The Journal of Infectious Diseases

PERSPECTIVE

The Importance of Cellular Immunity in the Development of Vaccines and Therapeutics for COVID-19

Jeffrey Schlom and Renee N. Donahue

Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 8B09, Bethesda, Maryland 20892, USA

It is important to develop vaccines that can also mediate T-cell responses to SARS-CoV-2 to limit severity of infections, and to analyze the cellular immunome in the use of anti-SARS-CoV-2 therapeutics.

Keywords. COVID-19; SARS-CoV-2; T-cell immunity; vaccines; immunology; human immunome.

For most viral infections, susceptibility is based on several factors, including (1) the presence of preexisting antibodies to the virus and/or the ability of the individual to rapidly mount de novo antibodies to the agent, (2) the innate cellular immune response, principally natural killer (NK) cells, and (3) the phenotype of the susceptible target cells in terms of receptors for viral entry. The severity of the infection, and the probability of reinfection, may depend on the level, type, and affinity of the antibodies developed to the virus, but perhaps more importantly, on the ability of the adaptive cellular immune response, principally mediated by T cells, working in concert with other cell types of the adaptive and innate immune systems, to destroy virus-infected cells. The generation of a T-cell response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may well also lead to a less severe outcome in cases of secondary exposure to the virus.

A great deal of progress has been made in cancer therapy in recent years with the discoveries and use of checkpoint inhibitor monoclonal antibodies for the treatment of a range of human cancers. This led to enormous efforts in academia, government, and the private sector to develop other immunotherapeutics and immune modulators for use in immune-oncology platforms, which in turn led to a much greater understanding of the human immunome and the complexities involving the interactions among multiple components of the adaptive and innate immune systems.

To date, well over 100 different phenotypes of human immune cells have been identified among the so-called parental cell types: NK cells, $CD8^+$, $CD4^+$, and NK T cells, regulatory $CD4^+$ T cells, conventional and plasmacytoid dendritic cells, myeloid-derived suppressor cells, B cells, and monocytes. For each of these parental cell types, multiple subtypes have been identified, many with defined activation and/or suppressive functions.

The assays to detect anti–SARS-CoV-2 antibodies and to detect serum or plasma cytokines, such as interferons, tumor necrosis factor α, and interleukin 6, 8, and 2, are readily available to screen large segments of the population who are apparently healthy, actively infected with SARS-CoV-2, or in convalescence. However, there is somewhat of a "paradigm paralysis" in vaccine development, with a tendency to concentrate on vaccines designed only to activate antibody responses to viral surface (coat) proteins and interfere with viral infection of cells. Although this should be the primary emphasis of a preventive vaccine, little attention has been given to designing vaccines that will also induce T-cell responses to internal components of the virus. The development of T-cell responses recognizing viral peptide– major histocompatibility complex (MHC) complexes on the surface of infected cells will in turn lyse those cells and limit the severity of infection.

The strategies and assays described herein are designed for use in clinical trials, to interrogate the cellular immunome of apparently healthy individuals, patients actively infected with SARS-CoV-2, or those in the convalescent stage to determine whether specific cellular immune subsets or combinations of subsets define which individuals are more likely to have limited versus severe infections, and which patients are more likely to succumb to secondary exposure to the virus.

ANALYSES OF >100 PERIPHERAL IMMUNE CELL SUBSETS

One assay [1, 2] is flow cytometry based and requires only 5×10^6 peripheral blood mononuclear cells (PBMCs), usually obtained from a single tube of blood. It interrogates multiple cell types and subtypes of both the innate and adaptive immune systems. After processing, cells can be frozen and stored so that analyses of a given individual's PBMCs obtained at different times can be assayed

Received 3 June 2020; editorial decision 2 July 2020; accepted 6 July 2020; published online July 11, 2020.

Correspondence: Jeffrey Schlom, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD (js141c@nih.gov).

The Journal of Infectious Diseases® 2020;222:1435–8

DOI: 10.1093/infdis/jiaa415

under identical conditions. Multiple prior studies have used this assay to detect the dynamics of 123 immune cell subsets, many of which have known biologic functions. Recently, additional immune cell subsets have been identified as having biologic functions [3]. The most recent assay being used can now simultaneously analyze 138 immune cell subsets. Table 1 displays the breadth and complexity within each of the parental immune subsets (ie, CD8, CD4, NK, etc).

