Immunological Reason for Mild Effection of Children to COVID-19, A Key Factor for Novel Solution of Vaccination and Medications

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ABSTRACT: Coronaviruses are important human and animal pathogens. We will show that probably antibodies don't have essential role in immunity against COVID-19 in long term, but a type of white globules named T cells may have critical role in immunity against COVID-19. T cells have long time memory to remain in blood. The most important point for investigation of such issue is mild effection of children to COVID-19. While the milder COVID-19 disease in children is remained secretly till this paper, but its understanding will provide important information about the disease. It may also suggest important protective mechanisms and targets for future therapies. Then a main factor in producing a vaccine for COVID-19 because of higher resistance of immune system of children comparing adults. We identified this could be because immunity of children is mostly based on innate immunity (phagocytes) while adults are based on antibodies. Our results show innate immune system including phagocytes contribute severely to the elimination of COVID-19 in both mouse model and human. Our results also show the elimination of COVID-19 required the activation of B cells by CD4+ T cells. CD4+ T cells play an important role in elimination of COVID-19 in primary effection. We measured IgM and IgG in human patients including adults and kids and found that IgM and IgG in kids' patients are much higher than other adults patients. It means production of much more natural antibodies in kids' bodies to protect them against COVID-19. We also identified in infections, the number of γδ T cells increases both locally and systemically in kids but decreases in adults with sever symptoms of COVID-19 a few days post-infection. The number of γδ T cells also increases in adult patients with mild symptoms of COVID-19. We also found that human peripheral blood γδ T cells can produce professional phagocytos. We found also T cells from recovered patients can target the virus. That is promising news for vaccine developers. We found antibodies disappear in blood of recovered pationes after 8 weeks then they don't play essential role for long term immunity but T cells compensate their role. Our observations have important ramifications for the development of novel vaccination and medicine strategies to alleviate COVID-19. For producing any vaccine for COVID-19, increasing and producing these factors must be included: 1) Phagocytes, 2) Natural antibodies, 3) T Cells, and 3) White Cells. All vaccine producers must consider those four factors seriously while immune antibodies don't have essential role in long term immunity. Therefore, vaccine should be based on T cells instead of antibodies plus booster of innate immunity including phagocytes. In the end a novel vaccine suggested which elicits a protective immune response against SARS-Cov-2.

Key Message: Antibodies don't have essential role in immunity against COVID-19 in long term, but T cells may have critical role in immunity against COVID-19. Immunity of children is mostly based on innate immunity (phagocytes) while adults are based on antibodies, that is reason for mild effection of children to COVID-19. T cells from recovered patients can target the virus. That is promising news for vaccine

developers. A vaccine should be based on T cells instead of antibodies plus booster of innate immunity including phagocytes.

Introduction

Coronaviruses are important human and animal pathogens. At the end of 2019, a novel coronavirus, was identified as the cause of a cluster of pneumonia cases in Wuhan, China. It rapidly spread, resulting in an epidemic throughout China, with sporadic cases reported globally. From December 2019 till now, the disease, which resulted in several millions cases with many deaths, was caused by a novel type of coronavirus, termed COVID-19. Patients with COVID-19 usually developed a high fever followed by clinical symptoms of cough and shortness of breath. Full-genome sequencing and phylogenic analysis indicated that the coronavirus that causes COVID-19 is a betacoronavirus in the same subgenus as the severe acute respiratory syndrome (SARS-CoV) virus (as well as several bat coronaviruses), but in a different clade [1]. The apparent structure of the receptor-binding gene region in COVID-19 is very similar to that of the SARS coronavirus, and there is speculation that it will be shown to use the same receptor for cell entry. Full-length genome sequences were obtained from five patients at the early stage of the outbreak show they are almost identical to each other and share 79.5% sequence identify to SARS-CoV [2]. Furthermore, it was found that COVID-19 is 96% identical at the whole-genome level to a bat coronavirus [2]. However, there has been no subsequent consensus regarding which treatment, it any, benefited COVID-19 patients during the outbreak. The development of an effective treatment and vaccination strategy for COVID-19 cases will require clarifying the precise mechanisms by which host immune responses control COVID-19 infection. Cumulative evidence suggests that patients who recovered from COVID-19 possessed specific acquired immunity based on both T and B cells [8]. However, the effector cells or molecules that act to eliminate COVID-19 during the acute phase of the infection remain unclear.

The immune system is a network of intricately connected cells to protect the body from internal and external threats. It is broadly classified into two sub-types: innate (or natural) and adaptive (or acquired). The key differences between the two are the specificity. The innate system is the first line of defence, capable of detecting many common infectious agents, such as viruses and bacteria, as soon as they find their way into the body. Although it may respond quickly, the innate system cannot always eliminate infectious organisms and it doesn't recognise all the pathogens.

Because of the intricate nature of the immune system, the innate system also provides cues in the forms of chemical signals (cytokines) or degraded products of infectious organisms (antigens) to activate the adaptive immune system, using a process known as "antigen presentation". Without these cues, the adaptive immune system cannot be activated.

The adaptive immune system has evolved to provide a more versatile and highly target-specific defence with an ability to distinguish very subtle differences in the make-up of infectious agents. But the adaptive immune system is slow and can take several days before two key cell types – B cells and T cells – are brought into play.

T cells are further divided into two sub-types, CD4+ and CD8+ cells. CD4+ are helper T cells that help the activity of other immune cells by releasing cytokines. The cytokines prime the maturation of B cells, which become plasma cells and produce antibodies to neutralise the pathogen. CD8+ cytotoxic T cells, on the other hand, directly kill infected cells.

