COLLAGEN-INDUCED ARTHRITIS IN RHESUS MONKEYS: EVALUATION OF MARKERS FOR INFLAMMATION AND JOINT DEGRADATION


Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, *Division of Vascular and Connective Tissue Research, TNO Prevention and Health, Leiden, The Netherlands and *Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, The Netherlands. Like experimental autoimmune encephalomyelitis [13].

SUMMARY

The objective of this study was to analyse parameters in rhesus monkey collagen-induced arthritis (CIA) with which the inflammation and destruction of the joints can be described in quantitative terms. CIA was induced in genetically susceptible and resistant monkeys, which can be distinguished on the basis of the dominant resistance marker Mamu-A26. The disease course was monitored daily using a semiquantitative scoring system. Plasma samples were collected once or twice weekly and analysed for C-reactive protein (CRP). Urines were collected overnight once a week and analysed for excretion rates of the collagen cross-links hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP). The results show that periods of active CIA are characterized by substantial weight loss and increased plasma CRP levels, followed shortly thereafter by increased excretion rates of the collagen cross-links HP and LP. Remission of the disease can be recognized by a decline in plasma CRP levels and especially an increase in body weight. The highest CRP levels were found in the most severely arthritic monkeys, indicating a possible relationship of the absolute plasma CRP levels to the severity of inflammation. During periods of active arthritis, increased excretion rates of collagen cross-links HP and LP in the urine were found. In particular, the major collagen cross-link in articular cartilage, HP, showed a strong increase (9- to 15-fold). The excretion rates of LP, which is considered as a bone-specific degradation marker, only increased 4- to 6-fold, thus indicating predominant destruction of cartilage and less of bone. In conclusion, the severity of CIA can be monitored in a quantitative manner using plasma CRP levels, urinary excretion rates of HP and LP, and body weights, superimposed on semiquantitative clinical scores. The parameters also facilitate a more objective assessment of the effect of anti-arthritic drugs in the model than with the clinical scores alone.

KEY WORDS: Collagen-induced arthritis, Rhesus monkey, Disease markers.

IMMUNIZATION of rhesus monkeys with bovine type II collagen (CII) induces a self-limiting monophasic polyarthritides, known as collagen-induced arthritis (CIA). This experimentally induced autoimmune disease shows several similarities with human arthritides [1–3]. Over the past few years, disease models in non-human primates have become increasingly important for safety and efficacy evaluation of new therapeutic modalities that cannot be tested in rodents [4]. The therapeutic activity and adverse side-effects of biological molecules depend on functional interaction with their target antigen. The poor reactivity of most human biologicals with the relevant target structures in rodent species makes disease models in rodents invalid in this respect. The phylogenetic relationship and consequent immunological similarity [6–11] between man and rhesus monkey makes this non-human primate CIA model an attractive test system for human-specific biological molecules with anti-rheumatic potential.

For the clinical evaluation of the severity of arthritis, a semiquantitative scoring system has been used, which is based on the degree of inflammation and deformation of the affected joints [5]. These semiquantitative clinical parameters are very useful for longitudinal monitoring of the CIA course in individual monkeys. However, they are too inaccurate for comparison of the disease severity between different experimental groups. For objective efficacy evaluation of anti-arthritic drugs in the model, quantitative disease parameters are needed.

The results of our study show that periods of active arthritis are characterized by substantial loss of body weight, increased plasma concentrations of C-reactive protein (CRP) and higher urinary excretion rates of the major collagen cross-links hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) present in the various joint tissues. The validity of these parameters as quantitative indicators of CIA, in particular of inflammation and joint destruction, is discussed.

MATERIALS AND METHODS

Animals

The rhesus monkeys (Macaca mulatta) that have been used in this study were housed at the Biomedical Primate Research Centre (Rijswijk, The Netherlands). All monkeys have been genotyped for class I (Mamu-A and -B) and II (Mamu-DR) antigens as described previously [11]. The essential data of all monkeys are summarized in Table I. CIA-susceptible monkeys were selected based on the lack of the MHC class I allele Mamu-A26 which seems to act as a dominant genetic marker for resistance to CIA in rhesus monkeys [12]. Notably, Mamu-A26 has no association with susceptibility or resistance to a non-related autoimmune disease like experimental autoimmune encephalomyelitis [13].

