Chapter 15
Rheumatoid Arthritis: History, Molecular Mechanisms and Therapeutic Applications

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15.1 Rheumatoid Arthritis: Background

Rheumatoid arthritis (RA), a chronic autoimmune inflammatory disorder of unknown etiology that occurs in approximately 1% of the population (Albani and Carson 1997). In all populations, RA is more prevalent among women than men, and usually develops in the fourth and fifth decades of life, with 80% of the total cases occurring between the ages of 35 and 50 (Kavanaugh and Lipsky 1996). The primary presenting symptoms are pain, stiffness, and swelling of the joints resulting in impaired physical function. These symptoms are often accompanied by constitutional symptoms such as fever and malaise (Grassi et al. 1998). Synovial inflammation underlies the cardinal manifestations of this disease, which include pain, swelling, and tenderness followed by cartilage destruction, bone erosion, and subsequent joint deformities. In RA, joint involvement is typically symmetric, a characteristic usually not found in other forms of arthritis (Muller-Ladner et al. 2005; Majithia and Geraci 2007).

Despite intensive research, the precise cause of RA remains elusive. Although a variety of cells play a role in RA disease progression, macrophages may be of particular importance. Once in the synovium, macrophages are capable of antigen presentation and T-cell activation. Moreover, the extent of macrophage infiltration into the synovium correlates with RA severity and progression (Maruotti et al. 2007). Macrophage-derived cytokines, such as tumor necrosis factor alpha (TNF-α), appear to play a critically important role in the induction and perpetuation of the chronic inflammatory processes in rheumatoid joints as well as in the systemic manifestations of this disease (Grossman and Brahn 1997). TNF-α is a key inflammatory mediator. This cytokine is overproduced in joints of patients with RA and triggers increases in synoviocyte proliferation and a cascade of secondary mediators involved in the recruitment of inflammatory cells and in the process of joint destruction (Camussi and Lupia 1998). Joint erosion is known to occur early in RA, affecting about 40% of the patients during the first year and 90% during the

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first 2 years (Plant et al. 1998). Elevations in inflammatory markers are antecedents of disease progression and joint destruction in early RA (Matsuda et al. 1998). Indeed, the rate of cartilage and joint damage is correlated with plasma elevations in inflammatory acute phase reactants, such as C-reactive protein (CRP) and vascular endothelial growth factor, and in the synovial concentrations of matrix metalloproteinase's, matrix digesting enzymes directly responsible for joint destruction (Paleolog 2002; Burrage et al. 2006; Varghese 2006).

The treatment of RA has undergone somewhat of a revolution over the last decade, with a strong consensus emerging in favor of early, aggressive therapy (Lee and Weinblatt 2001; O'Dell 2001; Goldbach-Mansky and Lipsky 2003; Scott and Kingsley 2006). There is now evidence that early treatment of the disease has a beneficial impact on treatment outcome. The goals to be achieved in managing RA are prevention or control of joint damage, prevention of loss of function, and reduction of pain (Sizova 2008). Non-steroidal anti-inflammatory drugs (NSAIDS), glucocorticoid joint injections, and/or low-dose prednisone may be considered for control of symptoms (Kirwan 1995; Lim and Conn 2001; Kavanaugh 2007).

