Histopathological Study of Arthritic Lesions Induced by Immunization with Type II Collagen in DBA/1J Mouse


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Eight male DBA/1 J mice immunized twice by intradermal injection of type II collagen were autopsied 12 weeks after the first immunization and analyzed for anti-type II collagen antibody level, and the limb joints were examined radiologically and histopathologically. Clinical onset of swelling and erythema in the limb joints occurred about 5 weeks after the first immunization and deformity of the limbs was observed in a few animals about 5 weeks later. Although there were marked individual differences, serum anti-type II collagen antibody levels were elevated in all animals. Histopathologically, the changes were similar to those seen in human rheumatoid arthritis and were characterized by proliferation of synovial lining cells, formation of granulation tissue with destruction of cartilage and subchondral bone, and ankylosis. Systematic examination of various joints of the fore-and hind-limbs revealed definitely that the sequence of arthritic lesions was not uniform. The knee joint was involved most frequently, but smaller joints such as the phalangeal joints were involved less frequently but exhibited severe changes. The significance of histopathological examinations in the evaluation of effects of anti-rheumatic drugs was discussed with reference to this model.

DBA/1J mouse is known to develop polyarthritis after immunization by intradermal injection of type II collagen [2, 4, 7, 16], and the resultant chronic polyarthritis is attracting interest as an animal model of human rheumatoid arthritis (RA). Recently this model has been also dealt with as an excellent tool for the evaluation of anti-rheumatic drugs [8, 9]. Histopathological examination of the limb joints in this model was carried out by Wooley et al. [19] and Holmdahl et al. [5]. Systematic examinations of various limb joints, however, have been rarely performed. The objective of this study was to investigate histopathological characteristics and the distribution of affected joints as a fundamental examination to evaluate the effects of anti-rheumatic drugs in this model and to correlate the morphological changes with serum anti-type II collagen IgG levels.

Materials and Methods

1. Animals and induction of arthritis : DBA/1J mice, 5 weeks of age, purchased from Jackson Laboratories (Bar Harbor, ME) were used. Eight animals immunized with type II collagen and the other 3 animals were remained untreated and served as controls. At 7 weeks of age, the animals in the immunized group were injected with an emulsion of type II collagen (100 μg/body) dissolved in 0.05 M acetic acid at 1mg/ml and an equal volume of complete Freund’s adjuvant under the skin at the base of the tail. The injection was repeated one week later. Control animals received the equivalent volume of complete freund’s adjuvant in 0.05 M acetic acid.

2. Clinical evaluation : The condition of each joint was grossly evaluated as follows : (Arth-
ritis score) 0: no swelling and erythema; 1: swelling and erythema of one interphalangeal joint; and 2: swelling and erythema in the entire area from the carpal or tarsal region to the periphery or swelling and erythema of two or more interphalangeal joints. The score for one animal was calculated as the total of the scores for all four limbs, and therefore the maximum score that any animal could receive was 8, and the minimum was 0.

3. Immunological examination: At the end of the test period all animals were sacrificed by decapitation and bled. After the blood was centrifuged, serum anti-type II collagen IgG levels were measured by enzyme-linked immunosorbent assay (ELISA). The 96-well micro-titer plates were coated with 100 µl of type II collagen (1 µg/ml in phosphate-buffered saline (PBS)), 100 µl of test serum was added, and the samples were incubated overnight at 4 °C. Then peroxidase-conjugated goat anti-mouse IgG was added. The amount of bound enzyme was estimated by addition of 100 µl of peroxidase-substrate, and the absorbance was measured at 410 nm using Titertek Multiscan MC (Flow Laboratories).

4. Radiological examination: After specimen fixation in 10% buffered neutral formalin solution, soft x-ray photographs of the limbs of some animals from both treated and control groups were taken.

5. Histopathological examination: All animals were autopsied 12 weeks after the first immunization, and the entire skeleton and major organs and tissues of each were collected. The limbs (right and left elbows, knees, carpal and tarsal bones, and fingers and toes) and the thoracic, lumbar and caudal vertebrae were decalcified with 5% formic acid. Paraffin sections were prepared by the routine method and histopathological examination was conducted. Histological lesions observed in the limb joints were graded in 4 steps according to the stage of disease.

Results

1. Clinical evaluation: In the immunized group, the clinical onset of arthritis was seen about 5 weeks after the first injection and all animals developed arthritis after a further one week (Fig. 1).