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Correspondence to: B. A. "T Hart, Department of Immunobiology, Biomedical Primate Research Centre, PO Box 3306, 2280 GH Rijswijk, The Netherlands.

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Monkeys in the experiment were observed daily for changes in behaviour, food intake and general appearance. When necessary for handling, the animals were sedated with 0.1 mg/kg ketamine (10 mg/ml; Chassot & Cie AG, Bern, Switzerland). During the studies, the monkeys received a standard diet of food pellets (Hope Farms) supplemented with rice and fruits or vegetables.

For the purpose of this study, new and historical data, collected over the past 9 yr, have been grouped to show a relationship between clinical evaluation, joint inflammation and destruction of joint tissues along with the histology of affected joints. The original literature references for animals which were part of previous studies are given in Table I in the last column. The monkeys in the table lacking a literature reference were positive for the dominant CIA-resistance marker Mamu-A26 are in bold.

**CIA induction**

CIA was evoked by immunization with 1–3 mg type II collagen (b-CII) from bovine hyaline cartilage. The arthritogenicity of each newly isolated batch was routinely tested in WAG/Rij rats (RT-1<sup>b</sup>) as described elsewhere [14]. b-CII was dissolved to a clear solution in 0.1 M acetic acid to 6 mg/ml and then emulsified in an equal volume of complete Freund’s adjuvant. Each monkey was injected intracutaneously on the back with 1 ml of the emulsion, which was distributed over 10 spots to reduce the formation of ulcerative skin lesions.

Medication during the experiment was given at the indication of the institute’s veterinarian. Anti-analgesic medication during the experiment was given at the indication of the institute’s veterinarian. Anti-analgesic medication during the experiment was given at the indication of the institute’s veterinarian.
medication consisted of an opiate (Temgesic), supplemented with acetylsalicylate (Aspegic). Medication for suppression of disease severity consisted of dexamethasone (mixed preparation of phenylbutazone and dexamethasone) or diadresone. In all monkeys, ulcerative skin lesions at the immunization sites were sprayed daily with acederm to prevent further contamination.

Disease parameters
Clinical evaluation. Apparent symptoms of arthritis, such as soft-tissue swelling and redness of affected joints, were diagnosed under the supervision of a trained veterinarian and scored on a modified version of the arbitrary scale published previously [5]: 0 = no disease symptoms; 0.5 = fever; 1 = apathy and loss of appetite, weight loss; 2 = weight loss, warm joints with pain, no apparent joint swelling; 3 = moderate soft-tissue swelling, but normal flexibility of affected joints; 4 = severe soft-tissue swelling with joint stiffness; 5 = such severe disease that euthanasia is necessary. Each time the monkeys were sedated for other purposes, they were weighed and the body temperature was recorded.

Inflammation. Serum or plasma CRP levels were determined at the diagnostic laboratory SSDZ (Delft, The Netherlands) using a turbidimetric standard test provided by Orion Diagnostica, Espoo, Finland. The assay uses an antiserum against human CRP and a human CRP reference standard.

