CYTOMETRY
Journal of Quantitative Cell Science PART A

SARS-CoV-2, the Virus that Causes COVID-19: Cytometry and the New Challenge for Global Health

Andrea Cossarizza,[1*] Sara De Biasi,[1†] Giovanni Guaraldi,[2] Massimo Girardis,[3] Cristina Mussini,[2] for the Modena Covid-19 Working Group (MoCo19)[#]

• **Key terms**
SARS-CoV-2; Covid-19; coronavirus; cytometry; CD4; CD8; T cells

Introduction: The Covid-19 Epidemic

In December 2019, patients affected by severe illness and a pneumonia of unknown origin were described in the city of Wuhan, Hubei province, China (1). One month later, different laboratories, first in China and then in different countries, were able to demonstrate that a novel coronavirus, genetically very close to that of a bat, first named "2019 novel coronavirus" (2019-nCoV), and then defined as "SARS-CoV-2" (Severe Acute Respiratory Syndrome Corona Virus-2), was the cause of the disease, defined as Covid-19 (2). The most common symptoms of this novel pathology are fever; fatigue; respiratory symptoms such as cough, sore throat, and shortness of breath; and rare intestinal symptoms such as diarrhea (3).

The virus is transmitted from human to human (4), and according to the most recent data, most infected persons (up to 80%) present very mild symptoms or no symptoms at all. Unfortunately, the virus has some peculiarities, among which: (1) it is very contagious; (2) it can be transmitted (at very low rate) even by asymptomatic individuals; (3) in the relevant percentage of people, the infection can be extremely severe, forcing patients to be hospitalized (typically in infectious disease clinics or, in the most serious case, in intensive care units) and that the rate of mortality is far from being negligible.

In a relatively short time, Covid-19 epidemics have spread all over Asia and presented itself in the United States (Snohomish County, Washington) on January 19 (5) and in Germany on January 24 (6), arriving from China through different routes. Italy, where a higher number of cases than in any other European country has been described in the first weeks of the epidemic, was different from many other countries in distributing and administering large numbers of SARS-CoV-2 tests, which were immediately provided free of charge (like all medical treatments and drugs, thanks to the public health system) after the first recognized case, in Lombardy, on February 20. Thus, in reality, the high rate of infection in Italy soon after the identification of the first case only reflected the high local rate of detection of a virus already spread over all Europe. Unfortunately, in several countries, such as Germany, France, UK, and United States, very few tests have been conducted at the beginning of the epidemic, so the real extent was initially missing. In some cases, tests are expensive, and it seems very unlikely testing will ever be free. Coupled with the fact that, in some countries, depending on the circumstances or on the type of job, workers do not have paid sick (or quarantine) leave, it is also likely that people who are asymptomatic or slightly sick, but infectious, will continue to go to work and will infect others. Clearly, this does not help in containing the Covid-19 epidemic. Most countries are now coping very heavily with the virus, and evidence has emerged that the virus is circulating within local communities so that border-based travel restrictions are now no longer able to control its unpleasant arrival.

[1]Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia School of Medicine, Via Campi 287, 41125, Modena, Italy

[2]Infectious Diseases Clinics, University Hospital, via del Pozzo 71, 41124, Modena, Italy

[3]Department of Anesthesia and Intensive Care, University Hospital, via del Pozzo 71, 41124, Modena, Italy

*Correspondence to: Andrea Cossarizza, Department of Medical and Surgical Sciences for Children and Adults, via Campi 287, 41125 Modena, Italy Email: andrea.cossarizza@unimore.it

[†]Sara De Biasi is a Marylou Ingram Scholar of the International Society for Advancement of Cytometry (ISAC).

[#]The members of the Modena Covid-19 Working Group are listed in the Appendix.

Published online 18 March 2020 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.24002

Coronaviruses and Cytometry

The community of cytometrists and scientists has already been heavily touched by this epidemic, not just because of the threat to our health. Indeed, the meeting "CYTO Asia," which should have been held in Shanghai in early September 2020, has been postponed to 2021. Similarly, in different parts of the world, a relevant number of scientific and nonscientific meetings are now being canceled. Moreover, several prestigious research centers and universities are prohibiting their members and employees not only from traveling to "dangerous" countries but also from participating in any sort of domestic or international meeting, for 60 days at least. Several countries have adopted similar decisions at the government level.

