Urological Research 7, 57-67 (1979)

Urological Research

REVIEW ARTICLES

Tumour Markers in Urology: Aids in Cancer Diagnosis and Management

Britta Wahren

Radiumhemmet, Karolinska Hospital, and Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden

In recent years substances have been discovered which occur in increased amounts in tumour cells as opposed to normal cells, or in raised concentrations in the blood of patients with advanced tumours. New techniques and the availability of purer reagents have permitted us to characterise these marker substances for diagnostic and prognostic use. Already several substances are being used to monitor disease activity. The present summary does not attempt to cover the established literature, but discusses some recent findings of potential interest to clinicians.

TYPES OF MARKERS

Morphology

Histopathologically, highly differentiated tumours have a near-normal growth pattern, a near-normal DNA pattern, cells and nuclei of slightly atypical size, little or no infiltration of surrounding tissue, and often a good prognosis when treated by surgery alone. In contrast, poorly differentiated or anaplastic tumours, have considerable variation in cell-size, DNA polymorphism, and often an infiltrative mode of growth.

Material from urogenital tumours for cytological examination is obtained by needle biopsy or exfoliation (67). Criteria, similar to those used in histology, such as variations in cell and nuclear size, are evaluated. It is probable that some tumour cells have reduced adherence to surrounding tissue. Hence, the cytological aspirate may be more representative than histopathology of the population of cells with the highest tendency to recur or metastasize. Cytology, therefore, adds a new dimension to diagnosis besides providing a non-invasive technique for preoperative analysis.

Microspectrophotometry and automated cytology of single cells are used for rapid identification of multiple parameters (19, 61, 79). This may be done on unstained native cell populations or after specific staining, or reaction with labelled immune sera. Computerisation of such data from a tumour cell population aids the description of its growth-pattern, antigenic properties and possibly also describes its aggressiveness towards the host.

Marker Substances

As the germ layers develop into organ systems, pluripotent cells differentiate in diverse directions. Repressor substances are thought to suppress areas of genetic information not required for further development or function of certain organs or tissues. Thereby, primitive phase-specific substances (23, 43) cease to be produced or production is drastically reduced. Substances similar to those normally found during embryonic life may also be produced by tumours (3). These fetal antigens are usually not immunogenic in the human host.

Tumours produce a range of hormone-related substances, either in tumours of normally hormone-releasing organs or ectopically. Adrenocorticotrophic hormone (ACTH), antidiuretic hormone (ADH) and hypercalcaemic agent may give disturbing clinical syndromes and the tumour or metastatic lesion can be traced that way. Other abnormal products, such as precursors, subunits, or break-down products, have little biological activity but still retain their antigenic activity. This has been appreciated with the de-

0300-5623/79/0007/0057/$02.20

velopment of highly specific radioimmunoassays (87, 88, 119). Hormones which are normally produced only by the placenta are of special interest, i. e., human placental lactogen (HPL), human chorionic gonadotrophin (HCG) and its subunits. Prostatic, renal and testicular tumours have all been associated with ectopic hormone production.

Enzyme determinations in serum, urine or tissue are useful when isoenzymes specific for a certain organ exist. Studies of these may reflect the extent of a tumour and help to evaluate progression and regression. A reversion towards a fetal isoenzyme pattern may take place in tumour tissue. One such example is the Regan placental alkaline phosphatase, which occurs in high amounts during pregnancy, but not at all in adult males or non-pregnant women. In the case of substances which are elevated in relatively few patients, a positive finding is clinically useful but a negative value is of no diagnostic help.

Other enzymes occur in many tissues: amylase and lipase are raised mainly in gastro-intestinal disease; aminopeptidase and γ-glutamyltranspeptidase in liver and bile duct disease; aldolases in brain and liver disease. These substances are raised in diseases other than cancer and are less specific for neoplasia.

The blood levels of acute phase proteins vary in response to injury, surgery, infarction, infection, collagenosis and neoplasms (4). These proteins include haptoglobin, complement components, C-reactive proteins, transferrin, ceruloplasmin, α_1-antitrypsin and α_1-antichymotrypsin.

The use of polyamines in marker studies of tumours was reviewed recently (24). These substances, like nucleosides, are byproducts of increased cellular metabolism. Urinary levels are raised with a number of malignancies. Urinary spermidine appears to be the most frequent abnormal substance with solid tumours.

In vivo studies in syngeneic animals have clearly revealed the existence of tumour-associated antigens. They are defined by eliciting an immune reaction of the tumour-bearing animal or patient. Following detailed and abundant studies in animals where tumour-specific and tumour-associated antigens have been defined, Hellström et al. first described organ-related antigens in many human tumours (56, 57). The importance of these antigens in tumour rejection is still being debated. Experimentally, antibodies formed to tumour antigens may be of a direct cytolytic type. Combined with an antigen, they might interfere with cell-mediated immunity.

Viruses induce tumours in animals, which reject the transplanted tumours due to new, virus-induced cellular antigens. This has been shown both with solid tumours induced by DNA viruses and with RNA virus-induced leukemias. Several observations suggest that human tumours (75) may be caused by viruses: a) certain viruses cause naturally occurring cancers in animals; b) evidence of infection with a certain virus is sometimes associated with the development of tumours; and c) viral genomes, similar to oncogenic viral genomes, can be identified in human tumour cells. It is possible that viruses and other carcinogens cooperate to initiate and promote neoplastic disease.

MARKERS IN SPECIFIC TYPES OF TUMOURS

Prostatic Carcinoma

Continuous cytological or histopathological assessment is possible with needle biopsies (31), and aspirated cells can provide material for DNA analysis. Higher degrees of heteroploidy are related to a more malignant type of differentiation (125) and this is thought to be of prognostic relevance.

In prostatic carcinoma, blood enzymes and hormone profiles permit specific evaluation of the patient's disease status. A good example of an efficient marker substance is acid phosphatase. With the use of substrates, such as β-napthyl-phosphate, β-glycero-phosphate, adenosine-phosphate or the inhibitor L(+)-tartrate (34, 41), measurements of acid phosphatase become more specific for prostatic cancer than when total serum acid phosphatase is measured. Still more sensitive radioimmunoassays are often used now (35). A sensitive assay may detect around 50% of the tumours before they metastasize. 75-90% of patients with prostatic carcinoma metastatic to bone have elevated levels of acid phosphatase (91), although further disease progression, as seen by scintigraphy or X-ray, may take place without further rise of the enzyme blood level. A statistical calculation of variables in 1824 patients shows that high acid phosphatase is a powerful indicator of poor prognosis (18). Decreased serum levels are an indication of response to treatment. Acid phosphatase assay of a bone marrow aspirate of the iliac crest can be used for staging (90).

