells may be derived from differentiated Th1, Th2, or Th17 effectors and retain their respective cytokine profiles on reactivation.

2.2.3 Migration and Localization of T Cells

After the T cells are primed for recognition they must move to the tissues. In the work of Spitael et al they note[15]:

In order to migrate from the LNs to peripheral tissues, activated T cells change the expression profile of homing molecules. Mature naïve T cells express lymphoid homing receptors CD62L and CCR7, which are necessary for migration to secondary LNs.

Once these T cells are activated after a DC encounter, they migrate to the site of infection. In order to get out of the LNs, downregulation of CD62L and CCR7, and upregulation of other receptors is necessary. It has already been shown that different T cell subsets express their own specific chemokine receptor repertoire after activation, which allows them to be recruited to different peripheral tissues.

Recruitment of activated T cells to the infected lung occurs via nonspecific and specific routes.

CD11a, which is a subunit of the integrin lymphocyte function-associated antigen-1 (LFA-1), is responsible for the nonspecific recruitment of activated T cells into the lungs, because this protein is upregulated in activated T cells, and its ligand ICAM-1 (Intercellular adhesion molecule-1) is expressed in peripheral tissues*.*

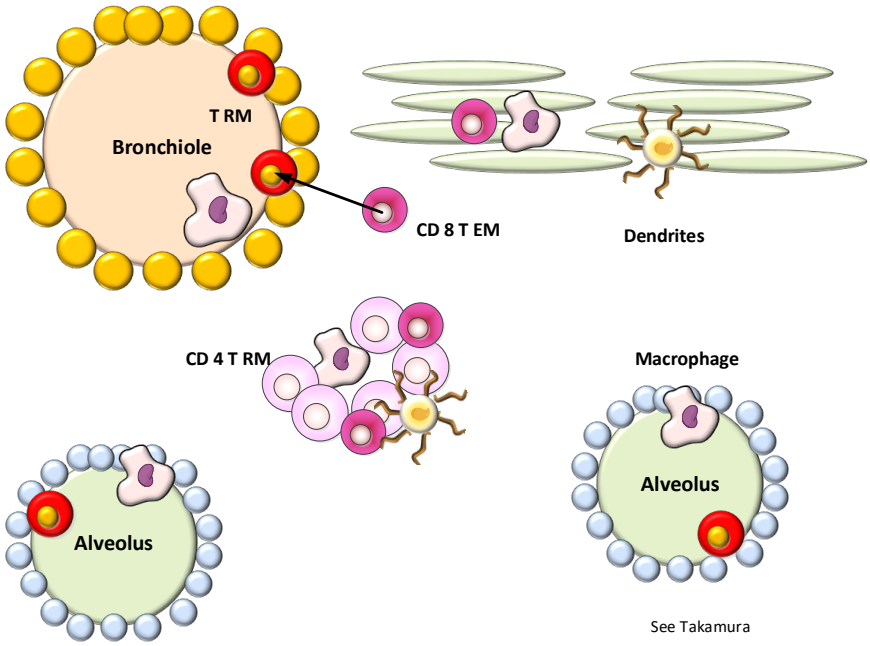
Specific recruitment of activated T cells is more complicated and less well understood. Mikhak and colleagues observed that lung DCs are responsible for the upregulation of chemokine receptor CCR4 on effector T cells, which allows for selective recruitment into the infected lung, where CCL2, CCL3, CCL5, CCL17 and CCL22, the ligands of CCR4, are produced.

However, it seems that this is not the case for CD8+ T cells, or at least that other mechanisms cannot be excluded. For this T cell population multiple recruitment mechanisms are implied to get the cells to the lung interstitium. Galkina and colleagues showed that migration of effector CD8+ T cells is promoted by expression of the chemokine CCL5 in the lung interstitium [70]. Slütter et al. on the other hand, have indicated that expression of CXCR3 on antigen-specific memory CD8+ T cells, from vaccinated mice, is critical for their migration to the airways [71]. Then again, Lim and coworkers recently reported the importance of the chemokine CXCL12 which is mainly produced by neutrophils, for virus-specific recruitment of CD8+ T cells and antiviral effector functions

2.2.4 MTC Activation in Lungs

Now the specifics of MTC in lung tissues has been examined for other viruses. We now consider the specific dynamics of that process. As Takamura notes in the Figure below:

[15] See the work of Hunter et al as well.



With the following commentary:

TRM niches in the in the lung. A majority of CD8+ TRM cells in the lung interstitium are maintained within the repair-associated memory depots (RAMD) that are temporarily created at the site of tissue injury, while CD8+ TRM cells are found sparsely in the unaffected areas.

A complex of niche factors, including signals via cognate antigen, TGF-β, Notch, and IL-7, are known to be involved in the formation of CD8+ TRM cells in the lung interstitium. CD8+ TRM cells are also present in the lung airways, the number of which is presumably maintained by continual recruitment of cells from the pool of CD8+ TRM cells in the lung interstitium. CD4+ TRM cells in the lung interstitium are maintained predominantly within the inducible bronchus-associated lymphoid tissues (iBALT).

Late antigen recognition triggers autocrine IL-2 signaling, which supports the proliferation and survival of CD4+ TRM cells. Homeostatic cytokines IL-7 and IL-15, and Notch signaling are also required for the maintenance of CD4+ TRM cells in the iBALT.

TEM cells are passing through the normal interstitium. Orange and blue cells indicate CD8+ and CD4+ TRM cells, respectively, unless otherwise stated. Red lines indicate the representative niche factors that influence the maintenance of TRM cells. …

Abbreviations: TRM, tissue-resident memory T cells; TEM, effector memory T cells.

