Collagen Diseases: 
Its Diagnosis, Pathogenesis and Treatments**

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Since Klemperer, Pollack and Baehr introduced the term "diffuse collagen diseases" based on the fibrinoid degeneration of the connective tissue in 1942, there was a tendency to use "collagen diseases" as a diagnosis, which sometimes resulted in misdiagnosis. Therefore, this term was replaced by the connective tissue disease.

Klemperer used the term fibrinoid degeneration in a purely descriptive sense. However, later studies have demonstrated the existence of immunological abnormalities in connective tissue diseases. At present, the collagen disease is classified as connective tissue disease from location of tissue injury, as rheumatic disease from articular manifestations and as autoimmune disease from pathogenetic mechanisms. The concept of collagen disease has changed with the expanding studies of the morbid process.

Collagen diseases apply to a group of six distinct illness; however the four classical collagen diseases, i.e. systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), polymyositis (PM) and polyarteritis (PM), tend to overlap in a particular patient and have important implications for prognosis. This paper is to present our study results on diagnosis, pathogenesis and treatment of these diseases.

**MATERIALS AND METHODS**

(1) Four hundred and eighty patients (SLE 274; PSS 95; PM 45; PN 26; overlap syndrome 40) seen at the Keio University Hospital were studied. The retrospective clinical analysis were conducted by controlled study matched for sex and ages. Some studies were performed prospectively.

(2) Immunological studies included tests for antinuclear and anticytoplasmic antibodies, immune complexes assayed by measurements of cryoglobulins and Raji cell tests. In vitro assay of lymphocyte functions was performed by reconstitute study of separated peripheral blood T and B cells. The effects of anti-lymphocyte antibodies were also investigated in vitro.

(3) The IgG fractions obtained from SLE patients were tested for inhibitory activities against reverse transcriptase of baboon endogenous virus.

(4) The materials obtained at autopsy or by biopsy were studied by light and electron microscopies. Some of the specimens were examined by immunofluorescence for depositions of immunoglobulins and complement components.

(5) The frequencies of major histocompatibility antigens were examined. The presence of cross reactive idiotypes in unrelated patients with anti-SS-B antibodies was studied.

**RESULTS**

I. Characteristic of Collagen Disease

(1) Clinical aspects

The clinical pictures of each disease were analyzed. The majority of the patients of each disease had multisystem involvements of skin, muscle, joints, kidney, GI tract, lung and serous membranes associated with constitutional symptoms.

The overlap syndrome was defined as the presence of more than two diseases fulfilling definite diagnostic criteria. Its incidence of
7.6% was considered to be apparently higher than expected, indicating the presence of common fundamental factors between the diseases.

However, the varied frequency and characteristics of organ involvements in each entity require to differentiate them as separate entities.

(2) Antinuclear antibodies (ANAs):

(a) Incidence of ANA

The incidence of fluorescent ANAs was studied in 497 patients. In SLE the incidence was 94%, PSS 78% and polymyositis only 19%. In 64% of active SLE patients, two or more precipitin lines of antibodies to nuclear acidic protein antigens (NAPA) were detected by immunodiffusion. Unexpectedly, similar findings were observed in other collagen diseases, and in polymyositis, the incidence of anti-NAPA antibodies was 50%.

(b) Patterns of appearance of ANA

Collagen diseases, except polyarteritis, presented many kinds of ANA which were classified into two groups; one was marker antibodies which have high specificities for a particular entity: (1) Antibodies to double stranded DNA, poly ADPR, Sm, Ki for SLE, (2) Antibodies to Scl-70 (Og) for scleroderma, (3) Antibodies to Jo-1 (Su) for polymyositis, (4) Antibodies to Ku for overlap syndrome. The other group was antibodies having close associations with certain symptoms observed in common with various entities; antibodies to RNP and SS-A antigens (Fig. 1).

c. Cellular immunity

Patients with active SLE showed negative skin reactions to PPD or DNCB. Numbers of circulating T lymphocytes and response to PHA stimulations were diminished. On the contrary, blastogenic response to DNA increased, indicating the abnormalities of cell mediated immunity. Patients with ANAs in the other collagen diseases had also hypofunction of suppressor T cells.

