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***Serum glycosaminoglycans from rheumatoid arthritis patients do not affect reactive oxygen species secretion by normal monocytes.***

**Brief Communication**

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**SUMMARY.**

**Introduction.** Several observations suggest that macrophages play an important role in the physiopathology of rheumatoid arthritis (RA). One of the most important features of RA is the serious cartilage destruction with consequent glycosaminoglycans (GAG) release. Here we studied whether GAG from RA patients can induce peripheral blood monocytes to secrete reactive oxygen species (ROS), thus being responsible for the inflammatory injury.

**Patients and methods.** 10 female healthy postgraduate student volunteers and 10 female patients with RA were included. GAG were isolated and purified according to Merhraban and Volpi methods. Mononuclear cells from 15 mL of peripheral blood were obtained and monocytes were identified by neutral red coloration and phagocytosis and purified by adherence to plastic. Chemiluminescence (CL) was measured on a LKB-1250 Bioluminometer at

37°C. The cuvettes contained  $5 \times 10^5$  monocytes from healthy donors resuspended in Hank's solution without phenol red at 37°C, pH 7.4. Different GAG concentrations from either normal volunteers (5, 40, 80, and 160 µg/mL) or RA patients (5 and 80 µg/mL) were resuspended in Hank's solution in a standard volume of 100 µL. To elicit CL emission 100 µL of a solution containing 4 mg/mL of opsonized zymosan were added and photoemission was measured every ten minutes during 60 minutes.

**Results.** The electrophoretic patterns of serum GAG obtained from normal volunteers and RA patients were comparable: in both cases chondroitin 4 sulfate and chondroitin 6 sulfate were identified. No chemiluminescent signal was emitted by monocytes in presence of luminal and GAG, suggesting that GAG are not able to elicit respiratory burst in those cells, neither via protein C kinase  $Ca^{++}$  influx nor via Fc receptors. CL emission by zymosan of peripheral

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monocytes was higher in presence of GAG from RA patients than that in presence of GAG from normal volunteers or without GAG (increase of 36%). However, the difference was not statistically significant.

**Conclusion.** Serum GAG from patients are not likely to play a role in the physiopathology of RA linked to the secretion of ROS by monocytes.

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**Key words:** glucosaminoglycan, rheumatoid arthritis patients, oxygen reactive species, monocytes.

## RESUMEN.

**Glucosaminoglicanos séricos de pacientes con artritis reumatoide no afectan la secreción de especies reactivas de oxígeno de monocitos normales.**

**Introducción.** Diversas observaciones sugieren que los macrófagos juegan un papel importante en la fisiopatología de la artritis reumatoide (AR). Uno de los más importantes hallazgos de la AR es la importante destrucción del cartílago con la consecuente liberación de glucosaminoglicanos (GAG). Nosotros estudiamos si los GAG de pacientes con AR pueden inducir a los monocitos de sangre periférica a secretar especies reactivas de oxígeno (ROS), siendo entonces responsables de la lesión inflamatoria.

**Pacientes y Métodos.** Diez mujeres voluntarias estudiantes de postgrado y 10 pacientes femeninas con AR fueron incluidas. El GAG fue aislado y purificado de acuerdo a los métodos de Merhraban y Volpi. Se obtuvieron células mononucleares de 15 mL de sangre periférica, los monocitos fueron identificados por la coloración de rojo neutral y fagocitosis y purificados por adherencia al plástico. La quimoluminiscencia (CL) fue medida en un Bioluminómetro LKB-1250 a 37°C. Los recipientes conteniendo monocitos en una cantidad de  $5 \times 10^5$  de los donadores sanos se resuspendieron en una solución de Hank sin rojo fenol a 37°C y un pH de 7.4. Diferentes concentraciones de GAG de voluntarios normales (5, 40, 80 y 160 µg/mL) o pacientes con AR (5 y 80 µg/mL) fueron resuspendidos en solución

de Hank en un volumen estandar de 100 µL. Para obtener la emisión de CL, fueron adicionados 100 µL de una solución que contenía 4 mg/mL de zymosan osonizado y la fotoemisión fue medida cada 10 minutos durante 60 minutos.

