demonstrated that T cells may secrete soluble suppressor factors (9), and T cells have been shown to exert a suppressor regulatory function in MLC comparable to T cell control of antibody synthesis (10). Thus, it is possible that a thymus-derived suppressor cell, activated *in vivo* during gestation, may be responsible for reduced stimulation in MLC. Alternatively, cells derived from the equivalent of the avian bursa may inhibit responder proliferation in the same way they suppress *in vivo* graft rejection (11).

REFERENCES

1. Olding, L. C., K. Bernischke, and M. B. A. Oldstone. 1974. Inhibition of mitosis of lymphocytes from human adults by lymphocytes from human newborns. Clin. Immunol. Immunopathol. 3:79.
2. Olding, L. C., and M. B. A. Oldstone. 1976. Thymus-derived peripheral lymphocytes from human newborns inhibit division of their mothers' lymphocytes. J. Immunol. 116:682.
3. Nabholz, M., J. Vives, H. M. Young, T. Meo, V. Miggiano, A. Rijmbeek, and D. C. Shreffler. 1974. Cell-mediated cell lysis *in vitro*: Genetic control of killer cell production and target specificities in the mouse. Eur. J. Immunol. 4:378.
4. Bradley, J. V. 1968. *In* Distribution-Free Statistical Tests. Prentice-Hall, Englewood Cliffs, N. J. Pp. 96–103.
5. Schendel, D. J., B. J. Alter, and F. H. Bach. 1973. The involvement of LD- and SD- region differences in MLC and CML: A three-cell experiment. Transplant. Proc. 5:1651.
6. Cudkowicz, G. 1965. The immunogenetic basis of hybrid resistance to parental bone marrow grafts. *In* Isoantigens and Cell Interactions. Edited by Jay Palm. Wistar Institute Press, Philadelphia. Pp. 37–56.
7. Murgita, R. A., and T. B. Tomasi, Jr. 1975. Suppression of the immune response by α-fetoprotein. II. The effects of mouse α-fetoprotein on mixed lymphocyte reactivity and mitogen-induced lymphocyte transformation. J. Exp. Med. 141:440.
8. Rich, S. S., and R. R. Rich. 1975. Regulatory mechanisms in cell-mediated immune responses. II. A genetically restricted suppressor of mixed lymphocyte reactions released by alloantigen-activated spleen cells. J. Exp. Med. 142:1391.
9. Tadakuma, T., A. L. Kuhner, R. R. Rich, J. R. David, and C. W. Pierce. 1976. Biological expression of lymphocyte activation. V. Characterization of a soluble immune suppressor (SIRS) produced by concanavalin A-activated spleen cells. J. Immunol. 117:323.
10. Gershon, R. K. 1974. T-cell control of antibody production. *In* Contemporary Topics in Immunobiology, Vol. III. Edited by M. Cooper, and N. Warner. Plenum Press, New York. P. 1.
11. Droege, W. 1975. Immunosuppressive effect of syngeneic thymus cells on allograft rejection. Proc. Natl. Acad. Sci. 72:2371.

THE JOURNAL OF IMMUNOLOGY
Vol. 119, No. 5, November 1977
Printed in U.S.A.

Unusual Prevalence of Epstein-Barr Virus Early Antigen (EBV-EA) Antibodies in Ataxia Telangiectasia[1]

JEAN JONCAS, NORMAND LAPOINTE, FRANCINE GERVAIS, AND MICHELE LEYRITZ

From the Department of Virology, Institut Armand-Frappier, Pediatric Research Center, Ste-Justine Hospital and the Department of Microbiology and Immunology and the Department of Pediatrics, University of Montreal and University of Quebec, Montreal, P. Q., Canada

EBV-EA antibodies are consistently present in the serum of patients with nasopharyngeal carcinoma (1, 2) (NPC)[2] and Burkitt's lymphoma (3) (BL) at least in the late stages of the disease. In contrast, only 50 to 70% of patients with infectious mononucleosis (4–6) and less than 10% of normal children and adults have detectable serum EA antibodies (4–6). These antibodies in almost all cases of infectious mononucleosis fall to undetectable levels within a year or two after the disease and in patients with Burkitt's tumor disappear during remission but will rise again if a relapse occurs (4–7). It is of interest, therefore, that a high prevalence of Epstein-Barr virus early antigen (EBV-EA) antibodies was found in patients with ataxia telangiectasia (AT) in whom the incidence of lymphoma is increased (8).

