Uric acid effects on *in vitro* models of rheumatoid inflammatory and autoimmune processes

ROBERT A. TURNER, EDWARD J. PISKO, CARLOS A. AGUDELO, GRACE B. COUNTS, SUSAN L. FOSTER, AND WILLIAM J. TREADWAY

From the Rheumatology Section, Department of Medicine, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC, USA

SUMMARY A neutrophil monolayer system was used to study the effects of uric acid on neutrophil-aggregate interactions important in rheumatoid inflammation. No effect on immunoglobulin G aggregate phagocytosis was seen, but hyperuricaemic levels of uric acid were associated with an enhancement of phagocytosis-induced release of the azurophilic granular enzyme β -glucuronidase. A trinitrophenyl-coupled mononuclear leucocyte rheumatoid factor plaque-forming assay was utilised to study uric acid effects on polyclonal activation of immunocompetent cells. Low levels of uric acid enhanced and high levels suppressed this system. Hyperuricaemia may enhance some aspects of rheumatoid inflammation, while uric acid may modulate an important component of rheumatoid autoimmunity.

Rheumatoid arthritis is a highly prevalent inflammatory disease characterised by autoimmune immunological processes. Gout is a commonly occurring metabolic disease associated with systemic hyperuricaemia which only occasionally coexists with rheumatoid arthritis.¹ The present study was performed to determine whether uric acid modulating effects on rheumatoid inflammatory and autoimmune processes occur, since these effects might help to explain the infrequent simultaneous occurrence of these 2 relatively widespread rheumatological conditions. Uric acid effects on rheumatoid inflammatory processes were examined in an *in-vitro* system utilising neutrophil monolayers and immunoglobulin G (IgG) aggregates. The effects of uric acid on immunocompetent cells were studied in a system in which cells producing rheumatoid factor (RF) were polyclonally induced by haptenated mononuclear leucocytes.

Materials and methods

All studies were approved by the institutional clinical research practices committee. Informed consent was obtained from all participating subjects and all research was carried out according to the principles of the Declaration of Helsinki.

Accepted for publication 1 April 1982.

Correspondence to Robert Turner, MD, Bowman Gray School of Medicine, Winston-Salem, NC 27103, USA).

For the *neutrophil-aggregate studies* normal human polymorphonuclear leucocytes were suspended at $5 \times 10^6/\text{ml}$ concentration in Dulbecco's phosphate buffered saline (PBS) containing 10% autologous serum. Monolayers were prepared by placing 2.5 ml of this cell suspension in 35×10 mm plastic Petri dishes, incubating them for 45 min at 37°C , and washing gently with room temperature PBS. Cells/monolayer were determined by counting cells/standardised grid under an inverted microscope. 1.5 ml of a solution containing PBS, 10% serum, and either, 0, 4, 8, or 12 mg/dl uric acid (obtained from Fisher Scientific Company) were placed on the freshly prepared cell monolayers, which were then incubated for 30 min at 37°C . The supernatants were then removed, and for the phagocytosis studies ^{51}Cr -labelled IgG aggregates (2.4 mg/ml) were then added to the monolayers, which were incubated for 30 min, and cells/monolayer and mg of aggregated IgG/monolayer were then determined by methods previously described.² For enzyme studies unlabelled IgG aggregates were used, and fluid was aspirated from the monolayers for measurement of supernatant enzyme activity. The cell layer and remaining fluid were detergent-lysed and assayed for enzyme release by standard methods previously described.³

Studies inducing *rheumatoid factor producing cells* were performed as previously described.⁴ Peripheral blood mononuclear leucocytes were obtained by venipuncture on healthy adult volunteers. 60 to 100 ml

of heparinised blood was obtained, and cells were isolated on a Ficoll-Hypaque gradient.⁵ These cells were washed twice in Hepes-buffered Hanks's balanced salt solution (Grand Island Biological Company, Grand Island, NY), pH 7.8. Wright stain morphology revealed the cells to be 90% lymphocytes and 10% monocytes. Cells were 98% viable as determined by trypan blue dye exclusion after separation and washing. Washed mononuclear leucocytes were coupled to 2,4,6-trinitrobenzene sulphonic acid (Sigma, St Louis, MO) by Seldin and Rich's modification⁶ of Shearer's technique.⁷ 5×10^6 normal mononuclear leucocytes were cocultured with 1.25×10^6 TNP-coupled mononuclear leucocytes in a 5% CO₂ humidified atmosphere at 37°C in 17×100 mm polystyrene culture tubes (Falcon 2001, Cockeysville, MD). Culture media consisted of 10 ml of Roswell Park Memorial Institute (RPMI)-1640 (Flow Laboratories, Rockville, MD) supplemented with 1% antibiotic-antimycotic (GIBCO, Grand Island, NY), 2 mM L-glutamine (GIBCO, Grand Island, NY), and 10% pooled, heat-inactivated (30-min, 56°C) human AB serum (GIBCO, Grand Island, NY). Uric acid was added to culture medium at a concentration of 4, 8, or 12 mg/dl. The medium was adjusted to pH 7.2 prior to the addition of cells and remained at this level throughout these and the previous monolayer studies. Cultures were fed 1 ml of supplemented media on days 2 and 5.

