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MINIREVIEW

Immune Responses to the MUC1 Mucin*

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MUC1 mucins are highly glycosylated glycoproteins expressed on the luminal surfaces of glandular epithelia. In breast and ovarian carcinomas, their expression is frequently upregulated and they may be secreted into the circulation of cancer patients. Early studies aimed at the production of anti-MUC1 monoclonal antibodies revealed that MUC1 was a potent immunogen in mice with many monoclonal antibodies raised defining epitopes within the protein core of MUC1. The immunogenicity of MUC1 has now been extended to human studies and it is apparent that patients with breast and ovarian malignant disease are able to mount immune responses against MUC1. These findings provide information on the mechanisms involved in the recognition of MUC1 expressing tumours. The utilisation of MUC1 related immunogens to stimulate immune responses to tumours could lead to the improved management of patients and the development of new immunotherapeutic strategies aimed at the eradication of MUC1 mucin expressing cancers. (Pathology Oncology Research Vol 1, No1, 27–31, 1995)

Key words: MUC1 mucin, immunogenicity, peptide epitopes

Introduction

Mucus glycoproteins or mucins are large, extensively glycosylated macromolecules which are expressed and often secreted by diverse epithelia. Apart from their major physiological functions as protective agents and biological lubricants, they are frequently elevated and/or altered in cancer and thus have potential as tumour markers.[8,22,30,31] In addition, accumulation or increased expression of mucins at the tumour cell surface (which, in the transformed cell, is no longer directional or orderly) may reduce cell aggregation by processes such as the masking of extracellular domains of adhesion molecules.[25] This facilitates detachment from the primary tumour and contributes to an increased potential for invasion and metastasis.

The MUC1 mucin protein core

Distinct families of mucins have now been identified. They are the products of different gene loci on separate chromosomes. A large region of the protein core of the mucin defined by the MUC1 gene located on chromosome 1q21[28] consists of variable numbers of a highly conserved 20 amino acid repeat unit, **PDTRPAPGSTAPPAHGVTSA**. Indeed, variable number tandem repeat (VNTR) units which give rise to an extensive polymorphism seen at the level of epithelial mucin DNA and protein[12,32] may be a common feature of several mucin families.[8,22]

In the normal individual, MUC1 mucins are particularly expressed on the luminal surfaces of the acini and ducts of the breast and other specialised glandular epithelia.[8,30] These may be viewed as immunologically priviledged sites which are essentially external to the body. In carcinomas including those of the breast, lung, colon, ovary and bladder, their expression is often up-regulated,[41] and their glycosylation is frequently altered as a consequence of neoplastic transformation. Post-translational changes in tumour-associated mucins include incomplete, or aberrant glycosylation leading to shorter and less branched oligosaccharide side chains, the accumulation of precursor structures[29,33,35] and the increased or *de novo* exposure of regions of the mucin protein core.[10] Thus, in the cancer patient, there may be exposure to elevated concentrations of altered MUC1 mucin which may be informative as a diagnostic tumour marker, but there may be additional immunological effects which ultimately may influence patient survival. The enhanced surface expression of mucins by tumour cells may aid their escape from immune surveillance by cytotoxic cellular effectors,[3,13] and indeed,

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even exclude the binding of antibodies.[36] In this way, the elevated expression of mucins appears to confer on the cancer cell selective advantages for growth and survival. On the other hand, novel determinants exposed in mucins processed by malignant cells have been invoked as targets for immune manipulation in the cancer patient since they may induce both cellular and humoral responses. The significance of these responses is as yet unknown, but recent observations in this area may offer a new basis for therapy of malignant disease.

Immunodominant epitopes in the MUC1 protein core

Early investigations upon the MUC1 mucin established that many clinically-relevant murine anti-mucin monoclonal antibodies (which were originally produced against human tumour cells or extracts and other complex immunogens) recognised epitopes within the **APDTRPAP** region of the protein core.[4,32] Hydropathicity and antigenicity calculations on the repeated VNTR sequence of the MUC1 protein core indicated that the first seven residues, **PDTRPAP**, comprised a particularly hydrophilic region which should be of high antigenicity according to the predictive algorithms.[32] Epitope mapping tests using synthetic peptides with sequences related to the MUC1 proteins established that all of the mucin core reactive antibodies recognised determinants of 3, 4 or 5 amino acids within this discrete domain and, in fact, the central **R** residue was present in the epitopes of all antibodies tested.[4,31,32]

In the mouse, therefore, the **PDTRPAP** region of the protein core is remarkably immunodominant. Even though MUC1 mucins are highly glycosylated, it is the exposed protein core which appears to be recognised by the murine immune system.