When the adaptive immune system destroys the invaders, a pool of T and B cells is built with long-term memory. These memory lymphocytes remain dormant until the next time they expose the same pathogen. But this time it creates a much faster and stronger immune response. Memory is the main feature of the adaptive immune system that causes long-term protection. Since most people are not exposed to the novel coronavirus, it can be safely assumed that infected people lack memory T and B cells and therefore do not have protection against COVID-19 infection. Technically, like any other infection, COVID-19 must producers an immune response and begin to proliferate anti-COVID T and B cells.

Around 15 million people have recovered from COVID-19, yet evidence of exactly how the adaptive immune system responds to the novel coronavirus has, so far, been scarce. But new information is emerging all the time.

Our study showed that infected people are able to produce specific T cells and B cells of COVID-19. Our study also showed that even some uninfected people had T cells to COVID-19, suggesting an overlap with the response to previous coronavirus infections – so-called cross-reactivity.

Also, recent research from the Karolinska Institute in Sweden [ ] showed that several COVID-19 patients with mild to no symptoms had generated T cells against the virus. This was even the case in patients who didn't have any detectable levels of antibodies against the virus. More importantly, the researchers also found evidence of memory T cells in convalescent patients. This suggests that COVID-19 elicits a strong memory T cell response, which can prevent recurrent episodes of severe COVID-19.

Disappearing antibodies

Over the years, the research and clinical data have shown that because children have an immature immune system, they are more prone than the adults to microbial infections, and have higher severity of symptoms, which is especially true for the newborns and very young children. The analyses of clinical data from the current COVID-19 pandemic caused by COVID-19 are conflicting in this respect. Many data indicate that children are rarely infected and have less severe symptoms. We will show that the answer lies in the differences between adult and child immune systems. Kids' immune systems are dominated by innate immunity (phagocytes), which are big cells that "eat" any foreign material, while adult immune systems employ more antibodies, which attack microbial invaders like X-Wings attack TIE Fighters. This difference between adults and kids may help for developing vaccination and future therapies.

While the immune system's B cells make antibodies that block the novel coronavirus, T cells provide another line of attack, according to our results.

Lethal disease in BALB/c mice infected with a mouse-adapted strain of COVID-19, showed a lack of activation of innate immune response, resulting in a barely detectable antivirus T cell response [13]. On the other hand, aged BALB/c mice that were infected with a human clinical isolate of COVID-19 successfully eliminated the invasive virus within 1 week post-infection; these mice exhibited high and prolonged levels of viral replication, signs consistent with clinical symptoms, and pathologic changes in the lung resembling those seen in elderly SARS patients [9]. Therefore, the infection of these aged mice is consider a model for the successful elimination of COVID-19 by host immune responses. A study reported that CD4+ T cells play an important role in the control of COVID-19 infection [14]. Our research also identified an important role for innate defense mechanisms in controlling COVID-19 infection, as demonstrated by the clearance of COVID-19 over 9 days post-infection (dpi) in BALB/c mice depleted of both CD4+ and CD8+ T cells [14].

Results

According to the pathological reports for COVID-19, it was shown that COVID-19 mainly caused inflammatory responses in the lungs [7]. Several studies showed that COVID-19 patients developed lymphopenia and rising pro-inflammatory cytokines in severe cases [8, 9]. Inflammation can be triggered when innate and adaptive immune cells detect COVID-19 infection. Innate T cells can provide a first line of defense against pathogens. However, how innate T cells respond to COVID-19 infection remains unclear.

We studied the identity and role of effector cells and molecules participating in the elimination of COVID-**19** during the acute phase of COVID-**19** in this work. In this study, we attempted to identify the types of immune cells that contribute to clearing COVID-**19** during the acute phase of the infection in both mice and human models. We demonstrate that phagocytic and T cells play an important role in the elimination of COVID-**19** in both mouse and human models of infection.

Our results suggest that both innate and adaptive immune responses are essential for controlling COVID-19 infection. Therefore, it shows also that the cooperation of antibodies and phagocytic cells plays an important role in the elimination of corona virus.Adult patients with mild covid-**19** infection also shows IgM and IgG similar to kids.We suggest a novel vaccine production and medicine strategies for COVID**-19** based on our unique results in this paper.

Our experiments and research will be in both mice and human models. We used 96 mice plus 213 human patients. We show firstly results of mice model.

Mice Model

The contributions of cellular versus humoral immune responses in the resolution of acute COVID-19 infection have not yet exactly been experimentally addressed. Therefore, using the infection of viral titers in the lungs of aged (>8 months old) BALB/c mice, young (<4 weeks old) BALB/c mice, and (8 weeks old) SCID mice following infection with acute COVID-19 a 42 years old male

patient's throat lavage, we aimed to determine immune responses during acute COVID-19 infection. Prior to intrabronchial COVID-19 inoculation, 32 aged BALB/c mice, and 32 young BALB/c mice, and 32 SCID mice were divided into four groups of animals each: (1) control; (2) CD8+ T cell depleted; (3) CD4+ T cell depleted; and (4) CD20+ B cell depleted. T and B cell depletion regimens were initiated 7 days prior to infection to ensure that the targeted lymphocyte population was not present on the day of infection (0 dpi).

