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Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover.

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Abstract

Background: The recent SARS-Cov2 pandemic, which has recently affected Italy since February 21, constitutes a threat for normal subjects, as the Coronavirus Disease 19 (COVID19) can manifest with a broad spectrum of clinical phenotypes ranging from asymptomatic cases to pneumonia or even death. There is evidence that older age and several comorbidities can affect the risk to develop severe pneumonia and possibly the need of mechanic ventilation in subjects infected with SARS-Cov2. Therefore, we evaluated the outcome of SARS-Cov2 infection in patients with inborn errors of immunity (IEI) such as X linked agammaglobulinemia (XLA).

Methods: When the SARS-Cov2 epidemic has reached Italy, we have activated a surveillance protocol of patients with IEI, to perform SARS-Cov2 search by nasopharyngeal swab in patients presenting with symptoms which could be a manifestations of COVID-19, such as fever, cough, diarrhea or vomiting.

Results: We describe two patients with X-linked agammaglobulinemia (XLA) of 34 and 26 years of age with complete absence of B cells from peripheral blood who developed COVID-19, as diagnosed by SARS-Cov-2 detection by nasopharyngeal swab, while receiving immunoglobulin infusions. Both patients developed interstitial pneumonia characterized by fever, cough and anorexia and associated with elevation of CRP and ferritin, but have never required oxygen ventilation or intensive care.

Conclusion: Our report suggests that XLA patients might present high risk to develop pneumonia after SARS-Cov2 infection, but can recover from infection, suggesting that B cell response might be important, but not strictly required to overcome the disease. However, there is need of larger observational studies to extend these conclusions to other patients with similar genetic immune defects.

Introduction

Since February 21st 2020, several cases of Coronavirus Disease 2019 (COVID19), caused from the β-coronavirus associated with human severe acute respiratory syndrome (SARS) officially named SARS-CoV-2, were identified in Northern Italy (1). The disease has broad spectrum of clinical phenotypes ranging from mild symptomatic cases characterized by influenza-like manifestations, which occurs in the majority of subjects (about 80%) to severe pneumonia requiring admission to intensive care units in 5% or invasive mechanical ventilation, or even death (2). Patients with severe disease usually present comorbidities and are older than those with non-severe disease. Most of COVID19 patients display lymphopenia, which is more pronounced in severe case, thrombocytopenia and elevation of C-reactive protein. The disease has an incubation period of 5-14 days and manifests with fever, cough, vomiting and diarrhea lasting up to 2-3 weeks.

Little is known about the mechanism of immune response against the SARS-CoV-2 , but the first reports suggest that active immunity requires the formation of antigen specific cytotoxic T cells and synthesis of neutralizing antibodies directed against the virus (3,4). There is no specific antiviral treatment for patients with the infection, but many anti-viral or immunomodulatory drugs are under evaluation (5,6). Indeed, COVID19 patients with severe disease have been successfully treated with immune plasma derived from convalescent patients, suggesting that virus-specific neutralizing antibodies are important for disease recovery (7,8).

Methods

After the identification of patients with SARS-Cov2 infection in Italy, we started a survey protocol on patients with chronic diseases who presented COVID19 to identify conditions which could constitute a risk factor for an unfavorable outcome. SARS-Cov2 infection was confirmed in all patients by detection of viral RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay in nasopharyngeal swab specimens obtained during the clinical manifestations. Patient care and research were conducted in compliance with the Case Report guidelines and the Declaration of Helsinki. This study was performed after ethics approvals (Ethics committee of Brescia, protocols NP4000 and NP4047).

Results

Herein we describe two patients with agammaglobulinemia and absence of circulating B cells due to *BTK* mutations who live in Northern Italy and have developed Covid19 during the 2020 outbreak.

Patient 1 is a 34 years old subject who was diagnosed with agammaglobulinemia at 4 months of age because of perianal abscess associated with low levels of immunoglobulins (IgG 161 mg/dl, IgA 6.5 mg/dl, IgM 10 mg/dl as compared to IgG 222-846 mg/dl, IgA 6-60 mg/dl, IgM 28-39 mg/dl in age-matched children) and lack of B cells in peripheral blood (Figure 1). He started intravenous immunoglobulin treatment at the time of diagnosis and switched to subcutaneous immunoglobulins in March 2013. *BTK* genetic analysis revealed a non-sense mutation in exon 2 (K19X) which will cause termination of protein synthesis prematurely. Chest computed tomography (CT) scan performed in Jan 2010 did not reveal bronchiectasis. On January 14th 2020, he performed outpatient blood tests (IgG 860 mg/dl) and received immunoglobulin treatment; then, he continued immunoglobulin therapy at home every 28 days.

