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EBV-TRANSFORMATION OF SURFACE IgA-POSITIVE HUMAN LYMPHOCYTES[1]

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Surface IgA-positive human lymphocytes were selected, separated, and immortalized with Epstein-Barr virus (EBV). Selection was carried out by rosetting the cells with ox erythrocytes, coated with rabbit anti-human IgA immunoglobulin, followed by separation on Ficoll-Isopaque. Stable monoclonal lines were established by cloning in agarose. This shows that EBV-transformation is not restricted to the IgM-positive B cell category but can be extended to minority classes of B lymphocytes. The method provides the means to establish other minority categories as continuous lines, depending on the efficiency of the preselection procedure.

Epstein-Barr virus (EBV)[3] infects human B lymphocytes and transforms ("immortalizes") them into permanent lymphoblastoid cell lines (LCL) (1). Established LCL can maintain the phenotypic properties of the original B cells from which they have been derived. LCL are polyclonal, as a rule, and contain various proportions of surface and secretory immunoglobulin positive cells. Most cells carry C receptors and EBV receptors (2, 3).

We have recently shown that preselection of specific antigen-binding B cells, followed by EBV-transformation, can lead to the outgrowth of LCL that produce the corresponding antibody. Using this approach, we have established lines that secrete specific antibodies against the haptens 4-hydroxy-3,5-dinitrophenacetic acid (NNP) (4), 2,4,6-trinitropheny (TNP) (5), and against streptococcal carbohydrate A (6).

Most of the polyclonal LCL produce IgM antibodies, with preference for the κ-chain (7). In the present paper, we have explored the possibility of establishing LCL that produce, at will, a minority immunoglobulin category by preselection, transformation, and cloning. As a first approach, we have focused on surface-IgA-positive cells. For target cells, we have chosen both peripheral lymphocytes and tumor-draining lymph node cells of nasopharyngeal carcinoma (NPC) patients, since it is known that the NPC is accompanied by a marked increase of the serum IgA level (8) and a characteristic predominance of anti-EBV antibodies of the IgA type (9). It is also known that the NPC tissue is often infiltrated with large numbers of IgA-producing lymphocytes (10). The approach proved successful and can be extended to other minority categories of B lymphocytes.

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[3] Abbreviations used in this paper: EBV, Epstein Barr virus; LCL, lymphoblastoid cell line; NPC, nasopharyngeal carcinoma; OE, ox erythrocytes.

MATERIALS AND METHODS

Lymphocytes. Peripheral blood and cervical lymph node lymphocytes were received from an African NPC patient (pseudonym: Dakiki). The lymphocytes were purified on a discontinuous Ficoll-Isopaque gradient (density 1.077).

EBV infection. Cellfree supernatants of mycoplasma-free B95-8 cultures (1) were used as the source of transforming EBV. Cells were incubated with 1 ml virus containing supernatant for 120 min in 37°C, after which, medium was added (RPMI with 10% FCS).

Coupling of rabbit immunoglobulins to ox erythrocytes. Rabbit anti-human immunoglobulins were bound to ox erythrocytes (OE) by the $CrCl_3$ method (11). OE were washed three times with saline, and one volume was mixed with two volumes of γ-globulin fraction of rabbit anti-human immunoglobulin (100 μg/ml) (Dacopatts, Denmark). Two volumes of "aged" 2.5 mM $CrCl_3$ (12) were added and the mixture was incubated for 5 min at room temperature, followed by three washes with PBS.

Selection. Lymphocytes were mixed with a 10-fold excess of rabbit anti-human Ig-coupled OE, centrifuged at 300 rpm, incubated at 37°C for 7 h and at 4°C for 14 h. Resuspended cells were layered onto Ficoll-Isopaque (density 1.077) and centrifuged to separate rosettes from nonrosetting cells.

Cloning. Cloning was done in 0.35% agarose on a feeder layer of human embryonic lung fibroblasts as described (13).

Surface immunoglobulin staining. 5×10^5 cells were washed and mixed with an 1/10 diluted FITC-conjugated Ig reagent. All reagents were from Dakopatts, Denmark. They included 1) rabbit anti-human IgG, IgA, IgM, κ, and λ; 2) rabbit anti-human IgM, μ-chain specific; 3) rabbit anti-IgG, specific for the Fc fragment; 4) rabbit anti-κ (Bence Jones); 5) rabbit anti-λ (Bence Jones); and 6) rabbit anti-IgA, specific for α-chains. The following cell lines were included as positive and negative controls: surface IgM κ-positive BJAB (14), IgM λ-positive Ramos (15), and IgG λ-positive Rael (16).

RESULTS

Ox erythrocytes coated with different rabbit anti-human immunoglobulin reagents were reliable indicators of the type and class of surface immunoglobulin expressed on the target cells (Table 1). By using a panel of cell lines with known surface-Ig expression, there was an excellent agreement between this method and earlier, more conventional methods of Ig-typing.

Rosettes were only obtained with ox erythrocytes coated with an immunoglobulin class known to be present on the target cell surface. Fc receptors did not interfere, in spite of the fact that cell lines K562, Namalwa, Rael, and Molt-4 were strongly positive for Fc receptors.

As the next step, human anti-IgA-coated erythrocytes were used to select surface IgA positive B lymphocytes.

