BCG

ANTHONY BUSUTTIL

INTRODUCTION

The urothelium or transitional epithelium lines the entire urinary tract from the renal pelvi-calyceal systems as far down as the larger part of the urethra, and the pathological changes which can be observed in it are essentially identical irrespective of the actual anatomical location. On light microscopy the urothelium in the bladder comprises 4 to 7 layers of cells, depending on its degree of distension. The layer which is adjacent to the basal lamina consists of mitotically active, 'reserve' epithelial cells and the next few layers are made up o polygonal cells with central nuclei, and they exhibit an orientation parallel to the basement membrane. The uppermost layer consists of larger cells which are often multinucleated and cover several of the underlying epithelial cells and are therefore known as 'umbrella' cells.

Over the last two decades, the incidence of urothelial carcinoma of the bladder has gradually increased on a worldwide basis with only minor ethnic variations, as for example a slightly decreased incidence among the black population of the United States. Perhaps this may be reflecting the trend towards increasing urbanisation and industrialisation with a consequent increase in smoking, in atmospheric pollution and exposure to possible carcinogenic agents which enter the body and are excreted in the urine. The urinary bladder is the site where there is maximal and most prolonged urothelial contact with urine and it is the area which is demonstrating this increase in neoplastic lesions.

Surgical resection of tumours through a cystoscope and diathermy are still the mainstays of the treatment of bladder cancers but once there is infiltration of the other layers of the bladder there is a tendency for spread to occur outwith the bladder. When spread has occurred, various treatment modalities have been used in controlling such tumours, and these have ranged from the instillation locally of chemotherapeutic agents, to systemic courses of single and multi-agent chemotherapy, to radiotherapy and even to local hyperthermia. The rationale for all these forms of treatment is a strenuous attempt to spare the patient a cystectomy with all its inherent inconveniences, side-effects and biochemical derangements.

An essential aspect of the treatment of bladder carcinomas is careful planning and tailor-making of the treatment to the needs of each individual patient (1). This can only be achieved if there has been an adequate and comprehensive assessment of the degree of differentiation and spread of each tumour through a joint clinco-pathological assessment. An integral part of such an assessment is the thorough examination of the other ' normal ' areas of the bladder which are not obviously involved by tumour (1;2;3). Each quadrant of the bladder needs to be biopsied on the same occasion that the tumour is resected. These biopsies may show premalignant, hyperplastic or atypical changes indicating that the urothelium elsewhere in the bladder is also involved in a ' field change ' of carcinogenesis and is unstable, being a potential source of further trouble to the patient. The importance of this phenomenon was originally brought home through the ' mapping ' studies on cystectomy specimens which were carried out by Koss first in Newcastle and later in the States (4,5).

UROTHELIAL CARCINOMA in SITU

In some of these dysplastic bladders the changes amount to intra-epithelial carcinoma or carcinoma in situ. This change was defined in the WHO manual as a 'lesion in which there is definite anaplasia of the surface epithelium without the formation of papillary structures and without infiltration'. In this context anaplasia refers to both a cytological change and also to an architectural abnormality with a disturbance in the polarity or orientation of the epithelial cells (6). Such superficially carcinomatous urothelial linings are usually frayed and irregular due to a loss of cohesion between the epithelial cells, and portions of them are detached free and are passed out with the urine. They can therefore be picked up on cytological screening, and this method is of great value in the diagnosis and follow-up of such patients.

Furthermore some patients exhibit the changes of carcinoma in situ in the absence of any other tumour in their bladders (7,8). Some of these patients give a lengthy history of non-specific urinary problems such as frequency and dysuria, in others the history is short. In both instances unless the condition is controlled, there is a rapid progression to multicentric invasive high-grade tumours, rendering the prospect of cystectomy ;more proximate. These changes in the epithelial lining are often accompanied by oedema and a marked increase in the vascularity of the underlying stroma to the extent that the appearances of such bladders are also pathognomonic on cystoscopic examination.

