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Interstitial Cystitis

An Epidemiological, Experimental and Clinical Study

■

ACADEMIC DISSERTATION

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CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

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V. **Leppilahti M**, Hellström P, Tammela TLJ: Effect of diagnostic hydrodistension and four intravesical hyaluronic acid instillations on bladder ICAM-1 intensity and association of ICAM-1 intensity with clinical response in patients with interstitial cystitis. Urology 60:46-51, 2002.

ABBREVIATIONS

APF	Antiproliferative factor
BCG	Bacillus Calmette Guerin
CD4	Clustered designation 4; T-helper cell
DMSO	Dimethylsulphoxide
DTPA	Technetium Tc-99m diethylenetriamine penta-acetic acid
FDA	Food and Drug Administration
GAG	Glycosaminoglycan
GP-51	Glycoprotein-51
HA	Hyaluronic acid
HLA-DR	Human leukocyte antigen allele DR
HB-EGF	Heparin-binding epidermal growing factor
HP	Hunner's patch
IC	Interstitial cystitis
ICAM-1	Intercellular adhesion molecule-1
ICDB	Interstitial cystitis database study
ICSI	Interstitial Cystitis Symptom Index
IL	Interleukin
KCl-test	Potassium sensitivity test
LFA-1	Lymphocyte function-associated antigen-1
MC	Mast cell
MHC	Major histocompatibility complex
NF-κB	Nuclear factor kappa-B
NIDDK	National Institute of Arthritis, Diabetes, Digestive and Kidney Disease
NHS	Nurses' Health Study
PPS	Sodium pentosan polysulphate
SCF	Stem cell factor
SS	Sjögren's syndrome
Th-1(2)	T-helper cell type 1 (2)
U.S.	United States
VAS	Visual analogical scale

ABSTRACT

Interstitial cystitis (IC) is an inflammatory bladder syndrome of obscure aetiology and pathogenesis. Its prevalence has remained enigmatic, and the diversity of empirical therapies has underscored the lack of understanding about its treatment.

The aim here were to evaluate the prevalence of urinary symptoms related to IC in Finnish women and compare these findings with those in patients with Sjögren's syndrome (SS), which are thought to make up a noteworthy sub-population of IC patients. Urinary symptoms were evaluated in the epidemiological studies by means of the validated O'Leary-Sant score. The prevalence of urinary symptoms corresponding to probable IC in the absence of urinary tract infection or pregnancy in the Finnish population was estimated to be 450/100,000 females. The age-standardized prevalence of probable IC was 15 times higher among the SS patients than the controls without SS, and a third of the probable IC cases in the reference population (2/6) reported SS.

An additional goal was to investigate experimentally the influence of the most widely used treatment method, transient overdistension of the bladder, on bladder wall morphology and enzyme histochemistry and to assess the duration of increased mucosal permeability after transient overdistension in rats. In the experimental trials, prolonged overdistension caused damage primarily to the urothelium, but the histological damage had almost completely healed within one week. Leakage from the urothelium continued longer, however, suggesting that despite temporary alleviation of the urinary symptoms, prolonged overdistension can even be harmful.

The objective of the clinical trial was to study the response of IC patients to diagnostic hydrodistension plus hyaluronic acid (HA) instillations and the possible role of ICAM-1 (intercellular adhesion molecule-1) receptors in the clinical response. HA binds and blocks the ICAM-1 receptor, which is a key molecule for leukocyte adherence and transendothelial migration. Diagnostic hydrodistension combined with HA instillations alleviated the symptoms in most patients, and although the majority needed continuously repeated instillations, a third obtained long-lasting relief with distension plus 4 weekly treatments. The clinical response was associated with the pre-treatment ICAM-1 index, which was 1.8 in the non-responders and 3.7 in the responders.

In summary, the findings indicate that the prevalence of probable IC is much higher than was previously supposed, and the close association of probable IC with SS proves that the latter is an important risk factor and suggests that IC and SS have shared susceptibility or pathogenetic mechanisms. Prolonged overdistension of the bladder causes damage primarily to the urothelium, and urothelial integrity is destroyed for over 1 week. Diagnostic bladder distension and HA instillations alleviate symptoms of IC in most patients fulfilling the NIDDK (National Institute of Arthritis, Diabetes, Digestive and Kidney Disease) criteria. The clinical response was associated with ICAM-1 intensity, suggesting that ICAM-1 may have an important role in the pathophysiological mechanism in most patients.

1. INTRODUCTION

Interstitial cystitis (IC) is a heterogeneous inflammatory bladder syndrome with symptoms that include pelvic and/or perineal pain, urinary frequency, urgency and nocturia (Hunner, 1915; Johansson and Fall, 1990; Wein et al., 1990). It is ten times more common among women than men (Hand, 1949; Oravisto 1975; Koziol et al., 1993). The diagnosis is made clinically, but currently there is no consensus on the specific findings required for this. The relative rarity of this syndrome and the lack of objective findings have made epidemiological studies difficult, and the prevalence of IC has also remained obscure, estimates ranging from 4.5 to 870/100,000 females (Miki and Yamada, 2000; Jones and Nyberg, 1997). Many aetiologies have been proposed, but none has been definitely proved and the pathogenesis has remained enigmatic.

The epithelial leakage theory was popularised by Parsons and coworkers (1990; 1991) as one explanation for IC, and many epithelial coating techniques, including sodium pentosan polysulphate (PPS), heparin and hyaluronic acid (HA) instillations, have been tested for treating these patients (Parsons et al., 1993; Parsons et al., 1994A; Morales et al., 1996). Overdistension of the bladder has nevertheless remained as one of the most widely used empirical modes of treatment, despite its cytodestructive effects (Bumpus, 1929; Dunn et al., 1977; Rovner et al., 2000).

Autoimmunity may also play a role in IC, although the abnormalities of the immune system observed in IC patients have been assumed to be secondary to chronic inflammation (Ratliff et al., 1995). Abnormal expression of human leukocyte antigen allele DR (HLA-DR) on the urothelium of IC bladder, for example, may nevertheless indicate a specific type of inflammation that involves an unusual pattern of cytokine networks (Liebert et al., 1991; 1993).

The object of this thesis was to evaluate the prevalence of IC-like symptoms among Finnish women and, more specifically, in patients with Sjögren's syndrome (SS), an autoimmune disease affecting the mucosal membranes. The effect of overdistension on the bladder wall and urothelium was assessed experimentally in rats, and the clinical response to diagnostic overdistension plus HA instillations was investigated in IC patients, together with an evaluation of the possible role of intercellular adhesion molecule-1 (ICAM-1) receptors in the clinical response, in an attempt to find explanations to challenge the epithelial coating theory.

2. REVIEW OF THE LITERATURE

2.1. History

Historically, IC has been a poorly recognised condition, and its description has been full of misconceptions. There are some significant milestones to be observed, however, since the term interstitial cystitis was first introduced by Skene (1878), who said aptly that if you neglect or fail to understand the particular symptoms of this affection, you will do justice neither to yourself nor to your patient. Another notable event was the report by Hunner (1915) on eight women having an ulcer as a feature of a disease that also involved a history of suprapubic pain, frequency, nocturia and urgency lasting an average of 17 years. He described haemorrhage following cystodistension and advocated surgical excision of the ulcer, which had yielded a satisfactory outcome in four out of five cases.

Throughout the 1920s and 1930s most inflammatory disorders such as IC were thought to arise from bacterial infections. Bumpus (1929) suggested that the condition was more common in women because of the spread of infection from the cervix, and reported that animals developed a similar bladder condition after inoculation with bacteria from cervical swabs. He reported an important observation that the disease involved not only the ulcer area but in fact most of the bladder, and also suggested that the descriptive term submucous ulcer should be replaced by interstitial cystitis.

Even Skene (1878) had described the creation of a vesico-vaginal fistula as an extreme treatment modality which relieves the inflamed and ulcerated bladder. Tait was the first surgeon to use urinary diversion (ureterosigmoideostomy) as a treatment for IC, but his success at relieving bladder symptoms was overshadowed by the 23% operative mortality (Counseller, 1937). Bumpus (1929) reported successful relief of symptoms in more than 100 cases by means of cystodistension at a pressure of 120mg Hg under general anaesthesia, and cystodistension and fulguration of ulcerated areas persisted as the most popular treatment into the 1940s.

Cristol and coworkers (1944), reviewing 78 cases of IC in men, concluded that its incidence was low and that it was often confused with symptoms of outflow obstruction. The first comprehensive paper on IC, reviewing 223 cases, was written by Hand (1949). Many of his epidemiological findings have held up over the years. He showed a female-to-male ratio of 11:1, and proposed that

the disease should be graded from 1 to 3, in which case 69% of his patients were of grade 1 and only 13% of grade 3, with a scarred, small-capacity bladder as described by Hunner (1915).

IC was described in children for the first time by McDonald and coworkers (1953), when a series of six children (all girls) aged 3 to 10 years were reported to have been treated with fulguration and cystodistension. The first significant Scandinavian report covered 59 patients treated at the Karolinska Institute over the period 1940 to 1956, among whom 39 had associated immunological disorders (Franksson, 1957).

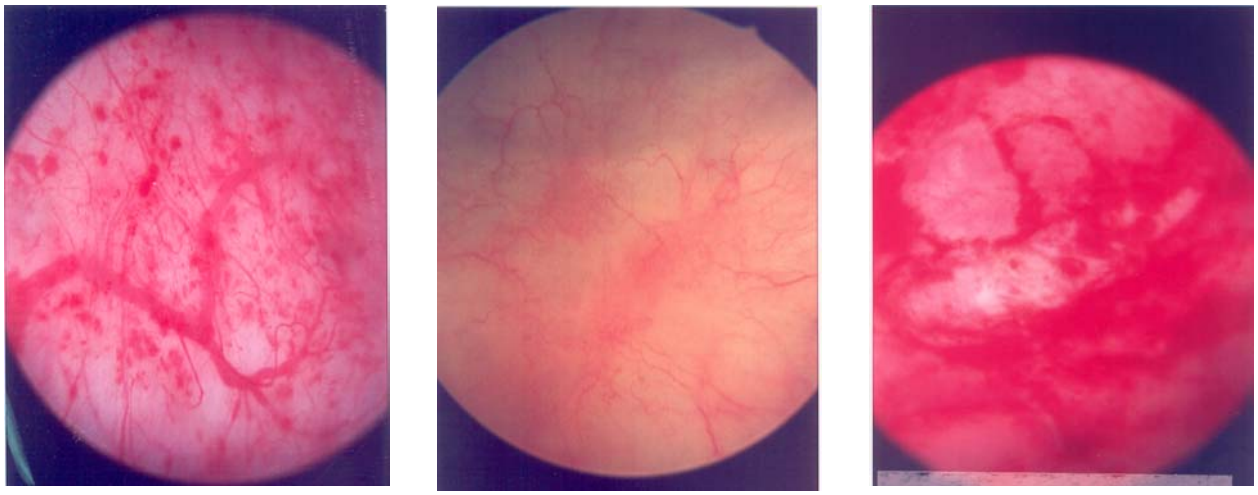
2.2. Definition

IC is still an enigmatic urological entity, diagnosis of which is based on its symptomatology. There are currently thought to be two types, of which the more common, non-ulcerative IC, accounts for 90% of all patients. This presents with petechial haemorrhages after hydrodistension of the bladder under anaesthesia (Figure 1A). Another form, classic IC, featuring Hunner's patches (HP), is present in approximately 5-10% of cases. An HP consists of a mucosal erythema with small vessels converging to a central pale scar, fibrin deposit or coagulum (Figure 1B-C). After hydrodistension, a central mucosal rupture occurs at this site, causing an artificial ulcer, called a "Hunner's ulcer" (Johansson and Fall, 1994).

