MICROBIOLOGICAL REVIEWS, Mar. 1983, p. 97–120
0146-0749/82/010097-24$02.00/0

Vol. 47, No.1

# Streptococcal Rheumatic Carditis

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## INTRODUCTION

Over the past few years, a large body of evidence has accumulated, which indicates that group A streptococci are involved in the pathogenesis of a number of disease processes in humans (83, 84, 67). Of such diseases, the two most prominent are rheumatic fever and acute glomerulonephritis.

Rheumatic fever, according to one definition, is "a delayed sequel to infection with Group A streptococci . . . an inflammatory disease which involves principally the heart, joints, central nervous system and subcutaneous tissues" (247). The classification of this clinical syndrome as a single disease entity is the culmination of the combined efforts of clinicians, pathologists, microbiologists, immunologists, and epidemiologists of the modern era of medicine, spanning the past four centuries. However, in spite of the scientific advances of this century, the pathogenesis of rheumatic fever and poststreptococcal sequelae remains tantalizingly elusive.

The major clinical importance of rheumatic fever is the potentially lethal effect on the heart occurring during acute rheumatic myocarditis. The severe symptomatology of chronic rheumatic heart disease is the result of fibrosis of the

heart's connective tissue "skeleton," primarily its valves. The heart is the direct target or is affected indirectly by the immunological events that follow streptococcal pharyngitis, perhaps due to certain unique properties that it possesses.

Rheumatic heart disease is a complication of upper respiratory infection with group A streptococci. Streptococcal products elicit a reaction in connective tissues, called "rheumatic inflammation." Sites of particular predilection are the heart and synovial membranes. The damaging lesions of rheumatic fever are: (i) toxic myocarditis in the acute phase of the disease (striking anatomical lesions are not the rule, even though the outcome may be profound and result in fatal heart failure); (ii) valvular lesions which result in permanent damage due to fibrosis; and (iii) persistent inflammation which occurs in chronic myocarditis, leading to chronic heart failure (250).

The group A streptococcus has been unequivocally proven to be the etiological agent of rheumatic fever. The pathogenesis of the lesions, however, is still unclear, which is why the role of various modifying factors is being currently researched (249).

Rheumatic fever is extremely diverse insofar as its functional and anatomical lesions are concerned. This makes it rather important to consider whether the disease could be due to a single pathogenetic process. It may be possible that the disease is diverse in its manifestations due to differences in the sites involved and to the consequences of their altered functions (72, 249).

It has been suggested that streptococcal antigens may elicit an aberrant immune response, i.e., the production of cross-reactive antibodies and cell-mediated immunity, which leads to the destruction of host tissue. However, the lack of a suitable animal model has made it difficult to identify the specific streptococcal components involved or to analyze the mechanisms of damage in vivo (55, 83, 146, 190, 212, 249, 267, 295).

This review will examine the relationship of group A streptococci to rheumatic fever and rheumatic carditis, the proposed role played by the immune system in the initiation and establishment of the cardiac lesions, and the experimental models to date that have been put forth to evaluate this problem. Some of the pathological mechanisms involved will also be covered in brief.

## A BRIEF HISTORY

With the development of the field of pathological anatomy in the early 18th century, descriptions of rheumatic lesions began to appear. Lancici, a papal physician, published the results of autopsy findings wherein he described valvular vegetations (145). Richard Pulteney, in 1761, is credited with the first clear description of the various manifestations of rheumatic fever and rheumatic heart disease, where the clinical findings of acute rheumatic fever (ARF) were related to the cardiac lesions observed at autopsy (203).

In the 19th century, Jean-Baptiste Bouillaud gained recognition for his treatise of 1836, "Nouvelles Recherches sur le Rheumatisme Articulaire." He described endocarditis of all types, early, subacute, and chronic (24, 25).

In 1904, Ludwig Aschoff described the specific rheumatic lesion in the myocardium named the Aschoff body in his honor (8). Romberg (219) had described similar lesions before Aschoff, but the specificity for rheumatic disease had not been established. Geipel (78) observed cellular lesions in a patient dying 5 weeks after an attack of rheumatic fever, which confirmed Aschoff's findings. Subsequently, a consensus was reached by a great number of authors: the rheumatic nodule goes through a cycle of development and retrogression, leading to a fibrous scar (93, 94, 139, 170). Various patterns were observed in different cases and in different situations. The pathogenesis of the lesions, especially the nature of the primary damage, remained unresolved.

According to Aschoff, the lesion was a cellular, proliferative process, followed by necrosis in the center, whereas Klinge (139) and Gross and Ehrlich (93, 94) were of the opinion that connective tissue damage occurred initially, with subsequent cellular proliferation. Geipel (78) and Talalajew (260) believed that both degenerative and proliferative changes could occur simultaneously in some cases (146).

Historically, the parallelism between the epidemiology of rheumatic fever and of streptococcal infections formed one of the strongest lines of evidence, which established the former as a sequel to the latter (248).

## ETIOLOGY: EVIDENCE FOR THE GROUP A STREPTOCOCCAL ETIOLOGY OF RHEUMATIC FEVER

Many investigators contributed to the identification of the group A streptococcus as the etiological agent of ARF. Schotmüller, in 1903, differentiated hemolytic from nonhemolytic streptococci on the basis of the blood agar technique he devised (232). Lancefield (143) classified the major pathogenic strains in one common group, group A, since they had the same antigenic cell wall polysaccharide. These were then subdivided still further into antigenically distinct strains which were distinguishable

from each other due to the type-specific nature of immunity to streptococcal infection (249).

Four major lines of evidence, clinical, epidemiological, immunological, and prophylactic, established the group A streptococcus as the sole infective agent causing initial and recurrent attacks of rheumatic fever (204, 248, 249, 251). None of the above lines of evidence are direct because group A streptococci have not been recovered from the lesions of rheumatic fever nor has a satisfactory experimental animal model of the disease been demonstrated to date. Hence, Koch's postulates are not fulfilled (249). Attempts to demonstrate an association of streptococcal antigens with lesions have yielded equivocal results (84, 147), as will be discussed below.

Clinical, epidemiological, and prophylactic evidence implicating group A streptococcal etiology in ARF has been put forth by various workers and has also been reviewed extensively (3, 7, 36, 41, 42, 44, 56, 60, 66, 143, 164, 202, 205, 227, 249, 252, 253, 270, 272, 283, 284). Immunological evidence to back up streptococcal etiology of rheumatic fever and its sequelae will be dealt with at some length.

### Immunological Evidence

Various streptococcal antigens have been studied in an effort to find out which one(s) played a major part in the pathogenesis of rheumatic fever. Some of these components include somatic antigens such as M-protein (13–16, 19–22, 37, 45, 57, 68–70, 83, 102, 110, 114, 116, 119, 135, 145, 172, 198, 201, 205, 222, 230, 242, 285, 287, 288, 299, 301), C-polysaccharide complexes of mucopeptide and polysaccharide (6, 27, 37, 50, 63, 131, 135, 188, 215, 216, 233–235, 237, 240, 245), mucopeptide (1, 84, 132, 167, 223, 224, 236, 302), cell wall components (2, 50, 82, 85, 86, 148, 238, 244, 271), and antigens cross-reactive with tissues (118, 119, 126, 129, 210, 249, 273, 296, 298, 299). Others include the cell membrane (43, 71, 84, 274) and L-forms (19, 43, 91, 103, 113, 166, 215, 244, 263, 297, 298). Of these various antigens, the ones with the most convincing experimental evidence are the: (i) M-protein; (ii) complexes of mucopeptide and polysaccharide; (iii) mucopeptide; (iv) persisting, degraded streptococci; and (v) streptococcal antigens cross-reactive with tissues.

**M-protein.** M-protein is one of the most important factors affecting virulence of group A streptococci. The M-protein has been identified as a part of the cell well structure. The immunology of M-proteins has been reviewed extensively (68, 144).

**(i) Clinical evidence.** Recent evidence has unearthed the fact that many human sera contain antibodies to a moiety(ies) of M-protein which is closely associated with, but distinct from, the type-specific determinant (13–16, 288). This protein is known as a non-type-specific M antigen(s) or M-associated protein. A variable proportion of normal human sera contains antibodies to M-associated protein, as do sera from most patients with recent streptococcal infections, ARF, or post-streptococcal glomerulonephritis. Assays that have been used to study these antibodies include platelet agglutination (14, 16, 288) and complement fixation (288). Titration of serum levels by the latter method have revealed that the highest titers are present in patients with ARF (13). Measurement of antibodies to M-associated protein is not presently a clinically applicable test. The topic of interest right now is whether or not there is a relationship between M-associated protein and the streptococcal surface antigen responsible for eliciting cross-reactive fluorescent anti-heart antibodies in rheumatic fever patients (19, 299).

