Histopathological Study of Arthritic Lesions Induced by Immunization with Type II Collagen in Lewis Rats

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(Received 30 April 1993/Accepted 16 July 1993)

Lewis rats were immunized with an intradermal injection of type II collagen and autopsied 40 days later for histopathological examination of the limb joints. An increased in paw volume in the hind limbs was observed from 11 days after immunization until autopsy. The joints of the hind limbs were more frequently and more severely affected than those of the fore limbs. The tarsal joints were especially affected, with a 100% morbidity rate, and more frequently exhibited the most advanced stage of lesions. —KEY WORDS: arthritic lesions, histopathology, rat, Type II collagen

The histopathological characteristics of collagen-induced arthritic lesions in animals are similar to those of rheumatoid lesions in humans, and are attracting interest as an animal model for evaluating the effects of anti-inflammatory or anti-rheumatoid drugs. Previously, we reported the histopathological characteristics of arthritic lesions and pathological evaluation of the effects of anti-rheumatoid drugs on collagen-induced arthritis in DBA/1J mice [8, 9].

However, systemic examinations taking into consideration the morbidity rate and severity of the collagen-induced arthritic lesions in rats have rarely been performed. The objective of the present study was to investigate the morbidity rate and characteristics of the articular lesions in Lewis rats.

Female Lewis rats at 4 weeks of age were purchased from Charles River Japan Inc. for use in the experiments. The rats were divided into two groups, immunized and control groups, with eight animals assigned to each group. At 6 weeks of age, the animals in the immunized group were intradermally injected with an emulsion of bovine type II collagen dissolved in 0.05 M acetic acid and incomplete Freund’s adjuvant (FIA) into the back (1.6 ml / head, containing 2 mg of collagen) under ether anesthesia. Control animals received an equal volume of FIA emulsion in 0.05 M acetic acid. All animals were autopsied 40 days after immunization following ether euthanization.

Paw volume: The paw volume of the hind limbs was measured with a plethysmometer (Unicom: Model TK-101), and was used as an indicator of swelling. The onset of swelling in hind limbs occurred from 11 days after immunization in the immunized group. The swelling increased rapidly until 2 weeks after immunization and thereafter plateaued until the time of autopsy, exhibiting a 1.4-fold increase compared with that of the control group (Fig. 1).

Histopathological examination: The limb joints of all animals were fixed in 10% buffered neutral formalin solution and decalcified in 5% formic acid. Paraffin sections were prepared for histopathological examination by routine
Arthritic changes were observed in all immunized animals and consisted of polyarthritis characterized by proliferative synovitis. However, morbidity rates of joints were not uniform. The finger, carpal, toe and tarsal joints of all animals were consistently involved, except for the right carpal joint of one animal (No. 001, Fig. 2).

The lesions were graded with reference to the grading system of Wooley et al. [18]. Namely, grade I is the early stage of exudation characterized by swelling and stratified proliferation of synovial lining cells (Fig. 3). Grade II is the advanced stage of proliferation of synovial lining cells with granulation formation manifesting as increased fibroblasts and capillaries (Fig. 4). Grade III indicates a more advanced stage of granulation tissue with destruction of bone or articular cartilage (Fig. 5). Grade IV, the most advanced stage, shows severe destruction of bone and articular cartilage and replacement by fibrous tissue, with narrowing or collapse of the joint cavity (fibrous ankylosis, Fig. 6).

The morbidity rate and the grade of each joint are shown in Table 1. The finger, carpal and elbow joints displayed early stage lesions mainly classified as grade I, while the toe and tarsal joints developed more or even the most advanced stages mainly classified as grade III or IV. The tarsal joints were especially affected, with a 100% morbidity rate, and 7 out of 8 rats (87.5%) had lesions of grade IV.

