Synovial Tissue Response to Treatment in Rheumatoid Arthritis

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Abstract: The recognition of the synovial tissue, as the primary target of inflammation in RA, has driven research in this field, not only to clarify the disease pathogenesis but also to evaluate local changes in response to treatment. Special interest has been given to the identification of sensitive synovial biomarkers that could be of help in demonstrating proof of principle in early stages of drug development. Synovial sublining macrophages have been shown to correlate with scores for disease activity in cross-sectional studies. Moreover, decreased disease activity as measured by the disease activity score evaluated in 28 joints (DAS28) after effective treatment, has consistently been associated with a reduction of the number of CD68+ synovial sublining macrophages across different therapies. This observation highlights a possible final common pathway in the mechanism of action of various therapies and supports the notion that macrophages have a central role in RA pathogenesis.

When considering experimental therapies, the study of serial synovial biopsies in relatively small numbers of patients, in the context of proof of principle trials, successfully distinguished between effective and ineffective treatments. This attractive approach can be used during early drug development for screening proposes, supporting which new treatments have higher probability to be beneficial in a large scale clinical trial.

In this paper we review the effects of RA treatments on the synovial tissue, including targeted therapies, with particular attention to their effect on synovial biomarkers.

Keywords: Rheumatoid arthritis, synovial tissue, treatment, biomarkers, clinical trial design.

1. INTRODUCTION

The aim of rheumatoid arthritis (RA) treatment is to reduce synovial inflammation and prevent joint destruction. The introduction of conventional disease-modifying antirheumatic drugs (DMARDs) and corticosteroids in RA management has been developed without a full understanding of their mechanisms of action. Specific targeted therapies have been introduced based on the increased knowledge of RA pathogenesis, highlighting a translation from serendipity to a selective interference with inflammatory and immune pathways.

Although a systemic disease, the hallmark of RA is chronic synovitis that affects multiple joints and invades cartilage causing bone erosions. Therefore, special interest has been given to the study of synovial tissue, not only to clarify the disease pathogenesis but also to evaluate local changes in response to treatment. First, such studies may provide insight into the mechanisms of action of a therapeutic intervention. Another goal of analysing synovial tissue is to identify molecular biomarkers that could be of help to predict response to treatment in individual patients. The ideal synovial biomarker would be able to discriminate at baseline between responders and non-responders to treatment, possibly leading to a more efficient and personalized treatment. Finally, the analysis of serial synovial biopsies is particularly attractive when analysing potential new therapies on the group level in proof of principle trials. As the main therapeutic target, changes in the synovial membrane can be used to detect early effects of treatment.

In this paper we review the effect of treatments on the features of the synovium *in vivo*. The *in vitro* and *ex vivo* experiments using different synovial cell lineages and tissue samples cultured *in vitro* will not be discussed, since they are beyond the scope of this article.

2. CORTICOSTEROIDS

The discovery of corticosteroids, more than 50 years ago, represents a remarkable progress in the treatment of inflammatory arthritis. Their anti-inflammatory effects are well known, including the impairment of pro-inflammatory cytokine synthesis and lymphocyte function as well as their adhesion to endothelial cells [1]. During the last decade, the mechanism of action of corticosteroids on the synovial tissue has been analysed, with compelling results.

In a randomized, double-blinded, placebo-controlled trial, patients given oral prednisolone 60 mg/day for one week followed by 40 mg/day in the second week (COBRA schedule), exhibited a significant decrease in the number of $CD68^+$ macrophages, but also $CD5^+$ (B and T cells), $CD4^+$

