



COMMENTARIES

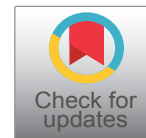
An Immunological Algorithm to Defeat Covid-19

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Abstract

Background: Local tissue destruction in Covid-19 patients is reported to be an inappropriate immune body response than viral loading. Very high levels of cytokines of mixed Th1/Th2 affinity have been demonstrated in critically ill patients.

Methods and results: Countries like Italy, Spain or Germany etc. with very low prevalence of tuberculosis and discontinued BCG immunization since decades, are witnessing the worst form of this global pandemic of Corona virus. It has also been seen that Covid-19 affected more of those countries who have robust and free influenza vaccination programme. High mortality of Covid-19 patients is probably result of the reduced Th1 immunity due to lack of past exposures to various infections and poor or absent local innate immunity due to the parental influenza vaccinations.

Conclusion: Influenza has been reported providing protection against the Mycobacteria. It is possible that a reciprocal cross immunity exists between the two pathogens. BCG vaccination should prove to be a great defence against Covid-19. Since BCG induces predominantly the cellular immunity which usually is peaked at 6-10 weeks, it may not yield instant protection as is needed. It may therefore be augmented with oral live-attenuated Salmonella vaccination to boost local mucosal immunity in mean time.

Keywords

Covid-19, BCG vaccine, Mycobacteria, Influenza, Salmonella

The modern human civilization with all its technical and scientific advancements, has suffered its worst ever defeat at the hands of Covid-19. Local tissue destruction in Covid-19 patients has been reported to be more due to an inappropriate immune body response than the viral loading. Immunity of an individual therefore, plays very important role in deciding outcome of Covid-19

infection both in terms of its morbidity and mortality. A dysregulated and enhanced immune response called cytokine storm than the virus itself is described to produce respiratory failure, acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS) leading to death in these patients [1].

Covid-19 after entering the respiratory tract, is trapped into the mucus which possess abundant antibodies like secretory IgA, or IgM providing an instant and broad-spectrum defence against the virus. Few viruses penetrate these protective barriers and enter the internal tissues and are confronted with a variety of chemical substances like transferrin, interferon, M-CSF or GM-CSF etc [2]. The next defence comprise of scavenger cells like granulocytes or monocytes that attack the virus directly or natural killer cells, which attack cells of the body harbouring the virus. M-CSF is reported to support expansion of the mononuclear phagocyte system, causing a substantial increase (up to 10-fold) in numbers of blood monocyte and resident macrophage while M-CSF augment the numbers of various tissue macrophage and monocyte population [3]. Macrophages release the cytokines interleukin-1 (IL-1) interleukin-6 (IL-6), GM-CSF and M-CSF to help fight the virus [4]. All of the aforesaid immune mechanisms compositely comprise the natural or the non-specific part of the immune response and is called the "Innate immunity".

After the innate immune system which evolves in about four to seven days, the adaptive immune response sets in and takes weeks to develop but is highly specific thus targets the pathogen more accurately. Another advantage of adaptive immune system is that it produces memory cells, thus remembers the antigens

and act quicker if it had been exposed to the antigen before also. This adaptive or acquired immunity mainly comprises of type 1 or type 2 immunity invoked through Th1 cells or Th2 cells. Th1 immunity is induced by intracellular pathogens like Mycobacteria, Salmonella, leishmaniasis, fungi and viruses etc. Th2 cells provide protection against extracellular pathogens such as multicellular parasites. The Th1 pathway releases various proinflammatory cytokines like IL1, IL2, IL12 and TNF- α , IFN- γ etc., while the Th2 pathway is seen to release various anti-inflammatory cytokines like IL4, IL5, IL6, IL10 etc. predisposing to several systemic autoimmune diseases [5-8]. Human population living in an hygienic environment, which has never or rarely been exposed to various antigenic infections grossly lacks in Th1 immunity thus over expressing Th2 immunity due to their cross regulation [8].

