**Visfatin/Pre-B-cell Colony-enhancing Factor (PBEF), a Proinflammatory and Cell Motility-changing Factor in Rheumatoid Arthritis**

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**Background:** The adipokine visfatin exerts proinflammatory effects in synovial fibroblasts of patients with rheumatoid arthritis (RA).

**Results:** Visfatin induces high amounts of chemokines, creating a microenvironment of enhanced fibroblast motility.

**Conclusion:** Visfatin is therefore very capable of contributing to the inflammatory state of RA.

**Significance:** Enlightening visfatin pathophysiology may lead to possible therapeutic targeting in the future.

Adipokines such as adiponectin and visfatin/pre-B-cell colony-enhancing factor (PBEF) have been recently shown to contribute to synovial inflammation in rheumatoid arthritis (RA). In this study, we evaluated the pathophysiological implication of visfatin/PBEF in the molecular patterns of RA synovial tissue, focusing on RA synovial fibroblasts (RASFs), key players in RA synovium. Expression of visfatin/PBEF in synovial fluid and tissue of RA patients was detected by immunoassays and immunohistochemistry. RASFs were stimulated with different concentrations of visfatin/PBEF over varying time intervals, and changes in gene expression were evaluated at the RNA and protein levels using Affymetrix array, real-time PCR, and immunohistochemistry. The signaling pathways involved were identified. The influence of visfatin/PBEF on fibroblast motility and migration was analyzed. In RA synovium, visfatin/PBEF was predominantly expressed in the lining layer, lymphoid aggregates, and interstitial vessels. In RASFs, visfatin/PBEF induced high amounts of chemokines such as IL-8 and MCP-1, proinflammatory cytokines such as IL-6, and matrix metalloproteinases such as MMP-3. Phosphorylation of p38 MAPK was observed after visfatin/PBEF stimulation, and inhibition of p38 MAPK showed strong reduction of visfatin-induced effects. Directed as well as general fibroblast motility was increased by visfatin/PBEF-induced factors. The results of this study indicate that visfatin/PBEF is involved in synovial fibroblast activation by triggering fibroblast motility and promoting cytokine synthesis at central sites in RA synovium.

Rheumatoid arthritis (RA) is a chronic polyarticular disease manifesting as painful inflammation of the synovial tissues and progressive destruction of the joints. Besides macrophages and T- and B-cells, synovial fibroblasts as resident cells are key players in mediating most of the relevant pathways in this process (1, 2). Activation of RA synovial fibroblasts (RASFs) in the synovium results in the production of proinflammatory cytokines and matrix metalloproteinases (MMPs). RASFs actively perpetuate inflammation as well as matrix degradation and invasion, leading to progressive destruction of the articular cartilage and the adjacent bone, thus determining the outcome of the disease (1, 3, 4).

The terms adipocytokine and adipokine are used for cytokine-like molecules synthesized by adipocytes, e.g. including adiponectin, leptin, resistin, and visfatin. There is growing evidence that adipose tissue is not only a repository for triglycerides or a passive connective tissue but, in fact, is an active endocrine organ regulating energy homeostasis and metabolism (5). Moreover, adipose tissue and obesity are connected with a low-grade state of inflammation and play a role in other chronic inflammatory disorders, including RA (6, 7).

Expression of the adipokine pre-B-cell colony-enhancing factor (PBEF) in the plasma, synovial fluid, and inflamed synovium of RA patients is elevated (8–11). In experimental settings like antigen-induced arthritis in mice, similar results have been seen (8). Thus far, it has been shown that during activation of immune cells such as macrophages, monocytes, dendritic cells, neutrophils, and T- and B-cells, visfatin/PBEF expression is increased (12–17). In turn, it has been reported that visfatin/PBEF up-regulates IL-1β, IL-1 receptor antagonist, IL-6, IL-10, and TNF-α in peripheral blood mononuclear cells and IL-1β, IL-6, and TNF-α in CD14+ monocytes (12). It is known that visfatin/PBEF induces different intracellular signaling path-
ways, e.g. AP-1 and NF-κB in RASFs (9). In CD14+ monocytes, the visfatin/PBEF-induced cytokine production can be reduced by inhibiting the p38 MAPK and MEK1 pathways (12). Given the fact that visfatin/PBEF acts additionally as the rate-limiting enzyme in the salvage pathway of NAD, recycling NAD from nicotinamide, it prevents apoptosis of neutrophils in experimental inflammation and clinical sepsis and promotes maturation of vascular smooth muscle cells, thus extending their life span (13, 18, 19).

