Patients with arthritis have been described in association with the class I major histocompatibility complex (MHC) antigen, HLA-B27. Classical ReA is triggered by two major types of bacterial infection, non-specific urethritis due to Chlamydia trachomatis, or gastrointestinal enteritis due to yersinia, salmonella, shigella, or campylobacter. A similar condition associated with B27 has been described in association with Clostridium difficile infection, though in this case it is unclear whether ReA is triggered directly by the organism or by secondary changes in bowel flora. The concept of ReA can also be expanded to include oligoarthritis triggered by a variety of other organisms, including streptococcus, borrelia, brucella, and mycobacteria. In these cases there is no association with HLA-B27 or with spondyloarthropic features and, except for comparative studies, they will not be further considered in this paper. The prognosis of ReA is generally considered to be good, at least in comparison with other forms of inflammatory arthritis like rheumatoid arthritis; however, most patients are affected for several months and a minority have chronic or recurrent disease. Treatment is mainly supportive, though second line antirheumatic drugs, especially sulphasalazine and methotrexate, are used in severe cases. Antibiotic treatment of the triggering infection has been shown to protect against the development of arthritis, but the role of long term antibiotic treatment of established ReA remains uncertain.

Unlike most forms of human arthritis, ReA has a known trigger with a clear point of onset, a defined genetic association, and a well described cellular and humoral immune response against the triggering pathogen in the joint. Thus as well as being of interest in itself, analysis of pathogenetic mechanisms in ReA should also provide a useful paradigm for other human inflammatory arthritides where these factors are not clearly defined. As is often the case, however, further research has raised more questions than it has answered. Early studies of ReA found that the triggering pathogen could not be cultured from the joint, hence the concept of ReA in contradistinction to septic arthritis; recent studies, have muddied the water as triggering bacterial antigens, and in some cases even bacterial DNA, have been identified in synovial cells by immunological and molecular techniques. The association with HLA-B27 has also become less clear cut, with some evidence from community based and epidemic studies that it acts as a severity rather than a susceptibility marker or that it is linked with spondyloarthropic features rather than synovitis alone. In addition, questions have been raised about the antibacterial immune response. It has long been clear that patients with ReA may not have specific antibacterial antibodies in their serum and that, conversely, because such antibodies are common in the general population, positive antibacterial serological tests may not be of predictive value in diagnosis. In terms of the cellular immune response, although T cells specific for the triggering bacterium are found in the ReA joint, their role in pathogenesis remains uncertain. With regard to the antigens they recognise, the question has again been raised of whether the entire pathologocal process can be attributed to persistent synovial bacterial antigen or whether non-bacterial joint autoantigens, or even HLA-B27 itself, may have a role, particularly in chronic disease.

In this report, and in the meeting abstracts which follow, these and other questions are examined and a state of the art pathogenetic hypothesis is proposed based on discussion at the workshop and other recent research. In the final section, diagnostic and treatment guidelines are reviewed together with other points of immediate value for clinical practice.

An overview

Reactive arthritis (ReA) is an asymmetrical inflammatory oligoarthritis of the lower limbs, associated in some patients with typical spondyloarthropathic features, such as enthesopathy and inflammatory low back pain. When the arthritis occurs in conjunction with urethritis and conjunctivitis the patient is considered to have classical Reiter’s syndrome; patients may, more rarely, display other systemic features, including iritis, mouth ulcers, keratoconjunctivitis sicca, and myocarditis.

The disease primarily affects young adults and there is a strong association with the class I major histocompatibility complex (MHC) antigen, HLA-B27. Classical ReA is triggered by two major types of bacterial infection, non-specific urethritis due to Chlamydia trachomatis, or gastrointestinal enteritis due to yersinia, salmonella, shigella, or campylobacter. A similar condition associated with B27 has been described in association with Clostridium difficile infection, though in this case it is unclear whether ReA is triggered directly by the organism or by secondary changes in bowel flora. The concept of ReA can also be expanded to include oligoarthritis triggered by a variety of other organisms, including streptococcus, borrelia, brucella, and mycobacteria. In these cases there is no association with HLA-B27 or with spondyloarthropic features and, except for comparative studies, they will not be further considered in this paper. The prognosis of ReA is generally considered to be good, at least in comparison with other forms of inflammatory arthritis like rheumatoid arthritis; however, most patients are affected for several months and a minority have chronic or recurrent disease. Treatment is mainly supportive, though second line antirheumatic drugs, especially sulphasalazine and methotrexate, are used in severe cases. Antibiotic treatment of the triggering infection has been shown to protect against the development of arthritis, but the role of long term antibiotic treatment of established ReA remains uncertain.

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Pathogenesis of reactive arthritis: the role of bacteria

Unlike most forms of arthritis, in ReA the role of an exogenous trigger is quite certain. However, the mechanism by which a distant bacterial infection of the gut or genitourinary tract can trigger arthritis is unknown; specific questions which were addressed at the meeting include whether the mechanism is the same for all the bacterial triggers, whether and in what form the bacteria are present in the joint, and what bacterial antigens are recognised by the immune response. Zinkernagel (Zurich, Switzerland) and Kauffmann (Ulm, Germany) discussed pathogenetic mechanisms and made the point that intracellular microbes can cause damage either by a direct toxic effect on the cells in which they live (cytopathic effect) or by initiating an antibacterial immune response which, while ineffective at eradicating the organism, does induce tissue damage (non-cytopathic effect). The most well known example of the latter is infection by Mycobacterium tuberculosis, and it is likely that this is the important mechanism in ReA too. There may be a difference, however, in the time scale over which this takes place between the various types of ReA. In ReA triggered by Chlamydia trachomatis (reviewed by Ward, Southampton, United Kingdom), there is clear and increasing evidence for persistence of live bacteria within synovial tissue. Not only has chlamydial DNA been found by polymerase chain reaction in the joint (Wilkinson et al and Bas et al presented at this meeting) but recent studies from Hudson and Schumacher (presented at the American College of Rheumatology Meeting, 1995) show that primary mRNA transcripts from chlamydia can also be identified. As primary transcripts have an extremely short half life and the rate of initiation of transcription is dependent on the...
growth rate of the organism, this finding shows that viable, metabolically active chlamydiae must be present, albeit in a form or in amounts which preclude their culture. The story for gastrointestinaly triggered ReA may be rather different. For some organisms, such as yersinia, the concept of a low level persistent infection is not improbable; however, observation that yersinia induced disease despite the presence of yersinia antigens by immunofluorescence. One possibility, suggested by Toivanen, is that yersinia do persist, but in some other organ of the body like the gut, and reach the joint in showers. Further analysis is required, with ultra-sensitive polymerase chain reaction systems, especially as in some cases in animal models Heesemann was able to culture yersinia yet polymerase chain reaction for yersinia was negative. In contrast with both chlamydia and yersinia, the life cycle of shigella (reviewed by Dehio, Tubingen) is such that persistent low level asymptomatic infection seems unlikely. One interesting mechanism mentioned by Toivanen was that certain organisms, notably Clostridium difficile, might not induce ReA directly but, because of their alteration of the bowel mucosa might enable normal bowel flora to trigger disease. An alternative approach to considering the role of bacteria, by examining their interactions with the cellular immune response, will be discussed further below in the section on T cells.

Pathogenesis of reactive arthritis: HLA-B27 and other genes

The observation that HLA-B27 is associated with ReA was first made over 20 years ago, shortly after the demonstration of an association with ankylosing spondylitis (AS); similar associations have since been shown for other spondyloarthopathies (SpA). As HLA-B27 is now known to have nine subtypes, one critical question is whether all predispose equally to SpA. Wordsworth (Oxford, United Kingdom) reviewed previously published data from the Gambia suggesting that HLA-B27:03 might not be associated with SpA in the context of more recent work. It has now been shown that a significant number of HLA-B27+ Gambian villagers carry the more usual HLA-B27:05 subtype, which is clearly associated in Europeans with AS. Yet, in the Gambia neither those who are HLA-B27:05 positive nor those carrying HLA-B27:03 develop SpA, suggesting that the explanation for the absence of SpA does not lie with the HLA-B27 subtype but rather with some other aspect of their lifestyle. A rare new HLA-B27 allele, HLA-B27:06, which is found in Thailand, has also not been described in association with AS, but again it may be that the population in which it occurs is not susceptible for other reasons. Wordsworth also considered whether other genes may be involved in the pathogenesis of SpA. No association has been shown, in a number of studies, with proteasome, transporter, or T cell receptor genes, but he suggested that there may be an association, at least in AS, with the class II MHC gene, HLA-DR1, or with an extended B27-DR1 haplotype. Clearly, however, HLA-B27 remains the strongest genetic risk factor so far identified.

Numerous explanations for the association with HLA-B27 have been proposed, yet none has gained universal acceptance. Unlike AS, where virtually all patients with the disease are HLA-B27 positive, in ReA a substantial proportion of patients do not carry this antigen, suggesting that it cannot be vital for pathogenesis. Leirisalo-Repo (Helsinki, Finland) presented two reviews at the meeting, the first on the strength of the association with HLA-B27 and the second on more general aspects of epidemiology and prognostic features in ReA, which will be discussed below. According to these studies, the frequency of HLA-B27 is much less in community based than in hospital based series, suggesting that B27 may determine disease severity rather than its initiation. Furthermore, the association also appeared stronger in those patients who had spondyloarthropathic features, such as iritis, enthesitis, and sacroiliitis, and in patients who went on to chronic disease. One possible interpretation of these data is that HLA-B27 may have relatively little role in acute ReA synovitis.

Inman (Toronto, Canada) examined further the idea that HLA-B27 on the surface of a cell reduced the ability of bacteria, such as salmonella and yersinia, to invade it. This effect was initially shown in HLA-B27 transfected cell lines in vitro but does not seem to occur in professional phagocytes. More recently, in studies with HLA-B27 transgenic mice, Inman’s group has shown that bacteria persist extracellularly and can be cultured from the gut and spleen for longer in transgenic mice than in controls; the transgenic mice, however, do not develop arthritis nor can bacteria be detected in their joints. Inman concluded that the modulation of invasion by HLA-B27 is subtle, especially since there is no evidence of an overwhelming infection risk in HLA-B27 positive subjects.

Probably the most popular current hypothesis for the association of HLA-B27 with SpA is the arthritogenic peptide theory in which it is suggested that HLA-B27 is, uniquely among class I MHC molecules, able to present an arthritis-inducing peptide or peptides to cytotoxic CD8+ T cells; initially it is proposed that the arthritogenic peptide would be derived from the initiating bacterium, though in more chronic cases common with an autoantigen may play a part (discussed further in the section on T cells). For this hypothesis to be correct in the case of ReA, several pieces of information are required; firstly, can HLA-B27 present peptides derived from the triggering bacteria, secondly, can bacteria specific HLA-B27 restricted CD8+ T cells be found in patients, and thirdly, are such T cells pathogenic? Studies reviewed at the meeting examined the first two aspects. Bowness (Oxford, United Kingdom) identified some 60 peptides, from chlamydial proteins whose sequence is known, which would be predicted to bind to HLA-B27 from their sequences and which could be shown to do so in an in vitro binding assay. So far, one of these peptides, derived from heat shock protein 70 has been shown to stimulate a T cell line derived from a patient with SpA. Märker-Hermann (Mainz, Germany) has previously identified bacteria specific, HLA-B27 restricted CD8+ T cell clones from the synovial fluid of patients with enteric ReA and AS; unlike CD4+ T cell clones from the same patients, these showed limited repertoire of their T cell receptors. The third question, of pathogenicity of specific T cells, is extremely difficult to address. In conclusion, there is some tantalising evidence in favour of the arthritogenic peptide hypothesis, but the difficulties encountered by the many groups trying to confirm it suggest that other mechanisms may be important.

One way in which further information may be gained on the association between HLA-B27 and SpA is by study of the various HLA-B27 transgenic animal models currently being developed. The most advanced of these is the model developed by Taurog and Hammer (Dallas, Texas, USA). In an update of this model Dr Hammer reminded us that Lewis or Fisher rats, made transgenic for the human HLA-B27 and human β2 microglobulin genes, develop arthritis, gut inflammation, epilidymitis, and skin/nail lesions; some of these features bear a strong resemblance to...
manifestations seen in human SpA. A high copy number of the HLA-B27 gene is required to develop these symptoms, which is hard to reconcile with the idea that HLA-B27 is simply acting as a restriction element for peptides. The condition does not develop in rats which are bred in pathogen free conditions, suggesting that bacteria have a role (though no specific bacterium has been singled out), nor can it be induced in nude mice, showing that T cells are required. Cell transfer experiments have, however, shown that the disease is best transferred to naive animals by HLA-B27 positive non-T cells from the bone marrow. Further examination provides a good chance of elucidating the role of HLA-B27 in this model. A more difficult task will be to confirm its relevance to human SpA.

Pathogenesis of reactive arthritis: T cells, bacterial antigens, and autoantigens

Early studies of the immune response in ReA focused on the humoral immune response, but more recent immunological and histological work has suggested that the cellular immune response is pathogenetically more relevant. It is now beyond doubt that a T cell proliferative response specific for the triggering bacterium is found in the ReA joint with little or no response in paired blood. Current studies are aimed at determining how such cells could induce synovitis.

Because of the association of ReA with HLA-B27 and, more specifically, the consequent arthritogenic peptide hypothesis, it is of major importance to determine whether the major response is found in the CD4+ or the CD8+ population. This question, which was addressed at the meeting by Gaston and Young (Birmingham) and by Marker-Hermann (Mainz), is more difficult to answer than it seems. Two approaches, immunohistological examination of sections from ReA joints and in vitro cell cycle studies, have been used. Immunohistological studies have shown that, as in the other inflammatory arthropathies, most T cells in the joint are CD4+. Many T cells in the joint, however, are not specific for the aetiopathological antigen but are “bystanders” migrating through the inflamed joint as they would through any inflamed tissue; such cells may make a contribution to perpetuating inflammation but are not the primary progenitors. In an attempt to refine the immunohistological approach, investigators have examined the presence of activation markers on T cells, suggesting that the most relevant subset would express more activation markers. Unfortunately, the results conflict both between different experimenters and between different activation markers so no firm conclusion can be drawn. Turning to the in vitro analyses, both in bulk cell cycle studies and in studies of T cell clone phenotype, the vast majority of cells which proliferate in response to the triggering bacteria are CD4+. Again, this cannot be considered the definitive answer as culture conditions may selectively allow CD4+ rather than CD8+ cells to grow. There is certainly clear evidence in mice that chlamydia and enteric bacteria can generate cytotoxic T cell responses. Furthermore, as mentioned above, using specific culture conditions and antigen presenting cells, Marker-Hermann derived bacteria specific CD8+ T cell clones from the ReA joint and interestingly, these, unlike their CD4+ counterparts, show a restricted T cell receptor usage. To complicate the issue further, it has become apparent that some CD8+ T cells in the ReA joint display MHC unrestricted but bacteria specific cytotoxicity; although some of these are T cell receptor αβ positive, others carry the γδ T cell receptor. The epitope specificity and role of these unusual cells remain to be established. Thus, although the idea that ReA synovitis may be induced by bacteria specific, HLA-B27 restricted CD8 T cells remains attractive, the situation is likely to be much more complex than this and the only definite conclusion which can be drawn is that both CD4+ and CD8+ cells are likely to play a part in pathogenesis.