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(subset of T cells) and $CD163^+$ (subset of resident macrophages) cells. An analysis of covariance model showed that for CD68+ sublining macrophages, the estimated effect of prednisolone was large. Patients receiving active treatment had markedly fewer macrophages after therapy compared with those receiving placebo. This study identified, from a large panel of synovial biomarkers, sublining macrophages as an optimal marker to evaluate clinical response to corticosteroids. Staining for cytokines showed a reduction of the expression of interleukin (IL)-1β and tumor necrosis factor (TNF), which are mainly produced by macrophages, and there was also a trend towards a reduction of markers associated with neoangiogenesis, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and αvβ3 integrin [2]. For patients treated with the same PDN regimen, it has been shown that real time quantitative-polymerase chain reaction (qRT-PCR) might provide additional information regarding treatment efficacy in small proof of principle clinical trials [3]. Prednisolone markedly decreased IL-8 and matrix metalloproteinases-1 (MMP-1) mRNA expression compared with placebo, and the confidentially intervals excluded the likelihood of no effect.

Methylprednisolone pulses were also associated with a rapid (within 24 hours) and significant reduction of inflammatory cytokines such as TNF and IL-8, but not of IL-1β or IL-1Ra. The adhesion molecules, E-selectin and inter-cellular adhesion molecule 1 (ICAM-1), were decreased, suggesting that this could be a possible mechanism of inhibiting neutrophils migration into the synovium [4, 5].

It has previously been demonstrated in randomized controlled trials that corticosteroids can improve radiographic progression [6]. In accordance with this clinical effect and the reduced MMP-1 mRNA expression after prednisone therapy, methylprednisolone pulses were able to reduce the expression of MMP-1 and of tissue inhibitor of metalloproteinases 1 (TIMP-1), possibly as a result of the reduction of the number of macrophages, albeit not reaching statistical significance at 24 hours. Of interest, there was no change in MMP3 expression, perhaps contributing to the relatively moderate effect of corticosteroids in preventing joint destruction [3, 7].

Intra-articular (IA) corticosteroids injection is a possible therapeutic approach when one or a few joints are affected, sparing patients from the systemic toxicity of oral administration. The mechanism by which IA corticosteroids suppress inflammation in the synovial membrane and the reason for an unpredictable clinical response in daily practice is still not fully elucidated. Previous work has shown that IA corticosteroid treatment results in decreased synovial inflammation [8]. In all patients the local treatment resulted in a decreased arthritis activity of the treated knee joint as measured clinically or histologically.

In another study of thirteen patients, 6 of which had RA, the administration of 40 mg triancinolone hexacetonide IA was accompanied by a significant reduction of the synovial receptor activator for nuclear factor κ B ligand (RANKL) expression, while osteoprotegerin (OPG) expression remained unchanged [9].

S100A12 (calgranulin C), a member of the phagocytic S100 family of calcium-binding proteins that is expressed and secreted by activated granulocytes, has been proposed as a synovial biomarker of clinical response to IA corticosteroids and also to infliximab. S100A12 is expressed in patients with active RA and its serum concentrations correlate with synovial fluid levels and disease activity. In a group of 14 patients, successful treatment with IA corticosteroids led to a decrease of S100A12 sublining expression two weeks after injection in the responders group, the same happening with infliximab at 8 weeks [10].

3. CONVENTIONAL DISEASE-MODIFYING ANTIRHEUMATIC DRUGS

DMARDs are able to decrease joint inflammation and prevent radiographic damage. Conventional DMARD therapy has been introduced for the treatment of RA and other forms of inflammatory arthritis as an empirical approach to RA management. Therefore, only a few trials have analysed the effect of these treatments in the synovial membrane.

Gold has been used in the treatment of RA for more than 60 years; however only a few studies looked at its effects on the synovium, with divergent results. In two studies, synovial T cells numbers were reduced after 6 months of gold treatment [11, 12]. Another study showed a decrease in CD68+ macrophage numbers as well as in the expression of macrophages-derived cytokines (TNF, IL-1β and IL-6), without changes in the number of T or B cells, 12 weeks after initiation of treatment with intramuscular gold or a combination of intramuscular gold with methylprednisolone [13].

