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HYPOTHESIS

How can a causal role for small bacteria in chronic inflammatory arthritides be established or refuted?

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The concept of septic arthritis in which bacteria replicate in joint tissue and cause inflammation, joint destruction, and sometimes death is well established. Equally, the idea that some forms of aseptic inflammatory arthritis, such as rheumatoid arthritis, may well be provoked or sustained by the direct or indirect effects of microbial infection is familiar and attractive, though the notion is underpinned more by logic than by data. Between these extremes, reactive arthritis appears to offer a new understanding of microbial pathogenesis as joints, conventionally aseptic, have been found to contain small numbers of bacteria. These bacteria, principally chlamydiae and mycoplasmas, may well provoke and maintain the arthritis. Much evidence has accumulated that they have proinflammatory components, induce immune responses, and could interact with HLA-B27 in a manner consistent with current theories of disease pathogenesis.

The issue of searching for bacteria in joint tissue, however, has become complex. When conventional cultures are positive, the effect of eradication of the micro-organism can readily be gauged, so that the significance of finding the bacterium in the joint can be established. But, when a small number of intra-articular bacteria can only be detected by using ultrasensitive molecular techniques, mainly those based on the polymerase chain reaction (PCR), and there is no discernible benefit from antimicrobial treatment, at least so far as current data show, how can we tell whether these bacteria are part of the normal milieu, have spread from elsewhere into an already inflamed joint as innocent passengers, have spread from elsewhere and contribute to disease persistence or, in fact, are the cause of the disease? By comparison, identifying *Mycobacterium tuberculosis* in diseased lung by culture provides satisfactory evidence of spe-

cific infection and using a PCR or other nucleic acid amplification technique may reduce the time for diagnosis in acute cases by 40-fold.¹ However, identifying DNA or RNA components of bacilli by a PCR in diseased or, particularly, apparently healthy lung, may raise concern about their role in pathogenesis. It is a different scenario, the significance of which has to be established.

PCR technology now allows us to search for minute numbers of bacteria, of any kind, in any tissue. Inevitably, there will be positive findings and some will be consistent with prejudices and data about arthritis development and some will not. There are temptations, therefore, to take up extreme views—either micro-organisms identified in a joint must be the cause of the arthritis or, alternatively, their presence may be seen as uninterpretable and therefore insignificant. Dispassionately, however, neither point of view is logical or tenable and the fundamental question remains: how can we decide whether bacteria identified in an apparently aseptic inflamed joint are or are not the cause of the arthritis? It is essential to take both the new and older observations seriously and not to dismiss them out of hand. Indeed, the time has come to consider in depth what needs to be done before accepting a micro-organism as a cause of arthritis. Kuipers and colleagues have recently set out one approach to understanding the role of bacteria in reactive arthritis.² We accept that the meaning of the term “reactive arthritis” has changed over time and that the original implication of an inflammatory joint response to an extra-articular rather than intra-articular micro-organism is no longer appropriate. However, it is not at all clear to us whether each of the bacteria on the list proposed by Kuipers and colleagues as triggering reactive arthritis do in fact do so. Such conclusions are based more on “accepted

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wisdom" than clear evidence so that there are very real risks of failing to consider alternative possibilities, however unlikely, which cannot currently be excluded. We have attempted to ask, therefore, what properties of the relation between individual bacteria and joint disorders might indicate that this relation between them is causal rather than incidental. In order to do this, we have not dealt with the issues of diagnostic criteria, with their inevitable pitfalls. We do not set out to review all the available literature, nor to unravel likely or possible pathogenic mechanisms; instead, drawing upon informative clinical circumstances, we have put forward what we believe is a rational set of suggestions which should help in efforts to judge causality.

