Transcriptomic profiling of osteoarthritis synovial macrophages reveals a tolerized phenotype compounded by a weak corticosteroid response

Cheng Wang¹,*, Ruben De Francesco¹,²,*, Lieke A. Lamers¹, Sybren Rinzema³, Siebren Frölich³, Peter L.E.M. van Lent², Colin Logie¹,*,# and Martijn H.J. van den Bosch²,*,#

¹. Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, the Netherlands.
². Experimental Rheumatology, Department of Rheumatology, Radboud university medical center, Nijmegen, the Netherlands
³. Department of Molecular Developmental Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, the Netherlands.

*These authors contributed equally; *corresponding authors; $present address: Plant System Physiology, Faculty of Science, Radboud University

Cheng Wang ORCID: 0000-0001-7886-2143
Siebren Frölich ORCID: 0000-0001-6925-8446
Colin Logie ORCID: 0000-0002-8534-6582
Martijn van den Bosch ORCID: 0000-0001-8074-826X

Corresponding authors information:

Martijn H.J. van den Bosch
Radboud university medical center, PO Box 9101, 6500HB, Nijmegen, the Netherlands
Email: martijn.vandenbosch@radboudumc.nl

Colin Logie
Radboud University, PO Box 9101, 6500HB Nijmegen, the Netherlands
Email: c.logie@science.ru.nl
Abstract

Objectives: It is well-known that long-term osteoarthritis prognosis is not improved by corticosteroid treatments. Here we investigate what could underlie this phenomenon by measuring the short term corticosteroid response of OA-Mf.

Methods: We determined the genome-wide transcriptomic response to corticosteroids of end-stage osteoarthritic joint synovial macrophages (OA-Mf). This was compared with LPS-tolerized and β-glucan-trained circulating blood monocyte-derived macrophage models.

Results: Upon corticosteroid stimulation, the trained and tolerized macrophages significantly alter the abundance of 201 and 257 RNA transcripts, respectively. By contrast, by the same criteria, OA-Mf have a very restricted corticosteroid response of only 12 RNA transcripts. Furthermore, while metalloproteinases 1, -2, -3 and -10 expression clearly distinguish OA-Mf from both the tolerized and trained macrophage models, OA-Mf Interleukin 1 (IL1), chemokine (CXCL) and cytokine (CCL) family member profiles resemble the tolerized macrophage model, with the exception that OA-Mf show high levels of CCL20.

Conclusion: Terminal osteoarthritis joints therefore harbor macrophages with an inflammatory state that closely resembles the tolerized macrophage state and this is compounded by a weak corticosteroid response capacity that may explain the lack of positive long-term effects of corticosteroid treatment for osteoarthritis patients.

Key messages

1. Corticosteroids work well to reduce pain, but fail to ameliorate long-term disease outcome in osteoarthritis.
2. Osteoarthritis macrophages show an inflammatory state that closely resembles LPS-tolerized macrophages for relevant factors.
3. Osteoarthritis macrophages respond very weakly to corticosteroids.

Key words: Osteoarthritis, Synovial inflammation, Macrophages, Immune paralysis, Corticosteroids, Cytokines and chemokines
Introduction

Osteoarthritis (OA) is the world’s most common joint disease. Patients suffer from severe pain, joint stiffness and they experience impaired mobility. It is a highly complex and multifactorial disease, with pathological processes in bone, cartilage, ligaments, menisci, peri-articular muscle and synovium [1]. Signs of synovial inflammation, characterized by hyperplasia, fibrosis and neo-angiogenesis, are seen in the majority of OA patients and are suggested as predictive factors for the progression of joint pathology [2-5]. Because no disease-curing drugs are available, current treatment options aim to suppress symptoms. For instance, synthetic corticosteroids can be injected into the articulation to suppress inflammation and relieve joint pain [6].