Received for publication April 5, 1977.
Accepted for publication July 21, 1977.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by Medical Research Council Grant MA5518 of Canada and the Department of Education of the Province of Quebec (FCAC).

[2] Abbreviations used in this paper: NPC, nasopharyngeal carcinoma; BL, Burkitt's lymphoma; EBV-EA, Epstein-Barr virus early antigen; AT, ataxia telangiectasia; VCA, viral capsid antigen; BUDR, bromodeoxyuridine; EBNA, EBV nuclear antigen; ACIF, anti-C immunofluorescence.

MATERIALS AND METHODS

Patients. All 16 patients had well documented AT with progressive cerebellar ataxia, oculo cutaneous telangiectases, and pedigrees consistent with autosomal recessive inheritance. Most had immunodeficiency. None of the patients were related to one another except four pairs of siblings in four families. One serum of each of these patients and of 42 unaffected members in the families of these patients was available for study. The sera of 16 controls matched for age and sex were randomly selected from a series of sera left over from previous seroepidemiologic studies (5, 6).

EBV serology. The EBV viral capsid antigen (VCA) antibody titers of the patient's sera were measured by indirect

immunofluorescence in 2-fold dilutions from 1/5 to 1/320 with the use of the HRIK clone of P3J lymphoblastoid cells as antigen-positive cells and Hyland fluorescein-conjugated goat antiserum to human IgG (heavy chain). Raji lymphoblastoid cells were used as antigen-negative control cells. Known positive and negative sera were used as control antisera (9, 6).

The EBV-EA antibody titers were measured by a similar indirect immunofluorescence test (10) on acetone-fixed Raji cells 6 days after bromodeoxyuridine (BUDR) induction of the EBV-EA antigens (6, 10, 11). Control tests included a VCA-positive, EA-negative serum on BUDR-activated Raji cells and a VCA-positive, EA-positive serum on normal antigen-negative Raji cells and on BUDR-activated Raji cells. The patients' sera were all tested on normal EA antigen-negative Raji cells.

The EBV nuclear antigen (EBNA) antibodies were measured by the anti-C immunofluorescence (ACIF) test (6, 12) by using EBNA-positive but EA and VCA-negative Raji cells as antigen-positive cells and MOLT 4 cells as EBNA and EBV genome-negative control cells. The first serum dilution was 1/2, the second 1/5, and the following were 2-fold dilutions up to 1/320.

Human serum C from a known EBV-negative patient was used in this three-stage ACIF test and fluorescein-conjugated anti-human B_1C/B_1A goat serum diluted 1/20 was added in the final stage.

Immunoglobulin determination. Immunoglobulin levels were measured by nephelometry by the Technicon Autoanalyser 2.

RESULTS

Eight of 16 patients (Table I) with AT had EA antibodies (titers 1:5, to 1:80). Six of these eight patients had EA antibody titers of 1:40 or 1:80. This prevalence was highly significant ($p < 0.005$) when compared with that in randomly selected controls matched for age and sex. Seven patients had EBNA antibodies and nine had VCA antibodies. The remaining seven patients with this disease were EBV negative. Therefore, eight of nine EBV-infected patients with AT were found to be EA positive. The prevalence of EBV-EA antibodies in the families of these cases of AT was 19% (eight of 42 unaffected members). Four of the eight were fathers or mothers of the 16 patients. This high prevalence of EA antibodies could be in part due to recent infection since two of the 16 patients and one of 42 family members were VCA positive but EBNA negative, this pattern being characteristic of recent EBV infection. More likely, however, the lack of EBNA antibodies, particularly in the two patients with AT, could be the result of an inability to develop these antibodies because of immunodeficiency, one of the features of this disease. We and others, (W. Henle, personal communication) have noted prolonged or permanent failure to develop EBNA antibodies in certain patients with congenital or acquired immunodeficiencies.

