HYPOTHESIS

Normal intestinal microbiota in the aetiopathogenesis of rheumatoid arthritis

P Toivanen

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A series of observations have led to the hypothesis that normal intestinal microbiota in patients with rheumatoid arthritis may harbour, for genetic reasons, bacteria with cell walls capable of inducing arthritis. Differences occur between bacterial species, and even between strains of a single species, because some cell walls induce experimental chronic arthritis, whereas some others induce only a transient acute arthritis or no arthritis at all. In susceptible subjects, with continuous seeding of bacterial products from the gut, the synovial inflammation is followed by erosion, exposition of cartilage antigens, and self perpetuating chronic arthritis.

The aetiology of rheumatoid arthritis has remained completely unsolved despite the several hypotheses presented. It has been suggested that both genetic and environmental factors are involved. The disease is known to be associated with certain HLA-DRB1 alleles, encoding a common sequence of five amino acids, "the shared epitope", in the hypervariable region. This epitope is found in 80–90% of Caucasoid patients with rheumatoid arthritis and in 40–50% of non-rheumatoid subjects. Disease heritability is estimated to be about 60%, of which HLA accounts for less than a half, and several identified loci outside the HLA region are responsible for the rest.[1 2] Among the potential environmental factors a role for infection and microbes has been one of the most popular alternatives.[3–6] My interest in the subject arose when the significance of the gastrointestinal pathogens in the aetiology of reactive arthritis became established. Among the gastrointestinal infections, those due to Yersinia, Salmonella, Shigella, and Campylobacter are the most common triggers of this disease, particularly in HLA-B27 positive subjects. On the basis of early studies on reactive arthritis it became known that degradation products of gastrointestinal pathogens can be present in the synovial tissues, resulting in a local inflammation. This was demonstrated in reactive arthritis triggered by Yersinia, Salmonella, or Shigella.[7–11] Such findings have led to consideration of the possibility that a similar phenomenon may occur also with bacteria normally harboured by the gastrointestinal tract, leading to synovial inflammation in genetically susceptible subjects. Furthermore, evidence has been presented that the composition of normal gastrointestinal microbiota can be affected by the host genotype.

HYPOTHESIS

I propose that the normal intestinal microbiota of people developing rheumatoid arthritis harbours bacteria, degradation products of which can induce chronic arthritis. Components of intestinal bacteria are normally found within circulating blood cells, and they end up in the synovial tissues. The same may occur also with the bacteria which have an arthritogenic ability, resulting in synovial inflammation. Depending on the persistence and other characteristics of the bacterial components, the inflammation is accompanied by production of cytokines, metalloproteinases, proteases, and prostaglandins. All these are known to play a part in the pathogenic process of rheumatoid arthritis, leading to a self perpetuating bone erosion and cartilage loss, with pannus formation and osteoclast activation (table 1). At the final destruction stage, the inciting bacterial components are not necessarily present any longer in the synovial tissue. Peptidoglycan, a crucial component of the bacterial cell wall, and several other microbial structures are known to stimulate production of rheumatoid factor, which may be the first sign of the pathogenic process.

SUPPORTING EVIDENCE

The hypothesis is based on four types of observations:

- Degradation products of bacterial cell walls are normally found within circulating blood cells, and they may end up also in joint tissue

Correspondence to:
Professor P Toivanen, Department of Medical Microbiology, Turku University, Kiinamyllynkatu 13, FIN-20520 Turku, Finland;
paavo.toivanen@utu.fi

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Table 1 Four phases in the pathogenesis of rheumatoid arthritis

Phase	Characteristics
Preinduction	Intestinal colonisation by arthritogenic bacteria
Induction	Degradation products of arthritogenic bacteria enter joint tissue
Inflammation	Antigen presentation. Production of metalloproteinases, proteases and prostaglandins
Destruction	Pannus formation. Osteoclast activation

- Cell walls of several bacterial species representing normal human intestinal microbiota are arthritogenic in animal experiments
- Evidence has been presented that patients with early rheumatoid arthritis have intestinal microbiota which differs from that in the control patients studied
- Considerable evidence also exists to indicate that the composition of gastrointestinal microbiota is genetically regulated.

These observations are briefly discussed below.

