Letters to the Editor

Aberrant expression of HLA-DR antigen in diffuse sclerosing variant of papillary carcinoma of thyroid

Diffuse sclerosing papillary carcinoma is a rare type of thyroid tumour, characterised by a diffuse disease in one or both thyroid lobes, with dense sclerosis, extensive lymphocytic infiltration and abundant psammoma bodies intermixed with islands of papillary carcinoma.[1] This type of tumour comprises 3% of papillary carcinomas and is associated with a more aggressive clinical course.[2] The pathogenic role of the lymphocytic infiltration and diffuse disease in this particular type of tumour remains unknown.

We recently studied a case of diffuse sclerosing papillary carcinoma in a 26 year old woman. The tumour exhibited diffuse spread throughout the left thyroid lobe. It showed dense sclerosis, lymphocytic infiltration, and abundant psammoma bodies intermixed with elements of papillary carcinoma with focal squamous metaplasia. To investigate the nature of the lymphocytic infiltration in this tumour we performed an immunohistochemical study with a large battery of antibodies. The lymphoid component was polymorphic and was composed of both B and T cells. The most striking immunohistochemical finding was the strong positivity of the tumour cells for HLA-DR antibodies. Normal follicular epithelial cells were negative for this antibody.

Little is known about the pathogenic importance of the lymphocytic infiltrate in diffuse sclerosing papillary carcinoma. It has been shown that the tumours contain numerous, S-100 positive, Langerhans', interdigitating reticulum cells scattered throughout the tumour islands and lymphoid infiltrate, suggesting an immunological reaction mediated by antigen-presenting cells.[3] In a recent study Kamma, Fuji and Oyata studied the importance of lymphocytic infiltration in nine juvenile thyroid carcinomas.[4] They found a clear correlation between HLA-DR expression by the tumour cells and the degree of lymphocytic infiltration. They suggested that lymphocytic infiltration is an immunological reaction induced by antigens from the carcinoma itself and that the reaction may progress according to tumour development. None of the tumours studied, however, was a diffuse sclerosing papillary carcinoma. The immunohistochemical findings in our case agree with their results and suggest that aberrant expression of class II histocompatibility HLA-DR antigens in tumour cells may have an important role in the immunological reaction in this type of tumour as it does in autoimmune thyroiditis.[5]

X MATIAS-GUIU
J ESQUIUS
Hospital de la Santa Creu i Sant Pau
Barcelona, Spain

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Cold Ziehl-Neelsen stain for Campylobacter in gastric biopsy specimens

Campylobacter can be identified in sections stained with haematoxylin and eosin but, as they stain relatively weakly and may be obscured by surface mucus, other stains are often used to visualise them.[1,2] Among the special stains used are silver methods (Warthin-Starry and Warthin-Faulkner), Romanowsky stains, the Gram Stain, and recently, the Gimenez stain.

The silver methods are complicated and time consuming and the Gram stain is unsatisfactory, leaving Giemsa or Gimenez as the best stains for routine use. We present an alternative method, based on cold carbol fuchsin as in the Gimenez technique but requiring less preparation.

Staining takes only about five minutes to complete, with the minimum of reagents, and the method has proved useful in a busy laboratory.

1 Sections to water via xylene and alcohol.
2 Stain in carbol fuchsin at room temperature for one minute.
3 Rinse in water to clear slide of stain.
4 Stain in Loefflers methylene blue at room temperature for one minute.
5 Rinse in water.
6 Wash in alcohol and dehydrate to xylene.
7 Mount in DPX.

Carbol fuchsin is made up as follows: basic fuchsin 1 g; phenol crystals 5g; isopropyl alcohol 10 ml; distilled water 100 ml.

Campylobacter stain blue and stand out clearly against a background of pale staining mucin. The mucosa stains shades of lilac, the nuclei being magenta. Coliforms also take the stain but Campylobacter are easily distinguished by their characteristic morphology.

JA HAWKINS
N GUBBAY
Pathology Department
Cheltenham General Hospital
Sandford Road
Cheltenham
Gloucestershire GL53 7AN

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Rectal carriage of *Chlamydia trachomatis* in women

The first reports of isolation of *Chlamydia trachomatis* from rectal mucosa in women in certain high risk groups showed the prevalence of rectal chlamydia infection to be 11·8%. Since then other investigators have found rates of 21% and 5·2%,[1] the difference being ascribed to the widely differing levels of admitted anal intercourse (60·9% and 4·1%, respectively) in the two groups. In a review of rectal infection in women with gonorrhoea the prevalence ranged from 26 to 63% [mean 44%].[2] As there was no history of anal intercourse in most cases autoinoculation with cervico-vaginal material was assumed.