We observed titers in the aged-mouse model were considerably higher than those seen in infected young BALB/c mice and very young SCID mice, which did not show apparent histological signs of pneumonia. In contrast, very young SCID mice, which lack functional T and B cells, were persistently infected with COVID-**19** during the experimental period. To investigate the effect of adaptive immune responses on clearance of pulmonary-infected COVID-**19**, either naïve splenocytes or sensitized splenocytes were adoptively transplanted into naïve very young SCID mice **1** day before COVID-**19** infection. The very young SCID mice and the BALB/c mice that received naïve splenocytes eliminated COVID-**19** from their lung early. Then very young SCID mice transplanted with COVID-**19**-sensitized splenocytes of BALB/c mice eliminated COVID-**19** more rapidly than mice transplanted with naïve splenocytes, although the initial pulmonary viral titers were effectively the same in all groups of very young SCID mice, with or without the transfer of splenocytes.

These results indicated that induction of adaptive immune responses is essential for the clearance of pulmonary-infected COVID-19 and young mice immune responses are higher than old mice.

To determine host protection(s) involved in the elimination of COVID-**19** in the lungs, we depleted CD4+ cells and / or CD8+ cells before and after COVID-**19** infection in BALB/c mice. Depletion of CD4+ cells or CD8+ cells was done by intravenous (i.v.) injection of monoclonal antibody; control experiments showed that the injection of these mAbs caused an almost complete depletion of the corresponding cell populations. Unlike peripheral blood, the incidence of CD**20** B cells was very low in BALB/c mice. In this study, we found that COVID-**19** constantly infects the lungs of nude mice (with T cell deficiency) as well as very young SCID mice (with T cell and B cell deficiency) without causing histological symptoms of pneumonia. Therefore, we examined (using one of the two models) that CD4+ T cells can directly eliminate COVID-**19** by secretion of IFN-y. The IFN-γ-deficient mice and the very young SCID mice that received splenocytes from IFN-γ deficient mice controlled the COVID-19 infection as well as the wild-type mice did. In summary, CD20 and CD8 T cell depletions were more profound (achieving ~100% loss by day 0) and lasted longer than CD4+ T cell depletion. Following depletion, recovery was very slow in all three lymphocyte subsets and the frequencies did not return to baseline by the end of the study. We found that loss of CD8+ T cells led to a very low peak viral

load and the loss of CD4+ T cells led to significantly higher viral loads and disseminated COVID-19. We found also the elimination of COVID-19 required the activation of B cells by CD4+T cells.

These results demonstrated the cellular immunity and more specifically CD4+ T cells are an essential cell type for the control of COVID-**19** infection, and that the effect of this cell fraction is indirect.

These observations have important ramifications for the development of novel vaccination strategies to alleviate COVID-19 associated diseases.

We found also phagocytosis serves as an important first line defense mechanism against invading pathogens. It is also essential for continuous clearance of dying cells, tissue remodeling, and acquisition of nutrients for some cells.

To identify the effectors involved in the elimination of COVID-19-infected pulmonary cells in our mouse models, we tested the contribution of several candidate effectors. COVID-19-infected BALB/c mice were depleted of the first candidate. These results demonstrated that the complement–antibody complex is not required for the control of COVID-19 infection in this model.

Other blood cells also may serve as effectors for the control of COVID-19 Specifically, elevated levels of alveolar macrophages, monocyte-derived infiltrating macrophages, and neutrophils that were observed also in many SARS patients [18]. To investigate the role of these myeloid cells in the clearance of COVID-19-infected pulmonary tissue, each subset of these myeloid cells was depleted by administration of a specific mAb or reagent. Consistent with other reports in SARS [19], alveolar macrophages were depleted for more than 5 days following i.n. administration of 100 μL of 33% clodronate liposome. Neutrophils (CD11b+ and Ly-6G hi) and Gr-1 int monocytes in blood and lung were significantly depleted for at least 3 days after i.p. treatment with 250 μg of anti-Gr-1 mAb, whereas neutrophils alone were depleted upon administration of anti-Ly-6G mAb (1A8). The Gr-1+ cell- and/or alveolar macrophage-depleted groups failed to eliminate the pulmonary COVID-19 infection, whereas the alveolar macrophage-depleted group showed partial elimination of the virus. In contrast, the neutrophil-depleted BALB/c animals (anti-Ly-6G mAb-treated mice) eliminated COVID-19 by 9 dpi. Importantly, the neutralizing Ab titer of these cell-depleted mice was comparable to that of untreated BALB/c mice. These results suggest that phagocytic cells, especially monocyte-derived infiltrating macrophages, cooperate with anti-COVID-19 Abs to provide control of COVID-19 infection in these mouse models while Abs individually have small role in control of COVID-19 infection.

Human Model

Phagocytosis is a critical part of the immune system. Several types of cells of the immune system perform phagocytosis, such as neutrophils, macrophages, dendritic cells, and B lymphocytes.

The majority of COVID-19 cases (about 80%) is asymptomatic or exhibits mild to moderate symptoms, but approximately the 15% progresses to severe pneumonia and about 5% eventually develops acute respiratory distress syndrome (ARDS), septic shock and/or multiple organ failure.

Surprisingly there is rare investigation about phagocytes cells role in COVID-19 patients while mild effection of children to this virus gives great idea about critical role of phagocytes cells in COVID-19.

We investigated **13** humanchildren in ages btween **4** and **10** who were in the first days of infection. We also investigated **33** human adults in ages between **18** and **67** with sever infection of COVID-19.

