Establishment of Cytotoxic T Lymphocytes Specific for Autologous Epstein-Barr Virus in HIV-Infected Patients: The Feasibility Study of EBV-Specific Immunotherapy for Patients with EBV-Associated Lymphoma

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Cytotoxic T lymphocytes specific for Epstein-Barr virus (EBV) have previously been successfully used in immunotherapy of Posttransplant lymphoproliferative disease (PTLD) and Hodgkin's disease. A similar strategy has never been employed in HIV/AIDS patients who also have high risk of developing EBV-associated lymphoma. A total of 5 HIV-infected patients were enrolled to evaluate their EBV-specific T cell responses by Interferon-gamma (IFNγ) ELISpot assays. Most patients had detectable T cell responses, mainly directed at Epstein-Barr nuclear antigen (EBNA-3). The authors wanted to see whether it was possible to augment magnitude and spectrum of the EBV responses by stimulating patient PBMC with cells presenting autologous EBV antigens. The authors successfully established spontaneously EBV-transformed lymphoblastoid cell lines (EBV_h-BCL) and used them for generation of EBV-specific CTL (EBV-CTL). The EBV_h-CTL lines established in the present study were not only highly cytotoxic against the autologous virus but also able to secrete IFNγ detected by ELISpot. The authors are now in the process of generating these lines in a large number and in a clinical grade for adoptive immunotherapy.

Keywords : *EBV, CTL, Adoptive immunotherapy*

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Epstein-Barr virus (EBV) is a gammaherpesvirus which infects more than 90% of the world population. Primary EBV infection occurs, particularly in developing countries, at an early age, and is generally asymptomatic. On the other hand, in developed countries where the primary infection is acquired during adolescence or later, the primary EBV infection can result in Acute Infectious Mononucleosis[1]. Following resolution of initial infection, EBV remains as a latent infection for lifespan of the host. Resting memory B lymphocytes are thought to be the site of EBV persistence. Whilst the infection is tightly controlled by EBV-specific cytotoxic T lymphocytes (EBV-CTL) in immunocompetent hosts, the reactivation of EBV infection in immunocompromised patients may result in EBV-associated diseases. Posttransplant lymphoproliferative disease (PTLD), for example, is perhaps one of the most important EBV-associated diseases occurring after transplantation, and is associated with a high mortality rate. Not only in transplant recipients, but HIV-infected individuals in the advanced stage are also at high risk of developing EBV-associated malignancies.

The EBV-CTL adoptive therapy has been demonstrated to be prophylactic and therapeutic useful in a number of PTLD trials[2-7]. All EBV-CTL exploited in these trials, however, were obtained from stimulation of T cells with marmoset EBV-transformed B lymphoblastoid cell lines (EBV_m-BCL)[6,7]. Although the CTL lines established based on this EBV_m-BCL protocol were cytotoxic against relevant target cells and of clinical benefit, the CTL line, in particular HLA context, might fail to kill human EBV-infected BCL. These can be explained by the genetic differences between marmoset (EBV_m) and human EBV (EBV_h). Indeed, one HLA-B8-restricted EBV_h epitope was proved to be absent in EBV_m strain in one study[8]. The genetic dis-

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crepancies of these two EBV strains are likely not limited to an HLA-B8-restricted epitope but also to other epitopes of various HLA restrictions[8,9]. Establishment of EBV-CTL against autologous EBV may, therefore, be an ultimate solution to these genetic variations problem. However, spontaneous transformation of BCL with autologous EBV, unlike transformation by B95-8 marmoset EBV, is infamous for being arduous and technical demanding. The process involves labour intensive limiting dilution of PBMC into 96-well plates to dilute the protective effect of EBV-specific T cell, and thus the latently EBV-infected B lymphocytes will be spontaneously transformed.

