

THE *IN VITRO* GROWTH PROPERTIES OF CELL LINES FROM EPSTEIN-BARR VIRUS-INDUCED TAMARIN TUMORS AND TAMARIN B CELLS TRANSFORMED BY EPSTEIN BARR VIRUS

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ABSTRACT

EBV-carrying human cell lines, depending on whether the cells are derived from Burkitt's lymphoma (BL) tumor biopsies or transformed by EBV *in vitro*, have different growth properties *in vitro*. In contrast, there are no clear differences between tamarin tumor lines and tamarin LCLs *in vitro*. Both types of tamarin cell lines could grow in agarose and formed colonies unlike human LCLs, although with a lower cloning efficiency than BL lines. The growth patterns of the tamarin tumor lines resemble more those of human LCLs than human BL lines, although the observation that tamarin LCLs can grow in agarose whereas human LCLs cannot may be significant. If it is accepted for arguments sake that Raji BL cells are representative of human EBV BL tumor cells, then both tamarin LCLs and tumor lines are more tumorigenic as judged by the single criterion of growth in agarose.

MJIRI, Vol. 15, No. 2, 89-92, 2001.

INTRODUCTION

The Epstein Barr virus (EBV) is an important human pathogen which is associated with a range of diseases affecting both lymphoid and epithelial cells. The most studied animal model is the cottontop tamarin (*Saguinus oedipus oedipus*) which consistently develops lymphoproliferative disease following injection of EBV.^{3,4} The lymphomas which arise resemble the EBV-positive tumors which occur in human immunosuppressed individuals. The tumors are frequently progressive and fatal; however, in a proportion of tamarins, spontaneous regression and recovery occurs.^{1,2} This work looked at one aspect of EBV infection in the cottontop tamarin, at the growth properties *in vitro* of cell lines derived from EBV-induced tamarin tumors in comparison with EBV-transformed tamarin lymphoblastoid cell lines (LCLs).

Cell lines derived from human tumor cells usually have different growth properties *in vitro* when compared to merely immortal cell lines such as LCLs. Tumors progress *in vivo* because the tumor cell no longer regulates its growth but also because the immune system can not recognize and/or destroy it. The outcome must reflect to a greater or lesser extent the combined effects of these two factors. *In vitro* growth properties associated with *in vivo* tumorigenicity include anchorage independent growth in agar. Previous work^{5,6} has compared the growth properties of different human cell lines by comparing human lines derived from tumors with cell lines established *in vitro* by EBV transformation (LCLs).

Nilsson *et al.*⁵ showed that EBV-positive Burkitt's lymphoma (BL) cell lines grew well in agarose whereas human LCLs did not form visible colonies. The purpose of the present study was to see whether there were any differences in *in vitro* growth proper-

Growth Properties of EBV-Induced Tumors

Table I. Characteristics of cell lines.

Cell line	Origin	Passage time
Human lines		
Raji	1	Late
Daudi	1	Late
Jijoye	1	Late
MR-LCL	2	Early <4 months
DH-LCL	2	"
B-LCL	2	"
Jill-LCL	2	"
CB-LCL	3	"
Tamarin lines		
B77-T	4	"
B77-LCL	5	"
R155-MLN	4	"
R155-LCL	5	"
B107-MLN	4	"
B107-LCL	5	"
R120-LCL	5	"
R165-LCL	5	"
B174-LCL	5	"

1. EBV+Burkitt's lymphoma lines (BL)
2. Adult human lymphocytes transformed by EBV
3. Cord blood lymphocytes transformed by EBV
4. Cell lines derived from tamarin tumors
5. Tamarin lymphocytes transformed by EBV

ties between cell lines obtained from EBV-induced tamarin tumors and EBV-transformed tamarin LCLs. The growth property studied was anchorage-independent growth in agarose.

MATERIAL AND METHODS

Cell lines

The cell lines used are summarized in Table I.

Cell culture

All cell lines were cultured in RPMI 1640 medium (Gibco Laboratories), supplemented with penicillin (100 IU per mL), streptomycin (100 µg per mL), 2mM L-glutamine and 10% fetal calf serum (FCS). The cells were incubated at 37°C, in 5% CO₂ in culture flasks. The cells were fed with sufficient medium twice a week to sustain maximum viability.

