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.
A novel application of delayed-type hypersensitivity reaction to measure cellular immune response in SARS-CoV-2 exposed individuals

Yvelise Barrios a, Andres Franco a, Inmaculada Sanchez-Machin b, Paloma Poza-Guedes b, Ruperto Gonzalez-Perez b, Victor Matheu b,*

a Immunology Lab, Central Lab, Floor - 1, Main Building, Hospital Universitario de Canarias, Ctra Ofra s/n La Cuesta, 38320 La Laguna, Tenerife, Spain
b Allergy Service, Floor-2, Outpatient Building, Hospital Universitario de Canarias, Ctra Ofra s/n La Cuesta, 38320 La Laguna, Tenerife, Spain

ARTICLE INFO

Keywords:
SARS-cov2
DTH
Skin test
Delayed-type hypersensitivity
T-cell response
COVID-19
Cell response
Humoral response

ABSTRACT

Objective: To understand the anti-virus adaptive immune response occurring during SARS-CoV-2 infection is necessary to have methods to investigate cellular and humoral components. The goal of this study has been to investigate the utility of a specific spike-DTH test using a coronavirus recombinant protein in COVID-19 patients.

Methods: DTH studies were performed by intradermal injection of a commercial recombinant spike protein from SARS-CoV-2 along with conventional serology studies.

Results: Fifty-one COVID-19 patients were studied showing 84.3% of concordance with spike-DTH and anti-RBD-IgG. Spike-DTH was superior to identify seven more COVID-19 individuals. A high specificity was found with no positive spike DTH reactions in the non-sick individuals. The skin test also showed more stable results over time while specific anti-RBD-IgG decreased gradually. Clinical severity groups also showed a progressive gradient of larger positive spike-DTH.

Conclusion: Specific spike DTH test seems to be an easy method to study cell immune response.

1. Introduction

The measurement of the immune response against SARS-CoV-2 has been a hot topic since the emergence of the pandemic situation. During this year, a lot of research has been directed to dissect the humoral response [1] and big efforts have been done to develop antibody test detection methods that could correlate well with the status of the immune response in the infected individuals. From these studies, it is currently accepted that there are in the market many reliable standard serological ELISAs, some of which even correlate with virus neutralization titers [2]. But an understanding of the critical in vivo T-cell responses to the SARS-CoV2 virus is lacking, mainly due to the difficult task of development of cellular assays to investigate the T-cell compartment. Several reports with limited number of participants have proposed different relationships between these two sides of the adaptive immune response [3,4]. Both ELISAs antibody methods and in vitro cellular assays require the extraction of a blood sample from the patient, what complicates possible massive analysis in large populations.

Particularly, technologies to study T-cell responses in vitro are too complex, tedious and time consuming to be applied to thousands of samples. For these reasons, an alternative method to evaluate the magnitude of anti-SARS-CoV2 cellular responses in vivo that could be easily accomplished in such high-throughput investigations is urgently needed.

Cutaneous antigen-recall models allow the study of human memory responses in vivo [5]. In this report we describe a feasible method, the classical delayed-type hypersensitivity (DTH) response to the intradermal injection of a recombinant protein representative of the SARS-CoV-2 virus to assess the T-cell mediated memory recall immune response. In our hands, the DTH reaction to the spike protein of SARS-CoV2 seems to be a good and simple tool to measure specific cellular response with a strong correlation with specific serological tests. DTH also seems to be highly specific and more stable over time after infection.

Abbreviations: COVID-19, Coronavirus disease 2019; DTH, Delayed-type hypersensitivity; ELISA, Enzyme-Linked Immunosorbent Assay; RBD, Receptor Binding domain; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

* Corresponding author.

E-mail addresses: vmatdel@gobiernodecanarias.org, vmatheu@ull.edu.es (V. Matheu).

https://doi.org/10.1016/j.clim.2021.108730
Received 5 March 2021; Received in revised form 13 April 2021; Accepted 14 April 2021
Available online 16 April 2021
1521-6616/© 2021 Elsevier Inc. All rights reserved.
2. Methods

2.1. Individuals

A total number of 65 individuals (43 female/22 male) with mean age was 47.9/46.7y-o were analyzed. Fifty-one individuals (36 female/15 male, mean age was 47.4y-o) were COVID-19-positive cases defined either by clinical or SARS-CoV-2 PCR-positive. Considering clinical phenotypes, 34 individuals were classified as asymptomatic/mild disease (group I), 13 moderate (group II) and 4 were severe/hospitalized (group III) with Pneumonia (E1). Fourteen individuals (7 female/7 male; mean age:49.2) were used as COVID-19-negative (non-infected) controls [6]. PCR was not performed in the controls (no clinical symptoms and negative serology).

