Biochemical and Biophysical Research Communications 642 (2023) 83–89

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Pro-inflammatory cytokine secretion induced by amyloid transthyretin in human cardiac fibroblasts

Christophe Magaud [a], Thomas Harnois [c], Stephane Sebille [a], Aurelien Chatelier [a], Jean-Francois Faivre [a], Patrick Bois [a, *], Guylene Page [d], Barnabas Gellen [b]

[a] Laboratoire PRéTI UR 24184, Université de Poitiers, France
[b] Groupe Elsan SAS Polyclinic Poitiers, France
[c] Laboratoire 4CS UMR 6041 CNRS, Université de Poitiers, France
[d] UFR Médecine et Pharmacie, Université de Poitiers, France

ARTICLE INFO

Article history:
Received 30 November 2022
Accepted 9 December 2022
Available online 17 December 2022

Keywords:
Senile systemic amyloidosis
Transthyretin
Human cardiac fibroblast
Cytokine

ABSTRACT

Extracellular aggregates of wild-type human transthyretin are associated with heart diseases such as wild-type transthyretin (TTR)-derived amyloidosis (ATTR-wt). Due to their strategic location, cardiac fibroblasts act as sentinel cells that sense injury and activate the inflammasome. No studies of the effects of TTR amyloid aggregation on the secretion of inflammatory factors by primary human cardiac fibroblasts (hCFs) have been reported yet. The intracellular internalization of TTR aggregates, which correspond to the early stage of ATTR-wt, were determined using immunofluorescence and Western blotting of cell lysates. A further objective of this study was to analyze the secretion of inflammatory factors by hCFs after analysis of TTR amyloid aggregation using X-MAP® Luminex Assay techniques. We show that TTR aggregates are internalized in hCFs and induce the secretion of both Brain Natriuretic Peptide (BNP) and N-terminal pro B-type Natriuretic Peptide(NT-proBNP). Also, pro-inflammatory mediators such as interleukin-6 (IL-6) and IL-8 are secreted without significant changes in the levels of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). In conclusion, these findings suggest that IL-6 and IL-8 play important roles in the development of ATTR-wt, and indicate that IL-6 in particular could be a potentially important therapeutic target in patients with ATTR-wt.

1. Background

Transthyretin amyloid cardiomyopathy (ATTR-CM) is characterized by the abnormal deposition and accumulation of an insoluble protein, transthyretin (TTR or prealbumin), in the form of myofibrils. This deposition induces progressive and deleterious heart failure in the long term. This amyloid fibril formation results from a destabilizing mutation in hereditary ATTR amyloidosis (FAC) or from an aging-related process. This last form of amyloidosis (also called in the past senile systemic amyloidosis or recently wild-type transthyretin (TTR)-derived amyloidosis (ATTR-wt)) is characterized by the deposition of amyloid fibrils derived from wild-type TTR. Amyloid deposition is mainly detected in the myocardium. Although it is generally accepted that the ATTRwt is confined in the ventricular tissue, some studies show that TTRwt can also directly affect the human atrium [1,2]. TTRwt deposits in these different cardiac tissues lead to fibrosis and stiffening, with subsequent arrhythmias giving rise to heart failure and ultimately death [3,4].

Cardiac fibroblasts (CFs) make up two-thirds of the cardiac cell population. They are commonly described as supporting cells of the cardiac network. They exert essential functions that are fundamental for normal cardiac activity as well as in pathological cardiac remodeling [5,6]. Due to their strategic location, CFs serve as sentinel cells that sense injury. They are also known to secrete pro-inflammatory and pro-fibrotic cytokines following episodes of myocardial infarction (MI) [7]. Tumor necrosis factor-a (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) are released subsequent to CF activation, while matrix metalloproteinase (MMP) stimulation and tissue inhibitors of metalloproteinases (TIMPs) regulation are also induced [8–10]. MMPs are a family of multigenic proteins that participate in both normal and pathological tissue remodeling [11].

Recently, transcriptomic and cytokine protein analyses

* Corresponding author.
E-mail address: patrick.bois@univ-poitiers.fr (P. Bois).

https://doi.org/10.1016/j.bbrc.2022.12.027
0006-291X/

suggested that TTR induces an inflammatory response in rat CFs in cell culture [12]. The purpose of the present study is to examine effects of TTR on the secretion of inflammatory factors by human primary CFs (hCFs), including peptides, cytokines, MMPs and TIMPS.

2. Materials and methods

2.1. Reagents and preparation of stock solutions

Recombinant human TTR was purchased from Sino Biological Europe GmbH (Eschborn, Germany). To prepare a stock solution of 150 μg/mL, 670 μL of sterile water were added to a vial containing 100 μg TTR endotoxin-free lyophilized in sterile endotoxin-free PBS (Phosphate buffered saline), pH 7.4, 5% trehalose, 5% mannitol and 0.01% Tween-80. This stock solution was aliquoted into smaller quantities for storage at −80 °C. Fifty μg of recombinant human TNFα lyophilized in 3 mM sodium phosphate, pH 8 and 20 mM NaCl (Peprotech, Neuilly-sur-Seine, France) were reconstituted in 287 μL of Dulbecco's Modified Eagle's Medium (DMEM; Ozyme, Saint-Cyr-l'École, France) supplemented with 0.5% fetal bovine serum (FBS; Eurobio Scientific, Ulis, France), 1% antibiotics (100 IU/mL penicillin-G-Na; 50 IU/mL streptomycin sulfate). This stock solution was aliquoted at 0.1 μM and stored at −20 °C. For experiments on cultured hCFs, the stock solution was diluted in low serum (0.5% FBS) DMEM media culture to a final concentration of 50 nM.

