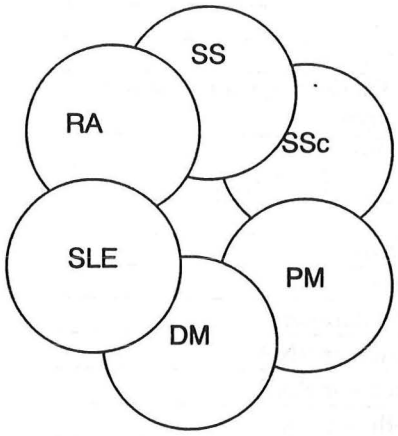
The Connective Tissue Diseases: Connected or Not?



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"...within the spectrum of lupus...there are examples of mixed forms of the disease which it is impossible to denote concisely without employing hybrid names"

Hutchinson, Br. Med. J., 1880

This to acknowledge that Salahuddin Kazi, MBBS has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program

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Acronyms in Rheumatology Used in this Protocol	
ACA	Anticentromere Antibodies
ANA	Antinuclear Antibodies
CREST	Calcinosis, Raynaud's, Esophageal Dysmotility, Sclerodactyly, Telangiectasia
CTD	Connective Tissue Disease
dcSSc	Diffuse Cutaneous Systemic Sclerosis
DM	Dermatomyositis
dsDNA	Double-Stranded DNA
ENA	Extractable Nuclear Antigens
HLA	Human Leukocyte Antigen
hnRNA	Heterogeneous nuclear RNA
hnRNP	Heterogeneous nuclear RNP
IIF	Indirect Immunofluorescence
IIM	Idiopathic Inflammatory Myopathy
La	La Antigen
lcSSc	Limited Cutaneous Systemic Sclerosis
LE Cells	Lupus Erythematosus Cells
MHC	Major Histocompatibility Complex
MCTD	Mixed Connective Tissue Disease
mRNA	Messenger RNA
PM	Polymyositis
PM/DM	Polymyositis/Dermatomyositis
RA	Rheumatoid Arthritis
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
Ro	Ro Antigen
rRNA	Ribosomal RNA
SCLE	Subacute Cutaneous Lupus Erythematosus
snRNP	Small Nuclear RNP
SLE	Systemic Lupus Erythematosus
SS	Sjögren's Syndrome
SSc	Systemic Sclerosis
ssDNA	Single-Stranded DNA
topo I	Topoisomerase I
tRNA	Transfer RNA
UCTD	Undifferentiated Connective Tissue Disease

My main impetus in preparing for these grand rounds was to scrutinize the concept of "mixed connective tissue disease", which has been the subject of much controversy since its first description by Sharp in 1972 [1]. This description of a disease that combined the features of several other well-established diseases forced rheumatologists to revisit the nosology of their diseases and to reflect on the demarcations that had been created. Overlapping diseases were increasingly recognized. These were further dissected with a two-fold objective: clearer delineation of diseases and their subsets and the discovery of etiologic clues.

My objective here is to explore this journey from its beginnings to the current prevailing concepts. To accomplish this task, I needed to ask and attempt to answer several questions: What constitutes a connective tissue disease (CTD)? What divides them, and with how much precision? Is there a unifying hypothesis for the CTDs? Should each disease be judged separately based on organ systems affected or should the CTDs be viewed as sharing a common etiology and pathogenesis? Are overlapping features indicative of such sharing or do they reflect incidental phenotypic convergence? Will the elucidation of a shared pathogenesis provide targets for more effective therapy?

Disease is a fact of nature. Diagnosis is an artefact constructed by human beings. Literally, the term 'disease' denotes a demonstrable lesion of cells, tissues, or organs; metaphorically, it may be used to denote any kind of malfunctioning, of individuals, groups, economies. Classic nosology was descriptive, based on somatic pathology. The diagnostician sought to anticipate and approximate the pathologist's findings at autopsy, with the aim of determining its material cause. Current nosology strives to be based on etiology. The subspecialties of medicine have had varying success in this endeavor. At one end lies the study of Infectious Disease, with well-characterized pathogens, dutifully fulfilling the postulates of Robert Koch. At the other is Psychiatry, still heavily dependent on diagnostic criteria, although increasingly peppered with advances in genetics and understanding of neurotransmitters. Rheumatology lies somewhere in between; still solidly embedded in classification by criteria, whilst increasingly employing knockout mice and transgenic animals, the modern surrogates for Koch's postulates, in an effort to base itself on firmer etiologic ground.

Evolution of the Concept of Connective Tissue Disease

Systemic lupus erythematosus is the prototypical connective tissue disease. The systemic nature of lupus was first recognized by Kaposi in 1872 [2], but the concept of lupus as a "connective tissue disease" was not uttered until 1942. Paul Klemperer, a pathologist at Mount Sinai Hospital in New York, is credited with this concept of "diffuse collagen or connective tissue disease". Driven by the lack of any distinctive features in autopsies performed on patients dying from lupus, Klemperer published a landmark paper in 1941 [3] that provided a detailed account of 35 autopsies of acute lupus erythematosus. He was struck by the ubiquitous "collagenization" of the ground substance in all tissues studied. Klemperer posed two questions: "Is there a common denominator in the localization of the process?" and "What is the nature of this process?" He noted that all elements of connective tissue (cells, fibers and ground substance) showed morphological evidence of injury. The mucoid ground substance, usually barely visible, became evident as a swollen homogeneous interfibrillar mass. The fibers were deeply eosinophilic and highly refractive (fibrinoid degeneration of connective tissue) and the fibroblasts underwent proliferation, degeneration and necrosis. Vascular changes, most commonly noted in the glomeruli were considered to represent "the most severe phase of connective tissue injury". In the spleen a "peculiar periarterial fibrosis limited to the central and penicilliary arteries" was consistently seen. Thus two types of alterations of collagen were recognized: fibrinoid degeneration and sclerosis. Klemperer admitted that "while often conspicuous, these features are not sufficiently distinctive to be considered characteristic". It was the "totality and universality" of these changes that lead Klemperer to conclude that "the morbid process in lupus erythematosus revolves about a well defined disturbance of collagen affecting all organs and tissues of the body". He further noted that such widespread changes in collagen "have been seen in no other disease save diffuse scleroderma". Klemperer refuted earlier concepts of lupus erythematosus as a single organ disease or as a diffuse disease of the peripheral circulation in favor of the concept of "widespread damage of collagen". The following year Klemperer articulated the concept of "diffuse collagen disease" in a commentary published in JAMA [4]. Rejecting the thesis of Morgagni that diseases reside in certain organs of the human body, and referring to the widespread changes in connective tissue in rheumatic fever,

lupus and scleroderma, Klemperer stated: "It is reasonable, therefore, to consider these maladies as systemic diseases of the connective tissues". He articulated further that "one may regard the connective tissues of the body as a whole as a well defined, widely dispersed colloidal system liable to a variety of injuries". Klemperer's suggestion of a non-organ directed systemic involvement of certain tissues as a common bond among identifiable disease entities led to a new perception of disease and indirectly prompted research efforts that have made enormous strides toward a more basic understanding of the connective tissue diseases. In 1949, the fourth edition of *Arthritis and Allied Conditions* listed the "collagen diseases" in a separate section. The authors suggested that these were diseases of mesenchymal origin and that skeletal and connective tissues were related to the cellular and humoral sources of immunity [5]. The Klemperer article of 1942 [4], in two short pages, legitimized an entire subspecialty.

What is a Connective Tissue Disease?

What have historically been referred to as the connective tissue diseases, with the exception of scleroderma, have as much to do with connective tissue as does any other disease. Perhaps the real connective tissue diseases would include keloids, Dupuytren's contracture, and genetic disorders of collagen and elastin. The term however, has "stuck". It has become firmly ingrained in the science and practice of rheumatology, which continues to be characterized by relatively uncommon, incompletely understood diseases. According to current wisdom, there are six diffuse connective tissue diseases (CTDs):

1. Systemic lupus erythematosus (SLE)
2. Systemic sclerosis (scleroderma) (SSc): limited or diffuse
3. Polymyositis (PM)
4. Dermatomyositis (DM)
5. Primary Sjögren's syndrome (SS)
6. Rheumatoid arthritis (RA)

What appears to unite the CTDs is their fundamentally autoimmune nature: the antigen driven production of autoantibodies, the major histocompatibility complex (MHC) and T-cell receptor restrictions, and the response to immunosuppression all provide strong circumstantial evidence for an autoimmune pathogenesis. What separates them from other autoimmune diseases, like type 1 diabetes, is their diffuse non organ-specific nature and their targeting of critical proteins of the nucleus. This division is by no means dichotomous; rather it is broad and overlapping.

