Synovial irritants: crystals, microbes and others—their implications for diagnosis, pathogenesis and therapy

H Ralph Schumacher Jr

Having accepted the above title for a catchy lecture subject, I proceeded to focus on a variety of visible factors that could be pathogenic in joints by lodging or precipitating in the synovium. These offer the possibility for ready diagnosis even in a routine pathology laboratory, though techniques not yet widely available can also expand diagnosis of such irritants. In addition, examination of how irritants may localise and cause disease provides an important opportunity to examine pathogenetic mechanisms and thus some new possible directions to be investigated for therapeutic approaches. Several structures and properties in synovial tissue appear to be important in localisation of potential pathogens and irritants to the joint.

At least some crystals seem most likely to precipitate in synovium in the matrix. Crystals and debris released from cartilage can also be sequestered in synovium; these are often seen in synovial lining cell and deeper cell vacuoles.

The prominent microvasculature of synovium may be important in emigration of bacteria and other microbes into the joint.¹ Circulating organisms, particles, and immune aggregates escape alone through venules, or can be carried into the synovium in phagocytic cells. Matrix molecules may influence sites of sequestration.

Less commonly, microbes or other irritants penetrate joints through overt or occult wounds. Foreign bodies may remain in synovium and produce intermittent arthritis. Prosthetic wear particles can produce chronic synovitis that contributes to implant loosening. Necrosis of joint tissues can provide endogenous irritants. Lipids and fatty acids released from fat in synovium can cause joint inflammation.

Elimination or prevention of some of these broadly defined irritants offers a potentially more direct way of controlling joint disease than treatment of the complex responses that they elicit.

Crystals

DIAGNOSIS

Although it should be possible, in general, to diagnose crystal associated diseases from careful examination of the synovial fluid (SF), there continue to be situations in which the diagnosis is first established at evaluation of the synovium removed at surgery or arthroscopy. Rarely, this is because crystals were not present in joint fluid because all crystalline material

was sequestered deep in the matrix of synovium or cartilage; much more often, fluid was not aspirated or examined. When episodes of arthritis have occurred, we virtually always find at least a few crystals in SF.

Tophaceous deposits of monosodium urate (MSU) in gout are best seen in frozen sections or in tissue processed with alcohol fixation. Urate crystals are water soluble and one can be left with only a tophus like site from which crystals have been dissolved; such sites have occasionally been confused with rheumatoid nodules. It is important to ascertain that crystals are clearly associated with cells and some reaction in the synovium. Bear in mind that a variety of artefacts can be birefringent. Deposits of calcium pyrophosphate dihydrate (CPPD) also can precipitate primarily in the synovium in tophus like aggregates. CPPD is not water soluble, but the crystals can be lost if the biopsy is decalcified. The amount of CPPD in the deposits is often small and can be ignored unless under specific consideration. Apatites and oxalate are the other crystals documented in synovial deposits. More studies are required on how these deposit; most often we have found them relatively superficially, as if they have been sequestered there after release perhaps from cartilage or other sites into the synovium. Apatites are not birefringent, but can be suspected if one sees haematoxyphilic clumps, further supported by calcium stains such as alizarin red, and confirmed by electron microscopy with the help of elemental analysis.²

PATHOGENESIS

At least two aspects of pathogenesis are of investigative interest in considering crystals in the synovium: mechanisms of development and localisation to the synovium, and mechanisms of production of synovial inflammation and joint damage.

MSU crystals deposit in a variety of connective tissues, including synovium; they appear to precipitate in the matrix in the presence of supersaturation of serum and tissue fluid with urate. Some local factors must be involved. The most common hypothesis is that some local change, perhaps related to loss of proteoglycans, eliminates an inhibitory factor and influences their localisation. Antecedent joint disease may favour their deposition in gout, but we have also found synovial microtophi during the first clinically detected attacks of gout. Concentration of IgG

has been noted in synovial tophi.³ Connective tissue is perfused with IgG;⁴ its apparent concentration in tophi, however, is still incompletely explained. IgG coating the crystals may increase the inflammatory response to crystals when they are released into the joint space where they can readily attract polymorphonuclear leucocytes.⁵ In synovium, crystals in tophi seem to induce a more chronic giant cell containing response around the tophi, and are usually asymptomatic while in the tophi.