Alkaline phosphatases (ALP) are often raised in the blood when metastasizing tumours involve liver or bone. In prostatic carcinoma higher levels are obtained with osteoblastic than osteolytic destructions. Changes in the levels can occur before any radiological response to therapy is seen.

The diagnostic value of carcinoembryonic antigen (CEA) in prostatic carcinoma has been compared to the acid phosphatase test (41). It

is probable that these assays may complement each other, since liver involvement gives raised CEA with greater frequency than raised ALP with all types of metastases, while skeletal rather than soft tissue involvement yields high acid phosphatases. The value of serial CEA determinations is questionable, since increases or decreases do not relate well to clinical changes (52). However, high levels of CEA are synthesized by both prostatic adenoma and carcinoma cells in culture (121). Perhaps an antibody to prostatic CEA would give more specific information than the currently used antibody to colonic carcinoma CEA.

The determination of hormone receptors on cells or in serum is still of little clinical significance, but may become important for decisions about hormonal therapy. The 5-α-dihydrotestosterone receptors are prostate-specific and regulate hormonal dependence (77). Immunochemical detection of receptors may become a sensitive means of following treatment of prostatic cancer and detecting recurrence. Hormone receptors also make possible the use of hormones as carriers for cytotoxic drugs or antibodies to selected sites. The presumed presence of oestrogen receptors in prostatic tumours was the theoretical basis for using an oestradiol-coupled nitrogen mustard (65). It is not known whether the receptor structures of malignant tissues are biochemically identical, immunologically similar, or even quantitatively comparable to those of the corresponding normal tissues.

Sex hormone binding globulin (SHBG) is induced by oestrogen; diminished induction of SHGB is presumably reflected by poor testosterone depression with oestrogen treatment.

Virus-like particles have been detected in prostatic carcinoma, or in derived tissue culture cell lines, but it has not been possible to establish their aetiological significance. Since virus-induced proteins are usually strong antigens such a discovery would be of great importance both for monitoring disease and aetiological studies. Centifanto et al. (21) found herpes simplex virus in 15% of prostatic secretions and suggested that the prostate may serve as a reservoir for the venereal spread of this virus. Other investigators have not confirmed such a high rate of isolation, but conflicting results concerning yield of virus isolates are also obtained with uterine cervical carcinoma patients. In animal experiments, viruses or other carcinogens have had to be combined with hormones, i. e. oestrogens, to induce prostatic tumours.

Ablin and coworkers (2) described cell-bound antibody activity of eluates from prostatic carcinoma. Antibodies to prostatic carcinoma benign prostatic hyperplasia, or bladder tumours have been found in patients and have the distribution of antibodies to organ-specific antigens (27). Delayed-type hypersensitivity has been evoked to autologous tumour extracts in a few patients with prostatic cancer, but not to benign hyperplastic tissue (12).

Assay of polyamines has been proposed as a tumour diagnostic aid. Inflammatory and regenerative conditions, however, also raise levels sufficiently to make differentiation difficult. Spermidine is often raised in urine, blood and CSF in neoplasia, and urinary spermidine levels seem to be related to degree of differentiation and tumour stage in prostatic carcinoma. Haptoglobins and α_1-acid glycoprotein are raised with advanced prostatic tumours or with metastases.

Testicular Tumours

Recently, the histopathology of testicular tumours has been reevaluated due to the detection of hormones and fetal antigens in certain germ cell tumours (104).

Human chorionic gonadotrophin (HCG) is synthesized by the normal trophoblast in pregnancy and by trophoblastic tumours. Ectopic HCG production has been described in many malignant tumours (78). The majority of tumours of non-gonadal origin producing HCG result in only low plasma levels, in the range 1-20 ng/ml. The greatest incidence, 90%, and the highest levels of HCG are found in patients with choriocarcinoma but raised values are also seen with teratomas. Renal failure, occurring in many patients with genito-urinary malignancy, may give raised α and β subunits and also biologically active HCG levels (13). Human placental lactogen (HPL), also normally synthesised by the placenta in pregnancy, can be demonstrated with trophoblastic neoplasms of gonadal or non-gonadal origin (94). HPL production by malignant trophoblasts is usually much less than HCG production.

Oestrone production with 5-10-fold normal levels has been described in teratomas together with low FSH, perhaps induced by the high oestrone levels (89). Leydig cell tumours, although rare, may excrete high levels of androgenic steroids: testosterone and androstenedione, leading to precocious sexual development in young males. Additional unusual cases of hormonal disturbances have been reviewed by Bodansky (10).

LDH-x, a lactate dehydrogenase isoenzyme, is the major LDH of mature spermatozoa and occasionally appears with testicular tumours (120). An alkaline phosphatase called the Regan isoenzyme, otherwise of placental origin, appears in detectable levels in some patients. When present, it reflects progression or regression of the tumour (33, 60).

Elevated serum levels of alpha-fetoprotein (AFP) occur in many patients with malignant teratomas, largely depending upon the extent of the disease (1, 83). Yolk sac tumours produce AFP

regularly (105,104), but so sometimes do teratomas of other differentiation. Sequential studies show that AFP serum levels are related to progression or regression of disease, but also that a recurring tumour may have lost the property to synthesize this marker (13, 116). Healthy males and patients with seminomas do not express fetal antigens in their normal testicular tissues, seminoma cells or serum. AFP, carcinoembryonic antigen (CEA) and ferritin have been found in different percentages of malignant teratoma cell populations. There is no relationship between the four parameters: AFP, ferritin, HCG and CEA, suggesting that they are not under the control of the same gene (116).It is therefore likely that functionally different sub-populations exist. For AFP and ferritin, quantitative determinations show that they parallel the course of the disease. An analysis of the natural behaviour of these tumours shows that although the histology is similar, the mean survival time is longer for patients with little serum AFP and/or ferritin ($\geq$ 48 months) than for patients with high levels (12 months). Therefore, the demonstration of high AFP or ferritin in serum is an indicator of poor prognosis.

