CHAPTER 4

DEMONSTRATION OF SPECIFIC AUTO-ANTIBODIES ADD NEW EVIDENCE TO AN AUTO-IMMUNE PATHOGENESIS OF INTERSTITIAL CYSTITIS

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Abstract:

Background. Interstitial cystitis (IC) is a long recognised, chronic benign bladder disease with debilitating symptoms. The disease has by definition an inflammatory nature, but its cause and etiology are still unknown.

Methods. Antibodies were isolated from three, randomly chosen, sera taken from IC patients. After labelling with biotin these antibodies were applied to sections made from autologous or heterologous bladder tissue. Binding of the biotinylated antibodies was visualised with a streptavidine-peroxidase staining procedure.

Results. We demonstrated the presence of specific auto-antibodies in all (n=3) tested IC patients. The auto-antibodies were directed against epithelium and muscle fibers of the bladder wall. Bladder tissue treated with control serum showed no response.

Conclusions. The in the present study employed direct immunostaining procedure shows the presence of auto-antibodies that have not been reported before in relation with interstitial cystitis. The specific auto-antibodies demonstrated in this study indicate the involvement of an auto-immune reaction in the pathogenesis of interstitial cystitis. If future studies confirm these findings it might cause a breakthrough in the understanding and treatment of this disabling, chronic bladder disease.

The following presentations were based on this chapter:


INTRODUCTION

Interstitial cystitis (IC) is a chronic idiopathic inflammatory bladder disease. Most patients report a subacute onset with similar symptoms as a urinary tract infection, some relate the start of symptoms to a preceding event, i.e. hysterectomy or post-operative urine retention. The symptoms pain, frequency of micturation and nocturia usually fail to respond to regular treatment with antibiotics after which the disease takes a chronic course. Many studies were set up to detect a causitive infectious microorganism but proved to be negative. Unfortunately the diagnosis IC is often overlooked, even by urologists. Misdiagnosis, inappropriate surgery to relief the pelvic pain, and social isolation contribute to the chronic debilitating course of the disease. Eventually, some type of surgery is performed in 10 - 40% of the IC patients to achieve symptomatic relief. A substantial minority ends up with a permanent abdominal urostoma, which reflects the persistence and severity of symptoms caused by this benign bladder disease.

The typical clinical findings in IC, suprapubic or perineal pain combined with frequency of micturation and nocturia, were first reported in detail by C.L. Hunner in 1914, describing a series of female patients between 20 and 40 years. These characteristic symptoms combined with the typical mucosal lesions of the bladder have remained the mainstays of diagnosis in IC. Largely due to efforts of patient advocate groups in the USA awareness for this mysterious bladder disease has increased. Epidemiological studies revealed unexpected large numbers of patients suffering from interstitial cystitis. At present prevalence of IC in the USA approximates a 500.000 cases and is considered a major health problem. Despite an exponential growth in clinical and basic research in the past decennium, there are still no definite answers to vital issues such as the etiology of IC.

Today the general opinion is that the etiology of IC is multifactorial. Infection, deficiency in the bladder glycosaminoglycan layer, toxic agents in the urine, local neuropathic mast cell stimulation in the bladder, and auto-immune reactions have been suggested as possible etiologic causes. Non-consistency of pathological and serological findings indicating an auto-immune reaction in combination with the variable responses to immunosuppressive treatment have been interpreted as an argument against a possible autoimmune pathogenesis of IC. However, many facets of IC are similar to well-known auto-immune diseases. Firstly, 90% of the patients is female, with a preferential age between 20 and 60 years. Secondly, the episodic waxing and waning of the disease. Thirdly, the onset of disease is often well memorised by patients even after many years, which indicates a subacute onset. Fourth, histopathological examination of the bladder wall shows the localised presence of dense mononuclear infiltrates in which lymphocytes are abundantly present.
demonstration of mast cells is characteristic in these biopsies\textsuperscript{9}. Fifth, a considerable number of IC patients has been co-diagnosed with well-known auto-immune diseases, i.e. lupus erythematosis, polyarteritis nodosa, rheumatoid arthritis, ulcerative colitis and M. Sjogren\textsuperscript{8,10,11}.

This study was designed to evaluate the presence of tissue-specific auto-antibodies in IC patients using a direct immunostaining technique not reported before in relation with IC patients.