In light of the therapeutic paradigm shift to early and aggressive treatment, the majority of patients with newly diagnosed RA are started on disease-modifying antirheumatic drug (DMARD) therapy within 3 months of diagnosis. Methotrexate, a powerful immunosuppressive and anti-inflammatory agent, is probably the most commonly used DMARD and is one of the most consistently effective ones (Lee and Weinblatt 2001). Furthermore, the past 7 years have seen the introduction of seven new DMARDs which include leflunomide, the highly specific and efficacious anticytokine agents, including adalimumab, etanercept, and infliximab, and recently, abatacept, and rituximab, and others (Goldbach-Mansky and Lipsky 2003; Scott and Kingsley 2006). These therapies are emerging as important and successful therapeutics for patients with early disease (O'Dell 2001; Goldbach-Mansky and Lipsky 2003; Scott and Kingsley 2006). Although effective in many patients, they are not without their drawbacks. Methotrexate and Leflunomide, require careful monitoring and can cause serious hepatic and pulmonary toxicities (Lee and Weinblatt 2001). The anti-TNF-α biological agents are costly, require parenteral administration, and have been associated with serious and opportunistic infections and lymphoma (Lee and Weinblatt 2001; Goldbach-Mansky and Lipsky 2003; Scott and Kingsley 2006). Furthermore as there are no 'cures', patients will require 30–40 years of ongoing therapy; although most of these agents do not remain effective in an individual for longer than 5 years. Thus, despite major recent advances in the treatment of RA, there is still need for convenient, safe, and effective therapies for many patients.

15.2 A_3AR Agonists: Anti-inflammatory Agents for the Treatment of RA

The findings showing up-regulation of the A_3AR in inflammatory tissues of patients with RA prompted studies which led to the utilization of this receptor as a therapeutic target (Ochaion et al. 2006; Bar-Yehuda et al. 2007; Madi et al. 2007).

To explore the anti-inflammatory effect of synthetic A_3AR agonists and to look at the mechanism of action mediated down-stream to receptor activation, *in vitro* and *in vivo* studies were conducted.

The anti-inflammatory effect of the agonists was first proved in *in vitro* studies in fibroblast like synoviocytes (FLS) derived from synovial fluid of patients with RA. The cells were cultured and served as the first screening to study the effect of the agonists on inflammatory cells. The CF502 A_3AR agonist (generically known as MRS3558) possessing high affinity and selectivity at the human A_3AR, induced a linear dose dependent inhibitory effect on the proliferation of FLS via inhibition of TNF-α (Tchilibon et al. 2005; Ochaion et al. 2008).

Earlier work in experimental animal models, demonstrated that the A_3AR is highly expressed in the inflamed synovial tissue in comparison to low expression in naïve animals. Moreover, the high receptor expression was also reflected in the peripheral blood mononuclear cells (PBMCs) of the arthritic animals (Fishman et al. 2006; Ochaion et al. 2006, 2008; Rath-Wolfson et al. 2006; Bar-Yehuda et al. 2007). Upon oral treatment with the selective A_3AR agonists CF101 (generically known as IB-MECA) and CF502, disease was ameliorated and a marked decrease in disease clinical manifestations was recorded. CF101 treatment reduced inflammation, pannus formation, cartilage destruction and bone lyses (Baharav et al. 2005; Fishman et al. 2006; Ochaion et al. 2006, 2008; Rath-Wolfson et al. 2006; Bar-Yehuda et al. 2007). The specificity of the response was evidenced when an antagonist to the A_3AR was introduced to the arthritic animals prior to each agonist treatment, neutralizing the anti-inflammatory response (Fishman et al. 2006). A point to note is that the animals were treated twice or thrice daily via an oral route, approving former assumption that the molecule is stable and systemically absorbed via the intestine (Bar-Yehuda et al. 2007). Interestingly, shortly after treatment, A_3AR protein expression levels were down-regulated in the synovial tissue, lymph node and spleen of the AIA animals, demonstrating that receptor was internalized and degraded within the cells. Upon chronic treatment with CF101, receptor turned to its high levels again after 8–12 h (Fishman et al. 2006; Ochaion et al. 2006, 2008; Rath-Wolfson et al. 2006; Bar-Yehuda et al. 2007). It is important to note that receptor de-sensitization was transient and have not led to receptor tachyphylaxis, maintaining the A_3AR a valid target along the treatment period.