2.2. Study design

The patients were seen in the medical consultation during the months of November and December 2020. Demographic details and the time where the infection was diagnosed by clinical symptoms or by RT-PCR were collected from all participants. Three different clinical groups were assigned depending on the symptoms of the exposed individuals: asymptomatic/mild disease or group I, moderate or group II and serious/hospitalized or group III of patients.

Each subject that intends to enter the study was given a written document called “Patient Information Sheet,” which contains relevant and necessary information for the patient to decide on their participation in the study. Treatment, communication, and transfer of personal data of all participating subjects comply with the provisions of Law 03/2018 of 5 de December, RG:2016/679 on protection of personal data. The protocol was approved by the ethical committee of the Hospital (CHUC 2020_92). The study is conducted in accordance with the requirements expressed in Law 737/2015 about biomedical research and the Declaration of Helsinki (revised Brasil, October 2013).

2.3. Serology studies

All serum samples from patients were sent to Immunology laboratory for SARS-CoV-2-IgG and IgA determination between November and December 2020 from outpatients and were frozen. Then, serum samples and necessary information for the patient to decide on their participation in the study. Each subject that intends to enter the study was given a written document called “Patient Information Sheet,” which contains relevant and necessary information for the patient to decide on their participation in the study. Treatment, communication, and transfer of personal data of all participating subjects comply with the provisions of Law 03/2018 of 5 de December, RG:2016/679 on protection of personal data. The protocol was approved by the ethical committee of the Hospital (CHUC 2020_92). The study is conducted in accordance with the requirements expressed in Law 737/2015 about biomedical research and the Declaration of Helsinki (revised Brasil, October 2013).

All 14 non-exposed controls were negative for SARS-CoV-2-IgG and IgA. The mean O.D.ratio of controls was 0.3. The distribution of the O.D.ratio of specific-antiRBD-IgG showed an increasing tendency along the three different clinical phenotypes (O.D. ratio 2.1 in group I, 3.1 group II and 3.2 group III) (Fig. 1A, red line). All 14 non-exposed controls were negative for specific-antiRBD-IgG (mean O.D.ratio 0.2).

Continuous variables are expressed with means and standard deviations, and categorical variables with frequencies and percentages. Differences between the distributions of continuous variables were evaluated using the Mann-Whitney U test. Proportions between groups were compared with chi-square or Fisher exact tests, as appropriated. Association between variables were assessed with Pearson and Spearman correlation tests, as appropriated. All P value lower than 0.05 was considered statistically significant. Statistical analysis was carried out with SPSS v.25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows. Armonk, NY).

3. Results

A total number of 65 individuals, 51 COVID-19-positive cases and 14 non-exposed controls, were analyzed (Fig. 1). COVID-19-positive cases were defined either by SARS-CoV-2 positive RT-PCR (48 patients) or clinically/serology suggested if PCR was not feasible at the time of the diagnosis (3 patients).

Among the COVID-19-positive individuals, 37 were considered as positive specific-antiRBD-IgG (31 with values >1.2 O.D.ratio (60.8%) and six (11.8%) with values between 0.8 and 1.1 O.D.ratio and 14 were negative (27.4%). The distribution of the O.D.ratio of specific-antiRBD-IgG showed an increasing tendency along the three different clinical phenotypes (O.D. ratio 2.1 in group I, 3.1 group II and 3.2 group III) (Fig. 1A, red line). All 14 non-exposed controls were negative for specific-antiRBD-IgG (mean O.D.ratio 0.2).

The patients were seen in the medical consultation during the months of November and December 2020. Demographic details and the time where the infection was diagnosed by clinical symptoms or by RT-PCR were collected from all participants. Three different clinical groups were assigned depending on the symptoms of the exposed individuals: asymptomatic/mild disease or group I, moderate or group II and serious/hospitalized or group III of patients.

Each subject that intends to enter the study was given a written document called “Patient Information Sheet,” which contains relevant and necessary information for the patient to decide on their participation in the study. Treatment, communication, and transfer of personal data of all participating subjects comply with the provisions of Law 03/2018 of 5 de December, RG:2016/679 on protection of personal data. The protocol was approved by the ethical committee of the Hospital (CHUC 2020_92). The study is conducted in accordance with the requirements expressed in Law 737/2015 about biomedical research and the Declaration of Helsinki (revised Brasil, October 2013).
group II 4.4 mm (5.8) at 12 h, 12.5 mm (8.2) at 24 h, 17.8 mm (10.5) at 48 h; in group III 3.0 mm (3.8) at 12 h, 9.2 mm (3.5) at 24 h, 13.0 mm (6.8) at 48 h (Fig. 1B). Twelve out of 14 control individuals were positive for the candida-DTH skin test. The kinetics of the positive ones were 2.6 mm (12 h), 7.0 mm (24 h) and 8.8 mm (48 h) after intradermal application.