Up to the late 1980s the definition of IC was a "hole in the air", and in an effort to define IC in such a way that patients in different geographical areas could be compared, the National Institute of Arthritis, Diabetes, Digestive and Kidney Disease (NIDDK) held a workshop at which consensus criteria were established to form a "research definition" (Gillenwater and Wein, 1988). A year later the criteria were revised (Wein et al., 1990; table 1). Due to the absence of specific findings or objective criteria, these criteria have also been used as a de facto definition of IC by clinicians. The traditional view of a severe complex of symptom nevertheless recognises only those patients with end-stage disease and probably identifies only a small proportion of the individuals afflicted.

Agarwal and coworkers (2001) proposed that the definition of IC should undergo drastic change in the new millennium, with a better understanding of the population affected by the disease, the ways in which it progresses and the factors by which it is provoked. They suggested that it is time to revise the name and diagnostic criteria of IC and try to clarify the pathophysiology and clinical characteristics of different subgroups of this painful multifactorial bladder syndrome. We still do

not, however at this moment, have any unambiguous objective tests or specific diagnostic criteria, and IC is perhaps best defined by the clinical symptoms of chronic pelvic pain in association with urinary frequency, urgency and nocturia in a patient who has no other definable pathological condition such as urinary tract infection, carcinoma, untreated gynaecological disorders (e.g. endometriosis) or cystitis induced by radiation or medication.



A B C

Figure 1. (A) Endoscopic view of petechial haemorrhages on the bladder mucosa after diagnostic hydrodistension with non-ulcerative IC, x10. Hunner's patch with classic IC **(B)** before and **(C)** after hydrodistension, x10.

Figure 2. Location of yellow fluorescent MC near varicose nerve fibres is demonstrated by the glyoxylic acid-induced fluorescence method, which is specific for the histochemical demonstration of catecholamines, x250.

Table 1. The NIDDK research definition of interstitial cystitis. (Some criteria (*) are only relative)

Required Criteria:

1. Glomerulations, or Hunner's ulcer in a cystoscopic examination
2. Pain associated with the bladder or urinary urgency

The examination for glomerulations should occur after distension of the bladder to 80-100 cm H_2O for 1-2 min under anaesthesia. The bladder may be distended once or twice before evaluation. Glomerulations must be diffuse and present in at least three quadrants of the bladder, at least 10 glomerulations per quadrant. Glomerulations must not be along the path of the cystoscope (to eliminate artefacts from instrumentation contact).

The presence of any one of the following criteria excludes a diagnosis of interstitial cystitis:

1. Bladder capacity greater than 350ml in cystometry when awake
2. Absence of an intense urge to void with the bladder filled to 100ml of gas or 150ml of water during cystometry, using a filling rate of 30-100ml/min
3. Demonstration of phasic involuntary bladder contractions in cystometry using a filling rate in the above range
4. Duration of symptoms less than 9 months*
5. Absence of nocturia*
6. Symptoms relieved by antimicrobials, urinary antiseptics, anticholinergics, or antispasmodics (musculo- tropic relaxants)*
7. Frequency of urination while awake less than eight times per day
8. Diagnosis of bacterial cystitis or prostatitis within a 3-month period*
9. Bladder or lower ureteral calculi*
10. Active genital herpes
11. Uterine, cervical, vaginal, or urethral cancer*
12. Urethral diverticulum*
13. Cyclophosphamide or any type of chemical cystitis
14. Tuberculous cystitis
15. Radiation cystitis
16. Benign or malignant bladder tumors
17. Vaginitis*
18. Age less than 18 years*

2.3. Epidemiology

2.3.1. Patient demographics

IC can start at any age, and more than a fourth of all patients are less than 30 years old at the onset of symptoms, the mean age at onset being 42.5 ± 0.8 years (Koziol et al., 1993; Figure 3). Oravisto (1975) reported that the symptoms of IC typically progress rapidly to the final state, mostly with little worsening thereafter. Held and coworkers maintained that symptoms manifest themselves fully within 1 month of onset in 33% of IC patients, while Koziol and coworkers (1993) reported this plateau in symptoms to occur within 5 years of onset. Although the symptoms of IC fluctuate, there was no evidence of any significant long-term change in overall disease severity in the Interstitial Cystitis Database (ICDB) -study (Propert et al., 2000).

Even though a high proportion of patients report psychological symptoms and difficulties with everyday tasks, these personality factors are not a consequence of IC but play an independent role in its course (Keltikangas-Järvinen et al., 1988). The female-to-male ratio is approximately 10:1 in most epidemiological surveys (Hand, 1949; Oravisto, 1975; Koziol et al., 1993). There have been few investigations into genetic associations, Oravisto (1980) reported IC developing in monozygotic twins and in a mother and daughter, and Warren and coworkers (2001) have now confirmed a greater concordance of IC among monozygotic than dizygotic twin pairs, suggesting the existence of genetic susceptibility.

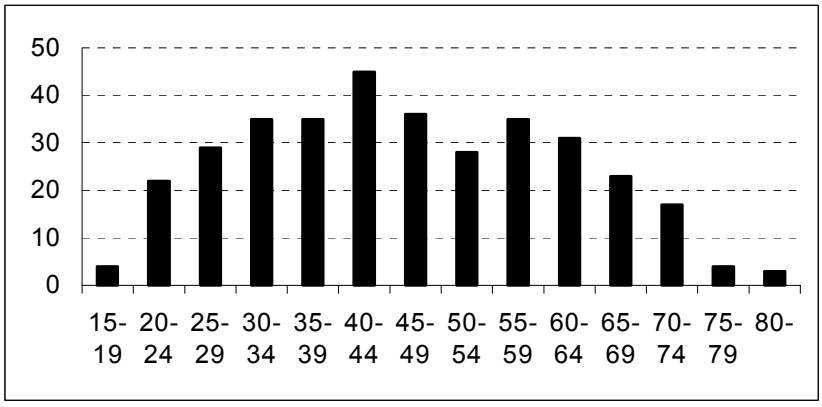


Figure 3. Age distribution of patients at the onset of IC, as reported by Koziol et al. (1993).

2.3.2. Population-based studies

Only a few epidemiological surveys of IC were reported during the last century, and without exception they had many shortcomings. The first urologist-based survey was conducted by Oravisto (1975), who estimated the prevalence of IC in the area served by Helsinki University Central Hospital at 18.1/100,000 females and confirmed that IC is ten times more common among women than men. A more recent study in the Netherlands gave a prevalence of 8-16/100,000 females (Bade et al., 1995), while an inquiry among 300 urologists in Japan yielded a prevalence of 4.5/100,000 females (Miki and Yamada, 2000). The major failing in these estimates, however, lies in the assumption that all IC patients are referred to a urology service and correctly diagnosed. A more comprehensive investigation, performed in the United States (U.S.) by Held and coworkers (1990), gave a prevalence for diagnosed IC cases that was approximately twice that reported by Oravisto (1975) in Finland. Interestingly, diagnosed cases represented only 20% of the patients with chronic painful bladder syndrome having sterile urine. The median age at onset was 40 years. Up to 50% of patients experienced spontaneous remissions that were probably unrelated to treatment. The patients with diagnosed IC had also had bladder problems during childhood 10 to 12 times more frequently than the controls. The time from onset of symptoms to diagnosis varied from two to five years. A further notable finding was that the IC patients scored lower in a quality-of-life test than patients with chronic renal failure undergoing dialysis.

The Nurses' Health Study (NHS) I and II in the United States estimated the prevalence of IC at 52-67/100,000 (Curhan et al., 1999), but these surveys again entailed a number of weaknesses, including inconsistencies in case definition. Altogether 357 patients in the NHS I population (0.4%) and 1354 in NHS II (1.4%) reported having been diagnosed by cystoscopy at some time as having IC (excluding urinary tract infection), but medical records were obtained for only 111 cases in NHS I (0.12% of all respondents), of which 47 were confirmed as IC (8 definite, 26 probable, 13 possible), and 106 in NHS II (0.11% of all respondents), of which 53 were confirmed (10 definite, 35 probable and 18 possible). The selected population and the low confirmation rate constitute potential sources of bias in the results.

The inconsistency of diagnostic methods and criteria is problematic, and each diagnostic approach has its inherent limitations. Chronic pelvic pain relieved by emptying the bladder is anyway the most distinctive symptom of IC, and the first large-scale epidemiological study of chronic pelvic pain in the U.S. was conducted as a telephone survey covering more than 17,000 households. Its

prevalence was 14.7%, and in 61% of cases the cause was unknown (Mathias et al., 1996). Another study (Zondervan et al., 1999), based on a general practice database, found an annual prevalence of pelvic pain of 38.3 per 1,000, with cystitis (31%) as the most common aetiology. Irritable bowel syndrome (29%) and pelvic inflammatory disease (10%) were other common causes. There were 28% of the positive respondents who did not receive any diagnosis, however, and it is particularly of interest that although genitourinary symptoms were present in 2.4% of the women, only 40% of them were referred to a specialist.

In the National Health Interview Survey, Jones and Nyberg (1997) used different methodology, relying on self-report of a previous diagnosis of IC. In this population-based study participants (20,561 adults) were asked if they ever had symptoms of a urinary tract infection that lasted 3 months or longer, and if they were told that they had painful bladder syndrome or IC. In this study, as high as 870/100,000 females self-reported painful bladder or IC.

2.3.3. Associated diseases

IC patients have been reported to suffer a higher incidence of other medical problems, such as allergies, migraines and certain autoimmune diseases (Sant and Theoharides, 1994). In a large-scale survey of 6783 patients having IC, 2,405 patients answered to the questionnaire. The data obtained from the responders (35% of all) was validated with information gleaned from 277 non-responders, showed allergies to be the most common disorder, with 41% having diagnosed allergies (Alagiri et al. 1997). A similar association to allergies was also reported by Hand (1949). Thirty percent of the individuals had irritable bowel symptoms, and fibromyalgia (typically associated with persistent fatigue, non-refreshing sleep, and generalized stiffness) was also over represented in the IC cases. As in IC, fibromyalgia also occurs mostly among women and only about 10% of patients are men (Yunus, 2002). The study also demonstrated a 30 to 50 times greater incidence of systemic lupus erythematosus in the IC group compared with the general population, while inflammatory bowel disease was found in over 7% (100 times higher than in the general population)(Alagiri et al. 1997).

There is also anecdotal evidence for an association between IC and SS, which is a systemic multiorgan autoimmune exocrinopathy with general mucosal dryness and systemic symptoms (Van de Merwe et al., 1993; Van de Merwe and Arendsen, 2000; Haarala et al., 2000). As with IC, 90% of SS patients are women and multifactorial influences contribute to the pathogenesis, but no large systematic studies have yet been published.

The pathophysiology of IC remains poorly understood, and several factors can contribute to development of its clinical symptoms. It is important, however, to study the connections with other medical problems as well, for progress in one may also help us to understand another. The exact relationships between IC and its associated disorders will not become clear until the cause and/or pathogenesis of each has been determined.

