PRP (PRGF technique) is injected arthroscopically into the sub-acromial bursa after rotator cuff repair, in dry conditions.
**Cytokines in Sports Medicine**

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Cytokines are relatively small protein molecules carrying signals from cell to cell. The nomenclature of cytokines is heterogenic. Interleukins (IL) were numbered in the order of their discovery: IL-1 to IL-37. Others were named according to one or more of their initially described functions (Tumour necrosis Factor = TNF, GCSF = Granulocyte Colony-Stimulating Factor) or primary cellular origin (Monokine = Monocyte Derived). Other cytokines are commonly referred to as growth factors (e.g. transforming growth factors (TGF-ßs), bone morphogenetic proteins (BMPs), platelet derived growth factor (PDGF), insulin like growth factors (IGFs)). Cytokines such as IL and interferons (IFN) have immunomodulatory effects, cytokines such as erythropoietin and thrombopoietin have different functions. Obviously the mentioned functions are of considerable interest in sports medicine. They appear to have a potential to act more specific than for example NSAID or Corticosteroids do. Especially tissue healing for tendons or cartilage may be less impaired than with the mentioned substances.

**Cytokines in acute inflammation**

Cytokines are essential in acute inflammation enabling the creation of an immune response. By activating leukocytes and endothelial cells in an insult, cytokines help to initiate a host of protective and reparative responses. IL-1, TNFa and IL-6 are cytokines playing an important role in formation of the acute phase response. This response, when transient, will eventually lead to a good outcome in tissue healing.

**Cytokines in chronic inflammation**

Chronic inflammation may have detrimental effects on tissues. In case of impairment of the local or systemic immune system (e.g. autoimmune disease, chronic osteoarthritis, chronic tendinitis) cytokines such as IL-1, and TNFa can cause and sustain chronic inflammation with no apparent injury left. This can impair the healing outcome of the tissues involved [Fig. 1].

![IL-1 in chronic inflammation](image)

**Local treatment of injury and the following acute or chronic inflammation**

Since 1980s, inhibition of pro-inflammatory cytokine production or receptors became major targets in research and, ultimately, treatment. Interleukin-1 is one of the main mediators in cartilage destruction and therefore a main target for new biological treatment methods. Interleukin-1 Receptor Antagonist (IL-1Ra) is the naturally occurring competitive receptor antagonist of IL-1 [Fig. 2]. It binds to the IL-1 receptor without turning on the inflammatory biochemical switches. IL-1Ra is virtually free of side effects, can be used in high doses and is eliminated within several hours by urinary excretion. Other cytokines such as IL-4, IL-10 and IL-13 have been shown also to exert significant anti-inflammatory action by increasing the synthesis of IL-1Ra and/or reducing pro-inflammatory cytokine production. Table 1 gives a -by far- complete list of important cytokines.

Production of cytokines with recombinant DNA techniques is technically demanding. A limited number of cytokines have been approved for clinical treatment in a wide range of clinical indications. These include IL-1Ra, PDGF BB, IL-2, EPO, IFN, G-CSF, GM-CSF, BMP2+7 and IGF-1. Other cytokine related approvals are for neutralizing antibodies or decoy receptors in a variety of immune diseases. Because of their strong potency these recombinant factors are to be used with care within the safe dosages. All pure recombinant growth factors are banned by WADA (World Anti-Doping Agency) as doping.
CURRENT CONCEPTS

<table>
<thead>
<tr>
<th>Pro inflammatory cytokines</th>
<th>Regulatory and anti-inflammatory cytokines</th>
<th>Growth factors</th>
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</thead>
<tbody>
<tr>
<td>IL-1α + IL-1β</td>
<td>IL-1Ra</td>
<td>FGF</td>
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<tr>
<td>IL-2</td>
<td>IL-4</td>
<td>PDGF</td>
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<td>IL-3</td>
<td>IL-6</td>
<td>VEGF</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-10</td>
<td>TGFβ</td>
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<tr>
<td>IL-7</td>
<td>IL-37</td>
<td>EGF</td>
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<td>IL-9</td>
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<td>HGF</td>
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<td>IL-12</td>
<td></td>
<td>IGF</td>
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<td>TNF</td>
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Other sources of cytokines are blood preparations often prepared at site of care utilizing official medical devices. They range from platelet concentrates (PRP) to autologous conditioned serum (ACS). The use of these preparations is exempt from WADA prohibition because they appear to have little effect on athletic performance. The authors of a recent study showing some circulatory increase of growth factors after PRP injection “believe that PRP will at best restore athletes to preinjury levels of competition but is unlikely to provide a competitive advantage relative to uninjured”. Some reviewers arrive to the conclusion that there is not sufficient evidence for the clinical effectiveness of PRP. However, given their apparent safety, autologous blood preparations are popular methods in the effort to supply athletes with a locally effective reparative stimulus in case of injury.