We studied rectal carriage of *C trachomatis* in 84 women attending consecutively at a sexually transmitted disease

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clinic. They either had diagnosed cervical chlamydial infection and were being seen at second visit, or were considered to be at risk for this infection because they had pelvic inflammatory disease or because they or their partner had gonorrhoea. Swabs were taken from the endocervix and, using a proctoscope, from the rectal mucosa avoiding gross faecal contamination. One of the swabs from the rectum was first used to make a smear for a direct immunofluorescence slide test (Imagen, NovoBiolabs) before being transferred to tissue culture transport medium. Specimens in their respective transport media were inoculated into McCoy cells treated with cycloheximide or were tested in a chlamydial antigen detecting ELISA (IDEIA, Mark 2; Boots-Celltech, Novo Biolabs). The latter specimens were also tested in the Mark 3 form of the test, modified to eliminate non-specific reactions with, for example, staphylococcal protein A.

None of the 84 women studied had overt proctitis. *C trachomatis* was isolated from the cervix of 39 [46·4%] and from the rectum of eight [9·5%] of the women, none of whom was in the group who admitted rectal intercourse. Positive rectal cultures were found only in those women who were also positive in the cervix, but cervical inclusion counts showed no correlation with rectal positivity, suggesting that factors in addition to autoinoculation, such as partiality of *C trachomatis* serovars for different sites,[3] may also be important. In all cases the rectal inclusion counts were less than 20, which leads us to suspect that we may be underdiagnosing rectal carriage, a situation which might be remedied by sonication of specimens before tissue culture inoculation. Nevertheless, the rate of rectal carriage found is significant and should be considered in determining the antibiotic regimens required to eradicate *C trachomatis* infection in women, particularly where pelvic infection is present, and in deciding which sites should be sampled in follow up.

Although chlamydial antigen detection tests are not recommended by manufacturers for diagnosis with specimens other than from the genital tract of adults, they have been and are being used for non-genital specimens, even in low risk groups.[4] Non-cultural methods of diagnosis were therefore included as a comparison with the direct culture method in non-genital specimens from this high risk group.

In the direct immunofluorescence test chlamydial elementary bodies were found in seven of the eight culture positive rectal specimens, although the numbers of elementary bodies bore little relation to the numbers of inclusions found in tissue culture. This result, however, has to be judged against the finding of 15% false positive smears from women who were culture negative in both cervix and rectum; and this, despite the rigorous application of criteria for numbers, size, shape, colour and intensity of fluorescence of elementary bodies.

The problem of specificity of the IDEIA Mark 2 when testing rectal/faecal specimens is well recognised,[5] so that finding 55% false positive results among rectal specimens from women who were culture negative in both cervix and rectum was not unexpected. Although this figure improved when the same specimens were tested in the modified IDEIA Mark 3, it was still too high at 20% to allow this test to be used in diagnosis of rectal chlamydial infection.

Under certain conditions the presence of *C trachomatis* in children indicates that sexual activity has occurred. If, in such low risk groups, cultural methods may result in underdiagnosis and antigen detection methods show high false positive rates,[4] the implications where sexual abuse is suspected may be serious.

BC PRATT,
I ANNE TAIT,
WI ANYAEGBUNAM
Departments of Medical Microbiology, and Genitourinary Medicine, Royal Liverpool Hospital, PO Box 147, Liverpool L69 3BX.

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Cytomegalovirus and autoimmune liver disease

There are three major liver diseases for which no infectious or toxic causal agent has been identified, but in which the hepatic parenchyma is damaged: primary biliary cirrhosis (PBC); autoimmune (lupoid) hepatitis; and primary sclerosing cholangitis (PSC). Autoantibodies are common in PBC and lupoid hepatitis, suggesting an autoimmune process. This has been considered for PSC as well, but evidence is scanty. Among other possibilities, it has been suggested that occult viral infection might have a role in initiating these three diseases. Cytomegalovirus (CMV), which is common in the general population, is a potential candidate, having already been implicated in a paucity of intrahepatic bile ducts in neonates and after orthotopic liver transplantation.[1,2] CMV also causes acute hepatitis in immunocompromised patients and, occasionally, in immunocompetent hosts,[3] and like PBC, can have granulomatous features.[4] Moreover, CMV probably causes disease not only by lysing infected cells, but also by interacting with and changing the host immune system.[5,6] Ways in which it might do this and thereby induce an autoimmune process include perturbation of cell membranes of non-productively infected cells, and expression of viral proteins by such cells, leading to a T cell response or to stimulation of local interferon release.[6] In such circumstances productive viral replication would not necessarily occur and the classic cellular enlargement and inclusion bodies of productive infection would not be recognised. Yet, for CMV to be considered pathogenic, it must be identified at the affected site. We therefore used both immunoperoxidase and in situ hybridisation techniques to look for early viral antigens (which may be produced without complete viral replication) and viral nucleic acid, respectively.

Biopsy specimens from eight patients with PSC, seven with autoimmune hepatitis, and 15 with PBC were examined. Two of the patients with PBC had two biopsy specimens each. Thus 32 formalin fixed, paraffin wax-embedded biopsy specimens were studied. Sections were examined using commercially available primary antibodies to early and late viral antigens (Dako Inc.) and DNA probes (Enzo Inc.). Biopsy specimens from patients with CMV oesophagitis were used as positive controls. These specimens had been processed in the same way as those from our study cases and had typical cytomegalic cells present in all levels as well as infected cells identifiable only by the special staining techniques. Appropriate method controls

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