Connective tissue abnormalities in MRL/1 mice

J C W EDWARDS,¹ A COOKE,² A R MOORE,³ C COLLINS,⁴ F HAY,²
AND D A WILLOUGHBY³

From the ¹Department of Rheumatology, University College Hospital, London; the ²Department of Immunology, Middlesex Hospital Medical School, London; the ³Department of Experimental Pathology, St Bartholomew's Hospital Medical School, London; and the ⁴Department of Morbid Anatomy, University College Hospital Medical School, London

SUMMARY Pathological changes in the connective tissue of the limbs of MRL/1 mice are described. Focal infiltrates of polymorphs or large mononuclear cells, or both, were seen both in synovial lining and subcutaneous tissue. Infiltrates were associated with vasculitis in some cases. Deposits of amorphous material were seen in and around joints and in foot pads. The material was more particulate and refractile than typical 'fibrinoid' and showed a positive Feulgen reaction. It was not surrounded by palisading cells and when seen in synovial tissue was not usually associated with changes in synovial lining cells. No obvious difference was seen between intra-articular and extra-articular lesions. Lesions in subcutaneous tissue occurred exclusively in the foot pads. Lymphocyte infiltration was not prominent at any site and no follicle formation was seen. Of two colonies studied, only one showed a significant increase in lining cell numbers in synovial tissue. Exercised animals had a similar distribution and severity of disease to those of matched controls. All lesions described were distinguishable from non-specific inflammatory lesions in normal control mice and MRL/++ mice on assessment of unmarked sections. The relation between these connective tissue lesions and the changes found in human chronic synovitis is discussed.

Key words: rheumatoid arthritis, animal models.

Connective tissue lesions which have been compared with those seen in human subjects with rheumatoid arthritis, have been described in MRL/1 mice, by Hang and coworkers.¹ An association was found between joint lesions and the presence of serum rheumatoid factors in the MRL/1 mice studied. These findings suggest that the MRL/1 mouse is the first animal example of a spontaneous rheumatoid syndrome of the arthritis type which is sufficiently consistent to be useful for experimental study.

The present study describes the connective tissue findings in two colonies of MRL/1 mice. The sites and detailed structure of these lesions suggest a pathogenesis which has certain parallels with human

rheumatoid arthritis, but also certain important differences.

Materials and methods

Two colonies of MRL/1 mice were set up in separate laboratories. Mice in colony 1 were obtained from the Kennedy Institute, London and mice in colony 2 from OLAC Ltd. Animals were kept at a temperature between 20 and 25°C and fed freely with either expanded diet (colony 1) or Dixon's FFG(m) pelleted diet (colony 2) and unlimited water. All animals studied were killed at six months of age. The MRL/1 mice weighed approximately 25 g.

Five male and five female mice from colony 1 were examined. Twenty male mice from colony 2 were examined and these were divided into two groups of 10. One group was kept in standard animal house conditions throughout and the other group underwent an exercise programme for the last

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Correspondence to Dr J C W Edwards, Department of Rheumatology and Rehabilitation, University College Hospital, Gower Street, London WC1E 6AU.

month of life. The latter group were transferred to a separate cage for eight hours a day, five days a week. The cage was constructed of fine mesh (4 mm × 10 mm) chicken wire with sides four feet in height. Food and water were placed at the top of the cage. From previous pilot studies it was noted that animals in this cage spent their time either clambering on or clinging to the sides. None of the mice remained on the floor of the cage. The cage therefore provided a different mechanical environment requiring constant gripping of narrow gauge wire. After three weeks of exposure to the cage environment mice were noted to have considerable difficulty in climbing up, and particularly down the sides of the cage. Even at this stage, however, all but one out of 10 of the animals were clinging to the sides at any one time.

Six Porton white mice kept in the same animal house as colony 2 and four MRL/++ mice kept in the same animal house as colony 1 were examined for comparison both of inflammatory changes and articular tissue architecture.

