

## RELATIONSHIP BETWEEN FOODBORNE BACTERIAL PATHOGENS AND THE REACTIVE ARTHRITIDES

ABSTRACT

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*Certain individuals suffer complications after diarrheic episodes caused by Campylobacter, Salmonella, Shigella, or Yersinia. One of these complications may be a sterile arthritis such as reactive arthritis, Reiter's syndrome and ankylosing spondylitis. Reactive arthritis and Reiter's syndrome have been shown to have a bacterial etiology; however, such etiology, while suspected, has not been definitely confirmed for ankylosing spondylitis. These arthritides show a strong familial association and here, reactive arthritis, Reiter's syndrome and ankylosing spondylitis are characterized and the relationship of the diseases to the major histocompatibility complex antigen, HLA-B27, is described. Studies have indicated that there may be molecular mimicry between certain microbial antigens and HLA-B27. Molecular mimicry is discussed in terms of its possible explanation of the etiology of the reactive arthritides. The roles of antibodies, lymphocytes, bacterial antigens, and stress proteins in the symptomology of the arthritides are reviewed.*

### INTRODUCTION

There appears to be a prevalent view that "A few salmonellae help keep you regular." However, it is not commonly recognized that *Salmonella* and other foodborne gastrointestinal pathogens may give rise to pathological problems that are far more serious than the temporary inconvenience of diarrhea. Certain immunocompetent individuals, after a bout of gastroenteritis, may suffer chronic joint diseases such as reactive arthritis, Reiter's syndrome, or ankylosing spondylitis. These chronic diseases appear to be triggered by infections with enteric

pathogens. Archer and his co-workers reviewed the relationship between diarrheal disease and chronic sequelae (Archer 1984, 1985; Archer and Kvenberg 1985; Bunning *et al.* 1988; Archer and Young 1988). These reviews indicated that chronic sequelae to diarrheal infections do occur and add to the health and economic burden of afflicted individuals.

Here, we discuss the role of foodborne bacteria and their antigens (including stress proteins) and the role of the major histocompatibility complex antigen, HLA-B27, and other host factors on the induction and symptomology of the reactive arthritides.

### **Reactive Arthritis, Reiter's Syndrome and Ankylosing Spondylitis**

The sterile reactive arthritides, which include reactive arthritis, Reiter's syndrome and ankylosing spondylitis, are characterized by disease of the sacroiliac joint, peripheral inflammatory arthritis and absence of rheumatoid factor. Other pathological effects may be seen in the entheses (sites of ligamentous insertion into bone), eye, aortic valve, lung parenchyma, and skin. There is a strong familial association. Clinically, the diseases have considerable overlap of symptoms.

Reactive arthritis is an acute, inflammatory, *sterile* arthritis that develops in joints due to a "triggering" infection originating at nonarticular sites. Reactive arthritis is generally triggered by an enteric or urethral infection. Infections by enteric pathogens such as *Campylobacter jejuni*, *Salmonella typhimurium*, *S. enteritidis*, *Shigella dysenteriae*, *S. flexneri*, *S. sonnei*, or *Yersinia enterocolitica* and venereal pathogens including *Chlamydia trachomatis* or *Ureaplasma urealyticum* may lead to reactive arthritis (Granfors *et al.* 1992; Hannu and Leirisalo-Repo 1988; Inman *et al.* 1988; Keat 1983; Lauhio *et al.* 1988; Merilähti-Palo *et al.* 1992). *Salmonella* other than *S. enteritidis* and *S. typhimurium* induce reactive arthritis but are rarely encountered (Maki-Ikola 1990). *Vibrio cholerae* and enteropathogenic or toxigenic *E. coli* have not been implicated in reactive arthritis (Aho *et al.* 1985). *Shigella* and *E. coli* form a single species on the basis of DNA relatedness and the genera are difficult to separate biologically (Brenner 1984). Therefore, it is surprising that strains of *E. coli*, particularly those that are enteroinvasive, have not been shown to trigger arthritides. Reactive arthritis also may be triggered by *Borrelia burgdorferi* (Weyland and Goronzy 1989) and *Giardia lamblia* infections (Woo and Panayi 1984).

Seven to 30 days after an intestinal infection, inflammatory polyarticular (average of three joints) arthritis usually involving the lower extremities may be seen in individuals susceptible to reactive arthritis. Generally the knee joint is affected and there may be massive effusion of fluid (> 100 ml). Smaller joints may be affected also and if wrist disease is present, often there is carpal tunnel syndrome (Firestein and Zvaifler 1987). Patients are seronegative for rheumatoid

factors. Spinal disease (lower back pain) may be found in which there is asymmetric sacroiliac joint involvement. Another distinctive feature of reactive arthritis is enthesopathy with tenderness and occasional swelling and redness at insertion sites. Ocular lesions may be present, also. The majority of patients improve regardless of therapy. In patients with persistent symptoms, Reiter's syndrome often develops (Aho *et al.* 1985; Firestein and Zvaifler 1987; Keat 1983).

Reiter's syndrome can be considered as a special case of reactive arthritis (Firestein and Zvaifler 1987). The disease is a triad of sterile arthritis, urethritis, and conjunctivitis occurring together or sequentially (Aho *et al.* 1985). The disease appears to be triggered by the same organisms that trigger reactive arthritis after either intestinal or venereal infection. Calin *et al.* (1987) describe reactive arthropathy and Reiter's syndrome in patients after they were vaccinated with heat-killed *Salmonella*. Thus, it appears possible that vaccination may induce reactive arthritis symptoms. If all three of the symptoms are not present, the disease is called incomplete Reiter's syndrome and it is difficult to draw the line between incomplete Reiter's syndrome and reactive arthritis symptomology. The arthritis in Reiter's syndrome is similar to that of reactive arthritis in that there is asymmetric arthritis affecting mainly the knees and ankles as well as the sacroiliac joint. Enthesopathy with inflammation of fingers and toes ("sausage digits") and Achilles tendon may be present. Urethritis manifested by dysuria and purulent discharge and ocular dysfunctions such as conjunctivitis are part of the Reiter's syndrome picture. Ulcers (circinate balanitis) of the glans penis and urethral meatus, skin lesions (keratoderma blennorrhagica) on the soles and palms and ulcers of the mouth also may be present (Firestein and Zvaifler 1987; Keat 1983; Willkens *et al.* 1981; Yu 1988).

Ankylosing spondylitis is a sterile arthritis of the sacroiliac joint and the vertebrae of the spine. In time, the spinal inflammation can lead to ankylosis (permanent stiffness and limitation of motion) and kyphosis (curvature of the spine) with limitation of chest expansion. In some patients, there is evidence of peripheral joint disease, generally of the lower limbs. Enthesopathic lesions such as tendinitis of Achilles tendon and "sausage digits" are seen. There may be extensive extra-articular involvement; ocular damage such as uveitis, and pulmonary and cardiovascular involvement may be present (Calin 1985; 1988; McGuigen *et al.* 1985).

