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ORIGINAL ARTICLE

Indirect Immunofluorescence Screening of Potential Arthritic Autoantibodies in Systemic Lupus Erythematosus

Malarvili Selvaraja¹, Che Ku Dahlan Che Ku Daud², Marsitah Abdul Jalil⁴, Anim Md. Shah³, Syafinaz Amin Nordin¹, Zamzarina Ahmad Bajari⁴, Chong Chou Min⁵, Maha Abdullah⁴

- ¹ Department of Medical Microbiology & Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
- ² Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
- Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
- Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
- ⁵ Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

ABSTRACT

Introduction: Joint involvement is common in systemic lupus erythematosus (SLE) patients, however, screening for joint specific autoantibodies in patients is not routinely performed. This may be due to the lack of known antigens and available tissue. The rat musculoskeletal tissue may be a suitable source of antigen to detect arthritic autoantibodies. **Method:** We tested plasma of SLE patients, with arthritis (N=9) and without arthritis (N=7) as well as plasma from normal individuals (N=7) on fresh sectioned tissue from rat plantar hind paw using indirect immunofluorescence method. **Results:** Binding of autoantibodies to striation in skeletal muscle cells in the tissue was clearly demonstrable in all samples from SLE with arthritis but not on slides incubated with plasma from normal or SLE without arthritis. **Conclusion:** Thus, rat plantar tissue may be suitable for detecting autoantibodies from SLE patients that may be involved in the pathogenesis of lupus arthritis.

Keywords: Autoantibodies, Lupus arthritis, Immunofluorescence

Corresponding Author:

Maha Abdullah, PhD Email: maha@upm.edu.my Tel: +603-89472375

INTRODUCTION

Arthritis is a general term for conditions that affect the joints and surrounding tissues induced by inflammatory processes leading to severe pain. There are more than 100 different forms of arthritis which are initiated by various factors. The most common arthritis are osteoarthritis and rheumatoid arthritis (RA). RA as well as systemic lupus erythematosus (SLE) are autoimmune diseases both presenting with symptoms of arthritis. There are similarities between arthritis in SLE and RA but these are unquestionably two different diseases. Severe erosive arthritis (EA) develop in the majority of RA but EA is only observed in 5% of SLE patients (1). Nevertheless, joint pain is one of the most common reasons for initial clinical presentation in SLE patients (2).

Autoimmune diseases are associated with autoantibodies

of which some are pathogenic. SLE has more than 100 different autoantibodies that target various cells and antigens of the body. Several auto-antigens associated with arthritis include nucleolar RNA helicase (Gu) protein seen in 18% SLE; RA33 in hnRNP complex associated with erosive arthritis reported in 5% of SLE; Ki [probably identical to sicca lupus (SL) system] found in 6-21% SLE; transfer RNA (tRNA) found in 3.5% SLE associated with non-erosive arthritis; FcyR (24-50% of SLE) associated with osteoarthritis and collagen (C) type I-VI highly prevalent in 15-85% SLE and possibly associated with deforming arthritis. Among cytoskeletal antibodies, antivimentin is most frequently detected (36-75%) in SLE and associated with arthralgia (3). However, screening for arthritic autoantibodies are not routinely performed.

Animal disease models are important to understand pathogenesis and evaluate potential drugs for therapy. Rats are highly susceptible to induction of arthritis (4). Various agents are used including complete Freund's adjuvant (CFA) to induce arthritic rats (5). Animal tissues including from rats are also commonly used in routine diagnostic tests to detect autoantibodies.

The objective of this study is to determine the potential of rat plantar tissue from hind paw as a source of antigen to detect arthritic autoantibodies in SLE patients. We used indirect immunofluorescence method to screen for autoantibodies in normal and SLE plasma on plantar tissue of normal rat.

MATERIALS AND METHODS

Plasma samples

Plasma samples were collected from SLE patients attending the Nephrology Clinic at Hospital Serdang, Kajang Selangor, after informed consent. This study was conducted from January 2016 to September 2016. Patients were diagnosed based on the existence of at least four out of 11 diagnostic criteria established by the American College of Rheumatology (ACR), 1997. SLE patients with arthritis were identified from clinical notes. Healthy controls, free from clinical evidence or a family history of any autoimmune diseases were also included. This study was approved by the Institutional Ethic Committee for Research Involving Human Subjects, Universiti Putra Malaysia, and the Medical Research and Ethics Committee, Ministry of Health Malaysia.