The two critical questions discussed by Marker-Hermann (Mainz), Gaston (Birmingham), Sieper (Berlin), and Campbell (London) is to determine which bacterial proteins and epitopes are recognised by the bacteria specific T cells in ReA. This is relevant for several reasons. Firstly, the epitope repertoire may be different from that seen in uncomplicated urethritis or enteritis, or epitope recognition may differ between acute and chronic ReA. Either of these would suggest that recognition of certain epitopes may be essential for rapid resolution or elimination of infectious organisms while, conversely, recognition of others may predispose to chronicity. Secondly, for chlamydia, the epitopes recognised can be compared with the genes expressed by the chlamydia in the joint. Thirdly, identification of bacterial epitopes may be of therapeutic relevance as induction of tolerance to harmful epitopes could be attempted. Initial approaches to the identification of potential epitopes previously relied on the application of biochemical or immunological separation techniques to bacterial preparations but have now been given a major boost by the increasing availability of recombinant bacterial proteins whose identity and purity is much more certain. Most of the work to date has been carried out on disease induced by yersinia or chlamydia. In yersinia, several groups agree that the 19 kDa urease β subunit, which has previously been shown to induce arthritis in mice, is of relevance. This is a cationic protein so will stick readily to cartilage, which may be of importance. The 14 kDa L23 ribosomal protein also remains to be a rea major target in chlamydia disease the 57 kDa heat shock protein, analogous to the 60 kDa heat shock proteins found in almost all bacterial and mammalian cells, has been found by two groups to be a major target antigen. Although at first sight the recognition of such a widely expressed heat shock protein might suggest that cross reactivity with human antigen was an important pathogenetic mechanism, in fact the epitope recognised has little similarity and no cross reactivity with its human counterpart. Clones have also been derived which recognise the 18 kDa histone protein, another cationic protein, and a 30 kDa antigen which has not been fully identified. In contrast, despite intensive studies by at least three groups, no such clone has been found to recognise the chlamydia major outer membrane protein, which is a major component of the protective immune response to chlamydia in normal individuals. Interestingly, chlamydia in the joint has been shown to express only low levels of major outer membrane protein but continues to express the 57 kDa heat shock protein. In further support of the idea that there may be different recognition in patients with ReA compared with those who develop only uncomplicated urethritis, a 23 kDa chlamydial antigen has been found that is recognised by patients with urethritis but not by those with ReA.

To date, all the effort in ReA has gone into identifying bacterially derived peptides that stimulate ReA synovial T cells. There has been no systematic search for autoantigens in this condition, though at least in the more chronic or persistent cases a transition from a purely infectious to a more autoimmune process seems plausible. Autoantigens, such as collagen type II, proteoglycans, or HLA-B27, have been suggested as stimulatory proteins in another spondylarthropathy, ankyllosing spondylitis. A presentation by Wucherpfennig (Boston), suggesting that the T cell expansion to myelin basic protein seen in multiple sclerosis might be initially virally driven, served to focus on the idea
that the infection-autoimmune transition should be more extensively investigated in ReA.

Pathogenesis of reactive arthritis: studies of cytokines
The role of cytokines in the pathogenesis of ReA and other forms of arthritis has been a recent topic of interest. As alluded to above, although it is relatively easy to establish the phenotype of the bacteria specific T cells in the joint, it is much more problematic to analyse the functional role of such cells. In this respect, cytokines can be used as surrogate markers. Suggesting that interferon γ was important in the elimination of intracellular bacteria while interleukin-4 and interleukin-10 might encourage bacterial persistence, Sieper (Berlin) investigated the presence of these molecules using a variety of methods, including immunohistology and polymerase chain reaction. Interestingly, most synovial tissue whether from patients with ReA or rheumatoid arthritis showed the presence of interferon γ, but interleukin-4 was found much more commonly in ReA, whatever the stage of the disease. Clearly, there is considerable scope for further analysis here looking at the many other cytokines which may contribute to the immune response in the synovium.

Pathogenesis of reactive arthritis: a comprehensive hypothesis
Based on the studies presented above, we have developed a hypothesis for the pathogenesis of ReA (figure). In this hypothesis the pathogenesis is divided into three phases: the initiation phase, the acute ReA phase, and the chronic ReA phase. In the initiation phase the subject is exposed to the triggering bacterium (chlamydia, yersinia, salmonella, shigella, campylobacter) and develops a local primary infection (gastroenteritis or urethritis) which may or may not be symptomatic. This primary infection resolves in most subjects without sequelae but in a minority of people ReA (or another secondary consequence such as pelvic inflammatory disease) ensues. The reason why this happens in some patients but not others is not known; however, both host specific and, possibly, bacterial strain specific factors may be important. In the acute ReA phase all subjects develop a peripheral synovitis, but classical spondyloarthropathic features are primarily seen in HLA-B27+ subjects. This dichotomy may suggest that different mechanisms are responsible for arthritis and spondyloarthropathic features. In acute arthritis the presentation of bacterial peptides to CD4+ T cells may be of primary importance, whereas other mechanisms more associated with B27-, such as presentation to CD8+ T cells or presentation of HLA-B27 derived peptides, may be relevant to the spondyloarthropathic manifestations. In the chronic ReA phase we postulate that the dichotomy between HLA-B27+ and HLA-B27- subjects continues, although those who are HLA-B27+ are more likely to develop persistent or recurrent disease. The factors which predispose to chronicity or recurrence are likely to include the host genetic and immune (especially cytokine) background, persisting bacterial antigen, and possibly the evolution of responses to cross reactive autoantigens (Sieper J, Kingsley G. Recent advances in the pathogenesis of reactive arthritis. *Immunol Today* 1996; 17: 160–3.).

Clinical aspects of reactive arthritis: epidemiology, clinical features, prognosis, and treatment
The last part of the workshop concentrated on more clinical aspects of ReA, including diagnosis, prognosis, and treatment. Much of what we know about the clinical aspects of ReA stems from the careful studies performed in the past in Scandinavia, especially in Finland. Leirisalo-Repo (Helsinki, Finland) presented interesting results from a 20 year follow up epidemiologic study in Finland. There had clearly been some change in the organisms triggering ReA. In sporadic ReA the incidence of chlamydia induced disease remained constant while the incidence of yersinia arthritis was decreasing, associated with a general decrease in the incidence of all yersinia infection. Conversely, the incidence of salmonella induced ReA was increasing in Finland. In general, the frequency of cases of ReA induced by a particular organism mirrors...
the frequency of primary infection with that organism within the population. However, an exception is campylobacter, which is a relatively frequent cause of gastroenteritis and which, purely seems to progress to ReA. Although similar changes are likely to have occurred in other countries, because the cause of ReA depends so closely on the local prevalence of the organism, the incidence of the various forms of ReA will vary from one locale to another. In epidemic disease the causative organisms again vary. In developed countries epidemic ReA is mainly due to salmonella, but shigella also plays a part. Although Shigella sonnei is the commonest strain in developed countries, its association with ReA is uncertain, and most ReA cases are probably associated with Shigella flexneri. World wide, especially in less developed countries, Shigella flexneri is, in any case, the major pathogen.

The study also provided some interesting information about prognosis. The 20 year prognosis was influenced by four major factors: the nature of the triggering infection, the presence of HLA-B27, the patient’s gender, and the presence of recurrent arthritis. When yersinia arthritis was compared with classical Reiter’s syndrome (presumed but not proved to be primarily due to Chlamydia trachomatis non-specific urethritis) it was found that the patients with Reiter’s syndrome had worse joint symptoms, more chronic or recurrent disease, and more joint erosions. Interestingly, the incidence of other features, such as sacroiliitis, spondylitis, and iritis, did not differ between the two groups. As already alluded to above, HLA-B27 patients had a worse prognosis. Recurrent arthritis, enthesopathy, sacroiliitis, and iritis, as well as progression to a chronic condition including ankylosing spondylitis was much commoner in these subjects. True spondyloarthropathic features, such as enthesopathy, sacroiliitis, and iritis, as well as progression to ankylosing spondylitis, were unknown in the HLA-B27 negative subjects. Sacroiliitis and progression to ankylosing spondylitis, but not other features, were worse in men with yersinia arthritis than in women; there were too few women in the group with Reiter’s disease to make a meaningful comparison. Finally, as might seem self-evident, patients who have disease recurrences get more chronic disease; interestingly, however, most still do not get erosive disease.

Major (Ghent, Belgium) presented an exhaustive analysis of their series of studies of colonoscopic changes in patients with spondyloarthropathy. In brief, they showed that many patients with various spondyloarthropathies have inflammatory changes in the gut, usually not associated with clinical features of intestinal disease. In some cases these lesions are histologically acute and disappear on repeat examination; other patients develop more chronic enteric disease (which appears to predispose to chronicity of the accompanying locomotor disease). Such changes are rarely seen in the general population. These data have normally been interpreted as showing that the gut has a causative role in many spondyloarthropathies. Recent studies, however, have shown that even patients with seronegative ReA have colonoscopic lesions in about 25% of cases, suggesting that in some cases these lesions represent yet another extra-articular manifestation of spondyloarthropathy.

Treatment of ReA, like that of most rheumatic diseases, remains largely empirical. Most patients who have self limiting disease receive only symptomatic treatment with anti-inflammatory agents and, where appropriate, intra-articular steroid injections. Disease modifying agents are increasingly prescribed, however, and Zeitler (Hannover, Germany) reviewed the rationale for their use. The consensus appears to be that disease modifying agents should be given to patients whose initial attack is prolonged or refractory and to those who develop recurrent or chronic disease. The second line agents that are usually considered are sulphasalazine and methotrexate, though azathioprine has also been used. Unfortunately, many studies of these drugs in ReA are short term, and uncontrolled and therefore firm treatment guidelines cannot be drawn up. Nevertheless, Zeitler proposed the following policies: sulphasalazine for a prolonged initial attack, methotrexate (or even azathioprine) if the disease became more severe and refractory, and longer term sulphasalazine for patients with recurrent disease. He also noted two particular points about methotrexate; firstly, it is especially efficacious in mucocutaneous lesions and, secondly, it should be avoided in HIV positive patients.

The role of antimicrobial treatment in ReA is another area of major interest. There are two related questions. Firstly, can antibiotic treatment for the primary infection prevent subsequent ReA and, secondly, can antibiotic treatment in established disease shorten its course. In sexually acquired ReA it is clear that the use of appropriate antibiotics to treat the initiating urethritis can substantially decrease the risk of subsequent ReA. Some studies have also suggested that prolonged tetracycline treatment after the onset of arthritis can decrease its duration and severity but this is far from being proved. In enteric ReA the situation is still less clear: for most of the primary infections, antibiotic treatment is generally considered inappropriate and such data as there are do not support antibiotic use after the onset of ReA. A trial with ciprofloxacin, however, which is ongoing in Berlin, and a pan-European study of azithromycin, which is just starting, should help to answer these questions. The more basic studies examining whether the triggering organisms for ReA persist in the joint or elsewhere in the body, and, if so, in what state, will also help to design appropriate antimicrobial treatment.

An alternative therapeutic modality which has become popular in the treatment of various immune rheumatic diseases is oral tolerance. Van Eden (Utrecht, Netherlands) reviewed the evidence for its effectiveness in rat adjuvant arthritis. He concluded that certain mycobacterial peptides, but not others, when given orally could make the animals tolerant to adjuvant arthritis. Of interest, the antirheumatic drug, Subreum, an Escherichia coli extract, which is also thought to be an oral tolerance, also suppressed arthritis in these rats, though less effectively than the mycobacterial peptides. The mechanism of action of oral tolerance remains hotly disputed by the various workers in the field, though one popular theory, supported by van Eden’s studies, is that it induces Th2 (interleukin-4, interleukin-10, and transforming growth factor β producing) T cells in the gut, which then migrate to the joint and suppress local immune activation. Sieper (Berlin, Germany) reviewed the use of oral tolerance in human rheumatoid arthritis where, once again, the data are conflicting, with some studies showing efficacy and others not. He further made the point that in any case oral tolerance might not be pertinent to ReA where, unlike rheumatoid arthritis, there appeared to be considerable local interleukin-4 production in the joint. In addition, if ReA were truly due to persistent bacterial antigen in the joint, no benefit would be anticipated as interleukin-4 would exacerbate bacterial persistence.

Clinical aspects of reactive arthritis: clinical and laboratory aids to diagnosis

In the final section of the workshop a series of consensus groups were held in an attempt to develop diagnostic guidelines of value for clinical practice.
CONSENSUS ON DIAGNOSTIC CLINICAL FEATURES

Various diagnostic criteria have been developed for the spondyloarthopathies, including ReA; those from the European Spondyloarthropathy Study Group have probably been the most extensively validated. However, all these criteria viewed ReA in the context of the spondyloarthropathy disease group. As discussed above, most patients with ReA, especially if they have HLA-B27 negative, may never develop spondyloarthropathy features. More recently the German Society of Rheumatology proposed that ReA should be diagnosed in patients with an asymmetrical predominantly lower limb oligoarthritis and a history or microbiological evidence of preceding infection. These criteria, however, have not been validated.

At the workshop Zeidler (Hannover, Germany) and Toivanen (Turku, Finland) proposed that the diagnosis of ReA should require the presence of typical clinical features (lower limb or asymmetrical oligoarthritis ± enthesopathy ± sacroiliitis) plus evidence of infection within the past four weeks with typical organisms such as chlamydia or yersinia; exclusion criteria would be required for other known rheumatic diseases, such as gout and other spondyloarthopathies. The cut off point of four weeks for preceding infection is somewhat arbitrary as some patients may have infection six or more weeks before arthritis; however, the extension of the time beyond four weeks makes these criteria less specific. They further suggested that in view of the weakening association of HLA-B27 with ReA its determination should play no part in diagnostic criteria. Other members of the group were uncertain how much emphasis should be placed on the presence of enthesopathic features because these features are only found in a minority of patients. The issue of whether ReA should be diagnosed in a patient with enthesitis but no peripheral arthritis remained unresolved. In patients with peripheral arthritis it was agreed that additional features, whether of spondyloarthropathy or of Reiter’s syndrome (conjunctivitis, skin lesions, and so on), should be recorded but not required for diagnosis.