**Resultados.** Los patrones electroforéticos de GAG obtenidos de voluntarios normales y pacientes con AR fueron comparables: en ambos casos fueron identificados condroitin 4 sulfato y condroitin 6 sulfato. No se emitieron signos de quimoluminiscencia por los monocitos en presencia de luminal y GAG, sugiriendo que los GAG no son capaces de generar una explosión respiratoria en esas células ni vía la entrada de  $Ca^{++}$  por protein-quinasa C ni vía los receptores Fc. La emisión de Cl por zymosan de monocitos periféricos fue mayor en presencia de GAG en pacientes con AR, que en presencia de GAG de voluntarios normales o sin GAG (aumento de 36%). Sin embargo, la diferencia no fue estadísticamente significativa.

**Conclusión.** El GAG sérico de pacientes no parece tener participación en la fisiopatología de la AR a través de la secreción de ROS por los monocitos.

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**Palabras clave:** glucosaminoglicanos, artritis reumatoide, especies reactivas de oxígeno, monocitos.

## INTRODUCTION.

Monocyte-macrophages are cells of the immune system with important functions which secrete more than 50 biologically active products including reactive oxygen species (ROS)(1). Under normal conditions, monocytes do not attack (self-structures of) the organism itself. However, cellular alterations can activate these cells and generate a full response against itself increasing tissue injury (2). Several observations strongly suggest that macrophages play an important role in the physiopathology of rheumatoid arthritis (RA). Affected joints are infiltrated by macrophages and several monocyte products are found in the synovial fluid (3). One of the most important features of RA is the serious cartilage destruction with consequent glycosaminoglycans (GAG) release (4).

### *Patient glucosaminoglycans not active on monocytes*

Some GAG have been reported to enhance ROS secretion of macrophages and to stimulate B-lymphocytes (5) and the glycan part of peptidoglycans can bind and activate monocytes through CD14 receptor (6, 7). We studied here whether GAG from RA patients can induce peripheral blood monocytes to secrete ROS, thus being responsible for the inflammation.

#### **MATERIAL AND METHODS.**

**Patients.** Written consent was obtained. Two mL of serum were taken from 10 healthy female postgraduate student volunteers and from 10 female patients with RA from the Rheumatology Department of the National Medical Center of Occident of the Mexican Social Security Institute, Guadalajara, Jalisco México, diagnosed according to the American Rheumatism Association criteria (8) and not having taken anti-inflammatory drugs during 10 days before the assay.

**Extraction and purification of serum glycosaminoglycans (GAG).** GAG were isolated and purified according to Merhraban and Volpi methods (9, 10).

**Monocyte collection.** Mononuclear cells from 15 mL of peripheral blood were obtained from 10 healthy volunteers and from the patients by centrifugation on density gradient with ficoll-hypaque, washed 3 times with Hank's solution without phenol red at 4°C. As previously described (11,12) monocytes were identified by neutral red coloration and phagocytosis and purified by adherence to plastic. Viability was determined by trypan blue exclusion (99% were viable cells).

**Chemiluminescence (CL).** Samples were measured on LKB-1250 Bioluminometer at 37°C. The cuvettes contained  $5 \times 10^5$  monocytes from healthy donors resuspended in 780  $\mu$ L of Hank's solution without phenol red at 37°C pH 7.4. Twenty  $\mu$ L of a luminal suspension were added ( $5 \times 10^{-6}$  M final concentration). Different GAG concentrations from either normal volunteers (5, 40, 80, and 160  $\mu$ g/mL) or RA patients (5 and 80  $\mu$ g/mL) were resuspended in Hank's solution without phenol red in a standard

volume of 100  $\mu$ L and added to the cuvettes. To elicit CL emission 100  $\mu$ L of a solution containing 4 mg/mL of opsonized zymosan was added and photoemission was measured every ten minutes for 60 minutes. Results were expressed in millivolts per minute (mV/min). Each test was done in triplicate (12).

