Intraarticular Injection of Platelet-Rich Plasma Reduces Inflammation in a Pig Model of Rheumatoid Arthritis of the Knee Joint

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Objective. Treatment options for rheumatoid arthritis range from symptomatic approaches to modern molecular interventions such as inhibition of inflammatory mediators. Inhibition of inflammation by platelet-rich plasma (PRP) has been proposed as a treatment for tendinitis and osteoarthritis. The present study was undertaken to investigate the effect of PRP on antigen-induced arthritis (AIA) of the knee joint in a large animal model.

Methods. Six-month-old pigs (n = 10) were systemically immunized by bovine serum albumin (BSA) injection, and arthritis was induced by intraarticular BSA injection. PRP was injected into the knee joints of 5 of the animals after 2 weeks. An additional 5 animals received no systemic immunization (controls). Signs of arthritis were documented by plain histologic analysis, Safranin O staining, and immunohistochemistry analysis for type II collagen (CII), interleukin-6 (IL-6), and vascular endothelial growth factor (VEGF). Interleukin-1β (IL-1β), IL-6, tumor necrosis factor α (TNFα), VEGF, and insulin-like growth factor 1 (IGF-1) protein content was measured by Luminex assay.

Results. In the pigs with AIA, plain histologic analysis revealed severe arthritic changes in the synovium. Safranin O and CII staining showed decreased proteoglycan and CII content in cartilage. Immunohistochemistry analysis revealed increased levels of IL-6 and VEGF in synovium and cartilage, and protein concentrations of IL-6, VEGF, IL-1β, and IGF-1 in synovium and cartilage were elevated as well; in addition, TNFα protein was increased in cartilage. Treatment with PRP led to attenuation of these arthritic changes in the synovium and cartilage.

Conclusion. We have described a porcine model of AIA. Experiments using this model demonstrated that PRP can attenuate arthritic changes as assessed histologically and based on protein synthesis of typical inflammatory mediators in the synovial membrane and cartilage.

Rheumatoid arthritis (RA) is the most common inflammatory joint disease and has significant societal consequences. RA is characterized by progressive destruction of cartilage and bone, which is generally considered to be caused by a chronic inflammatory process with periods of acute exacerbation. Histologic features include synovial hyperplasia and neovascularization, pannus formation, and subsequent loss of chondral and bone matrix. Inflammatory cytokines, mainly tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-6, play multiple roles in RA and seem to be involved in destructive as well as reparatory processes (1). The inflammatory cytokine TNFα is considered a pivotal mediator in chronic arthritis, and inhibition of TNF by specific antibodies has gained wide popularity as a treatment for RA (2). TNFα appears to be a master...
regulator of cytokines such as IL-1β and IL-6; hence, the overexpression of TNFα alone is sufficient to lead to arthritis in an animal model (3). A hierarchy of cytokines has been proposed, but cannot be universally applied for any state of arthritis or any model.

Arthritic changes can be demonstrated after systemic immunization and subsequent local application of methylated bovine serum albumin (BSA), in so-called antigen-induced arthritis (AIA). AIA has been applied in models using various small animals, such as mice and rabbits, and in beagles. The comparability of these animal models to humans is limited due to evolutionary genetic differences. In particular, the knee joint biomechanics in these models strongly differ from those in humans. In contrast, a close relationship and better comparability has been described for the knee of the pig (4).

AIA is considered to be the model in which the most pronounced histologic changes are consistently exhibited and has therefore been used to investigate cytokine levels in many studies. Several studies have shown that TNFα, IL-6, and IL-1β protein and messenger RNA (mRNA) levels correlate with clinical and histologic characteristics of arthritis (5,6). Notably, in an IL-6 knockout model, arthritic changes were elicited in animals with AIA but reduced in the knockout group, whereas levels of mRNA for TNFα and IL-1β appeared unaffected (7). TNFα, IL-6, and IL-1β are targetable by specific antibodies and therefore are certainly the most studied cytokines in arthritis. Cytokine up-regulation leads to destruction but also has a strong impact on angiogenesis and vasculogenesis, i.e., by the induction of vascular endothelial growth factor (VEGF). VEGF is probably the key regulator of neovascularization in inflammation that is produced within the synovium, by perivascular cells and synovial fibroblasts (8,9). Another growth factor that has been shown to have a critical impact on cartilage regeneration is insulin-like growth factor 1 (IGF-1). In osteoarthritis, IGF-1 resistance mediated by IL-1β and IL-6 is a phenomenon that maintains cartilage degeneration (10).

