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ANTITISSUE ANTIBODIES IN INTERSTITIAL CYSTITIS

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SUMMARY

Sera from thirty-three female patients with interstitial cystitis were studied for the presence of antitissue antibodies and a positive result was obtained in thirty-one cases (94%). Antinuclear antibodies detected by the immunofluorescence method were found in 85% of the sera in titres of 1:10 or higher. However, the LE-cell phenomenon was not seen in a single patient. Complement-fixing antibodies to crude kidney homogenate occurred in 48% of the sera. Antibodies, with an incidence not exceeding that expected in control groups, were against smooth muscle, thyroglobulin and gastric parietal cells. None of the patients had mitochondrial or thyroid cytoplasmic antibodies, rheumatoid factors or biologic false-positive reactions for syphilis.

Bladder specific antibodies could not be demonstrated by the double layer immunofluorescence method.

The results indicate that interstitial cystitis belongs to that group of autoimmune diseases in which the disease is restricted to one organ, whereas the autoantibodies are non-organ specific.

INTRODUCTION

Interstitial cystitis (IC) is a chronic inflammation of the bladder wall of unknown etiology. Some authors have emphasized those characteristics that the disease shares with autoimmune connective tissue diseases. Its similarity to and coexistence with chronic LE have been specially emphasized (Fister, 1938; Dees, 1953; Gil-Vernet *et al.*, 1960; Shipton, 1965), and it has even been claimed that IC might be a kind of variant or local manifestation of LE. On the other hand, Silk (1970) reported that he had found bladder-specific antibodies in IC patients. This finding suggests, in its turn, that IC belongs to the group of organ-specific autoimmune diseases.

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We have previously reported on a series of fifty-four patients with IC (Oravisto, Alfthan & Jokinen, 1970) also with several findings suggesting a similarity to autoimmune diseases. These findings included the particularly high proportion of female patients, the high incidence of drug reactions, good response to steroid therapy, and especially the similarity of histological findings. This prompted a search for antitissue antibodies in IC patients to further clarify the autoimmune characteristics of this disease.

MATERIALS AND METHODS

Patients

The series consisted of thirty-three female patients with IC. All had been included in the series of fifty-four patients which has been clinically described previously (Oravisto *et al.*, 1970). The disease was represented in all degrees of severity. Five patients were classified as belonging to severity class I (mild cases), twenty-five to severity class II (moderate cases), and three to severity class III (severe cases). None showed manifestations suggesting a systemic disease. The age range was 39–80 years, and the mean age was 61.

Serological methods

Antibodies to cell nuclei, mitochondria, smooth muscle and parietal cells were studied by the double layer IFT. A series of two-fold dilutions was made of the sera beginning with dilution 1:10 for the study of ANA and with undiluted serum for the study of the other antibodies. Human kidney and stomach, and rat liver were used as antigen substrates. Anti-HGG sera were made in rabbits and isolated γ-globulin conjugated with fluorescein isothiocyanate (Baltimore Biological Laboratories, Maryland, U.S.A.). Specific antisera to IgG, IgA and IgM were obtained commercially (Behringwerke, Mahrburg-Lahn, Germany). Thyroglobulin antibodies were detected by the TCH and thyroid cytoplasmic antibodies by CFT with thyrotoxic thyroid homogenate. RF was studied by the Latex slide test (Hyland Laboratories Eurobiochim, Brussels, Belgium) (Roitt & Doniach, 1969a). Non-organ-specific antibodies to rat kidney and liver homogenate were studied both by CFT (Roitt & Doniach, 1969a) and TCH (Gery & Davies, 1961) using the same antigens. The homogenates were also divided into particulate and soluble fractions by centrifuging them twice at 100,000 *g* for 1 hr. The Kolmer and VDRL tests were carried out according to the techniques described in the 1959 Manual of Serologic Tests for Syphilis (U.S. Department of Health, Education and Welfare, 1959).

LE-cell test

The test was performed by the technique of Magath & Winkle (1952).

Absorption studies

For establishment of bladder-specific antibodies, the sera were absorbed with lyophilized rat kidney and liver, and human uterine muscle homogenates, using 50 mg/ml each, for 1 hr at 37°C and then at 4°C overnight. Subsequently the antibodies were studied with double layer IFT (Roitt & Doniach, 1969a) using unfixed tissue sections of normal human bladder, obtained at operation, as antigen. The sections were not allowed to dry at any time during the treatment.

RESULTS

In thirty-one patients (94%) tissue autoantibodies were found. These were against both non-organ-specific and organ-specific antigens (Table 1).

Non-organ-specific antibodies

ANA in titres of 1:10 or higher were found in twenty-eight (85%) of the patients (Table 2). Seven had titres of 1:20–1:80 and seven, 1:160–1:1280. The fine-speckle pattern of fluorescence was the most common. With the serum dilution of 1:10, it was seen in eighteen cases.