The author continues:

Following the resolution of infection, substantial numbers of memory CD8+ T cells are maintained in both the lung interstitium and the airways for several months (153). We have recently shown that memory CD8+ T cells in both of these sites comprise a mixture of two distinct memory T cell populations: a major, stable population of TRM cells, and a minor, dynamic population of TEM cells that is continuously replenished by new cells from the circulation.

We also identified specific anatomical niches for CD8+ TRM cells around the bronchiole, which are temporarily created at sites of regeneration following tissue injury. We termed these sites repair-associated memory depots (RAMD). As with the epithelial layers in other mucosal surfaces, CD8+ TRM cells in the RAMD do not form clusters or lymphoid-like structures, but instead accumulate to relatively high densities in specific niches. By contrast, CD8+ TEM cells are widely, but sparsely, distributed throughout the unaffected lung interstitium.

This rigid compartmentalization of memory CD8+ T cell populations in the lung suggests that the two populations are maintained by separate signals.

It is also important to note that residual antigen-driven reactivation in the mediastinal LN plays a role in driving the continual recruitment of CD8+ TEM cells to the lung for several months after infection. Local instructive signals induced by pulmonary infection, such as IL-33 and TNF, presumably also contribute to the transient retention of circulating CD8+ TEM cells in the lung interstitium (157). A more detailed analysis of the factors and mechanisms that regulate the continual recruitment of memory CD8+ T cells to the lung

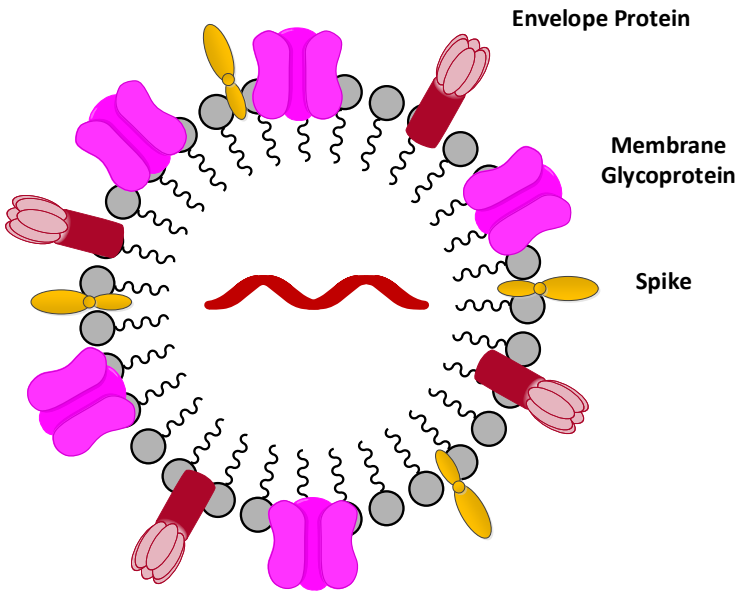
3 COVID

The current and ongoing viral pandemic has allowed for a significant examination of the MTCs. Specifically as non-infected individuals are vaccinated we ask if this results in the establishment of an MTC or does the MTC demand a direct cellular infection. As we have discussed above, it appears that the MTC are generated in the lymph nodes after the DCs bring the Ags to that site and from there T cells propagate to various sites. The issue is; can the activated T cells exit at the lung tissues and establish MTCs as we have been discussing.

3.1 COVID-19, BASICS

We present a brief summary of the COVID-19 corona virus which allegedly originated in some manner from Wuhan, China[16]. The actual provenance of the virus is yet to be adequately determined. In this section we provide a brief summary of the virus and its key issues[17].

The figure below is an example of the corona virus in generic terms. It is a single stranded RNA virus with a well-defined spike protein on the surface.



[16] In March 2020 we prepared a preliminary report on the virus discussing what was known to that data and examining the possible pandemic dynamics. In February 4, 2020 we declared this a pandemic despite the WHO and CDC delaying any prophylactic actions, the delay thus resulting in the current global pandemic, https://www.telmarc.com/Documents/White%20Papers/173Corona.pdf

[17] https://www.researchgate.net/publication/345813274_COVID-19_Vaccine_An_Update_and_Primer, https://www.researchgate.net/publication/349615892_COVID-19Variants_and_Vaccines _ https://www.researchgate.net/publication/348860153_COVID-19_Autoantibodies https://www.researchgate.net/publication/347270477_COVID-19_Multi-Organ_Sequellae https://www.researchgate.net/publication/348248952_COVID-19_Mutations_and_Infectivity

As Artika et al note:

The schematic diagram of coronavirus life cycle.

*The coronavirus infection is initiated by the **binding of the virus particles to the cellular receptors** leading to viral entry followed by the viral and host cellular membrane fusion.*

*After the membrane fusion event, the **viral RNA is uncoated in the host cells** cytoplasm.*

*The **ORF1a and ORF1ab** are translated to produce pp1a and pp1ab, which are subsequently processed by the **proteases encoded by ORF1a** to produce 16 non-structural proteins (nsps) which form the **RNA replicase–transcriptase complex (RTC)**.*

This complex localizes to modified intracellular membranes which are derived from the rough endoplasmic reticulum (ER) in the perinuclear region, and it drives the generation of negative-sense RNAs ((–) RNAs) through both replication and transcription.

*During replication, the **full-length (–)RNA copies of the genome are synthezied** and used as templates for the production of **full-length (þ)RNA genomes**.*

During transcription, a subset of 7–9 subgenomic RNAs, including those encoding all structural proteins, is produced through discontinuous transcription. In this process, subgenomic (–)RNAs are synthesized by combining varying lengths of the 30end of the genome with the 50 leader sequence necessary for translation.