Joint destruction. Urines were collected overnight with a fine-mesh covered tray placed under the cage of each individual monkey. After precipitation of contaminants by centrifugation, the clear urine samples were stored frozen at −20°C until analysis. In unhydrolysed urine samples, collagen cross-links were determined with reversed-phase high-performance liquid chromatography (HPLC) essentially according to Black et al. [15, 16]. After purification of urine samples by solid-phase extraction with CF1-cellulose, 3 ml of a 5 ml aqueous eluate were lyophilized and reconstituted in 1% (w/v) heptfluorobutyric acid (HFBA; 250 μl). After centrifugation, 50 μl aliquots were injected into the HPLC system, consisting of a Gynkotek pump (Model 300, delivering a flow of 1.0 ml/min), a Gastor GT-103 degasser, a Promis Autosampler, and a Jasco fluorometer (Model 821 FP, excitation and emission wavelengths set at 295 and 400 nm, respectively). All equipment was obtained from Separations Analytical Instruments, Hendrik Ido Ambacht, The Netherlands. The analytical C18-column (Tosohaas ODS-80TM, 4.6 × 150 mm; Bester, Amstelveen, The Netherlands) was equipped with a μBondapak C18 Guard-Pak column (Water-Millipore, Milford, MA, USA). Using a solvent select valve (Model SSV; Waters-Millipore, Bedford, MA, USA), the column was eluted for 15 min with 0.1% w/v HFBA containing 18% v/v methanol, followed by a 10 min column wash with 0.1% w/v HFBA containing 75% v/v acetonitrile. Fluorescence signals were converted to amounts of collagen cross-links with the Peakmaster 3 chromatography data system (Harley Systems Ltd, Bucks) based on peak heights. Collagen cross-links HP and LP were quantitated with a Pyd/DPD calibrator, obtained from Metra Biosystems Inc. (Mountain View, CA, USA) and expressed per mol creatinine.

Measurement of HP and LP in tissues. Tissue samples were hydrolysed in 6 N HCl at 110°C for 20 h. After hydrolysis and drying, samples were dissolved in water containing the internal standards pyridoxine (10 nmol/ml) and homoarginine (2.4 μmol/ml). The hydrolysates were analysed for their hydroxyprolines such as soft-tissue swelling and normal flexibility of affected joints; 4 = severe soft-tissue swelling with joint stiffness; 5 = such severe disease that euthanasia is necessary. Each time the monkeys were sedated for other purposes, they were weighed and the body temperature was recorded.

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per collagen molecule, assuming 300 hydroxyproline residues per triple helix.

**Histology**

At necropsy, the fingers and toes showing arthritic interphalangeal (IP) joints were cut off and fixed in 4% phosphate-buffered formalin. After fixation, the bones were decalcified for 3 weeks in Kristensen’s solution (17% formic acid in 1 m NaOH, pH 2.2). After a short wash in running tap water, the bones were trimmed longitudinally with a scalpel. After another wash in running tap water for 16 h, the bones were dehydrated in an ascending series of ethanol, followed by toluene and impregnation in liquid paraffin at 60°C. Subsequently, the fingers and toes were embedded in paraffin and cut into 2 μm sections. The sections were stained in HPS (haematoxylin–phloxin–safran) or Alcian Blue–PAS (periodic acid–Schiff).

**Statistics**

Statistical differences were determined with Student’s t-test (unpaired) using StatWorks (V1.2) for Macintosh computers, unless stated otherwise; P < 0.05 was considered significant. The correlation between CRP levels and the score for arthritis (Fig. 2C) was determined with the Spearman rank order correlation test.

**RESULTS**

**Body weight measurement as a parameter of CIA expression**

Four Mamu-A26-negative monkeys (BB115, BB103, BB106 and BB89) were immunized with b-CII in CFA and developed a monophasic polyarthritis. As could be expected from the fact that each rhesus monkey is genetically unique, a variable disease course is found

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**Fig. 2.**—Plasma CRP levels in CIA-susceptible and -resistant rhesus monkeys, and the relationship of CRP with CIA severity. Eleven monkeys were immunized with b-CII/CFA; six without (A) and five with the dominant disease-resistance marker Mamu-A26 (B). The CRP levels (mg/l) in venous blood plasma collected at the indicated time points were determined with routine diagnostic methods. Twenty-three monkeys, immunized with b-CII/CFA, were clustered based on the clinical diagnosis of their disease severity (C). The values of individual monkeys are given as dots, whereas the mean values per group are given with a bar. The numbers of animals in each group (n) are also indicated in the figure.