Previous studies have shown that CD14+ monocytes (Mo)/macrophages (Mf) are the most abundant cell type in the synovial tissue, followed by T cells and mast cells [7-10]. It is likely that disruption of normal Mo/Mf function contributes to OA progression since Mo/Mf orchestrate inflammation via the production of a plethora of cytokines, chemokines and enzymes [11]. In keeping with this concept, Mo/Mf depletion from cultures of OA synovial cells results in strongly decreased levels of various pro-inflammatory cytokines as well as of damaging matrix-metalloproteinases (MMPs) [12]. Moreover, depletion of Mf prior to induction of a preclinical OA model significantly decreases development of osteophytes and fibrosis. In addition, such depletion decreased MMP-mediated cartilage degradation, highlighting the involvement of CD14+ Mo/Mf in both the anabolic and catabolic processes that take place during OA [13, 14].

Past research mainly focused on the presence of pro-inflammatory, M1-like, and anti-inflammatory, M2-like, Mf in OA tissues [15-17]. In addition to the M1/M2 pro- or anti-inflammatory Mf polarization paradigm, a second logic emerged to model long-term macrophage polarization, namely Mf training and tolerization that respectively mimic non-specific vaccination [18, 19] and a form of Mf paralysis observed in sepsis [20].

Here we report global RNA profiles obtained in the presence and absence of pharmacological dose of the therapeutically active synthetic corticosteroid triamcinolone acetonide (TA) during four hours. To our knowledge this is the first direct transcriptomic comparison of OA-Mf obtained from synovial samples from terminal OA patient joint replacement surgery with in vitro trained and tolerized circulating blood monocyte-derived Mf.
Materials and methods

Samples

Blood donors gave written informed consent for epigenetic research (NVT0068.01). Disclosed post-operative acute OA sample metadata are as follows: Patient 1: 40 years old, Male, Hip; Patient 2: 79 years old, Male, Hip; Patient 3: 69 years old, Female, Hip; Patient 4: 69 years old, Female, Knee; Patient 5: 72 years old, Female, Hip. All experiments used protocols approved by the Sanquin Ethical Advisory Council, as previously described [21].

Cell purification and culture

Monocytes were purified from healthy blood donor buffy coats as described previously [21]. Monocyte-derived Mf were differentiated for 6 days in RPMI1640 medium with 10% human serum. Training and tolerization involved exposure to LPS (5 ng/ml), or β-glucan (10 μg/ml) during the first 24 hours of monocyte differentiation [19]. Synovial tissue from end-stage OA patients was obtained after joint replacement surgery. Post-operative biopsies from patients with end-stage OA were handed over to the Experimental Rheumatology laboratory of the RIMLS. The synovial tissue was separated from muscle, fat and ligament tissue and cultured overnight in RPMI1640 with 10% FCS at 37°C to let cells from a vascular origin leach out of the synovial tissue. The next day, the synovial tissue was first cut into small pieces and then digested using plain RPMI medium containing a final concentration of 0.1 mg/ml DNase I (Roche, 11284932001), 1 mg/ml Collagenase D (Roche, 11088858001) and 2.4 mg/ml Dispase II (Sigma, D4693-1G) on a roller at 37°C for an hour. Afterwards, a single-cell suspension was obtained by pipetting up and down to mechanically break down the tissue. Next, the suspension was washed and run through a 70 µm cell strainer, after which the digestion reaction was stopped by adding pure FCS. Erythrocytes were removed using lysis buffer (155 mM NH₄Cl, 12mM KHCO₃ and 0.1 mM EDTA) and the reaction was later stopped by PBS addition. To isolate CD14⁺ cells, MACS buffer (PBS, 1.5% acid citrate dextrose-A, 1% FCS) and CD14-coated microbeads were added to the sample, which was then run through a MACS (Magnetic-Activated Cell Sorting) column. After five washing steps, CD14⁺ cells were eluted following the manufacturer’s instructions and 200,000 obtained synovial cells were resuspended per 6 cm dish in RPMI medium supplemented with 10% FCS. Treatment with a final concentration of 1 µM TA (triamcinolone acetonide, Sigma, T6501) or only DMSO solvent (0.1% final concentration) lasted 4 hours for all cell types. As a positive control for the TA treatment, PBMC from a healthy blood donation buffy coat were exposed to TA under the same culture conditions as the isolated CD14⁺ OA-Mf using the same set of reagents and equipment.