TABLE 1. Autoantibodies in thirty-three patients with interstitial cystitis

Antibody	Number positive	Percentage positive
Antinuclear antibodies	28	85
CFT rat kidney homogenate	16	48
Smooth muscle fluorescence	2	6
Mitochondrial fluorescence	0	0
BFP reactions	0	0
Rheumatoid factors	0	0
Thyroid-specific antibodies	4	12
Gastric parietal cell antibodies	4	12
Total patients with antibodies	31	94

TABLE 2. Antinuclear antibodies in sera of thirty-three patients with interstitial cystitis, and controls

	Number of sera				
			With titre		
	Tested	Positive	1:10	1:20–1:80	1:160–1:1280
Interstitial cystitis	33	28 (85%)	14	7	7
Matched controls	33	2 (6%)	2	0	0
unselected female urologic Patients	60	5 (8%)	5	0	0

Seven sera gave a diffuse fluorescence and three sera a halo pattern. Nucleolar fluorescence combined with weak fine-speckle fluorescence was seen in four cases. In some cases it seemed as if the fine-speckle and the diffuse variety had combined. However, it was so difficult to evaluate these that they were classified according to the predominant fluorescence. Specific anti-IgG, -IgA and -IgM conjugates were used to study the ten sera with the highest

titres. In seven sera ANA was found to be present in all three classes, but in three sera, only in the IgG and IgM classes. The LE-cell test was negative for every patient.

Non-organ-specific CFT reactions were obtained with rat kidney homogenate in sixteen cases and with rat liver homogenate in twelve cases, four having titres of 1:256–1:512 (Table 3). The antibodies responsible for these reactions were almost solely to particulate components of homogenate, for when the soluble fraction of liver was used as antigen, only four sera gave a positive reaction and even then only in low titres. The incidence of positive reactions obtained by TCH, and the titres were slightly lower. The existence of the non-organ-specific cytoplasmic antibodies was also manifest when ANA was studied using rat liver sections as tissue substrate. They produced a typical diffuse cytoplasmic fluorescence.

To find out the proportions in which the patients produced the different antibodies, the titres of ANA and non-organ-specific cytoplasmic antibodies were compared, and a lack of correlation between them was established when rat kidney homogenate was used as antigen in CFT. Similarly, there was a lack of correlation between the ANA and TCH titres. Nor could any correlation be noted when titres of non-organ-specific cytoplasmic antibodies obtained with CFT and TCH were compared.

Smooth muscle antibodies were found in the serum of two patients (6%) with titres of 1:1 and 1:60, respectively. This is approximately the incidence expected among unselected hospital patients (Doniach *et al.*, 1966). No patient was found to have mitochondrial antibodies, rheumatoid factors or positive Kolmer or VDRL tests.

TABLE 3. Antibodies to rat organ homogenates in sera of thirty-three patients with interstitial cystitis

Method and homogenate	Number positive	Percentage positive	Titre		
			1:4–1:16	1:32–1:128	1:256–1:512
CFT kidney	16	48	10	3	3
CFT liver	12	36	6	2	4
CFT soluble fraction	4	12	4	0	0
TCH kidney	10	30	6	4	0
TCH liver	11	33	2	7	2

Organ-specific antibodies

Thyroglobulin antibodies in low titres (up to 1:250) were detected in four patients (12%) whereas none had thyroid cytoplasmic antibodies. Gastric parietal cell fluorescence with titres up to 1:160 were found in four cases (12%). These incidences correspond to those known to occur in women in the control groups of ages equal to those of the majority of the present patients (Hackett, Beech & Forbes, 1960; Anderson, Buchanan & Goudie, 1967).

Bladder specific antibodies were sought in the sera of six patients in whom the disease was clinically moderate or very severe (severity classes II and III) and in whom both ANA and the non-organ-specific cytoplasmic antibodies detected by CFT had been found. After absorption with rat kidney and liver, and human uterine muscle homogenates, no specific fluorescence could be seen in bladder sections by means of double layer IFT.

Correlation of antibodies with clinical features

Fig. 1 shows the ANA titres in the different severity classes of the patients. A certain correlation is seen in that in the mild cases the titre was relatively low and in the severe cases high. However variations within a severity class were wide, especially in moderate cases, the scale extending from negative values to the very highest recorded in the material (up to 1:1280). On the other hand, no correlation was noted between the classes of severity and the titres of non-organ-specific cytoplasmic antibodies, either with CFT or with TCH.

There were a few patients from whom serum samples could be obtained repeatedly during several months. In some the ANA titre remained on the same level but in others it fluctuated, or the antibodies disappeared and reappeared, without any essential change being clinically noticeable in the course of the disease.

DISCUSSION

In almost all of these patients some kind of antitissue antibodies were found (94%). Such a high incidence may be partly due to the fact that the disease, in the majority of the patients, was clinically either moderate or severe, and only five patients had it in the mild form.

