REVIEW ARTICLE

Ability of the immune system to fight viruses highlighted by cytometry and T-cell receptor clonotype assessment: lessons taken before the coronavirus disease 2019 pandemic outbreak

Emilia Jaskuła, Andrzej Lange

1 Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2 Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, Wrocław, Poland

KEY WORDS

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ABSTRACT

The intriguing aspects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are the high rate of spread and rapid progression of pneumonitis. Confronted with thousands of deaths daily worldwide, we have to quickly build the rationale behind the treatment, taking advantage of past analogues. When a new virus strikes, T-cell receptor γδ cells are in the first line of defense, activated by stress molecules and recognizing some epitopes in a process that is major histocompatibility complex–independent but still specific, eg, cytomegalovirus, as well as participating in the regulatory mechanism—both characteristics are useful in fighting SARS-CoV-2. Most deaths occur due to pneumonitis, in the course of which overwhelming inflammation impairs blood oxygenation, calling for artificial ventilation. In fatal cases of coronavirus disease 2019, the balance between the immune response and the inflammatory outcome fails and, therefore, patients at risk, mostly the elderly, show higher levels of anti–SARS-CoV-2 antibodies and enhanced inflammation in the lungs. Apparently, there is no feedback control over the antibody production. The investigational use of convalescent plasma, providing antibodies taken from patients who have recovered, was shown to be effective, likely through exerting idiotype-associated negative control of antibody production. Similarly, the use of mesenchymal stem cells may assist the body regulatory mechanisms, considering the anti-inflammatory potential of these cells. The use of these 2 immunotherapeutic tools is understandable based on basic immunology and this knowledge may direct the efforts of the medical community aimed at combating the virus.

Correspondence to:
Prof. Andrzej Lange, MD, PhD, FRCP, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Rudolfa Weigla 12, 53-114 Wrocław, Poland, phone: +48 71 783 13 75, email: andrzej.lange@hirszfeld.pl
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Introduction The immune system includes several compartments that guard against infection hits. This is a very complex immune system network, which needs to be well regulated to accomplish the task. As in normal life, each battle during fighting infection has a negative effect on the affected tissues. The regulatory network should set and then calm some immune system compartments defending the host against viruses. At first, natural immunity is activated with the inflammatory response being under negative control as soon as adaptive immunity is taking an action. The homeostasis of the immune system warrants an adequate immune response. If the regulatory mechanism fails, response against the microbial hit takes place at the cost of tissue injury. In this review, we discuss some aspects of this issue.

T cells involved in the immune response and the controlling mechanism induced by monocytic myeloid-derived suppressor cells The T-cell receptor (TCR) repertoire shows the ability to recognize some epitopes and also includes a proportion of naive cells ready to respond to unknown antigens. T-cell immunity is represented by the adaptive immunity exerted by cells having the TCR β-receptor and those reacting without help of major histocompatibility complex (MHC) antigens, ie, $TCR_{\gamma\delta}$ cells. The latter cells are triggered by stress molecules exerting cytotoxicity against

pathogens by producing proinflammatory cytokines. T-cell receptor γδ T cells may be identified by the detection of either $V_{\delta}1$ chain (usually acting in response to stress antigens) or $V_{\delta}2$ positivity, which are mostly present in blood.[1] In patients with rather poor adaptive immunity, $\gamma\delta^+$ cells are present in a greater proportion than in the competent ones. These cells may be active together with natural killer (NK) cells. In clinical practice, the detected balance between $TCR_{\alpha\beta}$ cells and $\gamma\delta^+$ cells is helpful in evaluating the level of adaptive immunity competence. In patients on immunosuppression after allogeneic stem cell transplantation (allo-HSCT), $TCR_{\alpha\beta}^+$ cells in blood, especially $CD4^+$, are poorly represented, but $TCR_{\gamma\delta}$ cells and NK cells are present in rather high proportions. Within the adaptive immunity cell compartment, terminally differentiated T cells prevail at the expense of naive cells. Therefore, in immunosuppressed patients, cells with a poor potential to adapt to new antigenic challenges prevail.