Koch's postulates—ancient and modern

Determining whether a micro-organism is a cause of a particular disease is, of course, hardly a new problem. Koch put forward his proposals (Koch's postulates) many years ago and these were admirably suited for the conditions he was tackling, where bacteria could be detected in clinical material in pure culture. Subsequently, the criteria were less easily fulfilled by those dealing with infections at a mucosal surface, at which there are multiple different organisms or at a site (joint) distant from the primary (mucosal) site of infection. Thus an extension of the postulates was considered appropriate recently in determining whether *Mycoplasma genitalium* is a cause of acute and chronic urethritis in man.³ The problem has been compounded by development of sensitive molecular techniques, and with it the more frequent detection of micro-organisms. Their detection in joints by such methods stimulated Kingsley to pose the question: how can an aetiological role for a micro-organism in human arthritis be confirmed?⁴ It was suggested, in keeping with Koch's postulates, that firstly, the micro-organism should be detected in most if not all patients; however, it was pointed out that this might not be strictly applicable because chronic arthritides may be triggered by a range of different micro-organisms. Secondly, the micro-organism should reproduce the disease in an animal model; however, it was noted that this might be impossible where pathogens are host-specific. Thirdly, an organism-specific immune response should occur; it was suggested that this provides circumstantial evidence for causality but not proof. All this tends to leave investigators in a quandary as to the paths they should follow in attempting to produce convincing evidence that a certain micro-organism is a cause of a particular type of arthritis. We may all agree that the original postulates do not meet current needs; if so, it is time to consider how investigations should be aimed so that we come to an understanding of the true causes of arthritis.

To help in defining a yardstick against which to assess causality, we have chosen to consider two types of human arthritis and two families of micro-organisms. Lyme arthritis and mycoplasmal arthritis in hypogammaglobulinaemia

are chosen because, though uncommon, there is wide consensus on their bacterial cause. Mycoplasmas and chlamydiae are chosen because their possible roles in several forms of arthritis have recently come under scrutiny.

Lyme arthritis

It was, in fact, the discovery that the apparently aseptic and sometimes chronic form of arthritis occurring in Lyme disease⁵ was linked to the presence of identifiable spirochaetes within diseased synovium that caused a radical rethink of the role of infectious agents in arthritis. Epidemiological studies clearly linked the development of arthritis with bites by ixodid ticks and a characteristic skin lesion.⁶ After the demonstration of transmission of a spirochaete, *Borrelia burgdorferi*, from the tick to the skin, it became clear that both the skin and joint lesion represented persistent bacterial infection⁷ and that it resolved with appropriate antimicrobial treatment.⁸

Although the distribution of arthritis is often oligoarticular and asymmetrical, the histological appearances of the synovial lesion resemble those of rheumatoid arthritis⁹ and the patterns of cytokine production are comparable with those found in rheumatoid synovium.¹⁰ However, the lesion is distinguished from other forms of joint disease by the identification of *B. burgdorferi* by immunohistochemistry,¹¹ culture,¹² and PCR¹³ techniques in joint tissue from patients with early disease.⁷ In addition, an antibody response to *B. burgdorferi* in patients with Lyme arthritis has been well characterised¹⁴ and is a diagnostic feature. Antibiotic treatment aimed at eradication of *B. burgdorferi* is usually curative in the early stage.⁸ Once arthritis has become chronic, however, the effectiveness of antimicrobial treatment and the likelihood of identifying *B. burgdorferi* in the joint are reduced.¹⁵ Thus it appears that sepsis is the major pathogenetic factor in early disease, but other mechanisms contribute to persistence of chronic disease.

In summary, epidemiological links, detection of the spirochaete in the joint, an antibody response, and response to antimicrobial treatment are key features in ascribing an aetiological role to the micro-organism.

Mycoplasmal arthritis in hypogammaglobulinaemia

Mycoplasmas are the smallest organisms capable of self replication, their small size being ascribable to the presence of only a very small number of copies of even the most important genes.¹⁶ They have no rigid cell wall, in this respect differing from conventional bacteria, and are contained only by a plasma membrane. As a consequence, they are insensitive to antibiotics that act on bacterial cell walls.¹⁷ They cause respiratory and genital tract diseases in humans and animals and their ability to cause naturally occurring and experimentally induced acute and chronic arthritides in animals has placed them under scrutiny as potential initiators of acute and chronic arthritis in humans,¹⁸ even though observations in animals

do not provide significant support for aetiological involvement in human arthritis.