2.4. Aetiology

2.4.1. Mast cells

Mast cells (MC) are multifunctional immune cells that originate from the bone marrow as undifferentiated cells and enter various tissues, where they mature to subtypes in response to growth factors, including stem cell factor (SCF) and also interleukin 3 (IL-3) and 4 (IL-4)(Galli, 1990). SCF is a major agonist for human MC, being normally derived from stromal cells, but also from dysfunctional urothelial cells and MC themselves (Aldenborg et al., 1998; Galli, 1993). Mucosal (atypical) MC are found mostly in the bladder and gastrointestinal tract, while connective tissue (typical) MC are present in the skin and lungs. Mucosal MC play a prominent role in many inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, and also in reactions to neoplasms, wound healing and fibrosis. Mucosal MC promote infiltration of neutrophils, T and B lymphocytes, monocytes and eosinophils (Kops et al., 1984). T lymphocytes are known to secrete substances capable of activating again mucosal MC in particular, thus perpetuating the cycle of inflammation (Kaplan et al., 1985).

MC lie adjacent to the epithelial cells, vessels and peripheral nerves (Keith et al., 1995; verified by personal observation, Figure 2), which makes their cell products available to a variety of cell types. Current evidence indicates that MC are involved in the pathogenesis of IC, as electron microscopy has demonstrated the presence of elevated MC counts, activated by piecemeal degranulation, in close proximity to nerves, particularly in the suburothelium (Ratliff et al., 1995; Sant and Theoharides, 1994; Letourneau et al., 1992, 1996; Theoharides et al., 1995). Similarly activated MC have not been observed in any other bladder conditions, but they have been observed in the intestinal mucosa in irritable bowel syndrome, which commonly coexists with IC (Pang et al., 1996). Moreover, a proliferation of nerve fibers in the bladder has been demonstrated in IC (Christmas et al., 1990), which suggests a possible pathogenetic role for neuronal stimulation of

local MC populations. Activated MC are observed most prominently in biopsies from areas of glomerulations following diagnostic hydrodistension, a procedure that is known to activate MC (Yun et al., 1992), and they may also be instrumental in the development of such glomerulations. The accumulation of MC is most prominent in biopsies from areas of "Hunner's ulcer" in classic IC (patches of reddened mucosa exhibiting small vessels converging on a central pale scar), suggesting that these are also the consequence of cumulative neuroimmunal inflammation mediated by MC.

Neuroimmunohormonal triggering of MC may play an unique role in the pathogenesis of IC. Increased expression of high-affinity estrogen receptors has been reported in MC of the bladder in patients with IC (Pang et al., 1995A). Estradiol augments histamine secretion, while the anti-estrogen tamoxifen appears to inhibit MC degranulation (Vliagoftis et al., 1992).

2.4.2. Epithelial permeability

The bladder epithelium is almost impermeable to irritants in the urine by virtue of its tight cell junctions, ion pumps and the mucin layer composed of diverse classes of proteoglycans [glycosaminoglycans (GAG), including HA, chondroitin 4 and 6-sulphates and heparin sulphates, coupled to protein cores]. It was Parsons and coworkers (1990; 1991) who hypothesised and popularised the concept that IC is the result of a defect in the epithelial permeability barrier of the bladder surface GAG. Despite its popularity, this GAG deficiency postulate has many shortcomings, however. Ultrastructural, biochemical and functional studies of bladder GAG have not supported this theory of increased mucosal permeability, and a sophisticated electron microscopy technique failed to demonstrate any differences in the ultrastructural morphology of the GAG between IC cases and controls (Nickel et al, 1993). Chelsky and coworkers (1994), who measured bladder permeability in IC directly in terms of the transvesical absorption of technetium Tc-99m diethylenetriamine penta-acetic acid (DTPA), found that although some IC patients had a more permeable bladder, the same was observed in some normal volunteers. Only three out of ten IC cases had greater absorption of DTPA than the controls, possibly representing a subpopulation of IC patients who do indeed have greater bladder permeability. In a potassium sensitivity test, KCl provoked symptoms in 70% of IC patients and 4.5% of normal subjects (Parsons et al., 1994B), and it has been proposed that this may indicate mucosal leakage. However, it is possible that heightened sensitivity to KCl may be responsible for positive test rather than increased permeability of the

covering GAG. Overall, it is probable that there is a subpopulation of IC patients with increased epithelial permeability, but they may be only a minority.

2.4.3. Neurogenic mechanisms

The documented intimate association of MC with neural endplates in the bladder suburothelium in cases of IC points to a likely substrate for neurogenic inflammation. Polymodal nociceptor activation generates axon reflexes in the terminal arborizations of primary afferent neurons, and these reflexes cause the C fibers to release neuropeptides that initiate inflammatory changes (Foreman, 1987). Neurogenic inflammation can generate a biologically very potent mediator soup of neuropeptides and MC mediators that can induce a wide range of tissue changes culminating in inflammation, cell damage and fibrosis. These changes are the product of individual series and interactive cascades of biological events that are potentially self-perpetuating and incorporate MC activation and epithelial and tissue injury via the MC-lymphocyte-cytokine cascade (Elbadawi, 1997; Galli, 1993). The nature and severity of the tissue changes following a neuroinflammatory episode would largely depend on which mediators are released. The key to all this is selective secretion of mediators from cytoplasmic granules of MC through piecemeal degranulation. Neurogenic inflammation may be the cause of some cases of IC, or it may be the result of other initiating aetiological events. Overall, it is compatible with the central role of MC and the leaky epithelium theory.

2.4.4. Immunological mechanisms

The mucosa

Urothelial cells are a part of the larger mucosal immune system and are capable of responding to various stimuli by expressing cell surface activation markers and generating cytokines. The polymorphic major histocompatibility complex (MHC) genes are the best documented genetic risk factors for the development of autoimmune diseases, and the most relevant MHC genes are those of Class II, most specifically HLA-DR and HLA-DQ alleles (Reveille, 1992; Merriman and Todd 1995). Normal cultured urothelial cells respond to cytokine stimulation by expressing the activation antigens HLA-DR and ICAM-1 on their surfaces (Liebert et al., 1991; 1993). The majority of IC patients have been shown to have abnormal expression of HLA-DR (without ICAM-1 expression) by the urothelium (Liebert et al., 1993; Christmas and Bottazzo, 1993). Furthermore, clustered designation 4 ($CD4^+$), an essential regulatory molecule of the immune system that is found predominantly on T-helper cells, is a receptor for the HLA-DR molecule that can respectively

stimulate $CD4^+$ lymphocytes to initiate destruction. The increased numbers of $CD4^+$ T cells within the urothelium and submucosa suggest that these cells also play an active role in the pathogenesis of IC (Christmas, 1994). $CD4^+$ T lymphocytes are also able to stimulate MC by releasing cytokines such as IL-3 and this may also be an important factor in the control of MC (MacDermott et al., 1991). Interestingly, depletion of $CD4^+$ T lymphocytes (with specific monoclonal antibodies) in experimental autoimmune cystitis significantly reduced the presence of inflammation and the numbers of MC in the detrusor muscle (Ratliff et al., 1993). In some cases of clinical IC the unusual urothelial response may be a reflection of a genetically controlled aberrant immune response to small numbers of normally non-pathogenic bacteria or other stimuli.

Autoimmunity

The possibility that IC may represent some type of autoimmune disorder has been under consideration for many years. The occurrence of circulating antibodies reacting with the bladder tissue in cases of IC was first reported by Silk (1970), who found bladder-specific antibodies in the serum of 8 out of 20 patients with IC. Jokinen and coworkers (1972) found antinuclear antibodies in 28 out of 33 IC patients, but they were not bladder-specific, while Keay and coworkers (1997A) found anti-epithelial cell antibodies in the urine of IC patients. These antibodies were bound to nuclear or cytoplasmic antigens, and also to epithelial cells from a variety of tissues. Certain aspects of IC suggest that autoimmunity may play a role in initiating or sustaining the evident chronic inflammatory response, but the lack of specificity indicates that the immunological findings are likely to be secondary to inflammation rather than being a primary cause (Ratliff et al., 1995). A bladder-specific cell-mediated autoimmune response could conceivably activate MC via the T-cell-derived antigen-binding factor (Kops et al., 1984), leading to various mediator-induced tissue changes.

2.4.5. Urine abnormalities

Since IC is a heterogeneous syndrome of pluricausal aetiology, it is unlikely that a single marker can be identified which will serve to diagnose or exclude the entire spectrum of the disease. Many urine components decrease or increase in some IC patients and may serve as objective IC markers, and in addition to clarifying the wavering diagnosis, it is hoped that such markers may be considered in order to obtain support for a certain theory of pathophysiology and predict the response to various treatments. The two urine markers which currently achieve the clearest

separation between IC and control groups are glycoprotein-51 (GP-51) and antiproliferative factor (APF).

The assumption that a change in urothelial permeability (mediated by aberrations in bladder surface mucin) can be a pathophysiological factor in patients with IC has focused attention on glycoprotein components of the mucin layer. GP-51, a glycoprotein in the urothelium that has been shown to be decreased in bladder biopsies from IC patients (Byrne et al., 1999), can also be isolated from urine and clearly serves to distinguish IC patients who meet the NIDDK criteria from control subjects (Moskowitz et al., 1994).

It has also been postulated that urine may contain factors capable of injuring the mucosa and causing increased permeability. Indirect evidence for the involvement of a urinary cytotoxin in IC was first reported by Clemmensen et al. (1988), who demonstrated a toxic reaction in skin patch tests with urine from IC patients. Keay and coworkers (1996) indicated the presence in IC of a urinary cytotoxin with a molecular mass <10,000 daltons which inhibits bladder epithelial cell DNA synthesis (identified as antiproliferative factor (APF)). A profound decrease in heparin-binding epidermal growing factor (HB-EGF) has also been documented in IC patients (Keay et al., 1997B). Later Keay and coworkers (2000) demonstrated that the decrease in urothelial cell DNA synthesis caused by urine from IC patients is the result of an alteration in HB-EGF mediated by a specific APF (with the same purification profile). This APF appeared to be specific to IC, with 94% sensitivity and 91% specificity for asymptomatic controls and also 88% specificity for bacterial cystitis. Like urine HB-EGF, serum HB-EGF was also significantly lower in IC, making it possible that IC may be a urinary tract manifestation of a systemic difference in growth factor regulation, and suggesting that IC may be endogenous and possibly genetic (Keay et al., 2001).

IC is believed to be a multifactorial syndrome, and it is possible that different aetiologies may produce specific changes. Urinary histamine (or its metabolites) may be specific to MC activation, for example, while markers such as APF or GP-51 that appear to be altered in most IC patients may reflect a final pathway that can be initiated by any of several IC aetiologies (Erickson, 2001). In future, urinary markers will probably prove to be valuable for identifying specific subsets of IC patients and focusing future research on specific pathophysiologies and treatments.

2.5. Histopathology

The histopathological findings associated with IC are mostly non-specific, and their role in diagnosis is primarily to exclude other known diseases. Light microscopy may in any case play a supportive role in the diagnosis of IC. The pathological findings are predominantly in the suburothelium, with variable lesions in the urothelium and little change in the muscular layer. Suburothelial oedema and haemorrhages with variable mucosal ruptures are the most consistently observed histopathological features, whether in classic or non-ulcer IC, but most reports concern specimens taken immediately after hydrodistension and observed features may equally well be purely iatrogenic. On the other hand, the IC bladder seems to be more vulnerable and sensitive to this kind of manipulation. Anyway, one can also see completely normal biopsies in the non-ulcerative IC group (Johansson and Fall, 1994).

