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A peptide derived from HSP60 reduces proinflammatory cytokines and soluble mediators: a therapeutic approach to inflammation

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Cytokines are secretion proteins that mediate and regulate immunity and inflammation. They are crucial in the progress of acute inflammatory diseases and autoimmunity. In fact, the inhibition of proinflammatory cytokines has been widely tested in the treatment of rheumatoid arthritis (RA). Some of these inhibitors have been used in the treatment of COVID-19 patients to improve survival rates. However, controlling the extent of inflammation with cytokine inhibitors is still a challenge because these molecules are redundant and pleiotropic. Here we review a novel therapeutic approach based on the use of the HSP60−derived Altered Peptide Ligand (APL) designed for RA and repositioned for the treatment of COVID-19 patients with hyperinflammation. HSP60 is a molecular chaperone found in all cells. It is involved in a wide diversity of cellular events including protein folding and trafficking. HSP60 concentration increases during cellular stress, for example inflammation. This protein has a dual role in immunity. Some HSP60-derived soluble epitopes induce inflammation, while others are immunoregulatory. Our HSP60-derived APL decreases the concentration of cytokines and induces the increase of FOXP3+ regulatory T cells (Treg) in various experimental systems. Furthermore, it decreases several cytokines and soluble mediators that are raised in RA, as well as decreases the excessive inflammatory response induced by SARS-CoV-2. This approach can be extended to other inflammatory diseases.

KEYWORDS

HSP60, APL, cytokines, inflammation, rheumatoid arthritis, CIGB-814/CIGB-258, COVID-19, Jusvinza

1 Introduction

Cytokines are molecules with relatively low molecular weights. These proteins are secreted by several immune cells (1, 2). Some cytokines are pro-inflammatory and others are anti-inflammatory. Consequently, they act mostly as regulators of immune and inflammatory responses (3–5).

Additionally, cytokines have important functions in a diversity of biological events including cell activation, differentiation, and proliferation (6, 7). Furthermore, cytokines play a crucial role in acute and chronic inflammation, and tumor progression, as well as in the onset and chronicity of autoimmune diseases (1, 6, 8).

The inhibition of proinflammatory cytokines has been used in the treatment of autoimmune diseases such as RA (9–11). Some of these drugs were repositioned in the management of COVID-19 patients (12, 13). However, controlling hyperinflammation with cytokine inhibitors is still a challenge, because these molecules are redundant and pleiotropic (14, 15). Likewise, these drugs are immunosuppressive and they can lead to the fall of the general condition of COVID-19 patients (16–18).

In this article, we focus on an original approach to the control of inflammation using an APL derived from HSP60. This therapeutic concept is focused on the induction of peripheral tolerance using a modified autoantigen implicated in RA pathogenesis. HSP60 was the autoantigen chosen for the APL design. Interestingly, this protein plays a dual role in immunity, i.e. certain soluble HSP60-derived epitopes induce inflammation and others are immunoregulatory (19–23).

The APL designed has anti-inflammatory properties and increases Treg in preclinical models of RA (24, 25). Clinical investigations in RA patients indicate that this molecule is safe and reduces inflammation (26–28).

Based on these results we decided to investigate whether this molecule could be useful in treating the hyperinflammation that distinguishes COVID-19 patients who are progressing to severe and critical conditions. The treatment with this peptide inhibited several cytokines and soluble mediators associated with hyperinflammation in COVID-19 patients (29, 30).

Given all these results, the anti-inflammatory effects of this molecule are assessed in other experimental models of inflammation.

2 HSP60, autoantigen selected for APL design

Among the autoantigens involved in RA pathogenesis, we selected HSP60 for the APL design. This selection was supported by the therapeutic potential of HSP60 for autoimmune diseases. We hypothesized that an APL derived from HSP60 would enhance these therapeutic effects.

HSP60 is extremely conserved in evolution (31, 32). This protein is classified as a chaperone and is located inside the mitochondria where, together with the co-chaperonin Hsp10, it assists in protein homeostasis (33, 34).

However, under certain physiological conditions, HSP60 is located in other cellular organelles and it can even appear in the extracellular space. At these sites, HSP60 participates in pathogenic events such as inflammation, autoimmunity and carcinogenesis (35, 36).