Collagen-induced arthritis has been produced in mice [4,7,11], rats [2,5,17] and
Table 1. The morbidity rate (%) of arthritis and histopathological characteristics of the lesions observed in each joint

<table>
<thead>
<tr>
<th>Joint</th>
<th>Finger&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Carpal</th>
<th>Elbow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
</tr>
<tr>
<td>Grade 0</td>
<td>58.3</td>
<td>54.2</td>
<td>54.2</td>
</tr>
<tr>
<td>Grade I</td>
<td>37.5</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>4.2</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Grade III</td>
<td>0</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Grade IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Joint</th>
<th>Toe&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Tarsal</th>
<th>Knee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Grade 0</td>
<td>0</td>
<td>0</td>
<td>4.2</td>
</tr>
<tr>
<td>Grade I</td>
<td>12.5</td>
<td>12.5</td>
<td>20.8</td>
</tr>
<tr>
<td>Grade II</td>
<td>12.5</td>
<td>6.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Grade III</td>
<td>12.5</td>
<td>25.0</td>
<td>16.7</td>
</tr>
<tr>
<td>Grade IV</td>
<td>62.5</td>
<td>56.3</td>
<td>45.8</td>
</tr>
</tbody>
</table>

<sup>a)</sup>: Three joints were examined in each finger and toe on each side, except for the first toe in which two joints were examined.  
L: Left joint. R: Right joint.  
: The highest morbidity rate of the most advanced stage.

monkeys [1]. The morbidity rate depends on the animal strain used, age at immunization, method of immunization, and amounts of collagen.

Rats of the Lewis, WFC and DA strains are highly sensitive to collagen-induced arthritis [6]. The optimal age of immunization is reported to be between 4 weeks [13] and 12 weeks [3], and the dose of collagen used varies from 0.2 mg/head [13] to 7.5 mg/head [12]. Moreover, it is known that even in animals of the same strain the morbidity rate varies with the breeder, breeding facility, infection, and stress [16].

Effective methods of producing collagen-induced arthritis in rats [10, 15] exist, but none of the methods thus far reported have induced arthritis in rats with the nearly 100% morbidity rate reported in mice [14].

In the present study, we succeeded in inducing arthritis at a 100% morbidity rate with a single immunization with 2 mg/head bovine type II collagen in female Lewis rats at 6 weeks of age. Although we cannot objectively determine the reason for the high morbidity rate in the present study, it is possible that the age at immunization, volume of collagen, and conditions in the breeding facility and our laboratory were optimal for inducing arthritis. The histopathological features of affected joints consisted of proliferative synovitis characterized by proliferation of synovial lining cells, infiltration of inflammatory cells and formation of granulation tissue with destruction and resorption of bone. These histopathological characteristics are similar to those of human rheumatoid arthritis.

Wooley et al. [18] evaluated arthritic changes histopathologically using a grading system, but did not mention differences in morbidity rates and severity among individual joints of the four limbs. With regard to the morbidity rate and the severity of changes in the joints examined in the present study, the joints of the hind limbs were more frequently and more severely affected than those of the fore limbs. In particular, the tarsal joints were affected at a 100% morbidity rate and more frequently displayed the most advanced stage of lesions. These observations support the
macroscopic finding reported by Takagishi [16] that collagen-induced arthritis in rats begins with swelling of the tarsus.

A previous histological study of arthritic lesions induced by type II collagen in mice [8] revealed that the knee joints were most frequently involved, whereas the present study in rats revealed that the tarsal joints were affected most frequently. Although the reason for such a discrepancy between rats and mice is obscure, it is of interest that the dominant site of the arthritic involvement differed between the two species.

We found that the tarsal joints were affected most frequently and exhibited the most advanced stage of collagen-induced arthritic lesions in rats. These findings are considered to be important for investigating pathogenesis and for histopathological evaluation of the effects of anti-inflammatory and anti-rheumatoid drugs on collagen-induced arthritis in rats.

References

II型コラーゲン感作 Lewis ラットの関節病変について

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Lewis 系ラットをII型コラーゲンで感作し、40日後
に四肢関節の病理組織学的検査を実施した。関節の腫
脹は全例に発現し、感作後11日から剖検時まで持続し
た。病理組織学的検査では、前肢と後肢の間に病変の
頻度および程度に差があり、特に足根関節では全例に
病変がみられ、末期病変が高い頻度で認められた。
Explanation of Figures

Fig. 3. Swelling and proliferation of synovial lining cells (arrow) in toe joint HE stain $\times 30$

Fig. 4. Infiltration of inflammatory cells and increased fibroblasts in knee joint HE stain $\times 160$

Fig. 5. Destruction of bone with granulation tissue in knee joint HE stain $\times 30$

Fig. 6. Fibrous ankylosis of toe joint HE stain $\times 15$