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Commentary

# COVID-19 vaccine and boosted immunity: Nothing *ad interim* to do?

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ABSTRACT

Today, Coronavirus Disease 2019 (COVID-19) is a global public health emergency and vaccination measures to counter its diffusion are deemed necessary. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiological agent of the disease, unleashes a T-helper 2 immune response in those patients requiring intensive care. Here, we illustrate the immunological mechanism to train the immune system towards a more effective and less symptomatic T-helper 1 immune response, to be exploited against SARS-CoV-2.



In the 60–80 years of last century, an experimental wave of immunological studies attempted to fight cancer by exploiting live or killed bacteria, among which the most investigated were the Bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, and *Corynebacterium parvum* (*C. parvum*), later renamed *Propionibacterium acnes* and then *Cutibacterium acnes* [1,2]. Administered percutaneously or into the neoplastic mass, they proved able to induce the tumor lysis at some extent, and to delay or arrest the cancer growth through the innate immunity potentiation [3,4]. BCG gained approval since 1977 and is currently the standard of care for patients with non-muscle-invasive bladder cancer by means of mucosal instillation, besides to be registered as an anti-tuberculosis intradermal vaccination [5,6]. As of March 2020, BCG vaccine is furthermore in phase III or IV trials to prevent Coronavirus Disease 2019 (COVID-19) among healthcare workers in the Netherlands, Australia, USA, Germany, France, Denmark, Colombia, Mexico, Egypt, and South Africa [7-16]. Additional trials in the Netherlands, Germany, Greece, and India are evaluating whether BCG vaccine provides protection against COVID-19 in the elderly and in middle age [17-20]; besides, randomized trials on volunteers over 18 years to test BCG vaccine in this context has been launched in Brazil and Canada [21,22]. *C. parvum* is an aerotolerant anaerobic rod-shaped Gram-positive bacterium largely commensal and part of the skin flora present on most healthy adults, but also associated to sarcoidosis and juvenile acne, hence its taxonomic renaming [23]. After an initial registration like immunoadjuvant and immunomodulator, *C. parvum* was added to the chemotherapy protocol for colon cancer by repeated injections in the form of formalin-killed freeze-dried vaccine preparation (Coparvax®, Wellcome Research Laboratories, Beckenham, UK); however, it was discarded because no partial remission, overall survival or significant benefits were achieved in the treated cohorts of patients [24]. At that time, one of us (Prof. Palmieri) had the chance to perform a clinical pilot trial with *C. parvum* administration into subcutaneous and lymph nodal metastases from lungs, thyroid and breast malignancies, noting local shrinking and colliquative effect in 48–72 h, accompanied by mild symptoms and occasional febrile peaks. In a few cases, very rapid regressions of concomitant herpes infections involving the head, the thorax

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and the genitals were incidentally observed. By searching on the English biomedical literature, several studies from the past fully support the *C. parvum* antiviral power on man and animal against, for example, influenza virus, hepatitis B, rabies, encephalomyocarditis virus, herpes zoster and human papilloma virus [25-30]. In addition, a scientific evidence of *C. parvum* vaccine protection against a coronavirus (mouse hepatitis virus type 3), dating back to 1981 murine model by Schindler and colleagues, is also reported [31]. Always working on a murine model, Teixeira and collaborators proved in 2018 that *C. parvum* enhances the immunogenicity of the HIVBr18 vaccine, a vaccine against 18 epitopes of the human immunodeficiency virus, subtype B [32]. In the same year, Hsu and coworkers discovered 16 short RNA sequences from *C. parvum* similar to Ebola virus microRNAs, capable to protect the human host by influencing the thrombospondin 4 expression, a multifunctional protein which plays also an initiating role towards cell-mediated immunity in the skin [33]. Palmieri dropped out his clinical trial on bacterial immune stimulation against cancer in 1980, but he followed up with anecdotical compassionate treatments of severe herpes, and others heavy viral infections, such as influenza, mumps, varicella and measles, by subcutaneous injections of *C. parvum* cultured and killed in our microbiological lab, after signing an informed consent out of a total of 40 patients, 30 males and 10 females, aged between 5 and 97 years (mean age 49 years). During our evolving experience, we modified the subdermal injection formula ($9 \times 10^9$ phenol-killed bacteria in 2 ml saline), adding high molecular weight hyaluronic acid as slow delivery system, having found aspecific virucide properties of this non-sulphated glycosaminoglycan, notoriously able to bind the cluster of differentiation 44 (CD44) receptors present on the surface of activated leukocytes [34,35]. The protocol varied from 1 single to 3–5 injections each other day or every 3 days accordingly with the clinical stage and response to the treatment. The *C. parvum* administration has been always safe and quickly effective showing clinical improvement after 24, 48, 72, 96 h, depending on the patient's performance status and the latency time between the infection outbreak and the beginning of the treatment (Fig. 1). As well known, naïve T-helper ($T_h0$) cells can respond to novel infectious agents never encountered before, like the specific case of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the newly identified betacoronavirus responsible for COVID-19, able to bind the angiotensin converting enzyme 2 receptors [36]. On the basis of the encountered pathogen, $T_h0$ then polarize the immune response into T-helper 1 ($T_h1$), the default response in immunocompetent subjects to intracellular or phagocytosable pathogens (*e.g.* viruses, bacteria, protozoa, fungi), mediated by macrophages and T-cytotoxic ($T_c$) cells (cell-mediated immunity), or into T-helper 2 ($T_h2$), classically directed against extracellular non-phagocytosable pathogens (*e.g.* helminths), whose main effectors are eosinophils, basophils, mastocytes and B cells (humoral immunity) [37]. In spite of this, severe SARS-CoV-2 infections are associated with marked $T_c$ lymphopenia [38]. During our researches on COVID-19, we have disclosed that the immune system is forced to mount in critically ill patients a $T_h2$ response, the only one still mountable in the attempt to counteract the viral load, rather than a $T_h1$ response, which would keep the infection under control by means of macrophages and $T_c$ cells [39,40]. Moreover, for the first time in worldwide literature, we have provide evidence that a life-threatening escalation from $T_h2$ immune response to type 3 hypersensitivity (immune complex disease) in COVID-19 vasculitis takes place, and that the inflamed smooth muscle cells of blood vessels concur to the «cytokine storm» via interleukin (IL)-6 [41]. Therefore, we have proposed that an effective vaccination strategy should be able to prevent or limit the systemic imbalance of $T_h2$ cytokines [42], inducing a protective $T_h1$ response to be exploited against SARS-CoV-2 (Fig. 2). Among the $T_h2$ cytokines, there are

