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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Editorial

Silencing of immune activation with methotrexate in patients with COVID-19

It is becoming apparent that patients with COVID-19 are developing a wide variety of autoimmune syndromes many of which involve the nervous system (Table 1). These syndromes most often occur when patients are recovering from the acute viral symptoms. Even in patients with active infection in the lungs, the inflammatory response to the viral infection can be overwhelming in some which is thought to be the major cause of acute respiratory distress syndrome or the viral pneumonia [1,2]. The inflammation is also thought to mediate a hypercoagulable state leading to thromboses in multiple blood vessels and organ systems including the brain [3,4]. Thus, these inflammatory syndromes are a major cause of morbidity and mortality in this patient population.

It is not unusual for viral infections to cause a massive immune response that overwhelms the host. However, the genetic factors that determine the susceptibility are not entirely clear. When a viral pathogen first infects a new host, there is a massive immune attack mounted by the immune system in an effort to control the organism, however the process of heightened immunity results in substantial injury to the host. This has been best studied in the context of retroviruses. For example, the simian immunodeficiency virus in the sooty mangabey macaques is non-pathogenic even though it produces very high titers of virus. There is a lack of immune response to the viral infection [5]. The same virus when inoculated into other species of macaques can cause an overwhelming inflammation and an AIDS like syndrome. However the adaptation to the host can take thousands of years and ultimately these retroviruses can become part of the mammalian genome itself [6]. Similarly, a number of viruses that cause little pathogenicity in bats when transmitted to humans can induce devastating syndromes. This has been attributed to the lack of stimulation of the interferon pathway in bats [7]. These include Ebola, Marburg, Nipah, Rabies, Hendra, MERS, SARS and now SARS-CoV-2 [8]. Massive inflammation accompanies most of these infections resulting in bystander injury. Several terms have been used to describe this remarkable inflammatory response that includes cytokine storm, viral sepsis and PANIC (Prolific Activation of a Network-Immune Inflammatory Crisis) [9–11].

At the early stage of severe COVID-19 infection, there are features of macrophage activation syndrome with increased C reactive protein, ferritin and cytokines like IL-1beta, IL-6, IL-8 and TNF-alpha [12]. Single cell sequencing of peripheral blood cells demonstrates a remarkable increase in inflammatory monocytes particularly CD14 + IL-1 beta+ cells at the early stage of recovery that normalizes later in the course of recovery [13]. Lymphopenia and lower numbers of CD4 and CD8 T cells if present in early COVID-19 infection [14] are associated with more severe disease and poor prognosis [15]. However, percentage of inflammatory CCL6+ Th17 cells increases and circulating regulatory T cells severely declines in these patients. [15,16]

In later stages of infection lymphopenia and atrophy of the spleen and necrosis of the lymph nodes has been noted at autopsy, In these autopsy cases macrophage infiltration is a key pathological finding in the lung [17]. Additionally, ACE2+ CD169+ macrophages are infected with SARS-COV2 in spleen and lymph nodes and lead to IL-6 production and lymphocyte apoptosis through FAS/FASL pathway [18]. Activation of alternative complement pathway and complement deposition in microvasculature of lung and other tissues has also been described [19] Use of an anti-inflammatory drugs in the presence of an active infection may sound counterintuitive. However, control of the inflammation is critically important to prevent catastrophic end organ damage. Even though the virus is the agent that triggers the immune response, control of the virus alone may not be sufficient for rapid dampening the immune response, which is driven by multiple positive feedback loops. For example, there is extensive experience in the use of anti-inflammatory drugs such as corticosteroids in patients with bacterial meningitis where it is given early in the course of infection along with antimicrobial drugs [20]. In the case of COVID-19, recent studies show that Remdesivir which is a nucleoside analog has a modest effect on the infection by decreasing the duration of hospitalization but no significant effect on mortality [21,22]. It was recently approved by the FDA for emergency use for treatment of the infection. Several immune modulatory drugs have been used in patients with COVID-19 and some are in clinical trials (Table 2). However, careful consideration needs to be given to the choice of anti-inflammatory drugs, since they all have different mechanisms of action and hence affect different arms of the immune system. For CNS syndromes additional consideration for penetration across the blood brain barrier needs to be considered as well. Hence a thorough understanding of the pattern of immune activation by SARS-CoV-2 is important. Many clinical studies are underway and immune manifestations of the infection will soon become well characterized. But even now it is clear that complement activation and macrophage mediated lung injury is a major concern. And in some patients antibody-mediated syndromes are becoming apparent. The cause of hypercoagulability is also thought to be immune mediated and antiphospholipid antibodies have been detected in these patients but this needs further investigation [3].

In the current manuscript, the authors provide strong rationale for the use of high dose methotrexate for treatment of the SARS-CoV-2 associated inflammatory syndromes [10]. As discussed by the authors, the primary mode of action of methotrexate is via inhibition of folate dependent pathways leading to inhibition of DNA synthesis in rapidly dividing tumor cells [10]. However the anti-inflammatory effects of methotrexate are broad particularly at high dosages and the mechanisms are not fully understood. Methotrexate significantly decreases IL-6 and TNFa in T cells and increase regulatory T cells [23]. Activated T cells undergo cell division so the effects of methotrexate on

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Table 1
Autoimmune and inflammatory syndromes associated with COVID-19.

