MEDICAL PRACTICE

Scientific Basis of Clinical Practice

Immunopathological Basis of Some Connective Tissue Disorders

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In the past two decades it has been established that patients suffering from some forms of rheumatic diseases (rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus, and related conditions such as progressive systemic sclerosis, Sjögren's syndrome, and dermatomyositis) show a high prevalence of immunological abnormalities. Some of the tissue lesions of these diseases (vasculitis, fibrinoid necrosis, lymphoid hyperplasia) resemble those accompanying experimentally produced allergic reactions. Nevertheless, the pathogenesis of the immunological abnormalities in human rheumatic diseases is still not fully understood. In view of the granulomatous nature of certain of the tissue lesions the hypothesis most widely favoured in the past, and still current, is that "cryptoinfection" by a viable external agent might be the initiating or persistent cause of some of these diseases and that this agent might give rise to the phenomena of hypersensitivity or autoimmunity as a concomitant but prominent component of the disease process.

The precise nature of the agent in each disease process is still conjectural. The range is still extending of the bacteria shown to be capable, as variants of their life cycle, of producing elementary infective particles ("L" forms). Some phages and "slow viruses" are now known to be able to enter cells and influence the host cell's genetic apparatus so as to induce the synthesis of "foreign" antigens. Certain strains of mycoplasma can produce a chronic polyarthritis in rodents and swine, while some viruses produce lymphoproliferative diseases with autoimmune accompaniments in several animal species.[1] Research is being actively pursued to see whether one or more of these agents may be responsible for some of the rheumatic diseases and what part they may play in inducing immunological phenomena in these conditions.[2]

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Rheumatic Fever

Perhaps the classic example of application of the concept that hypersensitivity may cause tissue reactions not directly attributable to bacterial invasion of the affected areas is provided by research on rheumatic fever.

Much work can be summarized by saying that it is now widely accepted that some of the manifestations of acute rheumatism are due to hypersensitivity to foreign antigens derived from Group A streptococci because of preceding streptococcal infections. Acute rheumatism is now relatively uncommon in Britain (perhaps because upper respiratory infections can be, and are, dealt with promptly by antibiotics). But Taranta[3] pointed out that, even in the heyday of its prevalence—though it had become axiomatic that *all* attacks of rheumatic fever followed streptococcal infections—nevertheless, only about 1-5% of streptococcal infections were followed by rheumatic fever. Patients who have suffered acute rheumatism show abnormally high levels of skin responsiveness to streptococcal extracts and high serum antibody titres to streptococcal antigens (streptolysin O, streptokinase, and streptococcal hyaluronidase). Nevertheless sensitivity is not confined to a sharply defined type of Group A strain nor to a particular antigen. Moreover, allergy of this kind is not in itself evidence for a causative role of hypersensitivity in the production of the granulomatous lesion (the Aschoff nodule of the myocardium) which is pathognomonic of the disease. It seems generally agreed that while certain tissue lesions resembling those of the natural disease can be reproduced experimentally by inducing hypersensitivity to streptococcal and other antigens, none of these is a convincing simulacrum of the Aschoff lesion.

On the other hand, other aspects of the acute illness may well be immunologically mediated since they resemble closely the

features of hypersensitivity to soluble antigens such as horse serum protein.

Acute serum sickness in man is characterized by fever, arthralgia, and joint and other serous effusions and sometimes by a rash identical with that seen in acute rheumatic fever. The nearest experimental immunological model appears to be the acute phase of so-called one-shot serum sickness. In this model after a single large injection of antigen, when antigen-antibody ratios approach equivalence, there is a sharp drop in serum complement and acute inflammatory lesions appear in the heart, blood vessels, kidneys, and joints.[4] Antigen, host complement, and host IgG (presumably as antigen-antibody complexes) are deposited in the lesions simultaneously with their development. The lesions themselves are characterized by endothelial proliferation, oedema, and focal tissue necrosis with "fibrinoid" change—resembling in these respects the lesions of acute rheumatic fever. But it is important to note that serum sickness is ephemeral; that the injured tissues may show no immunological relation to the initiating antigen; and that the process is not attended by granuloma formation.