The inflammatory changes are usually limited to the lamina propria and dominated by mononuclear cells (MC, lymphocytes, plasma cells). MC involvement in particular has been assumed to be pathognomic for IC (Larsen et al., 1982). These findings are most frequently reported in patients with classic IC (Johansson and Fall, 1990). The diagnostic significance of bladder mastocytosis remains controversial, however (Hanno et al., 1990), mostly due to the lack of uniformity in tissue preparation, staining and examination, and also depending on the inclusion or exclusion of appropriate controls. Patients with urothelial carcinoma (who are very often used as controls) have a form of mastocytosis similar to IC, for instance, although their MC are intact in electron microscopy, by contrast with the piecemeal degranulation of the MC in patients with IC (Theoharides et al., 1995). More severe inflammation, and even fibrosis of the muscle layer, may be evident in patients with reduced bladder capacity (Larsen et al., 1982). The presence of immunoglobulin deposits or complement in bladder biopsies has also been reported (Gillespie et al., 1990).

2.6. Pathogenesis

Neuroimmunohormonal activation of MC is thought to play a unique role in the pathogenesis of IC. The role and interactions of pathogenetic mechanisms are demonstrated in Figure 4.

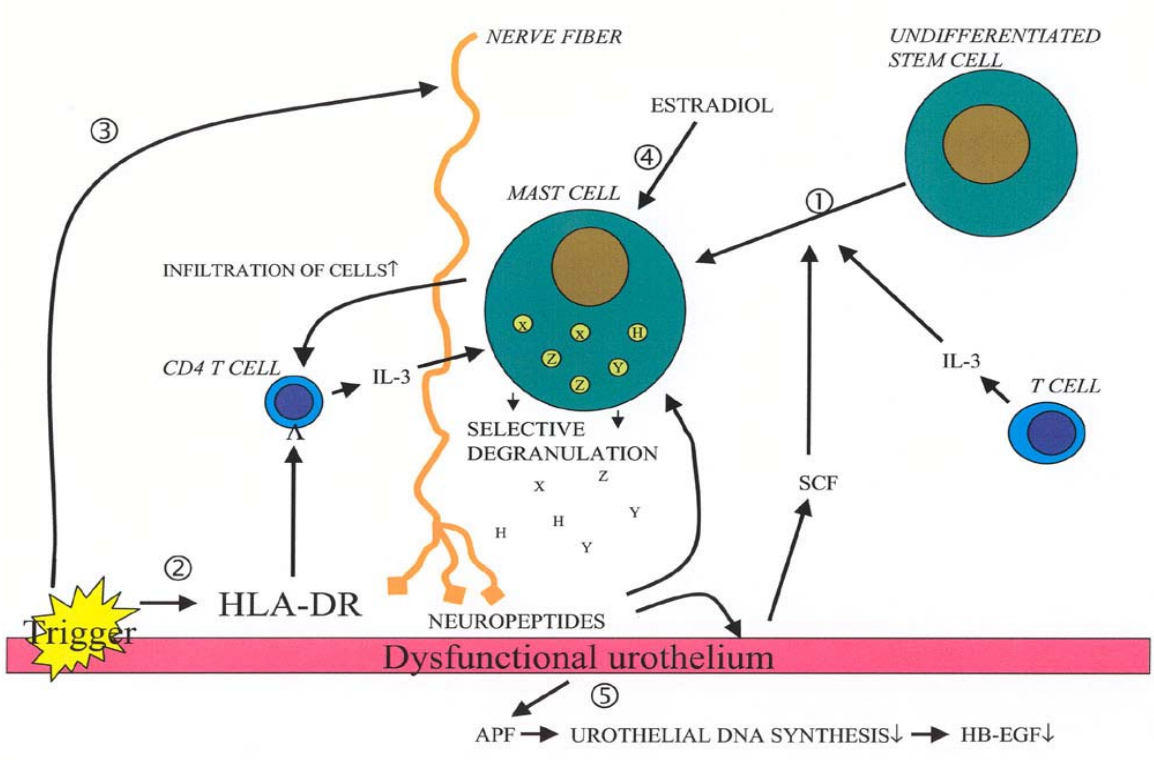


Figure 4. MC are submucosal multifunctional immune cells that lie adjacent to epithelial cells, vessels and peripheral nerves and can be activated by the bladder-specific cell-mediated autoimmune response via a T cell-derived factor, resulting in various mediator-induced tissue changes. **1.** MC originate from the bone marrow as undifferentiated stem cells that enter the tissues and mature to various subtypes in response to growth factors such as SCF (derived from dysfunctional urothelial cells), IL-3 and IL-4. **2.** A triggering factor (possibly microbial) activates abnormal expression of an HLA-DR molecule (possibly because of genetic susceptibility) that can in turn stimulate $CD4^+$ T cells to initiate tissue destruction. While recognising the HLA-DR molecule, the $CD4^+$ T cells are able to stimulate MC (and other inflammatory cells) through the release of cytokines (such as IL-3). **3.** The triggering factor may also have direct effects on sensory nerves, accelerating the release of neuropeptides which stimulate MC and accelerate a neurogenic inflammation mechanism. **4.** The MC have increased expression of high-affinity oestrogen receptors, and estradiol augments histamine secretion from them. **5.** Dysfunctional urothelial cells excrete specific APF, which reduces urothelial cell DNA synthesis and urinary HB-EGF.

2.7. Diagnostics

As the definition of IC has so far been based on the exclusion of recognizable diseases, it is not possible to suggest any accurate diagnostic criterion. The use of different criteria depends largely on the aim of the diagnosis. Inclusion in epidemiological series should be based on a symptomatology of urinary frequency, urgency and pain, while other known diseases are excluded. Kusek and Nyberg (2001) also proposed the more inclusive and less restrictive term chronic pelvic pain of the bladder (CPPB) to be used in future epidemiological studies. The strict diagnostic criterias of the NIDDK (Table 1) achieve high specificity and are valuable for research use, especially for studying the usefulness of new treatment methods, but it has been suggested that the diagnostic criteria used by experienced physicians in clinical practice are more comprehensive than those of the NIDDK (Propert et al., 2000). A thorough patient history, urine cultures and a cytological examination are the basic steps required to exclude other conditions.

2.7.1. Symptomatology

IC is characterised by urinary frequency, urgency and nocturia with pelvic pain. O'Leary-Sant's Interstitial Cystitis Symptom Index (ICSI, Appendix 1) was proposed by its authors as a uniform measure of IC (O'Leary et al., 1997). Its initial validation gave a sensitivity of 90% and a specificity of 97% at a cut-off level of 7 (or more), and its validity, reliability and responsiveness have been demonstrated more recently (Lubeck et al., 2001). These reports confirm that the ICSI is a useful tool for screening possible cases of IC at a cut-off level of 7. Urinary frequency can be measured more objectively by means of micturition diaries, which also reveal micturition volumes and nocturia.

2.7.2. Urodynamic studies

Urodynamic studies can help to assess bladder compliance and sensation and reproduce the patient's symptoms during bladder filling. It is also valuable for ruling out bladder instability (Siroky, 1994). Urodynamic testing was mandatory as a part of the work-up necessary for inclusion in the ICDB study (Simon et al., 1997), whereas cystoscopy was optional. One of the findings of this study was that the cystoscopic findings were significantly related to volume at the first desire to void and maximal urodynamic bladder capacity, the first desire to void being inversely associated to a high degree with the presence of Hunner's patch (94% of the patients with HP had a volume ≤ 50

cc) and the volume at maximum capacity in the urodynamics being positively associated with the volume at hydrodistension (Nigro et al., 1997).

Potassium sensitivity test

Parsons and coworkers (1994B) tried to popularise a KCl test to demonstrate increased permeability of the bladder mucosa in IC. The technique involves the subjects rating their subjective responses to the instillation 40 cc of water or 40 cc of potassium chloride solution (40 mEq per 100 cc water). Patients with IC are significantly more sensitive to the KCl test than healthy female controls (70% versus 4.5%; Parsons et al., 1994B), and sensitivity to KCl occurs in 16% of men with lower urinary tract symptoms, with significantly different urodynamic findings (urgency at lower volumes, lower bladder capacity) from those who have a negative test (Bernie et al., 2001). Teichman and coworkers (1999) supported the clinical usefulness of the KCl test by reporting a higher rate of improvement in patients with a positive test who were treated with PPS orally or heparin intravesically combined with tricyclic antidepressants. The diagnostic benefit of the KCl test has also been challenged, however (Chambers et al., 1999). Overall, potassium will depolarise the nerves, and a positive KCl test may be caused by abnormal leakage from the urothelium. On the other hand, bladder permeability in most IC patients is quite similar to that in controls (Chelsky et al., 1994), so that it is more probable that most patients with a positive KCl test will not have increased mucosal permeability but heightened sensitivity to potassium.

2.7.3. Cystoscopy

Cystoscopy under anaesthesia, together with hydrodistension of the bladder and a biopsy, are recommended for high-quality practice (Hanno, 1994A). Increased angiogenesis, in particular the appearance of tortuous, spiral vessels, which can be noted prior to distension, was discovered to be the most reliable endoscopic criterion (Gillespie et al., 1990). Glomerulations are not specific to IC, as they can be found in a variety of other disorders, and they may not always be present in IC (Fall et al., 1987), but their presence is still generally associated with a higher likelihood of IC. All the patients with HP in the ICDB study had some level of glomerulations, and none of the patients who did not have glomerulations had HP. Furthermore, the presence and increasing severity of glomerulations was positively associated with the presence of HP, reflecting a possible shared pathophysiology. Overall, glomerulations appeared in more than 90% of IC patients. Their severity was also correlated with a lower bladder capacity, and may point to the severity of the disorder (Nigro et al., 1997).

2.8. Treatment

The goal of curative therapy should be to neutralise the factors responsible for the disorder. As the aetiology and pathophysiology of IC are so far obscure, all treatment modalities are symptomatic, and there is no certain cure for IC at the present time. There is great diversity among IC treatment modalities, and as many as 183 types of therapy were recorded in the ICDB study (Rovner et al., 2000). It is difficult to achieve a critical assessment of the various therapies because up to 50% of temporary remissions are unrelated to them (Held et al., 1990). Furthermore, the use of subjective criteria for assessing the improvement may be expected to entail up to a 35% placebo effect. This may even induce that statistically significant results remain clinically marginal.