BCG THERAPY

The treatment of carcinoma in situ (CIS) occurring in isolation is even more problematic than that of multiple bladder tumours. Applying a treatment regime formerly used

A. Busuttil, MD FRCPath.
Regius Professor of Forensic Medicine.
University Medical School
Edinburgh.

with other neoplasms, various centres in North America, and elsewhere (including the Western General Hospital in Edinburgh) have attempted to control this condition by using instillations of commercially-available suspensions of BCG (Bacille Calmette-Guerin) into the bladders of these patients (9;10;11;12;13). Up to 80% successful control of urothelial C I S has been documented (14). BCG has also been tried, but with less consistent success, in widespread superficial bladder tumours which have escaped other conventional forms of therapy.

In successfully treated patients, the end-result of such treatment is very satisfactory in that the carcinomatous urothelium sloughs off and the bladder seems to grow a new urothelium which bears no resemblance to the atypical urothelium present prior to the commencement of therapy. The method by which such a suspension of Mycobacteria can achieve its effects is still controversial.

The strain of BCG used at the Western General Hospital, Edinburgh is the Evans strain (15) which is a lyophilised preparation containing 1 to 5 x 10 9 colony forming units in 60 mg. (14). This quantity is suspended in 60 cc. of saline and instilled into the bladder of the patient through an intra-urethral catheter. The patient is instructed to retain the suspension for a maximal period of two hours. This treatment is then repeated every week in succession for a total of eight weeks.

In a preliminary study on bladder biopsies removed from a small group of patients treated in this manner, there was an indication that after BCG treatment commenced, there were major alterations in the quantities and varieties of immunocompetent cells within such bladders, over and above the florid, organised granulomatous reaction which is seen in the submucosa of these biopsies after the first two weeks of therapy (16). These 'chronic inflammatory changes' were accompanied by a gradual denudation of the entire urothelium, as the further weeks of BCG exposure passed by (17).

PROSPECTIVE STUDY

A further prospective study (18) was therefore instituted in ten patients who were subjected to a similar treatment regime. In 9 of these patients there was evidence of carcinoma in situ with involvement of more than 50% of the entire bladder lining: in five of these patients, this was accompanied by superficial bladder tumour formation (T 1 or G 3 pTa). In the other patient there was widespread superficial tumour of intermediate malignancy (G 2 p T a).

The ages of these patients ranged from 44 to 80 years, with a mean age of 61.4 years. Biopsies were taken from the bladders before the commencement of treatment, between the fourth and fifth BCG instillation, and at two weeks after that the treatment course had been completed. Check cystoscopies were also carried out at three-monthly intervals and material was also taken for histological examination at these times. The four quadrants of the bladder were biopsied routinely in addition to any visually abnormal, heaped up or 'mossy' area which was identifiable cystoscopically. The biopsies were taken by the 'cold cup' method and after portions were removed for ordinary fixation and histological assessment, other portions were snap- frozen in liquid nitrogen at - 70 degrees Centigrade and stored at this temperature. Cryostat sections were then cut at 5 to 10 micron thickness at - 20 degrees Centigrade, air dried and fixed in acetone, and either used immediately or re-stored at - 20 degrees Centigrade covered in aluminium foil.

Controls were obtained from the bladders of organ transplant ' beating heart ' donors and from other cystoscopies carried out as part of the work-up of patients with urinary symptoms. Biopsy material was obtained and treated in a similar manner from patients with chronic non-specific cystitis, post-irradiation cystitis, follicular cystitis and from patients requiring a check-cystoscopy eight weeks after Mitomycin C instillation for superficial bladder tumours.

The immunohistochemical staining was carried out by using very specific commercially available, monoclonal antibodies directed at T lymphocytes and their subsets, B lymphocytes, macrophages and dendritic cells, and also at specific surface epitopes for major histocompatibility antigens and interleukin - 2 receptors. The reaction was enhanced by using a combined biotin-streptavidin technique in a multilayer 'sandwich' technique. The enzymatic marker for the reaction was alkaline phosphatase, developed by a tinctorial reaction with Fast Red TR / Naphthol AS-MX phosphate substrate at pH 9.2 in veronal buffer. The sections were counterstained with Mayer's haematoxylin, and mounted in glycerin jelly. Optimal dilutions of the antibodies were identified in preceding experiments. Each section was examined independently and blind by two observers.