The $CD4^+$ T-cell count is of value in diagnosis and monitoring of patients having their immune systems suppressed. It provides relevant information on the critical numbers of $CD4^+$ cells that need to be present to mount an adequate immune response on demand.[2] However, knowing only the number of $CD4^+$ cells, we cannot evaluate whether they can exert T-cell helper function. This ability largely depends on providing antigen-presenting cells, among which monocytes are of value. $CD14^+$ cells, whose proportion lacks human leukocyte antigen (HLA)–DR antigens, have a poor ability to present antigens, but, in addition, they may belong to the regulatory cell subset, that is, monocytic myeloid-derived suppressor cells (mMDSCs).[3,4] Importantly, these $CD14^+$ $HLA\text{-}DR^-$ cells represent mMDSCs, which impact the immune response. It is relevant not only during the initiation of the adaptive immunity response but also at the later stage, to calm the overwhelming response, which may injure the organs. The latter issue is raised in the case of patients with coronavirus disease 2019 (COVID-19), who suffer from lung damage likely due to overwhelming response.

Recent evidence has shown that a low $CD4^+$ cell count in patients with COVID-19 is associated with a poor humoral response as well as impaired cellular response at the site in the lung, which results in severe pneumonia.[5] A cytometric analysis of the basic blood cell populations is of value, considering the limitations of this approach, which is not well suited for the measurement of the minute numbers that we deal with working on naive cells. Cytometrically naive cells may be counted using multiparameter staining, which can divide the T-cell pool into a naive cell subpopulation having the CD45RA, CCR7, CD62L, and CD27 isoforms.[6] It is a difficult approach, as the profile of epitopes, which may characterize the naive cells, changes along the differentiation pathway.

To assess the ability of the host to combat new antigen(s), the information on the presence of naive cells should be precise, as the chance of matching largely relies on the number of naive cells in the immune system.

From the profile of blood cells, it is known whether an individual is numerically competent and which type of immunity prevails (adaptive, non–MHC-restricted, or natural) as well as whether there is any population of naive cells at disposal. There are no available data to answer the following questions: 1) whether there are any T-cell clones sharing the same TCR_{β}, which are ready to recall; and 2) how many cells of different clonotypes (the same complementarity-determining region 3 [CDR3] of TCR_{β}) are still available, being so far not triggered by an antigen. Next-generation sequencing helps to meet these needs.

Cytomegalovirus reactivation as a prototype phenomenon showing the adaptive immunity response To visualize the ongoing events that follow the immune response, we illustrate the situation seen when the infection or reactivation of cytomegalovirus (CMV) occurs. Cytomegalovirus is a widespread virus residing in about 80% of the adult population,[7] which is kept dormant under the surveillance of the immune response. When the immune response fails, not preventing the reactivation of the virus, we observe the presence of immunoglobulin M (IgM) antibodies in blood, which may further shift to immunoglobulin G (IgG) antibodies, witnessing the persistence of CMV ready to reactivate.[8] Reactivation events depend on the function of T-cells facing chronic viral infection:

1 Reactivation takes place not only when $CD4^+$ cells decrease in number but also when they are less efficient in interferon γ production. Our own study showed that a number of $CD4^+$ cells below 10% of all lymphocytes and an interferon γ genotype associated with a low generation potential constitute risk factors for CMV reactivation.[9]

2 At that time, the cellular immune response against CMV is on alert, which is reflected by an increase in the number of $CD57^+$ T-effector cells, which are highly differentiated and used to control CMV and some other viruses.[10,11] Indeed, $CD57^+CD8^+$ cells are effective in cytotoxicity (rich in perforins and good producers of interferon γ) and, following the differentiation pathways, evolve into terminally differentiated T cells.[12]

