Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Diagnostic Microbiology and Infectious Disease 98 (2020) 115148

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

COVID-19: Is there a role for Western blots and skin testing for determining immunity and development of a vaccine?

Charles S. Pavia [a,b,*], Gary P. Wormser [b]

Department of Biomedical Sciences, NYIT College of Osteopathic Medicine, New York Institute of Technology, Old Westbury, NY
Division of Infectious Diseases, New York Medical College, Valhalla, NY, USA

ARTICLE INFO

Article history:
Received 25 June 2020
Received in revised form 15 July 2020
Accepted 18 July 2020
Available online 13 August 2020

Keywords:
COVID-19
SARS-CoV-2
Serology
Western blots
Delayed-type hypersensitivity
Vaccine development

ABSTRACT

Infection with the virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) stimulates an immune response which can serve as a marker for current or past exposure to this pathogen, and possibly for resistance to re-infection. This response to COVID-19 can be monitored based on the production of antibodies, and thus, serologic tests have become available for diagnostic purposes. Despite progress in this area, concerns have been raised that too many of the commercially available serologic detection systems are not completely reliable. To address this issue, Western blots should be considered for confirming a positive or borderline-positive result from a screening test, such as an ELISA. An additional benefit of Western blots would be to identify antigens that could form the basis for developing a vaccine. Little is known about the cell-mediated immune response against COVID-19. One way to address this would be to use skin testing to measure the delayed-type hypersensitivity response in patients recovering from COVID-19.

© 2020 Elsevier Inc. All rights reserved.

1. Introduction

With regard to the coronavirus disease-19 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), this article is meant to be a basic but concise commentary on some of the subtle, but key, nuances associated with the use of immunologic based detection systems as additional diagnostic markers, along with shedding some light on the development of protective immunity to, and the design of vaccines against, this virus. Specifically, it is being proposed that the Western blot (immunoblot) technique be used to determine which protein antigens are recognized by sera from patients recovering from COVID-19 in order to better identify those components of the virus that should be part of a monovalent vaccine or a vaccine cocktail, especially if a live, attenuated virus is not used.

Additionally, measuring the delayed-type hypersensitivity (DTH) reaction following a skin test using SARS-CoV-2 antigens as the challenge inoculum might be a way to determine whether a long-lasting immune response has developed following a previous infection, and thus be an indicator of protective immunity.

2. Discussion

2.1. Use of the Western blot

Similar to most infectious diseases, infection with the coronavirus, SARS-CoV-2, elicits a relatively vigorous immune response, primarily in the form of antibody production (Guo et al., 2020; Infantino et al., 2020; Zhao et al., 2020). This has been demonstrated in clinical settings, when rapid, point-of-care tests are used on whole blood, or when the more precise and standard enzyme-linked immunosorbent assay (ELISA) is performed in multi-well plates using serum or plasma. Similar to what occurs with certain other natural infections and vaccines, it is hoped that these antibodies will provide long-lasting immunity to reinfection and have therapeutic value in the absence of effective drugs (Weiss and Wormser, 2020). However, shortly after widespread serologic testing began in the United States, reports started to circulate, primarily within the news media, that many of these serologic tests were not as accurate as the manufacturers claimed they were, which resulted in an unacceptable number of false-positive and false-negative tests. This was due, in part, because these tests did not undergo the usual rigorous evaluation that is typically required by the U.S. Food and Drug Administration (FDA), before they could become commercially available. This occurred as a by-product of the perceived urgency of the current pandemic which unfortunately precluded the strict oversight that is ordinarily taken by the FDA on the development of such tests.

* Corresponding author. Tel.: +1-516-686-3778.
E-mail address: cpavia@nyit.edu (C.S. Pavia).

https://doi.org/10.1016/j.diagmicrobio.2020.115148
0732-8893/© 2020 Elsevier Inc. All rights reserved.

