



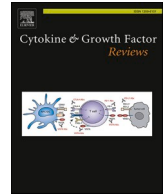
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Interferons and other cytokines, genetics and beyond in COVID-19 and autoimmunity

Ghislain Opendakker*, Jo Van Damme

Laboratory of Immunobiology and Laboratory of Molecular Immunology, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, University of Leuven, KU Leuven, Belgium

ARTICLE INFO

Keywords:

Interferon
Cytokine
Autoimmunity
COVID-19: antibody
Glycosylation
Proteolysis

ABSTRACT

Interferons are the best antiviral agents *in vitro* against SARS-CoV-2 so far and genetic defects in their signaling cascade or neutralization of alfa-interferons by autoantibodies come with more severe COVID-19. However, there is more, as the SARS-CoV-2 dysregulates not only innate immune mechanisms but also T and B cell repertoires. Most genetic, hematological and immunological studies in COVID-19 are at present phenomenological. However, these and antecedent studies contain the seed grains to resolve many unanswered questions and a whole range of testable hypotheses. What are the links, if existing, between genetics and the occurrence of interferon-neutralizing antibodies? Are NAGGED (neutralizing and generated by gene defect) antibodies involved or not? Is the autoimmune process cause or consequence of virus infection? What are the roles played by cytokine posttranslational modifications, such as proteolysis, glycosylation, citrullination and others? How is systemic autoimmunity linked with type 1 interferons? These questions place cytokines and growth factors at pole positions as keys to unlock basic mechanisms of infection and (auto)immunity. Related to cytokine research, (1) COVID-19 patients develop neutralizing autoantibodies, mainly against alpha interferons and it is not yet established whether this is the consequence or cause of virus replication. (2) The glycosylation of recombinant interferon-beta protects against breaking tolerance and the development of neutralizing antibodies. (3) SARS-CoV-2 induces severe inflammation and release of extracellular proteases leading to remnant epitopes, e.g. of cytokines. (4) In the rare event of homozygous cytokine gene segment deletions, observed neutralizing antibodies may be named NAGGED antibodies. (5) Severe cytolysis releases intracellular content into the extracellular milieu and leads to regulated degradation of intracellular proteins and selection of antibody repertoires, similar to those observed in patients with systemic lupus erythematosus. (6) Systematic studies of novel autoimmune diseases on single cytokines will complement the present picture about interferons. (7) Interferon neutralization in COVID-19 constitutes a preamble of more studies about cytokine-regulated proteolysis in the control of autoimmunity. Here we reformulate these seven conjectures into testable questions for future research.

1. Introduction

At the end of January 2021, the underestimated counts of COVID-19 patients and deaths are in excess of 100 and 2 million, respectively, and still increasing. Belgium comes with the sad record number of the highest death toll (more than 1800) per million inhabitants in the daily official COVID-19 statistics of the World Health Organization (WHO). Other countries in Europe and the Americas follow with similarly humbling numbers. This new situation, placed within the context of annual global death tolls by all infections (about 10 million citizens), is serious, alongside WHO-information about other diseases. Happily and

in contrast with equally important vaccine studies against other global infections (tuberculosis, malaria and HIV), coronavirus researchers have had more success in developing effective vaccines than their fellow colleagues [1–3], leaving patients with malaria and other microbial killers literally in the cold [4]. Another contrast is related to antiviral agents. Drugs against HIV were found thanks to the discovery of interleukin-2 by the group around Robert Gallo [5]. Thereby, permissive human leukocytes and cell lines could be grown *ad infinitum* for HIV replication and, hence, to screen for chemicals blocking virus replication, culminating in the discovery of tenofovir [6]. HIV has become a treatable disease with one-pill-a-day [7] and highly active antiretroviral

* Corresponding author.

E-mail address: ghislain.opdenakker@kuleuven.be (G. Opendakker).

<https://doi.org/10.1016/j.cytogfr.2021.01.004>

Received 8 December 2020; Received in revised form 22 January 2021; Accepted 24 January 2021

Available online 29 January 2021

1359-6101/© 2021 Elsevier Ltd. All rights reserved.

therapy has improved the lives of millions, including parents at reproductive age and children. In contrast, coronavirus infections are hardly treatable. For COVID-19, the publications of “successful” antiviral drugs are increasing exponentially, but their efficacies are modest [8]. At the Rega Institute, meanwhile more than 1.5 million compounds have already been tested *in vitro* in a sophisticated BSL3+ level and fully robotic facility (<https://rega.kuleuven.be/cmt/capsit>). Until now, the best compound against SARS-CoV-2 from this screening is human fibroblast interferon (IFN- β), the first cytokine ever discovered [9].

This paradox becomes even greater if we note that producing fibroblast interferon for antiviral therapy was already the dream of the virologists in the early 1960ies, when poliovirus was killing or driving patients into life-long paralytic diseases. That was the time when major breakthroughs in polio vaccine development met with double success: the FDA-approved killed polio virus (Salk) vaccine and the live attenuated polio (Sabin) vaccine [10,11], leading recently to global eradication of poliomyelitis. Natural IFN- β was first produced at semi-industrial scale at the Rega Institute on cultured human diploid fibroblasts around 1975 (Fig. 1). Till today, this represents the only interferon from non-transformed cells with authentic human glycosylation (*vide infra*) [12]. Once the cDNAs and genes of all interferons were cloned, recombinant expression and industrial production enabled global research activities [13]. With the exception of limited use for the treatment of a number of chronic viral diseases, antiviral therapy with interferons was not a great success [14]. For COVID-19 treatment too, the use of type I interferons comes with a number of reservations [15].