3 When the cellular adaptive immunity fails to control CMV reactivation, $TCR_{\gamma\delta}^+$ cells increase in number above the threshold value, and this increase is associated with the prevalence of the $V_{\delta}2^-$ family.[13]

Understanding the immune response against CMV is helpful in identifying the individuals at risk of reactivation. Fortunately, there is a drug in clinical use that is effective in mitigating the CMV spread. Cytomegalovirus notoriously reactivates, depriving the host of proper immune function (throwing a wrench in

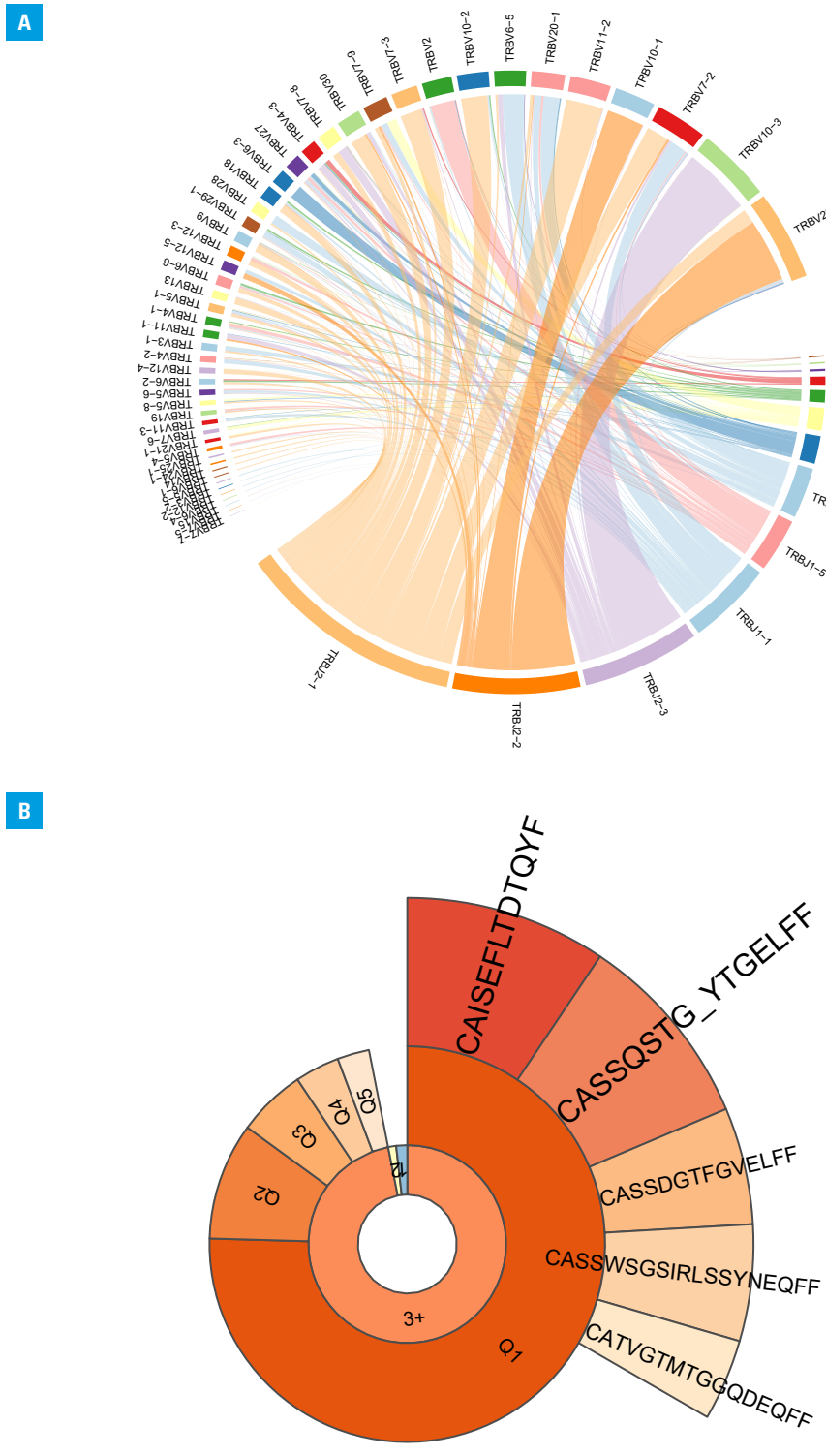

FIGURE 1 Next-generation sequencing tools allow us to estimate the structures of T-cell receptors of β chain, having them further grouped in subfamilies and paired with the joining (J) gene segment—clone determination (**A**). In the next step, all clones are ordered according to frequency and their complementarity-determining region 3 structures are established (**B**). Note the overwhelming representation of a few clones on the top of all (Q1 representing the top clones—20% of all clones). The 5 most frequent clonotypes cover almost half of Q1 quantiles. The other quantiles (Q2–Q5) are much less rich in frequent clonotypes. The naive clones, open to match with new antigens, are marginally present (light blue fraction).

the immune system according to Cicin-Sain et al)[14,15] and, as the epidemiological data show, is associated with a high death toll, especially in the elderly.[16]

The potential of next-generation sequencing in the analysis of the T-cell receptor β repertoire The dawn of next-generation sequencing provided a new tool to assess the T cell αβ cell repertoire, allowing one to determine the number of clones that

are dominant in a given situation and also those used less frequently, so that finally the naive cell population can be measured. In our own study, when we were boosting the antileukemic response in patients after allo-HSCT by infusing the donor cells,[17] we found that the immune system fighting leukemia is overwhelmingly concentrated on a low number of antigens, having not enough cells for other specificities, which