Also, α_1-antitrypsin (AAT) is slightly raised in 70% of patients with endodermal sinus tumour. As this marker does not rise to high levels (83, 103) it is not equally reliable in clinical use.

Bladder Tumours

Since urothelial cell tumours occur mainly in the bladder, little is known about tumour markers in the rarer forms occurring in the renal pelvis, ureter or urethra. Cytology of exfoliated cells has proved valuable in the diagnosis and monitoring of bladder carcinoma (32, 69). DNA analysis by cytofluorometry (79, 109, 110) shows the aneuploidy of malignant cell populations as compared to normal urothelial cells. Chromosomal defects also are correlated with progression of the cancer (73, 95) and often accompanied by hyperploidy and bizarre marker chromosomes. These findings are consistent with the DNA increase seen with other malignant tumours.

Electron microscopy has revealed alterations of surface membranes, cellular junctions and microvilli in papillary tumours (38, 68). Promising criteria for evaluation of precancerous changes are chromatin texture, staining density of the nucleus and optical density of the whole cell. Ultrastructural changes have been observed in chemical bladder carcinogenesis of rats. Pleomorphic villi may be related to invasiveness and it is conceivable that such investigations will lead to the diagnosis of precancerous lesions in humans (63). The relationship of such surface changes to superficially localized CEA of bladder tumour cells (118) should be interesting.

Lymphocyte cytotoxicity against primary human bladder tumours was studied originally by Bubenik and associates (16, 17). The questions they posed were: a) does a tumour-specific immune response occur in patients with urinary bladder cancer and, if so, b) do the tumour-specific antigens which give rise to this response crossreact with each other, or are they individually specific? These questions remain unresolved. Many investigations suggest that tumour-associated antigens are retained by bladder carcinoma cell lines in culture (7, 54, 111). A collaborative effort has been made to describe the cytotoxic reactions (6). A clear-cut disease-related cytotoxicity is not always seen (6, 9, 102) but most data suggest a reactivity to bladder carcinoma antigens of patients with this disease (7, 9, 11, 14, 111). Some patients have circulating lymphoid cells with bladder tumour-related cytolytic activity, and minimal but active disease has to be present for measurable cytotoxicity of this type (108). There is also, superimposed, some degree of non-selective cytotoxicity to a number of diverse cell lines. The latter type of cytotoxicity can occur in healthy persons which reflects the variety of antigens expressed in tumour cells that the lymphocytes of patients or healthy persons may respond to in vitro.

In the allogeneic reaction to established cell lines, K-cells, which have a receptor for the Fc-fragment of immunoglobulin, in cooperation with specific (and unspecific) antibody seem to be the effector cells (107, 112), while non-antibody dependent T-cells have been described to have a killer cytotoxic effect in the autologous situation. The clinical relevance of these reactions is still unclear. Reservations have been made about the applicability to the patient' s situation, mainly because further knowledge is needed to clarify the complex antigenic pattern of the studied bladder tumour cell lines.

A new series of surface antigenic determinants, Ek-1 to Ek-11, not obviously belonging to previously known HLA antigens, has recently been described (30). Among these, Ek-2 was the only Ek-determinant present on all nine bladder carcinoma lines but only found in 10 percent of other tumour lines.

An aetiological role of oncornavirus in cancer of the human bladder has been suggested (36). Chemical or physical carcinogens can induce the expression of RNA viruses of endogenous or exogenous origin, and this expression may result in cell transformation. Alternatively, cells actively producing viruses may be more susceptible to chemical transformation.

Virus-like particles have been described in the cytoplasm of transitional cell carcinomas of the human renal pelvis, ureter and bladder. Other workers have not isolated virus (86). Virus infections of the human urinary tract are known to be

common in e. g. patients with immunological impairment. One might speculate that bladder cancer, which occurs in persons exposed to certain chemical carcinogens, may result from chemical activation of endogeneous or passenger viruses present in the bladder epithelium.

Another virus of interest in the study of bladder cancer is cytomegalovirus, which is often found in the urine and is ubiquitous in the human population. Some herpes viruses, including cytomegalovirus, can transform cells in tissue culture (40). The common feature of herpes viruses and oncornaviruses is their capacity for latency within host cells.

Following Hall et al. (55), several authors (53, 92, 117) have found that urinary CEA is higher with more advanced bladder tumours and substances occur in chronic urinary infections which have the same immunoreactivity by radioimmunoassay. Provided that the urine is extracted with perchloric acid to avoid inhibitory substances and that severe bacterial infection can be excluded, sequential urinary CEA is a valuable additional method for monitoring patients with bladder carcinoma. Serum CEA levels in patients with bladder tumours are increased in 60% of the patients with stages T3 and T4, and with metastasizing bladder tumours the mean serum level is further increased. Monitoring of bladder carcinoma patients by both serum and urinary CEA determinations has shown a relationship to clinical stage, although not comparable to that with gastrointestinal tumours (44, 117). CEA has been isolated and characterised from bladder carcinoma tissue (126), and no CEA is found in normal urothelium (47, 118). It is therefore probable that urinary CEA is derived from the bladder cancer cells. Quantitative determination of cellular CEA content is a new way to characterize the cancer cell population (118).

Although many bladder carcinomas may be carcinogen- or parasite-induced, most have unknown aetiology. There is evidence of a role for some metabolites in carcinogenesis of the bladder (124). The urinary excretion of tryptophane metabolites is higher in bladder carcinoma than in healthy persons and loading tests increase the metabolites in patients whose level is already high (8). Other biochemical markers, excreted in the urine have also been considered for diagnosis. Pseudouridine, β-aminoisobutyric acid (62), and immunoglobulins (64) constitute a combination which, together with CEA, may become of value.

Renal Tumours

Preoperative thin needle biopsy and an evaluation of the malignancy grading may help in treatment planning, such as preoperative radiotherapy of poorly differentiated carcinomas (28, 97). Exfoliated tumour cells in urine are found only with tumours of the renal pelvis.

