PLASMACYTIC DIFFERENTIATION OF CIRCULATING EPSTEIN-BARR VIRUS-INFECTED B LYMPHOCYTES DURING ACUTE INFECTIOUS MONONUCLEOSIS*‡

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Infectious mononucleosis (IM)[1] is a usually benign lymphoproliferative disease caused by Epstein-Barr virus (EBV) (1). Atypical lymphocytosis, the major hematologic abnormality of the disease, is due largely but not exclusively to the proliferation of immunologically activated T lymphocytes which are presumably reactive against EBV-infected lymphocytes (2, 3). Since EBV transforms resting human B lymphocytes in vitro into continuously dividing cells (4, 5), the effects of the virus on cells in vivo have major importance, especially in view of the association of EBV with Burkitt lymphoma, nasopharyngeal carcinoma (6), and immunoblastic lymphoma (7).

Studies on EBV-infected cells in vivo have been limited. The presence of EBV genome-carrying cells in the circulation during IM was shown initially by the outgrowth of EBV-transformed lymphoblastoid cell lines from cultures of peripheral blood lymphocytes (8). It has been presumed that these cell lines originated from infected B lymphocytes because EBV has tropism for B cells in vitro, and cell lines from IM blood have B cell characteristics (9). However, these lines may not arise by direct outgrowth from circulating infected cells but from normal B cells secondarily infected by virus released in the culture (10). Lymphocytes expressing EB nuclear antigen (EBNA) have been seen in T cell-depleted peripheral blood lymphocytes during IM (11, 12), but these cells have not been further characterized. Thus, to date, direct evidence has been lacking that circulating EBV-infected cells are of B cell origin.

Recently we reported the case of a 4-yr-old girl who developed fatal lymphoma during infectious mononucleosis (13). The tumor cells consisted of several populations of EBV-transformed B lymphocytes which expressed EBNA and showed plasmacytic differentiation both by morphological characteristics and by the presence of cytoplasmic immunoglobulins detected by immunofluorescence. Several unusual features in this child suggested the presence of an immunodeficiency state permitting the uncontrolled proliferation of virus-altered cells. However, the plasmacytic nature of the EBNA-positive tumor cells suggested the possibility that the virus-induced lym-

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[1] *Abbreviations used in this paper:* cIg, cytoplasmic immunoglobulin; EBNA, EB nuclear antigen; EBV, Epstein-Barr virus; GVB, gelatin veronal buffer; HBSS, Hanks' balanced salt solution; IM, infectious mononucleosis.

phoproliferation resulted from a cell-virus relationship not present in the usual case of IM. These considerations led us to question whether EBNA-positive cells in patients with typical IM might also be B lymphocytes showing plasmacytic differentiation.

In this paper we report studies on the peripheral blood lymphocytes from otherwise normal patients with uncomplicated IM using a two-color immunofluorescence technique to identify EBNA and cytoplasmic immunoglobulin heavy chains in the same cells. The results indicate that the majority of circulating EBNA-positive cells during the acute phase of IM contain cytoplasmic immunoglobulins as in the patient with fatal disease.

Materials and Methods

Lymphocytes. Heparinized blood was obtained from six adult patients with infectious mononucleosis and processed within 3 h. The diagnosis was made on the basis of fever, lymphadenopathy, atypical lymphocytosis, and the presence of heterophil antibodies. Mononuclear leukocytes were isolated on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) (14), washed twice in Hanks' balanced salt solution (HBSS), and resuspended in medium RPMI-1640 (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) supplemented with 20% fetal calf serum, penicillin (50 U/ml), streptomycin (50 μg/ml), and amphotericin B (1 μg/ml). Adherent monocytic cells were removed by incubating the cells in 150-cm^2 plastic tissue culture flasks (Corning Glass Works, Science Products Div., Corning, N. Y.) for 1 h at 37°C. The lymphocytes were then mixed with neuraminidase-treated sheep erythrocytes (E_N), centrifuged for 8 min at 1,000 rpm, and incubated at 4°C for 15 min or at room temperature for 1 h (15). The pellet of lymphocytes and erythrocytes was resuspended gently and centrifuged on a second Ficoll-Hypaque gradient to separate rosette-forming cells (T lymphocytes) from the nonrosetting cells (B cells, null cells, and residual monocytes). The lymphocytes depleted of T cells were harvested, washed three times in HBSS, and suspended at 1–1.5 × 10^6 cells/ml in complete medium. These cells were either cultured overnight at 37°C in an atmosphere of 5% CO_2 or were used immediately to prepare cell smears. The T cell-depleted population regularly contained ≤ 4% T lymphocytes.