Rheumatoid-factor-producing cells were assayed on day 7 by a modification of the plaque-forming cell assay of Dosch and Gelfand.^{8,9} Human autologous red cells to be used as indicator cells in the assay were obtained at the time of lymphocyte separation and stored as a 50% solution in RPMI-1640 at 4°C until use. These cells were coupled to heat-aggregated IgG obtained by dissolving human pooled Cohn fraction II IgG (US Biochemical, Cleveland, OH) in normal saline at a concentration of 10 mg/ml and heating this solution at 63°C for 20 min. Heat-aggregated IgG at a concentration of 5 mg/ml was coupled to the red cells with chromic chloride by the method of Sweet and Welborn.¹⁰ Monolayers of these cells were prepared on flat bottom 96-well cluster plates (Dynatech Laboratories, Alexandria, VA) with poly-L-lysine (Sigma, St Louis, MO). Plates containing 50 µl of poly-L-lysine (25 µg/ml) per well were incubated at 37°C for 30 min and washed 3 times with PBS pH 7.4. 100 µl of the 5% red cell suspension was added to each well and plates were centrifuged at 1000 rpm for 5 min and then incubated at 37°C for 1 hour. Unbound red cells were eluted and monolayers were overlaid with 75 µl of RPMI-1640 until used. Monolayers were then inspected with an inverted microscope and only intact monolayers were used.

Cultures were used on day 7 if there was $\geq 90\%$

viability before the assay. Cell viability, pH, and yield were unaffected by the addition of uric acid to cultures. Cultured mononuclear leucocytes (10^4 to 10^5 cells) were added to each well containing the red cell monolayers after 2 washings with RPMI-1640. Guinea-pig serum was used as a source of complement and was obtained by cardiac puncture and stored at -70°C. This serum was adsorbed with the red cells at 0°C for 60 min, diluted 1:20 in normal saline, and 25 µl of this solution was added to each well. Plates were then incubated at 37°C for 1 hour. Plaques were counted with an inverted microscope. Five wells were counted per culture. Specificity of the assay for IgG was determined by successfully competitively inhibiting the assay with aggregated IgG.

Statistical analysis of experimental data was performed by standard methods. One-way analysis of variance was performed on the data, and Tukey's multiple comparison test was used to test the significance of all possible comparisons between groups.

Results

As shown in Table 1 adequate numbers (mean $\pm$ SEM $\times 10^6 = 3.44 \pm 0.82$) of neutrophils remained on the monolayers throughout the neutrophil-aggregate studies. As previously reported,¹¹ adherence of these cells was not significantly ($p < 0.05$) affected by incubation with IgG aggregates or by the addition of increasing concentrations of uric acid to the system. As shown in Table 1 the uptake of ⁵¹Cr-labelled IgG aggregates (specific activity 78 472 cpm/mg IgG) by neutrophil monolayers calculated as µg IgG/monolayer was not affected by the increasing concentrations of uric acid used in these studies. Additional calculations using µg IgG/neutrophil or cpm/monolayer or /neutrophil also showed no differences in the phagocytic capacities of neutrophils exposed to aggregates in the presence of 0, 4, 8, or 12 mg/dl of uric acid. (SI conversion: mg/dl $\times 0.01 =$ g/l.)

