

CD28 T lymphocytes and impact of viral infection in childrenNahla M Abd-Elaziz¹, Ateyat A Ateya², Amal Farouk³

Department of Clinical Pathology¹ and Pediatrics², Faculty of Medicine for girls, Alazhar University
 Clinical Pathology³, Faculty of Medicine, Ain shams University
nahlashankeer@yahoo.com

Abstract: Background and aim: Cellular innate immunity plays a crucial role in viral infection. During active viral infection, viral pathogens modulate host immune system leading to immuno-senescence and immuno-suppression. Viral illness is usually controlled by a range of innate and adaptive immune-effector mechanisms. Little details are available on the frequencies, phenotype and function of antiviral T lymphocytes in pediatric populations. It was demonstrated that differentiation of CD28⁺ T lymphocytes into CD28 null T lymphocytes result in immuno-senescence and impairment of immune response. Our study aimed to know the link between viraemia, CD28 T lymphocytes and immuno-suppression during childhood. **Patients and methods:** This is cross sectional comparative study conducted at Al-Zahraa University Hospital, between April 2012 and July 2012. The study included twenty acute viremic children, 20 chronic viremic children and 20 healthy control children. Using flow cytometry, we measured the percentage of the following phenotypes in the three studied groups, CD4 T helper lymphocytes, CD8 cytotoxic T lymphocytes and CD28⁺ T lymphocytes. In addition to the percentage of CD56⁺ natural killer cells. **Results:** We found that the mean percentage of CD28⁺ T lymphocytes of acute and chronic viremic groups was significantly lower than those of control group. Also, we observed significant difference between the three studied groups as regards CD4%, CD8% and CD4/CD8 ratio. Furthermore, chronic viremic group showed significantly lower frequency of CD28⁺ T lymphocyte than those of acute viremic group. Also, chronic viremic group showed strong correlation between the percentage of CD28⁺ T lymphocytes and CD4/CD8 ratio. **Conclusion:** The significant decrease in CD28⁺ naïve T lymphocytes of viremic group confirm the role of viremia in differentiation of CD28⁺ T lymphocytes into senescent CD28 null T lymphocytes. Also, striking reduction of CD28⁺ T cells in chronic viremic group revealed an association between chronic viremia and expansion of CD28 null T lymphocytes. Interestingly, the strong correlation between the percentage of CD28⁺ T lymphocytes of chronic viremic group and CD4/CD8 ratio which is considered as a predictor of immuno-efficiency clarify the interplay between viremia, CD28 molecules and immuno-suppression. Also, our study suggests that vaccine strategies may be available to support adaptive immune response against viral pathogens.

[Nahla M Abd-Elaziz, Ateyat A Attia, Amel Farouk: **CD28 T lymphocytes and impact of viral infection in children.** *J Am Sci* 2012;8(10):801-807]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 108

Key words: CD28, antiviral lymphocyte, viral infection

1. Introduction

Childhood viral infections are widespread in developing countries, the viral infections are self-limited in the vast majority of patients, although resolutions may take several weeks (*Varani and Landini, 2011*).

Some viral pathogens such as Epstein-Barr virus and cytomegalovirus (CMV) undergo latency and periodic reactivation causing chronic viremia (*Daniel et al., 2006*).

Within few days after onset of viral illness, T lymphocytes recognize replication of viral pathogens and develop immune response (*Kaushansky et al., 2011*).

The polyclonal expansion of T lymphocyte against viral pathogen is evidenced by reactive lymphocytosis which is considered as hematological hall mark of viral infections, but they are not always found (*Hudnall et al., 2003*).

The exclusively expression of CD4 or CD8 lymphocytes defines two major T lymphocytes subsets. Blood T lymphocytes that express CD8 had been designated as suppressor or cytotoxic or cytolytic T lymphocytes. While T cells that solely express CD4 are defined as T helper lymphocytes (Th) (*Kipps, 2011*).

T helper cells are divided into at least two subsets, Th₁ and Th₂, each of them is able to elaborate distinctive profile of cytokines. For example, Th₁ lymphocytes are the major source of γ interferon while Th₂ elaborate IL-4 (*Ogarra and Robinson, 2004*).

