

## CORRESPONDENCE

### Epstein-Barr virus and Hodgkin's disease

As an outsider I remain fascinated by the unfolding story of Epstein-Barr virus (EBV) and Hodgkin's disease, the latest chapter of which Jarrett *et al* have recently published in the Journal.<sup>1</sup> One aspect of the theories about an aetiological link between EBV and Hodgkin's disease continues to worry me. If a single cell, which by chance happens to be infected with EBV and has incorporated the EBV genome into its genetic material, then undergoes clonal expansion due to a stimulus or mutation independent of EBV, will the EBV genome in that clonally expanded population of cells also be clonal? If so, then could not the clonal EBV genome detected in Hodgkin's disease be an innocently carried passenger, as might be other clonal genetic material—for example, an aetiologically unimportant gene deletion, chromosome translocation, or even G6PD isoenzyme in a female?

Of course, Jarrett *et al* point out that clonal EBV genome is rare in a non-Hodgkin's lymphoma (NHL) but common in Hodgkin's disease, and that this observation supports the hypothesis that EBV infection predisposes to a particular type of malignancy. But might not that disparity simply tell us that the particular cell type from which Hodgkin's disease in children and older patients arises is more likely to be a carrier of EBV genome than the cell of origin of NHL or that of young adults with Hodgkin's disease, whether or not it actually has been infected by EBV?

Perhaps Jarrett *et al* could show that the clonally expanded EBV genome in their tissue is more typical of latent EBV infection than chance incorporation of a single copy into the human genome. Even with this information there may still be a problem distinguishing the "EBV infection of a single cell is responsible in part for progression to malignancy" hypothesis from the "malignancy may develop from an incidentally EBV infected cell and will therefore contain clonal EBV genome" hypothesis. I am keen to be persuaded that the former hypothesis is correct. I would find it more exciting than the latter, although those whose major interest is the nature of the cell of origin of Reed-Sternberg cells might take the other view.

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1 Jarrett RF, Gallagher A, Jones DB, *et al*. Detection of Epstein-Barr virus genomes in Hodgkin's disease: relation to age. *J Clin Pathol* 1991;44:844-8.

#### Dr Jarrett *et al* comment:

Recent data suggest a strong association between Hodgkin's disease and Epstein-Barr virus (EBV), although there is no proof that EBV has an aetiological role in this disease. One finding which lends support to the latter hypothesis is that the cells infected with EBV in Hodgkin's disease are clonal with respect to EBV—that is, they have arisen from a single infected cell.<sup>1-3</sup> In the accompanying

letter Reid points out that this result would be obtained if a cell infected by EBV were to undergo clonal expansion as a result of another stimulus. In the latter case the EBV would simply be a passenger virus. We agree that the data at present do not exclude this possibility and for this reason are cautious about stating that EBV has a causative role in Hodgkin's disease. This question is particularly difficult to address in the case of Hodgkin's disease as we do not know the nature of the Reed-Sternberg cell precursor and therefore cannot determine whether this cell type is latently infected by EBV in healthy persons.

We would, however, argue against the above suggestion for several reasons. First, we now have evidence that in most cases of Hodgkin's disease in which we can detect clonal EBV genomes EBV is localised to Reed-Sternberg cells and the EBV latent gene product LMP-1 is expressed (Armstrong *et al*, unpublished observations). The LMP-1 protein is known to have transforming potential, and more importantly, in the present context LMP-1 is a target for cytotoxic T cells. Using sensitive *in situ* hybridisation techniques EBV can be detected within lymphocytes in the reactive infiltrate in Hodgkin's disease tumours, but these cells do not express the LMP-1 protein (Armstrong *et al*, unpublished observations). Furthermore, in Burkitt's lymphoma the LMP-1 protein is down-regulated, perhaps allowing the tumour cells to escape immunosurveillance. We therefore believe that LMP-1 has some functional role within the Reed-Sternberg cells in Hodgkin's disease.

Secondly, our data suggest that older and paediatric cases of Hodgkin's disease are more likely to be EBV positive than young adult cases. If the cell type from which the Reed-Sternberg cells originate is the same in all cases of Hodgkin's disease, and the virus is a passenger virus, then one would not expect to see this difference in EBV positivity by patient subgroup.

Thirdly, if as Reid suggests, the cell of origin in young adults is different from that in older persons and the latter is more often a carrier of EBV then over 70% of these cells would have to be infected with EBV to explain our results. It would seem very unlikely that such a high percentage of this particular cell type is infected in this way, particularly as it is estimated that only 1 in 10<sup>5</sup> peripheral blood mononuclear cells is latently infected.

