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Ability of the immune system to fight viruses highlighted by cytometry and TCR clonotype assessments: lessons taken prior to COVID-19 virus pandemic outbreak

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Abstract

The intriguing aspects of SARS-CoV-2 virus are the high rate of spread and rapid progression of pneumonitis. Confronted with thousands of deaths daily worldwide, we have to build quickly the rationale behind the treatment, taking advantage from past analogues. When a new virus strikes, T-cell receptor (TCR) gamma/delta cells are in the first line of defence, activated by stress molecules and recognising some epitopes in a process that is major histocompatibility complex (MHC) independent but still specific, e.g. cytomegalovirus (CMV), as well as participating in the regulatory mechanism – both characteristics are useful in fighting SARS-CoV-2. The fatalities are mostly due to pneumonitis, in the course of which an overwhelming inflammatory process impairs blood oxygenation, calling for artificial ventilation. In fatal COVID-19 cases the balance between the immune response and the inflammatory outcome fails, due to which the patients at risk, mostly aged, have higher levels of anti-SARS-CoV-2 antibodies and an enhanced inflammatory process in the lung. 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 (MSC) may assist the body regulatory mechanisms, knowing the anti-inflammatory potential of these cells. The use of these two immunotherapeutic tools is understandable on the grounds of basic immunology, whose knowledge may direct the medical community in efforts to fight the virus.
The T-cell receptor (TCR) repertoire reflects the ability of recognition of some epitopes and also indicates the proportion of naïve cells ready to respond to unknown antigens. T cell immunity is represented by the adaptive immunity exerted by cells having the TCR beta receptor and those reacting without the help of major histocompatibility complex (MHC) antigens, i.e. TCR gamma/delta. The latter cells are triggered by stress molecules exerting cytotoxicity against pathogens by producing pro-inflammatory cytokines. TCR gamma/delta T cells may be identified by the detection of either V delta1 chain (usually acting in response to stress antigens) or V delta2 positivity, which are present mostly in blood [1]. In patients with rather poor adaptive immunity, gamma/delta positive cells are present in a greater proportion than in competent ones and may react in concert with natural killer cells. In clinical practice the detected balance between TCR alpha/beta cells and gamma/delta positive cells is helpful in evaluating the level of adaptive immunity competence. In patients on immunosuppression after allogeneic stem cells transplantation (alloHSCT), TCR alpha/beta positive 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 naïve cells. Therefore, in immunosuppressed patients, cells with poor potential to adapt to new antigenic challenges prevail.

The counting of CD4 positive T cells is of value in diagnosis and observation of patients having their immune system suppressed. It provides important information knowing the critical numbers of CD4+ cells which should be present to mount an adequate immune response on demand [2]. However, knowing only the numbers of CD4+ cells we do not know 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 which in a proportion lack HLADR antigens are poor as antigen presenting cells but in addition may
belong to the regulatory cells subset being monocytic myeloid-derived suppressor cells (M-MDSC) [3, 4]. Importantly these CD14+HLADR- cells represent monocytic myeloid suppressor cells which impact on the immune response is important in two phases during the initiation of the adaptive immunity response but also at later stage to calm overwhelming response which may injured the organs. The latter issue is raised in COVID-19 patients which suffer from the lung damage likely due to overwhelming response.

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

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

From the profile of blood cells it is known whether numerically an individual is competent and which type of immunity prevails (adaptive, non-MHC restricted, natural) as well as whether there is any population of naïve cells at disposal. We lack information based on the genetic level to know whether there are any T cell clones sharing the same TCR beta which are (i) ready to recall, and (ii) how many cells of different clonotypes (the same complementarity-determining region 3 (CDR3) of the TCR beta) are still available, being so
far not triggered by an antigen. Next-generation sequencing (NGS) helps in meeting these needs.

To visualize ongoing events which follow the immune response we illustrate the situation seen when the infection/reactivation of cytomegalovirus (CMV) occurs. CMV 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 in the blood the presence of IgM antibodies which may further shift to 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 when CD4+ cells decrease in number but also if they are less efficient in IFNgamma production. Our own study showed that a number of CD4+ cells below 10% of all lymphocytes and a IFNgamma genotype associated with a low generation potential constitute risk factors of 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 IFN gamma) and following the differentiation pathways evolve into terminally differentiated T cells [12].

3. When the cellular adaptive immunity fails in controlling CMV reactivation TCR gamma/delta positive cells increase in number above the threshold value, and this increase is associated with the prevalence of the V delta2 negative family [13].