Infection has long been regarded as a likely trigger of ankylosing spondylitis. Several features such as elevated serum IgA, clinical and immunogenetic similarities to reactive arthritis, and therapeutic response to sulfonamides suggest that bacterial infections may be involved (McLean *et al.* 1990). There have been numerous attempts to implicate *Klebsiella* in the etiology of the disease. Ebringer (1992) cited evidence that he considered as proof that *Klebsiella* causes ankylosing spondylitis; however, Russell and Suarez Almazor (1992) showed just

as convincing evidence that the organism has no role in the etiology of the disease. It is probable that the disease has a multifactorial cause involving the HLA-B27 antigen (see below), which interacts with unknown genetic and environmental factors. Various aspects of reactive arthritis, Reiter's syndrome and ankylosing spondylitis are compared in Table 1.

### **Association of HLA-B27 Antigen with the Reactive Arthritides**

There is a tendency for familial association with reactive arthritis, Reiter's syndrome or ankylosing spondylitis, which suggests that the genetic makeup of the afflicted individuals may predispose them to disease. This familial association is related to the possession of the gene that codes for the production of the HLA-B27 (HLA = human leucocyte antigen) antigen. The immune system recognizes self/nonself by utilizing the HLA molecules encoded by the major histocompatibility complex (MHC) genes. The MHC is a region of highly polymorphic genes (more than 40 common alleles are found for each gene) whose products — HLA molecules — are expressed on the surface of a variety of cells. The MHC is involved in regulation of transplant rejection or acceptance, in induction and regulation of the immune response and in the destruction of infected cells.

There are three classes of HLC molecules: (a) *Class I* HLA molecules (HLA-B27 antigen belongs to Class I) are polymorphic surface glycoproteins present on most nucleated cells. They are the major antigens recognized by cytotoxic T lymphocytes and thereby are involved in tissue graft rejection. In addition, Class I molecules have a role in restriction of cytotoxicity of target tissue cells containing foreign proteins and microorganisms to those cells bearing the same Class I antigens as the cytotoxic T lymphocytes. (b) *Class II* HLA molecules are polymorphic surface glycoproteins that have more limited distribution than Class I molecules and are found on B lymphocytes, macrophages, dendritic cells and endothelial cells. Class II molecules are involved in initiation and regulation of the immune response. Antigen-presenting cells (APC) take up foreign protein by endocytosis and the protein is processed by cellular proteases to peptides (10–20 amino acid residues). The peptides are bound to Class II molecules and the complex is expressed on the surface of the APC. The complex is recognized by helper T cells that are specific for the foreign peptide-Class II complex. Therefore, helper T cells can recognize antigen only when it is associated with Class II molecules (Class II restriction). The recognition of the antigen complex by the T cell leads to cytokine production which stimulates phagocytosis of the foreign protein and also stimulates specific antibody secretion by B lymphocytes. (c) *Class III* HLA molecules are a heterogeneous collection of proteins and include complement components, cytokines, HSP70 and other miscellaneous proteins (Abbas *et al.* 1991; Sargent *et al.* 1989).

TABLE 1.  
COMPARISON OF REACTIVE ARTHRITIS, REITER'S SYNDROME AND  
ANKYLOSING SPONDYLITIS<sup>a</sup>

|   | REACTIVE ARTHRITIS |                | REITER'S SYNDROME   |             | ANKYLOSING SPONDYLITIS |              |
|---|--------------------|----------------|---------------------|-------------|------------------------|--------------|
|   | male = female      | any age sudden | male ≥ female       | ≥ 20 sudden | male ≥ female          | ≥ 20 gradual |
| Sex distribution  |                    |                |                     |             |                        |              |
| Age at onset (years)  |                    |                |                     |             |                        |              |
| Onset rate  |                    |                |                     |             |                        |              |
| Triggered by microbial infection  | +                  | +              | +                   | +           | +                      | +            |
| Arthritis in peripheral joints  | lower > upper limb | +              | lower limb, usually | +           | lower limb, often      | +            |
| Spine involvement   | +                  | +              | +                   | +           | +                      | +            |
| Sacroiliitis - inflammation of the articulation of the sacrum and ilium                           | ++                 | ++             | ++                  | ++          | ++                     | ++           |
| Urethritis - inflammation of the urethra  | +                  | +              | +                   | +           | -                      | -            |
| Prostatitis - inflammation of the prostate gland  | ?                  | ?              | +                   | +           | +                      | +            |
| Conjunctivitis - inflammation of the mucous membrane covering the anterior portion of the eyeball | +                  | +              | +++                 | +++         | +                      | +            |
| Uveitis - inflammation of the uvea (iris, ciliary body and choroid)                               | +                  | +              | ++                  | ++          | +                      | +            |
| Skin involvement  | -                  | -              | +                   | +           | -                      | -            |
| Mucous membrane involvement   | -                  | -              | ++                  | ++          | -                      | -            |
| Enthesopathy - inflammation at sites of insertions (e.g., ligament into bone)                     | ? <sup>b</sup>     | ? <sup>b</sup> | +                   | +           | +                      | +            |
| Aortic regurgitation - leakage of blood through the aortic valve                                  | +                  | +              | +                   | +           | +                      | +            |
| Response to indomethacin or phenylbutazone  | +                  | +              | +                   | +           | +                      | +++          |
| Rheumatoid factor   | -                  | -              | -                   | -           | -                      | -            |
| Familial aggregation  | +                  | +              | +                   | +           | +                      | +            |
| HLA-B27 positive (% of cases)   | 80-90              | 80-90          | 80-90               | 80-90       | 80-90                  | 80-90        |
| % risk for HLA-B27 positive individual  | ~20                | ~20            | 10-20               | 10-20       | 10-20                  | 10-20        |

<sup>a</sup>Table modified from Calin (1988)

<sup>b</sup>Firestein and Zvaifler (1987) consider enthesopathy a distinctive feature of reactive arthritis.

The distribution of the HLA-B27 gene in healthy populations has been shown to be 6–10% in Caucasians, 1% in Japanese and 2–4% in North American Blacks. The gene is absent in pure (i.e., not mixed with Caucasian genes) African Blacks and Australian Bushmen (Benjamin and Parham 1990; Coppin and McDevitt 1986; Ebringer *et al.* 1985; Nickerson *et al.* 1990). Reactive arthritis or Reiter's syndrome develops in only about 2% of a population exposed to a triggering infection but approximately 20% of the exposed  $-B27^+$  population will succumb (Aho *et al.* 1985; Calin 1988). Roughly 80% of the patients with reactive arthritis or Reiter's syndrome are  $-B27^+$ , and they are likely to be more severely affected with multiple relapses than  $-B27^-$  patients (Firestein and Zvaifler 1987; McGuigan *et al.* 1985).