Immunofluorescence. Suspension cultures of the non-T lymphocytes were diluted with 4 vol of a 1% sodium citrate solution containing 1 mM $MgCl_2$ and $CaCl_2$ and smears were prepared on a cytocentrifuge. After being air dried and fixed in acetone-methanol (2:1) for 5 min at room temperature, the slides were stored at −70°C until use. Cells expressing the EBNA were identified by a modification of the anticomplement immunofluorescence test described by Reedman and Klein (16). Smears were incubated for 30 min at 37°C in a moist chamber with either an EBNA-positive human serum (RM) or an EBNA-negative serum (LH), each diluted 1:8 in gelatin-veronal-buffer (GVB) (17) and inactivated at 56°C for 30 min. Sera were aspirated from the slides and the smears were immediately covered with 6–8 drops of a 1:10 dilution of the EBNA-negative serum which was used as a source of complement. After incubation with complement for 30 min at 37°C, the slides were washed three times in GVB and then incubated 30 min at 37°C with a mixture containing rhodamine-conjugated goat anti-human C3 (Atlantic Antibodies, Westbrook, Maine) and fluorescein-conjugated rabbit antisera (Dako-Accurate Chemical and Scientific Corp., Hicksville, N.Y.) specific for each human immunoglobulin heavy chain class (IgA, IgM, IgG), each at a final dilution of 1:30 in GVB. The slides were washed three more times in GVB and mounted in glycerol-phosphate-buffered saline, pH 9.5. Smears were examined under a Zeiss Universal fluorescence microscope (Carl Zeiss, Inc., New York, N.Y.) equipped with a Ploemtype vertical illuminator.

Results

Smears of T cell-depleted lymphocytes from a series of six patients with IM were examined by two-color immunofluorescence for the presence of EBNA and cytoplasmic immunoglobulins (cIg). All six cases were studied during the first week of

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symptoms and four of these were studied at least once more during their course. The following determinations were made: (*a*) the proportion of the total cell population that expressed EBNA or contained each class of immunoglobulin heavy chains; (*b*) the fraction of the EBNA-positive cells that contained each class of immunoglobulin heavy chains; (*c*) the number of cIg-containing cells that expressed EBNA compared with the number that did not. The data are summarized in Tables I and II.

During acute illness (7 d of symptoms or less) 5.5–19.5% of the non-T lymphocytes expressed EBNA and 71–80% of the EBNA-positive cells showed brilliant staining for cIg (Table I and Fig. 1). Cells positive for both cIg and EBNA were morphologically heterogeneous; some had a small, round, eccentric nucleus and abundant cytoplasm (Fig. 1a and b) and others were blastlike cells with a large nucleus (Fig. 1c and d). We could not distinguish morphologically between infected cells in the blood of normal IM patients and the infected cells seen in the patient with malignant IM. The distribution of immunoglobulin heavy chains in the EBNA-positive cells varied among the patients. In two patients (B and E), IgA was present in 50–58% of the cells with antigen, and in three patients IgA and IgG were synthesized by nearly equal proportions of EBNA-positive cells. IgM was the least common heavy chain class synthesized by EBNA-positive cells, the only exception being patient F, in whom IgM was present in 25%, IgA in 38%, and IgG in 12% of the EBNA-positive cells. The subsets of EBNA positive cells that synthesized each class of immunoglobulin did not differ from each other with respect to cell size or morphology.