We investigated both innate and adaptive immune systems in all patients. Seroconversion curves of reference [24] were constructed from the data and showed that total antibodies and IgM and IgG isotypes antibodies were 100% detectable approximately one month after symptom onset. However, in the first week after developing signs of illness, antibodies were present in less than 40% of patients tested. The seroconversion rate and antibody levels rose quickly during the fortnight after symptom onset, and the cumulative seropositive rate was 50% on day 11 and 100% on day 39 [25].

Our purpose was to compare IgM and IgG isotype antibodies btween children and adults. As we expected their bodies first line of defense, the innate immune response, starts right after infection, like an infantry going after a foreign invader, killing the virus and any cells damaged by it. The second line of defense, the adaptive immune response, kicks in days later if any virus remains, employing what it has learned about the virus to mobilize a variety of special forces such as T cells and B cells.

We observed, in the early phases of infection in children, natural antibodies play a most important role. Natural antibodies, mostly of IgM isotype and generated independently of previous antigen encounters, have a broad reactivity and a variable affinity. They contain the infection during the 2 weeks necessary for production of high-affinity antibodies and MBCs that will clear the virus and prevent reinfection. In humans, natural antibodies are produced by innate or IgM MBCs, a population of MBCs that is generated independently of the germinal centres and is most abundant in children.

We measured IgM and IgG in all patients including adults and kids. As seen in Fig. 3 one may see IgM and IgG in kids patinets are much higher than adults patients in beginning days of infection but it becomes equal almost after 30 days. Higher IgM and IgG in beginning days cause production of much more natural antibodies in kids' bodies to protect them against COVID-19. This clearly shows why kids are more immune against COVID-19. It gives also idea about novel vaccination and medicine strategies to alleviate COVID-19.

We observed adult patients with sever COVID-19 showed lower percentage and count in CD4+ and CD8+ lymphocytes populations, strong predictive values for in-hospital mortality, organ injury, and severe pneumonia. We also observed significantly lower number of total T cells, both helper T cells and suppressor T cells.

Cytotoxic T-cells (CTLs) and Natural Killer (NK) cells are required to generate an effective immune response against viruses in a human body. Our studies in human found that increased cytokine levels (e.g. IL-6, IL-10, and TNFα) and lymphopenia (significantly reduced CD4+ and CD8+ T cells) correlate with disease severity of COVID-19. In addition to reduced T cell counts, the surviving T cells appear dysfunctional. Patients with severe COVID-19 present significantly lower lymphocyte, and higher neutrophil, counts in blood. Specifically, CD8+ lymphocytes and NK cells were significantly reduced in cases of severe infection compared to patients with mild infection and healthy individuals [21].

Among innate immune cells, γδ T cells proliferate rapidly and respond to pathogens by inducing apoptosis, mediating antigen presentation and immune regulation [10]. In healthy adult humans, γδ T cells represent 1%-10% of the total circulating lymphocytes, predominately displaying the CD4 and CD8 double negative phenotype [11]. However, in some cases, a fraction of γδT cells can express either CD4 or CD8 [12-14]. γδ T cells do not recognize classical peptide antigens, their TCRs are non-MHC restricted and they can respond to pathogen-associated molecular patterns and produce cytokines in the absence of TCR ligands [15].

In many infections, the number of γδ T cells increases both locally and systemically a few days post-infection. A study found that the ratio of γδ T cells among total lymphocytes in the lungs significantly increased in mice infected with influenza A (H1N1) virus three days post infection [16]. This observation suggests that γδ T cells play an important role in the host immune response.

To demonstrate how γδ T cells behave upon COVID-19 infection, we analyzed the PBMC samples from 38 patients and focused on the characterization of γδ T cell phenotypes. We showed that upon infection, the percentage of γδ T cells in the peripheral blood isolated from COVID-19 patients was drastically decreased when compared with healthy donors (Figure 1A). Although in the acute or early stages of other viral infections, the percentage of γδ T cells increased, we observed a decrease of γδ T cells in symptomatic patients. This might be explained that various types of virus impact γδ T cells in different ways. It is likely that γδ T cell response, including proliferation and cellularity, is dependent on the specific types of viral infections.

Interestingly, we found that in comparison to HD group, the percentages of CD4 γδ T cells within the γδ T cell population increased dramatically, while CD8 γδT remained unchanged in COVID-19 patients (Fig1 B and C). The increase of CD4 γδ T cells indicated that in response to SARS-CoV-2 infection, this particular subset of γδ T cells may play roles in antigen presentation and facilitation of activation of adaptive immune cells, which has been demonstrated in different models [19]. The data also suggested this subset of immune cells could immediately respond to viral infection similar to other innate immune

cells such as macrophages and dendritic cells, to provide the first line of immune defense. Therefore, γδT cells may act as a bridge between innate and adaptive immunity in response to COVID-19 infection[20].

In COVID patients, we further observed that γδ T cells exhibited a strong activation phenotype in COVID-19 patients based on CD25 expression (Fig2 B). However, the early activation marker CD69 showed no difference between the patients and healthy donor (HD) group (Fig2 A). It is possible CD69 is expressed strongly earlier during infection, followed by reversion to the quiescent state during prolonged recovery. Since we observed a decreased percentage of γδT cells, we suspected whether γδT cells underwent exhaustion. However, the expression of PD-1 did not differ in γδT cells between HD and COVID-19 patients (Fig2 C).