**Statistical analysis.** Results represent mean  $\pm$  standard deviation of 10 independent observations in each group. Differences between groups were calculated with the non-parametric Mann Withney U-test.

#### **RESULTS AND DISCUSSION.**

The electrophoretic patterns of serum GAG obtained from normal volunteers and RA patients were comparable: in both cases chondroitin 4 sulfate and chondroitin 6 sulfate were identified.

Since it has been reported that inert particles or biological molecules can elicit CL emission by phagocytic cells (1), we first studied if serum GAG from normal volunteers or from patients could also induce that secretion. Peripheral monocytes were incubated with luminal and different GAG concentrations, either from normal volunteers (5, 40, 80 and 160  $\mu$ g/mL) or from RA patients (5 and 80  $\mu$ g/mL). We measured photoemission during 10 minutes: no photoemission was observed.  $5.5 \pm 2.8$  mV/min correspond to CL emission background.

We also tested if the addition of GAG can modify CL emission of peripheral monocytes elicited by a phagocytic stimulus (opsonized zymosan). The addition of GAG from healthy volunteers did not modify the response ( $194.7 \pm 165$  mV/min versus  $171 \pm 110$  to  $225 \pm 165$  mV/min at the tested concentrations) (Table 1). The addition of GAG from RA patients at the concentration of 5  $\mu$ g/mL slightly increased the intensity of the emission without reaching statistical significance ( $264 \pm 215$  mV/min). Higher GAG concentration did not modify the response ( $210 \pm 145$  mV/min).

No chemiluminescent signal was emitted by monocytes in the presence of luminal and GAG, suggesting that GAG are not able to elicit respiratory

**Table 1**  
**Effect of serum GAG from either RA patients or healthy volunteers on the zymosan elicited chemiluminiscence of peripheral monocytes in healthy volunteers.**

	PROTEOGLYCAN CONCENTRATION (µg/ mL)					Rheumatoid arthritis	
	Healthy volunteers					5	80
	-	5	40	80	160		
MV/min	194 ± 87	225 ± 165	197.9 ± 115	171 ± 108	171 ± 110	264 ± 115	210 ± 145
Δ %		16	1.6	-11.8	-12	36	8
U-Mann Withney		NS	NS	NS	NS	NS	NS

**Peripheral monocytes from normal volunteers were incubated in luminol and different concentrations of GAG from either in healthy volunteers or rheumatoid arthritis patients.**

burst in those cells neither via protein C kinase, Ca<sup>++</sup> influx nor via Fc receptors, mechanism involved in the activation of respiratory burst of phagocytic cells (12).

CL emission by zymosan of peripheral monocytes was higher in presence of GAG from RA patients, than that in presence of GAG from normal volunteers or without GAG (increase of 36%). However the difference was not statistically significant. This observation may have resulted from variability between donors, although the statistical test used did not take the standard deviation into consideration. A dose-response curve was not obtained independently of which type of GAG used. These results suggest that GAG do not regulate phagocytic stimulus elicited respiratory burst of monocytes and do not show scavenger activity which would be interesting in RA (13).

Since GAG such as hyaluronic acid and heparin can modify immune response or such as heparin sulfate, an heterogeneous GAG, can induce cytotoxic activities in macrophages our results suggest that to obtain significant modifications in immune cell reactivity a critical GAG structure that may be altered by inflammatory process is necessary (5).

In conclusion, serum GAG from patients is not

likely to play a role in the physiopathology of RA linked to the secretion of ROS by monocytes.

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*Patient glucosaminoglycans not active on monocytes*

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