Many viruses have existed in their natural reservoirs for a very long time without causing relevant problems to humans. However, in the last decades, there has been an increased spillover of viruses from natural hosts (like the bat) to intermediate hosts (like market civets and dromedary camels) and then to humans, likely because of human activities, including modern agricultural practices and urbanization. Coronaviruses are broadly distributed in humans and in other mammals, and there are at least seven viruses that have been able to pass from animals to humans, crossing the species barriers and infecting humans (7). Some of them are, however, relatively innocuous and cause the common cold, while those quoted here below are extremely dangerous for human health as they are highly pathogenic, able to be readily transmitted from human to human, and able to spread to multiple continents in a very short time. Thus, to cope with this epidemic, and to substantially contribute to the fight against SARS-CoV-2, we have to learn the lesson derived from two recent outbreaks caused by two other highly pathogenic coronaviruses.

The first was due to the SARS-CoV, which started creating victims in Foshan, China, in November 2002, before being recognized a few months later in Hanoi, Vietnam, by the Italian doctor Carlo Urbani (who died of this disease) (8). SARS affected 17 countries and, during an epidemic of a few months, caused 774 deaths of 8,096 laboratory-confirmed cases (9). The second was due to the Middle East respiratory syndrome (MERS)-CoV that, in 2012, was first described in a man in Saudi Arabia (10). MERS has been found in 27 countries and is still present in many of them because of the continuing introduction from dromedary camels. Until last November 2019, MERS has caused 858 deaths of 2,494 laboratory-confirmed cases (11). The present epidemic due to SARS-CoV-2 has a much higher number of cases. As of the middle of March, there are over 100,000 confirmed cases all over the world, with over 3,500 deaths (for a continuous update, see https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6 (12)).

Several studies have described the biological and immunological features of the previous coronavirus epidemics, and obviously, most researchers have used flow cytometry to describe the changes in T and B lymphocytes, as well as the role of inflammatory cells in the immunopathogenesis of the disease (reviewed in Ref. (13)). Currently, very limited information is available on the host innate immune status of SARS-CoV-2-infected patients and regard the description of increased total neutrophils, reduced total lymphocytes, and increased serum levels of IL-6 and of C-reactive protein, which suggest a strong inflammatory response. This is indeed evidenced by reports detecting abnormally high plasma levels of innate cytokines such as IP-10, MCP-1, MIP-1A, and TNFα or of high levels of proinflammatory cytokines (including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα). Furthermore, mortality is higher in patients with elevated levels of IL-6 (14). The overall picture is similar to what happens during SARS and MERS and underlines the fact that leukocyte alterations and a cytokine storm that initiates a viral sepsis could be important in the pathogenesis of Covid-19. Unfortunately, to the best of our knowledge, as of middle of March 2020 no data have been published on the specific cellular immune response to SARS-CoV-2. The first data on changes in lymphocyte populations in patients severely affected by Covid-19 indicate a low T cells count, an increase in naïve helper T cells and a decrease in memory helper T cells (15). Thus, the immunopathogenesis of this disease is still largely unknown. However, several groups, including ours, are now investigating different molecular, cellular and immunological aspects. Confirming this previous report (15), Figure 1 shows our most recent data, related to the distribution of different subpopulations of peripheral blood CD4+ and CD8+ T cells from four aged patients in the symptomatic phase of the infection.

What we Need to Know, What we Have to Do

There are several crucial clinical questions that urgently await an answer and that require strong and highly dedicated work by a community such as ours that certainly remembers the terrific contribution that has been made and is still given to the fight against HIV/AIDS (17). Thus, to begin: why do some patients get infected but do not develop any disease, while others die from the infection? Which are the protective factors, and which are the biomarkers that clinicians could use to predict, and eventually modify, the course of the disease? Assuming that the scant, available data are reliable, why do children seem to develop a milder form of Covid-19, similar to what was described in the case of SARS? Can the immaturity of the immune system protect against immune-mediated damages that could occur during Covid-19? Which assays are useful to monitor the efficacy of an antiviral therapy? How can we help in developing a vaccine?

Needless to say, using adequate approaches, models, and methodologies (18), the starting point will likely be a deep characterization of the immune system in patients with different stage of the disease in order to understand, among others:

1. The role of different components of innate immunity, such as monocytes, macrophages, dendritic cells, NK cells, and different innate lymphocytes, and their ability in controlling the early phases of the infection.

