

The Adipokine Omentin in Late-stage Rheumatoid Arthritis and Endstage Osteoarthritis

To the Editor:

In the past several years, adipokines have gained a lot of attention in the field of rheumatic diseases¹. Rheumatoid arthritis (RA), for example, is associated with an altered production of adipokines¹, and there is some evidence of involvement of adipokines in the pathophysiology of RA as suggested by *in vitro*¹ and *in vivo* data^{2,3}. The term “adipokines” was originally used to describe cytokine-like factors secreted by adipose tissue, but it turned out that many adipokines are also produced at other sites including the joints^{1,4,5}, where they might have an influence on effector cells of RA or osteoarthritis (OA) pathophysiology.

As an adipokine, omentin was first found in omental adipose tissue from patients with Crohn disease⁶. Before this, it was identified as a secretory glycoprotein able to bind to galactofuranosyl residue on various microorganisms, suggesting a function in immune recognition of certain pathogens. It has also been described as a lactoferrin-binding protein with lactoferrin being a protein with multiple immunological functions. Omentin is highly abundant in human plasma⁶. It displayed antiinflammatory and antiatherogenic properties in obese patients⁷ and is negatively associated with inflammatory bowel disease (IBD)^{6,8} and metabolic syndrome⁹. Omentin levels in the synovial fluid (SF) of patients with RA were lower than in patients with OA⁵, while omentin serum levels were elevated in

children with juvenile idiopathic arthritis compared with healthy controls and positively associated with the presence and number of active joints¹⁰.

Based on these previous findings, we decided to examine omentin expression in the synovium of patients with RA and OA. Synovial tissue samples obtained from patients with RA (n = 12) and OA (n = 10) undergoing joint surgery were analyzed immunohistochemically with a specific antiomentin antibody to determine the synovial omentin expression pattern. Immunohistochemical staining revealed omentin expression in RA and OA synovium, especially within the lining layer and vessel walls (Figure 1), and the same expression pattern for RA (Figure 1A and Figure 1B) and OA synovium (Figure 1C and Figure 1D). This similar expression pattern may also appear because synovial biopsies were from patients with late- or endstage RA or OA.

The presence of omentin in SF⁵ and synovial tissue led us to the hypothesis that this adipokine may also be involved in the pathogenesis of RA. Therefore, we analyzed the effects of omentin on the following key cells of RA pathophysiology: human synovial fibroblasts (n = 7), macrovascular endothelial cells (EC) from varicose veins (n = 2), microvascular human umbilical artery EC (n = 1), lymphocytes and monocytes from human blood (n = 1), as well as chondrocytes from human cartilage (n = 1). Because it had previously been shown that within the joint adipokines are particularly expressed by rheumatoid arthritis synovial fibroblasts (RASf)¹, which can invade and destroy articular cartilage and subchondral bone, we focused our study on this cell type and started our analyses with RASf. All