facilitates reactivation of viruses, among which CMV reactivation is frequently seen. We observed that 30% of the patients after allo-HSCT had CMV DNA copies in blood, especially 16 weeks after transplant. Deep throughput sequencing of the TCR_{β} receptor after an appropriate mathematical analysis showed that 20% of all TCR_{β} clones occupied most of the patients' TCR repertoire (Q1), making the immune response focused on a limited number of antigens and rendering the patients defenseless against a variety of pathogens (FIGURE 1). Indeed, within their repertoire, the patients had only 0.1% of CMV-recognizing clones (according to the TCR motif VDJdb database)[18,19] and finally succumbed to CMV infection. In healthy individuals and in allo-HSCT patients in our study, the most frequently noted TCR_{β} repertoire included receptors recognizing epitopes for CMV, Epstein–Barr virus, and influenza. In healthy marrow donors, TCR_{β} clones recognizing CMV were present in 0.8% to 1.6% of the whole repertoire (median, 1.22%), and in allo-HSCT recipients confronting frequently reactivated herpes viruses, in 0.9% to 5.7% (median, 3.55%).

The recognition of a foreign antigen in the context of self-peptide MHC makes the immune responsiveness possible and finally effective. The recognition ability enabling infection to be fought depends on the number of naive T cells. The naive T cells are those which have not been or have been only once exposed to some foreign antigen at some time before, thus being open to matching a new foreign peptide. The pool of naive cells declines with age, which makes effective matching with new antigen(s) less probable. The TCR repertoire declines from about 40 years of age, with an interesting exception observed in individuals aged over 90 years, who may have a better repertoire than might be expected from their age.[20] This suggests that environmental selection is present during aging. Regarding COVID-19, there is a number of reports showing recovery from COVID-19 in patients over 90 years of age. However, if all patients over 60 years of age are considered, they are at high risk of a severe course of the disease.

