the proportion of BC-3-reactive aggrecan fragments generated by "aggrecanase" cleavage, with maximal stimulation seen with IL-1 concentrations 1 ng/ml and above. In contrast, IL-1 stimulation of newborn human articular cartilage resulted in a concentration-related increase in MMP-3, which was present exclusively in the zymogen or pro form, and GAG release into the culture media as well as an induction of BC-3-reactive fragment generation. The IL-1 concentration-response curves corresponded for induction of cleavage at the "aggrecanase" site and GAG release, but not for induction of MMP-3 (Figure). The concentration of IL-1 causing half-maximal stimulation (EC50) of MMP-3 induction was approximately 20 fold lower than that for GAG release whereas EC50 values were similar for GAG release and BC-3 fragment generation.

Discussion

These data support the induction of "aggrecanase" cleavage occurring in the absence of detectable MMP-3 up-regulation in adult human articular cartilage and suggest that stimulation with IL-1 causes an alteration in aggrecan catabolism that is not readily detectable by measuring the total amount of GAG release into culture media. The detection of increased GAG levels in the media from newborn cartilage stimulated with IL-1 along with the generation of BC-3-reactive fragments opens the possibility that the increased collagen crosslinking present in adult cartilage may result in retention of clipped aggrecan fragments within the matrix. In addition, in newborn cartilage there was a correlation between the IL-1 concentration-response curves for induction of cleavage at the "aggrecanase" site and GAG release, suggesting that "aggrecanase" may be responsible for aggrecan breakdown in this system.

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Immunogenetic profiles correlate with pro-MMP expression in rheumatoid arthritis

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Distinct HLA-DRB1 haplotypes may predispose patients with rheumatoid arthritis (RA) to a more aggressive disease course. This was suggested from data generated by retrospective analysis of disease patterns and profiles (Weyand et al. 1992). It has not been studied so far whether the presence or absence of DRB1*0401, 0404, 0408 and DRB1*0101 is associated with a difference in expression / up-regulation of effector molecules involved in articular matrix degradation.

Our aim was to explore whether certain HLA-DR haplotypes determine the course of RA by inducing different pathophysiological profiles as defined by a difference in pro-MMP expression / up-regulation.

Patients and methods

118 patients diagnosed for RA (1987 revised ACR criteria) were enrolled in this cross-sectional study. Subpopulations defined by disease duration and subgroups of patients defined by immunogenetic profiles

were analyzed for circulating pro-MMP-1 and pro-MMP-3 in relation to clinical indices of disease severity.

Patients (the total population, n = 118, and a subpopulation of 5-years disease duration, n = 53) were allocated to immunogenetically defined subgroups reflecting the presence of a single or a double dose or the absence of the HLA-DRB1* allele subtypes DRB1* 0101, 0401, 0404 and 0408. Circulating pro-MMP-1 and pro-MMP-3 levels were measured by ELISA (Taylor et al. 1994). Disease progression / severity was assessed clinically by an Articular Index Score (Thompson et al. 1987). For HLA-DRB1 allele typing mononuclear cells were isolated from EDTA blood by centrifugation using LeucoPREP tubes (Becton Dickinson), a Ficoll-Hypaque-like procedure. DNA was extracted with proteinase K and detergents (Tween-20, NP-40) using the Amplicor PCR reagents (Roche). DRB1 alleles were amplified by PCR, the products were blotted onto nylon membranes, hybridised to HRP-labeled oligonucleotides and visualized with TMB (Scharf et al. 1991). Serum levels of pro-MMP-1 / pro-MMP-3 and the Articular Index Score were compared between subgroups using the one-sided t-test.

Results

Circulating pro-MMP-1 levels did not show a significant difference between subgroups defined by immunogenetic profiles. There was a marked variability in the levels measured ranging from 10 ng/ ml to 60 ng/ml.

Serum pro-MMP-3 was markedly elevated in the majority of patients, in many patients up to the ten-fold compared to controls. This elevation to levels above the range reported for healthy controls (mean 24.3 [3.9–68.8] ng/ml) was seen more frequently in the subgroup presenting a double dose of HLA-DRB1 alleles (78% of the patients) or a single specific allele (70% of the patients) compared to the subgroup without specific alleles (57% of the patients). There was a significant difference in circulating pro-MMP-3 between patients with one or two of the suspected alleles (n= 77, mean pro-MMP-3: 131 ng/ml, and the subgroup bearing none of these alleles (n= 41, mean pro-MMP-3: 107 ng/ml).

In the subpopulation with 5-years disease duration pro-MMP-3 levels were significantly higher in the presence of one or two of the specific alleles than in the subgroup bearing none of these alleles. This difference was more pronounced in the presence of a double dose of the specific alleles compared to their absence ($p < 0.05$).The differences in circulating pro-MMP-3 shown for immunogenetically defined subgroups correspond with differences in the clinical manifestation of structural joint damage assessed by the Articular Index Score (AIS). Patients without severity-predisposing alleles had a lower AIS than those bearing a single or a double dose. In the subpopulation with 5-years disease duration a significant difference in AIS was demonstrated (p 0.02) between the subgroups bearing a double dose (mean AIS: 144) or none (mean AIS: 76) of the specific alleles.

Discussion

The results of this study present evidence for a link between immunogenetic profiles, up-regulation of pro-MMP-3, the latent form of stromelysin and disease severity assessed by a clinical score. Interestingly, circulating pro-MMP-1 is not elevated differently in the immunogenetically defined subpopulations in contrast to what would have been expected from immunohistochemistry data and results on synovial fluid TNF levels in correlation to pro-MMP-1 / pro-MMP-3 up-regulation suggesting a coordinated regulation of collagenase and stromelysin genes.

This patient population has also been analyzed for differences in circulating cytokines and cytokine inhibitors, soluble adhesion molecules and CRP levels in the immunogetically defined subgroups. The results suggest a difference in cytokine regulation (soluble TNF receptor expression and IL-1Ra up-regulation) in the presence or absence of distinct HLA-DRB1 subtypes which affects synovial production of effector molecules.

Immunogenetically determined differences in cytokine-inducible effector molecule expression perpetuating inflammatory synovitis and articular matrix degradation in rheumatoid arthritis correspond with clinical features of structural joint damage and indices of disease severity.

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