knee-joint. Moreover, the vulnerability of chondrocytes at various sites will determine the overall pattern of chondrocyte death.

Our dose response experiments made clear that chondrocyte death only occurs with the initial dose of 50 μCi. This probably is a relatively high dose as compared with the commonly used dose of 5 mCi in human knee-joints. So radiation damage to cartilage as a side effect of treatment of synovitis may be limited. However, studies on the effect of ^{90}Y on inflamed knee-joints are important in this respect. In that case chondrocytes are already affected by the inflammatory process [5] and may therefore be more vulnerable to radiation.

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Biochemical and clinical changes in rats with developing adjuvant arthritis

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Introduction

Rat adjuvant arthritis shares many features of human rheumatoid arthritis. Until now, biochemical experiments on this model have been limited to animals with established arthritis or, when the course of the arthritis was followed, a single parameter was evaluated. The aim of the present study was the comparative evaluation of several clinical and biochemical parameters throughout the development of arthritis with special reference to the SH plasma levels and to the amount of PGE_2 in the affected limbs.

Methods

Seventy male Sprague–Dawley rats weighing about 175 g were used. Thirty-five received 0.4 mg of *Mycobacterium tuberculosis* in the tail; the remainder were control animals. Five arthritic and 5 control rats were each sacrificed at 4, 8, 11, 15, 18, 25 and 32 days after the injection of *Mycobacterium*.

The following parameters were measured: body weight; index of polyarthritis (arbitrary score between 0 and 12, based on erythema, swelling, ankylosis of paws and ear nodules); weight of the hind paws, cut from the tibio-tarsal joint; SH plasma levels (Ellman reagent); *βN*-acetyl-glucosaminidase (NAG) activity in hind paws (spectrophotometric assay); amount of prostaglandin PGE_2 in hind paws (R.I.A., Institut Pasteur). The last two assays were performed on freeze-dried tissue homogenate (freezer mill SPEX). The differences between control and arthritic rats were evaluated by means of a binomial non-parametric test.

Results

The results shown in the figure demonstrate that the development of arthritis was accompanied by clinical changes: growth arrest, increased weight of paws, progressive increase in polyarthritis index. There were also significant changes in the biochemical parameters: decrease in reactive SH, increase in NAG and PGE_2.

By the 4th day following the injection of *Mycobacterium* the SH plasma level was significantly reduced, whereas significant changes in the other parameters only appeared 15 days after the injection.

A modest early decrease in PGE_2 levels was followed by a progressive increase after day 8.

The variations in the above parameters (weight of paws, index, PGE_2, NAG and SH) were closely correlated (see the Table).

Discussion and conclusion

The main change observed during the experiment was the decrease in SH plasma levels. This was noticeable as early as the 4th day, whereas no significant change in the other parameters occurred before the 15th day. This decrease, which may represent an oxidizing activity linked to activated phagocytes [1], is consistent with the concept that such mechanisms may be important in the early development of adjuvant arthritis, as has been proposed for rheumatoid arthritis [1, 2].

The rapid freezing and homogenizing techniques developed for this study made it possible to determine the amount of PGE_2 in the inflamed tissue. This level reflects the severity of the disease and especially the swelling. A similar result was obtained by PARNHAM, BONTA and ADOLFS [3] between the 10th and 22nd day, using a perfusate of the arthritic rat paw. The initial modest decrease in PGE_2 might be linked to the increase in PGE_2 urinary excretion noted by