In the present study, we analysed the EBV-specific T cell responses in asymptomatic HIV-infected patients by ELISpot assays to map and quantitate the responses to various EBV gene products. Moreover, the authors established, for the first time, the CTL lines against autologous EBV from HIV-infected patients. The present study provides not only an insight into EBV-specific immune responses against EBV infection in HIV-infected individuals, but also the EBV_h-CTL establishment protocol optimised for adoptive immunotherapy.

Material and Method

Patients

All patients were enrolled from the Anonymous Clinic, Thai Red Cross Society. Information for the present study was provided to all patients and informed consents were acquired. Approval of the present study was obtained from the Ethics Committee of Faculty of Medicine, Chulalongkorn University. The patients HIV status was initially determined by Double-check 2 (Firmer, Israel) and confirmed by Serodia particle agglutination (Fuji, Japan) and ELISA (Genscreen v2.0, BIO-RAD).

Quantification of EBV-specific T lymphocytes by Gamma Interferon ELISpot assays

A total of 40 ml heparinised blood was collected from the patients at the time of enrollment. The peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (GIBCO BRL, USA) density gradient centrifugation, and resuspended in 10% fetal bovine serum in RPMI (GIBCO BRL, USA) (R10). The ELISpot assays were performed as previously described [10]. Spots were counted under a dissecting microscope. EBV-specific T cell responses were calculated after subtracting their values from the control (wild type vaccinia) wells.

Establishment of EBV-specific cytotoxic T lymphocyte

EBV-specific cytotoxic T lymphocyte lines were established from PBMC of patients by co-culturing 2 x 10^6 PBMC per well of a 24-well plate with 5 x 10^4 irradiated autologous spontaneously EBV-transformed B lymphoblastoid cell lines (EBV_h-BCL). After 10 days, the cells were harvested on Ficoll-Hypaque density gradient centrifugation, subcultured in 24-well plates at 5 x 10^5 cells/well, and restimulated with 1.25 x 10^5 irradiated EBV_h-BCL per well. After 4 days, the cultures were fed with 50 units/ml of interleukin-2 (IL-2). Thereafter, the cultures were fed twice weekly, the first time with 50 units/ml of IL-2 and the second time with 50 units/ml of IL-2 supplemented with irradiated EBV_h-BCL (EBV_h-CTL: EBV_h-BCL ratio, 4: 1).

Immunophenotype analysis of EBV-CTL

Immunophenotype of EBV-CTL was characterised by fluorescence activated cell sorter (FACS) analysis. Several monoclonal antibodies were used to analyse the CTL including anti-CD3 FITC, anti-CD4 PE, anti-CD8 PE, anti-CD16/56 PE, anti-CD19 PE, anti-CD38 FITC and anti-CD95 PE. All monoclonal antibodies were obtained from BD Biosciences. The data was analysed using CellQuest software (BD Biosciences).

Cytotoxicity assays

Cytotoxic function of EBV-CTL was evaluated against Chromium-labeled targets in standard 4-hour chromium release assays. The target cells included autologous EBV-BCL and recombinant vaccinia containing EBV genes-infected autologous EBV-BCL. Percentage specific lysis was calculated as 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). Maximum release was obtained by adding 100 μL 5% Triton X-100 (Sigma, USA) to medium containing chromium-labeled target cells.

Result

Demographic and clinical information

Since HIV-infected patients have a relatively high risk of developing EBV-associated lymphoma, establishment of EBV_h-CTL from these patients may be useful for adoptive immunotherapy when needed. The authors, therefore, recruited 5 asymptomatic HIV-infected donors from the Anonymous clinic, Thai Red Cross Society to study EBV-specific CTL responses and the feasibility to establish the CTL lines suitable for adoptive CTL therapy. These patients were young with an age range of 21 to 34 years old, and 4 of them

were female. Although the majority of Thai adults have been infected with EBV, the authors confirmed their EBV serostatus by serodiagnosis of EBV infection. All of them were positive for anti-VCA IgG by ELISA (Organon Technika, the Netherlands). These patients had CD4 T cell counts ranging from 294 to 1030 cells/mm^3, with a median of 476 cells/mm^3. The HIV-RNA was in a range of 3,864 to 32,749 copies/ml, with a median of 9,758 copies/ml (Table 1).