2.2. Collection of human atrial trabeculae

Right atrial appendages were obtained during cannulation for cardiopulmonary bypass for coronary artery bypass surgery or aortic valve replacement from 16 patients (70.0 ± 5.8 years of age, 15 males and 1 female). All patients are anesthetized using pancuronium, propofol, and remifentanil. These patients were under treatment with various combinations of drugs, including beta-blockers, calcium-antagonists, nitrate, acetylsalicylic acid and angiotensin-converting enzyme inhibitors. All patients were sinus rhythm. This study conformed to the standards set down in the Declaration of Helsinki and was approved by the local ethics committee (Individual Protection Committee, CPP Ouest III, no. DC2008-565 for cardiac tissue collection) and included the consent of each subject.

2.3. Oligomerization of soluble TTR monomers

Aggregated TTR forms (oligotetramers) were generated by incubating the soluble recombinant protein in monomer form (0.15 mg/mL), with stirring, at room temperature and in the dark for 7 days [13]. Supplementary Fig. 1 shows the nature of structures obtained after stirring for 7 days; these consisted of monomers, dimers, tetramers and large oligomers. Thereafter, for the treatment of fibroblast cultures, these aggregated TTR forms were added in a final concentration of 1 or 1.5 μg/mL to low serum DMEM for 48 h.

2.4. Isolation and culture of human atrial fibroblasts

Fibroblasts from human atria were isolated by classical enzymatic digestion procedure that was previously reported [14,15]. In summary, cells were obtained by enzymatically digestion steps of atrial tissue in presence of collagenase (type V, Sigma-Aldrich Co., Saint-Quentin Fallavier, France), and protease (type XXIV, Sigma-Aldrich). Two filtrations were performed to remove cardiomyocytes, endothelial cells and smooth muscle cells, with 200 μm and then 20 μm cell strainers (Pluriselect, Life Science, Germany), as previously described [16]. Then, fibroblast pellet was resuspended in 4–6 mL of culture media, then seeded directly in 60 mm dishes (Nunclon, polystyrene surface).

These cultures (P0) were grown to 70–80% confluence over 8–9 days in DMEM supplemented with 10% FBS, and 1% antibiotics (100 IU/mL penicillin-G-Na; 50 IU/mL streptomycin sulfate). Cultures that were not 70–80% confluent after 8 days were excluded from the study. Experiments were performed on cells from passages 3–4 in low serum medium (0.5% FCS).

2.5. Sample and western blotting

Cells were treated or not with 1 or 1.5 μg/mL recombinant TTR proteins for 48 h. For western blot analyses, 10^6 cells were lysed in solution contained; 150 mM NaCl, 5 mM EDTA, 5 mM $MgCl_2$, 0.05% NP-40, 1% sodium deoxycholate, 1% Triton X-100, 50 mM Tris·HCl pH 7.4, 0.1% SDS, complemented with anti-phosphatase 1X and anti-protease (Roche, Complete Mini, EDTA-free). Cell lysates were parted by SDS-PAGE using 10% Bis-Tris polyacrylamide gels in a non-reduced condition. The generation of oligomers was performed in water, pH 7–8, with stirring for 7 days at room temperature. Each sample was lysed either in Laemmli loading buffer (Sigma/Millipore) for the reduced condition or in native buffer (62.5 mM Tris·HCl pH 7.4, 15% Glycerol, 2.3% SDS, 0.001% Bromophenol Blue) for the non-reduced condition and separated on 10% Bis-Tris polyacrylamide gels. The proteins were transferred to nitrocellulose membranes (0.20 μm-pore size,GE Healthcare, Little Chalfont, UK). The immunoblots were probed during one night at 4 °C in TBS-Tween (150 mM NaCl, 100 mM Tris–HCl, 0.1% Tween-20, pH 7.6, 3% fat milk) with anti-TTR antibodies (Santa Cruz). At that point, membranes were stored with anti-rabbit horseradish peroxidase-conjugated secondary antibodies during 1 h at 4 °C (1:5000, GE Healthcare, Little Chalfont, UK). The membranes washed during 5 min (three times) in TBS-Tween. ECL chemiluminescent substrate was used to detect bound antibodies (Immobilon, Millipore, Billerica, MA, USA) and results were examined with a GeneGnome XRQ chemiluminescence imaging system (SYNGENE Ozyme, Cambridge, UK).

2.6. Analysis of protein secretion

2.6.1. ELISA assay

Levels of BNP were measured in cell medium deficient in FCS using an electrochemiluminescence immunoassay (ELISA) (ThermoFisher Scientific, France). Briefly, fibroblasts were plated in 24-well plates containing DMEM medium supplemented with 10% FBS, 1% antibiotics (100 IU/mL penicillin-G-Na; 50 IU/mL streptomycin sulfate). Different initial cell densities were tested. After 7–8 days in culture, the cells were synchronized in the G0 phase in DMEM medium deficient in FCS (DMEM+0.5% FCS) for 24 h. The next day, the fibroblasts were incubated at 37 °C in the absence or presence of tetrameric TTR at concentrations of 1 and 1.5 μg/mL or TNFα at 50 nM. After 48 h of treatment, supernatants from the different cultures were collected. Samples were frozen at −80 °C until required for ELISA. BNP secretion levels were determined according to the manufacturer's recommendations. Results were read on a plate reader at 450 nm (Infinite F50, Tecan, Switzerland). Concentrations of BNP in the culture supernatants for the different treatment conditions for each atrial fibroblast culture for each patient were normalized to the average of the concentration of BNP in the culture medium of untreated fibroblasts (NT, No Treatment).