HSP60 has an interesting connection with innate and acquired immune response, which is related to its conservation through evolution and its chaperone function.

This molecule has been described as an innate signal for macrophages and dendritic cells. Macrophages in reaction to HSP60 produce pro-inflammatory molecules (37–39). HSP60 stimulates the maturation of dendritic cells (40).

This chaperone was identified as a dominant bacterial antigen during infections or vaccination. Both biological events are characterized by the secretion of antibodies to bacterial HSP60 (41). Moreover, autoantibodies to self-HSP60 are identified in several autoimmune diseases such as RA, lupus, inflammatory bowel disease and atherosclerosis (42–46).

Furthermore, autoantibodies against HSP60 are present in healthy subjects; for example, an Ig M iso-type autoantibody against HSP60 was identified within the blood of the umbilical cord of some newborns (47), and IgM and IgG autoantibodies against HSP60 were identified in healthy human beings (48).

On the other hand, epitopes derived from mycobacterial HSP65 that are identical to the HSP60 human peptide, induced cytotoxic T cell reactivity in healthy humans (20). Besides, T cells against HSP60 self-peptides were found in the blood of the umbilical cord of healthy neonates (39). These facts indicate that it is normal to find effector T cells to self-HSP60 from birth.

Cohen hypothesized that HSP may be included in the "immunological homunculus", which comprises several dominant antigens involved in an intricate biologic regulatory network (40).

Other authors have described that T cells to self-HSP60 are related to spontaneous remission in juvenile idiopathic arthritis (41, 42) and induce resistance to induction of experimental arthritis in Lewis rats (43–45). T cells against HSP60 that secreted IL-10 can be favorable in attenuating inflammation during autoimmune diseases and harmful in infections (42, 46, 47). In contrast, T cells vs HSP60 that produce proinflammatory cytokines can be damaging in autoimmune diseases and beneficial against pathogens (48).

Studies about HSP60 have progressively increased in recent years, mainly because of its potential as an approach for emerging therapeutic procedures in inflammatory diseases and severe chaperonopathies such as several kinds of cancer, as well as inflammatory and autoimmune diseases and neurodegenerative diseases (36, 49–52).

Particularly, since the regulatory effect of HSP60 on the immune response is well defined, several authors have proposed different approaches for its use in the treatment of autoimmune diseases (22, 23). Regardless of the potential risks of generating adverse inflammatory effects, diverse formulations of HSP60 and peptides derived from it, have been studied in experimental models without observing pathological autoimmunity (53).

Furthermore, reports show the use of the immunomodulatory properties of HSP60 and its peptides in clinical trials for

autoimmune diseases (54–57). However, none of these therapeutic candidates have become registered drugs.

We believe that there are key points in the success of the treatments based on peptides derived from HSP60 for their use in inflammation and the reduction of pro-inflammatory cytokines, i.e., the selection of a specific epitope, the biodistribution of this molecule and the frequency of its administration.