Long after the discoveries of interferons and in the era of the discoveries of colony-stimulating factors and interleukins, IFN- α and IFN- β again made headlines, when it was found that these delayed the

relapsing-remitting form of multiple sclerosis (MS) [16,17]. Gradually, cytokines and growth factors were mastered in the fight against autoimmune diseases and cancer and basic researchers created opportunities for biotech entrepreneurs [18]. We here review recent advances, provide alternative explanations, make links with autoimmune diseases and compare the effects of genetics with those of environmental factors, including virus infections. Finally, preparedness for the post-COVID-19 pandemic starts now with preventive measures to learn maximally from this catastrophe for future generations.

2. Seven pillars of wisdom to investigate, understand and treat COVID-19

2.1. Back to the blackboard: COVID-19 colors interferon in red

Recently, two research articles about susceptibilities of COVID-19 patients with obliteration or fading out of the interferon system made headlines. The authors of one manuscript detailed genetic defects of the interferon type 1 system [19] and the others described neutralizing antibodies against specific interferons [20]. The latter conditions represent true examples of autoimmune diseases [21] with specific interferon types as the culprit autoantigens. It is suggested that the neutralizing autoantibodies against interferons are the cause of the COVID-19 disease [20], but equally well these autoantibodies may be the consequence of this infectious disease. To be clear, we agree that shutting off of the type I interferon system by neutralizing autoantibodies may sever COVID-19 in specific patients. However, the possibility remains that the autoimmune process is caused or reinforced by the coronavirus, i.e. that the autoantibodies are the consequence of an immunity resetting by COVID-19. For instance, in two severe COVID-19 patients (of 101 subjects with neutralizing autoantibodies against type I interferons), the neutralizing antibodies were detectable in samples from before the COVID-19 outbreak. This information was used as proof that the coronavirus did not cause the autoantibody response [20]. However, these 2 citizens may have suffered antecedently from another (corona)virus infection with a resetting of their immune system. Coronavirus hunters have discovered related viruses [22] and, by probing SARS-CoV-2 peptide-recognizing T helper lymphocytes, immunologists detected adaptive CD4 + T cells against the C-terminal epitopes of the spike glycoprotein in 35 % of healthy donors [23]. The SARS-CoV-2 certainly has the potential to manipulate the immune system. Indeed, lymphopenia has been described as a hallmark of the COVID-19 syndrome [24,25]. In fact, COVID-19 pathogenesis is regarded as immune asynchrony or immune cacophony [26–28]. Several other elements, including genetic diseases (APS-1 and X-linked *Incontinentia pigmenti*) and gender bias [20] and epigenetic or environmental factors, including virus infections, may enhance the probability of autoimmune disease initiation/progression [21].

2.2. Immune tolerance enhanced by interferon oligosaccharides

Important solutions for medical problems may come by asking and answering the right questions. For instance, Karl Landsteiner was intrigued why blood transfusions sometimes fail and sometimes succeed and he provided the answers with the discovery of the ABO oligosaccharide antigens [29]. Another seminal example was the question in which ways antibodies in rheumatoid arthritis are different from those of healthy persons. After cloning and sequence analysis of numerous heavy and light chain cDNAs from both rheumatic and healthy cohorts, nothing of significance was found, until by the development of oligosaccharide sequence analysis researchers provided clear differences in the glycosylation of immunoglobulins [30]. Complementary studies [31] reinforced the thesis that glycosylation of immunoglobulins is critical in the regulation of inflammation. Today nobody negates anymore that antibodies and the immunological synapse are glyco-biological systems [32] and that even antigenic peptides may contain small



Fig. 1. Semi-industrial production plant of natural fibroblast interferon at the Rega Institute around 1975 and before cloning.

Left top: Glass and polystyrene roller bottle cultures of human embryonic dermal fibroblasts in conditioned rooms at 37 °C. At the back of the room, one remarks a rack with stationary fibroblast cultures and bacterial growth plates as sterility tests of used media, sera and end products. Right top: collection of superinduced conditioned cell culture media. Bottom left: first step in the downstream processing, i.e. adsorption of fibroblast interferon to controlled pore-glass beads. Bottom middle: the first batches of GMP-grade natural fibroblast interferon. Bottom right: skin reactivity of human fibroblast interferon, 24 h after intradermal injection. This type of skin inflammation is also observed in some patients with multiple sclerosis, who are treated with recombinant beta-interferon. Photographs: courtesy of Prof. Em. Dr. Alfons Billiau.

sugar side-chains [33]. What are the glycosylation differences between beta- and the alpha-interferons?

The authors of the recent interferon autoantibody study describe it as unlikely to break immune tolerance within only 1 or even 3 weeks [20]. However, such dogmas need to be avoided and new paradigms need to be tested, always humbly accepting that our null hypothesis is probably wrong. A primary antibody response takes about one week. During a florid coronavirus infection, interferons are induced instantaneously and at incredibly high local levels (of mainly IFN- β) at the virus entry and secondary sites [34,35]. In addition to these local events, viremia will unleash generalized leukocyte interferon production [36]. The latter various IFN- α subtypes are biologically and physicochemically different from the unique IFN- β . For instance, human IFN- β is the product of a single gene and the protein is N-glycosylated, i.e. relatively protected against proteolysis (Fig. 2). That such protection is relevant to understanding the formation of neutralizing antibodies against IFN- β comes from a meta-analysis of more than 30,000 patients with the relapsing remitting form of multiple sclerosis. In this study it was established that the IFN- β form without sugars yielded a higher percentage of neutralizing antibody formation than glycosylated recombinant IFN- β [37]. Recombinant glycosylated IFN- β is more resistant to degradation

