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Abstract This work is to investigate the levels of human xanthine oxidoreductase (HXOR), its antibodies, and microorganisms in synovial fluid of patients with untreated rheumatoid joint diseases. Synovial fluids were collected from sixty-four patients with rheumatoid joint diseases. Sixty-four age-matched individuals were included as control. Xanthine oxidoreductase (XOR) proteins level and anti-XOR antibodies were determined in the blood and synovial fluid, using human XOR as antigen, by enzyme-linked immunosorbent (ELISA) assay. Synovial fluids were cultured for bacteria and fungi. The titers of XOR protein in the synovial fluid of patients with rheumatoid arthritis were  $90.43 \pm 23.37$   $\mu\text{g/ml}$  (mean  $\pm$  SD,  $n = 29$ ) and up to  $62.42 \pm 8.74$   $\mu\text{g/ml}$  (mean  $\pm$  SD,  $n = 35$ ) in other joint inflammation. Anti-HXOR antibodies titers in patients were  $167.72 \pm 23.64$   $\mu\text{g/ml}$ ,  $n = 64$ , which was significantly higher in rheumatoid arthritis patients. The results indicated that anti-HXOR antibodies in synovial fluids have a protective role as high concentrations against XOR were detected in inflammatory arthritis. These antibodies play a role in eliminating XOR from synovial fluids. However, immune complex formation could activate complement and participate in propagating the inflammatory cycle. Synovial aspirate ordinary microbial cultures were negative for any bacteria or fungi, but that does not exclude organisms of special culture requirements.

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Keywords (separated by '-') Synovial fluid - Human xanthine oxidoreductase (HXOR) - ELISA - Rheumatoid joint diseases - Free radical

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Footnote Information N. Al-Muhtaseb and E. Al-Kaissi contributed equally.

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2 **The role of human xanthine oxidoreductase (HXOR), anti-HXOR**  
3 **antibodies, and microorganisms in synovial fluid of patients**  
4 **with joint inflammation**

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36

**Keywords** Synovial fluid · Human xanthine 37  
oxidoreductase (HXOR) · ELISA · Rheumatoid joint 38  
diseases · Free radical 39

**Introduction** 40

In recent years, free radicals reactions and other reactive 41  
intermediates produced in normal metabolic processes 42  
have been implicated in the pathogenic mechanism of a 43  
wide range of diseases, including inflammatory diseases [1] 44  
as acute coronary syndrome [2] and others cardiovascular 45  
diseases [3]. Similarly, pathogenic organisms such as par- 46  
vovirus B19, rubella, hepatitis B and C, herpes [4–10], 47  
Epstein–Barr virus [11], and retroviruses could also serve 48  
as infectious causes of rheumatoid arthritis–like disease. 49  
Synovial human T lymphotropic virus type 1 (HTLV-1) 50  
infection is associated with chronic arthritis [4]. 51

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Rheumatic joint disease is a chronic syndrome of ambiguous etiology and is characterized by non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues, and joint deformities. One particular type of tissue injury from free radicals is reoxygenation injury following reperfusion of ischemic tissue [12–17]. Rheumatoid synovitis is usually accompanied by increased synovial effusions. Damage to bone and cartilage of synovial tissue is mediated by metabolic free radical sources such as xanthine oxidoreductase (XOR) and others. Xanthine oxidoreductase (XOR) is a complex metalloflavoprotein, which plays a key role in catalyzing the oxidation of a wide range of substrates (purine, pterins, dinucleotides, and aldehydes) [18]. The enzyme is a homodimer of approximately 300KDa [19]. XOR exists into 2 different but interconvertible forms, xanthine dehydrogenase (XDH, EC 1.1.1.204) and xanthine oxidase (XO, EC 1.1.3.22) [19, 20]; the former is the dominant form. XOR produces reactive oxygen species (ROS), superoxide ( $O_2^-$ ), or hydrogen peroxide ( $H_2O_2$ ), respectively [21]. The capacity to generate such ROS has led to a great deal of interest in XOR as a pathogenic factor in many instances of ischemia–reperfusion injury in various organs [19, 22–25] including inflammatory joint and rheumatic diseases [15–17]. Xanthine oxidase is the first-registered biological source of oxygen free radicals and plays the leading role in the pathogenesis of tissue damage by the production of superoxide anion, hydrogen peroxide, and hydroxyl radical [26]. There is substantial evidence that XOR participates in the pathophysiology of many diseases [27].