With respect to therapeutic use in inflammation and degenerative tissue damage, platelet-rich plasma (PRP) has recently attracted interest in many medical specialties. PRP is an autologous thrombocyte concentrate that can be prepared from whole blood with relatively little effort. Autologous thrombocyte concentrates have gained popularity as an adjunct to a variety of surgical procedures. In the beginning of this century, the clinical application of PRP was considered a breakthrough in the stimulation and acceleration of bone and soft tissue healing. Platelets and the growth factors they release are essential for regulating the cellular events that follow tissue damage. They adhere, aggregate, form a fibrin mesh, and subsequently release a large variety of growth factors and cytokines. A platelet concentrate is believed to improve tissue healing by producing local stimulatory substances in higher-than-physiologic concentrations. Therefore, their benefit for use as an immunogenicity in arthritis of cases in which rapid healing and tissue regeneration is required seems self-evident. Several companies have developed kits and devices for the automatic preparation of PRP, e.g., during surgical procedures. To date PRP has been used predominantly in maxillofacial surgery as an autologous additive to bone grafts and soft tissue transplants (11). Other indications that have been proposed range from chronic diabetic ulcers to standard orthopedic procedures (12–17). The recent demonstration that PRP exerted antimicrobial properties in vitro (18,19) suggested another potential clinical application.

The regenerative capacity of PRP is attributed to at least 15 different factors that are known to be contained within platelets (20–22), including platelet-derived growth factor (PDGF) (BB, AB, and AA isoforms), transforming growth factor β (TGFβ) (β1 and β2 isoforms), VEGF, epidermal growth factor, and IGF. Most of these factors have been demonstrated to have an effect on bone and tissue regeneration (23–34), and in reports of 2 recent clinical studies, intraarticular PRP injection was proposed as a treatment for degenerative arthritis (35,36).

In this study we investigated the anti-inflammatory effect of PRP, administered as an intraarticular injection, in a large animal model for RA. For this purpose we established an arthritis model of immunogenic arthritis in the knee joint of pigs.

MATERIALS AND METHODS

Induction of arthritis in a porcine model. Fifteen pigs (Hungarpig) 6–6.5 months of age were housed in a swine stable in accordance with the current laws of Hungary, after ethics approval was obtained from the ethics commission of Budapest, Hungary. Arthritis was induced as previously reported for rabbits and beagles (37). Briefly, immunization was achieved by intravenous injection of 0.8 ml/kg BSA (Fractin V; Sigma) on day 0 and day 14. On day 28 and day 42, BSA (5 mg/ml in normal saline) was injected intraarticularly into the knee joint to induce arthritis. As a control, saline was injected intravenously and intraarticularly. The pigs were divided into 3 treatment groups (Table 1), as follows: 5 animals were not immunized and were used as controls for BSA injection and NaCl injection into the right knee and the left knee, respec-
Five animals were systemically immunized prior to injection of BSA and NaCl, into the right knee and the left knee, respectively. Five animals were systemically immunized and injected intraarticularly with BSA into both knees; the right knees were additionally injected with freshly prepared PRP 2 weeks and 4 weeks later, at which times the left knees were injected with NaCl. Animals were killed 2 weeks after the second intraarticular injection of normal saline or PRP.

**Production and injection of PRP.** PRP from the pigs was produced from 50 ml of whole blood obtained by puncture of the internal jugular vein. Whole blood was divided into a platelet-containing and a cell-containing fraction by centrifugation at 200 g. In a second, high-speed centrifugation (2,000 g), platelets were sedimented and subsequently rediluted in one-tenth of the initial blood volume. This method yields platelet concentrations of up to 1,000,000/µl. Five milliliters of the PRP was obtained and intraarticularly injected into the knee at 2 different time points after induction of arthritis, as described above. A suprapatellar lateral approach was used for injection.

