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Fc and C3 Receptor Patterns on Two EBV-Negative Burkitt Lymphoma Lines During Acute Exposure to EBV (P3HR-1 Substrain)*

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Abstract—*Exposure of the two EBV-negative Burkitt lymphoma lines, BJAB and Ramos, to the abortively cytopathic* P3HR-1 *substrain of EBV led to an increased expression of* C3 *receptors within the first twelve weeks. At the twelfth week,* 100% *of cells carried a high concentration of* C3 *receptors in both lines. Compared with the receptor pattern of BJAB and Ramos after chronic virus exposure it was seen that after the twelfth week some of the* C3 *receptors vanished while a certain number of Fc receptors reappeared. These changes in the surface markers are regarded as part of the multiple changes found during exposure to* P3HR-1 *virus. Apparently, the superinfection initiates a progressive maturation of the cells and the increase of* C3 *receptors is regarded as an expression of a 'switch to the right' in the B-cell differentiation pathway.*

INTRODUCTION

THE COMPLEMENT (C3) receptor of human B-lymphocytes (and derived lymphoblastoid lines) is closely associated with the Epstein–Barr virus (EBV) receptor, as demonstrated by the parallel expression of these two receptors on different cell lines and by cocapping, cross blocking, and ligand-induced shedding experiments [1–4]. The P3HR-1 clone of Burkitt lymphoma line Jijoye is known to release an abortively cytopathic EBV variant. We have previously found [5] that the release of this virus variant has induced the simultaneous disappearance of EBV and C3 receptors from the P3HR-1 cell lines. Both receptors are present on the original Jijoye that carries the non-cytopathic transforming virus prototype [6]. Sublines of P3HR-1 which have permanently turned off virus production reexpressed both receptors, but only after long periods of continuous passage [5, 6].

In two previous studies, we have examined the EBV-receptor [5] and C3 receptor [7] expression of two EBV-negative Burkitt lymphoma lines (BJAB and Ramos), before and after their conversion into EBV-carrying lines by two different EBV-substrains: the abortively cytopathic P3HR-1 and the non-cytopathic, transforming B95-8 variant, respectively. We found that the concentration of EBV and C3 receptor-positive cells was strongly reduced in P3HR-1 virus converted lines, but not in B95-8 virus converted lines. However, C3 receptor-positive cells still present after conversion had an increased number of C3 receptors per cell as estimated by a rosette technique, using isotope labelled marker cells [8].

We have attributed these changes to the 'internal selection' that takes place in lines which continuously release cytopathic virus. In the long run, this exposure leads to selection of cell variants that have lost both receptors.

The purpose of our ongoing study is to test receptor expression after acute exposure to

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P3HR-1 virus. This first report focuses on the C3 receptor. Corresponding EBV-receptor studies will be reported later.

MAERIALS AND METHODS

Cell lines

The origin and properties of EBV-negative Burkitt lymphoma lines, BJAB and Ramos, have been described previously [9–10]. Both lines were exposed to P3HR-1 virus as earlier described [5]. A concentrated EBV-containing P3HR-1 supernatant was used as the virus source in the final dilution of 1:5. The virus preparation was capable of inducing 15–25% early antigen–positive cells in the Raji indicator line after superinfection. All lines were propagated as stationary suspension cultures in RPMI medium with 10% fetal calf serum and were withdrawn for receptor studies before and at different times after virus exposure. Prior to the tests, the cells were washed twice in isotonic phosphate buffer and Hank's balanced salt solution, mixed 1:1, and adjusted to 4×10^6 cells per ml. The loss of cells was about 5% and no aggregation was seen.

Determination of Fc and C3 receptor-positive cell fractions

The percentage of lymphoma cells that carried surface Fc and C3 receptors (L^{Fc} and L^{C3}) was measured by rosette technique after incubation with EA and EAC marker cells, respectively; coated ox or sheep erythrocytes were used as marker cells [8, 11]. The number of rosettes found after incubation with both EA and EAC marker cells ($L^{Fc \cup C3}$) was also counted.