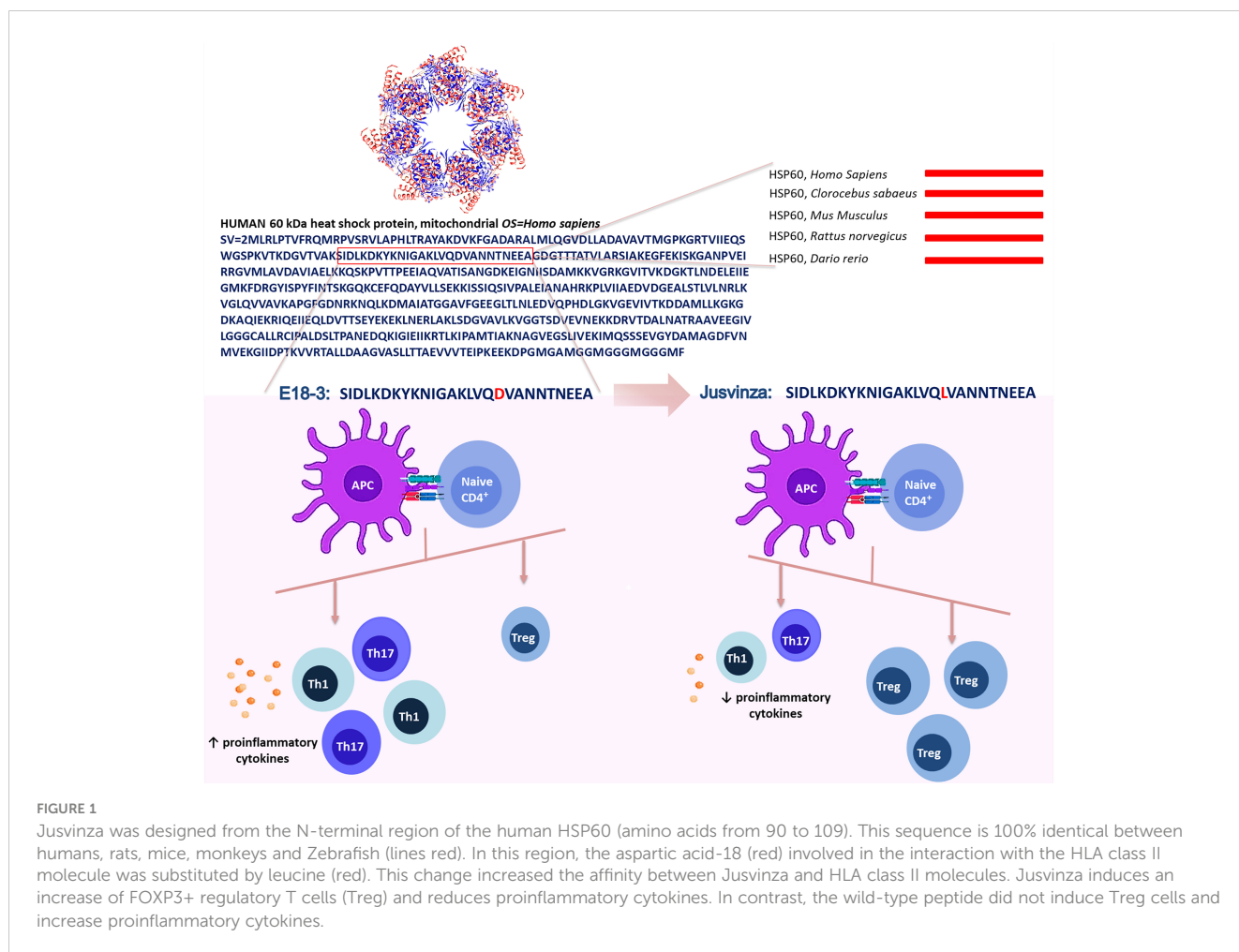# 3 An APL designed from HSP60 as an inductor of peripheral tolerance

The identification of an epitope from HSP60 is a critical factor for induced peripheral tolerance as a possible treatment for autoimmune diseases.

The potential of the APLs as tolerance inductors has been previously reported (58–60). APLs are similar to the wild-type peptide but with one or two mutations in the essential interaction positions with the T-cell receptor (TCR) or with the HLA class II molecules that modify the pathways for the activation of T cells. These APL can modify the response of autoreactive T cells by several mechanisms (60–64).

In contrast with other authors, we selected the N-terminal sequence from human HSP60 (amino acids 90 to 109). This region is much conserved, it is 100% identical in humans, monkeys, fish, rats and mice, but the match is 50% with *Mycobacterium tuberculosis* (*Mt*). In this sequence, the Propred computer algorithm (65) predicted new epitopes that would be interacting directly with the HLA class II molecule associated with RA. Aspartic acid 18 was substituted by leucine (Figure 1). This mutation increased the affinity between APL and HLA class II molecules, according to the bioinformatic prediction (24).

Furthermore, potential binding motifs (cores) of this APL and its wild type peptide (E18-3), and the affinity of both to HLA class II alleles related with RA, were analyzed by the NetMHCIIpan platform (66, 67). This algorithm indicated that APL has two possible overlapping epitopes. A notable feature is that the replacement of Asp-18 in E18-3 with a Leu raises the affinity of APL to HLA class II. Predictions using NetMHCIIpan advise that the novel peptide binds to more RA related HLA class II molecules and has a better affinity than E18-3 (68).

