Antibodies to EBV related antigens in West Malaysian children

by Dora S. K. Tan and *Gertrude Henle*

Virus Research Officer,
Institute for Medical Research,
Kuala Lumpur.

Professor of Virology,
Department of Pediatrics,
School of Medicine,
University of Pennsylvania and
Division of Virology,
The Children's Hospital of Philadelphia,
U.S.A.

Introduction

THE EPSTEIN-BARR VIRUS (EBV), a member of the herpes group of viruses, was originally discovered in continuous cultures of lymphoblasts derived from Burkitt lymphoma biopsies (Epstein and Barr, 1964). Transmission of EBV from such carrier cultures to other types of cultured human cells has not as yet been achieved. The virus was detected subsequently also in lymphoblast cultures initiated with peripheral leukocytes of healthy donors or patients with various diseases in many parts of the world.

Antibodies to EB viral capsid antigens (VCA) have been found in the serum of all African patients with Burkitt's lymphoma (Henle and Henle, 1966; Henle, et al., 1969) but also in many healthy control children and adults from all parts of the world. This ubiquitous virus turned out to be the cause of infectious mononucleosis (Henle, et al., 1968).

Antibodies to VCA are acquired, especially under low socioeconomic conditions, often early in life when primary EBV infections remain either silent or cause, as a rule, mild illnesses. In well-to-do segments of the population, seroconversion is frequently delayed until adolescence or later when primary infections are prone to result in infectious mononucleosis (IM). In West Malaysia, IM has been observed in Caucasians who were nonresidents, but not among Asians (except in 2 Eurasian children of well-to-do background) in spite of an intensive search over many years (Tan, 1967, unpublished). The present investigation was prompted by the possibility that West Malaysians, like E. Africans (Diehl, et al., 1969) acquire EBV infections in subclinical or mild form early in life so that few, if any, are still susceptible to EBV in adolescence, when primary EBV infections take a more severe turn and may cause IM.

Methods and Materials:

Sera were collected from Malaysian children ranging in age from 1 to 10 years and titrated in the indirect immunofluorescence test for antibodies to VCA (Henle and Henle, 1966) as well as for antibodies to the 2 components, D and R, of the EBV-induced early antigens (EA) complex (Henle, et al., 1971). For anti-VCA titration, acetone-fixed cell smears are prepared from virus-producing Burkitt tumor cell cultures by techniques described (Henle, et al., 1969).

TABLE I

Antibodies to EBV Related Antigens in West Malaysian Children aged 1-10 years

Age Group (Yrs.)	No. Tested	Anti-VCA titer			Anti-D	Anti-R
		Negative	Positive		Positive	Positive
		+1:10	1:10-1:80	+1:160	(1:10)	(1:10)
1-2	18	3 (16.7) *	12 (66.7)	3 (16.7)	0	1 (5.5)
3-4	19	1 (5.3)	17 (89.4)	1 (5.3)	0	2 (10.6)
5-6	18	1 (5.6)	17 (94.4)	0	0	0
7-8	20	2 (10.0)	17 (85.0)	1 (5.0)	0	0
9-10	20	0	20 (100)	0	0	0
Total	95	7 (7.3)	83 (87.4)	5 (5.3)	0	3 (3.2)

* % of age group

For the anti-D and anti-R titrations, smears are prepared from the normally non-virus producing Raji cell line which was experimentally exposed to EBV from a carrier culture 2 to 3 days previously. Under these conditions, an abortive infection of the cells is obtained; that is EA is synthesised but not VCA nor virus particles. These smears are either fixed in acetone, which preserves both the D and R components, or in methanol, which denatures R but not D. The 3 types of cell smears are first overlaid with test serum in various dilutions.

After incubation in a moist chamber for 45 minutes at 37°C and washing, the preparations are overlaid with fluorescein-conjugated antibodies to human immune globulin (anti-IgG) for 45 minutes at 37°C. Following rinsing, drying and mounting, the smears are then examined microscopically under ultraviolet illumination for detection of immunofluorescent cells; that is, cells containing antigen to which antibodies have attached and in turn the fluorescein-conjugated antibodies to human IgG. The last serum dilution yielding detectable, though weak fluorescence of the appropriate number of infected cells (from 5 to 15%) is taken as the titer.

Results

It is seen from the table that even in the 1-2 year age range, 83% of the children already had antibodies to VCA and in the older age groups, from 90-100% were positive. None of the children had antibodies to the D component of the early antigen complex and 3% to the R component. In IM, about 70% of the patients show a transitory anti-D response. The failure to detect anti-D in the series denotes that none of the children studied had undergone recent primary EBV infections. Anti-R is noted in healthy individuals only when they show relatively high anti-VCA titers, which was the case also in the series. It is suspected that relatively high anti-VCA levels accompanied by low titers of anti-R reflect the extent of the EBV carrier state which becomes established with a high degree of regularity in the lymphoreticular system after primary EBV infections.

Summary

The data presented explains why IM is not observed in West Malaysians. The vast majority of Malaysian children acquire antibodies to EBV in the first years of life. Thus, few, if any are

still susceptible when they reach adolescence when IM is the likely result of delayed primary EBV infections.

Acknowledgements

This investigation was supported by research grant CA 04568 and contract PH-43-66-477 within the Special Virus Cancer Program; National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

References

Epstein, M.A., Achong, B.G. and Barr, Y.M. (1964) Virus particles in cultured lymphoblasts from Burkitt's lymphoma.
Lancet, 1: 252-253.

Henle, G. and Henle, W. (1966). Immunofluorescence in cells derived from Burkitt's lymphoma.
J. Bacteriol. 91: 1248-1256.

Henle, G., Henle, W., Clifford, P. et al. (1969). Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups.
J. Natl. Cancer Inst. 43: 1147-1157.

Henle, G., Henle, W. and Diehl, V. (1968). Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis.
Proc. Natl. Acad. Sci. (U.S.A.) 59: 94-101.

Diehl, V., Taylor, J.R., Parlin, J.A. et al. (1969). Infectious mononucleosis in East Africa.
East African Med. J. 46: 407-413.

Henle, G., Henle, W. and Klein, G. (1971). Demonstration of two distinct components in the early antigen complex of Epstein-Barr virus-infected cells.
Int. J. Cancer, 8: 272-278.

Henle, W., Henle, G., Zajac, B.A., et al. (1970). Differential reactivity of human serums with early antigens induced by Epstein-Barr virus.
Science, 169: 188-190.

Tan, Dora S.K. (1967). "Absence" of infectious mononucleosis among Asians in Malaya.
Med. J. Malaya, 21: 358-361.