Helper and Suppressor T-Cells Regulating Killer Cells of EB Virus Infected Cells

NAOKO YOSHIMURA and JUNICHI YATA

Department of Pediatrics, School of Medicine, Tokyo Medical and Dental University, Tokyo 113

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— Using an in-vitro human lymphocyte system, a study was performed to determine whether helper T-cells and suppressor T-cells control the generation of killer cells against autologous B-cells transformed by Epstein-Barr (EB) virus. When T-cells were treated with mitomycin C and reacted in the presence of macrophages with target cells bearing HLA-DR, helper T-cells were induced but suppressor T-cells were not. In contrast, when T-cells were reacted in the absence of macrophages to target cells lacking HLA-DR, suppressor T-cells but not helper T-cells were induced. The culture supernatant of the T-cells which showed suppressor activity also suppressed the generation of killer cells. The thus-induced suppressor T-cells also suppressed the HLA incompatible allogeneic killer cells which were directed against autologous B-cells transformed by EB virus and also autologous killer cells directed against allogeneic target cells. These observations indicated that there is no HLA restriction between the suppressor T-cells and the killer cells and the suppression is antigen nonspecific. ———
helper T-cells; suppressor T-cells; EB virus; killer T-cells

Since Epstein-Barr virus (EBV) was first discovered in Burkitt's lymphoma (Burkitt 1958; Epstein et al. 1964), its etiological significance in human tumors such as Burkitt's lymphoma and nasopharyngeal carcinoma (De-The' et al. 1973; Reedman and Klein 1973; Yata and Tachibana 1973; Yata et al. 1974), has been intensively studied. It is now established that EBV is the causative agent of infectious mononucleosis. (Henle et al. 1968; Yata et al. 1973b; ZurHausen et al. 1974; Klein et al. 1976).

Killer T-cells are thought to play a major role in eliminating EBV-infected cells and actually the presence of killer T-cells specific for EBV-transformed cells was demonstrated in in-vitro experiments (Tanaka et al. 1980, 1982; Fukukawa et al. 1981; Moss et al. 1981; Tsoukas et al. 1981; Spits et al. 1982). It is possible that the generation of these killer T-cells is controlled by helper and suppressor T-cells, as in the case of other killer T-cells systems.

The purpose of this paper is to describe our experimental results indicating that helper T-cells and suppressor T-cells control the killer T-cells directed against autologous B-cells transformed by EBV, and that helper T-cells and

suppressor T-cells can be separately induced by changing the experimental conditions.

MATERIALS AND METHODS

Separation of lymphocytes, T-cells and non T-cells

Peripheral blood lymphocytes (PBL) were isolated from heparinized venous blood by using a Ficoll-Hypaque density gradient (Böyum 1968). PBL were mixed with sheep erythrocytes (Jondal et al. 1972; Yata et al. 1973a) at a cell ratio 1:100 in fetal calf serum (FCS). The cells were centrifuged at $300 \times g$ for 5 min and kept in ice water for 3 hr. The T-cells formed rosettes with sheep erythrocytes. The cells were gently resuspended and separated using the same density gradient. The rosette-forming cells (T-cells) were collected from the bottom of the centrifuge tube and non T-cells were obtained from the interface. The contaminating sheep erythrocytes were lysed with 0.84% NH_4Cl and removed.

Establishment of cell line from PBL infected with EB virus

Non-T-cells $1-2 \times 10^6$, were suspended in 1 ml of medium, RPMI 1640, supplemented with 10% fetal calf serum and added with 0.1 ml of the culture supernatant of B-95-8 cells which produce infective EB virus (EBV). B-cells were transformed by EBV and cell lines were established, usually after about 1 month. (Mizuno et al. 1974; Katsuki and Hinuma 1975; Yata et al. 1975).