Phagocytosis of the aggregates did not cause significant release of the cytoplasmic enzyme lactic dehydrogenase (LDH), and this release was not affected by increased concentrations of uric acid. There was, however, a significant ($p < 0.05$) effect on the release of the azurophilic lysosomal enzyme β -glucuronidase as shown in Fig. 1. This effect was apparent at 4 mg/dl and reached statistical significance ($p < 0.05$) at 8 and 12 mg/dl. Shown in Fig. 2 are the effects of similar concentrations of uric acid on the azurophilic and specific lysosomal enzyme, lysozyme. There is an apparent increase in release with increasing concentrations of uric acid, but this effect did not cause a statistically significantly different

Table 1 Effect of uric acid on neutrophil monolayer: phagocytosis of heat-aggregated IgG

Uric acid concentration (mg/dl)	μ g heat aggregated IgG/neutrophil monolayer			Mean $\pm$ SEM*
	Expt 1	Expt 2	Expt 3	
0	423 858	709 826	422 402	620 $\pm$ 67
4	763 1346	443 925	482 610	761 $\pm$ 137
8	1005 715	581 465	479 455	616 $\pm$ 88
12	991 1039 (3.36 $\pm$ 0.22)	674 610 (2.22 $\pm$ 0.06)	466 458 (4.92 $\pm$ 0.17)	706 $\pm$ 104 (3.44 $\pm$ 0.82)

() Neutrophils/monolayer $\times 10^6 \pm$ SEM for each of 3 separate experiments (expts).

*No statistically significant ($p < 0.05$) differences between groups.

SI conversion: mg/dl $\times 0.01 =$ g/l.

Fig. 1 The effect of uric acid on IgG aggregate-induced release of β -glucuronidase by neutrophil monolayers. Bars represent mean $\pm$ SEM enzyme release on 5 determinations in the absence (-) and presence (+) of heat-aggregated immunoglobulin G (HA IgG). Asterisks (*) indicate release statistically significantly different from control values.

Fig. 2 The effect of uric acid on IgG aggregate-induced release of lysozyme by neutrophil monolayers. Bars represent mean $\pm$ SEM enzyme release on 5 determinations in the absence (-) and presence (+) of heat-aggregated immunoglobulin G (HA IgG).

lysozyme release under the conditions of our study.

The results of experiments done to determine the effects of increasing concentrations of uric acid on the trinitrophenyl-coupled mononuclear-leucocyte-induced polyclonal activation of lymphocytes to produce rheumatoid factor are shown in Fig. 3. In these experiments rheumatoid-factor-producing cells were generated after coculturing normal and TNP-haptenated mononuclear leucocytes. Uric acid at a concentration of 4 mg/dl significantly ($p < 0.01$)

Fig. 3 The effect of uric acid on the induction of rheumatoid-factor-producing cells. The data are represented as the mean $\pm$ SEM of 3 experiments on separate individuals. Asterisks (*) indicate cultures statistically significantly different from controls.

increased the numbers of rheumatoid-factor-producing cells detected in these cultures when compared to control cultures without added uric acid. Cultures containing 8 mg/dl of uric acid were no different from control cultures containing no added uric acid, but cultures containing 12 mg/dl of uric acid had significantly ($p < 0.01$) less rheumatoid-factor-producing cells than control cultures without added uric acid.

Discussion

These studies suggest that hyperuricaemia does not affect the phagocytosis of IgG aggregates, which has been thought¹² to be an important contributing factor to the inflammation occurring in the joints of patients with rheumatoid arthritis. This finding is in keeping with that of Malawista *et al.*,¹³ who reported a similar lack of effect on phagocytosis using uric acid, human neutrophils, and different particles. Their study demonstrated augmented LDH release in the presence of silica crystals but not bacteria. They attributed this augmented release to inhibition by uric acid of the binding of albumin but not IgG to membrane surfaces and subsequent increased susceptibility to crystal-induced phagolysosome lysis and cell death. We have extended these studies in a system which does not cause early cell death but does result in the selective release of lysosomal enzymes. It is clear from our studies that high levels of uric acid enhance the release of azurophilic granular enzymes. This effect did not seem to be mediated through crystal formation as (1) none were detected by polarised light microscopic examination of test solutions; (2) there was no effect on enzyme release by the highest concentrations of uric acid used alone with the cells; and (3) crystallisation may not be expected to occur under the experimental conditions employed in these studies.^{14 15}

High levels of uric acid clearly inhibit the ability of our normal mononuclear cell system to be activated to produce rheumatoid factor. This activation may be a model for the response of the rheumatoid patient's immunological system to the factors responsible for the initiation and persistence of the rheumatoid process. This process often results in invasion of cartilage by masses of activated cells and the erosion of cartilage and bone by the rheumatoid pannus. Lussier and deMedicis¹⁶ studied the effects of hyperuricaemia on adjuvant arthritis which had been used as an in-vivo animal model for the study of rheumatoid joint disease processes. Their studies showed weak uric acid effects on the primary inflammatory stage but marked inhibitory effects on the secondary destructive stage of this experimental model. Our studies suggest similar findings with an enhancement of

inflammatory but an inhibition of immunological processes by high levels of uric acid. Although there have been a number of recent clinical reports¹⁷⁻²¹ of coexisting gout and rheumatoid arthritis, our study is the first to describe uric acid effects on in-vitro neutrophil and lymphocyte functional systems designed as models of rheumatoid inflammation and autoimmunity. Future clinical studies might prospectively examine the effects of hyperuricaemia on these aspects of rheumatoid disease processes and determine their effects on the x-ray progression which is seen in many patients with rheumatoid arthritis.

