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# Elevated PD-1 expression and decreased telomerase activity in memory T cells of patients with symptomatic Herpes Zoster infection

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**Abstract**

We investigated PD-1 levels on VZV-specific CD8+ T-cells of patients with zoster and the effect of PD-1 on the telomerase activity. CD3, CD8, CD137 and PD-1 expressions were analyzed on PBMCs from 9 symptomatic and 5 asymptomatic individuals. The effect of PD-1 blockade at the time of stimulation on the telomerase activity of non-senescent CD57-CD45RO+CD8+CD3+ memory T-cells was evaluated. PD-1 was elevated on CD8+ T-cells in patients. The frequency of PD-1+ and CD137- cells in total CD3+CD8+ T cells of patients was elevated compared to controls. Telomerase activity of non-senescent memory T-cells was lower than that of controls. Blockade of PD-1 at the time of stimulation increased telomerase activity of non-senescent memory T-cells, accompanied by increased CD137 expression. Low telomerase activity of the patients with reactivated zoster could be partially overcome by blocking PD-1 pathway.

***Key words:*** Memory, PD-1, T cell, Telomerase.

## Introduction

CD8+ T cells are committed to defend the body against pathogens and tumors. Frequent antigenic stimulation of CD8+ T cells due to high antigenic load, persistent inflammation and aging leads to two distinct but related processes, namely exhaustion and senescence, which result in impaired T cell function (1, 2) . Senescent T cells are terminally differentiated cells that express CD57, killer-cell lectin like receptor G1 (KLRG1), but do not express CD28 and CD27 molecules (2-4) . Shortened telomeres and decreased telomerase activity are the hallmarks of senescent cell (2, 4) . Cell cycle arrest, lack of proliferation, effector function integrity (cytokine secretion, killing target cell), DNA damage, high levels of p16, p21 and p53 molecules as well as increased expression of transcription factor T-bet, are other features of most senescent T cell (5) .

Major driver of CD8+ T cell exhaustion in effector/memory T cells is chronic antigenic stimulation that renders these cells functionally impaired. Loss of proliferation capacity, altered cytokine secretion and decreased cytotoxicity are accompanied by coexpression of several inhibitory receptors such as Program Death-1 (PD-1), Cytotoxic T lymphocyte Antigen-4 (CTLA-4), T cell immunoglobulin mucin-3 (Tim-3), Lymphocyte-activation gene 3 (LAG-3) and 2B4 in the exhausted T cells (6-8) . In contrast to senescent cells, exhausted T cells need constant antigenic stimulation for their survival (6) . There are reports indicating a relationship between these two processes, particularly emphasizing on shared phenotypic markers (2) . In uninfected aged mice the decreased T cell precursors and altered functional quality of T cells are reported. Such changes include altered transcription profile similar to exhausted CD8+ T cells and expression of PD-1, CD160, LAG-3 and 2B4 on $CD44^{Hi}$ CD8+ T cell (9) . The coexpression of exhaustion and senescence markers on CD8+ T cell may indicate the overlap between these pathways. In contrast, by considering the cause, transcription profile and expression of inhibitory receptors, other investigators have suggested that exhaustion and senescence are distinct failure mechanisms in T cell ontogeny and function (5) .

Since the first notion of exhausted T cells in chronic Lymphocytic choriomeningitis virus (LCMV) infection in mice, exhausted CD8+ T cells have been reported in several chronic viral infections in human and mice (10) . Not surprisingly, exhausted CD8+ T cells are investigated the most in chronic HIV infection where virus-specific CD8+ T cells express high level of PD-1, have impaired function, decreased cytokine production and granzyme-B secretion (7) .

Herpes Simplex virus-1 (HSV-1), a member of herpes simplex virus family goes through latency periods in human ganglia (11) . Recent evidence has shown that during latency, HSV-1 specific CD8+ T cells overexpress PD-1 and demonstrate impaired cytokine secretion capacity (12) . It is very likely that persistent antigenic stimulation by latent HSV-1 antigens may have led to CD8+ T cell exhaustion (12) . *Varicella zoster* virus (VZV), another member of herpes simplex virus family, is responsible for two distinct diseases; chicken pox in the childhood and herpes zoster in the

adulthood (13, 14) . *Varicella zoster* virus, too, becomes latent in human ganglia and continues to express a few antigens such as IE62 and IE63 during latency (15, 16) . However, there is not much information regarding the extent of T cell exhaustion and PD-1 expression on VZV-specific CD8+ T cells in zoster infection. On the contrary, elevated PD-1 expression is already reported on Hepatitis C virus (HCV), Hepatitis C virus (HBV), and Cytomegalovirus (CMV) specific CD8+ T cells (7, 17, 18) .

