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Effect of combined spa-exercise therapy on circulating TGF-β1 levels in patients with ankylosing spondylitis

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Einfluss von kombinierter Heilstollen-Behandlung auf die Serumkonzentration von TGF-β1 in Patienten mit ankylosierender Spondylitis

Zusammenfassung. *Ziel:* Um einen objektiven Indikator für das biologische Ansprechen von Patienten mit ankylosierender Spondylitis (AS, M. Bechterew) auf die Heilstollen-Behandlung in Badgastein zu finden, wurde die Rolle des anti-inflammatorischen Zytokins TGF-β1 (Transforming Growth Factor β 1) untersucht.

Methodik: 83 Patienten mit AS wurden 3–4 Wochen lang einer Therapie bestehend aus Hyperthermie, Exposition mit niedrigen Radon-Dosen und gymnastischen Übungen unterzogen. Das Ansprechen auf die Behandlung wurde durch das Ausmaß der Schmerzreduktion ermittelt. Als Kontrollen wurden 10 AS-Patienten, die eine konventionelle Therapie ohne Heilstollen-Exposition erhielten und 10 Patienten mit Rückenschmerzen ohne entzündliches Substrat (low back pain, LBP) in die Untersuchungen eingeschlossen. Vor und nach der Therapie wurden mittels Immunassays die Serumkonzentrationen von TGF-β1 bestimmt.

Resultate: Nach Therapie wurde im Serum der AS-Patienten ein signifikanter Anstieg von TGF-β1 (total und aktiviert) festgestellt. Die mittlere Konzentration des totalen TGF-β1 stieg von 28715 pg/ml auf 43136 pg/ml ($p < 0{,}01$) und die des aktivierten TGF-β1 von 77 auf 1096 pg/ml ($p < 0.001$). Bei Unterteilung der AS-Patienten hinsichtlich des Ausmaßes der Schmerzreduktion in zwei Gruppen, zeigte die Gruppe 1 (Schmerzreduktion: $n = 46$) einen 17-fachen Anstieg der Konzentration von aktiviertem TGF-β1 (96 bis 1654 pg/ml, $p < 0.0001$), während die Gruppe 2 (keine Schmerzreduktion: $n = 37$) nur einen 7-fachen Anstieg (53 bis 402 pg/ml, $p < 0.01$) aufwies. Bei LBP-Patienten wurde nur ein geringfügiger Anstieg des aktivierten TGF-β1 von 31 auf 42 pg/ml ($p < 0.01$) registriert und bei AS-Patienten, die lediglich einer konventionellen Therapie unterzogen wurden, fand sich keine signifikante Änderung der TGF-β1 Werte.

Schlussfolgerung: Nach Heilstollen-Therapie in Badgastein fand sich im Blut von Patienten mit ankylosierender Spondylitis ein signifikanter Anstieg des zirkulierenden TGF-β1. Das Zytokin könnte auf Grund seiner anti-inflammatorischen Eigenschaften auf den Heilungsprozess einwirken und somit zu einer Besserung der Schmerzen und der Mobilität der Patienten beitragen.

Summary. *Background:* Ankylosing spondylitis (AS) is a chronic inflammatory disease of the axial joints with no satisfactory therapy. Reduction of joint pain has been reported after a course of therapy at a spa, Gasteiner Heilstollen, in Badgastein in Austria. The mechanism underlying this beneficial effect is not clearly understood and objective evidence for the biological response to therapy is lacking. The aim of this study was to find evidence for a biological response to speleotherapy in patients with AS and to study the involvement of the anti-inflammatory cytokine TGF-β1 in this response.

Patients and methods: 83 patients with AS were treated in Badgastein for 3–4 weeks. Therapy included active exercises, hyperthermia and exposure to low doses of radon in a former mine. Response to therapy was assessed from measurement of morning pain and immunoassay of serum levels of TGF-β1 before and after therapy. Ten AS patients who received conventional therapy and 10 patients with low back pain (LBP) served as controls.