Rheumatoid arthritis (RA) is the most common chronic inflammatory arthritis that affects about 1% of adults [28]. There are no specific laboratory tests specific for RA; diagnosis depends on a constellation of signs and symptoms that can be supported by serology and radiographs [29–31]. Some RA patients show evidence of autoimmunity long before the appearance of clinical arthritis [32].

The presence of human anti-XOR antibodies was initially reported by Oster et al. [33]. Bruder et al. [34] confirmed the existence of such antibodies and showed them to be IgG. The amount of anti-XOR antibody in normal healthy humans represents 1–8% of total IgG. Levels of these antibodies were shown to be elevated in patients suffering from myocardial infarction [35]. Ng et al. [36] found by ELISA technique that the IgM anti-XOR antibodies in healthy humans varied from 0.32 to 1.8% of total IgM, whereas IgG anti-XOR level represented only 0.02–0.4% of total IgG. Ng and Lewis [37] reported the presence of circulating XOR–anti-XOR immune complexes (XORICs) in healthy humans. The complex containing antibodies of IgG and IgM classes, representing <20% of total anti-XOR antibodies. The levels of these complexes correlate with the anti-XOR antibody titers [38].

Rheumatoid arthritis (RA) is characterized by the appearance of autoantibodies of types IgM, IgG, and IgA known as rheumatoid factors (RF) that react essentially against autologous IgG [39]. Immune complexes activate complement. Complement activation induces inflammatory reaction [40], which contributes to the constitution of a vascular formation leading to cartilage destruction and bone erosion [41–43]. XOR present in the synovium could be liberated by synovium destruction and could play a role in post-ischemic reperfusion of rheumatoid synovium [17] contributing to the characteristics signs and symptoms of radical attack present in synovial fluid. XOR concentration is significantly raised in patients suffering from RA, up to 60 times in synovium [16] compared with healthy humans or patients with other non-rheumatic diseases.

The detection of xanthine oxidoreductase in human synovium is therefore of importance as a first step in the investigation of possible mechanisms of radical generation peculiar to a reperfusion phase of synovial injury. The aim of this work is to investigate the presence of human xanthine oxidoreductase, its antibodies in synovial fluid, and the possible microbial etiology of the inflammation in untreated patients suffering from rheumatoid arthritis (rheumatoid factor +ve) and other joint inflammatory diseases (rheumatoid factor –ve).

## Materials and methods

### Subjects

Sixty-four patient with rheumatoid joint disease and 64 age- and sex-matched controls were included in the study. All patients fulfilled the revised American College of Rheumatology (ACR) criteria for rheumatoid arthritis [23]. Consent for all procedures was obtained from each individual. The study was approved by the hospital ethics committee and was in accordance with the ethical standards. Preliminary evaluation consisting of a brief medical history, smoking, alcohol habits, and physical examinations was performed. Patients with any history of liver diseases, diabetes mellitus, respiratory disorders, and cardiovascular diseases were excluded. None had been treated with corticosteroids or disease-modifying antirheumatic drugs prior the study.

A pooled high-titer anti-XOR and immune complexes (XORIC) human sera from volunteer donors were served to build standard curves.