The mean values of arthritis scores in both groups are shown in Fig. 2. The arthritis score in the immunized group increased during the period from the clinical onset of arthritis to 10 weeks after the first injection and then plateaued near the 8-point level, which was the highest value. In a few animals deformity of the limbs was observed about 10 weeks after the first injection.

2. Immunological examination: Serum anti-type II collagen IgG was hardly detected in the control group. IgG was detected at high levels in all animals of the immunized group, however, there was considerable individual differences in anti-collagen IgG levels (Table 1).

3. Radiological examination: Soft x-ray photographs of the limb joints are shown in Fig. 3. In the animals of the immunized group, multiple changes were, for the most part, observed at the tarsal and metatarsophalangeal joints. Namely, the cortical bone at the epiphyses became thin and the surface of the joints exhibited irregular contours. Further-

Table 1. Comparison of arthritic lesions and serum anti-type II collagen IgG levels in individual mice

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>021</th>
<th>022</th>
<th>023</th>
<th>024</th>
<th>025</th>
<th>026</th>
<th>027</th>
<th>028</th>
<th>Correlation with IgG titer (Correlation coefficient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of affected joints</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>0.201</td>
</tr>
<tr>
<td>Total number of histological grades</td>
<td>23</td>
<td>20</td>
<td>27</td>
<td>24</td>
<td>31</td>
<td>25</td>
<td>22</td>
<td>35</td>
<td>0.540</td>
</tr>
<tr>
<td>Anti-collagen IgG titer (O.D. at 410 nm)</td>
<td>0.239</td>
<td>0.375</td>
<td>0.324</td>
<td>0.261</td>
<td>0.529</td>
<td>0.229</td>
<td>0.171</td>
<td>0.359</td>
<td>___</td>
</tr>
</tbody>
</table>

Note: In the case of fingers or toes, if any phalangeal joints in one leg developed arthritis, the finger or toe was judged as arthritic, and the histological grade of the finger or toe was designated to be equivalent to the most severe arthritic change at the phalangeal joints involved.
Fig. 1. Left hindlimb of an immunized animal

Fig. 2. Arthritis score of DBA/1J mice in both immunized and control groups

Fig. 3. Representative limb joints of DBA/1J mice in each group, soft X-ray photographs. Left: the forelimb of a control animal. Right: the hindlimb of an immunized animal. Destruction of the joint surfaces at the fourth metatarsophalangeal joint and joint deformity is observed (arrow).
Fig. 4. Stratified proliferation of synovial lining cells in the elbow joint (arrow) HE stain ×35

Fig. 5. Granulation tissue with destruction of cartilage and subchondral bone in the third metatarsophalangeal joint HE stain ×55

Fig. 6. Magnification of granulation tissue shown in Fig. 5. Fibroblastic cell (downward arrow), macrophage-like cell (upward arrow), neutrophil and mononuclear cell infiltration between these cells and absorption of the bone is indicated (arrowhead). ×220

more reactive osteogenesis was observed around the affected joints.

4. Histopathological examination: Arthritic changes in the limbs were observed in all animals of the immunized group. The lesions were graded according to the stage of disease. Namely, grade I is the early stage of exudation characterized by stratified proliferation of synovial lining cells on the medial surface of the articular capsule, and neutrophil and mononuclear cell infiltration in the synovium (Fig. 4). Grade II is the advanced stage of granulation consisting of proliferation of synovial lining cells, fibroblasts and capillaries, and destruction of articular cartilage and subchondral bone with neutrophil infiltration (Figs. 5 and 6). Grade III indicates a more advanced stage involving an increase in fibrous tissue and adhesion of granulation tissue (fibrous ankylosis, Fig. 7). Grade IV, the terminal stage of arthritis, involves cartilaginous or bony metaplasia of fibrous tissue (bony ankylosis, Fig. 8).

The arthritis is a "polyarthritis" and most of the joints (finger, carpal regions and elbows, or toes, tarsal regions and knees) of one leg were affected in the same way (Fig. 9). The morbidity rate of each joint in the forelimbs was about 50%. That in the hindlimbs was higher, and in particular the knee joint was consistently involved with a 100% morbidity rate (Fig. 10). As for the histological characteristics of the lesions however, lesions at the majority of phalangeal joints of both the forelimbs and hindlimbs showed a tendency to develop into grade III or IV, displaying ankylootic changes, while elbow and knee joints developed ankylootic changes only rarely and remained in the stages of granulation and destruction of subchondral bone.

