Basic and translational research

EXTENDED REPORT

Adiponectin isoforms: a potential therapeutic target in rheumatoid arthritis?

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ABSTRACT

Objectives Several clinical studies have suggested the adipocytokine adiponectin is involved in the progression of rheumatoid arthritis (RA). From this point of view, adiponectin might present a new therapeutic target. However, as adiponectin also exerts beneficial effects in the human organism, a strategy that would allow its detrimental effects to be abolished while maintaining the positive effects would be highly favourable. To elucidate such a strategy, the authors analysed whether the different adiponectin isoforms induce diverging effects, especially with regard to rheumatoid arthritis synovial fibroblasts (RASF), a central cell type in RA pathogenesis capable of invading into and destroying cartilage.

Methods Affymetrix microarrays were used to screen for changes in gene expression of RASF. Messenger RNA levels were quantified by real-time PCR, protein levels by immunoassay. The migration of RASF and primary human lymphocytes was analysed using a two-chamber migration assay.

Results In RASF, the individual adiponectin isoforms induced numerous genes/proteins relevant in RA pathogenesis to clearly different extents. In general, the most potent isoforms were the high molecular weight/middle molecular weight isoforms and the globular isoform, while the least potent isoform was the adiponectin trimer. The chemokines secreted by RASF upon adiponectin stimulation resulted in an increased migration of RASF and lymphocytes.

Conclusion The results clearly suggest a pro-inflammatory and joint-destructive role of all adiponectin isoforms in RA pathophysiology, indicating that in chronic inflammatory joint diseases the detrimental effects outweigh the beneficial effects of adiponectin.

With 1% prevalence worldwide, rheumatoid arthritis (RA) is a common form of arthritis that, although the onset of RA is more frequent later in life, can affect people at any age. Without adequate treatment, this severe chronic inflammatory joint disease inevitably causes loss of articular function and mobility. Even though effective therapeutics are now available against the progression of the disease, additional therapeutic options are still needed when current therapies fail or cause severe adverse effects. This is where the so-called adipocytokines may come into play.

The major source of adipocytokines is adipose tissue. It has now become evident that adipose tissue is not merely an immunologically inactive type of connective tissue but also an important immunoendocrine organ producing hormones and cytokines.[1–3] These factors have been collectively termed adipocytokines or, in short, adipokines. Adiponectin, leptin, resistin and visfatin are just a few examples of this growing number of highly bioactive substances with metabolic and immunological functions.[4 5]

Pathologically, adipokines appear to be involved in numerous chronic inflammatory diseases. This not only includes RA but also systemic lupus erythematosus, ankylosing spondylitis and systemic sclerosis.[6]

Synovial hyperplasia accompanied by substantial inflammation and degradation of joints[7] is a key feature of RA, and rheumatoid arthritis synovial fibroblasts (RASF) are a major player in these destructive processes.[8–10] This RA-specific cell type therefore presents a promising target for therapeutic intervention. For that reason, we investigated the effects of the adipocytokine adiponectin on RASF in order to find out how this may affect the pathogenesis of RA.

Adiponectin, a C1q/tumour necrosis factor (TNF) homologue,[11] lent itself to this question as its synovial fluid levels are significantly increased in RA patients compared with osteoarthritis patients as well as healthy controls,[12 13] and hyperadiponectinemia is associated with an increased incidence of joint destruction[14] or radiographic progression[15 16] in RA patients. Of note, adiponectin is not only produced by adipose tissue but also by synovial fibroblasts, endothelial cells, osteoblasts and cardiac myocytes.[17–19] In a previous study,[20] we were able to show that native adiponectin affects several RA effector cells.

Interestingly, adiponectin is not a homogenous entity but consists of several isoforms corresponding to different oligomers with a 'bouquet of flower' structure. Trimeric adiponectin, also called low molecular weight adiponectin, is composed of three full-length adiponectin monomers forming a collagen triple helix with a C-terminal globular gC1q domain (head domain).[21] Globular adiponectin consists of the head domain of trimeric adiponectin as a result of proteolytic cleavage.[22–24] The adiponectin hexamer, the so-called middle molecular weight (MMW) adiponectin, is a combination of two trimeric adiponectin molecules, while an assembly of 12–18 monomers is collectively termed high molecular weight

Table 1 Affymetrix microarray results: differential gene induction in RASF by adiponectin isoforms