PD-1, an inhibitory surface receptor belongs to the CD28 superfamily and is expressed on T cells, natural killer T (NKT) cells, T regulatory (Tregs), and B cells (19) . The molecule is by far the most respected marker of exhausted CD8+ T cells in chronic infections and tumors (20, 21) . Interaction of PD-1 with each of its two ligand, PDL-1 and PDL-2, inhibits T cell proliferation as well as IFN-γ and TNF-α cytokines secretion (22) . PD-1 inhibits T-cell functions by activation of phosphatase SHP2 that dephosphorylates and inhibits PI3K pathway (23, 24) .

While CD57+ senescent T cells have decreased telomerase activity and shortened telomeres, they still seem to be functional (25, 26) . Previous studies have shown that blockade of inhibitory molecules, KLRG1 and PD-1, cannot reverse the telomerase defect in these cells (27, 28) . The inhibition of p38 signaling in CD45RA+CD27- T cells, however, can significantly enhance the proliferation, telomerase activity and survival of these cells after T cell receptor (TCR) activation (29) .

Recent evidence demonstrates that repeated antigenic stimulation decreases telomerase activity of CD8+ T cells (30, 31) . In chronically HIV-infected individuals with the progressor phenotype, HIV specific CD8+ T cells express higher levels of PD-1, decreased telomerase activity and loss of telomere length as compared to HIV viral load controllers (30) . Blockade of PD-1/PDL-1 interaction restores telomerase activity and telomere length as well as IFN-γ secretion and proliferation capacity in HIV infection (30) . Therefore, PD-1 pathway may influence telomerase activity, proliferation and survival of CD8+ T cells in individuals chronically infected with other infective agents.

In this study, we investigated the levels of PD-1 on the IE62- and IE63-specific CD3+CD8+ T cells of VZV infected individuals and analyzed telomerase activity in non-senescent CD57-CD45RO+ memory CD3+CD8+ T cells in different TCR-stimulatory conditions. We found that the low telomerase activity of CD57-CD45RO+ memory CD3+CD8+ T cells can be partially rescued by PD-1 blockade.

## Materials and methods

### *Study subjects*

After informed consent, 12 ml heparinized blood was obtained by venipuncture from 9 patients with symptomatic herpes zoster infection (2 men and 7 women aged 38-71 yrs) as well as 5 asymptomatic control individuals with a history of chicken pox (2 men, 3 women aged 26-47 yrs). Peripheral Blood Mononuclear Cells (PBMCs) were isolated by density gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare Europe GmbH) and cryopreserved in 10% dimethyl sulfoxide (DMSO; Sigma- Aldrich) 90% fetal bovine serum (FBS Biosera, UK).

### *Peptide pool dilution*

Lyophilized peptide pools (15-mers with 4-aa overlaps) of *varicella zoster* virus Immediate Early (IE)62 and IE63 antigens (PepMix VZV IE62 pool A and B (Q8AZM1; Swiss prot, JPT, GmBH), PepMix VZV IE63 (Q77NN7-1; Swiss prot, JPT, GmBH) were used to stimulate PBMCs. IE62 pools A and B contained 163 peptides and IE63 pool consisted of 67 peptides. The peptides were diluted in 100% DMSO solution to the concentration of 50, 10, 1 mg /ml, respectively, and then diluted in fresh RPMI-1640 and used in final concentration of 200 ng/µl right before use.

### *Flowcytometry*

Thawed PBMCs ($5\times10^5$ cells) were washed and stimulated with 7 µg/ml of peptide in RPMI-1640 medium containing 10% FBS overnight at 37ºC. Non-specific stimulation of PBMCs was done using 250 ng/ml of phorbol myristate acetate and 50 ng/ml of Ionomycin (PMA/ION) overnight. Then the cells were washed and stained for surface markers using conjugated antibodies: anti-CD3-PerCP (BD Pharmingen), anti-CD8-PE (BD Pharmingen), anti-CD137-APC (BD Pharmingen), anti-PD-1-FITC (Abcam) and incubated in 4°C for 30 minutes. The cells were subsequently washed and resuspended in PBS containing 10% FBS. We acquired $10^5$ events for each sample by FACS-Calibur flowcytometer. Lymphocytes were gated by forward and side scatter and analyzed for surface marker expression (supplementary figure 1). Flowcytometry analysis was done by flowjo software (version 7.6.2).