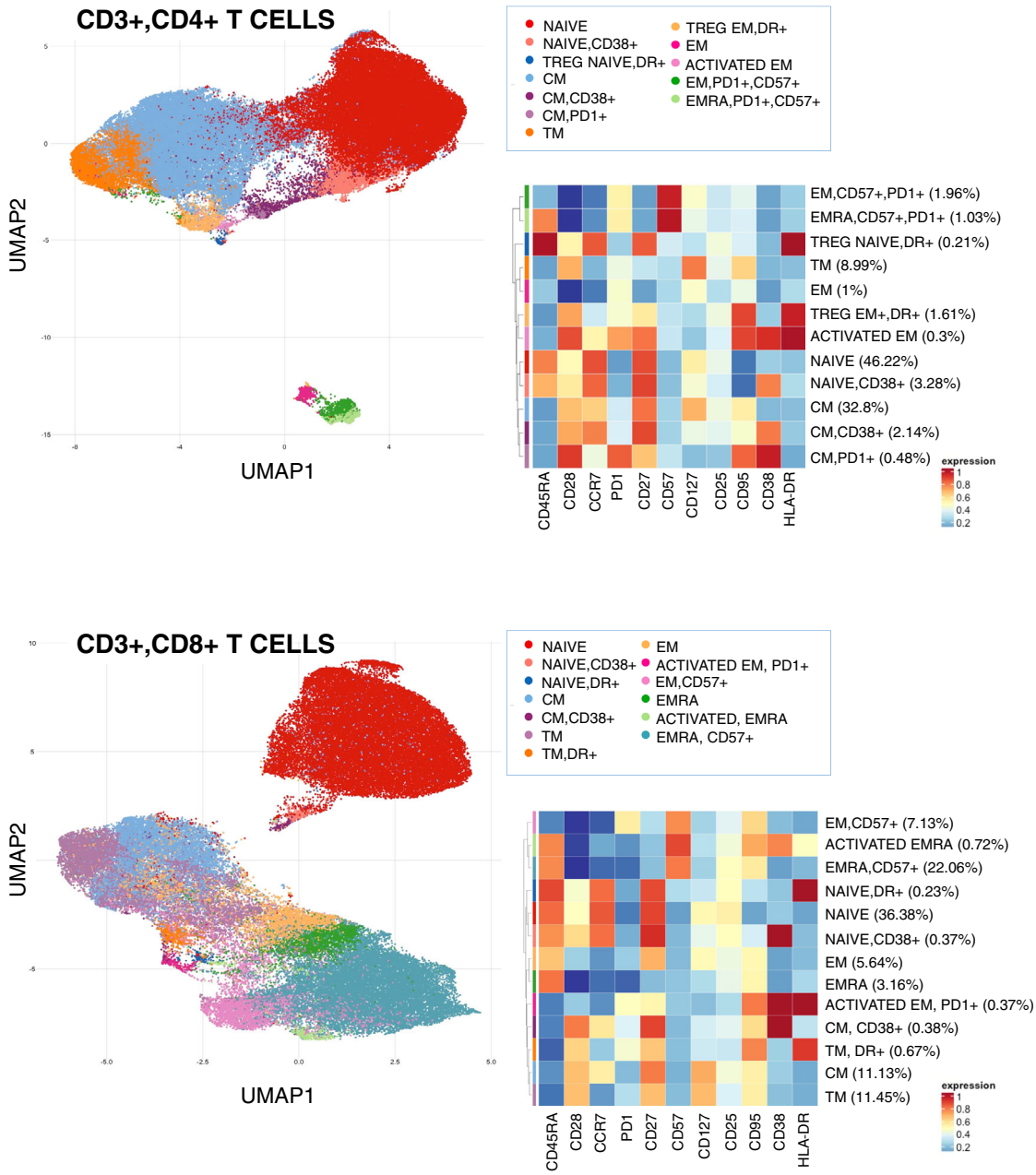

Figure 1. Differentiation, activation and exhaustion of CD4+ or CD8+ T-cell subsets in patients with Covid-19. Representation of an 18-parameter analysis of peripheral blood cells. Cells were stained with the Duraclone IM T cell panel (from Beckman Coulter, FL, USA) added with another five fluorescent mAbs and a marker of cell viability and analyzed on a CytoFLEX LX flow cytometer (Beckman Coulter). Beside side and forward scatters, markers were CD45 conjugated with Krome Orange, CD3 APC-A750, CD4 APC, CD8 AF700, CD27 PC7, CD57 Pacific Blu, CD279 (PD-1) PC5.5, CD28 ECD, CCR7 PE, CD45RA FITC, HLA-DR BUV661, CD127 BV650, CD25 BV785, CD95 BUV395, CD38 BUV496, and PromoFluor-840 (Promokine). Unsupervised analysis of electronically gated CD45+, CD3+, CD4+ or CD45+, CD3+, CD8+ T-cells was first performed by using the Catalyst package (Bioconductor) (16). Second, for both CD4+ and CD8+ T-cell analysis, 10,000 cells per sample were concatenated and transformed. FlowSOM was used to perform the metaclustering (K = 20); then, data were represented by the dimensionality reduction method named Uniform Manifold Approximation and Projection (UMAP). It is possible to observe the presence of 12 clusters among CD4+ and 13 among CD8+ T-cells and to see the high amount of naive cells in both T cell populations, and how the distribution of naive, memory, activated and exhausted lymphocytes is different among different types of CD4+ or CD8+ T lymphocytes.