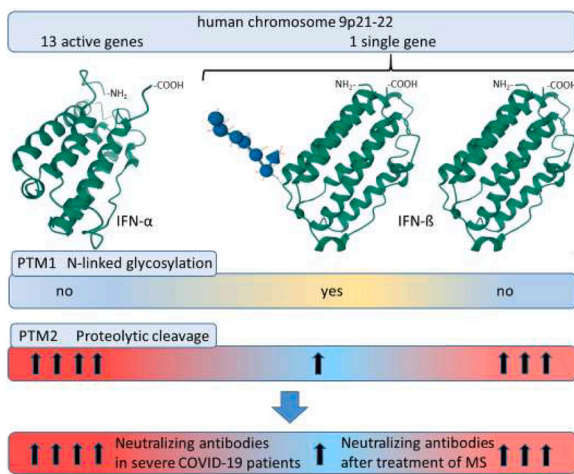


Fig. 2. Comparison of human alpha-interferons and IFN- β glycoforms after cloning.

Top: A total of 13 alpha-interferon (IFN- α) genes and a single beta-interferon (IFN- β) gene are grouped together with the interferons epsilon (IFN- ϵ), kappa (IFN- κ) and omega (IFN- ω) on the short arm of human chromosome 9 (Giemsa-stained band 9p21). The encoded multiple IFN- α proteins are not glycosylated, whereas the single IFN- β contains an N-linked oligosaccharide as a post-translational modification (PTM1). These molecules signal through two receptors (IFNAR1 and IFNAR2) with differences in affinities, in receptor internalization and recycling and in biological effects [36]. Natural IFN- β exists in various glycoforms and recombinant forms have been produced in Chinese hamster ovary (CHO) cells (with hamster cell-type sugars, one glycoform is indicated in the middle) and in bacteria (without oligosaccharides, i.e. aglycosyl proteoform, structure indicated at the right). Natural glycosylated IFN- β is not a single molecule but instead an ensemble of various glycosylation variants, called glycoforms, with variation in bioavailability, in receptor interactions and in protection against proteolysis as additional posttranslational modification (PTM2). Such proteolysis may be executed by gelatinase B/matrix metalloproteinase-9 (MMP-9) [38]. The concerted actions of both PTM1 and PTM2 leads to more remnant epitopes for IFN- α than for glycosylated IFN- β [21] and consequently to an increased probability to develop neutralizing antibodies in patients with severe COVID-19 disease. The comparison of neutralizing antibodies in severe COVID-19 patients (lower line, left) is from a recent study [20]. The higher prevalence of neutralizing antibodies against aglycosyl IFN- β versus glycosylated interferon was observed in a comparative study by collection of meta-data from clinical and serological studies of 32,026 patients with the relapsing remitting form of multiple sclerosis (MS) and treated with different recombinant IFN- β formulations [37] (lower line, right).

by matrix metalloproteinase-9 (MMP-9) than the aglycosyl form [38]. These studies may now be related to the observed “auto-immunizations” observed in severe COVID-19 patients. In these patients, neutrophils are activated and release considerable amounts of MMPs, including MMP-9, without its inhibitor [39] into the circulation. By extracellular proteolysis (in this case of unprotected non-glycosylated alpha-interferons) an excess of IFN- α peptides is produced for uptake and presentation to activate autoreactive T cells, assisting in the stimulation of rare autoreactive B lymphocytes (against IFN- α) [21].

2.3. If COVID-19 breaks tolerance, autoimmunity is on the lurk

By severe neutrophil-rich inflammatory reactions in infected tissues of COVID-19 patients and by resulting unencumbered proteolysis by neutrophil enzymes in the circulation, interferons and many other cytokines and other host molecules are broken down into remnant epitopes by extracellular proteases. These peptides may be further post-translationally modified into autoantigenic peptides [21]. Myeloid cell activation also occurs systemically in COVID-19 with concomitant coagulopathy, again driving systemic proteolytic events, including complement activation. If these processes evolve simultaneously with a cacophonous resetting of the immune system [26,27], COVID-19 patients are in an ideal state to boost their immune system towards IgG auto-antibody formation during the shortest possible time. All these elements are testable. For instance, what are the local and systemic levels of proteases and protease inhibitors after infection with SARS-CoV-2? Do patients with severe COVID-19 have generalized lymphadenopathy and splenomegaly as critical elements of autoantigen presentation and of circulatory lymphopenia? What are the T cell reactivities in epitope scanning analysis of IFN- α or IFN- ω peptides in patients with severe COVID-19 who develop neutralizing antibodies against interferon?

2.4. “NAGGED” antibodies against cytokines?

It is known that neutralizing antibodies, coined “neutralizing and generated by gene defect” or “NAGGED” antibodies, appear after substitution therapy in monogenic and X-linked diseases. The original concept, based on the studies of the ABO blood groups, has been proven with “spontaneous” neutralizing antibody formation in mammalian hosts with homozygous autosomal gene defects [40]. By the description of autoantibodies against interferons [20] and the discovery of primary immune deficiencies of the interferon system in relation with severity of COVID-19 [19], the concepts of nagged antibodies [40] and post-translational modification as triggers for autoimmunity [21] come again to the forefront. Various interesting research questions arise. For instance, are the named interferon genes and the genes of their regulatory network normal or aberrant in the COVID-19 patients with auto-antibodies [20] and did some, all or none of these subjects have normal or scrambled interferon-encoding and regulatory gene segments or not? Are some of the observed neutralizing antibodies in fact nagged antibodies, i.e. not autoimmune but instead by a gene defect? Alternatively, did the patients with primary immune deficiencies in the genetic studies [19] develop neutralizing antibodies against the missing protein sequences? Did any of the patients receive substitution therapy with interferon? If only a small sub-cohort of patients developing severe COVID-19 of the study population would suffer from underlying genetic defects and nagged antibodies, what may be the reason(s) for the neutralizing antibody response in those without gene defects? Does a link exist with immunoregulatory genes, with posttranslational modifications or are other possibilities playing a role? Furthermore, it remains critical to study innate immune mechanisms [41]. Indeed, it was found that epithelial cells can remember injury for 180 days [42] and that such epigenetic trained immunity may be involved as innate immune mechanism in COVID-19 [43]. If textbook immunology about innate immunity - in 2015 naming absence of memory as a characteristic of innate immune responses - needs some rewriting, it will be an additional