Results: A significant increase in TGF-β1 (total and active) was found in AS patients after spa therapy. Mean concentration of total TGF-β1 increased from 28,715 pg/ml to 43,136 pg/ml, ($P < 0.01$) and active TGF-β1 in-

creased from 77 pg/ml to 1096 pg/ml ($P < 0.001$). When the AS patients were divided into two groups according to pain reduction, group 1 (decrease in morning pain, responders: n = 46) exhibited a 17-fold increase of active TGF-β1 levels (96 pg/ml to 1654 pg/ml, $P < 0.0001$) whereas group 2 (no change or an increase in morning pain: nonresponders: n = 37), showed only 7-fold increase (53 pg/ml to 402 pg/ml, $P < 0.01$). There was a moderate increase in active TGF-β1 from 31 pg/ml to 42 pg/ml ($P < 0.05$) in patients with LBP and no significant change was observed in the patients treated with conventional therapy.

Conclusion: These results demonstrate a significant increase in circulating TGF-β1 in patients with AS after the combined spa-exercise therapy in Badgastein. The results also provide evidence for a biological response to speleotherapy and suggest that TGF-β, through its anti-inflammatory function, may play a role in this response.

Key words: TGF-beta, Ankylosing Spondylitis, Radon, Badgastein, Heilstollen.

Introduction

Ankylosing spondylitis (AS) is a chronic and progressive inflammatory disorder that leads to disabling bony ankylosis of the spine [1, 2]. Physical exercise and non-steroidal anti-inflammatory drugs have been the mainstay of treatment for this disease for many years [3, 4]. Major advancement has recently been achieved with the use of biological agents that inhibit the pro-inflammatory cytokine TNFa (tumor necrosis factor-α) [5, 6]. However, these treatments are insufficient, since they are unable to cure AS. There is therefore continuing need for additional therapeutic strategies. A special spa treatment, the speleotherapy in Badgastein, Austria, where 2,500 AS patients are treated every year, is known for its safe and satisfactory responses to therapy [7–10] and has become one of the largest treatment facilities as far as the number of patients is concerned. Van Tubergen et al. [11] have recently demonstrated that the combined spa-exercise therapy at Badgastein, which includes exercise, high temperature and radon exposure, has significant long-term benefits compared with standard treatment with drugs and physical therapy alone. However, the mechanisms underlying these beneficial effects are still not clearly defined [7] and the identification of objective indicators for a biological response to therapy is needed. Studies on pro-inflammatory cytokines such as IL-6 have not revealed significant correlations between IL-6 serum levels and changes in the variables of mobility after treatment [12].

On the other hand, transforming growth factor-beta (TGF-β) is a very potent immunomodulating and anti-inflammatory cytokine and plays a major role in tissue healing, bone remodeling and fibrosis [13, 14]. TGF-β is usually produced as a large latent complex, and the active form has to be released to produce biological effects [15]. In patients with AS, in situ hybridization studies of biopsies from sacroiliac joints have identified increased expression of TGF-β mRNA, particularly near the site of new bone formation, but not in the inflammatory infiltrates [16]. In addition, investigations in murine progressive ankylosis have shown an enhanced proliferative responsiveness of spinal ligament fibroblasts to TGF-β compared with normal fibroblasts [17]. Further, both production and activation of TGF-β have been shown to be stimulated by hyperthermia and exercise [18–21].

In this retrospective study we evaluated the effect of the combined spa treatment in Badgastein on serum levels of the anti-inflammatory cytokine TGF-β and studied the relation between response to therapy and variations in concentration of this cytokine.

Patients and methods

Patients and controls

In a retrospective study, 83 patients suffering from AS (ages 30 to 73 years, men: n = 62, women: n = 21, disease duration: 3–55 years) who received treatment at Badgastein health resort (Heilstollen, mine galleries) were investigated. In order to investigate the relation between concentration of circulating TGF-β1 and response to therapy, care was taken to include comparable numbers of patients who showed response to therapy in terms of reduction of morning pain (responders: n = 46) and those who did not show this response (non-responders: n = 37) at the end of 3–4 weeks of therapy. In addition, two control groups of similar ages to the 83 AS patients were included in the study. The first control group consisted of ten patients with low back pain (LBP) without signs of inflammatory disorders who received the same treatment at the spa as the 83 AS patients. The second control group comprised 10 AS patients who did not attend for therapy at Badgastein and were either untreated or received conventional therapy such as physiotherapy and anti-inflammatory drugs when required, at another medical center.

Treatment and assessments

Treatment consisted of a 3–4 weeks spa-exercise therapy, essentially as described by Falkenbach [12]. Briefly, patients were admitted 9–12 times to the gallery of the Gasteiner Heilstollen hospital. Each stay took about 1 hour (radon concentration up to 4.5 nCi/l; temperature 38–41 °C; relative humidity 70–98%). In addition, outdoor exercises, physiotherapy, hydrotherapy and massage were applied according to each patient's need.

