The enigma of memory T cells in viral infections (including SARS-CoV-2)
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ABSTRACT
Viral infections and vaccinations produce immune responses mostly through B cells (plasmocytes/antibodies) and effector T cells (helper and cytotoxic). After removing the antigen, 90-95% of the effector cells disappear, but the remaining ones turn into T cells with long memory. The maintenance of cellular memory, the mode of information storage and the lifespan of T cells are insufficiently known. After measles, resident T cells in the plasma will offer protection only against the measles virus, generating a long period of immunodepression (immune amnesia). After the flu, memory T cells generate immune protection for 1-2 years for secretory IgA and longer for serum IgG. In SARS-CoV-2 infection, memory T cells (B and T) respond quickly to reinfection for 8-10 months. In conditions of intense stimulation in SARS-CoV-2, SARS-CoV, MARS infection, marked leukopenia occurs with lymphopenia generating immunodepression and high mortality, disorders similar to septic shock. An important role in these disorders is played by the host’s genetic structure and epigenome.

Keywords: immune memory, measles, flu, SARS-CoV-2, cell exhaustion

Immune memory represents the ability of the immune system to recognize an antigen previously encountered and to generate a specific response in a short time interval. The immune system has 2 main lines of defense: innate and adaptive immunity. During viral infections, both types of response are involved with well-defined particularities

Innate immunity is largely based on inflammatory responses triggered in the first instance by macrophages, polynuclear leukocytes and mast cells, cells that destroy infectious agents in an inflammatory process. Some of these cells have the ability to block viral replication or to destroy cells infected with viruses, processes in which type I IFN and NK cells play an important role [1].

Adaptive immunity. In general terms, B cells produce antibodies (AC) and T cells provide help to other immune cells and directly eliminate infected cells. The control of the viral infection involves the populations of CD4+ (helper) and CD8+ (cytotoxic) cells by stimulating the production of antibodies and by apoptotic destruction of cells populated by viruses. CD4+ cells activate B cells that produce antibodies that act by blocking viral receptors, by blocking fixation on the host cell and by opsonizing viral particles. Clearance of the initial infection provides a basis for protection against subsequent infections through T and B cells, specific CD4+ and CD8+ virus populations and neutralizing antibodies.

Immune defense against viral infections is based on 2 coordinates: elimination of active infection and protection against subsequent infections. A series of clinical, epidemiological and immunological data show that sometimes the “textbook” scenario does not correspond to reality.

Measles – memory T-cell amnesia. Measles is a contagious disease produced by a single-stranded RNA lymphotropic virus. The widespread introduction of vaccination (note the active anti-vaccination currents)
which is safe, effective and accessible has led to the saving of millions of lives [4]. Measles virus (MV) initially infects the macrophage cells in the pulmonary alveoli through its tropism towards their membrane glycoproteins. Entering the cell, MV releases its own genome and induces viral multiplication. Infected macrophages and dendritic cells (DC), carrying the virus, transfer the virions to memory T and B immune cells and naïve B and dendritic cells (DC), carrying the virus, transfer the virions to memory T and B immune cells and naïve B cells of the immune system where the virus undergoes intense replication. In the following period, there is a firm stimulation of immunity against MV through the rapid expansion of specific B and T lymphocytes with the appearance of CD4+ cells producing IFN-γ and cytotoxic CD8+ cells [8]. After the peak of viremia, the elimination of infected lymphocytes takes place, highlighted laboratory through transient lymphopenia [5], but important, followed by marked immunodepression against various other pathogens [8]. It was found that the changes are determined by the appearance of cell clones with long-term memory only against the measles antigen [4,5,6] because the virus destroys the long-lived plasma cells stored in the bone marrow, holders of the immune memory pool [10,11]. The immunological imbalance during an extended period of immunodepression also called “immune amnesia” lasts 2-3 years or more after the measles episode [7,8,9]. Using BCR receptor sequencing of peripheral blood lymphocytes before and after MV infection, Petrova et al [7] identified 2 immunological consequences underlying immunodepression:

- Incomplete reconstitution of the naïve B cell pool generating immunological immunity
- Compromising the immune memory against previously encountered pathogens by reducing the proliferation of innate immunity cells, with a drastic effect on the risk of infection

Mina et al [10] examined infant mortality rates in the US, UK and Denmark in the pre-vaccine decades and after the introduction of the RV vaccine. In the pre-vaccination epidemic episodes, almost half of the children’s deaths from infections were preceded by the measles episode. Virtually all deaths occur within the first four days of illness, with the highest death rates occurring in the first 24 hours [14]. Children who survive the measles episode are not immune to the disease, as the virus induces an immune amnesia or “permanent immune paralysis” [5].