Table 1. Clinical and demographic characteristics of patients

	ID	Age (yrs)	Sex*	Anti-VCA	Number of CD4+ T cell (cell/mm^3)	HIV-RNA (copies/ml)
1	PP	34	M	+	1030	9,758
2	UK	27	F	+	531	12,009
3	RM	33	F	+	476	9,754
4	PL	24	F	+	376	32,749
5	LJ	21	F	+	294	3,864

* M and F are male and female, respectively

Quantitation of EBV-specific CD8+ T cells in PBMC

To evaluate if the enrolled patients had readily detectable EBV-specific T cells in the PBMC, the authors performed vaccinia-based IFNγ ELISpot assays based on the protocol previously described for HIV system[11]. The recombinant vaccinia containing EBV genes [Epstein-Barr nuclear antigen EBNA-1, EBNA-3A, EBNA-3B, EBNA-3C, LMP-1 and LMP-2] were kindly provided by Professor Alan Rickinson, University of Birmingham, UK. All patients had EBV-specific CD8+ T cells responses against at least one EBV protein. EBNA-3A was most commonly targeted, though the maximal magnitude of responses was mediated by EBNA-3B-specific T cells. LMP-1 protein, on the other hand, might be least immunodominant. Only 1 in 5 patients had detectable, albeit at a very low level, T cell responses against this protein. Although believed to be unprocessed protein, the Glycine-Alanine repeats (GAr) containing protein, EBNA-1, mediated detectable T cell responses in 2 patients. The maximal magnitude of overall EBV-specific T cell responses in each patient ranged from 46 to 1092 spot-forming units (SFU)/ 10^6 PBMC (Table 2).

Table 2. Enumeration of EBV-specific CD8+ T cells responses against various EBV gene products by vaccinia-based IFNγ ELISpot

	ID	EBNA -1	EBNA -3A	EBNA -3B	EBNA -3C	LMP -1	LMP -2
1	PP	10	56	0	206	0	0
2	UK	0	30	1092	12	0	14
3	RM	18	4	6	46	6	4
4	PL	0	8	304	40	0	10
5	LJ	0	120	78	0	0	4

Establishment and characterisation of EBV-specific cytotoxic T-lymphocytes (EBV-CTL) lines

The protocol to transform human B-LCL with marmoset EBV (EBV_m) is well established and the EBV_m-BCL lines derived from this protocol have been used in a number of EBV-CTL adoptive therapy trials. The human EBV (EBV_h) and EBV_m sequences are, however, different. EBV-CTL stimulated with EBV_m-transformed BCL may not, therefore, recognise all epitopes derived from natural EBV infection. Even though spontaneous transformation of B-LCL with autologous EBV is notoriously difficult, the authors were determined to establish this EBV_h-transformed BCL for establishment of ideal EBV_h-CTL lines suitable for adoptive T cell therapy. After a number of trials and errors, the authors have managed to establish spontaneously EBV_h-transformed BCL from all recruited patients following the protocol previously described with a slight modification (Hansasuta, P et al, manuscript in preparation). Most EBV_h-BCL lines required 4 weeks to be transformed and established.