A large proportion (96%) of Caucasian patients with ankylosing spondylitis are HLA-B27<sup>+</sup>, whereas only 80% of Mexican or Japanese and 50% of Black ankylosing spondylitis patients are positive (Keat 1986). Thus, the relationship between  $-B27^+$  and ankylosing spondylitis is striking and follows the distribution of  $-B27$  antigen in the population. HLA-B27<sup>+</sup> relatives of  $-B27^+$  ankylosing spondylitis patients are more likely to acquire the disease than  $-B27^+$  relatives of healthy  $-B27^-$  persons (Keat 1986), and it can be expected that approximately 20% of  $-B27^+$  individuals will develop ankylosing spondylitis (Calin 1985).

Why do only a portion of HLA-B27<sup>+</sup> individuals develop arthritis? Are  $-B27^+$  antigens heterogeneous? How do we explain the fact that there are patients with sterile arthritides who are not  $-B27^+$ ?

There are several subtypes of  $-B27$ : HLA-B2705 is the most common and is found in a number of racial groups. For example, 85–90% of Caucasians and 45% of Orientals who are  $-B27^+$  have the  $-B2705$  antigen. HLA-B2704 and  $-B2706$  are found only in Orientals,  $-B2702$  is found in Caucasians,  $-B2701$  is found in both Caucasians and Asiatic Indians and  $-B2703$  is found only in North American Blacks (Ahern and Hochberg 1988; Khan 1988; Lopez de Castro 1989). All subtypes of  $-B27$  appear to be equally susceptible to arthritis. Studies by Breur-Vriesdendorp *et al.* (1987) indicated that the  $-B27$  and ankylosing spondylitis correlation is not due to differences in the subtypes but is due to a common  $-B27$  determinant shared by the various subtypes. Many  $-B27^-$  spondylitis patients have other HLA antigens such as the  $-B7$ -CREG antigens, which cross react with  $-B27$  antigen (Ahern and Hockberg 1988; Keat 1986; Saag and Bennett 1987; Schwartz *et al.* 1979). Coppin and McDevitt (1986) have shown that the genes encoding  $-B27$  from a healthy individual and from an ankylosing spondylitis patient had the same nucleotide sequence. There is some evidence that suggests that ankylosing spondylitis disease is different in  $-B27^+$  individuals as compared to negative patients. Acute anterior uveitis and aortic regurgitation appear to be less common in  $-B27^-$  patients (Ahern and Hochberg 1988; van der Linden *et al.* 1983; Reynolds and Khan 1988); however, Singal (1988) has disputed this.

Thus, there do not appear to be any clear answers to the questions posed above. There is no doubt that possessing the –B27 antigen (or a CREG antigen) predisposes an individual to arthritis under the proper conditions. The –B27 gene may merely influence the clinical appearance of the disease or its severity without being necessary for development of the disease (Khan 1988).

Because of the close association of HLA-B27 and the seronegative reactive arthritides, molecular mimicry (the sharing of epitopes between distinct protein antigens) or cross-reactivity between microorganisms and –B27 molecules has been suggested as an explanation for the predominance of reactive arthritic disease in HLA-B27<sup>+</sup> individuals. Earlier studies on cross-reactivity between –B27 antigen and microorganisms have been reviewed (Benjamin and Parham 1990; Cavender and Ziff 1986; Inman 1986; Inman *et al.* 1986; Keat 1986; McGuigan *et al.* 1985; Nickerson *et al.* 1991; Saag and Bennett 1987).

Using monoclonal antibodies against HLA-B27 (B27M1, B27M2 and Ye-2), van Bohemen *et al.* (1984), Chen *et al.* (1987), Ogasawara *et al.* (1986), Rayborne *et al.* (1988), and Zhang *et al.* (1989) demonstrated cross-reactivity between –B27 and *E. coli*, *K. pneumoniae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Y. enterocolitica*, and *Y. pseudotuberculosis*. It is of interest that Inman *et al.* (1986) were unable to show cross-reactions between *Y. enterocolitica* or *C. trachomatis* and –B27 when they utilized B27M1 or B27M2 monoclonal antibodies. In general, the studies in which monoclonal antibodies were used suggest that there is cross-reactivity between the HLA-B27 molecules and enterobacterial strains, but there is notable inconsistency among the various studies. These inconsistencies may be due to differences in microorganism, procedure and the type of assay used.

Sullivan *et al.* (1988) failed to demonstrate specific cross-reactivity between bacteria and the HLA-B27 molecule itself. They found that antibodies raised to certain strains of *E. coli*, *Klebsiella*, *Shigella* and *Salmonella* recognized leucocytes from HLA-B27<sup>+</sup> ankylosing spondylitis patients but did not recognize cells from normal –B27<sup>+</sup> or –B27<sup>–</sup> individuals. Sera from patients with *Yersinia*- or *Chlamydia*-induced reactive arthritis were not cytotoxic to –B27<sup>+</sup> lymphocytes (Inman *et al.* 1986) and only 1 of 17 sera from *Yersinia*-induced reactive arthritis patients reacted against –B27<sup>+</sup> cells (Cavender and Ziff 1986). Cameron *et al.* (1987) found that antisera raised against two strains of *K. pneumoniae* were no more cytotoxic to peripheral blood mononuclear cells of –B27<sup>+</sup> ankylosing spondylitis patients than to normal –B27<sup>+</sup> controls. The studies by Cavender and Ziff (1986), Cameron *et al.* (1987), and Inman *et al.* (1986) suggest that there is little cross-reactivity between bacterial and –B27 antigens in arthritic patients.

A six amino acid peptide sequence has been shown to be similar in HLA-B27.1 subtype (i.e., subtype –B2705) and *K. pneumoniae* nitrogenase (Schwimmbeck *et al.* 1987; Schwimmbeck and Oldstone 1988). Sera from 29% of –B27<sup>+</sup> patients with ankylosing spondylitis and 53% of –B27<sup>+</sup> patients with Reiter's syn-

drome contain antibodies that reacted with -B27.1 peptide. Sera from healthy -B27<sup>+</sup> individuals or sera from -B27<sup>-</sup> reactive arthritis patients did not contain such antibodies (Schwimmbeck *et al.* 1987; Schwimmbeck and Oldstone 1988). In addition, ≤40% of the sera from -B27<sup>+</sup> ankylosing spondylitis and Reiter's syndrome patients contained antibodies that bound to the *K. pneumoniae* peptide. Antibodies raised against synthetic peptides from either HLA-B27 or *K. pneumoniae* nitrogenase containing the shared amino acid sequence reacted with sections of articular tissues (synovial lining cells, vascular endothelium and infiltrating inflammatory cells) from the joints of -27B<sup>+</sup> reactive arthritis and ankylosing spondylitis patients (Husby *et al.* 1989; Schwimmbeck and Oldstone 1988). Tsuchiya *et al.* (1989) showed that a proportion of -B27<sup>+</sup> ankylosing spondylitis patients had antibody to -B27.1 peptide but in contrast to Schwimmbeck *et al.* (1987) and Schwimmbeck and Oldstone (1988), Tsuchiya *et al.* (1989) were unable to show a corresponding antibody against *K pneumoniae* peptide. Interestingly, they found a high frequency (20%) of anti-B27.1 peptide antibody in -B27<sup>+</sup> normal females who had had at least one pregnancy; the reason is unknown but may be due to allosensitization by a fetus processing -B27 or a -B7-CREG antigen.