The MV vaccine is a vaccine containing killed measles virus. It is usually given at 12-15 months of age as part of the routine childhood immunization schedule in many countries. However, in some communities, the vaccine may be offered at a younger age or as a single dose instead of the standard two-dose schedule. The vaccine is very effective in preventing measles and can lead to a significant reduction in mortality and morbidity from the disease. It is estimated that the measles vaccine has reduced the global mortality rate from measles by 70% since its introduction in the 1960s and is considered one of the most successful vaccines ever developed.

The vaccine is typically given as a single injection, usually given intramuscularly into the upper arm. The vaccine is stable at room temperature for several years and does not require refrigeration, making it easy to transport and store in areas with limited access to refrigeration. It is safe and effective, with a very low risk of serious side effects. However, it is important to note that the vaccine may not be effective in all individuals who receive it, especially those who are immunocompromised or have certain medical conditions. It is also important to note that the vaccine does not provide protection against other infectious diseases, such as mumps, rubella, and varicella (chickenpox), which are caused by different viruses. Therefore, children who are vaccinated against measles should continue to receive other vaccines as part of the routine childhood immunization schedule to protect them against these other diseases.
Changes in the population of memory T cells during influenza infection. Influenza infects 5-15% of the world’s population every year. The flu virus constantly evolves through rapid and unpredictable mutations, producing new viral strains that bypass the humoral immunity generated by flu vaccines [13].

Post-vaccination immunity induces neutralizing antibodies against viral surface glycoproteins. Both hemagglutinin and neuraminidase often undergo mutations. Seasonal viruses have the ability to evade the immune system through the gradual acquisition of mutations of exposed hemagglutinin epitopes [14]. In this “war” current vaccines lose their effectiveness. For this reason, the vaccines also “adapt” by changing the composition frequently depending on the antigenic stimuli. Vaccination induces the formation of influenza A virus-specific T-cell reservoirs along the respiratory tract and consequently long-term immunity against circulating seasonal influenza strains [13].

Immunological imprinting The immune system is imprinted by antibodies (AB) produced in response to previous infections/vaccinations in life. Each vaccination produces new ABs specific to the new strain, but they degrade over time, returning to the repertoire existing before vaccination. However, over 70% of AB identified in the blood of donors remain unchanged for more than 5 years; 2/3 of them attack the invariable part of the virus. Lee J, Paparoditis P, Horton A et al. Persistent Antibody Clonotypes Dominate the Serum Response to Influenza over Multiple Years and Repeated Vaccination. Cell Host Microbe, 2019;25,3:367

Natural infection, in contrast, offers better protection against homologous and heterologous strains of the influenza virus through memory T cells from the pulmonary [13] and nasal [14] epithelium. They recognize the more stable internal viral components and can provide cross-protection against a wide range of virus variants [15]. Both primary infection and vaccination produce immediate and long-term immune responses through the differentiation of naïve T cells into effector B cells (plasmocytes/antibodies) and through effector T cells (CD4 helper and cytotoxic CD8) [14,16]. Memory T cells that remain resident in the lung provide strong protection against subsequent influenza A virus infections [13]. The longevity of antibodies varies between 1-2 years for secretory IgA and decays for serum IgG [17].

The role of memory T cells in SARS-CoV-2 infection. Immune memory is an attribute of both the B and T cell lines. Memory B cells show clonal expansion, the ability to differentiate and divide rapidly, the ability to produce specific antibodies (predominantly IgG) in response to the antigenic stimulus [18]. Memory T cells, differentiated as CD4 helper and cytotoxic CD8, react to the molecules expressed by SARS-CoV-2 and presented by dendritic cells (DC) by activating the Th1 line (IFN-γ, IL-2 and TNF-α) [19]. CD8 T cells recognize viral peptides on the surface of infected cells and trigger their apoptosis (programmed cell death). They are located in the blood, lymphoid organs and tissues. A number of responses are characteristic of memory T cells:

- Formation of a pool of cells reactive to the pathogen through specific receptors
- Fast and strong response to infection
- Pre-programming to generate an adapted set of effector cells
- The presence of memory T cells in the barrier tissues for rapid detection and control of infection [18].