All the patients underwent physical examination before and after entry into the gallery. Severity of morning pain was assessed according to a questionnaire answered by the patients and was based on a 5-point scale as follows: intolerable pain (1), severe pain (2), moderate pain (3), slight pain (4) or no pain (5). Response to therapy was evaluated in terms of the change of pain level at the end of therapy compared with pain level before therapy, as previously described [12]. For example, a pain level of +2 at the end of therapy indicated pain reduction of two scale units e.g. from intolerable to moderate. Similarly, a pain level of –2 indicated pain worsening by two units e.g. from moderate to intolerable. These values were used in the statistical analysis and to study the correlation between pain reduction and changes in TGF-β1 and C-reactive protein (CRP) in response to therapy.

TGF-β1 immunoassays

Blood samples were taken from AS patients and patients with LBP before and after the combined spa-exercise program or at two time points within a 3–4 week interval from the AS and LBP control groups. Sera were separated and stored frozen

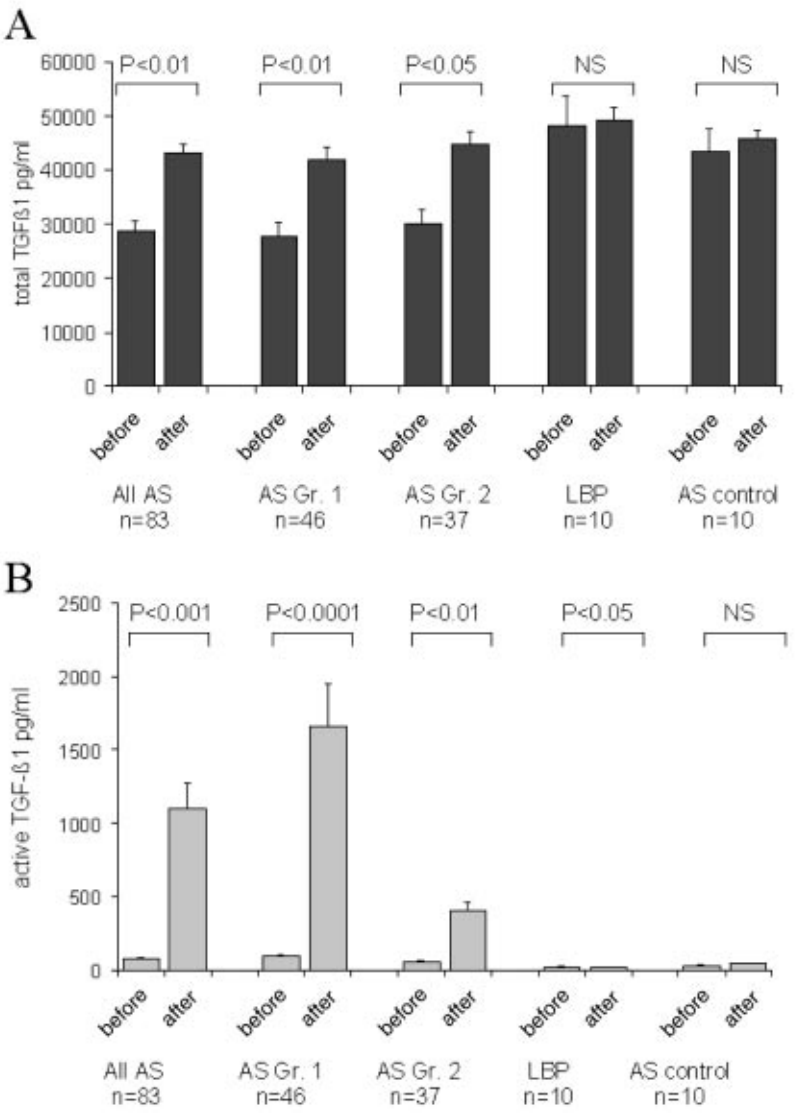

Fig. 1. Changes in serum levels of total (**A**) and active (**B**) TGF-β1 in patients with AS (n = 83) and the control groups. The first control group comprised 10 patients with non-inflammatory low back pain (LBP) and the second control group comprised 10 AS patients who were either untreated or received conventional therapy but not at Badgastein. TGF-β1 was measured before and after therapy in Badgastein in the AS and LBP groups. In the AS control group, TGF-β1 was evaluated on two occasions with a 3–4 week interval. AS patients were classified into two groups according to the response to therapy in terms of pain reduction and improved mobility; group 1 (patients who showed pain reduction at the end of therapy, n = 46) and group 2 (patients with no immediate response after therapy, n = 37). A remarkable increase particularly in active TGF-β1 was observed in group 1 AS patients (responders) compared with group 2 (non-responders). Data are given as mean ± SEM. *NS* non significant