Forty-three out of 51 positive-COVID-19 patients were positive for Spike-DTH skin test (Fig. 1B). The immediate reading from 15 min to the first 30 min was negative in all 51 cases. The kinetics of the positive cutaneous tests was: in group I, the mean was 2.6 mm (STD 3.1) at 12 h, 4.7 mm (5.3) at 24 h, 6.5 mm (6.2) at 24 h, 12.5 mm (12.9) at 48 h; in group II, 3.7 mm (4.7) at 12 h, 6.5 mm (6.2) at 24 h, 12.5 mm (12.9) at 48 h; in group III, 4.2 mm (5.6) at 12 h, 12.7 mm (11.7) at 24 h, 19 mm (18.2) at 48 h after the intradermal injection. The 14 control individuals were negative for the Spike-DTH skin test.

The concomitant analysis of the 51 exposed individuals for both serological (anti-RBD specific IgG) and Spike-DTH skin test showed a concordance in 43 patients (84.3%). Thirty-six of them were positive for both methods and 7 individuals were negative for both methods. One individual was positive for IgG and negative in skin test and seven were negative for IgG with positive Spike-DTH skin test (Fig. 2). All 14 individuals belonging to the non-exposed group were negative for both specific anti-RBD IgG and Spike-DTH skin test showing a concordance of 100%.

Because the individuals had been infected in different periods of time, one corresponding to the first wave in Europe (March–April 2020) and another group of individuals corresponding to the second wave in Europe (September–October 2020), serology and cutaneous test response was divided in two different groups: those who had been infected with SARS-Cov2, eight months before (group “+8” with 34 individuals) and those who had been infected more recently, two months before (group “+2” with 17 individuals).

Disaggregated analysis of both groups showed that in group “+8” there were 24 individuals with positive IgG (70.6%) and 28 individuals with positive skin test (82.4%) and in group “+2” thirteen individuals had positive IgG (76.5%) and 15 individuals (88.2%) were positive for the cutaneous test. Moreover, if we compare the positive specific IgG (n = 24 in group “+8” and n = 13 in group “+2”) vs positive-cutaneous test (n = 28 in group “+8” and n = 15 in group “+2”), the results showed that the cutaneous test positive individuals remains stable throughout the follow up whereas specific anti-IgG positive showed a decreased value (OD ratio) when compared between these two time points (Fig. 1D).
4. Discussion

DTH studies have been used in the past to assess immune function on HIV-infected patients [12]. The goal of this study has been to investigate the utility of a specific spike-DTH test in the COVID-19 infection and consequently the correlation with the anti-RBD serology. The RBD recombinant antigen has been decided based on published data that consider the receptor binding domain of the viral spike protein as the immunodominant target of antibodies in SARS-CoV-2 patients [13,14].

As reported by others [15], there is a clinical gradient of immune activation among the 51 exposed individuals. The results showed an increased value of specific anti-RBD IgG O.D.ratio on clinical groups I, II and III. The low number of individuals in clinical group III precludes any strong conclusion. This gradient is also present on the results of Spike-DTH skin test expressed as measurement (mm) of the diameter of the cutaneous reaction on three (12–24-48 h) different times after the intradermal application of the antigen (Fig. 1).

Comparisons between specific anti-RBD IgG and Spike-DTH cutaneous test to identify the exposed individuals showed a concordance number of 43 (84,3%) (36 double positive vs 7 double negative). Spike-DTH showed a superior capacity to identify exposed individuals because there are 7 that are positive for the cutaneous test but negative for anti-RBD specific IgG, whereas just only one individual positive for anti-RBD specific IgG was found negative for the Spike-DTH (candida skin test used as control of cellular immune competence was positive) (Fig. 2A). There are 7 exposed individuals that showed negative results for serology and skin test. Serum from these individuals were studied and 6 have at least one different antigen (anti-nucleocapsid protein) (data not shown) or isotype (IgA) positive antibody response (Fig. 1C). Specific anti-spike IgA determination in all participants was included, given the relevance of this isotype in the control of the virus at the respiratory level. To improve the cutaneous test, it can be implemented in the future the use of other antigen specificities that could cover these “false” negative results. Others have suggested a CD4+ T-cell response against epitopes located in the small M protein [16], that could be one of the reasons for the cutaneous negative individuals. An already ongoing large study (including more individuals and other recombinant proteins of the SARS-CoV-2 virus has started to fill these knowledge gaps.