exercise of humility to acknowledge that the workings of the adaptive immune system and our understanding of autoimmune processes may also need broader approaches against existing dogmas and into new paradigms.

2.5. Autoantibodies against single antigens or intracellular repertoires?

COVID-19-related discoveries of the interferon system are related to mechanisms of autoimmunity, clinically regarded as a spectrum from highly organ-specific to generalized autoimmune disorders, such as systemic lupus erythematosus (SLE) [21]. Often autoantigens in organ-specific diseases are extracellular (glyco)proteins, whereas those in systemic autoimmunity, including SLE, are commonly multiple intracellular proteins [44]. In fact, in SLE, antibodies against more than 100 self-molecules have been found and these include small nuclear ribonucleoproteins [45–47]. When cells die massively under pathological conditions, intracellular proteins are degraded extracellularly by proteases, e.g. MMPs or caspases. This results in astronomical amounts of autoantigens, ready for presentation to T lymphocytes or for direct B cell activation. Such conditions may occur in tumor lysis syndrome, in SLE [48] and, maybe also under conditions when a virus induces cytolysis. So far, cytolysis and the release of abundant intracellular proteins by virus infections and its effects on exposing intracellularly hidden autoantigens to the immune system have not been studied in COVID-19. However, it is straightforward to test this hypothesis by measuring released intracellular proteins and their remnant epitopes and autoantibodies in the circulation. What has already been achieved is the recent description of similarities between severe COVID-19 and SLE [49]. The latter study brings an additional link between COVID-19 and autoimmunity with the demonstration of pathogenic immune activation by extrafollicular B cell responses. These findings may even be linked with the above data about type I interferons. Indeed, it is well known that innate immune mechanisms, i.e. myeloid cells and a prominent interferon signature are involved in SLE pathogenesis [50] to the point of ascribing a causative role of interferons in SLE [51,52]. By integration of these recent insights, new questions evolve about causal inferences. Are severe COVID-19 patients, who develop neutralizing antibodies against type I interferons less, equally or more susceptible to develop SLE in comparison with those without such antibodies? What is the activation status of plasmacytoid dendritic cells, as analyzed by single cell RNA-sequencing, in patients with COVID-19?

2.6. Cytokines: from bC (before COVID-19) to aC (after COVID-19)

A pioneer in cytokine research, Jan Vilcek, once compared the interferon era before cloning (BC) and after cloning (AC) [53]. Walter Fiers (1931–2019), leading human interferon- β cDNA cloning [54,55], followed up on this *boutade* in a manuscript entitled “Cloning and expression of human interferon-beta: from bc to ac” [56]. Today, we witness in research the dogmas from before COVID-19 (bc) and how, with optimism, we need to look at new paradigms to stimulate research for after COVID-19 (ac). Not only interferons, but also colony stimulating factors (CSFs) [57], interleukins [5,58,59] and specific chemokines, in particular IL-8/CXCL8 [60] and SDF-1/CXCL12 [61] will further come in the picture as individual molecules and novel insights into COVID-19 pathogenesis will evolve. Neutrophils are understudied in COVID-19. Therefore, we urge researchers to study in-depth neutrophil-related cytokines and chemokines, such as G-CSF, GM-CSF, IL-1, IL-6, IL-17, IL-8/CXCL8 and other chemokines, always with a critical attitude to question whether the detections of their increased levels are the cause or effect of COVID-19. Indeed, wrong causative inferences will lead to personal and community losses. This was recently so well documented with the example of (hydroxy)chloroquine, claimed to be beneficial against COVID-19 because of *in vitro* data on antiviral activity against another coronavirus [62] and because of European rumors of efficacy and American presidential approval for use *in vivo*, whereas

large-scale clinical studies did not yield any effect *in vivo* [63,64]. The temporary deprivation of these drugs during the year 2020 may have an impact on morbidities and mortalities of patients in malaria-endemic regions.

2.7. Pro-inflammatory cytokines and proteases: a dangerous mixture in COVID-19 and beyond

Cytokine-regulated extracellular proteolysis and other post-translational modifications are critical in autoimmune diseases [21]. Balances between pro-inflammatory and anti-inflammatory cytokines, between proteases and inhibitors and between subsets of disease-promoting and -limiting leukocyte types determine autoimmunity [65]. These insights have led to some success for treatments [66, 67]. The same pro-inflammatory molecules with similar interactions, often at much higher levels, are at play in COVID-19. For instance, significant increases of MMP-9 and associations with disease severity were detected in a hamster model of COVID-19 [68]. Similarly, MMP-9 and other proteases are out of balance in the circulation of patients with severe COVID-19 and MMP-9 is a relevant biomarker for COVID-19-related mortality [69]. A number of questions come to the forefront. What will be the effect of COVID-19 infections, both clinical and subclinical, on the incidence and prevalence of autoimmune diseases? Will the present therapies used in autoimmune diseases be in the same ways applicable for post-COVID-19 patients with specific autoimmune diseases? Prevention of autoimmune diseases must be achieved by maximal avoidance of autoantigenic stimulation. In the event that autoantigens are presented to the host immune system, a fast and efficient therapy to block adaptive immune reactions, i.e. strong immunosuppression, needs to be instated [21]. Immunosuppressive treatments compromise antiviral host defense mechanisms in patients suffering from COVID-19. For this disease, the messages are nevertheless similar: prevention of infections and elaborate vaccination campaigns to protect populations and individuals with the hope that SARS-CoV-2 gets eradicated.