Erythropoietin is normally formed in the kidney, and renal tumours are a common cause of abnormally high secretion (106). Erythropoietin stimulates maturation of red blood cells and a polycythaemia may develop. Resection of the tumour leads to reduction or disappearance of the erythrocythaemia. Only about 3% of renal tumours have excess erythropoietin, while many more produce an elevated ESR. The reason for this has been ascribed to serum protein abnormalities of diverse kinds, such as raised haptoglobin or globulins.

Ectopic ACTH secretion, although most common with lung tumours, has also been noted in patients with renal or prostatic carcinomas (76). It is important to differentiate the Cushing's syndrome which may result from these tumours from that induced by primary adrenocortical tumours. Parathyroid hormone (PTH) production by renal cell tumours may explain the hypercalcaemia seen even in the absence of bone metastasis (45, 48). Other rare hormones produced by kidney tumours are enteroglucagon, which results in malabsorption (42), and prolactin, producing galactorrhoea (113). Rarer syndromes have been described by Ratcliffe et al. (87).

Renal tumours in frogs are induced by the Lucké virus (49). This is a herpes virus, readily isolated from the tumours at low temperature, and it leads to new tumour formation when inoculated into further frogs. During the summer, no frog tumours are seen, so that a proviral or latent form at higher temperatures has been postulated. Two papovaviruses, BK and JC, which have a known oncogenic capacity in animals, are of potential importance for renal malignancies. BK is frequently isolated after immunosuppressive therapy (39), perhaps due to reactivation. Papillomas of the larynx and warts are induced by papovaviruses, but no evidence has been found for papovaviruses as aetiologic agents of tumours of the urogenital tract (99).

Evidence of renal tumour antigens has been presented (15, 26, 101). Using the mixed lymphocyte-target interaction test, cellular immunity to renal carcinomas was studied in six patients (101). Renal carcinoma cells significantly stimulated the patients' own lymphocytes in three cases, while normal renal tissue did not induce the same reaction. These results seem to be confirmed by studies demonstrating leucocyte inhibition by renal tumour extracts (66). Cell-mediated reactivity to autologous tumour cells has also been described with nephroblastoma or Wilms' tumour (56, 70). Antibodies with membrane reactivity or cooperating in cell-mediated killing have been described in a few children with this tumour (71).

The morphology of nephroblastoma is reminiscent of the developing embryonic kidney. An interesting substance from this renal tumour of

childhood is a mucopolysaccharide that is also present in the blood and urine. It does not occur in the normal adult or fetal kidney, but is immunologically similar to calf fetuin (123). The clinical usefulness of this antigen has not yet been explored.

With renal tumours, CEA determinations in urine and serum were predictive of the disease status in less than 40-60% of the cases (22, 51). LDH and ALP may be found in the urine after removal of inhibitors and are signs of underlying renal or bladder disease, often of a malignant nature (5).

Immune complexes are deposited in the kidney in autoimmune and other diseases (122). Presumably not only antibodies reacting with a postulated renal tumour cell antigen can be deposited there, but also circulating immune complexes derived from other tumour antigen-antibody reactions (93).

CONCLUSIONS

The presence of unexpected enzymes, hormones, fetal or tumour-associated antigens suggests ectopic synthesis by tumour cells. One hypothesis assumes that genes repressed in normal healthy tissue become de-repressed, changed or deleted with neoplasia. De-repression is probably not totally random, since the incidence and concentration of a substance vary between different types of tumours. In virus-induced tumours, additional genes might be expressed in addition to the existing information. Multiple antigens may act as markers for cells of varying differentiation within a population, and thus also serve as indications for the choice of therapy.

Many patients with urogenital tumours (20, 82, 96) as well as patients with other types of cancer (29, 59) have been shown to have lymphocyte or myeloid defects, compared to healthy persons (50, 58). The evaluation of reactivities without known specificity have, in fact, often been of a predictive value, a depressed response indicating poor prognosis. Various explanations have been suggested for depressed cellular or humoral functions. Unspecific factors may be the debiliating disease, or the treatment itself, i. e. radiotherapy or chemotherapy.

Detection of specific antibodies or cell-mediated reactivity to tumour antigens, when in existence, is potentially a sensitive procedure for immunodiagnosis. Only lately, with the aid of K-cell assays, has the sensitivity reached high, discriminative levels (84). In syngeneic systems it is possible that T-cells may play a role. Animal experiments indicate that for T-cell cytotoxicity to virus-infected target cells for example, the cytotoxic cell and the target cell must have histocompatibility determinants in common (127). Thus, cytotoxic T-cell killing in vivo may occur largely as a function of similarities, not dissimilarities.

A variety of mechanisms have been proposed to explain why tumours can continue to grow in the presence of detectable immune reactivity. Failures may be found in the tumour cell, e. g. the antigens are weak and evoke a response, but too late. The antigens may be secreted (25), thereby blocking attacking antibodies or cells. Modulation - i. e. temporary loss of surface expression of a tumour cell surface antigen - is another mechanism.

On the responding side, cellular incapacities to exert an immune reaction may be due to intrinsic defects, blockage by soluble tumour antigens, or the appearance of suppressive agents of other kinds, such as pregnancy proteins or fetal AFP. T-cell response is diminished or suppressor cells are induced by AFP (80), pregnancy associated macroglobin (PAM) (100) or HCG (74). All are substances normally present in high amounts during pregnancy, which also constitutes a tolerance to an incompatible tissue. Such factors may depress the immune responses of a tumour host, although this hypothesis has been questioned.

An increased incidence of organ antibodies has been described in patients with bladder, prostate and kidney cancer (72), similar to the findings in other forms of tumours and in documented autoimmune disease. The interference of such antibodies in assays of antibody-mediated specific cytolysis must be taken into account. Another possibility, that tumour cells have reinduced Fc-receptor sites and therefore attract antibodies without tumour specificity, as in cells infected with herpes viruses, has not received enough attention. The induction of Fc-receptors on T-cells has lately been considered as taking part in the suppression of antibodies or antibody dependent effector cells (37).

In conclusion, tumours release substances which differ from the products of normal cells. At present, much emphasis is being placed on understanding the decline in immune functions with advanced malignancy, on purification of the measurable antigens and on characterisation of circulating immune complexes.

For cardiology, liver diseases and gynaecology, enzyme and hormone determinations have become important tools in diagnosis.