### Rheumatoid factor detection

Rheumatoid factor is detected by latex agglutination test using appropriate plates from Behring (Germany) according to the manufacturer's recommendations. Fifty microliters of latex

152	coated with human IgG was added to different dilutions from	195
153	each serum sample. Negative and positive sera were used as	196
154	controls. After 2 min, a clear agglutination is observed in the	197
155	positive, indicating the presence of RF. Sera with titer less	198
156	than 20 UI/ml were considered negative according to the	199
157	manufacturer's recommendation.	200
158	Milk and reagents	201
159	Human breast milk was kindly donated by mothers who	202
160	had that in excess at the special care unit of the maternity	
161	hospitals; human milk was freshly collected and stored at	
162	-20°C until use.	
163	Unless otherwise stated, all other reagents were pur-	
164	chased from sigma (Poole, UK).	
165	CRP latex agglutination was purchased from Genzyme	
166	diagnostics (Kent, UK).	
167	Purification of human xanthine oxidoreductase (XOR)	
168	Human XOR was purified according to the previously	
169	described protocol for human milk [22]. The purified	
170	enzyme was dialyzed overnight against 3 l of 50 mM	
171	sodium/Bicine buffer pH 8.3 and processed as reported by	
172	Godber et al. [44]. Enzyme activity tests were then carried	
173	out. Purified XOR enzyme was found to have more than	
174	75% in the oxidase form.	
175	Total protein estimation	
176	Protein estimation for XOR enzyme was carried out	
177	according to the method of Bradford [45]. The enzyme	
178	purity was assessed on protein/flavin ratio ( $PFR = A_{280}$	
179	$\text{nm}/A_{450} \text{ nm}$ ). An enzyme sample with a PFR value 5–5.2	
180	is widely accepted to be pure.	
181	Xanthine oxidase activity assay	
182	Total xanthine oxidase activity of the synovial fluid was	
183	determined by measuring the rate of oxidation of xanthine to	
184	uric acid spectrophotometrically at 295 nm in a Cary 100	
185	spectrophotometer, using a molar absorption coefficient ( $\epsilon$ ) of	
186	$9.6 \text{ mM}^{-1}$ [46]. Assays were performed at $25.0 \pm 0.2^\circ\text{C}$ in	
187	air-saturated 50 mM Na/Bicine buffer, pH 8.3, containing	
188	100 $\mu\text{M}$ xanthine. Total (oxidase plus dehydrogenase)	
189	activity was determined as above but in the presence of	
190	500 $\mu\text{M}$ $\text{NAD}^+$ . Dehydrogenase content of an enzyme sample	
191	was determined from the ratio of oxidase and total activities.	
192	C-reactive protein (CRP) concentration determination	
193	CRP concentration was determined as recommended by the	
194	manufacturer; 2.4 $\mu\text{l}$ of patient serum is added to 120 $\mu\text{l}$	
	buffer solutions (pH 8.5) and mixed with 120 $\mu\text{l}$ suspension	203
	of mouse anti-human CRP monoclonal antibody that is	204
	bound to latex (2 mg/ml) and incubated for 5 min. CRP	
	binds to the latex-bound antibody and agglutinates. The	
	resulting agglutination was measured spectrophotometri-	
	cally at 580 nm, negative and positive control samples	
	were included. Values higher than 9.4 mg/l for females and	
	8.55 mg/l for males were consider as positive.	
	Single radial immunodiffusion assay (SRID)	205
	to determine levels of total IgG and IgM	206
	Commercially available plates of agarose containing anti-	207
	human IgG or anti-human IgM (Behring, Germany) were	208
	used as described by Benboubetra et al. [38]. Serial dilu-	209
	tions of purified human IgM or IgG were used to generate	210
	standard curves. Human synovial fluids or sera were dia-	211
	lyzed against the commercial buffer and run against anti-	212
	human IgG or anti-human IgM on the same plates as the	213
	standard curves. Plates were placed in a humidified box and	214
	stored for 48–72 h at room temperature until the sizes of	215
	the precipitate rings were stable. Standard curve was	216
	plotted and used to determine total IgG and IgM anti-	
	human XOR contents in synovial fluids.	
	Enzyme-linked immunosorbent assay (ELISA)	217
	(a) For anti-HXOR antibodies	218
	Specific human anti-XOR antibodies were determined	219
	as previously described by Harrison et al. [35] and	220
	Benboubetra et al. [38] with slight difference in enzyme	221
	substrate. Orthophenylene diamine (OPD) [47] was used	222
	instead of 3,3,5,5-tetramethylbenzidine (TMB). In addition	223
	to synovial fluids, each plate (Coster, Spain) included serial	224
	dilutions (200–6,400 fold in PBS-Tween) of a standard	225
	high-titer pooled serum, in duplicate wells (100 $\mu\text{l}$ /well).	226
	The absorbance was measured at 492 nm, in each	227
	well using a 96-well plate reader (Diagnostics Pasteur	228
	LP200).	229
	(b) For XOR immune complexes (XORIC)	230
	To determine (XORIC) polystyrene, 96-well microtiter	231
	plates (Coster, Spain) were coated (100 $\mu\text{L}$ /well) with	232
	diluted (1 in 40) rabbit anti-human XOR serum in sodium	233
	hydrogen carbonate (pH 9.6), then incubated overnight at	234
	4°C, and processed as described by Stevens et al. [17].	235
	XORIC concentrations were calculated from plotted stan-	236
	dard curves of absorbance against log concentration of the	237
	standard serum for each plate, and the linear part of	238
	the curve was used to calculate the titers as a percentage of	239

