Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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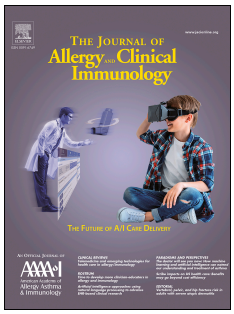

A possible role for B cells in COVID-19?: Lesson from patients with Agammaglobulinemia

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A possible role for B cells in COVID-19?: Lesson from patients with Agammaglobulinemia

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Short summary

COVID-19 had a mild clinical course in patients with Agammaglobulinemia lacking B lymphocytes, whereas it developed aggressively in Common Variable Immune Deficiency. Our data offer mechanisms for possible therapeutic targets.

Key words

Agammaglobulinemia; Common Variable Immune Deficiency; COVID-19; B lymphocytes; BTK

"To the Editor."

An epidemic of Coronavirus SARS-CoV-2 has become the focus of scientific attention[1]. The high infectivity of SARS-CoV-2 and rapid rise in number of patients affected reflects lack of pre-existing immunity as reported by the World Health Organization (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The clinical presentation of Coronavirus disease 2019 (COVID-19) is variable, ranging from lack of symptoms to severe respiratory distress, and multi-organ failure requiring intensive care unit admission and mechanical ventilation. Treatment of COVID-19 requires in-depth knowledge of the immune-mediated mechanisms of the disease. To date, we have identified seven Primary Antibody Deficiencies (PAD) patients with COVID-19 infection: five affected with Common Variable Immune Deficiencies (CVIDs) and two affected with Agammaglobulinemia, one with X-linked Agammaglobulinemia (XLA) and one with Autosomal Recessive Agammaglobulinemia (ARA)[2]. All Primary Antibody Deficiency patients have defective antibody production. Patients with Agammaglobulinemia lack B lymphocytes whereas patients with Common Variable Immune Deficiency have dysfunctional B lymphocytes. In patients affected with Agammaglobulinemias, the COVID-19 course was characterized by mild symptoms, short duration, with no need of treatment with the immune-modulating drug blocking IL-6, and had a favorable outcome. In contrast, patients affected with Common Variable Immune Deficiencies presented with a severe form of the disease requiring multiple drug treatment, including antiretrovirals agents and IL-6 blocking drugs, and mechanical ventilation (Table 1). The strikingly different clinical course of COVID-19 in patients with Agammaglobulinemia compared to CVIDs cannot be explained by the level of serum immunoglobulins which were similarly low in all PAD patients at diagnosis, and were maintained at adequate and comparable levels in all patients by immunoglobulin substitutive therapy (On line Repository, Table). A detailed COVID-19 clinical history, laboratory data, type and dosage of administered treatment, and disease timing, are provided for each patient in On line Repository, Case reports. Lung HRCT of a patient with Common Variable Immune Deficiency at hospital admission for COVID-19 showed extensive ground glass opacities associated with areas of alveolar consolidation in the upper and lower lobes where the alveolar component predominates over the interstitial one. (Fig. 1A). Upon treatment, lung HRCT showed reduction in extension of ground glass opacities and areas of alveolar consolidation. (Fig. 1B). Differently, lung HRCTs of a patient with Agammaglobuliniemia performed at the time of COVID-19 was unchanged with respect to lung HRCT performed one year earlier, and showed bronchiectasis and sequelae of right lung pneumonectomy done at the age of 18 (Fig. 1C, and 1D). All patients with primary antibody deficiencies are equally vulnerable to most bacterial infections since antibodies are important in blocking infectivity and preventing diseases. In addition, antibodies have a role in the immune response to viral infections[3]. Patients with Agammaglobulinemia are susceptible to a limited number of viral infections only, mainly norovirus and enteroviruses such as polioviruses[4] with an increased incidence of post-vaccination poliomyelitis due to the oral attenuated Sabin vaccine[5]. CVIDs patients are susceptible to rhinoviruses, noroviruses, and herpesviruses that on turn play a role in driving an underlying inflammatory condition. Since only Agammaglobulinemia patients had a mild course of COVID-19, we speculate on a possible role of B