Antibody raised against the B27.1 peptide reacted with a 19 kDa protein from *Y. pseudotuberculosis*; antibody binding was not inhibited by the *Klebsiella* peptide (Chen *et al.* 1987). The *Yersinia* protein apparently shares an amino acid segment similar to that in B27.1 peptide but not to *Klebsiella* peptide. A tetrapeptide sequence found in the outer membrane protein, Y op1, of arthritogenic strains of *Y. enterocolitica* and *Y. pseudotuberculosis* is also present in -B27.1 antigen (Tsuchiya *et al.* 1990a).

Strains of arthritogenic *S. flexneri* carry a 2 mDa plasmid, pHS-2, which encodes an epitope (inferred from the DNA sequence) homologous to a pentapeptide sequence present in B27.1 (Stieglitz *et al.* 1988, 1989). Sera from -B27<sup>+</sup> patients with reactive arthritis, Reiter's syndrome or ankylosing spondylitis contained antibodies to pHS-2 peptide and B27.1 but not to *K. pneumoniae* (Tsuchiya *et al.* 1990b).

Using HLA-B27<sup>+</sup> T-cells and sera from patients with reactive arthritides, Cavender and Ziff (1986), Inman *et al.* (1986) and Yu *et al.* (1985) were unable to find antibodies that would bind to the HLA-B27<sup>+</sup> cells. The -B27 antigen located on T cells would be in the native conformation (quite unlike synthetic peptides) and would appear to be a more realistic target for antibody binding.

Thus, there is a high degree of cross-reactivity with HLA-B27 and antigens from certain microorganisms. However, a note of confusion arises since some of these cross-reacting organisms — *E. coli* and *K. pneumoniae* — are not known to be associated with the reactive arthritides. In most of the studies, synthetic peptides were used and antibodies against these have been found in the sera of patients

with reactive arthritides but the significance of such antibodies is not at all clear. The finding of cross-reactivity between the bacterial and –B27 antigens or sequence homology between –B27 or a bacterial antigen does not necessarily mean that the mimicry is associated with the disease process itself (Nickerson *et al.* 1991).

A study by Kapasi and Inman (1992) shows an interesting relationship between HLA-B27 and gram-negative bacteria. The invasion of murine L cells transfected with the human Class I MHC genes was determined using *S. typhimurium*, *S. flexneri*, and *S. sonnei* isolates from reactive arthritis patients and an enteroinvasive *E. coli* strain isolated from a septicemic patient. The expression of –B27 on the L cells resulted in a marked decrease in invasion by the four bacterial strains as compared to L cells containing other Class I markers. With increasing surface –B27 expression, there was a corresponding decrease in the invasive capacity of *S. typhimurium*. Preincubation of the –B27 L cells with monoclonal antibody specific for human –B27 surface antigen resulted in significantly increased invasion of the –B27 L cells by *S. typhimurium*. Kapasi and Inman (1992), thus, have demonstrated that there is a bacterial:host cell interaction that is specific for –B27 as compared to other Class I HLA antigens and this interaction modulates the degree of invasion of pathogens into host cells. The implication of this work for the arthritic disease process is uncertain.

### **Role of Antibodies and Bacterial Antigens in the Sterile Arthritides**

Patients who develop *Yersinia*-induced arthritis usually have a less severe diarrhea than the individuals who do not develop arthritis; a mild diarrheic response leads to poor elimination of the infecting organism and thereby allows it to invade the intestinal mucosa and mesenteric lymph nodes (Toivanen *et al.* 1985). Intestinal biopsies indicated the presence of yersiniae in mucosal, submucosal and lymphoid tissue in the gut; in most cases, however, the bacteria were not in a cultivable state (de Koning *et al.* 1989). The deep location of the bacteria prevents them from being eliminated with feces and allows chronic stimulation of the gut-associated lymphatic system resulting in production of *Yersinia*-specific IgA and proliferation of specific T cells.

Toivanen *et al.* (1987), studying the different immunoglobulins in patients suffering from *Y. enterocolitica* enteritis, found that the large majority of patients had *Yersinia*-specific IgM, IgG and IgA at 1–2 months after infection. However, 12–16 months after infection, only 22% of the nonarthritic patients had *Yersinia*-specific IgM, 50% had specific IgG and 32% had specific IgA. In arthritic patients, 40% demonstrated *Yersinia*-specific IgM, 80% had specific IgG and 84% had specific IgA. Thus, the *Yersinia*-specific IgG and IgA were present at high levels in most of the arthritic patients for > 1 year after infection. The HLA-

B27 antigen was present in 76% of these arthritic patients as compared to only 17% in the nonarthritic patients. Granfors and Toivanen (1986) showed that *Yersinia*-specific secretory IgA and J-chain antibodies were significantly higher in patients with arthritis as compared to nonarthritic patients. In addition, levels of *Yersinia*-specific IgA1 and IgA2 were also elevated in arthritic patients. A later study by Granfors *et al.* (1989a) indicated that the increased level of IgA in *Yersinia*-induced reactive arthritis patients appeared to be antibodies against *Yersinia* lipopolysaccharide; more specifically, the antibodies were against the O-polysaccharide unit rather than the lipid-A core. The immunoglobulin classes of patients with sporadic *Salmonella* infections were studied by Maki-Ikola *et al.* (1991). At 4–14 months after infection with *Salmonella*, patients with reactive arthritis had higher levels of *Salmonella*-specific IgM, IgG and IgA than patients not suffering from arthritis. The long-term persistence of antibody appears to be a common feature in *Yersinia* and *Salmonella* triggered reactive arthritis and indicates that the pathogen or its components persist in the host.