**Protein analysis of PRP.** To analyze concentrations of different proteins in PRP, platelet-poor plasma (PPP) was used as a control. PPP was obtained by obtaining whole blood as described above and following the procedures described above through the high-speed centrifugation step, without subsequent redilution of the platelets. Concentrations of IL-1, IL-6, TNFα, PDGF-BB, and TGFβ in PRP and PPP samples were analyzed by Luminex assay according to standard protocols using LiquiChip200 (Qiagen). A Milliplex human cytokine multiplex immunoassay kit (MPXHCYTO-60K-01; Millipore) was used for protein detection.

**Specimens.** Two weeks after the final intraarticular injection, all animals were killed and knee joints were dissected to obtain samples for histologic and molecular examination. Tissue samples were collected from cartilage and synovial membrane. All samples were stored at −20°C until processed.

**Histology and immunohistology.** Tissue samples were decalcified in 0.5M EDTA (pH 7.4) and embedded in paraffin. Sections (5µ) were cut out of the central portion of the femoral condyle with a sliding microtome (HM 430; Microm International). They were placed onto Superfrost Plus microscope slides (Thermo Scientific) and left overnight at 60°C. Sections were routinely stained with hematoxylin and eosin (H&E). Safranin O staining was carried out for 6 minutes, using a 0.1% aqueous solution at pH 3.

In addition, immunohistologic staining for type II collagen (CII), IL-6, and VEGF was performed. Slides were digested with hyaluronidase (2 mg/ml; 30 minutes at 37°C), and Pronase E (5 mg/ml; 15 minutes) for CII antibodies or proteinase K (0.5 mg/ml; 10 minutes at 37°C) for VEGF and IL-6. Subsequently, slides were incubated with Protein Block Serum-Free (X0909; Dako) for 30 minutes at room temperature. For primary antibody incubation, the following anti-

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**Table 1.** Treatment regimens and histologic indicators of arthritic changes*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Treatment</th>
<th>Weeks 0–2</th>
<th>Weeks 4–6</th>
<th>Weeks 8–10</th>
<th>Synovitis</th>
<th>Safranin O staining</th>
<th>CII staining</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>Right knee</td>
<td>IV saline</td>
<td>IA BSA</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>--</td>
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<tr>
<td></td>
<td>Left knee</td>
<td>IV saline</td>
<td>IA saline</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>AIA (n = 5)</td>
<td>Right knee</td>
<td>IV BSA</td>
<td>IA BSA</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Left knee</td>
<td>IV BSA</td>
<td>IA saline</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>AIA + PRP (n = 5)</td>
<td>Right knee</td>
<td>IV BSA</td>
<td>IA BSA</td>
<td>IA PRP</td>
<td>--</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Left knee</td>
<td>IV BSA</td>
<td>IA BSA</td>
<td>IA PRP</td>
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* CII = type II collagen; VEGF = vascular endothelial growth factor; IV = intravenous; IA = intraarticular; BSA = bovine serum albumin; AIA = adjuvant-induced arthritis; PRP = platelet-rich plasma.

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**Figure 1.** Morphologic changes in synovial membrane from the knees of pigs with antigen-induced arthritis, and effects of plasma-rich protein (PRP). Left, Hematoxylin and eosin staining of representative synovial specimens, showing synovial hypertrophy and leukocyte infiltration when arthritis was induced by injection of bovine serum albumin and striking attenuation of these features after treatment with PRP. Bars = 50 µm. Right, Modified Goldberg score in each treatment group. Values are the mean ± SEM (n = 5 per group). * = P < 0.05 versus control.
bodies (all from Abcam) were used: for CII, ab54236 (prediluted), for IL-6, ab46154 (1:1,000), and for VEGF, ab6672 (1:500). Antibodies were diluted in antibody diluent (S3022; Dako) and incubated overnight at 4°C. The samples were then washed 3 times with Tris buffered saline (7 minutes for each wash). Secondary antibody incubation and alkaline phosphatase detection were performed with the LSAB + System-AP Kit according to the instructions of the manufacturer (Dako). After counterstaining with hemalum, the sections were mounted with Aquatex (Boehringer). Negative control experiments were performed by inactivating the primary antibody with specific blocking peptides. As positive controls, sections of pig cartilage (CII), ear (IL-6), and brain (IL-6, VEGF) were used. The sections were examined by light microscopy (Axioskop 40; Zeiss).

**Histologic scoring.** In order to classify arthritic changes 6 weeks after the initial BSA injection, synovial specimens were histologically analyzed using a previously described modification of the Goldberg scoring system (37,38). Since H&E staining of cartilage did not reveal differences with varying treatment, no histologic scoring was applied to cartilage.