Ureaplasma urealyticum organisms (ureaplasmas) are mycoplasmas, unique in their ability to metabolise urea.¹⁹ Their detection in the synovial fluids of hypogammaglobulinaemic patients by two groups working independently 20 years ago,^{20–21} as well as the isolation of *Mycoplasma pneumoniae* at this time,²² heralded the isolation of ureaplasmas and other mycoplasmas from synovial specimens on numerous occasions subsequently.^{23–31} Sometimes very large numbers of organisms have been recovered, often early in disease and often sequentially. Indeed, it has been determined that isolation can be made from at least two fifths of cases of arthritis occurring in hypogammaglobulinaemia.³² Molecular technology has been used to make the microbiological diagnosis,³³ but whether this approach would make a significant contribution to identifying mycoplasma-associated joint diseases in patients from whose joints mycoplasmas are not culturable is a moot point that needs to be determined. Antibody responses have not been recorded, which is not surprising in view of the paucity of antibody production in this group of patients. Cell mediated responses have not been sought, though they would seem unlikely in view of the strong polymorphonuclear leucocyte response in the joint, signifying the septic nature of the disease. Antibiotic treatment is successful, particularly when given early, but may fail owing to the development of resistance by the organisms.³⁴ A vaccine has never been given, but high titre-specific antibody given passively appears to have been helpful in the management of antibiotic resistant disease.³⁴

Overall, therefore, the features that have incriminated these organisms have been their recovery often early in disease and often repeatedly, a response to antibiotic treatment, and to passively administered antibody.

Proposals to be considered in defining causality

Considering the evidence reviewed above, we suggest that the following statements, which are a modification and extension of Koch's postulates, provide a sound and rational basis for judging a micro-organism as playing a causal role in a particular form of arthritis. It appears logical that a causal micro-organism should:

- Be found by use of a PCR or other molecular techniques more often in specimens (synovial fluid and/or membrane) from patients with the particular arthritis than in those from controls
- Preferably be found in joint specimens by a culture method also
- Be found in more than one joint specimen—that is, in sequential specimens, from the same patient, particularly in chronic disease and preferably in more than one site if more than one is involved
- Be found specifically in joint specimens from patients with early disease

- Be found by other investigators studying different groups of patients with the same disease, preferably in another geographical location
- Stimulate humoral antibody more often and in higher titres, particularly in synovial fluid, in people with arthritis than in those without
- Stimulate a specific cellular response in people with disease rather than in those without.

In addition, there should be clinical improvement after treatment with an antibiotic to which the micro-organism is sensitive, and the disease should be prevented or improved by a vaccine made against the micro-organism.

A rare micro-organism is unlikely to cause a common disease but, conversely, a common micro-organism might cause a rare disease. Thus a degree of epidemiological compatibility must be built into assessments of possible causality, though detailed epidemiological studies may not be helpful. We recognise that interpretation of data relating to some of these proposals may be problematic—such as determining the specificity of cellular immune responses—but such difficulties do not detract from the validity of the proposals themselves. Similarly, the extent to which immunological and other mechanisms contribute to the pathogenesis of the arthritis is outside the scope of this communication. Some antibiotics, especially tetracyclines, have anti-inflammatory properties and these must be taken into account in assessing their effect in arthritis.

Table 1 shows the reasons for believing that *B burgdorferi* causes Lyme arthritis and that mycoplasmas cause arthritis in hypogammaglobulinaemia. These form a yardstick against which it is then possible to consider the various small bacteria (chlamydiae and mycoplasmas) that have been found in the joints of patients with inflammatory arthritides. Against this it may be possible to form a judgment as to whether similar microbiological findings in these conditions support the same conclusions. These assessments are summarised also in table 1. For those proposals that have not been fulfilled, we discuss what might be achieved realistically in future studies.