Microbial antigens have been found in the joints of patients with reactive arthritis after infection by *Salmonella*, *Shigella* or *Yersinia*. *Yersinia* antigens (both cellular and lipopolysaccharide) were demonstrated in polymorphonuclear leucocytes or mononuclear phagocytes in synovial fluid from patients suffering from reactive arthritis induced by *Y. enterocolitica* (Granfors *et al.* 1989b). *Salmonella*-specific antigens were found in synovial fluid cells from patients with reactive arthritis caused by *S. enteritidis* or *S. typhimurium* (Granfors *et al.* 1990) and *Shigella* specific antigens were present in synovial fluid cells from patients suffering from *S. flexneri*-induced arthritis (Granfors *et al.* 1992). *Salmonella*-specific lipopolysaccharide was found in cells from both the synovial fluid and peripheral blood in *Salmonella*-induced reactive arthritis patients (Granfors *et al.* 1990). Viable bacteria were not found in the joints of any of these patients described by Granfors *et al.* (1989b, 1990, 1992). IgM and IgA immune complexes were present in the circulation and in the joints of some patients with *Yersinia*-triggered reactive arthritis (Lahesmaa-Rantala *et al.* 1987a,b); rheumatoid factor was not found in the joints of these patients. However, Inman *et al.* (1987) were not able to demonstrate circulating IgA complexes in patients with reactive arthritis or Reiter's syndrome (unspecified etiology). Thus, there appears to be some conflict concerning the presence of immune complexes in the reactive arthritides. Immune complexes may play a role in the pathogenesis of rheumatoid arthritis (Zvaifler 1988).

Using monospecific antibody against *Yersinia* outer membrane protein 1, Hammer *et al.* (1990) found whole cells of *Yersinia* in the synovial membrane (the site of inflammation) of patients with *Yersinia*-induced arthritis. The authors suggested that the bacterial cells persisted in the joints of arthritic patients; however, they did not actually test for the viability of the yersiniae present in the joint mem-

brane. Mononuclear cells containing *Yersinia* antigens were demonstrated in the synovial membrane of patients with *Yersinia* reactive arthritis (Merilahti-Palo *et al.* 1991). Both synovial fluid and membrane tissue were cultured for viable *Yersinia* but none were found. Viitanen *et al.* (1991), using the polymerase chain reaction, were unable to find *Yersinia* DNA in synovial fluid cells of patients, even though they were able to demonstrate *Yersinia* antigens in these cells. The absence of detectable bacterial DNA would argue against the presence of viable bacterial cells in synovial fluid cells.

The examination of proliferative responses (measured by incorporation of  $^3\text{H}$ -thymidine) in mononuclear cells from synovial fluid and peripheral blood of reactive arthritis patients revealed that the cells incorporated the greatest amount of isotope when they were exposed to the specific organism (*Campylobacter*, *Salmonella* or *Yersinia*) that had acted as the trigger for the arthritis (Gaston *et al.* 1989b). Maximum proliferative response was seen for synovial fluid cells, whereas the peripheral blood mononuclear cells gave only marginal response. The proliferative response was shown to be due to class II MHC-restricted helper T cells. Some cross reactions were seen between triggering organisms, suggesting that these microorganisms share antigenic epitopes recognized by the T cells.

Hermann *et al.* (1989) did a clonal analysis of synovial T lymphocytes in two patients (both HLA-B27<sup>-</sup>) with *Yersinia*-induced reactive arthritis. T lymphocytes reactive to *Yersinia* were present in synovial fluids. The *Yersinia*-specific proliferative responses were dependent on the presence of autologous monocytes as antigen presenting cells (APC) and were restricted by HLA class II antigens (HLA-DR). These workers also demonstrated two types of T lymphocyte clones: one type recognized only *Y. enterocolitica*, whereas the other type recognized both *Yersinia* and *S. typhimurium*.

Analysis of helper T cells isolated from synovial fluid from two HLA-B27<sup>+</sup> patients with *Yersinia*-induced reactive arthritis revealed that the T cells which recognized *Yersinia* antigens produced IFN- $\gamma$  (gamma interferon) and IL-2 (interleukin-2) but not IL-4 or IL-5 when activated (Lahesmaa *et al.* 1992). Thus, the *Yersinia* antigen selectively activated a subset of joint T cells that had a limited profile of cytokine secretion.

Peltz (1991), Romagnani (1991) and Street and Mosmann (1991) showed that human T cells recognizing antigens involved in the pathogenesis of several chronic inflammatory or allergic diseases exhibit a selective pattern of lymphokine secretion upon activation. The H<sub>H</sub>1 subset of CD4 T cells cloned from patients with chronic Lyme arthritis produce IL-2 and IFN- $\gamma$  but not IL-4, -5, or -6 upon activation. A similar picture of T<sub>H</sub>2 T cell response is seen in other allergies (Peltz 1991). It is probable that selective imbalance or inappropriate activation of either T<sub>H</sub>1 or T<sub>H</sub>2 T cell subsets leads to a disease. An immune-associated disease develops if the cellular response is inappropriately fixed in one or the other subset,

whereas a balanced response or response dominated by the correct subset does not lead to illness. Thus, the presence of *Yersinia* antigen and *Yersinia*-reactive T<sub>H</sub>1 T cells within the affected joint leads to selective expansion of T<sub>H</sub>1 cells with production of IL-2 and IFN- $\gamma$ , and these lymphokines probably play a role in generation and propagation of joint inflammation and arthritis (Lahesmaa *et al.* 1992).

In patients with *S. typhimurium*-induced reactive arthritis, Inman *et al.* (1989) demonstrated impaired lymphocyte proliferation to the triggering *Salmonella*. Peripheral blood lymphocytes were harvested from patients with reactive arthritis and from patients with *S. typhimurium* infection but without arthritis. The causative pathogens in these patients were of the same phage type, indicating that an outbreak had occurred that was caused by the same bacterial strain. The lymphocytes from the arthritis patients were three-fold less responsive to in vitro stimulation with the triggering organism than were those from nonarthritic patients. Addition of IL-2 during the in vitro proliferation of lymphocytes corrected the low proliferative response by cells from arthritic patients (Inman *et al.* 1989). The in vitro IgG, IgM and IgA production in cells from arthritic patients was several fold less after stimulation with *Salmonella* than that in cells from nonarthritic patients. Thus, there appeared to be decreased lymphocyte proliferation and lower levels of IgG, IgA and IgM in patients with *S. typhimurium*-induced reactive arthritis. An increased in vitro lymphocyte proliferation to *S. typhimurium* following addition of exogenous IL-2 suggests that there was minimal production of IL-2 in these arthritic patients. Ten of the 11 arthritic patients were either -B27<sup>+</sup> or -B7<sup>+</sup> (a CREG antigen), whereas none of the 11 nonarthritic (but *S. typhimurium* infected) patients were -B27<sup>+</sup> or -B7<sup>+</sup>. Previously, Leino *et al.* (1983) and Vuento *et al.* (1984) had demonstrated a lower proliferative response by T lymphocytes to *Yersinia* in reactive arthritis patients as compared to those patients with only *Y. enterocolitica*-induced diarrhea.