In order to demonstrate the feasibility of augmenting EBV_h-specific CTL responses in patients with a high risk of developing EBV-associated lymphoma, the authors established a total of 5 EBV_h-CTL lines from HIV-infected donors. Most EBV_h-CTL required approximately 4 to 5 weeks for establishment. Since Thais and other Southeast Asians were reported to have a relatively high number of natural killer (NK) cells[12], the authors characterised phenotypes of EBV_h-CTL lines to ensure that the established lines were not contaminated with NK cells. Staining with various monoclonal antibodies revealed that the majority of the stained cell line were CD3+ T cells. Indeed, the proportion of CD3+ T cells was more than 99%. On the other hand, by staining the cells with anti-CD16 and anti-CD56, no NK cells in the established CTL lines were demonstrated (Table 3). Moreover, the CTL lines established by this method were proved to produce mainly, except for one line (LJ), CD3+ CD8+ T cells. Whilst CD8+ T cells were demonstrated in a range of 48-94%, CD4+ T cells were in a range of 1-11% of total CD3+ T cells. In order to see whether these CTL are activated, the lines were stained with anti-CD38

monoclonal antibody. Interestingly, majority of the cells were shown to be activated. One may doubt whether these lines would survive when adoptively transfered into patients, since activated cells are prone to undergo apoptosis. However, when the authors stained these lines with anti-CD95, only a minority of the cells were positive to anti-CD95 (Table 3).

Quantification of EBV-specific CTL lines by Gamma IFN ELISpot assays

To determine if the established CTL lines were EBV-specific, IFNγ ELISpot assays were performed to quantify the T cell responses. The CTL lines were stimulated either with autologous BCL to evaluate overall EBV_h-specific responses (ELISpot1) or with autologous BCL transfected with recombinant vaccinia containing EBV genes (ELISpot2) to determine EBV protein-specific responses. All CTL lines were shown to be EBV-specific by ELISpot assays. The authors initially thought transfection of recombinant vaccinia virus containing EBV genes would increase expression of EBV antigens, hence augmenting magnitude of responses detected by IFNγ ELISpot assays. The CTL responses detected by ELISpot1 were, in contrast to what the authors believed, higher than the responses detected by ELISpot2. Compared to EBV-specific responses from PBMC, the CTL responses were increased by many folds. For example, EBV-CTL line from the patient PL, even at a low effector to target ratio (ET ratio) of 1:1, mediated responses as high as 3,080 spot-forming units/10^6 cells (Table 4). Interestingly, even after *in vitro* manipulation, EBNA3 are still the most immunodominant proteins targeted by these EBV-specific CTL lines. LMP-1 and LMP-2, even though are presented by EBV_h-BCL, the responses to these two proteins are not enhanced much (data not shown). The titration of EBV-specific T cell responses at ET ratio of 5:1, 2.5:1 and :1 were observed in all patients (Table 4).

Cytotoxic activity of EBV-CTL lines

Even though all CTL lines were demonstrated by IFNγ ELISpot to be EBV-specific, cytotoxic activity of these lines remained to be determined. Each EBV-CTL line was co-cultured with radioactive chromium-labeled autologous EBV_h-BCL to assess their cytotoxic function. All CTL lines showed very high cytotoxic activity with a specific lysis ranging from 48 to 100% at ET ratio of 50: 1 (Table 5). Spontaneous lysis was less than 25% in all experiments. The highest CTL cytotoxic responses, consistent with responses observed in ELISpot assays, were from patient PL, and indeed the cytotoxic activity remained high even at lower ET ratio (Fig. 1). On the average of 5 patients, EBV-CTL lines were shown to mediate as high as 67% lysis at an ET ratio of 50:1, 62% lysis at 25:1, 57% lysis at 12:1 and 49% at 6:1, respectively.