**What can we do now?**

There are many papers covering wildly varying PRP preparation and treatment techniques. Not only is the yield in platelets variable between preparations but also the yield in white blood cells and residual erythrocytes. Cells, even when autologous, leave a “footprint”. They either have to leave the site of injection or their debris be phagocytosed. This opens the field for speculation if amongst the possibly beneficial activities of cells like platelets and leucocytes there might be counterproductive aspects in case of their excessive presence [Fig. 3]. On the other hand controlled trials have been published that show effect, if variable, of different PRP preparations in osteoarthritis.

We shall discuss another, more controlled option that also makes use of autologous cytokines and growth factors from blood: Autologous Conditioned Serum (ACS). Table 2 shows the respective cytokine profiles in ACS and a single spin low platelet PRP version.

**Autologous Conditioned Serum (ACS)**

Publications about ACS treatment have increased in recent years. The basis is a technology that takes advantage of anti-inflammatory IL-1Ra generated from autologous blood at site of care. In 2003 a publication described the technique: strong IL-1Ra elevation in patient’s blood serum when whole venous blood is incubated at 37°C in a special device. At that time recombinant IL-1Ra had been shown to have potential in the treatment of osteoarthritis in dogs and other species. In ACS additional anti-inflammatory cytokines such as IL-4 and IL-10 accumulate along with growth factors. The cytokines so produced become available in the autologous blood serum, which is subsequently used for local injection into the injury. Several studies have shown good results for musculoskeletal indications. We shall summarize a few significant publications relevant for sports medicine.

**Intramuscular injection**

In a much cited clinical pilot study with professional athletes with a variety of muscle lesions and in a mouse model study, Wright-Carpenter et al. proved that muscle injuries can be treated successfully with local intra-lesional ACS injections. The clinical effect of ACS injections was compared to Actovegin® / Traumeel® injections. Return-to-full-training time for the athletes in the ACS group was reduced by a means of 5.7 days (16.6 vs 22.3 days). The mouse model (severe contusion injury to the gastrocnemius muscle) histologically showed at 30 and 48 hrs more activated satellite cells and accelerated tissue recovery when three local injections of ACS was used compared to three saline injections. After seven days histology of the ACS group displayed a much further developed muscle tissue. IL-1Ra contained in ACS may play an additional beneficial role, being able to block IL-1α that has been described to inhibit myogenic terminal differentiation.

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01 Interleukin 1 as a central player in acute and chronic inflammation. Schematic view of some of the pathologic effects IL-1 can have on tissues. IL-1Ra for this reason is a very interesting molecule.

02 Non complete list of cytokines playing an important role in acute and chronic inflammation and tissue regeneration. Note: IL-6 is an example of a pleiotropic cytokine that can have different functions. IL-6 amongst other functions is important in tendon, skin and liver regeneration. Interestingly, also TGFβ may act anti-inflammatory in addition to its growth factor function.
**Achilles tendon injection**

Majewski et al. showed that healing of Achilles tendon in a rat model improves when ACS is applied locally three times after dissection and re-suturing. Histologically, tendon tissue showed a remarkably superior orientation and deposition of collagen vs placebo. Biomechanically tested tendon strength in the ACS group was superior at four weeks but similar to the placebo (saline) group at eight weeks after the treatment. An interpretation is that tendon regeneration may not gain in speed but may ultimately gain in quality when inflammation is reduced and regenerative growth factors are supplied. Since single growth factors usually are not sufficiently effective or have side effects, Majewski et al. conclude that the complex combination like in ACS is possibly one key to the effects observed.

**Epidural peri-radicular injection for lumbar back pain**

Chronic inflammation of spinal nerve roots is a cause for radicular pain. Anti-inflammatory cytokine studies had shown that lumbar radicular pain may be influenced beneficially. A controlled clinical study by Becker et al. showed that injections of ACS show superior pain reduction after six months when compared to 2 groups (5mg and 10mg) Triamcinolone after application via epidural peri-radicular approach.

**Intra-articular injection after ACL Reconstruction**

Two studies by Darabos et al. describe the use of ACS after Anterior Cruciate Ligament (ACL) reconstruction. IL-1 has been implicated in bone lysis of the tibial tunnel after ACL surgery. Darabos et al. showed in an RCT study that intra-articular IL-1β concentration and tibial tunnel widening were reduced compared to placebo (saline) when ACS was injected post-surgically intra-articularly. The clinical significance of laxity is open to debate. However, substantial tunnel widening can become an issue during revision surgery. Darabos et al. showed that ACS with its strong anti-inflammatory action significantly reduces tibial bone tunnel widening at twelve months after surgery.