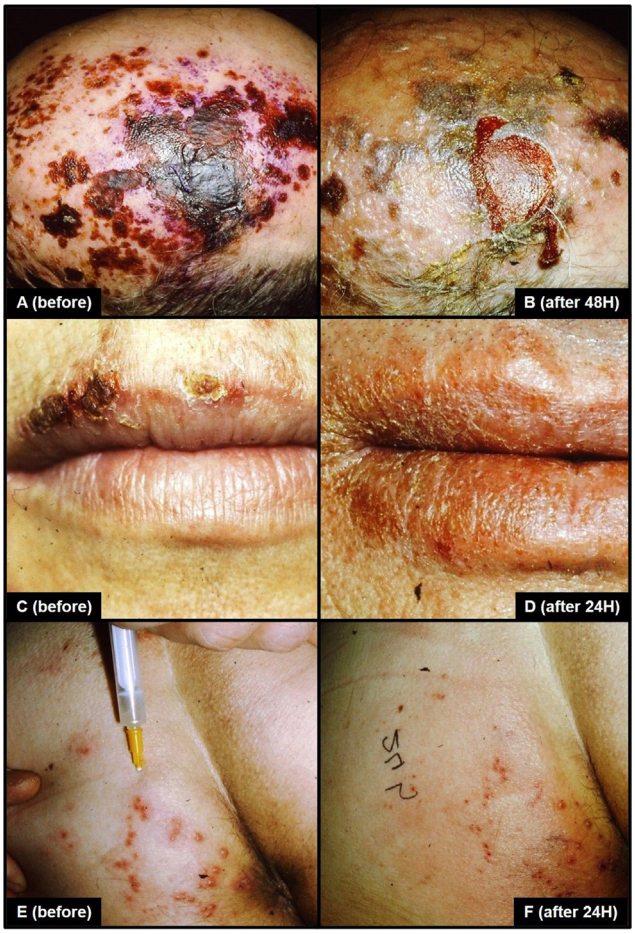


**Fig. 1.** Illustrative clinical photographs taken by Prof. Palmieri before and after *C. parvum* vaccination: herpes zoster of the scalp pre-injection (A) and 48 h post-vaccine (B); herpes labialis before the injection (C) and after 24 h from the treatment (D); genital herpes at the time of subdermal injection (E) and 24 h post-vaccination (F).

IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and IL-25, while IL-2, IL-12, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) are the master $T_h1$ cytokines [37]. Many researches have ascertained that *C. parvum* subcutaneous injection is able to induce a strong $T_h1$ response favoring the production of IL-2, IL-12, IFN-γ and TNF-α, in practice as BCG works, and that the characteristic allergic $T_h2$ response can be counterbalanced by *C. parvum* vaccination [3,43-48]; besides, it has been found a natural killers (NK) and dendritic cells activator [49-51]. In 1985 Cioffi and colleagues reported that *C. parvum* protects splenectomized Sprague Dawley rats from respiratory challenge with *Streptococcus pneumoniae*, without alter the number or activity of lavageable alveolar macrophages, and they hypothesized that *C. parvum* protection is more likely due to anincreasedclearance of blood-borne bacteria by the expanded and enhanced reticuloendothelial system [52]. If we transfer this murine model to man, a $T_h1$ cytokines release syndrome from activated pulmonary macrophages after *C. parvum* lysate subcutaneous injection, such as to aggravate a possible superimposed COVID-19, appears somewhat unlikely. Therefore, our long-standing experience lays the foundation to revalue *C. parvum* lysate as a further surrogate vaccine against COVID-19, in the attempt to prevent or mitigate the cumbersome pandemic morbidity and mortality. The accurate preparation of the lysate is a crucial time to avoid opportunistic bone implant-associated infections or acute septic polyarthritis, whose a single case has been described