240 the standard. Data for ELISA were expressed as  
241 mean  $\pm$  SD.

242 Data from patient groups were compared with each  
243 other using Student's *t* test. SigmaStat Software were used  
244 for statistical analyses. Probability values of 0.05 were  
245 considered significant.

#### 246 Microbiologic testing

247 Synovial fluids were cultured on Blood agar (BA),  
248 MacConkey's agar (MA), Chocolate Agar (CA), and  
249 Sabouraud's dextrose agar (SA) (Oxoid). BA and MA plates  
250 were incubated aerobically at 37°C, CA plates were incu-  
251 bated under microaerophilic condition (3% CO<sub>2</sub>), and SA  
252 plates were incubated at room temperature (25°C) for 72 h.  
253 Synovial fluids were centrifuged at 500 rpm for 5 min, and  
254 smears from the deposit were Gram and Acid fast stained.  
255 The slides were examined by light microscopy with oil  
256 immersion lens.

#### 257 Results

258 Latex agglutination used in determining IgM-RF factor  
259 showed that of the 64 patients, 29 were RA+ (seropositive)  
260 and 35 were RA- (seronegative). The Clinical and bio-  
261 chemical characters of the participated subjects are shown  
262 in Table 1.

263 The mean age and BMI for both rheumatoid arthritis and  
264 other joint inflammation and control subjects were com-  
265 parable. ESR (after 1 h) and CRP values were significantly  
266 elevated in patients with rheumatoid arthritis (RA+)  
267 compared with the values in patient with other joint  
268 inflammation (RA-) and to those of the control values.

269 The total IgG and IgM titers in the synovial fluid of  
270 patient with rheumatoid arthritis (RA+) and patients with  
271 other joint inflammation (RA-) as determined by single  
272 radial immunodiffusion assay (SRIDA) are shown in  
273 Table 2.

274 Total IgG and IgM (mg/ml) levels were significantly  
275 higher in patient with rheumatoid arthritis in comparison  
276 with the levels in patient with other joint inflammation,  
277 respectively.

278 Total IgG and IgM titers in blood circulation of patient  
279 with rheumatoid arthritis (RA+), other joint inflammation

(RA-), and in healthy volunteers using SRIDA are shown  
in Table 3.