USEFULNESS OF POTENTIAL DIAGNOSTIC TESTS

The remainder of the consensus session was devoted to the methods available for obtaining evidence of preceding infection. In what is probably a minority of patients this is simple; if the symptoms suggest infection, and patients report symptoms in the groin, the urethra, or the rectum they have been or are having urethritis or genitourinary infection. A more difficult group are those from whom no symptoms or signs of infection are elicit. In some patients no further tests are needed. However, many patients, particularly those with chlamydia infection, do not have any symptoms of their primary infection; this is probably the norm in women. Although this is rare in most forms of enteritis, it can occur; in yersinia infection, asymptomatic infection may be more usual. In addition, by the time patients present with arthritis the primary infection may have resolved so local culture is unsuccessful. In such cases, even if the patient had, for example, a urethritis, it will be unclear whether this was the initiating infection or merely a clinical manifestation of Reiter’s syndrome. In these circumstances, clinicians usually resort to serological testing; more recently, lymphocyte proliferation of synovial T cells and detection of bacterial antigen in the joint by immunological or molecular methods have been used in diagnosis. What is the true usefulness of such tests?

Even the interpretation of stool and urethral cultures may not be as clear cut as it seems. Although carriage of enteric organisms associated with ReA is rare, a substantial minority of the population, possibly as high as 20% in sexually active young people, are asymptomatic carriers of chlamydia. If chlamydia is cultured from a patient without recent genital symptoms it cannot be presumed with certainty to be related to their arthritis. Nevertheless, in the absence of a clear enteric trigger, urethral culture should be considered as treatment of genital chlamydia will avoid other consequences such as pelvic inflammatory.

Heesemann (Wurzburg, Germany) pointed out the major technical problems in detecting some organisms associated with ReA which are quite fastidious in their growth requirements. For yersinia, shigella, salmonella, and campylobacter, stool culture should be used, but selective or enriched media may be required. Salmonella may additionally be excreted in the urine. For chlamydia, urethral swab and culture is the traditional detection method, but this organism is often difficult to grow. In many laboratories, therefore, polymerase chain reaction for chlamydial DNA is being used on urethral swabs or even urine. The question of what cultures should be done in patients with an arthritis typical of ReA, but no symptoms of primary infection, left the group divided as there was no agreement on the pick up rate. It was generally thought to be quite high for chlamydia and lower for enteric organisms, but few hard data exist.

If the interpretation of culture results can be difficult, serological tests, by comparison, are a veritable minefield. Heesemann discussed the various antibody tests available. For yersinia there is a Widal-type agglutination reaction, but enzyme linked immunosorbent assay (ELISA) or immunoblotting for the YOP protein is considered more accurate. Again for salmonella, the Widal test remains in use but has been abandoned in other centres as too unreliable; an ELISA for salmonella lipo-poly saccharide is available from some laboratories. Immunoblotting is available for campylobacter, but for shigella there is currently no serological test. How should the finding of a significant titre of antibodies to an enteric organism be interpreted? Although there are few hard data directly concerning patients with ReA, it is clear that a correct interpretation requires some knowledge of the prevalence of such antibodies in the local population. For example, yersinia infection is relatively unusual in Britain compared with Finland; appropriate local microbiological advice should be sought in cases of doubt. Serological tests are available for Chlamydia trachomatis, including microimmunoﬂuorescence (which is time consuming and difficult) and the more usual ELISA techniques. Vischer (Zurich, Switzerland) pointed out the diagnostic usefulness of detecting IgM, IgA, and IgG antibodies in the serum and synovial fluid of patients with oligoarthritis. In serum, even when all three antibody isotypes were detected, this gave a positive predictive value of only 58% because of the high prevalence of such antibodies in the general population and the existence of patients with chlamydia induced ReA who do not make a humoral immune response against the organism. He concluded that serum antibodies were not really useful in daily clinical practice unless the patient came from an area where local antibody prevalence was unusually low. He also presented limited data suggesting that the detection of chlamydia specific IgA in the synovial fluid might be more helpful, but further studies are needed.

Regarding the more experimental diagnostic techniques of bacterial detection by immunofluorescence, bacteria specific synovial lymphocyte proliferation, and bacterial DNA detection by polymerase chain reaction, it was generally agreed that none was currently ready for use in the clinic. Granfors (Turku, Finland), who has enormous experience of the use of bacterial immunofluorescence to detect salmonella, shigella, and yersinia, felt that it would probably never be suitable for routine use because it was
Table 1 Diagnostic criteria for reactive arthritis

<table>
<thead>
<tr>
<th>Typical peripheral arthritis</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominantly lower limb, asymmetric</td>
<td>Patients with other known causes of mono/oligoarthritis,</td>
</tr>
<tr>
<td>oligoarthritis</td>
<td>such as other defined spondyloarthropathies, septic</td>
</tr>
<tr>
<td>plus</td>
<td>spondyloarthropathies, and reactive arthritis, Lyme</td>
</tr>
<tr>
<td></td>
<td>disease, and streptococcal ReA, should be excluded</td>
</tr>
</tbody>
</table>

Evidence of preceding infection
(a) Where classical diarthrosis or uveitis within preceding four weeks, laboratory confirmation is desirable but not essential
(b) Where no clear clinical infection, laboratory confirmation of infection is essential

Exclusion criteria

The diagnosis of ReA does not require the presence of HLA-B27 or extra-articular features of Reiter’s syndrome (conjunctivitis, iritis, skin lesions, non-infectious urethritis, cardiac and neurological and/or typical spondyloarthropic features (inflammatory back pain, alternating buttock pain, enthesitis, iritis) but, if present, should be recorded

Table 2 Laboratory tests for preceding infection

<table>
<thead>
<tr>
<th>Stool culture</th>
<th>Helpful if positive; should be routine if previous diarrhoea; stool culture in absence of diarrhoea rarely positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethral culture</td>
<td>Urethral culture often positive in absence of symptoms; need to interpret in light of local asymptomatic carriage rate</td>
</tr>
<tr>
<td>Urethral/urethral PCR*</td>
<td>These tests can be used, where available, instead of urethral culture or urethral immunofluorescence for bacteria</td>
</tr>
</tbody>
</table>

Serology

Chlamydia IgG of no value because high prevalence in community; rising titre or IgA antibodies useful in presence of non-specific urethritis history

Yersinia, salmonella, and campylobacter antibodies by ELISA: IgG antibodies—a fourfold change in titre or a strongly raised titre (difficult to specify) demands further workup

Serology for yersinia or salmonella are specific for recent infection but insensitive, especially for salmonella

Shigella serology is of no use because of cross reactivity with Escherichia coli

Immunofluorescence for bacteria in synovium and proliferation of synovial lymphocytes

These tests are labour intensive and technically difficult tests whose sensitivity and specificity is uncertain. They are unlikely ever to be suitable for routine diagnostic use

PCR for chlamydial DNA in the joint

A potentially valuable test which could be practicable for routine use. Its sensitivity and specificity are being further investigated. This test cannot currently be used for enteric ReA because it is uncertain whether DNA from enteric organisms reaches the joint

*PCR = polymerase chain reaction

Financial help was also received from Bayer, Kabi-Pharmacia, Tosse, Lederle, Ciba-Geigy, Smith-Kline Beecham, and Henning.

Key references


Genetics of reactive arthritis


Immunology of reactive arthritis


Clinical aspects of reactive arthritis

Abstracts of invited lectures

Interactions between the immune system and infectious agents: protection, immunopathology, autoimmunity, subversion

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Viruses have coevolved with vertebrate hosts and their immune systems to reach evolutionarily balanced states; therefore many of these virus-host arrangements show limiting facets of the immune system. For example, immunological unresponsiveness to viruses may be established by infection of the thymus, by sequestration of antigens exclusively in epithelia or neurons where they are ignored by T cells, or by exhaustive differentiation of T cells in the periphery. Induction of autoreactive T cells depends on proper antigen presentation in lymphoid organs where sufficient concentrations of cytokines prevails. Mechanisms of T cell mediated antiviral protection also vary with the virus. Cytotoxic viruses in either CD8+ or CD4+ T cell dependent cytokines or antibodies provide protection, dependent upon the site of viral replication. Against non-cytotoxic viruses cytokine-dependent CD8+ T cells are mandatory for virus elimination. B cell induction by viruses depends very much on antigen pattern and organisation; repetitive identical determinants with defined dispositions give rise to high avidity responses which may be responsible for "endosomal" or "cytotoxic" pathogens, respectively. In addition, γδ T cells seem to be required for efficient protection. We used genetically mutant mice to assess the role of different T cell populations in immunity against intracellular bacteria. Effective protection against the intracellular bacterium, L monocytogenes, BCG, and M tuberculosis, required all three subsets, though in varying degrees. Thus protection against the endosomal bacterium BCG not only involved CD4 but also CD8 T cells. Reciprocally, protection against the cytosolic pathogen, L monocytogenes, depended on CD4 T cells in addition to CD8 T cells. Although the contribution of γδ T cells to protection against L monocytogenes seemed small, these cells were essential for combat of M tuberculosis.

The Th1 cytokine, interferon γ, is central to protective immunity against intracellular bacteria. In contrast, the Th2 cytokines seem to exacerbate disease. Cytokines produced by the innate immune system directly influence the divergence into Th1 or Th2 dominated immunity. Thus interleukin-12 (IL-12) favours development of Th1 cells, whereas IL-4 promotes that of Th2 cells. Although the source of early IL-4 production remains enigmatic, recent evidence points to CD4+ natural killer 1+ (NK1.1+) T cell receptor αβ+ (TCRαβ+) lymphocytes. Infection with intracellular bacteria induced the disappearance of these lymphocytes from the liver and, as a consequence, abolished IL-4 production. We conclude that down regulation of CD4+ NK1.1+ TCRαβ+ lymphocytes by intracellular bacteria favours unconstrained antimiicrobial protection.

Key words

Bacterial persistence in reactive arthritis: how do chlamydiae evade the immune system and persist in the joint?

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Bas et al, using four different polymerase chain reactions (PCRs) for three different chlamydial gene targets, showed that Chlamydia trachomatis was present in the joints of 22 of 71 patients with various arthropathies. Other studies have shown the presence of chlamydiae in a layer of cells beneath the synovium and have suggested that the organism may be detected by reverse transcriptase PCR in synovial tissue from patients with arthritis. Chlamydiae, unlike yersinia, are obligate intracellular pathogens. Glycosaminoglycans, abundant in joints, help maintain chlamydial adhesion to target cells. Antibody directed against neutralising epitopes on the major outer membrane protein (MOMP) may block infection of host cells, though these epitopes are subject to antigenic drift (point mutations giving rise to serovarians) and antigenic shift (arising from recombination events). Once established in the host cell, chlamydiae will be protected from neutralising antibody, resisting oxidative stress by elaborating heat shock proteins. One of these, a 57 kilodalton protein, shares B and T cell epitopes with human heat shock protein 60 (hsp60) and is thought to be involved in chlamydial immunopathology. Some peptides on chlamydial hsp-60 are able to bind to HLA-B27. Once chlamydial infection becomes chronic, in vitro studies suggest that proteolytic cleavage of MOMP by a trypsin-like endopeptidase in the endosome, whereas, in vivo, proteolytic enzymes are generated by neutrophils and macrophages. Thus, the availability of hsp-60 on the surface of infected host cells may lead to a state of persistent infection, in which shedding of infectious elementary bodies is curtailed but aberrant chlamydiae are transferred at host cell division. This may be accompanied by up regulation of immunopathological hsp-60 and down regulation of the MOMP. Interferon γ probably has a protective and a pathological role, determined by the fragile balance between the cell mediated immune responses and chlamydial replication. In ocular chlamydial infection (trachoma), severe disease has been associated with anergic cell mediated responses to chlamydiae. Chlamydial antigen, particularly lipopolysaccharide, persist for long periods in macrophages. Chlamydial survival may be aided by a chlamydial homologue of the Legionella pneumophila monomeric adhesin protein. Chlamydial antigen is processed by both the exogenous and endogenous pathways. Animal studies suggest T helper 1 responses and interferon γ are more important for protection than cytotoxic T lymphocyte responses, but the role of the latter in immunopathology deserves further study, particularly in reactive arthritis.


Detection of Chlamydia pneumoniae and Chlamydia trachomatis in the joints of patients with arthritis

N Z Wilkinson+*, G Kingsley†*, M E Ward†
Whethervochondrolysis DNA is present in the synovial fluid of patients with reactive arthritis is controversial. Present reports are conflicting and it is unclear whether the whole organism or merely bacterial fragments are present. To clarify the discrepancies between results we developed polymerase chain reactions (PCRs) to detect C pneumoniae and C trachomatis, targeting the Ompl gene and the cryptic plasmid respectively. Subsequently, 85 synovial fluid samples from patients with a range of rheumatic diseases, but predominantly reactive arthritis and undifferentiated oligoarthritis, were tested.

Both PCRs could amplify about 10 fg of the relevant DNA, the equivalent of 10 organisms, and there was no cross reactivity. Thirty patients were positive for C trachomatis PCR and has been verified by sequencing, and no patients were positive for C pneumoniae. It is likely that C trachomatis is present in the joint of patients with reactive arthritis and undifferentiated oligoarthritis. C pneumoniae does not have a prominent role in reactive arthritis.

Comparisons between various infections triggering reactive arthritis: implications for the pathogenesis
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Infections triggering reactive arthritis can be grouped in two different ways. Firstly, most of them are either urogenital, enteric, or respiratory tract infections; in addition, several other more or less generalised infections may induce reactive arthritis, with the causative microbe identified or unknown.

Another, equally useful way to divide the microbes associated with reactive arthritis is by their direct or indirect influence on the presence of HLA-B27. A firm association between bacterial genera and B27 has been established in only six cases: campylobacter, chlamydia, clostridium, salmonella, shigella, and yersinia. The prevalence of B27 positive patients varies between 60 and 80% for any of these triggers. For instance, of 19 B27 typed patients with Clostridium difficile induced reactive arthritis reported on so far, 63% were B27 positive. On the other hand, because no clostridial structures have been demonstrated in the synovium, it remains unclear whether the arthritis is due directly to C difficile or to changes in the intestinal flora, parallel to that occurring, for example, in the intestinal bypass syndrome.