### Stress Proteins

Stress proteins (heat shock proteins), both microbial and self, are recognized by T-cells (Gaston 1992; Young 1990). Since the amino acid sequence of the stress proteins does not vary greatly between different biological species, a T-cell recognizing a microbial stress protein as antigen could act against a very similar self stress protein. Thus, there is possibility for the development of autoimmunity (Res *et al.* 1991). There has been recent interest in the role of stress proteins in arthritides and other autoimmune diseases (for reviews see: Cohen 1991; Gaston 1992; Winfield and Jarjour 1991; Young 1990, 1992). A discussion of the general aspects of stress proteins and the possible role of microbial stress proteins in the pathogenesis of the reactive arthritides should prove useful in gaining some

understanding of the relationship between certain foodborne pathogens and arthritis.

All organisms respond to heat shock with enhanced synthesis of highly conserved (i.e., a high degree of homology is found) proteins called heat shock proteins (HSPs) or stress proteins. The response appears to be universal in plants, animals and bacteria. Various aspects of HSPs have been reviewed by Lindquist (1986), Lindquist and Craig (1988) and Schlesinger (1990); monographs covering various aspects of heat shock and stress proteins have recently appeared (Kaufmann 1991; Morimoto *et al.* 1990; Nover 1991a). HSPs can be induced not only by heat shock but also by a variety of stresses. Thus, the proteins produced are more properly termed stress proteins. In *E. coli*, there are a number of inducers (stresses) for HSPs including (but not limited to) temperature shifts from 28°C to 42°C, ethanol, puromycin, virus (phage) infection, nalidixic acid, methylating and alkylating agents, CdCl<sub>2</sub>, and H<sub>2</sub>O<sub>2</sub> (Neidhardt and Van Bogelen 1987). Many of these agents will induce HSPs in higher organisms, also. Lindquist (1986), Nover (1991c) and Nover and Scharf (1991) discussed other HSP inducers that have been used with plant, animal and microbial systems.

Since the HSPs are called into play when a cell is exposed to heat shock, it is logical to assume that HSPs play some role in protecting the cell against devastation by heat. HSPs induced by heat shock coincide with the ability to tolerate higher temperatures and loss of thermotolerance coincides with the decay of HSPs. Proteins produced during heat shock also can induce tolerance to ethanol and other stresses. Yeast or mammalian cell lines selected for survival at high temperatures constitutively produce HSPs and thermosensitive bacterial mutants are defective in HSPs synthesis (Lindquist 1986; Lindquist and Craig 1988). Exceptions do occur and reports have appeared in which synthesis of HSPs does not lead to thermotolerance or in which thermotolerance is found in the absence of HSPs (Lindquist 1986; Lindquist and Craig 1988). For more complete discussions of HSPs and thermotolerance see Hahn and Li (1990) and Nover (1991b).

A stress that is important in terms of disease is infection; the metabolites produced by phagocytes ingesting invading microorganisms may induce HSP synthesis in both the microorganisms and phagocytes (Garbe 1992; Kaufmann 1990). The role of the HSPs during infection appears to be protective. HSPs are produced by microorganisms in an attempt to protect themselves from the noxious environment (reactive oxygen metabolites involved in the respiratory burst) of the phagocytic interior. Additionally, phagocytic HSPs may protect phagocytes against their own reactive metabolites (Garbe 1992; Kaufmann 1990).

Usually HSPs are defined on the basis of their approximate molecular weight. Cells are heat shocked and then incubated in the presence of radiolabeled amino acids. Newly synthesized proteins are identified by autoradiography after they have been separated on SDS gels and then the HSPs are grouped into families

of different approximate molecular weights (Kaufmann 1990). Members of a particular HSP family are approximately the same in size and are similar in sequence and function across species lines; thus, they are highly conserved polypeptides. Cognates of the HSP60 family (molecular weights are approximately 60 kDa), for example, perform similar functions in animal, plant, yeast and bacterial cells and share more than 50% amino acid sequence homology. A similar picture is seen for the family HSP70 (Kaufmann 1990).

HSPs have functions in normal cells, since HSPs and their relatives serve vital roles in cells in the absence of stress (Kaufmann 1990; Rothman 1989; Young 1990). HSPs are involved in the assembly and disassembly of oligomeric proteins complexes. They bind to incompletely or improperly folded proteins and redirect folding into the appropriate conformation. HSPs are involved in the traffic of specific proteins from one cellular compartment to another; they do this by unfolding the protein complex prior to translocation across the membrane and then refolding the protein when it has reached its new location. Thus, the HSPs are designed to protect, preserve and recover the functions of various protein complexes. When proteins are no longer salvageable, there are HSPs involved in degrading denatured and nonfunctional proteins. Thus, the protective role of HSPs during stress is merely an extension of their regular role in normal cells; refolding and reassembly of stress denatured proteins and dissociation and degradation of proteins that are damaged beyond repair (Kaufmann 1990; Rothman 1989; Young 1990). For a thorough discussion of the various functions of HSPs in normal cells, the monograph edited by Morimoto *et al.* (1990) should be consulted.

The functions that HSPs fulfill in the cell put them in the class of molecular chaperones. Molecular chaperones are a family of unrelated proteins that mediate the correct assembly of other polypeptides but do not themselves become components of the final function. They assist polypeptides in self-assembly by inhibiting unproductive assembly pathways that would lead to nonfunctional protein structures. Some, but not all, molecular chaperones are stress proteins, but it is possible that all stress proteins are molecular chaperones (Ellis 1991; Rothman 1989).

Archaeobacteria, cyanobacteria, eubacteria and rickettsiae as well as mitochondria and plastids have molecular chaperones called chaperonins, which have a high degree of sequence homology. It appears that these proteins are true evolutionary homologues. The chaperonins are constitutive proteins that increase greatly in amount after stresses such as heat shock or other physical and chemical stress, during bacterial infections (both the microorganism and macrophage produce HSPs in high concentrations during infection) and when there is an increase of unfolded protein in the cell (Ellis 1991). Thus, the response to stress by the chaperonins makes them potent immunogens. During infection, the high degree of homology of bacterial and mitochondrial chaperonins creates the potential development of

autoimmune disease. The chaperonins, as would be expected, bind to unfolded polypeptides to prevent them from misfolding, assist polypeptides in arriving at their proper conformation, transport proteins across membranes and permit proteases to degrade certain proteins (Ellis 1991).

HSPs are good immunogens (Young 1990). HSPs may be immunodominant in patients because these proteins are abundant in pathogens, especially under the adverse conditions imposed upon them by the host milieu. For example, HSP60 accounts for approximately 1.5% of the total *E. coli* protein under normal conditions; however, upon heat shock of the organism, HSP60 accounts for approximately 15% of the total cell protein (Neidhardt and Van Bogelen 1987). A similar picture is probably seen for invading pathogens. The presence of HSPs in the invading (and stressed) pathogen as well as in the stressed host ensures the continual restimulation of immunological memory for HSPs in the host. The "common antigen" is a major antigen in prokaryotes and eukaryotes; this antigen is a stress protein (Kaufman *et al.* 1991). Elevated antibody levels to species-specific and cross-reactive epitopes on "common antigen" are a feature of bacterial infections. The "common antigen" is the GroEL-related HSPs (60-65 kDa) found in bacteria and other cells. The 65HSP of mycobacteria species has approximately 50% homology with the corresponding HSP from *E. coli* (GroEL protein) and human cells (Born *et al.* 1990; Thole *et al.* 1988; Young *et al.* 1988). Thus, the omnipresence of shared epitopes by various pathogens may provide the immune system with a universal sign for infection (Kaufmann 1990).