Massell et al. published the results of streptococcal vaccination of the siblings of rheumatic patients (162). It was reported that three cases of rheumatic fever appeared among 21 siblings vaccinated with a type 3 streptococcal M-protein. This high rate contrasted with the observation that, in nonvaccinated siblings, 447 similar streptococcal infections occurred, of which only five cases turned out to have rheumatic fever. It must be mentioned, however, that each of the siblings in the vaccinated group who developed rheumatic fever had, in addition to receiving the type 3 vaccine, also experienced intercurrent group A streptococcal infections with high-titer anti-streptolysin O rises. The probability, therefore, that the vaccine could have caused rheumatic fever is negligible. This is in opposition to the contention by some workers that the M-protein used could have contained either antigens cross-reactive with the heart or other toxic substances that could have predisposed the children to the development of rheumatic fever (83).

**(ii) Experimental evidence.** Epidemiological and bacteriological evidence suggests that group A streptococci may vary in their potential to cause rheumatic fever. Hence, the terms "rheumatogenic" and "non-rheumatogenic" have arisen. Such a variability in rheumatogenicity is consistent with observed variations in the geographical and temporal distribution and incidence of ARF. In studies of outbreaks of streptococcal pharyngitis, it has been found that certain serotypes, such as type 5, very frequently give rise to acute rheumatic fever, whereas others, such as type 4 and most other opacity factor-positive serotypes, generally do not do so (45, 70, 110, 201, 205, 222, 287, 307). Bisno has provided an excellent review of the data relating

to the concept of streptococcal rheumatogenicity non-rheumatogenicity (22).

Mice were inoculated with acid-extracted M-protein from streptococcal types 1, 5, 19, and 12 (116), as well as the type 12 M-protein extracted from the cells after phage-associated lysis (172). The M-protein was found to localize in the endocardium, the lungs, the glomerular tufts of the kidney, the reticuloendothelial system of liver, spleen, and lymph node, and the adrenal glands of the inoculated animals.

M-protein accumulated in the reticuloendothelial system and serous surfaces of the spleen and liver, but not in the heart, lung, or kidney, of mice inoculated with virulent types 5 and 12. However, absesses of the liver and spleen showed the presence of M-protein (116).

Other studies showed that normal mice and rabbits exhibited no pathological alterations in the heart after exposure to M-protein (69, 116, 230). On the other hand, toxic reactions were observed in adrenalectomized rats that received sonically disrupted streptococci containing M-protein (242). However, in view of the fact that streptococcal cell wall components devoid of M-protein are definitely toxic to rabbits (19, 37, 135), it remains to be seen whether or not M-protein caused the toxic reactions described (83).

Intravenous injection of acid-extracted M-protein in mice induced severe hemorrhagic infarcts in the lungs (115). This streptococcal antigen was found to interact with, and to precipitate, mouse fibrinogen in vitro (114). M-protein–fibrinogen complexes must fix complement; this reaction is known to be essential for initiation of tissue damage (57). When whole streptococci are suspended in human plasma, the M-protein becomes coated by fibrinogen, and this fibrinogen prevents complement binding to the organisms (285). It is now commonly held that the M-protein of group A streptococci functions as a major virulence factor by virtue of its antiphagocytic property. Peterson et al. (198) provided evidence to support the concept that, in the absence of type-specific opsonic antibodies (nonimmune serum), M-protein inhibits phagocytosis. This is achieved by interference with the process of bacterial opsonization; possibly via the alternative complement pathway (198). Studies carried out by Bisno suggest that the M-protein retards interaction of alternative complement pathway components with structures present on the streptococcal cell surface, thereby explaining the contribution made by M-protein to group A streptococcal virulence (21).

Other in vitro tests carried out showed that purified M-protein isolated from types 6, 12, 24, 30, and 56 caused clumping of platelets and leukocytes (14). Lysis of polymorphonuclear neutrophils and migration inhibition of polymorphonuclear neutrophils in glass capillary tubes occurred with higher concentrations of M-protein (102). Similar toxic effects were produced by M-protein and antibody to M-protein complexes, as well as by suspensions of M-negative streptococci (type 26), but not by suspension of M-positive strains. Since the M-protein used was purified, its toxicity could not be due to contamination with mucopeptide, which is known to damage leukocytes (102).

Besides the M-protein, group A streptococci contain a number of other protein cell wall antigens, R and T being the ones best studied. Insofar as is known, these substances do not have any biological significance relating to streptococcal virulence (83).

**Complexes of mucopeptide and polysaccharide.** C-polysaccharide–mucopeptide complexes can be prepared from cultures of group A streptococci by sonication, mechanical breakage with glass beads, or lysis with muramic acid-splitting enzymes (83). Such preparations usually contain plasma membranes, intracellular cytoplasmic fractions, enzymes, and toxins in addition to the above-mentioned complex.

Mucopeptide-polysaccharide complexes of group A (37, 50, 135), C, and G and viridans streptococci (63) prepared by ultrasonication and mechanical disintegration induced myocardial and valvular lesions in rabbits (37, 135) and mice (50) when inoculated into these animals by the intravenous and intraperitoneal routes. The lesions obtained in mice were granulomatous in nature, with multinuclear giant cells, and were claimed to be similar to the cardiac lesions characteristic of rheumatic carditis in humans (50). The toxic components of such preparations (which caused cardiac lesions) were associated with neither the soluble cytoplasmic components nor the unfragmented cell wall. The cell wall components in the cardiac lesions were found to localize in the periphery of the granuloma (83). This pattern of localization was similar to that seen in the mouse muscle injected with fluorescence-labeled streptococci (215). Localization of the cell wall components was also found to occur in the reticuloendothelial system, as expected for a particulate substance (50, 131, 188). Besides cardiac injury, hepatic lesions also appeared within 12 h of injection of the sonicates (63, 245).

**Mucopeptide.** The mucopeptide is composed of repeating units of *N*-acetylglucosamine and *N*-acetylmuramic acid, linked to a tetrapeptide (L-alanyl-D-glutamyl-L-lysyl-D-alanine) through the carboxyl group of the muramic acid (167). Mucopeptide is usually prepared by extraction with formamide and is always contaminated with small amounts of C-polysaccharide. The

mucopeptide is immunogenic. It can be cleaved by lysozyme (167), but the presence of even traces of C-polysaccharide in the mucopeptide preparation abolishes the activity of lysozyme (1, 236).

Rabbits injected intravenously with solubilized mucopeptide prepared by ultrasonication of a mucopeptide preparation developed extensive granulomatous cardiac lesions (223, 224). On the other hand, particulate mucopeptide caused very mild cardiac lesions.

The mechanism by which mucopeptide induces tissue damage is not very well understood. Injury may be due to direct irritation to some connective tissue elements or to the effects of split products of the mucopeptide released by lysozyme (83). Most animal sera tested contained some antibodies to the mucopeptide that cross-reacted with mucopeptide of different bacterial species (132). Also, sera of patients with collagen diseases were found to contain antibodies to mucopeptide (302). Based on these facts, therefore, it is possible that hypersensitivity reactions could have participated in the process.

The role of the mucopeptide in the pathogenesis of streptococcal infection is still arguable. It is not known whether streptococcal cell wall degradation causes release of mucopeptide in sufficient amounts in vivo to be injurious. Another factor to be considered is that the mucopeptide is not specific for group A streptococci, which therefore creates some shadow of a doubt on its pathogenic role in the development of post-streptococcal sequelae (84).

**Persisting, degraded streptococci.** There appears to be a direct relationship between the persistence of streptococcal cell wall components in macrophages and the maintenance of a chronic inflammatory process in the tissues (83, 84). Such a persistence is probably due to insufficient cell wall antigen degradation by the enzyme systems of the macrophage. This persistence of cell wall structures within phagocytes coupled with their translocation by phagocytes to inflammatory sites that are induced by streptococcal toxin (82, 85, 86) may be an important factor in the pathogenesis of post-streptococcal sequelae.

Animal experimentation has revealed that mice sensitized with streptococci did not eliminate $^{14}$C-labeled streptococci from muscle granuloma any faster than did unimmunized mice (2, 83). Previous studies by Spector et al. on the elimination of cellular components from animal tissues has been carried out, using $^{125}$I-labeled *Bordetella pertussis* or *Mycobacterium tuberculosis* (244). Elimination was measured by the release of trichloroacetic acid-soluble radioactivity from the cells. Measurable elimination of cellular components from such cells was reduced by the addition of specific antibodies (244), implying that a state of immunization may not contribute to a more rapid degradation of bacterial cell walls.