### *Memory T cell isolation and telomerase activity*

Memory CD8+CD57-CD45RO+ T cells were purified from PBMCs by negative selection using Magnetic-activated cell sorting (MACS) technique (Miltenyi-Biotec GmbH, CD8+Memory T cell isolation kit # 130-094-412). The purity of cells was measured using anti-CD45RO-PE (Dako, Denmark), CD8-APC (BD Pharmingen), CD3-FITC (BD Pharmingen) and was documented to be more than 90%. After negative selection, the cells were suspended in RPMI-1640 medium containing 10% FBS and stimulated with plate coated anti-CD3 (BD Pharmingen) (1 µg/ml) with and without 1 µg/ml soluble anti-CD28 (BD Pharmingen) where indicated. PD-1 signaling was blocked at the time of stimulation using 1 µg/ml blocking anti-PD-1 antibody (BD Pharmingen). The cells were then incubated at 37°C in 5% CO2 atmosphere for 16 hrs. Cells were washed post-incubation and telomerase activity was measured using a PCR-ELISA assay (Telo TAGGG Telomerase PCR ELISA, Roche GmbH: Cat. No. 11854666910) according to manufacturer's instructions.

### *Statistical analysis*

Statistical analysis was performed using Prism GraphPad (version 5). Mann-Whitney test and Wilcoxon signed rank test were used for non-parametric comparison of the groups. P-values less than 0.05 were considered significant.

