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Letter to Editor

Biobetter Versions of Recombinant Human IFN-α2b for the Treatment of Viral Infections

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the infectious agent causing COVID-19 disease, whose pandemic has had far-reaching consequences on the global population. Since the detection of the first cases in late 2019, much has been learned about the mechanism of action of SARS-CoV-2 and the associated immune response to eradicate the infection. Recently, a clear correlation between disease severity and abnormal type I IFN response in patients has been established. Individuals with immune responses characterized by high concentrations of IFN-a2b and low blood levels of IL-6, TNF-alpha, and IL-1Ra were much less affected than those patients who exhibited an opposite scenario. Interestingly, recombinant human IFN-a2b (rhIFN-a2b) could mitigate the severity of symptoms, if given in the early stages of the disease, before reaching the inflammatory shock (cytokine storm) that characterizes the most severe cases. However, there are adverse effects associated with rhIFN-a2b-based therapy. Among them, the emergence of unwanted immune responses against the biologic can, in some cases, compromise the treatment's safety and efficacy. In addition, rhIFN-a2b is a small cytokine, which results in rapid clearance from the bloodstream. This quick plasma clearance poses the need for frequent high doses to achieve the desired effect, which may, in turn, exacerbate unwanted effects associated with therapy. In this article we will address the most relevant strategies for the development of biobetters versions of rhIFN-a2b, as promising candidates for the treatment of COVID-19 and other human viral diseases.

Keywords: SARS-CoV-2; COVID-19; IFN-a2b; Immunogenicity; Pegylation; Glycosylation; Biobetter.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the infectious agent causing COVID-19 disease, whose pandemic has had far-reaching consequences on the global population. Since the detection of the first cases in late 2019, much has been learned about the mechanism of action of SARS-CoV-2 and the associated immune response to eradicate the infection [1]. Recently, a clear correlation between disease severity and abnormal type I IFN response in patients has been established. Individuals with immune responses characterized by high concentrations of IFN-a2b and low blood levels of IL-6, TNF-alpha, and IL-1Ra were much less affected than those patients who exhibited an opposite scenario [2]. Interestingly, recombinant human IFN-a2b (rhIFN-a2b) could mitigate the severity of symptoms, if given in the early stages of the disease, before reaching the inflammatory shock (cytokine storm) that characterizes the most severe cases.

However, there are adverse effects associated with rhIFN-a2b-based therapy. Among them, the emergence of unwanted immune responses against the biologic can, in some cases, compromise the treatment's safety and efficacy. In addition, rhIFN-a2b is a small cytokine, which results in rapid clearance from the bloodstream. This quick plasma clearance poses the need for frequent high doses to achieve the desired effect, which may, in turn, exacerbate unwanted effects associated with therapy [3].

These limitations have prompted the development of rhIFN-a2b biobetters versions, mainly focused on improving its plasma stability. The two most successful approaches were protein pegylation and glycosylation. Both strategies aim to increase the protein's apparent size and, consequently, decrease the plasma clearance rate. For example, adding rhIFN-a2b of polyethylene glycol (PEG) molecules (12 kDa) allowed the development of a product marketed as PEGIntron®. Although this product possesses high relative antiviral activity, the improvement in pharmacokinetic properties of the molecule is still reduced [4]. Another pegylated version of rhIFN-a2b is commercially known as PEGASYS®, provided with larger PEG residues that confer to the molecule a longer half-life. However, the larger apparent size of the molecule is achieved to the detriment of the reduced antiviral activity exhibited by this product over the unmodified protein [5].

Glycoengineering of therapeutic proteins is another interesting approach that has led to longer plasma half-lives of certain biologics. Although glycan residues can be added in diverse ways, two strategies have achieved successful outcomes. For instance, N-glycosylation involves the addition of carbohydrates to Asparagine atoms, whereas, for O-glycosylated proteins, sugars are bound to Threonine or Serine residues [6]. Thus, a hyperglycosylated version of rhIFN-a2b, developed using the N-glycosylation strategy, exhibited a 25-fold longer elimination half-life than the non-glycosylated protein. In addition, O-glycosylation of the cytokine also allowed for improved plasma stability of the product, with an additional advantage associated with high retention of *in vitro* antiviral activity [7].

Pegylation and glycosylation strategies have made it possible to achieve substantial improvements in the in vivo rhIFN-a2b half-life and, consequently, to reduce the number of doses needed to reach the therapeutic window. However, these biobetter versions of the biologic do not solve the problems associated with the risk of immunogenicity [8].

For this reason, we recently proposed the development of hyperglycosylated versions of rhIFN-a2b with reduced immunogenicity, using a strategy based on the identification and elimination of epitopes potentially recognized by T cells. This approach, known as De-immunization for Functional Therapeutics (DeFT), combines powerful immune-informatics algorithms with *in vitro* and *in vivo* experimental platforms. First, we analyzed the immunogenicity of two hyperglycosylated rhIFN-a2b muteins, which were found to be more immunogenic than the unglycosylated protein. We then identified the most immunogenic residues by *in silico* analysis. These amino acids were then substituted to reduce the binding of rhIFN-a2b-derived peptides to human major histocompatibility complex (HLA) molecules and, consequently, the immunogenicity of these proteins. The *in silico* predictions were then validated by *in vitro* binding experiments to relevant HLA molecules and by *ex vivo* and *in vivo* assays. The new hyperglycosylated and de-immunized rhIFN-a2b versions exhibited a marked reduced immunogenicity and retained high residual antiviral activity [8-10]. Altogether, these results demonstrate the success of the strategy approached and highlight the new rhIFN-a2b de-immunized variants as promising antiviral candidates.

In conclusion, since its approval as an antiviral agent, significant progress has been made in the search for new rhIFN-a2b versions with improved properties in terms of stability and immunogenicity. Approaching these strategies in combination will allow further improvements in the development of biobetter versions of this potent antiviral agent.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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