REACTIVE ARTHRITIS

Association with *U urealyticum* and other mycoplasmas

Ureaplasmas have been associated with a few cases of reactive arthritis^{35–36} and recently were detected by a nested PCR technique in the joint fluid of two patients with reactive arthritis.^{37–38} Furthermore, these organisms and *Mycoplasma fermentans* were detected by a PCR technique and culture, respectively, in joint fluid specimens from a B27 negative woman who developed monoarthritis after an episode of cervicovaginitis.³⁹ In addition, synovial fluid lymphocytes, more than peripheral blood lymphocytes, have been shown to proliferate specifically in response to ureaplasma antigens.^{40–42} As ureaplasmas are associated with genital tract disease,³ it is reasonable to believe that they might trigger a sexually acquired reactive arthritis in some patients.

Table 1 Extent of fulfilment of potential criteria for determining whether a micro-organism is a cause of a particular type of arthritis

Criterion	Lyme arthritis	Arthritis in hypogammaglobulinaemia (mycoplasmas)	Reactive arthritis				Chronic inflammatory arthritides				Juvenile chronic arthritis C.pn.
			U.u.	Other mycoplasmas	C.t.	C.pn.	M.f.	U.u.	Other mycoplasmas	C.t.	
1. Detection by molecular method	++	++	+	+	+++*	—	+++	++	+	+	+
2. Isolation by culture	++	+++	—	+	—	—	++	—	+	—	—
3. Detection in sequential samples	?	+++	?—	?—	?—	?—	+	?	?	?	++
4. Detection in early disease	+++	+++	++	?	+++	?	++	?	?	++	+
5. Consensus among investigators	+++	+++	+	?	+	?	++	?	?	—	?
6. Specific antibody response	+++	NA	?	?	+++	?	?	?	?	?	++
7. Cellular proliferative response	+++	?	++	?	++	++	+	?	?	?	?
8. Response of arthritis to appropriate antibiotic treatment	++	++	?	?	+	?	?	?	?	?	?
9. Prevention or improvement of disease by appropriate vaccine	?	+	?	?	?	?	?	?	?	?	?

U.u. = *Ureaplasma urealyticum*; C.t. = *Chlamydia trachomatis*; C.pn. = *Chlamydia pneumoniae*; M.f. = *Mycoplasma fermentans*; —, +, ++, +++ = No, weak, moderate, strong fulfilment of criterion, respectively; ? = Still questionable because of little or no information; ?— = Questionably negative because of few opportunities for sequential samples; * = Also detected by direct immunofluorescence techniques.

However, whether or not this is so, remains unproved.

Evidence that *Mycoplasma genitalium* is a cause of urethritis is strong,³ so that the possibility that it might cause sexually acquired reactive arthritis exists. It has been detected in the synovial fluid of one such patient by PCR technology,⁴³ but obviously its role, if any, remains unclear. Further efforts should be focused on detection of this micro-organism in joint and other samples.

Association with *Chlamydia trachomatis*

Chlamydiae (*C. pecorum*) have long been recognised as a cause of arthritis in animals,⁴⁴ so are clear candidates for causes of human arthritis. As shown in table 1, a large proportion of the proposals to incriminate an organism as a cause of disease has been met with regard to the possible role of *C. trachomatis*. Initially, there was circumstantial serological evidence to implicate *C. trachomatis* as a cause of sexually acquired reactive arthritis,^{45–49} then direct evidence as a result of finding chlamydial antigen in joints by immunofluorescence or immunohistochemical methods,^{50–52} and then by molecular techniques in the hands of most,^{37 53–58} but not all investigators.^{59 60} *C. trachomatis* has also been demonstrated in the joints of patients with seronegative oligoarthritis.^{56 61–63} However, although a synovial lymphocyte response to *C. trachomatis* antigen is known to occur in patients with sexually acquired reactive arthritis,^{40 41 64–69} the response is not chlamydia-specific, so that this phenomenon cannot be used alone to implicate chlamydiae as a cause. Evidence that *C. trachomatis* antigens are presented by synovial fluid dendritic cells⁶⁹ indicates that these antigens provoke an immune response. This in itself is not proof of the causation of the arthritis, though an immunological basis for the induction of reactive arthritis by chlamydiae has been cogently argued.⁷⁰ However, there are gaps in our knowledge that need to be filled (table 1). It may never be possible to culture *C. trachomatis* from synovial specimens,⁷¹ but showing that the chlamydial genotype in the joint is the same as that detected in the genital site would be of further help in defining causality in acute cases. A close temporal relation between the development of arthritis and a