The proportion of the total cell population showing cIg synthesis during acute disease varied from 4.6 to 20.8% (Table II). By comparison, cIg was present in 0.66–2.8% (mean 1.55%) of the same population of cells from six normal adult individuals (Table III). IgA- and IgG-containing cells predominated in all of the IM patients, again with the exception of patient F in whom there was 10% of the total cell

TABLE I

Distribution of Cytoplasmic Immunoglobulins in EBNA-positive Cells in T Cell-depleted Lymphocytes during Infectious Mononucleosis

Patient	Days of illness	EBNA (+) cells*	Distribution of cIg heavy chains in EBNA (+) cells‡			
			IgA	IgG	IgM	IgA + G + M
		% of total		%		
A	6	6.5	29	33	9	71
	11	1.8	32	6	6	44
	18	0.6	4	ND§	0	4
B	6	18.0	58	18.5	2	79.5
	13	1.4	8	0	1	9
C	7	10.0	26	33	18	77
	14	0.7	24	7	0	31
D	7	19.5	37	30	5	72
	12	1.2	32	5	0	37
E	5	5.5	50	23	1	74
F	6	12.6	38	12	25	75

* 300–1,000 cells counted.

‡ Expressed as percent of EBNA (+) cells; 50–100 EBNA-positive cells counted.

§ ND, not determined.

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TABLE II

Heavy Chain and EBNA Expression in Cytoplasmic Immunoglobulin-containing Cells during Infectious Mononucleosis

Patient	Days of illness	Subpopulations of cIg-containing cells*					
		IgA*	IgG	IgM	EBNA (+)‡	EBNA (−)§	Total cIg (+) cells‖
				%			
A	6	1.8 (0.75)	1.8 (0.90)	1.0 (0.53)	3.5	1.1	4.6 (0.76)
	11	1.5 (0.50)	0.2 (0)	0.2 (0.60)	1.15	0.75	1.9 (0.61)
	18	0.1 (0.13)	ND	0.6 (0)	0.02	0.68	0.7 (0.03)
B	6	5.5 (1.00)	3.4 (1.00)	2.0 (0.50)	10.0	1.0	11.0 (0.91)
	13	0.1 (0.10)	0	0.1 (1.00)	0.11	0.09	0.2 (0.55)
C	7	4.0 (0.70)	3.0 (0.87)	1.2 (0.87)	6.5	1.7	8.2 (0.79)
	14	0.6 (0.60)	0.2 (0)	0	0.4	0.4	0.8 (0.50)
D	7	9.6 (0.90)	9.0 (0.90)	2.2 (0.70)	18.3	2.5	20.8 (0.88)
	12	0.1 (1.00)	0.1 (0.90)	0	0.19	0.01	0.2 (0.95)
E	5	9.6 (0.45)	4.2 (0.33)	1.6 (1.00)	8.4	8.0	16.4 (0.51)
F	6	5.3 (0.90)	1.3 (0.64)	10.0 (0.73)	12.6	4.0	16.6 (0.76)

* Expressed as percent of the total cells; 500–1,000 cells counted per slide. Numbers in parentheses indicate the fraction of cells containing cIg which were also EBNA (+).

‡ EBNA (+), percentage of total cells that express both cIg and EBNA: (no. IgA cells × fraction IgA cells with EBNA) + (no. IgG cells × fraction IgG cells with EBNA) + (no. IgM cells × fraction IgM cells with EBNA).

§ EBNA (−), percentage of total cells with cIg but lacking EBNA: total cIg (+) cells minus EBNA (+), cIg (+) cells.

‖ Total cIg (+) cells, sum of cells with IgA, IgG, and IgM.

population synthesizing IgM. No consistent pattern in the distribution of immunoglobulin isotypes in the control subjects could be distinguished. Most of the increase in the number of cIg-positive cells above control levels could be attributed to EBV-infected cells, since 75–90% of the cells with cIg were also antigen positive. However, in three patients (D, E, and F), there were increased numbers of cells that contained cIg but lacked EBNA (Table II). These data indicate that in early IM the majority of circulating EBNA-positive cells have differentiated toward plasma cells and that in some patients increased numbers of apparently noninfected lymphocytes also synthesize cIg.