There is not much evidence concerning the prolonged persistence of streptococci in human tissues. There are several controversial reports concerning the presence and persistence of streptococci in the heart and joints of rheumatic fever patients (271). It is generally accepted tht living streptococci are absent in Aschoff bodies. Experimental evidence has shown that streptococcal cell wall components localize in the hearts of mice (50) and that translocation of streptococci and inert particles to tissue sites in the heart occurs after injury by streptococcal toxin (85). In light of this evidence, therefore, the logical course to follow would be to examine human lesions for the presence of streptococcal cell wall antigens. Submicroscopic structures that resembled bacterial L-forms morphologically were found in Aschoff nodules from biopsy specimens of the left auricular appendage obtained at mitral valvulotomy (147). However, fluorescence or ferritin-labeling techniques, using a variety of polyvalent antisera to group A streptococci, have failed to detect the presence of streptococcal antigens (83). The nature of these pathological structures is unknown.

**Streptococcal antigens cross-reactive with tissues.** There is a large body of evidence to support the existence of antigenic structures common to both streptococci and mammalian tissues (273). Such cross-reactions have been demonstrated between the streptococcal wall, or protoplast membrane, and the heart and skeletal muscles, smooth muscles of blood vessels, kidney basement membrane, mucopolysaccharides of the heart valves, and connective tissue elements and with some transplantation antigens. These numerous studies have been reviewed comprehensively (119, 126, 210, 273, 296).

Immunofluorescence and a variety of other immunological techniques have revealed the frequent occurrence of high-titer antibodies in the sera of rheumatic fever patients. Studies carried out by Dudding and Ayoub have revealed the persistence of elevated levels of antibody to the streptococcal group A carbohydrate (A-antibody) in patients with chronic rheumatic valvular disease (58). During the same year, Goldstein and Trung found the C-polysaccharide of streptococci to be cross-reactive with valvular glycoprotein (91). Later studies conducted by Ayoub and Shulmann confirmed previous observations of patients with rheumatic valvular disease possessing elevated and persistent levels of A-antibodies. This A-antibody declined to normal levels in patients without rheumatic carditis or with other complications of group A streptococ-

cal infections. An additional finding in this later study was that the A-antibody in patients with transient rheumatic valvulitis declined almost as rapidly as in patients without carditis (10). Initially elevated levels of this antibody may be found in patients without rheumatic valvular involvement; hence, the causal relationship of this antibody to the pathogenesis of rheumatic valvulitis is questionable (10). This evidence lends further credence to the existence of common antigenic structures between streptococcal and mammalian cells. In some studies these anti-heart antibodies have been removed by absorption with streptococcal cellular extracts (129, 299). Furthermore, antibodies which bind to the sarcolemma and subsarcolemmal sarcoplasm of cardiac and other striated muscle, as well as to the smooth muscle of vessel walls and endocardium, have been found in rabbit antisera to group A streptococci (118, 298). Recently, Poul Christensen and his colleagues from Lund have published a series of papers indicating that group A streptococci have Fc receptors on their surfaces and raising questions as to whether many of the cross-reactive antibodies previously described in streptococcal research may not, in fact, have been due to nonspecific immunoglobulin binding (32, 39, 156).

There has been much debate over which particular or specific component of the group A streptococcus possesses this property of cross-reactivity. Suggested streptococcal cross-reactive antigenic components include the cell wall (closely associated with type-specific M-protein) or protoplast membrane or both. Dale and Beachey (51) have very recently provided definitive evidence that at least one protective antigenic determinant of a structurally defined polypeptide fragment of type 5 M-protein evoked antibody that cross-reacted with sarcolemmal membranes of human heart muscle. These studies also indicated that the heart cross-reactive determinant of type 5 M-protein was shared with type 19 M-protein. When this antibody was purified and used in an affinity column to isolate the sarcolemmal membrane protein, a cross-reactivity was observed between it and M-proteins of types 5 and 19.

## PATHOLOGY OF RHEUMATIC FEVER

### General Pathology

Rheumatic fever is characterized by diffuse, proliferative, and exudative inflammatory reactions in the connective tissues, especially those of the heart and joints (249). Collagen fibers are the first tissues to be affected, exhibiting exudative tissue lesions. Hence, rheumatic fever, like other rheumatic diseases with the same tendency, has been alluded to as a "collagen disease" or as a "connective tissue disease." However, there is a significant difference between what the pathologist sees as morbid anatomical changes and what the clinician observes as the signs and symptoms of the disease (249).

Rheumatic heart disease is a complication of upper respiratory infection with group A streptococci. The streptococci elicit a reaction in connective tissues, which is called "rheumatic inflammation." The heart and synovial membranes are the ones most apt to exhibit such a reaction. If the heart is the organ affected, it is quite likely that severe deformities may result, usually of the valves. In the heart, each of the major anatomic components, the pericardium (249), the myocardium (16, 46, 73, 134, 179–181, 225, 249, 264, 268, 278), the endocardium (249), and particularly the valves, may be involved. The persistent focal inflammatory lesions of the myocardium, without clear correlation with clinical manifestations of carditis, have led to a wide dichotomy between pathologists and clinicians in the definition of rheumatic activity.

Nonetheless, the disease has a distinct personality pathologically, both in its unique lesions of the heart and in its tendency to spare other organs from serious damage, particularly the joints, brain, kidneys, muscles, and so forth (249).

**The nature of the rheumatic lesions: the Aschoff body.** Rheumatic lesions have been observed in detail through studies of the histopathology of the myocardium in patients dying of acute and chronic rheumatic heart disease. Murphy (180) has made an excellent review of the history of these studies.

The first phase of rheumatic fever is the exudative-degenerative phase (259), which lasts for 2 to 3 weeks. The lesions are characterized by eosinophilia, along with the development of a meshwork of rigid, waxlike fibers in loose connective tissue areas (249). Lesions in this phase are not diagnostic (93, 94).

It is in the second phase that the most characteristic lesion of rheumatic fever, the myocardial Aschoff nodule, develops (8). According to the patterns created by the eosinophilic material, the cells, and the stage of development of the lesion, Gross and Ehrlich (93, 94) applied various descriptive terms for Aschoff bodies, such as coronal, syncytial-coronal, reticular, and mosaic (146). Strictly speaking, the term "Aschoff body" or "Aschoff nodule" should be reserved for myocardial lesions (146, 249). Lymphocytes and macrophages aggregate around fibrinoid deposits to form Aschoff bodies. Based on their distribution, their characteristic cells, and their histological configuration, Aschoff nodules are virtually pathognomonic of rheumatic fever (249). In spite of the Aschoff body being of such importance, it is ironic that its origin, functional

impact on the heart, and relation to the cause and severity of the rheumatic attack are obscure (249). McEwan (169) lists the suggested origins of fixed connective tissue cells. The two major theories are that (i) the Aschoff cells are nonmyogenic mesenchymal cells (9, 15, 139, 148, 187, 199, 243, 259, 276, 279) and (ii) they are myogenetic in origin (5, 18, 174, 180, 182, 183, 277). According to Becker and Murphy (18), Aschoff bodies are central to the pathology of rheumatic heart disease, which is in opposition to Stollerman's (249, 250) opinion that Aschoff lesions cannot account for the serious myocardial failure of severe rheumatic carditis. The basis of his logic is that ARF patients experience a rapid recovery of cardiac compensation after an attack of acute carditis. This phenomenon occurs in spite of the persistence of Aschoff nodules in such hearts.

After the above phase in the sequel to rheumatic fever, the proliferative and healing phases follow and last for a period of several months. Cells collect around and between the eosinophilic material. Initially, the cells are lymphocytic in nature, but soon after, larger cells appear with ragged basophilic cytoplasm (259). Sometimes these cells are multinucleated. Many of these cells possess characteristic nuclei of two types: the owl-eye nucleus and the Anitschkow myocyte (5). The former is a heavy eccentric chromatin dot with fine fibrillae radiating to the sharply clear nuclear membrane. The latter has a bar arrangement of chromatin, with a sawtooth edge, also called a fibrocytoid, lattice or caterpillar nucleus.

Lesions of different types, and apparently of different ages, may be present in the same heart (94, 170).