lymphocytes in the SARS-CoV-2 induced inflammation. We have already shown that children appear to contain better SARS-CoV-2 in the early phase of infection, possibly because their B cells are able to generate natural antibodies timely upon encounter with novel pathogens when compared to B cells from adults[6]. The role of inflammation in aggravating the clinical picture of subjects with COVID-19 has already been described. Treatment with drugs such as IL-6 inhibitors aimed at reducing the Cytokine Storm Syndrome (CSS) and lung inflammation associated with a profound increase of cytokines such as IL-6 and increased ferritin[7] have already been carried out, initially on an individual basis, and currently within clinical trials. Of note, our CVID patients (3 out of 5) that required IL-6 blocking treatment presented increased ferritin serum levels (Online Repository Material). It has been demonstrated that B cells produce IL-6 to drive germinal center formation. In patients unable to carry on the physiological immune response, IL-6 produced by B cells may increase the level of inflammation. Lack of B cell-derived IL-6 abrogates spontaneous autoimmune germinal center formation in a mouse model, resulting in protection from systemic autoimmunity[8]. Thus, it appears that CSS may play a significant role in the respiratory failure in COVID-19 infection. The role of B cells in determining lung inflammatory disorders is also demonstrated by the observation that granulomatous-lymphocytic interstitial lung disease (GLILD), occurring in 10% of CVID patients, can be treated with B cell depleting drugs[9]. COVID-19 treatments might contemplate the possibility to damper the inflammatory functions of B cells, and to block cytokine production by monocytes and dendritic cells. Our data represent the first description of COVID-19 in patients affected with primary antibody defects, offer useful insights in the putative mechanisms underlying the immunologic response to the infection, and suggest possible clues to novel therapeutic targets.

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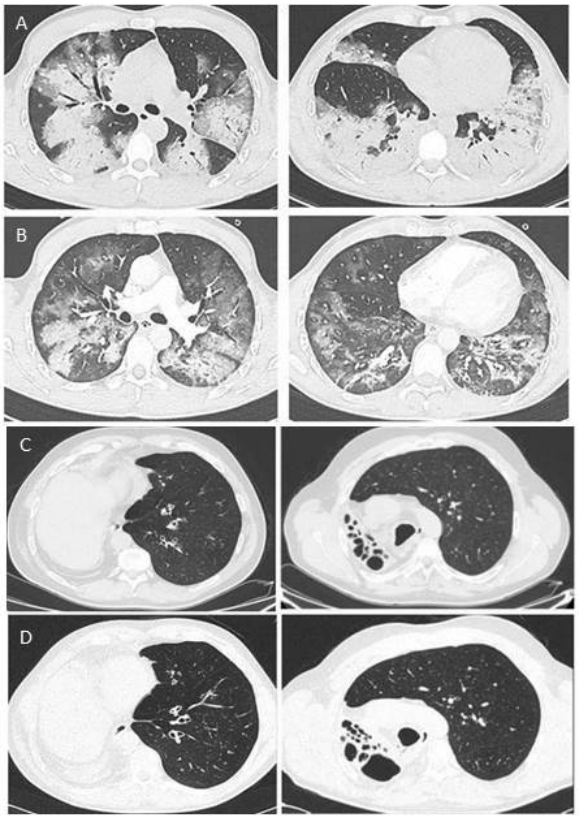
Table 1. Summary of data of the seven PAD patients with COVID-19.

Patient	PAD	Age	Sex	COVID-19				
				Clinical symptoms	Days	Treatment	ICU	Outcome
1	ARA	56	M	No symptoms	0	hydroxychloroquine, azithromycin, darunavir/cobicistat	no	recover
2	XLA	34	M	High fever	3	hydroxychloroquine, ceftriaxone, lopinavir/ritonavir	no	recover
3	CVID	59	F	High fever, dyspnea	20	hydroxychloroquine, azithromycin, tocilizumab	yes	death
4	CVID	32	F	High fever, dyspnea	16	hydroxychloroquine, darunavir/ritonavir, tocilizumab	no	recover
5	CVID	57	M	High fever, dyspnea	25	hydroxychloroquine, lopinavir/ritonavir, remdesivir methylprednisolone.	yes	recover
6	CVID	52	M	High fever, dyspnea	21	hydroxychloroquine, azithromycin, lopinavir/ritonavir,	no	recover
7	CVID	41	M	High fever, dyspnea	19	hydroxychloroquine, piperacillin/tazobactam, lopinavir/ritonavir, tocilizumab, remdesivir	yes	recover

Fig. 1. Lung HRCT in a patient with Common Variable Immune Deficiency at admission showing extensive ground glass opacities associated with areas of alveolar consolidation in the lower lobes where the alveolar component predominates over the interstitial one (A: left: mid upper: right: lower), and upon treatment showing reduction in extension of ground glass opacities and areas of alveolar consolidation. (B: left: mid upper: right: lower). Lung HRCT in a patient with Agammaglobulinemia. Axial sections showing bronchiectasis and sequelae of right lung pneumonectomy (C: March 2020; D: January 2019)



