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Tocilizumab for the Treatment of AA Amyloidosis

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1. Introduction

Amyloid A (AA) amyloidosis is a serious complication of chronic inflammatory, infectious and neoplastic diseases (Merlin & Bellotti, 2003). Insoluble amyloid fibril deposition resulting from the extracellular aggregation of proteolytic fragments of serum amyloid A (SAA), an acute-phase reactant protein, causes progressive deterioration in various organs (Obici et al., 2009; Perfetto et al., 2010). Moreover, long-term overproduction of the SAA protein is a key component of the resultant pathogenetic cascade (Pettersson et al., 2008). Recent fundamental analyses of SAA gene activation have revealed that proinflammatory cytokines such as IL-1β, TNFα and IL-6 are key players in SAA synthesis, leading to speculation that biological modifiers to block the activity of these cytokines may function as novel therapeutic drugs for AA amyloidosis. Some reports have dealt with the beneficial clinical effects of the IL-1 receptor antagonist, TNF inhibitors, and IL-6 receptor antibody (ab) on AA amyloidosis. In this chapter, we present evidence that tocilizumab, a humanized anti-interleukin-6 receptor ab, is a powerful inhibitor of SAA production and has the potential of becoming a first-line drug for AA amyloidosis.

2. Molecular mechanism of transcriptional activation of SAA1 gene

2.1 IL-6 plays a critical role in the synergistic induction of SAA1 gene by proinflammatory cytokines

SAA, which is mainly produced in the liver, is a precursor protein of amyloid A fibril in AA amyloidosis (Uhlar & Whitehead, 1999). The human SAA family consists of SAA1, SAA2, and SAA4. When the first two, also known as acute phase SAAs, are induced, they increase dramatically up to 1000 times during inflammation (Emery & Luqmani, 1993). The vast majority of human AA protein isolated from amyloid deposits are derived from SAA1 (Yamada et al., 1996).

The SAA2 gene is reportedly regulated by nuclear factor kB (NF-kB) and CAAT enhancer-binding protein β (C/EBPβ) in response to synergistic stimulation by IL-1β and IL-6 (Betts et al., 1993), although the exact induction mechanism of the SAA1 gene by proinflammatory cytokines remains unknown. An SAA isoform real time quantitative RT-PCR assay using the hepatic cell line HepG2 revealed that the combination of IL-6 and IL-1β or of IL-6 and TNFα, but not of IL-1β and TNFα displayed synergistic induction of SAA1 gene mRNA expression

(Hagihara et al., 2004). As shown in Fig. 1, anti-IL-6 receptor ab markedly diminished the synergistic induction of SAA mRNA by the triple stimulation of IL-6, IL-1β, and TNFα, which indicates that IL-6 plays a critical role in the synergistic induction of the SAA1 gene by proinflammatory cytokines.

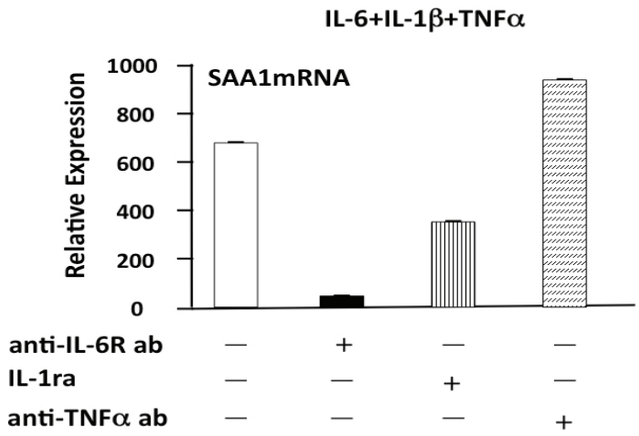


Fig. 1. Inhibitory effects of anti-IL-6R ab (25 μg/ml), IL-1 receptor antagonist (ra) (100 ng/ml), and anti-TNFα ab (4 μg/ml) on the synergistic induction of SAA1 generated by triple stimulation of IL-6, IL-1β, and TNFα. Each specific reagent was incubated with HepG2 cells for 30 min prior to cytokine stimulation. SAA1 mRNA in HepG2 cells was measured by means of real-time quantitative RT-PCR at 6 h after cytokine stimulation. Values shown represent the means + SD of duplicate measurements

Next, we studied the signal transduction pathway leading to the activation of the SAA1 gene. IL-6 was shown to have two main signal transduction pathways, the MAPK and Jak-STAT pathways (Akira, 1997). The Jak2 inhibitor AG490 reduced the synergistic induction of SAA1 to 30%, indicating that the Jak-STAT pathway plays an important role in the synergistic induction of the SAA gene (Hagihara et al., 2004). It was further shown that STAT3 binds to a γ-interferon activation sequence (GAS) like sequence (-TTNNNGAA) and that the C-reactive protein (CRP) gene, a major acute phase protein activated by IL-6, in fact has a STAT3 response element (RE) (-TTCCCGAA) in its promoter (D. Zhang et al., 1996; Nishikawa et al., 2008), while there is no STAT3 RE in the human SAA1 promoter (Uhlar & Whitehead, 1999). We therefore investigated what role STAT3 plays in human SAA1 promoter activity.