15.3 Anti-inflammatory Effect of A_3AR Agonists: Molecular Mechanism

The A_3AR is a 7 trans-membrane Gi-protein coupled inhibitory (Gi) receptor and its activation inhibits adenylyl cyclase activity and cAMP formation (Zhao et al. 2000). *In vitro* and *in vivo* studies show that treatment of inflammatory cells with A_3AR agonists induce modulation of signaling proteins, downstream to receptor activation, leading to de-regulation of the NF-κB signaling pathway and apoptosis of inflammatory cells. More specifically, in fibroblasts-like synoviocytes derived from RA patients as well as in synovial cells and in DLN derived from CF101

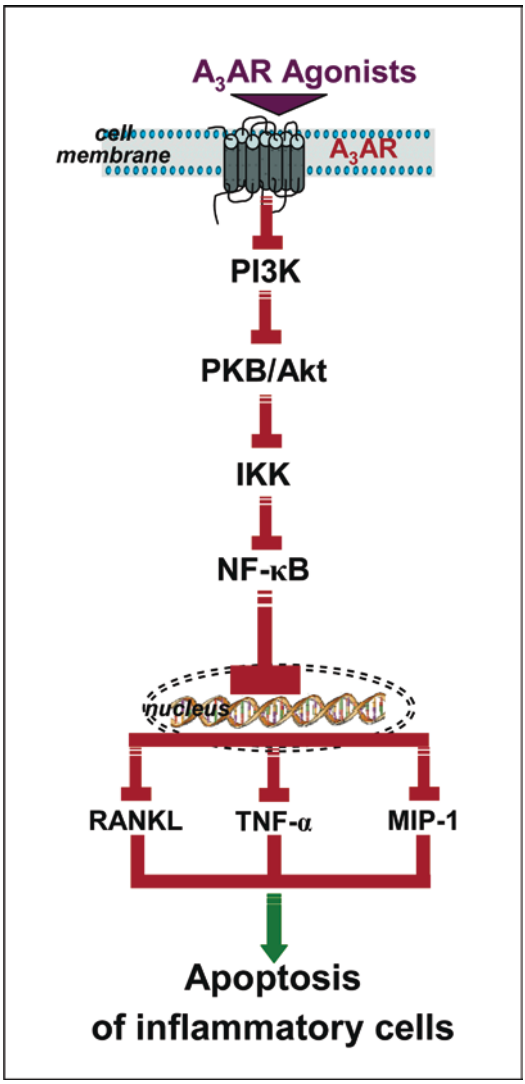
arthritic animals, a decrease in the expression levels of PI3K, phosphorylated PKB/Akt, IKKα/β, NF-κB and TNF-α protein was noted. In addition, RANKL was down-regulated as well, attributing to the ability of CF101 to prevent bone resorption (Baharav et al. 2005; Fishman et al. 2006; Ochaion et al. 2006, 2008; Rath-Wolfson et al. 2006; Bar-Yehuda et al. 2007). The expression level of the chemokine macrophage inflammatory protein 1 alpha (MIP-1α) was also decreased upon CF101 treatment (Szabó et al. 1998). Moreover, an increase in the expression level of caspase-3 took place, suggesting that apoptosis occurred upon CF101 treatment (Fishman et al. 2006; Rath-Wolfson et al. 2006; Bar-Yehuda et al. 2007). The extended lifespan of rheumatoid inflammatory cells such as neutrophils, lymphocytes, macrophages, fibroblasts and synoviocytes in the joints, and other inflammatory sites, is part of the pathogenesis of rheumatoid arthritis (Pap et al. 2000; Wang et al. 2003). One of the mechanisms that can contribute to this phenomenon is inhibition of apoptosis due to stimulation of the PI3K pathway, which leads to activation of PKB/Akt. The latter phosphorylates several proteins such as GSK-3β, FKHR and BAD, which then fail to induce apoptosis. It may also prevent the expression of caspase-9 and caspase-3, proteins pivotal in the apoptotic cascade. Over-expression and activation of PKB/Akt have been defined as the main barrier of apoptosis in the inflamed rheumatoid arthritis tissues (Stoica et al. 2003; Yang et al. 2003). PKB/Akt inhibition by CF101 and the increase in caspase-3 expression level in the CF101-treated animals, support the role of this pathway in ameliorating the inflammatory process (Scheme 15.1).