**Protein analysis of synovium and cartilage.** Frozen samples were chopped with an agate stone mortar using liquid nitrogen (−196°C), and IL-1, IL-6, TNFα, and VEGF concentrations in the homogenates obtained were analyzed by Luminex assay using LiquiChip200 (Qiagen) according to standard protocols. For detection of IL-1, IL-6, TNFα, and VEGF, the Milliplex human cytokine multiplex immunoassay kit was used.

**Gene expression analysis.** For gene expression analysis, frozen tissue samples (100 mg) were crushed in an agate mortar under liquid nitrogen. RNA from tissue was generated using TRIzol reagent according to the instructions of the manufacturer (Fermentas). Total RNA (1,000 ng) was used for reverse transcription and subsequent real-time quantitative polymerase chain reaction (PCR) with gene-specific primers. Reverse transcription was performed using an RNA-to-cDNA-Kit (Applied Biosystems). Real-time PCR was performed using Maxima SYBR Green qPCR Master Mix (Fermentas) on an iCycler IQ (Bio-Rad) with gene-specific primers. Primers were spanned over exon–exon boundaries in order to prevent contamination of genomic DNA in RNA extractions. The specificity of the amplification reaction was determined by analyzing the melting curves. We screened relative expression of various genes according to the ΔΔCt method, with 18S ribosomal RNA (rRNA) as an endogenous control. A threshold value of fluorescence was set in the exponential phase of amplification, and the number of PCR cycles needed for each sample to reach that level was recorded as the Ct value. We analyzed the expression of the following genes: IL-6 (forward GCCCTCGAGCCCACCAGGAA, reverse CCCAGGGAGAAGGCGACTG), VEGF (forward...
GAGCTTCCTACAGCACAAC, reverse CAAATGCTTTCTCCGCTC), and 18S rRNA (forward GTAACCCGTTGAACCCCATT, reverse CCATCCAATCGGTAGTAGCG).

**Statistical analysis.** Gaussian distribution of grouped data was tested by Kolmogorov-Smirnov test with Dallal-Wilkinson Lilliefors $P$ value. Mean ± SEM values were calculated, and the differences between means were assessed by Wilcoxon’s signed rank test. $P$ values less than 0.05 were considered significant.

**RESULTS**

In the first part of this study we established a large animal model of arthritis, using pigs. AIA was introduced by BSA injection as previously described for small animals. Arthritis induction was assessed in synovial specimens, with changes seen on H&E staining graded using a modification of the Goldberg score (37,38). Clear histologic features of arthritis were seen in animals with AIA (Table 1). All synovial specimens exhibited hypertrophy as well as leukocyte infiltration 6 weeks after local injection of BSA (Figure 1). The modified Goldberg score was significantly lower in control animals than in animals with AIA (mean ± SEM 0.71 ± 0.95 and 2.55 ± 0.88, respectively; $P < 0.05$). Cartilage sections were examined for arthritis induction by Safranin O staining for proteoglycan and immunohistochemical staining for CII. In samples from animals

![Figure 3](image-url)

**Figure 3.** Changes in the synovium after induction of arthritis and after administration of platelet-rich plasma (PRP). **a,** Immunohistologic analysis demonstrated enhanced staining for interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) in synovium from knees with antigen-induced arthritis (AIA). PRP injection reduced the intensity of staining for IL-6 and VEGF. **b,** Protein analysis by Luminex assay showed significantly elevated levels of IL-6 and VEGF after induction of arthritis. These elevated concentrations were reduced after administration of PRP. PRP injection did not significantly alter protein levels of tumor necrosis factor α (TNFα). Similar to the findings with IL-6 and VEGF, insulin-like growth factor 1 (IGF-1) and IL-1β protein concentrations were increased after induction of arthritis and decreased in PRP-injected knees. **c,** Gene expression analysis demonstrated that AIA caused increased synovial expression of IL-6 and VEGF. IL-6 expression levels were significantly reduced following PRP administration, whereas VEGF expression was not significantly altered. Values in **b** and **c** are the mean ± SEM (n = 5 per group). $\star = P < 0.05$ versus control; $\star\star = P < 0.05$ versus animals with AIA not treated with RPR.
with AIA, staining for both cartilage-specific markers in the superficial layers was reduced (Figure 2 and Table 1).