Induction of killer T-cells and measurement of killer activity

Target cells (autologous B-cells transformed by EBV) were treated with $75 \mu\text{g/ml}$ of mitomycin C (MMC) at 37°C for 60 min. 5×10^4 MMC-treated target cells were added to 5×10^5 PBL. The cells were suspended in RPMI 1640 medium supplemented with 10% human AB serum and cultured at 37°C for 3-4 days in a 5% CO_2 incubator. The killer activity (Cerottini and Brunner 1974) of the thus-treated cells was estimated by adding the cells to ^{51}Cr -labeled target cells. The target cells were incubated with Na_2CrO_4 ($100 \mu\text{Ci/ml}$) at 37°C for 60 min and washed with phosphate-buffered saline. 2.5×10^4 ^{51}Cr -labeled cells were mixed with 5×10^5 effector cells. The cells were suspended in 1 ml of the medium, centrifuged to get tight cell contact and incubated at 37°C for 4 hr. Then the cell free supernatant was obtained and its radioactivity was counted with a gamma counter (experimental release). The radioactivity of the supernatant of the same number of target cells totally lysed by 1 N HCl (total release) and that of target cells alone (spontaneous release) were estimated at the same time. The killer activity was expressed as % lysis according to the following formula.

$$\% \text{ Lysis} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total release} - \text{Spontaneous release}}$$

Induction of helper T-cells and estimation of their activity

It was assumed that helper T-cells recognize the combination of virus antigen and HLA-DR antigen of the target cells, and that their generation is not affected by MMC treatment (Okada et al. 1979), while suppressor cells are sensitive to MMC. PBL were treated with $25 \mu\text{g/ml}$ MMC at 37°C for 25 min. 1.5×10^6 MMC-treated PBL were mixed with 3×10^5 MMC-treated target cells. The cells were cultured at 37°C for 3 days in a 5% CO_2 incubator. These T-cells themselves did not show killer activity but worked as helper of killer cells as described below.

The PBL stimulated by the heat-treated target cells did not work as killers (Schellekens and Jsvooel 1970; Rollinghoff and Wagner 1975; Sondel et al. 1977). This can be explained by the fact that heat-treated target cells do not express HLA-DR and therefore cannot induce helper T-cells. 5×10^5 PBL were mixed with 5×10^4 heat (45°C , 60 min)-treated target cells and cultured for 3 days with or without 5×10^5 of the above

mentioned T-cells. When the killer activity was increased by the addition of the T-cells, it was judged that the T-cells had helper activity.

Induction of suppressor T-cells and estimation of their activity

Assuming that HLA-DR on the target cells and the presence of macrophages (Lutz and Fitch 1979; Taniyama and Holden 1979; Woodward et al. 1979) are needed for the induction of helper T-cells, T-cells free of macrophages were reacted with target cells whose HLA-DR had been destroyed by heat treatment, the objective was to induce suppressor T-cells (Finberg et al. 1979b; Chiu et al. 1980) but not helper T-cells. 1.5×10^6 T-cells were mixed with 4×10^5 target cells treated at 45°C for 60 min and cultured for 3 days in a 5% CO_2 incubator. 5×10^5 of these T-cells were added to 5×10^5 PBL stimulated with 5×10^4 MMC-treated target cells. Then it was studied whether the generation of killer activity in PBL was suppressed by addition of the T-cells.

Changes in the expression of HLA-DR antigen of target cells following various treatments

It has been reported that heat, glutaraldehyde, and ultraviolet (UV) treatment destroy the HLA-DR antigens of B-cells without affecting the expression of the HLA-A, B, C, antigens. A cell line of B-cells transformed with EBV was treated with $75 \mu\text{g/ml}$ MMC for 60 min, 45°C for 60 min, 0.01% glutaraldehyde for 3 min or UV (15 W, 10 cm) for 20 min. The cells were reacted with monoclonal antibody directed against framework of HLA-DR for 60 min, washed three times with PBS and further stained with anti-mouse IgG fluorescein conjugate for 60 min. The cells were observed under a UV microscope and the cells showing membrane-associated fluorescence were scored.

RESULTS

Expression of HLA-DR antigen of target cells variously treated

The cell line of B-cells transformed with EBV was stained for HLA-DR by immunofluorescence. 61% of untreated cells and 50% of MMC-treated cells showed fluorescence, while 0% of glutaraldehyde treated cells and 18.9% of UV-treated cells were positive (Table 1).

