ELSEVIER

FEMS Immunology and Medical Microbiology 19 (1997) 81–87

FEMS
IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Positive role of macaque cytotoxic T lymphocytes during SIV infection: decrease of cellular viremia and increase of asymptomatic clinical period

Isabelle Bourgault Villada [a,*], Lorenzo Mortara [a], Anne Marie Aubertin [b], Hélène Gras-Masse [c], Jean Paul Lévy [a], Jean Gérard Guillet [a]

[a] *Institut Cochin de Génétique Moléculaire, Laboratoire d'Immunologie des Pathologies Infectieuses et Tumorales, Institut de la Santé et de la Recherche Médicale Unité 445, Université René Descartes, Hôpital Cochin, 27 rue du Faubourg Saint Jacques, 75014 Paris, France*

[b] *Laboratoire de Virologie, Institut de la Santé et de la Recherche Médicale Unité 74, Université Louis Pasteur, 3 rue Koeberle, 67000 Strasbourg, France*

[c] *Laboratoire de Chimie des Biomolécules, Institut Pasteur de Lille, Centre National de la Recherche Scientifique, Université de Lille II, 1 rue du Professeur Calmette, BP 245, 59019 Lille Cedex, France*

Received 28 April 1997; revised 18 June 1997; accepted 26 June 1997

Abstract

We have measured cellular viremia and observed clinical outcome of macaques from two cohorts, the first including 12 macaques infected by SIVmac251 and the second including 12 macaques immunized by lipopeptides and then challenged by SIVmac251. In the first cohort (SIV-infected macaques), 3 patterns of CTL responders were determined: high, low and non-responders. In the macaques belonging to pattern of low and non-responders, cellular viremia, measured by growing the virus from PBMC, was continuously high during the first 6 months after infection, and five macaques developed AIDS within 14.4 ± 7.7 months. Conversely, in the six high-responder macaques, cellular viremia was constantly low and only one macaque developed AIDS at 19 months, the five others being alive at 24 months. After immunization with lipopeptides, 7/12 macaques showed CTL responses and among these, after SIV challenge, cellular viremia was continually low, and no disease was observed at 22 months of follow-up. Conversely, the five non-responder macaques displayed persistent high viremia and macaques developed AIDS within 12.6 ± 2.9 months after SIV challenge. These data strongly suggest that the presence of cytotoxic responses is inversely correlated with cellular viremia and correlated with overall survival and thus is an important component of the immune response in vaccinated individuals. It supports the idea that a strengthening of the CTL responses, if possible, might be beneficial in HIV-infected human beings.

Keywords: Animal model; Simian immunodeficiency virus; Cytotoxic T lymphocyte; Disease progression

1. Introduction

Cytotoxic T lymphocytes (CTL) are produced during viral infection. It is well established that they recognize viral peptides presented by major histocompatibility complex (MHC) class I molecules on the surface of target cells [1]. In vivo, the CTL probably have the capacity to kill virus-infected cells and

* Corresponding author. Tel.: +33 (1) 4407-1821; fax: +33 (1) 4407-1425; e-mail: bourgau@icgm.cochin.inserm.fr

0928-8244/97/$17.00 © 1997 Published by Elsevier Science B.V. All rights reserved
PII S0928-8244(97)00071-0

to clear the virus. This has been well demonstrated in perforin-deficient mice in which, in absence of functional CTL and natural killer cells, lymphocytic choriomeningitis virus (LCMV) persists at high titer after infection [2]. Virus clearance is, however, often associated with immunopathological consequences. Indeed, CTL have been shown to be deleterious to the host by killing infected viral cells. They induce an acute respiratory disease in the case of respiratory syncytial virus (RSV) [3], and bring about choriomeningitis in the case of LCMV [2,4]. In HIV or SIV infections, high levels of CTL responses have been regularly found [5–10]. A deleterious role has been suggested for these CTL, in particular in the nervous central system with destruction of infected microglial cells, astrocytes or oligodendrocytes [11,12] and in the lung with diffuse interstitial pneumonitis and pulmonary function abnormalities [13]. A positive role in vivo of CTL on viremia has been demonstrated only during HIV or SIV primary infections [14–16]. In the present study, we investigated during 2 years, the changes in viral load and the clinical outcome of 24 previously studied rhesus macaques, either directly infected by SIV [17,18] or immunized by lipopeptides [19] and then challenged by SIV. Our goal was to test for correlation between the presence of cytotoxic responses and viral load as well as clinical outcome.

