

This Week's Citation Classic™

Henle G & Henle W. Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriology* 91:1248-56, 1966.

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Immunofluorescence was used to detect the herpesvirus in cultured Burkitt's lymphoma (BL) and other lymphoblast cultures and to identify it as a previously unknown virus, now called Epstein-Barr virus. All sera from African BL patients elicited brilliant immunofluorescence, whereas sera from other donors gave weaker or no reactions. [The SC® indicates that this paper has been cited in over 1,100 publications since 1966.]

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"When C. Everett Koop, then surgeon in chief of the Children's Hospital of Philadelphia, now US Surgeon General, returned in 1963 from a conference in Africa, he urged us to work on Burkitt's lymphoma (BL) because of its presumed viral origin and relevance to our ten-year (*sic!*) research grant awarded by the National Cancer Institute under its Viral Oncology Program and entitled 'Interference phenomena in the detection of human cancer viruses.' Approaches to several physicians in Africa, among them D.P. Burkitt, revealed that all were already committed to other laboratories. A year later, M.A. Epstein and co-workers at Middlesex Hospital, London, reported growth of cell lines from BL biopsies and electron microscopic detection of herpesvirus particles in some of the cultured cells.^{1,2} As no known herpesvirus had been shown to be oncogenic, most virologists considered the indigenous virus a passenger of no concern. When Epstein turned to us for help, our chance to work on BL had come. After implantation on monolayers of human embryonic kidney

cells, cultured BL cells induced resistance to various viruses due to production of an interferon in line with the title of our grant.³ The virus was not transmissible to routine host systems suggesting that it was heretofore unknown,⁴ but immunology was needed for proof. Only immunofluorescence was feasible. As reported in this *Citation Classic*™, the virus-producing cells in BL (as well as other) lymphoblast cultures were readily identified by sera from BL patients but also by commercial human gamma globulin and many sera collected anywhere in the world. Some reactive sera had no antibodies to herpes simplex, varicella zoster, or cytomegaloviruses proving this ubiquitous virus to be indeed new. It was henceforth called Epstein-Barr virus (EBV) after the EB-1 culture in which it was first observed.

"The indirect immunofluorescence test continues to be widely used today for titration of antibodies to EBV, more specifically its viral capsid antigen (VCA). By demonstration of elevated antibody titers as compared to controls, this test was instrumental in linking EBV not only with BL, but also nasopharyngeal carcinoma (NPC) and infectious mononucleosis (IM). Initial clues were obtained when NPC sera included among controls reacted as well as BL sera in double diffusion precipitation assays with BL cell extracts⁵ and when an antibody-negative technician in our laboratory seroconverted in the course of IM.⁶ These clues were substantiated subsequently by demonstration of EBV DNA in BL and NPC biopsies and of the EBV-associated nuclear antigen (EBNA) in the tumor cells and in lymphocytes of IM patients (cf. reference 7). With identification of the EBV-coded diffuse and restricted early antigens in addition to VCA and EBNA, and differentiation of the Ig class of the various antibodies, patients with IM, BL, and NPC were shown to develop characteristic antibody spectra which differ from each other and from the pattern of healthy seropositive donors.⁷ EBV thus turned out to be the first human virus which is closely associated with human cancers."

1. Epstein M A, Achong B G & Barr Y M. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1:702-3, 1964.
2. Epstein M A. Citation Classic. Commentary on *Lancet* 1:702-3, 1964. *Current Contents/Life Sciences* 22(14): 10, 2 April 1979.
3. Henle G & Henle W. Evidence for a persistent viral infection in a cell line derived from Burkitt's lymphoma. *J Bacteriology* 89:252-8, 1965. (Cited 65 times.)
4. Epstein M A, Henle G, Achong B G & Barr Y M. Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. *J. Exp. Med.* 121:761-70, 1965. (Cited 205 times.)
5. Old L J, Boyse E A, Oettgen H F, de Harven E, Geering G, Williamson B & Clifford P. Precipitating antibody in human serum to an antigen present in cultured Burkitt's lymphoma cells. *Proc. Nat. Acad. Sci. US* 56:1699-704, 1966. (Cited 270 times.)
6. Henle G, Henle W & Diehl V. Relation of Burkitt tumor associated herpes-type virus to infectious mononucleosis. *Proc. Nat. Acad. Sci. US* 59:94-101, 1968. (Cited 730 times.)
7. Henle W, Henle G & Lennette E T. The Epstein-Barr virus. *Sci. Amer.* 241:48-59, 1979.