Summary I

(1) Collagen diseases have systemic inflammation with characteristic multiple organ involvements.

(2) Production of ANAs is the hallmark of their immunological abnormalities. These are divided into disease specific and symptom specific across different entities.

(3) Hypofunction of suppressor T cells is observed in patients with ANAs.

(4) The immunological abnormalities of polyarteritis is distinct from other entities and immunological studies from different aspect is indicated.

II. Collagen diseases of recent attention

(1) Overlap Syndrome and Mixed Connective Tissue Disease (MCTD)

Whether overlap syndrome is different entity from MCTD was studied. 103 out of 462 collagen diseases patients fulfilled multiple criteria. Among them, 33 patients with overlap syndrome were extracted. The same number of patients with MCTD were found:

<table>
<thead>
<tr>
<th>Kinds of ANAs</th>
<th>SLE</th>
<th>PSS</th>
<th>PM</th>
<th>PN</th>
<th>Overlap Syndrome</th>
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<tr>
<td>nucleus</td>
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<td>cytoplasm</td>
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Disease Specific:
- ds DNA (high level) (**)  (**)  (**)  (*)
- Poly ADPR ribose (**)  (*)  (*)  (*)
- Sm Ag  (*)  (*)  (*)  (*)
- Scl-70 (Og) Ag. (*)  (**)  (*)  (*)
- Ki Ag.  (*)  (*)  (*)  (*)
- Jo-1 (Su) Ag. (*)  (*)  (*)  (*)
- Ku Ag.  (*)  (*)  (*)  (*)

Symptom Specific:
- RNP Ag  (**)  (***)  (*)  (*)
- SS-A (Ro) Ag.  (**)  (***)  (**)  (***)
- SE B(2a,3a) Ag.  (*)  (*)  (*)  (*)
- Ne (PCNA) Ag.  (*)  (*)  (*)  (*)
- Si Ag.  (*)  (*)  (*)  (*)

*:10% positive
-:negative

Fig. 1. ANA specificity and corresponding entity in collagen diseases.
31 had incomplete overlapping (definite-probable or probable-probable).
1. In both overlap and MCTD, the combination of SLE-PSS was the most frequent; however, PSS-PM existed only in overlap syndrome.
2. In contrast to MCTD, patients with overlap syndrome had multiple ANAs.
3. The five year survival rate after diagnosis was 53% in overlap syndrome and 93% in MCTD.
4. The transition between the two groups was not observed.
5. In MCTD, frequency of HLA-B7 and DW1 increased, indicating the presence of different genetic backgrounds.
(2) CRST and CREST syndromes
The clinical significance was studied in patients with CRST characterized by angiomata-like telangiectasia and CREST syndrome.
From 166 patients with PSS, 12 had characteristics of CRST while 37 had characteristics of CREST syndromes. Both syndromes had milder skin involvements and better five year survival rate than the rest of the PSS patients. CRST syndrome had minimum skin involvement in particular and therefore, a subset of PSS is sometimes overlooked.
One case with primary biliary cirrhosis was found in CRST and two cases of primary pulmonary hypertension in CREST syndrome.
(3) Polymyalgia rheumatica
Polymyalgia rheumatica is a subset of polyarteritis. A typical case of 75 year old male was reported. The clinical significance of this entity should become greater in Japan as the aged people increase.
(4) Vascular diseases associated with cryoglobulinemia:
Among the patients with mixed cryoglobulinemia of IgG-IgM type, a 71 year old male with endoarteritis obliterans and antibodies to HB antigens, and a 44 year old female with polyarteritis with positive Wasserman antibody were observed.
In both cases, specific antibody activities in the cryoprecipitates were not detected, while close associations of obstructive vascular symptoms and serum cryoglobulin levels were demonstrated.

Summary II
(1) An attempt should always be made to differentiate overlap syndrome from MCTD.
(2) The CRST tends associate with primary biliary cirrhosis while CREST with primary pulmonary hypertension.
(3) Polymyalgia rheumatica is a ischemic disease of the aged people and will become more prevalent in future.
(4) Analysis of mixed cryoglobulins is indicated for the study of vasculitis.