2. Materials and methods

2.1. Animals

Twelve rhesus macaques (*Macaca mulatta*) were infected intravenously with SIVmac251 as previously described [17,18]. Briefly, SIVmac251 isolate, passaged only on macaque PBMC was kindly provided by R. Desrosiers (New England Regional Primate Research Center, Southborough, MA, USA). A stock of this virus was grown in rhesus macaque PBMC and cell-free virus was titrated in vivo by intravenous inoculation in rhesus macaques. Animal infective dose 50 (AID_{50}) was the dose of SIVmac251, able to infect 50% of inoculated macaques. One ml of stock virus contains 3.3×10^4 AID_{50} as determined by the Vacman program [20]. This strain was then used at 3, 30, 300 AID_{50}, respectively, in six macaques (RH 473, RH 477, RH 483, RH 493, RH 505, RH 1238), three macaques (RH 485, RH 495, RH 499) and three macaques (RH 471, RH 475, RH 501). Twelve additional uninfected rhesus macaques (RS 4, RS 6, RS 7, RS 17, RS 20, RS 21, RS 22, RS 23, Q 50, Q 51, Q 53, Q 54) were subcutaneously inoculated with a mixture of 7 lipopeptides (500 μg of each) in IFA to induce CTL as already described [19]. Briefly, 5 lipopeptides from NEF SIV protein were synthesized: LP1 amino acids (aa) 101–126, LP2 aa 125–147, LP3 aa 155–178, LP4 aa 201–225, LP5 aa 221–247. Two lipopeptides from GAG SIV protein were also produced: LP6 aa 165–195, LP7 aa 246–281. These peptides were chosen because of their immunogenicity in macaque. P-methyl-BHA-N-tert-butyloxycarbonyl-amino hexadecanoic acid resin was synthesized as previously described [21]. Synthesis of lipopeptides was performed with cleavage and deprotection of lipopeptidyl resins as already reported [19]. Purification and characterization of the lipopeptides were performed by reverse phase HPLC. Three immunizations of the 12 macaques were performed at day 0, 30 and 60. Thirteen months later, all macaques were boosted with the same mixture of lipopeptides. Fifteen days after the boost, challenge with 10 AID_{50} SIVmac251 was performed. Animal experiments were done in accordance with European Economic Community guidelines.

2.2. Lymphocyte preparation

PBMC were isolated by density gradient centrifugation on lymphocyte separation medium (Flow Laboratories, Glasgow, UK) and were either used immediately or stored at −180°C in liquid nitrogen.

2.3. Measure of cellular viremia

To quantify cellular viremia, 10^6 PBMC were cultured with 1.5×10^5 CEM×174 cells for 30 days. CEM×174 cell which is a fusion product of human B-cell line 721.174 and human T-cell line, is easily infected with SIV and particularly useful for SIV studies. Serial 5- or 10-fold dilutions of PBMC were performed and p27 antigen was detected by ELISA in the supernatants.

2.4. Neutralization assay

Antibody end point dilution assay was used to quantify neutralizing antibody titers in SIV-infected macaques. Virus suspension (75 μl) containing 10 tissue culture infectious doses ($TCID_{50}$) for SIV-mac251 was incubated with 75 μl dilutions of serum for 1 h at 37°C in Eppendorf tubes (Prolabo, Paris, France). After this incubation period, 25 μl of this mixture was taken and incubated with 25 μl of CEM × 174 (2.5×10^5) in RPMI 1640 supplemented with 10% of heat-inactivated healthy macaque serum (56°C for 30 min) in 96 well flat-bottom tissue culture plates (Costar, Cambridge, MA). After 2 h of incubation at 37°C, 100 μl of RPMI 1640 supplemented with 15% fetal calf serum was added in each well. On day 8, reverse transcriptase activity was measured as previously described [22]. The neutralization titer was defined as the dilution of serum that results in a 90-fold decrease of reverse transcriptase activity.