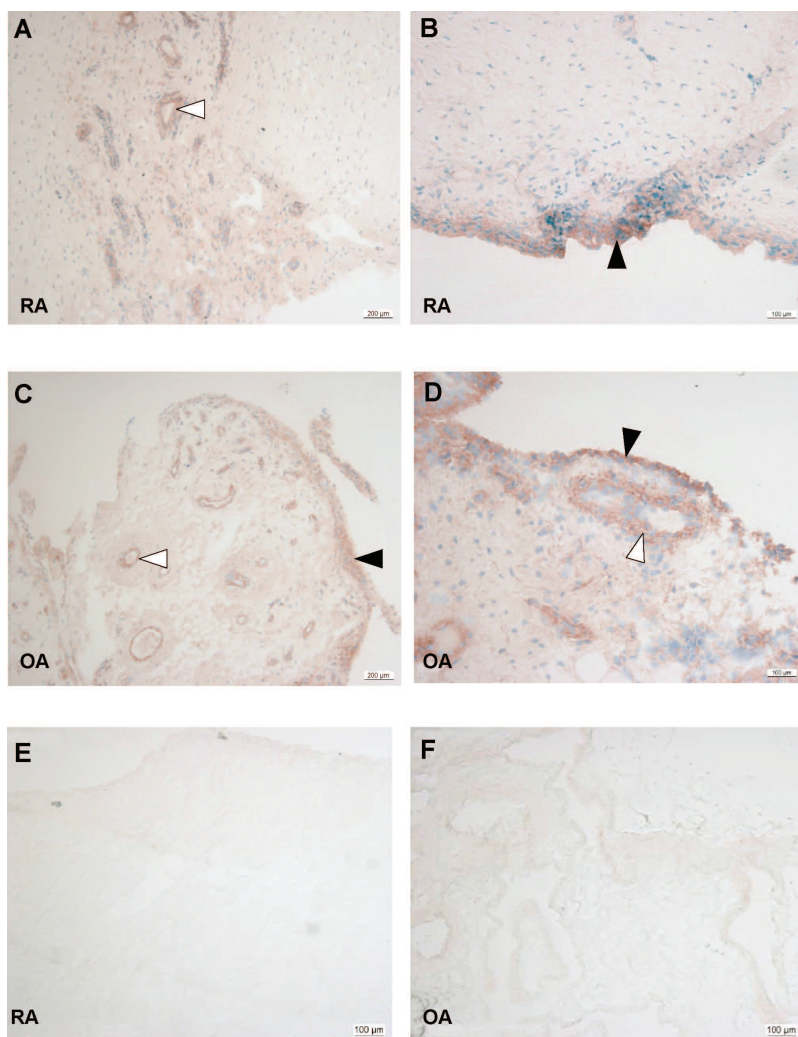


Figure 1. Expression of omentin in synovial tissue of patients with RA or OA. Immunostaining of omentin in synovial tissue was done with nuclear counterstaining (hematoxylin). Nuclei are stained in blue, and omentin is stained in red. A primary antibody isotype control is shown for RA and OA synovial tissue in (E) and (F). Expression of omentin in synovial tissue was most prominent within the synovial lining layer (black arrowheads) and perivascular (white arrowheads). RA synovial tissue (A and B) showed the same expression pattern of omentin as OA synovial tissue (C and D). RA: rheumatoid arthritis; OA: osteoarthritis.

cell types used were stimulated *in vitro* with 500 ng/ml omentin (BioVendor) for 15 h. We first screened for potential changes in gene expressions induced by omentin in RASF using Affymetrix microarrays (n = 1; human genome U133A oligonucleotide probe microarrays, Affymetrix) and antibody arrays (n = 1; Human Chemokine Antibody Array I kit, custom human cytokine antibody array, RayBiotech). The array analysis resulted in a small number of genes or proteins showing limited up/downregulation by omentin in RASF (maximum upregulation: 13.1-fold, maximum downregulation: -13.9-fold; Table 1). However, the differential expression could not be confirmed for the genes selected for verification at mRNA level by real-time PCR or at protein level by ELISA (Table 1). For the other cell types, appropriate variables were analyzed by real-time PCR or ELISA; interleukin (IL)-6 was quantified for all cell types. Additionally, the following factors were determined in selected cell types: IL-8 for lymphocytes and monocytes; tumor necrosis factor- α only for lymphocytes; vascular endothelial growth factor and vascular cell adhesion molecule 1 for EC; and aggrecan, cartilage oligomeric matrix protein, and matrix metalloproteinase 3 for chondrocytes.

Interestingly, none of these factors were changed by omentin stimulation. Of note, the small sample sizes, which were not extended because of the negative characteristic of the results, may be a limitation of our study.