Rat tissue

All procedures conducted on animals were in compliance with guidelines and recommendations set out by the Institutional Animal Care and Use Committee on Ethical Conduct in the Care and Use of Non-Human Animals in Research.

Healthy Sprague-Dawley rats weighing approximately 180g were dissected to collect tissue at the plantar pads of the hind legs. The dissected tissue were immediately transferred into a 15 mL tube and kept in -80°C freezer until further use.

(Tissues from paw of Sprague Dawley rats experimentally induced with arthritis using complete Freund's adjuvant following standard procedure were tested but found unsuitable as tissues were too damaged.)

Tissue processing

Tissue samples were defrosted at room temperature for 10 minutes. Specimen were cut into smaller sizes and placed on the specimen disc at the appropriate orientation and embedded with OCT (Embedding Matrix for Frozen Section) freezing medium (CellPath, UK). Specimen disc was placed into one of the holes of the quick freeze shelf on the cryostat and the specimen were left to freeze at low temperature (-25°C) for 2 to 5 minutes. Once the specimen was frozen, specimen disc was inserted into the specimen head of cryostat (LEICA, CM850, Germany) to begin sectioning. The block was trimmed until the specimen side was exposed and sectioning continued to obtain thickness of 10-15 micron, followed with sectioning at 4 micron thickness. Sectioned specimens were mounted on slides and left

at room temperature for 2 hours before proceeding with staining procedure.

Indirect immunofluorescence

Slides with tissue sections were placed into a Coplin jar and incubated with blocking solution (2% BSA in PBS) at room temperature for one hour. This was followed by incubation with 1:10 diluted plasma for one hour at 4°C. Slides with plasma substituted with PBS were also prepared to control for background fluorescence. Tissues were then washed by dipping slides into Coplin jars containing PBS three times at one minute intervals. Incubation with secondary antibody was performed with goat-anti-human IgG tagged to fluorescein isothiocyanate (Invitrogen, Germany) at dilution recommended by manufacturer (1:100) at room temperature for 30 minutes, in the dark. Slides were again washed for three times with PBS at one minute interval. Subsequently, tissues were counter stained with DAPI (Sigma-Aldrich-Merck, UK) solution, 1 μg/ml in PBS at 10 min incubation in the dark. Otherwise, slides were immediately mounted with 90% glycerol mountant. Finally, tissues were covered with a cover slip before proceeding to view under fluorescence microscope (Olympus BX51, Japan) with fluorescence filter set for fluorescein FITC and DAPI. Images were captured with a camera (Olympus XC50, Japan) using software Five Olympus Soft Imaging Solution (Soft Imaging system, Japan) for life science study. Exposure time was fixed for every viewing sessions.

RESULTS

Plasma samples from SLE patients with arthritis (N=9) and without arthritis (N=7) as well as normal plasma (N=7) were randomly selected for the study. All subjects were Malay, females with age range from 22-30 years old. Fig 1 shows fluorescence micrograph images from indirect immunofluorescence staining of rat plantar tissue with plasma samples. Fluorescence staining of normal samples was at background levels (Fig 1a-b). Slides of rat plantar tissue incubated with plasma from SLE without arthritis showed background or increased green fluorescence on tissue without staining of striated muscle except for weak staining on one sample, SLE-wA6 (Fig 1c). Presence of basal levels of autoantibodies in normal and thus SLE without arthritis is not unexpected. All slides incubated with samples from SLE with arthritis showed staining of muscle striation in skeletal muscle cells (Fig 1d).