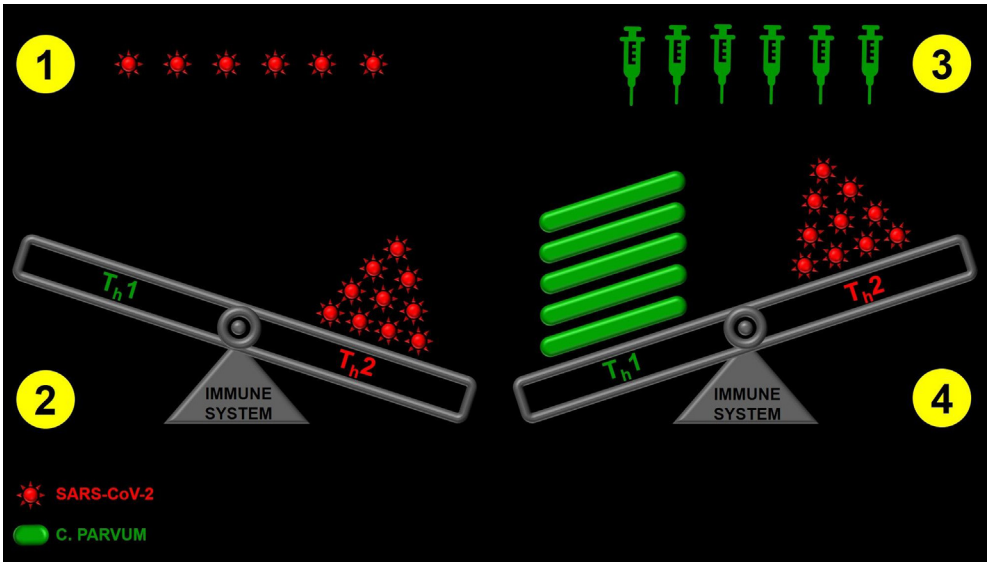


**Fig. 2.** Illustrative scheme of our vaccination rationale: following SARS-CoV-2 entry (point 1), the immune system is forced to mount a $T_h2$ response in those patients requiring intensive care, at the expense of a more effective and less symptomatic $T_h1$ response, compromised by the viral load (point 2). Through *C. parvum* or BCG vaccine (point 3), it is theoretically possible to train and calibrate the immune system towards a $T_h1$ response (point 4), able to prevent COVID-19 or to keep the disease under control in a paucisymptomatic or asymptomatic way thanks to activated reticuloendothelial system, NK, $T_c$ and dendritic cells.

in 1983 after *C. parvum* instillation for malignant pleural effusion; an episode of prolonged fever from immune system hyperactivation has also been reported [53-55]. Previous efforts to develop subunit vaccines against the most lethal human coronaviruses, for instance the Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), have failed because not enough protective; a recent study on SARS-CoV-2 has confirmed that neutralizing antibodies decline during the weeks or 2–3 months following infection at least in asymptomatic or paucisymptomatic patients [56], a biological behavior comparable to that of other known human coronaviruses [57], and which complicates the road to develop a specific long-term protective COVID-19 vaccine. By boosting $T_h1$ response and innate immunity rather than the humoral one, the disappearance of neutralizing antibodies and the adaptive mutational potential of SARS-CoV-2, limiting factors for the development of an effective subunit vaccine, could be so circumvent. In this regard, a further recent study on 10.022 SARS-CoV-2 genomes has identified 5.775 distinct genome variants, including 2.969 missense mutations, 1.965 synonymous mutations, 484 mutations in the non-coding regions, 142 non-coding deletions, 100 in-frame deletions, 66 non-coding insertions, 36 stop-gained variants, 11 frameshift deletions and 2 in-frame insertions, a series of genetic events which determine SARS-CoV-2 virulence, infectivity and transmissibility [58]. In conclusion, we have here illustrated our rationale for a strategic off-target vaccination against SARS-CoV-2, promptly available and safe for the patients.

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None.

## Authors contribution

LR conceived, designed and supervised the study, prepared figures and related legends, revised critically the first draft and wrote the final version of the manuscript; MV and VC analyzed and interpreted the data and performed the literature search; BP conceived and designed the study and wrote the first draft of the manuscript. All the authors approved the final version of the manuscript and attested they meet the ICMJE criteria for authorship.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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