2.2 STAT3 is essential for the synergistic induction of human SAA1 genes via the NF-kB RE containing region after complex formation with NF-kB p65

To examine the effect of STAT3 on SAA1 promoter activity, pEF-BOS dominant negative STAT3 Y705F (dn STAT3) or pEF-BOS wild type STAT3 (wt STAT3) were co-transfected with the pGL3-SAA1 promoter luciferase construct (-796/+24) (pGL3-SAA1) into HepG2 cells (Hagihara et al., 2005). dn STAT3 eliminated the transcriptional activity of pGL3-SAA1 even after stimulation with IL-1β+IL-6, while wt STAT3 enhanced the transcriptional activity of pGL3-SAA1 three times more than was attained with IL-6 or IL-1β+IL-6 stimulation.

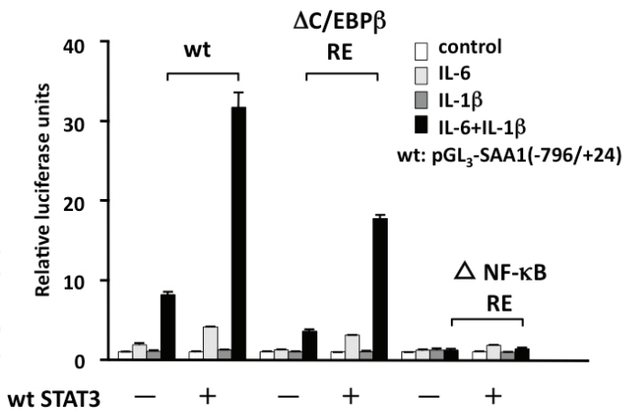


Fig. 2. STAT3 cannot augment the transcriptional activity of SAA1 without NF-kB RE. Wt STAT3 (0.5 μg) was co-transfected with 0.5 μg of pGL3-SAA1 (-796/+24) and pGL3-SAA1 DC/EBPβ RE, pGL3-SAA1 DNF-kB RE. Cytokine stimulation was performed with IL-6 (10 ng/ml) and/or IL-1β (0.1 ng/ml) for 3 h. The relative luciferase activity is expressed as means + SD of triplicate cultures and transfections

As shown in Fig. 2, the co-expression of wt STAT3 enhanced the transcriptional activity of pGL3-SAA1 and pGL3-SAA1 DC/EBPβ RE almost three-fold, but did not augment the transcriptional activity of pGL3-SAA1 DNF-kB RE. These results suggest that STAT3 is involved in the transcriptional activity of SAA, most likely through interacting with NF-kB RE. In the case of rat γ fibrinogen, the CTGGGAATCCC sequence was found to be responsive for transactivation by both STAT3 and NF-kB (Zhang & Fuller, 2000). We postulated that STAT3 might form a complex with NF-kB and contribute to the transcriptional augmentation of the human SAA1 gene. To examine our hypothesis, we performed IP-Western blotting of STAT3 and NF-kB. Fig. 3 clearly shows that STAT3 is associated with NF-kB p65 following IL-1β+IL-6 treatment but that no specific band of NF-kB p50 was detected.

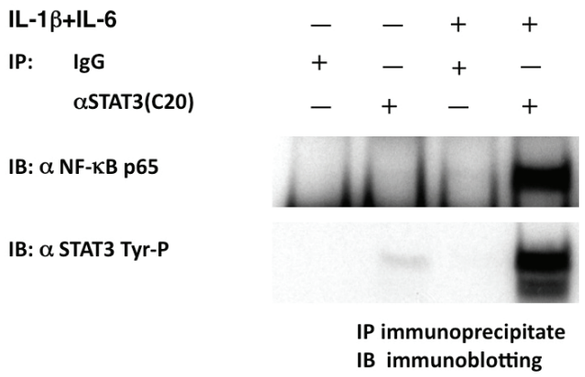


Fig. 3. Endogeneous STAT3 interacts with NF-kB p65 following IL-1β+6 treatment. Nuclear extracts of HepG2 cells stimulated with IL-1β and IL-6 were immunoprecipitated with the anti-STAT3 antibody. Western blots of immunoprecipitates with anti-NF-kB p65 or anti-STAT3 antibodies were performed

These findings indicate that crosstalk between STAT3 and NF-kB p65 contributes to the transcriptional augmentation of SAA1 by IL-1β+IL-6 stimulation.