This novel peptide was called APL-1 or CIGB-814 in preclinical and clinical studies in RA. This peptide was renamed CIGB-258 during clinical research in COVID-19 patients. Subsequently,

FIGURE 1
Jusvinza was designed from the N-terminal region of the human HSP60 (amino acids from 90 to 109). This sequence is 100% identical between humans, rats, mice, monkeys and Zebrafish (lines red). In this region, the aspartic acid-18 (red) involved in the interaction with the HLA class II molecule was substituted by leucine (red). This change increased the affinity between Jusvinza and HLA class II molecules. Jusvinza induces an increase of FOXP3+ regulatory T cells (Treg) and reduces proinflammatory cytokines. In contrast, the wild-type peptide did not induce Treg cells and increase proinflammatory cytokines.

CIGB-258 was granted Authorization for Emergency Use (AEU) by the Cuban Regulatory Authority for COVID-19 patients under the commercial name of Jusvinza.

4 Biodistribution and pharmacokinetics (PK) of Jusvinza

The biodistribution profile of a drug is essential in activating the molecular mechanisms that induced peripheral tolerance. Hence, depending on the organ where the drug is positioned, it may be able to interact with antigen-presenting cells (APC), T cells and cytokines that mediate the induction of tolerance.

The biodistribution of a peptide can be affected by the dose, as well as by the inoculation route. Jusvinza was mainly distributed in the stomach and small intestine, after being inoculated in Lewis rats. The levels of Jusvinza increased in lymph nodes (LNs) at 24 hours, compared to four hours postadministration. This peptide was likewise found in the liver, spleen, heart and lungs (Figure 2A). The biodistribution of Jusvinza was almost the same for the three routes studied (subcutaneous, intravenous and intradermal routes) (69). This biodistribution profile is interesting because the small intestine is specialized in the induction of tolerance (70).

In addition, the highest concentration of Jusvinza in the blood plasma of rats was found at 0.5 to 1 hour; and the half-life in the blood was calculated to be of six hours. These results agree with the PK profile identified in RA patients included in the Phase I Clinical Trial (27).

Recently, we have identified that Jusvinza binds only with apolipoprotein A-I (Apo-AI) and transthyretin in human plasma. The identification of these proteins were through affinity chromatography using as the matrix a pearl-shaped resin (ChemMatrix) - coupled to Jusvinza. The eluted proteins were accurately identified by mass spectrometry (nano ESI-MS). Apo-AI and transthyretin are involved in lipid metabolism, and changes in their conformation and concentration have been associated with diseases such as type II diabetes and atherosclerosis. These results agree with those of Cho et al, who demonstrated that Jusvinza enhanced HDL stability (71).

Future research is needed to elucidate the biological significance of the binding of Jusvinza to Apo-AI and transthyretin.

5 Jusvinza reduces inflammation and TNFα in two animal models for RA

The biological effect of Jusvinza was assessed in an experimental model in rats (72). Jusvinza has an epitope that could be bound to rat MHC class II molecules (RT1.BI); according to the MHC2PRED software (73). In this animal model, the disease is induced in rats
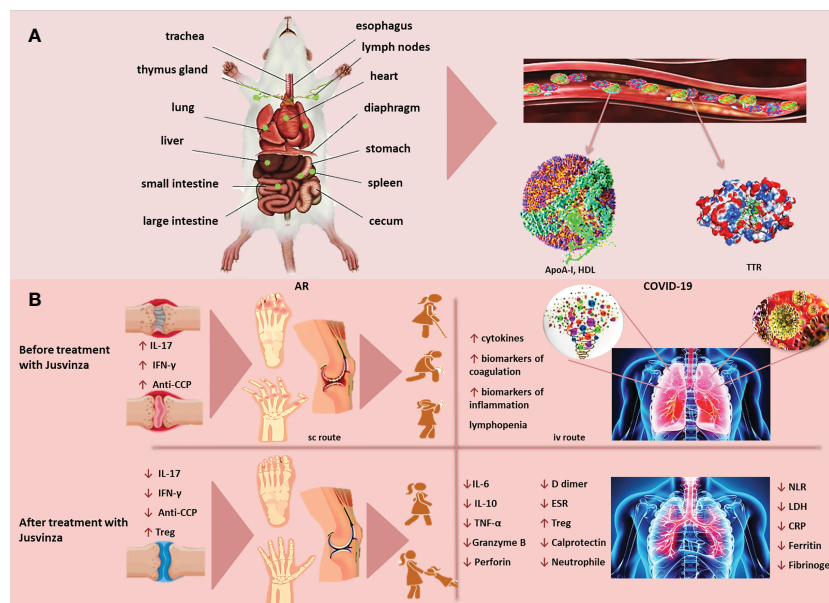