Table 3. Phenotype characterisation of EBV-CTL lines

	ID	CD3	CD4	CD8 +16	CD56	CD19	CD38	CD95
1	PP	100	11	88	0	0	72	6
2	UK	100	3	94	0	0	98	4
3	RM	100	1	91	0	0	82	3
4	PL	100	3	91	0	0	86	5
5	LJ	99	3	48	0	0	99	1

Table 4. Determination of EBNA3-specific T cells responses from EBV-CTL lines by IFNγ ELISpot assays (spot-forming units/10^6 cells) in 3 different ET ratio

No	ID	Autologous BCL with recombinant vaccinia			Autologous BCL		
		5:1	2.5:1	1:1	5:1	2.5:1	1:1
1	PP	4060	2180	1210	3400	1800	1220
2	UK	3280	3640	2000	3960	2440	3040
3	RM	2240	1700	1040	7320	4320	2840
4	PL	8760	4440	3080	4120	3680	2240
5	LJ	4140	2100	1500	4460	3720	2040

Table 5. Specific lysis of EBV-CTL lines at different ET ratio

	ID	% Specific lysis			
		50:1	25:1	12:1	6:1
1	PP	58	55	53	45
2	UK	73	69	58	52
3	RM	57	53	50	43
4	PL	100	99	86	70
5	LJ	48	43	38	36

Discussion

Epstein-Barr virus (EBV) is a member of the herpesviruses and is associated with a number of

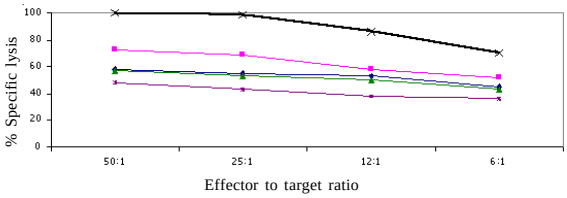

Fig. 1 EBV-specific CTL responses against autolgous EBV-BCL

diseases ranging from benign Infectious Mononucleosis to malignancies[13]. In a majority of immunocompetent hosts, EBV is kept checked by the immune system resulting in a benign course of infection. On the other hand, immunocompromised hosts are prone to have EBV-associated diseases. EBV-specific cytotoxic T-lymphocytes (EBV-CTL) were demonstrated to play a key role in the protective immune responses against EBV infection. Indeed, adoptive donor-derived EBV-CTL therapy was proved to be safe and immensely successful in bone marrow or solid organ transplant patients with EBV-associated lymphoma[3,14-16]. In contrast, the authors have no evidence of whether autologous EBV-CTL could be established and transferred to prevent or treat EBV-associated disease such as Primary CNS Lymphoma (PCNSL) in severely immunocompromised HIV/AIDS patients.

Cytotoxic T lymphocytes were shown to play a protective role in a number of viral infections[17-20]. The protective role of EBV-CTL may be clearly seen from many observations where drug-induced immunocompromised transplant recipients were shown to have a high risk of EBV-associated lymphoma[21]. This group of patients was proved to benefit from an adoptive transfer of EBV-CTL either as a prophylactic option or a therapeutic intervention. However, previous studies have never been performed in HIV-infected patients with a high risk of EBV-associated malignancies. The authors demonstrated here, for the first time, the establishment of EBV-CTL lines from HIV-infected individuals for adoptive therapy.

All the donors, even though immunocompromised, had EBV-specific T cell responses detected by IFNγ ELISpot assays. Consistent with previous observation in healthy donors, EBNA-3 are immunodominant proteins in these patients[19]. The magnitude of responses to EBNA-3 proteins did not seem to correlate with immune status reflected by CD4 T cell count. In fact, some of them had even higher EBV-CTL responses than healthy donors (Hansasuta, P. manuscript in preparation), this may be due to higher EB viral loads in HIV-infected patients.

Previous EBV-CTL adoptive therapy trials used supernatant of EBV-producing marmoset cell line (B95-8) to transform B-lymphoblastoid cell line for establishing EBV-CTL lines. Since the marmoset EBV (EBV_m) is not identical to human EBV (EBV_h), EBV-CTL established with this EBV-expressing B cell line may not efficiently recognise EBV_h-infected cells[8]. Despite the infamous difficulty in establishing spontaneously EBV-transformed B-LCL, the authors successfully set up the spontaneously-transformed BCL lines from all 5 asymptomatic HIV-infected patients. All BCL lines took approximately 4 weeks to develop, the duration of which was the same for conventional B95-8 EBV-transformed technique (data not shown). This may be not a big surprise considering the fact that these patients are relatively immunocompromised, and hence having relatively higher frequencies of circulating EBV-infected B-lymphocytes. Another 4 weeks were required to establish EBV-CTL lines ready for CTL analysis. Regarding EBV-CTL adoptive therapy, one may need a total of 10 to 12 weeks to obtain the CTL for such treatment. The authors are now, however, continuing the works to optimise and shorten the time needed for establishment of EBV-CTL.