Next, we performed immunohistologic staining for IL-6 and VEGF in synovium and cartilage. BSA injection led to a strong increase in IL-6 and VEGF content compared to that in controls, which was evident 6 weeks after induction of arthritis. Representative images of synovium and cartilage, respectively, are shown in Figures 3a and 4a, respectively.

Finally, we analyzed the content of IL-6, VEGF, IGF-1, IL-1β, and TNFα protein in synovium from BSA-injected and control knee joints. Luminex analysis showed a dramatic elevation of protein levels in the synovial membrane after induction of arthritis. The elevations of all of these proteins except TNFα were statistically significant ($P < 0.05$) (Figure 3b). Gene expression analysis revealed that induction of arthritis led to increased synovial expression of IL-6 and VEGF (mean ± SEM increases of $3.82 ± 1.1$-fold and $3.31 ± 2.0$-fold, respectively) (Figure 3c).

To further characterize the model, cartilage from BSA-injected and control knee joints was also examined by Luminex assay. Levels of the tested proteins (IL-6, VEGF, IGF-1, IL-1, and TNFα) in the BSA-injected animals were again markedly elevated compared to those in controls. The increase was significant for all proteins (Figure 4b).

After confirming the induction of arthritis in this large animal model, we examined the effect of PRP injection on arthritic changes in the knee. The same measures were applied to evaluate the effect of PRP on arthritic changes induced by BSA injection as were used for the characterization of arthritis. First, plain histologic staining (H&E) of synovium and cartilage was analyzed. In the synovium samples, PRP injection led to strikingly attenuated synovial hypertrophy and leukocyte infiltration compared to the arthritic samples (Figure 1), and the modified Goldberg score was lower than that of the arthritic samples (mean ± SEM $2.0 ± 0.8$ versus $2.55 ± 0.88$). Consistent with the findings in synovial tissue, Safranin O and CII staining of cartilage showed a recovery of the specific protein content when PRP was injected after induction of AIA (Figure 2).

The effect of PRP on the inflammatory markers IL-6 and VEGF, which were shown by immunohistol-
chemistry analysis to be elevated in the synovium and cartilage of pigs with AIA, was investigated. In both synovium and cartilage, staining for IL-6 and VEGF was markedly reduced after administration of PRP (Figures 3a and 4a). IL-6 expression levels were also significantly reduced following PRP application (from a mean SEM of 3.82 ± 1.1-fold over control to 2.91 ± 1.1-fold; P < 0.05), whereas VEGF expression was not significantly changed.

Finally, proteins were quantified by Luminex assay to confirm the histologic and immunohistochemistry findings. We observed an almost universal effect of intraarticular PRP injection on tissue concentrations of the inflammatory markers tested (Figure 3b). In synovial samples from pigs with AIA, the concentrations of IL-6, VEGF, IGF-1, and IL-1 were reduced to control levels and were significantly different from the increased levels obtained after BSA injection. Mean ± SEM concentrations of IL-6 in control animals, animals with AIA, and animals with AIA treated with PRP were 37.49 ± 7.57, 69.80 ± 43.60, and 50.38 ± 15.53 pg/ml, respectively, and concentrations of VEGF were 54.50 ± 11.52, 74.68 ± 34.74, and 60.87 ± 11.94 pg/ml, respectively. IGF-1 protein concentrations were 85.32 ± 199.30, 407.6 ± 390.9, and 212.9 ± 241.5 pg/ml, respectively, and levels of IL-1 were 31.72 ± 9.89, 47.49 ± 23.30, and 36.14 ± 15.26 pg/ml, respectively. The TNFα concentration (35.83 ± 13.04 pg/ml in control animals and 48.13 ± 16.45 pg/ml after induction of arthritis) was not altered by PRP injection.

Similar findings were obtained when cartilage was examined for content of the above-mentioned markers of inflammation (Figure 4b). All chondral protein concentrations returned to control levels when PRP was injected, and the reduction of each protein was significant compared to levels in the specimens with non–PRP-treated AIA (for IL-6, mean ± SEM 8.014 ± 3.898, 16.85 ± 17.44, and 4.16 ± 0.87 pg/ml in control animals, animals with AIA, and animals with AIA treated with PRP, respectively; for VEGF, 1,826 ± 1,815, 3,421 ± 6,111, and 1,232 ± 223.3 pg/ml, respectively; for IGF-1, mean ± SEM 0.30 ± 1.34, 18.92 ± 29.25, and 1.17 ± 0.87 pg/ml, respectively; for IL-1, 4.50 ± 2.14, 6.46 ± 6.04, and 2.23 ± 0.61 pg/ml, respectively; for TNFα, 6.45 ± 4.13, 9.09 ± 8.53, and 3.13 ± 0.46 pg/ml, respectively).