FIGURE 2
(A) Jusvinza is mainly distributed to the stomach and small intestine, as early as 4 hours post-administration. The concentration of Jusvinza increased in lymph nodes (LNs) at 24 hours post-administration. This peptide was also found in the liver, spleen, heart and lungs in Lewis rats. Jusvinza binds apolipoprotein A-I (Apo-AI) and transthyretin (TTR) in plasma. **(B)** Jusvinza reduces inflammation in animal models and RA patients. The processing and presentation of this APL by the antigen-presenting cells (APC) to the autoreactive T lymphocytes in the periphery could induce the expansion of Treg. These cells migrate to the swollen joints and attenuate autoreactive T cells responsible for arthritis pathogenesis. In addition, Jusvinza reduces TNFα, IL-17, interferon-gamma (IFN-γ), anti-CCP antibodies and neutrophil migration. Jusvinza reduces hyperinflammation in COVID-19 patients. Jusvinza can inhibit the activity of monocytes, macrographs and neutrophils. This inhibition may contribute to the decrease of IL-6, TNFa and IL-10, as well as the restoration of lymphocyte counts in patients. Additionally, this peptide can induce Treg. These activated cells migrate to inflamed sites and could cross-recognize the wild-type epitope from HSP60 expressed in the endothelial tissue and inhibit autoimmune damage induced during viral infection.

through immunization with *Mt* in incomplete Freund's adjuvant (AA). Jusvinza reduced the inflammation and pannus in these rats with arthritis.

However, the original epitope from HSP60 did not produce these therapeutic effects in rats. Besides, the treatment with Jusvinza significantly reduces TNFα levels in the spleen of treated animals compared to the placebo group. This clinical efficacy induced by Jusvinza was associated with an increase of Treg in the rats.

The AA model is characterized by the fact that immunization with *Mt* protects against subsequent attempts to induce this disease. This effect is due to the induction of Treg against a conserved epitope from HSP60 (aa from 256 to 270) and the secretion of regulatory cytokines (41, 74). These factors could favor the therapeutic effect of Jusvinza in the AA model. Hence, it was essential to assess the therapeutic effect of Jusvinza in another animal model, in which Treg cells are not induced against HSP60 in the course of the disease.

Consequently, the therapeutic effect of Jusvinza was evaluated in Collagen Induced Arthritis (CIA) in DBA/1 mice (75). Jusvinza monotherapy was able to reduce inflammation in these mice. The therapeutic effect was similar to mice inoculated with Jusvinza plus methotrexate (MTX) (25). Furthermore, pannus development was not detected in mice treated with Jusvinza. This indicates that the migration of macrophages and neutrophils did not take place in the joints of mice treated with this peptide.

These results were also linked to a decrease in TNFα levels. This cytokine is crucial for the onset and chronicity of RA. TNFα can stimulate proinflammatory cytokine production, increasing the expression of adhesion molecules and neutrophil activation, and enhancing antibody secretion by plasmatic cells (76–78).

Contrary to the effect in the spleen of AA rats treated with Jusvinza, Treg was not detected in CIA mice. Nevertheless, the treatment with Jusvinza plus MTX induced Treg in the spleen of CIA mice. The molecular mechanism of MTX is not associated with an induction of Treg in mice (79). But, the decrease of TNFα mediated by MTX can favor the induction of Treg by Jusvinza. The results suggest that MTX plus Jusvinza could have a molecular synergic effect in the CIA model.

Jusvinza reduced inflammation in AA and CIA models mediated by the expansion of Treg at the periphery. These cells could migrate to the swollen joints and decrease autoreactive T cell activity that perpetuates arthritis, blocking the production of proinflammatory cytokines and the consequent neutrophil migration.