CD28 receptors are co-stimulatory molecules that expressed on T lymphocytes, CD28 molecule may enhance intracellular signaling of T lymphocyte by stabilizing and prolonging the synapse between T lymphocytes and antigen presenting cells (APC). Also, CD28 molecules have an important role in

activation of B lymphocytes and antibodies production (*Kaushansky et al., 2011*).

According to the presence or absence of CD28 molecules, two T lymphocyte subsets were designated, CD28⁺ T cells and CD28 null cells (*Diaz et al., 2012*). The homeostasis between CD28⁺ and CD 28 null T lymphocytes depends on cascade of proliferation and differentiation of T lymphocytes in response to pathogens (*Mojumdar et al., 2012*).

Therefore, our aim was to clarify the relation between viremia, CD28 T lymphocytes and immuno-compression.

2. Patients and Methods

The study comprised 40 patients with viral infection. They were 21 males and 19 females

, their ages ranged from one to 12 years. Twenty age – and sex – matched healthy children were included in the study as controls. Exclusion criteria were chronic systemic disease, malnutrition, immuno-suppression drugs, acute and chronic bacterial infection.

Patients included in the study were attending the Pediatric Clinic at Al-Zahraa University hospital with manifestations of acute respiratory viral infection including low grade fever, coryza or runny nose, sore throat, strider and cough. Manifestations suggestive of chronic viral infections were fatigue, fever, malaise, sore throat, myalgia, headache, lymphadenopathy and hepatosplenomegaly. Patients with viral infection were subjected to the following investigations to confirm the diagnosis; CBC for lymphocytosis or reactive lymphocytes, -ve CRP and -ve throat culture. Specific viral serology, IgG and IgM were done for selected cases to confirm the diagnosis of cytomegaloviral, and Epstein-Barr viral infection.

The study was conducted at Al-Zahraa University hospital from April 2012 to July 2012. An informed consent was obtained from parents of all children included in the study. The viremic and control groups were evaluated for the percentage of CD4, CD8, CD28⁺ T lymphocytes and CD56 natural killer cells using flowcytometry.

Methodology:

Samples:

Venous blood was taken in sterile EDTA vacutainers. We used three test tubes for each sample. The 1st tube contain 20 microliteres of IO test conjugated fluoresceine isothiocyanate (FITIC) labeled CD4 monoclonal Abs second and 20 microliteres IO test conjugated phycoerythrin (PE) labeled CD8 monoclonal, 2nd tube contain 20 microliteres FITIC labeled CD28 monoclonal Abs. and third tube contain 20 microliteres FITIC CD56. Add 100 microliteres of the test sample to each tube, vortex the tubes gently. The tubes were incubated for 20 minutes at room

temperature in the dark then add 1 mL of Fix and lyse mixture, vortex immediately for one second and incubated again for 10 minutes in the dark at room temperature. Centrifugation at low (200g) speed for 5 minutes.

The supernatant were aspirated, the cell pellet resuspended in residual fluid, 2 mL of phosphate buffer saline was added to each tube and mixed with cell suspension. The suspension was centrifuged at 200 g for 5 minutes, the supernatant was aspirated then the samples were passed through the flow-cytometer. For analysis, gates were set around lymphocytes on the bases of the forward and side scatter profile.

3. Results

Forty virally infected children and 20 non-infected healthy children were studied. The virally infected children were divided into two groups, 20 children with acute respiratory viral infection and 20 children with chronic viral infection (CMV or EBV).

The descriptive statistics of control, acute viremic group and chronic viremic group were shown in tables and figures 1,2,3(respectively).

They included the percentage of CD4 (T helper) lymphocytes, CD8 (cytotoxic T lymphocytes), CD28⁺ T lymphocytes and CD56 natural killer cells.

Table & figure (4) show the results of acute viremic group as matched with control group. A significant decline in the percentage of CD4 T helper cells was observed ($p < 0.001$), while the mean percentage of CD8 T lymphocyte was significantly increased ($p < 0.002$).

CD4/CD8 ratio of acute viremic group showed significant decrease in comparison with control group ($p < 0.001$).