2.3 STAT3 acts on the SAA1 promoter by means of a newly discovered cis-acting mechanism

STAT3 is reportedly associated with p300 (Nakashima et al., 1999), which raises the possibility that heteromeric complex formation of STAT3, NF-kB p65 and p300 is involved in the transcriptional activity of the human SAA1 gene. To examine this hypothesis, we performed a chromatin immunoprecipitation (ChIP) assay using chromatin isolated from HepG2 cells. STAT3 and p300 were apparently recruited to the SAA1 promoter region (–226 /+24) in response to IL-6 or IL-1β+IL-6 and weakly recruited by IL-1β (Fig. 4), while NF-kB p65 was recruited by IL-1β or IL-1β+IL-6 and slightly recruited by IL-6. These results demonstrate that STAT3 forms a transcriptional complex with NF-kB p65 and p300 on the SAA1 promoter region. Moreover, we found that co-expression of p300 wt in pCMVβ with wt STAT3 dramatically enhanced the luciferase activity in a dose-dependent manner. These findings indicate that STAT3 interacts with p300 in the transcriptional activity of the human SAA1 gene. However, the question remained how STAT3 could bind to the promoter region of the SAA1 gene because there is no typical STAT3 RE. It is likely that STAT3 either binds indirectly to the promoter region of the SAA1 gene, or obtains binding affinity for an unknown DNA sequence in a complex with NF-kB p65. To answer this question, we performed DNA affinity chromatography using a wt SAA1 probe (–196/-73). Nuclear extracts specifically interacting with a biotinylated wt SAA1 probe (–196/-73) were collected with the aid of streptavidin Dynabeads and a magnet, and transcriptional factors were analyzed by Western blotting.

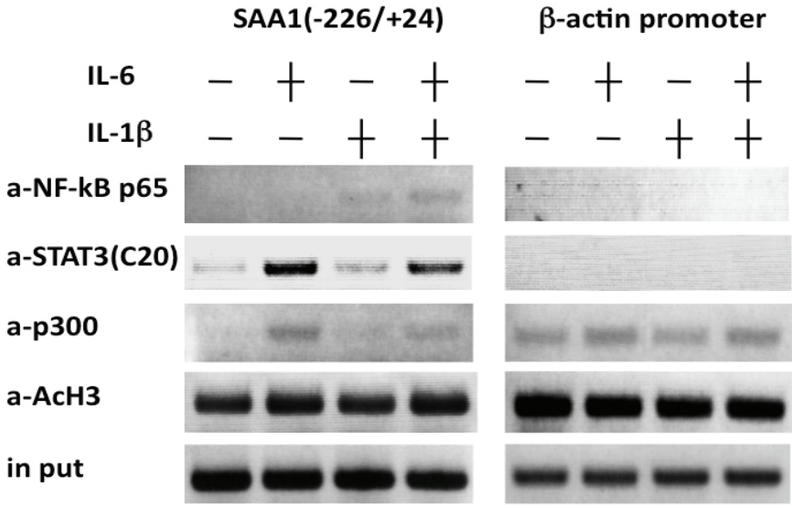


Fig. 4. Chromatin immunoprecipitation assays demonstrate recruitment pattern of STAT3, NF-κB p65, and p300 on the SAA1 promoter (-226/+24) from HepG2 cells treated with IL-6 (10 ng/ml) and/or IL-1β (0.1 ng/ml) for 30 min. Anti-AcH3 antibody was used as a positive control for this assay

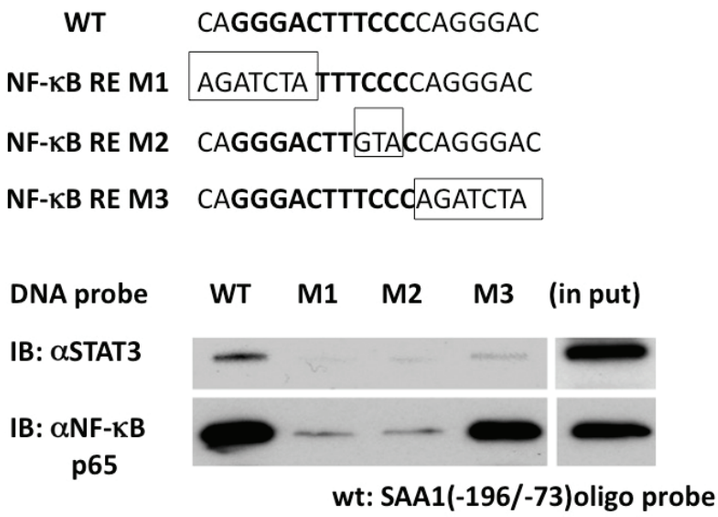


Fig. 5. DNA affinity chromatography shows that STAT3 can act on the SAA1 promoter after binding to NF-kB p65. The nuclear extracts (200 μg) from HepG2 cells after cytokine stimulation were mixed with 1 μg of biotinylated DNA probe, and 50 μl of streptavidin-Dynabeads was added to and mixed in with the samples and collected with a magnet. The trapped proteins were then analyzed by Western blotting. The SAA1 (–196/-73) mt NF-κB RE M1 and M2 probe lost its ability to interact with both STAT3 and NFκB p65, while the SAA1 (–196/-73) mt NFκB RE M3 probe maintained its binding affinity for NF-κB p65 but not STAT3