Gene name	Gene symbol	Fold change native Ad	Fold change Ad HMW/MMW	Fold change Ad trimer	Fold change gAd
Chemokines					
Chemokine (C-C motif) ligand 2 (CCL2)	MCP-1	4.8	4.7	3.4	5.8
Chemokine (C-C motif) ligand 5 (CCL5)	RANTES	24.0	24.7	3.8	73.2
Chemokine (C-C motif) ligand 7 (CCL7)	MCP-3	101.3	74.9	28.0	254.0
Chemokine (C-C motif) ligand 8 (CCL8)	MCP-2	25.8	11.2	3.5	297.3
Chemokine (C-C motif) ligand 20 (CCL20)	MIP-3α	1424.0	1547.0	82.9	728.3
Chemokine (C-X-C motif) ligand 1 (CXCL1)	GRO-α	29.4	33.3	17.7	32.8
Chemokine (C-X-C motif) ligand 2 (CXCL2)	GRO-β	33.5	43.9	12.6	19.7
Chemokine (C-X-C motif) ligand 3 (CXCL3)	GRO-γ	99.6	234.0	34.6	60.2
Chemokine (C-X-C motif) ligand 5 (CXCL5)	ENA-78	37.8	121.7	9.1	12.6
Chemokine (C-X-C motif) ligand 6 (CXCL6)	GCP-2	5.5	5.6	3.7	4.1
Chemokine (C-X-C motif) ligand 8 (CXCL8)	IL-8	43.4	50.5	28.2	47.6
Chemokine (C-X-C motif) ligand 9 (CXCL9)	MIG	9.3	36.7	−3.1	48.9
Chemokine (C-X-C motif) ligand 10 (CXCL10)	IP-10	161.0	78.6	2.1	1225.0
Chemokine (C-X-C motif) ligand 11 (CXCL11)	I-TAC	130.9	88.0	−4.4	728.3
Cytokines					
Interleukin 6	IL-6	6.4	6.5	6.6	8.9
Interleukin 11	IL-11	24.3	441.0	157.4	2.3
Other inflammatory molecules					
Prostaglandin E synthase	PTGES	3.4	4.6	2.4	9.4
Prostaglandin endoperoxide synthase 2/cyclooxygenase 2	PTGS2/COX2	19.9	17.9	7.1	26.1
Pre-B cell growth and B cell activation					
Bone marrow stromal cell antigen 2	BST2	200.8	100.4	28.0	1091.0
Receptors					
Interleukin 7 receptor	IL-7R	5.6	6.9	3.0	8.9
Interleukin 17 receptor B	IL-17RB	2.6	2.1	1.0	2.8
Proteinases and peptidases					
Matrix metallopeptidase 1 (interstitial collagenase)	MMP1	11.6	13.6	5.2	12.4
Matrix metallopeptidase 3 (stromelysin 1 progelatinase)	MMP3	62.5	115.6	36.3	16.2
Matrix metallopeptidase 10 (stromelysin 2)	MMP10	88.7	599.9	94.4	17.0
Matrix metallopeptidase 12 (macrophage elastase)	MMP12	49.9	222.7	10.1	12.6
Bone metabolism					
Stanniocalcin 1	STC1	19.8	23.4	14.5	24.6
Growth factors					
Fibroblast growth factor 10	FGF10	5.0	5.6	1.0	3.9

RASF (n=1) were treated with adiponectin isoforms or left untreated (control). Affymetrix microarray analysis (GeneChip HG U133A) was performed as described. The results indicate that genes from several functional groups are differentially regulated in RASF by adiponectin isoforms. The cut-off value for fold changes was ≤2 (repression) or ≥2 (induction), respectively. Strongly repressed genes were low in number and of no known or well-defined function.
Ad, adiponectin; gAd, globular adiponectin; HMW, high molecular weight; MMW, middle molecular weight; RASF, rheumatoid arthritis synovial fibroblasts.

(HMW) adiponectin.[21] Even though some studies have investigated selected adiponectin isoforms,[21] [25–28] no studies have yet analysed the potentially differential effects of adiponectin isoforms on effector cells involved in the pathophysiology of RA.

With adiponectin's important functions in energy metabolism and beneficial effects on the cardiovascular system,[29] [30] it might be unadvisable to modulate adiponectin levels systemically in order to prevent its disease-promoting effects in RA. Instead, inhibiting adiponectin locally at sites of joint destruction or targeting specific isoforms could be viable options. Therefore, in this study we investigated whether adiponectin isoforms differentially affect gene expression and protein secretion of RASF, and could thus provide targets for specifically inhibiting the detrimental effects of adiponectin while preserving its beneficial effects. As rheumatoid synovium is strongly infiltrated by lymphocytes and migrating RASF, which can additionally invade the synovium and cartilage,[8] we also analysed whether the factors induced by the different adiponectin isoforms in RASF have chemoattractive properties on RASF and lymphocytes.