Data files for all the BG-, LPS and OA-Mf RNA-seq samples are available in the NCBI Gene Expression Omnibus (GEO) series GSE124928.
**RNA isolation and sequencing**

RNA isolation and RNA sequencing libraries from the circulating blood Mo-derived Mf (BG-Mf, LPS-Mf) were generated as described previously [21]. For each OA-Mf sample, approximately $2.1 \times 10^5$ cells were lysed in 0.15 ml Trizol for RNA isolation. OA-Mf RNA-seq libraries were prepared using the KAPA RNA HyperPrep Kit with RiboErase (HMR) (Kapa Biosystems). Libraries were prepared according to the manufacturer’s protocol, with some adaptations. A total of 30 ng RNA was used as input material for library construction. RNA fragmentation was performed for 6 minutes at 94°C. For adapter ligation, 5 µL of 1.5 µM NEXTFLEX adapters (Bioo Scientific) was used. For OA-Mf library amplification, 15 PCR cycles were applied. Library amplification was followed by a 0.8X and a 1.0X KAPA bead-based PCR product clean-up and size selection step. Library concentration was measured using the dsDNA High Sensitivity Assay (DeNovix) and the average library fragment size was determined using the High Sensitivity DNA Chip on a Bioanalyzer 2100 (Agilent). Paired-end sequencing of the libraries was performed on an Illumina NextSeq500 to yield approximately 20 million reads per sample. Data files for the BG-, LPS and OA-Mf samples are available in the NCBI Gene Expression Omnibus (GEO) series GSE124928.

**RNA-seq data processing**

Pre-processing of reads was performed with seq2science v0.1.0 [22]. Genome assembly hg19 was downloaded from UCSC with genomepy v0.8.3 [23]. Paired-end reads were trimmed with trim galore! v0.6.4 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and cutadapt [24] with default options. Reads were aligned with hisat2 v2.1.0 [25]. Mapped reads were removed if they did not have a minimum mapping quality of 44, were a (secondary) multimapper or aligned inside the ENCODE blacklist [26]. Afterwards, duplicate reads were marked with picard MarkDuplicates v2.21.2 (http://broadinstitute.github.io/picard). Read counting and summarizing to gene-level was performed on filtered bam files using featureCounts v2.0.1 [27]. For quality control, fastq quality was measured by FastQC v0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). General alignment statistics were collected by samtools stats v1.10 [28]. Quality control metrics were aggregated by MultiQC v1.9 [29]. For visualization, bigwig files were generated, as previously described [21, 30]. Transcript abundance is expressed as transcripts per million transcripts values as computed with Salmon [31]. Raw data can be found in Supplementary Table S1, available at *Rheumatology* online.
**Bioinformatic analyses**

Differential gene expression analysis was performed using DESeq2 v1.26.0 [32]. Raw data can be found in Supplementary Table S2, available at *Rheumatology* online. The analysis compared the five OA-Mf samples exposed to triamcinolone acetonide to those exposed to DMSO vehicle. Batch correction was applied by including the patient identifiers as the first covariate in the design formula. To adjust for multiple testing the Benjamini-Hochberg procedure was performed with an FDR cutoff of 0.1. Counts were log transformed using the apeglm [33] shrinkage estimator. Only genes with a baseMean expression level of 20 or higher (N= 12,468 in BG-Mf, 10,810 in LPS-Mf and 14,354 OA-Mf, data for Figure 1) were considered. Genes exhibiting a fold-change of at least two-fold and an adjusted P-value below 0.05 were labelled as dynamic. For Figures 2 and 4, these restrictions were not applied so as to survey the entire human IL1, CCL, CXCL and MMP families. Of note, dropping out one OA patient at a time, even patient 3, did not result in the identification of more significantly TA responsive genes, indicating that none of the patient samples individually compromised the statistical power for differential gene expression detection.

