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Preparation of rat synovial membrane for studies of cytokine secretion

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Abstract: The objective of this work was to devise an in vitro system for studies on cytokine secretion by synovial membrane treated as a whole organ with various synoviocyte populations. Synovial membrane from knee joints of WAG rats was dissected and incubated in culture medium without serum for 4 - 48 h. The level of IL-1α was determined in synovial lysates and IL-6 in culture medium. The synovial membrane from left and right knee joint of the same rat produced similar amount of cytokines both in lysates and in the medium. Synovial membrane stimulated by LPS for 4 or 24 h gave significantly stronger cytokine response than the membrane from the opposite (control) knee. After 48 h incubation of synovial membrane drastic drop in cytokine level was noted, which indicated on deterioration of the membranes. The test may be useful in studies on factors affecting cytokine secretion by synoviocytes.

Key words: Synovial membrane - IL-1α - IL-6 - LPS

Introduction

The synovial membrane consists of macrophage-like type A cells and fibroblast-like B cells so called synoviocytes [2]. A functional characteristic of synoviocytes is usually done in cultures either of fibroblasts or macrophages obtained from normal or pathological synovial membrane. Both of these cell types produce a similar panel of cytokines [7,8] and may affect each other, therefore their combined response could differ from that produced by particular class of synoviocytes. Moreover, synovial membrane is highly vascularized and cytokines produced by endothelial cells [3] may also influence synoviocyte response. To study the combined response of various cell types present in synovial membrane we have devised a simple *in vitro* system enabling to study cytokines present in the synovial membrane dissected from the knee joints or secreted into the culture medium. In this work, using IL-1α and IL-6 as examples, we have demonstrated that Il-1α and IL-6 may be estimated in the non-stimulated membranes and that the membranes stimulated by LPS (lipopolysaccharides).

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Materials and methods

Animals. Inbred, 3 month-old WAG (Wistar Albino Glaxo) specified pathogen-free rats were obtained from the Animal Unit of the Mossakowski Medical Research Centre, Polish Academy of Sciences in Warsaw. The study and the methods used were approved by the Animal Ethical Committee of the Medical University of Warsaw.

Preparation and incubation of synovial membrane. Joint cavity was opened and the synovial membrane was excised together with patella, patellar ligament and joint capsule after detachment of patellar ligament from the upper part of the tuberosity of the tibia. Synovial membrane, together with infrapatellar fat pad, was separated from the patellar ligament and joint capsule and immersed into a culture medium After separation synovial membrane curled with synovial lining exposed to the medium (Fig.1). The medium consisted of RPMI and antibiotics (Sigma-Aldrich Chemie, D82041 Diesenhofen, Germany). No serum was added. Synovial membranes from the same rat were paired and put into the different wells of flat-bottomed 24-well plate (Corning, NY USA) in 1 ml of medium. The plates were put onto slow-motion shaker and incubated for 4, 24 or 48 h in 5% CO_2 in air at 37°C. Following incubation, the tissue was extracted on ice with lysis buffer (CHAPS 1% w/v, NaCl 150 mM, TRIS 50 mM, EDTA 5 mM, phenylmethylsulfonyl fluoride (PMSF) 1 mM, pH=7.8), according to Authier et al. [1] for 30 min. The lysates and the collected culture medium were stored at -25°C.

Experiments. In the first experiment synovial membranes from the same rat served as a control of each other, to estimate variation in cytokine concentration caused by excision technique (Table 1). In another experiment one synovial membrane from each pair was stimulated by addition to the medium of 10 μg/ml of Lipopolysac-