NF-kB p65 and STAT3 were both pulled down by the wt SAA1 probe from the nuclear extracts of HepG2 cells after IL-1β+IL-6 stimulation. In the case of rat γ fibrinogen, the CTGGGAATCCC sequence was identified as responsible for transactivation by both STAT3 and NF-kB (Zhang & Fuller, 2000). It was also reported that TCC was necessary for NF-kB binding and the CTGGGAA sequence for STAT3 binding because of the loss of transcriptional activity in an AGATCTATCCC mutant. To investigate whether STAT3 could bind to the CAGGGAC sequence of NF-kB RE on the SAA1 promoter region, we created two mutated constructs, the SAA1 NF-kB RE M1 probe (AGATCTATTTCCC) and the M2 probe (CAGGGACTTGTAC). We expected that STAT3 would bind to NF-kB RE M2, but not to NF-kB RE M1. However, neither NF-kB p65 nor STAT3 was detected by the two probes, indicating that the interaction between STAT3 and NF-kB p65 is essential for activation of the binding affinity of STAT3 to the wt SAA1 probe (Fig. 5). We assumed that the formation of the heteromeric complex of STAT3 and NF-kB p65 might confer STAT3 binding affinity to the SAA1 promoter region. On the basis of our results and those obtained with rat γ fibrinogen, we focused our attention on the 3′-site of NF-kB RE (CAGGGACTTTCCCCAGGGAC) as a candidate STAT3 binding site, because sequences contiguous to NF-kB RE could have influenced the binding affinity of STAT3. For vertification of our assumption, we created a SAA1 mt NF-kB RE M3 probe (CAGGGACTTTCCCAGATCTA). As expected, specific bands of STAT3 from the nuclear extracts of HepG2 cells after IL-1β+6 stimulation were markedly reduced by the SAA1 mt NF-kB RE M3 probe compared to the effect obtained with the wt SAA1 probe, although specific bands of NF-kB p65 found to be almost as intact as in the wild type (Fig. 5). These

results thus supported our assumption that binding affinity of STAT3 for the human SAA1 promoter region is the result of the formation of a heteromeric complex comprising STAT3 and NF-kB p65. Taken together, our findings demonstrate that STAT3 acts on the human SAA1 promoter via a newly discovered cis-acting mechanism, that is, the formation of a heteromeric complex containing STAT3, NF-kB 65 and p300, resulting in a schematic model which can explain the synergistic induction of human SAA1 gene by IL-1β+IL-6 stimulation (Fig. 6).

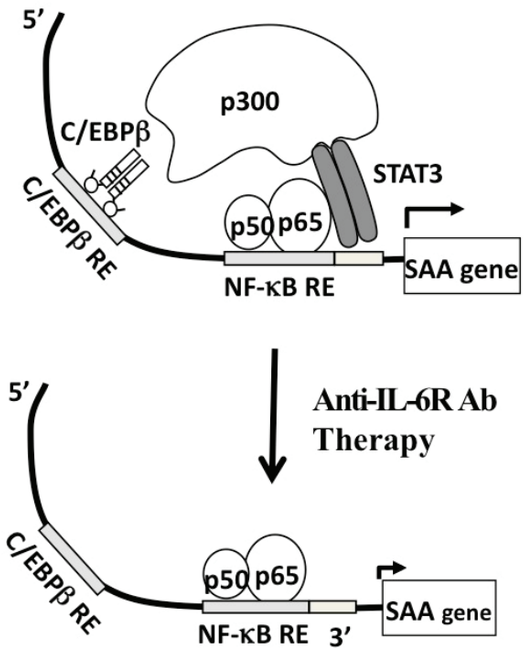


Fig. 6. A model of transcriptional regulation of the SAA1 gene. Cytokine stimulation caused the formation around NF-kB RE of a heteromeric complex with STAT3 and NF-kB p65. STAT3, which is assumed to interact with the 3'-site of NF-kB RE, recruited the co-activator p300, which then coordinated the interaction of NF-kB p65, STAT3, and C/EBPβ thus resulting in the augmentation of transcriptional activity of the human SAA1 gene. Anti-IL-6R ab therapy inhibited the activation of STAT3 and C/EBPβ, and eliminated the formation of the transcriptional complex on the SAA1 promoter