at –80 °C until assay for TGF-β1. The active form of TGF-β1 was measured using a quantitative sandwich enzyme immunoassay (Quantikine, R & D systems, Minneapolis, MN, USA). To determine total amounts of TGF-β1, the latent form was activated by transient acidification according to the manufacturer's instructions.

Statistical analysis

Data are expressed as means ± SEM. The results were analyzed for statistical significance using paired Wilcoxon tests and correlation was calculated according to Spearman, using the SPSS 10.0 program. A P value of < 0.05 was considered significant.

Results

Increased TGF-β1 levels after spa therapy at Gastein

The results demonstrated a significant increase in circulating total and active TGF-β1 in the majority of AS patients (68.7% and 91.6% respectively) after completion of the combined spa and exercise therapy. Figure 1 shows the mean values ± SEM and Fig. 2 the variation in each individual patient. The mean concentration of total TGF-β1 before therapy was 28,715 pg/ml (± 1879) and increased to 43,136 pg/ml (± 1626) after therapy (1.5 fold, $P < 0.01$). However, the changes in levels of the biologically active form of

TGF-β1 were more remarkable: a dramatic rise occurred in AS patients, from a mean level of 77 pg/ml (± 7.5) to 1096 pg/ml (± 179) after therapy (14-fold, $P < 0.001$).

In the first control group (patients with LBP who were treated in Badgastein and received the same therapy as AS patients), the increase in total TGF-β1 from 43,566 pg/ml (± 3938) to 45,634 pg/ml (± 1746) after therapy was not significant, but the rise in the active TGF-β1 from 31 pg/ml (± 1.8) to 42 pg/ml (± 2.8) was significant (1.4-fold, $P < 0.05$). It is noteworthy that the increase in active TGF-β1 in this group was considerably lower in magnitude (1.4 fold) compared with the increase in the AS group who were also treated at the spa (14-fold).

To investigate whether the observed increase in TGF-β1 levels in AS patients was indeed due to the combined spa-exercise therapy, TGF-β1 was measured at two different time points (within a 3–4 week interval) in sera of ten AS patients who were either untreated or treated with conventional therapy elsewhere. The results revealed no significant variation in total TGF-β1 (48,055 ± 2560 pg/ml vs. 49,106 ± 5623 pg/ml) or in active TGF-β1 (17 ± 3.4 pg/ml vs. 21 ± 4.5 pg/ml) in these patients.

These data suggest that the observed increase in circulating TGF-β1 levels in AS patients was indeed related to the combined therapy at Badgastein.