In follow-up specimens taken from four of the patients 13–18 d after the onset of symptoms, the number of EBNA-positive cells showed a marked decrease from levels observed in the 1st wk (Table I). At the same time there was also a dramatic decline in the proportion of EBNA-positive cells that synthesized cIg. This can be seen more clearly in Fig. 2, in which the data for patients A and B are shown graphically. In patient A on day 11 of symptoms the percent of EBNA-positive cells dropped to 1.8% from the day 6 level of 6.5%, and the portion of EBNA-positive cells containing cIg decreased from 71 to 44%, mainly because of a decrease in the fraction producing IgG. By day 18, EBNA-positive cells decreased further to 0.6% and the cIg containing subset made up only 4% of the cells with antigen. In patient B by day 13, the proportion of EBNA-positive cells fell to 1.4%, and only 9% of the antigen-positive cells contained cIg. The pattern in patients C and D was similar to that seen in patient A on day 11, i.e., the decrease in EBNA-positive cells staining for cIg was mainly in cells producing IgG. Although IgA remained the most frequently observed

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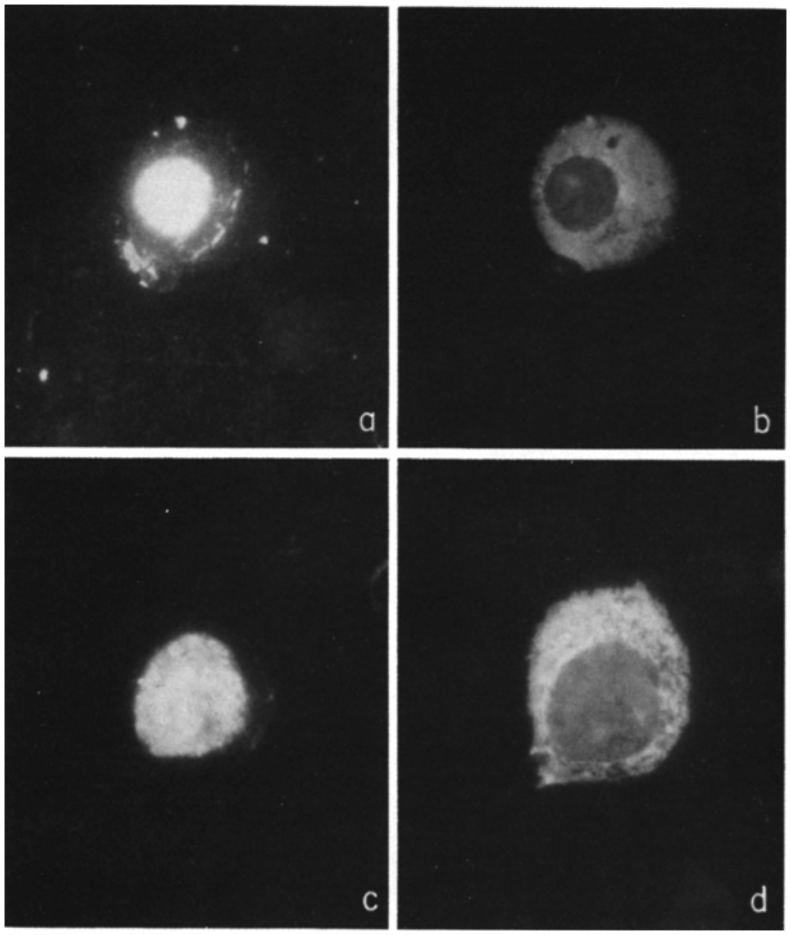

FIG. 1. (a) Small EBNA-positive cell stained with rhodamine-conjugated goat anti-human C3; (b) same cell stained for cytoplasmic IgA with fluorescein-conjugated antibody. (c) Large EBNA-positive cell stained with rhodamine-conjugated antibody; (d) same cell stained for cytoplasmic IgA with fluorescein-conjugated antibody. × 630.

isotype in antigen-positive cells in the 2nd or 3rd wk of illness, it is clear that the majority of infected cells no longer showed plasmacytic differentiation late in the disease.

Quantitative immunoglobulin levels determined on acute patients' sera were usually within normal limits; although several of the patients showed mild elevations of IgM, no elevation of IgG or IgA was seen. Thus there was no correlation between serum immunoglobulin levels and the distribution of immunoglobulin isotypes synthesized by lymphocytes in the circulation during the acute phase of IM.