"Online Repository"

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Case reports

Patient 1. Male, 53 years old with a diagnosis of Agammaglobulinemia in 1985, 18 years of age, two years after a right lung upper lobectomy for bronchiectasis. Since 1985 he received intravenous immunoglobulin replacement at a cumulative monthly dosage of 600 mg/kg. In 2005 a diagnosis of Autosomal Recessive Agammaglobulinemia was made, after exclusion of Bruton tyrosine (BTK) mutation. As in most ARA patients we did not find other mutation including μ chain mutation. Treatment notwithstanding, chronic sinusitis, COPD, and bilateral lung bronchiectasis progressively worsened. In 2019, therapy was shifted to

facilitated subcutaneous immunoglobulins. On March 12, after two SARS-Cov2 infections among family members were ascertained, an oropharyngeal swab was performed and the patient resulted SARS-Cov2 positive. At that time the patient was completely asymptomatic. The contact was likely his wife who was presumably infected on February 29 by a relative from Milan (an Italian high risk area), and who in turn infected both her mother, who died on March 20, and the patient. His wife developed anti-SARS-Cov2 antibodies (Chemtrue, Novel Coronavirus, 2019-nCov IgM/IgG antibody diagnostic kit, SIC, Italy) 15 days after a mild fever with anosmia. The patient never presented any clinical symptoms of COVID-19 and, one week after the first swab, a repeated nasopharyngeal swab turned negative. Lymphocyte counts stayed within the normal range (L 1600 cells/mm^3) as well as CRP (1 mg/L, normal range: <5), LDH 143 U/L, IL-6 1 ng/L serum levels. The lung High Resolution Computed Tomography (HRCT) performed on the same day was unchanged with respect to lung HRCT performed one year earlier (Fig. 1A and 1B). Since March 26 he is on hydroxychloroquine 200 mg bid, azithromycin 500 mg qd, darunavir/cobicistat 800/150 mg qd.

Patient 2. Male, 34 years old, with a diagnosis of with X-Linked Agammaglobulinemia established at the age of 1 year. He has been since on Ig replacement treatment at 400 mg/kg/dose every 3 weeks. His clinical history includes recurrent skin and respiratory infections. He developed bronchiectasis at the age of 16. Four months before the diagnosis of COVID-19, the differential blood count was normal. On March 13, he presented with fever >38°C. At home, he received paracetamol, ibuprofen, amoxicillin clavulanate and levofloxacin. On March 19, he was admitted to the ER. The patient did not show respiratory difficulty and his pulse oxygen saturation was 98%. His initial blood workout showed mild leukopenia (3840 cells/mm^3), slightly elevated CRP (26 mg/L, normal values <5.0). The chest X-ray showed mild interstitial alveolar infiltrates. His oropharyngeal swab resulted positive for SARS-Cov2. He was started on lopinavir/ritonavir 200/50 mg 2 cp bid, hydroxychloroquine 200 mg bid, and ceftriaxone due to previous allergic reaction to azithromycin. After two days, the fever resolved and the patient was discharged home on March 27.

Patient 3. Female, 59 years old, with a diagnosis of CVID. At the age of 48 years, low IgG, IgA and IgM serum levels were first detected after the occurrence of upper and lower respiratory tract infections, and a diagnosis of CVID was made after exclusion of secondary forms of hypogammaglobinemia. Since then she has been on immunoglobulin replacement with intravenous immunoglobulin at a cumulative monthly dosage of 400 mg/kg. With time, she has progressively developed chronic sinusitis, chronic bronchitis, and chronic gastritis. In 2009, she shifted to self-administered subcutaneous immunoglobulins. The patient resulted SARS-Cov2 positive in a nasopharyngeal swab performed on March 8 when she was hospitalized because of high fever, and dyspnea. The contact was not identified, as her husband and her son became positive five days later. Endotracheal intubation was performed in the intensive care unit (ICU) and therapy with tocilizumab (8 mg/kg/die) was started, but she never improved. She died on March 25.