3. Conclusions and perspectives

Above we described seven critical remarks and outlined pertinent questions to resolve in the coming years. With these insights one may evolve from 7 plagues of COVID-19 to 7 pillars of treatment optimism (Box 1). By education and solid research, with optimistic views related to recent studies [19,20,23], on the verge of having good vaccines [1–3], the COVID-19 pandemic has led to unprecedented collaborations to define new links between individual genomes, immune repertoires and autoimmune diseases. If we keep searching for real causes and consequences, if it is determined further that indeed a resetting of the immune system occurs in COVID-19 and that posttranslational modifications may influence breaking of immune tolerance, if we understand better whether and how viruses influence the initiation and progression of autoimmune diseases, this SARS-CoV-2 may become one day a useful tool to reset the immune system of individuals to better treat autoimmune diseases and organ transplantations. Then only, we will have succeeded in welding swords into plowshares, much in the same way as autoantibodies from patients with SLE became the critical tools to decipher the splicing machinery [70]. After this COVID-19 storm and successful vaccination campaigns, we will also be able to measure the collateral damages and record the effects of various vaccine formulations on the importance of cytokine regulation of protective adaptive immunity. Therefore, it is now the time to carefully store cohort and sequential patient samples to study on longer term causes and effects in autoimmune diseases. In such a way, the sufferings and deaths of too many fellow citizens will not be in vain, but will contribute to preventive medicine for the generations to come.

Box 1

Outstanding questions and methodological approaches.

Topic	Questions	Methods for investigation	References
Virus host interaction	Does SARS-CoV-2 induce autoantibody formation? What are the effects of this virus on the regulatory mechanism of immunity?	Permissible mammalian infection models, such as hamsters. Detection and titrations of autoantibodies in large human cohorts before and after infection and vaccination.	[20,28,52,68,69]
Posttranslational modifications of interferons and other cytokines	Is the formation of autoantibodies related to effects of glycosylation and citrullination on proteolysis?	Titrations of autoantibodies against cytokines with and without specific post-translational modifications.	[20,21,37,38]
Proteolysis of self molecules by host proteases	Are remnant epitopes generated in COVID-19?	Epitope scanning analysis to detect T cell reactivities and definitions of T cell subclasses	[21,23,44,48]
Host genetics	Do genetic defects of cytokines, receptors and signaling events exist which lead to nagged antibodies? What are the effects on COVID-19 severity?	Genetic analysis in association with autoantibody titrations. Generation of gene knockouts in hamsters with CRISP/Cas9 technology and study of COVID-19 susceptibility.	[19,21,40,61,68,69]
Cytolysis of viral entry cells versus inflammatory cells	Do cytolytic effects of the virus on host cells and death of inflammatory cells lead to circulating intracellular proteins and/or induce antibodies against these?	Detection of intracellular molecules (e.g. cellular actins or myeloperoxidase from neutrophils) in plasma. Detection of autoantibodies, classically searched for in lupus patients.	[25,28,44,45,48]
Innate immune factors	What is the role of neutrophils and neutrophil specific factors in COVID-19 and may these be modulated?	Detection of neutrophil numbers and differentiation stages with the use of molecular markers (e.g. CXCR1, CXCR2, NGAL, MMP-9).	[21,24,26,27,28,34,41,60,65]
Preventive measures	Are the frequencies of any autoimmune disease different after severe COVID-19 or not? Does vaccination prevent such possibility?	Comparisons of incidences and prevalences of specific autoimmune diseases in patients with clinical COVID-19, versus control and vaccinated cohorts.	[1–3,21,45,47–49]

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

Ghislain Opendakker (Laboratory of Immunobiology) and Jo Van Damme (Laboratory of Molecular Immunology) are at the Rega Institute for Medical Research, KU Leuven Belgium. They thank Professor Pauline M. Rudd (Dublin) for a critical review and the Research Foundation of Flanders (FWO-Vlaanderen, G0A3820 N) and KU Leuven (C1 funding 2017-2023) for financial support. GO is a member of the Royal Academy of Medicine of Belgium (KAGB) and sustaining member of the Belgian American Educational Foundation (BAEF), New Haven, CT, USA. This manuscript is dedicated to the memory of two Yale University mentors and friends of the Rega Institute: Professor Peter Lengyel and Professor Thomas Steitz, pioneers of research on mRNA translation, leading to the present state of interferon research.