In response to evolutionary pressures the immune system has developed tremendous diversity and intricate regulatory mechanisms to differentiate self antigens from foreign proteins. This is the principle of self tolerance. These delicately poised regulatory mechanisms are susceptible to seemingly minor perturbations that can lead to the abrogation of self- tolerance. Effector mechanisms, so efficient in eliminating foreign antigens, can paradoxically be recruited to propagate pathologic processes. The CTDs all involve the breakdown of self-tolerance, be it by failure of central deletion of autoreactive clones or by the loss of peripheral regulatory mechanisms. They share several epidemiologic and clinical features including a female preponderance, polyarthritis, Raynaud's phenomenon, myositis, interstitial lung disease, pleuropericarditis, and vasculitis (Table 1.). They also share autoantibodies and (MHC) associations. Additionally, each disease displays great heterogeneity in clinical expression. As a consequence rendering a specific diagnosis is often difficult, especially during the early stages of a disease. Thus concepts like "undifferentiated connective tissue disease"; "overlap syndromes" and "mixed connective tissue disease" have arisen and are the subject of lively debate. While advances in serology and immunogenetics have helped demarcate many of these diseases, new overlap syndromes have been spawned by such progress.

Discovery of the LE Cell

In 1943, Malcolm M. Hargraves, a hematologist at the Mayo Clinic, found what he termed "peculiar rather structureless globular bodies taking purple stains" in the Feulgen-stained marrow of a child with an undiagnosed illness. Similar findings in a three cases with SLE suggested that this finding was a feature of SLE. In 1948 Hargraves published his discovery of the LE cell [6]. Subsequently Hargraves also demonstrated the LE-cell in the buffy coat of centrifuged serum from patients with similar bone marrow findings. Haserick discovered that plasma from patients with SLE could produce the LE-cell after mixing with normal bone marrow [7]. The LE-cell was regarded as important advance in the diagnosis of SLE, especially in patients lacking characteristic cutaneous features. The LE-cell

or LE-phenomenon refers to observation of a mature neutrophil engulfing free nuclear material. The LE-cell phenomenon occurs in vitro during the incubation of peripheral blood or bone marrow aspirate. LE-factor, if present in the serum of the patient, can enter a traumatized neutrophil, and bind to the nuclear material, which swells and is extruded from the cytoplasm. The resulting free LE-body is engulfed by another neutrophil in the presence of complement. When highlighted by Wright's stain, the globular inclusion body appears as a homogeneous pale blue to purplish material, pushing the nucleus of the phagocyte to one side of the cell. The discovery of the LE-cell provided the impetus to the emerging concept of systemic lupus erythematosus as an autoimmune disease. Over the next decade, most of the historical connective tissue diseases moved steadily in to the autoimmunity sphere.

Table 1. Disease-specific and Overlapping Clinical Features of the CTDs

Disease	Frequency of ANA	Differentiated Features	Undifferentiated Features
SLE	99%	Glomerulonephritis	Pleuropericarditis
		Photosensitivity	Peritonitis
		Malar Rash	Certain Skin Rashes
		CNS Disease	Calcinosis
		Cytopenia	Non-destructive Arthritis
			Myositis
			Raynaud's Phenomenon
			Interstitial Lung Disease
			Pulmonary Hypertension
SSc	97%	Proximal Skin Thickening	Raynaud's Phenomenon
		Telangiectasia	Pleuropericarditis
		Sclerodactyly	Non-destructive Arthritis
		Esophageal Dysmotility	Myositis
			Interstitial Lung Disease
			Pulmonary Hypertension
			Calcinosis
PM	80%		Myositis
			Interstitial Lung Disease
			Raynaud's Phenomenon
			Non-destructive Arthritis
			Calcinosis
DM	80%	Heliotrope	Myositis
		Gottron's Papules	Interstitial Lung Disease
			Raynaud's Phenomenon
			Calcinosis
			Non-destructive Arthritis
SS	90%	Sicca Complex	Certain Skin Rashes
			Interstitial Lung Disease
			CNS Disease
			Non-destructive Arthritis
RA	20%	Erosive Polyarthritis	Interstitial Lung Disease

Autoantibodies

Reliance on the LE-cell for the diagnosis of lupus diminished after a few years, especially when its presence was demonstrated in rheumatoid arthritis, making it a no longer specific for SLE [8]. Additionally, the sensitivity of LE-cell for SLE diminished in when it was noted to be absent in a quarter of cases [9]. The LE-cell was replaced by the antinuclear antibody test (ANA) using the technique of indirect immunofluorescence [10]. From the beginning, several patterns of ANA immunofluorescence were demonstrated in the sera of patients with SLE and other connective tissue diseases [11]. These patterns reflect the heterogeneity of autoantibodies directed against discrete nuclear antigens (Table 2.). The next decade witnessed the resolution of this heterogeneity with the identification of the various nuclear antigens (Table 3.). Additionally, it was recognized that the sera of patients with the CTDs may also react against cytoplasmic antigens. These have been termed anticytoplasmic antibodies.

Table 2. ANA Immunofluorescence Patterns in the CTDs

Pattern	Related Antigen Specificities
Homogeneous	Chromatin, Histone, DNA, Ku
Peripheral or Rim	DNA, Lamins
Speckled	RNP, Sm, Ro, La, Ku, Topoisomerase I (Scl-70)
Nucleolar	RNA Pol 1, Fibrillarin, PM-Scl
Centromere	CENPs
Cytoplasmic	Ribosomal P, Aminoacyl t-RNA synthetases

Table 3. Milestones in the Discovery of Antinuclear and Cytoplasmic Antibodies

Year	Discovery	Reference
1948	LE Cells	Hargraves [6]
1957	Anti-DNA	Holman and Kunkel [12], Robbins et al [17], Seligman and Milgrom [18]
1966	Sm Antigen	Tan and Kunkel [13]
1969	Ro Antigen	Clark et al [14]
1971	nRNP Antigen	Mattioli and Reichlin [16]
1974	La Antigen	Mattioli and Reichlin [15]
1979	snRNPs	Lerner and Steitz [19]
1979	Anti-Topo I (Scl-70)	Douvas et al [20], Shero [21]
1980	Anti-Centromere	Moroi [22]
1984	Anti-Jo-1	Wasicek et al [23]
1985	Anti-Ribosomal P	Elkon et al [24], Francoeur et al [25]

In 1957, Holman and Kunkel [12] demonstrated that the basis of the LE phenomenon was antibody to chromatin (DNA-histone complex). Over the next fifteen years, using techniques of immunodiffusion, investigators discovered the Sm [13], Ro [14], La [15], and RNP [16] antigens.

Antibodies to Ro, La, Sm and RNP antigens often arise in grouped sets. This observation was initially made by Mattioli and Reichlin [26], when they demonstrated that the "nuclear RNA protein" (nRNP) and Sm antigen are physically associated. The Ro, La, RNP and Sm antibodies were suspected to react with RNA-containing complexes. A major breakthrough occurred in 1979 when Lerner and Steitz [19] established the molecular identity of the Sm and RNP antigens. They demonstrated that anti-Sm sera precipitated six small nuclear RNA molecules (snRNAs), while anti-RNA sera precipitated only two of these molecules. They argued that each of the six snRNAs exist in a separate small nuclear ribonucleoprotein (snRNP) complex. The work of Lerner and Steitz caused a reorientation of the field in the direction of molecular dissection of these autoantigens and provided a new understanding of the

biologic function of the snRNPs. In the 1980's it was established that the Sm and RNP complexes were central components in the splicing of precursor messenger RNAs.