Neurological
Acute disseminated encephalomyelitis [26]
Acute Necrotizing Hemorrhagic Encephalopathy [27]
Transverse myelitis [28]
Neuromuscular
Guillain Barre Syndrome [29]
Rhabdomyolysis [30]
Myocarditis [31]
Pulmonary
Acute Respiratory Distress Syndrome [17]
Systemic
Vascular occlusions [4]
Kawasaki Syndrome [32]
Chronic Fatigue Syndrome
Others
Purpuric rash [19]
Keratoconjunctivitis [33]
Orchitis [34]

Table 2
Anti-inflammatory Agents currently being used.

Corticosteroids: broad immunosuppressant effects but induces SARS-CoV-2 receptor, ACE2
Tocilizumab; Kevzara: inhibits IL-6 receptor and downstream pathways
Anakinra: inhibits IL-1 and downstream pathways
Eculizumab: blocks complement pathway
Plasmaphoresis: Removes soluble mediators; little effect on immune cells,; also removes antiviral antibodies; contamination of equipment, some patients are hemodynamically unstable

inflammatory T cells can also be explained by action on the folate pathway. In addition, the drug also binds directly to high-mobility group box 1 (HMGB1) protein and by doing so inhibits its interactions with RAGE leading to inhibition of cytokine production by macrophages [24]. High dose Methotrexate is shown to decrease monocyte proliferation and modify their function towards less inflammatory cytokine profile [25].

Administration of high dose methotrexate requires careful consideration of a number of factors. The authors provide a detailed protocol and list potential drug-drug interactions. The authors share their personal experience in treating neuroinflammatory disorders, which includes a single case of adenovirus associated encephalomyelitis and several other cases of autoimmune CNS diseases that had failed other modes of treatment [10]. Another advantage of methotrexate is that it is widely available, it is relatively inexpensive, there is extensive experience with the drug, it has good CNS penetration and can also be administered intrathecally if necessary.

In conclusion, select patient populations COVID-19 in whom cytokine storm or other autoimmune syndromes are a major consideration, methotrexate is a reasonable choice but should be done in the context of a clinical trial so that data on the effects on the virus and the immune system can be collected. The recent approval of Remdisivir as an antiviral agent, now makes it possible to treat patients with a combination of the two drugs. In fact combining Remdesivir with an anti-inflammatory agent will likely improve its efficacy.