Methotrexate, the so called anchor-drug in the treatment of RA, was shown to be associated with a decrease in the number of macrophages, T and plasma cells at 16 weeks of successful therapy. Interestingly, corroborating the previously described effect of oral prednisolone, a trend towards an association between clinical improvement and the reduction of $CD68^+$ macrophages scores was depicted [14]. This relationship was confirmed when other DMARDs were included in the analysis, such as leflunomide, sulphasalazine, hydroxychloro-quine and infliximab. The mean change in DAS28 (ΔDAS28) consistently correlated with the mean change in sublining CD68+macrophages (ΔCD68sl) suggesting that macrophages correlate with clinical improvement independently of the therapeutic strategy, probably reflecting a common final pathway regarding their mechanisms of action [15]. This correlation was confirmed in different centres indicating that the relationship between CD68+ macrophages and disease activity is reproducible across centres [16, 17]. The sensitivity to change of the number of CD68+ sublining macrophages was determined by calculation of the standardized response mean (SRM). After effective DMARDs treatment the SRM for this biomarker was high (>0.8) indicating high sensitivity to change while it was low when ineffective therapies (anti-CCL2 or anti-C5aR) were analysed. Consistent with previous studies, CD68+ macrophages are less susceptible to placebo effects than clinical evaluation (DAS28) indicating a high sensitivity in discriminating between effective and ineffective therapies or placebo [18-20].

Following DMARD treatment (mainly methotrexate and gold) a reduction of the expression of inflammatory cytokines

(IL-1β and TNF) and adhesion molecules (E-selectin, ICAM-1 and VCAM-1) has been described, particularly evident in the responder groups [14, 21, 22]. After 4 months of treatment with either methotrexate or leflunomide, the results were generally similar. Of importance, MMP-1 expression and the ratio MMP-1/TIMP-1 were also diminished after treatment. These changes were, again, more evident in the group that had achieved an ACR20 response [22]. In addition, successful treatment with DMARDs resulted in a reduction of RANKL and increase in OPG expression in the synovium. There was a significant correlation between RANKL, DAS28 and erosion scores, highlighting the benefits of DMARDs in improving radiographic outcomes [23]. The consistent relationship between clinical improvement, associated with protection against joint destruction, and decreased synovial inflammation after DMARD treatment, is in line with the observation that synovitis is a predictor of joint damage [24-26].

Examination of serial synovial tissue samples may also provide insight into the mechanisms underlying the resistance to treatment. Loss of efficacy detected with the long-term use of some DMARDs is well known by clinicians but the reasons for this secondary failure are poorly understood. Cell membrane–associated drug efflux transporters belonging to the family of ATP-binding cassette (ABC) proteins have been associated with acquired resistance to anticancer drugs as well as DMARDs. *In vitro* methotrexate, leflunomide and sulphasalazine can be exported from cells *via* the breast cancer resistance protein (BCRP). BCRP was recently found to be expressed in macrophages in RA synovial tissue. After 4 months of treatment with methotrexate or leflunomide, high BCRP expression was associated with persistence of infiltrating macrophages and higher DAS28 and C reactive protein (CRP) levels. Similarly, patients who did not respond to treatment had higher BCRP cell counts suggesting that BCRP might be responsible for the efflux of these drugs in macrophages, inducing resistance to therapy [27].

Taken together, the results above reinforce the importance of macrophages in the pathogenesis of RA (reviewed in *ref. 28 and ref. 29)* and suggest that the immunomodulatory action of DMARDs might be in part dependent on the down-regulation of cytokines and adhesion molecules with a consequent decrease of the inflammatory synovial cellular infiltrate [28, 29]. These studies have also shown the efficacy of DMARDs in modulating the RANKL/OPG ratio and reducing MMP expression and consequently joint destruction (reviewed in *ref. 30)* The number of synovial sublining macrophages has been demonstrated to be a reliable biomarker of therapeutic response to DMARDs in clinical trials [30].

