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LETTER

Response to: Cytokine profile of autologous conditioned serum for treatment of osteoarthritis, *in vitro* effects on cartilage metabolism and intra-articular levels after injection – authors' reply

Marijn Rutgers[1], Daniël BF Saris[1], Wouter JA Dhert[1,2] and Laura B Creemers*[1]

See related letter by Moser, http://arthritis-research.com/content/12/6/410, and related research by Rutgers *et al.*, http://arthritis-research.com/content/12/3/R114

We thank Moser for his comments [1] on the paper [2] we published in a previous issue of *Arthritis Research & Therapy*. To dispel any concerns about the proper use of the product, the autologous conditioned serum (ACS) in this study was prepared in a GMP (good manufacturing practice) facility in strict accordance with the guidelines supplied by the manufacturer (Orthogen, Düsseldorf, Germany) and was injected six times at 3-day intervals in accordance with the instructions given. As recommended, immediately before injection of ACS, synovial fluid was carefully aspirated to minimize ACS dilution. This synovial fluid was used to determine the cytokine levels before and during ACS treatment. We showed that, 3 days after injection, cytokine levels were at baseline and had no secondary effects on other cytokines. This finding is not in conflict with that of Darabos and colleagues [3], whose publication is cited by Moser; upon careful reading of that publication, none of the effects of ACS injection, including the alleged decrease in synovial fluid levels of interleukin-1 (IL-1), turned out to be statistically significant.

We certainly agree with Moser that *in vitro* and *in vivo* studies should be well controlled. However, in the case of ACS, the use of an autologous product is not required; this autologous product is devoid of cells, which are the only factors capable of evoking an immune response upon transfer of human materials to human recipients. We have used unconditioned serum as controls in our *in vitro* studies because serum in general seems to be more amenable to cartilage health than either saline or the synovial fluid the tissue is exposed to *in vivo* [4]. In this setup, we could not demonstrate any effect of conditioning the serum. The observation that IL-1 and tumor necrosis factor-alpha levels were upregulated in ACS, in contrast to the levels found previously upon characterization of ACS, may be due to the use of healthy subjects in the latter case, whereas we characterized the ACS prepared from osteoarthritis (OA) patients, the intended target population. Blood from OA patients was recently shown to contain cytokine profiles different from those of healthy subjects, suggesting a differential immune status [5].

Unconditioned serum, despite being the best control for ACS, has never been used in any *in vivo* study or clinical trial. Until now, the trials carried out with ACS have either used saline [6] or compared ACS with other treatments. However, the number of injections per treatment type was always dissimilar in these latter trials. For example, in the OA trial carried out by Baltzer and colleagues [7], six injections of ACS yielded more clinical improvement than three injections of hyaluronic acid, but the effect of multiple lavage sessions removing pro-inflammatory cytokines present in the synovial fluid cannot be ruled out here. In addition, concerns about the blinding of the patients to their treatment may be raised. In particular, in OA, more invasive treatment shows a stronger placebo effect than non-invasive therapies do [8].

It is questionable whether ACS has any positive effects with regard to *in vitro* and *in vivo* chondroprotection and clinical outcome. This notion is further corroborated by the fact that ACS therapy (Orthokine; Orthogen), to our knowledge, is not registered in any European country and that its 'wide use' is limited to clinical trials and some private clinics. We would encourage investigators to set

*Correspondence: L.B.Creemers@umcutrecht.nl
[1]Department of Orthopaedics, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands
Full list of author information is available at the end of the article

up ACS-based randomized controlled trials with full patient and observer blinding and with unconditioned serum as control in order to provide a final answer to whether this treatment merits registration.

Abbreviations

ACS, autologous conditioned serum; IL-1, interleukin-1; OA, osteoarthritis.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Orthopaedics, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. [2]Faculty of Veterinary Sciences, Utrecht University, Yalelaan 1, De Uithof, 3584 CL Utrecht, The Netherlands.

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