Patient 4. Female, 32 years old, with a diagnosis of CVID established at the age of 14 years when low IgG, IgA and IgM serum levels were first detected in a routine assessment. A diagnosis of CVID was made after exclusion of secondary forms of hypogammaglobinemia. She refused to be started on immunoglobulin

replacement. She was never diagnosed with respiratory infections, her clinical history includes endometriosis, celiac-like disease, allergy, and skin cancer (melanoma). On March 10, she developed cough and mild fever (temperature ranging from 37.3°C to 38°C). She went to the ER where nasopharyngeal, and oropharyngeal swabs resulted negative for SARS-Cov2. Nine days later, on March 19, cough and fever worsened, while dyspnea and chest pain appeared. She contacted again the ER and a new nasopharyngeal and oropharyngeal swabs resulted positive for SARS-Cov2. Despite respiratory symptoms, her room air oxygen saturation was fair (95%) and she was allowed to go home. After 4 more days, on March 23, her conditions worsened with a high fever (39°C), and room air oxygen saturation of 80%. She was admitted to the infectious diseases department. Laboratory tests showed leucopenia without lymphopenia (WBC 3200 cells/mm^3, L 1480 cells/mm^3, CRP 7.5 mg/L (normal value <5), ferritin 4000 ug/L, LDH 270 U/L. She started oxygen supplementation by venti-mask at 4 L/hr. A lung HRCT showed interstitial pneumonia. On day 1, she was on started therapy with darunavir 800 mg qd plus ritonavir 100 mg qd for 6 days, hydroxychloroquine 200 mg bid for 6 days. On day 2, intravenous tocilizumab was started (8 mg/kg/die) after a high IL-6 level (10.6 ng/L) was detected. On day 3, laboratory tests were as follow : WBC 3600 cells/mm^3, L 1670 cells/mm^3, PCR 3.9 mg/L, LDH 196 U/L. Fever abated to 37.3°C, and oxygen saturation increased to 98% (on supplemental oxygen 4 L/hr). On day 4, the patient was afebrile although a nasopharyngeal swab was still positive for SARS-Cov2. Her clinical conditions furtherly improved with reduction in cough, as well as in respiratory and heart rate. Oxygen saturation stayed at 98%. Up to March 27, she has received 2 doses of tocilizumab.

Patient 5. Male, 57 years old, with a diagnosis of CVID. At the age of 49 years the patient underwent laboratory investigations because of recurrent upper and lower respiratory tract infections. Low IgG, IgA and IgM serum levels were then detected, and a diagnosis of CVID was made after exclusion of secondary forms of hypogammaglobinemia. His clinical history includes asthma, hypertension, overweight/obesity (weight 106 kg, BMI 26.77). Since diagnosis he was started on subcutaneous immunoglobulins at a cumulative monthly dose of 400 mg/kg. The asthma treatment included oral steroids; anti-hypertensive drugs (Ca-antagonist, telmisartan ticlopidine); inhalation treatment with steroids and long acting Beta-agonists (fluticasone/salmeterol). On February 21, he developed dry cough and mild to moderate fever (temperature ranging from 37.5°C to 38.5°C), and myalgia. His General Practitioner ordered levofloxacine 750 mg/day. On February 24, mild dyspnea appeared. On February 26, because of worsening dyspnea, he was admitted to the ER where nasopharyngeal, and oropharyngeal swabs were positive for SARS-Cov2. The chest X-ray showed initial evidence of interstitial pneumonia and two opacities which were interpreted as alveolar consolidation. The initial laboratory investigations revealed lymphopenia (620 cells/mm^3), CRP 120 mg/dl, LDH 266 U/L. He was immediately started on high-flow nasal oxygen supplementation and methylprednisolone (80 mg/day iv). On February 27, therapy with lopinavir/ritonavir and hydroxychloroquine 200 mg bid was started. On February 29, laboratory tests showed decreased lymphocyte count (370 cells/mm^3), CRP 29 mg/dl, LDH 335 U/L. On March 1, his conditions worsened, blood oxygen saturation dropped below 85%, the patient underwent endotracheal intubation and was transferred to the

intensive care unit. Antiviral treatment was changed to remdesivir 200 mg iv/qd (first day) followed by remdesivir 100 mg iv qd, while continuing methylprednisolone 0.8 mg/kg. Broncho alveolar lavage resulted positive for beta-d-glucan and Candida. Vancomycin, meropenem, linezolid, caspofungin, cotrimoxazole were started. On March 3, laboratory tests showed lymphopenia (300 cells/mm^3), CRP 120 mg/dl, IL-6 11.8 mg/dl. On March 12, clinical conditions appeared markedly improved while lymphocyte count returned to normal (1.300 cells/mm^3), he was then extubated, and started on non-invasive ventilation which was maintained until March 17. On March 17, his laboratory investigations showed: L 1940 cells/mm^3, LDH 189 U/L, CRP 3.8 mg/dl. On March 26, he was discharged home in good clinical conditions after a nasopharyngeal swab resulted negative for SARS-Cov2. He is actually on home treatment with prednisone 25 mg/day and subcutaneous immunoglobulins.