Our results further lead to the conclusion that the serum SAA1 level is affected by the intensity of the interaction between STAT3 and NF-kB p65. At the same time this schematic model explains the effect of anti-cytokine therapy on the transactivation of SAA1. Anti-TNFα or anti-IL-1β therapy can reduce but not completely eliminate the NF-kB signaling pathway, because this pathway is also activated by other cytokines or stimulants to activate toll like receptors (Li & Verma, 2002). As a consequence, the transcriptional complex on the SAA1 promoter is thought to remain after anti-TNFα or anti-IL-1β therapy. On the other hand, IL-6 blocking therapy can inhibit the activation of STAT3 and C/EBPβ, and prevent the formation of the transcriptional complex on the SAA1 promoter (Fig. 6). This model therefore shows that SAA1 gene activation depends on IL-6 more than on other proinflammatory cytokines such as IL-1β and TNFα, thus suggesting that IL-6 blockade is superior to the IL-1 or TNF blockade for the suppression of SAA synthesis.

3. The inhibitory effect of tocilizumab and other biologics on serum levels of SAA

Tocilizumab is a humanized anti-IL-6 receptor monoclonal ab of the IgG1 class that is generated by grafting the complementarity determining regions of a mouse anti-human IL-6 receptor ab onto human IgG1. Tocilizumab blocks IL-6-mediated signal transduction through inhibition of IL-6 binding to transmembrane and soluble IL-6 receptor. So far, tocilizumab has been approved for the treatment of moderate to severe rheumatoid arthritis (RA) in more than 90 countries and of juvenile idiopathic arthritis and Castleman's disease in Japan and may be effective for other autoimmune, inflammatory and neoplastic diseases (Tanaka et al., 2010; Tanaka et al., 2011). For RA, the recommended posology in Japan and the EU is 8 mg/kg once every 4 weeks. In clinical terms, if the serum tocilizumab concentration is maintained at more than 1 μg/ml, CRP remains negative (Nishimoto et al., 2008). The concentration of CRP is therefore a hallmark for determining whether tocilizumab completely blocks IL-6 activity in vivo.

The goal of therapy for AA amyloidosis is treatment of the underlying disorder (Perfetto et al., 2010). Treatment that suppresses the inflammatory activity reduces circulating levels of the SAA protein, but since AA amyloidosis occurs as a complication of chronic inflammatory diseases treated with conventional regimen(s), it is clear that the current treatment of the underlying disorder is not adequate for the prevention of AA amyloidosis development. Another therapeutic strategy therefore needs to be developed. A sustained high concentration of SAA has been found to correlate with a rapid progression of renal amyloid diseases and a low concentration (below 10 mg/L) with a more favorable outcome (Gillmore et al., 2001). Chronic suppression of SAA levels thus leads to a notable regression or stabilization of amyloid load (Lachmann et al., 2007). This makes a strategy to suppress SAA production a rational approach for the treatment of AA amyloidosis and biologics including IL-1 receptor antagonist, TNF blockers and IL-6 receptor ab can be expected to serve as novel therapeutic drugs for AA amyloidosis.

Clinical studies of how tocilizumab affects RA and Castleman's disease have demonstrated its strikingly suppressive effect on serum concentrations of SAA. In an open label trial, 3 doses of tocilizumab (2, 4 or 8 mg/kg) biweekly for 6 weeks were administered to 15 patients with active RA (Nishimoto et al., 2003). In 12 of the patients whose serum tocilizumab level was detectable during the treatment period, SAA was completely normalized after 6 weeks. A multicenter, double-blind, placebo-controlled trial of tocilizumab for RA patients showed that the injection of tocilizumab at 8 mg/kg every 4 weeks resulted in a reduction of the mean concentration of SAA from 365 to 75 mg/L after 3 months (Nishimoto et al., 2004). In a multicenter double-blind, randomized, placebo-controlled, parallel group Phase III OPTION study, patients with RA were randomized to receive tocilizumab (4 or 8 mg/kg) or placebo intravenously every 4 weeks while MTX was continued at stable pre-study doses (10-25 mg/week) for 24 weeks (Smolen et al, 2008). The mean levels of SAA of the group treated with 8 mg/kg, with 4 mg/kg and the placebo-administered group were reduced from 70.4 mg/L to 6.1 mg/L, 69.2 mg/L to 26 mg/L and 64.4 mg/L to 62 mg/L, respectively. For patients with Castleman's disease, tocilizumab treatment at 8 mg/kg every 2 weeks caused a prompt reduction of SAA in 5 patients (Nishimoto et al., 2000) and a striking reduction of SAA in 27 patients at week 60 (Nishimoto et al., 2005).