Human antibody responses to the MUC1 protein core

Evidence is accumulating that the same short peptide sequence in the MUC1 mucin core defined by murine monoclonal antibodies is immunogenic in man. Human antibodies have been generated from Epstein-Barr virus immortalised B-cells from tumour draining lymph nodes of an ovarian cancer patient.[33] One of the B cell clones reacted with a synthetic peptide with the sequence **PDTRPAPGSTAP** containing the hydrophilic motif and this antibody, an IgM, was also positive with several MUC1 expressing cell lines.

A different approach was adopted by Kotera et al[24] who screened serum samples from cancer patients for reactivity with synthetic peptides 60, 80 and 105 residues long which corresponded to 3, 4 and 5.25 tandem repeats of human MUC1 mucin. Sera were obtained from patients with cancer of the breast, colon and pancreas, at various stages of disease, and these were tested using an enzyme linked immunosorbent assay (ELISA). Around 10% of the sera reacted well with the antigenic peptides, with reactivity being highest against the 105-mer. Serum blocking experiments established that short peptides containing the motif **APDTRPAP** were able to inhibit the positive cancer patient serum reactivity by greater than 60% whereas a control peptide was without effect.[24]

If there is a serological response to MUC1 in cancer patients, then it is reasonable to suggest that some patients at least may produce immune complexes of MUC1 mucin and specific antibody. The presence of circulating immune complexes in breast cancer patients has long been the subject of extensive investigation.[9,14,17,22] The findings would infer that breast tumour-associated antigens can elicit a humoral immune response in the host and that a proportion at least of the antigen released from the tumour becomes complexed with antibody. This proposal is supported by the results of a recent investigation showing that polyethylene glycol precipitates from breast cancer patients' serum samples (containing putative immune complexes) may be fractionated under conditions to release antigen from these complexes[5] and that MUC1 antigen can be identified in the antigen containing fractions. Furthermore, the major protein bands in the immune complex fractions, which were analysed by SDS–PAGE, migrated in the gels with the mobilities of IgG heavy and light chains.

The MUC1 antigen identified in immune complexes in breast cancer patient's serum was recognised by the highly specific antibody C595 which defines the tetrameric motif **RPAP** in the mucin protein core.[32] In breast cancer, positive antibody reactivity (in terms of binding to high molecular weight antigens in fractionated samples) was found in 7/10 subcellular membrane preparations of tumour and in 4/10 serum samples analysed by Western blotting, with no binding to samples from patients with benign breast disease.[5]

The MUC1 mucin is the major circulating tumour marker which is presently under investigation in breast cancer,[40] and if antigen is complexed in the blood with antibody, at least in a proportion of patients, then this might explain why a number of patients with a significant tumour burden appear to be negative for circulating MUC1. Preliminary evidence has now been presented to suggest that the formation of immune complexes containing the MUC1 mucin occurs in up to 30% of patients and that these factors may impair MUC1 immunoassay performance.[11,27]

The findings emphasise the need to devise immunoassays to quantify *total* MUC1 antigen in the circulation since this may be more informative in assessing the status of the patient than present tests which have been developed to measure free circulating antigen.