2.5. ELISA

Ninety-six well plates (Nunc, Roskilde, Denmark) were coated by incubating 10 μg/ml of each free immunizing peptide per well overnight at 4°C. PBS containing 0.1% Tween 20 and 1% BSA was used as blocking buffer. The plates were washed with PBS and dilutions of test sera were incubated in the coated wells for 2 h at 37°C. The plates were again washed and incubated with biotin-labeled goat anti-human IgG (Sigma, St Quentin Fallavier, France) followed by Extravidin-alkaline phosphatase (Sigma) for 1 h at 37°C. Alkaline phosphatase activity was detected using 4-methyl-umbelliferyl phosphate as a substrate (Sigma) and reading fluorescence at 360/460 nm in a Cytofluor 2300 (Millipore, Bedford, MA). Titers were derived from maximum plots and represent the reciprocal value of the serum dilution giving 50% of the maximal OD.

2.6. Statistical analysis

To compare the differences in viremia and clinical outcome between high CTL responder macaques and low or no CTL responders, we used a chi-square test. To compare titers of antibodies between high CTL responder macaques and low or no CTL responders, we used the Mann Whitney test.

Table 1
Time course of the cellular viremia in 12 infected macaques during the first 6 months post SIV infection; correlation with CTL activities and clinical outcome

Macaque #	Profile of CTL macaque responses[a] (Months 1–6)	Cellular viremia[b]			Clinical outcome[c]
		Month 1	Month 3	Month 6	
RH 471	high responder	10	1	1	alive month 24
RH 473	high responder	10	10	10	alive month 24
RH 477	high responder	nt	nt	0	alive month 24
RH 499	high responder	10	1	1	alive month 24
RH 505	high responder	nt	nt	0	AIDS month 19
RH 1238	high responder	1000	1	0	alive month 24
RH 483	low responder	100	10	1000	AIDS month 11
RH 495	low responder	10	1	100	AIDS month 21
RH 501	low responder	10	10	10	AIDS month 23
RH 475	non-responder	10	10	10	alive month 24
RH 485	non-responder	1000	100	dead	AIDS month 4
RH 493	non-responder	nt	nt	100	AIDS month 13

[a]CTL responses during the first 6 months after SIV infection were already described in Refs. [17,18]. Three patterns of macaques were found: pattern 1: high responders, pattern 2: low responders and pattern 3: non-responders.
[b]Cellular viremia was measured by coculture of PBMC with CEM × 174. The numbers indicate the dilution of PBMC up to which coculture was positive.
[c]The clinical status of these macaques was observed for 2 years.

Table 2
Titers of neutralizing antibodies in the 12 SIV-infected macaques; titers were the highest measured values during the first 6 months after SIV infection

Macaque #	Neutralizing antibodies
RH 471	100
RH 473	50
RH 477	nt
RH 499	25
RH 505	nt
RH 1238	0
RH 483	75
RH 495	400
RH 501	100
RH 475	200
RH 485	nt
RH 493	nt

3. Results and discussion

We have previously shown CTL activities in SIV-infected macaques [17,18]. Briefly, the CTL responses were present among PBMC in nine out of the 12 infected macaques while in the three remaining macaques (pattern 3: macaques RH 475, RH 485, RH 493) cytotoxic activity was never found. Two patterns were observed in the nine responding macaques. The first pattern was seen in six macaques (RH 471, RH 473, RH 477, RH 499, RH 505, RH 1238) and was characterized by a strong and sustained CTL response directed simultaneously against most of SIV proteins. The second pattern observed in responding macaques, concerned three macaques (RH 483, RH 495, RH 501) with poor recognition of a few SIV proteins with low level of lysis. In the macaques belonging to patterns 2 (low responders) and 3 (non-responders), we show in the present study that cellular viremia was continuously high during the first 6 months after infection, whereas five macaques developed AIDS with a mean time of 14.4 ± 7.7 months, the sixth macaque (RH 475) had an AIDS-related complex disease at 30 months (Table 1). Conversely, in the six macaques from pattern 1 (high responders), cellular viremia was constantly low and only one macaque developed AIDS at 19 months. The five other macaques were AIDS free and still healthy after 2 years of follow-up. Four animals were kept and were alive at month 30 without any disease. One macaque was still alive 5 years after infection (RH 471). Neutralizing antibodies were detected at low titers in all macaques and

Table 3
Correlation between presence or absence of CTL responses after lipopeptidic immunization, cellular viremia and clinical outcome

