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Changed Fc and C3 Receptor Pattern on Human EBV-negative Lymphoma Cells, Following *in vitro* Conversion by EB-virus*

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Abstract—*EBV-conversion of the originally EBV-negative Ramos and BJAB lymphoma lines has led to an increased frequency of C3 receptor positive and a decreased incidence of Fc receptor positive cells in the population. The concentration of C3 receptors per cell has increased as well. This finding supports the view that EBV-conversion leads to a modification of surface architecture which, in turn, may explain the changes in nutritional and other biological properties.*

INTRODUCTION

EPSTEIN–BARR virus (EBV) transforms normal B-lymphocytes into immortal lines that can acquire a certain oncogenic potential. It is important to explore the phenotypic effects of this transformation. In experimental oncogenic (transforming) DNA and RNA virus systems, the study of phenotypic changes at the cell level has given considerable insights into the mechanism of transformation.

One approach to explore the phenotypic consequences of EBV-transformation would be to compare normal, resting B-lymphocytes with EBV-transformed, established lines. However, EBV acts as a polyclonal B-cell activator; it induces immunoglobulin secretion and cellular DNA synthesis [1]. For this reason, comparisons between resting B-lymphocytes and derived, EBV-transformed lines, while informative, do not specifically focus on the phenotypic changes that are due to the presence of the viral genome as such. Subtle, virally induced changes can be obscured by the massive, pleiotropic activation process. A more sharply focused analysis can be performed by comparing EBV-negative human lymphoma lines of B-cell origin, with their own *in vitro* EBV-converted sublines.

We have previously described two EBV-negative but EBV-convertible lymphoma lines, BJAB and Ramos. The former is an exceptional, EBV-negative African Burkitt-like lymphoma [2], while the latter is a typical EBV-negative American Burkitt lymphoma [3]. We have established multiple *in vitro* EBV-converted sublines from both, by *in vitro* infection and selection [4–6]. Comparisons between the original BJAB and Ramos lines and their EBV-converted derivatives showed no detectable changes in chromosomal constitution, HLA expression or surface immunoglobulin markers. However, there were profound changes in nutritional and surface characteristics, i.e., increased resistance to saturation conditions, decreased serum dependence, decreased requirements for dialysable serum factors [7–9], decreased capping of various membrane constituents, increased lectine agglutinability [10–12], and increased activation of the alternate complement pathway [13]. The evidence suggests that EBV-conversion is accompanied by important modifications of surface architecture: it is intriguing that they resemble some of the changes associated with classical monolayer transformation by both RNA (Rous, MSV) and DNA (SV40, polyoma, adeno)-viruses.

In order to check the suggestive but largely indirect evidence of membrane rearrangements upon EBV-conversion from a previously unexplored angle, we have examined the expression of two typical lymphocyte markers, Fc and C3 receptors, by a semiquantitative rosetting technique with isotope labelled markers [14]. Pronounced changes were found in the expression of both receptors.

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MATERIALS AND METHODS

Cell lines

The origin and properties of the EBV-negative BJAB and Ramos lymphoma lines were described previously [2, 3]. We have used three EBV-converted, EBNA and EBV-DNA positive sublines of BJAB, BJAB/B95-8, BJAB/HR1K and E95A/BJAB and five EBV-converted sublines of Ramos, namely EHR-A/Ramos, Ramos/B95-8, II-WA-Ramos, AW-Ramos and Ramos/HR1K [5, 15]. All lines were propagated as stationary suspension cultures, on RPMI medium with 10% fetal calf serum.

Measurements of surface Fc and C3 receptors

Cultured cells were washed twice in an 1 : 1 mixture of Hank's balanced salt solution and isotonic phosphate buffer, pH 7.2 and suspended to a concentration of 4×10^6 ml. No more than 5% of the cells were lost during this procedure. There was no detectable aggregation. Fc and C3 receptors were assayed by the previously described isotope labelled marker cell technique [14]. The procedure can be summarized as follows:

Three test tubes were filled with 1 ml washed cell suspension, adjusted to the same concentration. One millilitre of EA marker cells (viz. sheep erythrocytes sensitized with 1/4 agglutinating titer of rabbit-anti-E-IgG-antibodies) was incubated with the target cells in one tube, to estimate the frequency of Fc receptor positive target cells $= L^{Fc}$.

One millilitre of EAC marker cells (viz. sheep erythrocytes sensitized with 1/4 agglutinating titer of rabbit-anti-E-IgM-antibodies plus Complement C3) were incubated with the target cells in the second tube, to estimate the frequency of C3 receptor positive target cell $= L^{C3}$.

In the third tube, the cells were incubated with 0.5 ml EA and 0.5 ml EAC marker cells, to assess the number of target cells with Fc and/or C3 receptors $= L^{Fc \cup C3}$.

Since Fc and C3 receptors are simultaneously expressed on the surface of some target cells, we have used the previously described formula [14] to compute the number of cells with only Fc receptors $= L^{|Fc|}$ the number of cells with only C3 receptors $= L^{|C3|}$ and the number of cells with both Fc and C3 receptors $= L^{Fc \cap C3}$.

Using radioactive ^{99}Tc labelled EA and ^{51}Cr labelled EAC marker cells, the mean number of marker cells per rosette was calculated as a semiquantitative measure of the number of Fc and C3 receptors on $L^{|Fc|}$, $L^{|C3|}$, and $L^{Fc \cap C3}$. Details of this calculation and various control experiments have been described by Jønsson and Christensen [14].

RESULTS

As shown in Tables 1 and 2, EBV-converted Ramos and BJAB cells showed an increase in the number of C3-receptors per EAC rosetting cell, both in the $L^{Fc \cap C3}$ and on $L^{|C3|}$ fractions.