6 Jusvinza induces Treg with suppression activity and reduces IL-17 secreted by Th17

Jusvinza induced Treg in draining lymph nodes and spleen from healthy BALB/c mice. At the same time, the wild-type epitope of HSP60 recruits more CD4+ FoxP3- T lymphocytes, showing that the mutation of the wild-type peptide was effective in inducing Treg and reinforces the beneficial potentials of Jusvinza for the management of RA patients (24).

In addition, experiments with peripheral blood mononuclear cells (PBMC) from RA patients indicate that Jusvinza induces Treg (24, 80). In contrast, the wild-type peptide did not induce Treg (24).

Furthermore, autologous cross-over experiments showed that Jusvinza-treatment had a significant effect in reducing IL-17 levels. Jusvinza can induce Tregs and its suppressive activity against antigen-specific T lymphocytes cells, whereas the activated T effector cells produce less IL-17 (68).

The change of sequence produced in Jusvinza could increase Treg in RA patients and specifically inhibit autoreactive T cells. Treatment with Jusvinza could help restore the healthy Th17/Treg balance for patients with RA and other autoimmune diseases.

7 Jusvinza reduces inflammation, proinflammatory cytokines and autoantibodies against cyclic citrullinated peptides (anti-CCP) in RA patients

The safety of Jusvinza was assessed in patients with RA, during an open phase I clinical trial. Twenty patients with moderately active RA were involved in this study. Three doses (1, 2.5 and 5 mg) of Jusvinza were evaluated by the subcutaneous route. This study included the restriction of the use of non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs and corticosteroids, as of four weeks before the start of the Jusvinza treatment. Clinical response in patients was assessed following the guidelines of the American College of Rheumatology and Disease Activity Score in 28 joints. Function and health-related quality of life, quantification of inflammatory biomarkers and radiographic changes in patients were also evaluated.

Jusvinza was well tolerated at all doses. The adverse effects detected were minor and reversible, essentially irritation at the inoculation site. Treatment with this peptide diminished disease activity and magnetic resonance imaging score in patients. This treatment enhanced the health-related quality of life of all patients included in the study. Moreover, Jusvinza significantly reduced IL-17 and interferon-gamma (IFN-γ) in patients (26).

RA is mediated by autoreactive T lymphocytes, with TH1 and TH17 phenotypes. However, antibodies against citrullinated self-proteins have a pathogenic role in this disease. Anti-CCP antibodies are related to a fast course of RA, primary erosive damage, increased inflammation and disability in patients (80).

Jusvinza induced a significant reduction of anti-CCP antibodies in patients (28). These results suggest that Jusvinza could inhibit B lymphocytes that produce these pathogenic antibodies.

Another explanation is related to the possible induction of Treg by Jusvinza in patients. This peptide increases Tregs in some experimental models (24, 25). Tregs have different mechanisms of action that may depend on cell contact, through which they can induce apoptosis on effector T cells and plasma cells secreting anti-CCP antibodies (81). Interestingly, Jusvinza decreased the viability of PBMC isolated from RA patients by inducing apoptosis (82).

Furthermore, Jusvinza might affect the citrullination process by decreasing IL-17. Interactions with viruses or bacteria that activate NETosis are the leading sources of protein citrullination in RA (83). NETosis is a type of regulated cell death dependent on the formation of neutrophil extracellular traps. Some studies describe that HSP60 disturbs the effector functions of neutrophils, i.e. HSP60 enhances the phagocytic activity of neutrophils (84); bacterial HSP GroEL from *Staphylococcus epidermidis* biofilms promoted ADN decondensation and induced NETosis (85); inflammatory cytokines can lead to NETosis in neutrophils from RA patients (86). Specifically, IL-17 has widespread inflammatory effects on the joints, inducing bone and cartilage erosions and promoting the migration of inflammatory molecules to the synovia (87). This cytokine plays an important role in the regulation of anti-citrullinated protein antibody secretion (88).

Jusvinza decreases IL-17 and soluble mediators as anti-CCP antibodies, and it can stimulate the activation of the suppressive activity of Tregs (24, 68) (Figure 2B). Therefore, all results confirm the beneficial effect of Jusvinza and its possible medical application in the reduction of inflammation in RA and other inflammatory diseases.