**Intra-articular injection for knee osteoarthritis**

Several studies on ACS relate to its wide spread use for joint disease. In a placebo-controlled equine study with a trauma induced osteoarthritis model, a clear clinical and histological superiority of the ACS group over placebo was reported and judged significant after 7 weeks. In another placebo-controlled clinical trial for osteoarthritis, the ACS treated group reported significant superiority in specific KOOS and KCRS parameters after one year. A randomized controlled osteoarthritis study comparing ACS injections to Hyaluronan and saline proved that ACS injection is significantly superior in effect size and has an effect duration of two years in osteoarthritis grades II-III. A recently presented case series with 118 ACS-treated patients by Baselga et al. showed outstanding symptomatic relief in osteoarthritic patients with initially high pain intensity (mean VAS 8 reduced to 3) and osteoarthritis grades I–IV when combined with physiotherapy.

ACS has shown potential for use in muscle injuries, Achilles tendon injuries, spinal nerve root inflammation, in osteoarthritis and after ACL surgery. It offers an easy and safe technique to use cytokines for the treatment of injuries and chronic syndromes. For further reading, published reviews are recommended.

**Table 1:**

<table>
<thead>
<tr>
<th></th>
<th>IL-1Ra pg/mL</th>
<th>IL-10 pg/mL</th>
<th>IL-6 pg/mL</th>
<th>EGF pg/mL</th>
<th>bFGF pg/mL</th>
<th>HGF pg/mL</th>
<th>IGF-1 ng/mL</th>
<th>PDGF AB ng/mL</th>
<th>TGFβ1 ng/mL</th>
<th>VEGF pg/mL</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>161.4</td>
<td>3.6</td>
<td>17.1</td>
<td>0.1</td>
<td>2.1</td>
<td>396</td>
<td>69.2</td>
<td>0</td>
<td>1</td>
<td>6.1</td>
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<tr>
<td>low PRP</td>
<td>250.3</td>
<td>4.5</td>
<td>18.9</td>
<td>289.2</td>
<td>33.6</td>
<td>550.1</td>
<td>81.9</td>
<td>18.6</td>
<td>34.5</td>
<td>177.8</td>
</tr>
<tr>
<td>ACS</td>
<td>1642.7</td>
<td>12.7</td>
<td>117.7</td>
<td>1019.2</td>
<td>14.2</td>
<td>1238.3</td>
<td>107.1</td>
<td>28.1</td>
<td>41.0</td>
<td>409.5</td>
</tr>
</tbody>
</table>
What may we be able to do in future?
Some of the clinically approved recombinant cytokines may have benefit for clinical use in sports injuries. Especially the IL-1Ra is to be judged extremely safe. As pointed out, single factor injection has so far not produced the striking improvements looked for. Obviously the correct cytokine has not been yet identified. There remains the fact that recombinant cytokines are still considered performance enhancing and not therapeutic by WADA. The use of blood born cytokines will therefore recommend itself to be improved in terms of cytokine profile and growth factor profile, in terms of dosage and in terms of purity of the compounds injected into a lesion.

For PRP this possibly means optimized fractionation of thrombocytes, of white blood cell populations and of plasma. The problem of dosage has occasionally been discussed. Too high concentrations of growth factors may have a quite paradox effect on tissue healing.

For ACS further research and studies should be performed to better define the mechanisms that make ACS such a promising option. It is desirable to more clearly define its clinical scope and limitations. In distinction to PRP, ACS delivers cell free, anti-inflammatory and growth factor rich blood serum for autologous use at site of care. Dosage does not appear to be a very big problem because no cells are concentrated above in vivo levels. Therefore local cytokine concentrations at injection site can hardly exceed physiological levels.

Ultimately, the often chronic nature of musculoskeletal diseases might be a good target for gene therapy approaches. Both for humans and animals first trials have been performed in the past. New studies and data are to be expected in coming years. IL-1Ra is a favorite cytokine gene, for it is very well tolerated, is cleared very rapidly and is capable of resolving chronic degenerative inflammation. The viral transfer vehicles for the IL-1Ra gene may probably be Adeno Associated Virus vectors. Expression of the transgene is stable over an extended period of time and it is expected to be an immunologically well tolerated transfer system. This leaves open the regulatory hurdles associated with an approval for gene therapy for a non-lethal disease. And, IL-1Ra gene therapy would be considered gene doping by WADA even if no performance enhancing effect is to be expected.

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