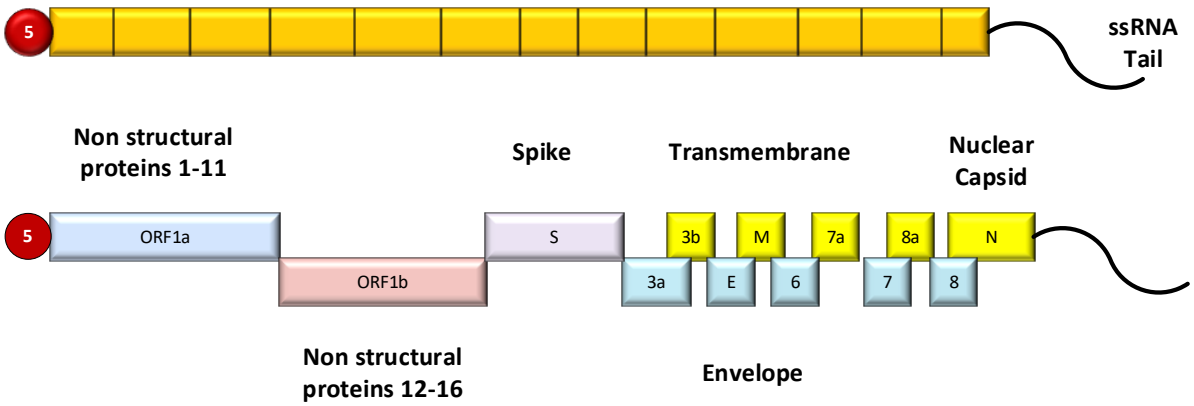
These subgenomic (–)RNAs are then transcribed into subgenomic (þ)mRNAs.

*The **subgenomic mRNAs are then translated**.*

The generated structural proteins are assembled into the ribonucleocapsid and viral envelope at the ER–Golgi intermediate compartment (ERGIC), followed by release of the newly produced coronavirus particle from the infected cell

3.2 COVID GENE

Various authors have discussed the general structure of a corona virus gene structure and we present this below. It is a positive single stranded mRNA virus and the mRNA has a form as shown below. It is approximately 30,000 nucleotides in length and the spike protein is approximately 3,000 nucleotides in length. As with most corona viruses it contains in the mRNA, via the non-structural proteins, the ability to reconstruct itself many times over by generating the structural genes and implanting a new copy of the mRNA.



The nonstructural proteins, NSP, for the replicase transcriptase complex. There are four structure proteins:

1. S is the spike forming protein, of which we shall speak of later in detail

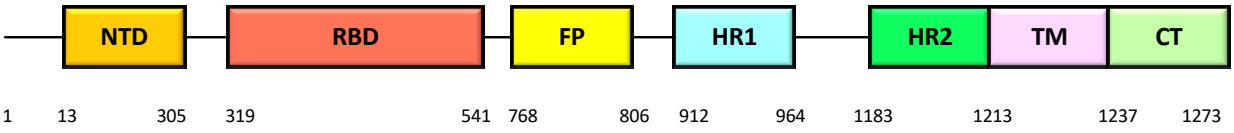
2. E is the envelope protein for the new virion

3. M is the membrane protein for the new virion

4. N is the nucleocapsid protein for the new virion

Overall we now have the two sets; those allowing for self-reproduction and those relating to construction of the new virion.

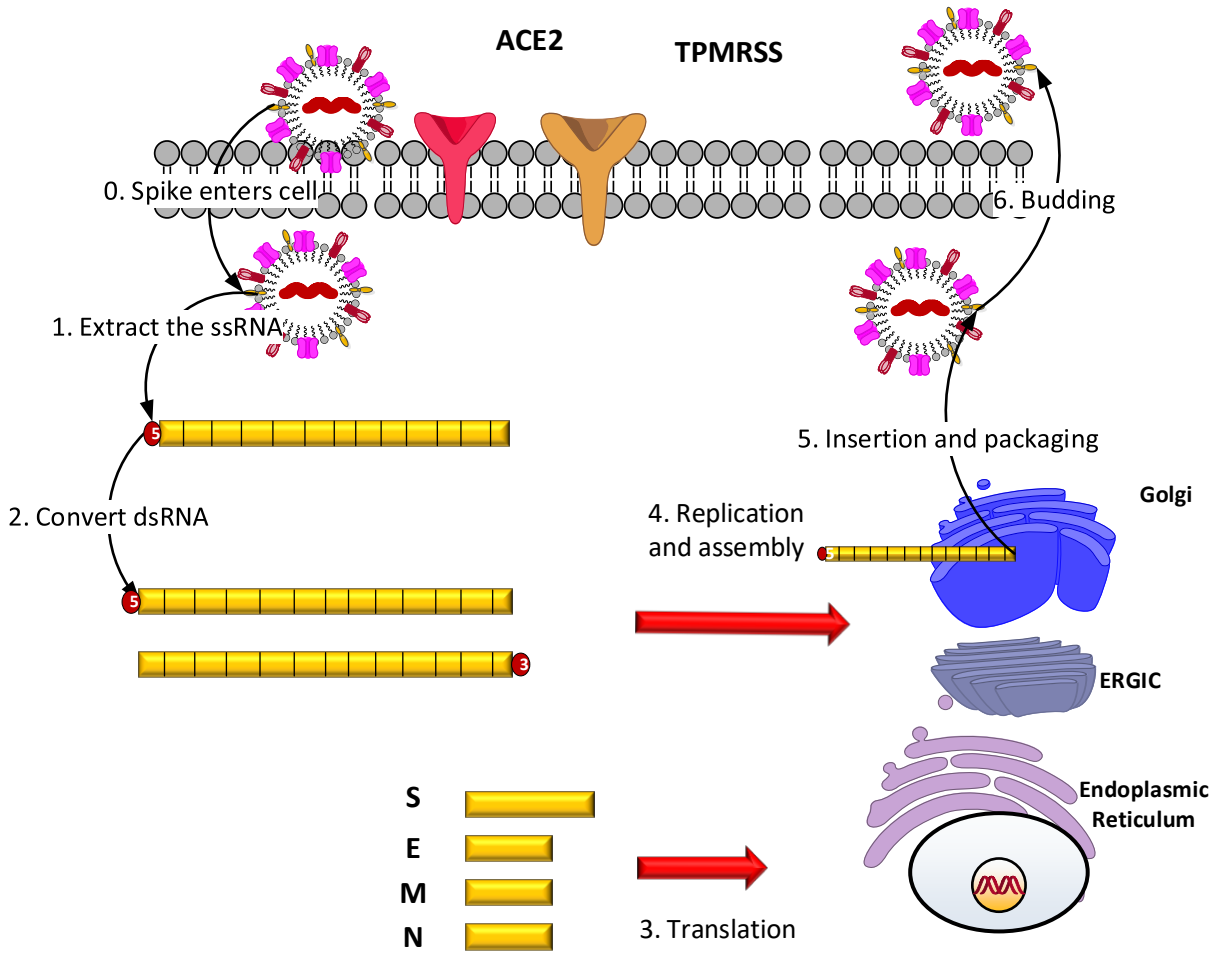
The following is the detail of the spike gene which we shall discuss later.