Animals were killed by cervical dislocation under ether anaesthesia. Hind limbs were severed with bone cutters at mid-tibia and mid-femur and forelimbs were severed at mid-humerus and mid-ulna. Slits were made in the skin of the dorsum of hindfeet to aid penetration of decalcifying agent and fixative. Specimens were fixed in formal saline for at least 48 h and transferred to ethylenediaminetetra-acetic acid (EDTA) solution for at least four weeks. Specimens were trimmed after decalcification so that each joint could be embedded in a standard orientation (for sectioning in the sagittal plane). Paraffin sections were stained conventionally with haematoxylin and eosin for standard assessment. Selected sections were also stained with the Feulgen reagents, toluidine blue (pH 3.5), van Gieson's solution, Martius scarlet blue (MSB), phosphotungstic acid-haematoxylin (PTAH), and a polyclonal antifibrinogen antibody (linked to peroxidase).

Unmarked sections were examined independently by two observers who were not aware of the groups from which the tissue sections were derived.

Results

NORMAL AND MRL/++ MICE

Normal Porton white mice showed a variety of cell infiltrates in subcutaneous tissue overlying the olecranon and the anterior tibia and in foot pads. All animals showed loose clusters of polymorphs at one or more of these exposed areas. Two animals showed small clusters of mononuclear cells in foot pads, in one case surrounding a group of giant cells.

In none of these lesions were more than 50 cells seen together in each section. These findings were thought to be consistent with minor trauma, superficial infection, or foreign body reaction.

No definite intra-articular abnormalities were seen. The apparent thickness of the synovial lining cell layer varied greatly, with five or more layers of cells covering short sections. This was thought to be consistent with fortuitous tangential sectioning. Not more than a quarter of the synovial surface appeared covered by multiple layers of lining cells. The transitional zone between synovial lining and cartilage was often extensive, with fibroareolar tissue overlying large areas of hyaline cartilage. The synovial and hyaline cartilage surfaces, however, formed a smooth continuum. The periosteal surface of intra-articular bone was not necessarily composed of smooth compact cortical bone. Spongy bone extended to the edge at certain sites, so that the bone margin was tortuous and invaginated. These invaginations were filled with soft tissue, which was in places contiguous with bone marrow. These findings did not appear to indicate disease but simply normal murine tissue architecture.

MRL/++ animals were not distinguishable from normals. Two loose clusters of polymorphs were seen in one animal (anterior tibial region and foot pad). None of the normal or MRL/++ mice were mistaken for MRL/1 mice by either observer, on reading unmarked sections.

MRL/1 MICE: COLONY 1

Two types of lesion could be recognised by both observers as characteristic of MRL/1 mice on study of unmarked sections. The first consisted of an area of increased cellularity surrounding deposits of amorphous deeply staining material (referred to below as 'amorphous material') (Figs 1-4). The second type of lesion consisted of collections of large numbers of cells, usually polymorphs, but sometimes chiefly mononuclear cells, in fascial planes around nerves, muscle bundles, and elsewhere, in the absence of amorphous material (Figs 5 and 6). These lesions differed from inflammatory infiltrates in normal animals by site and the number and density of cells (more than 50 closely packed inflammatory cells). They were consistently identified on unmarked sections by both observers. Vaculitis was seen in association with both types of lesion (see below: 'Arteritis and vasculitis').

Five male and five female mice were examined in detail, with eight blocks per animal (pairs of hindfeet, forefeet, knees, and elbows). Nine out of 10 animals showed characteristic lesions. All nine animals with lesions had these in foot pads in one or more feet. All nine animals had lesions in the

majority of sections and five had lesions in all sections studied. The unaffected animal was female.

AMORPHOUS MATERIAL

The amorphous material found in the first type of lesion is shown in Figs 1–4. All nine animals with abnormalities showed this material at one or more sites. The material was present in circumscribed, slightly refractile, round or ovoid masses. It was much more deeply staining than the surrounding tissue matrix with haematoxylin and eosin (H and E). Uptake of the two stains varied considerably

Fig. 1 Amorphous material (A) in one of two colony 1 MRL/1 mice showing an increase in synovial lining cells (S). Scattered neutrophils are mingled with histiocytic cells in the nearby tissue. (H and E, $\times 70$).