We therefore feel that at present the data favour the idea that EBV has some role in the pathogenesis of Hodgkin's disease, but further studies are required in order to clarify this issue.

- 1 Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol* 1987;129:86-91.
- 2 Anagnostopoulos I, Herbst H, Niedobitek G, Stein H. Demonstration of monoclonal EBV genomes in Hodgkin's disease and KI-1-positive anaplastic large cell lymphoma by combined Southern blot and *in situ* hybridization. *Blood* 1989;74:810-16.
- 3 Jarrett RF, Gallagher A, Jones DB, *et al*. Detection of Epstein-Barr virus genomes in Hodgkin's disease: Relation to age. *J Clin Pathol* 1991;44:844-8.

### Absence of human papillomavirus in cervical adenocarcinoma

The paper by Young and others produced some unusual results.<sup>1</sup> In the discussion the authors mention different results in one Australian article, then published together with articles from the United States of America. Since April 1991 two reports from other centres in Australia have been published, both showing by different *in situ* hybridisation methods the presence of HPV 16 or 18 in adenocarcinoma *in situ* of the endocervix.<sup>2,3</sup> These results from Australia are fairly consistent. The results from the United Kingdom are somewhat puzzling and probably highlight the need for more reports from British laboratories.

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- 1 Young FI, Ward LM, Brown LJR. Absence of human papilloma virus in cervical adenocarcinoma determined by *in situ* hybridization. *J Clin Pathol* 1991;44:340-1.
- 2 Nicklin JL, Wright RG, Bell JR, *et al*. A clinicopathological study of adenocarcinoma *in situ* of the cervix. The influence of cervical HPV injection and other factors, and the role of conservative surgery. *Aust NZ J Obstet Gynaecol* 1991;31:179-85.
- 3 Leary J, Jaworski R, Houghton R. *In situ* hybridization using biotinylated DNA probes to human papillomavirus in adenocarcinoma-in-situ and endocervical glandular dysplasia of the uterine cervix. *Pathology* 1991;23:85-9.

#### Drs Young and Brown comment:

Drs Wright, Samaratunga, and Jaworski draw attention to their recent results from Australia, showing human papillomavirus (HPV) DNA in 25-70% of cervical adenocarcinoma *in situ* (AIS) by *in situ* hybridisation.<sup>1,2</sup> Their results contrast with our failure to detect HPV DNA<sup>3</sup> in 21 invasive endocervical adenocarcinomas by *in situ* DNA hybridisation. In our discussion we drew attention to studies from the USA<sup>4,5</sup> and Australia<sup>6</sup> which detected HPV DNA or RNA in 70-88.6% of AIS and 42.5% of invasive endocervical adenocarcinomas. Wright *et al* suggest that further publications from United Kingdom centres are needed, and another study<sup>7</sup> has been published from a separate centre in the United Kingdom where a low incidence of HPV DNA was detected in both invasive endocervical adenocarcinoma (6.25%) and AIS (12.5%) by *in situ* hybridisation.

In one of the recent Australian studies it was suggested that the polymerase chain reaction (PCR) could be used to detect minute amounts of HPV DNA associated with endocervical glandular lesions,<sup>1</sup> and although this is possible,<sup>7</sup> it may not be as valuable as it seems. The PCR may give misleading results as the localisation of the signal given by *in situ* hybridisation would be lost. This is especially worrying in view of the coexistence of endocervical glandular neoplasia and cervical intraepithelial neoplasia as well as the high detection rate of HPV DNA in normal cervical tissue.

From the results available it seems that fewer cases of AIS or invasive cervical adenocarcinoma contain detectable HPV DNA in the United Kingdom than in Australia or the USA. Though this variation may be due to different sensitivity of the detection techniques, the United Kingdom

results originate in two independent centres. Leary *et al* suggest that as HPV DNA is not always present in glandular neoplasia<sup>1</sup> that HPV might be a cofactor rather than an initiator of endocervical glandular neoplasia. If this is so then HPV DNA need not necessarily be detected, possibly explaining the discrepant results from the United Kingdom and elsewhere.

To summarise, results from the United Kingdom<sup>2,7</sup> suggest that infection with HPV types 6, 11, 16, 18 and 31 does not necessarily have a major role in cervical glandular neoplasia.