**The nature of fibrinoid.** The term fibrinoid degeneration was introduced by Neumann in 1880 (187) to describe the structural changes perceived in the connective tissues of serous, synovial, and mucous membranes and of vascular intima and endocardium due to inflammation. The fibrinoid substance that he saw resembled fibrin and had the same staining properties.

Fibrinoid is observed in the exudative-degenerative phase of the lesion development. The ground substance is edematous, collagen fibers are fragmented, and cellular infiltrations of lymphocytes, histiocytes and a few polymorphonuclear neutrophils and eosinophils occur. The mucoid edema stains basophilic and is seen mainly in the endocardium and valves (249).

Earlier investigators therefore believed that fibrinoid was derived from fragmented collagen fibers. Later, investigators forwarded evidence to prove that fibrinoid is a complex mixture of substances including fibrin, gamma globulin (immunoglobulins G, M, and A), and other constituents such as complement (C1) (87, 148, 180, 275, 279).

Fibrinoid changes occur in all connective tissue diseases, hypersensitivity reactions, burns, and other inflammatory states. Such changes, therefore, are not specific for rheumatic fever.

## ROLE OF THE IMMUNE SYSTEM IN THE PATHOGENESIS OF RHEUMATIC FEVER

### Pathogenesis

In spite of rheumatic fever having been prevalent for centuries, its pathogenetic mechanisms have defied the onslaught of modern microbiology, immunology, and clinical investigation. Solution of this major enigma of modern medicine may yield implications for connective tissue diseases and other closely related diseases (249).

There are a few absolute requirements for the development of rheumatic fever: (i) the involvement of a group A streptococcus (258); (ii) a streptococcal antibody response, indicative that actual recent infection has occurred; (iii) persistence of the streptococcus in the pharynx for a sufficient length of time; (iv) location of the infection in the upper respiratory tract (249).

### Possible Role of Immunological Mechanisms in the Disease

The basic mechanisms of tissue destruction are relatively clear, despite which, however, the pathogenesis of characteristic lesions or clinical sequelae in rheumatic fever remains unsolved. Epidemiological and clinical studies have clearly established that antecedent streptococcal infection is a prerequisite to rheumatic fever (92, 165, 205, 206, 248). Widespread involvement of connective tissue structures, including endocardium, heart valves, myocardium, pericardium, lung and pleura, tendons, joints, subcutaneous tissue, and finally brain function, is supported by the clinical picture of the disease, as well as by numerous pathological studies. However, there is still some doubt as to the exact processes that translate streptococcal infection into subsequent acute pancarditis or severe choreiform episodes (290). It may be useful to review the evidence that has led most investigators to believe that immunological mechanisms were involved.

First, the average interval between streptococcal sore throat and the onset of rheumatic fever is about 3 weeks, which fits well with the time required for the development of an antibody response. Serum samples of patients were tested for the antibody response to several of the extracellular products of group A streptococci. Their sera were tested from the start of streptococcal infection and at periodic intervals after that throughout the course of the disease. It was

shown that the antibody titers rose sharply at the time of first appearance of symptoms of rheumatic fever and reached their peak 1 or 2 weeks afterwards (166).

A second set of observations supporting the role of immunological mechanisms in rheumatic fever relates to the finding by several groups that the mean antibody response to a number of different streptococcal antigens is higher in patients who develop rheumatic fever than in patients in the same epidemic who have uncomplicated streptococcal infection (164, 166).

A third type of evidence for an immune process in the pathogenesis of rheumatic fever comes from results of the Fort Warren studies, where it was shown that effective penicillin therapy of established streptococcal sore throat would prevent rheumatic fever (166, 280). Streptococci are eliminated by effective therapy which also usually suppresses the antibody response to streptococcal antigens. The implication here is that the risk of rheumatic fever in a susceptible subject is greatly reduced provided the antigenic stimulus is curtailed (166).

Less direct evidence to support immunological mechanisms in the pathogenesis of rheumatic fever has been obtained from certain analogies. Thus, the timing and some of the manifestations of serum sickness are like those of rheumatic fever. Furthermore, the lesions produced by certain kinds of immune injury in experimental animals simulate those found in the human disease (166).

### Immunological Theories

According to the most popular theory, rheumatic inflammation is some type of hyperimmune reaction, due to either bacterial allergy or autoimmunity. The hyperimmunity view is strongly supported by all of the immunological evidence which shows the rheumatic host to be acutely hyperimmunized by all streptococcal products which have been studied (249).

**Allergic theories.** Rheumatic fever has frequently been compared with serum sickness, ever since Schick (227) postulated the "Nachkrankheiten" of scarlet fever as a bacterial allergy. Investigators have postulated that the latent period of ARF is due to the lag in the appearance of specific streptococcal antibodies to persistent antigens. Serum sickness is like rheumatic fever in that both are produced by circulating immune complexes and also produce arthritis. However, unlike rheumatic fever, serum sickness is associated with angioneurotic edema and acute glomerulonephritis. The latent period of ARF does not decrease with repeated rheumatic attacks, as one would expect with repeated bouts of serum sickness (249). Other points of difference between the two are that, in ARF, there is an increase in the concentration of serum complement, whereas in serum sickness, when immune complexes are formed in large amounts, complement levels fall (64). Serum sickness can result from immune complex disease owing to a variety of causes, but ARF is unique to streptococcal infections only. Fibrinoid degeneration of collagen and arteritis occur in both ARF and serum sickness, but the latter never elicits the formation of Aschoff bodies in the myocardium.

Despite such differences between the two, however, it can be hypothesized that when a hypersensitized host succumbs to a virulent streptococcal pharyngeal infection large amounts of streptococcal antigen are absorbed by the host. A specific streptococcal product to which the host is very allergic is bound to the tissues affected by rheumatic fever. The same changes that one sees after collagen disruption due to other causes may then occur in the damaged myocardium and particularly the heart valves. The reason why streptococcal lesions occur in specific locations could be because of the close chemical similarities between streptococcal products and human host tissues, causing the products to bind to those tissues, ultimately resulting in lesions. Even though streptococcal antigens have been undetected in rheumatic lesions, this finding is open to interpretation, because immunological cross-reactions between group A streptococci and host tissues may obscure the identity of the streptococcal antigens (249).

Experimental evidence has been put forth to support the above theory. This evidence encompasses both humoral and cellular immune aspects.

**(i) Supportive experimental evidence—humoral immune aspects.** Immune complexes were detected in the sera of patients with ARF and carditis or chorea by Williams et al. (290). The techniques used were the Raji cell radioimmunoassay, originally described by Theofilopoulos et al. (265, 266), and the Clq solid-phase assay, performed according to a modification of Hay and co-workers (100). Such complexes were evident during the early phase of rheumatic activity. Patients' articular or periarticular complaints were ephemeral. Severe pain, heat, swelling, and minimal degrees of effusion were present on one day and gone the next. Studies in the past on joint fluids from such patients have revealed no signs of inflammatory synovitis. Hence, it is quite possible that circulating immune complexes do not account for all articular manifestations or joint symptoms during ARF (290).

It is noteworthy that in the above studies there was a failure to demonstrate streptococcal or other definite antigens in the complexes themselves. Attempts to visualize streptococcal anti-

gens on the Raji cell by direct or indirect immunofluorescence, using a variety of heterologous antistreptococcal antisera, had proved negative (290). However, there are now data indicating that circulating immune complexes in rheumatic fever do indeed contain streptococcal antigens (74).

**(ii) Supportive experimental evidence—cellular immune aspects.** There is a large body of evidence supporting the role of humoral immune mechanisms in ARF; however, relatively little is known of the role of cellular mechanisms in the disease. It has been noted (194, 207, 208) that ARF does not tend to occur in children before the age of 3 to 4 years, suggesting that the disease can be induced only after repeated streptococcal infections. Humphrey and Pagel (105) demonstrated that ARF patients have a heightened skin test reactivity to streptococcal antigens when compared with nonrheumatic controls. Stollerman (246) observed that ARF patients who were free of intercurrent streptococcal infections for 5 years after the initial attack rarely developed a second attack. This is another indication that repeated exposure to the streptococcus may be important in the development of the disease. These above-mentioned observations indicate that cellular reactivity to streptococcal antigens might also play an important role in the pathogenesis of ARF and carditis (213).