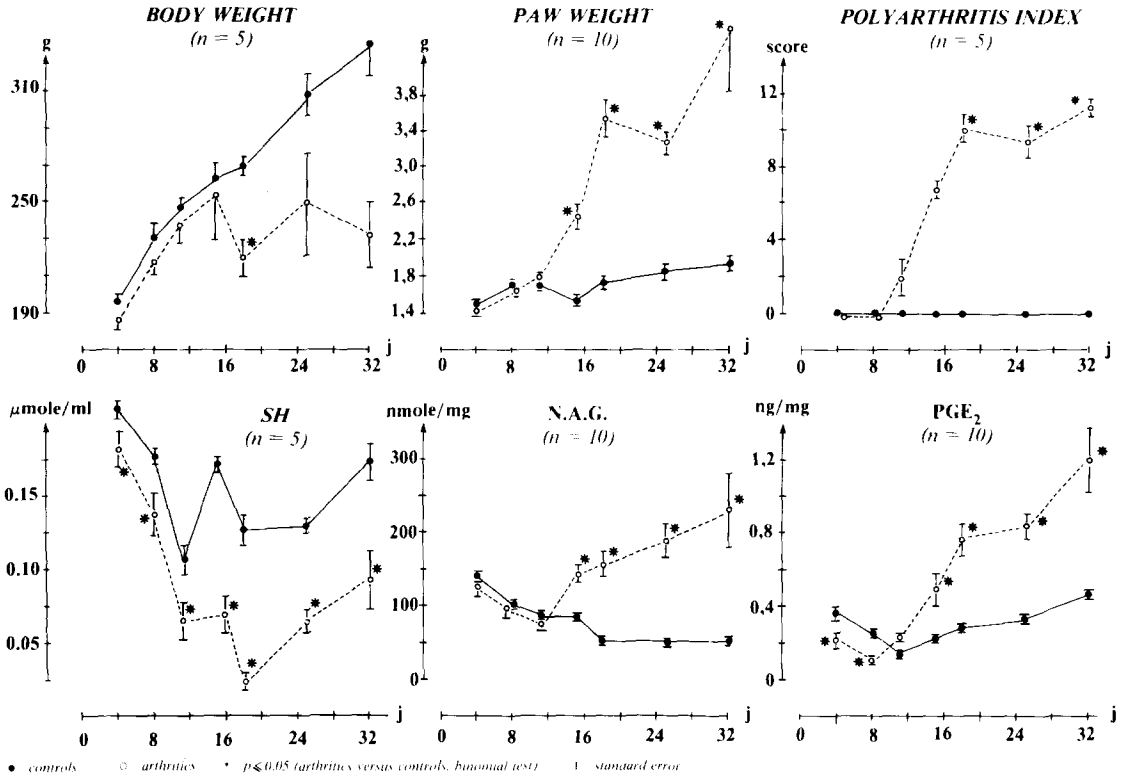

Figure
Clinical and biochemical changes during adjuvant arthritis development.

Table
Relationships between parameters. Correlation coefficients.

	Body weight	**Left paw weight**	**Right paw weight**	**Poly-arthritis index**	**SH**	**N.A.G.** *(Left paw)*	**N.A.G.** *(Right paw)*	**PGE2** *(Left paw)*	**PGE2** *(Right paw)*
Body weight	1.00								
Left paw weight	0.03	1.00							
Right paw weight	0.09	0.79	1.00						
Polyarthritis index	— 0.11	0.81	0.89	1.00					
SH	— 0.06	— 0.64	— 0.75	— 0.77	1.00				
N.A.G. *(Left paw)*	— 0.47	0.48	0.39	0.58	— 0.27	1.00			
N.A.G. *(Right paw)*	— 0.42	0.52	0.56	0.71	— 0.32	0.71	1.00		
PGE2 *(Left paw)*	— 0.04	— 0.75	0.80	0.78	— 0.49	0.55	0.62	1.00	
PGE2 *(Right paw)*	— 0.05	0.78	0.77	0.80	— 0.57	0.44	0.66	0.79	1.00

d.f. = 58 ; significant at p = 0.001 for r ≥ 0.41

HONDA, PUKAWA and SAWABE [4] between the 4th and 11th day.