2.6.2. X-MAP® Luminex Assay

To measure levels of secreted NT-proBNP biomarker, cytokines/chemokines (IL-1α, IL-6, IL-8, IP-10, CCL-3,CCL-5, CCL-20 and CXCL-

1), TIMP-1 and -2 and MMP-1,-3 and-9, the different magnetic Milliplex™ human analyte magnetic bead panel kits purchased from Merck Millipore (Saint Quentin Yvelines, France) used are listed in Supplementary Table 1. To determine levels of inflammatory factors secreted by hCFs under the different culture conditions, cells were seeded at 10^4 cells per well in 24-well plates as described above for the ELISA assays. After 48 h of treatment with TTR in FCS-deficient DMEM medium, supernatants of the different cultures were collected. Samples were frozen at −80 °C until analysis with a X-MAP® Lumix Assay. Detection of each analyte was determined in supernatants according to the manufacturer's recommendations. Concentrations of each analyte were measured using a Luminex-200® instrument and xPONENT® software. For each analyte, equation of standard range, established using Milliplex® Analyst Software, made it possible to convert median fluorescence intensities obtained to concentrations (pg/mL). Finally, cytokine/chemokine concentrations in the supernatants of the different cultures were normalized to the average analyte concentration in the culture medium of untreated fibroblasts (NT, No Treatment).

2.7. Statistical analysis

Results were analyzed with GraphPad Prism® software. Non-parametric tests were performed for small sample numbers. Comparisons between treated and untreated cultures were performed with a non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison test. The significance level was $P < 0.05$.

3. Results

3.1. Analysis of TTR oligomer forms in cardiac fibroblasts

Before studying the effects of WtTTR on the secretion of inflammatory cytokines/chemokines by hCFs, we controlled for the presence of oligomeric forms of TTR in these cells under the stated experimental conditions. As specified in the Materials and Method, WtTTR oligomer generation was achieved after TTR stirring for 7 days at room temperature (in water pH7-8). We performed western blot of hCFs maintained in the presence of TTR extracellular deposits for 48 h (Fig. 1). To investigate the nature of the oligomeric TTR structures prepared under these conditions, we analyzed TTR aggregates in samples by immunoblotting with anti-TTR antibodies. As expected, western blots obtained from cell lysates showed different forms of TTR, ranging from monomers to dimers and large oligomers including tetramers.

3.2. TTR induces production of the TTR amyloidosis natriuretic biomarkers, BNP and NT-proBNP

We initially focused on the impact of TTR on the secretion of BNP and NT-proBNP by fibroblasts, considered in clinical research as biomarker peptides of cardiac amyloidosis (BNP assayed by the ELISA technique and NT-proBNP assayed by Luminex® Technology). Secretion levels of BNP and NT-proBNP were increased in fibroblasts after incubation with TTR (at 1 and 1.5 μg/mL) compared to untreated cells (Fig. 2). As expected, treatment with 50 nM TNF-α increased BNP production. Nevertheless, and perhaps surprisingly, human TNF-α at the same concentration did not change the level of NT-proBNP secretion.

3.3. TTR-induced cytokine production by hCFs

Previous analysis of the secretome of quiescent cardiac fibroblasts showed that cytokines were responsible for 5% of the total protein released [17]. On this basis, we tested whether TTR present in the culture medium could modulate the production of pro-inflammatory cytokines or chemokines. We assessed the levels of eight cytokines (IL-1α, IL-6, IL-8, IP-10, CCL-3, CCL-5, CCL-20 and CXCL-1) by X-MAP® Luminex Assay after 48 h TTR treatment. Secreted IL-6 and IL-8 levels increased significantly after TTR incubation at 1.5 μg/mL compared with untreated cells (Fig. 3B and C). No significant changes were observed for IL-1α (Fig. 3 A) or for five chemokines (IP-10, CCL-3, CCL-5, CCL-20 and CXCL-1) in medium from fibroblasts treated with soluble tetrameric TTR at concentrations of 1 and 1.5 μg/mL (Table 2 supplementary data). These results suggest that TTR induces a pro-inflammatory state with both IL-6 and IL-8 production, the latter being a polynuclear neutrophil attractant. It is noteworthy that TNF-α treatment at 50 nM induced a significant elevation in the production by hCFs of cytokines such as IL-1α, IL-6, IL-8 (Fig. 3) and IP-10, CCL-3, CCL-5, CCL-20 and CXCL-1 (Table 2 supplementary data), suggesting the presence of functional TNF-α receptors.

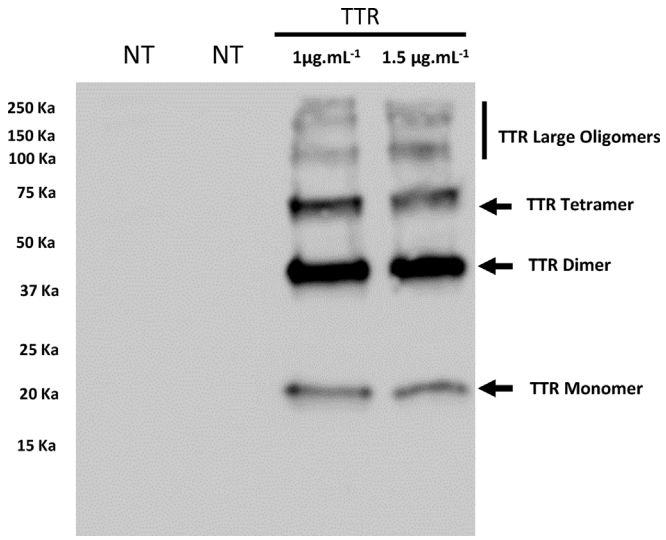

Fig. 1. Western blot analysis of TTR oligomers in human cardiac fibroblasts. Western blot of cell lysates of cardiac fibroblasts treated or not with TTR. The sample well in each lane was loaded with 30 μg of non-reduced and non-boiled sample. Note that generation of TTR oligomers was achieved after stirring for 7 days at room temperature, following which cardiac fibroblasts were incubated for 48 h with the TTR oligomers.