DISCUSSION

While there is abundant report on lupus nephritis, there are very few available for lupus arthritis. This may be due to the lack of specific therapy for this condition and the use of broad spectrum acting drugs in SLE patients. In contrast the pathogenesis of rheumatoid arthritis has been carefully and deliberately described from effects of

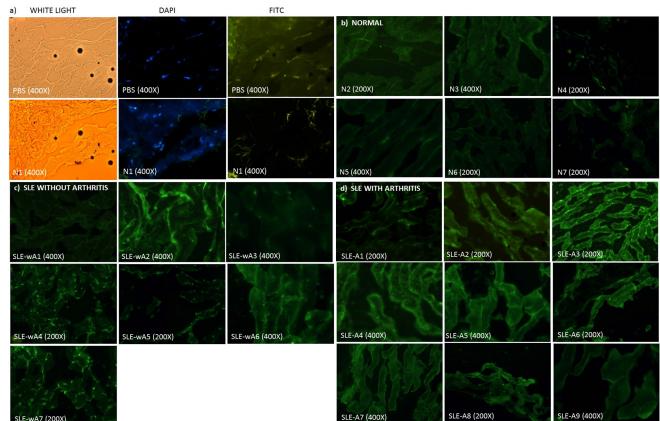


Figure 1: Fluorescence microscopy images of rat plantar tissue stained for a) background control (PBS) and a normal plasma sample. White light microscopy demonstrated elongated and tubular shape of the cell with the striated appearance typical of the skeletal muscle. DAPI staining revealed multiple nuclei located on the periphery of the cell. Also shown are green fluorescence staining patterns on rat tissue with b) additional plasma samples from normal individuals, total N=7 c) plasma from SLE without arthritis, N=7 and c) plasma from SLE with arthritis, N=9. Striking green fluorescence outlining striation on skeletal muscle cells was observed in slides stained with plasma samples from SLE patients with arthritis while weak staining could be detected in SLE without arthritis.

the environment to genetic factors leading to synovial hyperplasia and bone destruction (6, 7, 8).

Lupus patients with arthralgia and clinical evidence of joint swelling fall into the group with varying degrees of inflammation but no erosions or deformities. In general, the degree of inflammation and deformity i.e. Jaccoud's arthropathy, are lesser and present only on a small population of patients. Arthritis with prominent radiological erosion is uncommon, <5% (9).

Nevertheless, joint involvement in SLE is very common affecting ~90% of patients at some stage in the course of their disease. Small joints of the hand and wrist are usually affected (2). Musculoskeletal imaging show clearly the presence of not only significant bony but also soft tissue involvement leading to the suggestion that tendon involvement in SLE by MRI should consider tendonitis and tenosynovitis as candidates for inclusion in the diagnostic criteria (10). This is supported by a more recent study on musculoskeletal ultrasound examinations of hand and wrists in 108 SLE patients who had experienced musculoskeletal involvement in

the course of disease, which observed joint involvement in 38.8% and tendon involvement in 40.7% and both in 20.3% (11).

Thus, involvement of tissue exterior to the joints in SLE patients has been clearly demonstrated and may potentially include adjacent skeletal muscles. Tissue damage may be caused by autoantibodies targeting specific proteins in these cells. The F-actin specific smooth muscle autoantibody is most specific for the diagnosis of type 1 autoimmune hepatitis (AIH) and observed to bind to skeletal muscle striations (12). Joint pain is also a common symptom among AIH patients. However, actin autoantibody is only found in 9-26% of SLE patients (3) while F-actin activity is unclear.

We observed binding of autoantibodies from plasma of SLE patients with arthritis to skeletal cells in tissue of rat plantar. The number and location of the palmar and plantar pads in the rat have been found to be similar to those of humans and thus the rat is proposed as ideally suitable for experimental studies for morphology studies (13). It is highly possible that the rat may also be a

suitable model for histology study.

The current results were able to clearly differentiate the binding pattern of striation in skeletal muscle of rat between arthritic from non-arthritic SLE patients. This suggests presence of autoantibodies targeting joint tissue in SLE patients with arthritis. This study requires confirmation with a larger population of SLE patients. Future studies should include rheumatoid arthritis as an important control group.

The rat may be a useful model to examine the pathogenesis of lupus arthritis and evaluation of drug therapy.

CONCLUSION

Rat plantar tissue may be a useful substrate to detect autoantibodies that may be pathogenic in lupus arthritis.

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