4. BIOLOGICAL DISEASE MODIFYING ANTIRHEUMATIC DRUGS

4.1. Anti-TNF Therapy

Three TNF antagonists are at present widely used in clinical practice: infliximab, a chimeric monoclonal antibody, adalimumab, a fully monoclonal antibody and etanercept, a TNF-receptor Fc-fusion protein. As observed with conventional DMARDs, TNF antagonists clearly improve the signs and symptoms associated with synovial inflammation. Different mechanisms might contribute to their efficacy in decreasing the synovial infiltrating cells: the impairment of cell migration, cell proliferation and/or cell retention; the reduction of adhesions molecules, cytokines, chemokines and metalloproteinases expression; and the regulation of angiogenesis [31].

Studies on the effect of TNF antagonists on synovial cells have demonstrated that cell infiltration is significantly reduced as early as 48 hours, two and four weeks after the first infliximab infusion [32-34].

At 48 hours, the number of intimal CD68+ macrophages was significantly diminished and a trend towards a reduction of sublining CD68+ macrophages, T and plasma cells was also detected [32]. An enhancement of apoptosis and cytotoxicity, or an interference with cell trafficking have been proposed to explain the rapid reduction of synovial cellularity, but their exact role remains to be elucidated.

Synovial cell hyperplasia is a characteristic of pannus and resistance to apoptosis by fibroblast-like synoviocytes (FLS), T and B cells has been widely demonstrated [35, 36]. Of interest, using TUNEL assays, a sensitive method for detecting apoptosis induced DNA fragmentation, the number of TUNEL-positive cells in the infliximab treated group was similar to placebo at 48 hours and these results were confirmed by electron microscopy [32]. Similar findings were obtained at 1 and 24 hours after treatment, by active caspase 3 staining and other methods, making apoptosis induction as a mechanism underlying the reduction of synovial cellularity less likely [37].

Reduced synovial cell counts after TNF antagonist treatment could perhaps be explained by modulation of cell migration. Synovial tissue is most certainly a dynamic tissue in which the number of infiltrating cells is dependent on the balance between their influx, proliferation and efflux [38]. The interaction between inflammatory and endothelial cells is of great importance and is probably mediated by a wide range of cytokines and chemokines. RA synovial tissue endothelial cells express high levels of E-selectin, VCAM-1, ICAM-1 and ICAM-3 and the last 3 have also been identified in other synovial cells including macrophages, FLS and lymphocytes [39]. The synovial expression of E-selectin and VCAM-1 was shown to be decreased after infliximab treatment [34]. A reduction of serum level of E-selectin and ICAM-1 is associated with an increase of circulating lymphocytes early after TNF blockade, possible indicating reduced migration into the synovium [40]. In addition, the expression of IL-8 and monocytes chemotactic protein (MCP)-1 chemokines on synovial membrane is also down-regulated by anti-TNF therapy, suggesting that TNF antagonists might also act by diminishing chemokine-mediated attraction of leucocytes [39]. These data are further supported by the evidence of impairment of neutrophil-trafficking measured by labeled granulocytes gammagraphy after infliximab administration [33]. It is at present not clear if this endothelial deactivation is a direct effect of TNF blockade or whether it results from the decrease in macrophages numbers or expression of cytokines produced by the inflammatory cells.

Formation of new blood vessels is crucial for the maintenance of hyper-proliferative synovium, through nutrient supply and delivery of inflammatory cells and other inflammatory molecules. Pro-angiogenic factors including cytokines such as VEGF, bFGF, platelet derived growth

factor (PDGF), tumor growing factor (TGF) β, TNF, and chemokines such as IL-8 and Groα have been detected in synovial membrane [39]. However, the baseline expression of VEGF and bFGF, as well as of E-selectin, ICAM-1 and VCAM-1, were not predictive of response to infiliximab after 16 weeks of therapy [41].