genital tract infection is clearly an asset in associating *C. trachomatis* with arthritis. However, this temporal relation should not be regarded of itself as defining causality and, therefore, should not prevent further efforts to test the strength of other relations bearing on causality.

Association with *Chlamydia pneumoniae*

C. trachomatis is considered to disseminate from the genital tract and cause reactive arthritis; there seems no inherent reason why *C. pneumoniae* should not spread haematogenously from the respiratory tract and cause the same problem. It is genomically different from *C. trachomatis*,⁷² causes respiratory infections in children and adults,⁷³ and is known to disseminate haematogenously from the respiratory tract because it has been detected in arterial tissues.⁷⁴ However, there is the difficulty that a temporal relation between a respiratory infection caused by *C. pneumoniae* and arthritis is difficult to recognise, mainly because a specific diagnosis of the former is uncommon. Furthermore, in adults, current evidence for an association between this micro-organism and arthritis rests mainly upon a small number of case reports,^{75–80} a serological survey,⁸¹ and an exaggerated synovial lymphocyte response to *C. pneumoniae* antigens in a proportion of arthritic patients studied.⁸² One group has reported detection of *C. pneumoniae* 16S RNA sequences in joint tissue by use of the PCR technique,⁸³ but another found no evidence of *C. pneumoniae* outer membrane 1 gene, also using PCR technology.⁸⁴ Much has yet to be done before a case for causality can be made.

CHRONIC INFLAMMATORY ARTHRITIDES

Association with *M. fermentans*

The isolation of mycoplasmas, including *M. fermentans*, from synovial specimens through the use of cell cultures in the 1960s and early 1970s^{85–88} has to be viewed with scepticism in view of the fact that such cultures were and are frequently contaminated with mycoplasmas. The use of cell-free medium is the only approach that is likely to provide valid results. Nevertheless, the observations of Williams^{89 90} who, by using a sucrose density gradient to concentrate organisms before culture, claimed to have isolated *M. fermentans* from about 40% of synovial specimens from patients with rheumatoid arthritis, came under muted criticism,⁹⁰

particularly as others⁹¹⁻⁹² failed to repeat the observations and because *M fermentans* appeared to have a natural epidemiological history that was incompatible with it being a realistic contender for causing rheumatoid arthritis; that is, it was found by culture only very rarely in the genital tract, though, admittedly, serology suggested a more frequent occurrence.⁹³ However, the recovery of this mycoplasmal species from a small proportion of specimens by several other investigators⁹⁴⁻⁹⁸ and, even less often, other human mycoplasmal species too, seems beyond doubt. Most recently, *M fermentans* has been detected by the PCR technique in the throat of about 20% or more of the population⁹⁹⁻¹⁰⁰ and in 20% or more of synovial specimens from patients with various chronic inflammatory arthritides, including rheumatoid arthritis, but not in any specimens from patients with osteoarthritis, post-traumatic hyarthrosis or gout,⁹⁶⁻⁹⁸ observations which have heightened interest in their true role. This, however, has not been accomplished by all investigators¹⁰¹ and the reason for failure must be determined, preferably by exchange of methods and specimens. Because *M fermentans* has been detected in association with the peripheral blood mononuclear cells of about 10% of HIV negative patients attending a sexually transmitted disease clinic,⁹⁹ the view that this mycoplasma is present as a bystander in the joints of patients with chronic inflammatory arthritis and has no primary aetiological role, cannot be discounted. Of course, perpetuation of chronic disease, which had originally been initiated by some other stimulus, would be an important function. Finding *M fermentans* in a relatively large proportion of cases of early arthritis and in sequential samples would be in keeping with, but not prove, the former. Nevertheless, the answers to such questions as these would contribute materially to our understanding of the potential role of mycoplasmas in some forms of arthritis. Efforts should be made to provide the answers, as well as studying serological and cellular responses⁹¹⁻⁹² in greater detail; for example, antibody in synovial fluids. If evidence of local *M fermentans* antigen presentation can be found, and if the response of synovial lymphocytes turns out to be greater than that of peripheral blood lymphocytes and is specific to *M fermentans*, then it can be assumed to have some significance in terms of causality and certainly better than no response at all. Furthermore, the time is ripe to determine whether administration of antibiotics with anti-*M fermentans* activity, based on known antibiotic profiles,³⁻¹⁰² will convert synovial specimens that are *M fermentans* PCR positive to those that are PCR negative. This might be a prelude to, or run in parallel with, the undertaking of controlled antibiotic therapeutic trials with, rather than without,¹⁰³⁻¹⁰⁴ microbiological support, preferably on early cases. The latter may require collaborative efforts. Of course, it has to be recognised that no antibiotic is *M fermentans*-specific, so that even a successful outcome of such trials will not in itself provide evidence for the pathogenic

