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journal or publication title	Annals of the Rheumatic Diseases
volume	66
number	2
page range	242-245
year	2007
URL	http://hdl.handle.net/10097/51627

doi: 10.1136/ard.2006.054999

CONCISE REPORT

A non-major histocompatibility locus determines tissue specificity in the pathogenic process underlying synovial proliferation in a mouse arthropathy model

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Ann Rheum Dis 2007;66:242–245. doi: 10.1136/ard.2006.054999

Background: The incidence and characteristics of spontaneous ankylosis in the ankle of specific F₁ mice descended from two *Fas*-deficient strains were reported. Here the coincidence of synovial proliferation and ankylosis in the descendent F₂ mice is reported.

Aim: To clarify whether the two distinct manifestations are genetically different.

Methods: An arthropathic group of mice (MCF₂) were bred by intercrossing MRL/Mp.*Fas*^{pr}-*sap*⁻/*sap*⁻ and C3H/He.*Fas*^{pr} mice. All mice were killed by bleeding under anaesthesia when they were 6 months old. Pathological grades for synovial proliferation were determined by microscopical examination. To obtain a linkage locus, the whole genome of male MCF₂ mice was scanned by using 73 microsatellite markers.

Results: Synovial proliferation was equally observed in male and female MCF₂ mice. No correlation was observed between the grades of synovial proliferation and the ankylosis occurring in the MCF₂ mice. A suggestive susceptibility locus was shown in the middle of chromosome 11. This locus was an MRL allele with a recessive inheritance mode.

Conclusion: The pathogenic mechanisms of synovial proliferation and ankylosis are genetically different. The present locus is overlapped with some loci associated with rheumatoid arthritis and with others associated with experimental arthritides.

An inflammatory joint disease is usually recognised as an admixture of destruction and proliferation of the joint components. An imbalance of such counterprocesses may result in a pathological remodelling of joint structures giving rise to a unique clinicopathological outcome of the disease. Rheumatoid arthritis is characterised by synovial proliferation and erosion of joint cartilage due to chronic inflammation. The remodelling process in rheumatoid arthritis is associated with the contraction and destruction of joints. On the other hand, an ankylosing disease, another type of inflammatory joint disease, involves a distinct remodelling process causing joint ankylosis. Although the remodelling process for ankylosis has not been completely characterised, recent studies using rodent ankylosis models have evaluated the pathological proliferation of a particular region, where a joint capsule or ligament attaches to a bone.^{1–4} The histological characteristics are proliferation, cartilage formation and, subsequently, replacement of cartilage by bone, a process typical of endochondral bone formation. The process is called ankylosing enthesitis. A comparative study on the distinct joint remodelling processes may shed new light on the distinct and essential mechanisms of rheumatoid arthritis and ankylosing diseases.

We recently reported a mouse model with spontaneous and male-predominant onset of progressive ankylosis in ankle joints.⁵ It should be noted that this joint ankylosis occurred in a particular F₁ generation of mice, which descended from two

ankylosis-free strains—MRL/Mp.*Fas*^{pr}-*sap*⁻/*sap*⁻ (previously denoted as MRL/rpl)⁶ and C3H/He.*Fas*^{pr} (C3H/lpr). The MRL/rpl strain spontaneously arose from a lupus-prone MRL/Mp.*Fas*^{pr} (MRL/lpr) strain. It is noteworthy that the mutation on signalling lymphocyte activation molecule (SLAM)-associated protein (SAP) attenuated the development of autoimmune diseases as seen in MRL/Mp.*Fas*^{pr} (MRL/lpr) mice.⁶ These mice develop spontaneous arthritis characterised by synovial proliferation; therefore, they are considered as a model for rheumatoid arthritis. The onset of ankylosis in (MRL/rpl × C3H/lpr) F₁ mice was unexpected. A defect of SAP or a coupling of two predisposed genes descended from the two parental strains was a possible interpretation for this onset. To deal with this issue, we performed a linkage analysis using (MRL/rpl × C3H/lpr) F₂ (MCF₂) mice and arrived at two genetic mechanisms for the onset of ankylosis in these mice: the dissociation of the SAP defect and association of the single locus on chromosome 7 with the onset of ankylosis.⁵ Although the question is not completely answered, the results provided an opportunity to characterise a non-major histocompatibility complex (MHC)-linked locus associated with the pathogenesis of ankylosis.