In oncology too, measurements of tumour antigens or serum products have implications for diagnosis and monitoring of disease (81, 98, 114, 115, 120). The relative organ-specificity of some known antigens (46, 58) raises the hope of further identifying organ-associated antigens representative of different types of tumours. The contribu-

tion of viruses to carcinogenesis and resulting virus-associated antigens (85) is complex, and the importance of cofactors attracts renewed interest.

REFERENCES

1. Abelev, G.: Alpha-fetoprotein in ontogenesis and its association with malignant tumors. Advances in Cancer Research 14, 295 (1971)
2. Ablin, R. J., Gonder, M. J., Soanes, W. A.: Immunohistologic studies of carcinoma of the prostate. III. Elution of interepithelial antibodies from carcinomatous human prostatic tissue following cryoprostatectomy. Oncology 29, 329 (1974)
3. Alexander, P.: Fetal antigens in cancer. Nature 235, 137 (1972)
4. Alper, C. A.: Plasma protein measurements as a diagnostic aid. New England Journal of Medicine 291, 287 (1974)
5. Amador, E., Zimmerman, T., Wacher, W.: Urinary alkaline phosphatase activity. I. Elevated urinary LDH and alkaline phosphatase activities for the diagnosis of renal adenocarcinomas. Journal of the American Medical Association 185, 769 (1963)
6. Bean, M. A., Bloom, B. R., Herberman, R. B., Old, L. J., Oettgen, H. F., Klein, G., Terry, W. D.: Cell-mediated cytotoxicity for bladder carcinoma: Evaluation of a Workshop. Cancer research 35, 2902 (1975)
7. Bean, M. A., Pees, H., Fogh, J. E., Grabstald, H., Oettgen, H. F.: Cytotoxicity of lymphocytes from patients with cancer of the urinary bladder: Detection by a ^{3}H-proline microcytotoxicity test. International Journal of Cancer 14, 186 (1963)
8. Benassi, C. A., Perissinoto, B., Allegre, G.: The metabolism of tryptophan in patients with bladder cancer and other urological diseases. Clinica Chemica Acta 8, 822 (1963)
9. Bloom, E. T., Ossorio, R. C., Brosman, S. A.: Cell-mediated cytotoxicity against human bladder cancer. International Journal of Cancer 14, 326 (1974)
10. Bodansky, O.: Biochemistry of human cancer. New York: Academic Press 1975
11. O'Boyle, P. J., Cooper, E. H. Williams, R. E.: Evaluation of immunological reactivity in bladder cancer. British Journal of Urology 46, 303 (1974)
12. Brannen, G., Gomolka, D., Coffey, D.: Specificity of cell membrane antigens in prostatic cancer. Cancer Themotherapy Reports 59, 127 (1975)
13. Braunstein, G. D., McIntire, K. R., Waldmann, T. A.: Discordance of human chorionic gonadotropin and alpha-fetoprotein in testicular teratocarcinomas. Cancer 31, 1065 (1973)
14. Bubenik, J., Baresova, M., Viklichy, C., Jakoubková, J., Sainerova, H., Donner, J.: Established cell line of urinary bladder carcinoma (T-24) containing tumour-specific antigen. International Journal of Cancer 11, 765 (1973)
15. Bubenik, J., Jakoubková, J., Krákora, P., Baresová, M., Helbich, P., Viklichy, B., Malasková, V.: Cellular immunity to renal carcinomas in man. International Journal of Cancer 8, 503 (1971)
16. Bubenik, J., Perlmann, P., Helmstein, K., Moberger, G.: Immune response to urinary bladder tumors in man. International Journal of Cancer 5, 39 (1970)
17. Bubenik, J., Perlmann, P., Helmstein, K., Moberger, G.: Cellular and humoral immune responses to human urinary bladder carcinomas. International Journal of Cancer 5, 310 (1970)
18. Byar, D. P., Huse, R., Bailar, III, J. C., the Veterans Administration Cooperative Urological Research Group: An exponential model relating censored survival data and concomitant information for prostatic cancer patients. Journal of the National Cancer Institute 52, 321 (1974)
19. Caspersson, T., Lomakka, G.: Introduction to quantitative cytochemistry. Bahr, G., Wied, G., (eds.), Vol. 2. New York: Academic Press 1970
20. Catalona, W. J., Smoler, J. K., Harty, J. I.: Prognostic value of host immunocompetence in urologic cancer patients. Journal of Urology 114, 922 (1975)
21. Centifanto, Y. M., Drylie, D. M., Deardourff, S. L., Kaufman, H. E.: Herpesvirus type 2 in the male genitourinary tract. Science 178, 318 (1972)
22. Chu, T., Skulka, S. K., Mittleman, A. O., Murphy, G. P.: Plasma carcinoembryonic antigen in renal cell carcinoma patients. Journal of Urology 111, 742 (1974)
23. Coggin, J. H., Jr., Anderson, N. G.: Cancer, differentiation and embryonic antigens: Some central problems. Advances in Cancer Research 19, 105 (1974)
24. Cohen, S.: Conference on polyamines in cancer. Cancer Research 37, 939 (1977)
25. Currie, G.: The role of circulating antigens as an inhibitor of tumour immunity in man. British Journal of Cancer 28, Suppl. 1, 153 (1973)
26. Diehl, V., Jereb, J., Stjernswärd, J., O'Toole, C., Åhström, L.: Cellular immunity to nephroblastoma. International Journal of Cancer 7, 277 (1971)
27. Dmochowski, L., Maruyama, K., Ohtsuki,