**Fig. 3.**—Effect of cyclosporin A treatment on plasma CRP levels. Eight monkeys were immunized with b-CII/CFA. Four of the monkeys were treated with cyclosporin A to suppress CIA (solid lines), 25 mg/kg/day s.c. between days 7 and 25 a.i. and 12.5 mg/kg s.c. between days 25 and 32. Four other monkeys were sham treated (dotted lines). At the indicated time points, the monkeys were weighed (A) and venous blood plasma CRP levels (B) were determined.
in individual monkeys (Fig. 1A). Figure 1B shows that each monkey loses substantial body weight during the development of CIA. Monkeys BB115 and BB103 showed early onset of the first disease symptoms and substantial weight loss between 20 and 40 days after immunization (a.i.). They were therefore characterized as early responders. Monkeys BB89 and BB106 developed arthritis at a much later stage, and were therefore characterized as late responders.

Remission of arthritis in rhesus monkeys is difficult to assess due to severe deformation of affected joints and because the natural dark red colour of the skin obscures the reddening of inflamed joints. The recovery of the body weight of monkeys BB115 and BB103 from day 40 a.i. points to disease remission. During the study period, no remission of the arthritis was observed in BB89, whereas the very severe disease in BB106 necessitated euthanasia before remission could occur.

**Serum CRP levels as a quantitative parameter of inflammation in CIA monkeys**

In clinical practice, CRP is a commonly used parameter to monitor periods of active arthritis in RA patients. In the following experiment, the questions are addressed: (i) whether serum CRP levels reflect the severity of arthritis and (ii) whether CRP as disease marker is sensitive to anti-arthritis therapy, in this case with cyclosporin A.

Figure 2A and B show plasma CRP levels measured at different time intervals after immunization with b-CII in six Mamu-A26-negative and five Mamu-A26-positive monkeys. It is shown that high CRP levels were confined to Mamu-A26-negative, CIA-susceptible monkeys (Fig. 2A). CRP values remained at the background level in Mamu-A26-positive, CIA-resistant monkeys (Fig. 2B). For a second analysis, 23 monkeys of the panel were clustered based on the clinical diagnosis of CIA severity made by an experienced veterinarian in the field following the criteria described in Materials and methods. The highest measured CRP values during the disease period in groups of animals with similar macroscopic disease symptoms are given in Fig. 2C. The results show that monkeys with a more severe disease course are characterized by higher CRP peak values.

We have reported previously that cyclosporin A, when administered well after immunization but before the onset of CIA, suppressed the expression of arthritis in four out of four monkeys [17]. Figure 3A shows the
FIG. 4.—Histopathology of proximal interphalangeal (PIP) joints in rhesus monkey CIA. The histology of the PIP joints of three arbitrarily chosen monkeys is shown. (A) A sagittal section of a healthy PIP joint (×10). (B) A section of the cartilage surface in (A) (×100). (C) The PIP joint of a monkey during the height of CIA, showing synovial hyperplasia and overgrowth of pannus (×40). (D) The active destruction of cartilage and subchondral bone in the same joint at a higher magnification (×100). In end-stage CIA when the inflammation has already waned, the cartilage is severely eroded (E; ×10), which is shown in (F) at higher magnification (×100). (G) shows that active remodelling of the subchondral bone takes place at that stage (×100). Arrows marked with ‘a’ point to two osteoclasts. Between the arrows marked with ‘b’ a row of active osteoblasts is visible, lining the bone. In (A) and (E), the cartilage has been intensely stained with PAS. (B), (C), (D), (F) and (G) are HPS stainings.

TABLE II
Cross-links per collagen triple helix

<table>
<thead>
<tr>
<th>Tissue</th>
<th>HP</th>
<th>LP</th>
<th>HP/LP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>0.142</td>
<td>0.037</td>
<td>3.8</td>
</tr>
<tr>
<td>Tendon</td>
<td>0.443</td>
<td>0.018</td>
<td>24.6</td>
</tr>
<tr>
<td>Synovium</td>
<td>0.474</td>
<td>0.034</td>
<td>13.9</td>
</tr>
<tr>
<td>Ligamentum</td>
<td>0.174</td>
<td>0.015</td>
<td>11.6</td>
</tr>
<tr>
<td>Cartilage</td>
<td>1.323</td>
<td>0.024</td>
<td>55.1</td>
</tr>
</tbody>
</table>