Resident T cells with memory (Trm) settle in the lung, skin, being stable and not included in circulatory exchanges [20]; for this reason, they cannot be evaluated in blood samples collected in the laboratory. Natural infection and vaccination against SARS-CoV-2 produce specific neutralizing antibodies considered a success of immunization, but the level and duration of their protection remain in debate. Cellular immunity provides a different response, based as it has been shown, on memory T cells.

Memory storage in the T cell. The way of storing and maintaining information in cells against the infectious agent was elucidated relatively recently through epigenetic mechanisms [21,22,23]. Once naïve CD8 cells encounter an antigen, they go into a rapid proliferation phase and differentiate into effector T cells, being able to generate IFN-γ, TNF-α and other cytokines. After the antigen has been eliminated, 90-95% of the effector cells disappear, but those that survive are transformed into T cells with long memory [23] located mainly in the tissues/organs where the primary infection is located. The subsets of cells that give rise to memory cells acquire DNA programs that maintain the effector genes in a demethylated position, being associated with histone modifications that create the open configuration of chromatin [22]. These changes are maintained for a long period of time during subsequent cell proliferation, the inhibition of the programmed cell death program (programmed cell death protein-1 [PD-1]) also having a role [23]. The open profile of effector genes is maintained in the memory of isolated CD8+ T
cells even decades after vaccination, indicating that they retain the epigenetic imprint of their effector history and remain ready to respond rapidly to pathogen re-exposure as shown by yellow fever vaccination studies by Akondy et al [41]. Memory T cells were highlighted in healthy individuals exposed to SARS-CoV-2 infection suggesting their passage through an asymptomatic infection, 93% of them having a specific cellular response [24]. Asymptomatic infections could be quite widespread, especially since antibody testing underestimates the real prevalence of infection.

**Cell exhaustion and lymphopenia.** Under conditions of intense or sustained stimulation, memory T cells become non-functional, lose their ability to proliferate and produce cytokines, a process called cell exhaustion [23]. The decrease in the number of lymphocytes, lymphopenia, mostly transient, frequently reported in some viruses (measles, flu, infections from the SARS group, MERS) affects the populations of CD4+ T, CD8+ T, B cells and other immune cells [24]. Although the mechanisms responsible for the installation of lymphopenia are not well known, the damage to CD8 T cells is more severe and parallel to the severity of the evolution. Based on data from the literature, several hypotheses regarding the causes of severe lymphopenia in SARS-CoV-2 infection have been outlined [18,24,25,26,31]:

- The storm of cytokines would be the key factor, especially through TNF-α, IL-6 and Fas-FasL (receptor on the surface of apoptosis-inducing cells) directly influencing the cellular self-destruction process.
- The SARS-CoV-2 virus directly infects T cells. The hypothesis does not have wide support because the viral genes are not expressed in the leukocytes of patients with COVID-19, that is, the lymphocytes are not infected and the cytopathic effects are not at the origin of the lymphopenia.
- SARS-CoV-2 infection interferes with the expansion of T cells. In severe forms of infection, some genes involved in the activation and functions of T cells are inhibited, their expression returning to normal after the patients recover.
- The systemic inflammatory profile in severe forms of the disease and the hypersecretion of pulmonary chemokines induce the migration of lymphocytes to this organ and their depletion in the general circulation. The autopsy of some deceased COVID-19 patients showed in the bronchoalveolar lavage abundant infiltrates with T cells in the lungs.

Data from the period 2003-2004 show that in SARS-CoV (Severe Acute Respiratory Syndrome) and MERS-CoV (Middle East Respiratory Syndrome), both caused by coronavirus infections, there are severe changes in the number of leukocytes and lymphocytes. In the acute phase of SARS-CoV infection, Channanappanavar et al [32] mention marked leukopenia with lymphopenia (~80% of patients) with a marked decrease in CD4 cells (~90-100%) and CD8 (~80-90%). The decrease in the number of T cells was strongly correlated with the severity of the acute phase of the infection [33,34]. **Lymphopenia** is an evolutionary feature in severe bacterial sepsis and in infectious shock, immunosuppression being the result of immune cell reprogramming through the depletion of dendritic cells (DC), T and B cells through apoptosis [27,28] resulting in prolonged serious evolution and increase in late mortality [29]. It is possible that in the severe forms of SARS-CoV-2 infection and in lymphopenia septic shock, there are similar pathogenic changes.