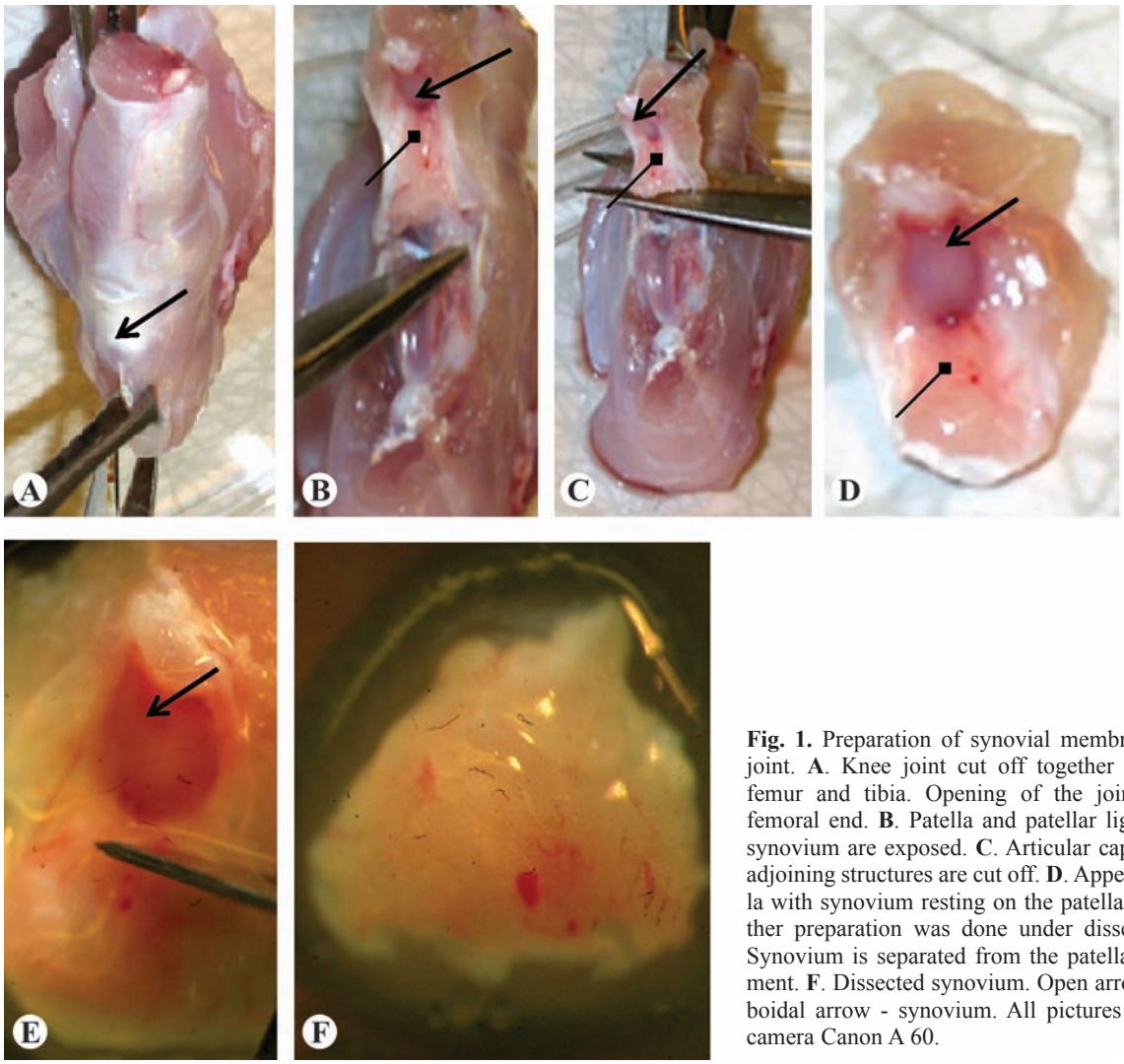

Fig. 1. Preparation of synovial membrane from rat knee joint. **A**. Knee joint cut off together with fragments of femur and tibia. Opening of the joint starts from the femoral end. **B**. Patella and patellar ligament covered by synovium are exposed. **C**. Articular capsule together with adjoining structures are cut off. **D**. Appearance of the patella with synovium resting on the patellar ligament. **E**. Further preparation was done under dissecting microscope. Synovium is separated from the patella and patellar ligament. **F**. Dissected synovium. Open arrow - patella; rhomboidal arrow - synovium. All pictures taken with digital camera Canon A 60.

charides (LPS) from E. coli 055: B5 (Sigma-Aldrich) [7] and incubated for 4, 24 and 48 h. In each experiment 6 rats were used. Moreover, synovial membranes from 6 rats were incubated as above, fixed in Bouin-Hollande solution and processed for light microscopy.

Cytokine immunoassays. The concentration of IL-6 in the incubation medium and IL-lα in the lysed material was determined using commercial ELISA kits (Biosource Int., Camarillo, Ca USA) according to the manufacturer's instruction. Detection thresholds were 3 pg/ml for IL-1α and 8 pg/ml for IL-6.