MATERIALS AND METHODS
Cell culture
Human primary synovial fibroblasts and primary lymphocytes were cultured as described in the supplementary material (available online only).
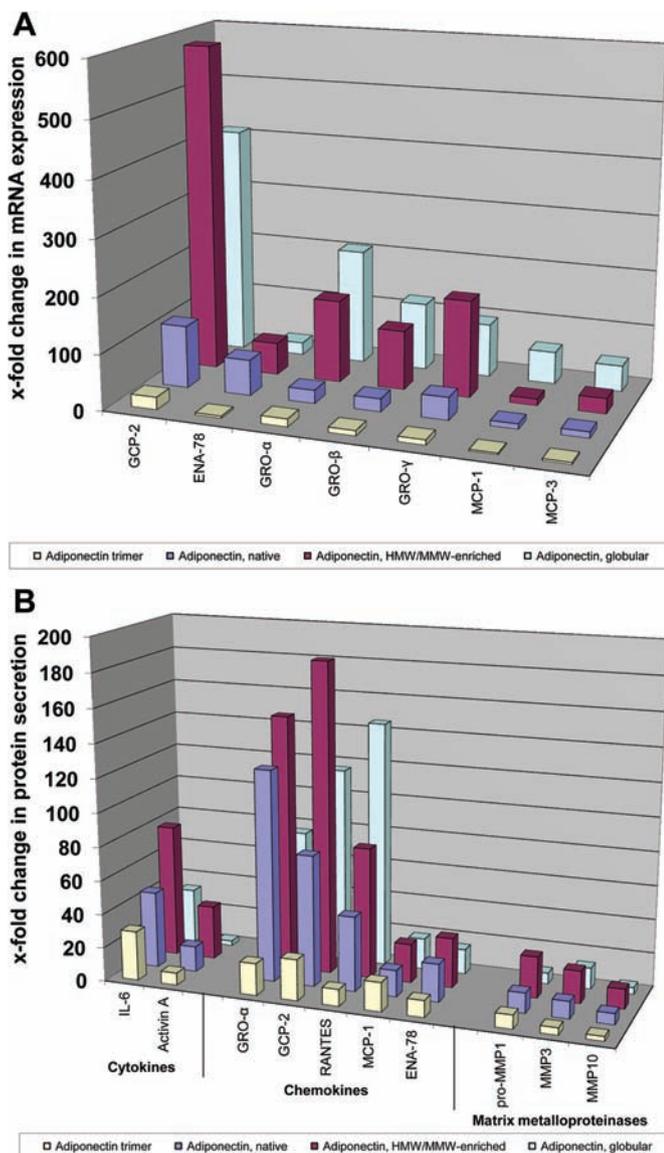
Isolation of synovial fibroblasts
Synovial tissue samples were obtained from synovial biopsy specimens from RA and osteoarthritis patients who were undergoing joint surgery. All specimens were obtained with the approval of the Ethics Committee of the Justus-Liebig-University of Giessen. All patients gave informed consent and fulfilled the criteria of the American College of Rheumatology.[31] [32] Following enzymatic digestion,[33] [34] primary synovial fibroblasts were isolated and cultured in supplemented Dulbecco's modified Eagle's medium as described previously.[20]

Isolation of lymphocytes from human whole blood
Lymphocytes were isolated by Ficoll-based density gradient centrifugation as described in more detail in the supplementary material (available online only).

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Figure 1 (A) Differential induction of chemokine mRNA expression in rheumatoid arthritis synovial fibroblasts (RASF) by adiponectin (Ad) isoforms (real-time PCR data). Multiple RASF populations (n=4) were stimulated with adiponectin isoforms or left unstimulated (control). X-fold changes in mRNA expression of several chemokines were determined by real-time PCR and are shown (as arithmetic mean) in comparison for all adiponectin isoforms used for stimulation. (B) Differential induction of cytokine, chemokine and matrix metalloproteinase (MMP) secretion by RASF upon stimulation with adiponectin isoforms. Multiple RASF populations (n=4–15, see table 3) were stimulated with adiponectin isoforms or left unstimulated (control). X-fold changes in protein secretion of cytokines, chemokines and MMP were determined by ELISA and are shown (as arithmetic mean) in comparison for all adiponectin isoforms used for stimulation. HMW, high molecular weight; MMW, middle molecular weight.

Stimulation of RASF and OASF

RASF and osteoarthritis synovial fibroblasts (OASF) from passages 3–8 were grown to 70–80% confluency and stimulated with 25 µg/ml of different human adiponectin forms (BioVendor, Heidelberg, Germany) for 15 h: native adiponectin (a mixture of different adiponectin isoforms; recombinantly produced in HEK 293 cells); HMW/MMW-enriched adiponectin (recombinantly produced in HEK 293 cells); trimeric adiponectin (recombinantly

produced in HEK 293 cells; prevented from further oligomerisation by a single amino acid mutation) and globular adiponectin (recombinantly produced in *Eschericia coli*). Sodium dodecylsulphate polyacrylamide gel electrophoresis analysis images of the commercially available adiponectin preparations, which were used, are shown in supplementary figure S1 (available online only). The stimulation time was chosen based on preliminary experiments that demonstrated optimal response after 15 h.[18] Unstimulated RASF and OASF were used as negative controls. Dose-response analyses were performed previously[18] and showed that the induction of interleukin (IL)-6 and pro-matrix metalloproteinase (MMP) 1 by adiponectin does not reach a plateau until a concentration of approximately 100 µg/ml. We additionally showed that potential lipopolysaccharide contaminations of recombinant adiponectin were not responsible for the effects observed after stimulation.[20]

Affymetrix gene chips

RASF (passage 5; n=1) were stimulated for 15 h with 25 µg/ml of the different adiponectin isoforms as described above. Affymetrix (Santa Clara, CA, USA) microarray analysis was performed as described in the supplementary material (available online only).

Real-time PCR

Reverse transcription of RNA and real-time PCR were performed as described in the supplementary material (available online only).

Immunoassays

The cytokine, chemokine, MMP and adiponectin levels in cell culture supernatants were measured using commercially available ELISA (R&D Systems, Wiesbaden, Germany).

Two-chamber migration assay

Media from adiponectin-stimulated RASF were analysed for their chemoattractive potential on RASF and lymphocytes using a two-chamber migration system. The procedure is described in detail in the supplementary material (available online only).

