C5a activation by monosodium urate crystals: effect of adsorption of serum and polymorphonuclear leucocyte homogenate

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Acute gout is characterised by intermittent episodes of acute inflammation of joints separated by symptom-free periods. This acute inflammatory response is not solely dependent on the presence of intra-articular monosodium urate crystals (MSU), as they can be found in asymptomatic joints, and the control mechanisms, particularly of the switch off, are unresolved. MSU crystals bind a wide variety of proteins, especially IgG¹ but also cytoplasmic and lysosomal constituents.² Adsorbed IgG may promote complement activation³ and increase polymorphonuclear leucocyte (PMN) lysosomal enzyme release.⁴ Alterations in the crystal coating subsequent to phagocytosis by PMNs may thus reduce or abrogate their phlogistic properties. In addition, it is possible that MSU crystals cause local depletion of pro-inflammatory factors by adsorption and thereby modify the inflammatory response.

MSU crystals activate serum complement, leading to the generation of the potent chemotaxin C5a,⁵ and a principal activity of PMN in response to acute inflammation is chemotaxis. We have, therefore, looked at the ability of MSU to generate chemotactic activity in serum and the effect of serum coating and PMN homogenate on this ability. We also looked at the ability of these crystals to activate serum depleted by adsorption with MSU.

Synthetic crystals of MSU were coated with normal human serum at 5 mg/ml 4°C for 60 min. Coated or plain MSU crystals were then incubated with normal human serum or adsorbed serum for 4 h at 37°C, with or without having been previously incubated for

60 min at 37°C in a PMN homogenate. Adsorbed serum was the supernatant left after coating the MSU crystals. PMN homogenate was prepared by dextran sedimentation of heparinised venous blood, RBC lysis, freeze-thawing and sonicating a suspension of 2×10^7 PMN/ml. MSU crystals were washed twice with MSU saturated sodium phosphate 0.0001 mmol buffer, pH 7.4, between all stages, and all incubations performed with constant mixing. Chemotactic activity was assayed by a modified agarose technique,⁶ all samples being heat inactivated at 56°C for 30 min. Migration distance was taken as the average distance travelled by the five most rapidly travelling cells towards the test well.

Coated MSU crystals were as capable of activating normal human serum and adsorbed serum as plain crystals. Comparing activation of adsorbed and non-adsorbed serum, significantly less chemotactic activity was generated in adsorbed than in normal human serum by coated MSU crystals in four of five experiments ($p = 0.01$) and by plain MSU crystals in two of five experiments ($p = 0.01$).

Generation of chemotactic activity in fresh serum by plain or coated MSU was unaffected by treatment with PMN homogenate, but the activation of adsorbed serum by coated MSU crystals was significantly increased after treatment with PMN homogenate in two of three experiments ($p = 0.01$).

Cell homogenate showed no significant activation of fresh or adsorbed serum in solution.

Adsorbed serum showed normal generation of chemotactic activity with zymosan (1 mg/ml 30 min 37°C)

compared with normal human serum.

Using a functional assay for C5a we found that coating MSU crystals in serum, where IgG is the principal adsorbed molecule,¹ had no effect on the activation of C5a.

The effect of treating coated MSU crystals with PMN homogenate in increasing the generation of chemotactic activity in adsorbed serum cannot be attributed to any direct activation by PMN homogenate and must be due to changes in the MSU coating.

It is interesting that adsorbed serum when exposed to coated MSU crystals failed to generate as much chemotactic activity as similarly treated fresh serum. As the coated crystals used to activate the adsorbed serum in these experiments were the same as those used in the initial adsorption, then no factors should have been depleted from this system. This suggests that binding of serum components to MSU crystal surfaces may produce changes in the adsorbed material that impairs the generation of chemotactic activity. This may be an important factor in the self limitation of acute gouty arthritis.

References

- 1 Kozin F, McCarty D J. Protein binding to monosodium urate monohydrate, calcium pyrophosphate dihydrate, and silicon dioxide crystals. I. Physical characteristics. *J Lab Clin Med* 1977; **89**: 1314-25.
- 2 Ginsberg M H, Kozin F, Chow D, May J, Skosey J L. Adsorption of polymorphonuclear leukocyte lysosomal enzymes to monosodium urate crystals. *Arthritis Rheum* 1977; **20**: 1538-42.
- 3 Hasselbacher P. C3 activation by monosodium urate monohydrate is enhanced by surface IgG. *Arthritis Rheum* 1979; **22**: 620.