It should be noted that all parameters, except body weight, were correlated. The most significant correlation observed was between the weight of paws and the polyarthritis index. The least striking correlations were between the SH plasma levels and every other parameter. This is due to the early decrease in SH, and also to the late increase. This secondary rise in plasma SH probably reflects the clinical improvement that has been previously noted after the 30th day [5].

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Role of Alpha$_2$-macroglobulin in joint inflammation: humoral and cellular aspects

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Alpha$_2$-macroglobulin (α_2M) is a large molecular plasma protein which has the unique property of binding and inhibiting virtually all proteinases (EC. 3.4.21–24), regardless of their specificity, serving as a mechanism for the removal of activated enzymes from the circulation. A dramatic conformational change occurs when native α_2M binds a proteinase [1]. In addition, a small mol. wt. peptide is released from α_2M. The complex state of α_2M is specifically recognized and cleared by highly discriminative receptors on fibroblasts and macrophages. A number of recent *in vitro* and *in vivo* findings indicate that native α_2M and α_2M · Proteinase complexes are involved in several aspects of humoral and cellular processes of joint inflammation, which are worth considering in the following paragraphs:

(1) Enhanced vascular permeability of inflammatory synovial tissue (RA) allows plasma α_2M to equilibrate into synovial tissue and fluid (SF), where its concentration rises to 30–50% of that of the plasma. Due to the broadest specificity of all inhibitors α_2M becomes the most important inhibitor of (serine-, metallo-) proteinases released and activated during joint inflammation. In addition, the key function of α_2M seems to be indicated by the stability of the native protein in altered biological fluids, the biologically efficient inhibition constants, the stability of the complex (the proteinase is trapped and covalently linked) and the specific clearance of the complex [1]. Apparently, the large mol. wt. (M_r = 725,000) of α_2M does not hamper the biological efficiency as evidenced by the demonstration of immunoreactive α_2M in the deeper layer of the synovium.

(2) Proteolytic enzymes were shown to be present on the surface of leukocytes and to play an important role in the regulation of cell responses and cellular interactions. Therefore it is likely that native α_2M interferes with cell surface-associated proteinases and modulate effector cells such as suppressor/cytotoxic T cells, NK cells and granulocytes. Application of immunohistochemistry to synovial tissue sections and synovial fluid cytosmears revealed the inhibitor mainly in association with macrophage-like cells. In tissue sections fibroblast-like cells were also identified to stain for α_2M. Immunoelectronmicroscopy revealed α_2M in secondary lysosomes of macrophages indicating the uptake of α_2M · P from the extracellular space [2]. Fibroblasts displayed the inhibitor in membrane-coated vesicles. In SF cytosmears other cell types associated with α_2M were found, namely neutrophils and lymphocytes. These cells were found to display membrane-associated α_2M rather than cytoplasmic α_2M. Conspicuously, α_2M-coated neutrophils showed morphological signs of necrobiosis. Thus, it seems conceivable that ectoenzyme-expressing cells, which bind α_2M, may be engulfed by macrophages, recognizing the complex state of α_2M (Reiter cell), see the Figure.

(3) It is now well established that α_2M · P complexes are generated during joint inflammation, although reports disagree with respect to the utilization of α_2M in SF. Previous investigations revealed a high degree of complexation of α_2M in SF. In our hands, using a solid phase immunosorbent assay, α_2M · P complexes did not significantly exceed 50% of the total amount of α_2M in few cases of RA. Therefore the bulk of α_2M in SF is functionally active, whereas α_2M · P complexes are very probably cleared by free- and tissue-fixed macrophages [2].

Some emphasis has been placed on the fact that α_2M · P complexes show slight proteolytic activity towards macromolecules. This phenomenon, however, is restricted to the α_2M · Plasmin complex which displays little fibrinolytic activity. The properties of the bound proteinases are summarized by saying that the enzymes retain the activity towards peptides of approximately $M_r \leqslant 10{,}000$. Several *in vitro* findings suggest that α_2M · P complexes may be