One observation that has emerged in the coronavirus disease 2019 (COVID-19) pandemic is the severity of infection seen between different age groups. A prior study [2] using this assay interrogated whether there were any differences in the immunome between apparently healthy individuals <40 versus >40 years of age; this arbitrary distinction was made because cancer incidence greatly increases after age 40 years. The results demonstrated that individuals >40 years old have significantly lower levels of $CD8^+$ T cells but, in contrast, significantly higher levels of NK cells (both $P = .009$). Clear differences were also observed in multiple other immune cell subsets between the 2 age groups. The younger population, for example, had significantly higher levels of $CD8^+$ cytotoxic T-lymphocyte antigen 4 $(CTLA\text{-}4)^+$, $CD8^+$ programmed cell death ligand 1 ($PD\text{-}L1^+$), naive $CD8^+$, and central memory $CD8^+$ T cells but significantly lower levels of T-cell immunoglobulin and mucin domain 3^+ B cells.

These studies were extended to look for differences between apparently healthy individuals versus age-matched patients with a range of different cancers. Although there was no difference in absolute lymphocyte count between the 2 groups, patients with cancer had significantly higher levels of 2 phenotypes of myeloid-derived suppressor cells, $CTLA\text{-}4^+$ regulatory T cells, and multiple phenotypes of $CD8^+$ T cells (eg, $CTLA\text{-}4^+$), while having significantly lower levels of B cells than apparently healthy individuals. This assay also has been used to study the effects of various immune modulators and so-called nonimmune-based therapeutics on the immune system. Thus, this assay or similar ones could be used to determine the effects of various antiviral agents on multiple components of the cellular immunome and to further determine whether they have any effect on secondary exposures to SARS-CoV-2.

DETECTION OF ANTIGEN-SPECIFIC T-CELL RESPONSES

The severity of SARS-CoV-2 infection in a given individual is most likely defined by the adaptive T-cell response to viral peptide/MHC complexes involving both $CD8^+$ cytolytic and $CD4^+$ helper T cells. Thus, an assay that simultaneously detects the levels and phenotypes of both $CD8^+$ and $CD4^+$ T-cell responses may well aid in the study of patients with COVID-19, by (1) determining whether apparently healthy individuals have "preexisting" T-cell responses to SARS-CoV-2, due to either prior exposure to SARS-CoV-2 virus or cross-reactivity of T cells directed against related coronaviruses as a consequence of prior exposure; (2) determining whether a given preventive vaccine can induce SARS-CoV-2–specific T-cell responses in addition to antibody responses; (3) aiding the development of a therapeutic anti–SARS-CoV-2 vaccine to be administered early after virus exposure; (4) better defining mechanisms of viral clearance after infection as they relate to the severity of disease; and (5) enabling comparisons of potential efficacy between different SARS-CoV-2 vaccines and/or antiviral therapeutics.

The assay described is flow cytometry based and uses 15-mer overlapping viral peptides and/or 9-mer peptides; it has been used in numerous cancer immunotherapy clinical studies [4–6]. It uses previously frozen PBMCs so that samples from the same patient obtained over time can be analyzed simultaneously. This assay [5–7] simultaneously defines $CD8^+$ and $CD4^+$ T-cell responses in terms of their expression of interferon γ, tumor necrosis factor α, interleukin 2, and/or CD107a (a marker of lytic potential), and it can thus simultaneously define the level of T-cell responses as well as their multifunctionality (ie, potency). This assay, which has been used to detect human papillomavirus–specific T-cell responses in patients with cancer, is also non-MHC restricted, and it thus detects all genotypes of MHC class I and class II alleles.

SOLUBLE FACTORS

The mainstay of analyses of serum/plasma samples from patients with COVID-19 will be the detection of SARS-CoV-2 virus or viral RNA, of antiviral antibody responses, and levels of various cytokines. There are, however, other soluble factors detected in serum or plasma that are produced as a consequence of cellular immune responses may help define patient responses to SARS-CoV-2; these include soluble CD27 (sCD27), CD40L (sCD40L), sPD-L1 and programmed cell death 1 protein. sCD27 is preferentially derived from activated $CD4^+$ T cells, and higher levels are seen in apparently healthy individuals than in patients with cancer [8]. Some studies have shown that immunotherapy can increase sCD27 levels in serum [8]. sCD40L is a functional trimer that is shed from activated T lymphocytes and, more likely, from platelets. Evidence has been provided that higher levels of sCD40L are seen in some patients with cancer and that these may have an immunosuppressive effect [9]. Studies have also shown that the ratio of sCD27 to sCD40L may be indicative of a therapeutic benefit [1].