Note: Decreased body weight was observed (BB59). For the entire cyclosporin-treated group, body weights measured at the start of the experiment did not differ significantly from those measured later on (P > 0.05). Body weights of the sham-treated monkeys were significantly lower than those of the cyclosporin-treated monkeys (P < 0.01; time points > 14 days). Figure 3B shows that high CRP levels were found in the four control monkeys, whereas a high CRP level was found only at one time point in only one of four cyclosporin-treated monkeys (8719). Statistical analysis showed that CRP levels at time points 14, 28 and 42 days are significantly higher in the sham-treated group than in the cyclosporin-treated group (P < 0.02). Furthermore, CRP levels in the cyclosporin-treated group at time points 14, 28 and 42 days were not significantly elevated as compared to baseline values recorded at the onset of the experiment.
CRP levels mark periods of active CIA, (ii) the absolute CRP level reflects the severity of arthritis and (iii) CRP as a disease marker is sensitive to arthritis suppression with cyclosporin A.

**Histology of CIA-affected IP joints**

The most common manifestation of CIA in rhesus monkeys is inflammatory swelling of the PIP joints of fingers and toes. Involvement of the large joints, elbows and knees is not always observed macroscopically. At autopsy, however, synovitis and joint destruction can often be observed in joints that appeared unaffected macroscopically.

Figure 4 shows representative examples of the healthy IP joints of a non-arthritic monkey (A/B), a monkey killed during a period of active arthritis (C/D) and one killed after the active disease period (E/F/G). In a healthy joint (A), the synovium is inactive, the cartilage has a smooth surface and the bone marrow has a normal appearance (B). During active CIA, ingrowth of the synovium into the joint cavity is observed (C) with pannus formation overlying the cartilage, which then has a rough surface (D). In some areas, subchondral degradation of bone by cells that have probably infiltrated from the bone marrow is observed as well (D). In advanced CIA, the cartilage is almost completely lost (E, F) and active bone remodelling by osteoblasts and osteoclasts is taking place (G). Cells resembling osteoclasts (a) and osteoblasts (b) are indicated in Fig. 4G with arrows.

These data show that joint erosion is an important feature of CIA in rhesus monkeys. We have therefore investigated markers for cartilage and bone erosion in the disease model.

**Urinary excretion rates of collagen cross-links as a marker of joint erosion in CIA monkeys**

The collagen cross-links HP and LP are present in different relative quantities in joint tissues, such as bone, cartilage, tendons, etc. (Table II). The highest levels of cross-links were found in articular cartilage. In the cartilage, >98% of the pyridinoline cross-links consisted of HP; the HP/LP ratio was 55. In tendons, synovium and ligaments, an intermediate HP/LP ratio was found, between 10 and 25. The lowest HP/LP ratio, four, was found in bone. To test the assumption that urinary excretion of cross-links reflects joint destruction, four male monkeys, two *Mamu-A26*<sup>+</sup> were immunized with b-CII/CFA. Three monkeys proved susceptible (BB58, BB67, BB78) and one resistant (BB77). The first disease symptoms and weight loss were observed in BB58, BB67 and BB78 around 3 weeks after immunization and remission was observed from week 8. The CIA-resistant monkey BB77 remained asymptomatic throughout the test period (Fig. 5A).

The urinary excretion rates of the collagen cross-link HP had already increased in responder animals BB58, BB67 and BB78 early in the disease, namely in the third week after immunization (Fig. 5B; paired t-test comparing week 2 and week 3: *P* < 0.001).