Bacterial components in joint

An essential component of the bacterial cell wall is the peptidoglycan layer, which is particularly thick in Gram positive bacteria. It consists of *N*-acetylmuramic acid and *N*-acetylglucosamine, layers of which are bound by peptide bridges. Muramic acid is not found in eukaryotic cells, and its detection indicates that bacteria or bacterial degradation products are present. Mass spectrometry shows that 60% of young adults have muramic acid within the circulating blood cells, with the frequency of positivity declining. Thus, with the present techniques, 2–3% of people at the age of 50–60 show circulating cells containing muramic acid.[12] In contrast, muramic acid is not observed in the circulation of newborns. Because newborns do not have bacteria within the gastrointestinal tract, it is apparent that muramic acid in the adult circulation is derived from the intestinal microbiota.[13] Muramic acid has also been observed in the spleen of healthy adult subjects.[14] Therefore, it is no surprise, as demonstrated by mass spectrometry or immunohistochemistry, that muramic acid may also end up in the synovial tissue, indicating the presence of bacterially derived peptidoglycan.[15–17] Evidence for bacterial nucleic acids has also been presented in studies on the synovial tissue from a variety of arthritides, including late stage rheumatoid arthritis and osteoarthritis.[18–20] Such findings indicate the presence of nucleic acids derived from a wide range of bacterial species, and they do not necessarily correlate with the presence of muramic acid in the synovial tissue.[17] Possibly, bacterial nucleic acids, which are quite often present in the blood circulation,[21] are trapped in the inflamed joint tissue. Transfer of bacterial products from the intestine to intact joints is understandable, because mucosal leucocytes are known to home specifically in the synovium.[22 23] It must be noted that the mere presence of bacterial structures within synovial tissues does not necessarily result in inflammation. Both experimental[24 25] and clinical[26–28] evidence shows that microbial components may end up in the synovial tissues without causing inflammation. For synovitis to develop the bacterial components have to be phlogistic.

Arthritogenic ability of bacterial cell walls

The ability of bacterial cell walls to induce chronic, erosive arthritis was first described in the rat by using *Streptococcus pyogenes*.[29] Self perpetuating arthritis, closely resembling human rheumatoid arthritis by histological criteria, develops in susceptible rat strains after a single intraperitoneal injection of the bacterial cell wall. In addition to *Streptococcus pyogenes*, several bacterial species representing Lactobacillus, Bifidobacterium, Eubacterium, Collinsella, and Clostridium have been observed to have a similar ability.[30 31] Surprisingly, most of these are anaerobic Gram positive rods belonging to the normal intestinal microbiota in man.

A typical bacterial cell wall arthritis has also been induced by using an extract from the human intestinal content.[32] In the bacterial cell wall, peptidoglycan has proved to be the decisive component for the induction of arthritis.[33] However, not all peptidoglycans are alike. Peptidoglycans of certain bacteria induce severe chronic arthritis, whereas others have peptidoglycan that induces only a transient, acute arthritis or no arthritis at all. Most clearly this has been demonstrated by using a pair of strains of *Collinsella aerofaciens*, both isolated from the human intestine.[34] These two bacterial strains are almost identical—100% identity is observed by polymerase chain reaction of 16S ribosomal genes and only a minor difference in the peptidoglycan structure.

> "Bacterial cell walls can induce chronic, erosive arthritis"

Despite their close similarity, only one of these two strains is arthritogenic. After enzyme digestion, degradation products of the arthritogenic Collinsella strain have a five- to eightfold increase in the ability to stimulate production of proinflammatory cytokines in comparison with the intact peptidoglycan. An opposite effect was observed with the degraded fragments of the non-arthritogenic Collinsella peptidoglycan—that is, its proinflammatory ability was significantly decreased by enzyme degradation.[34] These findings indicate that the nature of the bacterial cell wall peptidoglycan determines whether a particular cell wall can cause chronic arthritis or not. Normal microbiota usually coexists in a peaceful symbiosis with the host.[35] On the other hand, cell wall products including peptidoglycan, isolated from intestinal indigenous bacteria can mount human cytokine responses in vitro.[36] These responses are mediated by signalling Toll-like receptor 2 present on macrophages and synovial fibroblasts.[37 38] It should also be noted that immunisation with peptidoglycan or other persisting microbial products may lead to production of rheumatoid factor.[39]

Intestinal microbiota in rheumatoid arthritis

Analysis of human intestinal microbiota is not an easy task.[40] It has been estimated that, using traditional methods of culture and identification, complete analysis of such a sample comprising 400–500 different bacterial species would take one person-year of laboratory work.