In summary, γδ T cells are able to immediately respond to COVID-19 infection and upregulate the activation marker CD25. γδ T cells may act in parallel to other innate cells to mediate both direct and indirect defenses against COVID-19. In addition, the increased expression of CD4 in γδ T cells may serve as a biomarker for the assessment of COVID-19 infection.

Professional phagocytosis is considered to be performed exclusively by myeloid cell types. In this study, we found that a lymphocyte subset can operate as a professional phagocyte. By using confocal microscopy, transmission electron microscopy, and functional Ag presentation assays, we find that freshly isolated human peripheral blood γδ T cells can phagocytose Escherichia coli, leading to Ag processing and presentation on MHC class II. In contrast, other CD16 lymphocytes, i.e., CD16/CD56 NK cells, were not capable of such functions. These findings of distinct myeloid characteristics in γδ T cells strongly support the suggestion that γδ T cells are evolutionarily ancient lymphocytes and have implications for our understanding of their role in transitional immunity and the control of infectious diseases and cancer.

We identified also γδT cells may act as a bridge between innate and adaptive immunity in response to COVID-19 infection[20].

We found also T cells from recovered patients can target the virus. That is promising news for vaccine developers because it is "consistent with normal, good, antiviral immunity,"

Disappearing Antibiotics:

We know that antibodies to other coronaviruses (SARS and MERS) diminish over time (12 to 52 weeks from the time of infection). Our studies showed that COVID-19 antibodies can be detected for about 8 weeks in recovered patients. But given the huge variability of symptoms and immune responses among patients, the precise timeline is unclear.

Our study comparing groups of symptomatic with asymptomatic people showed that asymptomatic people had much lower antibody levels. And follow-up monitoring showed that about 40% of asymptomatic people had no detectable antibodies after eight weeks.

This suggests that antibodies to COVID-19 may not last very long. But this does not exclude the existence of memory T and B cells, capable of re-emerging from their dormant states to protect against re-infection. In other words, the antibodies that B cells make during initial exposure disappear in a few weeks, but the memory cells generated as a consequence of this persist for much longer.

As we mentioned before, while the immune system's B cells make antibodies that block the novel coronavirus, T cells provide another line of attack, according to our results. We found that T cells from recovered patients can target the virus. It compensates disappearing antibodies and that is promising news for vaccine developers because it is "consistent with normal, good, antiviral immunity,"

Discussion

The outbreak of COVID-**19** from December **2019** till now resulted in over **20** million cases, with about **3**% mortality. The worst symptoms might correlate with age-dependent defects of immune response, given that mortality exceeded **50**% in patients over **65** years of age. Retrospective analyses of recovered COVID-**19** patients suggests that patients who recovered from COVID-**19** possessed specific acquired immunity based on both T and B cells. Notably, most patients who recover from COVID-**19** had elevated and sustained levels of neutralizing Abs, and patients with longer illnesses exhibited lower levels of neutralizing Abs than did patients with shorter durations of illness.

Both T and B cell responses against COVID-19 are detected in the blood around 1 week after the onset of COVID-19 symptoms. CD8+ T cells are important for directly attacking and killing virus-infected cells, whereas CD4+ T cells are crucial to prime both CD8+ T cells and B cells. CD4+ T cells are also responsible for cytokine production to drive immune cell recruitment. The first autopsy of a patient with COVID-19 revealed an accumulation of mononuclear cells (likely monocytes and T cells) in the lungs, coupled with low levels of hyperactive T cells in the peripheral blood 57 . Together with reports of lymphopenia and reduced peripheral T cell levels in patients 6 , 95 , 96 , 97 , these findings suggest that T cells are attracted away from the blood and into the infected site to control the viral infection. In patients with COVID-19, increased T cell exhaustion and reduced functional diversity predicted severe disease 98. Despite the impaired response, patients who recovered from SARS-Cov infection developed coronavirus-specific memory T cells, which were found at least 2 years after recovery 99, 100 .

Cytotoxic T-cells (CTLs) and Natural Killer (NK) cells are required to generate an effective immune response against viruses [22], functional exhaustion of which results in disease progression [23]. Indeed, patients with COVID-19 presented with significantly lower lymphocyte and higher neutrophil counts in blood compared to healthy controls [24]. Specifically, CD8 + lymphocytes and NK cells were significantly reduced in severe infection compared to patients with mild infection and healthy controls [24] .

In this study, we found the lower control of the virus infection by mouse anti-COVID-19 antiserum when neutralization titers against COVID-19 were transferred into recipient mice. Therefore, it appears that the neutralization activity of Ab against COVID-19 does not correlate so much with the clearance efficacy of the virus from infected murine lung. On the other hand, some other antibody property (such as avidity) may differ, given that a polyclonal species derived from COVID-19-infected mice. This result suggests that the anti-infective activity of a neutralizing Ab is mediated primarily via prevention of COVID-19 invasion; the neutralizing Ab plays a lesser role in eliminating the virus after establishment of infection.