CONCLUSIONS

Multiple antiviral and other potential therapeutics have been and continue to be evaluated as ways to combat SARS-CoV-2 infections. These agents, however, may also have effects on different components of the innate and/or adaptive immune system and may thus influence clinical outcomes. Experience with different cancer therapeutics, such as small-molecule targeting agents, has revealed that many of these so-called

Table 1. Peripheral Immune Cell Subsets (n=138) Analyzed By Flow Cytometry[a]

1. Total CD4$^+$ T cells

- PD-L1$^+$ CD4 - *activation/inhibition*
- PD-1$^+$ CD4 - *activation/inhibition*
- CTLA-4$^+$ CD4 - *inhibition*
- Tim-3$^+$ CD4 - *inhibition*
- 41bb$^+$ CD4 - *co-stimulation*
- Ki67$^+$ CD4 - *proliferation*
- ICOS$^+$ CD4 - *activation*
  - ICOS$^+$ PD-L1$^+$ CD4 - *activation/inhibition*
  - ICOS$^+$ PD-1$^+$ CD4 - *activation/inhibition*
- Total naïve (CCR7$^+$CD45RA$^+$) CD4
  - PD-L1$^+$ naïve CD4 - *activation/inhibition*
  - PD-1$^+$ naïve CD4 - *activation/inhibition*
  - CTLA-4$^+$ naïve CD4 - *inhibition*
  - Tim-3$^+$ naïve CD4 - *inhibition*
- Total central memory (CCR7$^+$ CD45RA$^-$) CD4
  - PD-L1$^+$ CM CD4 - *activation/inhibition*
  - PD-1$^+$ CM CD4 - *activation/inhibition*
  - CTLA-4$^+$ CM CD4 - *inhibition*
  - Tim-3$^+$ CM CD4 - *inhibition*
  - Ki67$^+$ CM CD4 - *proliferation*
- Total effector memory (CCR7$^-$ CD45RA$^-$) CD4
  - PD-L1$^+$ EM CD4 - *activation/inhibition*
  - PD-1$^+$ EM CD4 - *activation/inhibition*
  - CTLA-4$^+$ EM CD4 - *inhibition*
  - Tim-3$^+$ EM CD4 - *inhibition*
  - Ki67$^+$ EM CD4 - *proliferation*
- Total EMRA (CCR7$^-$CD45RA$^+$) CD4
  - PD-L1$^+$ EMRA CD4 - *activation/inhibition*
  - PD-1$^+$ EMRA CD4 - *activation/inhibition*
  - CTLA-4$^+$ EMRA CD4 - *inhibition*
  - Tim-3$^+$ EMRA CD4 - *inhibition*
  - Ki67$^+$ EMRA CD4 - *proliferation*

2. Total CD8$^+$ T cells

- PD-L1$^+$ CD8 - *activation/inhibition*
- PD-1$^+$ CD8 - *activation/inhibition*
- CTLA-4$^+$ CD8 - *inhibition*
- Tim-3$^+$ CD8 - *inhibition*
- 41bb$^+$ CD8 - *co-stimulation*
- Ki67$^+$ CD8 - *proliferation*
- Total naïve (CCR7$^+$CD45RA$^+$) CD8
  - PD-L1$^+$ naïve CD8 - *activation/inhibition*
  - PD-1$^+$ naïve CD8 - *activation/inhibition*
  - CTLA-4$^+$ naïve CD8 - *inhibition*
  - Tim-3$^+$ naïve CD8 - *inhibition*
- Total central memory (CCR7$^+$CD45RA$^-$) CD8
  - PD-L1$^+$ CM CD8 - *activation/inhibition*
  - PD-1$^+$ CM CD8 - *activation/inhibition*
  - CTLA-4$^+$ CM CD8 - *inhibition*
  - Tim-3$^+$ CM CD8 - *inhibition*
  - Ki67$^+$ CM CD8 - *proliferation*
- Total effector memory (CCR7$^-$ CD45RA$^-$) CD8
  - PD-L1$^+$ EM CD8 - *activation/inhibition*
  - PD-1$^+$ EM CD8 - *activation/inhibition*
  - CTLA-4$^+$ EM CD8 - *inhibition*
  - Tim-3$^+$ EM CD8 - *inhibition*
  - Ki67$^+$ EM CD8 - *proliferation*
- Total EMRA (CCR7$^-$CD45RA$^+$) CD8
  - PD-L1$^+$ EMRA CD8 – *activation/inhibition*
  - PD-1$^+$ EMRA CD8 - *activation/inhibition*
  - CTLA-4$^+$ EMRA CD8 - *inhibition*
  - Tim-3$^+$ EMRA CD8 - *inhibition*
  - Ki67$^+$ EMRA CD8 - *proliferation*