As for TNF inhibitors, Elliott et al. first demonstrated the suppressive effect of infliximab on SAA levels in patients with RA (Elliott et al., 1993). Twenty patients with active RA were treated with 20 mg/kg of infliximab in an open phase I/II trial lasting 8 weeks and SAA concentrations were reduced from 245 mg/L to 58 mg/L after 1 week and to 80 mg/L after 2 weeks. In a later study, Charles et al. examined the effect of infliximab on 24 RA patients (Charles et al., 1999). After 24 weeks, SAA levels in patients treated with infliximab showed a significant reduction from 378 mg/L to 56 mg/L. Perry et al. reported on the changes in SAA levels during treatment with etanercept of 9 patients with AA amyloidosis complicated by inflammatory arthritis (Perry et al., 2008). In 7 out of 9 patients the median SAA level during ETA treatment was lower than levels before therapy, and in 5 patients, the median post treatment level dropped to below 11 mg/L. Further, the effect of etanercept on SAA in 92 patients with ankylosing spondylitis (AS) reportedly reduced the median pre-treatment level of 4.8 mg/L to 0.9 after 1 month and to 0.8 3 months later (Van Eijk et al., 2009). Similarly, anti-TNF treatment (either with 25 mg of etanercept twice a week or 50 mg once a week, or 5 mg/kg of infliximab every 6 weeks) of 155 AS patients led to a reduction of medial levels of SAA from 7.5 mg/L to 0.7 mg/L after 1 month and to 0.8 mg/L after 3 months (De Vries et al., 2009).

The marked suppressive effect of the IL-1 receptor antagonist, anakinra on serum SAA concentration was demonstrated for an autoinflammatory disease, Muckle-Wells syndrome (Hawkins et al., 2004). The family suffering from this disease was treated with anakinra 100 mg/day subcutaneously. The SAA levels of the mother, son and daughter decreased from 146, 264 and 193 mg/L to 1.3, 2.2 and 1.8 mg/L, respectively. Similarly, anakinra treatment reduced the SAA concentration from a median of 174 mg/L to 8 mg/L in 18 patients with neonatal-onset multisystem inflammatory diseases (Goldbach-Mansky et al., 2006), from a mean level of 133 mg/L to 5 mg/L in 15 patients with autoinflammatory disease associated with CIAS-1/NALP3 mutations (Leslie et al., 2006) and from a mean level of 645 mg/L to 3.4 mg/L in 5 patients with tumor necrosis factor-associated periodic syndrome (TRAPS) (Gattorno et al., 2008). Anakinra thus appears to be highly effective for the suppression of SAA levels in patients with autoinflammatory diseases but there have been no reports on whether anakinra can also inhibit serum concentrations of SAA in patients with inflammatory arthritides.

We compared the inhibitory effects of tocilizumab with TNF inhibitors including infliximab, etanercept and adalimumab, on serum SAAs in RA patients. The results are shown in Fig. 7. Mean SAA levels of patients treated with tocilizumab or anti-TNFs decreased from 194 mg/L to 27 mg/L or from 185 mg/L to 53 mg/L, respectively. In most of the patients who received tocilizumab, SAA immediately dropped to below 10 mg/L except for two patients. Since CRP levels of these two patients were also high, the failure of SAA normalization was due to the low concentration of tocilizumab. In another 25 patients, the mean concentration of SAA was reduced from 186 mg/L to 8.0 mg/L, indicating that, if the concentration of tocilizumab can be consistently maintained at more than 1 μg/ml by changing the infusion interval or dose, the SAA concentration can be expected to remain normalized.

These observations therefore indicate that biologics, including TNF inhibitors, IL-1 receptor antagonist and anti-IL-6 receptor ab are effective for the suppression of serum levels of SAA, while tocilizumab appears to be more effective than TNF inhibitors for the suppression of SAA.

