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.¹ 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.¹⁻³ 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 10⁵ 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.¹ 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.^{2,3} 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 infection 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.^{1,2} Their results contrast with our failure to detect HPV DNA³ in 21 invasive endocervical adenocarcinomas by *in situ* DNA hybridisation. In our discussion we drew attention to studies from the USA^{4,5} and Australia⁶ 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 study⁷ 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,¹ and although this is possible,⁷ 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