3. Total Tregs

- PD-L1$^+$ Tregs - *activation/inhibition*
- PD-1$^+$ Tregs - *suppression*
- CTLA-4$^+$ Tregs - *suppression*
- ICOS$^+$ Tregs - *suppression*
- CD45RA$^+$ Tregs - *highly expandable in vitro*
- CD49d$^-$ Tregs - *suppression*
- Ki67$^+$ Tregs - *proliferation*
- CD38$^+$ Tregs - *suppression*
- HLA-DR$^+$ Tregs - *suppression*

4. Total B cells

- PD-L1$^+$ B cells - *activation/inhibition*
- PD-1$^+$ B cells - *activation/inhibition*

5. Total NK

- PD-L1$^+$ NK - *inhibition*
- PD-1$^+$ NK - *activation/inhibition*
- Tim-3$^+$ NK - *activation/inhibition*
- Ki67$^+$ NK - *proliferation*
- NKp30$^+$ NK - *activation*
- NKp46$^+$ NK - *activation*
- NKG2D$^+$ NK - *activation*
- CD226$^+$ NK - *adhesion/activation*
- Total mature (CD16$^+$ CD56^{dim}) NK - *lytic*
  - PD-L1$^+$ mature NK - *inhibition*
  - PD-1$^+$ mature NK - *activation/inhibition*
  - Tim-3$^+$ mature NK - *activation/inhibition*
- Total functional intermediate (CD16$^+$ CD56^{br}) NK – *lytic, cytokine production*
  - PD-L1$^+$ functional intermediate NK - *inhibition*
  - PD-1$^+$ functional intermediate NK - *activation/inhibition*
  - Tim-3$^+$ functional intermediate NK - *activation/inhibition*
- Total immature (CD16$^-$ CD56^{br}) NK - *cytokine production*
  - PD-L1$^+$ immature NK - *inhibition*
  - PD-1$^+$ immature NK - *activation/inhibition*
  - Tim-3$^+$ immature NK - *activation/inhibition*
- Total unconventional (CD16$^-$ CD56^{dim}) NK – *non-lytic, non-cytokine production*
  - PD-L1$^+$ unconventional NK - *inhibition*
  - PD-1$^+$ unconventional NK - *activation/inhibition*
  - Tim-3$^+$ unconventional NK - *activation/inhibition*

6. Total NK-T

- PD-L1$^+$ NK-T - *inhibition*
- PD-1$^+$ NK-T - *activation/inhibition*
- Tim-3$^+$ NK-T - *inhibition*
- Ki67$^+$ NK-T - *proliferation*

7. Total cDC

- PD-L1$^+$ cDC - *inhibition*
- PD-1$^+$ cDC - *activation/inhibition*
- Tim-3$^+$ cDC - *inhibition*
- Ki67$^+$ cDC – *proliferation*

8. Total pDC

- PD-L1$^+$ pDC – *inhibition*
- PD-1$^+$ pDC - *activation/inhibition*
- Tim-3$^+$ pDC - *inhibition*
- Ki67$^+$ pDC - *proliferation*

9. Total MDSC

- PD-L1$^+$ MDSC - *inhibition*
- PD-1$^+$ MDSC - *activation/inhibition*
- CD16$^+$ MDSC - *immature/suppression*
- Total monocytic (CD14$^+$ CD15$^-$) MDSC
  - PD-L1$^+$ mMDSC - *inhibition*
  - PD-1$^+$ mMDSC - *activation/inhibition*
  - CD16$^+$ mMDSC - *immature/suppression*
- Total granulocytic (CD14$^-$ CD15$^+$) MDSC
  - PD-L1$^+$ gMDSC - *inhibition*
  - PD-1$^+$ gMDSC - *activation/inhibition*
  - CD16$^+$ gMDSC - *immature/suppression*
- Total lineage negative (CD14$^-$ CD15$^-$) MDSC
  - PD-L1$^+$ lin neg MDSC - *inhibition*
  - PD-1$^+$ lin neg MDSC - *activation/inhibition*
  - CD16$^+$ lin neg MDSC - *immature/suppression*

10. Total Monocytes

- Classical monocytes - *phagocytic*
- Intermediate monocytes - *phagocytic/proinflammatory*
- Non-classical monocytes - *proinflammatory*
- PD-L1$^+$ monocytes - *inhibition*
- PD-1$^+$ monocytes - *activation/inhibition*