As an approach, consideration should be given to follow an initial positive serologic test result with a supplemental or confirmatory test having optimal specificity such as a Western blot. There is ample precedent for taking this course of action which was deemed necessary, more than 25 years ago, to ensure the accuracy of an initial screening test for HIV infection, and which was subsequently recommended by the US Centers for Disease Control and Prevention for diagnosing the spirochetal infection, Lyme disease (CDC, 1995) (as shown in Fig. 1). Accordingly, the proposed algorithm and some of the key details for confirmatory testing for measuring serologic reactivity due to COVID-19 would rely on using the Western blot technique. A reactive ELISA, or an immunofluorescent antibody test, would be followed with a Western blot, which detects specific antibodies directed against various proteins. For Western blot testing, standard and well characterized techniques are used in which purified viral proteins are separated by electrophoresis on gels, transferred onto nitrocellulose membranes, and then reacted with the patient's serum that is usually diluted to the desired concentration. The reaction is completed by treating the sample with immunoglobulin (Ig) probes that might include IgM, IgG and/or IgA. Antibodies from the patient will bind to the specific viral proteins, which results in the visualization of discrete bands based on their molecular weight and migration patterns along the gels.

With regard to COVID-19, the median time to first appearance of antibodies occurred on day 12 for IgM, and day 14 for IgG (Infantino et al., 2020), although another study has shown that the majority of patients seroconverted between 7 and 11 days postexposure to the virus (Patel et al., 2020). A recent report (Haveri et al., 2020) showed that, when using the Western blot, there was a prominent response to the spike protein with convalescent patient sera, which further strengthens the potential role of the spike protein as a target for both serologic testing and vaccine development. In addition, reactivity to the envelope protein and to the nucleocapsid of SARS-CoV-2 by immune sera was detected on immunoblot, making these proteins also potential vaccine candidates (Haveri et al., 2020). On the other hand, it is also important to point out that, while the goal is to produce a safe and effective vaccine, clinical vaccine trials need to closely monitor test subjects for a paradoxical increase in susceptibility to COVID-19 in a manner similar to what occurred during the process of developing a vaccine for dengue fever (Katzelnick et al., 2017). This undesirable situation is based on the hypothesis that a previous immune response to one virus or pathogen can paradoxically render a person more susceptible to a subsequent infection with a slightly different, but antigenically or species-related virus (Tetro, 2020). This phenomenon is known as antibody-dependent enhancement whereby antibodies, rather than neutralizing the virus, actually facilitate viral uptake into the target cell (Modhiran et al., 2010). Lastly, an additional benefit of the Western blot would be to be able to identify unequivocally prospective donors of well characterized plasma to be used for therapeutic purposes in order to treat people most vulnerable towards developing a life-threatening infection. This is based on its almost unparalleled high level of sensitivity in identifying the key antigens that are evoking an immune response that is potentially protective in patients recovering from COVID-19.

In light of the preceding, how do we anticipate antibodies to be effective against COVID-19? The answer lies on what we already know about the pattern of inhibition that typically occurs and has been well recognized for many microbes, including most viruses. There are two main mechanisms. One mechanism involves neutralization of the infectivity of the virus by antibody binding to the proteins on the outer surface of the virus. This binding has two effects: (i) it can prevent the interaction of the virus with cell receptors, and (ii) it can cross-link the viral proteins so that uncoating does not occur. The virus, therefore, cannot replicate. Furthermore, antibody-coated virus may be more rapidly phagocytized by macrophages than non-coated virus, a process similar to the opsonizing effect of antibody on bacteria. A close in vitro analogy of these anti-viral processes is the plaque reduction neutralization test which can be used to quantify the titer of neutralizing antibody directed against the virus (Schmidt et al., 1976). Unfortunately, it is a somewhat

