

Burkitt lymphoma and the discovery of Epstein–Barr virus

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Summary

The chance germinal encounter with the first lecture outside Africa on Burkitt lymphoma is described together with the hypothesis of a viral cause. Repeated virological investigations on lymphoma biopsies proved negative, leading to the idea that a latent virus might be activated if lymphoma cells could be cultured, although no human lymphoid cell had at that time ever been maintained *in vitro*. A chance event reminding of the need for suspension culture with mouse lymphomas led to success. The cultured cells carried a morphologically unequivocal, strangely inert, herpesvirus shown later to be immunologically, biologically and biochemically unique. How this new agent acquired its name, Epstein–Barr virus, is explained.

Keywords: Burkitt lymphoma, Epstein–Barr virus, early electron microscopy, human lymphoid cell culture, oncogenic viruses.

Origin of the search for Epstein–Barr virus (EBV)

In 1961 I chanced to hear, at the Middlesex Hospital in London, an unknown surgeon from Uganda, D.P. Burkitt, give his first description outside Africa of a bizarre new children's cancer, which subsequently came to bear his name, Burkitt lymphoma (BL). I had been working at the Middlesex Hospital Medical School on the then seriously unfashionable cancer-causing viruses of chickens (Epstein, 1956, 1958), so when Burkitt mentioned the peculiar temperature and rainfall determined geography of the tumour I immediately considered the possible involvement of a human cancer-causing virus spread by a climate-dependent vector and decided, even as Burkitt was talking, to stop my current work and seek for such an agent. Although it later turned out that it was a co-factor which was arthropod-borne (Burkitt, 1969), my idea focussed correctly on the need to search for a viral cause.

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This hypothesis was quite unorthodox, but the then British Empire Cancer Campaign (now Cancer Research UK) generously provided funds for me to visit Uganda and arrange for lymphoma biopsies from Burkitt's patients to be flown to my laboratory in London.

Early investigations

For 2 years, standard virus isolation techniques were applied to lymphoma samples with depressing negative results. Direct electron microscopy, not generally available then, also proved fruitless, which was especially disappointing because not only had I had early access to it but in 1956 I had gone to the Rockefeller Institute (now University) in New York specifically to learn from George Palade (Nobel Prize 1974) (Fig 1) at the time of his outstanding contributions to the earliest phases of biological electron microscopy and to the very foundation of modern cell biology.

At this low point it occurred to me that if the tumour cells could be grown *in vitro* away from host defences a latent cancer virus might be activated, as I knew happened with certain



Fig 1. George E Palade, 1912–2008. Nobel Prize 1974 (By permission of Press Association, London).

chicken tumours (Bonar *et al*, 1960). But doing this with a human lymphoid tumour seemed dauntingly unlikely because no type of human lymphocytic cell had then ever been maintained in culture (Woodliff, 1964). Nevertheless, I tried repeatedly using many different techniques, which, predictably, all failed.

Successful culture of Burkitt lymphoma cells

On Friday 5 December 1963 a flight from Uganda was diverted to Manchester by fog and we were only able to retrieve the biopsy in the afternoon when the plane finally reached London. Unusually, the usual transit fluid was cloudy, suggesting bacterial contamination and that the sample was

useless but, although late and time to leave for the week-end, I examined the cloudy fluid under the microscope and was astonished to find the cloudiness was not due to bacteria but to huge numbers of viable, free-floating lymphoma cells shaken from the sample on the unusually long flight. I was immediately reminded that the mouse lymphoma programme at Yale University Medical School, which I had visited, had only been able to grow murine lymphomas by starting with suspensions of single cells (Fischer, 1957, 1958). Because of this, the free-floating cells from this delayed sample were set up in suspension and the first cell line duly grew out designated EB to distinguish it from HeLa, OMK, BHK and other cells in the laboratory. Suspension culture rapidly gave us more lines from the lymphoma biopsies and the technique was thereafter shown to be successful in several other laboratories.

This was the first time that any cells from the human lymphocytic series had ever been grown *in vitro* and when the account of the successful method was sent for publication, expert reviewers for a leading journal were unwilling to believe that such cells could be cultured at all. Yet suspension is the standard technique used world-wide for lymphocyte culture today, for a huge number of different purposes.