**Results**

OA-Mf were purified from synovium tissue of five end-stage OA patients undergoing surgical hip or knee joint replacements. In parallel, we employed previously established protocols to generate Mo-derived Mf with a long-term trained or tolerized phenotype, which we call, BG-Mf and LPS-Mf because they were respectively exposed to β-glucans and LPS during their first day of a six-day in vitro Mo/Mf differentiation protocol [19, 20]. To investigate corticosteroid responses, the cells were split into one sample that was exposed to the synthetic corticosteroid triamcinolone acetonide (TA) for 4 hours and one that was only exposed to its DMSO solvent.

**OA-Mf present a globally paralyzed TA response**

Plotting significance versus fold-change in so-called volcano plots reveals that TA best induces genes in BG-Mf while in LPS-Mf the repressive response is strongest, even though LPS-Mf show the most extensive TA response, with 257 significantly (2-fold change, Padj<0.05) impacted genes against 201 in BG-Mf (Figure 1A-B). By contrast, by the same criteria, the global TA response of OA-Mf is very poor, with only 12 induced RNA transcripts, of which five represent non-coding RNA transcripts emanating from genomic glucocorticoid receptor binding sites that we had identified previously in monocytes and unstimulated macrophages [21] (Table 1). Also, the significantly induced protein-coding mRNAs of OA-Mf are associated with genomic glucocorticoid receptor binding sites [21] indicating that these represent *bona fide* direct transcriptional targets of the ligand-activated glucocorticoid receptor in OA-Mf. We conclude that OA-Mf show a weakened response to corticosteroid stimulation.
Corticosteroids very weakly suppress inflammatory signaling molecule mRNAs in OA-Mf

Only one mRNA was significantly repressed (> 2-fold, P-adj < 0.05) by TA in OA-Mf, namely \textit{IL36RN}. This gene is located within the IL1 cluster on chromosome 2q14.1 together with the 10 other human interleukin 1 cytokine family members (Figure 2). \textit{IL36RN} tends to be co-regulated with its neighbors \textit{IL36G} and \textit{IL1RN}, which are ranked seventh and thirteenth of the most down-regulated mRNAs in TA-treated OA-Mf; respectively with mRNA level reductions of 1.7 and 1.6-fold (Figure 2A).

Notably, the tolerized LPS-Mf and OA-Mf much resemble each other when the IL1 cluster is considered as a whole, because both express more \textit{IL1B} than the dominant negative \textit{IL1RN} family member, whereas the converse is true in trained BG-Mf (Figure 2). However, contrary to OA-Mf, LPS-Mf efficiently suppress the levels of mRNAs for \textit{IL1B} 19-fold and \textit{IL1A} 16-fold when exposed to triamcinolone for 4 hours. The same picture of strong resemblance between LPS-Mf and OA-Mf, compounded by a weak corticosteroid response by OA-Mf, emerges when visualizing the C-C motif chemokine ligand (CCL) and CXCL chemo-attractants gene families, except that OA-Mf express \textit{CCL20} much more than LPS-Mf (Figure 2).

Altogether, this indicates that OA-Mf present an immunological state that is related to the memory of inflammation in LPS-Mf. However, in contrast to the \textit{in-vitro} monocyte-derived tolerized LPS-Mf, OA-Mf fail to efficiently suppress the mRNA levels of these pro-inflammatory genes upon TA exposure, further confirming that OA-Mf have a weak corticosteroid response.

Global expression patterns of LPS-inducible genes distinguish OA-Mf from trained and tolerized cells

The above analyses focused on the expression patterns of three major secreted pro-inflammatory protein families and revealed a striking similarity between tolerized and OA-Mf. To discover whether this extends to all the genes induced by LPS, we stimulated BG-Mf and LPS-Mf for 4 hours with LPS and/or TA and compiled a list of 1206 LPS-induced genes (two-fold induced, Padj<0.05), of which 690 are significantly affected in the trained BG-Mf, 190 in tolerized LPS-Mf and 326 in both Mf types.