Macaque #	Profile of CTL macaque responses[a]	Cellular viremia[b]						Clinical outcome[c]
		Day 15	Month 1	Month 3	Month 6	Month 12	Month 18	
RS 4	responder	1250	50	2	2	0	1	alive month 22
RS 6	responder	250	250	0	10	10	0	alive month 22
RS 7	responder	6250	250	0	2	1	nt	alive month 22
RS 17	responder	250	50	10	10	0	0	alive month 22
RS 20	responder	250	10	10	10	10	nt	alive month 22
RS 21	responder	1250	10	2	0	1	1	alive month 22
RS 23	responder	1250	1250	10	10	10	1	alive month 22
RS 22	non-responder	1250	250	10	50	250	dead	AIDS month 16
Q 50	non-responder	1250	1250	250	50	dead		AIDS month 11
Q 51	non-responder	1250	50	250	10	dead		AIDS month 11
Q 53	non-responder	250	50	50	250	dead		AIDS month 9
Q 54	non-responder	250	250	250	50	50		AIDS month 16

[a]Two patterns of macaques were defined after lipopeptidic immunization. The first pattern included macaque CTL responders and the second the non-responder macaques as previously reported [19].
[b]Cellular viremia was measured by coculture of PBMC with CEM × 174. The numbers indicate the dilution of PBMC up to which coculture was positive.
[c]The clinical status was observed for 22 months after SIV infection.

were not correlated with cellular viremia and clinical outcome within all patterns of macaques using the Mann Whitney test (Table 2).

We have also shown CTL activities against immunizing peptides in 7/12 lipopeptides vaccinated macaques [19]. Cellular viremia was measured regularly for 2 years in these all vaccinated macaques (Table 3). In those showing CTL responses after lipopeptidic immunization (RS 4, RS 6, RS 7, RS 17, RS 20, RS 21, RS 23), cellular viremia was continually low while non-responder macaques (RS 22, Q 50, Q 51, Q 52, Q 53, Q 54) displayed persistent high viremia. Furthermore, the clinical status of the macaques was observed for 2 years after challenge and appears correlated with the initial presence of CTL responses, suggesting a beneficial role of CTL. In this cohort, titers of antibodies against immunizing peptides are shown in Table 4. No correlation was established between levels of anti-NEF or anti-GAG antibodies and progression of the disease or cellular viremia except for antibodies anti-peptide GAG 246-281 which were higher in non-responder macaques than in responder macaques ($P < 0.05$ by the Mann Whitney test). The biological significance of this result has to be addressed.

There is strong evidence that CTL play a critical role in controlling HIV infection in humans. Indeed, it was demonstrated that early intense CTL responses are associated with the initial and sustained reduction of viremia [14,16]. CTL could perhaps prevent HIV infection by eliminating infected cells, as suggested in HIV-exposed but uninfected Gambian women [23]. In addition, $CD8^+$ lymphocytes can inhibit HIV replication in vitro [24–27] probably through soluble suppressor factor such as chemokines [28].

The macaque rhesus model is very interesting since longitudinal and vaccinal studies are easily performed. An inverse correlation was already reported between a vaccine-induced NEF-specific CTL precursor frequency and virus load measured for 8 weeks after challenge by SIV [15]. However, no correlation was established with the clinical outcome in this study, due to the lack of longitudinal observation. Therefore, our aim was to correlate the presence or absence of CTL with the cellular viremia over long periods of time and with the clinical outcome, in two cohorts of infected or vaccinated and then challenged macaques.

Altogether a favorable clinical outcome was observed in 12/13 CTL responders of these two cohorts, all but one being disease free for 24 months or more, while 1/13 developed AIDS at month 19. By contrast 10/11 low CTL responders had AIDS between 4 and 21 months post infection. There is a statistically significant difference between these two groups of macaques (responders or low/no responders) in terms of survival ($P < 0.001$).

In conclusion, this study suggests that vaccine-induced CTL responses have contained SIV replication without eliminating the virus. Despite a lack of exact determination of mechanisms of viral suppression, it

Table 4
Titers of antibodies anti-NEF and GAG peptides in the 12 immunized macaques with lipopeptides

Macaque #	Peptides[a]						
	NEF 101–126	NEF 125–147	NEF 155–178	NEF 201–225	NEF 221–247	GAG 165–195	GAG 246–281
RS 4	**2600**	450	960	600	**2000**	900	800
RS 6	470	470	**3200**	**1500**	**3300**	**3100**	**4000**
RS 7	460	450	440	440	500	450	920
RS 17	**5000**	600	420	**1500**	**50000**	**3000**	**4000**
RS 20	390	450	490	450	**6000**	**4800**	**4800**
RS 21	**2000**	600	420	440	430	**4200**	**3300**
RS 23	460	0	320	460	380	840	450
RS 22	**3800**	700	440	**2600**	**7000**	**4500**	**33000**
Q 50	**3400**	**1600**	**6000**	**1000**	**3000**	**3400**	**4700**
Q 51	**2500**	400	**3000**	450	**4000**	**3300**	**4900**
Q 53	**3400**	340	420	390	800	**3600**	**3000**
Q 54	500	380	400	410	**4000**	**2000**	**5000**