Beside the direct anti-inflammatory effect, CF101 was found to exert an immuno-modulatory effect in the AIA model. CF101 inhibited the proliferation of auto-reactive T cells derived from CF101-treated AIA rats (Bar-Yehuda et al. 2007). The drug also prevented the induction of arthritis in rats via adoptive transfer experiments. AIA can be transferred from one animal to the other upon engrafting spleen or lymph node cells from an arthritic animal to a naive one (Taurog et al. 1983; Spargo et al. 2006; Bar-Yehuda et al. 2007). Taken together, CF101 exerts its immuno-modulatory effect via inhibition of pro-inflammatory cytokine production and improvement of T cell function (Scheme 15.2).

15.4 The Clinical Development of CF101 as an Anti-inflammatory Drug to Combat RA

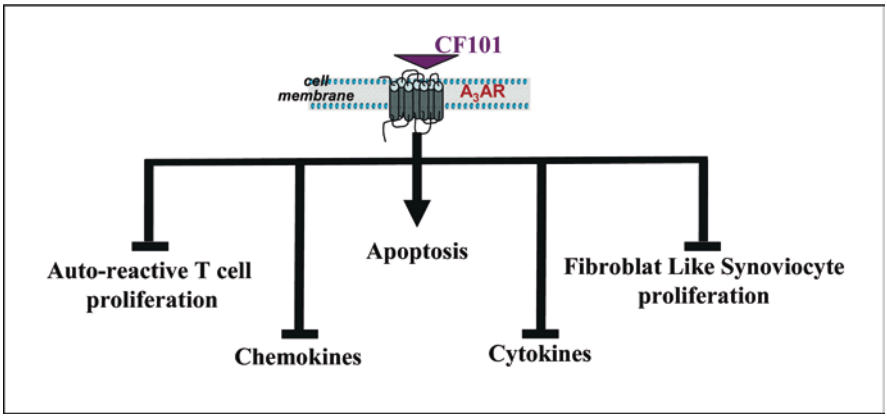
The excellent chemical and pharmacological profile of CF101 together with the findings showing up-regulation of the A_3AR in patients with RA, prompted the initiation of a clinical program aiming at the development of CF101 as a drug candidate for the treatment of RA.

Pre-clinical studies resulted in the development of a robust route of synthesis for CF101 in a large scale production. The active pharmaceutical ingredient was found to be highly stable for a long period of time. CF101 was slowly metabolized in mouse, rat, rabbit, monkey and human hepatocytes and studies with human hepatic



Scheme 15.1 Molecular mechanism involved with the anti-inflammatory effect of A_3AR agonists

microsomes indicated that no drug interactions, related to inhibition of the CYP450 metabolism, took place. Chronic toxicity studies in mice and monkeys revealed no cardiovascular risks and no toxicological issues (Bar-Yehuda et al. 2007). In a Phase I study in healthy subjects, CF101 was found to be safe and well tolerated with a linear pharmacokinetic activity (van Troostenburg et al. 2004). In a Phase IIa study conducted in patients with RA, CF101 administered twice daily for 12 weeks resulted in an improvement of disease signs and symptoms and appeared to be safe



Scheme 15.2 Immuno-modulatory effects mediated via the A_3AR

and well tolerated. Analysis of A_3AR expression levels at base line showed statistically significant direct correlation with patient response to CF101, suggesting A_3AR utilization as a biomarker to predict patients' response to the drug prior to treatment initiation (Silverman et al. 2008).

To conclude, the A_3AR which is highly expressed in inflammatory cells of AIA rats and patients with RA, is suggested as a biological marker and therapeutic target in RA. Synthetic agonists, which bind with high affinity and selectivity to the receptor, induce a marked anti-inflammatory effect mediated via de-regulation of the NF-κB signaling pathway, resulting in inhibition of pro-inflammatory cytokines and in apoptosis of inflammatory cells. The oral bioavailability of the A_3AR agonists and the clinical data produced in the Phase IIa human study support the development of these agents as anti-rheumatic drugs.

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