The mean percentage of CD28⁺ T lymphocytes was significantly lower than that of control group ($p < 0.001$).

The results of chronic viremic group are shown in table & figure (5). We found highly significant decrease of CD4 percentage and significant increase of CD8 percentage compared with control group ($p = 0.00$ and $p \leq 0.002$, respectively). CD4/CD8 ratio was reversed and showed significant decrease as matched with control ($p = 0.00$).

Also, chronic viremic group showed highly significant decrease in frequency of CD28⁺ T lymphocytes in comparison to control ($p = 0.00$).

On comparing the frequency of T lymphocytes subset (CD4%, CD8%, CD28%) and NK cells in acute and chronic viremic group (table & figure(6)), it was observed that chronic viremic group posses lower frequency of CD28⁺ T lymphocytes than acute viremic group ($p = 0.00$). Whereas, no significant difference was observed as regard CD4%, CD8%,

CD4/CD8 ratio and the percentage of natural killer cells ($p = 0.39, p = 0.38, p = 0.609$, respectively).

Correlation studies between CD28⁺ T lymphocyte and CD4/CD8 ratio in acute and chronic viremic groups showed strong correlation between 2

parameters in chronic viremic group ($p \leq 0.009$). While no correlation could be detected between the two parameters in acute viremic group, Table & Figure (7).

Table and figure 1: Descriptive statistics for the control group.

	Control			
	Mean	±SD	Minimum	Maximum
CD4%	44.71	5.99	40	60
CD8%	22.14	6.42	15	32
CD4/CD8 ratio	2.19	0.68	1.3	3.6
Cd56%	6.29	2.13	3	9
CD28⁺%	50.59	3.70	42	57

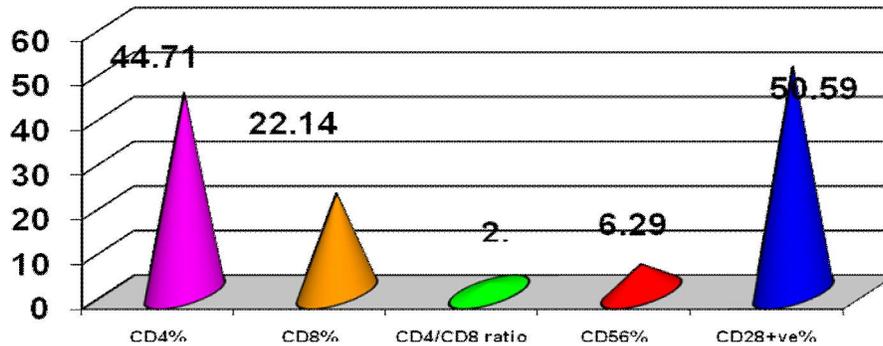


Table and figure 2: Descriptive statistics for the acute group.

	Acute			
	Mean	±SD	Minimum	Maximum
CD4%	32.21	7.53	22	45
CD8%	30.58	6.50	18	40
CD4/CD8 ratio	1.09	0.30	0.6	1.6
Cd56%	6.29	0.74	5.4	7.5
CD28⁺%	37.39	5.00	30.4	46.1

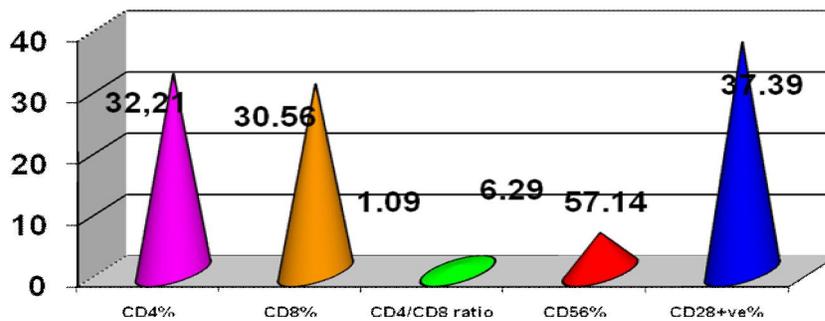


Table and figure 3: Descriptive statistics for the chronic group.

