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Endemic and Sporadic Cases of Epstein-Barr Virus-Positive Burkitt's Lymphoma: Immunological Characterization of Derived Cell Lines

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A. Introduction

Burkitt's lymphoma (BL) is a tumour which is endemic in regions of Africa and New Guinea where temperature and rainfall are high and malaria is holoendemic. BL also occurs worldwide (sporadic BL), but at a much lower incidence, and showing no association with specific climatic or geographical features. Some 98% of endemic tumours carry multiple copies of the Epstein-Barr (EB) virus genome, and it is possible that this virus has a causal relationship with BL. However, only 20% of sporadic cases are associated with the EB virus, and this would suggest that the endemic and sporadic forms of the disease may differ in pathogenesis.

In this work we have compared tumour cell lines established from endemic cases of BL with lines established from EB virus genome-positive sporadic tumours. The differences apparent between them suggest that the two diseases, even when both are EB virus-associated, may be distinct in terms of the cell of origin, and therefore in terms of pathogenesis.

New BL cell lines have been established from patients located in regions of Africa (seven cases), and New Guinea (two cases) where the tumour is endemic. Simultaneously, lymphoblastoid cell lines (LCL) were established from the normal, circulating B cells of these same patients, by in vitro infection with EB virus. BL/LCL pairs have likewise been established from sporadic cases of the disease arising in Algeria, France and La Réunion.

B. Phenotypic Analysis of Endemic and Sporadic BL Lines

In every case, BL cells were distinct from the autologous LCL cells, in being monoclonal in immunoglobulin expression, in showing specific chromosomal translocations, and in terms of cell surface phenotype and morphology [1]. More importantly there were interesting differences between individual BL lines (Table 1).

All nine BL lines of endemic origin initially grew as single cell suspensions, and, using a selected panel of monoclonal antibodies, they showed a characteristic pattern of reactivity which showed no resemblance to that shown by LCL. Endemic BL did not bind two "lymphoblastoid-specific" antibodies, AC2 and MHM6, nor two antibodies with known reactivity with Sternberg-Reed cells, Ki1 and Ki24, but they did show strong reactivity with J5, an antibody with specificity for the common acute lymphoblastic leukemia antigen (cALLA). This phenotype was called group 1. Only 2/9 sporadic BL expressed a group 1 phenotype, while the remaining 7 lines were reactive not only with J5, but also with Ki24, and showed a variable pattern of reactivity

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Table 1. Phenotypic analysis of endemic and sporadic BL cells

Cell line type	Patient	Early passage							Later passage						
		Growth pattern	Monoclonal antibody binding					Group	Growth pattern	Monoclonal antibody					Group
			MHM6	AC2	Ki1	Ki24	J5			MHM6	AC2	Ki1	Ki24	J5	
Endemic BL	WEW 2	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	0	+++	I
	WAN	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	0	+++	I
	CHEP	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	0	+++	I
	MAK	Single cells	0	0	0	0	+++	I	Small clumps	0	0	0	+	+++	II
	LIV	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	++	+++	II
	MUK	Single cells	0	0	0	0	+++	I	Small clumps	+	0	0	++	+++	II
	ELI	Single cells	0	0	0	0	+++	I	Small clumps	0	+	0	+++	++	II
	KYU	Single cells	0	0	0	0	+++	I	Small clumps	0	+++	++	+++	+++	II
	WEW 1	Single cells	0	0	0	0	+++	I	Small clumps	0	+	+++	+++	+++	II
Sporadic BL	LAT	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	0	+++	I
	TOL	Single cells	0	0	0	0	+++	I	Single cells	0	0	0	0	+++	I
	MON	Single cells	0	0	0	+++	+++	II	Small clumps	0	0	0	+++	+++	II
	OUS	Large clumps	+	+	0	++	+++	II	Large clumps	+	+	0	+++	+++	II
	Ls32	Large clumps	++	+++	++	+++	+++	II	Large clumps	+++	+++	+	+++	0	III
	Ls92	Large clumps	++	+	++	++	+++	II	Large clumps	+++	++	++	++	0	III
	PUY	Large clumps	++	++	+	+++	+++	II	Large clumps	+	+++	++	+++	0	III
	LOU	Large clumps	+	+	0	++	++	II	Large clumps	+	++	++	+++	0	III
	BOC	Large clumps	+	+++	++	+++	+	II	Large clumps	+	+++	+++	+++	0	III
Endemic and sporadic LCL	All patients	Large clumps	+++	+++	++/ +++	++/ +++	0		Large clumps	+++	+++	++/ +++	++/ +++	0	

Table 2. Endemic and sporadic BL cells differ in their immunogenicity to NK cells, and to allospecific cytotoxic T cells