- 1 Leary J, Jaworski R, Houghton R. In situ hybridization using biotinylated DNA probes to human papillomavirus in adenocarcinoma in situ and endocervical glandular dysplasia of the uterine cervix. *Pathology* 1991;23:85-9.
- 2 Nicklin JL, Wright RG, Bell JR, Samarutunga H, Cox NC, Ward BG. A clinicopathological study of adenocarcinoma in situ of the cervix. The influence of cervical HPV infection and other factors, and the role of conservative surgery. *Aust NZ J Obstet Gynaecol* 1991; 31:179-83.
- 3 Young FI, Ward LM, Brown LJR. Absence of human papillomavirus in cervical adenocarcinoma determined by in situ hybridisation. *J Clin Pathol* 1991;44:340-1.
- 4 Tase T, Okagaki T, Clark BA, *et al*. Human papillomavirus types and localization in adenocarcinoma and adenosquamous carcinoma of the uterine cervix: A study by in situ DNA hybridization. *Cancer Res* 1988;48: 993-8.
- 5 Tase T, Obagaki T, Clark BA, Twigg LB, Ostrow RS, Faras AJ. Human papillomavirus DNA in adenocarcinoma in situ microinvasive adenocarcinoma of the uterine cervix and co-existing cervical squamous intra epithelial neoplasia. *Int J Gynecol Pathol* 1989;8:8-17.
- 6 Farnsworth A, Laverty C, Stoler MH. Human papillomavirus messenger RNA expression in adenocarcinoma in situ of the uterine cervix. *Int J Gynecol Pathol* 1989;8:321-30.
- 7 Griffin NR, Dockey D, Lewis FA, Wells M. Demonstration of low frequency of human papillomavirus DNA in cervical adenocarcinoma and adenocarcinoma in situ by the polymerase chain reaction and in situ hybridization. *Int J Gynecol Pathol* 1991; 10:36-43.

### Radiation colitis is another mimic of chronic inflammatory bowel disease

We read with great interest the article written by Shepherd.<sup>1</sup> This informative review will be of great use to practising histopathologists when they face an avalanche of colorectal biopsy specimens with relatively little clinical information. The article should persuade both pathologists and physicians that clinical information is of great importance in reaching a histological diagnosis. The colorectal mucosa has limited ways of expressing itself in response to injury—a single brick from the Berlin Wall may look identical to one from the longstanding Wall of China.

The article does not define the histological features of chronic inflammatory bowel disease, but this diagnosis must be based on a combination of several morphological features such as crypt distortion, metaplasia (Paneth cells or pseudopyloric), fibrosis of the lamina propria associated with loss of crypts and/or significant increase in chronic inflammatory cells. On this basis we believe that radiation colitis is an important addition to the diagnostic possibilities. Radiotherapy is a common form of treatment for several pelvic carcinomas and the clinical features of radia-

tion enteropathy may appear after many years when the inheriting surgeon may be unaware that the patient has been irradiated. Radiation colitis in the chronic phase demonstrates a very significant crypt distortion, vascular telangiectasia, and fibrosis of the lamina propria, which can easily be misinterpreted as healed or quiescent chronic inflammatory bowel disease, unless the relevant information is available.<sup>2</sup>

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- 1 Shepherd NA. Pathological mimics of chronic inflammatory bowel disease. *J Clin Pathol* 1991;44:726-33.
- 2 Haboubi NY, Hasleton PS. *The causation and clinical management of pelvic radiation disease*. Berlin: Springer-Verlag, 19-35.

### Oestrogen receptors in conjunctival malignant melanoma

Paridaens *et al* claim to have demonstrated oestrogen receptors in paraffin wax sections of formalin fixed conjunctival malignant melanomas.<sup>1</sup> It is not unreasonable to expect that these lesions may be susceptible to endocrine factors, but the authors' results do not support their conclusions.

I have two reservations. First, the cytoplasmic staining they observed conflicts with the known nuclear location of oestrogen receptors.<sup>2</sup> Second, although the antibody to ER-D5 recognises an epitope on an oestrogen receptor related protein, several studies have shown that immunostaining with this reagent correlates poorly with the results of ligand binding assays for oestrogen receptors.<sup>3-5</sup> Furthermore, the authors are mistaken to believe that ER-D5 is "... present only in oestrogen receptor positive tissues."<sup>6</sup>

Finally, the statement that "... a nuclear binding assay, which identifies non-functional receptors, may be more appropriate" makes no sense. Surely it is more appropriate to identify functional receptors by, for example, seeking oestrogen regulated proteins, such as progesterone receptor and cathepsin D.