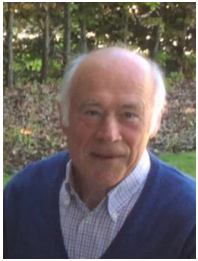
References

- [1] F. Krammer, SARS-CoV-2 vaccines in development, *Nature* 586 (2020) 516–527.
- [2] S.H. Hodgson, K. Mansatta, G. Mallett, V. Harris, K.R.W. Emary, A.J. Pollard, What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2, *Lancet Inf Dis* (2020), [https://doi.org/10.1016/S1473-3099\(20\)30773-8](https://doi.org/10.1016/S1473-3099(20)30773-8). Online ahead of print.
- [3] L. Sanchez-Felipe, T. Vercruyse, S. Sharma, J. Ma, V. Lemmens, D. Van Looveren, et al., A single-dose live-attenuated YF17D-vectored SARS-CoV2 vaccine candidate, *Nature* (2020), <https://doi.org/10.1038/s41586-020-3035-9>. Online ahead of print.
- [4] A. Pance, Diversify and Conquer: The vaccine escapism of *Plasmodium falciparum*, *Microorganisms* 8 (2020) 1748.
- [5] J.W. Mier, R.C. Gallo, Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media, *Proc Natl Acad Sci U S A*. 77 (1980) 6134–6138.
- [6] E. De Clercq, A 40-year journey in search of selective antiviral chemotherapy, *Annu. Rev. Pharmacol. Toxicol.* 51 (2011) 1–24.
- [7] E. De Clercq, Anti-HIV drugs, *Verh. K. Acad. Geneesk. Belg.* 69 (2007) 81–104.
- [8] A.K. Ghosh, M. Brindisi, D. Shahabi, M.E. Chapman, A.D. Mesecar, Drug development and medicinal chemistry, efforts toward SARS-coronavirus and Covid-19 therapeutics, *Chem Med Chem*. 15 (2020) 907–932.
- [9] A. Isaacs, J. Lindenmann, Virus interference. I. The interferon, *Proc. R. Soc. Lond., B, Biol. Sci.* 147 (927) (1957) 258–267.
- [10] J.E. Salk, Considerations in the preparation and use of poliomyelitis virus vaccine, *J. Am. Med. Assoc.* 158 (1955) 1239–1248.
- [11] A.B. Sabin, Immunization of chimpanzees and human beings with avirulent strains of poliomyelitis virus, *Ann. N. Y. Acad. Sci.* 61 (1955) 1050–1056.
- [12] A. Billiau, J. Van Damme, F. Van Leuven, V.G. Edy, M. De Ley, J.J. Cassiman, H. Van de Berghe, P. De Somer, Human fibroblast interferon for clinical trials: production, partial purification, and characterization, *Antimicrob. Agents Chemother.* 16 (1979) 49–55.
- [13] E.C. Borden, G.C. Sen, G. Uze, R.H. Silverman, R.M. Ransohoff, G.R. Foster, G. R. Stark, Interferons at age 50: past, current and future impact on biomedicine, *Nat. Rev. Drug Discov.* 6 (2007) 975–990.
- [14] G. Antonelli, C. Scagnolari, F. Moschella, E. Proietti, Twenty-five years of type I interferon-based treatment: a critical analysis of its therapeutic use, *Cytokine Growth Factor Rev.* 26 (2015) 121–131.
- [15] E. Aricò, L. Bracci, L. Castiello, S. Gessani, F. Belardelli, Are we fully exploiting type I Interferons in today's fight against COVID-19 pandemic? *Cytokine Growth Factor Rev.* 54 (2020) 43–50.
- [16] H.S. Panitch, G.S. Francis, C.J. Hooper, T.C. Merigan, K.P. Johnson, Serial immunological studies in multiple sclerosis patients treated systemically with human alpha interferon, *Ann. Neurol.* 18 (1985) 434–438.
- [17] L. Jacobs, J.A. O'Malley, A. Freeman, R. Ekes, P.A. Reese, Intrathecal interferon in the treatment of multiple sclerosis. Patient follow-up, *Arch. Neurol.* 42 (1985) 841–847.
- [18] J. Vilcek, M. Feldmann, Historical review: cytokines as therapeutics and targets of therapeutics, *Trends Pharmacol. Sci.* 25 (2004) 201–209.