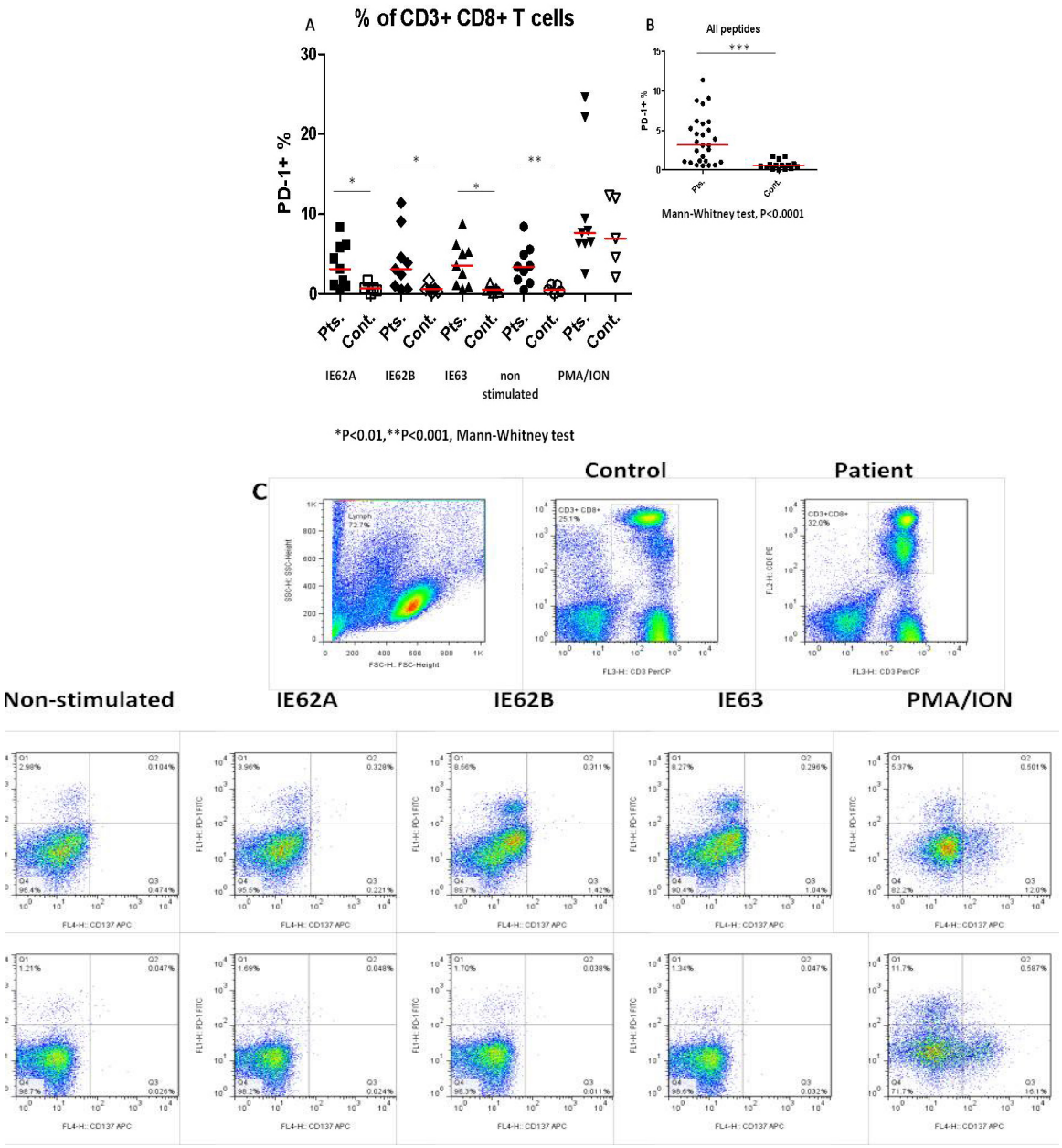


**Figure 1. Frequency of PD-1 expressing CD3+ CD8+ T cells of patients after stimulation with VZV peptides compared to controls. A.** Both in non-stimulated condition and after peptide stimulation the frequency of PD-1+ CD3+CD8+ T cells was higher in patients with reactivated herpes zoster infection compared to controls. However, non-specific PMA/Ionomycin stimulated T cells did not show a difference in the frequency of PD-1 expressing T cells. **B.** The Frequency of PD-1+ T cells stimulated by all VZV peptides in the total CD3+CD8+ PBMCs of patients was also significantly higher in patients compared to controls. **C.** Representing dot plots of the VZV peptide stimulated T cells of patients and controls. Peripheral blood mononuclear cells from 9 patients and 5 controls were stimulated with 7 µg/ml of each of the VZV IE63 and IE62 (A and B) pool overlapping 15-mer peptides or with 250 ng/ml of phorbol myristate acetate and 50 ng/ml of Ionomycin (PMA/ION) overnight. The cells were then stained with fluorochrome-conjugated antibodies against CD3, CD8, CD137 and PD-1 and the cell frequencies were acquired by a FACS-Calibur flowcytometer. The final concentration of each peptide in the pool was 200 ng/ml. The cell percentages in the total CD3+CD8+ PBMCs are shown.

## Results

### *The frequency of PD-1 expressing CD8+CD3+ T cells is higher in the peripheral blood of patients with reactivated zoster compared to controls*

We investigated the frequency of CD8+CD3+ T cells expressing PD-1 by four-color flowcytometry subsequent to stimulation with overlapping pool peptides (IE62A, IE62B and IE63). Our result indicated that both in non-stimulated condition (p=0.007) and after peptide stimulation (p= 0.0190 for IE62A, p= 0.0190 for IE62B, p= 0.0070 for IE63) the percentage of PD-1+CD3+CD8+ T cells was higher in patients with reactivated herpes zoster infection compared to controls (figure 1a). By considering the frequency of VZV stimulated CD8+CD3+ T cells by all peptide pools, we also found the difference in the PD-1+CD8+CD3+ T cells between patients and controls significant (figure 1b, p<0.0001). Figure 1c represents dot plots of stimulated T cells and the gating strategy used to detect the frequency of PD-1+ T cells.

### *PD-1 expression on CD8+CD3+ T cells increases after VZV peptide stimulation in the peripheral blood of patients with reactivated zoster compared to controls*

The PD-1 Mean Fluorescent Intensity (MFI) was measured on the CD8+CD3+ T cells of PBMCs from patients with reactivated herpes zoster infection and was compared to that of controls. The PD-1 MFI of CD8+CD3+ T cells increased after VZV peptide stimu-