Y., Seman, G., Bowen, J.M., Newton, W.A., Johnson, D.E.: Virologic and immunologic studies of human prostatic carcinoma. Cancer Chemotherapy Reports 59, 17 (1975)
28. Edsmyr, F., von Schreeb, T., Johansson, B., Esposti, P.: Radiation therapy of malignant renal tumors. Rad. Ther. Malignant Renal Tumors. Pathologie und Radiologie von Hochdruck- und Nierenerkrankungen. Kröpelin, T. (ed.), pp. 270. Stuttgart: Thieme
29. Eilber, F.R., Nizze, A., Morton, D.: Sequential evaluation of general immune competence in cancer patients in correlation with clinical course. Cancer 35, 660 (1975)
30. Espmark, A.: Tissue typing of cells in culture. IV. Cell surface antigens of tumor cell lines, not obviously belonging to the HLA-system. Tissue Antigens, in press (1978)
31. Esposti, P.: Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. Scandinavian Journal of Urology and Nephrology 5, 199 (1971)
32. Esposti, P.L., Zajicek, J.: Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. Acta Cytologica 16, 529 (1972)
33. Fishman, W.H.: Perspectives on alkaline phosphatase isoenzymes. American Journal of Medicine 56, 617 (1974)
34. Fishman, W., Lerner, F.: Method for estimating serum acid phosphatase of prostatic origin. Journal of Biological Chemistry 200, 89 (1953)
35. Foti, A.G., Herschman, H., Cooper, J.F.: A solid-phase radioimmunoassay for human prostatic acid phosphatase. Cancer Research 35, 2446 (1975)
36. Fraley, E.E., Elliot, A.Y., Castro, A.E., Cleveland, P., Hakala, T., Stein, N.: Ribonucleic acid virus associated with human urothelial tumors: Significance for diagnosis and treatment. Journal of Urology 111, 378 (1974)
37. Fridman, W.H., Fradelizi, D., Guimezanes, A., Plater, C., Leclerc, J.C.: The role of the Fc receptor (FcR) of thymus-derived lymphocytes. II. Presence of FcR on suppressor cells and direct involvement in suppression. European Journal of Immunology 8, 549 (1977)
38. Fulkner, M.J., Cooper, E.H., Tanaka, T.: Proliferation and ultrastructure of papillary transitional cell carcinoma of the human bladder. Cancer 27, 71 (1971)
39. Gardner, S.D., Field, A.M., Coleman, D.V., Hulme, B.: New human papovavirus (BK) isolated from urine after renal transplantation. Lancet 1, 1253 (1971)
40. Geder, L., Lausch, R., O'Neill, F.J., Rapp, F.: Oncogenic transformation of human embryo lung cells by human cytomegalovirus. Science 192, 1134 (1976)
41. Gittes, R.F., Chu, T.M.: Detection and diagnosis of prostate cancer. Seminars in Oncology 3, 123 (1976)
42. Gleeson, M.H., Bloom, S.R., Polak, J.M., Henry, K., Dowling, R.H.: Endocrine tumor in kidney affecting small bowel structure, motility and absorptive function. Gut 12, 773 (1971)
43. Gold, P.: Antigenic reversion in human cancer. Annual Review of Medicine 22, 85 (1971)
44. Gold, P., Wilson, T., Romero, R., Shuster, J., Freedman, S.O.: Immunology and colonic cancer: Further evaluation of the radioimmunoassay for carcinoembryonic antigen of the human digestive system as an adjunct in cancer diagnosis. Diseases of the Colon and Rectum 16, 358 (1973)
45. Goldberg, M.F., Tashjian, A.H., Jr., Order, S.E., Dammin, G.J.: Renal carcinoma containing a parathyroid hormone-like substance and associated with marked hypercalcemia. American Journal of Medicine 36, 805 (1964)
46. Goldenberg, D.M.: Oncofetal and other tumor-associated antigens of the human digestive system. Current topics in pathology 63, 290 (1976)
47. Goldenberg, D., Wahren, B.: Immunoperoxidase staining of carcinoembryonic antigen in urinary bladder cancer. Urological Research 6, 211 (1978)
48. Goodall, C.M.: On para-endocrine cancer syndromes. International Journal of Cancer 4, 1 (1969)
49. Granoff, A.: The Lucké renal carcinoma of the frog. The herpesviruses. Kaplan, A. (ed.), p. 627. New York: Academic Press 1973
50. Granström, M: Editorial. Myelopoiesis in human disease. Acta Medica Scandinavica 201, 401 (1977)
51. Guinan, P.D., Ablin, R.J., Dubin, A., Nourkayhan, S., Bush, I.M.: Carcinoembryonic antigen test in renal cell carcinoma. Urology 5, 185 (1975)
52. Guinan, P., Sadoughi, N., John, T., Ablin, R.J., Bush, I.M.: The prognostic value of carcinoembryonic antigen in carcinoma of the prostate. Urological Research 2, 79 (1974)
53. Guinan, P., John, T., Sadoughi, N., Ablin, R.J., Bush, I.: Urinary carcinoembryonic-like antigen levels in patients with bladder carcinoma. Journal of Urology 3, 350 (1974)
54. Hakala, T., Lange, P.: Serum induced lymphoid cell mediated cytotoxicity against human transitional cell carcinomas of the genitourinary tract. Science 184, 795 (1974)
55. Hall, R., Laurence, D., Darcy, D., Stevens, U., James, R., Roberts, S., Neville, M.: Carcinoembryonic antigen in the urine of patients with urothelial carcinoma. British Medical Journal 3, 609 (1972)
56. Hellström, I., Hellström, K., Pierce, G.E.,