Discovery of EBV

All efforts to show a virus in the cultured lymphoma cells using the current tests failed, so samples were then prepared for electron microscopy. However, this technique was not then accepted as a way of demonstrating viruses – dogma required they should be shown by positive biological effects or the finding of virus-determined antibodies. It was not credited that they could be recognized morphologically; indeed, at that time when electron microscopes were rare, many considered the images they gave of biological material were merely artefacts of preparation. But after my time with George Palade I was convinced that viruses could be identified and classified by their appearance, just as had been done for bacteria with the light microscope for over 100 years.

I examined the first preparation on 24 February 1964 and unequivocal virus particles were present in a cultured lym-

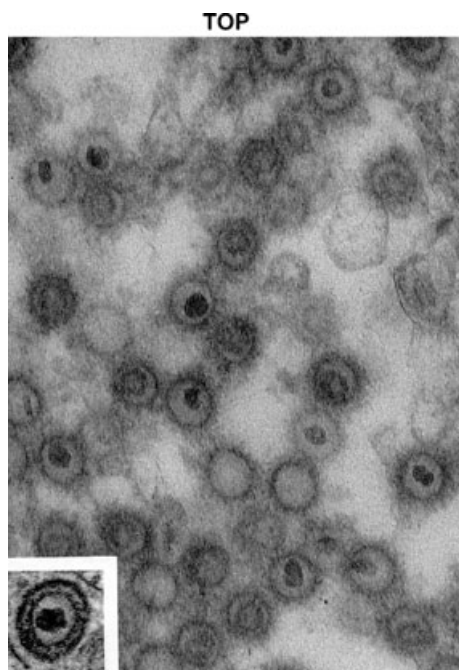


Fig 2. Electronmicrograph of thin sectioned EBV particles. Immature virions (above) cut in various planes in an infected cell. Inset (below) a mature enveloped particle. These images led to the virus being immediately recognized as a member of the herpesvirus family.



Fig 3. M A Epstein, B G Achong and Y S Barr in 1964.

phoma cell in the very first grid square (Fig 2). It was evident that this was a typical member of the herpes group but there was, of course, no means of knowing which herpesvirus it might be. But it did seem extraordinary that a herpesvirus was producing virions in a cell line without destroying the culture, as known herpesviruses would do. Accordingly, the discovery was rapidly sent for publication with my research assistants, Bert Achong, who helped with the electron microscopy, and Yvonne Barr, who helped with the cell culture (Fig 3), and the resulting paper (Epstein *et al*, 1964) became a citation classic in 1979.

The unusual inertness of the virus was confirmed when specific tests for herpesviruses were applied to the cells and again proved negative, at which point I became concerned that we might be doing something unnoticed in our procedures which was inactivating the virus. It thus became urgent to have the tests repeated in another laboratory.

Confirmation of the uniqueness of EBV

EB cells were therefore flown to the laboratory of Werner and Gertrude Henle in Philadelphia, where the lack of detectable biological activity was confirmed (Epstein *et al*, 1965). Shortly afterwards, the immunological uniqueness of the virus was demonstrated by my laboratory and the Henles, using quite different methods (Henle & Henle, 1966; Epstein & Achong, 1967), and its genetic singularity was established a year or two later (Zur Hausen *et al*, 1970).

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Subsequent knowledge of the very limited range of cells with receptors for Epstein–Barr Virus (EBV) makes the failure to show biological activity understandable but it was very puzzling at the time. It was indeed fortunate that work on the lymphoma cells and the search for virus was going forward at a centre where a rare electron microscope was in daily use, otherwise the extreme inertness could have left EBV undiscovered. It also appears that EBV was the first virus to have been discovered solely by electron microscopy.

How EBV got its name

As described above, not long after its discovery, the virus was sent to Philadelphia for study and confirmation of what had been found out about it. Subsequently, when the Henles came to publish further on it they began to refer to it as EBV (Henle *et al*, 1968) after the EB cells in which we had sent it to them and that name was generally adopted.

Acknowledgement

Sir Anthony Epstein is the sole author.

Conflict of Interest

The author has no conflict of interest.