CPPD crystals can also deposit initially in synovium in addition to cartilage. Our studies have suggested that most, if not all, primary deposits of CPPD are in areas of chondrometaplasia, with the smallest crystals lined up along degenerated collagen fibres.⁶ CPPD (and urates) can also be collected back in the synovium after bouts of synovitis. These tend to be in mononuclear phagocytes.

THERAPY

Treatment of gout with urate depleting agents provides the best proof that removal of the irritant from the joint space can be curative. Complete elimination of MSU may require smaller concentrations of serum uric acid (about 5 mg/dl) and longer durations than previously considered. Methods are not available to eliminate most other crystals. In renal dialysis patients with hyperphosphataemia causing apatites to deposit, decreasing the phosphate concentrations can resorb all apatite.

Infectious agents

DIAGNOSIS

For some time it has been recognised that many joint infections are present and perpetuated primarily in the synovium, rather than the SF, and thus are more readily diagnosed if synovial tissue is cultured or examined microscopically.⁷ This is most common with mycobacterial and fungal infections. We have one experience in which gonococci were easily cultured from synovium when SF cultures had been negative. How often this explains culture negative gonococcal arthritis is not known. In unexplained monoarthritis or oligoarthritis, we generally send synovial biopsy specimens for culture, and perform histological examination looking for granulomas or other clues to infection, tissue gram stains, acid fast stains, and Grocott stains for fungi. Possibly much more important is growing evidence that some agents which elude cultures and staining by light microscopy can be identified as likely causes of arthritis with the newer techniques of electron microscopy, immunohistology, immuno electron microscopy, polymerase chain reactions (PCR), molecular hybridisation and in situ hybridisation. Generally acceptable examples of such organisms are the *Borrelia* of Lyme disease,⁸ and *Chlamydia* in Reiter's syndrome and other 'reactive' or unclassified arthritis. With *Chlamydia*, the presence of chlamydial ribosomal RNA⁹ and electron microscope

evidence of intact elementary and reticulate bodies has led us and others to speculate that the organism is present in a viable, although perhaps latent, form. We have also found *Ureaplasma* using PCR in a patient with hypogammaglobulinaemia in whom cultures were negative.

In some other situations, evidence for infectious agent antigens can be found in the joint while there is as yet no evidence that whole organisms persist. This is currently the case with *Yersinia*, *Salmonella*, and *Shigella* in reactive arthritis. In some psoriatic arthritis we have found synovial peptidoglycan antigen, presumably of bacterial origin, using immuno electron microscopy. Continued searches for infectious material are needed in all the unexplained diseases. Electron microscopic demonstration of tubuloreticular structures in vascular endothelial and mononuclear cells may suggest a viral trigger. These tubuloreticular structures are known to be present in systemic lupus erythematosus and dermatomyositis, are generally related to production of interferon gamma, and are also common in HIV, other virus infections, and some malignancies.¹⁰

PATHOGENESIS

Infectious agents probably produce disease in joints by a wide variety of mechanisms including direct effects on invaded cells, toxins, and possibly the immune response that they elicit. The nature of the synovitis is influenced by the properties of the organism and its route of entry into the synovium. Although some organisms enter via penetration from the skin or invasion from bone, most arrive at the joint via the circulation: the synovium is a richly vascular tissue. There has been some evidence for years that bacteria and other circulating particles preferentially localise to joints. As with crystals, joints previously involved with some other disease are more likely to be infected. Some infectious agents may be carried to synovium in emigrating macrophages; endothelial adhesion molecules may be important in their localisation.