role of this mycoplasma. That will come only from an overall assessment of the collective data pertaining to each of the proposals that we have put forward.

Association with U urealyticum and other mycoplasmas

Ureaplasmas are a likely cause of some cases of non-gonococcal urethritis,¹⁷ particularly chronic disease. In addition, they are found in the urethra of about 20% of men without disease¹⁰⁵ and in vaginal specimens from at least 40% of subjects without symptoms.¹⁰⁶ The opportunity for spread from these mucosal sites exists and it is of interest that ureaplasmas have been detected by the PCR technique in synovial specimens from about 14% of subjects with various chronic inflammatory arthritides (Schaefferbeke *et al*, unpublished data). Furthermore, subjects with ureaplasma-associated disease were not the same as those with *M fermentans*-associated disease. The detection rate for ureaplasmas seems sufficient to question their role, rather than to disregard them, and this can only be done by testing the set of proposals for defining causality that we have outlined. Other mycoplasmas have been detected by culture (*M hominis*, *M orale*)⁹⁸ or by PCR technology (*M genitalium*)⁴³ so infrequently that trying to meet any set of potential criteria in their case is not a sensible proposition, at least presently. *M penetrans* has been sought but not found at all.⁹⁷

Association with C trachomatis

One group of investigators has presented some preliminary evidence, based on 16s RNA technology, to suggest that *C trachomatis* might be found in a sizeable proportion of synovial specimens from patients with early rheumatoid disease.¹⁰⁷ Others, previously⁵⁰⁻⁵³⁻⁵⁴⁻⁵⁶⁻⁵⁸⁻⁶¹ or subsequently,⁶²⁻⁶³ have not made this observation. Its validity needs to be established before it will be worthwhile attempting to assess further the relation between *C trachomatis* and rheumatoid arthritis.

JUVENILE CHRONIC ARTHRITIS

Association with C pneumoniae

This chlamydial species infects children,⁷³ whereas *C trachomatis* does not, except occasionally at birth. The possibility, therefore, that *C pneumoniae* might be responsible for some cases of juvenile chronic arthritis should not be ignored. It is noteworthy that a bacteria-specific synovial cellular immune response has been found to occur dominantly in late onset pauciarticular juvenile chronic arthritis (type II),¹⁰⁸ a disease occurring mainly in older HLA-B27 positive boys who may develop spondyloarthropathy. In this regard, it is of interest that DNA of the organism has been detected in synovial specimens from one patient with HLA-B27 positive spondyloarthropathy by the PCR technique and chlamydial joint involvement was identified serologically in a similar case.¹⁰⁹ Identifying a chlamydial respiratory infection before the onset of arthritis will always be difficult because children are subject to many respiratory infec-

tions of various causes, many of which may be asymptomatic or trivial. The question of controls in studying children is clearly important and it is fair to state that obtaining synovial specimens from age matched normal children is at best extremely difficult; identification of a *C pneumoniae*-associated arthritis in HLA-B27 positive children only, in the study mentioned above, does, however, provide an internal control and suggests that further studies should be directed particularly at children who are B27 positive. Such studies should be undertaken with the proposals for defining causality in mind.