MATERIALS AND METHOD
Peripheral blood samples were obtained from 20 consecutive interstitial cystitis patients participating in a placebo controlled therapeutic trial. All patients were diagnosed as having IC in accordance with the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (USA) criteria\textsuperscript{12}. All patients participated in a placebo-controlled study on the efficacy of pentosanpolysulfate intravesical instillations for the treatment of IC. Serum was collected before initiation of treatment and stored frozen at -20\textdegree C. In addition, coldcup bladder biopsies of each IC patient were snap-frozen in methyl-butane (-80\textdegree C) and stored at -80\textdegree C until use. The biopsies were used for standard histopathological examination and partly for the present study. The control serum sample consisted of pooled serum from 20 normal, healthy individuals. Sera samples of 3 IC patients were chosen at random. From these sera and the control serum batch, the IgG fraction was isolated by protein-G-column (Pharmacia, Uppsala, Sweden) chromatography. IgG was biotinylated by the addition of NHS-biotin (Pierce, ?, USA) in a mol/mol ratio of 20. Unbound NHS-biotin was removed by gelfiltration (sephadex 650, Pharmacia, Uppsala, Sweden). The final concentration of the biotinylated IgG preparation was set at 1 mg/ml. Various dilutions of these preparations in PBS containing 10\% normal human serum were incubated with cryostatsections of bladder tissue. Both, heterologous-normal and autologous bladder tissue was used. The bound antibody-biotin was visualized - with a standard streptavidine-peroxidase (DAKO, Denmark, dilution 1:50) staining using AEC as a substrate. Nuclei were weakly counter stained with haematoxylin. From the sera of the same three IC patients the antinuclear antibodies (ANA) titers were determined, using standard indirect immunofluorescence procedures.
Results
Antibodies isolated from all three IC-patient sera, but not from the control serum, showed a restricted reactivity pattern when tested on heterologous-normal bladder tissue specimens. When sections from autologous bladder biopsies were taken as a substrate similar specificities were noted. The antibodies appeared to react with the epithelial layer as well with muscle filaments. Detailed results are presented in Table 1. In one patient (no. 3, Table 2) also nuclear staining could be seen.

Table 1

<table>
<thead>
<tr>
<th>Summary of immunohistochemical findings</th>
<th>Lowest Ig concentration showing positive reaction</th>
<th>Test for anti-nuclear antibodies (ANA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1: Transitional Cell layer: positive; muscle: positive; B-cell follicles: some staining</td>
<td>25 µg/ml</td>
<td>Negative</td>
</tr>
<tr>
<td>Patient 2: Transitional Cell layer: positive; muscle: positive; B-cell follicles: some staining</td>
<td>25 µg/ml</td>
<td>Homogeneous staining; titer 40</td>
</tr>
<tr>
<td>Patient 3: Transitional Cell layer: positive; muscle: positive</td>
<td>100 µg/ml</td>
<td>coarse speckled staining pattern; titer 640</td>
</tr>
</tbody>
</table>

The clinical characteristics of the three IC patients are shown in Table 2. The tested IC patients were all ‘end stage’ patients with limited bladder capacity. Patients number 1 and 3 (Table 1) failed to obtain symptomatic relief from conservative treatment options and, eventually, underwent supravesical urinary diversion while the bladder remained in situ. All IC symptoms disappeared completely after urinary diversion. Patient no. 2 achieved only a symptomatic remission with pentosanpolysulfate instillations. One year after the collection of serum, she was treated for an allergic alveolitis caused by nitrofurantoin medication with high dose corticosteroids (Prednisolone). The alveolitis was cured and also caused a return to normal of her micturation frequency and voiding volumes.

The streptavidin-peroxidase mediated staining of the transitional cell layer in autologous bladder tissue following contact with biotinylated Ig isolated from the serum of patient no. 3 is shown in photomicrograph 1, while the result after contact with biotiny-
lated Ig isolated from the control serum is demonstrated with photomicrograph 2. Photomicrograph 3 presents the lamina muscularis in autologous bladder of patients no. 1 after contact with biotinylated Ig isolated from the serum in a magnification of 1:1000.

Table 2

Clinical characteristics of the three interstitial cystitis patients.