The proportion of $CD8^{+}CD57^{+}$ lymphocytes in blood increases with age.[21] These cells are reaching the stage of terminal differentiation, being unable to respond to a new antigenic challenge. They occupy a great part of the homeostatic space in the immune cell compartment, not leaving enough room for naive cells whose presence is necessary to cope with new antigenic challenges.[22]

How the immune system may confront severe acute respiratory syndrome coronavirus 2 All the above presented information is of value in assessing the risk of new pandemic viral diseases on the basis of the prevalence of terminally differentiated T cells (flow cytometry) or by using deep throughput TCR sequencing, which visualizes not only the prevalence of dominant clones (expanding in the course of chronic infections) but also the proportions of naive cells ready to respond. The ability to respond to new antigen(s) is greater, if the host has not been previously exhausted by chronic infections and is of younger age. Being aware of that, especially of the preconditions to effectively combat new viruses, may help in tailoring healthcare delivery to people confronting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The latter issue is crucial. In spite of global efforts to mitigate the pandemic spread, the rates of infection and human loss are high and success seems to be far away. There may be some hope for vaccination. The spread of SARS-CoV-2 infection is rather poorly evaluated worldwide, because the criteria for epidemiological surveillance differ from country to country. In Poland, as might also be the case in other countries, there is no universal policy on SARS-CoV-2 genetic testing. The media quite frequently forget that the sensitivity of genetic testing regarding the presence of the virus in the upper respiratory tract is about 40% to 70%, providing an analysis of the presence of 3 independent genes depending on the time after infection or disease onset when the nasal swabs were taken.[23,24] What we know better is that there is a death toll. Information from various sources indicates that fatal cases occur within the older population owing to severe comorbidities and likely because of the reduced ability to recognize new epitopes. A higher risk of severe infection is associated with defects in the immune system, which should be considered when designing a vaccine. The same defect that facilitates the virus spread may also hamper the response to a vaccine. In addition, SARS-CoV-2 is one of the RNA viruses that mutate easily.[25] Therefore, the vaccine must cover stable—but crucial for virus survival—epitopes. The effect of vaccination depends on the ability of the vaccine to initiate an adaptive immune response, within which the neutralization antibody plays a key role. Indeed, potent human neutralizing antibodies are elicited by SARS-CoV-2 infection.[5,26] The curves reflecting a relationship between the titer of neutralizing antibodies and the level of viral protection demonstrate the potential of these antibodies in disease prevention. The neutralizing antibodies are of germline and germline-divergent origin[27] and, due to the mutation rate, likely biased by environmental stress. Finally, there are diverse families of antibodies built from an array of heavy and light chains and their random associations. As a result, there are numerous antibodies differing with regard to their disease