Seroconversion to SARS-CoV-2 range from 91 to 99% in large studies [17,18], but little is known about the kinetics of virus-specific T-cell responses during SARS-CoV-2 infection and their behavior in different grades of disease severity. Reports about correlation between T-Cell and
anti-IgG showed contradictory results, some demonstrating a robust correlation [16,19], while others showed poor correlation [6].

To the best of our knowledge, this report is the first one showing a good correlation among cellular in vivo measurement/specific IgG response. It can be hypothesized that the use of an in vivo method, although less sophisticated it may reproduce a more real situation on these exposed individuals. Moreover, we have not found any positive DTH to SARS-CoV-2 S protein on the unexposed individuals, showing the high specificity of the method. However, PCR-confirmation of these exposed individuals was not done to rule out the remote possibility of some asymptomatic and negative serology individual. Another interesting finding is that while the serology tends to fall after few months (especially in the less severe group of patients), the cutaneous test remains stable (Fig. 2), reflecting a more homogenous in vivo T-cell response measurement. Possibly this reflects a shorter life span of the antibody producing plasma cells compared to the circulating anti-S T-cells.

In this report we have demonstrated that assessment of cellular immune activation through delayed hypersensitivity using a recombinant protein of the virus is an easy, affordable, and suitable method to study this new coronavirus infection. An extended use of this test in vaccinated population opens a new horizon for massive test of large populations that can be used as a screening method for assessment of cellular immunity to evaluate the efficacy of vaccination.

Funding

This research was self-funding and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure statement

Y-B and V.M. have filed provisional Utility Model applications related to DTH tests for cellular immunity against SARS-CoV-2. All authors declare that they have no competing interests.

Authorship

Y-B and V.M. participated in the conception of the idea and designed the study and drafted the manuscript. ISM, AF, PPG, RGPh participated in analysis and interpretation of data and revised the manuscript critically. All authors approved the final version to submit.

Ethical committee

All included subjects received full written and informed consent. The study was approved by the Ethical Committee with the code CHUC_2020_92.

Acknowledgements

We would like to thank to patients and Dr. Fernando Díaz-Espada, former Chief of Immunology Department in Hospital Clínica Puerta de Hierro for rich discussions to improve the project.

The authors want to thank the Immunology Laboratory Technicians (Tamara MJ Placer, Gloria Camacho, María Romero,Montserrat Padilla) and Allergy registered nurses (Sofía García, Antonio Pérez-Granados, Lila Pérez) for their help with the procedures and dedication, Alejandro Jiménez for statistical analysis and Miriam Hernández from Microbiology Service for helping with some negative patients.

Y-B and V.M. have filed (79241/P85474) Utility Model application related to DTH tests for cellular immunity against SARS-CoV-2.