Visfatin/PBEF was originally described as a cytokine involved in early B-cell development and was later renamed visfatin due to the fact that it is secreted mainly by visceral fat (20, 21). Recent studies showed that visfatin/PBEF is up-regulated in activated RASFs by inflammatory stimuli such as STAT-3-dependent IL-6 trans-signaling and poly(I-C)-mediated TLR-3 activation (8, 9). In turn, visfatin/PBEF induces IL-6 in RASFs, suggesting a positive feedback mechanism due to the proinflammatory activities of this protein (9). In this study, we investigated the effects of visfatin/PBEF on RASFs in detail, with the main focus specifically on the change in gene and protein expression mediated by visfatin/PBEF, signaling pathways involved in these processes, and alterations in fibroblast motility to further clarify the role of visfatin/PBEF in RA.

EXPERIMENTAL PROCEDURES

Tissue Specimens and Cell Culture

During routine synovectomies, synovial tissue was obtained from patients with RA and osteoarthritis (OA). Patients met the 1987 criteria of the American College of Rheumatology classification of RA and OA (22, 23). Written informed consent to use the synovium or synovial fluid for research purposes was obtained from each patient. The local ethics committee of the Justus Liebig University Giessen approved the study. RASFs were cultured as described recently (24–26). During routine synovectomies, synovial tissue was obtained from patients with RA and osteoarthritis (OA). Patients met the 1987 criteria of the American College of Rheumatology classification of RA and OA (22, 23). Written informed consent to use the synovium or synovial fluid for research purposes was obtained from each patient. The local ethics committee of the Justus Liebig University Giessen approved the study. RASFs were cultured as described recently (24–26). The synovial fluid of RA patients with articular effusion was aspirated. Primary human lymphocytes were isolated by Ficoll gradient centrifugation from Buffy coat samples of healthy donors. Primary human lymphocytes were cultured in RPMI 1640 medium (PAA Laboratories), 100 units/ml penicillin, 10 mM HEPES (PAA Laboratories) supplemented with 5% human serum (PAA Laboratories), and 10 mM HEPES (PAA Laboratories) at 37 °C and 5% CO2.

Stimulation Assays

Recombinant human visfatin/PBEF (subsequently referred to as visfatin/PBEF; 100 ng/ml; BioVendor R&D) was used throughout the experiments if not mentioned otherwise. To exclude the effects of stimulation-related cell division, cell counting was performed.

Dose-Effect Relationship—RASFs were stimulated with increasing concentrations of visfatin/PBEF (2.5, 5, 10, 50, 100, 250, 500, 2500, 5000, and 10,000 ng/ml). Unstimulated RASFs served as a negative control, whereas RASFs stimulated with adiponectin (25 μg/ml; R&D Systems) served as a positive control (27). IL-6 and IL-8 served as parameters.

Time-dependent Response—RASFs were stimulated with visfatin/PBEF for 4–48 h. IL-6 and IL-8 production was quantified over time.

Affymetrix GeneChip® Expression Analysis

RASFs were stimulated with 500 ng/ml visfatin/PBEF for 15 h. RNA was extracted using the RNeasy™ miniprep kit (Qiagen). Target preparation and hybridization for the Affymetrix human genome U133 Plus 2.0 GeneChip® (Affymetrix) were performed according to the recommended protocol. The results of the two-condition design were analyzed with GeneSpring microarray analysis software (Silicon Genetics) to obtain increase/decrease or no-change calls.

Real-time PCR

RNA was isolated using the RNeasy™ miniprep kit. Reverse transcription was performed using avian myeloblastosis virus reverse transcriptase (Promega) and random hexamer primers (Roche Applied Science). Primers (provided in the supplemental table (No. 1)) were designed, and the efficiency of each primer pair was tested using the standard curve method (E = 10–ΔC/E) considering 2.00 ± 0.05 as acceptable for experiments. Real-time PCR was performed using LightCycler® (Roche Applied Science) with SYBR Green I (Roche Applied Science) as the detection system. Melting curve analysis was used to confirm the specificity of amplification. 18 S RNA served as a reference gene. Results were analyzed using LightCycler® software.