Since Fc and C3 receptors are expressed simultaneously on some lymphoid cells [12, 13], our technique has been adopted to define cells with Fc receptors only ($L^{|Fc|}$), C3 receptors only ($L^{|C3|}$), and both Fc and C3 receptors simultaneously ($L^{Fc \cap C3}$) [8]:

$$L^{Fc} = L^{|Fc|} + L^{Fc \cap C3}$$
$$L^{C3} = L^{|C3|} + L^{Fc \cap C3}$$
$$L^{Fc \cup C3} = L^{|Fc|} + L^{|C3|} + L^{Fc \cap C3}.$$

The terms L^{Fc}, L^{C3}, $L^{Fc \cup C3}$, etc., all indicate percentage values of cell fractions, characterized by the receptors in question. The outcome of such rosette investigations is based on the common definition that a rosette is a lymphoid cell with three or more attached marker cells. Thus, the rosetting abilities of a lymphoid cell become an either–or phenomenon in spite of the fact that each rosette has a varying number of adsorbed marker cells and that each lymphoid cell has a varying number of receptors. In order to perform a more quantitative mapping of the receptors, we also tested the mean number of marker cells per rosette, using radiactive EA and EAC marker cells for Fc and C3 receptors as described below.

Measurement of Fc and C3 receptors per cell

EA and EAC marker cells were labelled with radioactive ^{99}Tc and ^{51}Cr, respectively [8]. The radioactivity per ml of marker cell suspension (C) and the number of marker cells per ml (M) were calculated. Pure fractions of $L^{|Fc|}$, $L^{|C3|}$ and $L^{Fc \cap C3}$ (N cells per ml) were prepared as described earlier [8]. The rosettes were isolated by step gradient centrifugation [14] and the radioactivity of the rosettes (P) was measured in a gamma counter. The mean number of marker cells per rosette was calculated according to the formula [8]:

$$\frac{M \times P}{C \times N}.$$

This estimate represents the mean number of EA and EAC marker cells per EA and EAC rosette as a semiquantitative expression of the mean number of Fc and C3 receptors per $L^{|Fc|}$, $L^{|C3|}$ and $L^{Fc \cap C3}$, based on the presumption that there is a proportional relationship between the number of marker cells per rosette and the number of receptors per lymphoid cell. Each bound marker cell of the rosette is assumed to indicate a certain number of receptors either bound to or covered by marker cells. As we discussed earlier [8], these measurements do not take into account such factors as potential heterogeneous distribution or capping of the receptors on individual lymphoid cells. Therefore, the measurements were performed under standard conditions and the marker cells were always adjusted to a uniform sensibility, corresponding to 1:4 agglutination titer.

Concerning the rosette technique for L^{C3} and $L^{|C3|}$, mainly C3d receptors were detected as we used complement from mouse serum or human serum treated with zymosan and C3b-inactivator [13, 15]. We prefered this technique, since C3d receptors are largely if not entirely specific for lymphoid cells while C3b receptors are found on several other types of cells as well [15].

RESULTS

As shown in Tables 1 and 2, the percentage of lymphoid cells with C3 (L^{C3}) and with C3 only ($L^{|C3|}$) increased in both BJAB and Ramos within twelve weeks of exposure to P3HR-1 virus ($P<0.01$). In Ramos there was already a significant increase after seven weeks (Table 2). Following chronic virus exposure for more than twenty weeks, there was a decrease of L^{C3} and $L^{|C3|}$ in both lines ($P<0.01$). The number of C3 receptors per

Table 1. Effect of P3HR-1 *virus treatment on* Fc *and* C3 *surface receptor expression in the* BJAB *lymphoma lines*

Parameter	BJAB without P3HR-1 virus		BJAB treated with P3HR-1 virus 12 weeks		Chronic
Percentage of cell fractions:					
L^{Fc}	35	↓	0	↑	18
L^{C3}	63	↑	100	↓	72
$L^{Fp \cup C3}$	87	↑	100	↓	83
$L^{Fp \cap C3}$	11	↓	0		7
$L^{\|Fc\|}$	24	↓	0		8
$L^{\|C3\|}$	52	↑	100	↓	65
Number of marker cells per single lymphoid cell:					
EA per $L^{\|Fc\|}$	7.9		0.0		4.0
EA per $L^{Fc \cap C3}$	5.6		0.0		4.1
EAC per $L^{Fc \cap C3}$	4.2		0.0	↑	10.7
EAC per $L^{\|C3\|}$	4.3	↑	37.0	↓	21.6

↑ = significant increase ($P<0.01$).
↓ = significant decrease ($P<0.01$).
(Two-tailed Mann–Whitney rank sum test.)

