Letter to the Editor

EBNA-1 Sequences in Endemic and Sporadic Burkitt’s Lymphoma

In their recent manuscript, Habeshaw et al. (4) used mutations in EBNA-1 to identify Epstein-Barr virus strains in immortalized cell lines and Burkitt lymphomas (BL) from different geographic regions. Like us (1, 2), Habeshaw et al. found a near absence of P-ala in BL from any part of the world but frequently identified V-leu and P-thr. However, whereas we concluded that some subtypes present in normal B cells are not found in BL, implying a differential pathogenicity in the context of BL, Habeshaw et al. concluded that within any geographic area, the EBNA-1 subtypes present in BL simply reflect those prevalent in the population. If the data on BL subtypes are similar in these studies, why are the conclusions different?

One problem is that Habeshaw et al. studied only three tumors from Europe (including the only P-ala-positive tumor in their series), with most of their data coming from East African tumors. The lack of European tumor data makes it impossible to demonstrate a geographical bias in the distribution of EBNA-1 subtypes in tumors, and the concordance of EBNA-1 subtypes between tumors and subtypes present in nonmalignant B cells cannot be proved, except in the context of East African BL. They will need to study additional tumors from Europe and show that P-ala is prevalent in such tumors to confirm their conclusion. If they were able to demonstrate this, it would contrast with our data, for we have not found P-ala in American BL.

A second problem is that Habeshaw et al. do not think it important that they used lymphoblastoid cell lines as opposed to peripheral lymphocytes (PBLs) as controls. It seems probable, from the sum total of the data, that lymphoblastoid lines do not permit identification of the complete virus repertoire in an individual. We and others have observed multiple EBNA-1 subtypes (and multiple subtypes based on other genetic loci) in single individuals as well as in T-cell nasal lymphomas and have proposed that some of these subtypes evolve postinfection (3, 5, 7).

We have also observed differences in the relative proportions of the major subtypes associated with BL (P-thr and V-leu) in BL from different regions, as is hinted at by Habe shaw’s data from New Guinea, but neither we nor Habeshaw et al. have observed other subtypes that can be detected in peripheral blood or saliva, namely V-val and V-pro, in BL from any of the combined regions we have examined. In contrast, we and others frequently find V-val in nasopharyngeal carcinomas (2, 3, 6). Habeshaw et al. did not find either V-pro or V-val in the lymphoblastoid lines they examined. This does not surprise us—we have found V-val only in saliva in nonimmunosuppressed individuals, and neither we nor anyone else, to our knowledge, has found V-pro in lymphoblastoid lines, suggesting that it is in some way transformation defective.

In patients from Europe and America, we have found V-leu (along with multiple other subtypes) in PBLs from individuals with infectious mononucleosis or with immunosuppression, but we have yet to find V-leu in PBLs from normal individuals. This could be a quantitative phenomenon, but it nonetheless suggests that it is not simply geography (as suggested by Habeshaw et al.) that dictates the distribution of EBNA-1 subtypes in tumors: host factors and the biology of the tumor cells themselves may well play a role and, indeed, may be more important than geography.

REFERENCES

5. MacKenzie, J. Personal communication.

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Authors’ Reply

We were cajoled into the study of EBNA-1 sequence polymorphism by the assertions Bhatia and colleagues made in their original paper (2). They studied two geographically heterogeneous groups of Epstein-Barr virus (EBV)-positive Burkitt lymphomas (BLs) comprising 24 cases from North and West Africa (of which only the latter is a BL-endemic area) and 12 from North and South America. These were compared with two control groups comprising 6 healthy carriers from West Africa and 24 carriers from the Americas; a further 21 North American controls were added in a subsequent study (3). On this basis, Bhatia and colleagues proposed (i) that a particular EBNA-1 sequence variant, designated V-leu on the basis of the identity of amino acid 487, was preferentially found in association with BL and not in healthy carriers and (ii) that another variant, P-ala, was rarely found in BL yet was prevalent in healthy controls.

Given the increasing evidence for geographically linked polymorphisms among EBV isolates (1, 7), we determined to compare BL and control samples within more circumscribed geographic areas (6). Our findings on the above two points are as follows (for consistency we use Bhatia et al.’s nomenclature, though that does oversimplify the actual polymorphisms observed).

(i) V-leu was the most common EBNA-1 sequence variant in endemic BL in East Africa (29 of 55 tumors). However, it was
also found in 18 of 32 control donors from the same area, hence refuting the notion of preferential association with the tumor. By contrast, only 1 of 32 European (Caucasian) control donor isolates carried a V-leu sequence and V-leu was not seen in any of the 3 EBV-positive European BLs available to us for analysis.

(ii) The P-ala variant was not found in any of the 55 East African BLs examined, but neither was it represented among the 32 East African control isolates. However, this EBNA-1 sequence was common among European controls, being seen in 14 of 32 cases, and was also present in 1 of the 3 European BLs. As Bhatia et al. point out, more European tumors need to be examined before any firm conclusions can be drawn; however, two more cases have recently been analyzed in another laboratory and both also have the P-ala sequence (8).

The evidence to date therefore suggests that, within any one area, the EBNA-1 sequence variants found in virus-associated BL reflect those seen within the community at large. Bhatia et al. also rightly point out that our analysis of virus strains in control donors was based on EBV isolation in vitro rather than on direct amplification of viral sequences in blood and/or throat washings. Given the potential importance of this issue, we recently used direct amplification to identify resident viral strains in healthy donors from whom in vitro isolates were also available. In the large majority of cases, the two assays identified the same resident EBNA-1 sequence. From the findings so far, therefore, we do not believe that a reliance on in vitro isolates has grossly distorted our analysis of viral variants present in control populations.

Bhatia et al. also extend their concept of disease-associated EBNA-1 variants to a second EBV-positive tumor, nasopharyngeal carcinoma, in which they and others detect a high frequency of the V-val sequence (5, 9). Noting that most of the tumors studied in that work were of Chinese origin, we recently analyzed virus isolates rescued from the blood of healthy Chinese donors and likewise found the V-val sequence in 7 of 11 cases; we infer that V-val is yet another example of a polymorphic marker common among Southeast Asian virus strains (4, 7). A recent independent study of EBV’s association with gastric carcinoma, this time in Japan, leads to an exactly similar conclusion (3).

A direct effector role for EBNA-1 in EBV-associated oncogenesis remains an interesting possibility. However, as our paper tried to emphasize, if one is looking for circumstantial evidence of such a role by analyzing EBNA-1 sequences in tumors, an equally rigorous analysis of viral strains in the appropriate control populations is essential.

REFERENCES


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