3.4. TTR had no impact on the production of MMPs and TIMPs by cardiac fibroblasts

MMPs are a family of zinc proteases which are responsible for breakdown of the extracellular matrix (ECM). These MMPs play important roles in the release of apoptotic ligands, the cleavage of cell surface receptors, cell proliferation or differentiation, and in chemokine activity. Stimuli such as adhesion molecules, growth factors, cytokines, or hormones, increase MMP expression. On the other hand, TIMPs control the regulation of MMP activity. Thus, both MMPs and TIMPs are key players in the maintenance of ECM homeostasis. To investigate whether TTR induces disruption of the MMP/TIMP balance in cardiac fibroblasts, levels of MMP-1, -3 and -9 and TIMP -1 and −2 were measured by X-MAP® Luminex Assay after 48 h treatment with TTR. Compared to untreated cells, levels of MMP-1, -3 and -9 and TIMP-1 and -2 were unchanged in the medium of fibroblasts treated with soluble tetrameric TTR at concentrations of 1 and 1.5 μg/mL (Fig. 4 A, B, C, D and E). These results suggest that TTR does not interfere with the MMP/TIMP balance. In

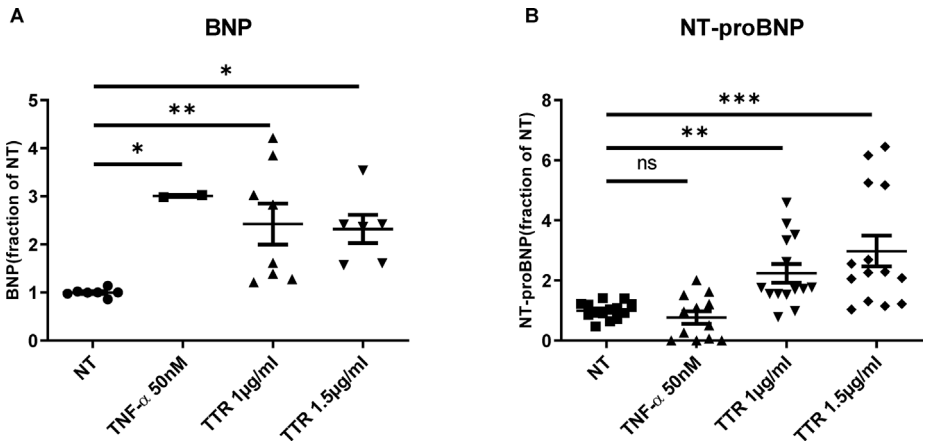

Fig. 2. TTR activation increased BNP and NT-proBNP biomarker production in human cardiac fibroblasts. Concentrations of the BNP and NT-proBNP biomarkers were measured by ELISA (for BNP, A) and by X-MAP® Luminex Assay (for NT-proBNP, B) in culture supernatants of untreated (NT) or TTR- or TNF-α-stimulated fibroblasts after 48 h' treatment. Significantly higher levels of BNP and NT-proBNP in culture media from fibroblasts treated with soluble tetrameric TTR at concentrations of 1 and 1.5 µg/mL were measured compared to untreated cells. TNF-α treatment at 50 nM induced a significant elevation in the production of BNP (A) but not in NT-proBNP (B). (A, number of patients = 3, n = 2–8 cell cultures; B, number of patients = 3, n = 12–14 cell cultures) *P < 0.05, **P < 0.01,***P < 0.005.

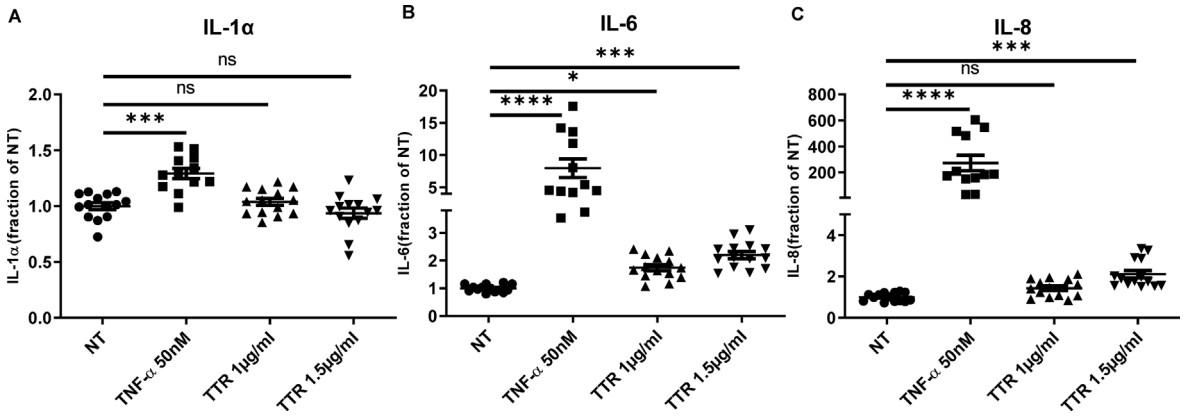

Fig. 3. TTR activation increases cytokine production in human cardiac fibroblasts. Concentrations of cytokines were measured by X-MAP® Luminex Assay in culture supernatants of untreated (NT) or TTR- or TNF-α-stimulated fibroblasts after 48 h' treatment. No significant changes in IL-1α concentrations were observed in medium from fibroblasts treated with soluble tetrameric TTR at concentrations of 1 and 1.5 µg/mL compared to untreated cells (A). Significantly higher levels of IL-6 production were observed in response to treatment with TTR (B). Treatment with TTR at a concentration of 1.5 µg/mL but not at 1 µg/mL induced a significant change in IL-8 secretion (C). TNF-α treatment at 50 nM induced a significant elevation of IL-1α, IL-6 and IL-8 production (A), (B) and (C). (Number of patients = 3; n = 12–14 cell cultures).*P < 0.05, ***P < 0.005,****P < 0.0001.

contrast, TNF-α treatment at 50 nM induced a significant elevation of MMP-1, -3 and -9 production by hCFs (Fig. 4C, D and E). These findings were associated with a downregulation of TIMP-2 production induced by TNFα (Fig. 4 B), whereas the level of secreted TIMP-1 did not change (Fig. 4 A).