Thus, while many of the acute features of rheumatic fever may be explicable as due to the damaging effect of circulating streptococcal antigen-antibody complexes (that is, a form of "immune complex" disease), the continuing process in the heart, which is the really serious outcome of this illness, cannot be adequately accounted for on this basis.

ASCHOFF BODY

The feature to which most attention has been paid, in seeking an explanation for the cardiac localization of subacute and chronic rheumatic fever, is the immunological cross-reactivity which the antigens of certain strains of group A streptococci exhibit with human heart tissue. This has given rise to two alternative hypotheses about the nature and origin of the Aschoff body.

The first is that the Aschoff body is the outcome of an autoimmune process caused by the cross-reacting antibodies to the streptococcal antigens producing damage to the immunologically similar antigenic material in heart muscle.[5,6] Against this is the observation that antibodies against heart antigens occur after myocardial infarction[7] and operations on the heart (that is, appear to be the result rather than the cause of myocardial damage) and Kaplan's own observations[8] that, in material taken from cases of rheumatic carditis, deposits of immunoglobulins and complement are demonstrable in association with sarcolemma, subsarcolemmal sarcoplasm, and structures in vessel walls but *not* in Aschoff bodies.

The alternative view is that cellular and immunological mechanisms eliminate streptococcal strains unrelated to those of human cardiac tissue in most people but are less effective in eliminating cross-reactive strains—which therefore persist as decapsulated and degraded (L-forms) in the heart in some people, being tolerated as "self" antigens at this site. In this latter view the Aschoff body is simply an indolent granuloma due to the persistence of infective particles of relatively low virulence in the tissues. In support of this, it was shown[9] that round or ovoid structures (resembling morphologically the appearance given by certain known L-forms of Group A streptococci following their ingestion by guinea-pig macrophages in culture) could be found in Aschoff cells in biopsies from the left auricular appendage from patients subjected to mitral valvulotomy for chronic rheumatic carditis. To confirm this possibility further by isolation of the organisms demands that necropsies should be conducted with strict aseptic precautions and cultures made using techniques and media suitable for the primary isolation of L-forms. This evidence is now difficult to obtain because of the considerable reduction in the total incidence of rheumatic fever, and in particular, of fatal cases of the florid illness.

Rheumatoid Disease

Evidence of autoimmune phenomena is even more prominent in rheumatoid disease, taking the form of autoantibodies such as the rheumatoid factor (an antibody to denatured γG globulin) and a range of anti-tissue antibodies (see Table II). As in the case of rheumatic fever, there is controversy whether such autoantibodies might be the mechanism for perpetuation of a disease process triggered off initially by an external agent, or whether they are merely epiphenomena, accompanying the disease but not central to its pathogenesis.

The clinical course, the nature of the pathological lesions, and the serological changes would all be quite in keeping with an infection by an organism capable of persistence against a background of varying reactivity. For example, in relation to onset, this may be an acute illness (especially in the juvenile form, Still's disease) initially without joint changes. More often the onset is insidious, with periods of exacerbation and remission in which arthritis becomes a progressively more important component of the clinical picture. But though arthritis becomes the component demanding attention and treatment, since it is, to the patient, the prime cause of pain and disability, it must be stressed that, whatever the mode of onset, other symptoms such as loss of weight, anaemia, a rise in the erythrocyte sedimentation rate and in the level of serum proteins, and changes in the skin and bones all attest to the presence of a generalized disease.

