Monocyte migration to the synovium in rheumatoid arthritis patients treated with adalimumab


Published in:
Annals of the Rheumatic Diseases

DOI:
10.1136/ard.2010.141549

Citation for published version (APA):
Monocyte migration to the synovium in rheumatoid arthritis patients treated with adalimumab

M M J Herenius,1 R M Thurlings,1 C A Wijbrands,1 R J Bennink,2 S E Dohmen,3 C Voermans,3 D Wouters,4 E S Izmailova,5 D M Gerlag,1 B L F van Eck-Smit,2 P P Tak1

ABSTRACT

Objectives The mechanism of action of treatment with tumour necrosis factor (TNF) blockers in rheumatoid arthritis (RA) is still not completely understood. The aim of this study was to test if adalimumab treatment could affect the influx of monocytes into the synovium.

Methods A novel technique was used to analyse the migration of labelled autologous monocytes before and 14 days after initiation of adalimumab treatment using scintigraphy. CD14 monocytes were isolated from patients with RA, using a positive selection procedure with magnetic-activated cell sorting, and labelled with technetium-99m-hexamethylpropylene-amine-oxime. Scintigraphic scans were made 1, 2 and 3 h after re-infusion.

Results As early as 14 days after the start of treatment with adalimumab a significant decrease in disease activity score evaluated in 28 joints was shown. There was no significant decrease in the influx of monocytes into the joint at this time.

Conclusions This study indicates that adalimumab treatment does not reduce the influx of monocytes into the synovium early after initiation of treatment. As previous studies showed a rapid decrease in macrophage infiltration after TNF-antibody therapy, which could not be explained by increased cell death, this points to an important role for enhanced efflux of inflammatory cells from the synovium.

INTRODUCTION

Disease control has been improved by the use of tumour necrosis factor (TNF) blockade in patients with rheumatoid arthritis (RA) and other immune-mediated inflammatory diseases. However, the mechanisms by which TNF antagonists exert their effect is still not completely understood.1 Anti-TNF antibody treatment has been shown to result in marked reduction of synovial inflammation in RA and psoriatic arthritis.2-4 This decrease in synovial cellularity could be observed as early as 24–48 h after initiation of treatment.5-7 This early reduction in synovial inflammation after TNF blockade could not be explained by apoptosis induction at the site of inflammation,6 7 leaving either reduced cell influx or enhanced cell efflux to explain this process. In one study infliximab treatment significantly decreased the influx of 111In-labelled granulocyte migration into affected joints of RA patients,8 and in another study adalimumab significantly reduced influx of 99Tc-labelled leucocytes, whereas no decrease in influx was seen in patients treated with placebo.9 Monocytes and macrophages are key players in the pathogenesis of RA.9 Furthermore, the decrease in macrophage numbers in the synovium is associated with clinical improvement after effective treatment.10 Therefore, the effect of adalimumab treatment on monocyte migration towards the synovial compartment was examined. The authors recently developed a procedure using a combination of immunomagnetic cell selection with CD14-coated beads, labelling with technetium-99m-hexamethylpropylene-amine-oxime and scintigraphy to visualise the migratory behaviour of autologous monocytes.11 Applying this method, a continuous migration of monocytes into the inflamed synovial tissue of RA patients at a slow macrophage-replacement rate was shown.12 The slow rate of monocyte influx into the synovial compartment suggests that the rapid effect of anti-TNF therapy on macrophage infiltration cannot merely be explained by blockade of cell influx, as previously thought. Hence, this novel imaging technique was used to directly test if adalimumab treatment could affect the influx of monocytes into the synovium.

PATIENTS AND METHODS

Patients Eight patients with established RA according to the revised American College of Rheumatology criteria for the diagnosis of RA13 were included. All patients had an indication for the use of anti-TNF therapy according to the guidelines of the Dutch Society for Rheumatology, which is active disease status (disease activity score evaluated in 28 joints (DAS28) ≥3.2) despite treatment with two conventional disease-modifying antirheumatic drugs. In this study all patients started with adalimumab (40 mg subcutaneously every other week) 24 h after the baseline scans. Three patients used maximally tolerable methotrexate at a stable dosage (10–25 mg/week). The others received adalimumab monotherapy. The use of concomitant non-steroidal anti-inflammatory drugs was permitted if stable for at least 1 month prior to baseline and was kept stable throughout the study. All patients provided written informed consent and approval was granted by the local medical ethics committee.