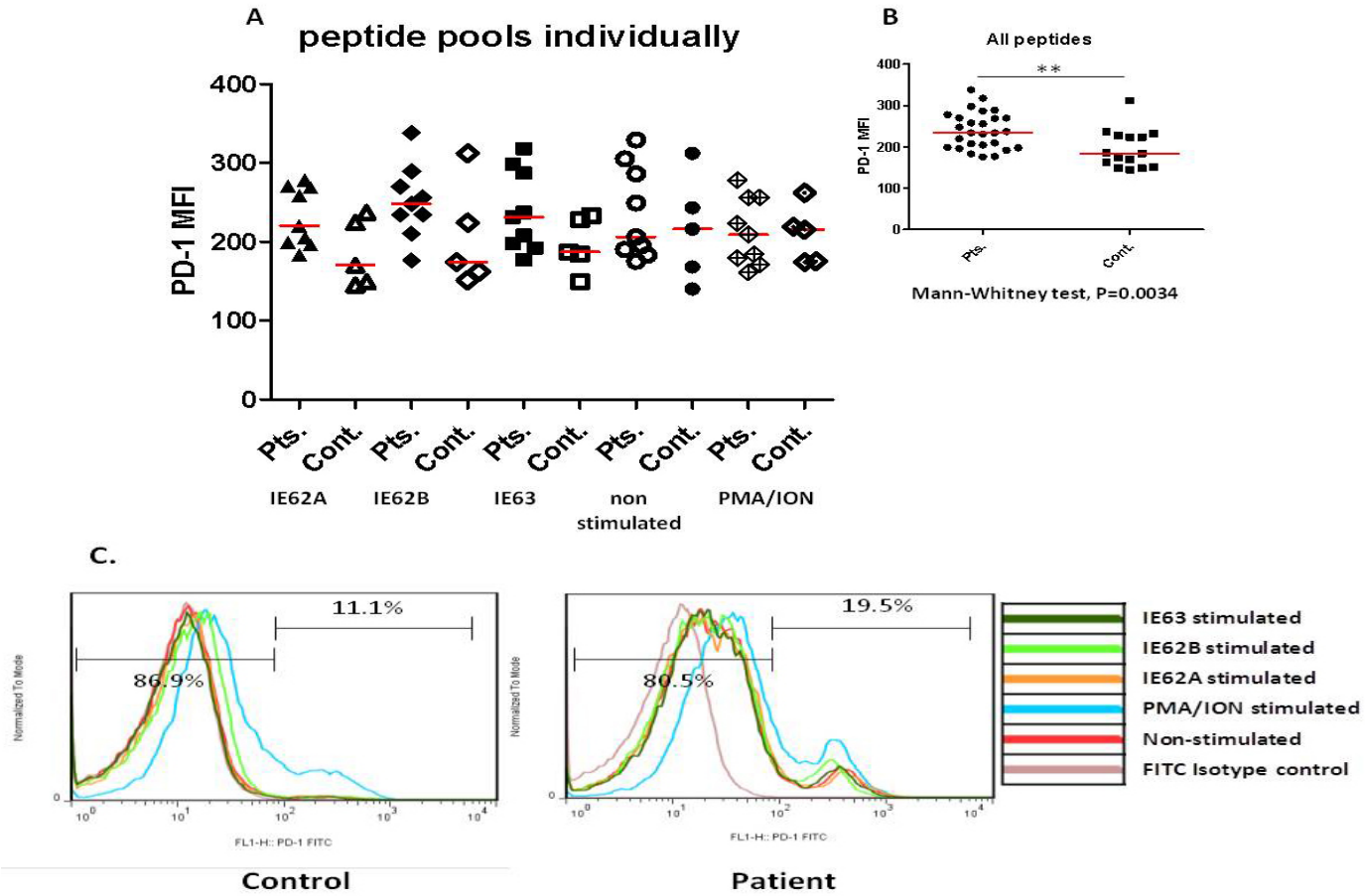


**Figure 2. PD-1 expression on CD3+ CD8+ T cells in patients compared to controls. A.** PD-1 MFI increased on the T cells of patients (n=9) after VZV peptide stimulation although this difference for individual peptide pools did not reach the significant level. The PD-1 MFI was not different between patients (n=9) and controls (n=5) in non-stimulated and after non-specific stimulation by PMA/Ionomycin. **B.** By considering the level of increase in the PD-1 MFI on T cells stimulated by all VZV pools as a whole, we could see a significant difference between patients and controls. **C.** Histograms showing PD-1 expression on the CD3+CD8+ T cells of patients with reactivated VZV infection and healthy individuals.
Peripheral blood mononuclear cells were stimulated with 7 µg/ml of each of the VZV IE63 and IE62 (A and B) pool overlapping 15-mer peptides or with 250 ng/ml of phorbol myristate acetate and 50 ng/ml of Ionomycin (PMA/ION) overnight. The cells were then stained with fluorochrome-conjugated antibodies against CD3, CD8, CD137 and PD-1 and the geometric mean of PD-1 level was calculated on the cell populations acquired by a FACS-Calibur flowcytometer using flowjo software. The final concentration of each peptide in the pool was 200 ng/ml.