To investigate the possible mechanisms of action of porcine PRP, we measured PDGF-BB, IL-6, IL-1α, TNFα, and TGFβ in PRP compared to PPP (Figure 5). A substantial amount of each protein was detected in all PRP samples (mean ± SEM 3,209.93 ± 2.21 pg/ml PDGF-BB, 8,423.38 ± 1,260.79 pg/ml IL-6, 223.86 ± 39.99 pg/ml IL-1α, 1,591.38 ± 755.49 pg/ml TNFα, and 85.97 ± 9.95 pg/ml TGFβ), but only PDGF-BB, IL-6, and TNFα were observed in higher concentrations in PRP than in PPP (concentrations in PPP 2.73 ± 2.21 pg/ml, 3,911.73 ± 554.93 pg/ml, and 1,053.48 ± 554.930 pg/ml, respectively). For the 2 proteins observed at similar levels in PPP and PRP (IL-1α and TGFβ), concentrations were generally low in PRP as well as in PPP.

**DISCUSSION**

In this study we developed a porcine model of immunologic arthritis and demonstrated that PRP could
attenuate the subsequent inflammatory response. To our knowledge this is the first large animal model for rheumatic joint disease of the knee. In accordance with published reports on the production of synovitis in small animal models (3,37,39,40), we conclude that our model can serve as a tool to analyze early inflammatory arthritis.

We chose the porcine model for investigating the effects of intraarticular PRP on knee joints because pig knees are more comparable to the human knee than are the knees of various small animals that have been used in well-established models. Knee biomechanics of the pig have previously been compared to those of humans and were shown to be comparable in terms of degenerative processes (4). In the first part of our study we demonstrated that BSA injection induced early changes of arthritis that are sufficiently comparable to those in human disease. The changes we observed included severe synovial reactions and loss of cartilage-specific Safranin O and CII staining. Similar findings have been reported by other authors, with chondral damage generally considered a characteristic feature of RA (41). Consistent with the pathophysiologic findings, protein analysis and immunohistochemical staining of cartilage showed enhanced levels of VEGF and IL-6 (42). Therefore, we clearly demonstrated arthritic changes in cartilage as well as in synovial membrane.

Of the molecular markers examined, IL-6 and VEGF have attracted the most attention in the past (43–45). VEGF plays a key role in hypervascularization of inflamed tissue and represents an early marker of arthritis and synovitis (8). Blocking of the effects of VEGF is a recently emerging concept for treatment of inflammation. In contrast, activation of the cytokine cascade downstream of TNFα is a mechanism that has long been recognized. This concept has led to in vivo and in vitro investigations aimed at specifically inhibiting IL-1β and IL-6 (46). As a consequence, IL-1β and IL-6 together with TNFα were a major focus of our evaluation of the porcine arthritis model. In accordance with the findings of Pohlers et al (6), we demonstrated increased levels of all markers except TNFα and increased gene expression of VEGF and IL-6 in synovial membrane. Other investigators have demonstrated up-regulation of cytokine production in chondrocytes of patients with arthritis (47). In the present study, IGF-1 protein concentrations were increased in both synovial membrane and cartilage from knees with AIA. IGF-1 has been shown to have a strong impact on chondrocyte regeneration and damage in arthritis, and has been found in increased concentrations in the synovial fluid of inflamed joints (10,48). Cytokines mediate the inflammatory process but IGF-1 regulates chondrocyte proteoglycan production (49), and as a consequence, the number of IGF-1 binding sites is increased in arthritis (50).

After adapting the immunologic model of early arthritis to pigs, we investigated the effect of plateletrich plasma, an autologous thrombocyte concentrate that has gained popularity within the last decade. PRP has been proposed as an almost omnipotent adjunct treatment for acute and chronic inflammatory conditions, but confirmatory clinical data are still rare.