TABLE 1. *Expression of HLA-DR antigen of variously treated EBV-transformed cells*

Treatment	% of HLA-DR(+)
Untreated	61.0
Mitomycin C	50.0
Heat	8.0
UV	18.9
Glutaraldehyde	0.0

Demonstration of helper T-cells

PBL stimulated with MMC-treated target cells showed strong killer activity against autologous B-cells transformed by EBV. However, killer activity did not appear when PBL were stimulated with heat-treated and thus HLA-DR lacking cells (Finberg et al. 1979a; Fyte and Finke 1979; Pilarski 1979) probably because of the failure of induction of helper T-cells which should recognize the HLA-DR antigen. The T-cells treated with MMC to block the generation of suppressor and killer activity and stimulated with HLA-DR positive MMC-treated

target cells in the presence of macrophages did not show killer activity by themselves, but helped the generation of killer activity from PBL stimulated with HLA-DR negative (heat-treated) target cells. (Fig. 1).

The T-cells stimulated with heat-treated target cells or unstimulated T-cells did not show such helper activity.

Demonstration of suppressor T-cells

T-cells were stimulated with heat-treated target cells (Schellekens and Jsvogel 1970; Sondel et al. 1977). Helper T-cells seem not to be induced in this case because macrophages are not present and the HLA-DR antigen is not

Fig. 1

Fig. 1. Induction of helper T-cells for the generation of killer T-cells against EBV-transformed autologous cells.

PBL, peripheral blood mononuclear cells (lymphocytes+monocytes); PBL + Heat EB cell, PBL stimulated with heat-treated target cells; MMC PBL+MMC EB cell, T-cells from PBL treated with MMC and stimulated with MMC-treated target cells; MMC PBL+Heat EB cell, T-cells from PBL treated with MMC and stimulated with heat-treated target cells.

Fig. 2. Suppression by T-cells stimulated with heat or glutaraldehyde (G.A.) treated stimulator cells of the generation of killer cells against EBV-transformed autologous cells.

PBL, peripheral blood lymphocytes; PBL+MMC EB cell, PBL stimulated with MMC-treated target cells; T+MMC (Heat, G.A.) EB cell, T-cells stimulated with MMC (heat, G.A.)-treated target cells.

Fig. 2

Fig. 3

Fig. 3. Suppression of cytotoxicity to EBV-transformed cells by the culture supernatant of T-cells stimulated with variously treated target cells. PBL, peripheral blood lymphocytes; PBL+MMC EB cell, PBL stimulated by MMC-treated target cells; Sup (T+Heat EB cell), culture supernatant from T-cells stimulated by heat-treated target cells.

Fig. 4

Fig. 4. Suppression of cytotoxicity to EBV-transformed cells by allogeneic T-cells stimulated with variously treated target cells. PBL, peripheral blood lymphocytes; PBL+MMC EB cell, PBL stimulated with MMC-treated target cells; allo T, allogeneic T-cells; allo T+MMC (Heat) EB cell, allo T stimulated with MMC (heat)-treated target cells.

expressed on the target cells. When these T-cells were added to PBL stimulated with MMC-treated target cells, the generation of killer activity in the PBL was remarkably suppressed (Fig. 2). This indicated the induction of suppressor T-cells. T-cells stimulated with glutaraldehyde-treated (Rollinghoff and Wagner 1975) target cells also showed a strong suppressor effect. T-cells stimulated with MMC-treated target cells or unstimulated T-cells did not show such suppression.

Suppressor effect of culture supernatant of suppressor T-cells

The culture supernatant of T-cells was collected at the end of culture, and 0.5 ml was added to PBL stimulated with MMC-treated target cells. The generation of killer activity in the PBL was also suppressed by this supernatant (Fig. 3). The culture supernatant of the T-cells stimulated with MMC-treated target cells or unstimulated T-cells did not show such suppression.

TABLE 2. *HLA antigen of suppressor cells and killer cells*

HLA of suppressor	HLA of killer	Suppression
AW 31, AW 33 B 15, BW 44 CW 3 DR 2	AW 31, AW 33 BW 44, BW 55 CW 1 DRW 9	+
AW 24, AW 31 B 15, BW 51 CW 3 DRW 9	AW 31, AW 33 BW 44, BW 55 CW 1 DRW 9	+
AW 24 BW 48, BW 52 DR 2, DR 4	AW 24 BW 54, BW 62 CW 1, CW 3 DR 4, DRW 8	+

Fig. 5

Fig. 6

Fig. 5. Suppression of cytotoxicity to EBV-transformed cells by the culture supernatant of allogeneic T-cells stimulated with heat treated target cells. Sup (allo T+Heat EB cell), supernatant of allogeneic T-cells stimulated with heat-treated target cells.