lation in the peripheral blood of patients with reactivated zoster but not controls (figure 2a), however, this difference reached the significant level only when all peptides were considered as a whole (P=0.0034, figure 2b). In the non-stimulated condition and non-specific stimulation by PMA/Ionomycin, the level of PD-1 expression was not different between patients and controls. Figure 2c represents the histograms showing the shift in the PD-1 expression after stimulation of CD8+CD3+ T cells in patients.

### *VZV-specific T cell subpopulations differ in the expression of PD-1 after in-vitro stimulation with peptides*

Higher frequency of PD-1+CD137-CD3+CD8+ T cells was observed in patients with reactivated herpes zoster infection compared to controls. Both in non-stimulated condition (p=0.0195) and after peptide stimulation the percentage of PD-1+CD137-CD3+CD8+ T cells was higher in patients with reactivated herpes zoster infection compared to controls (p= 0.0195 for non-atimulated, p= 0.019 for IE62A, p= 0.042 for IE62B, p=0.012 for IE63, figure 3). However, the percentage of PD-1+CD137-CD3+CD8+ T cells in each patient did not differ significantly before and after stimulation.

As indicated in figure 4, the baseline frequency of PD-1+CD137-CD3+CD8+ T cell population was higher than the PD-1-CD137+CD3+CD8+ and PD-1+CD137+CD3+CD8+ T cell populations in both patients and controls. Interestingly, the PD-1+CD137+CD8+ T cell population (activated PD-1+ CD8+ T cells) increased in controls but not in patients after stimulation with PMA/Ionomycin.

### *Increased telomerase activity of stimulated CD57-CD45RO+CD8+ non-senescent memory T cells of patients with reactivated herpes zoster infection in response to PD-1 blockade after TCR mediated stimulation and CD28 engagement*

Purified CD3+CD8+CD57-CD45RO+ memory T cells of patients showed low baseline levels of telomerase activity compared to control subjects (n=8, supplementary figure 2). Telomerase activity after stimulation of CD3+CD8+CD57-CD45RO+ memory T cells with anti-CD3 antibody as well as anti-CD3 and anti-CD28 antibodies in the presence and absence of blocking anti-PD-1 antibody was evaluated for six patients. As shown in figure 5, stimulation of CD3+CD8+CD57-CD45RO+ memory T cells in all of the conditions increased telomerase activity significantly, however, the highest increase was observed in the presence of blocking anti-PD-1 antibody and CD28 co-stimulation. It should also be noted that co-stimulation by CD28 pathway increased