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- 1 Paridaens DA, Alexander RA, Hungerford JL, McCartney ACE. Oestrogen receptors in conjunctival malignant melanoma: immunocytochemical study using formalin fixed paraffin wax sections. *J Clin Pathol* 1991;44:840-3.
- 2 King WJ, Greene GL. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* 1984;307:745.
- 3 Horne GM, Angus B, Wright C, *et al*. Relationships between oestrogen receptor, epidermal growth factor receptor, ER-D5, and P24 oestrogen regulated protein in human breast cancer. *J Pathol* 1988;155:143-50.
- 4 Giri DD, Dangerfield VJM, Lonsdale R, Rogers K, Underwood JCE. Immunohistology of oestrogen receptor and D5 antigen in breast cancer: correlation with oestrogen receptor content of adjacent cryostat sections assayed by radioligand binding and enzyme immunoassay. *J Clin Pathol* 1987;40:734-40.
- 5 van der Walt LA, Phillips JI, Afrika DJ. Oestrogen receptor assay by ER-D5 immunocytochemistry fails to correlate with ligand-binding assay in breast cancer. *South Afr Med J* 1988;74:581-3.
- 6 Jemec GB, Wojnarowska F. The distribution of p29 protein in normal human skin. *Br J Dermatol* 1987;117:217-24.

### Dr Paridaens *et al* comment:

We thank Professor Underwood for his comments on our paper. We disagree, however, with his statement that "the cytoplasmic staining they observed conflicts with the known nuclear location of oestrogen receptors". An alternative immunochemical approach in the detection of the receptor moiety of steroid-hormone receptor complexes or unliganded receptors is the use of antibodies directed against receptor proteins. We used a monoclonal antibody which has been shown to be specific to D5 antigen, a non-hormone-binding component related to cytosolic oestrogen receptors, and which does not recognise classic type 1 nuclear oestrogen receptor.<sup>1</sup> The cytoplasmic staining we observed therefore reflected recognition of the ER-D5 antigen which has been shown to be closely related to oestrogen receptors.<sup>2</sup>

Secondly, a study by Coffey *et al* showed a significant correlation ( $p < 0.001$ ) between D5 immunoradiometric assay (IRMA) value and oestrogen receptor sites in breast tumours assayed by [<sup>3</sup>H]oestradiol binding sites.<sup>2</sup> However, the correlation between ER-D5 immunohistochemistry and ligand-binding assays for oestrogen receptors has been in dispute<sup>3-5</sup> and as a result the predictive value of the immunocytochemical method using anti-ER-D5 should be interpreted with caution.

Thirdly, the distributors of the antibody (Amersham) indicate that the antigen ER-D5 is present only in oestrogen receptor positive tissues, a finding which was confirmed by King *et al*.<sup>6</sup>

Finally, the aim of our concluding statement was to highlight the importance of identifying the hormone receptors that are biologically active (functional as opposed to non-functional receptors) to predict response to hormonal treatment, because this cannot be assessed by immunocytochemistry alone.

- 1 Coffey AI, Lewis KM, Brockas AJ, King RJB. Monoclonal antibodies against a component related to soluble estrogen receptor. *Cancer Res* 1985;45:3686-93.
- 2 Coffey AI, Spiller GH, Lewis KM, King RJB. Immunoradiometric studies with monoclonal antibody against a component related to human estrogen receptor. *Cancer Res* 1985; 45:3694-8.
- 3 Horne GM, Angus B, Wright C, *et al*. Relationships between oestrogen receptor, epidermal growth factor receptor, ER-D5, and P24 oestrogen regulated protein in human breast cancer. *J Pathol* 1988;155:143-50.
- 4 Giri DD, Dangerfield VJM, Lonsdale R, *et al*. Immunohistology of oestrogen receptor and D5 antigen in breast cancer: correlation with oestrogen receptor content of adjacent cryostat sections assayed by radioligand binding and enzyme immunoassay. *J Clin Pathol* 1987; 40:734-40.
- 5 van der Walt LA, Phillips JI, Afrika DJ. Oestrogen receptor assay by ER-D5 immunocytochemistry fails to correlate with ligand-binding assay in breast cancer. *South Afr Med J* 1988;74:581-3.
- 6 King RJB, Coffey AI, Gilbert J, *et al*. Histological studies with a monoclonal antibody raised against a partially purified soluble estradiol receptor preparation from human myometrium. *Cancer Res* 1985;45:5728-33.

### Secretarial services to consultant microbiologists

A questionnaire on the use of secretarial services sent to 21 consultant microbiologists in Yorkshire in July 1991 produced a