There exists a huge variation in reported deaths due to Covid-19 ranging from 0.05 per million of population in Nigeria, Ethiopia and Sudan to 344 per million of population in Spain [9]. Covid-19's unique geographical prevalence based on the economic and financial strength of the countries forces us to analyse this disease's causation in a different perspective. Immunization rate against influenza are significantly higher in high income (92%) than in low income countries (8-47%) [10]. Influenza virus is uniquely characterized by frequent mutations due to antigenic drifts and antigenic shifts. The parenteral administration of inactivated influenza vaccines provides specific immunity against only those strains of viruses for which the vaccine has been developed with no innate mucosal immunity, while natural infection is able to induce both mucosal and systemic immunity with relatively broad-spectrum credentials [8]. It is possible that the broad spectrum innate immunity, best derived through natural infection somehow is not very well developed among people in economically developed countries due to their extensive and healthier vaccination policy including that of seasonal influenza. This gets supported from the observation that Covid-19 affected more of those countries who have robust and free influenza vaccination programme leading to reduced natural flu infection among their population [10-12]. It is suggested that boosting innate immunity with its broad spectrum or non-specific nature could be a novel concept to enhance host defence against such continuously mutating viruses and deserves further exploration.

In normal individuals with good immunity, Covid-19 virus like other influenza viruses in normal course should induce Th1 cells which in turn shall eliminate the virus in a time bound manner. Influenza virus in an experimental study on mice however, has shown to be lethal if it somehow induces a combined Th1 and Th2 response [13]. A similar pattern of diverse cytokines profile with very high levels of mixed Th1/Th2 affinity like IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNF α were seen in

ICU patients of Covid-19 infection [14]. What prompts this unusual Th2 response in these patients could be the key to understand and evolve protective measures against this huge threat to humanity. Other studies also reported very high levels of IL-6 and IL-10 in severe Covid-19 patients along with low levels of CD4+T and CD8+T [15]. It is hypothesized that insufficient natural infection and frequent influenza vaccination by parenteral route cause poor innate immunity and grossly deficient secretory IgA (sIgA) in respiratory mucosa etc. This leads to excessive production of the other immune mediators of the innate immune system like transferrin or GM-CSF causing worsening of Covid-19 patients. This excess production of transferrin gets attached to the iron ions absorbed through the gut and prevents iron from being delivered to red blood cells in blood. This is reflected clinically as prolonged and progressive hypoxia and rapidly decreasing O₂ saturation along with very high level of serum ferritin in all critically ill Covid-19 patients [1,16]. Growth factors like GM-CSF and M-CSF in normal quantity are reported to support expansion of the mononuclear phagocyte system [5], while same cytokines when present in excess quantity are reported to produce acute interstitial pneumonitis and fibrosis without pleural effusion due to the increased free radicals and also an increased IL4 and IL6 [17]. Cytokine storm with very high levels of GM-CSF, IL-6 and IL-10 [15] therefore, could well explain the steep clinical deterioration and symptoms of ARDS in severe Covid-19 patients. This could be the result of the combined effect of the reduced Th1 immunity due to lack of past exposures to various infections and the poor or absent local innate immunity due to the parental influenza vaccinations.

Natures every creation has a motive to serve- Be it ant, mosquito, earthworm or tuberculosis! It is our ignorance only not to recognise their useful motives. Countries like Italy, Spain or Germany etc. with very low prevalence of tuberculosis and typhoid etc. have shown a higher prevalence of Covid-19 while a higher prevalence of Covid-19 is also seen among countries where the routine BCG immunization has been discontinued since decades [18-20]. LowTh1 immunity due to poor or no exposure to strong Th1 antigens like Mycobacteria, Salmonella or even Bee stings etc. among these populations is probably the cause for such high morbidity and mortality following the Covid-19 infection [9].

BCG is the live attenuated vaccine used against tuberculosis prepared from *M. bovis* bacillus. It has an excellent safety profile, is part of national vaccine programme of more than 154 countries and is estimated to be given to more than 100 million children per year globally and thus has a well-established safety profile. BCG vaccinated children have demonstrated improved mortality rate associated with malaria, diarrhoea or attenuated yellow fever virus etc [21] and also the re-

duced risk of developing acute lower respiratory tract infections including adults and elderly population [22-25]. These non-specific beneficial effects of vaccination are believed to be the result of the generation of innate immune memory through epigenetic modification [26]. Protective effect of BCG wanes with time and is reported to disappear completely in 10-15 years [27]. Countries like Iran with policy of compulsory BCG vaccination at birth therefore, may provide protection to their population from childhood tuberculosis but the continued collateral benefits like what is being contemplated during the present Covid-19 pandemic, are possible only through the environmental exposure to Mycobacteria as seen in the tubercular high burden countries.