> "Patients with early rheumatoid arthritis have intestinal microbiota different from that in other subjects"

To overcome this problem, fatty acids derived from bacterial cell membranes have been analysed in stool samples by gas chromatography. Such a study showed that patients with early rheumatoid arthritis (disease duration <6 months) have intestinal microbiota significantly different from that in controls. The difference was best seen in patients with erosive or rheumatoid factor positive rheumatoid arthritis. Anaerobic bacteria, which form the overwhelming majority of the gastrointestinal microbiota, were mostly responsible for the difference observed.[41] It is also known that changes in diet induce changes in intestinal microbiota. When patients with rheumatoid arthritis underwent a dietary trial with fasting and vegan/vegetarian diet, the most significant changes in the intestinal microbiota were displayed by those showing clinical improvement.[42] Why would the patients with early rheumatoid arthritis have intestinal microbiota different from that in other subjects?

Genetics of intestinal colonisation

Only a few studies have been carried out to clarify whether the composition of gastrointestinal microbiota is influenced by the host genotype. van de Merwe *et al* analysed stool samples using anaerobic bacterial cultures and concluded that the composition of microbiota in identical twins was considerably more similar than in non-identical twins.[43] Interestingly, a

similar conclusion was also reached for bacteria present on the nasal mucosa.[44] More recently, Zoetendal *et al* used electrophoretic analysis of bacterial ribosomal RNA to suggest that the host genotype determines the composition of the bacterial community in the human intestinal tract, without defining the genes involved.[45] Our own studies carried out with congenic mouse strains indicate that the major histocompatibility complex (MHC) may have a prominent effect.[46 47] It is tempting to consider MHC molecules and bacterial colonisation and speculate about the potential role of immune elimination.[46] However, little is known about the effect of the MHC on antibacterial responses.[48 49] A more probable mechanism is bacterial adherence to intestinal epithelial surface as a requisite first step in the colonisation process. Bacterial surface molecules, adhesions, recognise proteins or glycoproteins on the epithelial cells. Bacteria which cannot adhere are shed. The specificity of the adherence leads to a restricted colonisation of the host. As an example, attachment of *Helicobacter pylori* to the human gastric epithelium is selectively mediated by blood group antigen Lewis[b].[50] Regarding MHC molecules and bacterial adherence, several immunoglobulin binding proteins have been demonstrated on the bacterial surfaces. One type of these, fibrous proteins called curli, has been shown to interact with the immunoglobulin-like domains of human class I MHC molecules.[51] The effect of different MHC genotypes was not studied. However, gastric inflammation induced by *Helicobacter felis*, which depends on bacterial attachment and colonisation, varies in severity in congenic mice with different MHC.[52]

The hypothesis does not imply that the genes known as rheumatoid arthritis susceptibility genes are necessarily those favouring the presence of arthritogenic bacteria in the intestinal microbiota. According to my thinking, intestinal colonisation by arthritogenic bacteria might also be regulated by so far undefined gene loci, either within the MHC or outside, or both (fig 1). Most probably, different genes encode on the intestinal epithelium for a variety of proteins and glycoproteins, allowing adherence of different bacteria. Therefore, the possibility remains that involvement of two or more susceptibility loci is required, some favouring colonisation by arthritogenic bacteria and others allowing inflammatory response to the appropriate bacterial components. In addition, random colonisation by arthritogenic bacteria has to be taken in account, owing to the estimated disease heritability of only about 60%.[1] It should be realised that random effects are feasible despite the genetic regulation of the colonisation, because the difference between an arthritogenic and non-arthritogenic bacterial strain may be minimal, not affecting the adherence required for the colonisation.[34]