Fig. 6. Suppression of cytotoxicity to allogeneic Raji cells by T-cells stimulated with variously treated autologous EBV-transformed cells. PBL, peripheral blood lymphocytes; PBL+MMC Raji, PBL stimulated with MMC-treated Raji cells; T, T-cells; T+Heat EB cell, T-cells stimulated by heat-treated autologous B-cells transformed by EBV.

HLA restriction between suppressor T-cells and killer cells

Allogeneic T-cells stimulated with heat-treated target cells, autologous to T-cells also suppressed the generation of killer cells directed against EBV-transformed autologous B-cells (Fig. 4).

Suppression was observed in HLA incompatible combinations of suppressor T-cells and killer cells (Table 2). These observations indicate that no HLA restriction exists between suppressor T-cells and killer cells. The culture supernatant of allogeneic suppressor T-cells was also equally suppressive of the generation of the killer cells (Fig. 5).

Antigen specificity of the suppressor T-cells

PBL were stimulated with MMC-treated Raji cells or BJAB cells and killer cells directed to these cells were induced. Raji and BJAB are cell lines derived from Burkitt's lymphoma. Raji cells lack surface antigens associated with EBV and BJAB cells lack the genome of EBV. Therefore, killer cells seem to react to the allo-antigen of the target cells. T-cells stimulated with heat-treated autologous B-cells transformed by EBV also suppressed the generation of these killer cells (Figs. 6, 7).

This suggests that these suppressor T-cells work on antigen in nonspecific fashion.

Fig. 7. Suppression of cytotoxicity to allogeneic BJAB cells by T-cells stimulated with variously treated autologous EBV-transformed cells. PBL+MMC BJAB, peripheral blood lymphocytes stimulated with MMC-treated BJAB cells; T+Heat EB cell, T-cells stimulated with heat-treated autologous B-cells transformed by EBV.

DISCUSSION

Not self cells such as virus-infected cells are destroyed by killer T-cells, and this process is controlled by helper T-cells and suppressor T-cells. It was intended to prove regulating the generation of killer T-cells in this study.

Helper T-cells recognize the combination of virus antigen and HLA-DR antigen (Ia antigen in mice) of the target cells, and macrophages (Lutz and Fitch 1979; Taniyama and Holden 1979; Woodward et al. 1979) are a prerequisite for their generation. Cell division (Okada et al. 1979) does not seem necessary for the induction of helper T-cells. Therefore, helper activity can be induced in T-cells treated with mitomycin C (MMC). In contrast, the presence of HLA-DR antigen on target cells and coexistence of macrophages are not needed for the induction of suppressor T-cells. And suppressor T-cells are usually sensitive to MMC treatment.

Thinking that these assumptions can be applied to helper and suppressor T-cells controlling the generation of killer T-cells directed against EBV infected cells, we tried to induce helper T-cells and suppressor T-cells separately. Lymphocytes were treated with MMC to block the appearance of suppressor activity and stimulated with target cells bearing HLA-DR antigen in the presence of macrophages. T-cells isolated from these lymphocytes did not show killer activity by themselves, but aided in the generation of killer cells from lymphocytes stimulated with target cells lacking HLA-DR. On the contrary, T-cells stimulated with target cells lacking HLA-DR in the absence of macrophages showed suppressor activity of the generation of killer cells from lymphocytes stimulated with HLA-DR positive target cells.

These observations indicate that helper T-cells and suppressor T-cells are actually present and control the generation of killer cells against EBV-infected cells. Which of these two kinds of T-cells having opposite functions is more strongly induced seems dependent on the expression of HLA-DR on the target cells, the coexistence of macrophages and the presence of agents inhibiting cell division. These conditions, therefore, might have an influence on the elimination of EBV-infected cells.

Various types of suppressor T-cells are known. Some need concordance of HLA antigen between suppressor cells and effector cells, but others do not. Some suppress only the killer cells directed against the same target cells that have induced the suppressor cells (antigen specific), but others also suppress killer cells against other target cells. The suppressor T-cells demonstrated in this paper seemed to have no HLA restriction and were antigen nonspecific. They suppressed HLA incompatible allogeneic cells reacting with not only EBV antigen but also with alloantigen(s).

References

- 1) Böyum, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.*, **21**, 97.