Yang, J. P. S.: Cellular and humoral immunity to different types of human neoplasms. Nature 220, 1352 (1968)
57. Hellström, I., Hellström, K. E., Sjögren, H. O., Warner, G. A.: Demonstration of cell-mediated immunity to human neoplasms of various histological types. International Journal of Cancer 7, 1 (1971)
58. Herberman, R. B.: Immunologic approaches to the diagnosis of cancer. Cancer 37, 549 (1976)
59. Hersh, E. M., Mavglit, G. M., Gutterman, J. V.: Immune evaluation of the cancer patient. Medical Clinics of North America 60, 623 (1976)
60. Holmgren, P., Stigbrand, T., Damber, M., von Schoultz, B., Wahren, B.: Determinations of placental alkaline phosphatase Regan isoenzyme in cancer sera by a sensitive radioimmunoassay. Scandinavian Journal of Immunology, Suppl. 8, in press (1978)
61. Horan, P. K., Wheeless, L. L., Jr.: Quantitative single cell analysis sorting. Science 198, 149 (1977)
62. Irving, C. C.: Biochemically detectable tumor markers in urine of bladder cancer patients. Cancer Research 37, 2872 (1977)
63. Jacobs, J. B., Cohen, S. M., Arai, M., Friedell, G. H.: SEM on bladder cells. Acta Cytologica 21, 3 (1977)
64. Johansson, B., Kistner, S., Norberg, R.: Proteinuria in patients with urinary tract tumors. Scandinavian Journal of Urology 5, 229 (1971)
65. Kirdani, R. Y., Müntzing, J., Varkarakis, M. J., Murphy, G. P., Sandberg, A. A.: Studies on the antiprostatic action of estracyt, a nitrogen mustard of estradiol. Cancer Research 34, 1031 (1974)
66. Kjaer, M., Christensen, N.: Ability of renal carcinoma tissue extract to induce leukocyte migration inhibition in patients with nonmetastatic renal carcinoma: Correlation with clinical and histopathological findings. Cancer Immunology and Immunotherapy 2, 41 (1977)
67. Koss, L. G.: Diagnostic cytology and its histopathologic basis. Philadelphia: Lippincott 1968
68. Koss, L. G.: Some ultrastructural aspects of experimental and human carcinoma of the bladder. Cancer Research 37, 2824 (1977)
69. Koss, L. G., Bartels, P. H., Bibbo, M., Freed, S. Z., Taylor, J., Wied, G. L.: Computer discrimination between benign and malignant urothelial cells. Acta Cytologica 19, 378 (1975)
70. Kumar, S., Taylor, G., Steward, J. K., Waghe, M. A., Pearson, A.: Cellular immunity in Wilms tumor and neuroblastomas. International Journal of Cancer 10, 36 (1972)
71. Kumar, S., Waghe, M., Taylor, G.: Tumour-specific antibodies reactive with cell-surface antigens in children with Wilm's tumour. International Journal of Cancer 19, 351 (1977)
72. Kurki, P., Linder, E., Miettinen, A., Althan, O., Heikkinen, A., Pasternack, A.: Tissue antibodies in malignant and benign urogenital disease. International Journal of Cancer 19, 332 (1977)
73. Lamb, D.: Correlation of chromosome counts with histologic appearances and prognosis in transitional cell carcinoma of the bladder. British Medical Journal 1, 273 (1967)
74. Lange, P. H., Hakala, T. R., Fraley, E. E.: Suppression of antitumor lymphocyte mediated cytotoxicity by human chorionic gonadotropins. Journal of Urology 115, 95 (1976)
75. Levine, P., Pearson, G., Huberman, R., Rabson, A., Burton, G.: Symposium on immunological control of virus-associated tumors in man: Prospects and problems. Cancer Research 36, 559 (1976)
76. Liddle, G. W., Nicholson, W. E., Island, D. P., Orth, D. N., Abe, K., Lowder, S. C.: Clinical and laboratory studies of ectopic humoral syndromes. Recent Progress in Hormone Research 25, 283 (1969)
77. Mainwaring, W. I. P., Milroy, E. J. G.: Characterization of the specific androgen receptors in the human prostate gland. Journal of Endocrinology 57, 371 (1973)
78. McManus, L. M., Naughton, M. A., Martinez-Hernandez, A.: Human chorionic gonadotropin in human neoplastic cells. Cancer Research 36, 3476 (1976)
79. Melamed, M. R., Kamentsky, L. A.: Automated cytology. International Review of Experimental Pathology 14, 205 (1975)
80. Murgita, R. A., Goidl, E. A., Kontiainen, S., Wigzell, H.: α-fetoprotein induces suppressor T cells in vitro. Nature 267, 257 (1977)
81. Neville, A. M., Cooper, E. H.: Biochemical monitoring of cancer. A review. Annals of Clinical Biochemistry 13, 283 (1976)
82. Olsson, L. A., Rao, C. N., Menozian, J. O., Byrd, W. E.: Immunologic unreactivity in bladder cancer patients. Journal of Urology 107, 607 (1972)
83. Palmer, P. E., Safaii, H., Wolfe, H. J.: $Alpha_1$-anti-trypsin and alpha-fetoprotein. Protein markers in endodermal sinus (yolk sac) tumors. American Journal of Clinical Pathology 65, 575 (1976)
84. Perlmann, P., Troye, M., Pape, G. R.: Cell-mediated immune reactions to human tumors. Cancer 40, 448 (1977)
85. Rapp, F., Reed, C. L.: The viral etiology of cancer. A realistic approach. Cancer 40, 419 (1977)
86. Rasheed, S., Gardner, M. B., Rongey, R. W., Nelson-Rees, W. A., Arnstein, P.: Human bladder carcinoma: Characterization of two new tumor cell lines and search for tumor viruses. Journal of the National Cancer Institute 58, 881 (1977)