Understanding the immune response against CMV is helpful in identifying the individuals at risk of reactivation. Fortunately, there is in clinical use a drug which is effective in mitigating CMV spread. CMV notoriously reactivates, depriving the host of proper immune function
(throwing a wrench in the immune system according to Luka Cicin-Sain et al. [14, 15]) and according to the epidemiologic data is associated with a high death toll, especially in the elderly [16].

The dawn of NGS provided a new tool to assess the T cell alpha/beta cell repertoire, allowing one to determine the number of clones which are dominant in a given situation and also those used less frequently, so that finally the naïve cell population can be measured. In our own study when we were boosting the anti-leukemic response in the patients after alloHSCT, by infusing the donor cells [17], we found that the immune system fighting leukaemia 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. Our observation documented that 30% of the patients after alloHSCT had CMV DNA copies in the blood, especially 16 weeks after transplant. Figure 1 shows the results of deep throughput sequencing of the TCR beta receptor after an appropriate mathematical analysis showed that 20% of all TCR beta clones occupy most of the patients’ TCR repertoire (Q1), making the immune response focused on a limited number of antigens and rendering the patients defenceless against a variety of pathogens. Indeed, the patient had within his repertoire only 0.1% of CMV recognizing clones (according to the T-cell receptor motif database VDJdb [18, 19]) and finally succumbed to CMV disease. In normal individuals and in alloHSCT patients, under our study, the most frequently noted TCR beta repertoire includes receptors recognizing epitopes for CMV, Epstein-Barr virus and influenza. In healthy marrow donors TCR beta clones recognizing CMV were present in the whole repertoire from 0.8% to 1.6% (median:1.22%) and in alloHSCT recipients confronting frequently reactivated herpes viruses from 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 ability of recognition enabling infection to
be fought depends on the number of the naïve T cells. The naïve T cells are those which have not been or have only once been exposed to any foreign antigen any time before, thus being open to matching a new foreign peptide. The pool of naïve cells declines with age, which makes effective matching with new antigen(s) less probable. The TCR repertoire declines with age from about 40 years of age, with an interesting exception in those aged over 90 years, who may have a better repertoire than might be expected from the age [20]. This suggests the presence of environmental selection while aging. Regarding COVID-19 there is a number of medial information highlighting recovery from COVID-19 patients over 90 years of age. However, if all patients over 60 years of age are considered there are at high risk of severe course of the disease.

With the age proportion of CD8+CD57+ lymphocytes in the blood increases [21]. These cells are reaching the stage of terminal differentiation being unable to respond to new antigenic challenge. They occupy the great part of the homeostatic space in the immune cells compartment not leaving enough room for naïve cells which presence is mandatory to cope with new antigenic challenges [22].