- 2) Burkitt, D. (1958) A sarcoma involving the Jaws in African children. *Brit. J. Surg.*, **46**, 218-223.
- 3) Cerottini, J.C. & Brunner, K.T. (1974) Cell mediated cytotoxicity allograft rejection, and tumor immunity. *Advanc. Immunol.*, **18**, 67-132.
- 4) Chiu, K.M., Franses, R.B. & Choi, Y.S. (1980) Activation of specific suppressor cells with heat-treated allogeneic tumor cells. *Cellular Immunol.*, **49**, 283-292
- 5) De-The', G.B., Ablashi, D.V., Llabueuf, A. & Mouraji, N. (1973) Nasopharyngeal carcinoma (NPC). Presense of an EBV nuclear antigen in fresh tumor biopsies. Preliminary results. *Biomedicine*, **19**, 349-352.
- 6) Epstein, M.A., Achong, B.G. & Barr, Y.M. (1964) Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet*, **1**, 702-703.
- 7) Finberg, D., Greene, M.I., Benacerraf, B. & Burakoff, S.J. (1979a) The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. I. Evidence for antigen-specific helper T-cells. *J. Immunol.*, **123**, 1205-1209.
- 8) Finberg, D., Burakoff, S.J., Benacerraf, B. & Greene, M.J. (1979b) The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. Evidence for antigen-specific suppressor T cells. *J. Immunol.*, **123**, 1210-1214.
- 9) Fukukawa, T., Hirano, T., Kaguchi, N., Teranishi, T., Tsuyaguchi, I., Nagao, N., Naito, N., Yoshimura, K., Okuda, Y., Tohda, H. & Oikawa, A. (1981) In-vitro induction of HLA-restricted cytotoxic T lymphocytes against autologous Epstein-Barr virus transformed B lymphoblastoid cell line. *J. Immunol.*, **126**, 1697-1701.
- 10) Fyte, D.A. & Finke, J.H. (1979) Soluble helper factor(s) participates in the generation of cell mediated cytotoxicity directed against syngeneic tumor cells. *J. Immunol.*, **122**, 1156-1161.
- 11) Henle, G., Henle, W. & Diehl, V. (1968) Relation of Burkitt's tumor associated herpes type virus to infectious mononucleosis. *Proc. nat. Acad. Sci. USA*, **59**, 94-101.
- 12) Jondal, M., Holm, G. & Wigzell, H. (1972) Surface markers of human T and B lymphocytes. I. A large population of lymphocytes forming non immune rosettes with sheep red blood cells. *J. exp. Med.*, **136**, 207-215.
- 13) Katsuki, T. & Hinuma, Y. (1975) Characteristics of cell lines derived from human leukocytes transformed by different strains of Epstein-Barr virus. *Int. J. Cancer*, **15**, 203-210.
- 14) Klein, G., Svedmyr, E., Jondal, M. & Persson, P.O. (1976) EBV-determined nuclear antigen (EBNA)-positive cells in the peripheral blood of infectious mononucleosis patients. *Int. J. Cancer*, **17**, 21-26.
- 15) Lutz, C.T. & Fitch, F.W. (1979) Accessory cell requirements for the generation of cytolytic T lymphocytes. *J. Immunol.*, **122**, 2598-2604.
- 16) Mizuno, F., Aya, T. & Osato, T. (1974) B lymphocytes as target cells for E.B. Virus transformation. *Brit. med. J.*, **3**, 689.
- 17) Moss, D.J., Wallace, L.E., Rickinson, A.B. & Epstein, M.A. (1981) Cytotoxic T cell recognition of Epstein- Barr Virus infected B cells. I. Specificity and HLA restriction of effector cells reactivated in vitro. *Europ. J. Immunol.*, **11**, 686-693.
- 18) Okada, M., Klimpel, G.R., Kuppers, R.C. & Henney, C.S. (1979) The differentiation of cytotoxic T cells in-vitro. I. Amplifying factor(s) in the primary response in Lyt 1 (+) cell dependent. *J. Immunol.*, **122**, 2527-2533.
- 19) Pilarski, L.M. (1979) Antigen-specific helper T cell are essential for cytotoxic T cells responses to metabolically inactivated stimulator cells. *Europ. J. Immunol.*, **9**, 454-460.
- 20) Reedman, B.M. & Klein, G. (1973) Cellular localization of an Epstein-Barr Virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer*, **11**, 499-520.
- 21) Rollinghoff, M. & Wagner. (1975) Secondary cytotoxic allograft response in-vitro. I. Antigenic requirements. *Europ. J. Immunol.*, **5**, 875-879.
- 22) Schellekens, P. Th.A. & Jsvoogel, E.I. (1970) Lymphocyte transformation in-vitro.