It has been shown that influenza provides significant protection against the Mycobacterial infection [28]. It is possible that a reciprocal cross immunity exists between the two pathogens. Mice co-infected with BCG and influenza A virus, exhibited reduced frequency and numbers of CD8 T cells specific to Mycobacterium (BCG) in the lungs when compared with mice infected with BCG alone. Viral specific CD8 T cells were seen to have special affinity towards BCG induced granulomas resulting into substantial accumulation of viral specific CD8 T cells there [29]. Such an affinity can be useful in patients with Covid-19 to divert its overactive immune response towards BCG granulomas. It is suggested that deployment of BCG specific CD8 T cells will occur at the cost of viral specific CD8 T cells thus reducing the magnitude of cytokine storm of the Corona infection.

BCG-specific CD4+ T-cell response is reported to be at peak 6-10 weeks after vaccination [30]. Administering BCG by oral or intranasal route which is likely to improve its capacity to induce innate immunity has been explored but with contradictory results. A number of studies have shown that delivery via mucosal routes may elicit a local respiratory mucosal immunity which may increase protection against Mycobacterial infection [31,32] while no significantly improved protection was reported in guinea pigs immunised with SL3261mycolacZ after the aerosol challenge with pathogenic *M. Tuberculosis* [33,34]. Due to its inability to induce an early innate immunity, BCGs prophylactic use may not be able to provide an early protection as is required in present pandemic time. A vaccine capable to induce good innate immunity with heterogeneous or non-specific predominance along with Th1 biased adaptive cellular immunity should probably be the need of the hour in addition to the BCG vaccine.

Salmonella vaccination which provides protection against the organism predominantly through Th1 biased cellular mechanism, when administered orally (strain Ty21a) showed activation of circulating monocytes and dendritic cells (DCs) for up to 96 hours and induced up-regulation of CD11b, CD11c, CD16, CD64, CD303, TLR-4, and TLR-5 among CD14+ monocytes for as long

as 3 months [35]. It was demonstrated that Vaccination with Ty21a altered cytokine production among various cell populations in response to stimulation with nonrelated pathogens as well. The authors suggested that vaccines such as Ty21a, which are low cost, extremely well tolerated, and easily administered, could be included in wider vaccination programs, even in non-typhoid- endemic regions, solely for their off-target, nonspecific beneficial effects [35].

The innate immune is evoked by varied antigens via members of structurally related receptors termed toll-like receptors (TLRs) [36]. Flagellin is a protein forming an appendage protruding from the cell body of certain bacteria including Salmonella and is a known potent inducer of innate immune responses against airway epithelial cells through binding to TLR5 expressed on them. TLRs do not possess fine specificity, such as that of BCRs, TCRs or adaptive immune receptors, but they can individually respond to a limited but specific number of microbial pathogen-associated molecular patterns (PAMPs) such as lipoteichoic acids and lipid peptides, double stranded RNA, lipopolysaccharides, flagellin etc [36]. Flagellin, as a TLR5 agonist, is an established mucosal adjuvant for enhancing mucosal IgA responses in lung epithelial cells and pneumonocytes by intra-nasal immunization [37]. Flagellin can be an excellent tool to induce nonspecific innate immunity to help to combat with the Covid-19 pandemic but somehow has not attracted attention on this aspect. Since use of flagellin is already in practice as an adjuvant anti-tumor and radioprotective agent, the wild thought may not be as elusive.

In defence against Covid-19, there is urgent need for an immuno-modulatory agent capable of boosting mucosal innate immunity but there are not much choices available. A study in healthy adult males demonstrated that live-attenuated *Salmonella Typhi* strain Ty21a increased the frequency of heterologous influenza virus-responsive CD4+ T cells by 5-fold while the frequency of heterologous influenza virus-responsive CD8+ T cells increased by 6-fold in vaccinated volunteers, compared with unvaccinated volunteers [38]. Under the circumstances, it may not be unwise to suggest use of oral live-attenuated Salmonella vaccination to attain the said objective.

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