Next we clustered our experimental samples at the hand of the expression patterns of these LPS-inducible genes. This reveals that OA-Mf have a distinct pro-inflammatory mRNA expression profile from either Mo-derived Mf models (Figure 3, Supplementary Figure S1, available at Rheumatology online). The same picture emerges when a short list of key genes that distinguish Mf states is used (Supplementary Figure S2, available at Rheumatology online), further underscoring that the OA-Mf are distinct from Mf derived \textit{in vitro} from healthy circulating blood monocytes.
We conclude that although OA-Mf closely resemble LPS-Mf but not trained BG-Mf when focusing on key secreted interleukin, cytokine and chemokine gene families (Figure 2), globally, the OA-Mf transcriptomes very reproducibly cluster together and apart from the *in vitro* derived Mf models.

Of note, the above analyses brought to light that our third LPS-Mf sample had failed to become tolerized, since it clusters away from the two other tolerized LPS-Mf samples and shows a very weak response to the 4-hour LPS treatment administered on day 6. We therefore elected *post-hoc* to not take the results from that sample along in our differential gene expression analyses, including Figures 1-3 above.

**MMP gene family analysis highlights a unique expression pattern by OA-Mf**

MMPs are key proteases involved in the breakdown of extracellular matrix, which is a main hallmark of OA pathology. Therefore, we investigated the MMP gene family in our datasets. This revealed that BG-Mf and LPS-Mf mainly express *MMP7, -9, -14* and *-19* mRNAs. On the other hand, OA-Mf showed particularly strong expression of *MMP1, -2, -3* and *-10* as well as expressing *MMP9, -14, and -19* (Figure 4).

This indicates that OA-Mf have an MMP expression profile that is consistent with a Mf cell identity, since they do express *MMP9, -14* and *-19*. However, OA-Mf are distinct from both Mo-derived LPS-Mf and BG-Mf as they also express high levels of *MMP1, -2, -3* and *-10* which are not expressed well by the healthy circulating blood monocyte-derived tolerized and trained Mf model cells.

**Gene-specific integration of the LPS and corticosteroid responses in tolerized and trained Mf**

To address whether the largely paralyzed corticosteroid response we observe in OA-Mf might have been caused by an acutely activated M1-like response during OA-Mf purification that in turn would have switched off their corticosteroid response, we exposed BG-Mf and LPS-Mf to solvent vehicle, TA, LPS or TA and LPS together for 4 hours. This revealed that the corticosteroid response does take place when LPS inflammatory signaling is stimulated at the same time, both in tolerized and trained Mf (Figure 5A). In fact, the combined treatment could be rather accurately predicted by the single TA and LPS treatments when focusing on 238 genes that are significantly affected by both LPS and TA individually (Figure 5B-C) whether or not LPS and the corticosteroid had concordant or opposing influences. We therefore conclude that the LPS and corticosteroid signals are integrated independently at individual Mf genes, rendering a generic scenario of global inhibition of TA responsiveness by acute proinflammatory LPS signaling unlikely.
Discussion

OA patients are often treated with high-dose (40 mg) intra-articular injections of the synthetic corticosteroid triamcinolone acetonide [34]. Whereas this works well to reduce pain, it is only effective for periods of about 12 weeks and does not ameliorate long-term prognosis [34, 35].

We recently reported that freshly isolated healthy circulating blood monocytes regulate the mRNA levels of more than 1000 genes at least two-fold within 4 hours of exposure to triamcinolone acetonide [21]. By contrast, six days later, Mo-derived Mf show a more modest response encompassing only 165 genes [21]. Notably, almost identical results were obtained when applying dexamethasone or prednisone [21].

Here we report that the beta-glucan-trained and LPS-tolerized Mf models [19, 20] display quantitatively distinct TA responses, indicating that corticosteroid responses are dependent on Mf polarization states. Furthermore, we show that the corticosteroid response is not inhibited by Toll-like receptor stimulation via LPS. The concept that each individual gene independently integrates corticosteroid and inflammatory signaling is in keeping with results reported by Oh et al [36] in mice.

Strikingly, we found that freshly isolated OA-Mf only weakly respond to corticosteroids, even though a handful of relevant genes is specifically induced in these cells (Table 1). It would be of interest to directly measure the impact of a pro-inflammatory treatment on the corticosteroid response of postsurgical synovial OA-Mf. However, the synovial OA CD14+ cell samples we studied may well be quite heterogenous in their CD14+ cell (sub)type composition, with different cell (sub)types potentially displaying different kinds of corticosteroid and/or pro-inflammatory responses. Resolving these two linked questions (number of responding cells, type of responding cell) will require applying single cell technologies that permit quantification of thousands of genes in thousands of individual cells. Only then, will it be possible to interpret LPS or S100A9 co-exposure experiments with corticosteroids.