TABLE I

Prevalence of EBV-EA antibodies in patients with ataxia telangiectasia (AT), other members of the families of these patients and in another group of matched controls

GROUP	EA positive/total	%	EBNA positive/total	%	VCA positive/total	%
AT	8/16	50	7/16	44	9/16	56
Family members	8/42	19	32/42	76	33/42	78
Matched controls	0/16	0	13/16	81	13/16	81

TABLE II

Immunoglobulin levels and EBV antibody titers in 16 patients with ataxia telangiectasia

Patient	Age in Years	VCA	EBNA	EA	IgA mg%	IgG mg%	IgM mg% Neph.[a]
1	3	1/10	<1/2	<1/2	<2	12	145
2	16	1/40	<1/2	1/40	<3	N[b]	N
3	43	1/160	1/10	1/40	4.3	N	N
4	40	1/160	1/80	1/80	4.3	N	N
5	26	1/320	1/40	1/40	47	N	N
6	10	1/320	1/40	1/40	82	420	226
7	16	1/320	1/10	1/80	540	N	N
8	3	1/20	1/40	1/5	233	N	111
9	9	1/10	1/10	1/5	205	N	N
10	9	Negative	Negative	Negative	4	N	N
11	26	Negative	Negative	Negative	7	N	N
12	10	Negative	Negative	Negative	45	N	N
13	12	Negative	Negative	Negative	190	N	200
14	12	Negative	Negative	Negative	220	N	N
15	11	Negative	Negative	Negative	162.5	N	181
16	9	Negative	Negative	Negative	90	N	185

Normal Ig levels mg %

Age	IgG	IgM	IgA
3- 5 ans	569-1597	22-100	55-152
6- 8 ans	559-1492	27-118	54-221
9-11 ans	779-1456	35-132	12-208
12-16 ans	726-1085	35- 72	70-229
Adults	569-1919	47-147	61-330

[a] Neph: nephelometry.

[b] N: normal Ig level for age.

It is of interest in this respect that the two EBNA-negative patients were tbe only two in the group who had profound hypogammaglobulinemia, IgG, and IgA with elevated IgM in one case and undetectable IgA in the other case. (Table II). Unfortunately no follow up sera were available to document definitely either a delayed EBNA antibody production characteristic of recent infection or a more permanent failure to produce these antibodies as a result of immunodeficiency.

DISCUSSION AND CONCLUSION

The interest of these observations stems from the fact that EA antibodies are rather characteristic of EBV-associated tumors, BL and NPC, as shown in many studies including our own study of a Canadian family (2) in which occurred two fatal NPC and one BL. In addition to the patients with tumors, only the mother had EBV-EA antibodies. In contrast 11 siblings in this large family were EBV-VCA and EBNA positive but EA negative. Secondly in both BL and AT, a translocation involving chromosome 14 has been reported (13, 14). The possibility should be considered that the target cell of the EBV infection in patients with BL and AT is a genetically defective lymphoid cell that could more easily undergo transformation into a malignant cell (with chromosomal aberrations) when exposed to EBV or other putative carcinogens. This could account for the increased incidence of lymphoma that has been found in patients with AT (8). Alternatively, a cell-mediated immunity being generally impaired in AT, a deficiency in the regulatory function of a T cell subpopulation of B cells could contribute to dysfunction of the target B cell of the EBV infection. "Target cell defective-

ness" or impairment of T cell regulatory function might result in a deficient control of the expression of EBV-EA and consequently a sustained EBV-EA antibody response. The possibility of a defective control of EBV-EA expression by certain cells, such as lymphoma cells, is suggested by our observations that in productive cell lines derived from BL, in contrast to cell lines derived from normal peripheral blood, hydrocortisone significantly increases EBV-EA (15, 16). Finally, the possible significance of persisting EA antibodies in the late outcome of the EBV infection in terms of tumor development deserves careful attention.