Murphy and Swift (183) produced experimental rheumatic-like cardiac lesions in rabbits by repeated skin infections with group A streptococci. According to them, crucial factors in this induction were repeated infections and heightened cutaneous reactivity to streptococcal products in these animals. More recently, using the in vitro technique of direct capillary migration inhibition, Read et al. (212) demonstrated that ARF patients were highly reactive to streptococcal antigens when compared with normal subjects. Although heightened cellular reactivity was obtained to both cell wall and membrane antigens, the latter elicited a greater and longer-lasting reactivity. Furthermore, Williams and co-workers (289) have noted that during the acute phase of the disease there was an increase in the number of lymphocytes primarily reactive with streptococcal membrane antigens. Reid et al. (213) reported that rheumatic fever patients exhibited a heightened reactivity to streptococcal antigens when compared with nonrheumatic controls. It was also shown that membrane antigens from strains associated with ARF were more stimulatory to the patients than were membranes from strains that caused nephritis.

Long (157) suggested that heightened reactivity to streptococcal antigens was due to a delayed hypersensitivity reaction. Tissue culture techniques were used to test the theory that cell damage in post-streptococcal diseases might be due to hypersensitivity of the delayed type (65, 281). The effect of streptococcal extracts on leukocytes was observed in vitro (65). It was found that there was no difference in the reaction of cells obtained from rheumatic fever patients or normal subjects (65). On the other hand, disintegrated streptococci inhibited the growth of fibroblasts obtained from skin and heart of patients with rheumatic fever, but not of those from healthy individuals (281).

Migration inhibition studies were conducted with streptococcal filtrates and extracts and leukocytes obtained from guinea pigs injected with group A streptococci in Freund adjuvant. Results indicated that leukocyte migration was inhibited by these streptococcal products (96). The nature of the filtrates used is unclear. No cardiac or renal lesions were seen in any of the animals immunized with streptococci. Since leukocyte sensitivity in the guinea pigs occurred only after the streptococci were injected with Freund adjuvant, the relationship of this model system to human diseases is questionable.

Lymphocytes obtained from rats sensitized to streptococci in complete Freund adjuvant caused distinct cytopathic changes in tissue cultures of rat hearts (75). Myocarditis was induced in rats that received lymphocytes sensitized to rabbit heart. However, no transfer experiments were done with lymphocytes sensitized to streptococci. Thus, there is no clear correlation between the in vitro results of the tissue culture studies and the actual disease process. The use of Fruend adjuvant for sensitization also raises questions about the validity of these results.

Preliminary data reported by Hutto and Ayoub (106) indicate that lymphocytes from patients with acute rheumatic carditis are cytotoxic in vitro to cells derived from the left cardiac atrial appendage grown in tissue culture. Target cells used in the study were a mixture of cardiac cells and fibroblasts. Results showed that there was definite target cell specificity. Target cells prepared from the first two subcultures were susceptible to the cytotoxic lymphocytes, as observed by the level of cytotoxicity achieved, 80 to 90%. It was found that when target cells were grown through three additional subcultures, they were no longer susceptible to lymphocyte cytotoxicity (106). The three additional subcultures had caused the cell monolayer to assume the characteristics of fibroblasts, which could have affected the degree of susceptibility. These findings are similar to those reported by Yang and co-workers (294) in studies with guinea pig cells. They suggest that whereas antigenic identity is maintained early in tissue culture, it is lost during successive subculture.

The observations of Halle and Wollenberger (98) on morphological and behavioral changes of embryonic and neonatal cardiac cells in tissue culture support the occurrence of these alterations (106).

These studies suggest that antibody or complement is not involved in cytotoxicity (106, 294). In their experiments, Hutto and Ayoub (106) did not test the sera of patients for the presence of antibodies to cardiac tissue. However, based on previous studies, one can assume that such antibodies were present (129, 274). Lymphocyte cytotoxicity was not enhanced by either homologous fresh plasma or serum. This suggests that antibody to cardiac tissue, with or without complement, does not participate in this cytotoxicity (106). It is quite possible that homologous plasma contains a blocking antibody that, by coating the large cell, inhibits access of the effector cell to the surface of the target cell, thereby resulting in a removal of cytotoxicity (84, 85).

These studies of Hutto and Ayoub (106) together with the earlier observations of Friedman et al. (75) and Yang and co-workers (294) suggest that lymphocyte-mediated cytotoxicity may play an active role in the pathogenesis of rheumatic myocarditis. Neither antibody nor complement appears to play a role in this activity in vitro, a fact that does not exclude their participation in vivo. However, the in vitro studies on the inhibitory activity of homologous plasma on lymphocyte cytotoxicity indicate that a circulating antibody may be blocking an antibody that mitigates the effect of cytotoxicity in vivo (106).

**Autoimmune theory.** For a disease to be classified as an autoimuune one, the following criteria must be met: (i) the demonstration of antibodies to host tissue in the area of injury, or the presence of lymphoid cells capable of reacting with or attaching to host tissue or cells; (ii) transfer of the disease with the patient's serum or lymphocytes; and (iii) production of the disease experimentally by immunization or sensitization with the specific tissue antigen (150).

For several decades investigators have pursued the theory that heart antibodies produced in rheumatic fever may cause carditis. Cavelti (33) described such antibodies in 1945. However, it was only after Kaplan's use of the fluorescence-labeled antibody method that modern interest was revived in the concept that autoimmune mechanisms were involved in the pathogenesis of rheumatic fever (48, 105, 118). The work supporting this view has been thoroughly reviewed (119, 120, 126). Evidence to support an autoimmune theory encompasses both humoral and cellular immune aspects.

**(i) Supportive experimental evidence—humoral immune aspects.** The two major lines of evidence put forth are concerned with the demonstration of heart antibodies in rheumatic fever patients and with cross-immunity of streptococcal antigens with heart tissue (249).

**(a) Heart antibodies.** Heart antibodies are gamma globulins specific for heart preparations, especially the sarcolemmal membranes. Complement component C3 is usually deposited in large amounts in those areas to which heart antibodies are bound. The frequency of occurrence of heart antibodies is higher in rheumatic fever patients who develop carditis than in those who do not (249). Less frequently, they also occur in patients with previous rheumatic fever, regardless of the presence or absence of residual heart disease (101). The frequency of such antibodies is very high (63% in some series) in patients who have recently undergone mitral commissurotomy (101, 126). These antibodies are true autoantibodies since they can be absorbed with the patient's own atrial tissue obtained by biopsy during the operation. Gamma globulin deposits in extremely high quantities have been found in the hearts of children who died of rheumatic carditis (123). The significance of such antibodies in the pathogenesis of rheumatic fever has diminished slightly by the growing understanding of autoantibody formation as a general response to tissue injury such as burns (249).

Cavelti (34) injected rats with a mixture of homologous heart extracts and killed group A streptococci and produced heart autoantibodies as well as myocardial lesions in them. Since Cavelti considered the antibodies to be at least partially directed against a component of connective tissue, the use of the term "autoantibodies" is justifiable. Pathological alterations in the valves and connective tissue of the heart occurred after the appearance of the antibodies in question. It was therefore assumed that these were heart autoantibodies. Since neither the specificity of group A streptococci in producing this effect nor the cytotoxic nature of such antibodies has been established, the role played by such antibodies in the initiation of tissue damage is not known. Several workers (104, 171, 196) were unable to confirm Cavelti's findings, despite following the same experimental protocol.

The induction of autoimmunity by immunization with homologous and heterologous cardiac tissue sometimes resulted in the appearance of cardiac lesions (interstitial myocarditis) which bore only little resemblance to the lesions of rheumatic carditis. The literature on this subject has been reviewed extensively (117, 126).

Streptococcal toxins have been postulated to alter heart tissues to create new hapten-antigen complexes, which, in turn, initiate the formation

of anti-heart immunoglobulins. That streptolysin O has a direct toxic effect on heart tissue ground substance indirectly supports this hypothesis. However, there is little factual support for a direct involvement of streptococcal toxins in rheumatic fever (12, 150).

Another possibility is that streptococcal products activate lymphocytes, especially at the sites of infection, with increased chances of mutation and activation of "forbidden clones" with autoimmune activity (84). A variation of this theme is that a streptococcal mitogen (261) could transform lymphocytes into blast cells which might damage the host tissue. This is analogous to the in vitro phenomenon of lymphocytes damaging syngeneic cells under the influence of nonspecific transforming agents (84). In fact, mitogenic activity has been found to be associated with streptococcal M1 protein, isolated from a type 1 group A streptococcus (197). This M1 protein stimulated human peripheral blood lymphocytes in short-term tissue culture to undergo a dose-dependent increase in DNA synthesis (197).