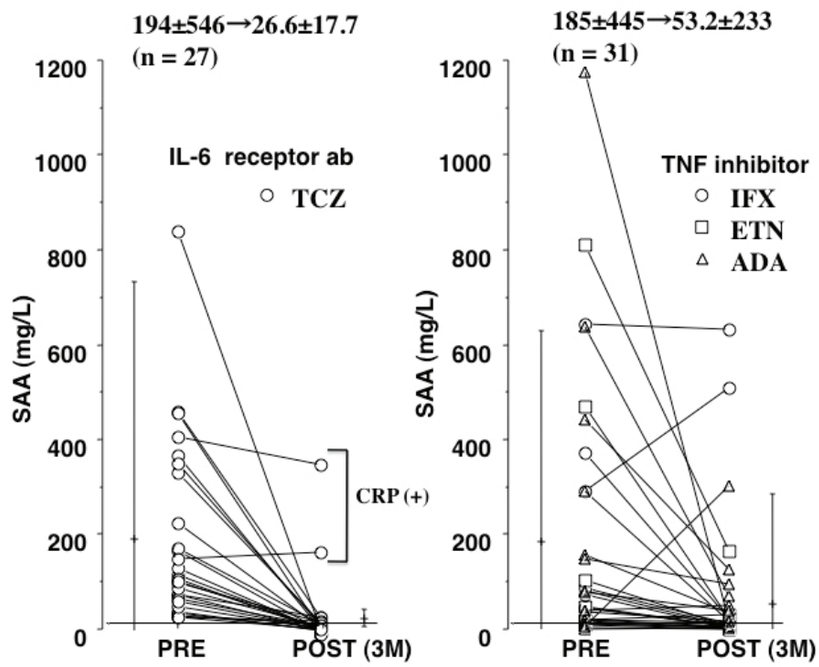


Fig. 7. The serum levels of SAA from patients with RA were more markedly suppressed by tocilizumab than by TNF inhibitors. SAA was measured before and at 3 months after biologics were administered. Twenty-seven patients were treated with tocilizumab (TCZ) and 31 patients anti-TNFs comprising infliximab (IFX), etanercept (ETN) or adalimumab (ADA). There was no significant difference between the baseline SAA of the two groups (p=0.09, Wilcoxon test). The median SAA in the tocilizumab treatment group was reduced from 114.5 mg/L to 6.5 mg/L, and in the anti-TNF treatment group from 72 mg/L to 16 mg/L. Three months after treatment, however, there was a significant difference in SAA between the two groups (p=0.017, Wilcoxon test)

4. The clinical effect of tocilizumab and other biologics on AA amyloidosis

The chronic suppression of SAA levels by anti-proinflammatory agents may lead to clinical amelioration of symptoms, prevention of progressive organ deterioration or recover from the damage, caused by amyloid A deposits. In fact, several studies have reported on the efficacy of biologics for the treatment of AA amyloidosis. In 2002, Elkayam et al. first reported that renal amyloidosis secondary to RA responded well to treatment with infliximab with the pre-therapy SAA level of 29 mg/L decreasing to 4.5 mg/L after 14 weeks. (Elkayam et al., 2002). In addition, rapid and complete clinical and laboratory remission of the nephrotic syndrome was observed within weeks, as well as stabilization of amyloid deposits confirmed by ^{123}I-labeled serum amyloid P (SAP) scintigraphy after 1 year. In 2003, Ortiz-Santamaria et al. reported the effect of infliximab on six patients with AA amyloidosis (complicated by RA in five cases and AS in one case), whose CRP levels improved compared with baseline values (Ortiz-Santamaria et al., 2003). Although three patients were withdrawn from the therapy in the first 2 months, reduced serum creatinine and proteinuria levels were observed in two of the remaining three patients. Gottenberg et al. reported that 15 patients with AA amyloidosis and renal involvement were treated with

TNF inhibitors (10 patients received infliximab, 4 received etanercept, and 1 underwent both types of treatment) (Gottenberg et al., 2003). Frequency of diarrhea was markedly reduced in 2 of the 3 patients with digestive tract amyloidosis, while amyloidosis progressed in 7 patients and was stabilized in 5 patients. Verschueren et al. described a patient with AA amyloidosis secondary to juvenile spondyloarthropathy treated with 3 mg/kg of infliximab (Verschueren et al., 2003). After six months, CRP returned to normal in conjunction with a reduction in proteinuria and after nine months urine analysis showed normal findings and protein excretion had been reduced. However, a renal biopsy after seven months of treatment detected equal amounts of amyloid fibrils in the mesangium, the subendothelial, and subepithelial space. In 2004, Ravindran et al. published a report of a case of RA with secondary Sjögren's syndrome, complicated by AA amyloidosis and nephrotic syndrome, which was treated with etanercept for 2 years (Ravindran et al., 2004). A significant reduction in proteinuria as well as a sustained stabilization of renal function were observed. In addition, a regression of AA amyloid, as quantified by ^{123}I-labeled SAP scintigraphy was established. As described elsewhere, etanercept treatment of patients with AA amyloidosis produced a decrease in SAA, but there were no significant changes in serum creatinine or proteinuria (Perry et al., 2008). In 2009, Kuroda et al. reported the effect of TNF inhibitors on 14 patients with AA amyloidosis associated with RA (Kuroda et al., 2009). For the 4 patients treated with infliximab and 10 patients with etanercept, creatinine clearance improved in 4 patients, did not change in 5 patients, and deteriorated in 3 patients. Urinary protein excretion significantly decreased in 3 patients, remained the same in 6, and increased in 3. The gastroduodenal biopsy from 9 patients showed significant reductions in amyloid deposits and these were no longer detectable in 2 patients. In 2010 it was reported that etanercept treatment of 14 RA patients with AA amyloidosis resulted in the AA amyloidosis improvement and stabilization of AA amyloidosis after 89 weeks (Nakamura et al., 2010). Further, proteinuria decreased from 2.24 to 0.57 g/day and SAA fell from 250 to 26 mg/L, while diarrhea secondary to gastrointestinal AA amyloidosis diminished, but serum creatinine levels did not improve as a result of the treatment. In another study, long-term TNF blockade treatment with infliximab, etanercept or adalimumab of 36 patients with AA amyloidosis as a complication of rheumatic diseases reduced the median levels of proteinuria by 59.7% during the first 24 months (Fernandez-Nebro et al., 2010), while both mean serum creatinine levels and creatinine clearance remained stable. Finally, anti-TNF treatment was also shown to be effective for clinical improvement in AA amyloidosis associated with Crohn's disease (Pettersson et al., 2008) or TRAPS (Drewe et al., 2000). Putting these various findings together indicates that the treatment of AA amyloidosis with TNF inhibitors is promising but that its efficacy appears variable. Anakinra has also been found to control the clinical symptoms or progression of renal amyloid disease in autoinflammatory syndromes such as TRAPS, familial cold autoinflammatory syndrome and cryopyrin-associated periodic syndrome (Pettersson et al., 2008).