Therefore, we focused on the cooperation between anti-COVID-**19** Abs and other effectors in the control of COVID-**19** infection. Candidate effectors include complement (e.g., C**3** and other members of the complement–antibody complex pathway), NK cells (mediators of the Ab-dependent cell-marriage cytotoxicity pathway), and Fc gamma receptor (FcγR)-bearing cells (notably alveolar macrophages, monocytes [monocytes-derived infiltrating macrophages], and neutrophils). We used both anti-Gr-**1** mAb and neutrophil-specific mAb (anti-Ly-6G mAb) to discriminate monocytes and neutrophils. We tested the role of these candidates by selective depletion of a mouse infection model for various factors using CVF (complement depletion), anti-IL-2Rβ mAb (TM-β1; NK cell depletion), clodronate liposomes (alveolar macrophage depletion), anti-Gr-1 mAb (monocytes/neutrophil depletion), or anti-Ly6G mAb (neutrophil depletion) before or after COVID-**19** infection. Notably, the groups administered with clodronate liposome or anti-Gr-1 mAb, but not those treated with anti-Ly-6G mAb, failed to eliminate COVID-**19** from their lungs by 9 dpi. Our results indicated that phagocytic cells such as monocyte-derived infiltrating macrophages and partially alveolar macrophages, but not neutrophils, play a crucial role in the elimination of COVID-**19**-infected pulmonary cells in mice.

We here demonstrated that both monocyte-derived macrophages (infiltrating-type) and partially alveolar macrophages (resident-type) contribute to the elimination of COVID-19-infected pulmonary cells in the presence of anti-COVID-19 Abs.

We investigated also both innate and adaptive immune systems in all human patients. We observed, in the early phases of infection in human, natural antibodies play a most important role. Natural antibodies, mostly of IgM and IgG isotypes and generated independently of previous antigen encounters, have a broad reactivity and a variable affinity. In humans, natural antibodies are produced

by innate or IgM, IgG MBCs, a population of MBCs that is generated independently of the germinal centres and is most abundant in children. It shows why children are more imune against COVID-19 [26].

In conclusion, we demonstrate a crucial role for cooperation of antigen-specific antibodies and phagocytic cells (monocyte-derived infiltrating macrophages and partially alveolar macrophages) in the elimination of CIVID-**19** in mouse models and human of infection. Our findings provide a better understanding of the mechanism(s) by which host defenses control COVID-**19** infection. Ideally, this information can contribute to the development of novel therapeutic protocols or treatments for COVID-**19 [27]**.

Interestingly, we found that in comparison to HD group, the percentages of CD4 γδ T cells within the γδ T cell population increased dramatically, while CD8 γδT remained. unchanged in COVID-19 patients (Fig1 B and C). The increase of CD4 γδ T cells indicated that in response to COVID-19 infection, this particular subset of γδ T cells may play roles in antigen presentation and facilitation of activation of adaptive immune cells, which has been demonstrated in different models [19]. The data also suggested this subset of immune cells could immediately respond to viral infection similar to other innate immune cells such as macrophages and dendritic cells, to provide the first line of immune defense. Therefore, γδT cells may act as a bridge between innate and adaptive immunity in response to COVID-19 infection[20].

In COVID patients, we further observed that γδ T cells exhibited a strong activation phenotype in COVID-19 patients based on CD25 expression (Fig2 B). However, the early activation marker CD69 showed no difference between the patients and healthy donor (HD) group (Fig2 A). It is possible CD69 is expressed strongly earlier during infection, followed by reversion to the quiescent state during prolonged recovery. Since we observed a decreased percentage of γδT cells, we suspected whether γδT cells underwent exhaustion. However, the expression of PD-1 did not differ in γδT cells between HD and COVID-19 patients (Fig2 C).

In summary, γδ T cells are able to immediately respond to SARS-CoV-2 infection and upregulate the activation marker CD25. γδ T cells may act in parallel to other innate cells to mediate both direct and indirect defenses against COVID-19. In addition, the increased expression of CD4 in γδ T cells may serve as a biomarker for the assessment of COVID-19 infection.

CONCLUSION

For most vaccines to work the body needs two cell types – B cells and T helper cells – to make antibodies. B cells are the antibody factories and the T helper cells refine the strength and accuracy of antibodies to home and attack their targets. A technique that identifies these helper immune cells could inform future vaccine design, especially for vulnerable populations. The T cells, along with antibodies, are an integral part of the human immune response against viral infections due to their ability to directly target and kill infected cells. We found T cell immunity in people who recovered from COVID-**19** while antibodies disappeared **8-10** weeks after recovery.

Usually the level of antibodies in the blood tells immunologists how well a vaccine is working, specifically, how many antibodies are made and how strongly they disable microbes and virus, but it is not true in COVID-19 because antibodies disappears in body of recovered pationts after 8-10 weeks.

The most presize scenario for elevation of COVID-**19** in kids and adults should go head like: producing phagocytes (IgM and IgG) for eliminary protection through innate immunity. Then production of γδT cells may act as a bridge between innate and adaptive immunity **[20]**. A small portion of long-lived T cells still remains for rapid response upon pathogen re-exposure. This kind of cells is called memory T cells, because memory T cells have been trained to recognize specific antigens, they will trigger a faster and stronger immune response after encountering the same antigen. Activation of B cells through T cells and contribution of antibodies for protection of patients. T memory cell will protect recovered or vaccinated patient for future infection to the virus.

In many infections, the number of γδ T cells increases both locally and systemically a few days post-infection. Although in the acute or early stages of other viral infections, the percentage of γδ T cells increased, we observed a decrease of γδ T cells in symptomatic patients.

Our observation suggests that γδ T cells play an important role in the host immune response.

Antibodies will have essential role only in presence of phagocytes, otherwise lesser role. Antibodies will be also disappeared in body of recovered patients after about **8-10** weeks then it should be considered in production of vaccine. However, memory T cells remain in body of recovered patients long time and this is promising for producing vaccination.