Fig. 2 Amorphous material (arrowed) in a footpad from a colony 2 MRL/1 mouse. The material is associated with polymorph infiltration around a vessel (V). (H and E, $\times 285$).

Fig. 3 Basophilic amorphous material (A) in synovium from a colony 2 MRL/1 mouse. There are no lining cell changes (S) or lymphocytic infiltrates nearby. (H and E, $\times 285$).

Fig. 4 Amorphous material (arrowed) in a knee from a colony 1 MRL/1 mouse. In this section stained with toluidine blue the amorphous material gave a sky blue (non-metachromatic) colour and mast cells (M, ringed) were absent from the immediate vicinity. ($\times 70$).

with the colour ranging from deep pink through purple to blue. The material differed from typical fibrinoid in its variable basophilia and semirefractile margin. It gave a positive reaction with the Feulgen reagents, suggesting the presence of nuclear material. Staining with MSB gave a variable colour. Smaller fragments of the material stained red. (These were usually basophilic with H and E.) Large refractile ovoid masses (variable on H and E) stained yellow. At the periphery of large aggregates some of the material stained grey-blue. Staining

with PTAH gave a dull blue colour. Immunoperoxidase staining for fibrinogen failed to stain the amorphous material at all. Staining was seen, however, in association with synovial lining cells at sites where H and E stained sections showed eosinophilic matter consistent with fibrin. The material was not metachromatic with toluidine blue and was not birefringent.

These results suggest that the material varies in composition from place to place and probably includes a mixture of substances including nucleic acid.

Seventy one more or less discrete deposits of amorphous material were identified in 10 mice. Of these, 14 were chiefly surrounded by polymorphs, 11 chiefly by mononuclear cells, 26 by both cell types in similar proportions, and 20 showed no surrounding cell infiltrate. No giant cells were seen in the vicinity of amorphous material. Twenty three lesions were associated with vascular wall infiltration with leucocytes. Mast cells were conspicuously absent from the vicinity of amorphous material on toluidine blue staining (Fig. 4).

The large mononuclear cells seen in association with amorphous material were chiefly ovoid cells with extensive cytoplasm. The nuclear configuration varied from a pale open chromatin pattern with prominent nucleoli to a denser chromatin pattern without distinguishable nucleoli. A proportion of these large cells contained material in their cytoplasm with the same staining characteristics as the amorphous material. This strongly suggests that these cells are avidly phagocytic and may be regarded as 'macrophages'. There is a possibility, however, that the amorphous material represents the degraded cytoplasm of these cells and that the material arises within the cells. In some areas no free amorphous material was seen but there was a high density of large mononuclear cells with strongly staining cytoplasm as described above.

Cells of lymphoid origin were scarce in all types of lesion. Small round cells with densely staining nuclei made up less than 20% of cells, and there were no recognisable follicles.

The arrangement of cells around amorphous material was either as a loose sheet or as a rounded cluster. These two appearances may partly reflect the relation of the plane of section to tissue planes. In either case no radial elongation or 'palisading' of cells was seen. No fibrous outer coating to the lesion was seen.

Lesions including amorphous material occurred repeatedly in two sites. Subcutaneous lesions were exclusively seen on the undersurface of the feet (Fig. 2). They did not occur in skin on the dorsum of the foot, or in skin overlying the calf, forearm, knee, or

elbow. Most characteristically, lesions were seen in the foot pads (soft protuberances at the base of the toes consisting of fibroareolar tissue).

Deeper lesions were seen within joints and between bone and muscle in close proximity to joints (Figs 1, 3, and 4). Other than in foot pads amorphous material was not seen more than 1 mm away from joints.

Mast cells are numerous in mouse connective tissue (approximately 5% of cells). Sections stained with toluidine blue showed mast cells to be conspicuously absent from the vicinity of amorphous material (Fig. 4). The significance of this finding is unclear.