When examining these proteins we must note that the numbers above are those of the nucleic acids whereas the nucleotides are three times the number. Thus the 1273 nucleic acids represent almost 3900 nucleotides. Mutations in any of these nucleotides can result in a mutation of the nucleic acids and thus the protein conformation.

3.3 COVID SYSTEM

We depict the process below for the entry and reproduction of the virion. This process starts with the attachment and entry of the virion and ultimately the release of a collection of new virions until the cell is depleted and dies.



We can summarize the steps as shown above.

1. The virion spike protein attaches to the ACE2 receptor protein. As we will note later this means that there is a selective capability in this specific spike protein. As we shall note from the literature later, strangely this receptor is weak in Chinese individuals and is a strong bond in Europeans and most other ethnicities.
2. The virion then enters the cell and disassembles. The ss mRNA is extracted
3. The structure RNA sequences are then translated in the inter ER/Golgi space into the constituent proteins.
4. The ss mRNA is completed to form a complete ds mRNA which will be used to generate multiple ss mRNAs to be inserted in other new virions. This may be a point for possible mutant changes.
5. The multiple ss mRNAs and the structural proteins are assembles in the Golgi apparatus and extracted.
6. The final result is a repackaged virion which is budded outwards.

These steps are very general and there are as of yet many holes as to exactly how all this is accomplished. Yet for our purposes the processes lay out the locations of the possible mutation sites.

3.4 COVID-19 AND MEMORY T CELLS

As Lipstich et al have noted:

SARS-CoV-2 infects epithelial cells of the upper respiratory tract (URT; including the nasal passages and throat) and the lungs (bronchi and lung alveoli). These sites are involved in different aspects of SARS-CoV-2 pathology and transmission.

Severe COVID-19 involves extensive lung infection, whereas SARS-CoV-2 URT infection is important for viral transmission and is associated with milder disease symptoms. Recent reports have shown that SARS-CoV-2 cross-reactive memory T cells are detectable in ~28–50% of individuals not exposed to SARS-CoV-2.

These studies consistently found cross-reactive CD4+ memory T cells in blood samples, but there was little evidence of cross-reactive CD8+ memory T cells.

Memory T cells can be classified according to their anatomical location and trafficking patterns.

Recirculating central memory T cells (TCM cells) and effector memory T cells (TEM cells) traffic through the blood and lymph nodes and are recruited to sites of infection by inflammatory signals. Tissue-resident memory T cells (TRM cells) permanently reside within a given non-lymphoid tissue such as the lung or URT12. TCM/TEM cells respond more slowly to infections than T RM cells and usually undergo proliferation for several days before trafficking into an infected tissue. CD4+ T cells can also be divided into distinct functional subtypes.

For example, T follicular helper cells (TFH cells) are a specialized subtype of CD4+ T cells required for B cell help and thus almost all neutralizing antibody responses13. T helper 1 cells (TH1 cells) and CD4 cells with cytotoxic activity (CD4-CTL cells) are subtypes of CD4+ T cells with direct antiviral activities in infected tissues. CD4+ T cell-mediated memory responses to a virus may involve TFH cell, TH1 cell and/or CD4-CTL cell types.

In a recent set of papers summarized by Canete and Vinuesa they note:

Understanding which arms of the immune response are responsible for protection against SARS-CoV-2 infection is key to predicting long-term immunity and to inform vaccine design. Two studies in this issue of Cell collectively suggest that, although SARS-CoV-2 infection may blunt long-lived antibody responses, immune memory might still be achieved through virus-specific memory T cells….

While a lot of attention has been placed in antibody-based immunity, there is increasing evidence that T cells play a major role in the resolution of COVID-19, but whether SARS-CoV-2 generates longterm memory T cell responses and whether these are important for lasting immunity are still unclear.

These questions are important because vaccines are generally less effective at eliciting CD8 T cell responses. In this issue of Cell, two separate studies address the formation of long-lived immunity to SARS-CoV-2. Kaneko et al. report that severe SARS-CoV-2 infections blunt the germinal center response, which is likely to dampen the generation of long-lived antibody responses (Kaneko et al, 2020). The authors set out to establish the root cause of the reported short-lived humoral response to SARS-CoV-2, which was also characteristic of related coronaviruses causing severe infection in humans such as SARS and MERS. For SARS infections, this was thought to be caused by a lack of germinal center (GC) responses (Gu et al., 2005). GCs are transient microanatomical environments that form after antigen-activated B cells receive help from a specialized CD4 T cell subset known as follicular T helper (TFH) cells. Within GCs, B cells undergo clonal expansion and affinity maturation and receive further help from TFH cells to differentiate into memory B cells or long-lived plasma cells. Kaneko et al. investigate GC B cell responses in individuals succumbing to SARS-CoV-2.