Main effort of all researchers on COVID-19 in future should be buster of innate immune system especially phagocytes what is main reason for better immunity of children against COVID-19. For producing vaccination main and essential role of T cells should be considered while antibodies role are not critical and they disappear after 2-3 months in blood of recovered patients.

Suggestion

According to results of this paper we suggest a vaccine which elicits a protective immune response against SARS-Cov-2. Designed engineered peptides that could strongly bind to the spike protein of coronavirus inside cells, and to use these peptides to trigger the cells to break down the viral proteins. In this vaccine the mRNA (encoding RiboNucleic Acid) encodes a stable prefused form (the form before being fused to the cell membrane of the host cell) of Spike protein (S). In this vaccine the main problem of short time remaining of antibodies of SARS-Cov-2 virus in body could be compensated by activation of T cells that causes long term

immunity because memory T cells remain in body at least 10 years. A peptide based vaccine with encoding mRNA of virus along the activation of T cells through MHC class-II which elicits protection against SARS-Cov-2 through immune cells from the lymph nodes process the mRNA and synthesize specific viral protein antigens so that other immune cells recognize them. In this vaccine that comprising peptide encoding mRNA along activation of T cells by MHC class-II which immunize body against SARS-Cov-2 are also described. Methods of protecting a host against coronavirus infection will be discussed. Our invented vaccine is type of mRNA vaccine (but combined with other components) against SARS-COV-2 is based on a relatively new genetic method that does not require growing the virus in the laboratory. The technique transforms the human body into a 'living laboratory'.

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Conflict of Interest Statement

There is no conflict of Interest for authors in this PAPER.

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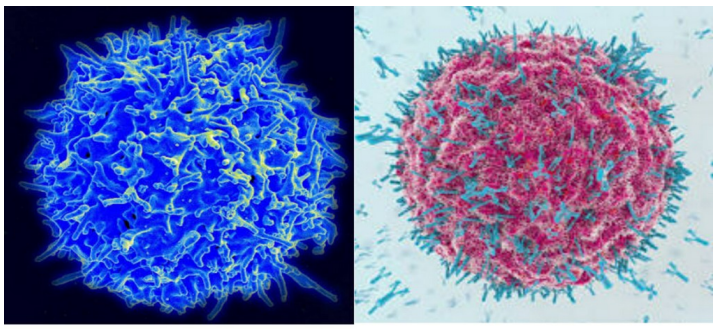


a) T cell **b) B cell**

Figure 1. a) T cell, b) B cell. Both contribute to the elimination of COVID-19.

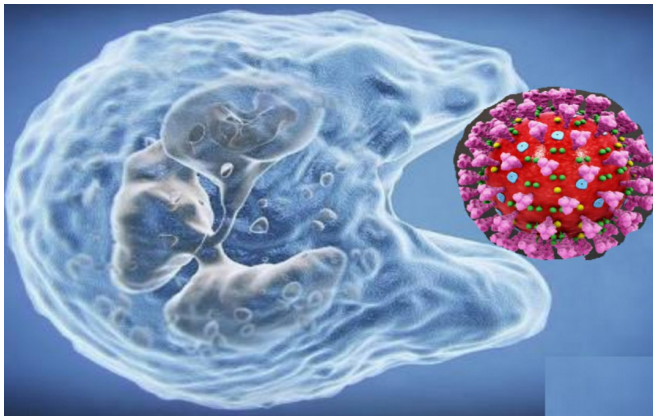


Figure 2. Phagocytes has essential role in mild effection of children by COVID-19.

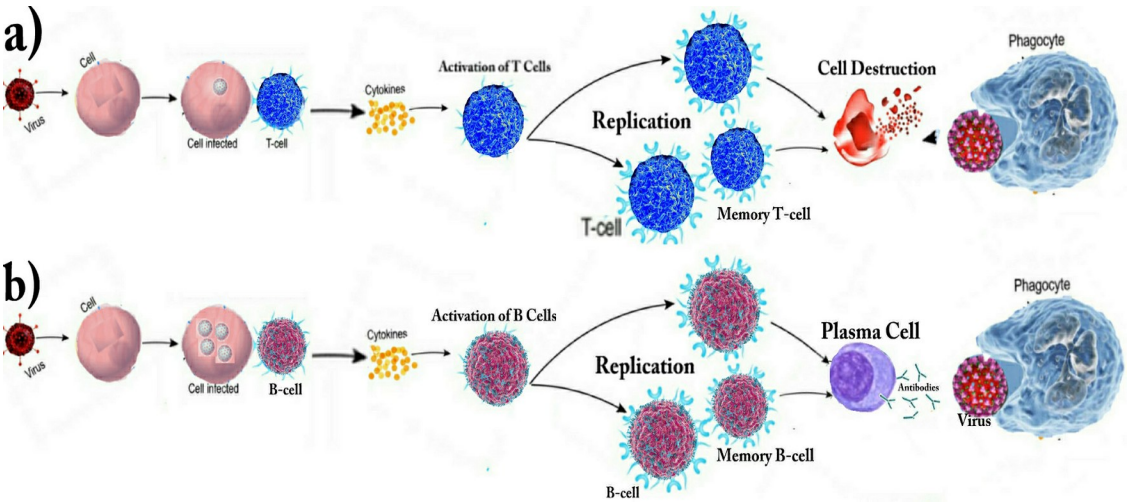
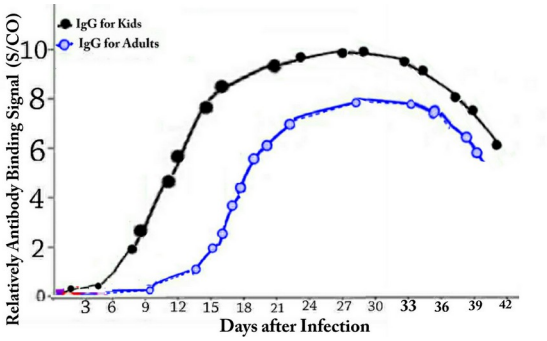