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References

1. Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc Natl Acad Sci U S A 1968; 59: 94-101.
2. Haque T, Taylor C, Wilkie GM, Murad P, Amlot PL, Beath S, et al. Complete regression of posttransplant lymphoproliferative disease using partially hla-matched epstein barr virus-specific cytotoxic T cells1. Transplantation 2001; 72: 1399-402.
3. Khanna R, Bell S, Sherritt M, Galbraith A, Burrows SR, Rafter L, et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. Proc Natl Acad Sci U S A 1999; 96: 10391-6.
4. Savoldo B, Goss J, Liu Z, Huls MH, Doster S, Gee AP, et al. Generation of autologous Epstein-Barr virus-specific cytotoxic T cells for adoptive immunotherapy in solid organ transplant recipients. Transplantation 2001; 72: 1078-86.
5. Comoli P, Labirio M, Basso S, Baldanti F, Grossi P, Furione M, et al. Infusion of autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. Blood 2002; 99: 2592-8.
6. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein- Barr-virus-related lymphoproliferation. Lancet 1995; 345: 9-13.
7. Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood 1998; 92: 1549-55.
8. Misko IS, Schmidt C, Honeyman M, Soszynski TD,

Sculley TB, Burrows SR, et al. Failure of Epstein-Barr virus-specific cytotoxic T lymphocytes to lyse B cells transformed with the B95-8 strain is mapped to an epitope that associates with the HLA-B8 antigen. Clin Exp Immunol 1992; 87: 65-70.
9. Apolloni A, Moss D, Stumm R, Burrows S, Suhrbier A, Misko I, et al. Sequence variation of cytotoxic T cell epitopes in different isolates of Epstein-Barr virus. Eur J Immunol 1992; 22: 183-9.
10. Appay V, Hansasuta P, Sutton J, Schrier RD, Wong JK, Furtado M, et al. Persistent HIV-1-specific cellular responses despite prolonged therapeutic viral suppression. Aids 2002; 16: 161-70.
11. Larsson M, Jin X, Ramratnam B, Ogg GS, Engelmayer J, Demoitie MA, et al. A recombinant vaccinia virus based ELISPOT assay detects high frequencies of Pol-specific CD8 T cells in HIV-1-positive individuals. Aids 1999; 13: 767-77.
12. Webster HK, Pattanapanyasat K, Phanupak P, Wasi C, Chuenchitra C, Ybarra L, et al. Lymphocyte immunophenotype reference ranges in healthy Thai adults: implications for management of HIV/AIDS in Thailand. Southeast Asian J Trop Med Public Health 1996; 27: 418-29.
13. Rickinson AB, Kieff E. Epstein-Barr Virus. In: Field BN, Knipe DM, Howley PM, editors. Field's Virology. Philadelphia: Lipincott-Raven; 1996: 2397-446.
14. Heslop HE, Perez M, Benaim E, Rochester R, Brenner MK, Rooney CM. Transfer of EBV-specific CTL to prevent EBV lymphoma post bone marrow transplant. J Clin Apheresis 1999; 14: 154-6.
15. Rooney CM, Roskrow MA, Smith CA, Brenner MK, Heslop HE. Immunotherapy for Epstein-Barr virus-associated cancers. J Natl Cancer Inst Monogr 1998; 23: 89-93.
16. Rooney CM, Roskrow MA, Suzuki N, Ng CY, Brenner MK, Heslop H. Treatment of relapsed Hodgkin's disease using EBV-specific cytotoxic T cells. Ann Oncol 1998; 9(Suppl 5): S129-32.
17. Rowland-Jones SL, Dong T, Dorrell L, Ogg G, Hansasuta P, Krausa P, et al. Broadly cross-reactive HIV-specific cytotoxic T-lymphocytes in highly-exposed persistently seronegative donors. Immunol Lett 1999; 66: 9-14.
18. Reusser P, Cathomas G, Attenhofer R, Tamm M, Thiel G. Cytomegalovirus (CMV)-specific T cell immunity after renal transplantation mediates protection from CMV disease by limiting the systemic virus load. J Infect Dis 1999; 180: 247-53.
19. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. Annu Rev Immunol 1997; 15: 405-31.
20. Posavad CM, Koelle DM, Shaughnessy MF, Corey L. Severe genital herpes infections in HIV-infected individuals with impaired herpes simplex virus-specific CD8+ cytotoxic T lymphocyte responses. Proc Natl Acad Sci U S A 1997; 94: 10289-94.
21. O'Reilly RJ, Small TN, Papadopoulos E, Lucas K, Lacerda J, Koulova L. Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts. Immunol Rev 1997; 157: 195-216.

