Scavenging of Interleukin 6 Receptor by Bioidentical Recombinant gp130 as Intervention in Covid-19 Exacerbation

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Keywords

coronavirus; SARS; covid-19; gp130; interleukin 6; cytokine storm

Abstract

Acute exacerbations in the form of cytokine storm are the main cause of organ failure caused by SARS-related coronaviruses and mediated by pro-inflammatory transcriptional programs in immune cells. While the effectors of these transcriptional changes have been sufficiently studied, there is a noticeable lack of interventional treatments for acute exacerbations. An important interface of innate immune signalling are the components of the JAK/STAT pathway, activated by interferons as well as pro-inflammatory cytokines such as interleukin (IL)-6. An intervention based on the IL-6 receptor antibody, tocilizumab, has already been proposed and is currently in clinical trial; however, the antibody does not differentiate between soluble and membrane-bound IL-6 receptors and thus may inhibit regenerative processes. Here, I propose utilising recombinant soluble protein gp130, also known as IL6ST, as a bioidentical scavenger molecule for activated soluble IL6R, while retaining the desirable functionality of membrane-bound IL6R. In light of

SARS patient expression profiles and of IL6R and gp130 tissue distribution, the clinical testing of soluble gp130 is a warranted alternative to monoclonal antibody therapy.

Introduction

Cytokine storm, with overproduction of pro-inflammatory cytokines and exaggerated response of pro-inflammatory immune cell types in the blood, is the most common cause of acute respiratory distress syndrome and organ failure in severe viral infection (Bhaskar *et al.* 2020). Two major pro-inflammatory interleukins (ILs) are required for cytokine storm formation: IL-1 and IL-6 (Norelli *et al.* 2018). The acute-response cytokine IL-1 β facilitates a sustained increase in IL-6, which subsequently activates the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway through the gp130 receptor, thereby perpetuating inflammation (Tisoncik *et al.* 2012). Blocking this cascade upstream of JAK/STAT, for instance at the IL-6 receptor, may be a valuable therapeutic option (Bhaskar *et al.* 2020).

Susceptibility to severe exacerbations of Covid-19 seems influenced by genetic disposition rather than simply by age; why some infections tend to present with severe clinical symptoms particularly in young, healthy adults is currently unknown (Tisoncik *et al.* 2012). To interfere with this exaggerated response, treatment with *i.v.* tocilizumab, the IL-6 receptor antibody, has been proposed and is currently in clinical testing. However, compared to approved indications for tocilizumab, such as rheumatoid arthritis (RA), the doses for *i.v.* application in acute hypercytokinaemia may have to be increased significantly, bearing the risk of severe adverse reactions and paradoxical immune stimulation (van Schie *et al.* 2015). Additionally, tocilizumab without distinction binds to soluble and membrane-bound IL-6 receptors alike, harbouring the risk

for additional off-target adverse effects. For instance, the most common (very common, > 10%) adverse reaction to *s.c.* and *i.v.* tocilizumab is upper respiratory tract infection (Scott 2017). Serious respiratory tract infections occur in 8.5% of tocilizumab monotherapy patients in approved indications, i.e., at low doses (Haraoui *et al.* 2017).

IL-6 as a neurokinin conveys its effect on cells through the ubiquitously distributed gp130 transmembrane receptor protein, which is also known as IL-6 signal transducer (IL6ST), immediately resulting in JAK/STAT activation. In most cases, IL-6 binds to the soluble IL-6 receptor (sIL6R) which then mediates gp130 homodimer activation on the cell surface (trans-signalling). On the other hand, some cell types have been found to express a membrane-bound form of the IL-6 receptor (mbIL6R): macrophages, neutrophils, some types of T-cells, and hepatocytes (Scheller *et al.* 2011). A multitude of studies has shown that the deleterious effects of IL-6 overexpression are caused by trans-signalling via the sIL6R, while the classic cis-signalling conveyed by mbIL6R is essential for anti-microbial effects and regeneration (Garbers *et al.* 2015). Consequently, inhibition of IL-6 signalling via the IL6R antibody tocilizumab, the direct IL-6 antibody siltuximab, or small molecule TYK2-antagonists would inevitably interfere with the desired, classic IL-6 signalling through mbIL6R. In contrast, application of sIL6R-scavenging recombinant soluble gp130 protein (sgp130) could theoretically reduce detrimental trans-signalling while keeping classic signalling intact.