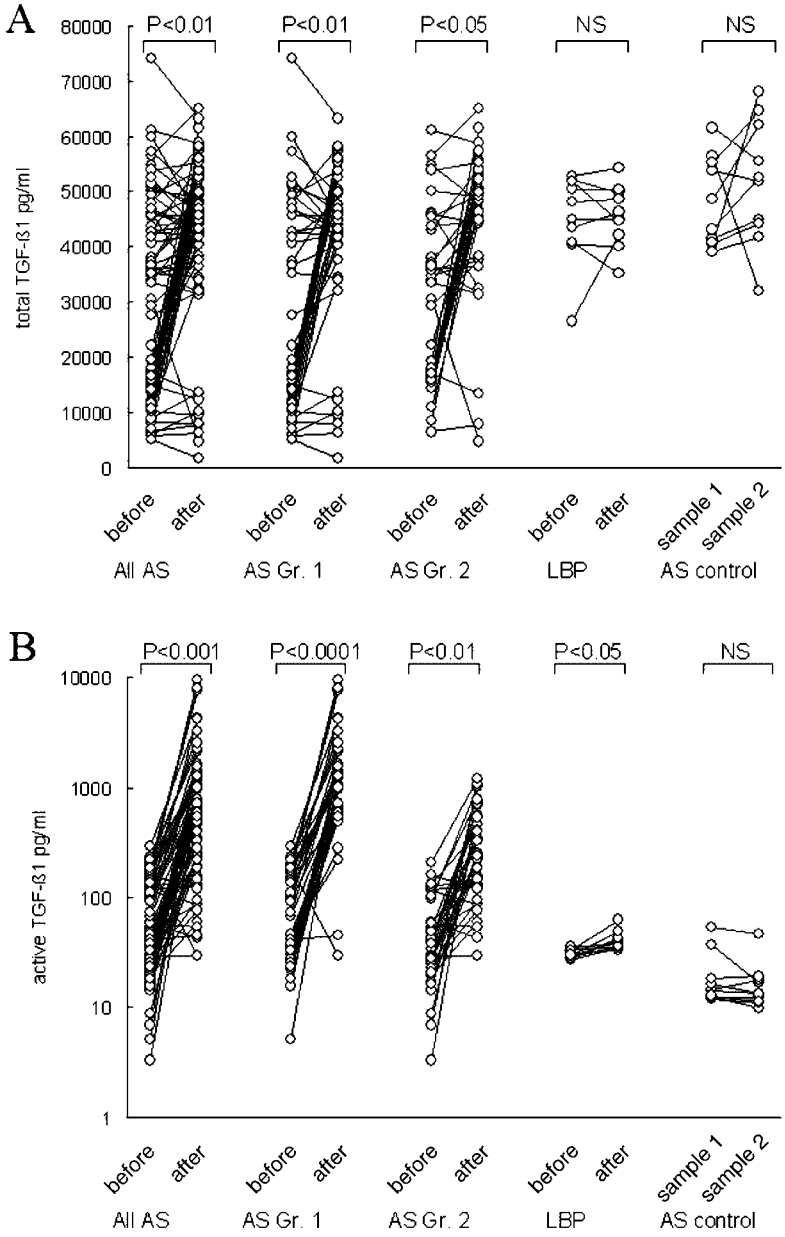

Fig. 2. The figure shows the changes in circulating active TGF-β1 in individual patients and the remarkable increase of TGF-β1 concentrations after completion of therapy in group 1 AS patients compared with group 2. The figure also shows the dramatic changes in active TGF-β1 in AS patients who were treated in Badgastein compared with the control groups

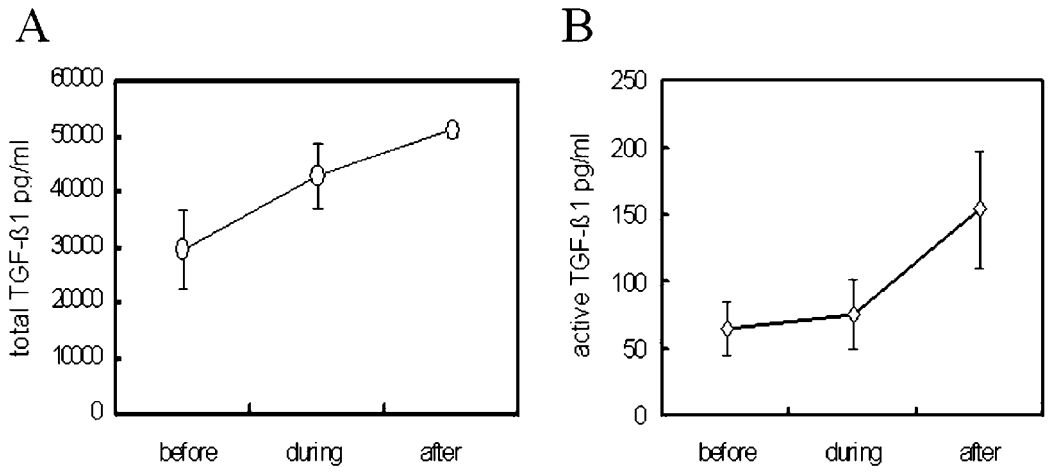

Fig. 3. Kinetics of TGF-β1 increase during therapy at Badgastein. Serum samples were collected from 4 patients with AS before therapy, 2 weeks after and at the end of therapy (4 weeks), and concentrations of circulating TGF-β1 (total and active) were measured using immunoassay. A gradual and steady increase in TGF-β1 is shown

