antibodies to parvovirus B19 results were further confirmed by Western blot.

Results In 28 of 50 patients with RA (56%) and in 7/26 of the controls (26.9%) IgG antibodies to parvovirus B19 were detected (x_2 , $p < 0.05$). In 9 of 24 patients with OA and in 7/26 of controls IgG antibodies to parvovirus B19 were detected (x_2 , $p > 0.05$). In 11 of 50 patients with RA (22%) IgM antibodies to parvovirus B19 were detected and the results were further confirmed by Western blot, while in none of the patients with OA and in none of the controls were IgM antibodies observed (x_2 , $p < 0.001$ and $p > 0.05$, respectively).

Conclusion The prevalence of antibodies to parvovirus B19 was found to be increased in patients with RA as compared to controls. In patients with OA the prevalence of antibodies to parvovirus B19 was found to be comparable to that in controls. These results indicate either that infection with parvovirus B19 may be implicated in the pathogenesis of RA, as previously discussed in the literature, or that some cases of chronic polyarthropathy due to parvovirus B19 may have been misdiagnosed as rheumatoid arthritis.

THU0109 THE DESTRUCTIVE PROCESS OF RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS (RA-SF) IS INDEPENDENT OF P53

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Background

Objectives The aim of this study was to investigate, whether the destructive process of synovial fibroblasts in rheumatoid arthritis is dependent on the expression of p53. Moreover, we searched for the expression of p53 in RA-SF *in vitro* and *in vivo*. In addition, we examined the inducibility of p53 in RA-SF by X-rays *in vitro*.

Methods Paraffin embedded synovial tissues from 14 RA, 3 osteoarthritis (OA), 3 normal synovia and a neurological p53 positive tumour were investigated with DO7 anti-p53 antibodies by immunohistochemistry. RA-SF from passages 2 to 12 ($n = 10$), OA-SF ($n = 2$), normal synoviocytes (N-SF, $n = 1$) and foreskin fibroblasts (FSFB, $n = 1$) from passages 4–8 were grown on chamber slides and analysed with the same primary antibodies utilising immunofluorescence. SV40 transformed RA-SF (HSE) and SV40 transformed N-SF (K4IM) as well as the SV40 transformed bronchial epithelial cell line BEAS 2B served as positive controls. The percentage of positive cells was evaluated by a scoring system. RA-SF and HSE cultured *in vitro* were co-implanted with human cartilage under the renal capsule or the under the skin of SCID mice and kept there for 60 days. Paraffin sections from these SCID mice experiments were stained for p53. In addition, sections from former SCID mouse experiments including retroviral and adenoviral gene transfer studies revealing a strong invasion of synovial cells into the cartilage were investigated for the expression of p53. Moreover, RA-SF ($n = 2$) and controls (FSFB, $n = 1$) were irradiated with 10 Gy (X-rays) and analysed time dependently for the expression of p53 by immunofluorescence.

Results *In vivo*, p53 was not abundantly expressed in RA synovial tissue and in particular not at sites of invasion. In general, less than 5% of the cells in RA and OA synovial tissues stained

positively for p53, while no staining in the normal synovia occurred. In contrast, the positive control revealed a strong nuclear p53 signal in almost 100% of the tumour cells. *In vitro*, the expression rate of p53 in RA-SF was comparable low, while the SV40 transformed cells demonstrated a strong and specific nuclear staining. Furthermore, RA-SF invading the human cartilage in the SCID mouse co-implantation model did not show any expression of p53 at sites of invasion, whereas the implanted SV40 transformed HSE revealed a strong p53 staining indicating that those cells survived at least for 60 days in SCID mice. RA-SF and FSFB cultured *in vitro* responded equally to the irradiation with X-rays by producing transiently p53, demonstrating an adequate and functional response of p53.

Conclusion We suggest that the expression of p53 plays only a limited role in the pathogenesis of RA and particularly not in the invasive process of RA-SF.

THU0110 V-ATPASE INHIBITOR BAFILOMYCIN A1 REDUCES PROTON SECRETION IN FIBROBLASTS

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Background Metabolic activation of synovial fibroblasts (SF,¹), prosthesis loosening fibroblasts (PLF,²) and osteoblasts (OB,³) results in an enhanced acid secretion as determined by cytosensor microphysiometer. In addition, PLF were shown to degrade bone in absence of infiltrating macrophages or osteoclasts.

Objectives We were interested to study if the proton secretion was associated with the activity of specific proton pump, especially with the activity of V-type H⁺ ATPases.

Methods Fibroblasts or osteoblasts were expanded in complete medium containing 10% FCS and antibiotics. For cytosensor analysis cells were seeded at 30 000 cells/ml in microcups, mounted into the cytosensor. For metabolic activation the medium was enriched with cytokines such as TNF-alpha, IL-1 (Roche) or different agents such as ionomycin or PMA (Calbiochem) at different concentrations. To block V-ATPase activity, cells were incubated with Bafilomycin A1 or Amiloride (Calbiochem).

Results Metabolic activation as determined by enhanced acid secretion was observed upon stimulation of the fibroblasts with TNF-alpha, IL-1, PDGF, ionomycin or PMA but not with IL-6 or bFGF. Osteoblasts were activated by TNF-alpha, ionomycin and PMA; bFGF, IL-1, IL-6 or PDGF are not tested yet. Acid secretion occurred as early as 20–30 min. after initial stimulation. Under identical conditions, immortalised synovial fibroblast clones secreted less acid when compared to normal fibroblasts. As metabolic activation stimulates glycolysis, the acidification may be simply a consequence of carbohydrate catabolism generating acetate, lactate or carbonate. Therefore fibroblasts were incubated in medium containing Bafilomycin A1, a specific V-type H⁺ ATPase blocker. Bafilomycin reduced the proton secretion in a time course experiment within 20 min. irreversibly. Interestingly, Amiloride inhibited the proton secretion reversibly.

Conclusion Metabolic activation of fibroblasts or osteoblasts by pro-inflammatory cytokines results in enhanced acid secretion. As pro-inflammatory cytokines enhance the expression of matrix degrading proteases as well, we conclude that the acidification of the pericellular milieu by mesenchymal cells accelerates matrix