This project was supported in part by grants from the Arthritis Foundation and the Easter Seal Research Foundation.

References

- 1 Ramay D, Abdalla A M, Newton D R L, *et al.* Coexistent rheumatoid arthritis and tophaceous gout: a case report. *Ann Rheum Dis* 1981; **40**: 427-9.
- 2 Turner R, Counts G, Mashburn H, *et al.* Drug and rheumatoid factor effects on the uptake of immunoglobulin G aggregates. *Inflammation* 1980; **4**: 55-63.
- 3 Turner R, Martin M, Schroff J, *et al.* Neutrophil enzyme activities in rheumatoid inflammation. *Inflammation* 1979; **3**: 319-27.
- 4 Pisko E J, Foster S L, Turner R A. Induction of autoantibody producing cells after the co-culture of haptenated and normal human mononuclear leukocytes. *Proc Soc Exp Biol Med* 1981; **168**: 131-136.
- 5 Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* 1968; **21** (suppl 97): 77-89.
- 6 Seldin M F, Rich R R. Human immune responses to hapten-conjugated cells. I. Primary and secondary proliferative responses in vitro. *J Exp Med* 1978; **147**: 1671-83.
- 7 Shearer G M. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. *Eur J Immunol* 1974; **4**: 527-33.
- 8 Dosch H M, Gelfand E W. In vitro induction and measurement of hemolytic plaque forming cells in man. *J Immunol Methods* 1976; **11**: 107-16.
- 9 Dosch H M, Gelfand E W. Generation of human plaque forming cells in culture: tissue distribution, antigenic and cellular requirements. *J Immunol* 1977; **118**: 302-8.
- 10 Sweet G H, Welborn F L. Use of chromium chloride as the coupling agent in a modified plaque assay cells producing anti-protein antibody. *J Immunol* 1971; **106**: 1407-10.
- 11 Turner R, Counts G, Johnson J, *et al.* Neutrophil interactions with particulate materials. In: Willoughby D A, Giroud J P, eds. *Inflammation: Mechanisms and Treatment*. Lancaster: MTP Press, 1980: 347-53.
- 12 Hollander J L, McCarty D J Jr, Astorga G, *et al.* Studies on the pathogenesis of rheumatoid joint inflammation. I. The 'R. A. Cell' and a working hypothesis. *Ann Intern Med* 1975; **62**: 271-80.
- 13 Malawista S E, Blaricom G V, Gretulla S B, *et al.* The phagocytic potential of urate in solution. Studies of phagocytic process in human leukocytes. *Arthritis Rheum* 1979; **22**: 728-36.
- 14 Fried F A, Vermeulen C W. Artificial uric acid concretions and observations on uric acid solubility and supersaturation. *Invest Urol* 1964; **2**: 131-44.

- 15 Klinenberg J R, Bluestone R, Schlosstein L, *et al.* Urate deposition disease. How is it regulated and how can it be modified? *Ann Intern Med* 1973; **78**: 99–111.
- 16 Lussier A, deMedicis R. Inhibition of adjuvant arthritis in the rat by an oxonate diet: sequential studies. *J Rheumatol* 1977; **4**: 369–76.
- 17 Talbott J H, Alyman R D, Yu T E. Gouty arthritis masquerading as rheumatoid arthritis or vice versa. *Semin Arthritis Rheum* 1978; **8**: 77–114.
- 18 Jessee E F, Toone E, Owen D S, *et al.* Coexistent rheumatoid arthritis and chronic tophaceous gout. *Arthritis Rheum* 1980; **23**: 244–7.
- 19 Rizzoli A J, Trujeque L, Bankhurst A D. The coexistence of gout and rheumatoid arthritis: case reports and a review of the literature. *J Rheumatol* 1980; **7**: 316–24.
- 20 Waterworth R F. The effect of allopurinol therapy on coexistent gout and rheumatoid arthritis. *Arthritis Rheum* 1981; **24**: 103–4.
- 21 Wallace O J, Klinenberg J R, Morhaim D, *et al.* Coexistent gout and rheumatoid arthritis. *Arthritis Rheum* 1979; **22**: 81–6.