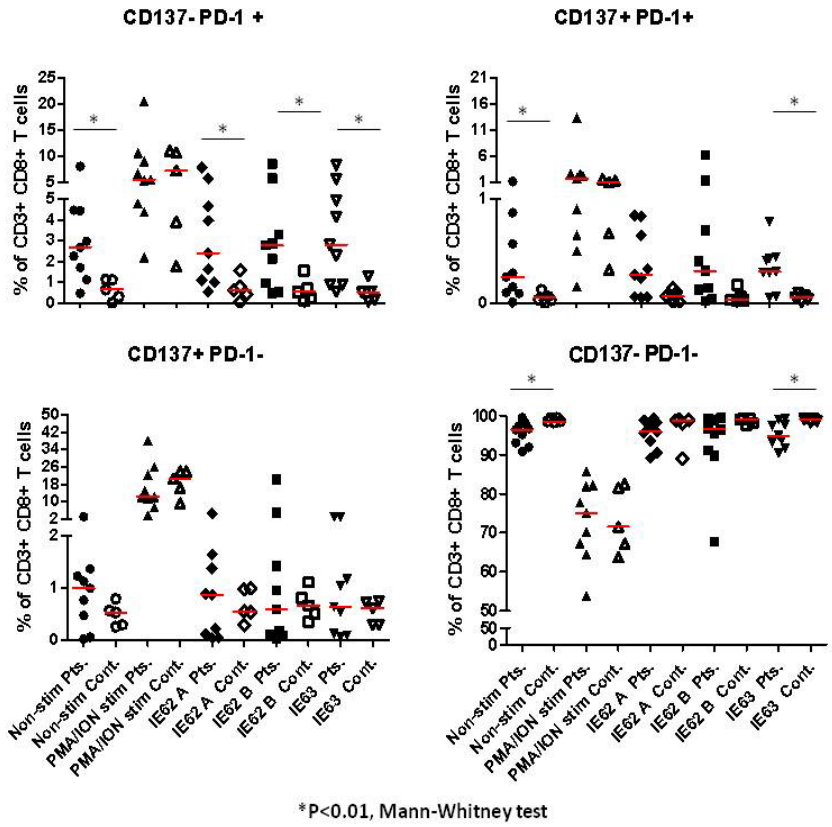


**Figure 3. Pattern of PD-1 and CD137 expression on CD3+ CD8+ T cells in patients compared to controls.** In non-stimulated condition and after peptide stimulation the percentage of PD-1+CD137-CD3+CD8+ T cells was higher in patients (n=9) with reactivated herpes zoster infection compared to controls (n=5). CD137 only marginally increased on PD-1+ T cells after stimulation with VZV peptides in patients compared to controls.

Peripheral blood mononuclear cells were stimulated with 7 µg/ml of each of the VZV IE63 and IE62 (pools A and B) pool overlapping 15-mer peptides overnight. The cells were then stained with fluorochrome-conjugated antibodies against CD3, CD8, CD137 and PD-1 and the cell frequencies were acquired by a FACS-Calibur flowcytometer. The final concentration of each peptide in the pool was 200 ng/ml. The cell percentages in the total CD3+CD8+ PBMCs are shown.

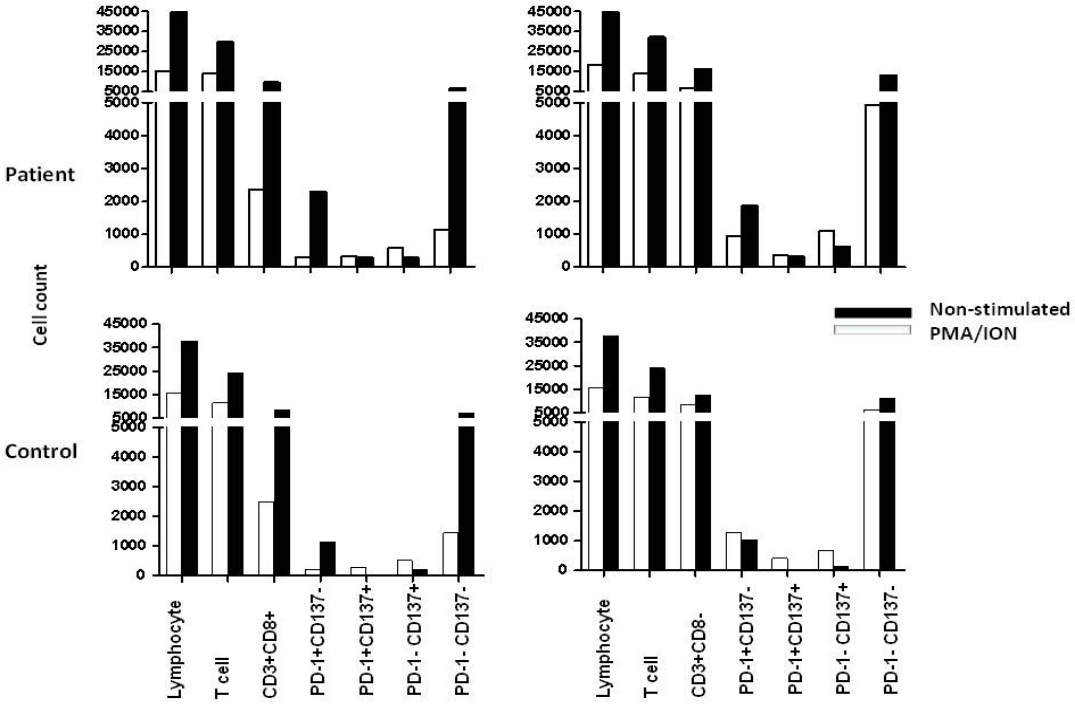


**Figure 4. Bar diagrams comparing different populations of lymphocytes before and after PMA/Ionomycin stimulation in patients and controls**. The baseline frequency of PD-1+CD137-CD3+CD8+ T cell population was higher than the PD-1-CD137+CD3+CD8+ and PD-1+CD137+CD3+CD8+ T cell populations in both patients (n=9) and controls (n=5). Interestingly, the PD-1+CD137+CD8+ T cell population (activated PD-1+ CD8+ T cells) increased in controls but not in patients. The cell stimulation and staining was performed as explained in figure 3. Mean cell frequencies are shown.