Discussion

Using two-color immunofluorescence we have demonstrated in this study that during the first week of IM most (71–80%) of the circulating EBNA-positive cells synthesize cIg. Thus during acute uncomplicated IM, infected cells in the blood show the same differentiated characteristics as were observed in the EBNA-positive tumor

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TABLE III

Cytoplasmic Immunoglobulins in T Cell-depleted Peripheral Blood Lymphocytes from Normal Individuals

Subject	Populations of cIg-containing cells*			
	IgA	IgG	IgM	IgA + G + M
		%		
1	0.4	0.4	0.2	1.0
2	1.6	0	0.8	2.4
3	0.4	0.4	0.4	1.2
4	0.8	0	2.0	2.8
5	0.25	0.25	0.75	1.25
6	0	0.33	0.33	0.66
Mean				1.55

* 500 cells counted per slide.

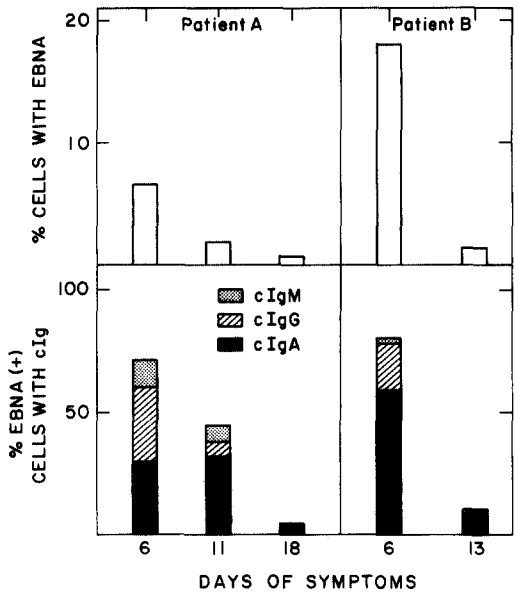

FIG. 2. Graphic presentation of data from Table II for patients A and B showing the decline of the percentage of EBNA-positive cells (top panels) and decreased fraction of EBNA-positive cells which synthesize cIg (lower panels) after the 1st wk of symptoms.

cells of the child who developed a fatal lymphoplasmacytic malignancy during IM (13). Furthermore, the distribution of immunoglobulin heavy chain classes in infected cells of patients with typical IM was similar to that seen in the fatal case; EBNA-positive cells with IgA and, to a slightly lesser extent, IgG, were more common than were infected cells with IgM. In the patient with fatal IM, proliferation of virus-altered cells was unchecked, whereas in the usual case of IM the proliferation of infected cells, which also can divide in vivo, is restricted to very low levels by host control mechanisms (18). Thus it is likely that the outcome in the patient with malignant IM resulted not from an unusual interaction of EBV with infected cells

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but from a host deficiency in controlling the same lymphoproliferative process normally initiated by EBV in infected cells during IM. The results of this investigation also demonstrate conclusively that the circulating EBV-infected cells in IM are of B cell origin.

In the present study, as in a previous report (18), EBNA was present in as much as 20% of the T cell-depleted lymphocytes of patients during the 1st wk of illness. These numbers are considerably higher than reported earlier by other investigators (11, 12); however, most of their patients were usually not studied until the 2nd or 3rd wk of illness. We found that by this time the number of EBNA-positive cells decreased to levels comparable to those in previous reports. We cannot explain the failure of Crawford et al. (19) to detect any EBNA-positive cells whatsoever during IM. The decrease of circulating EBNA-positive cells in the 1st wk of disease may be brought about by the action of cytotoxic T cells, since T cells that kill EBV-transformed cells in vitro have been shown to appear during IM (20, 21). Natural killer (NK) cells, which are also capable of killing EBV-transformed cells in vitro (22), and interferons, which augment NK activity (23), may also participate in host defense systems against EBV-infected cells in vivo. In addition, the in vivo action of cells suppressing EBV-induced transformation in vitro, which has been described by Thorley-Lawson et al. (24), is not known.

Together with the decrease in the number of EBNA-positive cells after the 1st wk of symptoms, the fraction of antigen-positive cells that contained cIg also decreased. These findings might be explained by a selective survival advantage of the 20–30% of EBNA-positive cells in acute IM blood which had not differentiated. Cells producing immunoglobulin may have a decreased capacity to divide or a greater susceptibility to cytotoxic mechanisms. It is also possible that the stage of differentiation might be altered by T cells that suppress immunoglobulin secretion, since suppressor cells have been reported to appear in peripheral blood during the 2nd wk of IM (25, 26).