280 Both IgG and IgM were significantly higher in rheumatoid  
281 arthritis patients than in the healthy controls. Similarly, the  
282 levels were higher in serum of patient with other joint  
283 inflammation than controls. IgG levels were more elevated in  
284 serum of patient with rheumatoid arthritis compared with  
285 other joint inflammation ( $P < 0.001$ ). On the other hand,  
286 total IgM was more elevated in serum of patients with other  
287 joint inflammation compared with rheumatoid arthritis.  
288

289 Human XOR protein, its activity, and anti-human XOR  
290 (IgG and IgM) in patient's synovial fluid were measured;  
291 the results are shown in Table 4.  
292

293 Human xanthine oxidase protein (HXO) concentrations  
294 were significantly elevated in patient with rheumatoid  
295 arthritis compared with the concentrations in patient with  
296 other joint inflammation ( $P < 0.01$ ).

297 Human xanthine oxidase (HXO) activity was also sig-  
298 nificantly elevated in patient with rheumatoid arthritis  
299 ( $100.00 \pm 47.24 \text{ nmol min}^{-1} \text{ mg}^{-1}$ ) compared with the  
300 activity in patients with other joint inflammation ( $81.42 \pm$   
301  $33.22 \text{ nmol min}^{-1} \text{ mg}^{-1}$ ) ( $P < 0.01$ ).

302 Both IgG and IgM anti-HXOR levels are remarkably  
303 high, representing, in the case of anti-HXOR IgM anti-  
304 bodies, 6.6% of the total IgM immunoglobulin in patient  
305 with rheumatoid arthritis and 4% in patient with other joint  
306 inflammation. However, anti-HXOR IgG was higher in  
307 patients with other joint inflammation compared with  
308 patients with rheumatoid arthritis.

309 Levels of free and immune complexes IgG and IgM  
310 anti-HXOR antibodies determined by ELISA are shown in  
311 (Table 5).

312 Both the free and immune complexes of anti-HXOR  
313 (IgG and IgM) were lower in patients with rheumatoid  
314 arthritis compared with the levels in patients with other  
315 joint inflammation.

316 Direct examination as well as aerobic and microaero-  
317 philic cultures for bacteria and fungi did not reveal the  
318 presence of microorganisms.

#### 319 Discussion

320 The elevated levels of ESR (erythrocyte sedimentation  
321 rate) and CRP (C-reactive protein) reported in this study

**Table 1** Clinical and biochemical characters of the participated subjects

Type of disease	No. of subjects	Age (years)	Height (cm)	Weight (kg)	BMI	ESR (mm)	CRP
RA+	29	39.1 $\pm$ 8.9	159.8 $\pm$ 5.8	69.6 $\pm$ 2.3	27.3 $\pm$ 9.2	66.6 $\pm$ 45.2	+
RA-	35	41.04 $\pm$ 9.1	161.6 $\pm$ 5.6	69.1 $\pm$ 6.0	26.9 $\pm$ 9.7	40.3 $\pm$ 30.4	+
Controls	64	42.04 $\pm$ 7.7	162.3 $\pm$ 4.7	68.7 $\pm$ 5.8	25.8 $\pm$ 2.3	12.8 $\pm$ 4.01	-

**Table 2** Total IgG and IgM titers in synovial fluid of patients with rheumatoid arthritis (RA+) and other joint inflammation (RA-), using SRIDA

Patients	Number of patients	Total IgG (mg/ml)	Total IgM (mg/ml)
RA+	29	13.71 ± 4.41 <sup>a</sup>	0.97 ± 0.34 <sup>b</sup>
Other joint inflammation	35	9.77 ± 2.71	0.55 ± 0.20

RA rheumatoid arthritis latex agglutination positive, SRIDA single radial immunodiffusion assay

<sup>a</sup>  $P < 0.001$  significantly higher with RA+ group

<sup>b</sup>  $P < 0.05$  significantly higher with RA+ group

**Table 3** Total IgG and IgM titers in sera of patients with rheumatoid arthritis (RA+), other joint inflammation (RA-), and healthy volunteers, using SRIDA