In this study, we observed sporadic incidences of synovial proliferation and ankylosis (enthesal proliferation) in MCF₂ mice. The relationship between the pathogenic mechanisms of synovial and enthesal proliferations has been discussed. We provide evidence that the two distinct joint manifestations independently develop and are controlled differently by distinct genetic loci.

METHODS

Mice

C3H/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) and bred under specific pathogen-free conditions in the Animal Research Institute, Tohoku University Graduate School of Medicine, Sendai, Japan. MRL/rpl, MCF₁ and MCF₂ mice were housed in the same condition. In all animal experiments, we observed the Tohoku University guidelines for animal experimentation.

Histopathological grading of ankylosis

All mice were killed at 6-months of age under ether anaesthesia. Ankle joints were fixed in 10% formalin buffered with 0.01 M phosphate (pH 7.2), decalcified in 5% formic acid and 5% formalin and embedded in paraffin wax. Tissue sections were stained with haematoxylin and eosin. The pathological grade of the synovial proliferation was determined according to the following criteria: normal (grade 0), multiplication of synovial lining layer (grade 1) and grade 1 with villous

Abbreviations: MCF₂, (MRL/rpl × C3H/lpr)F₂; SAP, signalling lymphocyte activation molecule-associated protein; SLAM, signalling lymphocyte activation molecule