[a]Plate wells were coated with each free peptide corresponding to immunizing lipopeptides.

appears that CTL could have the potential to reduce both early and late viral load. Although there has been only one previous indication that anti-NEF CTL responses would be especially advantageous [15], this protein may be a particularly good candidate to induce protective CTL since NEF is expressed early in infected target cells.

Acknowledgments

This work was supported by the Agence Nationale de Recherche sur le SIDA, and L.M. was supported by SIDACTION/Ensemble contre le SIDA and European Community fellowships.

References

[1] Rötzschke, O., Falk, K., Deres, K., Schild, H., Norda, M., Metzger, J., Jung, G. and Rammensee, H.G. (1990) Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature 348, 252–254.

[2] Kägi, D., Ledermann, B., Bürki, K., Seiler, P., Odermatt, B., Olsen, K.J., Podack, E.R., Zinkernagel, R.M. and Hengartner, H. (1994) Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature 369, 31–37.

[3] Cannon, M.J., Openshaw, P.J.M. and Askonas, B.A. (1988) Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. J. Exp. Med. 168, 1163–1168.

[4] Baenziger, J., Hengartner, H., Zinkernagel, R.M. and Cole, G.A. (1986) Induction or prevention of immunopathological disease by cloned cytotoxic T cell lines specific for lymphocytic choriomeningitis virus. Eur. J. Immunol. 16, 387–393.

[5] Gotch, F.M., Nixon, D.F., Alp, N., McMichael, A.J. and Borysiewicz, L.K. (1990) High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals. Int. Immunol. 2, 707–712.

[6] Koenig, S., Earl, P., Powell, D., Pantaleo, G., Merli, S., Moss, B. and Fauci, A.S. (1988) Group-specific, major histocompatibility complex class I-restricted cytotoxic responses to human immunodeficiency virus 1 (HIV-1) envelope proteins by cloned peripheral blood T cells from an HIV-1-infected individual. Proc. Natl. Acad. Sci. USA 85, 8638–8642.

[7] Lahmamedi-Cherradi, S., Culmann-Penciolelli, B., Guy, B., Kieny, M.P., Dreyfus, F., Saimot, A.G., Sereni, D., Sicard, D., Lévy, J.P. and Gomard, E. (1992) Qualitative and quantitative analysis of human cytotoxic T lymphocyte responses to HIV-1 proteins. AIDS 6, 1249–1258.

[8] Miller, M.D., Lord, C.I., Stallard, V., Mazzara, G.P. and Letvin, N.L. (1990) The GAG-specific cytotoxic T lymphocytes in rhesus monkeys infected with the simian immunodeficiency virus of macaques. J. Immunol. 144, 122–128.

[9] Yamamoto, H., Miller, M.D., Tsubota, H., Watkins, D.I., Mazzara, G.P., Stallard, V., Panicali, D.L., Aldovini, A., Young, R.A. and Letvin, N.L. (1990) Studies of cloned simian immunodeficiency virus-specific T lymphocytes. GAG-specific cytotoxic T lymphocytes exhibit a restricted epitope specificity. J. Immunol. 144, 3385–3391.

[10] Yamamoto, H., Miller, M.D., Watkins, D.I., Snyder, G.B., Chase, N.E., Mazzara, G.P., Gritz, L., Panicali, D.L. and Letvin, N.L. (1990) Two distinct lymphocyte populations mediate simian immunodeficiency virus envelope-specific target cell lysis. J. Immunol. 145, 3740–3746.

[11] Sethi, K.K., Näher, H. and Stroehmann, I. (1988) Phenotypic heterogeneity of cerebrospinal fluid-derived HIV-specific and HLA-restricted cytotoxic T-cell clones. Nature 335, 178–181.

[12] Jassoy, C., Johnson, R.P., Navia, B.A., Worth, J. and Walker, B.D. (1992) Detection of a vigorous HIV-1 specific cytotoxic T lymphocyte response in cerebrospinal fluid from infected persons with AIDS dementia complex. J. Immunol. 149, 3113–3119.