A remarkable characteristic of reactive arthritis triggered by these six B27 dependent bacterial genera is their true reactive nature—that is, these species do not usually induce bacterial arthritis. This is in contrast with the following bacteria (borrelia, brucella, burtnalles, leptospira, mycobacterium, neisseria, staphylococcus, streptococcus, ureaplasma, and vibrio) which are B27 independent and which may cause both reactive and bacterial arthritis. However, no evidence exists to indicate different pathogenetic mechanisms for reactive arthritis triggered by different groups of bacteria. An induction period of one to three weeks occurs always between the initial infection and development of arthritis. During this period the host becomes immunologically sensitised against the causative microbe. Whether the microbial components then enter the joint tissue, a local delayed-type hypersensitivity reaction manifests as an acute reactive arthritis. So far, synovial localisation of bacterial structures originating from the B27 independent species has been little studied.

Key papers

Shigella as a paradigm for bacteria that induce reactive arthritis
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Shigella species cause bacillary dysentery in humans by invading the intestinal mucosa of the colon. After infection 1-4% of patients develop reactive arthritis. Compared with reactive arthritis induced by other intracellular bacteria, this postdysenteric reactive arthritis is of special interest because (a) the association with HLA-B27 is particularly strong, (b) shigella strains show intra/interspecies variation in their ability to trigger reactive arthritis, and (c) postdysenteric reactive arthritis is the most common form of this arthritis in developing countries. It has been suggested that molecular mimicry of bacterial epitopes with epitopes of HLA-B27 may help to induce reactive arthritis. This popular hypothesis is supported by several reports on cross reactivity of monoclonal antibodies directed against HLA-B27 and bacterial antigens in both. A particular mimetic epitope to HLA-B27 was identified as part of a polypeptide sequence encoded by a 2 megadalton plasmid, which was found to be associated with a number of arthropathogenic Shigella flexneri strains. This plasmid was not present, however, in two non-arthropathogenic Shigella sonnei strains.

1 Stiegitz H, Lipsky P. Association between reactive arthritis and antecedent infection with Shigella flexneri carrying a 2-Md plasmid and encoding an HLA-B27 mimetic epitope. Arthritis Rheum 1993; 36: 1387-91.

What can animal models tell us about human reactive arthritis?
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Enteropathogenic bacteria, such as salmonellae, shigellae, and yersiniae, can induce reactive arthritis in humans. To study the pathogenesis of yersiniosis and its sequelae animal models are required. Fortunately, experimental yersiniosis of rodents resembles that of humans and thus offers suitable opportunities for studying the sequential steps of the infectious process and host response. To analyse the immune response we used yersinia susceptible Balb/c mice and resistant C57 BL/6 mice. As major components of the protective immune response we identified antibodies directed against the virulence proteins YadA and V antigen. Results obtained from the mouse infection model showed the protective role of T cells which produce interferon γ. Innate susceptibility of Balb/c mice to yersinia infection seemed to be induced by suppression or failure of interferon γ production of infected tissue (liver, spleen).

Lewis rats develop reactive arthritis-like disease after intravenous challenge with virulent Yersinia enterocolitica serotype O8. Yersinia mutants impaired in full virulence expression are significantly less arthritogenic. Microbiological and immunohistopathological examination of tarso-skeletal joints of yersinia infected Lewis rats revealed location and persistence of cultivable yersinia in synovial tissue accompanied by significant infiltration of αβ T cells during the chronic phase. The number of culturable yersinia drops. Surprisingly, treatment of rats with antibody against αβ T cell receptor did not suppress development of yersinia induced arthritis. whereas treatment with cobra venom factor (depletion of complement) did so.

From these results we conclude that reactive arthritis may be related (a) to an inappropriate immune response against arthritogenic microorganisms, (b) to synovial tissue susceptible for translocation of microbes and microbial antigen, and (c) to hyperreactivity of infected synovial tissue.


Are CD8+ T cells really the major players in reactive arthritis?
Elisabeth Mäker-Hermann
Abstracts of invited lectures

First Department of Medicine, University of Mainz, 55101 Mainz, Germany

One current theory trying to explain the association of HLA-B27 with reactive arthritis is the "arthritogenic peptide model". This model is based on the physiological antigen presenting function of HLA class I molecules. This theory proposes that "arthritogenic" bacteria produce a peptide that primes a cytotoxic T lymphocyte response restricted by HLA-B27. Consequently, synovial fluid CD8+ rather than CD4+ T cells should have a prominent pathogenetic role. The in vivo observation that reactive arthritis may occur in HIV infected individuals essentially devoid of functional CD4+ cells supports this hypothesis. The reactive arthritis synovitic infiltrate contains CD8+ lymphocytes, some of which are HLA-B27 restricted and specific for the triggering bacterium or for autoantigens. Furthermore, we have isolated CD8+ cytotoxic T lymphocytes that kill bacterial infected targets but are not restricted by HLA molecules. T lymphocytes themselves can present bacterial antigens to such HLA unrestricted CD8+ cytotoxic T lymphocytes and may thus contribute to the cytotoxic potential. Peptides derived from bacterial antigens may be recognised by B27 restricted cytotoxic T lymphocytes. We should consider the question whether mimicry of B27 presented peptides is necessary to explain autoimmune cytotoxic T lymphocyte-mediated phenomena and whether the number of T cells (either CD4+, CD8+ or y8+) of certain specificities or the demonstration of oligoclonal expansion of subpopulations in reactive arthritis is really decisive for disease induction.

T cells and the pathogenesis of reactive arthritis: is the CD4+ T cell response arthritogenic, regulatory—or irrelevant? J H Gaston

CCRIS, The Medical School, University of Birmingham, Birmingham B15 2TT, United Kingdom

CD4+ T lymphocytes specific for organisms which trigger reactive arthritis are readily demonstrable in the synovial fluid (SF) and membrane (SM) of affected joints; these cells have been cloned and their peptide major histocompatibility complex (MHC) specificities determined. However, given the association between reactive arthritis and the class I MHC antigen, HLA-B27, do these class II restricted CD4+ T cells have any pathogenic relevance?

Infections associated with reactive arthritis provoke a CD4+ T cell response, so their presence in SF and SM is not in itself surprising, as memory CD4+ T cells may be preferentially recruited to the joint. If bacterial antigens are present in the joint, however, these cells will not only be recruited but also activated. In these circumstances their activation is likely to be important in driving the inflammatory process. Evidence for the presence of antigen in the joint is increasing; for Chlamydia trachomatis, organisms (in small numbers) are present and may produce antigens, such as heat shock protein 60, which we have recently shown to be a target of the CD4+ T cell response. Antigen in SF (or on SF macrophages) is also suggested by the high "background" proliferative responses which are commonly seen in reactive arthritis SF T cells, and by the responses to autologous SF occasionally noted. Flow cytometry of reactive arthritis SF T cells also shows evidence of activation of CD4+ T cells, often to a greater extent than CD8+ cells. We have derived antigen specific T cells from SM biopsy samples cultured in interleukin-2, again suggesting activation (and interleukin-2 receptor expression) of CD4+ T cells in SM. Lastly, the antigenic targets of synovial CD4+ T cells have in several cases proved to be highly cationic proteins (for example, chlamydial Hc1), a property associated with arthritogenicity in experimental models of antigen induced arthritis.

In view of these findings it is unlikely that CD4+ T cells are irrelevant to the pathogenesis of joint inflammation. HLA-B27, in conferring susceptibility to reactive arthritis, may determine whether a response by CD4+ T occurs in the joint (for example, by influencing whether antigen gets to this site).

Key papers

Are y8+ T cells involved in the pathogenesis of reactive arthritis? J L Young, J S H Gaston

CCRIS, University of Birmingham, Edgbaston, Birmingham, United Kingdom

A defective or inappropriate cellular immune response has been postulated to be responsible for reactive arthritis. However, the precise disease mechanism remains to be elucidated. In this study we examined the in vitro responses of synovial fluid and peripheral blood mononuclear cells stimulated with autologous lymphoblastoid cell lines infected with Yersinia enterocolitica, a bacterium associated with triggering reactive arthritis. This procedure resulted in a profound expansion of y8+ T cells which displayed major histocompatibility complex (MHC) independent cytotoxicity for yersinia infected target cells. In the absence of B lymphoblastoid cell lines, bacteria fixed with formaldehyde, but not heat inactivated, also preferentially expanded y8+ T cell proliferation from mononuclear cell cultures. We obtained T cell clones after such stimulations—namely, CD2+, CD3+, CD4+, CD8+, T cell receptor y8+. As with cell lines, these clones killed both infected autologous and allogeneic cells. Infected cell lines deficient in MHC class I or class II expression, or both, were also lysed, including K562 (class I), C1R (HLA-A2, B8, C*), and Dauid (B2m-). Likewise, infected T2 cells, which have impaired antigen processing for both MHC class I and class II presented peptides, were susceptible targets. Interestingly, a lower level of cytotoxicity was observed when autologous rather than allogeneic targets were used. We have yet to determine if HLA-B27 is implicated in this reduced cytotoxicity. Our results suggest that antigen recognition by y8 T cells is independent of classical MHC molecules and their associated antigen processing pathways. Indeed, preliminary experiments suggest that at least one T cell clone can be triggered by intact Y enterocolitica.

Our study supports existing experiments in both humans and mice indicating that some y8 T cells can recognise antigens in a distinct manner from y8 T cells. Experiments in mice suggest that y8 T cells can influence the pathology of bacterial infections. Moreover, y8 T cells have been proposed to act as a first line of defence at the mucosal surface, the point of entry of bacteria associated with reactive arthritis. With our demonstration that y8 T cells with potent yersinia responsiveness can be found within the disease site, we suggest that a role for y8 T cells in the pathology of reactive arthritis should be considered.

Antigenic targets for T cells in reactive arthritis—therapeutic opportunities? Is there a role for autoantigens? Elisabeth Mäcker-Hermmann, Rainer Duchmann, Karl-Hermann Meyer zum Büschenfelde

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In enterogenic reactive arthritis the prevalence in the affected joints of T lymphocytes specific for the triggering bacteria is high compared with the prevalence of T cells from paired peripheral blood samples. However, retrieval of memory T cells of different specificities into the joint may accompany this response. There is increasing evidence that T cell reactivity to enterobacterial antigens varies depending on the stage of disease and may spread to other antigens. However, peripheral blood T cell responsiveness to enterobacterial antigens is often reduced in active arthritis, and that different T cell subsets may recognise different dominant antigens.

1. Specific T cell response to the triggering enterobacterium: Yersinia fluid T cells were shown to recognise dominantly the enterobacterial protein antigens Yersinia enterocolitica urease β subunit and Yersinia 50 S ribosomal protein L23. The Escherichia coli GroEII is recognised in reactive arthritis and other spondyloarthropathies. Cytotoxic T cells can also respond to peptides from some of these antigens.

2. "Secondary" T cell responses to autoantigens of intestinal flora: Our studies indicated that in inflammatory bowel disease, laminar propria T lymphocytes from inflamed intestine respond to autologous intestinal flora and thus break the selective tolerance to these bacteria. This mechanism may also be relevant in reactive arthritis, where T cells with specificity for autologous gut flora could migrate to extraintestinal (joint?) tissues and contribute to inflammation.

3. Autoantigens: Autoreactive CD4+, CD8+, and y8 T cells can be isolated from reactive arthritis synovial fluid. The significance of these findings is still unclear; cross reactivity to enterobacteria is not a prerequisite to explain the induction and in vivo expansion of such T cells.
Molecular mimicry in T cell mediated autoimmune relevance to spondyloarthropathies
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Viral and bacterial infections may be important in the initiation or progression of human T cell mediated autoimmune diseases. T cells specific for immunodominant epitope of human myelin basic protein (MBP, residues 85-99) is bound by HLA-DR2 and which of its amino acid side chains are important in contacting the T cell receptor, seven viral and one bacterial peptide were identified that activate MBP(85-99) specific T cell clones. Two principles were important in the identification of these mimicry peptides: (a) the MBP(85-99) binding motif is degenerate as structurally related amino acids can be substituted for the two anchor residues of the MBP(85-99) peptide, (b) the two primary T cell receptor contact residues of the peptide have to be conserved. The importance of these structural considerations is demonstrated by the fact that seven of eight mimicry peptides do not have obvious sequence similarity with MBP(85-99) and would not have been identified by sequence alignments. The fact that several different viral peptides can activate the same T cell clones suggests that different viruses may trigger the same autoimmune syndrome.

The approach also provide insights into the pathogenesis of rheumatic diseases, in particular to the pathogenesis of rheumatoid arthritis, which is associated with certain alleles of major histocompatibility complex (MHC) class II genes. A structural approach may also be useful for the identification of peptides that mimic MHC class I bound self peptides. However, a key difference between MHC class I and class II bound peptides will have to be considered: for MHC class II bound peptides the conformation of the peptide backbone is largely sequence independent as the peptide is fixed to the binding site by a number of hydrogen bonds along the entire peptide backbone. In contrast the conformation of MHC class I bound peptides is sequence dependent as the peptides are only fixed at their N and C terminuses to the MHC class I binding site.

Consideration of the structural aspects of MHC-peptide interactions will help in the identification of viral and bacterial peptides that may be responsible for the loss of self tolerance in human autoimmune diseases.


HLA and non-HLA genes in the spondyloarthropathies
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A genetic contribution of ankylosing spondylitis is clearly evident from the different accordance rates in monozygotic (60%) and dizygotic twins (12%). It has how been established beyond reasonable doubt that HLA-B27 itself is an important part of this genetic contribution but is apparent that other genes are also likely to play a part. Analysis of the nine B27 subtypes currently recognised suggests positive associations with B27*, B27*01, *02, *04, *05, *07, but there is emerging evidence that *03, *06, and *09 may not be associated with the disease or only weakly so.

In a study of 100 nuclear families with at least two affected sibling pairs we found evidence of an extended DRI/B27 haplotype overrepresented in patients compared with B27 controls (30% v 19%, p < 0.01), suggesting that another gene within the major histocompatibility complex (MHC) could also be involved. However, analysis of the TAP and LMI loci, in association studies, has excluded a significant role for these genes which are involved in the class I pathway of antigen presentation. Analysis of patterns of HLA haplotype sharing among 100 affected sibling pairs showed 6% sharing 0 HLA haplotypes. This compares with predicted sharing of 1.8-8% derived from estimates of sibling recurrence risk (7% in 134 families) and the population prevalence (0.5%). It compares with expected sharing of 25% if HLA was not linked with susceptibility to ankylosing spondylitis. These results therefore provide further support for the suggestion that HLA B27 is a significant genetic contribution comes from outside the MHC. Consequently, we have now embarked on a genomic screen for susceptibility genes in ankylosing spondylitis using a panel of 350 microsatellite DNA markers, which should result in a rough linkage map of susceptibility loci within the next 18 months.