	Chronic			
	Mean	±SD	Minimum	Maximum
CD4%	29.51	8.32	13.5	47
CD8%	33.44	10.38	17	48
CD4/CD8 ratio	0.97	0.45	0.5	2.2
Cd56%	6.14	0.86	5.1	7.8
CD28⁺%	20.51	5.85	10	28.5

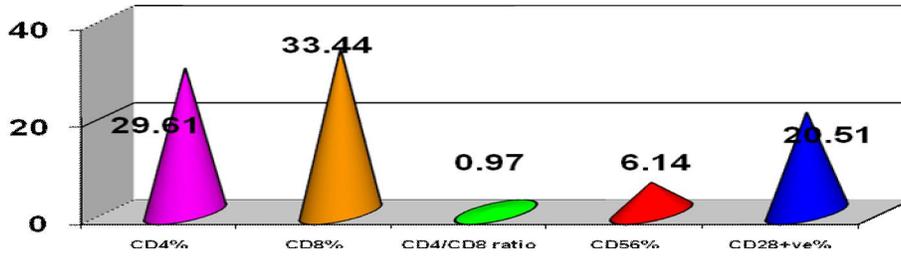


Table and figure 4 : Comparison between acute group and control group.

	Acute group		Control group		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
CD4%	32.21	7.53	44.71	5.99	-4.862	0.001
CD8%	30.56	6.50	22.14	6.42	3.450	0.002
CD4/CD8 ratio	1.09	0.30	2.19	0.68	-5.514	0.001
CD56%	6.29	0.74	6.29	2.13	0.012	0.991
CD28+%	37.39	5.00	50.59	3.70	-7.933	0.001

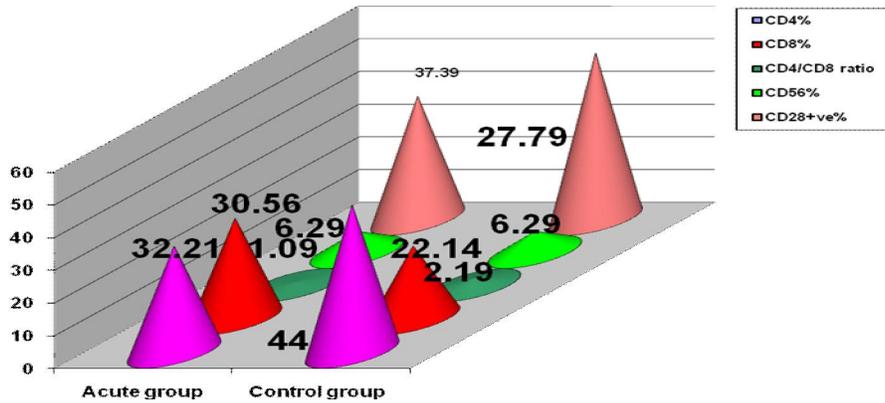


Table and figure 5: Comparison between chronic group and control group.

	Chronic group		Control group		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
CD4%	29.61	8.32	44.71	5.99	-5.514	0.000
CD8%	33.44	10.36	22.14	6.42	3.467	0.002
CD4/CD8 ratio	0.97	0.45	2.19	0.68	-5.550	0.000
CD56%	6.14	0.86	6.29	2.13	-0.245	0.809
CD28+%	20.51	5.85	50.59	3.70	-16.266	0.000

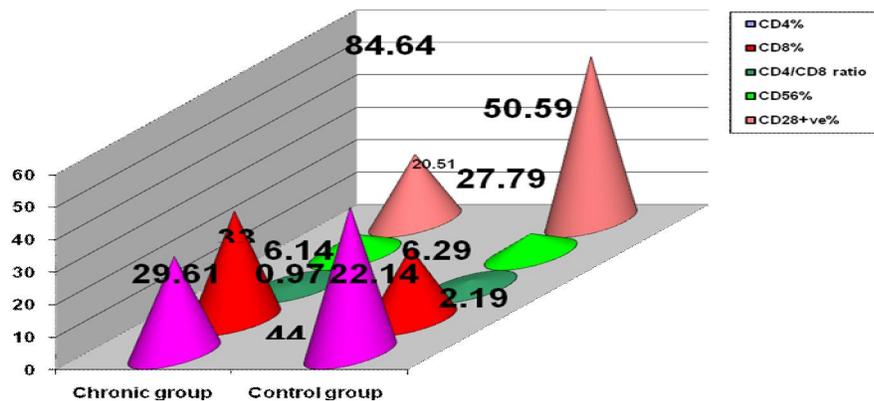


Table and figure 6 : Comparison between Acute group and Chronic group.