A remarkable array of autoantigens have been characterized in the CTDs [27] and some of the important ones are listed in Table 4:

Table 4. Important Autoantigens in the Connective Tissue Diseases

Antigen	Structure	Function	Disease
dsDNA	Double Helix	Template for Transcription	SLE (40-70%)
Histone	H1, H2A, H2B, H3, H4	Components of Chromatin	Drug-induced SLE (95%) SLE (50-70%)
Ro (SSA)	52, 60kDa Proteins	Unknown	SLE (24-60%) SCLE (70-90%) Neonatal LE (>90%) SS (up to 95%) RA (up to 10%) PM/DM (5-10%)
La(SSB)	48 kDa Protein	Transcription Termination Factor	Generally accompanies Ro
Sm	B, B', D, E Proteins	Spliceosome Components	SLE (15-30%)
U1 nRNP	Small Nuclear RNA	Pre-mRNA Spicing	SLE (30-40%) MCTD (100%) SSc (up to 5%)
Ku	70 and 80 kDa Proteins	Repair DNA Termini	SLE (up to 20%) PM/SSc Overlap (26%) SS (20%)
Ribosomal P	Ribosomal Proteins	Protein Synthesis	SLE (10-20%)
DNA Topoisomerase I (Scl-70)	Topoisomerase I	Relaxation of Superhelical DNA	SSc (20-35%)
Centromere	CENP-A, B, C	Kinetochore Function in Mitosis	lSSc (60-80%)
PM-Scl	Nucleolus Protein Complex	Pre-Ribosomal Formation	SSc (2-5%) PM/SSc Overlap (24%)
Fibrillarin (U3 nRNP)	Nucleolar Protein	Ribosomal RNA Processing	SSc (6-8%)
RNA Pol I,II and III	RNA Polymerases	RNA Synthesis	SSc (5-45%) lSSc (6%)
Jo-1, PL-7, PL-12, SRP	Aminoacyl Transfer RNA Synthetase	"Charging" t-RNA	PM (up to 40%) DM (10-30%)
Mi-2	Undifferentiated Protein	Unknown	DM (10%)
hnRNP	A2/RA33	Post-translational mRNA Processing	RA (35%) SLE MCTD

Clustering of Autoantibodies: The Case for Epitope Spreading

While the characterization of autoantibodies in the CTDs have provided valuable tools for the diagnosis, prognosis and treatment of specific CTDs and their subsets, the application is confounded by their multiplicity and variations in test sensitivity and specificity. The clustering of autoantibodies and their occurrence across apparently clinically distinct syndromes raise interesting issues: Why do autoantibodies appear in linked sets? What clues do these phenomena provide in disease pathogenesis? Is there a linked thread between the various CTDs? If so, what is the basis of this apparent linkage?

Despite their abundance, autoantibodies in the CTDs are directed against a limited number of nuclear and cytoplasmic antigens. This suggests an antigen driven process. Interestingly, these autoantibodies often arise in grouped sets. Hardin hypothesized that these targets are available to the immune system as intact rather than as individual particles [28]. The suggestion was that once immune tolerance to the intact particles was broken down, the autoantibody response could diversify to the individual components via recognition of new epitopes within the intact complex. Until recently, direct evidence for this hypothesis in the autoimmune diseases was lacking. Most of the current knowledge in this area relates to systemic lupus erythematosus, but similar processes likely occur in the other CTDs as well as in other autoimmune diseases.

Immune focusing vs. diversification

There are two opposing tendencies that characterize the functioning of the immune system. On the one hand, there is the propensity towards immunodominance. On the other, there is a drive towards diversification and broadening of specificity. Immunodominance results from the multitude of steps involved in the process of antigen recognition and the shaping of the immune response to it. Macromolecules theoretically have hundreds of possible binding motifs, yet only a few ultimately succeed in gaining the attention of the immune system. Successive selections occur at the levels of antigen processing, presentation, and B and T cell responsiveness. Thus the resulting response no longer reflects the full potential of the immune system but rather is focused.

The opposite tendency, diversification of the immune response, is increasingly recognized as a critical process in autoimmunity. Diversification refers to the process by which an immune response to a whole antigen starts by recognizing a restricted antigenic focus and then broadens to recognize many epitopes. The term "epitope spreading" was introduced to describe how a self-directed response induced by a single peptide could spread to include other epitopes on the same autoimmunogen (intramolecular spread) as well as epitopes on other self molecules in the vicinity (intermolecular spread) [29]. Epitope spreading in autoimmunity was first described in experimental allergic encephalomyelitis (EAE), a murine model of multiple sclerosis. In this model, immunization of susceptible mice with myelin basic protein (MBP) induces a demyelinating disorder resembling multiple sclerosis. During the inductive phase of the disease, the T-cell response is initially directed to a single immunodominant MBP peptide, but the response eventually diversifies to include reactivity to several newly revealed cryptic peptides of MPB [30]. This switch from cryptic to revealed is thought to arise from cycles of antigen exposure and lymphocyte activation [29]. Epitope spreading is likely to depend on a number of factors, including the physical form of the antigen, genetic influences including MHC restriction, and levels of established immunological tolerance.

The vast majority of the literature has focused on epitope spreading in the autoimmune diseases. However, epitope spreading is a fundamental mechanism of the immune system that has evolved for the survival of organisms, and is not just a pathological mechanism in autoimmune processes. Ironically the same mechanisms that generate protective diversity may also amplify autoimmune pathology when the focus of the immune system is self-antigen or self-tissue.

Systemic Lupus Erythematosus: Self-Antigens and Epitope Spreading

SLE is a prototypic multiorgan systemic autoimmune disease. The disease usually begins with involvement of a few organs and evolves in to a multisystem disorder. For example patients may present with hematologic, skin or joint problems and later develop disease in brain, kidneys or other organs. Similarly, the autoantibody response may diversify over time. SLE is characterized by the presence of a wide variety of autoantibodies with distinct specificities. This diversity does not occur randomly. In fact the majority of autoantibodies recognize nucleic acids and proteins associated with DNA replication and transcription. Targets of antinuclear autoantibodies in SLE include ribonu-

cleoproteins (RNPs) such as small nuclear ribonucleoproteins (snRNPs) involved in the processing of precursor messenger RNAs; Ro and La cytoplasmic RNPs that help process the small RNAs, and Sm, that is involved in the splicing of pre-mRNA. Particularly important are autoantibodies directed against chromatin and it components, dsDNA and histones since they may be directly pathogenic.

Autoantibodies in lupus often arise as linked sets. Anti-dsDNA and anti-histone antibodies are typically seen together, and anti-La almost always accompanies anti-Ro antibodies. Over time, patients with SLE may produce autoantibodies that were not present at disease onset. Rabbits immunized with Sm antigen-derived octapeptides develop antibodies that not only bind these octapeptides, but also subsequently bind many other octapeptides derived from Sm. [31] Eventually the rabbits immunized with one octapeptide develop autoantibodies that bind other spliceosomal proteins. Any mechanisms that operate to maintain tolerance or anergy for the spliceosome are thus overcome. Features considered typical of human systemic lupus erythematosus are also found in these peptide-immunized animals, such as antinuclear antibodies, anti-Sm precipitins, anti-double-stranded DNA, thrombocytopenia, seizures, and proteinuria [31].

The aggregation of these autoantibodies and the source of the triggering autoantigens was a mystery until it was realized that most of the lupus target autoantigens are clustered in distinct structures at the surface of apoptotic cells [32]. These blebs contain nucleosomal DNA, Ro, La, and the snRNPs. Abnormalities in apoptosis in SLE have been demonstrated in both humans [33] and in mouse [34] models of SLE. Interestingly, nucleosome-specific antibodies have been demonstrated in patients with SLE without detectable anti-dsDNA antibodies [35]. Thus the following scheme can be proposed for the presence of multiple linked sets of autoantibodies in SLE: Due to as yet undefined genetic or environmental factors in SLE, there is an increased tendency for apoptosis which leads to the release of large numbers of nucleosomes. These are internalized by antigen presenting cells, which then process the individual components of the nucleosomes and present them to T cells. Each cycle of this process presents new epitopes to which specific autoantibodies are produced. Thus, the most evident explanation for the observed clustering and diversity of autoantibodies is epitope spreading. It is therefore unlikely that SLE is merely a collection of independent immune responses to individual proteins. The more plausible explanation is that the response originates in a single epitope and then spreads in an intra and intermolecular fashion to multiple related epitopes in a manner that is consistent with the concept of epitope spreading.

However, not all patients with SLE develop all autoantibodies or involvement of all organs. The clinical and autoantibody patterns usually fall into distinct subsets, some of which correlate with HLA and other genes [36-38]. The specificity of many such autoantibody subsets is shaped by the MHC Class II phenotype of the host. This influence of the MHC Class II molecules is important not so much in predisposition to autoimmunity, but in the shaping of the autoantibody repertoire of the individual and can direct autoimmunity to specific target organs.

Refining the Connective Tissue Diseases

There are several possible approaches that one may take towards understanding the protean clinical characteristics of the CTDs. The most popular approach is to consider the six clinically defined CTDs as distinct entities with clear demarcations. But given the frequent overlap of clinical, serologic and immunogenetic features, such a scheme would be fraught with indistinct boundaries, gray zones, and redundancy. A more interesting scheme would involve selecting specific immunologic features and tracing their associations with specific diseases. Autoantibody production and its relationship to specific genes of the major histocompatibility complex is the best-characterized immunological feature of the CTDs and is the format I have selected for this discussion. There are several justifications for such an approach. Dr Eng Tan has summarized this in four statements [39]:

1. The autoantibody response in systemic autoimmune diseases is antigen driven
2. Autoantigens are typically components of multimolecular subcellular particles
3. Autoantigens are involved in important cell functions
4. Autoepitopes are frequently functional regions or catalytic domains of subcellular particles

Advances in autoantibody characterization and in the immunogenetics of the CTDs have led to clearer definitions of these diseases. These advances have permitted an enhanced understanding of disease heterogene-

ity and have facilitated the elucidation of disease subsets. Autoantibodies have been increasingly associated with specific MHC Class II molecules and precise target organ involvement. While the CTDs continue to be mainly defined by clinical criteria, specific subsets more closely associated with MHC and autoantibody subtypes have emerged.

