Failure to Immortalize Human "Null" Cells by Epstein Barr Virus (EBV) "In Vitro"

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It es now well established that Epstein Barr Virus (EBV) specifically immortalizes B lymphocytes 'in vitro' to continously growing lymphobalstoid cultures (1, 2, 3). The same virus failed to induce T lymphocytes into longterm lymphoblastoid cell cultures suggesting that T cells lack EBV specific receptors (1, 2). We would like to report on experiments in which we failed to immortalize a subpopulation of human 'Null' cells in the presence of EBV. This subpopulation is detectable in lymphocyte preparations isolated by IgG-anti-IgG columns.

Peripheral blood lymphocytes were isolated from 13 healthy persons and 14 patients with malignant melanomas by a four step purification procedure (4). All donors were EBV sero-reactive with a mean VCA titer of less than 1:20. From 100 ml of defibrinated blood, first a crude lymphocyte preparation was prepared by Ficoll-Urografin density gradient centrifugation *(fraction F)*.

In a second and third step fraction F lymphocytes were depleted of iron phagocytosing macrophages and cells adhering to plastic surface. The remaining non-phagocytic, non-adherent lymphocyte population was referred to as *fraction FFF*, while the plastic adherent cells were termed *fraction AD*. The final purification step consisted of a passage of FFF lymphocytes over IgG-anti-IgG columns (5). By this procedure all B cells and the majority of Fc-Receptor carrying lymphocytes ('K' cells) were removed, leaving in the post-column fraction FFF-C approximately 70 % T cells and a subpopulation of 'Null' cells with low affinity for IgG-anti-IgG columns. A small proportion of these 'Null' cells formed EA-rosettes, whereas EACrosettes ranged below 1.0 %. Table 1 summarizes the cell composition of the different lymphocyte fractions. Since there was no significant difference between melanoma patients and control persons the results of both groups were presented together.

'K' cell activity was measured in whole blood and lymphocyte fractions F, FFF and FFF-C by an antibody dependent cellular cytotoxicity reaction (ADCC) utilizing as target cells a human melanoma cell line (IGR3), sensitized with rabbit anti-melanoma IgG (4). The results obtained with three leukocyte target cell ratios are shown in Table 1; details are presented elsewhere (4, 6). It can be seen that the B cell free lymphocyte fraction FFF-C retained significant ADCC activity, although it was strongly reduced compared to the activities measureable in fractions F and FFF. Furthermore, when lymphocytes of fraction FFF-C were separated into E-rosettes forming lymphocytes (T cells) and 'Null' cells, ADCC activity was only found in the 'Null' cell population. These results, together with the immunological and morphological criteria, suggested that the 'Null' cell

Table I: Morphological and immunological characteristics of purified human peripheral lymphocytes, ADCC activity and rate of establishment of lymphoblastoid cell cultures in the presence of EBV

	Lymphocyte Fractions			
	F	FFF	AD	FFF-C
Lymphocytes	93,1 ± 1,8[a]) (11)	96,1 ± 1,2 (23)	38,3 ± 3,2 (17)	99,2 ± 0,4 (23)
Monocytes	2,6 ± 1,0 (11)	2,1 ± 0,9 (23)	45,4 ± 3,2 (17)	0,6 ± 0,3 (23)
Granulocytes	4,2 ± 1,0 (11)	1,7 ± 0,3 (23)	15,5 ± 2,5 (17)	0.5 (23)
E-Rosettes	54,8 ± 3,6 (20)	54,1 ± 4,1 (20)	n. t.	69,9 ± 3,3 (17)
EA-Rosettes	12,2 ± 3,0 (10)	12,3 ± 2,2 (10)	n. t.	1,5 ± 0,7 (9)
EAC-Rosettes	10,0 ± 1,6 (10)	8,3 ± 1,7 (10)	n. t.	1.0 (10)
Surface Ig Pos.	10,0 ± 0,8 (18)	4,0 ± 0,4 (27)	27,3 ± 1,5 (10)	0 (27)
ADCC[b]) 12:1	46,6 ± 1,6 (7)	53,0 ± 8,0 (14)	n. t.	11,7 ± 2,1 (12)
25:1	51,2 ± 6,5 (7)	65,0 ± 7,9 (14)	n. t.	15,0 ± 4,8 (12)
50:1	63,5 ± 5,8 (7)	72,8 ± 7,4 (14)	n. t.	26,5 ± 4,3 (12)
Cultures				
without EBV	0/11[c])	0/15	0/17	0/18
with EBV	3/11 (27 %)	2/15 (13 %)	11/17 (65 %)	0/18

a) Percentage of total cell population; mean ± standard error (n).
b) ADCC = antibody-dependent cellular cytotoxicity against 51 Cr labeled IGR 3 melanoma cells, tested at 3 leukocyte to target cell ratios.
c) Cultures established/cultures started.

compartment in our post column fraction FFF-C represents a subpopulation of 'K' cells with low affinity FC-receptors.

The question, whether this cell type would be susceptible to EBV induced blast formation, was investigated by setting up 18 cultures of fraction FFF-C, together wirth a total of 56 cultures of the other lymphocyte fractions, in the presence of EBV. The initial cell inoculum was 2 x 10^6 cells per culture vial (25 ml Falcon plastic screw cap bottles) in 5 ml RPMI-1640 medium supplemented with 20 % fetal calf serum, penicillin and streptomycin.

EBV was derived from supernatants of B-95-8 cultures (marmoset lymphoblasts) (7) and added to the cultures in a final concentration of 1:10. The results are summarized in the lower part of Table 1. Without addition of exogenous

EBV no cultures were established from any one of the four fractions. By contrast, in the presence of EBV 3 out of 11 cultures with fraction F (27 %), 2 out of 15 cultures with fraction FFF (13 %) and 11 out of 17 cultures with fraction AD (65 %) gave rise to continously growing cell lines. None of 18 trials with cells of fraction FFF-C was successful. Irrespective of the presence or absence of exogenous EBV, lymphocytes of fraction FFF-C died within 10 days. A feeder-layer of allogeneic human embryonic fibroblasts prolonged the survival of FFF-C lymphocytes up to 2 months, but even under these conditions EBV induced blastoid transformation was not observed. Although the number of 'Null' cells present in fraction FFF-C was small (10–30 %), the prolonged survival by means of a feeder layer should have provided sufficient time to allow a virus – 'Null' cell – interaction to take place. However, the preliminary experiments do not exclude the possibility that through a decreased EBV receptor affinity 'Null' cell immortalization would need a higher EBV multiplicity or increased numbers of 'Null' cells. To exclude a mere threshold sensitivity for EBV, experiments are now in progress to increase the number of 'Null' cells in fraction FFF-C before exposure to EBV (8).

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