- [19] Q. Zhang, P. Bastard, Z. Liu, J. Le Pen, M. Moncada-Velez, J. Chen, et al., Inborn errors of type I IFN immunity in patients with life-threatening COVID-19, *Science* 370 (6515) (2020) eabd4570.
- [20] P. Bastard, L.B. Rosen, Q. Zhang, E. Michailidis, H.H. Hoffmann, Y. Zhang, et al., Autoantibodies against type I IFNs in patients with life-threatening COVID-19, *Science* 370 (6515) (2020) eabd4585.
- [21] G. Opendakker, A. Abu El-Asrar, J. Van Damme, Remnant epitopes generating autoimmunity: from model to useful paradigm, *Trends Immunol.* 41 (2020) 367–378.
- [22] J.F. Drexler, V.M. Corman, C. Drosten, Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS, *Antiviral Res.* 101 (2014) 45–56.
- [23] J. Braun, L. Loyal, M. Frensch, D. Wendisch, P. Georg, F. Kurth, et al., SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19, *Nature*. 587 (7833) (2020) 270–274.
- [24] T.A. Khartabil, H. Russcher, A. van der Ven, Y.B. de Rijke, A summary of the diagnostic and prognostic value of hemocytometry markers in COVID-19 patients, *Crit. Rev. Clin. Lab. Sci.* 57 (2020) 415–431.
- [25] A.P. Spihlman, N. Gadi, S.C. Wu, V.R. Moulton, COVID-19 and systemic lupus erythematosus: focus on immune response and therapeutics, *Front. Immunol.* 11 (2020), 589474.
- [26] T. Zhou, T.T. Su, T. Mudianto, J. Wang, Immune asynchrony in COVID-19 pathogenesis and potential immunotherapies, *J. Exp. Med.* 217 (2020), e20200674.
- [27] E.J. Giamarellos-Bourboulis, M.G. Netea, N. Rovina, K. Akinosoglou, A. Antoniadou, N. Antonakos, et al., Complex immune dysregulation in COVID-19 patients with severe respiratory failure, *Cell Host Microbe* 27 (2020) 992–1000, e3.
- [28] C.N. Gruber, R.S. Patel, R. Trachtman, L. Lepow, F. Amanat, F. Krammer, et al., Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C), *Cell* 183 (2020) 982–995, e14.
- [29] K. Landsteiner, A.S. Wiener, Studies on an agglutinin (Rh) in human blood reacting with anti Rhesus sera and with human isoantibodies, *J. Exp. Med.* 74 (1941) 309–320.
- [30] R.B. Parekh, R.A. Dwek, B.J. Sutton, D.L. Fernandes, A. Leung, D. Stanworth, et al., Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG, *Nature* 316 (6027) (1985) 452–457.
- [31] R.M. Anthony, F. Nimmerjahn, D.J. Ashline, V.N. Reinhold, J.C. Paulson, J. V. Ravetch, Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc, *Science* 320 (5874) (2008) 373–376.
- [32] P.M. Rudd, T. Elliott, P. Cresswell, I.A. Wilson, R.A. Dwek, Glycosylation and the immune system, *Science* 291 (5512) (2001) 2370–2376.
- [33] P.E. Van den Steen, B. Grillet, G. Opendakker, Gelatinase B participates in collagen II degradation and releases glycosylated remnant epitopes in rheumatoid arthritis, *Adv. Exp. Med. Biol.* 564 (2005) 45–55.
- [34] W. Sungnak, N. Huang, C. Bécaivin, M. Berg, R. Queen, M. Litvinukova, et al., SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes, *Nat. Med.* 26 (2020) 681–687.
- [35] A. Gupta, M.V. Madhavan, K. Sehgal, N. Nair, S. Mahajan, T.S. Sehrawat, et al., Extrapulmonary manifestations of COVID-19, *Nat. Med.* 26 (2020) 1017–1032.
- [36] P. Génin, A. Vaccaro, A. Civas, The role of differential expression of human interferon- α genes in antiviral immunity, *Cytokine Growth Factor Rev.* 20 (2009) 283–295.
- [37] T.R. Einarson, B.G. Bereza, M. Machado, Comparative effectiveness of interferons in relapsing-remitting multiple sclerosis: a meta-analysis of real-world studies, *Curr. Med. Res. Opin.* 33 (2017) 579–593.
- [38] I. Nelissen, E. Martens, P.E. Van den Steen, P. Proost, I. Ronsse, G. Opendakker, Gelatinase B/matrix metalloproteinase-9 cleaves interferon-beta and is a target for immunotherapy, *Brain*. 126 (2003) 1371–1381.
- [39] V.C. Ardi, T.A. Kupriyanova, E.I. Deryugina, J.P. Quigley, Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis, *Proc Natl Acad Sci U S A* 104 (51) (2007) 20262–20267.
- [40] G. Opendakker, P.E. Van den Steen, G. Laureys, K. Hunninc, B. Arnold, Neutralizing antibodies in gene-defective hosts, *Trends Immunol.* 24 (2003) 94–100.
- [41] S. Mukherjee, Before virus, after virus: a reckoning, *Cell* 183 (2020) 308–314.
- [42] S. Naik, S.B. Larsen, N.C. Gomez, K. Alaverdyan, A. Sandoel, S. Yuan, et al., Inflammatory memory sensitizes skin epithelial stem cells to tissue damage, *Nature* 550 (7677) (2017) 475–480.
- [43] A. Mantovani, M.G. Netea, Trained innate immunity, epigenetics, and COVID-19, *N. Engl. J. Med.* 383 (2020) 1078–1080.
- [44] B. Cauwe, E. Martens, P. Proost, G. Opendakker, Multidimensional degradomics identifies systemic autoantigens and intracellular matrix proteins as novel gelatinase B/MMP-9 substrates, *Integr. Biol. (Camb)* 1 (2009) 404–426.
- [45] Y. Sherer, A. Gorstein, M.J. Fritzler, Y. Shoenfeld, Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients, *Semin. Arthritis Rheum.* 34 (2004) 501–537.
- [46] E.A. Lerner, M.R. Lerner, J.A. Hardin, C.A. Janeway Jr., J.A. Steitz, Deciphering the mysteries of RNA-containing lupus antigens, *Arthritis Rheum.* 25 (1982) 761–766.
- [47] E.A. Lerner, M.R. Lerner, C.A. Janeway Jr., J.A. Steitz, Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease, *Proc Natl Acad Sci U S A* 78 (1981) 2737–2741.
- [48] B. Cauwe, G. Opendakker, Intracellular substrate cleavage: a novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases, *Crit. Rev. Biochem. Mol. Biol.* 45 (2010) 351–423.