protection potential. This is an issue in view of several lines of clinical data on the positive effect of transfusing convalescent plasma in patients suffering from severe COVID-19. The positive effect of plasma transfusion as passive immunization depends on the potential of convalescent serum antibodies to block the virus. However, the assessment of antibodies for their neutralization activity is complex and relies on the use of an animal model or cultured cells. Infection evokes a more complex humoral response including SARS-CoV-2 antibodies that lack a neutralization potential but are effective in armoring NK cells and granulocytes. The antibodies are bound to the cells by the Fc part, having that recognizing antigen part protruding outside and directing the cells to the target. Armored cytotoxic cells, connected with the recognized epitope, exert their lytic activity (antibody-dependent cellular cytotoxicity). Antibodies formed in the course of the infection can be measured using enzyme-linked immunosorbent assay (ELISA). Enzyme-linked immunosorbent assay used in patients with COVID-19 is helpful in the diagnostic workup, being positive against the SARS-CoV-2 spike protein in the IgM class as soon as 8 days after infection, then the antibody production switches to the IgG class,[27] which may persist for at least 1 year, as suggested by the observation of IgG antibodies specific for SARS-CoV-1.[28] Importantly, the level of ELISA-measured IgG SARS-CoV-2 antibodies correlates well with the level of neutralizing antibodies. Therefore, good responders to virus epitopes seen in ELISA in patients who have recovered from the disease are likely rich in the neutralizing antibodies. Enzyme-linked immunosorbent assay should therefore be used in the search for convalescent plasma donors organized by several blood banks. The United States Food and Drug Administration approved the use of convalescent plasma in COVID-19 treatment.[29] Shortly after that, the Polish Transfusion Centers launched an initiative to request patients who had recovered from COVID-19 to donate plasma for patients in need. However, it is not an easy task to do. An intriguing aspect of the COVID-19 research is the discrepant association between the level of antibodies and the risk of a severe course of the disease.[30] In addition, it is reported that the elderly are better producers of antibodies than younger individuals.[27] The discrepancy lies as to the relation in the outcome of the disease and the level of the antibodies. Older people who are good producers of antibodies suffer more frequently from target organ injury. The offered explanation is that the patients at risk of severe pneumonitis suffer from the outcome of poor regulation within the immune system, which results in a higher production of antibodies and poor control of inflammation. In view of that, the positive effect of the plasma treatment may depend on feedback control exerted, according to the Niels K. Jerne's immune network hypothesis,[31] by the anti-idiotypic antibody likely present in infused plasma. The variable region of the IgG molecule is characterized by a peculiar composition of aminoacids, which may evoke idiotypic autoantibody formation. These autoantibodies mitigate further antibody production simply mimicking an antigen, thus blocking CDR3 on B cells in a way it works in patients with immune thrombocytopenic purpura and other autoimmune diseases.[32,33]

A clue issue is to maintain a proper balance between the immune response and associated inflammatory reaction. Physiologically, inflammation induces differentiation of mMDSCs, which may originate from both monocyte and granulocyte lineages. These mMDSCs should control the overproduction of antibodies as well as the inflammatory process.[3,4] This is our hypothesis to support attempts to use mesenchymal stem cells (MSCs) for COVID-19 treatment. Mesenchymal stem cells constitute a stroma for myeloid cell differentiation and have some distinguishing features including lack of HLA on the membrane and the ability to support the balance between immune system reactivity and the extent of inflammation. This may be exemplified by the positive effect of MSCs on acute and chronic graft-versus-host disease. This life-threatening complication of allo-HSCT is caused by tissue injury prevailing over the benefit deriving from the immune response, even if a virus underlies the response. The effect of inflammation is disastrous. Infused MSCs can successfully calm the response. This ability of MSCs was used by Leng et al[34] in the treatment of COVID-19 patients with severe pneumonitis. To our knowledge, many trials have already been registered to use MSCs for COVID-19 treatment, including a company producing MSCs.[35] The results are promising and stem cell therapy is regarded as a candidate to be the best therapeutic agent restoring the proper balance between the disastrous effect of the inflammatory response and the positive effect of the immune response measured by the blood level of antibodies. The mortality rate of COVID-19 is much higher than that of influenza A. Mortality is associated with the viral attacks upon pneumocytes, which are rich in angiotensin-converting enzyme 2, and the injury of pneumocytes causing a severe inflammatory exudate that blocks and consequently damages alveolar vessels. The fatal course of the disease is associated with a high level of interleukin 6. Therefore, in the first attempt to use MSCs in the treatment of COVID-19 pneumonia, 10^6 cells of MSC characteristics/kg of body weight were injected in patients with COVID-19 pneumonia manifested with high fever, dyspnea, and poor oxygen saturation. Importantly, no adverse effects were reported, and the authors claimed that all patients improved within 2 days.[34]

Conclusions Still, we are at the beginning of the way in the search for the optimal COVID-19 prevention strategy and treatment. The key issue

is to develop a medicine blocking virus replication and to improve our understanding of the immune system confronting the virus. This knowledge can reveal weak points of the system that facilitate the infection and may also hamper the vaccination effect if not managed in a timely manner.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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