How strong is the association of reactive arthritis with HLA-B27?
Marjatta Leirisalo-Repo*, Leena Mattilat†, Anja Siitonen‡, Saia Koskimies, Kaija Granfors§
*Department of Medicine, University of Helsinki; †National Public Health Institute; ‡Finnish Red Cross Blood Transfusion Service Helsinki; §National Public Health Institute, Turku, Finland

HLA-B27 is considered as the most important risk factor for the development of reactive arthritis. It is present in 60-80% of patients from different centres,1 patients with chlamydia as the triggering infection usually having slightly lower prevalence. Most of the reports come from academic institutions or from rheumatological centres, which might cause bias in the patient selection, on the more severe cases being referred to the specialists.

Patient data from a single outbreak would be more informative. Although such epidemiological data concern only a small number of patients with reactive complications, recent epidemiological studies on patients collected during salmonella outbreaks in Finland2 confirmed the data from different sources.3 The incidence of joint symptoms varies from 2 to 30%, but the prevalence of HLA-B27 in such patients is considerably lower than previously reported (table). Most such reports concern salmonella outbreaks, and calculations from the reports show that an average of 29% of the patients have HLA-B27.

The discrepancy between different reports may be due to differences in the arthritogenic properties of the bacteria, in the antigenic load in different outbreaks, different populations, or due to bias in patient selection. The most reasonable explanation would be a bias in the patient selection.

It was concluded that as shown before, HLA-B27 positive patients have more severe disease, with more complicated acute disease, and worse long term prognosis than HLA-B27 negative patients.4 Thus patients with HLA-B27 may have more severe disease, with enrichment of HLA-B27 positive cases. The mild cases are HLA-B27 negative, recover quickly, and have no need to be referred to specialists.


HLA-B27 restricted peptide presentation to cytotoxic T cells in reactive arthritis
Paul Bowness, Rachel Allen, Andrew J McMichael

Molecular Immunology group, Institute of Molecular Medicine, Neuffield Department of Medicine, John Radcliffe Hospital, Oxford

In an attempt to identify HLA-B27 restricted cytotoxic T cells from the joints and blood of patients with reactive arthritis we used a reverse immunogenetic approach based upon knowledge of the unique peptide binding properties of HLA-B27s. One hundred and twenty potential HLA-B27 binding peptides of eight to 10 amino acids length, identified by a database search of known chlamydial proteins, were synthesised. Sixty two were found to bind to HLA-B27 in vitro. These peptides were used in pools to screen synovial fluid and peripheral blood mononuclear cells from patients with reactive arthritis, other HLA-B27 associated spondyloarthropathies, and healthy controls. CD8 positive HLA-B27 restricted synovial T cell lines were grown from blood and synovial fluid of
two patients (one with reactive arthritis, one with ankylosing spondylitis). In one case a long-term culture in vitro culture was maintained and shown to be specific for a single nonamer peptide derived from chlamydial heat shock protein 70. Cytotoxic T cell responses to this or other peptides were not detected in three patients with other HLA-B27 associated arthropathies, one HLA-B27 negative patient with reactive arthritis, or from the blood of one HLA-B*2705 positive healthy control.

Control of the availability of HLA-B27 peptides at the levels of the proteasomes, TAP and HLA-B27
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The ability of HLA-B27 to present a particular peptide to the immune system depends on several factors: the transscription of the HLA-B27 gene, the expression of the HLA-B27 protein, the generation of the peptide by the proteasome complex, the translocation of the peptide into the endoplasmic reticulum, and the affinity of the peptide for the HLA-B27 molecules.

We studied the effect of interferon γ on the transcription of the genes of HLA-B27 as well as of the LMP2 and LMP7 of the proteasome complex using RT-PCR; on the expression of total HLA-B27 molecules as well as those with certain specific peptides using immunohistochemistry with specific monoclonal antibodies to HLA-B27. We also studied the residue specificities which control the ability of a peptide to translocate into the endoplasmic reticulum using a competition assay with a labelled reporter peptide, and which control the affinity of a peptide for the HLA-B27 molecules using a stabilisation method.

The results showed that for a bacterial peptide to become presented by HLA-B27 it has to overcome several hurdles of control, the factors of some of which can be analysed with certain criteria of algorithms.


Influence of HLA-B27 on host-microbial interactions
R D Inman
The Toronto Hospital and the University of Toronto, Toronto, Canada

The clinical features and immunological studies on reactive arthritis have raised the possibility that B27 may have a role in this disease other than the classical function of peptide presentation to CD8+ cytotoxic T cells.

We examined direct microbe major histocompatibility complex interactions using an in vitro invasion assay, in which arthritogenic Gram negative bacteria invade L cells transfected with various class I HLA alleles.

Role of T cell cytokines in initiation and maintenance of chronic inflammatory arthritis
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Most information on this topic has been obtained from study of cytokine mRNAs and cytokine proteins in synovial fluid. Some cytokines produced by peripheral blood lymphocytes and T cells clonally escaped from synovial fluid. So far these studies have dealt only with established disease. We hope to extend them to reactive arthritis, so as to encompass the initial triggering infection. A prospective study of this sort could be carried out only in a population with a high level of the triggering infection factor T cells, so that individuals could be recruited that would be large enough to generate a reasonably large sample of overt arthritis.

With colleagues in London, Paris, and Delhi we plan to carry out a study in the International Centre of Research in Diarrheal Diseases, Bangladesh. We have chosen to examine only shigella infections owing to their high prevalence in developing countries, the high levels of encountering reactive arthritis driven directly by bacteria in the joint. The indications are that Dhaka will have a European-type prevalence of B27. Our aim is to determine the factors in the infecting pathogen (and in the host response which favour triggering of reactive arthritis).

We believe that the major histocompatibility complex (MHC) associations provide a powerful tool for probing the role of these cytokines. Negative associations (protective effects) have been described in RA and in early onset pauciarticular juvenile rheumatoid arthritis, though not as yet in reactive arthritis. With Monika Brunner we have recently described a protective effect of the H2A* allele in collagen induced arthritis, which parallels the immunosuppressive effect of this allele in other collagen responses.1 In one of these parallel responses (to the antigen allo-4-hydroxy-phenyl-pyruvate-dioxygenase) the operation effects via suppression of an early (>24 h) burst of interleukin-4 (IL-4) production,2 and we are currently testing whether a similar burst is needed to establish chronic inflammatory arthritis. When our data for IL-4 are compared with those of Fournier et al.,4 and with those of Rödel et al.5 in whom inflammatory arthritis emerges as a disease in which Th2 cells are required for initiation of the disease, but become beneficial later in the disease course. Our working hypothesis is that polymorphisms of the regulatory regions of MHC class II genes is conserved because it results in differential expression in different types of antigen presenting cells (macrophages, dendritic cells, B cells, activated T cells), and thus in preferential activation of either Th1 or Th2 cells. Sequencing of mouse upper regulatory regions by our colleagues R Lauster and M Janitz demonstrates a high level of polymorphism in synovial T cells.

Oral tolerance, as described elsewhere in this workshop by Sieper, is another way of probing for T cell cytokine effects. We are impressed by the immunological impact of oral type II bovine collagen arthritis in Berlin rats.

In the highest treatment group (10 mg/day) the level of antihuman type II collagen dropped significantly among those patients with reactive arthritis who showed a clinical response.

Macrophages constitute a major part of the normal synovial lining. Their apparent role appears to lie in scavenging debris originating from joint material as well as the elimination of microorganisms entering the joint. In chronic inflammatory joint diseases, the synovial macrophages may also play a role in the generation and propagation of an autoimmune tissue reaction, perhaps like that which is seen in rheumatoid arthritis, but monocytes-macrophages may also be involved in the subsequent pathogenesis of these diseases, possibly spreading the inflammatory agents or their antigenic material throughout the body or providing a cytokine profile supporting the onset of joint inflammation, or both. This distinct local cytokine composition may direct the subsequent T cell response by leading to type I or II like reactions, and it may therefore be responsible for either antibody or cell-mediated immune reactions.

One particular example of an infectious arthritis is Lyme arthritis where, despite the identification of Borrelia burgdorferi as the inciting organism, the pathogenesis is still incompletely understood. A failure in the immune response to the infectious agent, or the autoimmune mechanism triggered by infection, microbial escape into protective tissue sites, and even intracellular persistence of B burgdorferi may be involved in this process.

To disclose a possible failure in the first line of defence, human mononuclear phagocytes were incubated with B burgdorferi. Light and electron microscopy showed that the presence of the spirochete induced distinct alterations in the phagocytes, including coiling phagocytosis, the formation of leaky lysosomes, the invagination of large membrane areas, the extracellular and cytoplasmic degradation of internalised B burgdorferi cells, and finally the formation of mononuclear syncytial cells and homotypic cell clusters. Further analyses showed distinct cytokine profiles with regard to interleukin-1, tumour necrosis factor α, IL-10, and IL-12 released by monocytes. In conclusion, as exemplified in Lyme disease the monocyte-macrophage system may direct the subsequent specific immune system by (a) altered antigen processing and presentation, (b) changes in cytokine integrity, and (c) a distinct cytokine milieu.

Lymphocyte traffic in reactive arthritis
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Spondyloarthropathies refer to a group of disorders with overlapping manifestations, characterised by common clinical and genetic features, of which reactive arthritis is an example. The presence of subclinical gut inflammation (ileitis) in patients with spondyloarthropathies and its evolution in some patients to clinically overt inflammatory bowel disease (Cohn's disease) strongly suggest a pivotal role of the gut immune apparatus in the development of this disease complex. T cells are believed to have an important role in the pathogenesis of spondyloarthropathies. However, at present it is unknown if the same lymphocytes are involved in the development of articular and gut manifestations. Recent data on the peripheral blood suggest new interesting insights into the gut-synovium homing relation. Indeed, mucosal immunoblasts can bind (in vitro) to both reactive arthritis and spondylarthropathy and gut endothelial venules (HEV), but not to peripheral lymph node HEV.

We investigated the role of the integrin α4β7 in the proposed gut-synovium re-circulation of lymphocytes. It is expressed on lymphocytes with specific homing affinity for Peyer's patch HEV (the ligand being MadCAM-1). In addition, this integrin has been reported to be overexpressed in synovium, compared to normal synovium. The quantitative expression of α4β7 on inter leukin-2 responsive T cell lines from ileum and colon in normal controls and patients with inflammatory bowel disease (IBD) was assessed by flow cytometry, using the Act-1 monoclonal antibody (a gift from Dr A Lazaroits). T cell lines from patients with IBD were significantly lower expressors of α4β7 than normal controls. These data may either suggest that the expression of α4β7 in IBD are low α4β7 expressors or that this integrin is down regulated by inflammatory mediators in the IBD process.

Epidemiology and prognostic features of reactive arthritis
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Within the past 20 years Reiter's syndrome, for which Chlamydia trachomatis is a major cause, has remained a major form of reactive arthritis. In Scandinavia, reactive arthritis due to yersinia infection has been common, while reactive arthritis induced by salmonella infections has been uncommon. Within the past 10 years, however, there has been a worldwide increase in salmonella infections, which is reflected also in the frequency of salmonella arthritis. At the same time there seems to be a decrease in yersinia infections and yersinia arthritis in Scandinavia.

We analysed the long term prognosis of reactive arthritis to determine the role of the triggering infection, genetic factors, gender of the patients, and incidence of recurrent arthritis. The study included 50 patients with previous salmonella arthritis followed up for a mean of 11 years, and 75 patients with yersinia arthritis and 75 patients with reactive arthritis, both groups followed up for 20 years.

Cytokine patterns in reactive arthritis
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Reactive arthritis is caused by obligate or facultative intracellular bacteria which have been shown to persist inside the joint. For other intracellular bacteria, like Mycobacterium tuberculosis, it is known that Th1 cells, mainly secreting IFNγ, are necessary to fight this kind of infection while Th2 cells, secreting, among others, interleukin-4 (IL-4) inhibit an effective immune response. Therefore the question was investigated whether elimination of bacteria might be hampered by a dominant Th2 response in reactive arthritis.

We have shown earlier by using polymerase chain reaction in situ hybridisation to detect T cell cytokines in synovial membrane (SM) that more IL-4 can be found in reactive arthritis SM than in rheumatoid arthritis SM. We confirmed these results by investigating more patients, applying in addition immunohistochemical methods. We also showed that IL-4 positive cells are located in different areas of the SM than interferon γ positive cells, and that both in very early reactive arthritis as well as with a disease duration of less than one week, one patient two weeks) and late reactive arthritis (one year) IL-4 is present in the SM during active disease. When synovial fluid T cells from patients with reactive arthritis were stimulated, either with phytohaemagglutinin or specific bacterial antigen, more IL-4 was produced than for patients with rheumatoid arthritis. In one patient IL-4 production was investigated in serial synovial fluid samples and a decrease of IL-4 correlated with clinical improvement. In conclusion, IL-4 is present either in the same or greater amount as interferon γ in joints of patients with reactive arthritis. The presence of the Th2 cytokines might inhibit an effective immune response against bacteria which trigger reactive arthritis.

The monocye-macrophage in infectious diseases: simply a phagocyte or the director of the immune response?
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Long term prognosis of reactive arthritis

<table>
<thead>
<tr>
<th>Patients</th>
<th>SA* Y* RS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean follow up time (years)</td>
<td>11 20 20</td>
</tr>
<tr>
<td>Chronic arthritis (%)</td>
<td>16 7 24</td>
</tr>
<tr>
<td>Ankylosing spondylitis (%)</td>
<td>9 16 23</td>
</tr>
<tr>
<td>Chronic tenosynovitis (%)</td>
<td>0 1 5</td>
</tr>
<tr>
<td>Radiology</td>
<td></td>
</tr>
<tr>
<td>Erosions (%)</td>
<td>ND 10 26</td>
</tr>
<tr>
<td>Sacroiliitis (≥2 grade)</td>
<td>14 22 26</td>
</tr>
</tbody>
</table>

*SA = salmonella arthritis; Y = yersinia arthritis; RS = Reiter's syndrome.

Patients with Reiter's syndrome had the most severe prognosis with respect to the development of chronic disease (table). They also had a higher frequency of recurrent arthritis (found in 22% of patients with salmonella arthritis, in 28% of those with yersinia arthritis, and in 53% of patients with Reiter's syndrome). HLA-B27 positive patients more often had recurrent arthritis, enthesopathy, iritis, and they developed radiological sacroiliitis, chronic tenosynovitis, and ankylosing spondylitis more commonly than B27 negative patients. Most patients with Reiter's syndrome were male. The role of gender was therefore analysed in patients with salmonella arthritis triggered by enteric infections. In male patients the disease progressed more often to ankylosing spondylitis than in female patients.