Statistical analysis

Biological or experimental replicates were used to calculate arithmetic means and standard errors of the mean (SEM). Data are presented as the mean±SEM. In order to assess the significance of differences, a Student's two-tailed t test was performed for pairwise comparisons. For multiple comparisons, analysis of variance including Tukey's post-hoc test was performed. p Values less than 0.05 were considered significant. Statistical calculations were performed using Microsoft Excel and GraphPad Prism.

RESULTS

Differential induction of chemokines in RASF by adiponectin isoforms

RASF are an RA-specific cell type capable of driving inflammation and joint destruction,[9] of invading into cartilage,[35] and of migrating from joint to joint.[8] Inhibiting their destructive activity is a desirable goal in RA therapy. Factors that promote or inhibit this activity are thus of substantial interest as potential therapeutic targets. We therefore analysed the effects of the different adiponectin isoforms on RASF gene expression, focusing on finding out whether there are differences in the effects of the adiponectin isoforms and to what degree each isoform might be involved in RA pathogenesis.

Table 2 Differentially induced chemokine mRNA expression in RASF by adiponectin isoforms

Gene name	Symbol	Ad Isoform	Fold change	SEM	n	p Value
Chemokine (C-X-C motif) ligand 1 (CXCL1)	GRO-α	Native	23.9	8.9	4	0.036
		HMW/MMW	148.0	85.4	4	0.009
		Trimer	14.1	8.1	4	0.021
		Globular	206.8	134.9	4	0.009
Chemokine (C-X-C motif) ligand 1 (CXCL2)	GRO-β	Native	23.3	8.0	4	0.054
		HMW/MMW	106.2	57.5	4	0.008
		Trimer	9.3	4.5	4	0.021
		Globular	121.1	68.8	4	0.008
Chemokine (C-X-C motif) ligand 1 (CXCL3)	GRO-γ	Native	66.3	50.8	4	0.020
		HMW/MMW	77.7	36.7	4	0.005
		Trimer	4.9	1.9	4	0.072
		Globular	33.3	13.4	4	0.008
Chemokine (C-X-C motif) ligand 5 (CXCL5)	ENA-78	Native	70.8	41.9	4	0.006
		HMW/MMW	57.7	24.7	4	0.002
		Trimer	3.8	0.6	4	0.002
		Globular	22.2	8.4	4	0.054
Chemokine (C-X-C motif) ligand 6 (CXCL6)	GCP-2	Native	112.0	45.7	4	0.019
		HMW/MMW	584.6	327.7	4	0.003
		Trimer	22.3	14.8	4	0.025
		Globular	410.2	233.7	4	0.003
Chemokine (C-C motif) ligand 2 (CCL2)	MCP-1	Native	7.2	1.9	4	0.008
		HMW/MMW	11.9	3.0	4	0.005
		Trimer	2.7	0.5	4	0.013
		Globular	57.9	20.2	4	0.005
Chemokine (C-C motif) ligand 7 (CCL7)	MCP-3	Native	16.3	9.1	4	0.015
		HMW/MMW	17.8	7.9	4	0.007
		Trimer	3.9	2.4	4	0.151
		Globular	56.1	38.1	4	0.077

Multiple populations of RASF (n=4) were stimulated with different adiponectin isoforms. RNA was isolated, reverse transcribed to complementary DNA and quantified by real-time PCR. Fold changes in mRNA expression (as compared to an unstimulated control), biological variability indicated by the (SEM), number of populations analysed (n), and the p values are presented.
Ad, adiponectin; HMW, high molecular weight; MMW, middle molecular weight; RASF, rheumatoid arthritis synovial fibroblasts.

First, Affymetrix microarray analysis (GeneChip HG U133A) was performed in order to compare the gene expression of RASF stimulated with the different adiponectin isoforms or RASF left unstimulated. As large amounts of messenger RNA are required for Affymetrix microarrays and patient material was limited, one RASF population (n=1) was analysed exemplarily in this experiment to screen for changes in gene expression. The variability of different RASF populations was later accounted for by verifying selected results with higher n numbers. Chemokines were the largest group of dysregulated genes and were differentially induced by the adiponectin isoforms (table 1). Verification of selected chemokines (GRO-α/-β/-γ, ENA-78, GCP-2, MCP-1, MCP-3) by real-time PCR confirmed the differential induction of mRNA expression in multiple RASF populations (table 2 and figure 1A). Using immunoassays, we confirmed that chemokine secretion (GRO-α, ENA-78, GCP-2, IL-8, MCP-1, RANTES) was also differentially regulated by the individual adiponectin isoforms (table 3 and figure 1B). I-TAC (CXCL11) and MIP-3α (CCL20) protein, however, could not be detected in either cell culture supernatants or cell lysates (data not shown). In particular, within the real-time PCR and immunoassay results, we could identify a distinct pattern regarding the effect of the different adiponectin isoforms on RASF; overall, HMW/MMW-enriched and globular adiponectin were the most potent isoforms, while the adiponectin trimer was the least effective. Native adiponectin, which has not been enriched for any isoform, mostly held a middle ground but was rather variable in its potency depending on the regulated gene or protein. These observations are illustrated in figure 1.

Differential induction of cytokines, MMP and other RA-related genes in RASF by adiponectin isoforms

Not only chemokines, but also pro-inflammatory cytokines, MMP and inflammation-related enzymes play a major role in RA pathogenesis. Their regulation is therefore crucial.