Figure 3. Scenario for infection of cell by SARS-Cov2 and prduction and activation of a) T cells, b) B cells



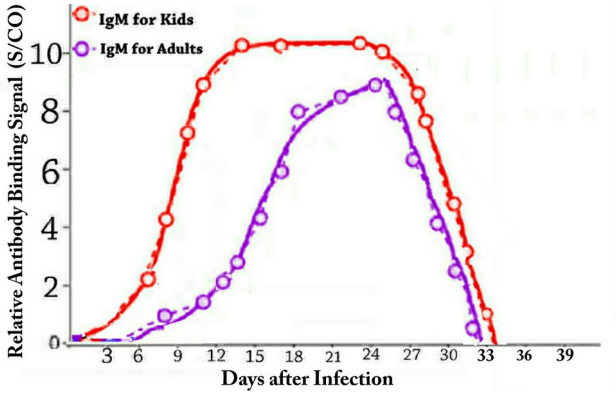


Figure 4. Average IgM and IgG in kids (13 kids in ages between 4 and 10) and adults patients (33 adults between ages 18 and 67) blood per days after infection to COVID-19. One may see IgM and IgG in kids patients are much higher than adult's patients in beginning days of infection but it becomes equal almost after 30 days. Higher IgM and IgG in beginning days cause production of much more natural antibodies in kids' bodies to protect them against COVID-19. This clearly shows why kids are more immune against COVID-19. It gives also idea about novel vaccination and medicine strategies to alleviate COVID-19.

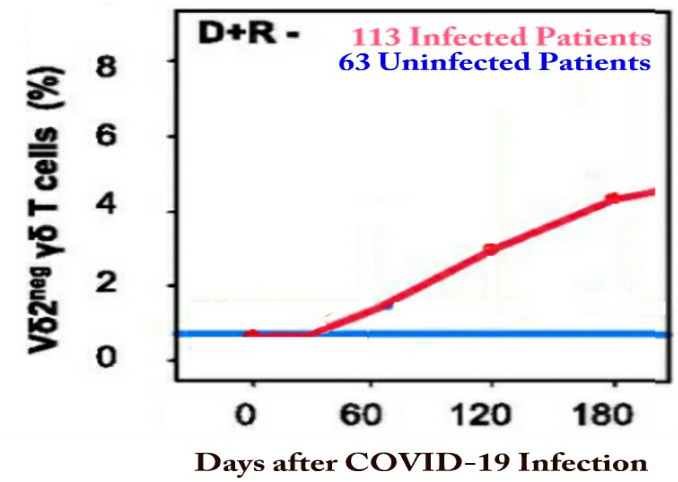


Figure 5. **γδ T cells after infection of COVID-19 in human patients.**

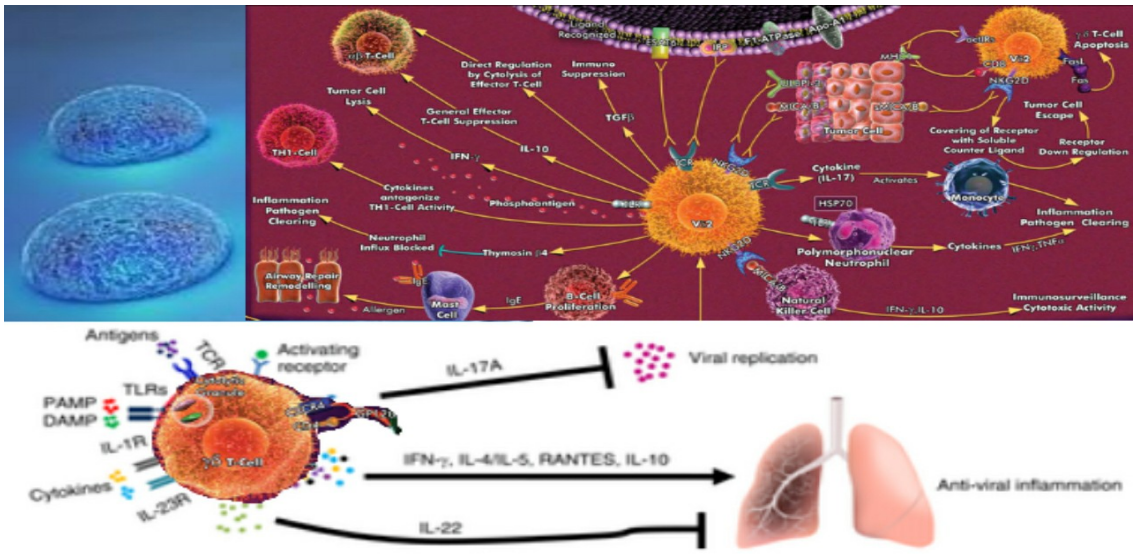


Figure 6. During viral infection of COVID-19, activated lung **γδ T cells produce several types of cytokines which among some inhibit virus replication and some induce of inhibit lung inflammation.**