Whether these processes play a role in exacerbation of Covid-19 infection is unclear. To clarify human interleukin-related reaction to coronavirus exacerbation and feasibility of sIL6R scavenging by sgp130 in these cases, I re-analysed expression data of a cohort of SARS-CoV patients, and

assessed the tissue distribution of interleukin-related transcripts involved in their responses to the infection.

Results

IL6R is elevated in SARS patients prior to exacerbation, whereas IL-1 β is not

Re-analysis of a publicly available dataset of 70 samples from peripheral blood of SARS-CoV patients and controls from the Toronto area in 2007 (Cameron *et al.* 2007) shows significant interleukin involvement in blood cell transcriptional processes surrounding exacerbation (Fig. 1). IL6R was up-regulated (~33%, $p = 0.036$) in patient blood pre-, but not post-exacerbation (indicated by arterial oxygen saturation nadir) compared to healthy controls, while IL-6 levels remained unchanged. The major pro-inflammatory cytokine IL-1 β and its receptors were down-regulated in patients pre- and post-exacerbation. In contrast, IL6R, IL17RE and IL22RA1 were up-regulated.

Bronchial epithelial cells express high amounts of IL6R

To get a better picture of IL6-related gene expression across relevant cell types, tissue-specific regulatory circuits of immune cells ($n = 17$) and control tissues ($n = 16$) were analysed, based on a dataset of transcriptional information from 808 human samples (Marbach *et al.* 2016). Since mbIL6R expression has been observed in immune and liver cells exclusively (Scheller *et al.* 2011), bronchial epithelial tissue was included as a control. Surprisingly, however, bronchial epithelia showed fourth-highest expression of the IL6R mRNA according to these data, more than liver cells, and surpassed only by monocytes and regulatory T cells (Supplementary Fig. 1).

82 Discussion

83 In a recent study performed on a humanised mouse model of hypercytokinaemia (utilising CAR T-
84 technology), human monocyte-derived IL-1 and IL-6 were the drivers of cytokine release syndrome
85 (Norelli *et al.* 2018). Conversely, differential expression in the Toronto cohort implies that
86 hypercytokinaemia as a cause of clinical exacerbation of SARS-CoV may not be mediated by IL-1 β .
87 IL-1 β was decreased pre-exacerbation and did not increase above control levels even post
88 exacerbation; IL-1 receptors were similarly down-regulated. Notably, most patients received
89 glucocorticoids orally or intravenously, at a median of six days after symptom onset (DSO), which
90 may have interfered with cytokine levels. Still, three pro-inflammatory cytokines and/or their
91 receptors were elevated around the time of exacerbation (which occurred at a median of eight
92 DSO): IL-6, IL-17, and IL-22. IL-17 and IL-22 have been described as relevant players in airway
93 inflammation, and may control pro-inflammatory response as well as recovery parameters via
94 complex interaction. However, their basal expression in the assessed tissues is extremely low, and
95 their description in the literature is sparse.

96 IL-6, on the other hand, is a well-described pro-inflammatory cytokine whose receptors show high
97 basal expression. IL6R expression was elevated in patients pre-exacerbation; its highest expression
98 in monocytes implies that in human coronavirus infection, monocytes may also play a pivotal role
99 in life-threatening exacerbations. Additionally, and surprisingly, IL6R basal expression in bronchial
100 epithelia is very high. Respiratory endothelial cells have previously been identified as mediators of
101 cytokine overexpression, which was ameliorated by sphingosine-1-phosphate (S1P₁) receptor
102 agonism (Teijaro *et al.* 2011). The rescue of mice from hypercytokinaemia in this study may have
103 been caused by the observed drastic reduction in circulating IL-6.