2.8.1. Overdistension of the bladder

Overdistension of the bladder under anaesthesia is the oldest (Bumpus, 1929) and usually still the first and most widely used therapeutic modality employed, often as a part of the diagnostic evaluation (Rovner et al., 2000). The method has not been validated and the mechanism of its effect is hypothetical. One modality of overdistension is a Helmstein's balloon dilatation, in which a special balloon is placed within the bladder and inflated to mid-systolic pressure for a certain length of time (Helmstein, 1972). Prolonged distension for up to 3 hours by this method has given good results in some instances (Dunn et al., 1977). Hydrodistension with fluid supplied at a pressure of 80-100cmH_2O is currently the most frequently used method. Many patients report transient relief of their symptoms even following diagnostic hydrodistension for 1 to 2 minutes at a pressure of 80 cmH_2O. Prolonged hydrodistension has been shown in animal experiments to cause damage to the submucosal nerve plexus, which is thought to reduce pain and frequency (Tammela et al., 1990; Lasanen et al., 1992). Furthermore, even diagnostic hydrodistension has an effect on MC and urinary markers, which may play a partial role in the response and in some cases even temporarily aggravate symptoms (Chai et al., 2000). Hydrodistension of the bladder can stimulate histamine release through activation of the MC (Pang et al., 1995B) and the histamine released can in turn stimulate the sensory nerves to induce neurogenic inflammation. Hydrodistension can also directly stimulate the release of neuropeptides from the sensory nerves (Ruggieri et al., 1994).

2.8.2. Oral medical therapy

Several IC therapies with oral drugs have been tried, and on many occasions the initial results have been promising, but mostly the efficacy has later proved disappointing. Some drugs have nevertheless established their position in this field.

Amitriptyline

Amitriptyline, which has been successfully used at a relatively low dose for many chronic pain syndromes, has also become a staple form of oral treatment for IC. Pharmacologically it has anticholinergic and beta-adrenergic bladder effects, sedative characteristics and a strong antihistaminic activity with respect to H_1-receptors, and it is therefore ideal for IC. Therapy is started at a dose of 25 mg at bedtime, gradually increasing to 75 mg over two weeks. The benefits should be apparent within 4 weeks. Hanno (1994B) reported that two thirds of the patients who could tolerate the drug obtained significant symptomatic relief, and that the drug seemed to achieve the best results in patients with bladder capacities over 600 ml under anaesthesia.

Hydroxyzine

Although the MC play an obvious role in the pathogenesis of IC (Theoharides et al, 1995), the results of antihistamine therapy have been far from dramatic. Encouraging results have nevertheless been reported for the H_1-receptor antagonist hydroxyzine, which blocks neuronal activation of the MC and inhibits their degranulation (Simmons, 1961). Hydroxyzine is the only antihistamine that has this effect (Theoharides, 1994). The recommended medication is 25 mg at bedtime, increasing to 50 mg at night and 25 mg in the morning over 2 weeks. In an open study of 40 cases, two thirds of the patients reported a sharp reduction in the burning sensation and pain (Theoharides, 1994).

Sodium pentosan polysulphate

Since Parsons (1977) popularised the theory of a defective GAG layer as an aetiological factor and reported successful results achieved with PPS (Parsons et al., 1993), this drug has been widely used, on the premise that it may replenish the defective GAG layer. In a randomized, placebo-controlled trial with 100 mg three times per day, 32% of the IC patients showed significant improvement compared with 16% of the controls receiving a placebo. The mucosal leakage theory has been challenged, however, and an alternative explanation for the benefit acquired from PPS in cases of IC may lie in direct inhibitory action on the MC, related to the inhibition of inositol triphosphate, and subsequent increases in intracellular calcium ions (Chiang et al., 2000).

Immunosuppression

While autoimmunity is one of the aetiological theories, immunosuppressive drugs have also been tried for IC therapy. Systemic steroids have not been found useful (Pool, 1967). Oravisto and Alfthan (1976) reported improvements in half of their patients treated with azathioprine and chloroquine derivatives, but there were significant side effects. Cyclosporine is a well tolerated and effective immunosuppressive drug which is widely used for a number of disorders mediated by immunological mechanisms. By preventing the expression of cytokines, cyclosporine selectively and reversibly inhibits T cell activation. In a preliminary study cyclosporine gave encouraging results in patients with severe IC, although a third of these patients later needed cystectomy (Forsell et al., 1996).

2.8.3. Intravesical therapy

Dimethylsulphoxide

Intravesical pharmacotherapy has a certain advantage over systemic therapy in that high drug concentrations can be achieved locally with no significant systemic side-effects. Dimethylsulphoxide (DMSO) is probably the drug most widely used for the instillation treatment of IC, because of its affordability and proven safety. The frequency and duration of treatment are empirical matters, from four to eight instillations mostly being given at 1 to 2-week intervals, with subsequent instillations depending on the severity of the symptoms and the temporal response to each treatment. Sant and La Rock (1994) also recommended monthly prophylactic instillations for patients with more severe symptoms. DMSO has many properties (anti-inflammatory, analgesic, MC stimulation, muscle relaxant and collagen dissolving) that make it appealing for the treatment of IC. It inhibits cell migration, modulates the cell-mediated immune response (Bartfeld and Goldstein, 1975) and may also prevent painful nerve irritation by scavenging radicals. Its efficacy has been demonstrated in controlled trials (Perez-Marrero et al., 1988) and it was also approved by the FDA (Food and Drug Administration) in 1978 for the treatment of IC. Both the classic and non-ulcerative forms of IC respond to DMSO treatment with 50% to 70% symptomatic improvement after repeated instillations of 50 ml of a 50% DMSO solution (Bartfeld and Goldstein, 1975; Sant and La Rock, 1994). Relapse rates after a 4 to 8-week course of treatment are about 40%, but up to 60% of relapse cases respond to additional treatment (Sant and La Rock, 1994). Suitably motivated patients have also used intermittent self-catheterization for DMSO instillation.

Hyaluronic acid

HA is an important GAG, and also a component of the normal GAG layer in the bladder. Intravesical HA (40mg 4 times weekly, then monthly) has been used to treat IC, by virtue of its possible replenishment of bladder GAG, with response rates of up to 70% (Morales et al., 1996). Alternatively HA may inhibit bladder inflammation in many other ways. HA inhibited stress-induced MC activation in rat experiments (Boucher et al., 2002), and, with a molecular weight above 1.2MDa, it can also inhibit nuclear factor-κB (NF-κB) activation and IL-6 expression in epithelial cells (Neumann A et al., 1999). It may also bind to ICAM-1, which is often expressed in response to MC activation (Walsh et al., 1991).

Bacillus Calmette Guerin

Intravesical administration of the Tice® strain of Bacillus Calmette Guerin (BCG) has proved in recent pilot studies to be safe and efficacious for the treatment of IC in some patients (Zeideman et al., 1994). A 60% BCG response rate was noted, compared with a 27% placebo rate (Peters et al., 1997), and 90% of the patients who responded favourably continued to have an excellent response over follow-up periods ranging from 24 to 33 months (Peters et al., 1999). The patients who achieved remission remained free of IC-related symptoms for more than 2 years, suggesting good durability of its effect (Peters et al., 1998). Aberrant HLA-DR expression is found in the bladder of patients with IC (Liebert et al., 1993), and $CD4^+$ T lymphocytes (activated by HLA-DR) presumably play an important role in the pathogenesis of IC. $CD4^+$ T-cells are divided into two subsets, T-helper cell type 1 (Th-1) and Th-2, of which the Th-2 response is thought to be associated with inflammation, favouring antibody formation and tissue necrosis, while Th-1 has protective properties (Pellegrini et al., 1996). The Th-2 response is mediated by IL-6, and the urine of patients with IC has been found to have a five-fold enrichment of IL-6 (Lotz et al., 1994). Inhibitors of IL-2, which stimulate a Th-1 response, are also found in the urine of patients with IC (Shingleton and Fleischmann, 1991). These findings support the hypothesis that IC is a $CD4^+$/Th-2-mediated disease. BCG is known to stimulate the Th-1 cytokine pathway (Jackson et al., 1995), and by altering the immune response profile it may lead to a long-term improvement in symptoms.

2.8.4. Surgical therapy

Major surgery for IC is accompanied by significant adverse effects (such as urostoma, a possible need for self-catheterization or operative complications) and is to be considered only when all other treatment options have failed. A thorough patient evaluation is mandatory, and reconstruction must

be planned individually. IC is not a fatal disease, but the urologist should also bear in mind that in severe cases it can influence the patient's quality of life very markedly, so that nihilism does not do justice to the patient.

Supratrigonal cystectomy and cystoplasty has been a popular surgical procedure for IC, but the results have not always been fully successful. Flood and coworkers (1995) reported an excellent outcome for only 10 out of 18 IC patients (56%), and the results have been similar in many other reports. Trigonal biopsies have also been recommended before cystoplasty (Nielsen et al., 1990; Nurse et al., 1991), but this is at least questionable, because IC has no pathognomic histological findings and even mastocytosis of the "affected" trigone may be missed if the activated MC have emptied their granules. Nurse and coworkers (1991) reported an active disease in trigonal biopsies from 13 out of 25 patients, but 2 of the 12 patients with apparently disease-free trigonal remnants also had poor results after supratrigonal cystectomy. Most patients need also permanent, clean intermittent catheterization after subtotal cystectomy (Flood et al., 1995). Bejany and Politano (1995) reported excellent results with total bladder replacement and recommended neobladder reconstruction. Total cystectomy with bladder replacement or urinary conduit is currently the only available curative treatment, but even this form of surgery requires considerable preoperative planning and careful follow-up to optimise the quality of life for a group of patients who often appear to have an unsalvageable problem.

3. AIMS OF THE PRESENT RESEARCH

Interstitial cystitis is a chronic inflammatory bladder condition, the epidemiology, aetiology, pathophysiology and appropriate treatment of which have remained elusive. Due to the absence of specific findings and objective diagnostic criteria, the true prevalence of IC is also obscure, with estimates ranging from 4.5 to 870/100,000 females. It has been suggested in previous pilot studies that IC may have a connection with SS. One of the most widely accepted theories assumes that increased permeability of the urothelium allows leakage of urine into the bladder wall, followed by irritative symptoms and late inflammatory changes. On the other hand, the bladder histology, showing infiltrates of mast cells, eosinophilic leukocytes and T lymphocytes, suggests that the disease is mediated by the immune system. As the factors causing IC remain to be identified, any therapies will necessarily be symptomatic. Overdistension of the bladder is the oldest and still the most widely used treatment to relieve symptoms temporarily, and a number of epithelial coating techniques have also been introduced for clinical use.

The aims of the present research were:

1. to evaluate the prevalence of urinary symptoms related to IC in Finnish women,

2. to study the occurrence of urinary symptoms related to IC in connection with SS by comparison with the normal population,

3. to investigate experimentally the influence of transient overdistension on bladder wall morphology and enzyme histochemistry in rats,

4. to study the duration of increased mucosal permeability of the urinary bladder after transient overdistension in rats, and

5. to study the clinical response of IC patients to diagnostic hydrodistension plus HA instillations and the possible role of ICAM-1 receptors in the vessels and inflammatory cells in the clinical response.

4. MATERIALS AND METHODS

The work was carried out at the Department of Surgery, Seinäjoki Central Hospital (I-V), and the School of Health Care, Seinäjoki Polytechnic (II), Seinäjoki, at the Department of Urology (I-V), Tampere University Hospital, and Medical School, and the School of Public Health (I-II), University of Tampere, Tampere, at the Department of Surgery (III-IV), Department of Urology (V), Department of Forensic Medicine (III) and Department of Pathology (IV), Oulu University Hospital and the University of Oulu, Oulu and at the Department of Obstetrics and Gynaecology, Turku University Hospital (II), Turku.

4.1. Epidemiological studies

4.1.1. Subjects (Papers I and II)

Two thousand Finnish-speaking women aged 18-71 years were randomly selected from the Finnish Population Register as a reference group, and a further group of women aged 18-71 (N=1,214) were identified from the membership files of the Finnish Sjögren's Syndrome (SS) patients' organization.

