Monoclonal antibodies in bladder cancer cytology

D.K. Chopin and J.-C. Laurent

Service d’Urologie, Hôpital Henri Mondor, 94000 Créteil and Centre Régional de Transfusion Sanguine, F-13005 Marseille, France

Summary. Conventional urinary cytology is the only routine noninvasive test accepted for the early detection and follow-up of bladder cancer. However, accuracy is achieved only in high-grade tumors and the method requires an experienced cytopathologist. Hybridoma technology has been developed to identify molecules or antigenic epitopes associated with malignant transformation. This report discusses the various monoclonal antibodies that have been generated against transitional-cell carcinoma (TCC) of the bladder and can be used to detect exfoliated cancer cells in urine. Several antibodies that recognize epitopes related to Lewis X carbohydrate molecules react with TCC cells of various grades, with the detection rate ranging from 70%–90%. Further studies are required to standardize the methodology and to determine the value of immunocytology in the management of bladder cancer patients. However, this approach shows great promise for improving diagnostic accuracy and for predicting biological aggressiveness and response to therapy.

Current knowledge of the natural history and biology of urothelial tumors points to the need for accurate, noninvasive, cost-effective methods of detecting bladder tumor markers, with a view to improving diagnostic accuracy and predicting biological behavior and response to treatment. Indeed, with the widespread adaptation of intravesical prophylaxis of superficial bladder tumors using chemotherapy or biological response modifiers (bacille Calmette Guérin) and the trend towards conservative management, such tools are becoming essential. In addition, although some patients with superficial disease may benefit from simple surveillance or prophylaxis, specific patient subgroups at the other end of the spectrum may require immediate radical locoregional therapy.

Urinary cytology might provide information closely reflecting field changes associated with malignant transformation in the urinary bladder. Several approaches are currently being used in an attempt to improve the biological information provided by urinary cytology, including immunocytochemistry, flow cytometry, and image analysis, alone and in combination. The present paper reviews the monoclonal antibodies that are available for the study of exfoliated urinary bladder cells and their clinical relevance to the conservative management of bladder cancer patients.

Monoclonal antibodies used in urinary cytology

Hybridoma technology was initially applied to tumor immunology with the hope of defining products intimately linked to neoplastic transformation. Bladder cancer was theoretically a good model since several lines of evidence suggested that it had immunogenic properties. However, hybridomas obtained using xenogenic immunization against cancer cells have failed to generate tumor-specific molecules. Furthermore, cell transformation is associated with changes in the expression of normal molecules and the emergence of modified molecules known as tumor-associated antigens (TAA). Monoclonal antibodies have been raised against these determinants, most of which are present at the cell surface [4, 13]. Despite a lack of tumor...
specificity (i.e. cross-reactivity with normal cells of other lineages or other tumors), a panel of antibodies is now available that overlaps the different grades of bladder tumors. Some of these antibodies have been evaluated for the detection of markers on exfoliated urinary cells (Table 1). We discuss below the spectrum of reactivity of these antibodies with bladder tumors as determined by immunohistochemistry and flow cytometry and the correlation of tumor binding with stage, grade, and, in some cases, DNA content.

G4/E7 antibodies were obtained by immunizing BALB/c mice with a human bladder-cancer cell line and are of the IgM subclass [2]. They recognize a cell-surface glycoprotein of 200 kDa, binding to the carbohydrate moiety. These antibodies have been successfully used to stain frozen and deparaffinized sections by indirect immunoperoxidase staining. Their reactivity has been evaluated by the present authors [2] and by other groups [10] in > 150 specimens. The epitope recognized by these antibodies is preferentially expressed on high-stage, high-grade, and aneuploid tumors; 80% of invasive or grade III tumors are stained as compared with 20% of low-grade, noninvasive tumors. In flow cytometry, G4 reacts preferentially with tumors exhibiting a bimodal DNA profile [10]. Both antibodies cross-react with umbrella cells (normal urothelium), granulocytes, intestinal goblet cells, and proximal renal tubules.

Antibody BL2-10D1 was obtained by immunizing BALB/c mice with a mixture of the RT4 human bladder-cancer cell line and a crude extract of grade II and III bladder cancers and is of the IgM subclass [14]. It also reacts with frozen and deparaffinized sections. This antibody, tested on 109 specimens, has been found to react with 80% of superficial grade I and II TCC, 41% of grade III papillary TCC, 14% of invasive TCC, and 100% of CIS. BL2-10D1 reacts with 5%–10% of umbrella cells. In flow cytometry, this antibody reacts preferentially with TCC cells exhibiting a unimodal DNA profile. It cross-reacts with umbrella cells, granulocytes, goblet cells, and proximal renal tubules. The antigen recognized by BL2-10D1 is of the glycolipid type.

Antibody 486P-2, developed by Arndt and Huland [1], was obtained by immunizing mice with a human bladder-cancer cell line (486P) and is of the IgM subclass. Immunohistochemistry using an alkaline phosphatase conjugate gave a reactivity of 89.5% in 19 TCC specimens. Negative staining was preferential in the least differentiated tumors (grade III). Reactivity with some normal urothelial cells and fetal bladder umbrella cells and granulocytes was also found. The antigen recognized by this antibody is a glycoprotein in the range of 200 kDa; the antibody reacts with the carbohydrate moiety.

Antibody P-12, established by Rettig et al. [19], is directed against the Lexis X antigen and is also a mouse monoclonal IgM. P-12 was found to react with 91% of 33 TCCs tested. The antibody also cross-reacts with granulocytes, intestinal goblet cells, and proximal renal tubules.

A panel of monoclonal antibodies against TCCs (T43, T138, M344, and 19A211) has been established by Fradet et al. [7, 8], covering the spectrum of urothelial tumors. They have been tested on an extensive panel of bladder tumors (n = 158). T43 and T138 were obtained by immunizing BALB/c mice with human bladder-cancer cell lines [6, 7], whereas the other two were obtained using extracts of human TCCs and coimmunization with mouse antisera against normal human urothelium [8, 9]. T43 and T138 react preferentially with high-grade, invasive aneuploid tumors, whereas M344 and 19A211 react with low-grade, superficial diploid tumors.

Results obtained using monoclonal antibodies for the detection of exfoliated TCC cells in urine

G4/E7 antibodies have been evaluated on bladder washings from 75 patients [2]. Specimens were processed immediately. Urothelial cells were spun, washed in phosphate-buffered saline (PBS), and incubated overnight with antibodies. Control samples were run in parallel. The cells were then spun onto slides. Detection of target cells was performed using a peroxidase-antibody conjugate after fixation with 0.1% glutaraldehyde. These antibodies identified 12/12 patients with high-grade lesions and 2/6 subjects with low-grade disease, giving an overall detection rate of 78%. Positive staining occurred in 0%,