Growth in agarose

The aim of these experiments was to investigate whether there was any difference between the two types

of cell lines from EBV-induced tamarin tumors and tamarin B cells transformed by EBV *in vitro* with regard to capacity for colony formation in agarose (soft agar) *in vitro*. Four separate experiments were performed. In three experiments one tamarin tumor line and one tamarin LCL were studied. In experiment III, CB-LCL (human cord blood lymphocytes transformed by EBV) was added. In experiment IV, three different tamarin LCLs were compared. Raji cell line Burkitt's lymphoma (BL) and one human LCL (MR-LCL, DH-LCL, or Jill-LCL) were used as controls.

All cultures were observed twice a week for up to 4 weeks and the growth of cells, colony sizes, and the percentage of cells forming colonies were counted. Cultures were fed with fresh agarose once a week. The colony size was measured using 1 mm² grid in the eyepiece of the inverted microscope. The grid contains 100 large squares (0.1×0.1 mm²) and the colony size is indicated by the number of large squares it covers. The percentage of cells forming colonies was measured as the mean (±SD) of 3 separate cultures.

RESULTS

Table II shows 4 separate experiments. In all experiments, the Raji BL cell line in both concentrations grew as large colonies in soft agar and its cloning efficiency was at a very high level ranging from 45% to 80%. In contrast, human LCLs did not form large visible colonies in soft agar. MR-LCL and Jill-LCL had no visible growth at all whereas DH-LCL showed a few small clusters after 3 weeks and CB-LCL cells appeared to coalesce and some small colonies were seen after 3 weeks. The cloning efficiency of human LCLs was at a very low level (<1%). These data are consistent with earlier work.⁶

As shown in Table II, three tamarin tumor lines were tested for their ability to grow in agarose. Two of the lines (B77T and B107-MLN) only formed small colonies, whereas one tumor line (R155-MLN) formed large colonies approaching the size of the large colonies obtained from Raji, the human tumor line. However, surprisingly all six tamarin LCLs also grew as large colonies, five of the six forming macroscopically visible colonies of the same order of size as Raji. The cloning efficiency of the tamarin tumor lines was similar to that of the tamarin LCLs. The range of cloning efficiencies varied from 7% to 17%, therefore being greater than that for human LCLs (<1%) but less than that for Raji (range 45% to 80%).

DISCUSSION

The experiments described here were preliminary studies with the aim of seeing whether there were any

Table II. Growth of tamarin cell lines in agarose (tumor lines and tamarin LCLs).**Experiment I**

Cell lines	Cell density per dish	Colony size in agarose*	Cells (%) forming colonies**
Raji	1×10 ⁵	53.3 ± 11.5	80% ± 10
Raji	0.5 × 10 ⁵	44.3 ± 5.1	60% ± 13.2
MR-LCL	1 × 10 ⁵	0	0
B77T	1 × 10 ⁵	2 ± 1	13% ± 3.6
B77-LCL	1 × 10 ⁵	40 ± 10	15% ± 1.7

Experiment II

Cell lines	Cell density per dish	Colony size in agarose*	Cells (%) forming colonies**
Raji	1×10 ⁵	40.3 ± 5.5	71% ± 5.1
Raji	0.5 × 10 ⁵	34.3 ± 5.1	72% ± 3.6
DH-LCL	1 × 10 ⁵	2.2 ± 0.8	<1%
R155- MLN	1 × 10 ⁵	32.3 ± 2.5	16% ± 3.4
R155-LCL	1 × 10 ⁵	33.7 ± 4.7	15% ± 4.5

Experiment III

Cell lines	Cell density per dish	Colony size in agarose*	Cells (%) forming colonies**
Raji	1×10 ⁵	48.3 ± 7.6	65% ± 5.6
Raji	0.5 × 10 ⁵	34 ± 4	60% ± 5
Jill-LCL	1 × 10 ⁵	0	0
CB-LCL	1 × 10 ⁵	3.3 ± 1.5	<1%
B107-MLN	1 × 10 ⁵	1.3 ± 0.6	8% ± 2.6
B107-LCL	1 × 10 ⁵	9 ± 1	7% ± 2

Experiment IV

Cell lines	Cell density per dish	Colony size in agarose*	Cells (%) forming colonies**
Raji	1×10 ⁵	58.3 ± 10.4	77% ± 6
Raji	0.5 × 10 ⁵	36 ±	45% ± 4
MR-LCL	1 × 10 ⁵	0	0
R120-LCL	1 × 10 ⁵	63 ± 14.7	8% ± 2.6
B165-LCL	1 × 10 ⁵	55 ± 5	11% ± 4
B174-LCL	1 × 10 ⁵	48 ± 9.8	17% ± 3

4 separate experiments were performed. In experiments I, II, and III, one tamarin tumor line and one tamarin LCL were studied. In experiment IV, three tamarin LCLs were compared. In each experiment Raji and one human LCL were used as controls. Human CB-LCL was added in experiment III.