Including or excluding candidate micro-organisms

Apart from identifiable micro-organisms, bacterial degradation products have been detected in the joint. Muramic acid, a marker of bacterial cell wall peptidoglycan, was reported several years ago to occur in synovial fluids from patients with acute inflammatory arthritis, some of which were culture negative.¹¹⁰ Since then, the ability to detect trace amounts of muramic acid in synovial fluids of patients with septic arthritis has been brought about through the development and use of gas chromatography-tandem mass spectrometry,¹¹¹ a method 1000-fold more sensitive than used previously. In addition, bacterial peptidoglycan-polysaccharide has been detected in synovial tissues by immunohistological staining.¹¹² Although it would be churlish to suggest that such findings are uninteresting, the problem is that without being able, in most cases, to relate a bacterial product to a specific micro-organism there is no means of proceeding with the proposals that we have outlined. So far as these are concerned, we feel that they cannot be regarded as strict criteria for determining whether a micro-organism is a cause of arthritis. This would assume that they have to be met in each case, which, though desirable, may not be possible for each potential causative micro-organism. Rather than as strict criteria, the proposals should be seen as guidelines that investigators should attempt to follow. Furthermore, some may consider that our exclusion of one of Koch's postulates—namely, production by a micro-organism of disease in an animal model which is similar to that occurring in the human species is an error and they may wish to pursue such a line of investigation. However, the production of arthritis in a small animal model, as opposed to a subhuman primate (chimpanzee), after experimental inoculation with an organism isolated from a human joint, does not necessarily point to it causing the human condition, particularly if injected directly into the animal joint and even if given extra-articularly. The production of acute or chronic arthritis in mice by various mycoplasmas¹⁸ is of only superficial interest so far as determining whether they are a cause of rheumatoid arthritis. That is why we have not included the production of disease in an animal model as a requirement that has to be met in recognising causality. Admittedly, if causation is apparent

through fulfilment of the proposals outlined, then the model might provide hints about questions that can be posed in attempting to unravel the mechanisms of pathogenesis and other features.

Those involved in the areas of investigation covered in this review should be aware that the ubiquity of a micro-organism is in favour of, not against, it having a role in arthritis. If the natural history of infection points to the micro-organism being found rarely at either the respiratory or genital mucosal site, then it cannot be regarded as a contender. In addition, demonstrating a temporal relation between peripheral mucosal infection and micro-organism-associated arthritis may not always be feasible, particularly if the micro-organism in contention is regarded as a commensal at the peripheral site. In this regard, there seems no reason to believe that such perceived behaviour at this site excludes it from being a pathogen in the joint. Although desirable, it may not be possible to detect organisms in joints other than the knee if smaller ones are affected simply owing to the difficulty of obtaining specimens. Whether detection of a large number of organisms rather than a small number in a joint is important is unknown. Only by quantitative culture or by application of quantitative PCR technology will this be determined. In doing this, the use of universal primers, with the possibility of detecting any bacterial agent, is not necessarily better than the use of specific primers, particularly when an investigator is obtaining positive results with the latter. There is room for the use of all the techniques that are available and, in the current state of knowledge, those providing investigative support should not turn a blind eye to this. Of course, the detection of a micro-organism or its DNA in a joint does not automatically make it the cause of the arthritis, as pointed out before.⁴ However, none of the findings should be dismissed as irrelevant or trivial so long as they constitute a progressive move towards meeting criteria concerned with causation. Finally, we must emphasise that it is not possible to state categorically how many of the proposals need to be met before a micro-organism can be regarded unequivocally as a cause of arthritis. They should be regarded as guidelines for those attempting to define causality and, at least, raise the level of debate on an issue that, to a large extent, remains enigmatic.

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