The weak induction of anti-inflammatory glucocorticoid receptor target genes by OA-Mf may explain why corticosteroid treatment has no beneficial effects for OA patients in the long term. We therefore speculate that short term symptom alleviation by corticosteroids in OA patients might be brought about by effects on other synovial cell types than CD14+ macrophages, although this remains to be established.
Interestingly, the synovial OA-Mf closely resemble the tolerized model Mf by a number of criteria, namely the mRNA expression patterns for the interleukin 1 family, CCL cytokines and CXCL chemokines, all of which are much more expressed by tolerized than by trained Mf. A specific example is IL8 (CXCL8); it is the most highly expressed protein-coding gene in OA-Mf and it is the second best expressed gene in tolerized Mf, while IL8 only ranks at position 526 in the trained Mf transcriptome. Intriguingly, the IL8 CXCL chemokine has been reported to promote neoangiogenesis [2], which is one of the hallmarks of advanced stage OA.

How OA-Mf acquire their tolerized phenotype is an open question, although TLR4-stimulation via S100A9 [37] may tolerize these cells in a similar fashion as LPS-stimulated TLR4 in our tolerized Mf model.

In keeping with previous reports, we found that OA-Mf express high levels of MMP1, -3 and -10 as well as CCL20 [12, 38, 39]. Hence, despite the striking resemblances in IL1, CCL cytokine and CXCL chemokine family member expression patterns between OA-Mf and tolerized Mf, the OA-Mf differ from the tolerized Mf in many aspects.

Our results suggest that seeking to restore the corticosteroid response of OA-Mf might be a key to enable more effective long-term treatment of pain and swelling symptoms in patients suffering from osteoarthritis. However, at this stage, we cannot formally exclude the possibility that past exposures of the OA patients to synthetic corticosteroid therapy or other drugs underlies the weak TA responses we observed in OA-Mf. Currently, treatments including corticosteroid injections are not given in the 3-6 months prior to surgery and beneficial corticosteroid effects are of short duration. We therefore consider past synthetic corticosteroid exposure unlikely to have affected our results but this remains an open question.

Intriguingly, because we know that LPS-induced Mf tolerization can be reversed by β-glucans ex-vivo through epigenetic reprogramming [20], the present results do raise the question as to whether it will be possible to epigenetically manipulate and even reverse the tolerized-like inflammatory phenotype of synovial OA-Mf in situ by other means than through synthetic corticosteroid treatments.
Disclosure statement: The authors have declared no conflicts of interest.

Contributors: PVL, CL, MVDB conceived and designed the experiments. CW and RDF performed the experiments. CW, RDF, LL, SR and SF analysed the data. CW and MVDB drafted the manuscript. All authors thoroughly reviewed the manuscript.

Acknowledgments: The authors would like to thank Simon van Heeringen for access to his databases and expertise.

Funding: Lieke Lamers is supported by the Oncode Institute, which is partly funded by the Dutch Cancer Society (KWF). Cheng Wang is supported by a PhD scholarship from the China Scholarship Council (CSC) number 201306740062. Martijn van den Bosch is funded by the ZonMW/VENI research program (project number 09150161810015), which is financed by the Dutch Research Council (NWO).

Data availability: Data files for all the BG-, LPS and OA-Mf RNA-seq samples are available in the NCBI Gene Expression Omnibus (GEO) series GSE124928.