Disease Specific Autoantibodies

Disease specific autoantibodies are seen in systemic lupus erythematosus, in systemic sclerosis and in subsets of myositis. However they vary in sensitivity and thus their absence cannot be relied on to exclude these diseases. None of the other CTDs have truly disease specific autoantibodies, although some typically dominate the given disease in question.

Autoantibodies considered specific for SLE: Anti-dsDNA, Anti-Sm and Anti-Ribosomal P

Antibodies to DNA are of two general types, those that recognize single stranded DNA (ssDNA) and those that recognize double stranded (dsDNA) or native DNA. Anti-ssDNA antibodies are not specific for SLE and occur in many rheumatic diseases, as well as in normal individuals. Antibodies to dsDNA occur in 40-70% of patients with SLE and are considered specific for this disease. They are useful in establishing the diagnosis of lupus and are one of the three immunological disorders that appear in the American College of Rheumatology criteria for the classification of systemic lupus erythematosus, which were revised in 1997 [40; 41]. The further importance of these antibodies stems from their direct pathogenicity. Some types of anti-dsDNA antibodies cause glomerulonephritis. High titres of antibodies to dsDNA have been correlated with disease activity and with glomerulonephritis in many studies while others have shown that the association is weak (for a recent review see [42]). The first MHC Class II association with anti-dsDNA antibodies was reported with HLA-DR3 [43], and later with HLA-DR2 [44] and HLA-DR7 [45]. Interestingly three HLA-DQ alleles, DQ2, DQ6, and DQ3, which are in linkage dysequilibrium with HLA-DR2, DR3 and DR7, and all share an isoleucine in position 26, had the strongest association with anti-dsDNA antibodies, suggesting a critical residue for this autoimmune response [46].

Anti-Sm antibodies are also considered specific for SLE and like anti-dsDNA, are included in the American College of Rheumatology criteria for the classification of SLE [40; 41]. While very specific for SLE, they are insensitive, occurring in 20-30% of cases, although they occur more frequently (30-40%) in African-Americans and in Asians [47]. Clinical correlations are not strong but have been shown for renal and central nervous system disease in certain subpopulations [48]. An association with HLA-DR2 and more closely with a linked DQ6 subtype has been shown in African-Americans [49].

Anti-Ribosomal P antibodies have been found to be highly specific for SLE, but occur in only 15% of unselected patients, although frequencies are higher in Chinese patients [50]. These antibodies correlate with lupus psychosis [51; 52], lupus hepatitis [53; 54] and nephritis [53]. They have been found in association with HLA-DR2 and an HLA-DQ6 subtype

Autoantibodies considered specific for Systemic Sclerosis: Anti-DNA Topoisomerase I (Scl-70), Anti-Fibrillarin (U3RNP), Anti-RNA Polymerase I, II and III, Anti-Th/To, Anti-Centromere Antibodies

Antinuclear antibodies are detected in over 95% of patients with systemic sclerosis (SSc) [55] and interestingly seem to target the structures of the nucleolus. These autoantibodies are valuable tools for clinicians since they often correlate with specific subsets of patients with SSc and can provide helpful diagnostic and prognostic information. These relationships are summarized in the Table 5:

Table 5. Disease Specific Autoantibodies in Systemic Sclerosis and their Clinical Associations

Autoantigen	Associated SSc Subset	Associated Organ Involvement
DNA Topoisomerase I (Scl-70)	Diffuse Cutaneous	Pulmonary Interstitial Fibrosis and Peripheral Vasculopathy
RNA Polymerases I, II, III	Diffuse Cutaneous	Renal Disease, decreased frequency of Pulmonary Interstitial Fibrosis
Centromere Proteins (CENPs)	Limited Cutaneous	Raynaud's Phenomenon, Calcinosis and Telangiectasia, Pulmonary Hypertension
Fibrillarin (U3RNP)	Diffuse Cutaneous	Combination of isolated Pulmonary Hypertension and Diffuse Cutaneous Disease
Th/To	Limited Cutaneous	Puffy Fingers, Small Bowel Disease, Hypothyroidism

Adapted from Okano, 1996 [56]

Immunogenetic studies in patients with systemic sclerosis have also revealed interesting HLA Class II associations, especially with regard to anti-topoisomerase I. Initial studies had revealed associations with HLA-DR2 in Caucasians [57; 58], and with HLA DR5 in Japanese patients [59]. These alleles were shown to be in linkage dysequilibrium with several HLA-DQ allelic subtypes, all of which have in common tyrosine in position 30. [60]. This held true across Caucasian, African American and Japanese patients. The Choctaw Native Americans in Oklahoma, who have a high prevalence of systemic sclerosis also, showed a similar association with specific HLA-DQ alleles [61].

Anti-centromere antibodies (ACA) have been associated with limited cutaneous involvement or CREST syndrome and depending on the definition of this subset have varied in frequency from 44% to 98%, and are only rarely found in diffuse cutaneous disease. Patients with limited cutaneous involvement who are positive for ACA have a ten-year survival rate of 92% [62]. ACAs have also been detected in 25% of patients with Raynaud's disease (Raynaud's phenomenon without any other signs of connective tissue disease) [63]. Additionally, the presence of ACAs has been demonstrated in some patients with Sjögren's syndrome, rheumatoid arthritis and SLE, but all the patients in these studies had Raynaud's phenomenon [63-65]. This indicates that the specific finding with ACA correlates with Raynaud's phenomenon. ACAs are also found in up to 30% of patients with primary biliary cirrhosis [66], a disease whose hallmark is antibodies to the M2 mitochondrial antigen [67]. The majority of such patients also had clinical features associated with limited cutaneous variants of systemic sclerosis (Raynaud's phenomenon, telangiectasia, and calcinosis) [68]. Thus there seems to be a distinct entity of systemic sclerosis with limited cutaneous involvement and primary biliary cirrhosis characterized by the coexistence of ACA and anti-mitochondrial antibodies.

Myositis-Specific Autoantibodies (MSAs): The Anti-Synthetases, Anti-SRP, Anti1-Mi-2

In polymyositis-dermatomyositis, both considered part of the idiopathic inflammatory myopathies (IIM), more than 80% of patients have autoantibodies to nuclear and/or cytoplasmic antigens [69]. Approximately half of these patients have been shown to have myositis-specific antibodies [70]. These myositis specific autoantibodies (MSAs), with the exception of anti-Mi-2, recognize intracytoplasmic molecules involved in protein synthesis. Thus, they are not "antinuclear antibodies" and often display a diffuse cytoplasmic staining pattern on indirect immunofluorescence. The MSAs are particularly exciting since each autoantibody is associated with a specific clinical syndrome with a group of common clinical features, strong HLA associations, a characteristic disease onset and response to therapy.

There are several MSAs; the most important of which are the anti-synthetases (Anti-Jo-1 and others), anti-SRP and anti-Mi-2. The anti-synthetases are directed at aminoacyl-transfer RNA synthetase enzymes that catalyze the binding of amino acids to their cognate t-RNAs for incorporation into growing polypeptide chains. Nishikai and Reichlin characterized the first antisynthetase (anti-histidyl-tRNA synthetase) in 1980 [71]. It was named anti-Jo-1 after the first patient in whom it was discovered. Several authors [72; 73] then

described its association with specific clinical features, collectively known as the "anti-Jo-1 syndrome", characterized by myositis, interstitial lung disease, nonerosive arthritis, mechanic's hands, fever and Raynaud's phenomenon. With the discovery of other aminoacyl-tRNA anti-synthetases (threonyl-, alanyl-, isoleucyl- and glycyl-tRNA synthetases), the anti-Jo-1 syndrome was renamed the "anti-synthetase syndrome" [74]. The association of the antisynthetases with interstitial lung disease (ILD) is very strong. Patients with polymyositis are much more likely to have ILD (50%-100% vs. 10%) if they have antisynthetase antibodies. Occasionally, ILD is the dominant clinical problem, with scant [75; 76] or no evidence [77] of myositis

Two other MSA autoantibodies, anti-SRP and anti-Mi-2 have also been associated with specific syndromes. Anti-SRP is a cytoplasmic autoantibody directed against the signal recognition particle, which binds to the signal sequence of newly formed proteins. Patients with anti-SRP antibodies develop acute, severe myositis often with cardiac involvement [70; 78]. Raynaud's phenomenon, interstitial lung disease, arthritis and mechanic's hands are not seen. Anti-Mi-2 is unique amongst the MSAs in that it is directed against a nuclear antigen [79]. It is exclusively associated with DM rather than PM [70; 79]. Patients with anti-Mi-2 have classic dermatomyositis, with the 'V' and 'shawl signs', and cuticular overgrowth.