The frequently intermittent progression of the disease may also be paralleled by many known chronic infections—for example, brucellosis, leprosy, and tuberculosis. We are woefully lacking in our knowledge of the crucial factors affecting host/parasite relationships, which presumably govern both the occurrence and the course of infective disease. At one end of the scale, we recognize that the herpes simplex virus can establish an almost perfect symbiosis with the host—while, at the other end, we are aware of highly virulent organisms producing clinical symptoms in a high proportion of those attacked. But in between these extremes, immunological methods such as skin tests have shown that for certain organisms, despite a high attack rate, the occurrence of frank clinical disease may be relatively low (for example, brucellosis, tularaemia, and even tuberculosis). At present we do not know which antigen to use for this kind of screening purpose in rheumatoid disease, though possibly some examples of seronegative "benign" polyarthritis may be examples of the successful elimination of the postulated initiating agent.

The histopathological lesions as seen in the rheumatoid nodule or in the affected joint are not in themselves pathognomonic, again presenting the picture of a granulomatous process. A granuloma, in pathological terms, is a lesion associated with the presence in the tissues of indigestible material that cannot be removed by polymorphs. The continuing presence of such material usually stimulates the arrival of macrophages and lymphocytes, the latter often becoming "sensitized" to the foreign material. The foreign material itself may be living in origin—that is, bacterial or fungal—or even non-living such as silica or beryllium salts.

Where material is thus not eliminated and a chronic granuloma is established there are several known accompaniments:

Humoral.—In addition to antibody specific to the antigen, with continuing hyperimmunization due to persistent foreign material and continuous tissue breakdown there develop antiglobulins and anti-tissue antibodies (Tables I and II).

TABLE I—*Incidence of Rheumatoid-factor-like Antiglobulins in Nonrheumatoid Conditions*

Condition	Reference*
Incidence between 10 and 20%	
Trichinosis	Epstein *et al.* (1957)
Acute viral hepatitis	Ziff (1957)
Rubella	Johnson and Hall (1958)
Syphilis	Peltier and Christian (1959)
Sarcoidosis	Kunkel *et al.* (1958)
Kala-azar	Kunkel *et al.* (1958)
Chronic hepatitis (? viral)	Kunkel *et al.* (1958)
Leprosy	Cathcart *et al.* (1961)
Tuberculosis	Singer *et al.* (1961)
Viral influenza	Svec and Dingle (1965)
Trypanosomiasis	Klein and Mattern (1965)
	Houba and Allison (1966)
Incidence between 50 and 65%	
Subacute bacterial endocarditis	Witebsky and Milgrom (1960)
	Williams and Kunkel (1962)
Interstitial pulmonary fibrosis	Turner-Warwick and Doniach (1965)
	Ward and Stalker (1965)

*For full references see Walton

TABLE II—*Incidence of Anti-tissue Antibodies in Rheumatoid Arthritis (R.A.) and Systemic Lupus Erythematosus (S.L.E.)*

Antibody	Percentage Incidence		
	Normal	R.A.	S.L.E.
Anti-nuclear factor	2	24	98
Anti-mitochondrial	2	8	12
Anti-smooth muscle	0	4	0
Anti-cardiac muscle	1	22	15
Anti-epithelial basement membrane	2	28	2
Anti-duct epithelium	10	21	10
Anti-endothelial basement membrane	4	11	15

Cellular.—The sensitization of lymphocytes, presumably circulating through the sites of lesions, produces the skin reactivity of delayed hypersensitivity. This may wax and wane (anergy) with the progress of the disease.

All these aspects of the immune response, which are present in greater or lesser degree in known chronic infective conditions, are seen in rheumatoid arthritis.

Other.—Another concomitant of chronic infection and hyperimmunization is the development of hyperglobulinaemia and of amyloidosis. This feature is again characteristic of rheumatoid disease of protracted duration.

Localization of Lesions

In active rheumatoid disease subcutaneous nodules tend to develop at points of pressure or friction. Active movement may also determine the involvement of joints—for example, joints are usually involved symmetrically in previously active people; on the other hand, in people in whom an arm or leg is paralyzed *before* the onset of rheumatoid disease the paralyzed member is spared, whereas the active limb shows arthritic change. So does altered vascular permeability in subcutaneous tissues or joints due to minor trauma allow the release into these sites of a damaging agent? Since typical subcutaneous nodules have been observed in cases of rheumatoid arthritis occurring in hypogammaglobulinaemic individuals most probably humoral antibodies or rheumatoid factor are not essential in the histogenesis of these lesions.