The response of the immune system to HSPs would appear to be two-fold. The rapidity of the response to HSPs may provide a first line defense before immunity to pathogen specific antigens develops, and the immune response to HSPs may provide the host with a means to eliminate stressed host cells. By such a mechanism, the host is able to eliminate both microorganisms and stress-damaged host cells. However, depending on genetic background, hormones and environmental stimuli, epitopes shared by the pathogen and host may be the link between an infective and autoimmune response, with unfortunate consequences to the host (Kaufmann 1990; Res *et al.* 1991).

### **The Role of Stress Proteins in Reactive Arthritides**

Synovial T cells from reactive arthritis patients gave the greatest proliferative responses when stimulated by triggering organisms (*C. jejuni*, *C. trachomatis*, *Salmonella agona*, or *Y. enterocolitica*) but also there was marked recognition of the recombinant HSP65 from *Mycobacterium leprae* and the purified protein derivative of tuberculin (PPD), which contains HSP65 of *M. tuberculosis* (Gaston *et al.* 1989a; Life *et al.* 1991). When synovial T cells from a *Salmonella*-induced reactive arthritis patient was exposed to SDS-PAGE fractionated antigens of *S.*

*agona*, *E. coli* or a strain of *E. coli* with a plasmid for GroEL (HSP60), there was marked stimulation of T cell proliferation by the enterobacterial fractions in the 55–65 kDa range; in addition, lower molecular weight antigens were stimulatory. These low molecular weight antigens may be fragments of HSP60 or GroEL (Gaston *et al.* 1989a; Life *et al.* 1991).

Synovial fluid T cells, from a *Salmonella*-associated arthritis patient, were cloned in an antigen specific manner using SDS solubilized *S. agona* antigens or mycobacterial recombinant HSP65 (Life *et al.* 1991). The cloning procedure resulted in two types of clones: (a) those specific for *Salmonella* antigens that were induced by either *Salmonella* antigens or mycobacterial HSP65 and (b) those induced by either antigen that were cross-reactive with both *Salmonella* antigens and mycobacterial HSP65. Mycobacterial HSP65-specific clones were not found (Life *et al.* 1991). Further investigation revealed that cross-reactive clones did not respond to PPD from *M. tuberculosis* as well as they did to mycobacterial HSP65, in spite of the fact that both antigenic products contained HSP65. The authors interpreted this finding as indicating that the clones were reacting against *E. coli* antigens present in the recombinant HSP65 (Life *et al.* 1991). Res *et al.* (1988), studying other types of arthritis, also cautioned that reactivity to recombinant mycobacterial HSP65 may well be due to reaction against *E. coli* contaminants.

Some work has been done to determine if synovial fluid T cells reactive toward bacterial HSPs also react to human HSPs. Jarjour *et al.* (1991) demonstrated that only 2/32 patients with Reiter's syndrome had IgG antibodies against human HSP90, HSP73 or HSP60; 0/32 of these patients had IgM activity against the stress proteins. None of 17 ankylosing spondylitis patients had IgG or IgM antibodies against the human HSPs. Thus, IgM and IgG antibodies against human stress proteins are quite uncommon in patients with Reiter's syndrome and ankylosing spondylitis. Using synovial fluid T cells from a patient diagnosed with reactive arthritis that did not appear to have been triggered by *Campylobacter*, *Chlamydia*, *Salmonella* or *Yersinia*, Gaston *et al.* (1990) found that PPD or mycobacterial recombinant HSP65 stimulated T cell proliferation. Clones raised to a series of synthetic peptides corresponding to the whole sequence of HSP65 indicated that all of the clones recognized a single epitope in the nonconserved portion of the mycobacterial stress protein, thereby indicating that joint inflammation was not due to bacterial-induced T cell clones cross-reacting with self HSP65.

Synovial fluid T cells isolated from a reactive arthritis patient gave proliferative responses to mycobacterial GroEL (recombinant HSP65) and to *E. coli* GroEL (Lamb *et al.* 1989). These T cells also were reactive to mycobacterial recombinant HSP70, *E. coli* HSP70 and HSP70 from heat shocked human peripheral blood mononuclear cells. Thus, T cells from the inflamed joint of a reactive arthritis

patient responded to self stress proteins (Lamb *et al.* 1989). Hermann *et al.* (1991), using T lymphocytes from a patient with *Yersinia*-induced acute Reiter's syndrome (HLA-B27<sup>-</sup>), demonstrated that cells from synovial fluid responded to *E. coli*, *S. typhimurium*, *Y. enterocolitica*, human HSP65 and *Mycobacterium bovis* HSP65. A *Yersinia*-reactive T cell clone was isolated, JP1.2, which showed a strong response to *Y. enterocolitica*, recombinant mycobacterial HSP65 and human HSP65 with lessened response to *E. coli* and *S. typhimurium*. The clone also gave a strong response to *Mycobacterium tuberculosis*, thereby indicating that the clone's response to mycobacterial HSP65 was specific for the mycobacterial antigen and not to contaminants present in the vector medium. The human HSP65 antigen was a  $\beta$ -galactosidase fusion product and the JP1.2 clone gave only a weak response to  $\beta$ -galactosidase of the vector. Thus, the clone responds to conserved epitopes of HSP65 of enterobacteria, mycobacteria and human origin. Hermann *et al.* (1991) found that there was a specific proliferative response of clone JP1.2 to host heat-stressed (42C for 2 h) antigen presenting cells. Also, nonheat stressed synovial fluid cells from the inflamed joint of the patient stimulated the clone, but stressing the synovial cells at 42C for 2 h gave enhanced stimulation. The stimulatory action of the heat-stressed host cells on the clone was probably due to HSP65 expressed by these cells. Thus, Hermann *et al.* (1991) were able to isolate a T cell clone specific for human HSP65 from the inflamed joints of a Reiter's syndrome patient. In addition, this clone demonstrated activity to the patient's heat-stressed cells.

Limited data suggest that synovial T cells from joints of patients with reactive arthritis give proliferative responses to HSPs and indicate that stress proteins induced in the invading pathogen by cells of the host may well be the antigens that trigger arthritis. The few studies that have been done on the role of self-HSPs in the reactive arthritides are conflicting, since the results of Gaston *et al.* (1990) and Jarjour *et al.* (1991) indicate that self-HSPs are not involved, whereas the work of Hermann *et al.* (1991) and Lamb *et al.* (1989) suggests that self-HSPs may be important in these arthritides.