Similarly, the elevated amounts of circulating activated sIL6R in human Covid-19 patients may be reduced through scavenging by *i.v.* recombinant soluble gp130 protein (sgp130), thus reducing cytokine storm risk or ameliorating its consequences. Importantly, this proposed scavenging mechanism would interfere only with the deleterious trans-signalling of IL-6, keeping intact the classic, pro-recovery cis-signalling (Garbers *et al.* 2015). This is an advantage over the IL6R-antibody tocilizumab, which may without distinction interfere with any IL6R-mediated function in all cells reached by the antibody; indeed, tocilizumab therapy conveys a high risk of severe respiratory infection as its main adverse effect, even in RA basal therapy (Haraoui *et al.* 2017; Scott 2017). In addition, pathological studies revealed that tissues infected by SARS mainly comprised airway epithelia, endothelia, monocytes, and macrophages (Gu *et al.* 2005). Thus, targeting of all IL6R molecules regardless of cell type may worsen pathology by inhibiting the bodily response and regenerative abilities.

A natural form of sgp130 is expressed via alternative splicing of the gp130 mRNA in normal human blood (Müllberg *et al.* 1993). Human recombinant gp130 protein is readily available for scientific purposes from a number of biotechnological suppliers, and a soluble gp130-analog coupled to the Fc portion of IgG (Olamkicept, Ferring Pharmaceuticals) is in Phase II clinical trial against inflammatory bowel disease (IBD). Thus, production capacities for clinical investigation are readily available. However, for an acute intervention in hypercytokinaemia (as opposed to chronic treatment of RA or IBD), a non-modified bioidentical human recombinant sgp130 may be preferable because of a lower immunogenicity. In these short-term scenarios, prolonged or increased activity (as e.g. achieved by hybridisation to an antibody Fc portion) may not be needed.

An open question is the timing of IL6R scavenging via *i.v.* sgp130. Since IL6R was elevated in patients pre-exacerbation and the onset of cytokine storm as clinical phenomenon is rather rapid and unpredictable (Tisoncik *et al.* 2012), there is need for a reliable biomarker of cytokine storm onset. Several chemokines of the CCL and CXCL families may be sufficient markers because of their significant up-regulation and accessibility via ELISA (Cheung *et al.* 2005). In spite of these uncertainties, the expected toxicity of the bioidentical protein is low and thus, first-stage testing is warranted. The details of clinical investigation of *i.v.* sgp130 in Covid-19 exacerbations will require additional investigation into the kinetics and dynamics of cytokine storm, for instance via longitudinal follow-up of patients with and without hypercytokinaemia, and adjustment of the therapeutic algorithm in regard to other common treatment options such as antipyretics and glucocorticoids (Bhaskar *et al.* 2020).

Declaration: no interests.

Methods

Differential expression analysis

Array data were downloaded as provided by the original authors from NCBI GEO, accession number GSE5972. The original cohort (Cameron *et al.* 2007) included 50 SARS patients (22 males, 28 females) “without bias to age, sex, or previous medical history” from the Toronto area and 10 healthy volunteers (five male, five female). Patients had a median age of 48 years (range 24-83) and presented to the hospital at a median of two days after symptom onset (DSO). 57% of non-severe and 100% of severe cases received glucocorticoid treatment, with first dose given at a median of six DSO. Given were either oral prednisone at a median peak daily dose of 100 mg, or intravenous methylprednisolone at a median peak daily dose of 125 mg. For microarray analyses, 60 RNA samples (from peripheral blood) were collected from 40 patients at various time points throughout the illness (1 sample from 30 patients and 2 to 5 samples from 10 patients). According to time point, patients were stratified into three groups: pre-exacerbation, post-exacerbation, and convalescent (exacerbation was determined by arterial oxygen saturation nadir); exacerbation occurred at a median of eight DSO. Ten RNA samples (from peripheral blood) were collected from the ten control subjects, adding up to 70 samples total. More detailed patient information can be obtained from Table 1 of Cameron *et al.* (Cameron *et al.* 2007) and from subject metadata of GSE5972.