It is concluded that triggering infection in the urogenital tract, new attacks of arthritis, male gender, and HLA-B27 are markers of development of late sequel in reactive arthritis.


Gut inflammation in different forms of reactive arthritis: prevalence and long term evaluation

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The concept of spondyloarthopathy gathers together a group of chronic diseases with common clinical, biological, genetic, and therapeutic characteristics. Eileenoscopic studies showed the presence of histological signs of gut inflammation in a relatively great number of patients with different forms of spondyloarthopathy (ankylosing spondylitis, reactive arthritis, psoriatic arthritis, etc.). The histological gut lesions could be subdivided into "acute" lesions, resembling acute bacterial enteritis, and "chronic" lesions, bearing resemblance to the picture of inflammatory bowel disease, especially Crohn's disease.

Ileoncoscopy was performed in 224 patients with spondyloarthopathy, in which psoriatic arthritis, inflammatory bowel disease, and ankylosing spondylitis were excluded. Thirty patients had a form of urogenital reactive arthritis (mostly chlamydia triggered), 19 patients an enterogenic reactive arthritis (yersinia, salmonella, or campylobacter triggered), and 175 patients were diagnosed as undifferentiated forms in which no triggering agent was found, but presenting the same clinical picture. Inflammatory gut lesions were found in all but one patient with enterogenic reactive arthritis (95%), most of them (69%) presenting the acute type of inflammation. In the enterogenic reactive arthritis, gut lesions were found in six patients (20%), all but one featuring the acute type of inflammation. In undifferentiated forms, the lesions were found in 65% of patients, with acute and chronic gut lesions equally distributed. This finding suggests that the gut is implicated in the pathogenesis of most cases of reactive arthritis.

Seventy one of these original 224 patients were clinically reviewed four to nine years later. Forty three patients (60%) were in clinical remission, presenting during at least one year without any signs of inflammation, complaints, or any other symptoms. This finding confirms the good prognosis of reactive arthritis.

Four patients (6%) had developed a psoriatic arthritis during the follow-up of these patients had been previously diagnosed as undifferentiated reactive arthritis, and one patient as yersinia reactive arthritis. All four patients had also developed ankylosing spondylitis and all initially presented histological gut inflammation.

In 19 of the 71 patients a secondary ileoncoscopy was performed at review. Of the 12 patients who were in clinical remission at that moment, none presented inflammatory gut lesions, whereas these lesions were found in six of the seven patients with persistent inflammatory joint symptoms. The finding of a close relationship between gut and joint inflammation in reactive arthritis. All patients with active gut inflammation at the second ileoncoscopy also presented lesions at the first investigation; they all showed chronic inflammatory gut lesions, three presenting the histological and clinical signs of Crohn's disease.

These findings substantiate the concept of spondyloarthopathy as an interrelated group of disorders in which one subdisease can evolve into another. Gut inflammation seems to have an important role in the pathogenesis and evolution of reactive arthritis, and the presence of gut involvement seems to be a good prognostic factor for the evolution of this disease.

Key papers


Conventional disease modifying treatment for reactive arthritis: the role of sulphasalazine and methotrexate

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Conventional disease modifying treatment for reactive arthritis is hardly discussed in rheumatological textbooks. Only a few recent reviews have summarised the relevant literature in more detail. 1 This review is based on the literature search published by Creemers et al. and Mielants et al., and more recent studies published in Medline from 1993 to June 1995.

Although no generally accepted criteria for the indication of disease modifying treatment in reactive arthritis are available at present, most authors consider these drugs for patients with severe early disease refractory to non-steroidal anti-inflammatory drugs but more commonly in patients with prolonged, flaring, or chronic disease. The most recent studies are based on uncontrolled clinical observations: the beneficial effect in placebo controlled trials is not always significant owing to the high placebo response, there is a lack of long term follow up studies, and, additionally, outcome variables are not standardised. Nevertheless, the efficacy of sulphasalazine in the treatment of peripheral arthritis has established that it should be the only drug to be accepted from a scientific point of view. 2 If further clinical experience is included, based mainly on observations in individual patients, case reports, and uncontrolled cohort studies, then methotrexate can also be recommended in cases where sulfasalazine fails or produces side effects. Especially in the case of severe mucocutaneous lesions methotrexate may be advantageous in the treatment of spondyloarthropathies. In chronic Reiter's syndrome a placebo controlled crossover study showed that azathioprine reduce the joint score and non-steroidal anti-inflammatory drug intake. Bromocriptine could, in the last but one trial, be accepted as a treatment of choice. Finally, the most recent positive case reports of treatment with the 5-aminosalicylic acid compounds, mesalazine and olsalazine, warrant further study to investigate the possibility of using these drugs as an alternative to sulfasalazine.

In conclusion, the treatment of reactive arthritis with conventional disease modifying drugs is poorly defined in view of the indications, and only the efficacy of sulfasalazine has been sufficiently established in the treatment of the peripheral arthritis. The results from these trials and other studies are on case reports and clinical experience. There is an urgent need for controlled studies to be undertaken.

Can antimicrobial drugs really cure reactive arthritis? evidence from studies of ciprofloxacin and other antibiotics

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Reactive arthritis can occur after preceding bacterial infections, mainly in the urethra owing to Chlamydia trachomatis and in the gut owing to Yersinia enterocolitica and others. Bacteria cannot be cultured from the joints, but bacterial antigens and bacterial DNA have been detected in the synovial membrane and the synovial fluid of these patients. The course of reactive arthritis is generally benign: most cases are healed within one year. However, chronic courses have been reported in 20–30% of patients. There are no established prognostic criteria for reactive arthritis, but the presence of other features of spondyloarthropathy, such as enthesisopathy and arthritis, and HLA-B27 positivity, has been reported to be associated with chronicity.

Antibiotic treatment in reactive arthritis is directed towards the eradication of bacteria preceding the arthritis. Therapeutic aims are effective prophylaxis, complete cure, and shortening of arthritic symptoms. There is evidence that the short term treatment of arthritis with an antibiotic appropriate to eradicate C. trachomatis prevents the onset of arthritis in a significant percentage of patients with chronic Reiter's syndrome. A three months' long term treatment with lymecycline shortened the disease course in some patients with chlamydia induced reactive arthritis but not in those with reactive arthritis triggered by enterobacteria.

In contrast, there is no evidence that short or long term treatment with antibiotics has any influence on the course of reactive arthritis following enteritis. A placebo controlled randomised study, in which a three months' course of 1 g ciprofloxacin daily was given to 120 patients with reactive arthritis, is currently being terminated in Berlin. A European study examining the effect of 1 g azithromycin given once weekly to patients with reactive arthritis has just been started. So far, the effectiveness of long term treatment of reactive arthritis with antibiotics has not been established.

Oral tolerance in the treatment of arthritis

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In animal models oral tolerance has been proved to be effective in preventing and curing autoimmune diseases by feeding organ specific antigen. It is assumed that local immunosuppression is mediated by secretion of Th2-like cytokines such as transforming growth factor β and interleukin-4 by T cells which have been primed by the orally fed antigen in the Peyer's patches of the gut. Therefore oral tolerance could be effective in all diseases in which an inhibition of a deleterious Th1 immune response by a Th2 response is required.

In rheumatoid arthritis a T cell mediated response to an unknown self antigen is believed to be dominant. So far, three studies for the treatment of rheumatoid arthritis (one in Berlin, two in Boston) have been conducted with oral collagen type II, an antigen abundant in the cartilage of the joint. The results of these studies are promising but not yet conclusive. The study in Boston with locally persistent bacterial antigen drives a T cell mediated response which seems to be responsible for the immunopathology. It can be speculated that the induction of a Th2-like response by oral tolerance would hamper an effective elimination of bacteria and therefore might lead to a more chronic inflammation.

Therefore, oral tolerance is not likely to be a therapeutic option in reactive arthritis. However, there may be exceptions such that hypersensitivity against otherwise harmless bacteria in the joint, or even an autoimmune response triggered by bacteria, is responsible for the local inflammation. In such a case oral tolerance might be favourable, with bacterially conserved proteins such as heat shock proteins being likely candidates.

Restoration of peripheral tolerance by immunisation with bacterial heat shock proteins: a possible mode of action of the antirheumatic drug Subreum®

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The manipulation of peripheral tolerance by relevant autoantigens may be used for the immunotherapy of certain autoimmune diseases. The proper definition of the relevant antigen has, however, remained elusive in many cases. Our most recent studies have offered evidence that heat shock proteins (hsp) can be useful regulatory antigens in rheumatic diseases.

Synthesis of hsp is enhanced in cells under conditions of cellular stress as is typically seen at sites of inflammatory arthritis. In a primed (auto)immune arthritis this has been shown for hsp-60 in the inflamed synovium of the arthritic joint, both in man and in experimental (rodent) models.

Recently, it has become clear that such overexpressed hsp-60 may be a potential target molecule for regulatory (or suppressive) T cells.

Experimental arthritis can be prevented by immunisation with mycobacterial hsp-60. This was found to be caused by T cells specific for highly conserved sequences in the molecule. Cloned T cells responsive to such conserved sequences were cross reactive with the mammalian hsp-60 as well as with stressed (heat shocked) cells in the absence of added antigen. Resistance was transferable with the latter cloned T cells and not with T cells that recognised non-conserved (guinea pig hsp-60) epitopes. Immunisation with the conserved microbial hsp-60 peptide, induced protection against arthritis, both in the mycobacteria and the non-microbiologically induced model. Together, these findings indicate that T cell recognition of conserved bacterial hsp-60 epitopes contributes to protective immunoregulatory events, on the basis of cross recognition of bacterial epitopes as expressed at the site of chronic inflammation.

Studies in juvenile chronic arthritis have now suggested that similar mechanisms are operating during disease flares of chronic arthritis. T cell responses to human hsp-60 were absent in patients with systemic and polyarticular forms of progressive disease, whereas responses were present in patients with oligoarticular remitting forms of the disease. In longitudinal follow up it appeared that remission was preceded by temporary responsiveness to human hsp-60.

We have now found that immunisation of rats with Subreum® elicited T cell immunity directed at hsp-60 (Gro-EL) and hsp-70 (DnAκ) of Escherichia coli. Furthermore, Subreum® was found to be protective against arthritis induced upon oral administration. Possibly, the induction of active peripheral tolerance (Th2?) for bacterial hsp is one of the mechanism of action of Subreum® as an antirheumatic drug.

Chlamydia trachomatis antibody isotypes in reactive arthritis: specificity and sensibility

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Studies of results on the clinical value of serological methods in patients with reactive arthritis are equivocal and the possibility cannot be excluded that Chlamydia trachomatis infections (apparent and hidden) in general populations is relatively high. Few studies, however, have evaluated the serology. We report results on a well defined patients. We therefore determined isotype anti-chlamydia antibody concentrations in the serum of patients with various arthropathies.

Antibodies were detected by an enzyme linked immunosorbent assay (ELISA) using as antigen an elementary body enriched fraction prepared from Verocells infected with chlamydia. The same fraction prepared from uninfected cells was used as control antigen. An alkaline phosphatase labelled F(ab')2 fraction of goat anitihuman immunoglobulin isotype was used for detection. Normal values were obtained through 100 serum samples from blood donors, and values at least three standard deviations higher than the mean antibody concentration of these samples were defined as positive. Patient groups included patients with sexually acquired reactive arthritis with proven C. trachomatis origin, uroarthritis without proven C. trachomatis origin, seronegative rheumatoid arthritis, rheumatoid arthritis, crystal induced arthritis and mechanical arthropathies. Sensitivity, specificity, negative and positive predictive values were calculated using the results from patients with C. trachomatis skin test positive, sexually acquired reactive arthritis as expected
Abstracts of invited lectures/Submitted abstracts

579

posives and the results from patients with rheumatoid arthritis, crystal induced arthritis, and mechanical arthropathies as expected negatives.

In all groups studied, positive values were found for all three isotopes.

If any of the three isotope determinations was positive, sensitivity was 73% and specificity 78%, with a positive predictive value of 56% and negative predictive value of 80%. Thus isotope specific antibody determinations with the method used, have a limited value in clinical practice owing to the high prevalence of antibodies to chlamydia in the general population and to the absence of antibodies in some of patients with well defined chlamydia induced reactive arthritis.

Bacterial detection by immunochemistry: is it practicable?
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Structures of causative microbes have been recently detected by immunochemical techniques in inflamed joints in reactive arthritis: in synovial fluid, synovial fluid cells, and synovial membrane. Structures from Chlamydia trachomatis, Yersinia enterocolitica O:3, Salmonella typhimurium, Salmonella enteritidis, and Shigella flexneri have been demonstrated by immunofluorescence and in some cases also by enzyme linked immuno- sorbent assay (ELISA), electron microscopy, and immunoblotting techniques. Polyclonal and monoclonal antibodies have been successfully used, and specific bacterial structures detected are lipopolysaccharide, heat shock protein, and outer membrane proteins of these bacteria. Peripheral blood cells from patients with salmonella and yersinia triggered infection of the joints have been studied also by immunofluorescence and immunoblotting, and bacterial lipopolysaccharide and heat shock protein have been detected.

In reactive arthritis after intestinal infections bacterial structures in the joints and in peripheral blood are in highly degraded form; no live bacteria or bacterial DNA have been detected. This means that the structures disappearing in different tissues might not be easily detected by immunochemical techniques as bacterial epitopes recognised by antibodies might have disappeared.

Although published reports on microbial structures in the inflamed joints in patients with reactive arthritis have shown that most samples are positive, immunochemical detection is difficult, especially owing to the small amount of microbial material in tissues to be studied and to the highly processed form of microbial structures. Antibodies used so far have been prepared by different groups working in the field. Any attempts to find a homogeneous distribution of them has been organised. Owing to these problems bacterial detection by immunochemistry cannot be recommended as a diagnostic test for reactive arthritis.

Both ankylosing spondylitis and reactive arthritis are strongly associated with HLA-B27, a class I product of the major histocompatibility complex. To explain this association it has been suggested that the unpaired cysteine residue at position 67 of the B27 chain of B27 could form a disulfide bond with thiol-containing agents, causing a conformational change in B27, thereby triggering autoimmune cytotoxic T lymphocyte (CTL) responses.

