Letters

Established in Aberdeen and that receptor concentrations are virtually identical to those found in Edinburgh, where assays have been performed routinely for 12 years. The percentage of tissues that were receptor positive (71–73%) is comparable with those reported by other laboratories using the charcoal adsorption assay. The two centres differed on only four of 59 tumours, these being tumours of low receptor content (< 30 fmol/mg protein). Lessons were learnt in both centres from these discrepancies.

To our knowledge this kind of comparison between two centres using the same method of receptor assay has not been reported previously. The good correlation (r = 0.97) confirms our ability to collect, store, transport, and assay specimens independently and reproducibly. This kind of control in setting up a new receptor assay laboratory is not only feasible, but is also the minimum which should be undertaken. Once established, each laboratory should also undertake its own long term quality control under such a scheme as that organised for the United Kingdom by Dr RE Leake.11

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References


Ca antigen and urothelial antibacterial defences: hypothesis

The Ca antigen is a glycoprotein that can be detected in a wide range of malignant human tumours 1–3 but which is absent from normal tissues with the exception of the urothelium; 4 the luminal epithelium of the fallopian tube; 5 the epithelium of apocrine sweat glands and ducts of eccrine sweat gland; 6 and trophoblast. 7 Initial enthusiasm for this antigen as an immunohistological marker of malignancy waned when it was realised that it also occurred in non-malignant pathological conditions, including benign ovarian tumours, 8 benign breast disease, 9 and on the surface of activated type II pneumocytes. 10 The recognition of this antigen is, however, still of extreme clinical and theoretical interest.

The Ca glycoprotein occurs as a thin layer on the luminal surface of superficial urothelial cells. 7 It has been suggested that its function is to protect the urothelial cells from extremes of pH and osmolality found in the urine, and to protect tumour cells from an acid microenvironment produced by glycolytic metabolism. 3, 4 Most, however, if not all, epithelial cells produce surface glycoproteins which provide physical protection, and it is not clear why tumour cells do not show this preference for the urothelial variety: gastric juice can achieve a lower pH than urine; the interstitium of the renal medulla has a higher osmolality than urine; and the most extreme acid microenvironment achieved within tissue probably occurs in the vicinity of striated muscle following anaerobic metabolism during exercise. Cells cope with these conditions without the aid of the Ca glycoprotein.

We offer an alternative possible explanation, which is that urothelial glycoprotein prevents bacterial colonisation of the bladder and that Ca glycoprotein in tumours, by an entirely analogous method, prevents immune rejection of the tumour.

Hypothesis

In a study of frozen hydrated non-neoplastic human urothelium using low temperature scanning electron microscopy we noted a focal thin layer of material on the surface of the urothelium that might contain the Ca glycoprotein. This layer is not visible in fixed or dehydrated urothelium viewed by conventional scanning electron microscopy. Parsons et al 11 produced evidence that the bladder secretes a mucin layer that impairs bacterial adherence, and we suggested that the focal surface layer seen by low temperature scanning electron microscopy could be the Ca glycoprotein.
perature scanning electron microscopy might be a mucin barrier to bacterial infection.\textsuperscript{8} Fresh urothelium stained by dilute Hale's colloidal iron (a mucin stain) and subsequently examined in the frozen hydrated state using low temperature scanning electron microscopy and x-ray microanalysis showed a thin layer of iron over the entire surface of superficial cells with focal accentuation in the extracellular surface layer (unpublished observations). This leads to the hypothesis that the superficial cells secrete a surface layer which is continuously shed and is only visible by low temperature scanning electron microscopy where the shed secretions pool. Furthermore, synthesis and shedding must be coordinated so that a thin surface layer is always present.

The bladder is remarkable in that it has a large epithelial lined surface without a resident bacterial flora. The microbial flora on other surfaces probably survive because of specific bacterial adhesins which combine with epithelial surface antigens.\textsuperscript{11} If, however, the bladder sheds its surface layer in the coordinated way that we suggest then any bacteria which adhere to it will be shed and it will remain free of bacterial colonisation.