Statistical analysis. Differences between groups were evaluated by Wilcoxon matched-pairs test.

Results and discussion

Preparation of synovial membranes from rat knee joints requires certain experience, but once it is mastered, the dissection of 12 specimens lasts no more than one hour. To assess quality of dissection, difference in the cytokine production between synovium from left and right knee joint of the same rat was evaluated, and it was found not to be significant in Wilcoxon matched-pairs test (Table 1). Marked difference was seen only in rat number 4 in Table 1. It was prob-

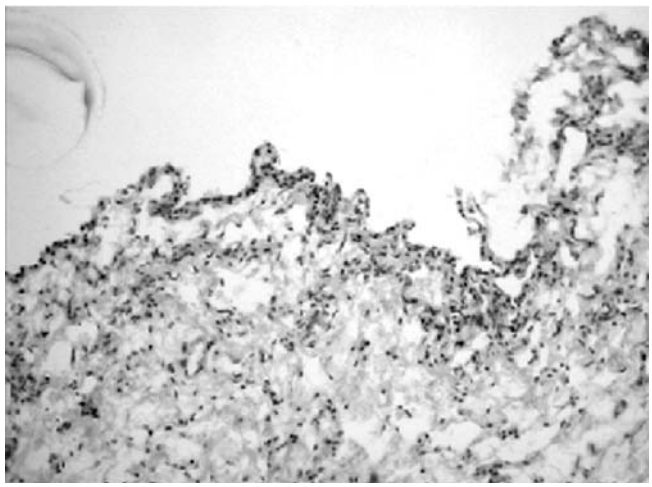

Fig. 2. Synovial membrane cultured with LPS for 24 h. H.E. Magn. × 200.

Table 1. Concentration of IL-1α in synovial membrane lysates and IL-6 in the incubation medium from knee joints of the same rat, after 4 h incubation

Rat number	*IL-1α		*IL-6	
	Left knee	Right knee	Left knee	Right knee
1	40	38	164	128
2	38	37	162	150
3	50	53	282	248
4	45	11	262	168
5	32	40	208	218
6	44	29	192	224
Mean ± SE	41.5 ± 6.3	34.7 ± 14	211.7 ± 50.2	189.3 ± 43.3

*In pg/ml; + differences between synovial membrane from left and right leg were not significant in Wilcoxon matched-pairs test.

Table 2. Concentration of IL-1α in synovial membrane lysates and IL-6 in the incubation medium from control and LPS-stimulated knee joint synovial membranes

Incubation lasting									
4h					24h				
Rat number	*IL-1α		*IL-6		Rat number	*IL-1 α		*IL-6	
	C	LPS	C	LPS		C	LPS	C	LPS
1	25	367	160	1368	7	53	1076	847	2593
2	47	374	190	1194	8	31	996	242	2366
3	25	368	210	1466	9	60	1086	677	2734
4	37	380	200	1504	10	28	1017	219	2522
5	42	392	170	1438	11	63	1000	367	2503
6	45	376	168	1444	12	35	1010	344	2769
Mean ± SE	36.8 ± 9.8	376.2 ± 3.7	183 ± 8.1	1402 ± 45.4	Mean ± SE	45 ± 6.3	1031 ± 16.2	449.3 ±103.9	2581 ± 61.8

*In pg/ml mean; + differences between control (C) and synovial membrane exposed to LPS were significant in Wilcoxon matched-pairs test at $p<0.05$.

ably caused by the damage inflicted during dissection. Dissected synovial membranes strongly responded to stimulation with LPS lasting for 4 or 24 h (Table 2) and remained morphologically well preserved (Fig. 2). After 48 h incubation in the cell culture medium production of cytokines by rat synoviocytes markedly dropped (not shown), accompanied by morphologically observed deterioration of tissue, presumably caused by the lack of serum in the incubation medium. The serum was not added since several samples of tested serum contained endotoxin which strongly stimulated synovial membrane and masked effect of LPS.

Up to now *in vitro* studies on the reaction of the synovial membranes on different stimuli were limited to the pathological material either from patients with rheumatoid arthritis [4,6] or from hind feet joints of rats with adjuvant arthritis [5,9,10]. Thus, as far as we could establish, we are presenting for the first time a method of culture of normal synovium and also we demonstrate that synovium produces *in vitro* cytokines and responds to stimulatory action of LPS.

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