2. The production and utilization of cytokines, chemokines, and their receptors, which are crucial in the initial cytokine storm.

3. The kinetics of the humoral response and production of antibodies against the virus including those with neutralizing activity.

4. The importance of different types of B and plasma cells, considering the problems related to short- and long-term memory.

5. The role of T-cell immunity, with the identification of eventual gross changes in CD4+ and CD8+ T cell populations that could have a prognostic meaning.

6. The specific T-cell response to different viral epitopes, which could allow a better characterization of the most antigenic parts of the viral proteins.

7. The importance of regulatory T-cells in modulating the response and controlling, or favoring, immunoactivation and suppression

Clearly, this is only a very partial list of the possible contributions that we can offer to the fight against SARS-CoV-2, but it is full of gaps that need to be filled as soon as possible. Our community has all the technical skills, scientific capabilities, and strength to help in significantly improving the knowledge of the main molecular and cellular aspects of this new infection and to help in developing a cure, based on drugs or, even better, innovative vaccines, with no fear of the SARS-CoV-2 threat.

NOTE: Modena Covid-19 Working Group (MoCo19) includes:

Cristina Mussini, Giovanni Guaraldi, Erica Bacca, Andrea Bedini, Vanni Borghi, Giulia Burastero, Federica Carli, Giacomo Ciusa, Luca Corradi, Gianluca Cuomo, Margherita Digaetano, Giovanni Dolci, Matteo Faltoni, Riccardo Fantini, Giacomo Franceschi, Erica Franceschini, Vittorio Iadisernia, Damiano Larné, Marianna Menozzi, Marianna Meschiari, Jovana Milic, Gabriella Orlando, Francesco Pellegrino, Alessandro Raimondi, Carlotta Rogati, Antonella Santoro, Roberto Tonelli, Marco Tutone, Sara Volpi, and Dina Yaacoub (*Infectious Diseases Clinics, University Hospital, via del Pozzo 71, 41124 Modena, Italy*);

Massimo Girardis, Alberto Andreotti, Emanuela Biagioni, Filippo Bondi, Stefano Busani, Giovanni Chierego, Marzia Scotti, and Lucia Serio (*Department of Anesthesia and Intensive Care, University Hospital, via del Pozzo 71, 41124 Modena, Italy*);

Andrea Cossarizza, Caterina Bellinazzi, Rebecca Borella, Sara De Biasi, Anna De Gaetano, Lucia Fidanza, Lara Gibellini, Anna Iannone, Domenico Lo Tartaro, Marco Mattioli, Milena Nasi, Annamaria Paolini, and Marcello Pinti (*Chair of Pathology and Immunology, University of Modena and Reggio Emilia, Via Campi, 287, 41125 Modena, Italy*).

ACKNOWLEDGEMENTS

We kindly acknowledge Dr. Leonardo Beretta, (Beckman Coulter, Milan, Italy) for his continuous support.

REFERENCES

1. WHO, Novel coronavirus - China Jan 12, 2020. https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/
2. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet 2020;395(10224):565–574.
3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China Lancet 2020;395(10223):497–506.
4. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020. https://doi.org/10.1056/NEJMoa2001316.
5. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med 2020;382:929–936. https://doi.org/10.1056/NEJMoa2001191.
6. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke K, Guggemos W, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 2020;382:10–11. https://doi.org/10.1056/NEJMc2001468.
7. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(3):181–192.
8. Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G, Lim W, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003;362(9380):263–270.
9. https://www.who.int/csr/sars/en/
10. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367(19):1814–1820.
11. https://www.who.int/emergencies/mers-cov/en/
12. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet 2020;19. https://doi.org/10.1016/S1473-3099(20)30120-1.
13. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol 2020. https://doi.org/10.12932/AP-200220-0772 Epub ahead of print.
14. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020. https://doi.org/10.1007/s00134-020-05991-x.
15. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis, published ahead of print March 12, 2020. https://doi.org/10.1093/cid/ciaa248.
16. Crowell H, Zanotelli V, Chevrier S, Robinson M (2019). CATALYST: Cytometry dATa anALYSis Tools. R package version 1.10.1, https://github.com/HelenaLC/CATALYST.
17. Cossarizza A, De Biasi S, Gibellini L, Bianchini E, Bartolomeo R, Nasi M, et al. Cytometry, immunology, and HIV infection: Three decades of strong interactions. Cytometry 2013;83A(8):680–691. https://doi.org/10.1002/cyto.a.22318.
18. Cossarizza A, Chang HD, Radbruch A, Acs A, Adam D, Adam-Klages S, Agace WW, Aghaeepour N, Akdis M, Allez M, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol 2019;49(10):1457–1973. https://doi.org/10.1002/eji.201970107.