Table 6. Myositis-Specific Autoantibodies: Clinical and Immunogenetic Associations

Autoantibody	Frequency in IIM	MHC Class II Association	Clinical Features
Antisynthetases	Anti-Jo-1 (20%) Others (<5%)	DR3, DQA1*0501, DQA1*0401 [80]	Arthritis, ILD, fever, mechanic's hands, Raynaud's. Onset in Spring Moderate response to therapy
Anti-SRP	4%	DR5, DQA1*0301 [81]	Cardiac involvement Very acute onset Poor response to therapy
Anti-Mi-2	15% - 20% (DM only)	DR7, DQA1*0201 [82]	Classic DM with 'V' and "shawl" sign, cuticular overgrowth. Good response to therapy

The Recognition of Overlap Syndromes

Despite remarkable progress in understanding the pathophysiology of the CTDs, their precise etiology is best characterized as unknown. This has resulted in the continuous need to redefine these diseases, as new information becomes available. The formulation of internationally accepted diagnostic criteria allows for the selection of reasonably homogeneous patient populations for epidemiologic studies. But this approach has limited utility for the classification of individual patients seen in the clinic, which often depends on discerning particular clinical and laboratory patterns. The problems in rheumatology are heightened by the tendency of one disease type to merge into another. This has resulted in a continuous spectrum of clinical features among the CTDs. As many as 25% of patients with the CTDs exhibit overlapping clinical features. While this frequent overlap has created diagnostic dilemmas, it has also provided an opportunity to uncover etiologic and pathogenic clues for this mysterious group of diseases.

Autoantibodies that are not Disease Specific

Disease-specific autoantibodies have been very useful in providing a specific CTD diagnosis and in elucidating distinct disease subsets. Conversely, several autoantibodies are detected in more than one CTD and are rightly considered non-specific. However many of these autoantibodies define distinct shared or overlapping disease features. Analyses of large groups of patients have helped dissect some of these relationships.

Several such autoantibodies have conspicuous target organ and immunogenetic associations that have clarified the nature of these clinical intersections. These associations are best understood if the antibodies, per se, are directly responsible for the clinical manifestation. Evidence for such a phenomenon is rare. One exception is neonatal lupus, where anti-Ro antibodies may be directly pathogenic.

Anti-PM-Scl, Anti-Ku: Myositis/Systemic Sclerosis Overlap Syndromes

Low-grade muscle involvement is not uncommon in scleroderma, occurring in 50% to 80% of patients [83]. Usually, it is clinically insignificant. In some cases, overt myositis is evident. In such instances, two specific autoantibodies have been associated with SSc/PM overlap: anti-PM-Scl and anti-Ku.

The PM-Scl antigen appears to be involved in preribosomal formation. Anti-PM-Scl antibodies have been reported in 3% of patients with SSc, 8% of patients with PM and 50% of patients with the scleroderma/myositis overlap syndrome [55; 84]. Marguerie et al [85] identified 32 patients with anti-PM-Scl antibodies: all had Raynaud's phenomenon, 31 had SSc, 28 had PM, and 25 had ILD. All of these patients expressed HLA-DR3. In another study, 75% of patients with this autoantibody had the HLA-DR3-DQ2 haplotype, while two other patients had the HLA-DR7-DQ2 haplotype, suggesting that DQ2 (DQB1*0201) is the predisposing allele [86]. Anti-PM-Scl seems to occur uniquely in Caucasians. A large series of Japanese patients has failed to report anti-PM-Scl antibodies [87]. This absence may result from the rarity of the HLA-DR3 and HLA-DQ2 in the Japanese population.

The Ku autoantigen is a ubiquitous heterodimeric protein that binds dsDNA termini [88]. Anti-Ku antibodies were first described in Japanese patients with SSc/PM overlap [89]. Such autoantibodies were found in 55% of patients with the SSc/PM overlap syndrome and are associated with the HLA-DQB1*0501 allele [90]. In American patients the association seems to be with SLE and MCTD [91]. Interestingly, anti-Ku antibodies have been detected in 23% of patients with primary pulmonary hypertension [92].

Anti-Ro, Anti-La: Systemic Lupus Erythematosus/Sjögren's Syndrome Overlap

Anti-Ro/SSA antibodies are strongly associated with Sjögren's syndrome, systemic lupus erythematosus and neonatal lupus. Autoantibodies to Ro (SS-A) recognize a ribonucleoprotein complex composed of small single-stranded RNAs (hYRNAs) that are transcripts of RNA polymerase III [93]. Four molecular forms of this complex have been differentiated based on the nature of the peptide: a lymphocyte and an erythrocyte Ro with a 60 kDa peptide, a lymphocyte Ro with a 52 kDa peptide and an erythrocyte Ro with a 54 kDa peptide [94]. The function of the Ro complex remains unknown, but its ability to bind nucleic acids and the fact that it shares homologies with gene regulation proteins suggest that it may participate in RNA transcription processes. A number of environmental factors (exposure to ultraviolet radiation, viral infections) may cause translocation of the Ro complex to nucleocytoplasmic and membrane sites where it is not normally found, thereby leading to the development of autoimmunity.

Anti-Ro/SSA antibodies encompass several phenotypic syndromes that range from the asymptomatic states to systemic lupus erythematosus as shown in Table 7. There are several shared features, including cutaneous disease, cytopenia, and neonatal lupus syndromes. The HLA allelic associations of anti-Ro antibodies (DR2, DR3) are constant without regard to the clinical entity in which they occur. Patients with primary Sjögren's syndrome (SS) will often fulfill criteria for systemic lupus erythematosus (SLE) [95]. Subacute cutaneous lupus erythematosus (SCLE) can occur as an isolated skin disease or it can be associated with both disorders [96]. Neonatal lupus syndromes occur in asymptomatic anti-Ro donors or can occur in patients with either SLE or SS. The cutaneous lesions of neonatal lupus resemble SCLE. Patients with homozygous C2 deficiency develop a lupus-like picture with SCLE, mild systemic disease and almost uniformly express anti-Ro antibodies [97]. Persons with anti-Ro antibodies followed longitudinally can develop any of these disorders [98]. This suggests that SS, SCLE and SLE are not truly separable and most patients with anti-Ro antibodies can be found somewhere on a continuous spectrum of disease expression.

Table 7. Clinical and Immunogenetic Associations of Anti-Ro/SSA Antibodies

Disease/Subset	Frequency of Anti-Ro	Associated Features	Associated HLA Alleles
Asymptomatic Blood Donors	0.44%	Neonatal Lupus Syndrome	DR3, DQA1*0501, DQB1*0502 haplotype
Sjögren's Syndrome (SS)	70%	Lymphadenopathy, Vasculitis, Purpura, Cytopenia, Hypergammaglobulinemia	DR2, DR3
Systemic Lupus Erythematosus (SLE)	35%	Photosensitivity, Interstitial Pneumonitis, Nephritis, Cytopenia, Complement Deficiencies	DR2, DR3, heterozygosity for DQ1/DQ2 DQA1 alleles with glutamine at position 34
Subacute Cutaneous Lupus Erythematosus (SCLE)	80%	SLE (50%), SS(12%)	DR2, DR3
Neonatal Lupus Syndrome	95%	Congenital Heart Block, Neonatal Lupus Dermatitis, Hepatitis, Thrombocytopenia	DR3, DQA1*0501, DQB1*0502 haplotype
ANA Negative Lupus	60%	SCLE	DR3

Anti-La/SSB antibodies recognize an RNP involved in the correct and efficient termination of RNA polymerase III transcription [99]. Anti-La antibodies share many features with anti-Ro antibodies because of their co-occurrence in patient sera and the physical relationship between the Ro and La antigens. Anti-La antibodies are present in approximately 50% of patients with SS but only occur in 15% of patients with SLE. Two groups of patients with SLE can be distinguished based on the carriage of anti-Ro alone, or in combination with anti-La. If anti-Ro occurs alone, there is a higher frequency of serious renal disease and anti-dsDNA production, whereas patients with both anti-Ro and anti-La have a lower prevalence of renal disease and anti-dsDNA antibodies [100]. The presumed role of anti-La antibodies in "protecting" against the development of renal disease is still unclear. Anti-La antibodies almost invariably accompany anti-Ro antibodies in neonatal lupus syndromes [101]. The HLA associations of anti-La antibodies are with DQB1 alleles that share leucine in position 26 [102].