- [49] M.C. Woodruff, R.P. Ramonell, D.C. Nguyen, K.S. Cashman, A.S. Saini, N. S. Haddad, et al., Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19, *Nat. Immunol.* 21 (2020) 1506–1516.
- [50] M.D. Catalina, K.A. Owen, A.C. Labonte, A.C. Grammer, P.E. Lipsky, The pathogenesis of systemic lupus erythematosus: harnessing big data to understand the molecular basis of lupus, *J. Autoimmun.* 110 (2020), 102359.
- [51] M.K. Crow, M. Olfertiev, K.A. Kirou, Type I interferons in autoimmune disease, *Annu. Rev. Pathol.* 14 (2019) 369–393.
- [52] M.L. Eloranta, L. Rönnblom, Cause and consequences of the activated type I interferon system in SLE, *J. Mol. Med.* 94 (2016) 1103–1110.
- [53] J. Vilcek, Interferon research BC (before cloning), *Curr. Top. Microbiol. Immunol.* 316 (2007) 9–22.
- [54] R. Derynck, J. Content, E. De Clercq, G. Volckaert, J. Tavernier, R. Devos, W. Fiers, Isolation and structure of a human fibroblast interferon gene, *Nature* 285 (5766) (1980) 542–547.
- [55] R. Derynck, E. Remaut, E. Saman, P. Stanssens, E. De Clercq, J. Content, W. Fiers, Expression of human fibroblast interferon gene in *Escherichia coli*, *Nature*. 287 (5779) (1980) 193–197.
- [56] W. Fiers, Cloning and expression of human interferon-beta: from bc to ac, *Verh K Acad Geneesk Belg* 71 (2009) 43–50.
- [57] L.J. Bendall, K.F. Bradstock, G-CSF: from granulopoietic stimulant to bone marrow stem cell mobilizing agent, *Cytokine Growth Factor Rev.* 25 (2014) 355–367.
- [58] J. Van Damme, M. De Ley, G. Opendakker, A. Billiau, P. De Somer, J. Van Beeumen, Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1, *Nature* 314 (6008) (1985) 266–268.
- [59] J. Van Damme, G. Opendakker, R.J. Simpson, M.R. Rubira, S. Cayphas, A. Vink, A. Billiau, J. Van Snick, Identification of the human 26-kD protein, interferon beta 2 (IFN-beta 2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor, *J. Exp. Med.* 165 (1987) 914–919.
- [60] J. Van Damme, J. Van Beeumen, G. Opendakker, A. Billiau, A novel, NH2-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive, and granulocytosis-promoting activity, *J. Exp. Med.* 167 (1988) 1364–1376.
- [61] L.E. Heusinkveld, S. Majumdar, J.L. Gao, D.H. McDermott, P.M. Murphy, WHIM syndrome: from pathogenesis towards personalized medicine and cure, *J. Clin. Immunol.* 39 (2019) 532–556.
- [62] E. Keyaerts, L. Vijgen, P. Maes, J. Neyts, M. Van Ranst, In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine, *Biochem. Biophys. Res. Commun.* 323 (2004) 264–268.
- [63] Z. Kashour, M. Riaz, M.A. Garbati, O. Aldosary, H. Tlayjeh, D. Gerberi, et al., Efficacy of chloroquine or hydroxychloroquine in COVID-19 patients: a systematic review and meta-analysis, *J. Antimicrob. Chemother.* 76 (1) (2020) 30–42.
- [64] A. Elavarasi, M. Prasad, T. Seth, R.K. Sahoo, K. Madan, N. Nischal, et al., Chloroquine and hydroxychloroquine for the treatment of COVID-19: a systematic review and meta-analysis, *J. Gen. Intern. Med.* 35 (2020) 3308–3314.
- [65] G. Opendakker, J. Van Damme, Probing cytokines, chemokines and matrix metalloproteinases towards better immunotherapies of multiple sclerosis, *Cytokine Growth Factor Rev.* 22 (2011) 359–365.
- [66] L.M. Metz, D.K.B. Li, A.L. Trabouise, P. Duquette, M. Eliasziw, G. Cerchiaro, et al., Trial of minocycline in a clinically isolated syndrome of multiple sclerosis. Minocycline in MS study team, *N. Engl. J. Med.* 376 (2017) 2122–2133.
- [67] A.M. Abu El-Asrar, A. Dheyab, D. Khatib, S. Struyf, J. Van Damme, G. Opendakker, Efficacy of B cell depletion therapy with rituximab in refractory chronic recurrent uveitis associated with Vogt-Koyanagi-Harada disease, *Ocul. Immunol. Inflamm.* (2020), <https://doi.org/10.1080/09273948.2020.1820531>. Online ahead of print.
- [68] R. Boudewijns, H.J. Thibaut, S.J.F. Kaptein, R. Li, V. Vergote, L. Seldeslachts, et al., STAT2 signaling restricts viral dissemination but drives severe pneumonia in SARS-CoV-2 infected hamsters, *Nat. Commun.* 11 (2020) 5838.
- [69] M.S. Abers, O.M. Delmonte, E.E. Ricotta, J. Fintzi, D. Fink, A.A. de Jesus, et al., An immune-based biomarker signature is associated with mortality in COVID-19 patients, *JCI Insight* 6 (1) (2020), 144455.
- [70] M.R. Lerner, J.A. Steitz, Snurps and scyrps, *Cell* 25 (1981) 298–300.



Ghislain Opendakker is professor of immunology. Together with several colleagues, he introduced molecular biology technology at KU Leuven. After postdoctoral training with Peter Lengyel at Yale University, he switched to the study of cytokine-regulated modifications of autoantigens. Studies on proteolysis by gelatinase B, alias matrix metalloproteinase-9, on glycosylation - together with Raymond Dwek and Pauline Rudd at the University of Oxford - and on citrullination led to the concept of extracellular remnant autoantigens in infection and autoimmunity.



Jo Van Damme is emeritus professor of immunology. He established a large-scale mammalian cell culture facility at the Rega Institute (KU Leuven, Belgium). In the pre-cloning era, in this facility he mastered, together with Alfons Billiau and Piet De Somer, the production of the first GMP-grade batches of natural interferon-beta from human fibroblasts and of Bowes melanoma-derived tissue-type plasminogen activator (t-PA), a serine protease used in the first successful clinical test for thrombolysis. Thereafter, he discovered interleukin-1, interleukin-6, interleukin-8 and many other chemokines as pro-inflammatory regulators in infections, cancer and autoimmune diseases.