The physical bond between bacterial adhesin and epithelial surface receptor is equivalent to the bond between antibody and its specific antigen, or lymphocyte surface receptor and target cell antigen. Thus if tumour cells synthesise and shed Ca glycoprotein in the same way as urothelial cells then any tumour specific antibodies, or specifically armed lymphocytes, or macrophages, which adhere to their surface, will be shed, like bacteria in the bladder, before they do harm. Furthermore, the shed antigen will act as decoy molecules and might induce immunological tolerance. Thus tumours that copy the urothelial bacterial clearance mechanism are more likely to avoid immune rejection and produce clinical cancer.

Discussion

The urothelium is special, although not unique, in not having a resident microbial flora. It obviously must have an efficient system for clearing bacteria, and it is our contention that tumour cells copying this mechanism will be particularly effective at avoiding immune attack. There are other body surfaces that are not normally colonised by bacteria, including the alveolar lining cells of the lung and ducted glands. It is therefore of interest that Ca glycoprotein is found on both activated type II pneumocytes\textsuperscript{7} and in eccrine and apocrine glands.\textsuperscript{4} It is also likely, however, that there are other glycoproteins, which are effective in clearing bacteria in these sites, and some of the tumours, which do not exhibit Ca glycoprotein, might copy these mechanisms. The fallopian tube, which leads directly into the peritoneal cavity, also needs an effective system of bacterial clearance, and interestingly, it too secretes the Ca glycoprotein.

Trophoblast is a foreign tissue, which like tumour cells, avoids immune rejection. The observation that it also produces the Ca glycoprotein\textsuperscript{6} provides further support for this hypothesis.

One of the problems with this explanation is that in some tumours there is only focal expression of the Ca antigen.\textsuperscript{2} This could be because of the relative insensitivity of immunoperoxidase techniques in formalin fixed tissue failing to detect a thin cell surface layer. It is possible, however, that once immune tolerance develops to shed antigens, clones of cells might arise which lack the mechanism. It is certainly possible that the mechanism is maximally operative when the tumour is first establishing itself in the body.

An important corollary of the idea that shedding of surface glycoprotein protects against immune rejection is the obvious conclusion that in the absence of the mechanism rejection would occur. This implies that there are antigens, perhaps more firmly bound as integral membrane proteins, which are sufficiently tumour specific to allow an immune attack.

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Comparison of API-10S strips and tube tests for biotyping Haemophilus influenzae

Biotyping strains of \textit{Haemophilus influenzae} according to the scheme devised by Kilian\textsuperscript{10} has proved useful for epidemiological purposes. Biotypes I and II, for example, are those most commonly reported in meningitis and septicaemic illness, although geographical differences are evident. We have previously reported on the value of the technique when applied to isolates from patients with cystic fibrosis.\textsuperscript{2}

The procedure has been simplified by the use of API-10S strips for detecting the formation of urease, ornithine decarboxylase and beta-galactosidase (ONPG), and the formation of indole. We have, however, found that the technique recommended by API and based on the report of Mehtar and Aminafshar\textsuperscript{3} has very serious limitations. Briefly, this technique entails an inoculum prepared by suspending one colony of \textit{H. influenzae} in 3·0 ml of deionised water (pH 7·0) containing one standard XV disc. We compared results using this API-10S strip method with those obtained with a similar inoculum in sterile 3·0 × ½ inch tubes using double strength media in 0·25 ml amounts. We also compared both test systems using heavy inoculum of 1·25 × 10\textsuperscript{8} colony forming units suspended in sterile distilled water. Modified Christensen's medium was used for urease detection\textsuperscript{4} and modified Farlow's medium for ornithine decarboxylase. Indole formation was detected with Kovac's reagent.

References


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