#### **Animal Models for Reactive Arthritis**

In the early 1970s, Volkman and Collins (1973, 1975, 1976), using LBN rats, developed a model for *Salmonella*-associated arthritis. Injection of viable *S. enteritidis* intravenously led to chronic polyarthritis; however, rats injected with heat-killed salmonellae did not develop lesions. Injection of viable salmonellae directly into the joints of naive or immunized rats did not lead to chronic arthritis, but injection of heat-killed salmonellae into joints of immunized rats did induce arthritis. The authors concluded that joint damage was induced by host factors and not by the infecting organism per se.

Hill and Yu (1987) using the Lewis strain of rats and Merilahti-Palo *et al.* (1992)

using SHR rats, consistently induced a sterile arthritis in rats injected intravenously with viable *Y. enterocolitica*. Other strains of rats were not susceptible. Both groups of workers found that systemic infection persisted for significantly longer periods in arthritis-prone animals than in control animals not susceptible to arthritis. In these two rat models, susceptibility to arthritis was not dependent on MHC genetic makeup but rather depended on susceptibility to *Y. enterocolitica*-induced death. The arthritis-susceptible rat strains were killed more readily by the organism than nonsusceptible rats. In both rat models, it was necessary to inject viable cells to induce arthritis, and there was a close association between poor elimination of the pathogen and the development of arthritis.

Toivanen *et al.* (1986) used SHR rats as a model for human *Yersinia*-triggered reactive arthritis. Intravenous injection of viable cells led to arthritis in the rats; however, the authors were able to isolate viable organisms from affected joints. Desferrioxamine-treated mice developed arthritis very similar to reactive arthritis when orally infected with *Y. enterocolitica* (de los Toyos *et al.* 1990). *Yersinia* lacking the Ca<sup>++</sup>-dependent virulence plasmid did not induce arthritis. However, de los Toyos *et al.* (1990) were able to isolate viable organisms from the joints of the arthritic mice. The ability to culture organisms from the arthritic joints would indicate that these animal models may not be suitable for the study of reactive arthritis.

When HLA-B27 transgenic mice and their normal siblings were intravenously injected with viable *Y. enterocolitica*, the -B27<sup>+</sup> mice developed complete hind limb paralysis (47% in -B27<sup>+</sup> as compared to 8% in -B27<sup>-</sup> animals). The -B27<sup>+</sup> mice were more susceptible to *Yersinia*-induced death than the controls (Nickerson *et al.* 1990). Only 27% of positive mice survived 30 days after injection of the organism as compared to 92% of the -B27<sup>-</sup> mice. Unfortunately, arthritis was not seen, and the authors postulated that the transgenic mice died before arthritis could develop. These transgenic mice, since the -B27 gene is present, could provide an ideal model system for determining the roles of the triggering microorganism, the -B27 gene and the animal immune system in reactive arthritis. However, the system obviously needs refining so that arthritic symptoms can be seen. Thus, it is apparent that there are animal models suitable for studying reactive arthritis but these model systems are not used extensively.

## CONCLUDING REMARKS

Reactive arthritis and Reiter's syndrome are induced by certain foodborne microorganisms such as *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia*. Ankylosing spondylitis has not yet been shown to be triggered by infections, but probably is, given the similarity of the syndrome to reactive arthritis and Reiter's syndrome.

There is a close relationship between the reactive arthritides and possession of the HLA-B27 antigen. Why this should be so is not at all clear. An enormous amount of effort has been expended on proving that molecular mimicry exists between -B27 and bacterial antigens, but these studies do not appear to have increased our understanding of why -B27<sup>+</sup> individuals are more susceptible to arthritis. Saario *et al.* (1992) have suggested that there may be increased permeability of the gut mucosa in -B27<sup>+</sup> individuals and thus, there would be increased uptake of substances (bacterial antigens, for example) from the intestinal lumen of the terminal ileum into the blood stream.

IgA and IgG antibodies persist much longer in patients with arthritis than in those who merely suffer from diarrhea. Since arthritic patients appear to have a milder diarrhea, there may be poorer elimination of the infective agent from the gut and the pathogen is not eradicated. de Koning *et al.* (1989) have shown the persistence of yersiniae in the tissues of the gut. This persistence allows chronic stimulation of the immune system with production of IgA and IgG. Bacterial antigens but not viable bacteria have been detected in the synovial fluid and cells of the joints of arthritic patients. How these antigens are disseminated from the gut tissues to the specific joints found in arthritis is unknown.

Investigators using antibodies from arthritic patients have been unable to show antibody binding to -B27 cells. Since synovial T lymphocytes from arthritic patients respond in vitro to infecting organisms, it is possible that arthritis is mediated by T cells and not by antibody. The T cell response is not Class I restricted but is actually Class II restricted (Gaston *et al.* 1989a,b). Since HLA-B27 is a Class I antigen, it is not apparent why -27<sup>+</sup> individuals are more susceptible to the reactive arthritides. Reiter's syndrome occurs in patients with acquired immunodeficiency syndrome (AIDS) in spite of the fact that helper T cells are severely compromised. If arthritis is mediated by T cells, they cannot be those that are adversely affected in AIDS (Winchester *et al.* 1987).

One of the more interesting areas of research in arthritides is the role of HSPs. The field is still in its infancy, and very little is known about HSP involvement in reactive arthritis, Reiter's syndrome and ankylosing spondylitis. Evidence indicates that there is direct stimulation of joint T lymphocytes by antigens from triggering microorganisms. The individual antigens have not been defined, but it is possible that they are stress proteins. HSP65 may be important since synovial T cells from reactive arthritis patients recognize microbial HSP65 in in-vitro proliferative assays. It is not clear whether synovial T cells from arthritic patients recognize self HSPs, since conflicting results have been obtained.

Studies on the reactive arthritides have been hampered by the failure of investigators to develop or use animal models. Admittedly, the few animal models that have been investigated do not give a complete clinical picture as seen in humans, but that might be too much to expect. For example, the use of HLA-B27 transgenic mice as described by Nickerson *et al.* (1990) would increase our

knowledge concerning the intertwined relationship of triggering microorganism, –B27 antigen and host immune system to the induction and symptomology of reactive arthritides.

The unfortunate consequences to patients suffering from one of the reactive arthritides triggered by diarrhea-producing foodborne microorganisms would indicate that diarrheic food poisoning episodes should not be dismissed lightly. Food processors, food service establishments and even home food preparers should exercise vigilance in preventing the introduction of foodborne pathogens into foods. In addition, they should be vigilant about storing foods under the proper conditions in order to prevent growth of foodborne pathogens.

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