The authors conducted extensive multicolor histological assessments of post-mortem thoracic lymph nodes and spleens. As for SARS, they found that GCs were also largely absent during the acute phase of COVID-19. The lack of GCs was accompanied by an absence of BCL6-expressing B cells or TFH cells, which are indispensable for the generation of GCs. Furthermore, an analysis of CD4 T cell composition in situ revealed an enrichment of TBET-expressing T cells with a concomitant increase of TNF-a.

As Lipsitch et al note:

Immunity is a multifaceted phenomenon. For T cell-mediated memory responses to SARS-CoV-2, it is relevant to consider their impact both on COVID-19 disease severity and on viral spread in a population. Here, we reflect on the immunological and epidemiological aspects and implications of pre-existing cross-reactive immune memory to SARS-CoV-2, which largely originates from previous exposure to circulating common cold coronaviruses. We propose four immunological scenarios for the impact of cross-reactive CD4+ memory T cells on COVID-19 severity and viral transmission. For each scenario, we discuss its implications for the dynamics of herd immunity and on projections of the global impact of SARS-CoV-2 on the human population, and assess its plausibility.

In sum, we argue that key potential impacts of cross-reactive T cell memory are already incorporated into epidemiological models based on data of transmission dynamics, particularly with regard to their implications for herd immunity. The implications of immunological processes on other aspects of SARS-CoV-2 epidemiology are worthy of future study

4 CANCER

Immunotherapy has been explosive over the past decade[18]. Targeting cancer cells and inhibiting the factors stopping the immune attack have successfully been demonstrated in a multiplicity of cancers.

4.1 CANCER MICRO ENVIRONMENT

The cancer tumor micro environment, TME, has received extensive investigation and this allows for a better understanding of what cells may mitigate the new cancers and which one may actually support it. We have examined multiple issues here over the years and such cells as fibroblasts and macrophages may actually assist the malignant cells to proliferate[19]. As Hiam-Galvez et al note:

Cancer is a systemic disease, and prolonged inflammation is a hallmark of cancer. Whether this inflammation initiates tumorigenesis or supports tumour growth is context dependent, but ultimately the global immune landscape beyond the tumour becomes significantly altered during tumour progression. Over the last decade, targeting the immune system with immunotherapy has revolutionized cancer therapy. Modulation of the existing patient immune system through immune checkpoint inhibitors (ICIs) such as anti-CTLA4, anti-PD1 and anti-PDL1 has led to durable remissions across a wide variety of different tumour types. Moreover, infusion of expanded autologous tumour-specific T cells or chimeric antigen receptor T cells has proven effective in patients with leukaemia. Despite these successes, immunotherapy remains ineffective for most patients with cancer.

To date, most immunotherapies have largely been used in patients with advanced cancers, and therefore the response rate in less advanced disease remains to be fully determined. Further progress towards more broadly effective immunotherapeutic strategies requires a deeper understanding of the immunological relationships between tumours and their hosts across the body.

The tumour immunology field has focused heavily on local immune responses in the tumour microenvironment (TME), yet immunity is coordinated across tissues.

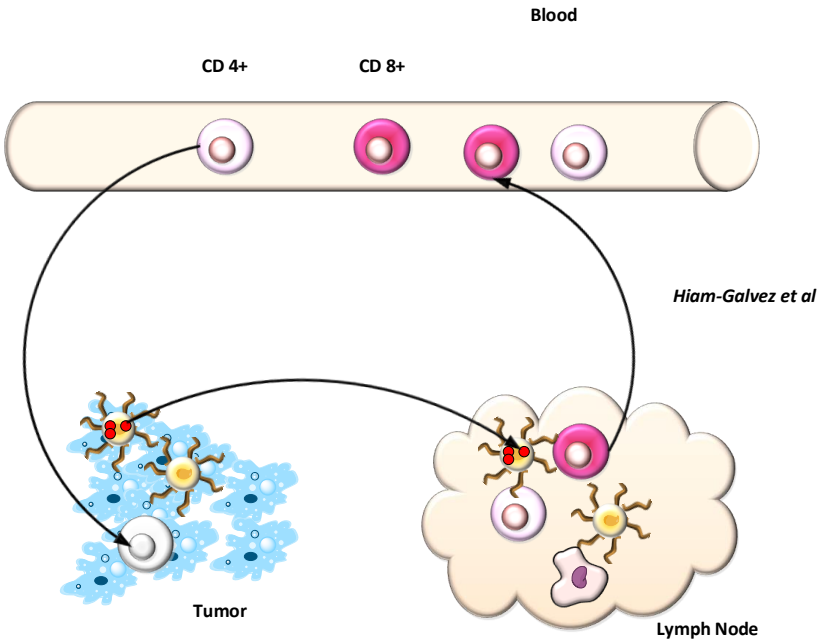
For example, many myeloid cells are frequently replenished from haematopoietic precursors in the bone marrow, and critical T cell priming events typically occur in lymphoid tissues. The localized antitumour immune response cannot exist without continuous communication with the periphery. Furthermore, virtually every subset of immune cell has been implicated in cancer

[18] https://www.researchgate.net/publication/314090163_Cancer_Immunotherapy_A_Systems_Approach

[19] https://www.researchgate.net/publication/330222973_EMT_and_Cancers, https://www.researchgate.net/publication/341788660_Fibroblasts_and_Cancer_The_Wound_That_Would_Not_Heal , https://www.researchgate.net/publication/336116071_Tumor_Associated_Immune_Cells_On_the_one_hand_and_on_the_other_hand

biology. Therefore, a thorough understanding of immune responses to cancer must encompass all immune cell lineages across the peripheral immune system in addition to within the TME.