8 Jusvinza reduces cytokines involved in the "cytokine storm" and soluble mediators of inflammation in COVID-19 patients

The clinical spectrum of COVID-19 is very widespread and complex. Patients may range from asymptomatic cases to those showing a rapid progression toward acute respiratory distress syndrome (ARDS) and death (89). Patients progressing to severe stages present an exacerbated inflammatory response, evidenced by the increase in the serum concentration of inflammation biomarkers (90). A group of these patients progresses to cardiovascular collapse, multiple organ failure, and death. Under these conditions, the treatments used for inflammatory chronic diseases have been repositioned to reduce hyperinflammation in COVID-19 patients (91, 92).

The anti-inflammatory effects of Jusvinza in several experimental models of RA are associated with TNFα reduction and Treg induction. At the same time, the therapeutic effect and the reduction of IL-17, IFNγ and autoantibodies against citrullinated self-proteins in RA patients (24, 26, 28, 68, 80) were the rational bases in the proposal of this molecule for the management of hyperinflammation in COVID-19 patients. Consequently, the Cuban Regulatory Authority approved the exploratory use of this peptide for COVID-19 patients in critical and serious conditions.

Exploratory studies in COVID-19 patients revealed that this APL promotes clinical and radiological improvement linked to a decrease in systemic inflammation biomarkers and IL-6, TNFα and IL-10 (29, 93). The treatment with this peptide was granted the AEU by the Cuban Regulatory Authority for COVID-19 patients (94). After this AEU, Jusvinza was included in the Cuban guidelines

for the management of COVID-19 patients with signs of hyperinflammation (95).

High levels of IL-6, associated with disease severity, have been widely described in COVID-19 patients (96, 97). IL-6 is considered one of the most important cytokines in infections, along with IL-1 and TNF-α (98). Jusvinza reduced IL-6 levels linked to the clinical improvement of patients (29).

TNF-α stimulates an ongoing inflammatory response associated with autoimmune diseases (99, 100). TNF-α is a pro-inflammatory cytokine that can support T cell apoptosis by interacting with its receptor (101, 102). This cytokine is essential in the pathogenesis of lung fibrosis and SARS-CoV-2 infection (103). Interestingly, TNF-α is significantly reduced with Jusvinza therapy (29).

On the other hand, critically ill COVID-19 patients display an increase of IL-10 related to a worsening of this disease (104). IL-10 is a cytokine with many pleiotropic properties in immunoregulation and inflammation (105). The coronaviruses have intricate mechanisms that use the immunoregulatory function of IL-10 for immune evasion, helping virus replication (106). An association between a high concentration of IL-10 with the fall and functional exhaustion of CD8+ and CD4+T cells has been found in COVID-19 patients (107), indicating that this cytokine plays a key role in SARS-CoV-2 pathogenesis. The Jusvinza treatment led to IL-10 reduction after 96 hours of therapy. This reduction was associated with the clinical improvement of patients (29). IL-10 is secreted by macrophages, monocytes and T cells. However, T cells can not contribute to the high concentration of IL-10 because these patients are characterized by a marked lymphopenia, typical of COVID-19. Jusvinza diminishes monocyte and macrophage counts under cell stress (82). This is possibly the mechanism through which IL-10 is reduced in COVID-19 patients treated with Jusvinza. Besides, the reduction of IL-6 and TNF-α could downregulate IL-10.

Furthermore, high concentrations of calprotectin (S100A8/A9) are associated with negative clinical results in COVID-19 patients (108). Jusvinza reduces calprotectin in sera from COVID-19 patients and this reduction was correlated with the contraction in neutrophil count. These results are interesting because calprotectin is also increased in patients with chronic inflammatory diseases, inducing cytokine production (109).

Lymphopenia, with significantly reduced numbers of T lymphocytes, has been described in severe COVID-19 patients. This lymphopenia is related to the functional activities of cytotoxic T lymphocytes in COVID-19. Natural killer cells, total T cells, as well as CD8+ T cells, are lower in patients showing severe disease than in healthy persons (110).

Likewise, Granzyme B and perforin are increased in CD8+ T cells in severely ill patients (111). Both proteins decreased in the serum 96 hours after the treatment with Jusvinza. This reduction was related to the stabilization of lymphocyte and neutrophil counts in the patients (29).