4.1.2. O'Leary-Sant score (Papers I and II)

The O'Leary-Sant score (Appendix 1) is considered to detect the most important voiding and pain symptoms experienced by IC patients and to assess the severity of the perceived problem (O'Leary et al., 1997). The validity, reliability and responsiveness of the index have been demonstrated by Lubeck and coworkers (2001). The O'Leary-Sant score focuses on the four symptoms which define IC - urgency, frequency, nocturia and pain – and score them on a scale from 0 to 5 to provide a diagnostic index. Pain is scored only on a five-point scale, with the score 1 being omitted in order to emphasise the importance of pain in IC. The problem arising from each of these four symptoms is then scored from 0 to 4. Thus the total symptom score ranges from 0 to 20 and the total problem score from 0 to 16. Although these indices were not initially developed for screening purposes, only a few IC patients scored less than 7 on the symptom index (5/45) or the problem index (6/45) in the validation study, whereas only 2/67 healthy controls had a symptom score of 7 or higher and 1/67 a problem score of that magnitude. All the controls had scores less than 12 on the two indices combined (O'Leary et al., 1997). The validation therefore suggests that the score is a valid tool for

epidemiological studies, with 90% sensitivity and 97% specificity at a cut-off level of 7 and a sensitivity of 50% and a specificity of 100% at a cut-off level of 12.

4.1.3. Data collection (Papers I and II)

A questionnaire including the necessary data for obtaining an O'Leary-Sant score was mailed to the subjects on April 10, 2000, and an identical questionnaire six weeks later to those not initially responding. The questionnaire also contained items on age, education, smoking, number of deliveries and diagnosis of SS made by a physician. An additional question on urinary tract infections treated with antibiotics during the past month was included to eliminate symptoms related to an infection.

4.1.4. Categorization of urinary symptoms (Papers I and II)

On the grounds of the original validation, we categorized the O'Leary-Sant score into four groups: no symptoms (0-3, only 2% of possible IC cases), mild symptoms (4-6, 10% of possible IC patients), moderate symptoms (7-11, 90% sensitivity and 97% specificity at a cut-off level of 7, 40% of IC patients) and severe symptoms (≥ 12, 50% of IC patients, with 100% specificity). IC was classified in terms of the symptom and problem scores as possible (≥ 7) or probable (≥ 12), with nocturia and pain ≥ 2 but without infection or pregnancy. The reference group comprised 27 cases of SS. When analysing the risk of IC among SS patients, these cases were excluded from the reference group. Two of these 27 patients had severe symptoms that fulfilled the criteria for probable IC.

4.2. Experimental studies

4.2.1. Animals (Papers III and IV)

A total of fifty-four 3-month-old female rats of the Sprague-Dawley strain weighing 245-320g were used for the experimental study of bladder overdistension. The bladders of 44 rats were over-distended and the remaining 10 rats were anaesthetized only as controls (III, IV).

4.2.2. Anaesthesia and bladder distension (Papers III and IV)

The animals were anaesthetized with pentobarbital sodium, 35mg/kg of body weight, injected intraperitoneally. The bladder was catheterised with a Fogarty (3F) arterial embolectomy catheter (Baxter, Santa Ana, U.S.), the balloon being filled with 0.05 ml water and pulled into the bladder neck. The rats were given furosemide, 12 mg/kg of body weight, intramuscularly and Ringer solution, 12 ml/kg of body weight, intraperitoneally to induce diuresis and maximal bladder distension, which was palpated as a hard resistance in the lower abdomen. After distension for 3 h, the bladder was emptied (range 2.0-2.5 cc) and the rats were allowed to recover. They were then watched carefully to check bladder emptying, and buprenorfine, 0.1-0.3 mg/kg of body weight was injected subcutaneously in cases of pain. Infection prophylaxis was achieved with intramuscular cefuroxime, 30 mg/kg of body weight.

4.2.3. Specimens (Papers III and IV)

Using the same anaesthesia as above, full-thickness samples were taken transversely from the wall of the bladder dome and posterior corpus of 30 rats 12, 24, 48 h and 7 days after distension for histological examination and enzyme histochemistry. Six rats at a time point were used, and six anaesthetized rats without bladder distension served as controls. Half of each specimen was fixed in buffered 10% formalin (pH 7), embedded in paraffin, sectioned at 4μm and stained with haematoxylin-eosin, and the other half was immediately frozen in liquid nitrogen and then stored at -70 °C. Three enzyme reactions (alkaline phosphatase, lactate dehydrogenase and adenosine triphosphatase) were performed on these sections to demonstrate degenerative changes (III).

Another 20 rats and four controls were anaesthetized 0, 24, 48 h, 7 days and 21 days after distension and 0.5 ml of a 2% solution of Trypan blue in 0.9% NaCl solution was instilled and left in the bladder for up to 1 h. The dye was then removed and the bladders washed four times with 0.5ml saline. Full-thickness samples were taken as previously described, buffered in formalin and embedded in paraffin, and blocks were photographed at 10x magnification in addition to histological examination (IV).

4.3. Clinical studies

4.3.1. Patients and treatment (Paper V)

The patient material consisted of two men and nine women fulfilling the rigorous criteria for IC described by the NIDDK (Wein et al., 1990) who were being treated at three urological centres (Seinäjoki Central Hospital, Oulu University Hospital and Tampere University Hospital). Diagnostic hydrodistension was performed under general anaesthesia, the bladder being distended to maximum capacity for 1 minute at a pressure of 80 cm water. All IC patients were treated with 4 weekly intravesical HA instillations (Healon GV® 40mg/50ml for 2 hrs).

4.3.2. Clinical examinations (Paper V)

The eleven IC patients kept voiding diaries for 7 days. A urine culture, urine cytology and urodynamic studies were performed to exclude other recognizable bladder diseases, and a visual analogical scale (VAS) was used to assess the severity of pain. Cystoscopy was performed under general anaesthesia and the bladder was distended. Deep-bladder biopsies were taken from petechial areas and stored at -70°C. The clinical examinations were repeated 2 weeks after intravesical HA instillation treatment, and then at 4.5, 8 and 12 months for check-up purposes. Control biopsies were taken 2 weeks after the last instillation.

4.3.3. Determination of ICAM-1 receptors (Paper V)

Antibodies

Previously described monoclonal antibodies against human ICAM-1 (5C3, mouse IgG1) and CD31 (2C8, mouse IgM) were used (Airas et al., 1993). A rabbit polyclonal antibody against Factor VIII was purchased from Atlantic Antibodies, and CD31 and Factor VIII served as endothelial markers. A monoclonal antibody 3G6 (mouse IgG1) against chicken T cells was used as a negative control. Peroxidase-conjugated antibodies against mouse and rabbit immunoglobulins were obtained from Dako. All the antibodies were used at predetermined optimal dilutions.

Immunohistochemical staining

Staining was performed as previously described (Salmi and Jalkanen, 1992). Briefly, 5μm frozen sections were cut from the tissue samples, fixed in acetone and overlaid with 100 μl primary antibody for 20 min and then with an appropriate second-stage antibody for another 20 min. The

colour reaction was developed with 3,3-diaminobenzidine in hydrogen peroxide as the chromogen. Slides were washed for 2x5min in phosphate-buffered saline between all incubations. The sections were finally lightly counterstained in haematoxylin, dehydrated in a graded alcohol series, cleared in xylene and mounted in Depex to obtain permanent records.

Determination of the ICAM-1 index

Two independent observers without any knowledge of the clinical data read all the sections. Sections from a given tonsil were stained with each antibody on the same day in all the staining batches to ensure that there were no significant changes in staining intensities between the days. Moreover, all the samples from one patient (representing different time-points) were always included within the same staining batch. A semi-quantitative scoring system was used to evaluate the staining results. The intensity of endothelial cell staining with anti-ICAM-1 mAb and the intensity of lymphocytic ICAM-1 expression were scored as follows: 0 = negative, 1 = low, 2 = moderate, and 3 = strong. The final score for each parameter was obtained by counting the mean of the estimates from the two readers. The ICAM-1 index was obtained by summing the numerical values for the intensity of lymphocytic ICAM-1 expression and the intensity of endothelial cell staining with anti-ICAM-1 mAb.

4.4. Statistical methods (Papers I, II and V)

The age-standardised prevalence ratios in papers I and II were determined according to the world population. Exact 95% confidence intervals were defined under the Poisson assumption.

The significance of the association between the ICAM-1 index and the clinical response in Study V was tested using the non-parametric Mann-Whiney's U test.

5. RESULTS

The results are given in detail in the appropriate papers I-V. They may be summarized as follows:

5.1. Epidemiological results

5.1.1. Response rates (Papers I and II)

Of the 2,000 women originally approached as a reference population, 1074 (53.7%) replied to the first mailing and 269 (13.5%) to the second, giving a total of 1,343 respondents (67.2%). The proportions of each score category (no symptoms-severe symptoms) were similar in both mailings. After further exclusions (incomplete questionnaire, subject institutionalised for chronic illness, unwilling to co-operate), the final reference group comprised 1331 women (66.6%). Of the female members of the Finnish SS organization originally approached (N=1,214), 827 (68.1%) replied to the first mailing and 163 (13.4%) to the second, giving a total of 990 respondents (81.5%), of whom 870 had a diagnosis of SS confirmed by a physician. The prevalence of severe symptoms was higher among the SS respondents in the first mailing than in the second.

5.1.2. IC-like symptoms in the reference population (Paper I)

Overall, 73% of the women in the reference group had few or no symptoms (score 0-3), 15% had mild symptoms (4-6), 9% moderate symptoms (7-11) and 3% severe symptoms (≥ 12). Mild to moderate symptoms were most common between the ages of 18 and 30 years, while the prevalence of moderate and severe symptoms increased slowly after 30 years (from 5% to 11%, and from 1% to 4%, respectively). Seventy-eight percent of the population experienced no problems (0-3), 13% had mild problems (4-6), 7% moderate problems (7-11) and 2% severe problems (≥ 12). The proportion of moderate and severe problems also increased after the age of 30 years (I, table 2). The prevalences of severe symptom/problem scores were slightly increased in current or former smokers, but the difference was not statistically significant. Urinary symptoms were not associated with the number of deliveries.

After exclusion of the women receiving antibiotic treatment for urinary tract infection during the past month (10 patients with moderate symptoms and four with severe symptoms), the number of patients receiving moderate or severe symptom scores combined with moderate or severe problem

scores (≥ 7) was 86/1331 (6.5%). Eleven out of the total of 1,331 patients (0.8%) reported severe symptoms and problems (≥ 12; I, table 3), and six out of these (0.45%, 95% CI 0.2-1.0%) fulfilled the criteria for probable IC, five of them having a pain score ≥ 3.

5.1.3. IC-like symptoms in patients with SS (Paper II)

Twenty-eight percent of the SS cases currently had moderate symptom and problem scores (7-11), while 12% gained severe symptom scores and 11% severe problem scores (≥ 12). There were no substantial age differences in the severity of symptoms (p= 0.29). After exclusion of the pregnant women and those with an assumed urinary tract infection during the past month, the prevalence of the SS patients reporting moderate or severe symptom and problem scores (≥ 7) combined with pain and nocturia ≥ 2 (possible IC cases) was 15%. Five percent of the SS cases (95% CI= 3.7-6.4%) fulfilled the criteria for probable IC (symptom and problem scores ≥ 12 with pain and nocturia ≥ 2, but without urinary tract infection or pregnancy), while 0.3% of the subjects in the reference group without SS fulfilled these criteria.