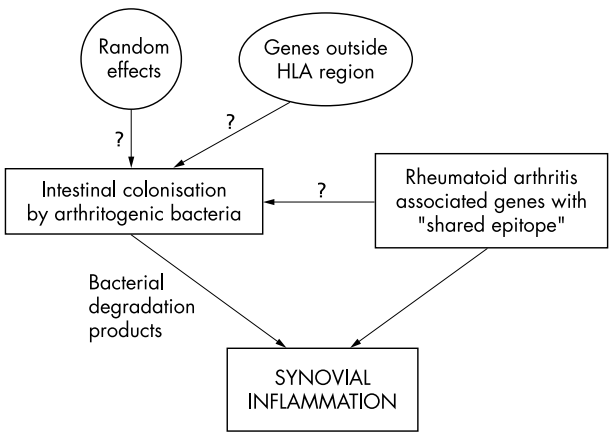

Figure 1 Intestinal colonisation in the pathogenesis of rheumatoid arthritis; contribution of environmental and genetic factors.

TESTING THE HYPOTHESIS

To test the hypothesis new techniques allowing quantitative determination of the 400–500 bacterial species present in the human gastrointestinal tract are needed. Methods which could be applied to the large number of samples required for reliable conclusions are not yet available. It should also be noted that all the bacterial species concerned have not yet been identified, or they may be extremely difficult to culture.[40] Mass spectrometric analysis of bacterially derived fatty acids already used in a mass scale does not allow quantitative conclusions.[41 42] At present, 16S rRNA probes specific for certain bacterial species seem promising tools, but further development is required before they can be used to evaluate numerous samples simultaneously.[53] The same holds true for collecting samples from different parts of the gut.

When suitable bacteriological methods become available, they should be applied to healthy people with rheumatoid arthritis associated genes. The subjects should be monitored for potential development of the disease and compared with a control population. Because rheumatoid arthritis may start with rheumatoid factor positivity several years before any other signs or symptoms are evident,[54] the clinical monitoring should be long lasting. In addition, composition of the gastrointestinal microbiota should be studied as early as possible at the onset of the disease, before the start of specific antirheumatic treatment. If these studies should indicate that the bacterial species found in patients susceptible to rheumatoid arthritis differ from those found in other groups, then the next step would be a search, as early as possible during the disease development, for specific bacterial components in the synovial tissue, and experimental studies to prove their arthritogenicity.

CONCLUSIONS

This hypothesis provides answers to a few unexplained findings in the aetiopathogenesis of rheumatoid arthritis. Monozygotic twins show a concordance rate of only 15–30%, which leaves considerable space not only for somatic diversification but also for environmental effects—that is, diverse intestinal colonisation. On the other hand, the concordance rate increases with time from the day when the first twin develops rheumatoid arthritis to about 40% during a 30 year follow up,[55] which well fits with the combined effect of genetic and environmental factors. The prevalence of rheumatoid arthritis is remarkably similar world wide, with estimates varying between 0.5 and 1.0%. However, a prevalence of 5% has been reported in American Indians and the disease is absent in areas of rural Nigeria. All these findings could be explained by the worldwide occurrence of a variety of intestinal bacteria, including aberrations in a few isolated areas. Variable occurrence of the arthritogenic bacteria explains the diversity of the clinical symptoms. In fact, many believe that rheumatoid arthritis is not a single disease. Even monozygotic twins and sibling pairs concordant for rheumatoid arthritis show enormous diversity in the clinical appearance of the disease.[56] Risk factors for rheumatoid arthritis include coffee and smoking.[57 58] It is not difficult to imagine that they both might influence bacterial colonisation. Mention of smoking in this context implies also that the role of commensal microbiota elsewhere than in the gut should not be excluded. However, the bacterial content in the intestine is overwhelming in comparison with other parts of the body.

Components of some bacteria can stimulate production of rheumatoid factor, and others cannot,[39] the latter giving rise to seronegative rheumatoid arthritis. Likewise, the early production of rheumatoid factor, antedating the clinical appearance of the disease,[54] is understandable owing to the preinduction period, which may last for years (table 1). No pathogenic role is here attributed to the rheumatoid factor; it

is considered to be a side phenomenon of the host immune response. This hypothesis does not directly explain the greater female incidence of rheumatoid arthritis, but it is in line with the suggested influence of reproductive and hormonal factors. Finally, with the introduction of antibiotics, it has been reported that both the incidence and severity of rheumatoid arthritis have declined in comparison with the pre-antibiotic era.[59–61]

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