Additionally, Tregs are significantly reduced in severely ill COVID-19 patients (112, 113). Jusvinza induces Tregs in seriously ill COVID-19 patients (29). Gammazza et al. identified an epitope from HSP60 that is common with the SARS-CoV-2 replicase polyprotein 1ab. These authors suggested that post-

translational modifications during metabolic stress produced by hypertension and diabetes could disturb HSP60 localization and induce an endothelial injury (114). The beneficial result of Jusvinza in COVID-19 patients could be facilitated by the induction of Treg. These cells migrate to inflamed tissues and could cross-recognize the wild-type peptide from HSP60. This new "cross-talk" could induce regulatory mechanisms that would reduce autoimmune damage in the endothelium produced by viral infection. Moreover, Jusvinza could inhibit the activity of neutrophils, monocytes and macrographs. This inhibition may influence the decrease of several proinflammatory cytokines and soluble mediators of inflammation, as well as the increase of lymphocyte counts (Figure 2B). All these effects could resolve exacerbated inflammation and contribute to a positive outcome for the patients.

These results are the premise for the evaluation of the anti-inflammatory effect of Jusvinza in other experimental models of inflammation.

9 Anti-inflammatory activity of Jusvinza against acute toxicity induced by carboxy methyl lysine in Zebrafish

High levels of inflammatory cytokines have characterized severe stages of COVID-19 (113), autoimmune diseases and inflammatory chronic diseases (115). The non-enzymatic glycation of proteins and carbohydrates induces some glycation end products, related to hyperinflammation (116). The glycation of high-density lipoproteins (HDL) is associated with the dysfunctional activity of this lipoprotein (117), which could enhance inflammation. Glycated HDL were found to be toxic in different human cells (118, 119), and in Zebrafish and its embryos, producing reactive oxygen species and showing a slower development rate (120).

Zebrafish (Danio rerio) is a routinely used model to assess the toxicity of advanced glycation end products (120) and to evaluate the anti-inflammatory effect of therapeutic candidates.

Recently, Cho et al. studied the therapeutic effect of Jusvinza against inflammation induced by N-"-carboxymethyl lysine (CML) in Zebrafish embryos and adults (71). High concentrations of CML have been identified in the serum of patients with diabetes mellitus and atherosclerosis (121, 122). These patients also showed extremely high inflammatory cytokines, such as IL-1 and TNFα, indicating that a high CML concentration is linked to a pro-inflammatory condition (123). CML produced an important glycation and aggregation of HDL that disturbs the structure and function of HDL, which is linked to a reduction in apolipoprotein A-I stability (118).

Microinjection of CML in Zebrafish embryos produces high embryonic death rates, where survival is of only 18% of the fish showing developmental defects. However, Jusvinza co-injection induces a significant increase in survival and normal development of the fish. Furthermore, an intraperitoneal inoculation of CML in adult Zebrafish produced acute paralysis, sudden death, and affected its swimming capacity through hyperinflammation. But, a co-inoculation of Jusvinza caused a quicker recovery of swimming

capacity and a higher survivable rate. Interestingly, the group treated only with CML showed a survival rate of 49%, while the group treated with CML and inoculated with Jusvinza had a survival rate of 97%, with a significant decrease in liver inflammation.

On the other hand, these authors compared the efficacy of Jusvinza, Infliximab (Remsima®), and Tocilizumab (Actemra®) in the model of the acute death of Zebrafish induced by CML. The results showed that the Jusvinza group had a quicker recovery and swimming capacity, with a higher survivable rate than the Remsima® group (71).

10 Conclusions and perspectives

This review describes an important translational research outcome; from the design of an APL for the induction of tolerance in RA, followed by its assessment in experimental models, and finally the treatment of the inflammation that characterizes RA and COVID-19 patients.

The anti-inflammatory effect of Jusvinza constitutes an attractive therapeutic approach for an extensive variety of diseases, characterized by inflammation and high levels of proinflammatory cytokines, such as autoimmune diseases, COVID-19, atherosclerosis, diabetes and neurodegenerative diseases.

Author contributions

Conceptualization: MdCD-H; Writing – original draft: MdCD-H, AS-D, and MH-C; Writing – review & editing: MdCD-H, AS-D, MH-C, GM-D, and GG-N. All authors contributed to the article and approved the submitted version.

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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