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Original Contribution

Strong homology between SARS-CoV-2 envelope protein and a Mycobacterium sp. antigen allows rapid diagnosis of Mycobacterial infections and may provide specific anti-SARS-CoV-2 immunity via the BCG vaccine

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ABSTRACT

The vaccine BCG has been reported to offer protection against SARS-CoV-2 infection. It has been hypothesized this is based on nonspecific enhancement of innate immunity. This study addressed whether there is strong homology between a SARS-CoV-2 capsid protein and a Mycobacterium bovis protein that would allow for stronger, more specific immune protection. The study also showed the utility of immunohistochemistry in the diagnostic pathology laboratory for elucidating this information. Immunohistochemistry documented that an antibody directed against the SARS-CoV-2 envelope, but not the spike or membrane proteins, strongly cross hybridized to 11/11 Mycobacterial species tested, including M. bovis. BlastP analysis showed high homology of the SARS-CoV-2 envelope protein with 12 consecutive amino acids of the protein LytR C, which is a consensus protein unique to Mycobacteria. Six additional cases of human tuberculosis with few organisms showed that the viral envelope specific antibody (5/6) was more accurate than the AFB stain (2/6) for diagnostic purposes. These data indicate BCG vaccination induces a specific immunity against SARS CoV-2 that targets the viral envelope protein that is essential for infectivity. Thus, a concurrent booster or first use of the BCG vaccine may reduce the severity of the current COVID-19 pandemic. The data also suggests the value of using the SARS-CoV-2 envelope antibody in the diagnosis of Mycobacterial infections in formalin fixed, paraffin embedded tissues by the diagnostic pathologist.

1. Introduction

SARS-CoV-2 is the causative agent of coronavirus disease 2019 (COVID-19) the worst worldwide pandemic since the H1N1 Spanish flu of 1918 [1]. The pathology of severe COVID-19 is one of a pauci-inflammatory complement mediated thrombotic microangiopathy affecting the lung's alveolar septal capillaries and other organs including the skin, heart, and liver [2].

Many countries mandate Bacillus Calmette-Guerin (BCG) vaccination, a live attenuated strain of Mycobacterium bovis, to newborns due to its effectiveness against tuberculosis and leprosy [1,3,4]. It has been reported that countries with long-standing mandatory BCG vaccination have less cases of COVID-19 per capita and a lower death rate compared to countries such as Italy, Spain, and the United States where BCG vaccines are rarely given [3]. Hence, BCG vaccination is being touted as a treatment for reducing the complications of SARS-CoV-2 [1,3-5]. It has been assumed that the protective effect of BCG vaccination is due to an epigenetic enhanced “trained innate immunity” whereby macrophages and natural killer cells are primed from the exposure to Mycobacterium bovis to eliminate any micro-pathogens [6-9].

Another possibility is that there may be heterologous immunity due to a protein in SARS CoV-2 that is homologous to a protein in Mycobacterium bovis. Such heterologous immunity has been documented between other pathogens, such as adenovirus and hepatitis C virus [10]. The presence of such immunity between a SARS-CoV-2 capsid protein and a protein of Mycobacterium bovis would allow for a much stronger and specific immunity against the virus subsequent to BCG vaccination. Immunohistochemistry is a powerful method well suited to detect such potential heterologous immunity since it allows for specific cellular localization of the relevant antibody-antigen complex in the context of the known distribution of the latter, in this case defined by the AFB stain [11]. Signal intensity between the primary
antibody and heterologous antigen is related to the degree of homology and co-localization documents that the primary antibody and antigen are within an area of 150 nm [11,12].

