Toxoplasma was detected in two of the seven cases by histology and in five cases using PCR.

Conclusions

Previous studies have shown that a single toxoplasma organism can be detected by PCR and that the method is highly specific.³ Contamination of the reactions leading to false positive results was unlikely as control samples of known toxoplasma free tissue repeatedly failed to produce amplification products. Consequently the enhanced detection of toxoplasma by PCR compared with histology may reflect the superior sensitivity of the former technique. In its present form the PCR assay will not distinguish quiescent cysts from active infection with free trophozoites of *T gondii*. This problem may be overcome by the development of stage specific primers. As the distribution of toxoplasma in infected tissue is uneven, sampling error could result in discordant histological and PCR findings. The optimal amount of tissue which should be examined by

each technique is not established.

Our results indicate that PCR can be used to detect *T gondii* in cardiac biopsy samples taken from transplant recipients with primary, organ graft related toxoplasmosis. This method may be more sensitive compared with conventional histological examination. Further studies are in hand to define the application of PCR in organ transplantation for the diagnosis of primary infection and the identification of organ grafts which contain the parasite.

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Specificity of colon specific antigen and colon ovarian tumour antigen

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Abstract

Sections (5 µm thick) from 101 primary adenocarcinomas (including ovarian, colorectal, gastric, breast, oesophageal, prostatic, pancreatic, endometrial and gall bladder) were incubated with anti-colon specific antigen (CSA) and anti-colon ovarian tumour antigen (COTA) antibodies using the peroxidase antiperoxidase technique with positive and negative controls. Anti-CSA positivity was seen in 19 of 20 colonic adenocarcinomas, but it was also seen in a large number of the other tumours. While anti-COTA staining was positive in 16 of 20 colonic adenocarcinomas and 20 of 30 ovarian adenocarcinomas, it was also positive in a large number of the tumours.

Anti-CSA and anti-COTA are not adequately specific in the identification of a colonic or ovarian origin of an adenocarcinoma and cannot reliably be applied to the identification of a metastatic adenocarcinoma of unknown primary site.

A patient with metastatic adenocarcinoma of unknown primary site is a relatively common clinical problem. The identification of an antigen specific for a primary site, such as colon or ovary would have the benefit of minimising invasive and expensive investigations in untreatable patients and would also indicate the surgical and chemotherapeutic regimens in potentially curable patients.

Since the discovery of carcinoembryonic antigen (CEA) in colon cancer other more allegedly specific colon cancer associated antigens (colon specific antigen (CSA) and colon ovarian tumour antigen (COTA)) have been identified.

CSA is a heat stable, mucin associated antigen present in normal colonic epithelial cells and is expressed in greater quantities in colonic adenocarcinomas.

COTA is a heat stable antigen present in colonic adenomatous polyps and adenocarcinomas but not in normal colonic epithelium and it is also present in mucinous ovarian tumours.¹

In 1986 Pant *et al* described the sensitivity and specificity of goat anti-COTA antibody to

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Table 1 Anti-CSA staining results

	Negative	Weakly positive	Strongly positive
Colon (n = 20)	1	9	10
Ovary (n = 30)	10	13	7
Mucinous (n = 9)	4	2	3
Serous (n = 16)	5	7	4
Endometrioid (n = 5)	1	4	0
Stomach (n = 9)	3	3	3
Breast (n = 10)	4	5	1
Oesophagus (n = 5)	0	4	1
Prostate (n = 10)	5	5	0
Pancreas (n = 13)	2	9	2
Gall bladder (n = 1)	0	0	1
Endometrial (n = 3)	0	3	0

Table 2 Anti-COTA staining results

	Negative	Weakly positive	Strongly positive
Colon (n = 20)	4	6	10
Ovary (n = 30)	10	12	8
Mucinous (n = 9)	4	2	3
Serous (n = 16)	4	8	4
Endometrioid (n = 5)	2	2	1
Stomach (n = 9)	4	3	2
Breast (n = 10)	7	2	1
Oesophagus (n = 5)	0	5	0
Prostatic (n = 10)	5	5	0
Pancreas (n = 13)	2	10	1
Gall bladder (n = 1)	0	0	1
Endometrial (n = 3)	0	3	0

colonic and ovarian mucinous tumour tissue in 5 μ m paraffin wax embedded tissue sections.² The study included eight cases of colonic adenocarcinomas which were positive, four adenomatous polyps one of which was positive, and one hyperplastic polyp (positive). It also included 12 normal colons or inflammatory conditions of which three were positive. Seventeen non-colonic or ovarian primary carcinomas from various sites were negative in their study. In 1988 Ueda *et al* found these antigens to be absent in normal endometrium. They noted a tendency for an increased positivity in malignant epithelial tumours of the ovary.³ The aim of this study was to determine how specific CSA and COTA are to colonic and ovarian tumours using a commercially available monoclonal antibody (Biogenex Laboratories)¹ applied to a large number of adenocarcinoma of various primary sites.

Methods

A representative 5 μ m section from 101 primary tumours were stained with anti CSA and anti COTA (Biogenex).

The cases included 20 cases of primary colorectal carcinomas and 30 ovarian tumours (including nine mucinous cystadenocarcinomas, 15 serous cystadenocarcinomas, and six endometrioid carcinomas). The other tumours included nine gastric, 10 breast, five oesophageal, 10 prostatic, 13 pancreatic, three endometrial and one gall bladder.

The cases were collected from the files of the pathology departments of Beaumont Hospital, The Royal College of Surgeons in Ireland, and St James's Hospital, Dublin.

The sections were placed on poly-L-lysine coated slides and rehydrated through graded alcohols. Having quenched endogenous peroxidase activity by incubating with 3% H₂O₂, the

slides were incubated with anti-CSA and anti-COTA antibodies proprietary kit (Biogenics) using the peroxidase anti-peroxidase streptavidin technique at a dilution of 1. Each batch included a positive control (colonic or ovarian carcinoma). A negative control (non-immune serum; Biogenics) was also used.

Positive staining was identified in the presence of granular cytoplasmic staining of viable cells. It was separated into three categories: negative, weakly positive, and strongly positive.

In the negative cases slides had either no positive staining or positivity was confined to inflammatory cells, necrotic cells, or non-granular staining of mucin pools. Weak positivity was defined as staining of 1–15% of tumour cells with +, ++, or +++ or 1–40% of cells with + or ++. Strong positivity was defined as +++ of more than 15% of tumour cells or + or ++ involving more than 40% of tumour cells.

Results

The results of the staining patterns seen are set out in tables 1 and 2.

Discussion

Distinguishing between weak and strong staining patterns does not reliably distinguish colonic or ovarian tumours from other adenocarcinomas (tables 1 and 2). Four of nine ovarian mucinous tumours were negative with both anti-CSA and anti-COTA, and 11 of 16 serous tumours and four of five endometrioid tumours were positive with anti-CSA and 12 of 16 serous and three of five endometrioid tumours positive with anti-COTA.

Varying numbers of cases of non-colorectal/ovarian tumours were anti-CSA and anti-COTA positive (tables 1 and 2). Anti-COTA staining was seen in three of 10 breast carcinomas, five of 10 prostatic carcinomas, 11 of 13 pancreatic carcinomas and all three endometrial carcinomas. Anti-CSA positivity was seen in six of 10 breast carcinomas, five of 10 prostatic carcinomas, 11 of 13 pancreatic carcinomas and all three endometrial carcinomas. Anti-CSA and anti-COTA were not either wholly sensitive or specific to colonic and mucinous ovarian tumours and could not reliably be applied to the identification of a metastatic adenocarcinoma of unknown primary site.

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