	Acute group		Chronic group		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
<i>CD4%</i>	32.21	7.53	29.61	8.32	0.870	0.392
<i>CD8%</i>	30.56	6.50	33.44	10.36	-0.879	0.388
<i>CD4/CD8 ratio</i>	1.09	0.30	0.97	0.45	0.857	0.399
<i>CD56%</i>	6.29	0.74	6.14	0.86	0.518	0.609
<i>CD28+%</i>	37.39	5.00	20.51	5.85	8.210	0.000

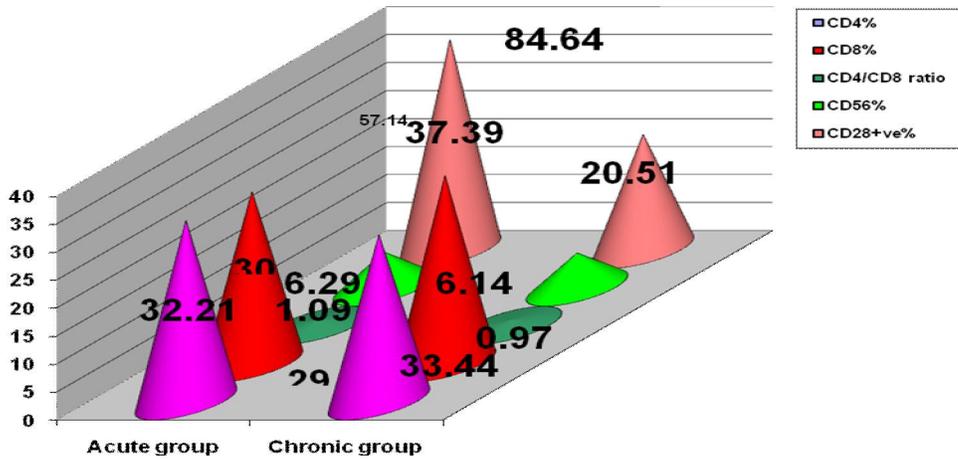


Table and figure 7: Correlation between CD4/CD8 and CD28 in the chronic group

	CD28	
	r	p-value
<i>CD4/CD8</i>	0.669**	0.009

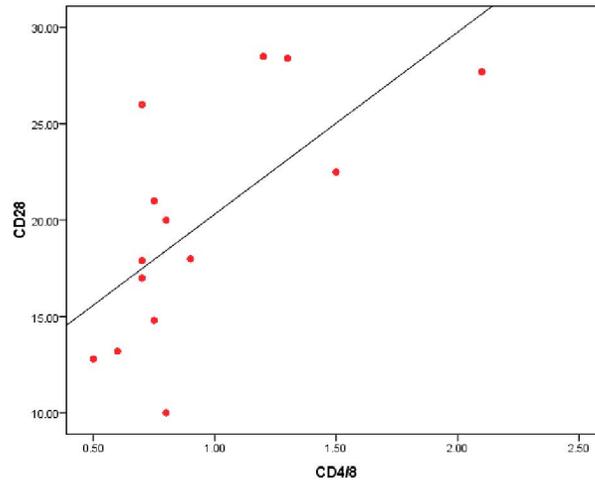


Table 8: Correlation between CD4/CD8 and CD28 in the acute group

	CD28	
	R	p-value
<i>CD4/CD8</i>	-0.025	0.933

4. Discussion

The aim of our study was to define the relation between viral infections, T lymphocytes subsets, mainly CD28⁺ T lymphocytes and immuno-suppression in pediatric population.