Our results showed that cytokines, MMP and other RA-related molecules were also regulated to very different extents depending on the particular adiponectin isoform (tables 1 and 3, figure 1B). For example, secretion of the pro-inflammatory cytokine IL-6 was most strongly induced by HMW/MMW-enriched adiponectin, while the weakest response was seen with trimeric adiponectin. Similar differential inductions by the individual adiponectin isoforms could be observed for the inflammation-related enzyme cyclooxygenase 2 (COX2) as well as the MMP 1, 3, 10 and 12.

Biological variability of RASF cell populations in response to adiponectin stimulation

Different RASF cell populations, ie, synovial fibroblasts obtained from different RA patients, showed highly variable responses to stimulation with adiponectin. Adiponectin upregulated gene expression or protein secretion in all cell populations that were analysed but to very different extents, which is illustrated in figure 2A.

Response of OASF to adiponectin stimulation in comparison with RASF

Synovial fibroblasts from RA patients and osteoarthritis patients responded similarly to stimulation with adiponectin isoforms, but OASF generally showed a weaker mean response than RASF,

Table 3 Differentially induced cytokine, chemokine and MMP secretion in RASF by adiponectin isoforms

Protein name	Symbol	Ad isoform	Fold change	SEM	n	p Value
Chemokines						
Chemokine (C-X-C motif) ligand 1 (CXCL1)	GRO-α	Native	125.1	43.0	9	0.024
		HMW/MMW	150.9	44.8	5	0.023
		Trimer	19.0	12.5	5	0.223
		Globular	75.8	26.4	5	0.027
Chemokine (C-X-C motif) ligand 5 (CXCL5)	ENA-78	Native	22.5	6.2	13	0.005
		HMW/MMW	29.5	5.4	8	0.001
		Trimer	9.9	3.9	8	0.056
		Globular	14.8	5.2	8	0.033
Chemokine (C-X-C motif) ligand 6 (CXCL6)	GCP-2	Native	58.5	23.1	15	0.026
		HMW/MMW	164.1	36.4	14	0.001
		Trimer	22.8	6.1	14	0.003
		Globular	110.3	35.8	14	0.009
Chemokine (C-X-C motif) ligand 8 (CXCL8)	IL-8	Native	611.3	193.2	14	0.008
		HMW/MMW	953.2	275.0	14	0.004
		Trimer	135.6	50.8	14	0.020
		Globular	570.5	223.1	14	0.024
Chemokine (C-C motif) ligand 2 (CCL2)	MCP-1	Native	15.8	3.6	13	0.001
		HMW/MMW	23.0	4.15	8	0.001
		Trimer	17.2	7.9	8	0.081
		Globular	18.5	6.9	8	0.040
Chemokine (C-C motif) ligand 5 (CCL5)	RANTES	Native	44.4	25.1	8	0.127
		HMW/MMW	77.5	17.8	8	0.004
		Trimer	10.0	7.8	8	0.381
		Globular	145.3	35.6	8	0.005
Cytokines						
Activin A	INHBA	Native	15.1	6.0	9	0.047
		HMW/MMW	32.0	9.8	4	0.099
		Trimer	7.3	2.2	4	0.065
		Globular	2.8	1.2	4	0.212
Interleukin 6	IL-6	Native	31.2	9.7	12	0.010
		HMW/MMW	57.0	16.9	12	0.007
		Trimer	20.7	7.1	12	0.019
		Globular	26.8	9.9	12	0.024
Proteinases & Peptidases						
Matrix metallopeptidase 1, propeptide	pro-MMP1	Native	11.9	8.1	13	0.206
		HMW/MMW	24.4	15.9	13	0.014
		Trimer	8.2	5.1	13	0.185
		Globular	6.5	1.8	13	0.028
Matrix metallopeptidase 3	MMP3	Native	10.3	3.5	10	0.025
		HMW/MMW	19.2	4.1	8	0.001
		Trimer	4.3	1.5	8	0.063
		Globular	12.6	3.8	8	0.014
Matrix metallopeptidase 10	MMP10	Native	4.4	1.1	11	0.009
		HMW/MMW	11.6	2.1	11	0.002
		Trimer	2.7	0.5	11	0.006
		Globular	4.7	1.5	11	0.043

Multiple populations of RASF (n=4–15) were stimulated with different adiponectin isoforms. Secreted chemokines, cytokines and matrix metalloproteinases were quantified by immunoassays. Fold changes of secretion (as compared to an unstimulated control), biological variability indicated by the (SEM), number of populations analysed (n), and the p values are presented.

Ad, adiponectin; HMW, high molecular weight; MMP, matrix metalloproteinases; MMW, middle molecular weight; RASF, rheumatoid arthritis synovial fibroblasts.

demonstrating the special phenotype of RASF (figure 2B). However, due to the high biological variability of the cell populations, statistical significance for the differences between RASF and OASF responses could not be reached in most cases. Although the differences in the response towards the different adiponectin isoforms were not as prominent as for RASF, differences could also be detected for OASF.