4. Discussion

In this study, we provide evidence that TTR is able to induce the secretion in hCFs of BNP, NT-proBNP and pro-inflammatory mediators such as IL-6 and IL-8, without significant changes in the levels of MMPs and TIMPs.

BNP and NT-proBNP are produced by the atria and ventricles of the heart in response to increased wall pressure and stretching of the heart muscle [18]. Most of the BNP in the heart is secreted by cardiac fibroblasts. While NT-proBNP has no known physiological function, the secretion of BNP is considered to represent an adaptive mechanism of the heart to maintain cardiac performance during atrial arrhythmias and ventricular overload [19]. The cardioprotective effects of BNP, like natriuresis, diuresis and inhibition of the renin–angiotensin–aldosterone system have been shown in healthy individuals and in patients with heart failure, suggesting that elevated BNP expression and secretion is an adaptive mechanism of the heart that contributes to maintain cardiac performance during ventricular overload.

Clinical data show that the log concentration of BNP in patients with ATTR-wt was significantly correlated with diastolic cardiac function and the biochemical markers such as log C-reactive protein and log high-sensitivity troponin T [20]. That study confirmed the utility of plasma BNP as a prognostic marker of cardiac function in ATTR-wt. By inducing cellular stress, the accumulation of TTR in cardiac fibroblasts seems to stimulate BNP synthesis and secretory pathways.

Upon TTR stimulation, human CFs in culture consistently produced IL-6 and IL-8, while TNF-α, IL1-α and IP10 were not significantly modified. IL-6 is a pleiotropic cytokine that is essential in various cell types and organs (including the heart) for numerous cell processes such as angiogenesis, cell growth, differentiation, apoptosis and survival. The dysregulated continuous synthesis of IL-6 exerts a pathological effect in chronic inflammatory and