III. Early diagnosis of collagen diseases
Patients suspected to have collagen disease do not always meet the diagnostic criteria. The study was conducted to determine whether these unclassified collagen diseases constitute the special subset or earlier cases of collagen diseases.
(1) Follow-up study
168 patients were followed for 3 years who had a clinical syndrome of suggestive collagen disease at their first visit. Patients with multiorgan involvements with fever fulfilled the diagnostic criteria more frequently (49/63 patients) than those without fever (44/93 patients). In constrast, none of those suspected to have collagen disease based on the characteristic mono-organ involvement met the criteria. During the follow up period, out of 75 patients not fulfilling criteria at first visit, 25 showed new manifestations and satisfied the criteria. 21 remained unchanged at the end of the study (Fig. 2).
(2) Studies of ANAs
The diagnostic significance of ANAs in 75 patients who did not meet the criteria was studied. Among 41 patients with ANA, 21 showed new manifestations related to specific antibodies and satisfied the criteria. Five were diagnosed as SLE with three items of the ARA criteria, 6 were unclassified collagen disease who had anti-RNP antibodies and sclerodactyly with Raynaud's
phenomenon. In contrast, out of 34 ANA negative patients only 4 cases were classified as PSS and 20 cases dropped from the study (Fig. 2).

Ninety cases who met the criteria at their first visit were analyzed. In several cases, diagnoses had to be changed because of new symptoms. They had antibodies only to RNP with initial diagnosis of PSS or PM which were changed to MCTD. The similar situations were observed in patients with antibodies to Sm, Og or Ku antigens. However, no such cases were found among ANA negative patients.

It is concluded that ANAs can be detected prior to the appearance of specific symptoms.

Summary III

The patient with ANAs who did not fulfill the diagnostic criteria at their first visit included those with early stage of disease and those with unclassified collagen diseases with anti-RNP antibodies alone. The demonstration of specific ANAs proved to be a powerful measure for early diagnosis.

IV. Pathogenesis

The studies of pathogenesis have been limited to SLE.

(1) a. Some patients presented symp-

toms similar to human serum sickness.

b. Significant associations with organ non-specific (9.6%) and organ specific (12.3%) autoimmune diseases were found.

c. Hypergammaglobulinemia was present in 79.9%.

d. Depositions of gammaglobulins and complement components at the renal glomerular lesions was found. The amount of these deposits correlated with severity of the disease.

e. 28% of active cases had IgG-IgM mixed cryoglobulins in their sera.

f. All active patients had circulating immune complexes measured by Raji cell test, which disappeared within three months.

By gel filtration analysis of immune complex positive serum, activities of antibodies to NAPA were demonstrated in the fractions with higher molecular weight than IgM.

(2) ANAs have close associations with specific clinical expressions of SLE. Anti-DNA antibodies were associated with leukopenia, azotemia and cylinduria. Each of the other antibodies showed significant associations with each specific symptom (Fig. 3).

The complex clinical manifestations of overlap syndrome could be understood as cumulative results of various symptoms associated with multiple ANA present in
antibodies at 37°C was separated from unreactive lymphocytes, utilizing the petri dish coated with rabbit anti-human gammaglobulin antibodies. Analysis by the fluorescent activated cell sorter confirmed effective separation of SLE+ cells (reactive with anti-lymphocyte antibodies at 37°C) from SLE- cells. The SLE+ cells showed weak response to PHA and strong inhibitory activity by Con A stimulation. These findings were further investigated in a collaborative study with Doctor Schlossman at the Harvard Medical School. The elimination of suppressor T cells from normal human lymphocytes by monoclonal antibody, OKT8, greatly reduced numbers of lymphocytes reactive with anti-lymphocyte antibody. On the contrary, lymphocytes deprived of helper T cells by OKT4 reacted with anti-lymphocyte serum.