Western Blot Analysis of p38 Phosphorylation

Cell extracts were harvested in 0.5% Nonidet P-40, 1 mM EDTA, 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 10% glycerol containing 1× cComplete protease inhibitor mixture (Roche Applied Science). Protein was separated on an SDS-10% (w/v) polyacrylamide gel and blotted onto nitrocellulose membrane (Bio-Rad). Proteins were detected by antibodies against phosphorylated and total p38 (Cell Signaling Technology). Detection was performed using horseradish peroxidase-conjugated secondary antibodies (Dako) and an enhanced chemiluminescence detection kit (ECL® Western blotting detection system, GE Healthcare). All Western blots were probed for cyclophilin B (Abcam) to ensure equal loading of samples.

Enzyme Immunoassay and ELISA

Commercially available ELISAs and enzyme immunoassays (R&D Systems and Phoenix Europe) were performed in accordance with the recommended protocols. Absorption was measured at 450 nm, and data were analyzed using Magellan software (Tecan).

Inhibition of Signal Transduction Pathways

Cultured RASFs were preincubated for 1.5 h with chemical inhibitors of signal transduction pathways: 1) p38 MAPK inhibitor SB203580 (20 μM; Sigma-Aldrich), 2) cell-permeable myristoylated PKC inhibitor 20–28 (40 μM; Calbiochem), 3) cell-permemeable myristoylated PKA inhibitor 14–22 (2 μM; Calbiochem), and 4) cell-permeable NF-κB activation inhibitor (40 μM; Calbiochem). Subsequent to the preincubation, cells were stimulated with visfatin/PBEF for 6 and 15 h in the presence of inhibitors. Visfatin/PBEF-stimulated RASFs served as a positive control, and unstimulated RASFs with or without inhibitors served as a negative control.

Proinflammatory Cell Motility-changing Factor PBEF in RA
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Immunohistochemistry

Snap-frozen RA and OA synovial tissue sections were prepared. Non-specific binding was blocked with 2% bovine serum albumin, followed by overnight incubation in a moist chamber at 4 °C with rabbit anti-human visfatin antibodies (10 μg/ml; Bethyl Laboratories) (9). Endogenous peroxidase activity was blocked with 0.3% H₂O₂ in 100% methanol. Sections were then incubated with Histofine Simple Stain MAX PO (MULTI) (mouse/rabbit primary antibodies; Nichirei Biosciences) for 30 min. Color development with 3-amino-9-ethylcarbazole substrate (Vector Laboratories) at room temperature was stopped after microscopic examination, followed by counterstaining of the nuclei using hematoxylin. Rabbit isotype-matched IgG sera (Santa Cruz Biotechnology) served as an isotype control, and mouse anti-human vimentin antibody (Dako) served as a positive control. A negative control experiment without primary antibody was carried out.

Chemotaxis

Migration of RASFs was measured using a 48-well Boyden microchemotaxis chamber (Neuro Probe, Inc.). Conditioned medium was prepared incubating RASFs for 16 h in DMEM without FCS. In contrast, stimulated media were acquired by stimulation for 16 h with 1) visfatin/PBEF (100 ng/ml), 2) visfatin/PBEF (100 ng/ml) and p38 MAPK inhibitor (20 μM), and 3) p38 MAPK inhibitor (20 μM), all in DMEM without FCS. In addition to using different cell populations, experimental replications were also performed.

20,000 cells in 50 μl of conditioned medium were placed in the upper chamber. In the lower chamber, 30 μl of conditioned medium (migration base line), medium with 10% FCS and without FCS. In contrast, stimulated media were acquired by stimulation for 16 h with 1) visfatin/PBEF (100 ng/ml), 2) visfatin/PBEF (100 ng/ml) and p38 MAPK inhibitor (20 μM), and 3) p38 MAPK inhibitor (20 μM), all in DMEM without FCS. In addition to using different cell populations, experimental replications were also performed.

Statistics

Statistical analysis was performed using Student’s t test. Means ± S.E. were calculated. Issues were regarded as significant for p < 0.05 (*), p < 0.01 (**), and p < 0.001 (**). Statistical evaluation was performed using GraphPad Prism 5.