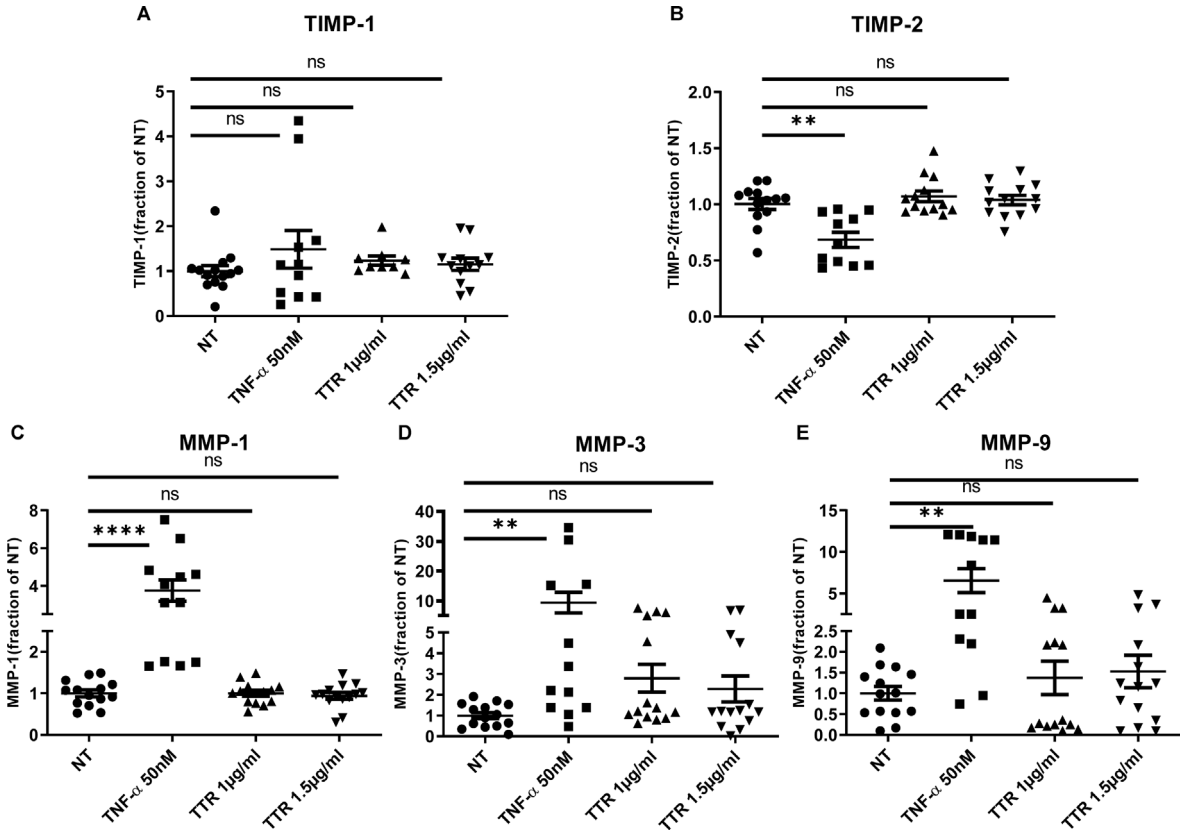

Fig. 4. TTR does not alter the MMP/TIMP balance in human cardiac fibroblasts. Concentrations of MMP-1,-3 and -9 and TIMP-1 and -2 were measured by X-MAP® Luminex Assay in culture supernatants of untreated (NT) and TTR- or TNF-α-stimulated fibroblasts after 48 h' treatment. No significant changes in MMP or TIMP levels were observed (A, B, C, D and E) in medium from fibroblasts treated with soluble tetrameric TTR at concentrations of 1 and 1.5 µg/mL compared to untreated cells. There was a significant downregulation of TNF-α-induced TIMP-2 (B) but not of TIMP-1 (A) production. TNF-α treatment at 50 nM induced a significant elevation of MMP-1,-3 and -9 production (C, D and E). (Number of patients = 3, n = 12–14 cell cultures).**P < 0.01, ***P < 0.005.

autoimmune processes [21,22]. Many studies have shown that IL-6 is a key element in cell-cell interactions that occur between endothelial cells, fibroblasts and cardio-myocytes [23,24]. Moreover, IL-6 is an important regulatory factor during the progression of cardiac hypertrophy and heart failure *in vivo* [25]. IL-6 therefore appears to be an interesting therapeutic target for the treatment of ATTR-wt. Indeed, it has been shown that among the most effective currently available treatments, IL-6 inhibitors appear to be more efficacious agents compared to TNF-α inhibitors [26] in amyloidosis AA (which is characterized by the aggregation and deposition of amyloid fibrils composed of serum amyloid A protein).

The Japanese survey of patients with AA amyloidosis [27] showed that tocilizumab, an anti-human IL-6 monoclonal antibody, successfully lowered SAA levels. This drug, due to a marked and long-lasting regression of AA protein deposits, improved the clinical symptoms of AA amyloidosis. As illustrated in the present work, TTR also significantly increases the level of IL-8 secretion. The main activity of IL-8 is to act as a polynuclear neutrophil attractant. IL-8 is present in a number of diseases with neutrophil infiltration. The sustained activation of neutrophils is the predominant factor that governs over-activated inflammation in acute heart failure (HF), and long-term outcomes in chronic HF [28]. It remains nevertheless difficult to specify the precise role of IL-8 secreted by cardiac fibroblasts in the presence of TTR. A better characterization of neutrophil infiltration kinetics in the cardiac tissue of patients with ATTR-wt is necessary.

As actors in the reparative fibrosis of infarcted cardiac tissue (and/or mechanical stretch or oxidative stress), CFs have a strong ability to produce ECM-associated proteins by modifying the balance of MMPs and their TIMP inhibitors [29]. Unexpectedly, we observed no change in MMP/TIMP ratios, in particular MMP-2/TIMP-2 and MMP-9/TIMP-1, unlike that reported by Tanaka (2013) [30] in patients with cardiac amyloidosis. In this clinical study, MMPs and IMPs concentrations were measured from the serum of patients who present an advanced amyloidosis ATTR-wt and hereditary forms. In our study the extracellular matrix factors concentrations are estimated at the scale cellular in experimental conditions mimicking the early stages of cardiac ATTR-wt (48 h TTR treatment). As expected, TNF-α had an inducible effect on the production of various protein factors by hCFs; these included BNP, IL-1α, IL-6, IL-8, IP-10, TIMP-2 and MMP-1,-3,-9, suggesting that TNF-α receptors were functional and that cells had a normal viability.

In addition to the human fibroblast cell line NIH3T3 having the ability to take up TTR intracellularly. These fibroblasts may also have the capacity to degrade TTR aggregates and fibrils extracellularly by secreting MMPs [31]. On the other hand, in cardiomyocytes, there is a close relationship between the cellular integration of the TTR and calcium homeostasis [32]. Further studies on hCFs are needed to clarify the multiplicity of cellular and molecular mechanisms in the clearance of TTR aggregates and in intracellular calcium regulation.

In conclusion, the results of this study suggest that treatment strategies targeting IL-6 inhibition seem promising in wild-type transthyretin (TTR)-derived amyloidosis.

Ethics approval and consent to participate

All patients were informed and consent for the use of their tissue samples. The design of this study was in agreement with the statements of the Declaration of Helsinki and was approved by the local ethics committee at Poitiers CHU Hospital (Number DC-2008–565).

Consent for publication

Not applicable.

Availability of data and materials

The data in this study are available from the author for correspondence upon reasonable request.

Funding

This study was supported by ELSAN grants.

Authors' contributions

Guarantor of integrity of the entire study: MC and TH, GP; experience and analysis, J-FF and AC; manuscript review, CM, BG and PB; manuscript preparation and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their appreciation to C. Bauer and J. Habrioux for their excellent technical assistance. ELSAN and the University of Poitiers supported this work.