Patients	Number of patients	Total IgG (mg/ml)	Total IgM (mg/ml)
RA+	29	24.52 ± 9.53 <sup>a</sup>	2.23 ± 1.25 <sup>a</sup>
Other joint inflammation	35	19.37 ± 5.6 <sup>b</sup>	3.42 ± 0.06 <sup>b</sup>
Pools from healthy individuals	60	13.5 ± 4.41	1.5 ± 0.52

RA rheumatoid arthritis latex agglutination positive, SRIDA single radial immunodiffusion assay

<sup>a</sup>  $P < 0.0001$  significantly differ from the control subjects

<sup>b</sup>  $P < 0.01$  significantly differ from the other joint inflammation, Results are means ± S.D in milligrams per deciliter

**Table 4** Human xanthine oxidase protein, (HXOR) activity and anti-HXOR (IgG and IgM) in patient synovial fluids

Type of disease	No. of patients	HXOR protein (µg/ml)	HXOR activity nmol min <sup>-1</sup> mg <sup>-1</sup>	Anti-HXOR (IgG) µg/ml	Anti-HXOR (IgM) µg/ml
RA+	29	90.43 ± 23.37	100.00 ± 47.24	36.37 ± 22.35	131.35 ± 30.39
Other joint inflammation	35	62.42 ± 8.74	81.42 ± 33.22	46.43 ± 23.62	105.6 ± 31.36
<i>P</i>		<0.01	<0.01	<0.05	<0.001

**Table 5** IgG and IgM anti-HXOR antibodies, free (HXORAb) and immune complexes (HXORIC) determined by ELISA (µg ml<sup>-1</sup>) in patient synovial fluid

	No. of patients	HXORAb		HXORIC	
		IgG	IgM	IgG	IgM
RA+	29	36.77 ± 22.35	131.35 ± 30.39	77.06 ± 38.74	90.34 ± 74.96
Others joint inflammation	35	46.43 ± 23.62	141.09 ± 31.36	86.13 ± 14.30	134.94 ± 43.61
<i>P</i>		<0.001	<0.01	<0.05	<0.001

322 indicate the presence of inflammation in both patient  
323 groups. Patients positive for rheumatoid factor were  
324 labeled as rheumatoid arthritis (RA+), while seronegative  
325 patients were labeled as other joint inflammation (RA-).

326 The level of human xanthine oxidase protein and its  
327 activity is significantly high in the synovial fluid of patient  
328 with rheumatoid arthritis (90.43 µg/ml, 100.00 nmol  
329 min<sup>-1</sup> mg<sup>-1</sup>) (Table 4). The normal level in healthy indi-  
330 vidual as reported by others [48, 49] is 0.16–0.38 mU/g.  
331 This may be due to upregulation of the enzyme by char-  
332 acteristically high levels of cytokines and hypoxic nature of  
333 rheumatoid synovium. Xanthine oxidase is the first-

334 registered biological source of oxygen free radicals and  
335 plays the leading role in the pathogenesis of tissue damage  
336 by the production of superoxide anion, hydrogen peroxide,  
337 and hydroxyl radical. The roles of XOR in cytokine  
338 induced bone erosion promoting vasculitis have been well  
339 documented by others [15, 16].

340 The slightly higher levels of total IgG and IgM titers in  
341 patients sera determined by SRIDA in RA+ patients  
342 compared with the levels in other joint inflammation and to  
343 the levels in the serum of control healthy are in agreement  
344 with [35, 37, 50]. This is expected because of the auto-  
345 immune nature of the disease. On the other hand, the

346 results showed that synovial fluids of patients suffering  
347 from rheumatoid arthritis and other joint inflammation  
348 contained lower levels of total IgG and IgM compared with  
349 the levels of these immunoglobulins in patients serum. This  
350 disagrees with the finding of others [35, 37, 50]. This may  
351 indicate that some of those immunoglobulins were bound  
352 to the tissue or are bound in immune complexes, which  
353 need to be detected by other techniques. In fact, the  
354 pathology of rheumatoid arthritis is attributed to an Arthus-  
355 like reaction in which immune complexes mediate the  
356 crucial role.