It has been shown that cardiac damage by rheumatic, traumatic, or ischemic heart lesions leads to antigenic changes which are manifested by the production of autoantibodies (59, 101, 109, 124). Myocardial infarction and post-pericardiotomy syndromes, for instance, have been associated with increased levels of heart antibodies (126). It is possible, then, that heart antibodies in rheumatic carditis represent the result of tissue injury rather than the cause of it. Some other immunological event could cause the release of native antigens or altered tissue substances to which the host is not immunologically tolerant, resulting in myocardial damage. In fact, this may even be a normal immunological mechanism for clearing damaged tissues (249).

**(b) Cross-reactions between streptococci and human heart.** The data demonstrating cross-reactions between streptococci and human heart lend more credence to the concept of the autoimmune pathogenesis of rheumatic fever (249). Kaplan's classic studies demonstrated the following. (1) Rabbit antisera against group A streptococci reacted with human heart preparations. Techniques used were immunofluorescent (118, 127) complement fixation and other serological techniques (72, 159, 298). (2) Goat antisera to human heart tissue precipitated streptococcal extracts (128). (3) Some human sera that contain precipitating antibodies against streptococcal extracts can be absorbed with human heart preparations (127). However, not all human autoantibodies to heart can be absorbed with streptococcal antigens (130).

The cross-reacting antigen(s) between group A streptococci and human myocardium has been under investigation by several research groups. Kaplan's work had implicated an M-protein-related moiety (118, 128) which was not the type-specific determinant itself. Zabriskie and Freimer (298) had produced cross-reacting heart antibodies in rabbits by using protoplast (cell) membranes of group A streptococci that were free of type-specific M-protein. Beachey and Stollerman (14, 16) showed cross-reactions between antibodies to non-type-specific M-protein and protoplast membranes. Similar studies were conducted by Widdowson and Maxted (288). Both antigens were shown to absorb heart antibodies from human sera (16). The possibility was therefore considered that the same antigen(s) was present on protoplast membranes as well as on the fimbriae-like structures that protrude through the cell wall and that contain M-protein (256). It has been speculated that membrane antigens (fimbriae) are present at the outer surface of the cell, a location that might contribute to their antigenicity. Fimbriae could possibly represent slender extensions of the membrane that penetrate the cell wall. This particular cellular location of the membrane antigens might explain why past attempts to clearly separate wall and membrane antigens have not been fruitful (166, 256). Dale and Beachey (51) recently showed that a purified preparation of group A streptococcal type 5 M-protein contained an antigen within it which elicited heart reactive antibody, implying that it was the component responsible for cross-reactivity. According to Kaplan (122) both the cell wall and the cell membrane may contain the same antigenic cross-reactive determinant, but the serological specificity of the absorption tests is determined by different carrier proteins in each case, analogous to carrier-hapten specificity (120, 249). Contrary to this, it may be possible that both the cell wall and cell membrane contain different cross-reactive determinants related to different antigens in myofiber and smooth muscle. A considerable amount of variation was observed in the relative efficacy of preparations from different streptococcal strains in permitting autoantibody absorption. This was especially true when the sera tested were undiluted or at low dilution.

Lyampert described four different antigens in acid extracts of streptococcal cells and four different myocardial antigens (158, 159). The heart-reactive antibody has been shown to be absorbed by washed heart homogenates, isolated sarcolemmal membrane, and acid extracts of heart (61). Cross-reactions have been described between glycoproteins of heart valves and C-polysaccharide of group A streptococci (90), between human valve fibroblasts and group A streptococcal cell membranes (122), and be-

tween streptococcal hyaluronic acid preparations and articular tissue (226). Kingston and Glynn (137) described cross-reactions of rabbit antistreptococcal sera with a number of tissue antigens, including fibroblasts of heart valves and skin, synovial membrane, astrocytes, and endothelial cells. Studies have also shown that streptococcal antigens cross-react with glomerular basement membranes (161) and with skin tissues (38). Streptococcal antibodies cross-react with both striated and smooth muscle.

The question then arises as to whether such a cross-reactive relationship has any pathological significance. Correlative studies were carried out in rheumatic fever patients for evidence of valve fibroblast antibodies with valvular disease. No evidence for such a correlation could be demonstrated (122). These results, according to Kaplan (122), do not necessarily rule out the possibility of a cross-reactive system in the pathogenesis of valvular disease. It may be that this disease represents an indicator system of a more directly involved cellular immune or combined humoral-cellular immune mechanism directed to valve fibroblast injury. Postmortem examination of rheumatic fever hearts has revealed the presence of massive deposits of immunoglobulins and complement in the myocardium (123, 125, 149, 299). Autoantibody to heart was detected in these immunoglobulin deposits by elution studies (121). Atrial appendage biopsies of patients with rheumatic heart disease have shown immunoglobulins to be deposited in focal sites, in and around cardiac myofibers and vessel walls (125). These observations are at least consistent with derivation of immunoglobulin deposits, in part, from autoantibodies (122).

Cardiac lesions have not been reported to occur in experimental rats and rabbits, in which streptococcal cross-reactive autoantibodies were induced by immunization with streptococcal membranes, cell walls, or whole cells (122). In analogous experimental studies, in which autoantibodies to hearts were induced in rats or rabbits by immunization with homologous or heterologous heart tissue homogenates, only scattered foci of muscle necrosis were observed (54, 124). Repeated doses of heart tissue were administered over periods of several months to maintain circulating antibody levels to heart for long periods of time. In spite of this, few lesions could be detected (122).

Studies by Zabriskie et al. (298, 299) do not show any clear relationship between the presence of heart antibodies in ARF and the development of rheumatic heart disease. Stollerman does not consider there to be a causal relationship between cross-autoimmune reactions and rheumatic carditis (249). Cross-reactions of microbial agent antigens with host tissues is very common in infections and may or may not have a bearing on the production of tissue injury. For instance, the cardiolipin host antigen cross-reacts with treponemal antigen without apparent heart damage in syphilis. This, in fact, forms the serological basis for diagnosis of this disease. On the other hand, cold hemolysis in syphilis can be regarded as an autoimmune disease (249).

**(ii) Supportive experimental evidence—cellular immune aspects.** Immune mechanisms in the inflammatory processes of rheumatic carditis have been implicated for 30 years. The autoimmune hypothesis of the etiology of rheumatic heart disease has lacked an adequate experimental model (295). Neither in vivo nor in vitro systems using cross-reacting antibody as an effector of pathogenesis have been successful (295). An alternate approach was put forth by Yang et al. (294) which involved the study of cell-mediated immune processes triggered by autoantigens of streptococci. They demonstrated a lymphocytotoxic mechanism in animals immunized with streptococcal cell membranes: immune injury could be demonstrated against embryonic myofibers in tissue culture exposed to sensitized lymphocytes (294).

Then, in 1980, an in vitro system was devised based on the fact that lymphocytes can be cytotoxic to allogenic target cells, expressing surface antigens to which the lymphocytes have been sensitized (295). Primary cultures of embryonic guinea pig cardiac myofibers were used as specific targets for adult guinea pig lymphocytes sensitized to streptococcal antigens. Results of these studies demonstrated that lymphocytes were cytotoxic to the target cells. Cytotoxicity was measured by $^{51}Cr$ released from target cells stimulated by lymphocytes sensitized in vivo with group A whole cells, cell walls, and purified protoplast membranes emulsified with complete Freund adjuvant. Lymphocytes sensitized to group C streptococcal antigens in complete Freund adjuvant or complete Freund adjuvant alone were found not to express much cytotoxic activity. Target cells of cultured fetal skeletal muscle, liver, or skin were relatively refractory to effector cell cytotoxicity. Immunofluorescence confirmed the existence of a cross-reactive antigen in membranes of the cultured heart cells and group A streptococcal cellular antigens. These data give some additional support to the autoimmune hypothesis of rheumatic carditis, particularly with the participation of cell-mediated immune mechanisms leading to tissue injury (295).

Rapaport et al. (210) showed that circulating antibody specific for group A streptococcal membranes caused an accelerated graft rejection. Kyogoku found that rabbit antisera to

sonicated streptococci were cytotoxic for rat heart cells in culture (142). On the other hand, Yang et al. (295) could not demonstrate any evidence for cytotoxicity in rabbit or guinea pig antisera prepared against whole organisms, ruptured cells, or protoplast membranes. However, it was shown that in the presence of guinea pig complement rabbit antiserum to guinea pig heart homogenates exerted a cytotoxic activity on cultured guinea pig heart cells (295).