Here we report that homocysteine can modify B27 through disulfide bonding and that modified cells can be specifically recognised by CTLs. The modification occurs in a pre-Golgi compartment in the cell and we suggest that the altered B27 binds different peptides from native B27. We have also identified homocysteine specific CTLs in a B27 negative patient with ankylosing spondylitis. Possibly, B27 is more easily modified by homocysteine through Cys67, whereas other HLA class I may also be modified through cysteine-containing peptides bound to them. This suggests that homocysteine-CTL responses in patients with reactive arthritis have shown that about 10% of patients with ankylosing spondylitis and 30% with reactive arthritis are B27 negative. Homocysteine-CTL responses were found in 11 out of 24 patients with reactive arthritis and in only 2/21 healthy controls. It is therefore proposed that these CTLs could contribute to the pathogenesis of this form of arthritis.

Conserved bacterial proteins: implication for the pathogenesis of reactive arthritis
Sanja Ugrinovic*, Andreas Merz*, Jurgen Braun*, Joachim Sieper†
* Klinikum Benjamin Franklin, Free University of Berlin; † Deutsches Rheumaforschungszentrum, Berlin, Germany

Reactive arthritis is triggered by infection with obligate (chlamydia) or facultative (yersinia, salmonella, shigella) intracellular bacteria. Examination of a group of patients with reactive arthritis shows that 60-70% of them are HLA-B27 positive. Yet the inter-action of environmental and genetic factors in the pathogenesis of reactive arthritis is not well understood.

We investigated which bacterial antigens are immunodominant for CD4+ or CD8+ T cells and whether they can be presented by HLA-B27.

Conserved ribosomal proteins L2, L3, Gro-EL, the 19 kilodalton urease subunit of yersinia, the 18 kilodalton histone-like protein, and the 57 kilodalton heat shock protein of chlamydia are candidate antigens for immunodominance in reactive arthritis. Recombinant and native forms of these proteins were first used in a proliferation assay. In four patients with chlamydia induced reactive arthritis, 57 kilodalton protein was positive (SI: 5, 7, 50, 800), whereas in two patients with yersinia induced reactive arthritis the 57 kilodalton protein was negative. The 18 kilodalton protein did not induce any proliferation.

The nonamer peptides with HLA-B27 binding motif were selected from these conserved proteins, tested in a standard HLA assay, and used for evaluation of CD8 responses.

Submitted abstracts

Homocysteine specific cytotoxic T lymphocytes in patients with ankylosing spondylitis and reactive arthritis
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Note: The abstracts were submitted to the Arthritis Research & Therapy conference, and the results presented here are not final.
We generated yersinia specific, synovial fluid derived cytotoxic T lymphocyte lines from three B27 positive patients and one B27 negative patient, using autologous macrophages infected with live yersinia as antigen presenting cells. A line from one patient, used for further investigation, showed a specific cytotoxic response to L23 and Gro-EL (15% and 25% above background at an effectortarget ratio of 50:1 respectively), but not to the 19 kilodalton derived peptides. Using Epstein-Barr virus transformed lines matched for B27, but mismatched for other class I molecules as targets, indicates that these responses are B27 restricted. Therefore, epitopes from conserved proteins seem as well as those to be relevant for a B27 restricted CDB response.

The ongoing investigation with chlamydia specific cytotoxic T lymphocyte lines may tell us if these CDB epitopes are shared between different bacteria associated with reactive arthritis.

**Induction of cytotoxic T cells in Chlamydia trachomatis infected HLA-B27+28m homozygous transgenic CBA mice**


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Chlamydial species are intracellular living bacteria that cause a variety of human infections. It has been shown in mice that infected animals can develop a CD8+ cytotoxic T cell response against chlamydia. In this study we considered whether C trachomatis can induce a cytotoxic T lymphocyte (CTL) response in HLA-B27+28m homozygous transgenic mice. The ability of C trachomatis specific immune spleen cells to lyse infected cells was investigated in a standard five hour 3HCr release assay. Our experiments with bulk culture derived CTLs showed that CTLs can be raised in infected transgenic mice and that lysis was only observed for C trachomatis infected targets. Preliminary blocking experiments in vitro with mononuclear antibodies suggest that the bulk derived CTLs are restricted by both murine major histocompatibility complex class I antigen and the transgenic human HLA-B27 class I molecule.

At present we are in the process of isolating CD8+ T cell lines and clones, particularly those that are HLA-B27 restricted, to characterise in more detail the nature of the immune response. Moreover, in preliminary experiments we investigated the role of the chlamydial heat shock protein hsp-57, which has been claimed to have a crucial role in CD4+ T cell mediated chlamydial inflammatory processes in the guinea pig model. In our transgenic animal model chlamydial hsp-57 seemed not to be recognised by CTLs, i.e. evoking the observed CTL response in C trachomatis infected animals. Taken together, our murine animal model offers the opportunity to isolate HLA-B27 restricted lines and clones specific for chlamydial antigen. Isolation and identification of the so far unknown antigenic peptide(s) could lead to a better understanding of the supposed role of the HLA-B27 antigen in bacterial infection and disease association.

**Response of synovial fluid derived T cell clones to recombinant Chlamydia trachomatis protein antigens**

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We have previously reported the initial characterisation of a panel of T cell clones derived from an HLA-B27 negative patient with chronic reactive arthritis. Subsequently, we tested the potential of these clones to proliferate against two other antigens, C trachomatis and L23, and to a panel of recombinant C trachomatis protein antigens.

T cell clones were made on two occasions from the synovial fluid of a 23 year old man with classical Reiter's syndrome by limiting dilution. Proliferation of the T cell clones was tested with C trachomatis, C pneumoniae, and a panel of C trachomatis recombinant proteins, including the major outer membrane protein (MOMP), Gro-EL, Gro-ES, an 18 kilodalton histone-like protein, and control vectors. The HLA-DR restriction of the clones was also investigated using irradiated HLA typed antigen presenting cells. Clones were either HLA-D3R or 1301 restricted.

We characterised four further T cell clones. All recognised C trachomatis and three also recognised C pneumoniae. One of the eight C trachomatis/C pneumoniae reactive clones, recognised Gro-EL. None of the other seven clones recognised any of the other recombinant antigens. No proliferation to the MOMP has been shown in 13 other partially characterised clones.

It is concluded that the recognition of Gro-EL is consistent with other reports of responses to heat shock proteins and the up regulation of these proteins within the synovium. In addition, we extensively looked for MOMP recognition, as this is an immunodominant antigen, but so far unsuccessfully. The lack of recognition, however, correlates with reports of the MOMP being down regulated in the synovium.

**Comparison of T cell receptor repertoire in blood and synovial fluid of patients with reactive arthritis**

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If reactive arthritis is a T cell mediated disease it may be possible to detect populations of T lymphocytes as sites of inflammation. Such expansions may be identified by a bias in T cell receptor (TCR) V gene segment usage.

We used a colour flow cytometry with a panel of 1 TCR V gene segment specific antibodies to investigate the T cell repertoire of CD4 and CD8 positive T cells in synovial fluid and blood of seven patients with reactive arthritis and one patient with peripheral oligoarthritis complicating ulcerative colitis. CD4/CD8 ratios and levels of v8 T cells were also examined.

We identified several minor expansions in the blood or synovial fluid, or both, of our patients by comparison with T cell repertoires of healthy individuals of similar age. Different V gene segments identified expansions in different patients, and expansions were seen in both CD4 and CD8 positive cells. One patient had V813 expansions in both CD4 and CD8 cell subsets in synovial fluid as well as V813 expansions in a positive peripheral blood lymphocytes. Another patient showed a 10-fold expansion of V812 in synovial fluid CD8 positive cells compared with CD8 positive peripheral blood lymphocytes. There was no common pattern of expansions between patients and no major expansions were observed.

We aim to use V gene specific polymerase chain reactions to determine the clonality of these expansions. Expanded T cell populations may also be analysed for activation markers using three colour flow cytometry. No significant differences were detected in the level of CD8+ T cells between blood and the synovial fluid, and there was no strong evidence for the involvement of a superantigen as suggested previously for rheumatoid arthritis.

**Superposition of hydrophathy plots from T cell receptor β chain CDR3 for detection of conserved properties of corresponding antigenic peptides: a study in HLA-B27 restricted T cells**

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Bacterial and autologous peptides presented on HLA-B27 molecules may be crucial in the induction of CD8+ T lymphocyte (CTL) mediated inflammation in the spondyloarthropathies.

In a previous study we showed the presence of HLA-B27 restricted cytotoxic T lymphocytes with specificity for bacterial or autologous antigens in the synovial fluid (SF) of HLA-B27+ patients with reactive arthritis.7 Seventeen HLA-B27 restricted CTL clones, four two-clonal lines, and 14 of the HLA-B27 restricted controls were grown from the site of inflammation of three patients or from peripheral blood lymphocytes of a B27+ healthy donor. We analysed their T cell receptor (TCR) β chain rearrangements by RT-PCR using primers for 24 different TCR Vβ families and solid phase DNA sequencing and showed limited Vβ usage in HLA-B27 restricted CTLs.3 Owing to the absence of information about TCR corresponding antigenic peptides, special attention was paid to the peptide contacting hypervariable CDR3. Functional and sequence homology in CDR3 of T cells with identical specificity are often used to identify TCR sites that contact antigenic peptide epitopes. However, it is known that identical peptide epitopes can be found in different homologous CDR3 as this region arises from
random processes and is not genetically encoded.

Nevertheless, peptide epitopes might restrict the physicochemical variability of corresponding residues in the TCR CDR3 by charge-charge interaction or hydrophobic interaction and might thereby reflect their own properties. We thus examined the distribution of polar and non-polar residues of both CDR3 amino acid sequences and known B27-bonding peptides by generation and construction of hydrophobicity plots. Such hydrophobicity overlay plots from control clones showed random distribution of hydrophilic and hydrophobic amino acids over the entire segment. In contrast, clear hydrophobic overlay plots from both types of HLA-B27 restricted CTLs showed profiles that were restricted and characteristically dissimilar from each other. None of the CDR3 from 14 autoreactive clones used charged amino acids at three distinct positions (5, 8, and 9 residues distant from a constant Cys), whereas such residues were present in yersinia specific CDR3. Here, in turn, hydrophobic residues were absent from P9, 10, and 12 positions which were heterogeneous in CDR3 from autoreactive CTLs. Accordingly, hydrophobicity overlay plots of known HLA-B27 binding self- and bacterial nonapeptides closely mirrored the characteristic TCR CDR3 profiles of such TCRs that exhibited the corresponding specificity.

Thus our data lead us to the conclusion that this method of comparative graphical analysis might be useful for detecting structural and functional concordances which would have remained cryptic using simple sequence alignment.

1 Herrmann E, Yu D T. HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. *Lancer* 1993; 342: 646–50.

Yersinia enterocolitica: a cause of chronic polyarthritis

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Yersinia macrocolla is associated with reactive arthritis. Persistent polyarthritis affects about 2% of patients with yersinia induced reactive arthritis. As cultures are often negative after the acute phase, the cause may remain undetected. We attempted to demonstrate yersinia antigens in biopsy specimens from two patients with chronic polyarthritis, who had circulating antibodies to yersinia outer proteins. Responses of synovial T cell clones against yersinia were also investigated.

Two patients were studied: (1) a 57 year old woman with arthritis of knees and elbows, but no gastrointestinal complaints and (2) a 44 year old woman with arthritis of knees, ankles, and hands, and bouts of diarrhea. Both patients were HLA-B27 negative. The only lead was the demonstration of circulating IgG and IgA antibodies to yersinia outer proteins.

Immunofluorescence using polyvalent anti-YadA antibodies directed against yersinia recombinant and synthetic C-terminal proteins of synovial tissue. T cells were cloned by limiting dilution in an aspecific manner. Thirty seven clones from patient 1 and 53 clones from patient 2 were screened for proliferative responses to yersinia, *Chlamydia trachomatis*, and *Salmonella typhimurium*.

Yersinia antigens were demonstrated in synovial and tissue fluid of both patients. Four CD8+ clones from arthritis with a CD4+ from patient 1 and six CD4+ from synovial and tissue fluid of patient 2 showed a positive response to *Y enterocolitica*. One clone cross reacted with *C trachomatis*.

It is concluded that the demonstration of bacterial antigens in situ and the high percentage of *Y enterocolitica* reactive T cell clones support the hypothesis that bacterial antigens are presented locally in the joint. This may be one of the causes of chronic polyarthritis.

Experimental reactive arthritis: effect of ciprofloxacin

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Significance of antibiotic prophylaxis and treatment of reactive arthritis, following infections by a variety of micro-organisms, has remained an unsolved issue. As controlled human studies are not easy to perform we used a rat model. *Yersinia enterocolitica* O:8, when injected intravenously into Lewis rats, causes in 70% of the animals a sterile arthritis closely resembling human reactive arthritis. Arthritis develops in one to two weeks, in some of the animals it remains chronic, and exacerbations occur.

With this model we studied the effect of a seven day treatment with ciprofloxacin, using two dosages (20 or 100 mg/kg/day) and four different timings. A seven day course with the higher dosage, started on day 3 after the bacterial inoculation, completely prevented development of reactive arthritis and eliminated yersinia during the 60 day experiment. If antibiotic treatment was started on day 5, the preventive effect was still observed but less pronounced. If treatment was started at the peak of the arthritic development, no effect on arthritis was found.

The results suggest that patients with infections known to induce reactive arthritis should be considered for early antibiotic treatment. This applies particularly to those with chronic arthritis and, in the case of enteric infections (yersinia, salmonella, campylobacter), HLA-B27 positive individuals and patients with a history of reactive arthritis.

Amplification of plasmid and chromosome chlamydia DNA in synovial fluid of patients with reactive arthritis and undifferentiated seronegative oligoarthritis

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Interferon γ induced growth inhibition of *Chlamydia trachomatis* in the human monocytic cell line THP-1 is not mediated by tryptophan deficiency

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Recent data suggest that *C trachomatis* can persist in monocytes of the synovial fluid and membrane, and liberated chlamydial antigens may trigger the inflammatory process in the joint. In human fibroblasts persistence of *C trachomatis* has been attributed to a cytokine mediated induction of indole-2,3-diaminooxygenase, a tryptophan degrading enzyme. The mechanism of chlamydial persistence in human monocytes, however, is not known.

Tissue plasminogen activator (TPA) differentiated THP-1 cells (human monocytic cell line) and HeP-2 cells (human larynx carcinoma) were preincubated with or without interferon γ (IFNγ) and/or tryptophan (200 μM/ml) for 24 hours and infected with *C trachomatis*. The number of inclusion bodies was determined by immunofluorescence microscopy and the number of infective chlamydiae by titration of the lysates of infected cells on Hep-2 cells.