Moderate or severe symptoms were more frequent among the current smokers in the SS cases than among the former or non-smokers (p=0.035, RR=2.3, 95% CI=1.9-2.8), whereas no such relation had been found in the reference group. Urinary symptoms were not associated with the number of deliveries in either group. A urinary tract infection treated with antibiotics during the past month was reported by 4.4% of the SS patients vs. 2.3% of the controls (RR 1.9, 95% CI=1.2-3.1; age-standardized prevalences).

Moderate symptoms were almost four times as common among the SS patients as in the reference group (RR=3.7, 95% CI=3.0-4.5) and severe symptoms five times as common (RR 5.0, 95% CI=3.3-7.8) (age-standardized prevalences), while moderate or severe problems were similarly five times as common in the SS cases. Severe pain (scores 4-5), which is perhaps the most important differential diagnostic symptom of IC, was as much as 14 times as common among the SS patients than in the reference population. The median symptom index was five for the SS patients and two for the reference cases, the corresponding median problem index being five for the SS patients and one for the controls.

The age-standardized prevalences (according to the world population) for possible and probable IC were 10% and 4% respectively in the SS patients and 2% and 0.2% in the control cases without SS, the prevalence rate ratios being 5.3 (95% CI=3.4-8.2) and 15 (95% CI=4.8-50).

The prevalence of probable IC in these women with SS was 7.4%, as against only 0.3% in the women without SS, giving a prevalence rate ratio of 24 (exact 95% CI 0.9-590). Two out of six patients in the probable IC group reported a diagnosis of SS, while such a diagnosis was reported by 27/1,331 women in the reference population (2.0%), of whom five (19%) fulfilled the criteria for possible IC and two (7%) for probable IC.

5.2. Experimental results

5.2.1. Histology (Papers III and IV)

Hyperaemia and oedema in the submucosa and muscular layer reached their maximum 48 h after distension, when large numbers of granulocytes were seen in the submucosa and the urothelium was disrupted and vacuolated in many places. By 7 days after distension the oedema and hyperaemia had disappeared, there were no granulocytes and the urothelium was continuous (III).
Following Trypan blue instillation 0, 24 and 48 h after overdistension the bladder wall was deep blue throughout, and there was still considerable staining of the bladder surface urothelium and subjacent connective tissue at 7 days, although staining of the muscle was very mild. There was no longer any evidence of staining of the bladder wall or urothelium 21 days after overdistension (IV).

5.2.2. Enzyme histochemistry (Paper III)

Lactate dehydrogenase showed a strong even reaction in the urothelium and muscle layer in control animals. Granular spreading of staining was seen in the urothelium 12 h after distension, but the subepithelial capillaries were normal in appearance and inflammatory cells were equally as common as in the controls. Twenty-four hours after distension some vacuoles were seen in the urothelium, which showed some small disruptions, and a few ruptures were seen in some fibres of the muscle layer. By 48 h, the reaction in the urothelium was irregular and vacuolated and there were dark cells (macrophages) in the submucosa, but the subepithelial capillaries were normal. Enlarged nuclei were seen in the cells of the urothelium 7 days after distension, but the urothelium itself was smooth and the number of inflammatory cells had normalised (III).

Alkaline phosphatase was especially used to study the inflammatory reaction. At 48 h the previously strong reaction in the most superficial cells had faded and the epithelium was seen vacuolated. The number of inflammatory cells was most abundant by this time. The adenosine triphosphatase reaction was strong in the muscle, and this was used to locate damage to the muscle layer. A few ruptures were seen in some fibres, and reaction in the muscle fibres had partly faded in intensity by 24 h and had entirely disappeared in some muscle fibres at this checkpoint.

5.3. Clinical results

5.3.1. Effects of hydrodistension and HA instillations on clinical symptoms (Paper V)

The patients were divided into long-term or short-term responders and non-responders on the basis of the degree of pain relief. No marked differences were found in the pre-treatment parameters between responders and non-responders. VAS measurements showed pain to be reduced by 75% in the responders 2 weeks after the final instillation. Short-term responders (5 patients) were again in pain by the 4.5 month checkpoint, whereas the long-term responders (3 patients) were still painless even after 8 months. The non-responders (3 patients) obtained no alleviation of pain. The long-term responders (1 man and two women) needed no additional treatment during the follow-up of one year, whereas the short-term responders (5 women) experienced some relief from the symptoms but needed additional treatment at the 4.5 month check-up. Mean micturition volumes were increased by 65% in the long-term responders and by 21% in the short-term responders 2 weeks after the final instillation, but there was no change in non-responders. Frequency was reduced by 40% in the long-term responders and by 26% in the short-term responders, but by only 6% in the non-responders.

5.3.2. Effect of hydrodistension and HA instillations on ICAM-1 intensity (Paper V)

The ICAM-1 intensity of the leukocytes in the pre-treatment deep bladder biopsies was low 0.5 (range 0-1) in the non-responders, while the index was 2 (range 1-3) for the long-term responders and 1.5 (range 0.5-2) for the short-term responders. The difference between the responders and non-responders was statistically significant ($p = 0.048$). The overall ICAM-1 index in the pre-treatment state was 3.7 (range 3-4) in the responders (long-term and short-term), while it was 1.8 (range 1.5-2) in the non-responders ($p = 0.012$). Two weeks after the final instillation the index was 3.5 (3-4) in the responders (long-term or short-term), while it had increased to 2.8 (2.5-3.5) in the non-responders.

6. DISCUSSION

The prevalence of IC was investigated here by means of a questionnaire including the data necessary for determining the O'Leary-Sant score. Special attention was paid to SS patients as a possible subgroup of IC having similar immune mechanisms in their pathophysiology. The effect of overdistension on bladder wall morphology and the duration of increased mucosal permeability after distension were investigated experimentally, while clinical interest was focused on the response to hydrodistension plus intravesical HA instillations (assumed to possess a mucosal coating mechanism) and the effect of this treatment on ICAM-1-mediated inflammation.

6.1. Epidemiological observations

6.1.1. Diagnostic and differential diagnostic aspects

Epidemiological investigations into IC are difficult to perform due both to the lack of specific diagnostic criteria and to the rarity of the condition. Consensus criteria have been established for its diagnosis (Gillenwater and Wein, 1988; Wein et al., 1990), but those actually used by investigators have varied, and estimates of the prevalence of IC vary from 4.5/100,000 females (Miki and Yamada, 2000) to 870/100,000 females (Jones and Nyberg, 1997). There are few, if any, pathognomic findings in IC, and each diagnostic approach has its inherent limitations. Chronic pelvic pain relieved by emptying the bladder, in association with urinary frequency, urgency and nocturia, are the most distinctive symptoms once other diseases have been excluded (Agarwal et al., 2001). The overlapping of IC with overactive bladder and urinary tract infection means that it cannot be differentiated totally in epidemiological surveys without the performing of clinical examinations. To minimise misdiagnoses, fourteen patients were excluded according to criteria from our series because they had received antibiotic treatment for a urinary tract infection during the last month and 51 other cases because they did not experience pain and/or nocturia ≥ 2, or because they were pregnant.

6.1.2. Dependability of the O'Leary-Sant score

The present survey was the first to use the validated O'Leary-Sant questionnaire to assess the frequency of IC symptoms. To overcome selection bias, the target population was randomly selected and thus representative of the general population. The fact that the reported prevalence of

IC symptoms was comparable between the first and second mailings would suggest that selection bias is unlikely. The O'Leary-Sant score is planned to capture the most distinctive symptoms of IC, and its validity, reliability and responsiveness have been demonstrated previously (O'Leary et al., 1997; Lubeck et al., 2001). Earlier results indicated that 97% (65/67) of the controls had symptom and problem indices < 7, while 89% (40/45) of the IC patients had indices ≥ 7, 50% having both indices ≥ 12, while all the subjects in the control group had lower indices (Jones and Nyberg 1997). The score is therefore a valid tool for epidemiological studies, with 90% sensitivity and 97% specificity at a cut-off level of 7 and a sensitivity of 50% and a specificity of 100% at a cut-off level of 12.

6.1.3. Prevalence of IC in the reference population

A bothersome urgency-frequency syndrome was found to be relatively frequent in our population, as almost a third of the subjects aged 18-71 years suffered from these symptoms and more than 20% experienced some degree of problem. Mild to moderate urgency-frequency symptoms may be due to a number of factors (Kurowski, 1998; Richardson, 1990). An active sex life (postcoital urethral syndrome) and menstrual disturbances (pain or burning in the bladder) may explain the increased prevalence of these symptoms among the youngest of the women (18-30 years) compared with the older age groups, and pregnancy was also an understandable cause of frequency and nocturia in some cases.

We used the same cut-off level of 7 as a criterion for possible IC and 12 for probable IC, with the additional inclusion criterion of nocturia and pain ≥ 2, but excluding instances of antibiotic treatment or pregnancy. The prevalence of the most obvious IC-like symptoms was 450/100,000 (95% CI 100-800) and given the estimated sensitivity of the O'Leary-Sant score (O'Leary et al., 1997), the true prevalence is likely to be even higher, up to 50% higher. This would come close to the figure reported in the National Health Interview Survey (870/100,000), in which 20,561 adult participants were asked if they had ever had symptoms of urinary tract infection that lasted 3 months or longer and if they had ever been told by a physician that they had painful bladder syndrome or IC (Jones and Nyberg, 1997). This was based on self-reporting without any verification by reference to medical records. There is a possibility of confusion over the difference between IC and infectious cystitis, but interestingly, the survey gave a similar estimate to the O'Leary-Sant score used by us.

6.1.4. IC among patients with SS

Five percent of the SS patients had findings compatible with probable IC, which meant that the age-standardized frequency of these symptoms was 15 times higher among the SS patients than in the reference population without SS. To avoid selection bias, the reference population had been randomly selected from the population register and is thus representative of the general source population. A high participation rate was also achieved, which further improved the representativeness.

It is becoming increasingly accepted that IC is not a single disease entity but a clinical syndrome of multifactorial aetiology. Increased antinuclear antibodies have been detected in about one third of patients (Ochs et al., 1994; Oravisto, 1980), but these autoantibodies are not specific to the bladder epithelial cells (Keay et al., 1997A). The significance of this finding is unclear, as autoantibodies have been detected in many chronic diseases and may only represent a secondary phenomenon. Interestingly, antinuclear antibodies are also an important diagnostic criterion for SS, and it is possible that IC cases with autoantibodies constitute a subgroup of IC patients related to SS or other autoimmune diseases. SS patients have also autoantibodies against muscarinic receptors, which may affect the muscarinic receptors of the bladder, attracting inflammatory cells through activation of complement proteins, with the result that late inflammatory changes may occur (Van de Merwe and Arendsen, 2000; Jonsson et al., 2000).