- 4 Kozin F, Ginsberg M H, Skosey J L. Polymorphonuclear leukocyte responses to monosodium urate crystals: modification by adsorbed serum proteins. *J Rheumatol* 1979; **6**: 519-26.
- 5 Russell I J, Mansen C, Kolb L M, Kolb W P. Activation of the fifth component

of human complement (C5) induced by monosodium urate crystals: C5 convertase assembly on the crystal surface. *Clin Immunol Immunopathol* 1982; **24**: 239-50.

- 6 Nelson R D, Quie P G, Simmons R L. Chemotaxis under agarose: a new and

simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. *J Immunol* 1975; **115**: 1650-6.

Inflammatory effect of aluminium phosphate

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Microcrystalline-induced arthritis is known to occur in patients undergoing haemodialysis for chronic renal insufficiency. The following microcrystals have been implicated so far in these inflammatory phenomena: apatite, calcium pyrophosphate dihydrate (CPPD), and sodium monourate.

In preliminary study,^{1,2} using scanning electron microscopy (SEM) and wavelength dispersive microanalysis (WDM), we found aluminium deposits associated with phosphate in synovial tissue from a patient undergoing haemodialysis because of chronic renal insufficiency, who had been taking aluminium hydroxide for five years. Aluminium concentrations measured with a sensitive and reproducible pulse polarography method were raised in the synovial fluid ($87 \mu\text{g/l}$), synovial tissue (3.7 and $2.2 \mu\text{g/g}$ wet weight) and in the cartilage ($22 \mu\text{g/g}$ wet weight) compared with controls: synovial fluid: $10.8 \pm 5 \mu\text{g/l}$ ($n = 6$), synovial tissue: $0.8 \pm 0.2 \mu\text{g/g}$ ($n = 4$), cartilage: $8.31 \pm 2.0 \mu\text{g/g}$ ($n = 4$). Transmission electron microscopy (TEM) revealed electron dense material of microfibrillar appearance in lysosomes of macrophagic synovial cells.

In a second part we developed experimental models to appreciate the prophlogistic properties of aluminium

phosphate using two different animal models: (a) paw oedema after subcutaneous injection in the rear foot pad of Sprague Dawley rats and (b) experimental arthritis after intra-articular injection in rabbits.

Tribasic aluminium phosphate was compared to calcium hydrogen phosphate dihydrate previously found during destructive chondrocalcinosis in humans³; natural diamond powder and lambda carrageenan were chosen as negative and positive controls.

In the first model, inflammation induced by aluminium phosphate reached its maximum as soon as 15-30 min after injection but lasted only 24 hours. The response to calcium hydrogen phosphate dihydrate was only minor whereas carrageenan-induced inflammation appeared after two hours but was far greater and lasted more than 24 hours.

The second experimental proceeding was used to evaluate the histological consequences of the injection of these compounds on synovial tissue and cartilage. Rabbits were injected in the knee joints every day for three days. Aluminium phosphate can induce, like carrageenan, inflammatory lesions of the synovial tissue and erosive damage of the articular cartilage. With TEM, lysosomal inclusions of phagocytosed material with microfilaments were seen. Through SEM coupled with a

wavelength dispersive microprobe analysis, aluminium associated with phosphate was found in cellular elements. These features are similar to those observed in the patient in our preliminary study and those described in cerebral tissue from patients with aluminium intoxication.

The inflammatory effect demonstrated in this study suggests that in addition to apatite, calcium pyrophosphate dihydrate and sodium monourate, aluminium compounds could play a part in clinical manifestations observed in patients with chronic renal insufficiency undergoing haemodialysis and receiving aluminium gels.

References

- 1 Netter P, Burnel D, Hutin M F, Kessler M F, Faure G. Aluminium in joint tissues of patient taking aluminium hydroxide. *Lancet* 1981; **i**: 1056-7.
- 2 Netter P. *L'aluminium dans l'os, le cartilage, la membrane synoviale et le liquide synovial*. University of Nancy, Nancy, 1980. Thesis.
- 3 Royer R J, Delongueas J L, Netter P, et al. Inflammatory effect of aluminium on rat paws. *Pathol Biol (Paris)* 1982; **30**: 211-5.
- 4 Faure G, Netter P, Malaman B, Steinmetz J. Monocrystalline calcium hydrogen phosphate dihydrate in destructive arthropathies of chondrocalcinosis. *Lancet* 1977; **ii**: 142-3.