The diagnosis of Mycobacterium species by the anatomic pathologist can be problematic because of the relatively few numbers of organisms in a given sample. There are two main tools available to the anatomic pathologist to diagnose Mycobacterial infection: the AFB stain and direct fluorescent microscopy using an antibody directed against Mycobacterial species. A study, using bacterial culture and PCR-based confirmation of Mycobacterial infection in 55 human samples, found that the AFB test gave false negative results in 64% of cases and direct fluorescent microscopy missed the diagnosis in 20% of cases [13]. Although Mycobacterial infection in patients with AIDS often have many organisms, it is still clear that better tests are needed by the diagnostic pathologist to diagnose Mycobacterial infections in either formalin fixed, paraffin embedded tissues or cytology specimens that can easily be fixed and processed for immunohistochemistry.

This study documented the strong cross reactivity between a SARS-CoV-2 protein and a consensus protein of Mycobacteria. The data offers a more sensitive test to diagnose Mycobacterial infections for the diagnostic pathologist and, clearly, indicates the BCG vaccine can offer immediate, specific immunity that could potentially much reduce the increasing death rate in the current COVID-19 pandemic.

2. Methods

2.1. Tissue samples

Formalin fixed, paraffin embedded tissues from cases confirmed to contain Mycobacterial infections were obtained from various sources. Eleven such cases were identified and were positive for: Mycobacterium bovis (n = 2), Mycobacterium leprae (n = 2), Mycobacterium avium-intracellulare (n = 3), and Mycobacterium tuberculosis (n = 4). Also studied were six cases of PCR-documented Mycobacterium infection chosen as they were reported to contain very few microorganisms. All samples were obtained prior to 2018, and, thus, could not have contained SARS-CoV-2.

2.2. Immunohistochemistry

Our immunohistochemistry method for the detection of SARS-CoV-2 proteins has been published [2]. The automated Leica Bond Max platform was used with DAB as the chromogen. The optimal conditions included antigen retrieval for 30 min with the EDTA solution from Leica, dilutions of 1:4000 (spike Ab), 1:500 (membrane Ab), 1:250 (envelope Ab, each from ProSci, Poway, CA) and the use of the horse-radish peroxidase conjugate from Enzo Life Sciences in place of the equivalent product from Leica as this reduced background [11,12]. In selected cases the chromogen Fast Red (with the alkaline phosphatase reporter enzyme) was used in place of DAB by using the Leica Fast Red kit. Positive and negative controls were lung tissues from people who had died of COVID-19 and normal lung tissue obtained prior to 2018.

3. Results

3.1. Immunohistochemistry with SARS-CoV-2 capsid antibodies and Mycobacterial positive samples

First, the question as to whether any of the SARS-CoV-2 capsid antibodies targeting the spike, envelope, and membrane proteins respectively would be able using immunohistochemistry to detect Mycobacteria in the formalin fixed, paraffin embedded tissues from the eleven cases that were each strongly AFB positive was addressed. The testing was done blinded with regards to the specific viral capsid protein on eleven pre-COVID-19 archival tissue samples of Mycobacterial infection (four of Mycobacterium tuberculosis, three Mycobacterium avium-intracellulare, and two each of Mycobacterium bovis plus Mycobacterium leprae). Acid-fast bacilli (AFB) stains indeed highlighted Mycobacterial organisms in each tissue. As seen in Fig. 1, antibodies targeting the SARS-CoV-2 spike or membrane proteins yielded no signals in the Mycobacterial samples. However, 11/11 (100%) of the
**Fig. 2.** BLASTP analysis indicating region of homology between SARS-CoV-2 envelope protein and *Mycobacterium* sp. A stretch of 12 amino acids (17–29 of YP_009724392 sequence) of SARS-CoV-2 envelope protein (dark red line) has high homology to LytR C-terminal domain-containing proteins of *Mycobacterium* taxa and variants as indicated. Also note the strong homology among the different species of *Mycobacteria*. An identical amino acid residue between the SARS-CoV-2 envelope and *Mycobacteria* is shown with an *r*: a marks a residue with strongly similar properties, and a denotes weakly similar properties. Sequence identities as follows: envelope protein (SARS-CoV-2) - YP_009724392; 1-WP_003873789; 2-WP_156147206; 3-YP_009357768 variant bovis AF2122/97; 4-CAL70455 M.tub. variant bovis BCG strain Pasteur 1173P2; 5-AHM06124, M.tub. variant bovis BCG strain ATCC 35743; 6-WP_003898448 M.tub. H37Rv tuberculin-like peptide; 7-WP_031685486; 8-AYP10944. Alignment was done using CLUSTAL O (1.2.4) multiple sequence alignment at EMBL-EBI [12]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**3.2. Direct comparison of the AFB test and immunohistochemistry using the SARS-CoV-2 envelope specific antibody for detection of *Mycobacteria* in formalin fixed, paraffin embedded tissues**