We hypothesize that, viremia lead to senescence of T lymphocytes and immuno-suppression via loss of CD28 molecules and subsequent differentiation of active CD28⁺ T lymphocytes into senescent CD28 null T lymphocytes. To confirm this hypothesis, we studied the frequencies of CD28⁺ T lymphocytes in three groups (acute viremic children, chronic viremic children and healthy children). Also, CD4 T helper, CD8 cytotoxic T lymphocytes and CD4/CD8 ratio were measured as predictor of immuno-efficiency.; moreover the percentage of CD56 natural killer cells was measured in the three studied groups to detect their role in immuno-pathogenesis of viral illness.

Our results showed that CD4% as well as CD4/CD8 ratio was significantly decreased in both acute and chronic viremic groups matched with control group which agreed with previous studies by *Lang et al. (2003) and Kaushansky et al. (2011)*.

CD4 percentage and CD4/CD8 ratio were powerful indicators for efficiency of immune system. Immune suppression was evident by CD4 depletion and reversed CD4/CD8 ratio (*Kipps, 2011, Lederman et al., 2011*).

Also, increased frequency of CD8 was observed in both viremic groups as compared to control group. Many previous researches have considered CD8 cytotoxic T lymphocytes as virus specific lymphocyte (*Dunne et al., 2003; Komatsu et al., 2006 and Diaz et al., 2012*).

As regard CD56⁺ natural killer cells (NK), our findings showed no significant difference between studied groups. Those findings were contradictory to the previous experimental observations that postulated an important role of natural-killer cells in immuno-pathogenesis of viral infections (*Trinchieri and Lanier, 2011*).

Based on analysis of CD28 expression on T lymphocytes, CD28⁺ T cells are considered to be naïve T cells whereas CD28 null T cells are described as senescent T lymphocytes (*Diaz et al., 2012*).

In acute viremic group, CD28⁺ naïve T lymphocytes were significantly decreased as compared to healthy control. Also, striking decline was observed in CD28⁺ T lymphocytes of chronic viremic group as matched to healthy control group. Other authors showed that cytomegalovirus infection (CMV) elects expansion of CD28 null T lymphocytes and subsequent premature aging of immune system (*Komatsu et al., 2006; Daniel et al., 2006 and Varani & Landini, 2011*).

On comparing both viremic groups, chronic viremic group had a lower frequency of CD28⁺ T

lymphocytes than acute viremic group. A study by *Diaz et al. (2012)*, supported a role of chronic viremia in exhaustion and senescence of T lymphocytes during childhood.

Comprehensive survey of T cells phenotype highlights the complex interaction between CMV infection, premature senescence of T lymphocytes and immune suppression (*Appay et al., 2011*).

In addition, *Sirnivasula et al. (2011)* demonstrated the differential effect of HIV load on senescence of naïve active T lymphocytes.

In chronic viremic group, the correlation studies showed significant correlation between the decline in the percentage of CD28⁺ T cells and CD4/CD8 ratio. Those findings confirmed the role of persistent viremia in excessive differentiation of CD28⁺ T cells into exhausted CD28 null T cells and immune-suppression that agree with previous studies reported by *Varani and Landini (2011), and Saucte et al. (2011)*.

Our results together with previous observations clearly indicate the vital role of CD28⁺ T lymphocytes in induction of immune response and controlling viremia (*Marcel et al., 2005 and Komutsu et al., 2006*).

Also, those data confirmed that chronic viremia and prolonged exposure to viral pathogens will compromise the viability of effector cells via replicative senescence of CD28⁺ naïve T lymphocytes into CD 28 null T lymphocytes (*Effros et al., 2008; Deeks et al., 2009; Sasaki et al., 2011 and Deeks et al., 2012*).

The expansion of CD28 null T cells pool leads to failure of immune response to new pathogens and immuno-compromised host (*Sauce et al., 2009; Dock et al., 2011; Deeks et al., 2012 and MoJumdar et al., 2012*).

In conclusion, all data proved the role of CD28 molecules in immunological consequence of viremia which may suggest CD28 molecules vaccine strategies aimed at priming and boosting T cells against viral pathogens.