Table 2. Effect of P3HR-1 *virus treatment of* Fc *and* C3 *surface receptor expression in the Ramos lymphoma lines*

Parameter	Ramos without P3HR-1 virus		Ramos treated with P3HR-1 virus 7 weeks		12 weeks		Chronic
Percentage of cell fractions:							
L^{Fc}	9		14	↓	1		5
L^{C3}	84		89	↑	100	↓	76
$L^{Fc \cup C3}$	89	↑	100		100	↓	78
$L^{Fc \cap C3}$	4		3		1		3
$L^{\|Fc\|}$	5		11	↓	0		2
$L^{\|C3\|}$	80		86	↑	99	↓	76
Number of marker cells per single lymphoid cell:							
EA per $L^{\|Fc\|}$	14.7	↓	4.1		2.0	↑	17.3
EA per $L^{Fc \cap C3}$	8.6		4.0		4.8	↑	13.8
EAC per $L^{Fc \cap C3}$	14.7	↑	25.6	↓	3.9	↑	25.5
EAC per $L^{\|C3\|}$	23.2		27.7	↑	44.0	↓	33.4

↑ = significant increase ($P<0.01$).
↓ = significant decrease ($P<0.01$).
(Two-tailed Mann–Whitney rank sum test.)

$L^{|C3|}$ (designated EAC per $L^{|C3|}$ in the tables) rose and fell in parallel with the change of L^{C3} and $L^{|C3|}$. It is concluded that repeated exposure to P3HR-1 virus for twelve weeks leads to a predominance of cells with a high concentration of C3 receptors, corresponding to 37 EAC marker cells per $L^{|C3|}$ in BJAB (Table 1) and 44 EAC per $L^{|C3|}$ in Ramos (Table 2). Due to the large increase of C3 receptors, the percentage of $L^{Fc \cup C3}$ increased at week twelve ($P<0.01$) in spite of a significant reduction of L^{Fc} and $L^{|Fc|}$ in both cell lines.

After the twelfth week, the mean number of C3 receptors per $L^{|C3|}$ decreased in both BJAB and Ramos ($P<0.01$). Some cells lost so many receptors that they could no longer be recognized as $L^{|C3|}$ or $L^{Fc \cup C3}$ any more. The cells that still carried a sufficient concentration of C3 receptors to be classified as $L^{|C3|}$ had as a rule less receptors than at the twelfth week, but more than the original untreated cells. In parallel with the loss of C3 receptors after week twelve, the L^{Fc}, $L^{|Fc|}$ and $L^{Fc \cap C3}$ fractions reappeared in small proportions that were not always significant, while the number of Fc receptors per $L^{|Fc|}$ and $L^{Fc \cap C3}$ and the number of C3 per $L^{Fc \cap C3}$ increased.

DISCUSSION

Previously, we have shown that conversion of BJAB and Ramos lines to a permanent EBV-carrying state by the P3HR-1 virus selects for EBV/C3 receptor-negative or low expressor sublines. Conversion with the transforming B95-8 virus had no such effect. We interpreted this as the selective survival of subsets of cells that were less sensitive to the cytopathic effect of P3HR-1 virus, due to their diminished receptor concentration.

In the present study, we wished to study the short-term effects of P3HR-1 virus superinfection of Fc and C3 receptor expression. Virus exposure over a few weeks does not lead to conversion of the cells into stable EBV-DNA and EBNA positive sublines, but rather to mixed populations, where EBV negative cells are still the predominating majority. We found under these circumstances that cells of both lines responded with an increased expression of C3 receptors. This was manifested both in the total number of C3 positive cells and in the average number of C3 receptors per cell.

The effect of virus exposure on C3 receptor expression can be seen as part of the multiple membrane changes that we have previously identified in the system. They include increased complement consumption [16], increased serum dependence [17], decreased capping and increased lectin agglutinability [18–20]. Most recently, we have found [21] that EBV-superinfection of the Ramos line can 'switch' the expression of surface immunoglobulin determinants from IgM + IgD − to IgM + IgD +, and can also increase the concentration of insulin receptors considerably. We have interpreted this as a 'switch' to the right within the B-cell differentiation pathway.

While the lymphoma lines studied represent monoclinical malignancies [9, 10] they do not seem to be entirely 'frozen' in their differentiation as they can still be affected by such manipulations as EBV conversion. In view of the fact that EBV is a polyclonal B-cell activator, this would not be surprising in itself, except in so far as the findings demonstrate that the 'switch' can affect established, malignant B-lymphoma lines as well.

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