**Longevity of memory T cells in the context of the life span of other types of cells**

<table>
<thead>
<tr>
<th>Table 4. Longevity of some human cells (synthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
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<tr>
<td>----------</td>
</tr>
<tr>
<td>Neurons</td>
</tr>
<tr>
<td>Crystalline</td>
</tr>
<tr>
<td>Ova</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
</tr>
<tr>
<td>Intestinal cells (exclusively absorptive)</td>
</tr>
<tr>
<td>Skeletal muscles</td>
</tr>
<tr>
<td>Fat cells</td>
</tr>
<tr>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>Hepatocytes</td>
</tr>
<tr>
<td>Immune memory T cells</td>
</tr>
<tr>
<td>SARS 11 years</td>
</tr>
</tbody>
</table>

The substrate of the host-viral infectious agent relationship. The genetic predisposition to severe infections is manifested both in the antigen recognition phase and in the body’s response phase [45]. Some genetic variants have been identified as severity or resistance factors in different viral infections. During the SARS-CoV-2 pandemic, a wide individual variability of the evolution was evident, the aggravating factors being the male gender, advanced age, smoking, comorbidities. Previously healthy patients, without notable pathology, sometimes developed severe forms of the disease and lasting complications. The suspected
genetic factors involved the ABO blood group, viral receptor genes, inborn errors of IFN type 1, antibodies to interferon [47]. On the other hand, it was found that the presence of a rare variant of the gene that codes for ACE 2 (angiotensin converting enzyme 2) is protective by reducing the expression of the enzyme by 37% [47,48]. In this complicated puzzle must be included Table 5.

**Table 5. Duration of immunity in different viral infections**

<table>
<thead>
<tr>
<th>Affection</th>
<th>Infectious agent</th>
<th>Virus type</th>
<th>Humoral immunity (the B)</th>
<th>Cell immunity (T cell)</th>
<th>Bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox</td>
<td>Smallpox virus</td>
<td>DNA</td>
<td>Tested up to 75-80 years with neutralizing ACs</td>
<td>20-30 years decreases slowly over time</td>
<td>35 36 37</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Yellow fever virus</td>
<td>RNA</td>
<td>AC neutralizers, duration?</td>
<td>25 years through CD4+ T cells apparently unchanged</td>
<td>38 39 44</td>
</tr>
<tr>
<td>Measles</td>
<td>The measles virus</td>
<td>RNA</td>
<td>10 years after 2 vaccination doses</td>
<td>Central role in maintaining immunity</td>
<td>40</td>
</tr>
<tr>
<td>Flu</td>
<td>Influenza virus</td>
<td>RNA</td>
<td>Effective ~6 months. AC persistent over 5 years</td>
<td>?</td>
<td>14 17 41</td>
</tr>
<tr>
<td>COVID-19 infection</td>
<td>SARS-CoV-2 coronavirus</td>
<td>RNA</td>
<td>AC neutralizers 5-8 months (6-7 months)</td>
<td>The CD4+ and CD8+ over 10 months after infection</td>
<td>42 43</td>
</tr>
</tbody>
</table>

Table 6. Genetic factors that influence the evolution of some viruses According to Oladejo et al [46]

<table>
<thead>
<tr>
<th>Viral infection/pathology</th>
<th>Genetic variants</th>
<th>The type of answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza virus (severe pneumonia)</td>
<td>IRF7, IRF9, TLR3, IFITM3, SFPA/B</td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Rhinovirus (severe pneumonia)</td>
<td>JFN1</td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Immune deficiency virus</td>
<td>CCR5, HLAB57</td>
<td>Resistance</td>
</tr>
<tr>
<td>Herpes simplex virus (encephalitis)</td>
<td>TLR3, TRIF, TRAF3, IRF3, TBK1,</td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Norovirus and rotavirus</td>
<td>FUT2</td>
<td>Resistance</td>
</tr>
<tr>
<td>Respiratory syncytial virus (bronchiolitis)</td>
<td>IL4, IL4RA, IL 8, IL10, IL13, SFPA/D</td>
<td>Susceptibility</td>
</tr>
</tbody>
</table>

REFERENCES


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16. How the immune system remembers viruses. Technical University Munich, November 2020