Chemoattractive effect of adiponectin-induced factors on RASF and lymphocytes

As outlined above, adiponectin isoforms induced numerous chemokines. We therefore investigated to what extent this leads to a functional chemoattractive effect on RASF and lymphocytes, two key cell types in RA. A two-chamber migration assay was performed with RASF and primary human lymphocytes. Conditioned media from RASF cultures incubated with the different adiponectin isoforms were used as potential chemoattractants against medium from unstimulated RASF incubated in parallel. RASF were allowed to migrate for 15 h, lymphocytes for 4 h. Cells that actively passed the membrane of the two-chamber migration system were counted. The gradient-free baseline was set to 100%.

Here, we observed an increased migration for RASF and lymphocytes towards conditioned medium from adiponectin-stimulated RASF, indicating that the adiponectin-induced factors have a significant chemoattractive effect on RASF (n=3)
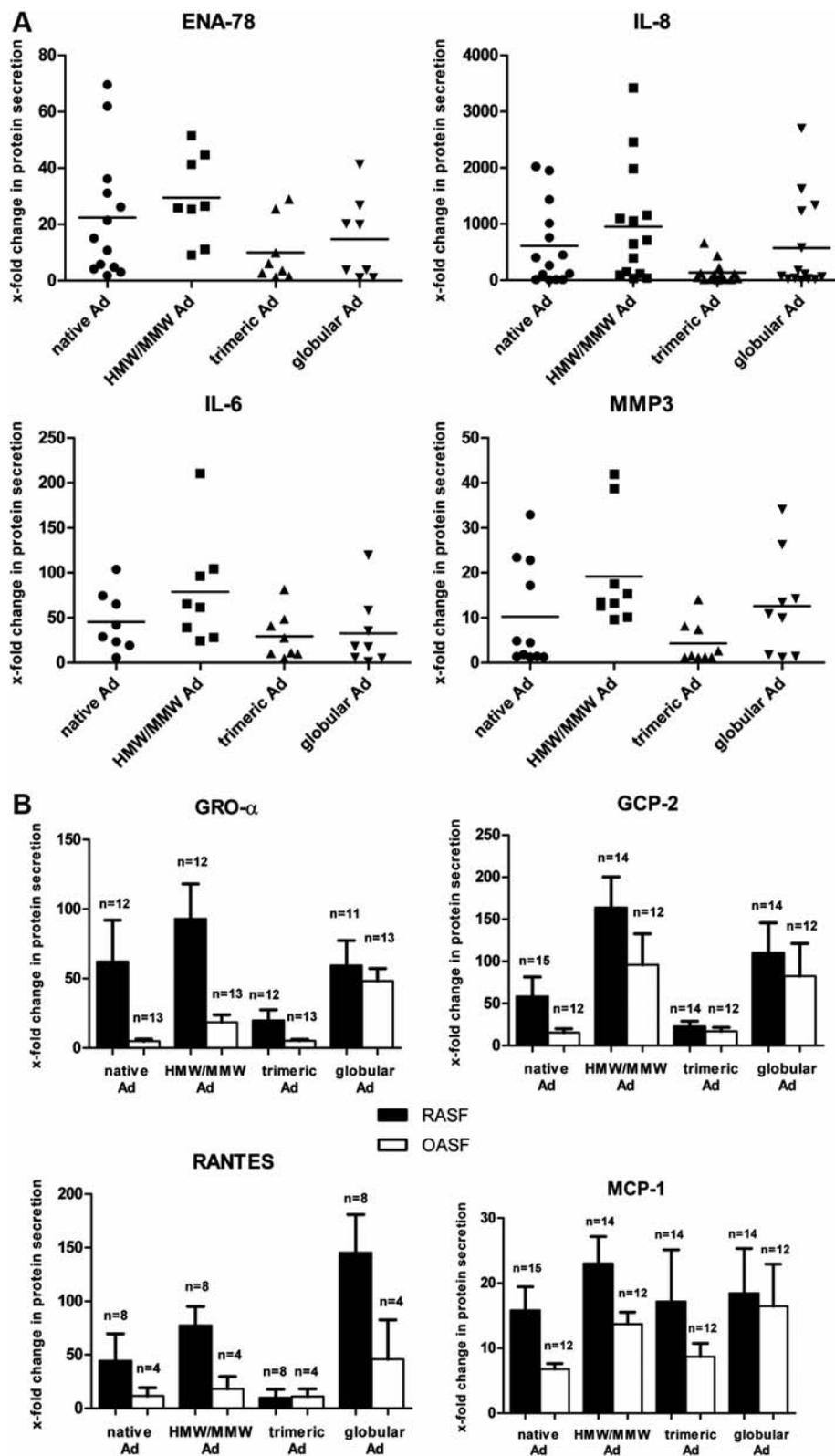
Figure 2 (A) Biological variability of rheumatoid arthritis synovial fibroblasts (RASF) cell populations in response to adiponectin (Ad) stimulation. Chemokine (ENA-78 and IL-8), cytokine (IL-6) and matrix metalloproteinase (MMP3) secretion was quantified by ELISA after adiponectin stimulation of cultured RASF from different rheumatoid arthritis (RA) patients. To illustrate the biological variability of RASF populations in response to adiponectin stimulation, the individual results (x-fold changes in protein secretion) are shown as dots. The arithmetic mean is displayed as a bar. (B) Response of osteoarthritis synovial fibroblasts (OASF) to adiponectin stimulation in comparison with RASF. Multiple OASF populations (n=4 for GRO-α; n=12 