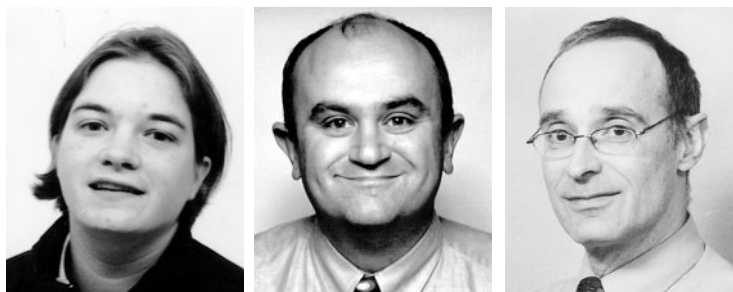


Editorial

Should Infection Still Be Considered as the Most Likely Triggering Factor for Rheumatoid Arthritis?



Although genetic factors are important in the development of rheumatoid arthritis (RA), not all those who are genetically susceptible develop the disease. Twin studies in the United Kingdom and Australia have shown disease concordance rates in monozygotic twins of between 15 to 21%^{1,2}. Earlier studies also showed only modest concordance for autoantibody and immunoglobulin production within twin pairs^{3,4}. There is weak evidence, however, of an increased concordance of RA, rheumatoid factor, and other autoantibodies within spouse pairs⁵. These observations have encouraged the search for environmental triggers, such as infection. In this issue of *The Journal*, for example, a report from Finland has highlighted the high prevalence of prior infection in patients newly presenting with all forms of inflammatory arthritis⁶.

Evidence that infection is the likeliest environmental trigger for RA is considered in this review: First, we look at the different ways in which infection might act as an etiological agent in RA. We then discuss whether the descriptive epidemiology of RA is consistent with an infective trigger. In conclusion, we review evidence that various specific infections play a role in the causation of the disease.

Historically, demonstrating that an infectious agent plays a key role in disease has required the fulfilment of Koch's postulates. These derive from a model of infectious disease whereby the etiological organism has a direct and continuing role in pathogenesis. However, the relationship between infection and a disease such as RA may be more subtle and complex, and different types of experimental design and evidence may therefore be required to confirm any given organism's role in the disease. Koch's postulates have recently been reformulated to define the criteria that need to be fulfilled to determine whether an organism might have an etiological role in RA⁷. It is important therefore to

consider the various ways in which infection might be associated with RA:

1. Nature of the organism: Is the putative trigger a novel organism or a previously described, well characterized entity?
2. Uniqueness: Should the search focus on one agent or could there be multiple triggers?
3. Attack rate: Is the triggering infection a common (or even ubiquitous) organism that is idiosyncratically handled, leading to RA in a minority, or an agent that leads to RA in most of those affected?
4. Delay: Is the interval between infection and disease short or perhaps many years long?
5. Persistence: Is a triggering organism present in active replicating form in RA synovium, playing a role in perpetuation of the disease or acting as a trigger for autoimmunity (perhaps by molecular mimicry), the organism being undetectable in established disease?
6. Site: Is the triggering infection sited in the synovium, or at a distant site (rheumatic fever or reactive arthritis being examples of how distant infection may trigger joint disease)?

Recent secular trends in the epidemiology of RA are consistent with a role for infection as a susceptibility factor, although many other explanations are possible. Historically, it has been suggested that in developed countries RA is a disease of recent onset⁸. Skeletal remains from North American populations suggest that the disease was present over 4000 years ago⁹. The hypothesis was posed that onset of RA is related to transmission of a relevant infection from the native population of the Old World to the colonizers⁹. RA is declining in incidence¹⁰, with recent, fairly dramatic, falls in disease occurrence in populations thought to be genetically predisposed, such as the Pima Indians¹¹, as well

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as those of North Americans of European origin¹². Although this may reflect changes in many environmental factors, falling rates of infection are a plausible explanation. There is also a suggestion of a birth cohort effect, with evidence of declining incidence with successively recent birth cohorts. Consistent with this are data suggesting a shift towards an old age at peak onset¹³. Such an effect would be consistent with a decline in infection in early life, which reduces the risk in such a group as they age.