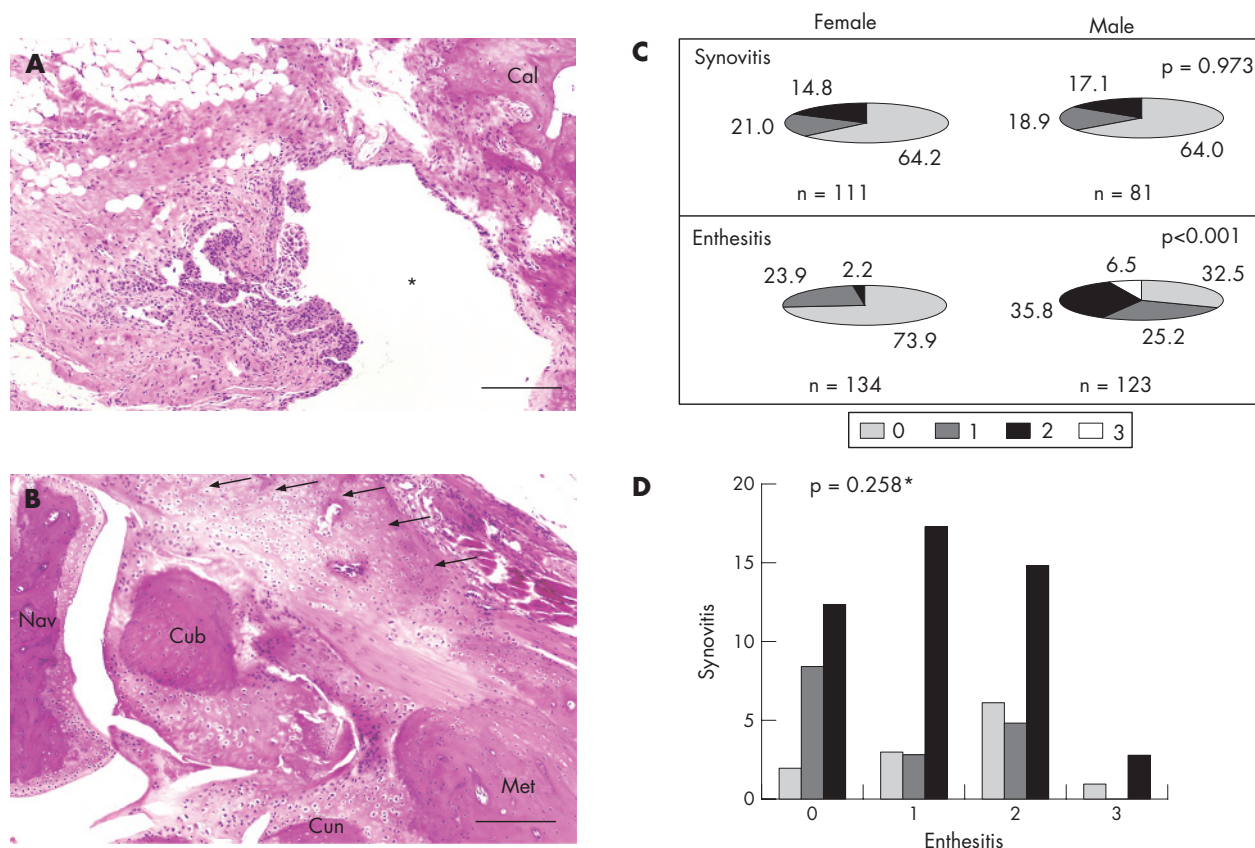


Figure 1 Specific manifestations of the synovitis or enthesitis developed in MCF₂ mice. Representative microscopic manifestations of synovitis (A) and enthesitis (B) in aged MCF₂ mice. Asterisk in (A) indicates a synovial cavity; arrows in (B) indicate the enthesial proliferation of ankle-associated tendon sheath (haematoxylin and eosin). Bars indicate 200 μ m. (C) Percentage distribution of grades of synovitis and enthesitis in aged MCF₂ mice. The number of the mice analysed and p values provided by the χ^2 test are indicated. (D) The bar graph indicates the number distribution of grades of enthesitis and synovitis in the 81 male MCF₂ mice. No correlation is shown between the onsets of synovitis and enthesitis. p Value was determined by Fisher's exact test with standard 3 \times 4 contingency matrices. Cub, cuboidal bone; Met, metatarsal bone; Nav, navicular bone; Cal, calcaneus; Cun, cuneiform bone.

proliferation (grade 2). The grade of an individual mouse was defined as a maximal grade determined in six longitudinal sections of bilateral hind paws. Samples inappropriate for microscopical examination, mainly because of the absence of synovium in accountable sections, were precluded from further analysis. The number of female and male MCF₂ mice accounted in this study was 111 and 81, respectively. The grade of ankylosis was determined previously.⁵

Genomewide screening for susceptibility loci to synovial proliferation

We searched association loci in the whole genome using 81 male MCF₂ mice and 73 microsatellite markers, which were all able to distinguish the parental genotypes and provided a

coverage of the mouse genome with an average distance of 23.3 cM apart. The genetic positions of microsatellite markers and genes were determined on the basis of the Mouse Genome Informatics provided by The Jackson Laboratory.

Statistical analysis

A correlation between the grades of synovial proliferation and ankylosis was evaluated by Fisher's exact test with standard 3 \times 4 contingency matrices. An association of the onset of synovial proliferation and the genotype at each marker position was evaluated by the χ^2 test with standard 2 \times 3 contingency matrices. A significant or suggestive association was estimated by $p < 0.000052$ or 0.0016 , respectively, as recommended by Lander and Kruglyak.⁷

Table 1 Association of microsatellite genotype and the incidence of synovitis in MCF₂ male mice

Marker	Position (CM)	Incidence of synovitis						χ^2 test	p Value
		Negative			Positive				
		MM	MC	CC	MM	MC	CC		
D11Mit71	1.1	13	25	14	7	16	6	0.48	0.79
D11Mit263	55.6	9	32	11	17	8	4	14.8	0.001
D11Mit334	68	11	29	12	12	13	4	3.92	0.14
D11Mit338	75	10	30	12	11	12	6	3.51	0.17

Genotypes MM, MC and CC indicate MRL/MRL homozygote, MRL/C3H heterozygote and C3H/C3H homozygote, respectively.

RESULTS

Incidence of synovial proliferation

In addition to the onset of ankylosis, we observed a thickening of the synovial lining layer, with mild lymphocytic infiltration and proliferation of small vessels in MCF₂ mice (fig 1A,B). Villous formations were evident in the advanced stage of the synovial proliferation. Neither lymphoid aggregation in the synovial lesion nor bone erosion with pannus-like formation was detected in all the mice examined. The microscopical incidence of synovial proliferation was 12/25 (48%) in MRL/rpl, 12/26 (46.2%) in C3H/lpr, 14/69 (20.3%) in MCF₁ and 69/192 (35.9%) in MCF₂ mice. The synovial lesion was detected equally in male and female MCF₂ mice ($p=0.973$) (fig 1C). No significant correlation was obtained between the progression of ankylosis and synovial proliferation in male-only ($p=0.258$, fig 1D) and all MCF₂ mice (data not shown).