The prototypical model for the interaction between cancers and the immune system may be shown below. Simply the flow is between tumor, lymph node blood stream and to the tumor. The recent immunotherapeutic efforts have been to block receptors such as PD-1 and CTLA4 and allow the immune system to function. However, this does not always work and if it does, it may not be permanent.



The above paradigm also points out several other issues.

1. We have the issue of getting the T cells from the blood stream into the targeted malignant cell. We know fairly well how this is accomplished with neutrophils but with the T cells it is a bit more complex. Now one process that may adhere is that the tumor presents an Ag which is identified by a dendritic cell then taken to a lymph node and processed into a T cell line and sent back to the blood stream where it moves into various cells.

2. The lymph node process of activating the dendritic cells activation is known but the details and efficiency is problematic.

3. The putative antigen, Ag, target may be understood or it may be inferred by examining the cells.

4. Cancer cells have the ability to mimic normal cells and thus inhibit attack by the immune system. Many of the current immunotherapies address this issue by blocking the self-identifiers such as PD-1.

4.2 RESIDENT MEMORY T CELLS

We can now examine the details of the MTC, or specifically the resident memory T cells. Now Amsen et al note:

Solid tumors frequently contain CD8+ T cells with the following T RM cell characteristics: expression of CD103 and (although it is less frequently analyzed) VLA-1 (CD49a) and CD69.

Although these markers do not by themselves unequivocally identify TRM cells, genome-wide transcriptional profiling has documented that a T RM cell transcriptome signature is often found in tumor-infiltrating lymphocytes (TILs).

Tumor epithelium generally shows enrichment for CD8+ T cells expressing CD103, the characteristic marker of epithelial TRM cells, whereas CD103– CD8+ T cells are found more in tumor stroma.

Infiltration of the tumor epithelium is a favorable prognostic sign and, correspondingly, the abundance of CD103+CD8+ T cells correlates with longer disease-free and overall survival of patients with breast cancer, lung cancer, endometrial adenocarcinoma, ovarian cancer, cervical cancer or urothelial carcinoma of the bladder. In fact, some studies have reported that disease progression correlates more favorably with tumor infiltration by CD103+CD8+ T cells than with tumor infiltration by total CD8+ T cells.

Among patients with non-small cell lung carcinomas (NSCLCs) that had a similar degree of infiltration by CD8+ T cells, those whose tumors exhibited high CD103 expression fared better than those whose tumors exhibited low CD103 expression. In a few cases in which no such correlation was found expression of CD103 was not examined in conjunction with that of the co-receptor CD8, which complicates interpretation of the results, as CD103 is also expressed by other cell types, such as regulatory T cells.

The proposal that TRM cells have a critical role in tumor control is supported by functional studies. Among CD8+ TILs isolated from human NSCLC, those that are CD103+ and thus at least resemble epithelial T RM cells kill autologous tumor cells more effectively than do their CD103– counterparts from the same tumors. Increasing the number of T RM cells by overexpression of Runx3, moreover, results in better control of melanoma in mice.

Perhaps most convincingly, vaccines that elicit T RM cells more potently suppress the growth of melanoma and tumors in mucosal tissues than does systemic immunization.

The above observation appears to elicit the fact that vaccines can produce MTCs (T RM). Just how and when this occurs appears yet to be answered. Finally:

The tissue-resident nature of the protective immunity involved has been documented by parabiosis experiments and by treatment with the S1P antagonist FTY720, which depletes the circulation of T cells and thereby prevents non-resident immunity from participating in the anti-tumor response.

In a similar manner Dumauthioz et al have noted:

Tissue resident memory T cells (Trm) *are a subset of memory T cells mainly described in inflammation and infection settings.*

Their location in peripheral tissues, such as lungs, gut, or skin, makes them the earliest T cell population to respond upon antigen recognition or under inflammatory conditions.

The study of Trm cells in the field of cancer, and particularly in cancer immunotherapy, has recently gained considerable momentum. Different reports have shown that the vaccination route is critical to promote Trm generation in preclinical cancer models. Cancer vaccines administered directly at the mucosa, frequently result in enhanced Trm formation in mucosal cancers compared to vaccinations via intramuscular or subcutaneous routes. Moreover, the intratumoral presence of T cells expressing the integrin CD103 has been reported to strongly correlate with a favorable prognosis for cancer patients.

In spite of recent progress, the full spectrum of Trm anti-tumoral functions still needs to be fully established, particularly in cancer patients, in different clinical contexts*.*

In this mini-review we focus on the recent vaccination strategies aimed at generating Trm cells, as well as evidence supporting their association with patient survival in different cancer types. We believe that collectively, this information provides a strong rationale to target Trm for cancer immunotherapy….

Tumor-infiltrating lymphocytes (TILs) frequently remain tolerant or display an exhausted phenotype favored by the tumor microenvironment. Thus, two of the main challenges of current immunotherapy against cancer are generating specific T cells that may effectively target tumor cells and ensuring the induction of long-term anti-tumor protective immune responses.

Therapeutic strategies to promote the development of immunological memory have for the most part focused on circulating memory T cells, such as central memory (Tcm) or effector memory (Tem) but have failed so far to consider resident memory cells (Trm).

Trm cells are a long-lasting population frequently characterized by the expression of CD103, CD69, and CD49a surface markers and by the absence of the lymph node homing receptors CD62L and CCR7.

The differentiation toward a residency memory program is known to be regulated by TGFβ and IL-15 cytokines, which promote the expression of the transcription factors Hobbit and Blimp1. The upregulation of these molecules induces the silencing of other transcription factors such as KLF2 and TCF1 and proteins involved in tissue egress such as S1PR1.