Correlation between the increase in circulating active TGF-β1 and pain reduction

When the relation between the concentrations of circulating TGF-β1 (total and active) and the severity of morning pain was analyzed, no significant correlation was found between these two parameters either before or after therapy (not shown). However, marked differences in pain reduction in response to therapy were revealed when the patients were classified into two groups: group 1 (46 patients with significant reduction in morning pain at the end of therapy: responders) and group 2 (37 patients with no immediate improvement in morning pain after therapy: non-responders). The increases in total TGF-β1 were similar in the two groups (1.5 fold) (Fig. 1). However, group 1 exhibited a 17-fold increase in active TGF-β1 levels (mean 96 pg/ml to 1654 pg/ml) ($P < 0.0001$), whereas group 2 showed only a 7-fold increase (mean 53 pg/ml to 402 pg/ml) ($P < 0.01$).

We also studied the variation of total and active TGF-β1 levels in individual patient**s.** Among the AS patients treated at Badgastein, more group 1 patients showed an increase in TGF-β1 than those in group 2 (Fig. 2). For total TGF-β1, 67% of patients (30/46) in group 1 showed an increase and 33% (16/46) showed a decrease; **i**n group 2, 73% of patients (27/36) showed an increase and 27% (10/37) showed a decrease. For active TGF-β1, 95.6% of group 1 patients (44/46) showed an increase and 4.4% (2/46) a decrease; in group 2, however, 86% of patients (32/36) exhibited an increase and 14% (5/36) a decrease after therapy.

These results suggest that the response to the treatment procedures at Badgastein, in terms of reduction of morning pain, is related to an increase in TGF-β1 and is particularly influenced by the magnitude of increase of the active form of this cytokine.

Kinetics of TGF-β variation during therapy

To study the rate of TGF-β1 variation during the course of therapy, serum samples from four randomly selected patients were evaluated for TGF-β1 concentrations on three occasions: before therapy, 2 weeks after the beginning of therapy, and at the end of 4-weeks therapy.

The results showed a gradual increase in both total and active TGF-β1 as follows: total TGF-β1: 29,453 (± 6975), 42,635 (± 5837) and 50,814 (± 861) pg/ml; active TGF-β1: 66 (± 19), 76 (± 25) and 104 (± 7) pg/ml respectively (Fig. 3). The gradual and steady increase in both forms of TGF-β1 might be due to the accumulation of circulating TGF-β1 or to the increased number or efficiency of TGF-β1-producing cells, as well as to the activation process. This issue remains to be clarified and is currently under investigation in our laboratory.

Relation between TGF-β1 and other inflammatory parameters

To gain insight into the relation between TGF-β1 concentrations and the inflammatory process in AS patients, levels of circulating TGF-β1 and CRP in serum samples of AS patients were compared before and after therapy. No significant correlation was found. However, when we studied the increase or decrease of these parameters after therapy in AS patients of group 1 compared with their respective concentrations before therapy, significant inverse correlation was found between the increase in active TGF-β1 and the decrease in CRP ($r = -0.332$, $P < 0.05$). Group 2 patients did not show this inverse correlation (Fig. 4).

Collectively, these data demonstrate the close relation between the response to therapy, in terms of pain reduction, and the increase of circulating anti-inflammatory cytokine TGF-β1 together with the decrease in the inflammatory parameter CRP. The data also indicate the involvement of TGF-β1 in response to therapy.

Discussion

Pain reduction is reported after a combined spa-therapy (exercise, hyperthermia and exposure to low doses of radon) at Badgastein [7–11]. However, an objective indi-

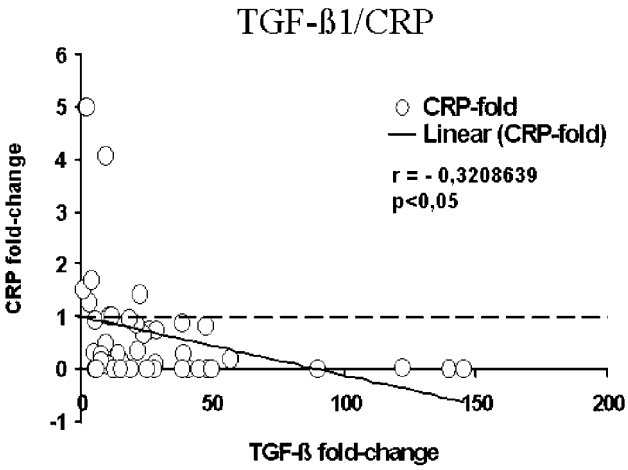

Fig. 4. Correlation between the change of active TGF-β1 and CRP. Changes in TGF-β1 and CRP in each patient were calculated by dividing the concentrations of TGF-β1 and CRP after therapy by their respective concentrations before therapy. Values > 1 indicate fold-increase and values < 1 indicate fold-decrease. Although no significant correlation between changes in TGF-β1 and CRP was found in group 2 AS patients (non-responders) (not shown), significant negative correlation was found in group 1 (responders)