The extensive plasmacytic differentiation of EBNA-positive cells seen in early IM may also result from the influence of immunologic mechanisms on EBV-infected cells, but of an opposite nature. Recent evidence suggests that EBV can stimulate polyclonal immunoglobulin synthesis in human B lymphocytes in vitro as measured by direct or indirect plaque assays (27–29). This effect appears to require biologically active virus and to occur independently of helper T cells (28, 29). However, preliminary results in our laboratory indicate that cIg synthesis detectable by immunofluorescence occurs in relatively few EBNA-positive cells 4–7 d after human T cell-depleted lymphocytes are inoculated with virus. (Robinson and Smith, unpublished observations.) Thus during acute IM the influence of the EBV genome per se may not account for the large proportion of EBNA-positive cells which exhibit plasmacytic differentiation. It is possible that the interaction of virus-altered cells with T lymphocytes in vivo may initiate release of factors that can stimulate immunoglobulin synthesis in both infected and noninfected B cells. Such helper factors are known to be produced in vitro in the early phase of allogeneic and autologous mixed lymphocyte reactions (30). Since these factors can induce immunoglobulin synthesis in leukemic and normal B lymphocytes (30), they may affect differentiation of EBV-transformed cells as well.

Alternatively, the virus might infect cells in vivo that are already differentiated. Such cells could be infected in the lymphoid or secretory tissues of the oropharynx, an area rich in plasma cells, especially those secreting IgA. This area is also the portal of

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entry for the virus and the proposed site of initial viral replication (31). However, it is not known whether B cells with plasmacytic characteristics are susceptible to EBV infection. Previous studies show that resting B cells with complement receptors are preferentially transformed in cultures of umbilical cord blood lymphocytes (5), but few plasma cells are present in this population of lymphocytes. Further study in this area is needed.

All the patients studied early in their disease had increased numbers of blood lymphocytes which synthesized immunoglobulins by comparison to control individuals. These results agree with but are not directly comparable to those from several laboratories (25, 26, 29) which demonstrated increased numbers of immunoglobulin-secreting cells in the circulation of a few acutely ill IM patients detected by direct or reverse hemolytic plaque assays on short-term cultures of blood lymphocytes. Our data show that a majority of the cIg-synthesizing cells were EBNA positive. It would be attractive to view EBV-infected cells as the likely source of the hypergammaglobulinemia and the many seemingly inappropriate antibodies, such as heterophil and autoreactive antibodies, which are characteristic of the disease; but such a conclusion is premature. The heterophil and other unusual antibodies produced during IM are mainly of the IgM class (32), and serum IgM levels in our patients and in other series (33) were elevated to a greater extent than were IgA or IgG levels. By contrast most EBNA-positive cells produced either IgA or IgG and few produced IgM. It is probable that the major source of serum immunoglobulins is either the bone marrow (34) or other lymphoid tissues which might contain larger proportions of cells producing IgM. The increased number of cIg-synthesizing cells observed in acute IM was not confined to cells containing EBNA, since in some patients the number of antigen-negative plasmacytic cells exceeded control levels. Some cells may have actually been infected but escaped detection due to leakage of antigen during slide preparation (35) or simply because the test lacks adequate sensitivity. However, T cell helper factors could stimulate immunoglobulin synthesis in normal B cells and these cells might also be a source of the unusual antibodies seen in IM.

Summary

During the acute phase (1 wk of symptoms or less) of infectious mononucleosis (IM), 70–80% of circulating Epstein-Barr virus nuclear antigen (EBNA)-positive cells have differentiated toward plasma cells. Thus the characteristics of the infected cells in the majority of IM patients during early disease are indistinguishable from EBNA-positive tumor cells of a previously reported child who developed lymphoma during IM. IgA and IgG were the most frequent and IgM the least frequent immunoglobulin isotypes detected in EBNA-positive cells. In acute disease EBNA was present in 5.5–20% of T cell-depleted blood lymphocytes but in the 2nd or 3rd wk of illness the number of EBNA-positive cells sharply decreased to 0.4–1.4%. At the same time the fraction of antigen-positive cells containing cytoplasmic immunoglobulins also diminished, suggesting either that differentiation of infected cells was altered during the disease or that nondifferentiated antigen-positive cells had a survival advantage. Both the high proportion of plasmacytic EBNA-positive cells seen during acute disease and the apparent loss of differentiation by these cells later in disease may be regulated by host immunologic factors.

