Human Autoantibodies to CA-125
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Introduction
CA-125 (MUC16) is a 200-2000kDa non-mucinoid glycoprotein normally expressed by tissues of Mullerian duct origin (1). CA-125 is an indicative tumour marker of advanced epithelial ovarian cancer patients, where serum levels are elevated in ~80% of patients (2). CA-125 levels are also often seen raised in patients with breast cancer (3).

Tumour markers such as CA-125 are detected and measured using mouse monoclonal antibodies to monitor disease progression. These mouse antibodies however are neither sufficiently sensitive or cancer-specific for use in early diagnosis.

Autoantibodies against tumour-associated proteins such as MUC1 and p53 have been described (4,5). These antibodies are produced as part of an early immune response to the cancer, and may provide an in vivo amplification of an early carcinogenic signal.

Identification of such tumour-specific antibodies may therefore provide a method for earlier diagnosis of such cancers.

Aims
• Purify normal and cancer-associated CA-125, and human anti-CA-125 antibodies.
• Investigate whether there are any immunologically identifiable differences between the normal and tumour-associated forms of the antigen, by comparing antibody responses to these different CA-125 proteins.
• Investigate the presence of circulating autoantibodies to CA-125 in the sera of patients with primary breast cancer.

Materials and Methods
CA-125 Antibody Purification
CA-125 preparations from normal sera (n=1) an ovarian cancer cell line, OVCAR-433 (n=2), and breast cancer pleural effusion (n=2), were affinity purified using a mouse monoclonal antibody specific for CA-125, VK-8 (kindly provided by KO Lloyd), bound to a CNBr Sepharose matrix (6).

Human CA-125 Autoantibody Purification
CA-125 autoantibodies were affinity purified from a breast cancer patient pleural effusion with known reactive antibodies to CA-125 (as identified by ELISA), using CA-125 (purified from OVCAR-433 cells) immobilised through oxidised sugar groups, to CarboLink™ coupling gel (Pierce).

Comparison of Human and Mouse anti CA-125 Antibodies
CA-125 antigen preparations were adsorbed on to 96-well plates overnight in sodium carbonate buffer. Plates were blocked with HSB/Tween for 1 hr before probing with biotinylated VK-8 (5ug/ml) or biotinylated human antibody to CA-125 (as identified by ELISA), bound to a CNBr Sepharose matrix (6).

CA-125 Autoantibody Assay
Affinity purified CA-125 (OVAR-433) was diluted 1:10 in sodium carbonate buffer and adsorbed overnight onto 96-well plates. Plates were blocked as above with HSB/Tween and samples (normal, PBC) were diluted 1:100 and controls (1:1000) in HSB, and incubated for 1.5 hrs at room temp. Plates were then probed with anti-Human Ig HRP for 1hr. Plates were developed with TMB substrate and read at OD 650nm.

Conclusions
1. Human antibodies to CA-125, purified from breast and ovarian sources show a greater specificity for tumour-derived antigen than currently available mouse antibodies.

Such discrimination suggests that there is a biological difference between cancer-associated and normal forms of the protein. Further studies are underway to investigate the biological differences between these molecules, and isolate the B-lymphocytes responsible for the production of these tumour-specific antibodies.

2. Autoantibodies to CA-125 can be detected in the serum of patients with primary breast cancer. Identification of such tumour-specific antibodies may be useful to aid early diagnosis of breast and ovarian cancer, reducing the need for surgery and radical treatment regimes.

References

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