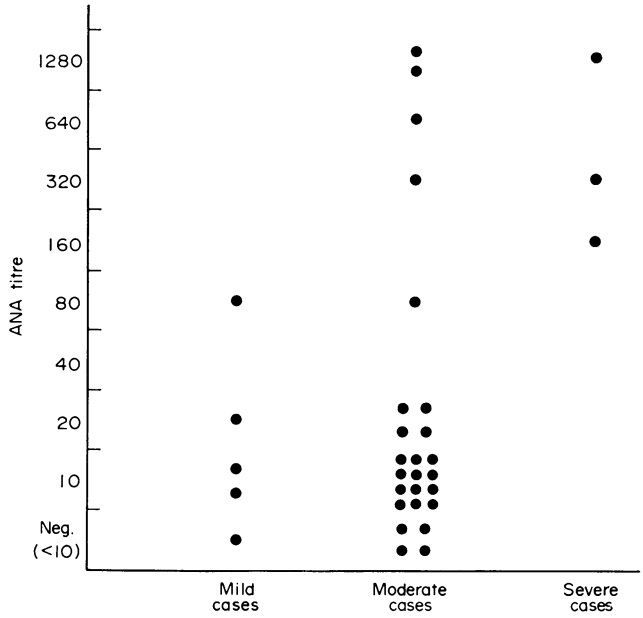

FIG. 1. ANA titres in different severity classes of thirty-three patients with interstitial cystitis.

Furthermore, the patients' mean age was high (61 years). The low number of mild cases and young patients may perhaps be due to the fact that it is difficult to diagnose IC, and that patients perhaps do not seek treatment as long as the discomfort is still only slight. In the present series the interval from the first clinical manifestations to the correct diagnosis was, on the average, about 6 years (Oravisto *et al.*, 1970).

From the present material it appears that the examination of ANA is useful in reaching a diagnosis of IC, since these antibodies are present in the majority (85%) of the IC patients. However, the level of the titre in individual cases does not seem to justify conclusions concerning the degree of severity of the disease. For screening purposes, it may be useful to examine ANA in all obscure cases of dysuria, especially in women.

The results obtained do not suggest that IC should belong to the group of organ-specific autoimmune diseases. The frequent combination of these diseases is a known fact, and antibodies to specific antigens of other organs simultaneously has been noted in a higher percentage of cases than in controls (Roitt & Doniach, 1969b). By contrast, in our patients the incidence of thyroid and parietal cell antibodies did not exceed those recorded in unselected patient materials for women of the age groups to which the vast majority of the present patients belonged. They reflect the known frequency of focal thyroiditis and atrophic gastritis in these age groups. Nor have we been able to show bladder-specific antibodies in the sera of our patients. We were thus unable to confirm the results of Silk (1970) who in nine out of twenty IC sera found such antibodies. Further evidence against the assumption that IC is an organ-specific autoimmune disease is that cystitis could not be produced in experimental animals by immunization with bladder incorporated to Freund's complete adjuvant (Silk, 1970).

In its clinical picture, IC shares certain characteristics with connective tissue diseases, especially with chronic LE (Fister, 1938; Dees, 1953; Gil-Vernet *et al.*, 1960; Shipton, 1965). The similarity of the histological findings has also been emphasized. On the basis of such observations it has been considered possible that IC might be a certain variant of LE. When this aspect is reviewed in the light of autoantibodies in the present patient material a similarity can be seen in that antibodies to cell nuclei and unidentified cytoplasmic components, which are typical of LE, were also common in the present patient material. It is known, however, that these antibodies are common in a wide variety of conditions with autoimmune phenomena. But dissimilarities are numerous. For example, the BFP reactions, present in LE in about one-tenth of the patients with the discoid variety and about one-third of those with the systemic variety of the disease (Dubois, 1966), are completely absent from our series. The antibody giving mitochondrial fluorescence was missing although, according to Doniach *et al.* (1966), it is noted in 29% of the patients with systemic LE. Nor did we, by using the Latex test, discover in any patient the RF which in discoid LE is found in about a quarter, and in systemic LE about half, of the patients (Dubois, 1966). Another important difference is that although in 85% of the present patients ANA, in titres of 1:10 or higher, was recorded, the LE-cell phenomenon could not be shown in a single patient. Its incidence in discoid LE, according to Dubois (1966), is 8% and in systemic LE 76%. The results concerning tissue autoantibodies therefore suggest, as do our earlier clinical observations (Oravisto *et al.*, 1970), that IC is not a kind of local manifestation or variant of LE but a separate clinical entity.

In our series, the tissue antibodies with a higher incidence than would be expected in patients of this age in an unselected material, were against non-organ-specific antigens, *viz* cell nuclei and unidentified cytoplasmic components. On the other hand, no manifestations indicative of systemic diseases were found in our patients. On the basis of these findings, it seems probable that IC belongs to the group of autoimmune diseases that lies between organ-specific and non-organ-specific systemic diseases. The disease is confined to one organ, but the tissue antibodies are non-organ-specific.

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