(7) a. The family members of SLE patients had higher serum immunoglobulin levels (1.19±0.35 vs. 1.05±0.26 g/dl) and higher incidence of positive ANAs (10.6 vs. 8.0%) than controls.

b. Familial occurrence of SLE was observed in 15 out of 260 patients (5.8%).

c. Analysis of major histocompatibility antigens revealed significant association of anti-DNA antibodies with BW35 and anti-RNP antibodies with B7 and DW1.

d. The anti-idiotypic antibodies of anti-SS-B antibodies were raised by injecting antibodies with restricted heterogeneity into a rhesus monkey. Inhibition test of solid phase radioimmuno-assay showed cross reactive idiotypes in one additional patient.

(8) a. In SLE, incidences of antibodies to synthetic double stranded RNA (poly I: C) (42%) and measles (15%) were higher than sex, age matched controls.

b. Incidence of anti-lymphocytes antibodies was higher in consanguineous than nonconsanguineous relatives (40 vs. 32%) and in unrelated spouse than normal controls (25 vs. 6%).

c. C57Bl/6 mice inoculated with Rauscher leukemia virus produced ANA from early stage.
d. Five out of 24 IgG preparations from SLE patients demonstrated significant inhibitory activity of reverse transcriptase.

(9) The activity of poly ADPR synthetase in lymphocytes from SLE patients was significantly lower than in those of normal controls.

**Summary IV**

SLE is considered as an immune complex disease with ANAs which have closely related with clinical manifestations.

As possible mechanisms of autoantibody production, hyper-reactivity of B cells and hypofunction of suppressor T cells were suggested. Antibodies to suppressor T cells were demonstrated in patients with active SLE. The genetic factors and retroviral infections might conceivably develop such immunological abnormalities. Metabolic abnormalities of chemically defined antigens such as poly ADPR should be studied to understand pathogenetic mechanisms.

**V. Prognosis**

The survival rates were calculated by the life table method of SLE patients divided into three groups according to their time of first visit (stage I 1964–1968, stage II 1969–1973, stage III 1974–1978). Marked improvement of 5 year survival rate was observed in stage III group, (stage I 68%, stage II 88%, stage III 96%). This improvement is attributable to the increase of relative proportion of non-renal SLE subset with good prognosis during these periods of years.

Prognosis of PSS and PM was also markedly improved during the recent five years. Similar to SLE, patient numbers belonging to the subset with good prognosis were increased.

**VI. Treatment**

Polyarteritis patients who were not diagnosed have succumbed within 8 months after onset and patients who were diagnosed and received appropriate treatments by steroid and immunosuppressive drugs showed prolonged survivals.

SLE patients without persistent proteinuria and the serum C3 level higher than 40 mg/dl showed infrequent appearance of renal disease (less than 10%) regardless of initial dosage of steroid therapy. In patients with depressed C3, administration of high dose steroid resulted in less frequent deterioration of renal disease than small dose therapy (13 vs. 48%). In this group, however, patients with antibodies only to RNP, progression of renal disease was not observed. Therefore, in patients who have not decreased serum complement or who have antibodies only to RNP despite of decreased complement, small amount of steroid is indicated.

Similar studies were conducted in groups of persistent proteinuria and nephrotic syndrome. These results were summarized on Fig. 4 as a guidance for treatment of lupus nephritis. As a general rule, appropriate initial dosage from among 30, 40 and 50 mg must be determined in accordance with the recommendation in Fig. 4.

**CONCLUSION**

Each collagen disease is regarded as a separate entity based on characteristic clinical manifestations and presence of specific ANAs.

At present, early diagnosis is made by suspecting collagen disease and testing for ANAs.

Based on the enormous progresses in pathogenesis studies during past 38 years, further studies on the causes of collagen disease should be continued.

The recent improvement in prognosis of collagen disease is remarkable. This might be attributed to the changing patterns of subset in each entity and improved methods of treatments. Unfortunately, we could not suggest the effective treatment of PSS. However, we have seen a patient with PSS, whose skin manifestations was markedly improved after complicated with miliary tuberculosis but returned to previous condition following antituberculous therapy. We would like to continue the research with a
belief that there should exist the specific therapeutic methods to cure the collagen disease.

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