ISOLATED CELL INFILTRATES

Cellular infiltrates in the absence of amorphous material were seen in all nine animals showing abnormalities. These infiltrates occurred chiefly in planes of fibroareolar tissue and within and around muscle (Figs 5 and 6). Perineural collections were common but may simply have reflected the presence of nerves in most sheets of connective tissue in mice. Pure collections of polymorphs occurred as well as circumscribed lesions in muscle and connective tissue planes. In contrast, collections of mononuclear cells tended to be diffuse and extend with variable cell density over large tracts of areolar tissue.

ARTERITIS AND VASCULITIS

Three animals showed major arteritis of either brachial, popliteal, or a major tributary of the popliteal artery. The arterial wall was grossly thickened and contained dense collections of leucocytes, most of which were polymorphonuclear (Fig. 7). Some vessels showed fibrinoid material. Throm-

Fig. 5 Polymorph infiltrate between nerve (N) and muscle (M) in a colony 2 MRL/l mouse. (H and E, $\times 140$).

Fig. 6 Necrosis of muscle in a colony 2 MRL/1 mouse showing the nuclear chains of regenerating muscle cells (arrowed). (H and E, $\times 285$).

Fig. 7 Arteritis of a popliteal vessel in a colony 1 MRL/1 mouse showing a mixed cell infiltrate (I) in the vessel wall and fibrinoid necrosis (F). (H and E, $\times 70$).

bosis was not seen in major arteries and intimal changes were slight.

Vasculitis of smaller vessels was seen in association with cell infiltrates as described above. Vessel walls were infiltrated with polymorphs. Vessels were surrounded by pyknotic and fragmented cells. In some areas there was necrosis of vessel walls, intraluminal thrombus formation, and recanalisation.

Focal disease was found in skeletal muscle, including fibre necrosis, with evidence of regeneration, infiltrates of polymorphs between fascicles, and vasculitis within the muscle. These changes in

skeletal muscle do not appear to have been reported previously. They are probably consistent with the degree of vasculitis present (Figs 5 and 6).

MRL/1 MICE: COLONY 2

Pathological changes in the second colony of mice were similar to those in the first. Local cell infiltrates, amorphous deposits, and arteritis were all seen. Typical lesions were seen in 16 out of 20 animals. (The proportion is not directly comparable with colony 1 since only one block per animal was examined rather than the eight joints examined for colony 1 animals.)

Comparison of exercised and non-exercised animals (10 in each group) showed no significant difference in the type or degree of abnormality.

ARTICULAR TISSUES

The appearances described above were seen both in subcutaneous and articular connective tissues. Certain other features which were specific to joint tissues are described below. Although some animals showed swelling of hindfeet, this proved to be due to subcutaneous disease. The synovial lesions described below were not associated with macroscopic signs.

APPEARANCES OF MRL/1 MOUSE JOINTS

Colony 1

Areas of amorphous material and cell infiltrates as described above occurred in many joints. Synovial tissue appeared otherwise normal in all but two joints. The appearance of synovial lining cells could not be distinguished from that of normal mice on unmarked sections except in the two cases where one or more lesions with amorphous material was present and involved the surface cell region. In these cases a mixture of cells and fragmented eosinophilic matter was seen free in the joint cavity. There was a generalised increase in lining cell numbers and size (Fig. 1). Amorphous material occurred chiefly in folds of loose areolar tissue at variable depths from the tissue surface.

No significant bone erosion could be recognised by either observer, though the irregular outline of intra-articular bone in normal mice made assessment difficult. Cartilage surface abnormalities were seen in four joints (out of 80 blocks). In one case soft tissue (pannus) was seen to lie over the physiological cartilage surface in an abnormal fashion (Fig. 8). Incomplete lacunae at the cartilage-pannus junction suggested recent destruction of cartilage matrix. In other cases fronds of fibrous soft tissue were seen adherent to or arising from the cartilage surface. These contained amorphous material but few cells.

Colony 2

In contrast with colony 1, an increase in the numbers of synovial lining cells was seen in joints and tendon sheaths in colony 2 (Fig. 9). Of the 20 animals studied, increased lining cell numbers were seen in 13, chiefly in knee joints and sporadically in tendons and joints in the hindfeet. This increased cellularity was only directly associated with amorphous material in one case (the knee of a non-exercised animal). In other cases the synovial tissue was either normal or contained a diffuse infiltrate of polymorphs in deeper zones.