for GCP-2; n=4 for RANTES; n=12 for MCP-1/native adiponectin and n=8 for MCP-1/other adiponectins) were stimulated with adiponectin isoforms in parallel with multiple RASF populations (see table 3 for n numbers). Chemokine secretion was quantified by ELISA. Black bars indicate x-fold changes in protein secretion for RASF, while white bars indicate x-fold changes in protein secretion for OASF (each compared with unstimulated controls). Data are shown as the mean±SEM. HMW, high molecular weight; MMW, middle molecular weight.
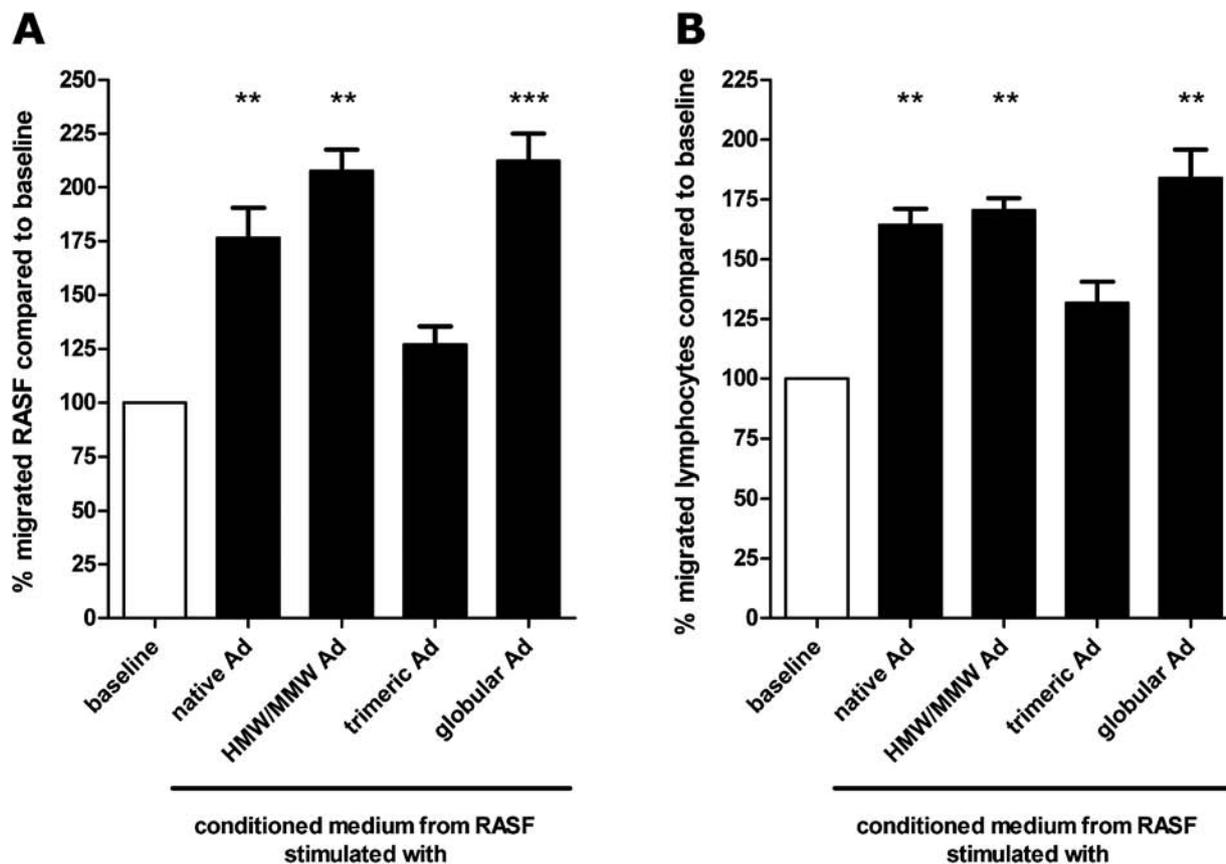
Figure 3 (A) Chemoattractive effect of adiponectin (Ad)-induced factors on rheumatoid arthritis synovial fibroblasts (RASF). Medium from RASF stimulated with different adiponectin isoforms was used in a two-chamber migration assay as a chemoattractant for RASF (n=3). The baseline (without chemotactic gradient) was set to 100%. Migration of RASF is expressed relative to the baseline. Using serum-free medium as a chemorepellent decreased RASF migration to 38%, while using 10% fetal calf serum medium as a positive control increased RASF migration by 278% (data not shown). (B) Chemoattractive effect of adiponectin-induced factors on lymphocytes. Medium from RASF stimulated with different adiponectin isoforms was used in a two-chamber migration assay as a chemoattractant for lymphocyte (n=3) and analysed as described above. Using serum-free medium as a chemorepellent decreased lymphocyte migration to 35%, while using RANTES (10 ng/ml) plus SDF (Stromal Cell-Derived Factor) (100 ng/ml) as a positive control increased lymphocyte migration by 345% (data not shown). Data are shown as the mean±SEM. *p<0.05; **p<0.01; ***p<0.001. HMW, high molecular weight; MMW, middle molecular weight.