Matrix molecules seem likely to be involved as influences on sites of sequestration. Recent studies suggest that *Borrelia* and *Chlamydia* can persist in viable but relatively inactive forms resistant to clearance, in deep synovial macrophages and fibrocytes. *Chlamydia* may be poorly expressing their major outer membrane protein and other surface markers and thus elude immune surveillance.¹¹ After latent periods, they seem to be able to produce new cycles of infectious elementary bodies and become symptomatic again.

TREATMENT

In most classical infections, antibiotic treatment and adequate drainage or debridement can be successful in eliminating the irritant and providing a cure for the synovitis. Resistant forms of some mycobacteria can be a concern. Recent evidence suggests that *Borrelia* and

Chlamydia may be especially difficult to eradicate. Although Finnish and German studies have shown shortening of episodes of *Chlamydia* associated 'reactive arthritis' with tetracycline,¹² our own experience is that organisms (and disease) can persist even after aggressive antibiotic treatment. Perhaps the ideal antichlamydial agents remain unavailable, though azithromycin deserves further use. Perhaps, also, problems lie in a genetically determined cellular ability to kill the organisms.

Very similar chronic inapparent infection in Lyme synovitis has recently been suggested by PCR studies showing persistent borrelial DNA in inflamed joints, even after antibiotics. If persistent infection is suspected, it seems wise to avoid immunosuppressive treatments that may exacerbate the problem of clearing the organisms. Some 'post infectious' synovitis seems to be caused by an immune response to lipopolysaccharide and other residual matter from dead organisms. Although this may last for months, resolution generally does occur.

Other irritants

DIAGNOSIS

Careful microscope evaluation of the synovial membrane can identify some other less common but potentially important irritants. Foreign bodies penetrating through the skin usually are cleared from the synovial fluid into the synovium. A high index of suspicion may be needed to detect the dark, non-birefringent particles of lead from a bullet, or the very brightly birefringent irregular fragments from sea urchin spines. An intact plant thorn or even small splinters are usually identified by the plant cell structure. Foreign bodies tend to be phagocytised and surrounded by a giant cell containing reaction.¹³ Foreign materials in the synovium can also come from deliberately implanted materials such as joint replacements. Grainy metal particles can be inconspicuous but easily detected by electron microscopy with elemental analysis. Polymethyl methacrylate can be seen; polyethylene droplets are birefringent and may actually be the most persistent and important wear particles. Silicone from spacers can produce a dramatic giant cell response. Interestingly, though arthritis does occur in some patients with leakage from silicone breast implants, we have not found evidence of silicone in the synovium.

Necrotic components of normal synovium can occasionally be identified as a cause of arthritis. Smudgy necrosis of synovial fat can be seen as a cause of release of necrotic lipid into the joint space in patients with pancreatic disease and increased concentrations of lipase.¹⁴

PATHOGENESIS

Investigation of the mechanisms by which foreign bodies and wear particles produce disease is an important area for study, as these

agents seem to be possibly more prominent and persistent in the synovium than in joint fluid. Lectins from plant thorns could be considered as stimulants for the surrounding immune cells. Wear particles are phagocytised and lead to release of cytokines such as tumour necrosis factor (TNF) and of prostaglandin E, which may be important in osteolysis and loosening of implants. Size and surface properties of particles influence how wear fragments are handled.

TREATMENT

Isolated foreign bodies can be removed, but the massive numbers of small wear particles seen after joint implants are much more problematic. One therapeutic strategy is to develop biomaterials that do not wear, or that produce particles of either extremely small or larger size that are less biologically active. Surface properties might also be manipulated to decrease or alter binding of proteins which, as with crystals, may accentuate inflammation. Drug therapy requires consideration: some of our preliminary work suggests that non-steroidal anti-inflammatory drugs used for symptomatic treatment can increase TNF production and potentially increase implant loosening. An experimental system has indicated that this may be blocked by misoprostol.¹⁵

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