Anti-U1nRNP and Mixed Connective Tissue Disease

The U series (uridine rich) of small nuclear ribonucleoproteins (snRNPs) are components of the spliceosome, which is involved in preribosomal RNA processing [103]. Clinically important antibodies to the snRNPs include anti-Sm, anti-RNP (anti-U1RNP) and anti-fibrillarin (anti-U3RNP).

In 1972, Sharp and his colleagues described "an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA)" characterized by features of SLE, SSc, RA and PM/DM that they termed mixed connective tissue disease (MCTD) [1]. Subsequent studies showed that extractable nuclear antigen contained both the Sm and RNP antigens and that these antigens were all small nuclear ribonucleoproteins. The RNP antigen resided on the U1 RNP complex, whereas the Sm antigen was found on U1, U2, U4, U5 and U6 complexes [19; 104]. The sera of the MCTD patients described by Sharp were shown to react to RNP and not Sm [105]. Analysis by immunoblotting demonstrated that MCTD sera recognized antigens on U1RNP. Thus anti-RNP changed its name to anti-U1RNP. Anti-U1 RNP has also been detected in 30-40% of patients with SLE [47]. Although these antibodies may occur alone in SLE, they usually accompany anti-Sm antibodies. It is rare to see anti-Sm alone in SLE [106]. In SLE the intact U1 snRNP particle acts as an autoimmunogen: initial responses occur to the U1 RNP epitopes and later spread to recognize Sm epitopes [31]. Anti-U1RNP antibodies are also detected in a small fraction of patients with SS, RA, SSc and PM

[27]. When anti-U1RNP occurs alone and in high titer, the major clinical association is with MCTD.

Sharp's 25 original patients had features of SLE (cutaneous disease, fever, hepatomegaly, splenomegaly, lymphopenia, anemia, hypergammaglobulinemia and serositis), of PM/DM (heliotrope, Gottron's sign, and proximal muscle weakness), of RA (arthritis) and of SSc (skin changes, Raynaud's phenomenon, and puffy hands and esophageal dysmotility). All patients had high titers of a hemagglutinating antibody to ENA. Noting the excellent response to corticosteroids and absence of renal disease, Sharp proposed that antibody to ENA was "protective" [1].

Rheumatologists initially welcomed this new entity because of its relatively benign prognosis and favorable therapeutic response. In 1980 Nimelstein published a revaluation of 22 of Sharps original 25 patients, reassessed in 1976 and 1977 [107]. Eight patients had died. The direction of clinical evolution in many cases was away from inflammatory rheumatic disease toward noninflammatory SSc. Fever and serositis was absent. Objective arthritis was seen in only three patients. No patients had active skin cutaneous disease. Inflammatory muscle disease was less frequent. By contrast, features of SSc were more persistent. Almost half of the living patients had sclerodactyly (some had extensive sclerodermatous skin changes); esophageal dysmotility and the majority had persistent Raynaud's phenomenon. Renal disease remained infrequent. Symptomatic pulmonary disease was also uncommon, although sensitive testing was not performed. It appeared that corticosteroid responsive features (fever, serositis, and myositis) had resolved, whereas corticosteroid resistant features (Raynaud's phenomenon, sclerodactyly and esophageal dysmotility) had persisted and dominated the subsequent clinical picture. With 8/25 deaths the prognosis of MCTD was not as benign as previously contended. Subsequent studies on patients with MCTD demonstrated that renal disease was seen in 10-50% of patients followed longitudinally [108]. Other studies indicated an increased incidence of deforming arthropathy [109], pulmonary hypertension [110] and neuropsychiatric disease [111], all casting doubts on the previously asserted benign course of this disease. The *coup de grâce* was that anti-U1RNP antibodies, the sine qua non of MCTD, were shown to be far from 100% sensitive and specific [55] thus failing to fulfill the potential of the perfect diagnostic test for this disorder.

On the basis of such findings, rheumatologists seriously questioned the distinctness of MCTD and preferred the designation undifferentiated connective tissue disease or overlap syndrome, citing the many other overlap syndromes seen in clinical rheumatology [112-114]. The arguments against the uniqueness of MCTD are summarized by Venables [115] as follows:

- If a disease is characterized by a serological reaction (anti-U1RNP), it is a fallacy to claim that the antibody constitutes a distinctive feature of the disease
- Many patients with anti-U1RNP have typical features of relatively well-defined diseases such as SLE
- A substantial proportion of MCTD patients evolve into typical cases of SLE or SSc after follow-up
- There is no homogeneity in prognosis or response to treatment
- Some patients with typical features of MCTD have autoantibodies other than anti-U1RNP.

Most of the objections listed above would also be valid for accepted entities like SLE or SS. As detailed earlier, SLE has considerable clinical and serological heterogeneity, with distinct subsets recognized, often correlating with specific autoantibodies and HLA alleles. Interestingly, SLE patients with muscle involvement often have Raynaud's phenomenon, a lower risk of renal manifestations, and often U1RNP antibodies, all of which are also features of MCTD. Perhaps MCTD has come under excessive scrutiny. Despite all the controversy, the term MCTD has survived and diagnostic criteria have been proposed [116-118]. The simplest to use are the ones proposed by Alarcon-Segovia [116]. All patients must have anti-U1RNP antibodies at a titer of ≥ 1:1,600 and three of five clinical criteria (edema of hands, synovitis, myositis, Raynaud's phenomenon and acrosclerosis). All patients must have either synovitis or myositis.

In one study, patients with high-titer anti-U1RNP antibodies who did not fulfill criteria for any CTD, including MCTD, were considered to have UCTD. Interestingly, the majority of such patients evolved into MCTD within 2 years. In contrast, patients with low-titer U1RNP antibodies developed other well-defined CTDs [109; 119; 120]

There are three unique proteins on U1RNP (70K, A, C) that are recognized by three separate antibody populations (anti-70K, anti-A and anti-C) and may occur

together or singly in a given patient. Anti-70K antibodies occur more frequently in MCTD than in SLE. When patients with SLE and MCTD are grouped together, anti-70K antibodies appear to correlate with myositis, esophageal dysmotility, Raynaud's phenomenon, lack of nephritis and the HLA-DR4 phenotype [121]. Thus, anti-70K antibodies, and anti-U1RNP antibodies in general, occur in both MCTD and SLE. They may be markers for MCTD when they occur in high-titer, and may correlate with overlap features in patients who are otherwise thought to have SLE.

U1RNP is part of the spliceosome. Other nucleoproteins in the spliceosome include the heterogenous nuclear RNPs (hnRNPs) [122]. Patients with SLE, MCTD and RA produce antibodies to hnRNP, especially to hnRNP-A2/RA33. In RA such antibodies may occur alone, in MCTD they are accompanied by anti-U1RNP, and in SLE anti-U1RNP and anti-Sm antibodies accompany them [123]. This suggests that the initial antibody response to the intact spliceosome may be followed by varying patterns of epitope spreading, depending on the disease in question.

Table 8. Autoantibodies to the Spliceosome

	SLE	MCTD	RA
Anti-Sm	+	-	-
Anti-U1RNP	+	+	-
Anti-hnRNPA2/A33	+	+	+

The immunogenetics of MCTD has also provided some insights. If MCTD has features of SLE (HLA-DR2, DR3), PM/DM (HLA-DR3), SSc (HLA-DR5) and RA (HLA-DR4), the HLA associations should be quite varied. MCTD patients that evolve into other CTDs may show such HLA associations. The strongest HLA association for MCTD is with HLA-DR4 [124], which is quite uncommon in SLE. MCTD patients that evolve into SSc have HLA associations with HLA-DR5 while those who do not express HLA-DR4 [124]. Interestingly irrespective of evolution into SSc, patients with MCTD who develop pulmonary fibrosis have associations with HLA-DR3 [124]. These HLA associations are similar to those seen in patients with PM and SSc who develop pulmonary fibrosis. [125; 126].

Taken together, the combined serologic and immunogenetic associations of MCTD seem to bolster the notion that MCTD is a distinct disease with subsets that are similar to clinical and serologic overlaps that occur in the other CTDs. [127].