RESULTS

Detection of Visfatin/PBEF in RA Synovial Fluid and Synovium

The mean concentration of visfatin/PBEF in RA synovial fluid was 76.26 ± 7.22 ng/ml (n = 24) (Fig. 1A). Strong expression of visfatin/PBEF in RA synovium (n = 3), mainly in the synovial lining layer, in lymphoid aggregates and perivascular areas was observed, indicating contribution to the inflammation of the synovium by local visfatin/PBEF production independent of adipose tissue. Visfatin/PBEF protein was expressed in OA synovium (n = 2) as well but to a lesser extent and mainly in regions of interstitial vessels (Fig. 1B).
Identification and Confirmation of Genes Regulated by Visfatin/PBEF in RASFs

To elucidate the effects of visfatin/PBEF on RASFs in a broad approach, the changes in gene expression and protein production were analyzed using AFFX (n = 1) as a screening method and real-time PCR (n = 7–9) and ELISA (n = 4–5) for confirmation. Of note, the -fold induction was different, as expected, between the non-quantitative AFFX and the quantitative real-time PCR and ELISA due to the different stimulation concentrations (500 versus 100 ng/ml).

Several genes were strongly altered by visfatin/PBEF (Table 1). Among them, chemokines of the CCX and CC families were predominantly represented and showed the highest -fold changes. Chemokines (CXCL-1–3, CXCL-5, CXCL-6, CXCL-8, CCL-2, CCL-5, CCL-13, and CCL-20) were therefore quantified at the RNA and protein levels, and their regulation could be confirmed in each case (Table 1). Verification of the observed regulation was also performed at the protein level for IL-6 and MMP-3. In addition, several cytokines, including IL-1β, IL-6, IL-7, IL-8, IL-15, IL-32, IL-33, and the IL-1 receptor antagonist, as well as the adipokines adiponectin and PBEF itself, were induced in AFFX (see supplemental table (No. 2)). A variety of key factors in RA pathophysiology were not regulated at the protein level in RASFs by visfatin/PBEF: pro-MMP-1, activin A, osteoprotegerin, IGFBP-2, IGFBP-3, TIMP-1, and TIMP-2 (n = 3–4) (see supplemental figure (No. 3)). IL-1β and TNF-α levels were below the detection level (data not shown).

Establishment of Dose-dependent Relationships and Time-dependent Responses for Visfatin/PBEF Stimulation of RASFs

With regard to the variability of visfatin/PBEF concentrations in the synovial fluids of RA patients, we determined whether the concentration of visfatin/PBEF plays a critical role in the production of proinflammatory markers such as IL-6 and IL-8. We further investigated whether the incubation period of RASFs with visfatin/PBEF leads to an additional increase in or suppression of cytokine production over time.

Dose-dependent Relationships—The effect of visfatin/PBEF on IL-6 and IL-8 production of RASFs was dose-dependent. The basal concentration of IL-6 was 23.3 pg/10^3 cells, which was increased up to 477.96 pg/10^3 cells by visfatin/PBEF stimulation (100 ng/ml). IL-8 was increased from 0.72 pg/10^3 cells up to 199.49 pg/10^3 cells (Fig. 2A). For subsequent stimulation experiments, 100 ng/ml visfatin/PBEF was used, which was located in the linearly increasing section of the curve and showed a significant effect on the production of proinflammatory cytokines. Further increase in the visfatin/PBEF concentration did not result in an additional increase in cytokine production and led to a plateau in the dose dependence curve (Fig. 2A).

Time-dependent Responses—After stimulating RASFs for 15 h with visfatin/PBEF (100 ng/ml), the basal concentration of IL-6 was increased from 14.9 pg/10^3 cells up to 106.04 pg/10^3 cells. For IL-8, the basal concentration was increased from 3.97 pg/10^3 cells up to 67.42 pg/10^3 cells. Extending the incubation period up to 48 h resulted in reduction of cytokine production, perhaps due to counter-regulation (Fig. 2B). However, 15-h incubations were used throughout the stimulation experiments to avoid secondary regulatory mechanisms taking place after longer incubation periods in vitro.

Signaling Pathways Involved in Visfatin/PBEF-mediated Production of IL-6 and CCL-2

Inhibition of the intracellular pathways of p38 MAPK (12, 30), NF-κB (9), PKA (31), and PKC (32, 33) in RASFs was performed to observe whether the induction of proinflammatory cytokines (i.e. IL-6) and chemokines (i.e. CCL-2) by visfatin/PBEF depends on them.