The initial eleven Mycobacteria positive cases had many *Mycobacterium* cases showed strong immunoreactivity with SARS-CoV-2 specific envelope antibody with a similar distribution and staining intensity as the AFB stain, indicative of strong homology. No signal was evident between the SARS-CoV-2 envelope antibody and bacteria including *Escherichia coli*, *Bacillus cereus*, *Streptococcus faecalis*, and *Lactobacillus* species (Fig. 1). Using a published protocol [2,12], co-localization experiments documented that the SARS-CoV-2 envelope protein and AFB signals co-expressed (Fig. 1).

BlastP analyses documented that SARS-CoV-2 envelope protein from the region used to generate the antibody shares high homology with a stretch of 12 amino acids present in *Mycobacterium bovis*, called tuberculin-like proteins, also known as the LytRC-terminal domain-containing protein (Fig. 2). Mycobacterial LytRC proteins are highly conserved in different *Mycobacterium* species (Fig. 2). This LytRC domain is rarely found in gram positive or negative bacteria [15] and does not show homology with other non-Mycobacterial species (data not shown). The AFB stain, specific for the mycolic acid-rich Mycobacterial cell wall, gave no signal with SARS-CoV-2 cases (data not shown).

The envelope protein is integral to the pathogenicity of the virus [1-3]. Despite these three important points for the diagnostic pathologist, clearly the most significant finding in this study is that the BCG vaccine, derived from *Mycobacterium bovis*, should indeed be effective against the current COVID-19 pandemic. SARS-CoV-2 has four major structural proteins: the spike, nucleocapsid, membrane and envelope proteins. The envelope protein is integral to the pathogenicity of the virus [1-3]. The data in this paper strongly suggests that BCG vaccination induces a specific immunity directed against the SARS-CoV-2 envelope protein that, in turn, is integral to the pathogenicity of the virus. Repeat BCG vaccination to those who have already received it, typically in childhood, can within several days reactivate the immune system memory directed against the Mycobacterial antigens that would include the heterologous immunity against the SARS-CoV-2 envelope protein [16]. Large numbers of new COVID-19 cases are still being reported daily. Thus, prospective trials examining whether there is an inverse correlation between a concurrent booster BCG vaccine in countries where this vaccine is mandatory at birth, as well as concurrent BCG vaccination to those who never have had the vaccine, and the incidence/severity of SARS-CoV-2 disease may well underscore the specific value of
vaccination against *Mycobacterium bovis* to reducing the severity of the current pandemic.

In sum, BCG vaccination is being touted as a treatment for reducing the complications of SARS-CoV-2 [1,3-5]. The main clinical finding of this study is that BCG vaccination offers a specific heterologous immunity against infection by SARS-CoV-2 by inducing an adaptive immunity response against a protein essential to the virus’s infectivity. This allows one to predict that BCG vaccination boosters should induce a strong anti-viral protection specifically against the disease COVID-19.

**CRediT authorship contribution statement**

GJN (hypothesis, testing, manuscript), EM (hypothesis and testing), LH (testing), DS (sample contribution, manuscript), ET (BlastP analyses, manuscript), CMM (testing, manuscript).

**Declaration of competing interest**

The authors have no competing interests to report.

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