1.8×10^6 peripheral lymphocytes and 4.2×10^6 lymphocytes collected from the tumor-draining neck gland of the same NPC patient ("Dakiki") were rosetted with OE, coated with a rabbit anti-human IgA reagent, specific for α-chains and secretory piece. Rosettes were separated on Ficoll-Isopaque, infected with EBV, and cultured. Since the peripheral and the cervical lymph node-derived LCL were quite similar with regard to the different categories of surface Ig-positive cells, they were mixed and rerosetted three times (with an interval of 3 weeks between each), with OE-coated with anti-human IgA. The resulting polyclonal line contained 73% surface IgA and less than 1% surface IgM-positive cells (Table II, section B).

Three clones were isolated from the culture. Two were surface IgA-λ and one was IgM-κ-positive (Table II; section C).

TABLE I

Rosette formation between rabbit anti-human immunoglobulins coated OE and various human cell lines

Cell Line	Reference	Surface Ig[a]	OE Coated with Rabbit Anti-human Ig[b]				
			μ	Fc(γ)	α	κ	λ
1301	(17)	–	–	–	–	–	–
Molt-4	(18)	–	–	–	–	–	–
K562	(19)	–	–	–	–	–	–
Rael	(15)	IgG, λ	–	+	–	–	+
Ly28	(20)	IgG, κ	–	+	–	+	–
Namalwa	(15)	IgM, λ	+	–	–	–	+
Raji	(21)	IgM, κ	+	–	–	+	–
Ramos	(14)	IgM, λ	+	–	–	–	+
BJAB	(13)	IgM, κ	+	–	–	+	–

[a] Direct staining with FITC-conjugated rabbit anti-human Ig reagents.

[b] +, Rosette formation with 50 to 90% of the cells; –, no detectable rosette formation.

TABLE II

Surface Ig staining of the Dakiki (NPC) derived LCL after one or several selections for surface-IgA positive cells

Staining Reagent: Rabbit Anti-human	% Stained Cells					
	A		B	C		
	Polyclonal LCL (One selection followed by EBV transformation)[a]		Polyclonal LCL after three additional selections[b]	Clones derived from B		
	I	II		1	2	3
Polyvalent (IgM, IgG, IgA, κ, λ)	>90	>90	>90	>90	>90	>90
μ	85	53	1	0	81	0
Fc (γ)	11	7	0	0	0	0
α	17	35	73	61	0	89
κ	62	64	57	0	41	0
λ	41	21	43	72	0	80

[a] Cultures I and II have been derived from peripheral lymphocytes and neck gland lymphocytes, respectively, of the NPC patient "Dakiki". Surface-IgA-positive lymphocytes were selected by one rosetting, followed by Ficoll-Isopaque separation and transformed subsequently (see text).

[b] Derived from a mixture of I and II, three times re-selected for surface IgA-positive cells.

TABLE III

Surface Ig staining of the Dakiki (NPC)-derived LCL after selective enrichment of cells with surface κ-, λ-, and γ-chains, respectively

Staining Reagent: Rabbit anti-human	% Stained Cells		
	Polyclonal LCL after three IgA re-selections[a] and κ selection a	and λ selection b	Polyclonal LCL I + II selected for Fc (γ)[b] c
Polyvalent (IgM, IgG, IgA, κ, λ)	>90	>90	>90
μ	55	2	4
Fc (γ)	0	0	53
α	14	90	2
κ	95	2	86
λ	3	92	19

[a] Derived from polyclonal LCL that was re-selected three times for surface IgA-positive cells (Table II, B) and selected either for κ- or λ-light chains with OE coated with rabbit anti-human κ or λ antibodies.

[b] Derived from the mixture of LCL I and LCL II (Table II, A) and selected for Fc (γ) with OE coated with rabbit anti-human Fc (γ).

After three reselections for surface IgA cells (Table II, B) (with an interval of 3 weeks between each) the polyclonal LCL contained nearly the same percentage of κ- and λ-positive cells. Cells carrying surface Ig with the two different light chains were selectively enriched by rosetting them with OE, coated with either rabbit anti-human κ or λ antibody. The resulting cultures contained more than 90% of the cells with the corresponding light chain (Table III, a, b). The same principle was also used to select surface IgG-positive cells. The mixture of polyclonal LCL I and II (Table II, A) was rosetted with rabbit anti-human Fc (γ) coated OE. As shown in Table III c, 53% of the cells were surface IgG-positive in the derived culture.

DISCUSSION

This study shows that minority categories of surface immunoglobulin-positive B cells can be established as continuous lymphoblastoid cell lines by pre-selection, EBV transformation, and repeated re-selection for the appropriate marker. We could enrich selectively surface IgA, IgG, κ- and λ-positive cells. To obtain stable lines, re-selection and cloning was clearly necessary, due to the fact that the selected cells were initially only a small fraction of all B lymphocytes transformed by EBV. This is also clearly shown by the fact that one of the three clones that we isolated carried surface IgM, rather than IgA.

NPC patients have usually high serum IgA levels (8) and their anti-EBV antibodies regularly include the otherwise unusual IgA class (9). Our choice of NPC-infiltrating lymphocytes was based on the fact that IgA-producing cells are abundant in this tumor (10), and EBV selectively transforms Ig-secreting lymphocytes (7).

With this approach, it should be possible to establish EBV-transformed monoclonal human cell lines with virtually any surface marker provided that the marker is expressed on B cells and that corresponding antibodies are available to design the selective system. If the initial number of the desired lymphocytes is very small, it may be advantageous to immortalize the cells first without any selection, and perform all selective procedures after immortalization.

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