Isolation and labelling of monocytes Isolation and labelling of monocytes was performed as described previously (see supplementary text).

Scintigraphy and signal calculations Scintigraphy and signal calculations were done as described previously (see supplementary text). This procedure was done at day –14, baseline and day 14.
**DISCUSSION**

The results of this mechanistic study indicate that CD14 monocyte influx into the synovium is not decreased 2 weeks after the start of adalimumab treatment. Earlier, TNF blockade was shown not to induce apoptosis in the synovium early after initiation of treatment, although the possibility cannot be excluded that more long-term treatment would lead to a more pro-apoptotic state, being a result of dampening of inflammation rather than its cause. This suggests that the rapid decrease in synovial macrophage numbers observed after anti-TNF treatment cannot be explained by an immediate effect on monocyte influx, as previously hypothesised. These data strengthen the recent observation that monocytes migrate towards the inflamed RA synovium at a slow macrophage-replacement rate, which already suggested that blockade of monocyte migration would be insufficient to explain the rapid reduction of macrophage numbers.

**RESULTS**

**Patient characteristics**

Half of the patients were IgM rheumatoid factor positive and all had erosive disease. Their median age was 53 (48.5–57) years and median disease duration was 162 (119–273) months. Individual patient characteristics are shown in table 1.

**Monocyte influx is stable and not markedly decreased early after initiation of adalimumab treatment**

Scans were made 14 days prior to the start of adalimumab treatment, at baseline and 14 days after the start of adalimumab treatment (see supplementary figure 1). The number of labelled monocytes in the joint of interest per patient was comparable 1, 2 and 3 h after re-infusion. This was true for day −14, baseline and day 14 (see supplementary table). Monocyte influx per patient at 3 h postinfusion is shown in figure 1. Of interest, there was no significant change in monocyte influx 1, 2 and 3 h after re-infusion from day −14 to day 1, which was before the start of adalimumab treatment (p=0.33, p=0.67, p=0.21 respectively). The heterogeneity between patients is consistent with changes in synovial macrophages that were found in serial biopsies of patients treated with placebo. Importantly, the influx of monocytes did not decrease after re-infusion of monocytes comparing day 14 to baseline, indicating that adalimumab treatment did not affect the influx of monocytes early after initiation of treatment (figure 1).

**Clinical benefit of adalimumab treatment**

The change in DAS28 from screening to day 84 is shown in figure 2. The median DAS28 at baseline was 5.9 (4.8–7.2). The DAS28 did not change significantly between screening and baseline (p=0.58). There was a statistically significant decrease in median DAS28 from baseline to day 14 of 1.2 (0.72–1.8, p=0.01). At day 84 the median DAS28 had decreased to 2.8 (2.5–3.6) (figure 2). Three patients were European League Against Rheumatism (EULAR) moderate responders to treatment and five were EULAR good responders. There was no correlation between the decrease in DAS28, erythrocyte sedimentation rate or C reactive protein and the change in monocyte influx into the joints at 1, 2 or 3 h after re-infusion from baseline to 14 days (see supplementary figure 2).

**Table 1 Baseline patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>TJC of 28 joints</td>
<td>4</td>
<td>6</td>
<td>25</td>
<td>16</td>
<td>12</td>
<td>23</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>SJC of 28 joints</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>20</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>VAS general disease activity</td>
<td>47</td>
<td>19</td>
<td>87</td>
<td>71</td>
<td>59</td>
<td>89</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>35</td>
<td>6</td>
<td>97</td>
<td>14</td>
<td>10</td>
<td>43</td>
<td>58</td>
<td>16</td>
</tr>
<tr>
<td>C reactive protein (mg/l)</td>
<td>4.6</td>
<td>7.2</td>
<td>85.6</td>
<td>5.9</td>
<td>5.1</td>
<td>17.5</td>
<td>34.7</td>
<td>3.0</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.75</td>
<td>3.58</td>
<td>7.58</td>
<td>5.91</td>
<td>5.06</td>
<td>7.82</td>
<td>6.03</td>
<td>5.3</td>
</tr>
<tr>
<td>Sex (0=male)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59</td>
<td>53</td>
<td>39</td>
<td>58</td>
<td>47</td>
<td>53</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>114</td>
<td>264</td>
<td>132</td>
<td>42</td>
<td>456</td>
<td>166</td>
<td>276</td>
<td>156</td>
</tr>
<tr>
<td>Rheumatoid factor (1=positive)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Erosive disease (1=yes)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