There was also a switch, as a rule, from 'non-marginal' to 'marginal' situations, previously defined as a higher number of Fc receptor per $L^{Fc \cap C3}$ than per $L^{|Fc|}$, suggesting a change of the cells from L^{Fc} via $L^{Fc \cap C3}$ to L^{C3} [14].

The appearance of marginal situations after conversion implies that the percentage number of L^{Fc} and $L^{|Fc|}$ decreased in the converted BJAB populations while the mean number of determined EA marker cells per $L^{|Fc|}$ and per $L^{Fc \cap C3}$ decreased in the converted Ramos lines. In two converted sublines, BJAB/B95-8 and EHR-A/Ramos, the changes were so pronounced that the whole L^{Fc} fraction disappeared. In the II-WA-Ramos subline, the same tendency was so strong that we could record the highest number of C3 receptors per $L^{|C3|}$ ever seen corresponding to 46.9 EAC marker cells per $L^{|C3|}$, together with complete obscuring of the $L^{Fc \cap C3}$ fraction.

In both EBV negative cell lines the relative frequency of L^{C3} and $L^{|C3|}$ was higher than of L^{Fc} and $L^{|Fc|}$. However, BJAB contained a relatively larger frequency of Fc receptor positive cells and a higher percentage of $L^{Fc \cap C3}$ than Ramos. Ramos had a higher concentration of C3 receptors than BJAB. This may explain why the cells of the converted Ramos sublines had more C3 receptors than the converted BJAB cells.

DISCUSSION

We have shown that EBV conversion has brought about a change in the surface receptor expression of both the BJAB and the Ramos cells. The two lines differed at the point of departure since Ramos showed a higher expression of C3 receptors and BJAB expressed relatively more Fc receptors. In spite of this difference, EBV-conversion changed both cell lines in the same direction,

Table 1. Fc and C3 receptor frequency on BJAB cells and its EBV-converted sublines

Target cells	Percentage of						Number of receptors				Marginal situation
	L^{Fc}	L^{C3}	$L^{Fc \cup C3}$	$L^{Fc \cap C3}$	$L^{\|Fc\|}$	$L^{\|C3\|}$	EA per $L^{\|Fc\|}$	EA per $L^{Fc \cap C3}$	EAC per $L^{Fc \cap C3}$	EAC per $L^{\|C3\|}$	
I. EBV-negative (n=1)											
BJAB	35	63	87	11	24	52	7.9	5.6	4.2	4.3	No
II. EBV-positive (n=3)											
BJAB/B95-8	20	98	98	20	0	78	0.0	5.7	10.0	17.2	Yes
BJAB/HR1K	18	72	83	7	11	65	4.0	4.0	10.7	21.6	No
E95/BJAB	13	92	99	6	7	85	5.2	13.4	11.6	15.9	Yes
Mean	17	87	93	11	6	76	4.6	7.7	10.8	18.2	
Ratio $\frac{\text{II mean}}{\text{I mean}}$	0.5	1.4	1.1	1.0	0.3	1.5	0.6	1.4	2.6	4.2	
Suggested effect of EBV conversion	↓				↓	↑			↑	↑	No→Yes

n = number of tests.
Marginal situation, see text.
Suggested effect: $1.5 \leqq$ ratio II/I $\leqq 0.5$.

Table 2. Fc and C3 receptor frequency on Ramos cells and its converted sublines

Target cells	Percentage of						Number of receptors				Marginal situation
	L^{Fc}	L^{C3}	$L^{Fc \cup C3}$	$L^{Fc \cap C3}$	$L^{\|Fc\|}$	$L^{\|C3\|}$	EA per $L^{\|Fc\|}$	EA per $L^{Fc \cap C3}$	EAC per $L^{Fc \cap C3}$	EAC per $L^{\|C3\|}$	
I. EBV-negative (n=3)											
Ramos	8	84	89	3	5	81	16.0	7.8	12.7	22.8	No
Ramos	11	88	93	6	5	82	13.5	11.9	13.3	21.0	No
Ramos	7	81	85	3	4	78	14.7	6.0	18.2	25.8	No
Mean	9	84	89	4	5	80	14.7	8.6	14.7	23.2	
II. EBV-positive (n=5)											
EHR-A/Ramos	2	95	95	2	0	93	0.0	3.1	37.2	42.7	Yes
Ramos/B95-8	6	96	99	3	3	93	2.0	3.2	29.7	37.8	Yes
II-WA-Ramos	5	82	87	0	5	82	8.3	0.0	0.0	46.9	Yes
AW-Ramos	12	94	98	8	4	86	4.2	3.9	18.6	31.3	No
Ramos/HR1K	÷X5	76	78	3	2	73	17.3	13.8	25.1	36.8	No
Mean	6	89	91	3	3	85	8.0	4.6	22.1	39.1	
Ratio $\frac{\text{II mean}}{\text{I mean}}$	0.7	1.1	1.0	0.8	0.6	1.1	0.5	0.5	1.5	1.7	
Suggested effect of EBV conversion							↓	↓	↑	↑	No→Yes

with a relative increase of C3 receptors and a relative decrease of Fc receptors per cell. This was reflected by the change in the total proportion of receptor positive cells and also by the receptor density per cell.

These observations clearly support the view that EBV-conversion leads to membrane rearrangements. Conceivably, the change in membrane structure may be responsible for the changed nutritional requirements. In Holley's view [16], the changed serum requirements are due to a changed expression of appropriate surface receptors.

The increased expression of C3 receptors may be related to the increased ability of the EBV-converted cell lines to activate the alternate complement pathway [13].

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