The TNF like weak inducer of apoptosis (TWEAK) has recently been implicated in inflammatory arthritis pathogenesis. Through the interaction with its receptor, the fibroblast growth factor inducible 14 (Fn14), TWEAK can drive FLS to produce cytokines and chemokines, promote bone destruction and develop pro-angiogenic effects. TWEAK and Fn14 are widely expressed in RA synovium, namely in the lining, sublining and perivascular regions, and their co-localization could be detected in FLS and macrophages. Interestingly, infliximab treatment did not modulate TWEAK and Fn14 expression, underlining the additional potential benefits of blocking their activation as a new therapeutic target [42].

Anti-TNF therapy could finally contribute to the clearance of immune cells from the synovial membrane by increasing their traffic into the lymphatic draining system. Consistent with this hypothesis lymphatic vessels are abundantly present in patients with RA and their formation is increased after infliximab treatment [43].

Despite the excellent response obtained by most of the patients after anti-TNF treatment, 40% do not respond to these therapies and some of the initial responders develop secondary failure [44, 45]. Baseline TNF expression has been found to be, at least in part, a determinant of the primary clinical response to infliximab, with responders exhibiting high levels of TNF in the lining and sublining comparing to non responders [41]. Positivity for lymphocyte aggregates further increased the power to predict the clinical response, when analyzed in a prediction model that included baseline disease activity, anti-cyclic citrullinated peptide antibody positivity, and synovial TNF expression [46]. Consistent with these results responders to anti-TNF therapy have higher expression levels of genes directly involved in inflammation in the synovial tissue, including those related to immunity and defence, T-cell mediated immunity, cell adhesion, cytokine and chemokine mediated signalling pathway, and macrophage-mediated immunity [47].

4.2. Rituximab

Rituximab, a chimeric monoclonal antibody against the B lymphocyte surface marker CD20, has been used in the treatment of RA for the last 7 years with encouraging results [48-50]. The recognition of its efficacy in controlling RA manifestations has driven research in this field aiming to explain the role of B cells in RA pathogenesis. Rituximab may cause circulating B cell depletion through antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and apoptosis [51].

The synovial B cell infiltrate in RA patients is very heterogeneous regarding the number of cells and its distribution. B cells might be found as a scarce or diffuse infiltrate, in follicles or integrated in germinal centers as those seen in the lymphoid tissues [52, 53]. The almost complete peripheral B cell depletion and the lack of correlation between the degree of depletion and clinical response raises the hypothesis of residual non depleted resident B cells in non-peripheral compartments, including lymphoid and synovial tissues [51]. Analysing the effect of rituximab on synovial cell populations, a significant decrease in the number $CD22^+$ B cells has been detected in some but not in all treated patients, 4 weeks after the first rituximab infusion. In addition, different patterns of depletion were observed, from a complete clearance of B cells to a partial decrease in synovial B cell number [54]. These results where further confirmed at 16 weeks post-treatment with a trend towards a more pronounced reduction of B cells in patients who had persistent B cell counts at 4 weeks. Moreover, a significant decrease in the number of T cells, macrophages and lymphoid aggregates was also depicted at the group level and a marked reduction of plasma cells in a subset of patients [55]. Other studies have addressed this same question. A complete depletion of $CD20^+$ cells at 12 weeks in 88% of patients after rituximab treatment has been identified but the concomitant use of prednisolone might have influenced these results [56]. Another study confirmed the variable reduction of synovial B cells after rituximab treatment [57].

Taken together, these studies have shown marked B cell depletion in the peripheral blood, whereas synovial B-lineage cells may persist in some patients. Local protective factors, such as B lymphocyte stimulator (BLyS) and CD55 (decay-accelarating factor (DAF)), and more efficient B cell depletion in the peripheral compartment might explain these results [55]. The secondary decrease in macrophages and T cells highlight the role of B cells in orquestrating synovial inflammation and suggest that the clinical response to rituximab could be predicted by changes in synovial cell types other than B cells.