The problem of antigen-binding cells has great relevance to the whole question of autoimmune disease (290). Previous studies of antigen-binding cells in both thyroid disorders and systemic lupus erythematosus had indicated the presence of a low, but definite, number of antigen-binding cells in normal subjects that were capable of binding to thyroglobulin or native DNA (290). Williams et al. (290) showed that the number of lymphocytes binding to autologous erythrocytes sensitized with group A streptococcal membranes increased greatly during the acute phase of both rheumatic fever and post-streptococcal glomerulonephritis (290). According to their studies, antigen-binding cells appear to be B-cells bearing surface immunoglobulin, at least a large proportion of them. Thus, B-cells exist with a potential for synthesizing various types of autoantibody. Further evidence along these lines comes from several laboratories working with polyclonal B-cell activators, such as lipopolysaccharides, or various bacterial or mycoplasmal antigens (20, 49, 99). It is therefore quite likely that various infections, or products of infectious agents, are capable of directly activating potentially harmful autoreactive B-cells on their precursors. This may well be the case with ARF (290).

Stollerman is of the opinion that autoimmunity is nothing more than an attractive hypothesis for the pathogenesis of rheumatic fever and that it is by no means the only possible one (249). One thing is certain, and that is that the pathogenesis of rheumatic fever is definitely not clear-cut and well defined.

## EXPERIMENTAL MODELS FOR THE PATHOGENESIS OF RHEUMATIC HEART DISEASE

To quote Lewis Thomas, "Perhaps nowhere else in the field of experimental pathology has a particular morphological lesion achieved the absolute eminence of the Aschoff body as the identifying hallmark of a disease with so little being known about either its nature or meaning" (267). A great deal of importance has been attached to the significance of the Aschoff body, the general consensus being that a satisfactory experimental model of rheumatic fever must demonstrate the presence of the Aschoff body. With this criterion in mind, it can be safely said that there are no available models for rheumatic fever (267).

In their effort to produce a satisfactory experimental model of rheumatic fever, pathologists have experimented with various manipulations carried out in accord with one or another of the dominant theories concerning the etiology of the disease. None of the experimental lesions have met the standards of classic pathologists, but some have the distinct advantage of being reproducible and open to experimentation. Other groups, believing that rheumatic fever is an immunopathological disorder, have tried to reproduce cardiac lesions by immunological modifications of the classical model of serum sickness (267).

Burch and associates (31) proposed a viral etiology of rheumatic carditis and used mice and cynomolgus monkeys to show that coxsackieviruses are capable of producing pancarditis, including valvular damage. This may mean that some human cases of so-called "chronic rheumatic heart disease," including mitral stenosis, may, in actuality, be the result of viral infections quite unrelated to the events in rheumatic fever.

A number of procedures have been followed in an attempt to find an experimental model for rheumatic fever, some of which include the injection of various fluids and tissues derived from ARF patients into laboratory animals. None of the lesions produced have been accepted as rheumatic (93). Swift (257) used ground-up skin nodules with no apparent results.

### The Murphy-Swift Model

Murphy and Swift (183, 184) initiated the most intensive study of streptococcal rheumatic carditis in the late 1940s, and numerous related studies have been done since that time (17, 18, 56, 179–182). This work has demonstrated that (i) when rabbits are infected by group A streptococci, they develop focal cardiac lesions characterized by acute and chronic inflammation; (ii) certain genetic strains of rabbits are more susceptible than others; and (iii) repeated infections with certain serological types by the intradermal route are more likely to produce lesions (267). These observations were confirmed by Kirschner and Howie (138) but not by Robinson (218).

Some of the drawbacks of this kind of an approach for yielding new information about rheumatic fever are that, first, the lesions have rarely occurred in more than a few rabbits. Second, it is quite impossible to predict in advance which animals will develop lesions. The lesions produced contain focal inflammatory cell aggregates, with occasional necrosis of the myofibers (which Murphy considers as the origin of the multinucleated cells of the Aschoff body). In

spite of this, they are not considered to be as significant as Aschoff bodies are in rheumatic fever (267). There is absolutely no question about the crucial role of streptococci in human rheumatic fever, but this does not obviate the need for an animal model with a much higher incidence of clear-cut lesions to allow study of the way in which the disease takes its course. A shortcoming of the Murphy-Swift model is that it has not been unequivocally ascertained that it was indeed the group A streptococcus responsible for producing the lesions in rabbits. A similar study of comparable duration and intensity should be conducted to determine the effects of repeated exposure of rabbits to other varieties of bacteria (267).

### Other Models That Use Streptococci or Their Components

Cardiac lesions have been frequently produced by using viridans streptococci (26, 40, 107) but they have not been regarded as rheumatic.

Streptococcal culture filtrates have been used, but again, whereas areas of myocardial necrosis, myocarditis, and endocarditis have been reported, the resemblance to rheumatic lesions has been slight (217). Kellner and Robertson (136) used crystalline streptococcal proteinase to produce myocardial fiber necrosis. Char and Wagner (37) produced nonrheumatic focal lesions with sonicated streptococci (146). Various group A streptococcal constituents have reportedly produced lesions, with some features of delayed hypersensitivity (267). Cromartie and Craddock (50) and Ohanian et al. (188) used streptococcal cell wall components to produce inflammation and granulomatous tissue reactions in various tissues, including the myocardium and heart valves.

Attempts have been made to induce rheumatic fever by the pharyngeal lymphatic route of infection, based on the fact that the disease does not occur after systemic streptococcal infections by other routes, including septicemia. Mice, rabbits, and monkeys injected with group A streptococci by the pharyngeal route developed scattered myocardial inflammatory reactions, with only slight resemblance to Aschoff bodies (267). Morse et al. (177) and Ginsburg and Trost (86) demonstrated lesions in rabbits subsequent to inoculation with group A streptococci. The lesions were characteristic of rheumatic fever and included myocarditis, endocarditis, and arthritis (267).

### Models That Use Immunological Procedures

**Experimental systems to elucidate the allergic nature of rheumatic carditis.** Myocardial lesions have been produced by sensitization to killed streptococci or streptococcal products, but these are not considered to be rheumatic (146). Masugi produced carditis and diffuse glomerulonephritis in 33 rabbits by injecting them with duck anti-rabbit heart sera (163). Rich and Gregory (214) and Kataumi et al. (133) produced rheumatic-like cardiac lesions in rats by carrying out experiments of the type used to induce serum sickness. However, the major type of lesion produced was a panarteritis. Experiments in which bovine albumin (79) and bovine gamma globulin (175) had been substituted for whole serum also produced similar lesions. Granulomatous endocarditis was produced in unilaterally nephrectomized rabbits by injecting them with massive doses of gamma globulin (176).

Thomas et al. (269) investigated the cardiac lesions of the generalized Schwartzman reaction in rabbits that received group A streptococci and found that the lesions did not resemble rheumatic carditis.

Cardiac lesions were produced in rats that received a mixture of rat heart extract and dead hemolytic streptococci (108). These lesions were characterized by primary myocardial fiber degeneration as well as secondary inflammation. When rabbit heart extract emulsified with paraffin was used to sensitize rabbits, it was found to produce localized degradation of myocardial fibers followed by chronic inflammation (109). When the antiserum was tested with extracts of various organs by a precipitation reaction in agar, it was shown that precipitation occurred only with myocardial and skeletal muscle extracts and not with any other organ extract. Kaplan, on the other hand, failed to produce antibody in rabbits by injection of fresh rabbit heart tissue coupled with Freund adjuvant (117). Gery et al. (80) conducted a similar experiment and produced antibodies with relative organ specificity, although cardiac lesions could not be produced. Majima and Otaka (160), using isologous heart homogenate as the antigen along with Freund adjuvant, succeeded in producing cardiac lesions in rabbits. However, the number of lesions produced was small and the incidence of such changes in the experimental animals was low (189). Pashinyan et al. (191) studied the effects of antilymphocyte serum on rats previously inoculated with group A streptococci and challenged with a second dose. Changes in blood characteristics were observed, as was the presence of bone marrow lesions, but the value of such changes from a diagnostic standpoint is arguable.

**Autoimmunization models.** No in vivo or in vitro model system that uses cross-reacting antibody as an effector of pathogenesis has been successful to date (267). A few investigators have used heart antigens or streptococcal prod-

ucts for immunization, together with Freund adjuvant (33, 34) and have produced myocardial inflammatory lesions and myofiber necrosis. The major drawback to these experiments has been a lack of reproducibility (267). Yang et al. (295) presented an in vitro model system comprised of primary cultures of embryonic guinea pig cardiac myofibers which constituted specific targets for adult guinea pig lymphocytes sensitized to streptococcal antigens. In this model, cell-mediated autoimmune mechanisms were suggested to explain the inflammatory processes of rheumatic carditis.