Redefining the Connective Tissue Diseases

We had started with the six classic connective tissue diseases, SLE, SSc, PM, DM, SS and RA. We then acknowledged considerable clinical heterogeneity in many of these clinical entities and recognized both overlap syndromes and clinical subsets that in many instances could be defined by autoantibodies and HLA associations. We reluctantly accepted a new disease, MCTD, because the arguments against its distinctness are equally applicable to the original CTDs. The term undifferentiated connective tissue disease (UCTD) was originally spawned by the debate about the existence of MCTD as a distinct entity [113]. But the term UCTD never succeeded in replacing MCTD and actually acquired a meaning of its own. It is now reserved for patients that have some features of a CTD but fail to fulfill diagnostic criteria for any established disease including MCTD. The term "undifferentiated" is now taken to represent early disease that has not yet evolved into a traditionally recognized CTD. Such patients typically have non-specific symptoms like Raynaud's phenomenon and arthritis and have low titers of autoantibodies. Prospective evaluation of many such cohorts has shown that the majority of such patients either underwent spontaneous remission or remained "undifferentiated", while a minority evolved into a traditionally recognized CTD [128; 129].

The following clinical terms are generally agreed upon:

- Connective Tissue Disease (CTD): Includes SLE, SSc (Limited and Diffuse), PM, DM, SS (Primary) and RA. Some authors will place MCTD in this category
- Overlap Syndromes: A combination of major features of more than one CTD occurring in the same patient, either simultaneously or sequentially. Many authors will place MCTD in this category.
- Undifferentiated Connective Tissue Disease (UCTD): Patients with clinical features insufficient to fulfill diagnostic criteria for any established CTD. Authors in the past tried to place MCTD here.

Bywaters [130] has very colorfully characterized the CTDs as follows:

"Thus instead of the old Victorian family of well-classified diseases, these connective tissue diseases resemble more a typical hippy commune, a hitherto forbidden clone sharing a common mystery of origin

...widely misunderstood, difficult in control and treatment, with multisystem involvement, but local manifestations and happenings, misunderstood by the body politic, error-prone, over-reactive sometimes to familiar antigens like DNA, parental influence and medical authority, given to strange drugs and stranger labels, difficult to distinguish from each other and adding a few mixed-up syndromes to their number from time to time.

"As they have grown up, this hippy colony has spread apart, and each sub-colony now manifests more individuality ...Occasional overlaps between the subgroups are seen and often rather confused and promiscuous connections, such as exist, are between these pathologic protective processes themselves."

Connecting the Connective Tissue Diseases

Naming diseases and using classification criteria will continue to be important in studying the natural history of disease and in providing uniform patient populations for clinical research. We continue to be challenged by patients who present with an incomplete clinical picture or with overlap syndromes that do not obey the constructs of conventional nosology. It is worth paying special attention to such patients, because it provides the opportunity to uncover common denominators of their etiology and pathogenesis. In 1942 Klemperer suggested that the common denominator for lupus and scleroderma was a "widespread damage of collagen". Today we recognize that common denominator as "autoimmunity".

A number of genes contribute to the predisposition to autoimmunity. These genes act in diverse ways, some swaying the immune response or shaping the immune repertoire, other genes contributing to the regulation of the immune response, and still others affecting the susceptibility of target organs. The concept of an autoimmune diathesis is best explained as resulting from the accumulation of a number of diverse susceptibility genes in a single subject. The cumulative load of genetic risk in some individuals may place them on the "brink of autoimmunity" while in others a strong environmental trigger is needed for its initiation. One can envision that once critical susceptibility to autoimmunity has been achieved, a sequence of genetic influences govern the direction autoimmunity may take and ultimately guide the expression of clinical features. Genetic predispositions to autoimmunity can be visualized to operate at various levels as described by Wakeland [131]: Level 1 represents genes that confer generalized immune hyperresponsiveness. In SLE such a gene is located on chromosome 1, is conserved in both mice and humans and has been demonstrated in every ethnic group studied [132]. Level 2 corresponds to genes responsible for selective targeting of individual autoantigens. These include MHC genes, and possibly other genes influencing the T cell receptor assembly. In SLE and in other CTDs the HLA Class II alleles have been linked to the production of specific autoantibodies as detailed earlier. The multiplicity of such autoantibodies and their appearance in linked sets is best explained by the phenomenon of epitope spreading. Level 3 involves genetic elements that influence a wide variety of events subsequent to immune activation and include genes encoding complement components, the Fcγ receptors, cytokines or genes involved in apoptosis. In SLE such genes have been demonstrated to be operant [133]. Level 4 represents genes that influence end-organ vulnerability. In the relatives of African-American SLE patients who had nephritis, familial aggregation of end-stage renal disease has been observed to be independent of the cause of renal failure [134].

Current treatment of the CTDs is largely based on nonspecific immunosuppression. Advances in the understanding of immunologic watersheds and key "downstream" events should provide an opportunity to interrupt these processes more selectively. The trimolecular complex of antigen-specific T cell receptor, antigenic peptide and the MHC confers the specificity to the interaction between the T cells and antigen presenting cell. Targeting this interaction could conceivably provide highly antigen-specific immunotherapy. All three elements could be targeted for intervention: the T cell receptor can be inhibited by peptide vaccination, the MHC peptide-binding cleft can be blocked with peptides, the antigen can be given in excess to induce tolerance. These approaches have been attempted in rheumatoid arthritis with clinical improvement [135; 136].

The connective tissue diseases may seem clinically discrete, but their overlapping manifestations suggest that downstream events tend to follow genetically guided paths that often conflict with what classic nosology predicates. Understanding these autoimmune pathways may lead to a more etiological based classification of the CTDs and reveal targets for more effective therapy.

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THE ACR 1982 REVISED CRITERIA FOR THE CLASSIFICATION OF SYSTEMIC LUPUS ERYTHEMATOSUS (Criterion 10 was updated in 1997)

Criterion	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration usually painless, observed by a physician
Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
Serositis	a) Pleuritis-convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion OR b) Pericarditis-documented by ECG or rub or evidence of pericardial effusion
Renal disorder	a) Persistent proteinuria greater than 0.5 grams per day or disorder greater than 3+ if quantitation not performed OR b) Cellular casts-may be red cell, hemoglobin, granular, tubular, or mixed
Neurologic	a) Seizures-in the absence of offending drugs or known metabolic disorder derangements; e.g. uremia, ketoacidosis, or electrolyte imbalance OR b) Psychosis-in the absence of offending drugs or known metabolic derangements. e.g., uremia, ketoacidosis, or electrolyte imbalance
Hematologic Disorder	a) Hemolytic anemia-with reticulocytosis OR b) Leukopenia-less than 4,000/mm3 total on 2 or more occasions OR c) Lymphopenia-less than 1.500/mm3 on 2 or more occasions OR d) Thrombocytopenia-less than 100,000/mm3) in the absence of offending drugs
Immunologic Disorder	a) Anti-DNA: antibody to native DNA in abnormal titer OR b) Anti-Sm: presence of antibody to Sm nuclear antigen OR c) Positive finding of antiphospholipid antibodies based on 1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2) a positive test result for lupus anticoagulant using a standard method, or 3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
Antinuclear Antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation

Sensitivity and specificity 96%

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, RothfieldNF, Schaller JG, Talal N, Winchester RJ: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271-1277, 1982

Hochberg MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997 Sep;40(9):1725

The 1987 ACR Revised Criteria for the Classification of Rheumatoid Arthritis

Criterion	Definition
1. Morning Stiffness	Morning stiffness in and around the joints lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously with soft tissue swelling or joint fluid observed by a physician. The 14 possible areas are (right or left): PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3. Arthritis of hand joints	At least 1 area swollen in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous joint involvement of the same joint areas on both sides of the body (bilateral involvement of PIP, MCP, or MTP acceptable without perfect symmetry
5. Rheumatoid Nodules	Subcutaneous nodules over bony prominences or extensor surfaces, or in juxtaarticular regions, observed by a physician
6. Serum rheumatoid factor	Abnormal amount of serum rheumatoid factor by any method for which the result has been positive in <5% of control subjects
7. Radiographic changes	Erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints, (osteoarthritis changes excluded), typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs

For classification purposes a patient is said to have rheumatoid arthritis if 4 of 7 criteria are satisfied. Criteria 1-4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

Sensitivity 89%, specificity 74%

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988 Mar; 31(3):315-24

1980 Preliminary Criteria for the Classification of Systemic Sclerosis (Scleroderma)

For the purposes of classifying patients in clinical trials, population surveys, and other studies, a person shall be said to have systemic sclerosis (scleroderma) if the one major or two or more minor criteria listed below are present. Localized forms of scleroderma, eosinophilic fasciitis, and the various forms of pseudoscleroderma are excluded from these criteria.