These studies have also analysed the correlation between changes in the synovial cell infiltrate, disease activity and response to treatment. Although the baseline characteristics of the synovium do not appear to predict the response to treatment, the secondary reduction of plasma cells and intimal macrophages between 4 and 16 weeks after initiation of rituximab treatment was associated with the clinical response at 24 weeks. Interestingly, the decrease in plasma cell numbers was also correlated with a reduction of serum anti-citrullinated protein antibodies (ACPA) levels at 16 weeks. In addition, the decrease in circulating ACPAs could be related, at least in part, to changes in synovial short-lived plasma cells, derived from B cells and responsible for autoantibody production [55]. In line with these observations, other investigators found an association between persistence of synovial $CD79a^+$ $CD20^-$ B-lineage cells, exhibiting plasma cell morphology, and disease activity after rituximab treatment [56, 58].

4.3. Abatacept

Abatacept, a human cytotoxic T-lymphocyte antigen (CTLA)-4 and Fc-IgG1 fusion protein that blocks the co-stimulatory signal between CD28 and CD80/CD86, is approved for the treatment of active RA patients, refractory to MTX or to TNF antagonists. The only study that determined the effects of abatacept on the synovium, demonstrated a moderate, although statistically significant decrease of $CD20^+$ B cells at day 120 in RA patients who

failed TNF antagonists. The lack of reduction of T cell counts is probably consistent with a more immunomodulatory effect on T cell activation rather than a direct effect on cell depletion; binding of abatacept to CD80/CD86 on B cells should also be considered. In parallel to the mild changes in synovial cell infiltrate there was a marked decrease in pro-inflammatory gene expression in the synovial tissue, including interferon γ, IL-1β, MMP-1 and MMP-3, which was also statistically significant when comparing responders to non responders [59].

5. EXPERIMENTAL TARGETED THERAPIES

5.1. Complement Blockade

5.1.1. C5a Blockade

Complement has been considered to be implicated in RA pathogenesis and recently it was shown that, *in vitro*, ACPA are able to activate complement [60]. Complement proteins and its receptors can be locally produced and activated in the RA synovial membrane [61].

Different molecules that block the possible role of C5a in recruiting and activating synovial inflammatory cells have been developed. One of these is PMX53 (Promics Ltd), an orally active hexapeptide that selectively antagonizes CD88, the C5a receptor. A double-blind, placebo-controlled proof of principle phase 1b trial demonstrated no evidence of clinical efficacy. Consistently, PMX53 did not reduce the synovial inflammatory cell infiltrate (including C5aR+cells and macrophages) or the expression of inflammatory cytokines (IL-6, IL-1β and TNFα) [62].

5.2. Chemokine Blockade

Chemokines are a group of chemotactic proteins that exhibit a similar basic structure. They are key mediators of cell migration but also stimulate cells to release inflammatory mediators and matrix metalloproteinases. Therefore, chemokines and chemokine receptors are thought to be key players in the inflammatory response observed in RA synovium.

5.2.1. CCL2 Blockade

CCL2/ monocyte chemotactic protein (MCP)-1 is the ligand for CCR2 on monocytes, T cells, dendritic cells, basophils and natural killer cells. It is highly expressed in RA synovium where it is mainly produced by macrophages [63, 64]. Based on the concept that blocking inflammatory cell migration into the synovial would improve arthritis in RA patients, the efficacy of a human monoclonal antibody directed against CCL2/MCP-1 (ABN912; Novartis Pharma AG) was tested [65]. ABN912 treatment was not associated with any clinical or immunohistologic improvement and in fact dose dependent increases in C-reactive protein concentrations, sublining macrophage numbers and CCL2/MCP-1 serum levels were detected. MLN202, an anti-CCR2 antibody (Millenium Pharmaceuticals Inc.) was also evaluated in a proof of principle trial, but again no clinical or synovial improvement was identified, suggesting that using these compounds in larger clinical trials would not translate into any clinical benefit [66].