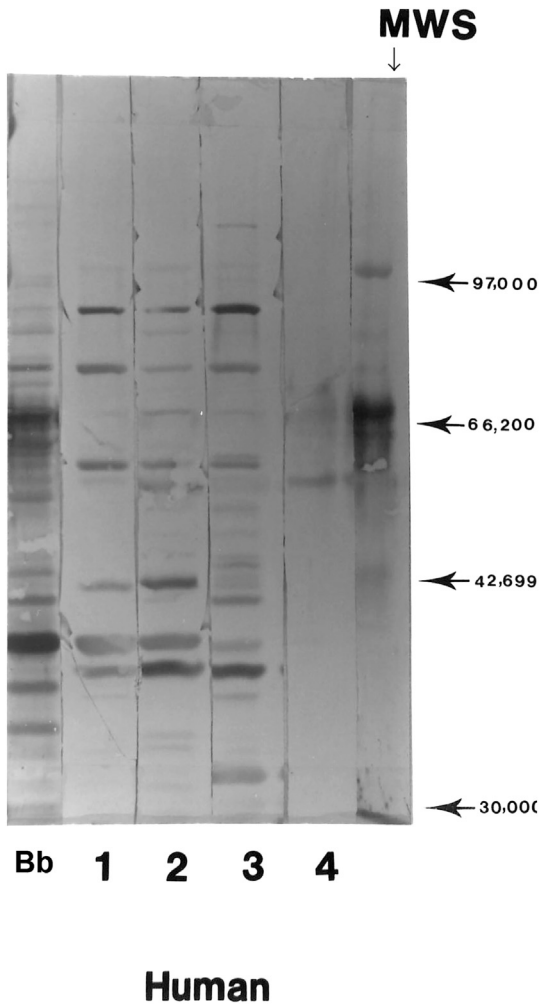

Fig. 1. Western blots of sera derived from 2 patients that were diagnosed with Lyme carditis (Lanes 1 and 2) or with Lyme arthritis (Lane 3). The reactivity patterns shown here are consistent with the CDC guidelines (CDC, 1995) for serological confirmation of Lyme disease. Lane 4: Western blot of serum from a normal healthy control. Lane Bb shows the banding pattern of electrophoresed proteins of *Borrelia burgdorferi* alone. Molecular weight standards (MWS), which serve as marker proteins, are shown to the far right.

cumbersome test, takes several days before results can be obtained and, given the pathogenicity of SARS-CoV-2, extremely high level containment facilities would be needed to perform this procedure. The second mechanism involves the lysis of virally-infected cells that are forming in the infected host, in the presence of antibody and complement. Antibody binds to newly produced virus-specific antigens on the cell surface and then binds complement, which enzymatically degrades the cell membrane. Because the host cell is killed before the full yield of virus is produced, the spread of virus is significantly reduced.

However, another less favorable possibility needs to be considered whereby SARS-CoV-2 could evade either of the two preceding mechanisms. Some viruses, such as herpesviruses, can spread from cell to cell across intercellular bridges eluding the neutralizing effect of antibody (Lodmell et al., 1973).

2.2. DTH reactions

DTH responses are a component of the type IV hypersensitivity reaction category of cell-mediated immunity. Unlike types I–III, which involve various forms of antibody-mediated activities, only effector T

cells and activated macrophages participate in DTH responses. These responses are often associated with the host response to intracellular pathogens. Their detection is best exemplified by the well-known tuberculin test (also known as the Mantoux or purified protein derivative [PPD] skin test). When a patient previously exposed to *Mycobacterium tuberculosis* is injected with a small amount of PPD (tuberculin) intradermally, there is little reaction in the first few hours. Gradually, however, induration and erythema develop which reaches a peak at 48–72 hours. A positive skin test indicates that the person has been infected with the agent, but it does not necessarily confirm the presence of current disease. However, if the skin test result converts from negative to positive, it suggests that the patient has been recently infected with *M. tuberculosis* (or an antigenically related mycobacterium).

It is noteworthy that delayed hypersensitivity reactions to antigens of microorganisms occur in many infectious diseases other than tuberculosis, and it has been used as an aid in diagnosis, especially for tuberculoid leprosy and for certain fungal infections, such as coccidioidomycosis and histoplasmosis. Cell-mediated immunity/hypersensitivity also develops in many viral infections including mumps, herpes and, to some extent, measles. If this occurs with SARS-CoV-2, testing for DTH responses could be used to detect people who were exposed to COVID-19, especially for those who no longer had detectable antibody levels. Also, in this context, if, instead of using purified/recombinant-derived antigens, live attenuated SARS-CoV-2 virus particles were to be used as an accepted vaccine, testing for DTH reactions could be a valuable tool for measuring the durability of immunity (either temporary or long-lasting) in vaccinees. This is based, in part, on the concept of "replicating antigen" (R. J. North, personal communication)) which leads to persistent stimulation of the immune system (primarily T cells), thus closely mimicking a natural infection (Parham, 2009), until the live vaccine is neutralized. This type of immunization strategy also leads to the emergence of clones of long-lasting and potent memory T cells, which would tend to elicit a strong protective immunologic response against a subsequent infection. Memory T cells (and to some extent B cells for antibody production), as the term implies, endow our host defenses with the ability to respond rapidly and vigorously for many years after the initial exposure to a microbe or after immunoprophylaxis with the appropriate vaccine. In this regard, for cellular immune/DTH responses, a memory response to COVID-19 would evoke several beneficial outcomes as follows: (i) more memory T cells relative to naïve T cells become activated making production of cytokines faster and be released in greater quantities, leading to enhanced interactions between effector T cells and macrophages; (ii) memory cells live for many years or have the capacity to reproduce themselves when re-stimulated; and (iii) memory cells are activated by smaller amounts of antigen and require less costimulation than do naïve, inactivated T cells. In this regard, data from a recent clinical study (Weiskopf et al., 2020) has provided evidence for the development of cellular immunity. In this study, strongly reactive CD4+ and CD8+ T cells were detected in a small group of COVID-9 patients, and these T cells produced a wide variety of cytokines which, under optimal conditions, should be part of a protective response and not be part of the detrimental "cytokine storm" (Tetra, 2020).