cator for the response to therapy has not yet been demonstrated. We have now shown that pain reduction is due to a biological response to therapy and is associated with a significant increase in serum levels of the anti-inflammatory cytokine TGF-β1, particularly in its active form.

The response to the combined therapy at Badgastein, in terms of reduction of morning pain and the significant increase in serum levels of TGF-β1, appears to be closely related to the chronic inflammatory conditions in AS, since in patients with non-inflammatory LBP a similar response could not be observed. This response in the AS patients also seems to be a consequence of the spa therapy rather than due to physiological fluctuations of TGF-β1 levels, because no significant changes were observed in AS patients who received conventional therapy elsewhere. Furthermore, within the group of AS patients treated at Badgastein, the magnitude of the TGF-β1 increase influenced the extent of response to therapy: in the responders (group 1) the increase in TGF-β1 levels was highly significant and markedly higher than in the non-responders (group 2).

Although there was a correlation between the increase in TGF-β1 levels and pain reduction in AS patients, there was no direct correlation between serum concentrations of TGF-β1 and the severity of pain. This might be explained by the rapid and dynamic changes of TGF-β1 production and activation in comparison with the slower process of inhibition of inflammation, tissue repair and pain reduction. In other words, in some patients the increase in TGF-β1 production or activation may take place before significant tissue healing and pain reduction can be observed. It is noteworthy that AS patients often experience the beneficial effect a few weeks after completion of treatment [7]. The question of why under the same therapeutic conditions lower or nonsignificant increases of TGF-β were observed in LBP patients might be explained by the different nature of the two diseases and the absence in LBP of inflammatory reactions and bone formation characteristic for AS.

The present study demonstrates that the response to therapy in the Gasteiner Heilstollen is not only based on a subjective parameter such as pain reduction but is also due to a biological response, as shown by the increase in the amount and activation of the anti-inflammatory cytokine TGF-β1. The precise mechanism leading to the significant increase in circulating TGF-β1 in AS patients remains unclear at present but might be attributed to a complex biological response at the cellular level. Among the factors that could be responsible for this effect are the exposure to radon, hyperthermia and physical exercises or a combination of these factors. It has been shown that exposure of mice to radiation leads to activation of latent TGF-β through a mechanism that involves the generation of reactive oxygen species (ROS) [22, 23], and α-particles, emitted from radon and radon daughters, increase the intracellular ROS in lung fibroblasts [24]. It is therefore possible that similar mechanisms account for the activation of TGF-β in AS patients. Hyperthermia may also induce the expression of TGF-β mRNA and protein production, as has been demonstrated in rat cardiac fibroblasts *in vitro* and *in vivo* [18] and in pancreatitis rat models [19]. Furthermore, physical exercise was found to increase the production and activation of TGF-β in peripheral blood mononuclear cells in humans [20] and to induce the expression of TGF-β mRNA in skeletal muscle of mice [21]. TGF-β mRNA has been reported to be increased near the sites of new bone formation in AS patients [16], thus it is conceivable that the treatments at the Badgastein health resort (radon, exercise and hyperthermia) may cooperate to increase the activation and release of TGF-β into the circulation from these sites or from other cellular components such as synovial fibroblasts or immune cells. The finding of VanTubergen et al. [11] that the combined treatment at Gastein is better than hyperthermia and humidity alone strongly suggests that exposure to radon could be a determining factor in response to therapy. Although the present study was not sufficiently powered to detect the determining factor responsible for pain reduction, it appears that exposure to radon may play an important role in enhancing TGF-β secretion or activation, as suggested by *in vitro* and *in vivo* studies [22–24].

In conclusion, our data show a significant increase in circulating TGF-β1 in patients with AS after the combined treatment at Badgastein and demonstrate objective evidence for a biological response during this therapy. The data also suggest an important role for TGF-β in the pathophysiology of AS.