Expression variance was analysed with R/quantro (Hicks & Irizarry 2015) to guide the choice of normalisation. Global differences were detected (via *quantro()*) in the four analysed groups ($p = 0.0198$), and thus, no further quantile normalisation was applied. Permutation testing of the result (1000 permutations) confirmed global differences with $p < 0.001$ (Supplementary Fig. 2).

160 Differentially expressed genes were then sought using R/limma (Ritchie *et al.* 2015) using a model
161 of all four groups (control, pre-exacerbation, post-exacerbation, convalescent), controlling for sex
162 and age of subjects. Intensity ratio versus average intensity was assessed via *plotMA()*, and
163 differential expression was assessed via the *topTable()* function, utilising Benjamini-Hochberg
164 correction of p-values for all groups versus controls.

165 All code is available at <https://github.com/slobentanzer/il6r-scavenging-by-sgp130>.

166 Tissue-specific gene expression

167 For basal expression of interleukin-related genes in immune and control tissues, the “tissue-specific
168 regulatory circuits” of Marbach *et al.* (Marbach *et al.* 2016) were used. Briefly, for each tissue
169 regulatory circuit, transcriptional activity towards each gene was aggregated across all individual
170 transcriptional activators, i.e., the sum of weights of incoming edges of each gene of interest; the
171 authors have shown that this cumulative activity correlates with tissue-specific expression of each
172 gene (Marbach *et al.* 2016). Aggregated values were treated as expression matrix and further
173 analysed using R, including visualisation via R/ggplot2 (Wickham 2016).

174 The code also is available at <https://github.com/slobentanzer/il6r-scavenging-by-sgp130>.

Figure 1: Differential expression of interleukin-related genes in a cohort of SARS (CoV 1) patients from the Toronto area. Displayed are log₂ fold expression values of interleukin (IL)-related signalling molecules that exhibit differential expression (adjusted p-value < 0.05) in at least one phase of the disease. The major pro-inflammatory cytokine IL-1 β is decreased in patients pre-exacerbation and slowly recovers until convalescence. The IL-1 β receptors IL1R1 and IL1RL1 are likewise decreased. In contrast, IL6R is elevated pre-exacerbation, and then returns to normal and almost sub-normal levels during and after exacerbation. Downstream of IL receptor activation,

184 STAT5A and STAT6 are decreased pre- and post-exacerbation, and return to normal levels upon
 185 convalescence. *: $p < 0.05$.

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 187 **Supplementary Figure 1:** Basal gene expression profiles of IL6R, gp130 (aka IL6ST), IL1R1,
 188 IL1RL1, IL17RE, and IL22RA, as derived from “tissue-specific regulatory circuits” (Marbach *et*
 189 *al.* 2016). As previously described, monocytes and regulatory T cells express highest amounts of
 190 IL6R (solid line). Most non-immune control tissues, on the other hand, express very low levels of
 191 IL6R (marked by italic font). However, bronchial epithelial cells express high amounts of IL6R,
 192 even in comparison with hepatocytes, the only non-immune cells reported to show high mbIL6R
 193 expression (marked by underscore). IL1R1 and its homolog IL1RL1 are also highly expressed,

194 particularly in non-immune tissues, whereas IL17RE and IL22RA1 show almost no basal
195 expression in the analysed tissues.

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197 **Supplementary Figure 2:** Permutation test of quantro analysis of global differences in the
198 Toronto SARS cohort.

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