On the basis of the findings of the central role of IL-6 in the SAA1 gene activation and the powerful suppressive activity of tocilizumab on SAA, it is anticipated that tocilizumab may serve as an innovative drug for the treatment of AA amyloidosis. In a murine AA amyloidosis model, anti-IL-6 receptor ab (MR16-1) produced marked suppression of amyloid deposition in various organs when administered either preventively or therapeutically (Mihara et al., 2004). While reports regarding the efficacy of tocilizumab on AA amyloidosis are limited, Okuda & Takasugi were the first to report an excellent clinical response to treatment with tocilizumab in a patient with AA amyloidosis complicating

juvenile idiopathic arthritis (Okuda & Takasugi, 2006). Tocilizumab immediately normalized the SAA level, followed by the disappearance of gastrointestinal symptoms such as diarrhea and abdominal pain after 1 month and resolution of proteinuria after 2 months of the treatment. Moreover, serial gastrointestinal biopsy specimens showed marked and lasting regression of AA protein deposits. We also reported that in an RA patient with AA amyloidosis who had been refractory to treatment with prednisolone, disease modifying anti-rheumatic drugs or TNF inhibitors including etanercept and infliximab, tocilizumab administration promptly stopped the diarrhea and diminished the disease activity of RA (Nishida et al., 2009). Surprisingly, three months after the tocilizumab treatment amyloid A protein deposits had completely disappeared. Subsequently, two other cases of AA amyloidosis associated with RA also showed the ameliorative clinical effect of tocilizumab on gastrointestinal symptoms due to intestinal amyloidosis (Sato et al., 2009; Inoue et al., 2010) and in one of the two cases amyloid A fibril deposits were found to have disappeared after three courses of tocilizumab treatment. Moreover, Kishida et al. reported observing the clinical ameliorative effect of tocilizumab on a patient with adult-onset Still's disease complicated by AA amyloidosis as well as marked regression of amyloid A protein in duodenal mucosa and submucosa after tocilizumab treatment (Kishida et al., 2010). These dramatic effects of tocilizumab on AA amyloidosis raise the possibility that tocilizumab may become an innovative drug for the treatment of AA amyloidosis, although further clinical studies are required to evaluate its efficacy and safety.

5. Conclusion

Recent analyses of molecular mechanisms regulating SAA production have revealed that proinflammatory cytokines such as IL-1β, TNFα and IL-6 are key players in this process. Biological modifiers including IL-1 receptor antagonist, TNF inhibitors and IL-6 receptor ab, on the other hand, have been demonstrated to be effective for the suppression of serum levels of SAA and for clinical improvement associated with AA amyloidosis. Our findings showed that among proinflammatory cytokines IL-6 appears to play a major role in the induction of the SAA1 gene, so that it is anticipated that IL-6 blockade may constitute the most powerful strategy for the treatment of AA amyloidosis. Indeed, the treatment of chronic inflammatory diseases with tocilizumab could markedly reduce serum concentrations of SAA and several recent case reports described how tocilizumab treatment led to the disappearance or marked regression of amyloid fibril. Tocilizumab may therefore be suitable as a first-line drug for patients who are complicated with, or at high risk of developing AA amyloidosis, although further clinical evaluation will be needed.

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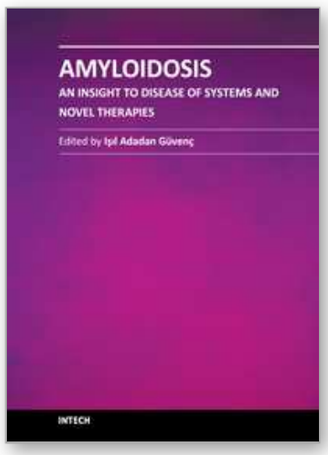
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Amyloidosis is a benign, slowly progressive condition characterized by the presence of extracellular fibrillar proteins in various organs and tissues. It has systemic or localized forms. Both systemic and localized amyloidosis have been a point of interest for many researchers and there have been a growing number of case reports in the literature for the last decade. The aim of this book is to help the reader become familiar with the presentation, diagnosis and treatment modalities of systemic and localized amyloidosis of specific organs or systems and also cover the latest advancements in therapy.

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