<table>
<thead>
<tr>
<th>gender</th>
<th>age</th>
<th>disease-history</th>
<th>cystometric capacity</th>
<th>frequency per 24 hr</th>
<th>nocturia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 1</td>
<td>F</td>
<td>6 years</td>
<td>210cc</td>
<td>26x</td>
<td>8x</td>
</tr>
<tr>
<td>Pt 2</td>
<td>F</td>
<td>3 years</td>
<td>125cc</td>
<td>18x</td>
<td>5x</td>
</tr>
<tr>
<td>Pt 3</td>
<td>F</td>
<td>4 years</td>
<td>75 cc</td>
<td>25x</td>
<td>6x</td>
</tr>
</tbody>
</table>

In two patients (no 1 and 2, Table 2) the presence of antinuclear antibodies (ANA) was demonstrated. Details are presented in Table 1.

Discussion

In this study we demonstrated the presence of specific autoantibodies in all (n=3) tested interstitial cystitis (IC) patients. The autoantibodies were directed against the transitional cell layer and muscle fibres of the bladder wall. Tissue treated with control serum showed no response. No difference was observed between autologous and heterologous bladder tissue. In addition to organ-specific antibodies we detected anti-nuclear antibodies in two out of the three IC patients.

Autoimmune disorders may be characterised by the presence of organ-specific or non-specific antibodies such as antinuclear antibodies. The first bladder-specific autoantibodies in interstitial cystitis were reported by Silk in 1970. It initiated a decade of intense interest in the study of immune phenomena in IC. Unfortunately, these studies were rather inconsistent in both methodology and results. In addition, the interpretation of these studies is hampered by the absence of uniform diagnostic or histopathological inclusion criteria for the inclusion of interstitial cystitis patients. The study of Ochs et al. in 1994 is the first to employ the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (USA) criteria. However, Ochs et al. tested the presence of specific antibodies on a T24 human epithelial cell line with a negative outcome. The most consistent finding concerning auto-antibodies in IC patients is the presence of ANA. However, this might be a rather aspecific corollary of auto-immune disease. Due to these negative, inconclusive or aspecific data concerning the presence of auto-antibodies the notion of an autoimmune etiology in interstitial cystitis remained a speculative hypothesis.
The findings in this study provide new evidence for an auto-immune involvement in interstitial cystitis. Although not all sera were tested, the positive outcome with the three at random chosen sera should be considered highly significant. In addition, the pattern of reaction was very similar with all three tested sera. Whether the interpretation of this finding hint at a primary or a secondary auto-immune pathogenesis is rather speculative, presently. On the one hand, a primary auto-immune involvement in IC, similar to other organ-specific autoimmune diseases such as thyroiditis and diabetes, is supported by similarities in histopathology. The reduced capacity, high compliance and fibrosis of the bladder in chronic IC patients could also be consistent with a primary auto-immune reaction. And the absence of symptoms in IC patients with in-situ bladder after urinary diversion does not contradict a primary auto-immune origin, since symptoms might well be related to a functioning bladder. On the other hand, the subacute onset of the disease could be coherent with a secondary auto-immune response to injury of the mucous lining or the bladder wall. In addition, the urine or agents in the urine of IC patients might be perpetuating a (secondary) auto-immune reaction. Which is in line with the observed allergic skinreactions in human volunteers and cystitis-like features in rats, both after injection with urine from IC patients.

A possible auto-immune pathogenesis has triggered the use of immunosuppressiva for the treatment of IC long before. In 1971, Badenoch used prednisolone and observed sustained improvement in 19 out of 25 cases. Oravisto, 1976, reported on the use of Azathioprine which banished the symptoms almost completely in 22 out of 38 patients. Other reports on the use of immunosuppressiva in IC patients are largely anecdotal case-reports. The potential dangerous side-effects of these medicines might be explanatory and indeed, warrant a solid pathogenetic-based indication for a properly designed study.

CONCLUSIONS

To our knowledge, the direct immunostaining procedure employed in this study has not been reported before in relation with interstitial cystitis. The specific auto-antibodies demonstrated in this study indicate the involvement of an auto-immune reaction in the pathogenesis of interstitial cystitis. If future studies confirm these findings it might cause a breakthrough in the understanding and treatment of this disabling, chronic bladder disease.
REFERENCES


Photomicrograph 1

Streptavidin-peroxidase mediated staining of the transitional cell layer in autologous bladder tissue following contact with biotinylated Ig isolated from the serum of patient no. 3.
Photomicrograph 2

Streptavidin-peroxidase mediated staining of the transitional cell layer in autologous bladder tissue from patient 3, following contact with biotinylated Ig isolated from the control serum.
Magnification of 1:1000 from streptavidin-peroxidase mediated staining of the lamina muscularis in autologous bladder tissue following contact with biotinylated Ig isolated from the serum of patient no. 1.