Mapping of susceptibility loci associated with synovial proliferation

In this mapping study, we analysed the same MCF₂ mice that were used in the previous mapping study on ankylosis. The genomewide search using 81 male mice suggested a single marker *D11Mit263* (55.6 cM) on chromosome 11 that was significantly associated with the onset of synovial proliferation ($p<0.001$; table 1). The incidence of synovial proliferation in the MRL homozygote group (17/26, 65.4%) in association with *D11Mit263* was higher than that in the MRL/C3H heterozygote (8/40, 20%) and C3H homozygote (4/15, 26.7%) groups. This indicates that this MRL-derived locus regulates the onset of synovial proliferation in a recessive inheritance manner. No significant association was observed between synovial proliferation and any marker on the X chromosome where the SAP gene exists.

DISCUSSION

Recent studies have reported several mouse models with spontaneous ankylosis. DBA/1 is a spontaneous ankylosis model and an induced synovitis model with immunisation of type II collagen. The two DBA/1-related arthropathies develop similarly in a male-predominant fashion, although they are histopathologically different. It has been of particular interest to determine whether the pathogenic mechanisms of the distinct arthropathies in the DBA/1 mice are overlapped.

A cross mating of the DBA/1 strain with a particular lupus-prone strain such as BXSB or MRL/Mp resulted in an acceleration of the onset and increase in the severity of ankylosis in F₁ mice.²⁻⁴ These facts may suggest a correlation between lupus susceptibility and the pathogenesis of ankylosis. The MCF₂ mice used in this study also shared lupus-prone and synovitis-prone genetic backgrounds with MRL/lpr mice to varied extents. Previous studies have shown some MRL alleles significantly linked to the onset of spontaneous synovitis in the *lpr* constraint.⁸⁻⁹ Therefore, there is a reason for the onset of synovitis in MCF₂ mice. This study showed the onset of synovitis in MCF₂ mice, although inflammation and erosive changes were present to a lesser extent than those in the original MRL/lpr mice. We thought that this was a countereffect of ankylosis on synovitis; however, this notion turned out to be erroneous. This may be partly due to the difference in bleeding condition and genetic effects derived from the C3H/lpr strain. Our results totally revised these considerations and enforced a notion that proliferating enthesitis entail specific mechanisms for the development of ankylosis.

Our results suggest a susceptibility locus in the middle of chromosome 11. As the number of markers and samples were not sufficient to detect complex loci, some loci may have remained undetected. However, the detected locus has a major

effect on the development of synovial proliferation. This locus contains several genes that are highly conserved in a particular interval of human chromosome 17. To date, a high-resolution linkage and association mapping study identified a susceptibility locus for rheumatoid arthritis in this region.¹⁰ The central part of mouse chromosome 11 also contains several loci associated with experimental autoimmune encephalomyelitis,¹¹⁻¹² collagen-induced arthritis¹³ and proteoglycan-induced arthritis.¹⁴ This region is noteworthy when considering the genetic predisposition of rheumatoid arthritis.

A previous study using a larger number of backcross mice prepared from the same parental strains of MRL/Mp and C3H/He indicated that the five linkage loci are associated with the onset of arthritis.⁹ None of these loci overlap with the present linkage region. This discrepancy is possibly due to a difference in the mouse generation used (N₂ or F₂), a difference in the housing condition or a suppressive effect of the Y chromosome derived from C3H/lpr as discussed previously while purporting a possible reason for the onset of new cases of ankylosis.⁵

ACKNOWLEDGEMENTS

We thank Ms N Yamaki, Dr N Tanda and Dr H Umeda for technical assistance, Mrs N Fujisawa for secretarial assistance and Dr M Nose for critical comments.

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Funding: This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan to SM (number 15390607) and MO (number 16390113).

Competing interests: None.

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Accepted 10 July 2006

Published Online First 25 July 2006

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Ann Rheum Dis 2007 66: 242-245 originally published online July 25, 2006
doi: 10.1136/ard.2006.054999

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