If infection was an important trigger, it might be expected that RA would be more frequent in situations where transmission of an infectious agent was more likely, and the epidemiology of RA would display patterns in time and space typical of an infectious disease. Surrogate markers of infection might include spatial and temporal clustering, poor social conditions¹⁴, family size, and factors such as contact with animals or other biological hazards. No consistent pattern emerges from this type of study. Geographical clustering is a common feature of infectious disease, and provided early evidence that Lyme disease was due to infection¹⁵. However, although clustering has been described in RA¹⁶, the vast majority of cases occur sporadically¹⁷. There is also no evidence that overcrowding per se is associated with an increased risk of onset¹⁸. RA may occur more frequently in those from a large sibship, although this is not a consistent finding¹⁹. Pet ownership is more likely prior to the onset of RA²⁰, and this is perhaps particularly true in relation to cats rather than dogs²¹. An incidence-based case control study of early RA suggested that prior blood transfusion might be a risk factor²², with the transfusion often happening several years previously. Subjects with new onset arthritis are no more likely to report immediately preceding infection than the general population²³, but long latency from infection to disease onset might explain such a negative finding.

Studies looking for specific infective triggers for RA have generally relied on serological methods to detect prior exposure or culture or molecular methods to detect the organism or its genetic material in blood or synovial tissue. These have usually been cross-sectional studies in subjects with established disease. The results of such studies need to be treated with caution. First, many such studies lack appropriate controls. Second, the disease itself or its treatment might increase the likelihood of infection. Third, the detection of an organism in synovium (even in viable, replicating form) is not necessarily indicative of an active role in pathogenesis: there is evidence that synovial tissue may nonspecifically trap bacteria and viruses^{24,25}, and others may enter the inflamed synovium because they are parasitic residents of cells entering the joint (such as Epstein-Barr virus in B cells).

A large number of candidate organisms, both bacterial and viral, have been investigated and these studies are reviewed below. Detection of a previously undescribed

trigger would be a far greater scientific challenge. There are, however, several molecular approaches that have been used to search for novel organisms. For example, synovial tissue can be screened for non-eukaryotic enzyme activity such as reverse transcriptase or heavily conserved components of the bacterial genome (such as 16S ribosomal genes). Subtraction techniques can also be used to search for nonhuman nucleic acid sequences.

BACTERIA

Mycobacteria. Over 70 years ago the histological similarity between rheumatoid synovitis and tuberculosis led Forestier and others to speculate that RA may be a mycobacterial infection, and many forms of mycobacteria are known to be arthritogenic²⁶. This hypothesis has been revisited many times since then, with the focus switching to atypical mycobacteria. Studies have shown elevated antibodies to mycobacterial heat shock proteins in sera²⁷ of patients with RA, suggesting the possibility of a cross-reactive epitope. Yet this has not been a consistent finding²⁸. Further, extensive experimental studies have failed to show any evidence of mycobacterial infection by culture and/or molecular techniques, and trials of antimycobacterial chemotherapy have failed to produce consistent improvement in disease²⁹⁻³¹.

Mycoplasmas. *Mycoplasmas* cause arthritis in some animal species that can be erosive, are associated with rheumatoid factor, and can last for at least a year. *Mycoplasmas* also cause a destructive arthritis in subjects with primary antibody deficiency. Numerous culture and polymerase chain reaction (PCR) based studies in both blood and synovium have been performed to look for evidence of infection by a variety of *Mycoplasma* species in human RA. Early studies are difficult to interpret, as until recently mycoplasmas were common unrecognized contaminants in many tissue culture systems. Most, but not all, recent studies report an increased frequency of *Mycoplasma* infection in RA compared to controls, but a similar prevalence for infection is also reported in other chronic inflammatory rheumatic diseases³²⁻³⁵. The prevalence of infection in these studies is usually in the range 5–30%. These data do not provide strong support for any obligate role for *Mycoplasma* in RA. Clinical trials that demonstrate a partial response to tetracycline treatment in RA may not support a role for *Mycoplasma*, as the former might be due to the anticytokine action or the inactivation of metalloproteinases by this class of antibiotics³⁶.

Other bacteria. Ebringer has developed the hypothesis that *Proteus mirabilis* may trigger RA, based on serological studies and sequence similarity between a *Proteus* protein and the HLA-DRB1 shared epitope³⁷. No convincing evidence has yet been presented, however, to show a link between persistent carriage of *Proteus* and RA³⁸.

Carson, *et al*³⁹ suggested that *Escherichia coli* heat shock protein dnaJ displayed the QKRAA amino acid sequence

present in the HLA-DRB1 shared epitope. Indeed, synovial cells from patients with early RA exhibited a marked response to the antigen, suggesting a cross-reactivity between that and activated T cells. Using combinatorial library techniques the same group demonstrated that IgG monoclonal anti-DNA antibody in a patient with RA showed homology with antibodies resulting from staphylococcal and *Hemophilus* infection⁴⁰.