The protein analysis of PRP revealed a content of typical secretory proteins that have been associated with modulation of inflammation. The content of pig PRP seems comparable to that of PRP from humans. IL1α is considered the classic catabolic proinflammatory cytokine involved in cartilage degeneration (51), and the finding that it was present in relatively low concentrations in PRP supports its use for antiinflammatory purposes. In contrast, IL-6 has multiple roles in tissue regeneration and inflammation and is generally considered an immune modulator. PDGF-BB and TGFβ have long been recognized as tissue-regenerating growth factors in PRP that can be considered beneficial for cartilage healing when injected intraarticularly (11). The finding of TNFα in high concentrations in PRP was unexpected. A possible explanation might be that inflammation as such can be destructive, but also promotes tissue regeneration, at least in the early phase of inflammation.

We found reduced levels of all growth factors measured after PRP treatment, which suggests one possible mechanism for the positive effect of PRP on cartilage. However, with specific regard to IGF-1, it remains unclear whether down-regulation of this growth factor in AIA constitutes an independent mechanism of action of PRP. IGF-1 down-regulation in this study may have been closely related to the actions of many other growth factors and cytokines that orchestrate the beneficial effect of PRP on inflamed articular surfaces.

Among the clinical indications for PRP are treatment of chronic wounds and nonunion fractures and reconstructive procedures in the oral cavity. Recently, intraarticular injection of PRP for the treatment of degenerative osteoarthritis of the knee was reported by Sanchez et al and by Kon et al (35,36). The results of both clinical studies were encouraging but preliminary, and limited by the small number of animals studied. Nevertheless, their findings are supported by our present results. We have shown that clear signs of arthritic
changes improve when PRP is injected after induction of arthritis with BSA. Based on a modified Goldberg score (37,38), used to assess the severity of AIA as well as to verify the effect of PRP administration, synovitis as an early histologic change was reduced by PRP. In addition, recovery of proteoglycan and CII in cartilage was documented.

Gene expression analysis revealed a decrease in expression of IL-6 following PRP injection, but no decrease in VEGF expression. We can only hypothesize as to the reason for this. Possibly, VEGF is produced by cartilage but mediates its reparatory effect at a site different from where it is produced, and PRP application does not alter the expression rate.

The consistent reduction of levels of all markers tested after treatment with PRP leads to our assumption that cellular protein turnover is affected by inflammatory stimuli and modified by intraarticular PRP injection. Such an effect of intraarticular injection would be particularly beneficial if it helps to maintain the integrity of the chondral surface and thereby facilitates joint movement. Moreover, our results show that the effect of PRP on production of inflammatory mediators generally seems stronger in cartilage than in synovium. In this respect the present findings support the use of PRP in cartilage damage, possibly in particular during the early stages of degenerative as well as rheumatoid arthritis.

The relatively low number of animals included in the study weakens the results but is a common problem with large animal models because of ethical considerations and administrative requirements. Nevertheless, we believe the consistency of the results with regard to induction of arthritis and effects of PRP outweigh this weakness. Therefore, we consider the number of animals used to be appropriate for drawing the conclusions reported.

In summary, we have constituted a novel large animal model of AIA, in which we have documented microscopic synovial changes and an increase in levels of inflammatory mediators in synovial and chondral tissue. Further, we have demonstrated that PRP injection can attenuate the chondral and synovial changes seen in AIA. Thus, PRP injection might be a novel therapeutic option in acute rheumatoid arthritis; our analysis of common mediators of inflammation in arthritis provides data supporting the notion that PRP has promise as an intraarticularly injectable antiinflammatory agent. Possibly by the release of secretory proteins such as PDGF-BB, IL-6, and TNFα, the collagen content of cartilage can be maintained by PRP injection. To our knowledge this is the first scientific report on the histologic and molecular effects of PRP in immunologic arthritis.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Varoga had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Lippross, Moeller, Tohidnezhad, Steubesand, Wruck, Kurz, Seekamp, Pufe.

REFERENCES

EFFECT OF PRP ON AIA IN A PIG MODEL


44. Schluter B, Konig B, Bergmann U, Muller FE, Konig W. Interleukin 6—a potential mediator of lethal sepsis after major thermal trauma: evidence for increased IL-6 production by peripheral blood mononuclear cells. J Trauma 1991;31:1663–70.