RESULTS

These studies confirmed that the normal bladder wall contains very few T and B cells. Lymphocytic infiltration is also not increased significantly in bladders showing malignancy. As expected, B cells were more prominent in those bladders showing cystitis. The control bladders and the carcinomatous bladders prior to treatment expressed HLA - ABC major histocompatibility antigens on their surfaces but no HLA - DR class II histocompatibility epitopes (Table 1).

After BCG treatment the HLA - DR antigens become very prominently expressed on the epithelial cells. No such changes were observed after mitomycin C therapy. The change in the expression of the surface histocompatibility epitopes was accompanied by a major influx of T cells, with a less pronounced infiltration with B lymphocytes and macrophages. When an analysis was made of the subsets of the infiltrating T lymphocytes there appeared to be a reversal of the T 4 to T 8 ratio with an increasing preponderance of T 8 (cytotoxic) T cells which also showed a marked increase in their interleukin - 2 surface expression, indicating that they were active cells (Table 2, 3).

HYPOTHESIS

On the basis of these findings and

other published work, it appears that the action of BCG is through the recruitment of T cells to the area. Indeed nude mice which are congenitally deficient in T cells and into whose bladders urothelial carcinoma cell lines had been explanted, will not react with BCG when this is inserted into their bladders unless they also receive a T cell transfusion (19). The T cells which infiltrate these bladders are memory cells resulting from previous sensitisation of the patient to the BCG or to the tubercle bacillus. The degree of lymphocytic and macrophage infiltration can indeed be correlated with the skin response to P P D (20). It is also essential that local contact is established between the organisms and the urothelium as has been shown when BCG was used formerly to treat solid tumours like melanoma, and the BCG has to be administered locally if it is to have any effect (21).

The cytotoxic action on the malignant cells is mediated by T lymphocytes and the subsequent activity of macrophages. The T lymphocytes which are present are active cytotoxic cells as shown by their interleukin - 2 receptors. There is also evidence that gamma-interferon and interleukin-2, which are produced during such activity, appear in the urine of these patients, in substantial quantities (22).

BCG also appears to alter the surface components of the urothelial cells either directly, or through the activity of the locally produced lymphokines such as interferon, with some prior antigenic processing through the action of macrophages. These epithelial cella are rendered more inter-reactive by exposing on their surfaces of HLA - DR major histocompatibility antigens. This transforms them into cells with which T lymphocytes can react, and thereby a direct antitumour cell cytotoxic reaction is facilitated. 'Normal' reserve cells which do not belong to the malignant 'clone' are preserved and eventually proliferate to cover up the sloughed off neoplastic urothelium.

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RESULTS

EXPRESSION OF HLA EPITOPES

UROTHELIAL CELLS	HLA-ABC	HLA-DR
Normal Bladder	STRONG	NOT expressed
Chronic Cystitis	STRONG	Patchy
Post-MITOMYCIN C	STRONG	NOT expressed
PRE-BCG treatment	PATCHY	Very PATCHY
POST-BCG treatment	STRONG	Very INTENSE

*possibly due to fraying of urothelium

TABLE 1

INFILTRATE WITHIN THE BLADDER SUBUMUCOSA

	TLympho.	BLympho.	Macropha.	NK cells	Dendritic
NORMAL BLADDER	+ (1.36/HPF)	-	+	-/+	+
CHRONIC CYSTITIS	(VARIABLE)				
POST-MITOMYCIN C	+	-	+	+/-	+
PRE-BCG instill.	++ (38.8/HPF)	+	++	+/-	+
POST-BCG instill. (at six months)	++++ (139/HPF) +++ (81.3/HPF)	++	+++	+/-	+

TABLE 2

III

T LYMPHOCYTE INFILTRATE IN THE SUBMUCOSA

	Subset analysis XT4:T8 Ratio	Mean IL2 Receptor Expression	Class II MHC-HLA-DR
NORMAL BLADDER	0.68	-	-
PRE-BCG TREATMENT	4.96	9%	+
POST-BCG TREATMENT	3.13	28.2%	++

TABLE 3