References

1. **Appay V, Fastenackels S and Katlama C (2011):** Old age and anti-cytomegalovirus immunity are associated with altered T cell reconsultation in HIV. *AIDS*; 25: 1813-1822.
2. **Danile L, Wherry E and Zhu B (2006):** Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*; 439: 682-686.
3. **Deeks S, Verdin E and McCune J (2012):** Immunosenescence and HIV. *Current opinion in Immunology*; 24: 1-6.

4. **Deeks SG and Phillips AN (2009):** HIV infection, antiviral treatment, aging and non-AID related morbidity. *BMJ*; 338 (a): 3172.
5. **Diaz L, Mendez G and Correa R (2012):** Detectable viral load aggravates immunosence features of CD8-T-cell subset: in vertically HIV-infected children. *J Acquir Immune Defic Syndr*; 60 (5): 447-454.
6. **Dock JN and Effros RB (2011):** Role of CD8 T cell replicative senescence in human aging and in human aging and HIV mediated immunosence. *Aging Disease*; 2: 382-397.
7. **Dunn W, Chou C and Paterson D (2003):** Functional profiling of human cytomegalovirus genome. *Prac Natl Acad SCI, USA*; 100: 14223-14228.
8. **Effros RB, Fletcher CV and Gebo K (2008):** Aging and infectious diseases. *Clin Infect Dis*; 47: 542-553.
9. **Hudnall SD, Patel JU and Schwab H (2003):** Comparative immunophenotypic features of EBV positive and EBV negative atypical lymphocytosis. *Cytometry*; 55 (B): 22.
10. **Kaushansky K, Lichtman M and Beutler E (2011):** Function of T lymphocytes: T-cell receptors for antigen. 8th ed. *Williams Hematology*; 3 (78): 1118-1130.
11. **Kipps J (2011):** Lymphocytosis and lymphopenia. *Williams Hematology*; 3 (81): 1141-1151.
12. **Komatsu H, Inui A, Sogo T and Fujisawa T (2006):** Large scale analysis of pediatric antiviral CD8⁺ T cell populations reveals sustained, functional and mature responses. *Immunity and Aging*; 3 (11): 1-11.
13. **Lang CG; Lederman MM and Medvic K (2003):** Nadir CD4⁺ T cell count and numbers of CD28⁺ CD4⁺ T cells predict functional response to immunization in chronic HIV infection. *AIDS*; 17: 2015-2023.
14. **Lederman MM, Ca'abrese L and Funderburg NT (2011):** Immunological failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis*; 204: 1217-1226.
15. **Marcel W, Vamer T and Bruce S (2005):** CD28 is required for optimal induction, but not maintenance, of vaccine-induced immunity to *Blastomyces dermatitis*. *Infection & Immunity*; 73 (11): 7436-7441.
16. **MoJumdar K, Vajpayee M and Chauhan NK (2012):** Altered T cell differentiation associated with loss of CD27 and CD28 in HIV infected Indian individuals. *Cytometry B Clin Cytom*; 82: 43-53.
17. **Ogarra A and Robinson D (2004):** Development and function of T helper 1 cells. *Adv Immunol*; 83: 133.
18. **Sasaki S, Sullivan M and Narvaez CF (2011):** Limited efficacy of inactivated influenza vaccine in elderly individuals is associated with decreased production of vaccine-specific antibodies. *J Clin Invest*; 121: 3109-3119.
19. **Sauce D, Larsen M and Fastenackels S (2009):** Evidence of premature immune aging in patients thymectomized during childhood. *J Clin Invest*; 119: 3070-3078.
20. **Sauce D, Larsen M and Fastenackels S (2011):** HIV progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood*; 117: 5147-5151.
21. **Sirnivasula S, Lampickj RA and Adelsberger JW (2011):** Differential effects of HIV viral load and CD4 count on proliferation of naïve and memory CD4 and CD8 T lymphocytes. *Blood*; 118: 262-270.
22. **Trinchieri G and Lanier L (2011):** Function of natural killer cells. *Williams Hematology*; 3 (79): 1131-1135.
23. **Varani S and Landini M (2011):** Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpes Viridae*; 2 (6): 1-14.

9/29/2012