### Virus Models

Several research groups have considered the possibility that infectious agents other than group A streptococci may be involved in the etiology of rheumatic fever (267). A number of attempts have been made to demonstrate the presence of viruses in rheumatic carditis and to reproduce the disease by the injection of various filterable agents. All of these attempts yielded negative results with the exception of that of Schlesinger and others (228), who claimed to have detected viral particles in rheumatic lesions. The claims have apparently not been subsequently confirmed (267). Burch and collaborators (31) suggested that all cases of rheumatic valvular disease may not have resulted from an initiating infection with group A streptococci. Many cases of chronic valvular disease occur without a prior history of ARF, which is substantiated by the lack of serological evidence at the time of valve disease. According to them, it is a mere assumption on our part that because many cases of ARF progressively result in chronic rheumatic valvular disease, all cases of the latter must have arisen from the former. Burch contends that this may not be the case. He used coxsackieviruses to infect mice and cynomolgus monkeys and showed, quite convincingly, that coxsackieviruses produce myocarditis, pericarditis, and valvulitis (254).

There appear to be two major possibilities, therefore, that need to be investigated further. (i) There may be some instances where virus infections (especially coxsackievirus) of human beings may be followed by chronic heart disease which have been misinterpreted as the sequelae of ARF; (ii) The evidence showing that this virus has a predilection for the valves as well as other parts of the heart suggests that the experimental pathology of rheumatic fever itself needs a more thorough examination (267).

It is quite possible that, if a viral infection were under way before a streptococcal infection occurred, the reactivity of the heart to the latter may be quite different. A worthwhile line of investigation would be to study the pathogenesis and results of experimentally arranged complex infections involving either viruses and streptococci or viruses and streptococcal L-forms. Studies of this nature have been conducted in the past with influenza virus but not with coxsackievirus. Results of these studies were not encouraging, but perhaps the coxsackievirus family possesses tissue tropisms that are more relevant to rheumatic heart disease (267), warranting further exploration of the concept.

## IMMUNOGENETIC ASPECTS OF RHEUMATIC FEVER

The concept of a genetic susceptibility to rheumatic fever on the part of the host is based on the following observations: (i) the pattern of inheritance of rheumatic fever in families is suggestive of a dominant gene with limited penetrance (262); (ii) the fluctuating rate of streptococcal infections has no effect on the incidence of the disease, which remains a constant (200); (iii) rheumatic fever-susceptible individuals exhibit an unusual cellular and humoral response to certain streptococcal antigens (212) that share antigenic determinants with mammalian tissue antigens (118, 299).

The laws of transplantation and the concept of histocompatibility were formulated by Snell, who together with Goren discovered the major histocompatibility complex, viz., *H-2*, in mice (255). Each vertebrate system has its own major histocompatibility complex together with a large number of minor histocompatibility systems. The HLA region is also known as the major histocompatibility system and refers to a genetic region on a chromosome which plays a dominant role in the survival of grafted tissue. The letter H in HLA designates human, and L designates leukocytes—the first cells shown to carry antigens of this complex (52, 53). The letter A originally stood for the designation of a locus (195). There are currently four loci for the leukocyte specificities—A, B, C, and D (286). The A, B, and C loci may be detected serologically, whereas the D-locus antigens are identified in a one-way mixed-lymphocyte culture reaction (195). Other characteristics such as specific erythrocyte groups, as well as complement factors (4, 76), have been found to be closely linked to HLA. The HLA-antigen determinants are located within the surface membranes of a cell and are present on leukocytes, including both T and B lymphocytes (292), on platelets, and indeed on all nucleated cells that have been examined. The antigens or their components may be shed into the plasma and other body fluids during metabolism. They develop early in fetal life and are present throughout the lifetime of an individual. Their biological functions are most likely related to cell recognition and immunolog-

ical response (23). The HLA system has been used to provide genetic markers for the study of linkage and to study genetic relationships of human populations (195). B-cell alloantigens are recognized by testing with alloantibodies that develop as a result of immunization with paternal antigens during pregnancy (192). Since most of these alloantisera recognize alloantigens that are extensively homologous with the region I antigens of the murine histocompatibility system, they are referred to as "Ia-like" or simply "Ia" (111). A few of the Ia alloantigens that are closely related to HLA-D alleles, have been designated HLA-DR (192). Other B-cell alloantigens appear to be quite unrelated to HLA-D alleles and seem to be particularly useful in demonstrating the immunogenetic associations of particular diseases (81, 291).

There appears to be a marked excess of individuals with A and B blood groups and a deficiency of ABH secretors in patients with rheumatic fever and rheumatic heart disease (178). There is a slightly increased frequency of Lewis group secretors (192). In spite of these observations, no specific marker in the patient group has been successfully identified as associated with rheumatic fever (192). No consistent difference was observed in the distribution of HLA-A and -B allotypes when compared with controls (62, 185, 211).

Brewerton and Albert contend that post-streptococcal rheumatic fever is not related to B27 and that the possibility of an association with other HLA antigens is controversial (29). Falk et al. (62) reported an increase of shared antigens in the parents. This was followed by the finding that there was an increase of BW17 in Europeans in New Zealand (35). In their study of 80 patients before cardiac surgery, Joysey et al. (112) found an increase of BW15, whereas Leirisalo et al. (153) detected a slight increase of BW35 and a decrease of B5 in recurrent cases. This is interesting in light of the report by Greenberg et al. (89) that individuals with B5 are more likely to have increased in vitro responses to streptococcal antigens.

Recent studies have revealed the existence of a genetically intricate system of serologically defined alloantigens that are selectively expressed on B lymphocytes. These alloantigens are chemically similar to the murine Ia molecules (193). Patarroyo et al. (193) discovered a particular B-cell alloantigen and found a significant association between it and patients who developed rheumatic fever. It conferred a relative risk of 12.9, evident in two distinctly different clinic populations, one from New York City and the other from Bogota, Colombia. The following studies were used to detect B-cell alloantigens: (i) indirect immunofluorescent tests were performed on mononuclear cells of rheumatic patients, the cells having been stimulated by pokeweed mitogen for a period of 4 to 6 days before the tests were conducted (293); (ii) B cells isolated by depletion of T lymphocytes were used in a modified Amos two-stage microcytotoxicity test (81).

A panel of 40 cell lines from individuals homozygous for defined D-locus alleles was tested for the presence of the B-cell alloantigen. No positive results were obtained (191, 193). However, the fact that it occurs among D-locus heterozygotes makes it seem likely that the B-cell alloantigen is linked to a D-locus allele that is as yet unrecognized (193).

The manner in which HLA-disease associations occur remains unresolved. Since the immune response genes are located between the HLA-B and -D loci (79, 155, 168), it could be speculated that the disease susceptibility gene might be a defective immune response gene which, on contact with an environmental factor such as an infectious agent, gives rise to a pathological immune response, resulting in disease (29). Read et al. (211) postulate that the individual could be either hyperresponsive or hyporesponsive to one or several streptococcal antigens. For instance, if there were a poor protective response to group A streptococcal M-protein antigens, streptococcal infections would be eradicated rather inefficiently. This would result in prolonged antigenic exposure, and the individual might ultimately react with a selflike cross-reactive antigen, with the production of appropriate pathology.

## CONCLUDING REMARKS

Decades of research in experimental pathology aimed at reproducing rheumatic-like cardiac lesions have not yielded conclusive information on the mechanism of this disease. It may be possible that the experimental animals are simply incapable of developing Aschoff bodies in response to streptococcal infection, for reasons not understood. Or it may be that the problem has been oversimplified by the various theories propounded, in particular when it is implied that the right kind of streptococcal infection accompanied by the right kind of immunopathological host response will give rise to rheumatic heart disease (267). The relative roles of cellular and humoral immunity in this pathogenesis needs to be delineated. Viral factors in the etiology of rheumatic fever provide another avenue to be explored. The possibility that genetic factors play a role in the host immune response by predisposing patients to the development of rheumatic heart disease subsequent to group A streptococcal infection cannot be ignored. Unravelling the mechanism of HLA-disease associ-

ations would most definitely be productive in terms of acquiring new knowledge about the pathogenesis of these diseases, especially that of rheumatic fever. Perhaps if the pathogenesis of rheumatic fever and rheumatic carditis was elucidated, it would lead to novel approaches to prophylaxis and therapy.

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