- Mechanism of stimulation in the mixed lymphocyte culture. *Clin. exp. Immunol.*, **7**, 229-239.
- 23) Sondel, P.M., Jacobson, M.W. & Bach, F.H. (1977) Selective activation of human suppressor cells by a non-proliferative stimulus. *Europ. J. Immunol.*, **7**, 38-40.
  - 24) Spits, H., Ijssel, H., Terhorst, C. & de Vries, J.E. (1982) Establishment of human T lymphocyte clones highly cytotoxic for an EBV-transformed B cell line in serum-free medium. Isolation of clones that differ in phenotype and specificity. *J. Immunol.*, **128**, 95-99.
  - 25) Tanaka, Y., Sugamura, K., Hinuma, Y., Sato, H. & Okuchi, K. (1980) Memory of Epstein-Barr virus specific cytotoxic T cells in normal seropositive adults as revealed by an in-vitro restimulation method. *J. Immunol.*, **125**, 1426-1431.
  - 26) Tanaka, Y., Sugamura, K. & Hinuma, Y. (1982) Heterogeneity of allogeneic restriction of human cytotoxic T cell clone specific for Epstein-Barr virus. *J. Immunol.*, **128**, 1241-1245.
  - 27) Taniyama, T. & Holden, H.T. (1979) Requirement of histocompatible macrophages for the induction of a secondary cytotoxic response to syngeneic tumor cells in-vitro. *J. Immunol.*, **123**, 43-49.
  - 28) Tsoukas, C.D., Fox, R.I., Solvin, S.F., Carson, D.A., Pellegrino, M., Forg, S., Pasquali, J.L., Ferrone, S., Kung, P. & Vaughan, J.H. (1981) T lymphocyte-mediated cytotoxicity against autologous EBV-genome bearing B cells. *J. Immunol.*, **126**, 1742-1746.
  - 29) Woodward, J.G., Fernandes, P.A. & Daynes, R.A. (1979) Cell mediated immune response to syngeneic U.V. induced tumor. Requirement for an Ia(+) macrophage in the in-vitro differentiation of cytotoxic T lymphocytes. *J. Immunol.*, **122**, 1196-1202.
  - 30) Yata, J. & Tachibana, T. (1973) The significance of lymphocytes in the etiology of E.B. virus associated tumor: Implication for serological epidemiology. In: *Analytic and Experimental Epidemiology of Cancer*, edited by W. Nakahara, T. Hirayama, K. Nishioka & H. Sugano, University of Tokyo Press, Tokyo, p. 161.
  - 31) Yata, J., Desgranges, Tachibana, T. & De-The', G. (1973a) Separation of human lymphocytes forming spontaneous rosettes with sheep erythrocytes. *Biomedicine*, **19**, 479-483.
  - 32) Yata, J., Desgranges, C., De-The', G. & Tachibana, T., (1973b) Lymphocytes in infectious mononucleosis properties of a typical cells and origin of the lymphoblastoid lines. *Biomedicine*, **19**, 479-483.
  - 33) Yata, J., Desgranges, C., De-Thé, G., Mourali, N., Ellouz, R., Tachibana, T. & Brugere, J. (1974) Nasopharyngeal carcinoma, B and T lymphocytes in the circulating blood and in tumor tissue. *Biomedicine*, **21**, 244-250.
  - 34) Yata, J., Desgrandes, D., Nakagawa, T. & Farre, M.C. (1975) Lymphoblastoid transformation and kinetics of appearance of viral nuclear antigen (EBNA) in cord blood lymphocytes infected by Epstein-Barr Virus (EBV). *Int. J. Cancer*, **15**, 377-384.
  - 35) ZurHausen, H., Schu He-Holthausen, H., Wolf, H., Dorries, K. & Egger, H. (1974) Attempts to detect virus-specific DNA in human tumor, Nucleic acid hybridizations with complementary RNA of human herpes group virus. *Int. J. Cancer*, **13**, 657-664.
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