On March 5th he presented fever and cough which required medical evaluation and following treatment with oral antibiotics. Because patient general conditions were maintained with normal breathing and unremarkable chest x-ray, he was advised to remain at home in self-isolation to reduce contacts with potential sources of SARS-Cov-2 infection. However, on march 13th , fever and cough reappeared and he was admitted to the hospital suspecting SARS-Cov2 infection, which was confirmed by naso-pharyngeal swab. Because chest radiography revealed interstitial pneumonia with bilateral infiltrates (Figure 2A), we started empirical treatment with lopinavir/ritonavir associated hydroxychloroquine, and subcutaneous infusion of immunoglobulins (400 mg/kg), but he never needed oxygen ventilation. During his stay in the hospital, he developed leukopenia (WB cells 2910 cells/ul), increase of CRP and ferritin (Table 2) which gradually improved in the following days. He was always able of spontaneous breathing and was discharged on March 27th in good health. Analysis of lymphocyte subpopulation during the infection revealed normal T lymphocyte subsets and low levels expression of the activation marker HLA-DR on both CD4 and CD8 subsets (Table 1), while B cells were about 0.03% of total lymphocytes (Figure 1).

Patient 2 is a 26 years old patient who was diagnosed with agammaglobulinemia at sixteen months of age because of respiratory tract infections and low immunoglobulins (IgG 63 mg/dl, IgA 7 mg/dl, IgM 12 mg/dl as compared to IgG 264-1509 mg/dl, IgA 17-178 mg/dl, IgM 48-337 mg/dl in age-matched children), virtual absence of B cells in the blood and *BTK* mutation (S578Y). Since then, he received regular intravenous immunoglobulin infusions every 28 days and stayed in good health as chest CT scan in June 2010 did not reveal bronchiectasis. In the following years, he moved to

London for work reasons, and returned occasionally to see his family in Northern Italy. In February 2020, he visited his family living in Milan just a few weeks before the SARS-Cov2 epidemics. On March 23rd, he performed blood test to determine IgG levels , which were normal (IgG 923 mg/dl), before receiving immunoglobulin infusion. On April 1st he presented anorexia, asthenia and vomiting, without fever or diarrhea. Execution of nasopharyngeal swab at a local hospital in Milan area revealed SARS-Cov2 infection; chest x-ray showed an interstitial pneumonia (Figure 2B) while IgG levels were 863 mg/dl. In the following days, the patient presented fever, but he never required oxygen supplementation. We started treatment with hydroxychloroquine, azithromycin and ceftriaxone and administered immunoglobulin infusion at 400 mg/kg. In the following days his conditions have been improving. Flow cytometry analysis of lymphocyte subpopulations, which was performed 7 days before the appearance of the first symptom, showed normal distribution of T and NK subsets (Table 1).

Discussion

We describe two patients with XLA who have been exposed to SARS-Cov2 during and developed interstitial pneumonia and lymphopenia. Despite the absence of B cells, both subjects could recover without need of intensive care or oxygen ventilation. This observation suggests that T cell response is probably important for immune protection against the virus, while B cell response might be unessential. This is in agreement with preliminary studies which have shown that in normal subjects infected by SARS-Cov2, the number of cytotoxic T cells expressing activation markers such as HLA-DR and CD38 increase during infection(4). However, the risk to develop pneumonia after SARS-Cov-2 infection is quite low in subjects at the same age of the two XLA patients, suggesting that lack of antibody production is probably contributing to disease severity. Moreover, there is also evidence that passive antibody administration through transfusion of convalescent plasma constitutes a feasible strategy to confer immunization to patients with severe COVID-19 and contributes to improve the clinical manifestations and the outcome of these subjects, suggesting that production of neutralizing antibody might also be an important step for disease recovery (7,8). Therefore, lack of neutralizing antibodies in XLA patients might place these subjects at higher risk of disease relapse, suggesting the need of careful monitoring during convalescence.

The observation that patients with x-linked agammaglobulinemia can recover from SARS-Cov2 infection suggests that human immune system could use multiple paths to counteract to viral infection and that a normal T cell immune response can be sufficient to defeat the virus in subjects who cannot synthesize antigen-specific immunoglobulins.