IL-6—Incubation with the inhibitor of the p38 MAPK pathway resulted in a strong reduction of visfatin/PBEF-mediated IL-6 production in RASFs. IL-6 production was reduced from 439.3 ± 103.09 pg/10^3 cells to 150.4 ± 50.31 pg/10^3 cells (reduction of 66%; p = 0.033; n = 3) (Fig. 3A) after 15 h and from 88.16 ± 22.17 pg/10^3 cells to 24.47 ± 5.29 pg/10^3 cells (reduction of 72%; p = 0.087; n = 3) (Fig. 3B) after 6 h compared with visfatin/PBEF-stimulated RASFs without the p38 MAPK.
Proinflammatory Cell Motility-changing Factor PBEF in RA

Visfatin/PBEF Influences Synovial Fibroblast and Lymphocyte Motility

Next, we analyzed whether the induction of proinflammatory factors and chemokines induced in RASFs via visfatin/PBEF leads to a cytokine environment of increased directed (chemotaxis assay) and/or general (scrape assay) cell motility of RASFs and/or lymphocytes. The migration index for RASFs was 2.73 ± 0.45 (n = 2) (Fig. 5A). Supplemental experiments showed that migration to visfatin/PBEF-stimulated media could be nearly reduced to the base line (1.08 ± 0.59, n = 2; 60% reduction) if preincubated with the p38 MAPK inhibitor. The p38 MAPK inhibitor itself reduced to some extent the migratory potential of RASFs (0.39 ± 0.31, n = 2) (Fig. 5A). Simultaneous with the effects of visfatin/PBEF-induced factors on RASFs, lymphocyte migration was increased but could be reduced via p38 MAPK inhibition (n = 2) (Fig. 5B). To see whether the increased migration was indeed caused by visfatin/PBEF-induced factors and not by visfatin/PBEF itself, we measured lymphocyte migration toward medium containing 100 ng/ml visfatin/PBEF and found no significant change in migration (n = 2) (Fig. 5C).

DISCUSSION

In this study, we investigated the potential of visfatin/PBEF to act as an effector molecule in RA. The significant changes in gene expression of RASFs mediated by visfatin/PBEF, especially the induction of a variety of chemokines and proinflammatory and matrix-degrading factors, could be confirmed at the RNA and protein levels. This supports the hypothesis that visfatin/PBEF...
PBEF creates an inflammatory molecular environment of increased fibroblast and leukocyte motility within RA synovial tissue.

Inflammation of the synovium is a hallmark in RA, and activated RASFs play a central role in local pathophysiological mechanisms. In the inflamed synovium of RA patients, accumulation of visfatin/PBEF was most dominant at the site of cartilage invasion (9), in the lining layer, in lymphoid aggregates, and around interstitial vessels, confirming that fibroblasts, lymphocytes, and endothelial cells express visfatin/PBEF in the synovial tissue and are exposed to increased concentrations of visfatin/PBEF in return (8, 20, 34).

The lining layer contains high numbers of activated RASFs characterized by a high basal production of IL-6 and MMPs advancing chronic inflammatory responses, contributing to T- and B-cell activation and progressive cartilage destruction (35). Visfatin/PBEF enhances these effects by increasing the production of IL-6 and MMP-3, MMP-10, MMP-12, and MMP-19. Therefore, visfatin/PBEF intensifies the aggressive phenotype of RASFs. This holds also true for visfatin-mediated stimulation of chondrocytes, resulting in higher amounts of prostaglandin E₂ and MMP-3, and CD14⁺ monocytes, expressing higher amounts of IL-6, IL-1β, and TNF-α, as reported recently (12, 36). By elucidating the change in gene expression of RASFs after visfatin/PBEF stimulation, we showed that a broad variety of chemokines of both the CXC and CC clusters are strongly upregulated, suggesting that visfatin/PBEF mediates chemotraction in RA synovium to a significant extent. In addition, visfatin/PBEF elevates the expression of adhesion molecules such as VCAM-1, ICAM-1, and ICAM-2, enabling RASFs to increase attachment to cartilage but also to contribute to cell migration (1). Chemoattraction is mediated via enhanced cell motility. Our results support the idea that visfatin/PBEF operates as a cell motility-increasing molecule for RASFs in vitro in a similar way as was shown for CD14⁺ monocytes and CD19⁺ B-cells under other pathophysiological conditions (12). The visfatin/PBEF-induced cytokine environment exerts chemotactic properties promoting directed fibroblast motility to the site of visfatin/PBEF expression. Furthermore, the visfatin/PBEF-induced cytokine environment contributes to lymphocyte recruitment. Therefore, visfatin/PBEF may contribute to accumulation of RASFs in the lining layer as well as at sites of cartilage invasion (1, 37), resulting in increased matrix destruction and remodeling at the invasion zone, a characteristic of hyperplastic RA synovium.