Patient 6. Male, 52 years old, with a diagnosis of CVID made in 1996. His clinical history included immune thrombocytopenia, polyclonal lymphoproliferation (polydistrectual lymphadenopathy and splenomegaly), recurrent infections (giardiasis, pneumonia due to Haemophilus influenzae) and interstitial lung disease (HRCT finding of macro nodules, ground-glass opacities, mediastinal lymphadenopathies, and negative mycobacterium screening) with maintained lung function. Since diagnosis he was started on subcutaneous immunoglobulins at a cumulative monthly dose of 400 mg/kg. On March 12, the patient developed fever (maximum temperature 39.2°C) and a mild exercise-induced dyspnea. One day later, his wife and one of his two daughters showed milder general symptoms (remittent fever without cough or dyspnea). According to the current Italian guidelines for the management of the COVID-19 epidemics, since symptoms were still present six days from their appearance, the patient's General Practitioner arranged for the patient admission to the Infectious Disease Unit appointed to perform the emergency nasopharyngeal swab for SARS-CoV-2 nucleic acid detection and a lung HRCT. Nasopharyngeal swab resulted positive for SARS-CoV-2, and lung HRCT showed a bilateral interstitial pneumonia. Therapy with lopinavir/ritonavir 400/100 mg qd, azithromycin 500 mg qd, hydroxychloroquine 200 mg bid, was started. No oxygen-supplementation was required during the course of the disease, as peripheral oxygen saturation was constantly above 90%. Fever and dyspnea completely resolved five days after the beginning of the treatment. A new nasopharyngeal swab performed 9 days after the beginning of therapy resulted negative, while no plasma viral replication was detected. As a relevant improvement of interstitial pneumonia was documented, patient was discharged, and a 14-days home isolation period was ordered.

Patient 7. Male, 41 years old, with a diagnosis of CVID established at the age of 14. Secondary causes of hypogammaglobulinemia were excluded. During childhood, he suffered from recurrent respiratory infections and measles-associated pneumonia. His clinical history was complicated by recurrent sinusitis and mild eczema. The patient received Ig replacement treatment at 400 mg/kg/dose every 4 weeks with intravenous immunoglobulins until 2017 when he switched to facilitated subcutaneous preparations. On March 8, the patient presented with high fever, cough, and dyspnea. At home he received paracetamol, ibuprofen and amoxicillin clavulanate. On March 16, as his conditions deteriorated he was admitted to the ER. Pulse

oxygen saturation was 80%, and the patients was started on Non invasive-ventilation with continuous positive airway pressure. His initial blood workout showed lymphopenia (800 cells/mm^3) with elevated CRP (315 mg/L, normal values <5.0). Chest x-ray showed diffuse interstitial alveolar infiltrates. Lung HRCT at admission confirmed extensive infiltrates (Fig. 2A). Oropharyngeal swab resulted positive for SARS-CoV-2. He was started on lopinavir/ritonavir 400/100 mg qd, hydroxychloroquine 200 mg bid, and piperacillin/tazobactam. After admission, his respiratory condition worsened dramatically and he was started on mechanical ventilation. Laboratory tests showed increased ferritin (7200 ug/L; normal values <400), and LDH serum levels (495 U/L; normal values <225). Therapy with tocilizumab (8 mg/kg/die) was started. After two days of mechanical ventilation, the patient was switched to remdesivir 200 mg iv/qd (first day) followed by remdesivir 100 mg iv qd. The clinical condition, and lung HRCT improved (Fig. 2B), and 72 hours later he did not require any more mechanical ventilation. He is still hospitalized, with a steady improvement of clinical conditions, and laboratory values.

Table 2. Summary of immunological data of the seven PAD patients collected 1-6 months before COVID-19.

#	**Date of last investigation**	**IgG (mg/dL)**	**IgA (mg/dL)**	**IgM (mg/dL)**	**Lymphocytes (mm3)**	**CD19 (mm3)**	**CD3 (mm3)**	**CD4 (mm3)**	**CD8 (mm3)**	**NK (mm3)**
1	January 2020	750	0	0	1300	0	1247	460	739	25
2	November 2019	800	0	0	1700	0	1600	900	700	28
3	January 2020	897	30	33	1600	400	1030	672	338	46
4	January 2020	500	0	153	2050	200	1800	950	850	30
5	October 2019	550	40	44	3400	96	3200	2767	1658	21
6	December 2019	662	11	8	890	55	750	274	258	85
7	September 2019	700	10	30	1800	278	1500	800	700	15