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References

[1] Bradley BTMH, R. Johnston, et al., Histopathology and ultrastructural findings of fatal COVID-19 infections, MedRxiv (2020), https://doi.org/10.1101/2020.04.17.20058545.
[2] X. Cao, COVID-19: immunopathology and its implications for therapy, Nat. Rev. Immunol. 20 (2020) 269–270.
[3] Y. Zhang, M. Xiao, S. Zhang, et al., Coagulopathy and antiphospholipid antibodies in patients with Covid-19, N. Engl. J. Med. 382 (2020) e38.
[4] F.A. Klok, M. Kruip, N.J.M. van der Meer, et al., Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis, Thromb. Res. S0049-3848 (2020) 30157–30162.
[5] D. Palesch, S.E. Bosinger, G.K. Tharp, et al., Sooty mangabey genome sequence provides insight into AIDS resistance in a natural SIV host, Nature 553 (2018) 77–81.
[6] M. Garcia-Montojo, T. Doucet-O'Hare, L. Henderson, A. Nath, Human endogenous retrovirus-K (HML-2): a comprehensive review, Crit. Rev. Microbiol. 44 (2018) 715–738.
[7] J.L. Xie, Y.; Shen, X., et al., Dampened STING-dependent interferon activation in bats, Cell Host Microbe (2020), https://doi.org/10.1016/j.chom.2018.01.006.
[8] L.F. Wang, D.E. Anderson, Viruses in bats and potential spillover to animals and humans, Curr. Opin. Virol. 34 (2019) 79–89.
[9] H. Li, L. Liu, D. Zhang, et al., SARS-CoV-2 and viral sepsis: observations and hypotheses, Lancet 395 (2020) 1517–1520.
[10] E.M. Frohman, E. Melamed, R.A. Cruz, et al., High-dose methotrexate with leucovorin rescue therapy for severe COVID-19: an immune stabilization strategy for SARS-CoV-2 induced 'PANIC'* attack, J. Neurol. Sci. (2020), https://doi.org/10.1016/j.jns.2020.116935 [Epub ahead of print].
[11] E.M. Frohman, E. Melamed, R.A. Cruz, et al., SARS-CoV2 triggered "PANIC"* attacck on severe COVID-19, J. Neurol. Sci. (2020).
[12] McGonagle DSK, A. O'Regan, C. Bridgewood, The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease, Aotoimmun Rev. 19 (6) (2020) 102537, https://doi.org/10.1016/j.autrev.2020.102537 Jun.
[13] W. Wen, W. Su, H. Tang, et al., Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing, Cell Discov. 6 (2020) 31.
[14] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.
[15] C. Qin, L. Zhou, Z. Hu, et al., Dysregulation of immune response in patients with COVID-19 in Wuhan, China, Clin. Infect. Dis. (2020), https://doi.org/10.1093/cid/ciaa248 Online ahead of print.
[16] D. Wu, X.O. Yang, TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor fedratinib, J. Microbiol. Immunol. Infect. S1684-1182 (2020) 30065–30067.
[17] Z. Xu, L. Shi, Y. Wang, et al., Pathological findings of COVID-19 associated with acute respiratory distress syndrome, Lancet Respir. Med. 8 (2020) 420–422.
[18] Chen YFZ, F. Bo, et al., The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly decimates human spleens and lymph nodes, MedRxiv (2020), https://doi.org/10.1101/2020.03.27.20045427.
[19] C. Magro, J.J. Mulvey, D. Berlin, et al., Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases, Transl. Res. S1931-5244 (2030070-0) (2020 Apr 15), https://doi.org/10.1016/j.trsl.2020.04.007 [Epub ahead of print], .
[20] F. McGill, R.S. Heyderman, S. Panagiotou, et al., Acute bacterial meningitis in adults, Lancet 388 (2016) 3036–3047.
[21] Wang YZD, G. Du, et al., Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial, Lancet (2020), https://doi.org/10.1016/S0140-6736(20)31022-9.
[22] H. L. Hopes rise for coronavirus drug remdesivir, Nature (2020), https://doi.org/10.1038/d41586-020-01295-8.
[23] A.P. Cribbs, A. Kennedy, H. Penn, et al., Methotrexate restores regulatory T cell function through demethylation of the FoxP3 upstream enhancer in patients with rheumatoid arthritis, Arthritis Rheum. 67 (2015) 1182–1192.
[24] Y. Kuroiwa, Y. Takakusagi, T. Kusayanagi, et al., Identification and characterization of the direct interaction between methotrexate (MTX) and high-mobility group box 1 (HMGB1) protein, PLoS One 8 (2013) e63073.
[25] A.H. Gerards, S. de Lathouder, E.R. de Groot, et al., Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis, Rheumatology (Oxford) 42 (2003) 1189–1196.
[26] T.R. Zhang, M.; Hirsh, E., COVID-19-associated acute diseeminated encephalomyelitis: a case report, MedRxiv (2020), https://doi.org/10.1101/2020.04.16.20068148.
[27] N. Poyiadji, G. Shahin, D. Noujaim, et al., COVID-19-associated acute hemorrhagic necrotizing encephalopathy: CT and MRI features, Radiology 201187 (2020).
[28] Zhou KHJ, D. Dai, Y. Feng, L. Liu, S. Nie, Acute myelitis after SARS-CoV-2 infection: a case report, (2020), https://doi.org/10.1101/2020.03.16.20035105v1.full.pdf.
[29] G. Toscano, F. Palmerini, S. Ravaglia, et al., Guillain-Barre syndrome associated with SARS-CoV-2, N. Engl. J. Med. (Apr 17 2020), https://doi.org/10.1056/NEJMc2009191 Online ahead of print.
[30] M. Jin, Q. Tong, Rhabdomyolysis as potential late complication associated with COVID-19, Emerg. Infect. Dis. 26 (2020).
[31] C. Chen, Y. Zhou, D.W. Wang, SARS-CoV-2: a potential novel etiology of fulminant myocarditis, Herz 45 (3) (May 2020) 230–232, https://doi.org/10.1007/s00059-020-04909-z.
[32] A.S. Harahsheh, N. Dahdah, J.W. Newburger, et al., Missed or delayed diagnosis of Kawasaki disease during the 2019 Novel coronavirus disease (COVID-19) pandemic, J. Pediatr. S0022-3476 (2030556-4) (May 3 2020), https://doi.org/10.1016/j.jpeds.2020.04.052 Online ahead of print.
[33] M. Cheema, H. Aghazadeh, S. Nazarali, et al., Keratoconjunctivitis as the initial medical presentation of the novel coronavirus disease 2019 (COVID-19), Can. J.

Ophthalmol. S0008-4182 (2030305-7) (Apr 2 2020), https://doi.org/10.1016/j.jcjo.2020.03.003 Online ahead of print.
[34] J. Xu, L. Qi, X. Chi, et al., Orchitis: a complication of severe acute respiratory syndrome (SARS), Biol. Reprod. 74 (2006) 410–416.

Farinaz Safavi, Avindra Nath*
Section of Infections of the Nervous System, National Institute of Neurological Disorders and stroke, National Institutes of Health, Bethesda, MD, United States of America
E-mail address: natha@ninds.nih.gov (A. Nath).

* Corresponding author at: Bldg 10; Room 7C-103, 10 Center Drive, Bethesda, MD 20892, United States of America.