Funding sources: Lieke Lamers is supported by the Oncode Institute, which is partly funded by the Dutch Cancer Society (KWF). Cheng Wang is supported by a PhD scholarship from the China Scholarship Council (CSC) number 201306740062. Martijn van den Bosch is funded by the ZonMW/VENI research program (project number 09150161810015), which is financed by the Dutch Research Council (NWO).
Figure legends

Figure 1. Global corticosteroid response of trained and tolerized Mo-derived Mf and of synovial OA-Mf. (A) Volcano plots comparing the extent and significance of induction and repression of mRNA levels upon a 4-hour exposure to TA in trained (BG-Mf) and tolerized (LPS-Mf) macrophages as well as synovial OA-Mf. (B) Venn Diagrams indicating the overlaps of the TA responses of BG-, LPS- and OA-Mf as a function of the number of dynamic genes that changed at least 2-fold with an adjusted p-value below 0.05.

Figure 2. IL1, CCL and CXCL gene clusters display tolerized expression patterns in OA-Mf. RNA expression signal for all the human family members of the IL1 interleukins and intercrine CCL and CXCL cytokines organized per chromosome cluster. Levels are indicated for LPS-Mf (green), BG-Mf (orange) and OA-Mf (black). The presence of TA for 4 hours is indicated by a lighter color shade. Units are transcripts per million (tpm) projected on a log_{10} scale.

Figure 3. OA-Mf display a unique and reproducible expression pattern of LPS-inducible genes. Hierarchically clustering of the reported Mf cells using 1206 protein-coding genes that are inducible by LPS in monocyte-derived Mf (Padj < 0.05, FC > 2). Note that these analyses indicates that the third LPS-Mf samples were not properly tolerized by LPS on day 1 and barely responded to LPS on day 6, leading to rejection from our analyses.

Figure 4. Matrix Metalloproteinase (MMP) family expression patterns. RNA expression signal for all the human members of the MMP family. Levels are indicated for LPS-Mf (green), BG-Mf (orange) and OA-Mf (black). The presence of TA for 4 hours is indicated by a lighter color shade. Units are transcripts per million (tpm).

Figure 5. Independent integration of corticosteroid and LPS-induced gene expression. (A) Heatmap displaying row-normalized expression values for 457 TA-responsive macrophage genes (this work and [21]) for 6 clusters of TA responding genes, namely down (top three clusters) or up (bottom three clusters) and common (blue and orange) or specific to BG-Mf (red purple) and LPS-Mf (yellow and green). Note that LPS-MF3 samples (labeled as donor 6 in this analysis) are ‘passengers samples’ as they were not used to generate the TA-responsive gene clusters. (B and C) Scatter plots comparing observed outcomes of 4-hour TA and LPS combined treatments with outcomes predicted from the single treatments. The dots represent 238 human genes. Axes are log_{2} fold-change.
References


12. Bondeson J, Wainwright SD, Lauder S, Amos N, Hughes CE. The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases,


Table 1. All significantly affected transcripts in TA-treated OA-Mf.

<table>
<thead>
<tr>
<th>Genetic feature</th>
<th>Biotype</th>
<th>Relative expression</th>
<th>Log2(FoldChange)</th>
<th>P-adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL36RN</td>
<td>mRNA</td>
<td>69</td>
<td>-1.36</td>
<td>1.51E-06</td>
</tr>
<tr>
<td>RHOH</td>
<td>mRNA</td>
<td>177</td>
<td>1.69</td>
<td>9.98E-21</td>
</tr>
<tr>
<td>RP11-184M15.1</td>
<td>GR eRNA chr4q28.2</td>
<td>48</td>
<td>1.66</td>
<td>1.11E-06</td>
</tr>
<tr>
<td>SLAMF1</td>
<td>mRNA</td>
<td>338</td>
<td>1.66</td>
<td>3.60E-38</td>
</tr>
<tr>
<td>RP11-95M15.2</td>
<td>GR eRNA chr6q23.3</td>
<td>68</td>
<td>1.66</td>
<td>4.33E-11</td>
</tr>
<tr>
<td>AL031666.2</td>
<td>GR eRNA chr20q13.12</td>
<td>86</td>
<td>1.51</td>
<td>1.76E-11</td>
</tr>
<tr>
<td>LPL</td>
<td>mRNA</td>
<td>1403</td>
<td>1.47</td>
<td>8.48E-25</td>
</tr>
<tr>
<td>RP11-243J16.7</td>
<td>GR eRNA chr20q11.21</td>
<td>25</td>
<td>1.39</td>
<td>0.00285</td>
</tr>
<tr>
<td>RNF175*</td>
<td>mRNA TLR2 3’end</td>
<td>120</td>
<td>1.29</td>
<td>6.58E-11</td>
</tr>
<tr>
<td>FKBPS</td>
<td>mRNA</td>
<td>1313</td>
<td>1.28</td>
<td>1.58E-05</td>
</tr>
<tr>
<td>AC145110.1</td>
<td>GR eRNA chr8p12</td>
<td>56</td>
<td>1.24</td>
<td>4.34E-05</td>
</tr>
<tr>
<td>SMIM3</td>
<td>mRNA</td>
<td>131</td>
<td>1.02</td>
<td>1.37E-05</td>
</tr>
<tr>
<td>TSC22D3</td>
<td>mRNA</td>
<td>5129</td>
<td>0.92*</td>
<td>0.00012</td>
</tr>
</tbody>
</table>