The granulomatous synovitis of rheumatoid disease can be mimicked by the instillation of poorly resorbed materials such as carrageenin.[10] But joints are sites capable of an immunological response—in other words, animals can be sensitized to several antigens solely by intra-articular instillation. Glynn and his associates[11-13] have shown that after a single injection of bovine serum albumin or heterologous fibrin into the joint of *pre-immunized* animals a persistent arthritis can be induced, accompanied by evidence of autoimmune phenomena. This has led to the suggestion that, though the primary process may be a simple inflammatory one due to some extrinsic cause, the persistence of the process is mediated immunologically in animals with an intact immunological response mechanism.

Systemic Lupus Erythematosus (S.L.E.)

This is a multisystem disease characterized by a plethora of autoantibodies active against erythrocytes, leucocytes, platelets, blood coagulation components, and cytoplasmic components (Table II). The most commonly encountered are those directed against nuclear constituents—for example, as the L.E. cell factor directed against nucleoprotein and giving rise to the L.E. cell test, or as antinuclear factor, which by immunofluorescence may give various nuclear staining patterns.

Almost certainly the antibodies directed against circulating cells and against clotting factors are pathogenic and are responsible for the haemolytic anaemia, leucopenia, thrombocytopenia, or the clotting defects which may occur in the disease. The feature mainly responsible for mortality in S.L.E. is the frequent occurrence of renal disease. The renal lesion shows variable pathological characteristics. There is now evidence that DNA-anti DNA complexes may contribute to the lesions which occur.[14]

During exacerbations of S.L.E. circulating DNA can be found in the serum.[15] and the appearance of this coincides with the decrease or disappearance of anti-DNA antibodies and serum complement.[16] This suggests the formation of immune complexes, which localize in the kidney and other sites [17]—that is, S.L.E. is another example of immune complex disease.

Hughes *et al.*[16] further showed that the release of DNA into the circulation is a fairly common phenomenon after major cardiac surgery and other processes involving tissue destruction. It has been shown that anti-DNA antibodies (antinuclear factor) and other anti-tissue antibodies increase with age, perhaps reflecting the increased "wear-and-tear" associated with age and degenerative processes. In S.L.E. there is often evidence of considerable tissue breakdown.[18] The cause of this is conjectural, but possibly a part may be played by virus-like particles, which have been found in renal and other biopsies from some cases showing active progression of the disease.[19 20]

An experimental model for S.L.E. in man is provided by a condition characterized by autoimmune haemolytic anaemia, renal disease, and systemic connective tissue lesions in a particular strain of mice (the New Zealand Black or NZB strain). Virus particles can be found in the tissues of these mice, while cell-free extracts of organs containing such particles induced lymphoid cell hyperplasia and renal changes in other strains of mice.[21] East *et al.*[22] showed that autoimmune reactions (such as positive Coombs tests and development of antinuclear antibodies) occurred even in NZB mice reared in germ-free conditions, suggesting possible transplacental transmission of an agent with "slow virus" properties.

In the pathogenesis of human S.L.E. the evidence suggests that genetic factors play a part, while in some people an S.L.E.-like syndrome may be induced by sensitization to some drugs—notably hydrallazine (Apresoline) and procaineamide. The overall immunological picture in S.L.E. may be regarded as a heightening of the normal process of response to tissue wear and tear by the production of anti-tissue antibodies—but to much higher titre and a much wider range of specificities than is encountered in health. Some pathological features (notably the renal changes) in S.L.E. are consistent with those of "immune complex disease." Nevertheless, the process actually initiating the tissue damage and setting the immunological responses in motion in so accentuated a fashion is still conjectural.[23]

Table I is reproduced from the *International Review of Experimental Pathology* with permission of the editor and publishers. I acknowledge gratefully the kindness of my colleague, Dr. N. Williamson, in allowing me to incorporate his unpublished data in Table II.

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