Trm cells are mainly localized in peripheral lymphoid and non-lymphoid tissues such as lung, skin, gastrointestinal and genitourinary tracts. Their permanence in these tissues is mainly mediated by the expression of the integrins CD103 and CD49a that bind E-cadherin and

collagen respectively. The homing properties of Trm cells can vary depending on the tissue and the chemokine receptor expression patterns. The presence of CCR5 and CXCR3, for instance, is essential for the recruitment of CD8+ Trm cells to the lungs in cancer and infection.

Furthermore Amsen et al note:

Immune responses in tissues are constrained by the physiological properties of the tissue involved. Tissue-resident memory T cells (TRM cells) are a recently discovered lineage of T cells specialized for life and function within tissues. Emerging evidence has shown that TRM cells have a special role in the control of solid tumors. A high frequency of TRM cells in tumors correlates with favorable disease progression in patients with cancer, and studies of mice have shown that TRM cells are necessary for optimal immunological control of solid tumors. Here we describe what defines TRM cells as a separate lineage and how these cells are generated. Furthermore, we discuss the properties that allow TRM cells to operate in normal and transformed tissues, as well as implications for the treatment of patients with cancer. …

TRM cells are a separate lineage with their own transcriptional program *The transcriptomes of TRM cells isolated from different locations exhibit tissue-specific adaptations. For example, intestinal TRM cells express the chemokine receptor CCR9, whereas CCR4 and CCR8 are found in skin-resident T RM cells*

TRM cells are a separate lineage with their own transcriptional program *The transcriptomes of TRM cells isolated from different locations exhibit tissue-specific adaptations. For example, intestinal TRM cells express the chemokine receptor CCR9, whereas CCR4 and CCR8 are found in skin-resident TRM cells. Nonetheless, all TRM cells share a transcriptional signature that defines their universal characteristics and differs from that of other T cell lineages, which suggests that TRM cells truly constitute a separate lineage.*

How are TRM cells generated*? The precursors of CD8+ TRM cells reside within a KLRG1– T cell memory precursor population, from which TCM cells and TEM cells also derive11,31,37,44. A single T cell clone can contribute to both the T EM cell lineage and TCM cell lineage45,46. Whether the same clone can also give rise to TRM cell progeny has not been determined. Singlecell transcriptome analysis indicates that memory T cell precursors form a uniform population early after infection47. However, TE cells isolated from the spleen 7 days later can generate circulating memory T cells but not TRM cells4*

TRM cell maintenance *TRM cell populations are stably maintained for many months in murine skin and intestinal mucosa and can persist for decades in human tissues. In the absence of infection, the maintenance of CD8+ TRM cell populations involves slow turnover, possibly by a subpopulation with stem-cell properties. The recruitment of circulating precursor cells does not contribute to this in most tissues, except apparently in the lungs. Persistent infection is not required for the maintenance of CD8+ TRM cells and might even be detrimental for it. This suggests that this process is independent of antigen, although the possibility of a role for antigen depots cannot currently be excluded.*

A role for TRM cells in the control of solid *tumors Solid tumors frequently contain CD8+ T cells with the following T RM cell characteristics: expression of CD103 and (although it is less frequently analyzed) VLA-1 (CD49a) and CD69. Although these markers do not by themselves unequivocally identify TRM cells, genome-wide transcriptional profiling has documented that a T RM cell transcriptome signature is often found in tumor-infiltrating lymphocytes (TILs)79,80. Tumor epithelium generally shows enrichment for CD8+ T cells expressing CD103, the characteristic marker of epithelial TRM cells, whereas CD103– CD8+ T cells are found more in tumor stroma.*

Infiltration of the tumor epithelium is a favorable prognostic sign2 and, correspondingly, the abundance of CD103+CD8+ T cells correlates with longer disease-free and overall survival of patients with breast cancer, lung cancer, endometrial adenocarcinoma, ovarian cancer, cervical cancer or urothelial carcinoma of the bladder

Maintenance of TRM cells in tumors *A possible role for TRM cell–maintenance signals inside the tumor environment has not specifically been investigated, but some indirect evidence of this does exist. For example, deletion of the gene encoding IL-15 in colorectal carcinoma is associated with a poor prognosis. That is consistent with a role for IL-15 in the maintenance of intestinal T RM cells, although of course other cell types also depend on this cytokine, such as natural killer cells, type I–like innate lymphoid cells and type I innate-like T cells*

TRM cell metabolism in tumors *TE cells and TEM cells rely on an aerobic glycolytic metabolic program in which glucose is catabolized only partially for energy generation to preserve incompletely degraded products for anabolic purposes. The concentration of glucose is low in tissues such as the epidermis or the lung mucosa, which forces TRM cells to use other energy-metabolic programs than those used by TE cells and TEM cells. Indeed, TRM cells in the skin 'preferentially' catabolize free fatty acids, which are abundant in that tissue. These cells possess superior ability to take up free fatty acids.*

How do TRM cells control tumors? *Precisely how TRM cells control tumors has not specifically been determined. However, given the mechanisms used by TRM cells to control microbial infection, reasonable speculation is possible. TRM cells can directly kill tumor cells, and those expressing CD49a were in fact the most potent killers among T cells in a mouse model of melanoma. CD8+ TRM cells often also secrete IFN-γ, a cytokine associated with a favorable prognosis for patients with cancer. IFN-γ can suppress tumor-cell division directly but also promotes the activation of other immune cells and inhibits the resistance to chemotherapy conferred by tumor-associated fibroblasts. It furthermore mobilizes chemokines and adhesion molecules in the tissue to recruit auxiliary 'troops' from the circulation*

5 OBSERVATIONS

We now consider several observations and extensions of the issues presented herein.

