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THE EPSTEIN-BARR VIRUS RECEPTOR ON TWO NASOPHARYNGEAL CARCINOMA MODEL CELL LINES

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Summary. — It was reported that the OKB7 monoclonal antibody to C3d receptor could directly inhibit Epstein-Barr virus (EBV) attachment to and infection of B-lymphocytes. So we tested whether the OKB7 could inhibit superinfection of two epithelial NPC model cell lines (D98/HR-1 and NPC-KT) with EBV. Pretreatment of B-lymphocytes with the OKB7 significantly inhibits EBV infection. However, pretreatment with the OKB7 had no effect on superinfection of D98/HR-1 and NPC-KT cells. These data suggest that an EBV receptor, unrelated to C3d receptor, exists.

Key words: *C3d receptor*; *monoclonal antibody*; *EBV receptor*; *NPC model cell lines*

Previous studies have suggested that the Epstein-Barr virus (EBV) receptor (EBVR) was closely related with, if not identical to, C3d complement receptor (CR2), a 140 000 to 145 000 molecular weight membrane glycoprotein (Fingeroth *et al.*, 1984; Jonsson *et al.*, 1982; Frade *et al.*, 1985). Several human cell lines either coexpress the EBVR and CR2 or simultaneously show the absence of these receptors (Jondal *et al.*, 1976). It is possible that the reason for some past difficulties in showing routine EBV-infection of human epithelial cells, unlike human B-lymphocytes, may not only be related to the EBVR, but also may be dependent on the strain of EBV used (Sixbey *et al.*, 1983). Young *et al.* (1986) recently showed that epithelial cells derived from the human ectocervix and pharynx are positive for CR2 by using two monoclonal antibodies (MoAbs) against CR2, HB-5, and anti-B2. In addition, they identified cell cultures of ectocervical epithelium showing specific binding of HB-5 MoAb and specific absorption of EBV particles to similar sites of the membrane.

In our previous studies, it was possible to demonstrate that nontransforming P3HR-1 EBV (but not transforming B95-8 EBV) could superinfect epithelial/Burkitt's lymphoma hybrid (D98/HR-1), primary nasopharyngeal carcinoma (NPC) tumour cells, and normal squirrel monkey nasopharyngeal epithelial cells (Glaser *et al.*, 1976 and 1977). We also found that the D98/

Table 1. Inhibition of superinfection or infection of different cells with EBVs after pretreatment with MoAb 1

Pre-incubation of cells with	EBV isolate	EA-positive cells (%)			EBNA-positive cells (%)[2]		
		Raji	D98/ HR-1	NPC-KT	CBLs	BJAB	Ramos
Culture medium	B95-8	N.T.	N.T.	N.T.	7.8	28	15
	HR-1	23	1.5	1.1	N.T.	7.3	5.9
	NPC	18	1.1	<0.05	3.5	11	13
	Mock	<0.05	0	<0.05	0	0	0
MoAb to C3b receptor[3]	B95-8	N.T.	N.T.	N.T.	7.5	29	14
	HR-1	21	1.4	1.2	N.T.	7.0	6.0
	NPC	18	1.0	N.T.	3.8	12	12
Anti-B2 MoAb[4]	B 5-8	N.T.	N.T.	N.T.	7.5	26	15
	HR-1	23	1.4	1.1	N.T.	7.8	5.5
	NPC	17	1.1	N.T.	3.5	10	12
OKB7 MoAb[5]	B 5-8	N.T.	N.T.	N.T.	0	0	0
	HR-1	<0.05	1.3	1.0	N.T.	0	0
	NPC	<0.05	1.0	N.T.	0	0	0
	Mock	<0.05	0	<0.05	0	0	0

[1] Cells (10^6) were pretreated with MoAb for 1 hour at 4 °C
[2] Assayed 2 days postinfection
[3] Obtained from Dakopatts, Denmark
[4] MoAb to CR2 obtained from Coulter Immunology
[5] Ortho Diagnostics N.T. = Not tested

HR-1 cells lacked detectable CR2 as measured by rosetting and using an anti-B2 MoAb (Glaser *et al.*, 1977; Takimoto *et al.*, 1986). In addition, we recently established an epithelial/epithelial hybrid cell line (NPC-KT) derived from the fusion of EBV genome-positive NPC epithelial tumour cells with an epithelial cell line of human adenoid origin (Takimoto *et al.*, 1984*a*). The NPC-KT cells produce infectious EBV (NPC virus) with both transforming and early antigen (EA)-inducing properties (Takimoto *et al.*, 1984*b*). We were able to demonstrate that the D98/HR-1 cells can be superinfected with both P3HR-1 and NPC viruses and superinfected with both P3HR-1 and NPC viruses and superinfection of the NPC-KT cells could be accomplished with P3HR-1 virus only (Takimoto *et al.*, 1986), suggesting that there may be a difference in the binding of different EBV isolates to virus receptors. The NPC-KT cells, like D98/HR-1 cells, lack detectable CR2 as measured by using anti-B2 MoAb (Takimoto *et al.*, 1986). We have continued to investigate the relationship between CR2 and the EBVR.

Since there is a report that the OKB7 MoAb against CR2 can directly inhibit EBV attachment to and infection of human B-lymphocytes (Nemerow *et al.*, 1985), we tested whether the OKB7 MoAb, along with the anti-B2 and an anti-C3b receptor MoAbs, can inhibit superinfection of two epithelial

NPC model cell lines (D98/HR-1 and NPC-KT cells) with P3HR-1 and NPC virus isolates. The results are shown in Table 1. Treatment of the D98/HR-1 and NPC-KT cells with any of the three MoAbs had no effect on superinfection. Pretreatment of human cord blood lymphocytes (CBLs), EBV genome-negative B cell lines (BJAB and Ramos), and EBV genome-positive B cell line (Raji) with the OKB7 MoAb significantly inhibited EBV infection as measured by the number of EBV-associated nuclear antigen (EBNA)-positive cells or EA-positive cells after infection or superinfection with three different isolates of EBV (B95-8, P3HR-1, and NPC), whereas pretreatment of the same cells with anti-B2 MoAb or C3b receptor MoAb did not inhibit infection or superinfection. Especially, the NPC-KT cells are an epithelial hybrid cell line that was prepared by fusing primary NPC epithelial cells with an epithelial cell line derived from human adenoid tissue. These data suggest that the EBVR on the NPC-KT cells, unrelated to CR2, is comming from epithelial cells. Whether these observations have implications regarding the susceptibility of human epithelial cells to EBV remains is to be explored.

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