All the above information is of value in assessing the risk of new pandemic viral diseases on the basis of prevalence of terminally differentiated T cells (flow cytometry) or by using deep throughput TCR sequencing, which visualizes not only prevalence of dominant clones (expanding in the course of chronic infections) but also the proportions of naïve cells ready to respond. The ability to respond to new antigen(s) is greater if the host was not previously exhausted by chronic infections and is of younger age. Knowing that, especially the preconditions for effectively combating new viruses, may help in tailoring health care delivery to people confronting SARS-CoV-2 infection. The latter issue is important. In spite of global efforts to mitigate the pandemic spread, the rate of infection and human losses are high and success seems to be far away. There may be hope for vaccination. SARS-CoV-2
infection spread is globally evaluated rather poorly because criteria for epidemiologic 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. It is quite frequently forgotten, when presenting the data through the media, that the sensitivity of genetic testing of presence of the virus in the upper respiratory tract is about 40% to 70%, providing an analysis of the presence of three independent genes depending on the time after infection or illness onset that the nasal swabs were taken [23, 24]. What we know better, there is a death toll. Information from various sources indicates that fatal cases occur within the aged population due to the presence of severe co-morbidities and likely to the reduced ability to recognize new epitopes. Higher risk of severe infection is associated with defects in the immune system, whose presence should be considered when designing a vaccine. The same defect which facilitates the virus spread may also hamper the response to a vaccine. In addition, SARS-CoV-2 is one of the RNA viruses, which mutate easily [25]. Therefore the vaccine must cover rather 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 neutralisation antibody plays a main role. Indeed, potent human neutralising antibodies are elicited by SARS-CoV-2 infection [5, 26]. The curves reflecting the presence of a relationship between the titre of neutralising antibodies and the level of viral protection demonstrate the potential of these antibodies in disease prevention. The neutralising antibodies are of germline and germline-divergent origin [27], 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 different antibodies regarding their disease protection potential. This is an issue in view of the presence of several lines of clinical information on the positive effect of transfusing COVID-19 convalescent plasma into patients suffering from severe COVID-19. The positive effect of plasma transfusion as passive
immunization depends on the potential of convalescent sera antibodies in blockage of the virus. However, the assessment of antibodies for their neutralisation 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 which lack neutralisation potential but are effective in armouring NK cells and granulocytes. The antibodies are bound to the cells by the Fc part, having that recognizing antigen part protruding outside in an effect directing the cells to the target. Armoured cytotoxic cells after connection 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 used in COVID-19 patients is helpful in the diagnostic process, being positive against 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 the presence 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 the ELISA in patients who have recovered from the disease are likely rich in the neutralizing antibodies. ELISA should therefore be used in the search for convalescent plasma donors organized by several blood banks. The Food and Drug Administration in the USA approved the use of convalescent plasma in COVID-19 treatment [29]. Shortly after that the Polish Transfusion Centres launched an initiative to request patients who had recovered from COVID-19 to donate plasma for patients in need. However, there is no an easy task. An intriguing aspect of the COVID-19 research is the rather discrepant association between the level of antibodies and the risk of a severe course of the disease [30]. In addition, it is reported that aged patients 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 higher production of antibodies and poor control of the inflammatory process. In view of that the positive effect of the plasma treatment may depend on a feedback control exerted, according to the Niels Jerne immune network hypothesis [31], by the presence anti-idiotypic antibody likely present in infused plasma. The variable region of immunoglobulin G molecule characterises by a peculiar composition of amino-acids which may evoke idiotypic autoantibody formation. This auto-antibodies mitigates further antibodies production simply mimicking an antigen thus blocking CDR3 on B cells how it works in immune thrombocytopenic purpura and other autoimmune disease patients [32, 33].

A clue issue is to maintain a proper balance between the immune response and associated inflammatory reaction. Normally the inflammation induces differentiation of myeloid-derived suppressor cells (M-MDSC), which may originate from both monocyte and granulocyte lineages. These M-MDSC should control the overproduction of antibodies as well as the inflammatory process [3, 4]. This is our hypothesis, which we are expressing openly just to support our and other attempts to use mesenchymal stem cells (MSC) as a tool in COVID-19 treatment. MSC constitute a stroma for myeloid cells’ differentiation, having some distinguishing features including lack of human leukocyte antigens (HLA) on the membrane and an ability to support a balance between immune system reactivity and the extent of the inflammatory process. This may be exemplified by the positive effect of MSC on acute and chronic graft-versus-host disease GvHD. This life-threatening complication of alloHSCT is due to the tissue injury prevailing over the benefit deriving from the immune response, even if a virus is behind the response. The effect of inflammation is disastrous. Infused MSC can successfully calm the response. This ability of MSC was used by Zikuan Leng et al. [34] in
the treatment of COVID-19 patients with severe pneumonitis. To our knowledge, almost many trials have already been registered to use MSC for COVID-19 treatment, including by a company producing MSC [35]. The results are promising, and there is a belief that stem cell therapy is 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 level of antibodies in the blood. The mortality rate of COVID-19 is much higher than that of influenza A. The fatalities are associated with the virus attacks upon pneumocytes, which are rich in angiotensin converting enzyme 2 (ACE2), and the injury of pneumocytes causes severe inflammatory exudate which causes a block in the alveolar vessels with consequent damage and also noncompliance in the severely affected. The fatal course of the disease is associated with the level of IL-6. Therefore, in the first attempt to use MSC in the treatment of COVID-19 pneumonia, 10E06 cells of MSC characteristics/kg of body weight were injected into patients with COVID-19 pneumonia manifesting with high fever, shortness of breath, and poor oxygen saturation. Importantly, no side effects were reported, and the authors claim that all patients improved within 2 days [34].

Still we are at the beginning of the road in the search for the optimal prevention and treatment of patients with COVID-19. The key issue is to develop a medicine blocking virus replication and also to improve our understanding of the immune system confronting the virus to reveal weak points of the system that facilitate the infection and may also hamper the vaccination effect if not considered timely.

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

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