Although omentin is expressed in RA and OA synovium and present in SF, it has no or limited effects on central effector cells of RA pathophysiology, in contrast to the effects observed with other adipokines^{1,4}. Our data suggest that the potentially antiinflammatory effect of omentin in diseases such as obesity⁷ and IBD⁸ does not occur in RA and OA, at least regarding the evaluated cell types.

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Table 1. Results of mRNA/protein expression analysis of omentin-stimulated RASF. RASF stimulated with 500 ng/ml omentin for 15 h and untreated controls were analyzed by Affymetrix (AFFX) oligonucleotide microarrays (GeneChip HG U133A) and protein arrays (RayBio "Custom Human Cytokine Antibody Array" and "Human Chemokine Antibody Array I"). The table shows a selection of functionally classified genes that according to the arrays were induced or repressed by omentin with fold changes of ≥ 2 (induction) or ≤ -2 (repression), respectively. For verification, selected genes/proteins were quantified by real-time PCR and ELISA.

Gene/Protein	Symbol	Fold Change AFFX	Fold Change Real-time PCR	Fold Change Protein Arrays	Fold Change ELISA
Chemokines					
Chemokine (C-C motif) ligand 3 (CCL3)	MIP-1a	-7.6	—	2.7	n.d.
Chemokine (C-C motif) ligand 7 (CCL7)	MCP-3	8.5	—	4.6	n.d.
Chemokine (C-C motif) ligand 8 (CCL8)	MCP-2	\approx	—	5.2	—
Chemokine (C-C motif) ligand 18 (CCL18)	PARC	\approx	—	4.3	—
Chemokine (C-C motif) ligand 20 (CCL20)	CCK1	\approx	—	5.0	—
Chemokine (C-X-C motif) ligand 9 (CXCL9)	MIG	\approx	—	5.5	n.d.
Chemokine (C-X-C motif) ligand 10 (CXCL10)	IP-10	-3.9	n.d.	\approx	—
Chemokine (C-X-C motif) ligand 11 (CXCL11)	I-TAC	-5.5	n.d.	\approx	—
Cytokines					
Interleukin 3	IL-3	4.5	—	\approx	—
Interleukin 11	IL-11	5.6	—	\emptyset	—
Interleukin 17C	IL-17C	3.7	—	\emptyset	—
Tumor necrosis factor- α	TNF- α	\approx	—	4.7	n.d.
Miscellaneous inflammatory molecules					
Prostaglandin endoperoxide synthase 1	PTGS1	4.8	—	\emptyset	—
Pre-B cell growth and B cell activation					
Bone marrow stromal cell antigen 2	BST2	3.0	\approx	\emptyset	—
Receptors					
Chemokine (C-C) motif receptor 9	CCR9	-6.1	—	\emptyset	—
Fibroblast growth factor receptor 3	FGFR3	3.7	—	\emptyset	—
Proteinases and peptidases					
Matrix metalloproteinase 9 (gelatinase B)	MMP9	3.2	—	3.0	—
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	3.2	—	2.5	—
Matrix metalloproteinase 14	MMP14	-4.0	—	\emptyset	—
ECM and cell surface molecules					
Collagen, type XXIV, alpha 1	COL24A1	-13.9	—	\emptyset	—
Collagen, type VII, alpha 1	COL7A1	-4.8	—	\emptyset	—
Glypican-3	GPC3	13.1	—	\emptyset	—
Bone and cartilage metabolism					
Bone morphogenetic protein 8b	BMP8B	3.0	—	\emptyset	—
Growth factors					
Endothelial cell growth factor 1 (platelet-derived)	ECGF1	5.0	—	\emptyset	—
Insulin-like growth factor 1 (somatomedin C)	IGF-1	2.0	—	10.8	n.d.
Vascular endothelial growth factor	VEGF	\approx	—	3.6	—

RASF: rheumatoid arthritis synovial fibroblasts; ECM: extracellular matrix; \approx : no change (i.e., less than ± 2 -fold change); \emptyset : not present on array; n.d.: not detectable.

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