Maximal excretion rates were observed around the fifth week in BB58 and BB78, and in BB67 in the eighth week, excretion rates being 8 to 16-fold higher than baseline values. Excretion rates of LP followed a
similar time course, although no significant increase was observed yet in week 3 (Fig. 5C). LP levels started to increase in the fourth week after immunization and reached a peak level in the sixth (BB58 and BB78) or eighth week (BB67), after which the levels of this established marker of bone turnover declined. The maximal increase in excretion rates of LP was significantly smaller (4- to 7-fold; mean 5.0 ± 1.3) than that of HP (8- to 16-fold; mean 12.3 ± 3.3; \( P < 0.05 \)). The larger increase in excretion rate of HP compared to LP also becomes apparent by analysing the change in HP/LP ratio with time. In the responder animals BB58, BB67 and BB78, HP/LP ratios from week 3 onward (12.2 ± 1.9) were significantly higher than values obtained during the first 2 weeks of the study (mean 5.3 ± 2.6; \( P < 0.001 \)). In the disease-resistant monkey BB77, no change in rates of cross-link excretion was observed throughout the study.

In a second experiment, the time course of plasma CRP levels and urinary excretion rates of HP was monitored in the three CIA-susceptible monkeys BB115, BB103 and BB89. The results in Fig. 6 show that in the two early responder monkeys BB115 and BB103 (see also Fig. 1) an early peak level of CRP was already found in the serum at \(~20–25\) days after immunization (Fig. 6A). At that time, a substantial weight loss was also recorded in these animals (Fig. 1). The peak levels of urinary HP excretion in these two monkeys were found 10 (BB115) or 20 (BB103) days later. In the late responder monkey BB89, a different situation was found, namely a gradual increase in the plasma CRP level and at the same time a gradual increase in the urinary excretion rate of HP. This observation suggests that in hyperacute CIA, inflammation is already found before substantial joint destruction takes place. In the more chronic progressing form of CIA, of which monkey BB89 is an example, inflammation and joint destruction may be more closely associated.

**DISCUSSION**

The rhesus monkey CIA model is of particular interest as a test system for the safety and efficacy evaluation of new therapeutic modalities based on human-specific biotechnology products for which rodent models are invalid, such as monoclonal antibodies, cytokines or cytokine antagonists [4]. To be able to assess the value of a new therapy and to compare its efficacy with existing ones, the effect on the disease process must be measured not only in a qualitative, but also in a quantitative, manner. A semiquantitative scoring system that is based on clinical diagnosis [5] has several limitations for the rhesus monkey. First, at autopsy, inflammation and cartilage erosion can be found in joints which appeared normal at clinical inspection. Second, the natural dark-red colour of the rhesus monkey skin obscures the reddening of inflamed joints. Hence, the onset of inflammation can only be estimated on the basis of warmth and swelling of the joint. For the same reasons, remission of the arthritis cannot only be estimated on the basis of warmth and swelling of the joint. In conclusion, there is a need for objective and quantitative parameters for monitoring the arthritis.

In this study, the validity of three parameters for different aspects of the disease was investigated, namely the body weight as a measure of general disease expression, plasma CRP levels to monitor inflammation and urinary excretion rates of collagen cross-links representing destruction of joint tissues.

**Body weight as a clinical marker in CIA**

In small rodent models, the onset of CIA is characterized by a substantial decrease in the body weight, whereas disease remission is associated with recovery of the body weight. The results of this study show that also in rhesus monkey the onset of CIA is associated with a decrease in body weight. It is shown that periods of substantial weight loss coincide with periods of high plasma CRP levels. When the inflammation subsides, as deduced from low plasma CRP levels, the body weight increases. In addition, when the arthritis was
suppressed with cyclosporin A, reduction of the body weight was also prevented. Such observations lead us to the conclusion that body weight measurement provides a sensitive indicator for the onset and remission of CIA.

**CRP as a marker of joint inflammation**

CRP is a marker of the acute-phase reaction which is commonly used in clinical studies in RA patients as a marker of active inflammation [18–21]. The results of the present study show that high CRP levels are confined to arthritic monkeys. A necessary condition for CRP as a quantitative disease marker in rhesus monkey CIA is that it should (i) reflect the disease severity and (ii) respond to anti-arthritic therapy. It is shown in this report that higher peak levels of CRP and C-reactive protein (CRP) are associated with more severe expression of arthritis. Furthermore, treatment with cyclosporin A for suppression of CIA clearly reduced plasma CRP levels.