Cell line type	Patient	Group	NK activation	Susceptibility to NK cells	Allo activation	Susceptibility to allo-killing
Endemic BL	WEW 1	1	BL≪LCL	BL<LCL	BL≪LCL	BL<LCL
	WEW 2	1	BL≪LCL	BL≪LCL	BL≪LCL	BL≪LCL
	WAN	1	BL≪LCL	BL=LCL	BL≪LCL	BL<LCL
	MAK	1	ND	BL<LCL	BL≪LCL	BL<LCL
	CHEP	1	ND	BL=LCL	ND	ND
	LIV	1	BL≪LCL	BL=LCL	ND	ND
	MUK	2	BL=LCL	BL=LCL	BL<LCL	BL<LCL
	ELI	2	BL</≪LCL	BL=LCL	BL<LCL	BL<LCL
Sporadic BL	LAT	1	BL<LCL	BL=LCL	BL≪LCL	BL≪LCL
	TOL	1	ND	ND	ND	ND
	MON	2	ND	ND	ND	ND
	OUS	2	BL<LCL	BL<LCL	BL>LCL	BL>LCL
	PUY	2	BL=LCL	BL>LCL	BL>LCL	BL=LCL
	Ls32	3	BL=LCL	BL>LCL	BL<LCL	BL<LCL
	Ls92	3	BL=LCL	BL=LCL	BL<LCL	BL<LCL
	Lou	3	BL=LCL	BL>LCL	BL>LCL	BL>LCL
	BOC	3	BL=LCL	BL=LCL	BL<LCL	BL=LCL

with Ki1, AC2 and MHM6. These lines were classified as group 2.

Within 20 in vitro passages, a number of lines were seen to change both in morphology and in phenotype: 6/9 endemic BL acquired a group 2 phenotype, and began to grow in small clumps, individual cells being less uniformly spherical. This growth pattern was quite distinct from that of the sporadic lines in group 2. Group 2 sporadic lines grew as large tight clumps, and although this growth pattern remained unaltered, with subsequent passage they lost their reactivity with J5, thus acquiring a group 3 phenotype, which was more similar to that of LCL.

Differences in growth pattern, and in quantitative expression of the monoclonal antibodies MHM6, AC2 and Ki1, suggest that the stable group 2 into which the endemic group 1 BL lines progress, is probably distinct from the unstable group 2 in which sporadic BL can arise. Thus, BL appears to arise in at least two separate B cell subsets; endemic BL is restricted to just one of these subsets, while sporadic BL may arise in a range of B cell differentiation states.

C. Immunological Analysis of Endemic and Sporadic BL Lines

Endemic and sporadic cell lines in groups 1, 2 and 3 are being characterized in terms of their performance in a number of immunological assays (Table 2). First, in their ability to induce "activated NK" cells, and in their susceptibility to "activated NK" cell-mediated cytolysis. Induction of "activated NK" cells was achieved after 4–6 days of coculture of γ-irradiated BL or LCL cells, with unfractionated mononuclear cells from seronegative donors, at a low responder: stimulator ratio of 4:1. Effector cells were harvested and tested for killer activity against chromium-labelled target cells (Table 2).

Cell lines in group 1, either sporadic or endemic, were poor inducers of activated NK cell activity in comparison with LCL, or with sporadic BL lines in group 2 or group 3. Only 1/4 endemic lines which had progressed into group 2 was capable of inducing NK cell activity which was comparable to that induced by LCL. There was little difference in the sensitivity of cell

lines in any one group, and LCL to NK cell-mediated cytolysis.

Second, the BL lines were tested for their ability to induce alloantigen-specific cytotoxic T cells (CTL), and for their susceptibility to lysis by these allospecific CTL. Allospecific CTL were induced by 9–10 days coculture of γ-irradiated BL or LCL cells, at a responder : stimulator ratio of 40 : 1. T cells were isolated by rosetting with sheep red blood cells, and soon after, a T cell growth factor-dependent cell line was established. These effector cells were tested in 5-h chromium release assays, against HLA-matched and mismatched target cell lines.

All seven endemic lines tested were poor inducers of allospecific CTL activity in comparison with the autologous LCL: BL < LCL, two pairs (group 2); BL $\ll$ LCL, four pairs (two in group 1, and two in group 2). One sporadic line in group 1 was tested, and this line was a very poor inducer of allospecific CTL. However, the activity of sporadic lines in groups 2 and 3 was more comparable to that of LCL: BL < LCL, 3/6 pairs; BL $\leqq$ LCL 3/6 pairs. Generally the susceptibility of each line to allospecific cytotoxicity reflected its capacity for alloactivation.

Currently, the susceptibility of these lymphoma lines to EB virus-specific T cell-mediated cytotoxicity is being tested. The outgrowth of virus-infected tumour cells in BL may be due, either to a failure of the patient's immune responses, or to a lack of sensitivity of the tumour cells to cell-mediated immune lysis. The latter hypothesis may be important in endemic cases of BL, while a depressed immune response may be a factor in most sporadic cases. Further studies of the immune responses of BL patients are required to answer these questions.

Reference

1. Rowe M, Rooney CM, Rickinson AB, Lenoir GM, Rupani H, Moss DJ, Stein H, Epstein MA (1985) Int J Cancer 35/4