[13] Guillon, J.M., Autran, B., Denis, M., Fouret, P., Plata, F., Mayaud, C.M. and Akoun, G.M. (1988) Human immunodeficiency virus-related lymphocytic alveolitis. Chest 94, 1264–1270.

[14] Borrow, P., Lewicki, H., Hahn, B.H., Shaw, G.M. and Oldstone, M.B.A. (1994) Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. 68, 6103–6110.

[15] Gallimore, A., Cranage, M., Cook, N., Almond, N., Bootman, J., Rud, E., Silvera, P., Dennis, M., Corcoran, T., Stott, J., McMichael, A.J. and Gotch, F. (1995) Early suppression of SIV replication by CD8+ nef-specific cytotoxic T cells in vaccinated macaques. Nature Med. 1, 1167–1173.

[16] Koup, R.A., Safrit, J.T., Cao, Y., Andrews, C.A., McLeod, G., Borkowsky, W., Farthing, C. and Ho, D.D. (1994) Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. 68, 4650–4655.

[17] Bourgault, I., Villefroy, P., Beyer, C., Aubertin, A.M., Lévy, J.P. and Venet, A. (1993) Cytotoxic T-cell response and AIDS-free survival in simian immunodeficiency virus-infected macaques. AIDS 7, S73–S79.

[18] Venet, A., Bourgault, I., Aubertin, A.M., Kiény, M.P. and Lévy, J.P. (1992) Cytotoxic T lymphocyte response against multiple simian immunodeficiency virus (SIV) proteins in SIV-infected macaques. J. Immunol. 148, 2899–2908.

[19] Bourgault, I., Chirat, F., Tartar, A., Lévy, J.P., Guillet, J.G. and Venet, A. (1994) Simian immunodeficiency virus as a model for vaccination against HIV. Induction in rhesus macaques of GAG or NEF specific cytotoxic T lymphocytes by lipopeptides. J. Immunol. 152, 2530–2537.

[20] Spouge, J.L. (1992) Statistical analysis of sparse infection data and its implications for retroviral treatment trials in primates. Proc. Natl. Acad. Sci. USA 89, 7581–7585.

[21] Martinon, F., Gras-Masse, H., Boutillon, C., Gomard, E.,

Chirat, F., Deprez, B., Tartar, A. and Lévy, J.P. (1992) Immunization of mice with lipopeptides bypasses the prerequisite for adjuvant: immune response of Balb/c mice to human immunodeficiency virus envelope glycoprotein. J. Immunol. 149, 3416–3422.

[22] Moog, C., Wick, A., Le Ber, P., Kirn, A. and Aubertin, A.M. (1994) Bicyclic imidazo derivatives, a new class of highly selective inhibitors for the human immunodeficiency virus type I. Antiviral Res. 24, 275–288.

[23] Rowland-Jones, S., Sutton, J., Ariyoshi, K., Dong, T., Gotch, F., McAdam, S., Whitby, D., Sabally, S., Gallimore, A., Corrah, T., Takiguchi, M., Schultz, T., McMichael, A. and Whittle, H. (1995) HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Med. 1, 59–64.

[24] Kannagi, M., Chalifoux, L.V., Lord, C.I. and Letvin, N.L. (1988) Suppression of simian immunodeficiency virus replication in vitro by CD8+ lymphocytes. J. Immunol. 140, 2237–2242.

[25] Mackewicz, C.E., Ortega, H.W. and Levy, J.A. (1991) CD8+ cell anti-HIV activity correlates with the clinical state of the infected individual. J. Clin. Invest. 87, 1462–1466.

[26] Tsubota, H., Lord, C.I., Watkins, D.I., Morimoto, C. and Letvin, N.L. (1989) A cytotoxic T lymphocytes inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes. J. Exp. Med. 169, 1421–1434.

[27] Walker, C.M., Moody, D.J., Stites, D.P. and Levy, J.A. (1986) CD8+ lymphocytes can control HIV infected in vitro by suppressing viral replication. Science 234, 1536–1566.

[28] Cocchi, F., DeVico, A.L., Garzino-Demo, A., Arya, S.K., Gallo, R.C. and Lusso, P. (1995) Identification of RANTES, MIP-1a, and MIP-1b as the major HIV-suppressive factors produced by CD8+ T cells. Science 270, 1811–1815.