References

[1] N. Baumgaertl, J. Nikolisch-Zugich, F.E. Lee, D. Bhattacharya, Antibody responses to SARS-CoV-2: Let’s stick to known knowns, J. Immunol. 205 (9) (2020 Nov 1) 2342–2350, https://doi.org/10.4049/jimmunol.2000839, Epub 2020 Sep 4. PMID: 32887754; PMCID: PMC7578055.
[2] A.P. Espejo, Y. Akgun, A.F. Al Mana, Y. Tjandra, N.C. Millan, C. Gomez-Fernandez, C. Hugel, Review of current advances in serologic testing for COVID-19, Ann J Clin Pathol. 154 (3) (2020 Aug 5) 293–304, https://doi.org/10.1093/ajcp/aqaa112. PMID: 32538352; PMCID: PMC7337672.
[3] K.E. Lineburg, S. Srirat, M. Alfar, S. Swaminathan, A. Panikkar, J. Raju, P. Crooks, et al., Rapid detection of SARS-CoV-2-specific memory T-cell immunity in recovered COVID-19 cases, Clin Transl Immunology 9 (12) (2020 Dec 7), e1219. https://doi.org/10.1002/cti.21219. PMID: 33331265; PMCID: PMC7720530.
[4] C.J. Reynolds, L. Swadling, J.M. Gibbons, C. Pade, M.P. Jensen, M.O. Diniz, et al., Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection, Sci Immunol. 5 (54) (2020 Dec 23), eabf6968, https://doi.org/10.1126/sciimmunol.abf6968. PMID: 33361161.
[5] A.R. Ahmed, D.A. Blox, Delayed-type hypersensitivity skin testing: a review, Arch. Dermatol. 119 (1983) 934–945.
[6] WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection, A minimal common outcome measure set for COVID-19 clinical research, Lancet Infect Dis. 20 (2020 Aug) e192–e197, https://doi.org/10.1016/S1473-3099(20)30483-7. Epub 2020 Jun 12. Erratum in: Lancet Infect Dis. 20 Oct;20(10):e250. PMID: 32539990; PMCID: PMC7292605.
[7] C. Vidal, D. Antolín, M. Reano, A. Valero, J. Sastre, Safety and quality recommendations in allergy medicine (Spanish acronym, RESCAL), A Investig Allergol Clin Immunol 28 (Suppl. 1) (2018) 1–39, https://doi.org/10.18176/jiac.0267.
[8] R. Badaro, B.A.S. Machado, M.S. Duthie, et al., The single recombinant M. tuberculosis protein PPD0 provides enhanced performance of skin testing among HIV-infected tuberculosis patients, AMB Expr 10 133 (2020), https://doi.org/10.1186/s13568-020-01606-4.
[9] H. Stavri, N. Bucurenci, I. Ulea, A. Costache, L. Popa, M.I. Popa, Use of recombinant purified protein derivative (PPD) antigens as specific skin test for tuberculosis, Indian J Med Res. 136 (5) (2012 Nov) 799–807. PMID: 23287127; PMCID: PMC3573601.
[10] K.T. Yeo, X. Zhu, H.L. Kirchner, A.D. LaBeaud, A. Mandalakas, Candida skin testing is a poor adjunct to tuberculin skin testing in international adoptees, Pediatr. Infect. Dis. J. 28 (11) (2009) 1020–1021, https://doi.org/10.1097/INF.0b013e3181e90d43.
[11] S.M. Reda, R.H. El-Owaidy, N.M. Lotfy, S.A. El-Toukhy, Reliability of candida skin testing, Clin. Infect. Dis. 26 (6) (1998 Jun) 1330–1334, https://doi.org/10.1086/315352. PMID: 9636588.
[12] K. Yuki, M. Fujisoya, S. Koutsoyannakis, COVID-19 pathophysiology: a review, Clin. Infect. Dis. J. 28 (11) (2020) 108427, https://doi.org/10.1097/INF.0b013e3181e90d43.
[13] L. Premkumar, B. Segovia-Chumbez, R. Jadi, D.R. Martinez, R. Raut, A. Markmann, et al., The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients, Sci Immunol. 5 (46) (2020 Jun 11), eabc8413, https://doi.org/10.1126/sciimmunol.abe8413. PMID: 32527802; PMCID: PMC7925055.
[14] B. Sun, Y. Feng, X. Mo, P. Zheng, Q. Wang, P. Li, et al., Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients, Emerg Microbes Infect 9 (1) (2020 Dec) 940–948, https://doi.org/10.1093/emi/mzaa151. PMID: 32357808; PMCID: PMC7273175.
[15] A. Grifoni, D. Weiskopf, S.I. Ramirez, J. Mateau, J.M. Dan, C.R. Moderbacher, et al., Targets of T cell responses to SARS-CoV-2 in humans with COVID-19 disease and unexposed individuals, Cell 181 (7) (2020 Jun 25) 1489–1501, e15, https://doi.org/10.1016/j.cell.2020.05.015. Epub 2020 May 20. PMID: 32473172; PMCID: PMC72737901.
[16] D.F. Guðbjartsson, A. Helgason, H. Jonsson, O.T. Magnusson, P. Melsted, G. L. Norddahl, et al., Spread of SARS-CoV-2 in the Icelandic population, N Engl J Med. 382 (24) (2020 Jun 11) 2302–2315, https://doi.org/10.1056/NEJMoa2006100. Epub 2020 Apr 14. PMID: 32289214; PMCID: PMC7175425.
[17] A. Wajnberg, F. Amat, A. Pipic, D.R. Altman, M.J. Bailey, M. Mainz, et al., Robust neutralizing antibodies to SARS-CoV-2 infection persist for months, Science 370 (6521) (2020 Dec 4) 1227–1230, https://doi.org/10.1126/science.abd7728. Epub 2020 Oct 28. PMID: 3315920; PMCID: PMC7810037.
[18] Y. Peng, A.J. Mentzer, G. Jin, Y. Yao, Z. Yin, D. Dong, et al., Broad and strong memory CD+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19, Nat. Immune. 21 (11) (2020 Nov) 1336–1345, https://doi.org/10.1038/s41590-020-0782-z. Epub 2020 Sep 4, 32887977.