Abbreviations: cDC, conventional dendritic cells; CM, central memory; CTLA-4, cytotoxic T lymphocyte-associated protein-4; EM, effector memory; EMRA, terminally differentiated effector memory; FoxP3, forkhead box P3; gMDSCs, granulocytic myeloid derived suppressor cells; ICOS, inducible T cell co-stimulator; lin neg MDSCs, lineage negative MDSCs; mMDSCs, monocytic MDSCs; NK, natural killer; pDC, plasmacytoid DC; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; Tbet, T box expressed in T cells; TCR, T cell receptor; Tim-3, T cell immunoglobulin and mucin domain-3; Tregs, regulatory T cells.

[a]Ten parental phenotypes are identified as well as refined subsets of each relating to maturation and function. Expected function based on expression of specific markers within each subset is indicated in italics.

non-immune–based therapeutics can have profound effects on multiple components of the human immune system. Some have predicted a possible second wave or multiple waves of SARS-CoV-2 infection. The effects of antivirals on immune subsets may have important consequences for subsequent exposures to SARS-CoV-2.

Multiple preventive vaccine clinical studies are currently ongoing, with the goal of inducing anti–SARS-CoV-2 antibody responses. These vaccines will principally be monitored for the level and type of antiviral antibodies induced. It is proposed that the assays described herein should also be used in clinical studies to define which vaccines also enhance various adaptive and innate cellular immune responses. These assays may also help explain why certain age groups or other demographic groups, such as patients with preexisting conditions (eg, cancer and diabetes), differ in response to SARS-CoV-2 infections, resulting in more severe infection and/or complications.

It is thus proposed that—in addition to the search for antivirals, the passive administration of hyperimmune immunoglobulin, and the development of vaccines to induce anti–SARS-CoV-2 antibodies—a serious effort also be made to develop vaccines that can mediate an adaptive T-cell response to components of the SARS-CoV-2 agent. It is further proposed that the assays described herein, or similar assays, be used in clinical trials to monitor various COVID-19 interventions and elucidate the pathogenesis of both primary and secondary exposures to SARS-CoV-2.

Notes

Acknowledgments. For detailed standard operating procedures for the assays described herein, contact R. N. D. (renee.donahue@nih.gov).

Financial support. This work was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health (internal no. ZIA BC 010598).

Potential conflicts of interest. Both authors: No reported conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Donahue RN, Lepone LM, Grenga I, et al. Analyses of the peripheral immunome following multiple administrations of avelumab, a human IgG1 anti-PD-L1 monoclonal antibody. J Immunother Cancer **2017**; 5:20.
2. Lepone LM, Donahue RN, Grenga I, et al. Analyses of 123 peripheral human immune cell subsets: defining differences with age and between healthy donors and cancer patients not detected in analysis of standard immune cell types. J Circ Biomark **2016**; 5:5.
3. Fabian KP, Padget MR, Donahue RN, et al. PD-L1 targeting high-affinity NK (t-haNK) cells induce direct antitumor effects and target suppressive MDSC populations. J Immunother Cancer **2020**; 8:e000450.
4. Gatti-Mays ME, Redman JM, Donahue RN, et al. A phase I trial using a multitargeted recombinant adenovirus 5 (CEA/MUC1/brachyury)-based immunotherapy vaccine regimen in patients with advanced cancer. Oncologist **2020**; 24:1–6.
5. Gatti-Mays ME, Strauss J, Donahue RN, et al. A phase I dose-escalation trial of BN-CV301, a recombinant poxviral vaccine targeting MUC1 and CEA with costimulatory molecules. Clin Cancer Res **2019**; 25:4933–44.
6. Heery CR, Palena C, McMahon S, et al. Phase I study of a poxviral TRICOM-based vaccine directed against the transcription factor brachyury. Clin Cancer Res **2017**; 23:6833–45.
7. Strauss J, Gatti-Mays ME, Cho B, et al. Phase I evaluation of M7824, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with human papillomavirus (HPV)-associated malignancies (abstract). Cancer Res **2019**; 79(13 Supp):CT075.
8. Huang J, Jochems C, Anderson AM, et al. Soluble CD27-pool in humans may contribute to T cell activation and tumor immunity. J Immunol **2013**; 190:6250–8.
9. Huang J, Jochems C, Talaie T, et al. Elevated serum soluble CD40 ligand in cancer patients may play an immunosuppressive role. Blood **2012**; 120:3030–8.