(figure 3A) and lymphocytes (n=3) (figure 3B). Additional controls with adiponectin (25 μg/ml) added just before the start of the migration assay showed that adiponectin itself does not have any chemoattractive properties on the cell types analysed (data not shown).

In summary, factors induced by adiponectin isoforms had a differential effect on RASF and lymphocyte migration, thus reflecting the individual effects of the respective adiponectin isoforms on protein secretion by RASF.

DISCUSSION

The primary objective of this study was to investigate if the different isoforms of the adipokine adiponectin have differential effects on RASF, a key cell type in RA pathogenesis. Previous data[14–16 20] have suggested that adiponectin may be rather detrimental in RA and involved in disease progression. However, as available data have indicated that adiponectin is beneficial for metabolic and cardiovascular health,[29 30] systemic elimination in order to avoid the harmful effects in RA might not be a favourable option. Based on initial data,[27 28] researchers concluded that mainly HMW adiponectin is responsible for the vascular-protective effects of adiponectin. On the other hand, available data have suggested that adiponectin promotes RA progression[14–16] and does this most likely by inducing the secretion

of pro-inflammatory molecules (eg, IL-6, COX-2), chemokines (eg, IL-8, MCP-1) and matrix-degrading enzymes (eg, MMP3).[20] Adiponectin is thus able to mount and sustain a pro-inflammatory response in various pathophysiologically relevant cell types in RA and osteoarthritis, including chondrocytes[20 36 37] and RASF,[20] both of which share the common characteristics of mesenchymal-derived cells.

These results led to the hypothesis that inhibition of specific adiponectin isoforms might help circumvent the problem of reducing the harmful effects of adiponectin in RA while maintaining its beneficial effects. However, our results showed that even though the individual adiponectin isoforms have different potencies to modulate gene expression of RASF they do not have opposing effects or no effect at all in the setting of RA pathophysiology. Nonetheless, our results suggest that certain isoforms of adiponectin are more detrimental in RA than others. Therefore, when considering adiponectin as a progression or activity marker for RA, it may be best to look at the most potent isoforms.

With regard to functional aspects of adiponectin isoforms, we were able to show that adiponectin-induced factors promote the migration of RASF and lymphocytes in vitro, which in vivo may lead to increased synovial lymphocyte infiltration and additional influx of RASF to sites of inflammation and cartilage

degradation. Inhibition of these processes by blocking the local effects of specific adiponectin isoforms within the joints could therefore lead to reduced disease progression and activity.

Another interesting observation was the high variability of RASF in response to adiponectin isoform stimulation, which may be attributed to different genetic profiles[38 39] as well as epigenetic variations between RASF populations.[40] This is also in line with clinical findings showing that there are considerable differences in how RA patients respond to the different available medications. RASF possess a special phenotype reflected not only in their ability to migrate and invade into cartilage,[8] but also in their ability to respond to external stimuli such as adiponectin, which was illustrated here by the weaker response of OASF to adiponectin compared with RASF.

When considering strategies for modulating the effects of adiponectin, there are other conceivable options besides modulating adiponectin itself: targeting adiponectin receptors[41–43] or co-receptors,[44–47] and inhibiting the oligomerisation of adiponectin isoforms by small molecule inhibitors that prevent the assembly into higher molecular weight isoforms.

With respect to animal models, the viability of adiponectin knock-out mice indicates that, at least in mice, adiponectin is not vital, but results regarding the effects of adiponectin knock-out or overexpression in vivo are controversial. While Shinoda et al[48] found no abnormalities regarding bone mass and turnover in Ad⁻/Ad⁻ mice, Williams et al[49] as well as Oshima et al[50] found an increased bone density. Conversely, adiponectin overexpressing mice had increased bone mass, parameters of bone resorption and bone erosion were not affected.[51] Contrary to what we would have expected based on our results, adenovirus-mediated systemic expression of human adiponectin in collagen-induced arthritis mice reduced clinical disease activity scores of collagen-induced arthritis.[52] Most likely, this result reflects the distinct phenotype of human RASF and the difference between human and murine arthritides.

Several groups also analysed the overexpression or knockdown of adiponectin in mouse models in the metabolic and vascular context.[53–57] Under special nutritional conditions (high-fat and/or high-glucose diet) or on an obesity background (ob/ob), antidiabetic and anti-atherogenic properties were observed for the overexpression of adiponectin, while adiponectin knockout resulted in insulin resistance and impaired glucose metabolism. Therefore, it is always important to consider the experimental environment when looking at the in-vivo effects of adiponectin.

Also, as yet nothing is known about the role of adiponectin isoforms in mice, their occurrence and distribution. It therefore remains questionable to what extent the existing adiponectin knock-out mouse models are able to provide hints on how adiponectin isoform deprivation would affect human RA.

In conclusion, while adiponectin may present an interesting therapeutic target in RA, more research is required to elucidate whether adiponectin isoforms can be targeted specifically and respective inhibitors can be used to provide new therapeutic approaches. Nonetheless, the clearly different potencies of adiponectin isoforms in RA suggest that considering the isoforms may be of value when utilising adiponectin as a marker for risk, activity or progression of RA.

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Adiponectin isoforms: a potential therapeutic target in rheumatoid arthritis?

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