DAS28, disease activity score in 28 joints; ESR, erythrocyte sedimentation rate; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale.
numbers found after anti-TNF treatment. Consistent with the hypothesis that TNF blockade does not interfere with high levels of monocyte influx into the synovial compartment, numbers of peripheral blood monocytes were not increased after initiation of infliximab treatment. Of note, there is no clear difference in replacement rate for monocytes compared to monocytes, as large numbers of monocytes traffic into the synovial fluid. In addition, the difference might be explained in part by the differential use of adhesion molecules between monocytes and monocytes. Very late antigen-4/vascular cell adhesion molecule-1 (VLA-4/VCAM-1) dependent rolling is seen mostly in monocytes, whereas adhesion of monocytes is very much dependent on lymphocyte function-associated antigen 1/intercellular adhesion molecule-1. The reduced serum levels of sICAM-1 but not sVCAM-1 (reflecting expression of adhesion molecules by endothelial cells) after administration of infliximab could perhaps explain the sustained influx of monocytes while neutrophil influx is reduced.

In contrast to sVCAM-1 levels in peripheral blood, VCAM-1 expression in the synovial tissue is reduced after anti-TNF treatment. VCAM-1 is especially highly expressed by fibroblast-like synoviocytes, and may play a crucial role in retention and survival of infiltrating monocytes/macrophages. Similarly, ICAM-1 expression is decreased in the tissue after TNF blockade. Thus, decreased expression of adhesion molecules as well as chemokines in the tissue may facilitate macrophage egress from the synovium, resulting in decreased macrophage numbers in the synovial tissue. In line with increased cell egress after anti-TNF treatment, it was found that anti-TNF therapy results in increased lymphatic vessel formation in the synovium.

Taken together, these data suggest that at this early time point the anti-inflammatory effect of treatment with TNF blockers can only partly be explained by downregulation of vascular adhesion molecules on endothelial cells and subsequent reduction in migration of inflammatory cells into the joint. Anti-TNF treatment may diminish RA disease activity by decreasing inflammatory cell retention in the synovial tissue, for example, by reducing integrins expressed by fibroblast-like synoviocytes. Accordingly, treatments aimed at integrins such as anti-LFA-1 (efalizumab) and anti-VLA-4 were shown to be effective treatment in inflammatory diseases, although serious infectious side effects were reported.

In conclusion, this study indicates that treatment with adalimumab does not reduce the influx of monocytes into the synovium 2 weeks after the start of treatment. In previous work early reduction in synovial inflammation after TNF blockade could not be explained by apoptosis induction at the site of inflammation. Based on these studies, it is hypothesised that efflux of inflammatory cells is a major contributor to the rapid reduction of cellularity after initiation of anti-TNF antibody therapy.

Acknowledgements The authors thank the clinical team of the Division of Clinical Immunology and Rheumatology, and Nuclear Medicine and the staff of the Laboratory for Stem Cell Transplantation at Sanquin Research for their assistance.

Funding This study was funded by Millennium: The Takeda Oncology Company.

Competing interests Millennium: The Takeda Oncology Company participated in the design of the study, monitored the clinical study and participated in the analysis and interpretation of the clinical study.

Ethics approval This study was conducted with the approval of the Academic Medical Center/University of Amsterdam.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

Monocyte migration to the synovium in rheumatoid arthritis patients treated with adalimumab

M M J Herenius, R M Thurlings, C A Wijbrandts, et al.

*Ann Rheum Dis* 2011 70: 1160-1162 originally published online February 22, 2011
doi: 10.1136/ard.2010.141549

Updated information and services can be found at:
http://ard.bmj.com/content/70/6/1160.full.html

*These include:*

**Data Supplement**
"Web Only Data"
http://ard.bmj.com/content/suppl/2011/01/27/ard.2010.141549.DC1.html

**References**
This article cites 21 articles, 6 of which can be accessed free at:
http://ard.bmj.com/content/70/6/1160.full.html#ref-list-1

**Open Access**
This paper is freely available online under the BMJ Journals unlocked scheme, see http://ard.bmj.com/info/unlocked.dtl

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Open access (263 articles)
- Immunology (including allergy) (3373 articles)
- Connective tissue disease (2872 articles)
- Degenerative joint disease (3125 articles)
- Musculoskeletal syndromes (3362 articles)
- Rheumatoid arthritis (2164 articles)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/