**Collagen cross-links as markers of joint erosion**

Thus far, efficacy evaluation of experimental therapies on joint destruction in rhesus monkeys has been difficult to perform. As in RA patients, radiographic analysis of affected joints can be performed, but clear alterations can be found only in advanced stages of the disease. An additional problem is that the cartilage, which is the main target tissue of CIA, is not directly visible with radiography. Magnetic resonance imaging (MRI) is more suitable for direct visualization of the articular cartilage (personal unpublished observation), but this is a time-consuming and costly procedure, and not suitable for routine monitoring of large numbers of animals. Finally, the necessity to transport the monkeys to an MRI facility and the long sedation time during scanning are a serious inconvenience to the monkeys. Hence, we have searched for a more convenient and non-invasive method.

Several markers have been studied in synovial fluid as feasible indicators of joint destruction [22–24]. Whereas many of these parameters serve their purpose in synovial fluid, systemic monitoring of joint destruction (in blood or urine) is hampered by the fact that these markers are extensively metabolized, resulting in low or undetectable concentrations in serum or urine. The pyridinoline cross-links in collagen (HP and LP) do not show this limitation and represent degradation of mature extracellular matrix. We therefore focused on the urinary excretion rates of these cross-links as indicators of joint destruction.

The present study shows that the urinary excretion rates of the collagen cross-links HP and LP were substantially increased during active arthritis, but not in the Mamu-A26-positive animal that lacked arthritis. Pyridinoline cross-links are present in collagen proteins in mature bone, cartilage and other joint tissues (see Table II). Articular cartilage contains predominantly the cross-link HP. In bone, HP as well as LP are present in a characteristic ratio of 4:1. As such, urinary excretion of LP is considered the best available biochemical marker of bone resorption [25, 26].

Since type II collagen in cartilage contains 9-fold higher levels of HP than type I collagen in bone, one might speculate that urinary excretion rates of HP may (partly) reflect cartilage degeneration. Cross-sectional studies revealed that excretion rates of HP and LP are increased in patients with joint diseases such as rheumatoid arthritis and osteoarthritis [27–29]. However, the HP/LP ratio does not deviate from the normal value of about four. Thus, excretion rates of collagen cross-links in joint diseases are considered to be indicative of bone turnover and not of degradation of other joint tissues. In our longitudinal study, which has the advantage of having baseline values of cross-link excretion before the onset of arthritis, urinary excretion of HP increased twice as much as that of LP (Fig. 5B and C). Thus, in this animal model, about half of the amount of HP excreted in urine may be derived from bone. The other half probably results from degradation of other joint tissues, in particular the cartilage, which is almost completely eroded in advanced CIA (Fig. 4).

This was confirmed by evaluation of the HP/LP ratio. In the non-responder monkeys, the HP/LP ratio was around five throughout the experiment. In contrast, in the three responder monkeys, the initial HP/LP ratios of five doubled within 3 weeks to about 12. In addition, urinary excretion rates of HP are already significantly elevated at week 3 after immunization, which is earlier than excretion rates of LP start to increase. This is consistent with observations in rodent CIA models that cartilage erosion starts earlier than erosion of bone.

An important question in the recent literature is how closely inflammation and joint destruction are connected. It is generally assumed that joint destruction is initiated by synovitis, but becomes uncoupled at later stages of the disease [29–32]. Looking in more detail at inflammation (BB58 < BB67 = BB78) vs cross-link excretion (BB78 < BB67 < BB58), our data suggest that CIA inflammation and destruction are also uncoupled processes in rhesus monkey.

The main conclusion is that by monitoring, in addition to the clinical evaluation, the body weight, serum CRP concentration and urinary excretion of the collagen cross-link HP, the processes of joint inflammation and joint destruction can be described in quantitative terms. This adds substantial strength to the rhesus monkey CIA model as a test system for evaluating the efficacy of new therapies.

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