5.1 T CELLS ARE ACTIVATED BY B CELLS AND ANTIBODIES. ANTIBODIES ARE ACTIVATED BY ANTIGENS.

There is a generally well understood sequence in the progression of the immune cells. However the complexity of the T cells leaves a bit to be desired in both identifying them and understanding their progression. The details are in the genetic expression evolution and the internal pathway dynamics. We can see by the surface markers that as the T cells go from naive to effective and then onto the MTC cells types we have cells that are changing by internal expression. The details of that process do not seem yet to be fully understood. In addition controlling the process may be the basis for additional therapeutic options.

5.2 COMPLEXITY OF THE TME

The TME is a complex environment of cells and signalling. Many of the putative immune cells and stroma elements can help or hinder in a cancer environment. The question then is what do they do in a viral environment. In addition, the issue also is how extensive if the TME? Is it just proximate to the lesion or does it include a wider environ?

5.3 VACCINATION VERSUS INFECTION

The question we had posed earlier was; can MTC be the result of just a vaccination or does a localized infection demand the requisite markers for localization. As Sadarangani et al have recently reported:

BBV152 (Bharat Biotech) *(6µg protein, 2 doses, 28 days apart): SARS-CoV-2 grown in Vero cells, inactivated with β-propiolactone and adsorbed onto aluminium hydroxide and an imidazoquinoline molecule (TLR7/ TLR8 agonist): Strong bias towards a TH1 cell phenotype (IFNγ and TNF), with minimal TH2 cell responses (as measured by IL-5 and IL-13) after in vitro stimulation.* ***Increase in CD4+CD45RO+ memory T cells by day 76 after second dose***

They further note:

Virus-specific CD8+ and CD4+ T cells, including CD8+ memory T cells, are present in patients who have recovered from COVID-19*,*

but their importance in protection against future infection and/or severe disease remain uncertain.

Interferon-γ (IFNγ)-producing T helper 1 cells (TH1 cells) are produced during acute infection, and it has been suggested that this T H1 cell-biased phenotype is associated with less severe disease — an important consideration given that current COVID-19 vaccines have been

designed to induce responses skewed towards the T H1 cell phenotype. There are indications that individuals with higher levels of IFNγ-secreting T cells (measured by enzyme-linked immunosorbent spot) against the S protein, nuclear proteins and membrane proteins of SARS-CoV-2 may have better protection from disease. Moreover, individuals with mild disease favour more efficient T follicular helper cell responses in the germinal centre, which supports an increase in plasmablast numbers and enhances antibody production.

Park and Kupper have noted:

The observation that pathogenic virus can be rapidly eliminated by TRM cells in animal models, even in the absence of antibody, has led to a burgeoning interest in the induction of TRM cells as a goal of vaccination.

Viruses show tissue tropism, with influenza specific for lung, rotavirus specific for gut and HSV specific for skin and other stratified squamous epithelia. TRM cell–based vaccination would direct pathogen-specific TRM cells to the relevant epithelial tissue88. Currently, the titer of neutralizing antibodies generated by a vaccine is considered a proxy for its efficacy. But for viruses invading barrier tissues, the process of infection of a resident cell and subsequent hijacking of the cell's program to make more virus is largely insensitive to extracellular antibody. In contrast, such infected cells express viral peptides on cell-surface class I molecules, making them ready targets for CD8+ TRM cells. Vaccination at epithelial surfaces, rather than intramuscularly, is thus a more effective way to generate robust TRM cells.

Promising approaches in lung for influenza and in other mucosal tissues have been reported recently39,40. As proof of principle, in animal models, vaccinia virus (VACV) immunization of skin and lung, influenza infection of lung and Listeria immunization through oral administration have all led to the generation of highly effective tissue-resident TRM cells.

A recent HIV vaccine engineered to generate TEM cells showed great promise, and although the investigators focused on blood, they did find memory T cells in mucosal tissue. The wisdom of generating lung TRM cells specific for conserved portions of the influenza virus or anogenital mucosal TRM cells specific for conserved portions of HIV is clear. Virally infected cells could be targeted by TRM cells for elimination shortly after exposure. The challenge with this approach to vaccination is at the level of practicality—how to immunize through an accessible tissue (such as skin) and generate TRM cells in other distant barrier tissues that are specific to the infectious virus. One of several promising approaches involves using VACV vectors delivered via skin scarification; this has been shown to trigger the generation of protective lung TRM cells in one model. Also, because skin immunization in general generates both skin TRM cells and a TCR-identical population of TCM cells in lymph node38, sequential skin and peripheral tissue immunization (to convert the TCM cells into tissue-relevant TRM cells) is a possible approach.

Although most work on TRM cells has been done in the setting of viral infection, this approach should be applicable to other tissue-selective pathogens. Mycobacterium tuberculosis, Listeria, Vibrio cholerae and Mycobacterium leprae are all candidate pathogens. What remains to be understood is what collection of factors in regional lymph nodes govern the acquisition of tissue-homing markers on effector T cells and how to ensure that these T cells that enter tissue remain

as long-lived TRM cells, poised to respond to pathogens through the appropriate environmental interface.

5.4 MALIGNANCIES: GENERATING MTC EX VIVO AND EX POST

Cancer therapeutics have taken on a multiplicity of dimensions. For example, cytokine induced killer cells, CIK, are NK cells that are enhanced ex vivo and then placed back in the patient[20]. The question then is; can we do the same with MTC in cancer cells. Will this merely, at best attack the local lesion or will it flow through the entire immune system and effect systemic cures?

[20] See
https://www.researchgate.net/publication/280627292_MDS_METHYLATION_AND_THE_EPIGENETIC_PARADIGM
and
https://www.researchgate.net/publication/280627289_MDS_PATHWAYS_AND_DNMT1_CONTROL

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