357 The high-titer anti-HXOR in RA patients may be due to  
358 elevated serum and synovial fluid XOR level. In most  
359 cases, more than 50% of synovial XOR is present as oxi-  
360 dase form [51]. The detection of relatively high concen-  
361 tration of XOR enzyme in its oxidative form in rheumatoid  
362 arthritis patient synovial fluids compared with the con-  
363 centration of the enzyme in other joint inflammation indi-  
364 cates that the enzyme XOR is liberated with lysosomal  
365 enzymes from the synovium and could be involved in  
366 cartilage destruction and bone erosion suggesting a positive  
367 correlation between the level of the enzyme and the  
368 severity of the disease.

369 The anti-HXOR (IgM) titers, in the synovial fluid  
370 samples, were found to be significantly higher in other joint  
371 inflammation than in RA+ patients. This finding that is  
372 also reported by others [50] could be explained by the fact  
373 that IgM anti-XOR is more efficient in immune complex  
374 formation and consequently damage in RA+ patients due  
375 to activation of the complement system.

376 The levels of IgM-containing XORICs were consider-  
377 ably higher than those of IgG, reflecting the relative levels  
378 of the free specific antibodies. In the case of both IgM and  
379 IgG, a high proportion of specific antibodies were in the  
380 complex form (90.34 and 77.06, respectively). It is worth  
381 noting that, despite the relatively high levels of circulating  
382 (complexes) XOR, corresponding enzymic activity is sel-  
383 dom detectable, which most probably reflects the low  
384 specific activity of the human enzyme. This was also  
385 reported by Abadeh et al. [52] and Yamomoto et al. [53].

386 Synovial aspirate were never positive for any bacteria or  
387 fungi. This was also reported by other [54]. A blood culture  
388 may be more revealing if an association between RA dis-  
389 order and infection with a microorganism exists. Some  
390 have reported improvement after treatment of RA patient  
391 with antibiotics [55] suggesting a microbial role. It may be  
392 useful to look for unconventional organisms such as  
393 Chlamydia and Mycoplasma as suggested by other [4, 56].

394 Rheumatoid arthritis may have complex pathology. In  
395 addition to the obvious effect of the rheumatoid factor,  
396 *Proteus mirabilis* was also incriminated. Newkirk et al. [57]  
397 have found that IgM and IgA anti-*Proteus mirabilis*  
398 antibodies were significantly higher in patients with

rheumatoid arthritis RA+ compared with RA-arthritis, 399  
spondyloarthropathy, and undifferentiated arthritis. Simi- 400  
larly, Tani et al. [58] have found that patients with rheuma- 401  
toid arthritis showed elevated levels of antibodies to *Proteus* 402  
*mirabilis* compared with ankylosing spondylitis and con- 403  
trols. They suggested an amino acid homology between an 404  
outer membrane hemolysin protein of *Proteus mirabilis* and 405  
susceptibility sequence in HLA-DR<sub>1</sub> and DR<sub>4</sub> subtypes. 406  
Also, Senior et al. [59] found that 33% of patients with 407  
rheumatoid arthritis had asymptomatic insignificant bacte- 408  
riuria compared with 4% of age- and gender-matched con- 409  
trols. Similarly, patients had significantly higher levels of 410  
IgA, IgM, and IgG antibodies to *Proteus mirabilis* in blood 411  
and urine than controls. It is suggested that this may be the 412  
trigger for the rheumatoid arthritis condition. 413

## 414 Conclusions

415 These findings suggest that XOR may play an important  
416 role as a source of ROS in RA and in other joint inflam-  
417 mation, which participate in self-maintenance of the dis-  
418 eases. Antibodies against XOR may play a major role in RA  
419 by inhibiting both xanthine and NADH oxidase activities of  
420 XOR. They may also play a key role in eliminating XOR  
421 from serum and synovial fluid. However, immune complex  
422 formation could activate the complement system and partici-  
423 pate in self-maintenance of the diseases. 424

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