However, BTK expression is not limited to B cells, and it also expressed in myeloid lineages where is involved in the toll-like receptor mediated production of proinflammatory cytokines, such as IL-6 or TNF-α, which are produced in large amount during viral infections, including in patients with SARS-Cov-2 infection (9). Therefore, lack of BTK in myeloid cells of XLA patients might provide a subordinate advantage for these patients by preventing the development of the inflammatory stage of the disease which has been associated with the possible fatal outcome of COVID-19.

However, we cannot draw final conclusions from the description of these two cases of XLA, because the clinical and immunological features of patients with this disease can be very heterogeneous. Indeed, Bruton's tyrosine kinase is required for many checkpoints during B cell differentiation and *BTK* mutations might also have variable effects on B cell precursors survival (10). In one of the two agammaglobulinemic patients, we detected an extremely low number of B cells at various stages of differentiation, suggesting that these cells might play a role in the immune response against the virus. Moreover, both subjects have received immunoglobulin infusions before the infection and have been further supplemented with immunoglobulins at the time of infection. Infusion of polyclonal immunoglobulins derived from pools normal donors has been used to treat COVID-19 patients (11), but there is limited evidence of efficacy. One might speculate that immunoglobulin pools might contain antibodies which could cross-react with SARS-Cov2 proteins by exerting a priming effect on host immune response. Alternatively, immunoglobulins could provide an immunomodulatory action on monocytes and tissue resident macrophages which are involved in the so called "cytokine storm" in the advanced phases of the infection in severe COVID19 patients (12).

Overall, our report provides evidence that B cell response can be dispensable in the immune response against SARS-Cov2 infection, but clinical decisions should be based on data derived by more broad survey of patients with IEI.

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Table 1

	Patient 1	Patient 2	Normal values
$CD3^+$ T lymphocytes (%)	88.4	94.7	(57.1 - 87.6)
$CD3^+$ (cells/µl)	1040	1791	(721 – 2562)
$CD4^+$ T cells (%)	42.1	44.9	(28.5 - 65.6)
CD4+ (cells/µl)	495	849	(273 – 1882)
CD8+ T cells (%)	43.3	30.6	(10.5 - 37.7)
CD8+ (cells/µl)	509	578	(177 – 783)
$\gamma\delta^+$ T cells (%)	3.8	32.4	(0.9 - 11.2)
B cells $CD19^+$ (%)	-	-	(5.8 - 22.1)
$CD19^+$ (cells/µl)	-	-	(86 – 684)
NK cells ($CD3^-CD56^+$. $CD3^-CD16^+$, %)	11.6	5.3	(3.4 - 28.4)
$CD3^+CD4^+$ (%)			
$HLADR^+$	3.1	2.4	(1.6 - 12.2)
Naïve	68	63.7	(20.4 - 63.6)
RTE*	41.2	46.9	(11.4 - 48.1)
RTE abs	204	398	(115 – 913)
Central memory	10.4	22.1	(18.7 - 46.2)
Effector memory	17.2	12.2	(7.1 - 38.0)
Terminally differentiated	4.4	2	(0.3 - 9.1)
$CD3^+CD8^+$ (%)			
$HLADR^+$	7.2	7.2	(2.7 - 31.7)
Naïve	29.7	30.6	(13.1 - 66.5)
Central memory	0.5	4.2	(2.6 - 24.5)
Effector memory	15.5	14	(10.1 - 47.4)
Terminally differentiated	54.3	51.2	(5.2 - 63.5)

*RTE: recent thymic emigrants

Table 2

Clinical chemistry values of two XLA patients with COVID-19

	Patient 1		**Patient 2**		**Normal values**
	19-mar	27-mar	03-apr	10-apr	
C-reactive protein (mg/L)	26	78	3,6	1,5	(< 5)
Lactate dehydrogenase (U/L)	170	194	235	248	(135-225)
Fibrinogen (mg/dl)	598	737	424	517	(170-410)
Ferritin (µg/l)	362	469	603	774	(30-400)
Aspartate transaminase (U/L)	24	22	30	65	(18-54)
Alanine transaminase (U/L)	17	19	26	230	(10-50)