Immunoglobulin-producing EBNA-positive cells may be the source of heterophile

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antibodies and other seemingly inappropriate antibodies usually found in serum during IM; however, increased numbers of noninfected plasma cells were present in some patients and may also be a potential source of these unusual antibodies.

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References

1. Henle, G., W. Henle, and V. Diehl. 1968. Relation of Burkitt tumor associated herpes-type virus to infectious mononucleosis. *Proc. Natl. Acad. Sci. U. S. A.* **59:**94.
2. Sheldon, P. J., M. Papamichael, E. Hemsted, and E. Holborow. 1973. Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet.* **I:**1153.
3. Giulano, V., H. Jasin, and M. Ziff. 1974. The nature of the atypical lymphocyte in infectious mononucleosis. *Clin. Immunol. Immunopathol.* **3:**90.
4. Pope, J., M. Horne, and W. Scott. 1968. Transformation of foetal human leukocytes *in vitro* by filtrates of a human leukemic cell line containing herpes-like virus. *Int. J. Cancer.* **3:**857.
5. Henderson E., J. Robinson, A. Frank, and G. Miller. 1977. Epstein-Barr virus: transformation of lymphocytes separated by size or exposed to bromodeoxyuridine and light. *Virology.* **82:**196.
6. Klein, G. 1975. The Epstein-Barr virus and neoplasia. *N. Engl. J. Med.* **293:**1353.
7. Purtilo, D. T., I. Szymanski, J. Bhawan, J. P. S. Yang, L. M. Hutt, W. Boto, L. DeNicola, R. Maier, and D. Thorley-Lawson. 1978. Epstein-Barr virus infections in the X-linked recessive lymphoproliferative syndrome. *Lancet.* **I:**798.
8. Diehl, V., G. Henle, W. Henle, and G. Kohn. 1968. Demonstration of a herpes group virus in cultures of peripheral leucocytes from patients with infectious mononucleosis. *J. Virol.* **2:** 663.
9. Jondal, M., and G. Klein. 1973. Surface markers on human B and T lymphocytes. II. Presence of Epstein-Barr virus receptors on B lymphocytes. *J. Exp. Med.* **138:**1365.
10. Rickinson, A. B., J. W. Jarvis, D. H. Crawford, and M. A. Epstein. 1974. Observation on the type of infection by Epstein-Barr virus in peripheral lymphoid cells of patients with infectious mononucleosis. *Int. J. Cancer.* **14:**704.
11. Klein, G., E. Svedmyr, M. Jondal, and P. O. Persson, 1976. EBV-determined nuclear antigen (EBNA) positive cells in the peripheral blood of infectious mononucleosis patients. *Int. J. Cancer.* **17:**21.
12. Katsuki, T., Y. Hinuma, T. Saito, J. Yamamoto, Y. Hirashima, H. Sudoh, M. Deguchi, and M. Motokawa. 1979. Simultaneous presence of EBNA-positive and colony-forming cells in peripheral blood of patients with infectious mononucleosis. *Int. J. Cancer.* **23:**746.
13. Robinson, J. E., N. Brown, W. Andiman, K. Halliday, U. Francke, M. Robert, M. Andersson-Anvret, D. Horstmann, and G. Miller. 1980. Diffuse polyclonal B-cell lymphoma during primary infection with Epstein-Barr virus. *N. Engl. J. Med.* **302:**1293.
14. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(suppl. 97):77.
15. Weiner, M., C. Bianco, and V. Nussenzweig. 1973. Enhanced binding of neuraminidase treated sheep erythrocytes to human T lymphocytes. *Blood.* **42:**939.
16. Reedman, B., and G. Klein. 1973. Cellular localization of an Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and nonproducer lymphoblastoid cell lines. *Int. J. Cancer.* **11:**2499.
17. Gershon A., Z. Kalter, and S. Steinberg. 1976. Detection of antibody to Varicella-Zoster virus by immune adherence hemagglutination. *Proc. Soc. Exp. Biol. Med.* **151:**752.