Abbreviations

AA amyloidosis	Amyloid A amyloidosis
ACE inhibitors	Angiotensin-converting enzyme inhibitors
ATTR-CM	Transthyretin amyloid cardiomyopathy
ATTR-wt	ATTR wild-type
BNP	Brain natriuretic peptide
BSA	Bovine serum albumin
CCL-20 (MIP-3α)	C–C motif Chemokine ligand 20 macrophage inflammatory protein 3-alpha)
CCL-3 (MIP-1α)	C–C motif Chemokine ligand 3 (macrophage inflammatory protein 1-alpha)
CCL-5 (RANTES)	C–C motif Chemokine ligand 5 (regulated upon activation normal T cell expressed and secreted)
CPP	Committee for Personal Protection
CXCL-1 (GRO)	CXC motif chemokine ligand 1 (growth-related oncogene)
DAPI	4′,6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle's Medium
DPBS	Dulbecco's Phosphate-Buffered Saline
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycol-bis(β-aminoethyl)-N,N,N',N'-tetraacetic Acid
ELISA	Electrochemiluminescence immunoassay
FAC	Familial amyloid cardiomyopathy
FBS	Fetal bovine serum
hCF	human primary Cardiac fibroblast
HF	Heart failure
IL-1	Interleukine-1
IL-1α	Interleukine-1 alpha
IL-6	Interleukine 6
IL-8	Interleukine-8
IP-10	Interferon gamma-induced protein 10
MFIs	Median fluorescence intensities
MI	Myocardial infarction
MMPs	Matrix metalloproteinases
NT	NoTreatment
NT-proBNP	N-terminal pro-Brain natriuretic peptide
P_0	cellular Passage number 0
PBS	Phosphate buffered saline
RIPA	Radio immunoprecipitation assay
rpm	rounds per minute
SAA	serum amyloid A protein
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBS-Tween	Tris buffered saline with Tween
TIMPs	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor-alpha
TTR	Transthyretin

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.12.027.

References

[1] C. Röcken, B. Peters, G. Juenemann, W. Saeger, H.U. Klein, C. Huth, A. Roessner, A. Goette, Atrial amyloidosis: an arrhythmogenic substrate for persistent atrial fibrillation, Circulation 106 (2002) 2091–2097, https://doi.org/10.1161/01.cir.0000034511.06350.df.

[2] R.Y. Kwong, B. Heydari, S. Abbasi, K. Steel, M. Al-Mallah, H. Wu, R.H. Falk, Characterization of cardiac amyloidosis by atrial late gadolinium enhancement using contrast enhanced cardiac magnetic resonance imaging and correlation with left atrial conduit and contractile function, Am. J. Cardiol. 116 (2015) 622–629, https://doi.org/10.1016/j.amjcard.2015.05.021.

[3] E. González-López, M. Gallego-Delgado, G. Guzzo-Merello, F.J. de Haro-del Moral, M. Cobo-Marcos, C. Robles, B. Bornstein, C. Salas, E. Lara-Pezzi, L. Alonso-Pulpon, P. Garcia-Pavia, Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction, Eur. Heart J. 36 (2015) 2585–2594, https://doi.org/10.1093/eurheartj/ehv338.

[4] F.L. Ruberg, M. Grogan, M. Hanna, J.W. Kelly, M.S. Maurer, Transthyretin amyloid cardiomyopathy, J. Am. Coll. Cardiol. 73 (2019) 2872–2891, https://doi.org/10.1016/j.jacc.2019.04.003.

[5] S. Chacar, N. Farès, P. Bois, J.-F. Faivre, Basic signaling in cardiac fibroblasts: basic signaling in cardiac fibroblasts, J. Cell. Physiol. 232 (2017) 725–730, https://doi.org/10.1002/jcp.25624.

[6] E.R. Olson, J.E. Naugle, X. Zhang, J.A. Bomser, J.G. Meszaros, Inhibition of cardiac fibroblast proliferation and myofibroblast differentiation by resveratrol, Am. J. Physiol. Heart Circ. Physiol. 288 (2005), https://doi.org/10.1152/ajpheart.00763.2004. H1131–H1138.

[7] F.A. van Nieuwenhoven, N.A. Turner, The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction, Vasc. Pharmacol. 58 (2013) 182–188, https://doi.org/10.1016/j.vph.2012.07.003.

[8] D. MacKenna, S.R. Summerour, F.J. Villarreal, Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis, Cardiovasc. Res. 46 (2000) 257–263, https://doi.org/10.1016/s0008-6363(00)00030-4.

[9] N.A. Turner, P.K. Aley, K.T. Hall, P. Warburton, S. Galloway, L. Midgley, D.J. O'Regan, I.C. Wood, S.G. Ball, K.E. Porter, Simvastatin inhibits TNFalpha-induced invasion of human cardiac myofibroblasts via both MMP-9-dependent and -independent mechanisms, J. Mol. Cell. Cardiol. 43 (2007) 168–176, https://doi.org/10.1016/j.yjmcc.2007.05.006.

[10] N.A. Turner, A. Das, D.J. O'Regan, S.G. Ball, K.E. Porter, Human cardiac fibroblasts express ICAM-1, E-selectin and CXC chemokines in response to proinflammatory cytokine stimulation, Int. J. Biochem. Cell Biol. 43 (2011) 1450–1458, https://doi.org/10.1016/j.biocel.2011.06.008.

[11] S. Corda, J.L. Samuel, L. Rappaport, Extracellular matrix and growth factors during heart growth, Heart Fail. Rev. 5 (2000) 119–130, https://doi.org/10.1023/A:1009806403194.