The inflammatory process in RA synovium is also driven by the influx of inflammatory cells into the synovial tissue, and activated interstitial vessels play a key role in the extravasation of leukocytes. Increased expression of visfatin/PBEF around interstitial vessels and a strong induction of IL-8, VEGF, and endothelial cell growth factor, factors with a strong pro-angiogenic effect (38, 39), were observed. It is likely that visfatin/PBEF promotes also RA synovial angiogenesis to some extent. This process has been demonstrated for visfatin/PBEF-mediated activation of ERK1/2 pathways as well as for increases in FGF-2 in human endothelial cells (40–43).

Because IL-6 and MMP-1 production is closely linked to PKC and p38 MAPK activation (30–32, 44–46) and because initia-

![FIGURE 4. Visfatin/PBEF influences synovial fibroblast motility. A, scrape assay images represent the difference in cell motility over time between cell culture wells of the negative control and wells of the sample (25 ng/ml visfatin/PBEF). Images from the start of the experiment and after 8 and 15.5 h are shown. Scrape assays were performed as described under “Experimental Procedures” (n = 3). Results are expressed as comparison of samples to base line (percentages). The graphs show enhanced cell motility in the samples after 8 and 15.5 h for 5 ng/ml (B), 25 ng/ml (C), and 50 ng/ml (D) visfatin/PBEF. *, p < 0.05.](image-url)
The effects of adipokines are not restricted to adipose tissue but inflammatory chemokines in RASFs, matrix-degrading enzymes, and RA, specifically by contributing to the production of proinflammatory and immunomodulating properties. The plasticity of dendritic cell responses to pathogens and their components. Lander, E. S., and Hacohen, N. (2001) The plasticity of dendritic cell reprogramming. Cell 106, 294–304. NADdependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. Circ. Res. 25–34. Dahl, T. B., Yndestad, A., and Skjelland, M., Øie, E., Dahl, A., Michelsen, A., Damås, J. K., Tunheim, S. H., Ueland, T., Smith, C., Bendz, B., Tonstad, S., Gullestad, L., Frøland, S. S., Krohg-Sørensen, K., Russell, D., Aukrust, P., and Østgård, H., Steinmeyer, J., Gay, S., Schülerich, J., and Büchler, C. (2006) Role of adipose tissue as an inflammatory organ in human diseases. Endocr. Rev. 27, 449–467.

The proinflammatory cell motility-changing factor PBEF in RA. Of interest, p38 MAPK inhibition in animal models of arthritis leads to improvement of the disease severity, thus reflecting its central role in arthritis (47, 48). Of interest, p38 MAPK inhibition in animal models of arthritis leads to improvement of the disease severity, thus reflecting its central role in arthritis (47, 48). Of interest, p38 MAPK inhibition in animal models of arthritis leads to improvement of the disease severity, thus reflecting its central role in arthritis (47, 48).

FIGURE 5. Visfatin/PBEF influences synovial fibroblast motility. A. RASF chemotaxis assays were performed (see “Experimental Procedures”), and the migration index was calculated (base line set to 1; ratio of sample to base line). Means ± S.E. are displayed as bars (n = 2). B, demonstration of lymphocyte migration to conditioned medium of RASFs (n = 2; the total number of migrated lymphocytes is shown). Results are expressed as means ± S.E. (see “Experimental Procedures”). C, incubation with 100 ng/ml visfatin/PBEF had only a slight effect on lymphocyte migration (n = 2). -Fold increase in migration is shown.

Taken together, the results of the study show that visfatin/PBEF is a strong effector molecule in the pathophysiology of RA, specifically by contributing to the production of proinflammatory chemokines in RASFs, matrix-degrading enzymes, and pro-angiogenic molecules. The data also support the idea that the effects of adipokines are not restricted to adipose tissue but are operative in chronic inflammatory joint diseases.

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