3. Conclusion

Combining serologic testing, that includes Western blots, along with skin testing using SARS-CoV-2 antigens for measuring DTH reactions, could be useful for diagnostic purposes and for assessment of immunity. Furthermore, the Western blot technique would also be helpful for identifying immunogenic antigens that might be used for vaccine development when analyzing the humoral immune response in patients recovering from COVID-19.

Funding

None.

Disclosures

Dr. Pavia – no disclosures. Dr. Wormser reports receiving research grants from Immunetics, Inc., Institute for Systems Biology, Rarecyte, Inc., and Quidel Corporation. He owns equity in Abbott/AbbVie; has been an expert witness in malpractice cases involving Lyme disease; and is an unpaid board member of the American Lyme Disease Foundation.

References

Centers for Disease Control and Prevention (CDC). Recommendation for test performance and interpretation from the second National Conference on serologic diagnosis of Lyme disease. Morb Mortal Wkly Rep 1995;46:S20–1.

Guo L, L, Ren L, Yanf S, et al. 2020. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19) [published online ahead of print, 2020 Mar 21] Clin Infect Dis 2020; ciaa310

Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveill 2020;25(11):2000266. https://doi.org/10.2807/1560-7917.ES.2020.25.11.2000266. 32209163.

Infantino M, Damiani A, Gobbi FL, Grossi V, Lari B, Macchia D, et al. Serological assays for SARS-CoV-2 infectious disease: benefits, limitations and perspectives. Isr Med Assoc J 2020;22:203–10. 32286019.

Katzelnick LC, Gresh L, Halloran ME, et al. Antibody-dependent enhancement of severe dengue disease in humans. Science 2017;358:929–93.

Lodmell DL, Niwa A, Hayashi K, Notkins AL. Prevention of cell-to-cell spread of herpes simplex virus by leukocytes. J Exp Med 1973;137:706–20. https://doi.org/10.1084/jem.137.3.706. 4347596.

Modhiran N, Kalayanarooj S, Ubol S. Subversion of innate defenses by the interplay between DENV and pre-existing enhancing antibodies: TLRs signaling collapse. PLoS Negl Trop Dis 2010;4, e929.

Parham P. The immune system, 3rd edition, p 438. Garland science. Taylor & Francis Group: Abington, UK; 2009.

Patel R, Babady E, Theel ES, Storch GA, Pinsky BA, St. George K, et al. Report from the American Society for Microbiology COVID-19 international summit, 23 March 2020: value of diagnostic testing for SARS-CoV-2/COVID-19. mBio 2020;11:e00722. 20 https://doi.org/10.1128/mBio.00722-20.

Schmidt NJ, Dennis j J, Lennette EH. Plaque reduction neutralization test for human cytomegalovirus based upon enhanced uptake of neutral red by virus-infected cells. J Clin Microbiol 1976;4:61–6.

Tetro JA. Is COVID-19 receiving ADE from other coronaviruses? Microb Infect 2020;22:72–3.

Weiskopf D, Schmitz SS, Raadsen MP, Grifoni A, Okba NMA, Endeman H, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol 2020;48:eabd2071. https://doi.org/10.1126/sciimmunol.abd2071.

Weiss SH, Wormser GP. COVID-19: understanding the science of antibody testing and lessons from the HIV epidemic. Diagn Microbiol Infect Dis 2020. https://doi.org/10.1016/j.diagmicrobio.2020.115078.

Zhao J, Yuan JQ, Wang H, et al. Antibody responses to SARS-CoV-2 specific antibody in patients of novel coronavirus disease 2019. Preprint in medRxiv on March 2020;03:2020.