A. Major Criterion

Proximal scleroderma: Symmetric Thickening: Symmetric thickening, tightening, and induration of the skin and fingers and the skin proximal to the metacarpophalangeal or metatarsophalangeal joints. The changes may affect the entire extremity, face neck, and trunk (thorax and abdomen).

B. Minor Criteria

1. *Sclerodactyly*: Above-indicated skin changes limited to the fingers
2. *Digital pitting scars or loss of substance from the finger pad*: Depressed areas at tips of fingers or loss of digital pad tissue as a result of ischemia
3. *Bibasilar pulmonary fibrosis*: Bilateral reticular pattern of linear or lineonodular densities most pronounced in basilar portions of the lungs on standard cheat roentgenogram; may assume appearance of diffuse mottling or "honeycomb lung". These changes should not be attributable to primary lung disease.

Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980 May;23(5):581-90

Sensitivity 97%, specificity 98%

Proposed Criteria for Classification of Sjögren's Syndrome

Primary SS

- Symptoms and objective signs of ocular dryness
  - Schirmer test less than 8 mm wetting per 5 minutes
  - Positive Rose Bengal or fluorescein staining of cornea and conjunctiva to demonstrate keratoconjunctivitis sicca
- Symptoms and objective signs of dry mouth
  - Decreased parotid flow rate using Lashley cups or other methods
  - Abnormal biopsy of minor salivary gland (focus score of ≥ 2 based on an average of 4 evaluable lobules
- Evidence of systemic autoimmune disorder
  - Elevated Rheumatoid factor ≥ 1:320
  - Elevated antinuclear antibody ≥ 1:320
  - Presence of anti-SS-A (Ro) or anti-SS-B (La) antibodies

Secondary SS

Characteristic signs and symptoms of SS (described above) plus clinical features sufficient to allow a diagnosis of RA, SLE, polymyositis or scleroderma

Exclusions: sarcoidosis, pre-existent lymphoma, acquired immunodeficiency disease and other known causes of keratitis sicca or salivary gland enlargement

The diagnosis of "definite SS" would be made when all 3 criteria are met; the diagnosis of "possible SS" would be made when 2 criteria are present
Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV: Sjögren's syndrome. Proposed criteria for classification. Arthritis Rheum 1986 May;29(5):577-85

Proposed Classification Criteria for Polymyositis and Dermatomyositis

1. Skin lesions
   a) Heliotrope rash (red purple edematous erythema on the upper palpebra)
   b) Gottron's sign (red purple keratotic, atrophic erythema, or macules on the extensor surfaces of finger joints)
   c) Erythema on the extensor surface of extremity joints: slightly raised red purple erythema over elbows or knees
2. Proximal muscle weakness (upper or lower extremity and trunk)
3. Elevated serum CK (creatine kinase) or aldolase level
4. Muscle pain on grasping or spontaneous pain
5. Myogenic changes on EMG (short-duration, polyphasic motor potentials with spontaneous fibrillation potentials)
6. Positive anti-Jo-1 (histadyl tRNA synthetase) antibody
7. Nondestructive arthritis or arthralgias
8. Systemic inflammatory signs (fever: more than 37°C at axilla, elevated serum CRP level or accelerated ESR of more than 20 mm/hr by the Westergren method)
9. Pathological findings compatible with inflammatory myositis (inflammatory infiltration of skeletal muscle with degeneration or necrosis of muscle fibers; active phagocytosis, central nuclei, or active regeneration may be seen)

At least 1 item from1 and at least 4 items from 2 to 9 = DM. Sensitivity is 94.1% (127/135), and specificity of skin lesions against SLE and SSc is 90.3% (214/237). At least 4 items from 2 to 9 = PM. Sensitivity is 98.9% (180/182) and specificity of PM and DM against control diseases combined is 95.2% (373/392).

Tanimoto K, Nakano K, Kano S, Mori S, Ueki H, Nishitani H, Sato T, Kiuchi T, Ohashi Y: Classification criteria for polymyositis and dermatomyositis. J Rheumatol 1995 Apr;22(4):668-74

Classification and Diagnostic Criteria for Mixed Connective Tissue Disease

A. Serologic
   1. Anti-RNP at a hemagglutination titer of ≥ 1:1,600

B. Clinical
   1. Edema of the hands
   2. Synovitis
   3. Myositis
   4. Raynaud's phenomenon
   5. Acrosclerosis

Serologic criteria plus at least 3 clinical criteria including either synovitis or myositis

Alarcon-Segovia D, Villareal M: Classification and diagnostic criteria for mixed connective tissue disease. In *Mixed connective tissue diseases and antinuclear antibodies*, Edited by Kasukawa R, Sharp GC. Amsterdam: Elsevier; 1987:33-40.

Nomenclature of MHC Class II HLA DR and DQ Alleles

DR Alleles (DNA sequencing)	DR Specificities (serologic)	Workshop (w) assignment	DQ Alleles (DNA Sequencing)	DQ Specificities (Serologic)
*DRB1*0101*	DR1	Dw1	*DQA1*0101*	—
*DRB1*0102*	DR1	Dw20	*DQA1*0102*	—
*DRB1*0103*	DR "BR"	Dw "Bon"	*DQA1*0103*	—
*DRB1*1501*	DR15 (DR2)	Dw2	*DQA1*0104*	—
*DRB1*1502*	DR15 (DR2)	Dw12	*DQA1*0201*	—
*DRB1*1503*	DR15 (DR2)	Dw2	*DQA1*0301*	—
*DRB1*1504-*1505*	DR15 (DR2)	Various	*DQA1*0302*	—
*DRB1*1601*	DR16 (DR2)	Dw21	*DQA1*0401*	—
*DRB1*1602*	DR16 (DR2)	Dw22	*DQA1*0501*	—
*DRB1*1603-*1605*	DR16 (DR2)	Various	*DQA1*0502*	—
*DRB1*0301*	DR17 (DR3)	Dw3	*DQA1*0503*	—
*DRB1*0302*	DR17 (DR3)	Dw "RSH"	*DQA1*0601*	—
*DRB1*0303*	DR18 (DR3)	—	*DQB1*0501*	DQw5 (w1)
*DRB1*0304-*0305*	DR17 (DR3)	(MIT)	*DQB1*0502*	DQw5 (w1)
*DRB1*0401*	DR4	Dw4	*DQB1*0503*	DQw5 (w1)
*DRB1*0402*	DR4	Dw10	*DQB1*0504*	DQ5 (w1)
*DRB1*0403*	DR4	Dw13	*DQB1*0601*	DQw6 (w1)
*DRB1*0404*	DR4	Dw14	*DQB1*0602*	DQw6 (w1)
*DRB1*0405*	DR4	Dw15	*DQB1*0603*	DQw6 (w1)
*DRB1*0406-*0422*	DR4	Various	*DQB1*0604*	DQw6 (w1)
*DRB1*1101*	DR11 (DR5)	Dw5	*DQB1*0605-*0609*	DQw6 (w)
*DRB1*1102*	DR11 (DR5)	Dw "JVM"	*DQB1*0201*	DQ2
*DRB1*1103-1122*	DR11 (DR5)	Various	*DQB1*0301*	DQ7 (w3)
*DRB1*1201-1203*	DR12 (DR5)	Various	*DQB1*0302*	DQ8 (w3)
*DRB1*1301*	DR13 (DR6)	Dw18	*DQB1*0303*	DQ9 (w3)
*DRB1*1302*	DR13 (DR6)	Dw18	*DQB1*0304*	—
*DRB1*1303-*1322*	DR13 (DR6)	Various	*DQB1*0305*	—
*DRB1*1401*	DR14 (DR6)	Dw9	*DQB1*0401*	DQ4
*DRB1*1402*	DR14 (DR6)	Dw16	*DQB1*0402*	DQ4
*DRB1*1403-1421*	DR14 (DR6)	Various		
*DRB1*0701*	DR7	Dw17		
*DRB1*0801*	DR8	Dw8.1		
*DRB1*0802-0811*	DR8	Various		
*DRB1*0901*	DR9	Dw23		
*DRB1*1001*	DR10	—		
*DRB3*0101*	DR52a	Dw24		
*DRB3*0201*	DR52b	Dw25		
*DRB3*0202*	DR52c	Dw26		
*DRB4*0101*	DR53	—		
*DRB5*0101*	DR15 (DR2)	Dw2		
*DRB5*0102*	DR15 (DR2)	Dw12		
*DRB5*0201*	DR16 (DR2)	Dw21		
*DRB5*0202*	DR16 (DR2)	Dw22		

Adapted from: Arnett, 1997 (Dubois's Lupus Erythematosus, 5th Edition, Williams & Wilkins, Baltimore)