6.1.5. Immune mechanisms shared between IC and SS

It is possible that IC and SS may involve certain shared immune mechanisms. IC has been shown to be associated with abnormal expression of HLA-DR molecules in the urothelium in most cases (Liebert et al., 1993), and the increased numbers of $CD4^+$ T cells within the urothelium and submucosa suggest that these cells may play an active role in its pathogenesis (Christman et al, 1994). Sixty percent of SS patients also have HLA-DR positive epithelial cells, and HLA-DR has been identified as a possible marker of a more active immune response in patients with primary SS (Kerttula et al., 1996). Furthermore, activated $CD4^+$ T cells are the predominant infiltrating cell type in the affected tissues of subjects with primary SS (Spadaro et al., 2001).

$CD4^+$ is an essential regulatory molecule in the immune system and is found predominantly on T-helper cells. It is thought to be a receptor for the HLA-DR molecule, which could in turn stimulate

$CD4^+$ lymphocytes to initiate destruction. T-helper cells are also able to stimulate MC through the release of cytokines such as IL-3, and this may be an important factor in the control of MC. Interestingly, depletion of $CD4^+$ T lymphocytes (with specific monoclonal antibodies) significantly reduced the presence of inflammation and the numbers of MC in the detrusor muscle of experimental autoimmune cystitis (Ratliff et al., 1993). Increased numbers of MC have also been demonstrated in mucous membranes of patients with SS (Amin et al., 2001).

6.2. Experimental observations

6.2.1. Effect of distension on the morphology and mucosal permeability of the female rat urinary bladder

Cystoscopy with hydrodistension of the bladder has been described as one of the most important diagnostic tools for assessing the status of IC patients (Gillenwater and Wein, 1988), and it has also been used for treatment of irritative bladder symptoms in these patients (Dunn et al., 1977; Rovner et al., 2000). Some patients experience symptomatic relief following distension (Hanno and Wein, 1987), presumably due to damage to the sensory (Tammela et al., 1990) or cholinergic innervation (Lasanen et al., 1992), and in any case, even diagnostic hydrodistension can reduce inflammatory parameters in some IC patients (Zuraw et al., 1994; Lynes et al., 1987), although a rapid reappearance of the symptoms is often observed after initial success with distension therapy for IC (Dunn et al., 1977).

Enzyme histochemical reactions are used to demonstrate changes in the metabolic activity of tissues, being sensitive means of showing cellular damage (Raekallio, 1973). Overdistension was found here to cause leaking of enzymes in the urothelium within 12 h, and degenerative changes (mostly in the urothelium) were most visible at 24 h. Recovery was nearly completed during the first week (III). Penetration of the Trypan blue dye through the urothelium does not occur until the mucosal surface is damaged (Monson et al., 1991, Tammela et al., 1993). Urothelial integrity was nevertheless destroyed for several days after the distension. Overdistension of the bladder with consequent overstretching of the blood vessels could play an important role in generating circulatory disturbances, as thrombosis of the small vessels could cause perfusion disturbances of quite long duration, even though the subepithelial capillaries may retain a strong, even reaction to enzyme histochemical staining, demonstrating that there has been no permanent damage. Metabolic activity in the muscle also remained strong throughout, disappearing only occasionally.

6.2.2. Clinical importance of urothelial degeneration after bladder distension

The origin of IC is still obscure, but a leaking urothelium is considered a pathophysiological factor in inflammatory conditions of the bladder in these patients (Parsons et al., 1991). A healthy transitional epithelium maintains a barrier between the bladder wall and the urine, and a healthy urothelium has a very good ability to regenerate the endogenous proteoglycan layer. The protective function of the urothelium is lost if the GAG layer is removed, e.g. by a dilute acid or detergent (Gill et al., 1982), but the healthy urothelium has a very good ability to regenerate the endogenous GAG layer, and the mucin layer is fully regenerated in 24 h after acid instillation (Parsons et al., 1977). According to results of this study the overdistension destroyed not only the mucin layer but the urothelial cells were also damaged for several days and the restoring of mucosal integrity took more than one week, even in an otherwise healthy bladder. It is therefore possible that overdistension of the bladder in patients with IC may increase leakage in the urothelium and thereby accelerate inflammatory reactions. This raises the question of whether it is acceptable at all to use prolonged overdistension as a method for relieving irritative bladder symptoms in patients with IC, and also of whether protective instillations of HA may be indicated even after diagnostic overdistension.

6.3. Clinical observations

6.3.1. Hydrodistension and HA instillations

Four HA instillations combined with diagnostic short-term hydrodistension gave an initial response in eight out of eleven IC patients fulfilling the NIDDK criteria (70%), three of whom (30%) experienced a long-lasting effect over one year of follow-up. A greater ICAM-1 intensity was found in the responders than in the non-responders.

Although it is possible that overdistension may partly explain even quite a long period of symptom relief in the long-term responders, ICAM-1 activity did not decrease after distension plus HA treatment, and further HA instillations were needed to maintain the response. It is therefore possible that the inflammatory stimulus may still have been present but that HA was blocking the response by interfering with inflammatory cell adhesion mediated by ICAM-1 receptors. The up-regulation of ICAM-1 in the non-responders was probably a consequence of trauma caused by the hydrodistension and biopsies.

Overdistension destroyed urothelial integrity for up to 1 week in our animal experiments (III, IV), and mucosal restoration in IC patients may take even longer. Thus it is possible that, in addition to preventing ICAM-1-mediated inflammation, the HA instillations may also have had a temporary urothelial coating effect, which cancelled out the increased permeability subsequent to hydrodistension. It can be speculated that the combination of four HA instillations with diagnostic hydrodistension might be significant to prevent any harmful effects attributable to impaired integrity.

6.3.2. Clinical significance of ICAM-1

ICAM-1, which is of great importance for immune responses, inflammation and wound healing, is expressed in endothelial cells and macrophages (Poston et al., 1992). ICAM-1 is also recognised in lymphocytes and granulocytes and it controls interactions between leukocytes and endothelial cells during an inflammatory response. It mediates cell-to-cell adhesion as a ligand for LFA-1 (lymphocyte function-associated antigen-1) and the macrophage-associated antigen Mac-1 (Makgoba et al., 1988; Diamond et al., 1990), and it is also a key molecule for leukocyte adherence and transendothelial migration (Dustin et al., 1986; Springer, 1994). Under normal conditions the level of ICAM-1 is low, but an increase is seen in many pathological conditions. ICAM-1 expression is induced by cytokines such as IL-1 and tumor necrosis factor-α (TNFa) (Dustin et al., 1986). The molecule is believed to play an important role in pathogenesis of rheumatoid arthritis and other autoimmune diseases, cardiac infarction, stroke, cancer and the rejection of transplanted organs, but very little is known as to its role in the pathogenesis of IC. ICAM-1 functions as a major cell surface receptor for HA (McCourt et al., 1994), which could provide a better explanation for the biological effects of HA in IC than reinforcement of the GAG layer. This binding obstructs ICAM-1 receptors and prevents leukocyte activation. Cell-bound HA also provides protection against various harmful agents such as oxygen radicals.

The semi-quantitative ICAM-1 index used here was high in both long-term and short-term responders, but lower in non-responders. ICAM-1 expression was surprisingly high in this material compared with a previous report (Liebert et al., 1993), as all the patients had a positive intensity to some extent. This probably reflects the heterogeneity of patient material of different studies. Our findings constitute evidence that blocking of the ICAM-1 receptor forms at least part of the pathophysiological mechanism by which HA relieves the symptoms of IC.

6.4. Future aspects

Population-based epidemiological surveys create a basis for future clinical studies aimed at clarifying the real characteristics of cases with possible IC and specifying the proportions of possible subgroups of IC. The close association between IC and SS is interesting, and suggests shared aetiological factors. Future investigations into these patients would be valuable, as genetic susceptibility to cellular immunity could form a very important part of the aetiology of these syndromes. Further research would also be essential to clarify the role of ICAM-1-mediated inflammation. A useful future approach would be to perform bladder distension on all patients and then randomise them to receive either four HA instillations a week followed by monthly instillations or a placebo. This would at the same time provide data on the clinical effects of hydrodistension.

7. CONCLUSIONS

1. Adopting diagnostic criteria based on the validated O'Leary-Sant symptom and problem scores, the prevalence of urinary symptoms corresponding to probable IC in the Finnish female population was estimated to be 450/100,000 (95% CI 100-800). This prevalence finding is 7-25 times higher than in the earlier studies.

2. The age-standardized prevalence of this painful bladder syndrome with urgency and nocturia, in the absence of urinary tract infection or pregnancy, was 15 times higher among female SS patients than in controls without SS. In addition, a third (2/6) of the probable IC cases in the reference population reported SS.

3. Overdistension of the female rat urinary bladder caused damage primarily to the urothelium, and only to a lesser extent to the muscular layer, while the vessels remained intact. The histological damage healed almost completely within one week, but enlarged nuclei were still seen in urothelial cells as a possible mark of increased metabolic activity, which suggest that dysfunctional disturbances continue longer in mucosa.

4. Overdistension of the urinary bladder in rats destroyed urothelial integrity for over 1 week, which made it possible for urine to penetrate into the bladder wall. It is thus possible that overdistension in patients with IC may further damage the leaking urothelium and even accelerate the inflammatory reaction, rendering this therapy potentially harmful.

5. Diagnostic bladder distension and HA instillations alleviated the symptoms of IC in most patients who fulfilled the NIDDK criteria. Whether the good results came from the distension, the HA instillations or both remains uncertain. Although the majority of patients needed constantly repeated instillations, some obtained quite long-lasting relief even with distension and 4 weekly treatments. The clinical response was associated with the ICAM-1 index, which suggests that ICAM-1 plays an important role in the pathophysiological mechanism of IC. HA probably down regulated the inflammation by blocking the ICAM-1 receptors in the case of responders.

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APPENDIX

A. INTERSTITIAL CYSTITIS SYMPTOM INDEX

During the past month, how often have you felt the strong need to urinate with little or no warning?

0. *Not at all.*
1. *Less than one time in five.*
2. *Less than half the time.*
3. *About half the time.*
4. *More than half the time.*
5. *Almost always*.

During the past month, have you had to urinate less than 2 hr after you have finished urinating?

0. *Not at all.*
1. *Less than one time in five.*
2. *Less than half the time.*
3. *About half the time.*
4. *More than half the time.*
5. *Almost always.*

During the past month, how often did you most typically get up at night to urinate?

0. *None.*
1. *Once.*
2. *Two times.*
3. *Three times.*
4. *Four times.*
5. *Five or more times.*

During the past month, have you experienced pain or burning in your bladder?

0. *Not at all.*
2. *A few times.*
3. *Fairly often.*
4. *Usually.*
5. *Almost always*

Total Score:_____

B. INTERSTITIAL CYSTITIS PROBLEM INDEX

During the past month, how much has each of the following been a problem for you?

Frequent urination during the day?

0. *No problem.*
1. *Very small problem.*
2. *Small problem.*
3. *Medium problem.*
4. *Big problem.*

Getting up at night to urinate?

0. *No problem.*
1. *Very small problem.*
2. *Small problem.*
3. *Medium problem.*
4. *Big problem.*

Need to urinate with little warning?

0. *No problem.*
1. *Very small problem.*
2. *Small problem.*
3. *Medium problem.*
4. *Big problem*.

Burning, pain, discomfort, or pressure in your bladder?

0. *No problem.*
1. *Very small problem.*
2. *Small problem.*
3. *Medium problem.*
4. *Big problem.*

Total Score: ____

ORIGINAL COMMUNICATIONS