*The colony size was measured using a 1 mm² grid in the eyepiece of the inverted microscope. The gride contains 100 large squares (0.1 × 0.1 mm²) and the colony size is indicated by the number of large squares it covers. The colony size is measured as the mean (±SD) of 3 cultures.

**The percentage of cells forming colonies is measured as the mean (±SD) of 3 cultures.

Raji= EBV + Burkitt's lymphoma line

MR-LCL, DH-LCL, Jill-LCL= Adult human LCLs

CB-LCL= Human cord blood lymphocytes transformed by EBV

B77T, R155-MLN, B107-MLN= Tamarin tumor cell lines

B77-LCL, R155-LCL, B107-LCL, R120-LCL, B165-LCL, B174-LCL= Tamarin LCLs

differences in the *in vitro* growth properties of cell lines derived from EBV-induced tamarin tumors in comparison with EBV-transformed tamarin LCLs.

Earlier work¹² had shown that EBV gene expression of both tamarin tumor cell lines and tamarin LCLs was identical (for the viral proteins studied), both types of cell line expressed the range of EBV latent proteins and the lytic cycle antigens viral capsid antigen (VCA), early antigen (EA) complex and gp 340. The present work aimed to see whether their *in vitro* growth properties were also similar or whether the cell lines derived from tamarin tumors might exhibit any features in common with human tumor cell lines namely anchorage-independent

growth in agarose.^{5,6}

The first set of experiments investigated anchorage-independent growth in agarose (Soft agar). Previous work studying human EBV-positive cell lines⁶ showed that human BL tumor lines grew well in agarose (Soft agar) forming visible colonies with a high cloning efficiency and human LCLs at a low passage did not form visible colonies; however, some LCLs did form a few small clusters at a frequency of <1% of the cells. The same findings were observed in the present work, although only Raji was used as a representative BL cell line. Four different human LCLs were studied.

Unexpectedly, both tamarin tumor lines and tamarin

Growth Properties of EBV-Induced Tumors

LCLs grew in agarose, the percentage of cells forming colonies ranging from 7 to 17%. Three different tamarin tumor lines were investigated. Only one of these lines grew as large colonies like Raji, and the other two grew only as small colonies. However, five of the six tamarin LCLs (including two LCLs derived from the same tamarin as two tumor lines studied) grew as large macroscopically visible colonies like Raji. The percentage of cells forming colonies from both tamarin tumor lines and LCLs was intermediate between that observed for human LCLs and the human tumor line Raji. Therefore, even though only a small number of cell lines were studied, it appeared that tamarin tumor lines did not grow more readily in agarose than tamarin LCLs; indeed, the trend observed was to the contrary. The reason for the differences in ability to grow in agarose for human and tamarin LCLs is not known. Tamarin LCLs are known to be more permissive for the replication of EBV but other characteristics, for example comparison of cell surface markers, have been carried out. Of course, it is not clear if these observations are related to the oncogenicity of EBV in the tamarin.

It is unclear why the tamarin is susceptible to EBV-induced lymphoma, but then it is not known whether the tamarin is more or less susceptible to EBV-induced lymphoma than humans. Would humans develop lymphoma if they received a similar dose of EBV in a similar manner? Perhaps the tamarin has a defective immune system and it can not control EBV infection or lymphomas like those that develop in severely immunocompromised AIDS patients and those undergoing immunosuppressive therapy following organ transplant surgery. In possible support of this theory it has been shown that there is limited major histocompatibility complex (MHC) class I and II polymorphism^{7,10,11} in tamarin cells. This limited MHC class I and II polymorphism may play a role in the tamarin's susceptibility to EBV infection, although there is no reason to think that tamarins are unusually susceptible to viral infections in general. Indeed it is most unlikely that such an unfavourable path of evolutionary development would have been followed. Certainly it appears that the large dose of EBV given to tamarins overwhelms their immune system and EBV-positive lymphomas result.^{8,9}

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