87. Ratcliffe, J.G., Podmore, J., McIlroy, B.: Ectopic hormone secretion and its clinical relevance. Cancer related antigens. Franchimont, P. (ed.), Amsterdam: North Holland 1976
88. Rees, L.H., Ratcliffe, J.G.: Ectopic hormone production by non-endocrine tumours. Clinical Endocrinology 3, 263 (1974)
89. Reiter, E.O., Kulin, H.E.: Suppressed follicle stimulating hormone in men with chorionic gonadotropin secreting testicular tumors. Journal of Clinical Endocrinology and Metabolism 33, 957 (1971)
90. Reynolds, R.D., Greenberg, B.R., Martin, N.D., Lucas, R.N., Gaffney, C.N., Hawn, L.: Usefulness of bone marrow serum acid phosphatase in staging carcinoma of the prostate. Cancer 32, 181 (1973)
91. Reynoso, G.: Cancer medicine. Holland, J.F., Frey, E. (ed.). Philadelphia: Lea and Febiger 1973
92. Reynoso, G., Chu, T., Guinan, P., Murphy, G.: Carcinoembryonic antigen in patients with tumors of the urogenital tract. Cancer 30, 1 (1972)
93. Robins, R.A., Baldwin, R.W.: Editorial. Immune markers on cancer cells. Cancer Immunology and Immunotherapy 2, 205 (1977)
94. Rosen, S.W., Weintraub, B.D., Vaitukaitis, J.L., Sussman, H.H., Hershman, J.M., Muggia, F.M.: Placental proteins and their subunits as tumor markers. Annals of Internal Medicine 82, 71 (1975)
95. Sandberg, A.A.: Chromosome markers and progression in bladder cancer. Cancer Research 37, 2950 (1977)
96. Schnellhamner, P.F., Brachen, R.B., Bean, M.A., Pinsky, C.M., Whitmore, W.F.: Immune evaluation with skin testing. A study of testicular, prostatic and bladder neoplasms. Cancer 38, 149 (1976)
97. von Schreeb, T., Franzén, S., Ljungqvist, A.: Renal adenocarcinoma. Evaluation of malignancy on cytologic basis. A comparative cytologic and histologic study. Scandinavian Journal of Urology and Nephrology 1, 265 (1967)
98. Schwartz, M.K.: Laboratory aids to diagnosis - enzymes. Cancer 37, 542 (1976)
99. Shah, K.V., Daniel, R.W.: Human bladder tumors and papovaviruses. Abstract, 7, National Bladder Cancer Conference, National Cancer Institute, Florida 1976
100. Stimson, W.H.: Studies on the immunosuppressive properties of a pregnancy-associated α-macroglobulin. Clinical and Experimental Immunology 25, 199 (1976)
101. Stjernswärd, J., Almgard, L.-E., Franzén, S., von Schreeb, T., Wadström, L.B.: Tumour-distinctive cellular immunity to renal carcinoma. Clinical and Experimental Immunology 6, 963 (1970)
102. Takasugi, M., Mickey, M.R., Terasaki, P.I.: Studies of specificity of cell-mediated immunity to tuman tumors. Journal of the National Cancer Institute 53, 1527 (1974)
103. Talerman, A., Haije. W.G., Baggerman, L.: Alpha-1 antitrypsin (AAT) and alpha fetoprotein (AFP) in sera of patients with germ-cell neoplasms: Value as tumour markers in patients with endodermal sinus tumour (yolk sac tumour). International Journal of Cancer 19, 741 (1977)
104. Teilum, G.: Special tumors of ovary and testis. Philadelphia: Lippincott 1971
105. Teilum, G., Albrechtsen, R., Nørgaard-Petersen, B.: Immunofluorescent localization of alpha-fetoprotein synthesis in endodermal sinus tumor (yolk sac tumor). Acta Pathologica et Microbiologica Scandinavica 82, 586 (1974)
106. Thorling, E.B.: Paraneoplastic erythrocytosis and inappropriate erythropoietin production. A review. Scandinavian Journal of Haematology Suppl. 17, 1 (1972)
107. O'Toole, C., Perlmann, P., Wigzell, H., Unsgaard, B., Zetterlund, C.G.: Lymphocyte cytotoxicity in bladder cancer. No requirement for thymus-derived effector cells? Lancet I, 1085 (1973)
108. O'Toole, C., Unsgaard, B., Almgard, L.E., Johansson, B.: The cellular immune response to carcinoma of the urinary bladder: Correlation to clinical stage and treatment. British Journal of Cancer 28, 266 (1973)
109. Traganos, F., Darzynkiewicz, Z., Sharpless, T., Melamed, M.R.: Nucleic acid content and cell cycle distribution of five human bladder cell lines analysed by flow cytofluorometry. International Journal of Cancer 20, 30 (1977)
110. Tribukait, B., Esposti, P.L.: Quantitative flow-microfluorometric analysis of the DNA in cells from neoplasms of the urinary bladder. Correlation of aneuploidy with histological grading and the cytological findings. Urological findings. Urological Research 6, 201 (1978)
111. Troye, M., Perlmann, P., Larsson, A., Blomgren, H., Johansson, B.: Cellular cytotoxicity in vitro in transitional cell carcinoma of the human urinary bladder: ^{51}Cr-release assay. International Journal of Cancer 20, 188 (1977)
112. Troye, M., Perlmann, P., Pape, G.R., Spiegelberg, H.L., Näslund, I., Gidlöf, A.: The use of Fab-fragments of rabbit anti human immunoglobulin as analytic tools for establishing the involvement of immunoglobulin in the spontaneous cytotoxicity of lymphocytes from patients with bladder carcinoma and from healthy donors. Journal of Immunology 119, 1061 (1977)

113. Turkington, R. W. : Ectopic production of prolactin. New England Journal of Medicine 285, 1455 (1971)
114. Vaitukaitis, J. L. : Peptide hormines as tumor markers. Cancer 37, 567 (1976)
115. Wahren, B. : Editorial. Fetal antigens in human tumors. Acta Medica Scandinavica 198, 337 (1975)
116. Wahren, B., Alpert, E., Esposti, P. : Multiple antigens as marker substances in germinal tumors of the testis. Journal of the National Cancer Institute 58, 489 (1977)
117. Wahren, B., Edsmyr, F., Zimmerman, R. : Measurement of urinary CEA-like substance: An aid in management of patients with bladder carcinoma. Cancer 36, 1490 (1975)
118. Wahren, B., Esposti, P., Zimmerman, R. : Characterization of urothelial carcinoma with respect to the content of carcinoembryonic antigen in exfoliated cells. Cancer 40, 1511 (1977)
119. Weintraub, B. D., Rosen, S. W. : Competitive radioassays and specific tumour markers. Metabolism 22, 1119 (1973)
120. Wilkinson, J. H. : Isoenzymes. London: Chapman and Hall Ltd. 1975
121. Williams, R. D., Bronson, D. L., Elliot, A. Y., Fraley, E. E. : Production of carcinoembryonic antigen by human prostate epithelial cells in vitro. Journal of the National Cancer Institute 58, 1115 (1977)
122. Wilson, C. B. : Immunopathology of glomerulonephritis. Textbook of Immunopathology, Vol. 1. Miescher, P. A., Müller-Eberhard, H. J. (ed.), p. 529. New York: Grune Stratton 1976
123. Wise, K. S., Allerton, S. E., Trump, G., Powars, D., Beierle, J. W. : A fetuin-like antigen from human nephroblastoma. International Journal of Cancer 16, 199 (1975)
124. Yoshida, O., Brown, R. R., Bryan, G. T. : Relationship between tryptophan metabolism and heterotopic recurrences of human urinary bladder tumors. Cancer 25, 773 (1970)
125. Zetterberg, A., Esposti, P. L. : Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. Acta Cytologica 20, 46 (1976)
126. Zimmerman, R., Hammarström, S. : Isolation of CEA-like material from urinary bladder carcinoma. Urological Research 6, 215 (1978)
127. Zinkernagel, R. M. : H-2 restriction of virus-specific cytotoxicity across the H-2 barrier. Journal of Experimental Medicine 144, 933 (1976)

Dr. Britta Wahren
Radiumhemmet
Karolinska Sjukhuset
S-105 21 Stockholm
Sweden