GR eRNA denotes enhancer RNAs that do not code for a protein and originate from glucocorticoid receptor-bound enhancers as reported previously [21]. Note that *RNF175 is in fact bleed-through mRNA signal from the TLR2 RNA 3’UTR which is itself induced via a proximal enhancer bound by GR in Mf. TLR2 is on position 48 (ranked by FC, minimum baseMean expression of 20) of patient TA-induced RNAs with a Log2(FC) of 0.56 (~1.5 fold)). *TSC22D3 is on position 15 of patient TA-induced RNAs with a Log2(FC) of 0.92 (~1.9 fold) and is shown here as a positive control gene.
Figure 1. Global corticosteroid response of trained and tolerized Mo-derived Mf and of synovial OA-Mf. (A) Volcano plots comparing the extent and significance of induction and repression of mRNA levels upon a 4-hour exposure to TA in trained (BG-Mf) and tolerized (LPS-Mf) macrophages as well as synovial OA-Mf. (B) Venn Diagrams indicating the overlaps of the TA responses of BG-, LPS- and OA-Mf as a function of the number of dynamic genes that changed at least 2-fold with an adjusted p-value below 0.05.
Figure 2. IL1, CCL and CXCL gene clusters display tolerized expression patterns in OA-Mf. RNA expression signal for all the human family members of the IL1 interleukins and intercrine CCL and CXCL cytokines organized per chromosome cluster. Levels are indicated for LPS-Mf (green), BG-Mf (orange) and OA-Mf (black). The presence of TA for 4 hours is indicated by a lighter color shade. Units are transcripts per million (tpm) projected on a log10 scale.

95x127mm (300 x 300 DPI)
Figure 3. OA-Mf display a unique and reproducible expression pattern of LPS-inducible genes. Hierarchically clustering of the reported Mf cells using 1206 protein-coding genes that are inducible by LPS in monocyte-derived Mf (Padj < 0.05, FC > 2). Note that these analyses indicates that the third LPS-Mf samples were not properly tolerized by LPS on day 1 and barely responded to LPS on day 6, leading to rejection from our analyses.
Figure 4. Matrix Metalloproteinase (MMP) family expression patterns. RNA expression signal for all the human members of the MMP family. Levels are indicated for LPS-Mf (green), BG-Mf (orange) and OA-Mf (black). The presence of TA for 4 hours is indicated by a lighter color shade. Units are transcripts per million (tpm).
Figure 5. Independent integration of corticosteroid and LPS-induced gene expression. (A) Heatmap displaying row-normalized expression values for 457 TA-responsive macrophage genes (this work and [21]) for 6 clusters of TA responding genes, namely down (top three clusters) or, up (bottom three clusters) and common (blue and orange) or specific to BG-Mf (red purple) and LPS-Mf (yellow and green). Note that LPS-Mf3 samples (labeled as donor 6 in this analysis) are ‘passengers samples’ as they were not used to generate the TA-responsive gene clusters. (B and C) Scatter plots comparing observed outcomes of 4-hour TA and LPS combined treatments with outcomes predicted from the single treatments. The dots represent 238 human genes. Axes are log2 fold-change.

95x127mm (300 x 300 DPI)