Inoculation of TPA differentiated THP-1 cells with *C trachomatis* resulted in a low productive infection. During the culture period of 10 days inclusion bodies were observed in 1–2% of the THP-1 cells. In addition to the inclusion bodies, atypical chlamydiae were found in the monocyctic phagocytes by electron microscopy after a incubation with IFNγ (25 U/ml) reduced the number of inclusion bodies by 90% in THP-1 cells and productive infection was converted into a non-infective state. After IFNγ treatment atypical chlamydiae were still seen in phagosomes of THP-1 cells. In THP-1 exogenously added tryptophan (200 μM/ml) did not reverse the antichlamydial effect of IFNγ, in contrast to controls treated with Hep-2 cells. The IFNγ mediated growth inhibition of *C trachomatis* was completely prevented by tryptophan.

These data show that (a) *C trachomatis* can persist in human monocytic THP-1 cells and (b) in contrast with fibroblasts, the persistent chlamydial infection in THP-1 cells is not mediated by tryptophan deficiency. Thus the persistence of *C trachomatis* is differentially regulated in monocytes and fibroblasts.
Oligoarticular synovitis of undetermined origin can closely resemble an incomplete form of reactive arthritis without history of an initial infectious event. Reactive arthritis can be related to an infectious cause, but an important question is whether whole microorganisms are present in the joint or only antigens. Different studies have been carried out with the aim of looking for chlamydia nucleic acids, with variable results.

To clarify the discrepancies between these results we attempted to optimize the conditions for DNA amplification with polymerase chain reaction, in synovial fluid. After multiple unsuccessful attempts to find a specific primer we used four different amplification systems on samples from 71 patients with various arthropathies. Detection was targeted both at the chlamydia cryptic plasmid and at the chromosomal DNA. Specificity was improved in two ways: the use of nested primer sets that primed within the first sequence amplified, and the use of an internal labelled hybridisation probe to detect a specific fragment amplified by a polymerase chain reaction in a Southern blot.

We amplified different sequences of chlamydia DNA from four patients with Chlamydia trachomatis sexually acquired reactive arthritis (CT-SARA), from six with SARA, from 11 with undifferentiated seronegative mono/oligoarthritis, and from one control patient. Although this method detects only small parts of the genome, different DNA pieces were found in the samples. Therefore, it is improbable that they correspond to residual genetic material from non-viable organisms. These results indicate that the presence of intra-articular viable chlamydia in certain cases of SARA and undifferentiated mono/oligoarthritis is probable.

**Dominant recognition of a *Borrelia burgdorferi* outer surface protein A peptide by T helper cells in patients with treatment resistant Lyme arthritis**

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Lyme arthritis is a tick-borne infection caused by *Borrelia burgdorferi sensu lato* spirochaetes. Whereas most patients with Lyme arthritis are cured with antibiotic treatment, some 10% of the patients have persistent arthritis after treatment. In an earlier study we found that T cell lines from patients with treatment resistant Lyme arthritis preferentially recognised *B. burgdorferi* outer surface protein A (OspA), but T cell lines from patients with treatment responsive arthritis only rarely recognised this protein. Dominant T cell recognition of an arthropotoxic epitope is one possible way in which the immune response against OspA might be involved in the pathogenesis of treatment resistant Lyme arthritis.

To test this hypothesis, we carried out epitope mapping of 31 OspA-specific T cell lines and five T cell clones derived from the synovial fluid or peripheral blood of three patients with treatment resistant Lyme arthritis. Although each patient's T cell line recognised a broad array of OspA peptides with different individual patterns, two regions of OspA were dominantly recognised. Each patient's T cell line dominantly recognised a C terminal epitope of OspA ranging from amino acids 214-233 in one patient to 244-263 in another, and all three patients' T cell lines dominantly recognised an epitope between amino acid 84 and 113. Thus the region of OspA between amino acid 84 and 113 was the dominant T cell epitope shared by these three patients with treatment resistant Lyme arthritis. If the T cell response to OspA plays a part in the pathogenesis of treatment resistant Lyme arthritis the epitope 84-113 is a candidate arthropogenic epitope.


**Phagocytosis of Borrelia burgdorferi by human dendritic cells**

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Phagocytosis of Borrelia burgdorferi by human dendritic cells

Although phagocytosis by dendritic cells has been reported before, there still are controversies about the phagocytic capacity of these cells and the uptake mechanism used by them. This study aimed at investigating the phagocytosis by dendritic cells of *Borrelia burgdorferi* in an in vitro system to test the current investigations.

**Different interleukin-4 production in the synovial fluid of patients with reactive arthritis and rheumatoid arthritis**

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Previously our group reported divergent T cell cytokine patterns in inflammatory arthritis. It was shown in the report that in synovial tissue, rheumatoid arthritis samples express a Th1-like pattern whereas in reactive arthritis this move towards a Th2 pattern.

To analyse further the discrepancy of cytokine pattern, the interferon γ (IFNγ) and interleukin-4 (IL-4) production of peripheral blood (PB) and synovial fluid (SF) monocellular cells from patients with reactive arthritis and rheumatoid arthritis stimulated with optimal concentration of phytohemagglutinin were measured by enzyme linked immunosorbent assay (ELISA). In PB the IFNγ production from patients with rheumatoid arthritis and the healthy donor's control group was slightly lower than that in the group with reactive arthritis, but for IL-4 production both groups showed similar potential, though some rheumatoid arthritis samples (three out of seven) produced less. However, in SF samples from patients with reactive arthritis and rheumatoid arthritis there was a difference in IL-4 production.

The IL-4 production from the group with RA (1-8 (SD 2-6) pg/ml) was clearly lower than that in reactive arthritis samples (13-3 (4-3) pg/ml). Next, the same SF samples from patients with reactive arthritis were stimulated with the triggering bacterium, *yersinia* or *chlamydia*. All the samples showed similar increased IL-12 production (yersinia 1183 (217) pg/ml, chlamydia 1048 (270) pg/ml) compared with phytohagglutinin (135 (35) pg/ml). In contrast, only three out of 14 samples produced a small amount of IL-4. Interestingly, one case included in this study, examined at three different times showed a decrease of IL-4 production after stimulation with phytohemagglutinin which correlated with improvement of disease.

Taken together, the Th2-type cytokines may be the main reason for persisting bacterium in the joints of patients with chronic reactive arthritis. These results further confirm the data from the synovial tissue mentioned above.

Persistent knee synovitis in reactive arthritis: aetiological and clinical correlations
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Knee joint involvement is one of the main clinical manifestations in reactive arthritis, and a common event in patients with chronic evolution of the disease. Measurement of synovial effusion and proliferation in the knee joint of patients with reactive arthritis can be useful for an objective assessment of the severity and of changes in local disease activity.

We conducted a prospective study to compare the ultrasound pattern with the gross appearance of knee synovitis and the histological picture. At study entry synovial proliferation was evaluated (location, extent, and morphological patterns) by a 10 MHz mechanical sectorial transducer and by scanning three recesses of the knee (suprapatellar, lateral, and infrapatellar). Findings were then compared with arthroscopic visualisation as the "gold standard" in six patients with persistent knee synovitis undergoing arthroscopic synovec-tomy. Moreover, to monitor progression and response to treatment, ultrasound joint effusion and synovial thickness were measured in four of these patients before and up to six months after arthroscopic synovec-tomy.

Ultrasound of localisation, morphology, and degree of synovial proliferation corresponded with arthroscopic gross appearance in every case. The predominant morphological pattern on ultrasound and subsequently confirmed by arthroscopic visualisation was villonodular (highly developed hypertrophic villi) in four knees, and uniform thickening (uniform synovial pannus with tightly crowded villi) in two. The lymphocytic infiltrate, vascularisation, and fibrosis of these two patterns also differed histologically. Immunohistological studies are now in progress.

Four male patients with reactive arthritis (three with urogenital forms and one with a gastrointestinal form), with a mean age of 38.6 (SD 4.9) years and a synovitis duration of 26.5 (12.6) months, received sulphasalazine treatment for more than six months and were then treated with arthroscopic synovec-tomy; a significant reduction in both the global clinical index (p = 0.03) and the ultrasound index (p = 0.02) was seen at six months.

Although preliminary, these data seem to confirm the persistence and severity of proliferative knee joint synovitis process in reactive arthritis. Our ultrasound procedure enabled an accurate morphological and quantitative definition of the synovial proliferation, as confirmed by arthroscopic visualisation. Therefore, in addition to histological evaluation, we suggest that ultrasound can be a useful, objective method for monitoring the progression of knee joint synovitis, and the response to treatment, with the advantage of non-invasiveness and low cost.

HLA-B27 subtypes and ankylosing spondylitis in the UK.
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The association of HLA-B27 and ankylosing spondylitis is well known. It is less clear, however, whether the risk for the development of ankylosing spondylitis is the same for the respective subtypes. Therefore we typed 284 white patients with ankylosing spondylitis and 1010 healthy white controls for B27 by the polymerase chain reaction. 94% of the patients with ankylosing spondylitis and 9-4% of the controls were positive for B27 (odds ratio (OR) 161, 95% confidence interval (CI) 113 to 230, p < 10^-6), confirming the previously reported strong association of B27 with ankylosing spondylitis. HLA-B27 typing was performed on 172 of the 284 patients.

Characterisation of the HLA-B27 linked mica gene
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The HLA-B27 antigen is known to be associated with ankylosing spondylitis and related spondyloarthropathies. Several hypotheses have been proposed to explain this association, based upon the function of the class I molecules of the major histocompatibility complex (MHC) as receptors for peptide ligands and as restriction elements for cytotoxic T cells. They propose a unique ligand presenting function of the HLA-B27 antigen, stimulating a local T cell response. Nevertheless, the presence of linked or additional predisposing genes within the HLA B region cannot be ruled out. Novel polymorphic class I genes (MIC) within the human MHC have recently been identified which are mainly transcribed in epithelial cells. These genes appear to be associated with a unique MICA allele. In addition, we are analysing the mRNA expression pattern of MICA in affected tissues.

Analysis of HLA-B27 subtype frequencies in patients with rheumatic disease: allelic typing by polymerase chain reaction and sequence specific oligonucleotides
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This study aimed at investigating whether HLA-B27 typing by polymerase chain reaction (PCR) can provide better typing results than those commonly obtained with serological typing (microlymphocytotoxicity assay). Furthermore, all HLA-B27 positive patients with rheumatic disease or uveitis, or both, should be subtyped by sequence specific oligonucleotides in search for a possible association between HLA-B27 subtypes and certain types of HLA-B27 associated disease.

Three hundred and ninety eight consecutive patients with rheumatic disease and 97 patients with uveitis were HLA-B27 typed by lymphocytotoxicity assay (LCA) and PCR. Amplified sequences (exon 2 and 3) of HLA-B27 positive patients were then tested with eight sequence specific oligonucleotides to distinguish all HLA-B27 subtypes.

In patients with rheumatic disease 61/398 patients tested HLA-B27 positive. PCR microlymphocytotoxicity assay. When a PCR technique was used 78 patients tested HLA-B27 positive. Eighteen patients with a weak signal or possible cross reaction (HLA-B7) in microlymphocytotoxicity testing gave a positive PCR result in 11 cases. Six patients with a negative serological typing result for HLA-B27 and a positive PCR result were sequenced, and the positive PCR result was confirmed in all cases.

Sequence analysis of HLA-B27 and HLA-B7 positive patients were sequenced for HLA-B27 (LCA/PCR). Subtyping in 144 HLA-B27 positive patients showed 24x = B2702, 4x = B2704, and 116x = B2705. Three patients tested positive for B2704 and B2705. Sequencing showed subtype B2705 in all three patients. No significant association between any HLA-B27 subtype and a certain type of HLA-B27 associated disease could be found.

The PCR technique is a reliable and reproducible method for HLA-B27 typing and can improve the common serological typing. Compared with LCA testing, the PCR technique is able to classify uncertain results (weak positive LCA results, cross reaction with HLA-B27). HLA-B27 subtyping, so far, seems to have no clinical relevance for making a diagnosis.
an alternative or additional predisposing gene for HLA-B27 associated diseases.

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HLA class II associations of ankylosing spondylitis

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Ankylosing spondylitis is clearly associated with the HLA class I gene, B27, but class II associations have also been reported with DR1, DR2, and DR7. Many investigators have found no association between class II alleles and ankylosing spondylitis, but in all these studies the number of patients and controls has been small.

We studied the HLA class II allele frequencies in 110 cases of familial ankylosing spondylitis (more than one case in the index generation), 70 patients with sporadic ankylosing spondylitis, and 147 B27 positive controls (71 blood donors, 47 healthy individuals, 18 cadaveric organ donors, 11 patients with chronic lymphocytic leukaemia). Although these patients were not known to have ankylosing spondylitis, as they had not been screened for this condition, it is possible that some cases would have been included in the control samples. This could result in an underestimate of the relative risk of any disease susceptibility gene. Class II typing was carried out by polymerase chain reaction (PCR)/sequence specific oligonucleotides in all patients with ankylosing spondylitis, whereas the controls were typed by a mixture of serology and PCR/sequence specific primers. Familial cases of ankylosing spondylitis were also typed for B27 and B60 by PCR.

A significant association between DR1 and ankylosing spondylitis was found in familial cases (proband DR1 allele frequency 30%, relative risk (RR) 3-5, 95% confidence interval (CI) 2-6 to 4-7) and in sporadic cases of ankylosing spondylitis (24% DR1+, RR 2-9, 95% CI 1-7 to 4-7) but not in the general population (11% DR1+). However, this may be due to linkage disequilibrium between B27 and DR1 as the frequency of DR1 in our population was significantly higher than in the general population (19% v 11%, χ² = 15-8, p = 7-2 × 10⁻⁵). When compared with our B27 positive controls, the association between DR1 and ankylosing spondylitis was found (patients v controls; OR 2-7, 95% CI 1-8 to 3-7) but not in the group with sporadic ankylosing spondylitis (patients v controls; OR 1-3, 95% CI 0-8 to 2-1). No other class II allele was associated with ankylosing spondylitis. DR1 was not independently associated with ankylosing spondylitis in the small subgroup of nine B27 negative patients (DR1+22%, when compared with general population RR 2-3, 95% CI 0-77 to 6-6).

Where haplotypes could be assigned in the familial cases, B27 and DR1 were mostly inherited linked on the same haplotype (19 linked, seven not linked, 11 indeterminate). B60 was significantly associated with ankylosing spondylitis in both our familial and sporadic cases of ankylosing spondylitis (familial ankylosing spondylitis 15% B60+, RR 1-9, 95% CI 2-6 to 23-8; sporadic ankylosing spondylitis 9% B60+, RR 5-5, 95% CI 1-5 to 19-9). There was no difference in the DR1 allele frequency between those who were B60 positive or negative. These results raise the possibility of a second ankylosing spondylitis susceptibility gene on an extended B27/DR1 haplotype (probably not DR1), the effect of which is seen most clearly in the familial cases.