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18. Robinson, J., D. Smith, and J. Niederman. 1980. Mitotic EBNA-positive lymphocytes in peripheral blood during infectious mononucleosis. *Nature (Lond.).* **287:**334.
19. Crawford, D. H., A. B. Rickinson, S. Finerty, and M. A. Epstein. 1978. Epstein-Barr (EB) virus genome-containing EB nuclear antigen-negative B-lymphocyte populations in blood in acute infectious mononucleosis. *J. Gen. Virol.* **38:**449.
20. Svedmyr, E., and M. Jondal. 1975. Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients with infectious mononucleosis. *Proc. Natl. Acad. Sci. U. S. A.* **72:**1622.
21. Hutt, L. M., Y. T. Huang, H. E. Dascomb, and J. S. Pagano. 1975. Enhanced destruction of lymphoid cell lines by peripheral blood leukocytes taken from patients with acute infectious mononucleosis. *J. Immunol.* **115:**243.
22. Jondal, M., and H. F. Pross. 1975. Surface markers on human and T lymphocytes. VI. Cytotoxicity against cell lines as functional marker for lymphocyte subpopulations. *Int. J. Cancer.* **15:**596.
23. Trinchieri, G., D. Santoli, and H. Koprowski. 1978. Spontaneous cell-mediated cytotoxicity in humans: role of interferon and immunoglobulins. *J. Immunol.* **120:**1849.
24. Thorley-Lawson, D. A., L. Chess, and J. L. Strominger. 1977. Suppression of *in vitro* Epstein-Barr virus infection. A new role for adult human T lymphocytes. *J. Exp. Med.* **146:** 495.
25. Haynes, B. F., R. T. Schooley, C. R. Payling-Wright, J. E. Grouse, R. Dolin, and A. S. Fauci. 1979. Emergence of suppressor cells of immunoglobulin synthesis during acute Epstein-Barr virus-induced infectious mononucleosis. *J. Immunol.* **123:**2095.
26. Tosato, G., I. Magrath, I. Koski, N. Dooley, and M. Blaese. 1979. Activation of suppressor T-cells during Epstein-Barr virus-induced infectious mononucleosis. *N. Engl. J. Med.* **301:** 1133.
27. Rosen, A., P. Gergely, M. Jondal, G. Klein, and S. Britton. 1977. Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes *in vitro*. *Nature (Lond.).* **267:**52.
28. Kirchner, H., G. Tosato, R. M. Blaese, S. Bruder, and I. Magrath. 1979. Polyclonal immunoglobulin secretion by human B lymphocytes exposed to Epstein-Barr virus *in vitro*. *J. Immunol.* **122:**1310.
29. Bird, A. G., and S. Britton. 1979. A new approach to the study of human B-lymphocyte function using an indirect plaque assay and a direct B cell activator. *Immunol. Rev.* **45:**41.
30. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1979. Induction of human antibody responses *in vitro* with emphasis on allogeneic helper factors. *Immunol. Rev.* **45:**219.
31. Morgan, D., J. Niederman, G. Miller, H. Smith, and J. Dowaliby. 1979. Site of Epstein-Barr virus replication in the oropharynx. *Lancet.* **II:**1154.
32. Langhorne, J., and T. Feizi. 1977. Studies on the heterophil antibodies of infectious mononucleosis. I. Separation of four antibody populations, one of which contains lymphocytotoxic activity. *Clin. Exp. Immunol.* **30:**354.
33. Wollheim, F. A., and R. C. Williams, Jr. 1966. Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM) increase in infectious mononucleosis. *N. Engl. J. Med.* **274:**61.
34. Hijmans, W., and H. Schuit. 1972. Immunofluorescence studies on immunoglobulins in the lymphoid cells of human peripheral blood. *Clin. Exp. Immunol.* **11:**483.
35. Henle, W., G. Henle, and C. A. Horwitz. 1979. Infectious mononucleosis and Epstein-Barr virus-associated malignancies. *In* Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. E. H. Lennette and N. J. Schmidt, editors. American Public Health Association, Inc. Wash., D. C. 455.

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