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References

1. Kim TH, Uhm WS, Inman RD (2005) Pathogenesis of ankylosing spondylitis and reactive arthritis. Curr Opin Rheumatol 17: 400–405
2. Ellegast HH (1974) Ankylosing spondylitis – radiological manifestations (author's transl). Wien Klin Wochenschr [Suppl] 28: 5–10
3. Dagfinrud H, Kvien TK, Hagen KB (2005) The Cochrane review of physiotherapy interventions for ankylosing spondylitis. J Rheumatol 32: 1899–1906
4. Boulos P, Dougados M, Macleod SM, Hunsche E (2005) Pharmacological treatment of ankylosing spondylitis: a systematic review. Drugs 65: 2111–2127
5. Braun J, Baraliakos X, Brandt J, Sieper J (2005) Therapy of ankylosing spondylitis. Part II: biological therapies in the spondyloarthritides. Scand J Rheumatol 34: 178–190
6. Gorman JD, Sack KE, Davis JC Jr (2002) Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor alpha. N Engl J Med 346: 1349–1356
7. Falkenbach A (2001) Combined radon and heat exposure for treatment of rheumatic diseases: a clinical approach. In: Kosaka M (ed) Thermotherapy for neoplasia, inflammation and pain. Springer, Berlin Heidelberg New York Tokyo, pp 495–503
8. Falkenbach A, Kovacs J, Franke A, Jorgens K, Ammer K (2005) Radon therapy for the treatment of rheumatic diseases – review and meta-analysis of controlled clinical trials. Rheumatol Int 25: 205–210
9. Falkenbach A, Jager U (2001) Is chronic lymphocytic leukemia a contraindication for radon and thermotherapy? – a case report. Wien Klin Wochenschr 113: 73–75
10. Sandri B (1974) Therapeutic results in ankylosing spondylitis in the Gasteiner Heilstollen (author's transl). Wien Klin Wochenschr [Suppl] 28: 11–12
11. van Tubergen A, Landewe R, van der HD, et al (2001) Combined spa-exercise therapy is effective in patients with ankylosing spondylitis: a randomized controlled trial. Arthritis Rheum 45: 430–438
12. Falkenbach A, Herold M (1998) In ankylosing spondylitis serum interleukin-6 correlates with the degree of mobility restriction, but not with short-term changes in the variables for mobility. Rheumatol Int 18: 103–106
13. Centrella M, Horowitz MC, Wozney JM, McCarthy TL (1994) Transforming growth factor-beta gene family members and bone. Endocr Rev 15: 27–39
14. Shehata M, Schwarzmeier JD, Hilgarth M, Hubmann R, Duechler M, Gisslinger H (2004) TGF-beta1 induces bone marrow reticulin fibrosis in hairy cell leukemia. J Clin Invest 113: 676–685
15. Annes JP, Munger JS, Rifkin DB (2003) Making sense of latent TGFbeta activation. J Cell Sci 116: 217–224
16. Braun J, Bollow M, Neure L, et al (1995) Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum 38: 499–505
17. Krug HE (1998) Fibroblasts from mice with progressive ankylosis proliferate excessively in response to transforming growth factor-beta 1. J Investig Med 46: 134–139
18. Flanders KC, Winokur TS, Holder MG, Sporn MB (1993) Hyperthermia induces expression of transforming growth factor-beta s in rat cardiac cells in vitro and in vivo. J Clin Invest 92: 404–410
19. Weber H, Wagner AC, Jonas L, et al (2000) Heat shock response is associated with protection against acute interstitial pancreatitis in rats. Dig Dis Sci 45: 2252–2264
20. Smith JK, Dykes R, Douglas JE, Krishnaswamy G, Berk S (1999) Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. JAMA 281: 1722–1727
21. Gavin TP, Spector DA, Wagner H, Breen EC, Wagner PD (2000) Nitric oxide synthase inhibition attenuates the skeletal muscle VEGF mRNA response to exercise. J Appl Physiol 88: 1192–1198
22. Barcellos-Hoff MH, Derynck R, Tsang ML, Weatherbee JA (1994) Transforming growth factor-beta activation in irradiated murine mammary gland. J Clin Invest 93: 892–899
23. Barcellos-Hoff MH, Dix TA (1996) Redox-mediated activation of latent transforming growth factor-beta 1. Mol Endocrinol 10: 1077–1083
24. Narayanan PK, Goodwin EH, Lehnert BE (1997) Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. Cancer Res 57: 3963–3971

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