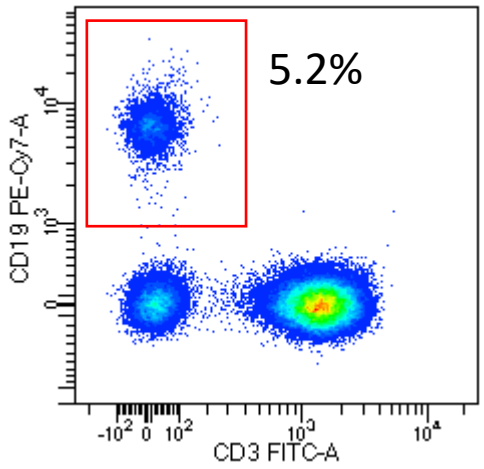
Figure legend

Figure 1. Flow cytometry dot plot of T and B cell subsets in two XLA patients and one control subject.

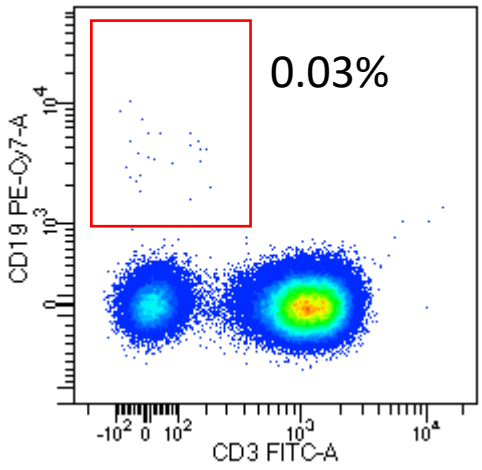
Figure 2. Chest radiography of two XLA patients (Patient 1 in panel A and patient 2 in panel B) with COVID-19.

Figure 1

Control



Patient 1



Patient 2

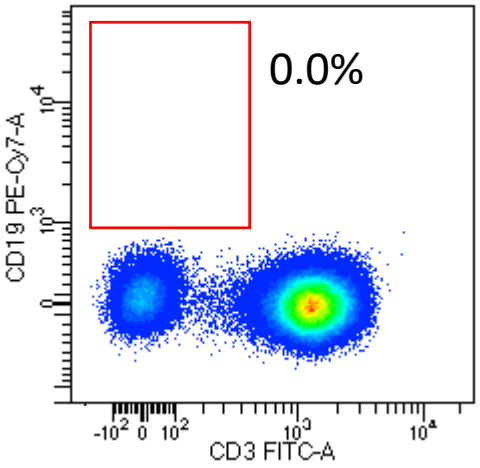
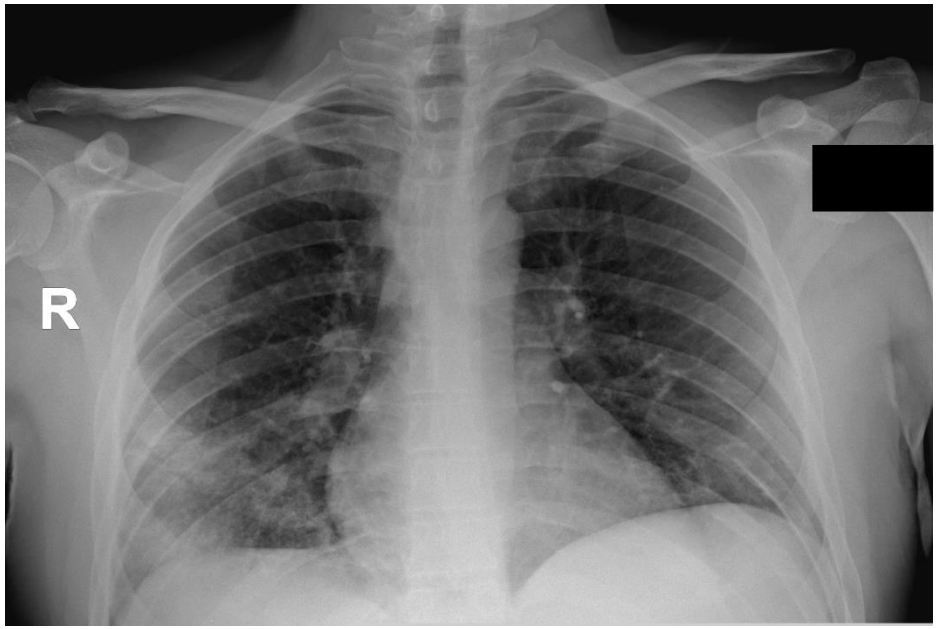


Figure 2

A



B