Several studies have used PCR based techniques to look for heavily conserved sequences of bacterial DNA in synovial samples from a variety of rheumatic diseases. DNA from a number of bacterial species have been detected in synovial fluid, although no consistent relationship between organism and disease is apparent from these studies^{24,41}, except in cases of septic arthritis or post-chlamydial reactive arthritis. DNA from a wide variety of organisms, including *Hemophilus*, *Bordetella*, and *Acinetobacter* were isolated from synovial fluid from patients with RA. This is perhaps most consistent with inflamed synovium acting as a nonspecific trap for bacterial fragments (which might potentially play a role in disease perpetuation).

VIRUSES

Parvovirus. Parvovirus B19 infection is undoubtedly associated with short-lived arthropathy, with clinical similarities to RA. There is also some evidence to suggest that a chronic rheumatoid pattern arthritis can develop following the acute illness. However, serological evidence of human parvovirus infection was very infrequent in a large cohort of patients with very recent onset of arthritis⁴², and persistence of Parvovirus DNA in synovium cannot be reliably linked to RA⁴³.

Epstein-Barr virus. Epstein-Barr virus (EBV) is a potent stimulator of polyclonal B cell proliferation, and can induce the proliferation of autoantibody-producing B cells, including those producing rheumatoid factors. Serological studies from the 1970s also suggested that autoantibodies found in patients with RA might cross-react with EBV nuclear antigen. Sequence similarity has also been described between an EBV glycoprotein and the shared epitope. Serological and molecular evidence has been produced revealing coexistence of EBV infection and RA, but these studies are hard to interpret because of the high seroprevalence of EBV in most populations⁴⁴⁻⁴⁶. EBV, however, has been frequently detected in the synovial tissues of RA patients (in contrast to its absence in osteoarthritis synovium)⁴⁷. Further recent work has suggested that RA is associated with a decreased T cell response to EBV glycoprotein 110. This latter protein is important in the control of the viral replication, suggesting that RA is associated with persistence of EBV infection⁴⁸.

Retroviruses. Caprine arthritis-encephalitis virus (CAE) infection in goats is characterized by adult onset chronic progressive arthritis with bursitis, tenosynovitis, and extraarticular involvement. The causative retrovirus, of the

Lentivirus type, infects cells of the monocyte/macrophage lineage, although only a minority of synovial macrophages contain virus⁴⁹. HTLV-1 infection has been associated with chronic arthritis, particularly in Japanese subjects⁵⁰. The site of infection in HTLV-1 infection is the synovial fibroblast, which becomes transformed in a tumor-like fashion. HTLV-1 and CAE virus are not associated with RA⁵¹, but these observations have led to a search for other retroviruses in RA, particularly as retrovirus-like particles have been observed in RA synovium by electron microscopy. A novel retrovirus, human retrovirus-5 (HRV-5), was first isolated from salivary gland. Subsequent PCR studies have revealed HRV-5 proviral DNA in 53% of RA synovial samples, and in 10% of peripheral blood samples from patients with RA. The virus was found in a similar proportion of peripheral blood samples from patients with systemic lupus erythematosus, but not in blood or tissue from other rheumatic diseases⁵².

Other unknown viruses. A number of studies have used DNA subtraction technology to look for genes that are expressed in blood or tissues in RA, but not in health or in other disease states. These studies have, to date, failed to identify any sequences suggestive of novel pathogens, and usually identify genes encoding proteins involved in inflammation⁵³⁻⁵⁵.

What can be concluded from the studies reviewed above? It is clear that no overwhelming evidence exists to link any single known infectious agent with RA, although some candidate organisms seem particularly worthy of closer study, such as mycoplasmas and HRV-5, and the search for novel organisms needs to continue. However, future studies will produce more valid results only if greater rigor is applied to experimental design: prospective, inception based cohort studies are preferable to retrospective studies (although undeniably more expensive), and great care must be taken with choice of controls. Studies need to be designed to examine specific models of interaction between RA and infection, addressing variables such as attack rate, delay in onset, and persistence of infection. Progress is most likely to be made by close collaboration between epidemiologists and molecular microbiologists. Finally, the need for further study should not close our eyes to the possibility that RA is unrelated to infection.

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