REFERENCES

1. Henle, W., H. C. Ho, G. Henle, and H. C. Kwan. 1973. Antibodies to Epstein-Barr virus-related antigens in nasopharyngeal carcinoma. Comparison of active cases with long-term survivors. J. Natl. Cancer Inst. 51:361.
2. Joncas, J., E. Rioux, J. P. Wastiaux, M. Leyritz, L. Robillard, and J. Menezes. 1976. Nasopharyngeal carcinoma and Burkitt's lymphoma immunoglobulins. Can. Med. Assoc. J. 115:858.
3. Henle, W., G. Henle, P. Gunven, G. Klein, P. Clifford, and S. Singh. 1973. Patterns of antibodies to Epstein-Barr virus-induced early antigens in Burkitt's lymphoma. Comparison of dying patients with long-term survivors. J. Natl. Cancer Inst. 50:58.
4. Henle, W., G. Henle, J. C. Nierderman, E. Klemola, and K. Haltia. 1971. Antibodies to early antigens induced by Epstein-Barr virus in infectious mononucleosis. J. Infect. Dis. 124:58.
5. Joncas, J., J. C. Gilker, and A. Chagnon. 1974. Limitations of immunofluorescence tests in the diagnosis of infectious mononucleosis. Can. Med. Assoc. J. 110:793.
6. Joncas, J. 1976. Laboratory diagnosis of infectious mononucleosis Epstein-Barr virus and the heterophil test. *In* Proceeding of an ASM symposium on Modern Methods in Medical Microbiology. University Park Press. Baltimore, Md.
7. Henle, W., and G. Henle. 1974. Epstein-Barr virus and human malignancies. Cancer 34:1368.
8. Fraumeni, J. F., Jr., and R. W. Miller. 1967. Epidemiology of human leukemia: Recent observations. J. Natl. Cancer Inst. 38:593.
9. Henle, G., and W. Henle. 1966. Immunofluorescence in cells derived from Burkitt's lymphoma. J. Bacteriol. 91:1248.
10. Henle, G., W. Henle, and G. Klein. 1971. Demonstration of two distinct components in the early antigen complex of EBV infected cells. Int. J. Cancer 8:272.
11. Gerber, P. 1972. Activation of EBV by 5-bromodeoxyuridine. Proc. Natl. Acad. Sci. 69:83.
12. Henle, W., A. Guerra, and G. Henle. 1974. False negative and prozone reactions in tests for antibodies to Epstein-Barr virus-associated nuclear antigen. Int. J. Cancer 13:751.
13. Zech, L., U. Haglund, K. Nilsson, and G. Klein. 1976. Characteristic chromosomal abnormalities in biopsies and lymphoid cell lines from patients with Burkitt and non-Burkitt lymphomas. Int. J. Cancer 17:47.
14. McCaw, B. K., F. Hecht, D. G. Harnden, and R. L. Teplitz. 1975. Somatic rearrangement of chromosome 14 in human lymphocytes. Proc. Natl. Acad. Sci., 72:2071.
15. Leyritz, M., and J. Joncas. 1976. The interaction of hydrocortisone, lymphoid cells and the Epstein-Barr virus. Abstract of paper (RT 14). Seventy-seventh Annual ASM meeting New Orleans, Louisiana, May 8 to 13, 1977.
16. Leyritz, M., and J. Joncas. 1978. The interaction of hydrocortisone, lymphoid cells and the Epstein-Barr virus. Proceedings of the VIIIth International Symposium on Comparative Leukemia Research, Amsterdam. August 22 to 26, 1977. Elsevier/North-Holland Biomedical Press. In press.