Pannus was seen in two knee joints in colony 2,

Fig. 8 Erosion of cartilage by pannus in the knee of a colony 1 MRL/l mouse. Pannus has grown over the physiological cartilage surface (arrowed) and invaded a lacuna (L). (H and E, $\times 285$).

Fig. 9 Gross hypercellularity of tenosynovium in a colony 2 MRL/l mouse. No clear distinction is seen between synovial lining cells (S) and deep (D) cells. (H and E, $\times 285$).

Fig. 10 Synovial tissue (ST) overlying cartilage (C) in a colony 2 MRL/l mouse knee. The physiological cartilage surface is still visible (arrows). Chondrocytes show pairing and eroded lacunae. (H and E, $\times 285$).

consisting of soft tissue overlying cartilage on normally bare articular surfaces, with an irregular cartilage-soft tissue interface and invasion of lacunar walls (Fig. 10).

Discussion

These observations confirm the original findings of Hang *et al*¹ in that widespread connective tissue lesions have been found in MRL/l mice. Some of these lesions are subcutaneous and others are intra-articular. Abnormalities of cartilage have also been found. Many of the lesions seen in the present study are very similar to those illustrated by Hang *et al*.¹ This suggests that the MRL/l mouse is a consistent model of spontaneous polyarthritis in association with genetically determined autoimmunity.

Comparison of the results from the two colonies suggests that a similar general pattern of disease may be expected in colonies in different laboratories, but that individual features may vary. The occurrence of increased synovial lining cell numbers in one colony and not the other indicates the importance of matched controls in any study of these animals.

Despite the fact that the lesions described occur at sites which might be expected to be exposed to high mechanical stress, the exercise programme did not alter the pattern of disease. Any relation between mechanical forces and localisation of connective tissue lesions in these animals must therefore remain uncertain.

The combination of rheumatoid factor production and polyarthritis with an immunogenetic basis suggests a parallel with the pathogenesis of human

rheumatoid arthritis. The two types of lesion most specifically associated with serum rheumatoid factors in the human are chronic, often erosive, synovitis, and nodular foci of fibrinoid necrosis surrounded by radially arranged connective tissue cells.² Although nodular lesions chiefly occur at subcutaneous pressure points, they may coexist with synovitis, especially in the walls of bursae and occasionally within joints.³

The lesions described above in the MRL/1 mice resemble the nodular lesions seen in the human to the extent that there is an accumulation of amorphous material in the tissue which is suggestive of some form of cell necrosis. The amorphous material in human rheumatoid nodules, known as fibrinoid, is a mixture of a number of macromolecules, including collagen, fibrin, immunoglobulin, phospholipid, and cholesterol, together with cytoplasmic and nuclear debris.⁴⁻⁶ The material deposited in the tissues of MRL/1 mice may have a similar mixed composition, but the basophilia of at least some of the deposits suggests a much larger component of nuclear material. Immunochemical staining suggests that fibrin is not present in significant amounts. More specifically, the cellular reaction is quite different. The typical structure of the human nodule with radially arranged cells is not seen.

The lesions seen in MRL/1 mice in this study also differ from those of human rheumatoid disease in that no lymphoid aggregates are present. The abnormalities seen in our laboratories suggest that the MRL/1 mouse is not a model for in situ rheumatoid factor formation within synovial tissue.⁷ It is possible, however, that rheumatoid factor manufactured in lymphoid tissue binds either in the circulation or in the connective tissues, and the complexes thus formed contribute to the local connective tissue lesions.⁸ This may parallel at least part of the role of rheumatoid factors in the human.

In summary, these findings suggest that MRL/1 mice develop connective tissue abnormalities with certain similarities to those of human rheumatoid arthritis (cartilage erosion in particular), but also with important differences. These animals are known to show a very wide spectrum of autoimmune disease, including nephritis, vasculitis, and lymphoid infiltrates in salivary glands.⁹⁻¹¹ The contribution to disease made by rheumatoid factors and rheumatoid factor containing immune complexes in these animals remains unknown.

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