การเตรียมไซโตทอกซิค ที ลิมป์โฟไซต์ ที่จำเพาะต่อไวรัสเอปสไตน์-บาร์ของตนเองในผู้ป่วยติดเชื้อเอชไอวี: การศึกษาความเป็นไปได้ในการใช้ภูมิคุ้มกันจำเพาะต่อไวรัสเอปสไตน์-บาร์ในรักษาผู้ป่วยโรคมะเร็งต่อมน้ำเหลืองที่มีความสัมพันธ์กับไวรัสเอปสไตน์-บาร์

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มีนักวิทยาศาสตร์แสดงให้เห็นว่าสามารถนำ Cytotoxic T lymphocyte (CTL) ซึ่งจำเพาะต่อ Epstein-Barr virus (EBV) มาใช้ในการรักษาโรคมะเร็งต่อมน้ำเหลืองหลังจากการปลูกถ่ายอวัยวะ (Posttransplant lymphoproliferative diseases, PTLD) และ Hodgkin's disease แต่การรักษาโรคในลักษณะดังกล่าวยังไม่เคยถูกทดสอบในผู้ป่วยติดเชื้อเอชไอวีที่มีความเสี่ยงต่อมะเร็งต่อมน้ำเหลืองที่มีความสัมพันธ์กับ EBV ผู้วิจัยได้ศึกษาภูมิคุ้มกัน T cells ต่อ EBV ในผู้ป่วยติดเชื้อเอชไอวีจำนวนห้าราย พบว่าผู้ป่วยส่วนใหญ่มีการตอบสนองของ T cells ที่ตรวจพบได้ด้วยวิธี IFNγ ELISpot assays และโดยมากมักจะเป็นการตอบสนองต่อโปรตีน EBNA-3 ต่อจากนั้น ผู้วิจัยได้พยายามเพาะเลี้ยง CTL ที่จำเพาะต่อ EBV ที่เป็น autologous virus ของผู้ป่วยแต่ละราย เพื่อศึกษาความเป็นไปได้ของการเพาะเลี้ยงเซลล์ดังกล่าวในห้องปฏิบัติการ และพบว่าเซลล์ที่เราเพาะเลี้ยงได้ มีความสามารถในการฆ่าเซลล์เป้าหมายซึ่งเป็น autologous EBV-transformed lymphoblastoid cell line ขณะนี้ผู้วิจัยกำลังทดลองเพิ่มจำนวนเซลล์ดังกล่าวให้ได้ปริมาณมาก และมีคุณภาพเพื่อใช้งานในระดับคลินิกต่อไป