The number of joints affected, total histological score and serum anti-type II collagen IgG levels are listed for individual animals in Table 1. In the animals with high antibody levels (#025 and #028), both the number of arthritic joints and the total histological score
Fig. 7. Joint narrowing of the tarsal joint. HE stain × 30

Fig. 8. Cartilagenous metaplasia (A) and bony metaplasia (B) in the tarsal joint. HE stain × 30
Fig. 9. The distribution of histopathological lesions in individual mice

Fig. 10. The morbidity rate of arthritis and histopathological characteristics of the lesions observed in each joint

Discussion

Polyarthritis induced by immunization with type II collagen (collagen-induced arthritis; CA) has been produced in rats [1, 3, 17], mice [2, 4, 7] and monkeys [10]. In mice, CA has been reported mainly in DBA/1J strain. Development of CA is considered to be closely associated with humoral and cellular autoimmunity to type II collagen [13, 14, 18]. In the present study, the coincidence of elevation of serum anti-type II collagen IgG and development of polyarthritis was recognized in all animals of the immunized group.

Histological changes were characterized by proliferation of synovial lining cells, formation of granulation tissue with destruction of cartilage and subchondral bone, and ankylosis. As for the histological changes in RA, it is known that the characteristics of the changes in the early stage are exudation in the synovial membrane, subsequent proliferation of synovial lining cells, and destruction of cartilage and proliferation of granulation tissue are marked in the advanced stages, and result finally in the development of ankylosis [6, 11, 12]. Thus the histological changes of CA in DBA/1J mice are essentially similar to those of RA.

Wooley et al. [19], who conducted histopathological evaluation of arthritic changes using a grading system, did not mention any differences in the severity and morbidity rates between individual joints of the four limbs. The present systematic examination revealed that most of the phalangeal joints developed ankylosis changes, but the elbow and knee joints, in contrast, did not do so. The difference was considered to be related to the size and morbidity of each joint. With respect to the distribution and severity of arthritic lesions, the joints of the hindlimbs were more frequently and more severely involved than were those of the forelimbs. These observations of the distribution of histological lesions supports the macroscopic findings of Stuart et al. [14].

With regard to the correlation between the...
number of affected joints, the total histological score and serum anti-type II collagen IgG levels in individual animals, no correlation between the morphological changes and serum IgG level was recognized. The inconsistency of these two parameters was believed to be derived mainly from the considerable variation in the serum anti-type II collagen IgG levels. Holmdahl et al. [5] also reported that no direct relationship could be found between any manifestation of disease detected during macroscopic observation over time and anti-type II collagen titers [5]. According to Stuart et al. [15] and Wooley et al. [20], in the manifestation of CA, it would seem that immune cells other than antibody-producing cells are the most critical.

Thus there were individual variations in serum anti-type II collagen antibody levels in DBA/1 J mice with induced arthritis, and these were not necessarily correlated with the manifestations and progress of the disease. Therefore using this experimental model, histopathological examination is considered to be most important for the assessment of anti-rheumatic drugs because the morphological changes observed are believed to be the cardinal and constant signs of this autoimmune-mediated disease.

References

Ⅱ型コラーゲン感作 DBA/1 Jマウスの関節病変について

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Ⅱ型コラーゲン皮内注射により感作した8匹の雄 DBA/1 Jマウスを12週後に剖検し、抗Ⅱ型コラーゲン抗体価の測定と四肢関節のX線学的および病理組織学的検査を実施した。感作後5週ごろから四肢関節の発赤腫脹が認められ、10週ごろには一部の動物で四肢の変形が観察された。血清抗Ⅱ型コラーゲン抗体価は個体差はあるものの全例とも著しい上昇を示した。病理組織学的検査では関節病変は滑膜細胞の増殖および関節軟骨および骨組織の破壊を伴う肉芽組織の形成と関節強直で特徴づけられ、ヒトの慢性関節リウマチの関節病変と類似していた。四肢の関節を系統的に観察した結果、関節の病変の進行状態には部位により相違があり、膝関節には最もしばしば病変が見られるが、最も激しい病変を呈するのは趾関節であることが明らかとなった。この関節炎モデルを用いた抗リウマチ薬の薬効評価法における病理組織学的検索の意義について考察した。