5.2.2. CCR1 Blockade

In RA patients the percentage of CCR1 and CCR5 positive peripheral blood monocytes cells is decreased when comparing to healthy controls, but they are highly expressed in the synovial tissue [64]. The potential benefits of blocking CCR1, using CP 481,715 (Pfizer Inc.), an oral CCR1 antagonist, were studied in a small-placebo controlled proof of principle trial. Two weeks after treatment there was a significant reduction of synovial macrophages and CCR1 positive cells together with a trend towards clinical improvement as compared to placebo [67]. The use of another CCR1 antagonist, MLN3897, Millennium Pharmaceuticals Inc.), did not result in clinical improvement in RA [68]. Thus, it is at present unclear whether blockade of CCR1 at optimal levels of receptor occupancy might be sufficient to induce amelioration of RA.

5.3. Cytokines

5.3.1. IL-10

The systemic administration of human recombinant IL-10 in active RA patients showed no improvement of cell infiltration or cytokine expression in accordance with a lack of clinical benefit [18].

5.3.2. Interferon β

Evaluation of the effect of systemic treatment with interferon β (IFN β) on the synovial tissue from RA patients showed a modest reduction of CD3+ T cells, but no statistically significant change in CD68+ macrophages, in 11 RA patients; this effect was lost 3 months after therapy [69]. Consistent with these results, a double-blind, placebo-controlled clinical trial in 209 RA patients showed no improvement after treatment with either 2.2 mg or 44 mg of IFNβ, given subcutaneously three times weekly for 24 weeks compared to placebo [70]. It remains to be determined whether other dosing regimens or other routes of administration may be more effective.

5.4. Targeting T Cells

5.4.1. Alemtuzumab

Alemtuzumab (Campath-1H), a humanized antibody against CD52 that is broadly expressed on lymphocytes and macrophages, did not decrease synovial T cells in two patients with recurrent synovitis after treatment, despite inducing profound depletion of circulating lymphocytes (mainly CD4+ cells) [71]. The further evaluation of alemtuzumab for treatment of RA was discontinued due to anxieties related to prolonged therapy-induced lymphopenia.

5.4.2. Anti-CD4

The chimeric anti-CD4 monoclonal antibody cM-T412, when administered intravenously, for five days, to 7 patients, significantly decreased the synovial T cells, the expression of adhesion molecules and peripheral CD4+ cells counts. There was however no statistically significant reduction of CD68+ macrophages and there was no clinical improvement [72]. The lack of efficacy was convincingly shown in a double-blind, placebo-controlled multicenter trial in 64 patients receiving concomitant methotrexate [73].

6. CONCLUSIONS

The synovium is the major target tissue in RA and other inflammatory arthritides. The evaluation of serial synovial biopsies has proven to be advantageous to improve knowledge regarding RA pathogenesis, new molecular targets and major mechanisms of action of RA therapeutics. In addition, CD68+ sublining macrophages have been recognized as a synovial biomarker that may help to distinguish on the group level between effective and ineffective treatment in an early stage of drug development. The correlation between sublining CD68+macrophages and disease activity as measured by DAS28 has consistently been shown across different therapies, suggesting a possible common final pathway in their mechanism of action.

Analyzing synovial samples in proof of principle trials was demonstrated to be helpful for screening proposes. The absence of significant changes in the number of CD68+ sublining macrophages after therapeutic interventions paralleled the lack of clinical benefit. This was for instance shown for systemic treatment with IL-10, IFNβ, a C5a receptor antagonist, anti-CD4 antibodies, anti-CCR2 antibodies and anti-MCP-1 antibodies. The lack of correlation between changes in peripheral blood compared to synovial tissue highlight the importance of considering the inclusion of synovial biomarkers in clinical trials. The profound depletion of peripheral T and B cells after alemtuzumab or rituximab treatment, respectively, did not concur with the same profound changes in the synovial compartment. Collectively, these results clearly support the usefulness of examination of synovial biopsies in proof of principle trials.

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CONFLICT OF INTEREST

None declared.

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