As well as interfering with the sensitivity of diagnostic immunoassays, circulating antibodies may exert effects which might limit tumour growth or the development of metastases. Complement dependent cytolysis, particularly as mediated by IgM antibodies, and/or antibody dependent

cellular cytotoxicity may be invoked as possible mechanisms. The potential destructive nature of an anti-mucin antibody in destroying human tumour cells has also been highlighted by Kotera et al.[34] It was suggested that influencing more effective cognate T cell help through carefully designed mucin vaccines may change the nature of the humoral response into a very powerful one, considering its tumour specificity. This was illustrated by reference to a recent report on the detection of circulating anti-mucin antibodies in 5 of 19 patients with ulcerative colitis.[15] The reactivity here is directed to the same epitope in the mucin tandem repeat, expressed in this case on inflamed colonocytes. Moreover, it is associated with the sites of inflammation suggesting its pathogenic role, and interestingly, the dominant isotype of the anti-mucin antibody in this destructive disease is not IgM but rather IgG.[15]

Human cellular responses to the MUC1 protein core

Recent findings have indicated that some cancer patients may have an active cellular immune response to tumours which involves the recognition of the MUC1 mucin. Analysis of tumour reactive cytotoxic T lymphocytes (CTLs) isolated from patients has identified populations of these cells which are able to kill tumour cells through recognition of MUC1 expressed at the malignant cell surface. It has been reported that HLA-unrestricted and MUC1 reactive CTL could be induced from tumour-draining lymph node lymphocytes.[1,20] These included patients with pancreatic and breast carcinoma. That the response was directed against the protein core was suggested by the demonstration that the murine SM3 antibody, which recognises the motif **PDTRP**, blocked the response of the cytotoxic effectors. This particular antibody has previously been shown to exhibit preferential reactivity with MUC1 mucin derived from malignant cells,[10] and other anti-mucin core antibodies failed to inhibit CTL cytotoxicity. The suggestion is therefore that the CTL response displayed a specificity which was most evident against tumour-associated MUC1 determinants.

A T cell line which was established from a human multiple myeloma has been employed by Takahashi et al[38] to further probe the MUC1 recognition event by CTLs. This line expressed T cell receptor (TCR) α/β, CD3 and CD8 molecules, and its cytotoxicity for tumour cell targets was inhibited by treatment with anti-CD3 but not anti-HLA antibodies. The data strongly indicates that the activity of at least some populations of tumour reactive CTLs is through recognition of MUC1 mucins and that recognition occurs in an HLA-unrestricted manner. The exact nature of this type of event is largely unexplained although the repetitive nature of epitopes within the MUC1 mucin protein core may allow cross-linking of the TCR to occur upon mucin reactive T cells. Additional evidence has suggested that the anti-tumour reactivity of CTLs from patients with ovarian cancer is directed against the MUC1 mucin protein core.[19] However, recognition of MUC1 core related peptides by CTLs and the induction of killing occurred in an MHC-restricted manner, so that the involvement of the MHC in these events requires further evaluation.

Discussion

The ability of determinants in the MUC1 mucin to stimulate cells of the immune system to evoke anti-tumour responses makes these molecules candidates for use in active specific immunotherapy.[39] Trials using a synthetic MUC1 core related peptide administered in admixture with BCG have already begun. Results are awaited with interest.[26] In our laboratories, we have noted that circulating MUC1 mucins are considerably elevated in the third trimester of pregnancy and MUC1 levels soon return to normal values *post partum*. It is feasible therefore that sensitization to protein core epitopes in MUC1 mucins during pregnancy may contribute to the protection against breast cancer observed in multiparous women.[33]

Other strategies for the activation of cellular immunity against MUC1 mucin include the development of anti-idiotypic antibodies as antigen mimicing the binding to so-called regulatory idiotopes on B or T cells.[2,21] The synthesis of novel epitope constructs involving T cell epitopes linked to the immunodominant B cell motif of the MUC1 core is also being explored as a means to enhance antibody responses to MUC1 mucins.[6,7]

Finally, MUC1 mucins expressed on ovarian tumours have been exploited as targets for radioimmunotherapy using anti-protein core monoclonal antibodies.[23] An unexpected anti-tumour therapeutic bonus was detected in patients receiving such treatment. Mouse antibodies administered to patients generated T cells capable of recognising the antibodies and thus focusing the host's cellular immune response against the targeted tumour. It was suggested that multiple treatments could lead to the induction of T cells with specificity for the idiotypic component of the administered antibody. It remains to be seen whether this approach can be developed as an alternative strategy in the therapy of MUC1 expressing tumours.

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