[12] K.T. Dittloff, A. Iezzi, J.X. Zhong, P. Mohindra, T.A. Desai, B. Russell, Transthyretin amyloid fibrils alter primary fibroblast structure, function, and inflammatory gene expression, Am. J. Physiol. Heart Circ. Physiol. 321 (2021), https://doi.org/10.1152/ajpheart.00073.2021. H149–H160.
[13] P.F. Teixeira, F. Cerca, S.D. Santos, M.J. Saraiva, Endoplasmic reticulum stress associated with extracellular aggregates. Evidence from transthyretin deposition in familial amyloid polyneuropathy, J. Biol. Chem. 281 (2006), 21998, https://doi.org/10.1074/jbc.M602302200. –22003.
[14] A. Chatelier, A. Mercier, B. Tremblier, O. Thériault, M. Moubarak, N. Benamer, P. Corbi, P. Bois, M. Chahine, J.F. Faivre, A distinct de novo expression of Nav1.5 sodium channels in human atrial fibroblasts differentiated into myofibroblasts, J. Physiol. 590 (2012) 4307–4319, https://doi.org/10.1113/jphysiol.2012.233593.
[15] A. El Chemaly, R. Guinamard, M. Demion, N. Fares, V. Jebara, J.-F. Faivre, P. Bois, A voltage-activated proton current in human cardiac fibroblasts, Biochem. Biophys. Res. Commun. 340 (2006) 512–516, https://doi.org/10.1016/j.bbrc.2005.12.038.
[16] C. Louault, N. Benamer, J.-F. Faivre, D. Potreau, J. Bescond, Implication of connexins 40 and 43 in functional coupling between mouse cardiac fibroblasts in primary culture, Biochim. Biophys. Acta Biomembr. 1778 (2008) 2097, https://doi.org/10.1016/j.bbamem.2008.04.005. –2104.
[17] H. Bräuninger, T. Thottakara, J. Schön, S. Voss, V. Dhople, S. Warnke, K. Scherschel, B. Schrage, P. Kirchhof, S. Blankenberg, U. Völker, D. Westermann, E. Hammer, D. Lindner, Cytokine-mediated alterations of human cardiac fibroblast's secretome, IJMS 22 (2021), 12262, https://doi.org/10.3390/ijms222212262.
[18] H.H. Chen, J.C. Burnett, The natriuretic peptides in heart failure: diagnostic and therapeutic potentials, Proc. Assoc. Am. Phys. 111 (1999) 406–416, https://doi.org/10.1111/paa.1999.111.5.406.
[19] C. Hall, Essential biochemistry and physiology of (NT-pro)BNP, Eur. J. Heart Fail. 6 (2004) 257–260, https://doi.org/10.1016/j.ejheart.2003.12.015.
[20] H. Usuku, K. Obayashi, M. Shono, T. Oshima, M. Tasaki, H. Yasuda, H. Ogawa, Y. Ando, Usefulness of plasma B-type natriuretic peptide as a prognostic marker of cardiac function in senile systemic amyloidosis and in familial amyloidotic polyneuropathy, Amyloid 20 (2013) 251–255, https://doi.org/10.3109/13506129.2013.844122.
[21] T. Tanaka, M. Narazaki, T. Kishimoto, IL-6 in inflammation, immunity, and disease, Cold Spring Harbor Perspect. Biol. 6 (2014) a016295, https://doi.org/10.1101/cshperspect.a016295. –a016295.
[22] T. Tanaka, M. Narazaki, A. Ogata, T. Kishimoto, A new era for the treatment of inflammatory autoimmune diseases by interleukin-6 blockade strategy, Semin. Immunol. 26 (2014) 88–96, https://doi.org/10.1016/j.smim.2014.01.009.
[23] P. Camelliti, Spatially and temporally distinct expression of fibroblast connexins after sheep ventricular infarction, Cardiovasc. Res. 62 (2004) 415–425, https://doi.org/10.1016/j.cardiores.2004.01.027.
[24] I. Banerjee, J.W. Fuseler, A.R. Intwala, T.A. Baudino, IL-6 loss causes ventricular dysfunction, fibrosis, reduced capillary density, and dramatically alters the cell populations of the developing and adult heart, Am. J. Physiol. Heart Circ. Physiol. 296 (2009) H1694–H1704, https://doi.org/10.1152/ajpheart.00908.2008.
[25] S. Fredj, J. Bescond, C. Louault, A. Delwail, J.-C. Lecron, D. Potreau, Role of interleukin-6 in cardiomyocyte/cardiac fibroblast interactions during myocyte hypertrophy and fibroblast proliferation, J. Cell. Physiol. 204 (2005) 428–436, https://doi.org/10.1002/jcp.20307.
[26] Y. Okuda, AA amyloidosis – benefits and prospects of IL-6 inhibitors, Mod. Rheumatol. 29 (2019) 268–274, https://doi.org/10.1080/14397595.2018.1515145.
[27] Y. Okuda, T. Yamada, M. Ueda, Y. Ando, First nationwide survey of 199 patients with amyloid A amyloidosis in Japan, Intern. Med. 57 (2018) 3351–3355, https://doi.org/10.2169/internalmedicine.1099-18.
[28] V. Kain, G.V. Halade, Role of neutrophils in ischemic heart failure, Pharmacol. Ther. 205 (2020), 107424, https://doi.org/10.1016/j.pharmthera.2019.107424.
[29] D. Fan, A. Takawale, J. Lee, Z. Kassiri, Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease, Fibrogenesis Tissue Repair 5 (2012) 15, https://doi.org/10.1186/1755-1536-5-15.
[30] K. Tanaka, E.E. Essick, G. Doros, K. Tanriverdi, L.H. Connors, D.C. Seldin, F. Sam, Circulating matrix metalloproteinases and tissue inhibitors of metalloproteinases in cardiac amyloidosis, JAHA 2 (2013), e005868, https://doi.org/10.1161/JAHA.112.005868.
[31] Y. Misumi, Y. Ando, N.P. Gonçalves, M.J. Saraiva, Fibroblasts endocytose and degrade transthyretin aggregates in transthyretin-related amyloidosis, Lab. Invest. 93 (2013) 911–920, https://doi.org/10.1038/labinvest.2013.83.
[32] L. Sartiani, M. Bucciantini, V. Spinelli, M. Leri, A. Natalello, D. Nosi, S. Maria Doglia, A. Relini, A. Penco, S. Giorgetti, E. Gerace, G. Mannaioni, V. Bellotti, S. Rigacci, E. Cerbai, M. Stefani, Biochemical and electrophysiological modification of amyloid transthyretin on cardiomyocytes, Biophys. J. 111 (2016) 2024–2038, https://doi.org/10.1016/j.bpj.2016.09.010.