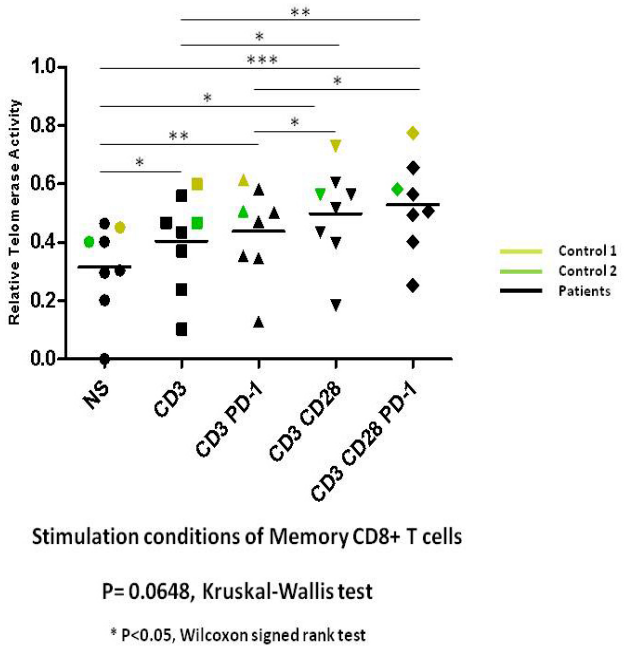


**Figure 5. TCR Stimulation increased telomerase activity of non-senescent memory T cells in patients (n=6).** The highest increase in the telomerase activity of non-senescent memory T cells was observed in the presence of CD28 co-stimulation and PD-1 blockade. Memory CD8+CD57-CD45RO+ T cells were purified from PBMCs by negative selection using MACS technique. The purity of cells was measured using anti-CD45RO-PE, CD8-APC, CD3-FITC. After negative selection, the cells were stimulated with plate coated anti-CD3 (1 µg/µl) with and without 1 µg/µl soluble anti-CD28 where indicated. PD-1 signaling was blocked at the time of stimulation using 1 µg/µl blocking anti-PD-1 antibody. The cells were then incubated at 37°C in 5% CO2 atmosphere for 16 hrs. Cells were washed post-incubation and telomerase activity was measured using a PCR-ELISA assay.

the telomerase activity per se. The effects were noticed both as the median of 6 observations (figure 5) and also for each patient individually (supplementary figure 2).

### *Blockade of PD-1 at the time of stimulation increased CD137 expression of CD3+CD8+ T cells of patients with reactivated VZV infection*

In addition to increased telomerase activity, an increase in the frequency of CD137 expression was observed when PD-1 blockade was accompanied by stimulation of CD8+ T cells through CD3 and CD28 pathways compared to activation by anti-CD3 antibody alone or anti-CD3 and anti-CD28 together (figure 6). However, due to the paucity of cells, we could only examine samples from two patients in this test.

## Discussion

One of our findings in this study was elevation of PD-1 on VZV-specific peripheral blood CD8+ T cells and enrichment of PD-1+ cells in the CD137- CD3+CD8+ T cell subpopulation. PD-1 inhibitory molecule exerts negative effect on cell proliferation and cytokine secretion through upregulation of different transcription factors (32) . The main inducer of prolonged PD-1 expression is repeated antigenic stimulation and TCR ligation during chronic viral infections such as HIV, HCV, HBV, and CMV (7, 8, 33) . However, in case of acute infections or ceased antigenic expression, the PD-1 transcription is rapidly downregulated (34) . In addition to chronic virus infections, viruses that have latency features can also cause T cell exhaustion and PD-1 overexpression (35) . HSV-1 antigens (LAT) are expressed in latency period and activate antigen specific CD8+T lymphocytes,

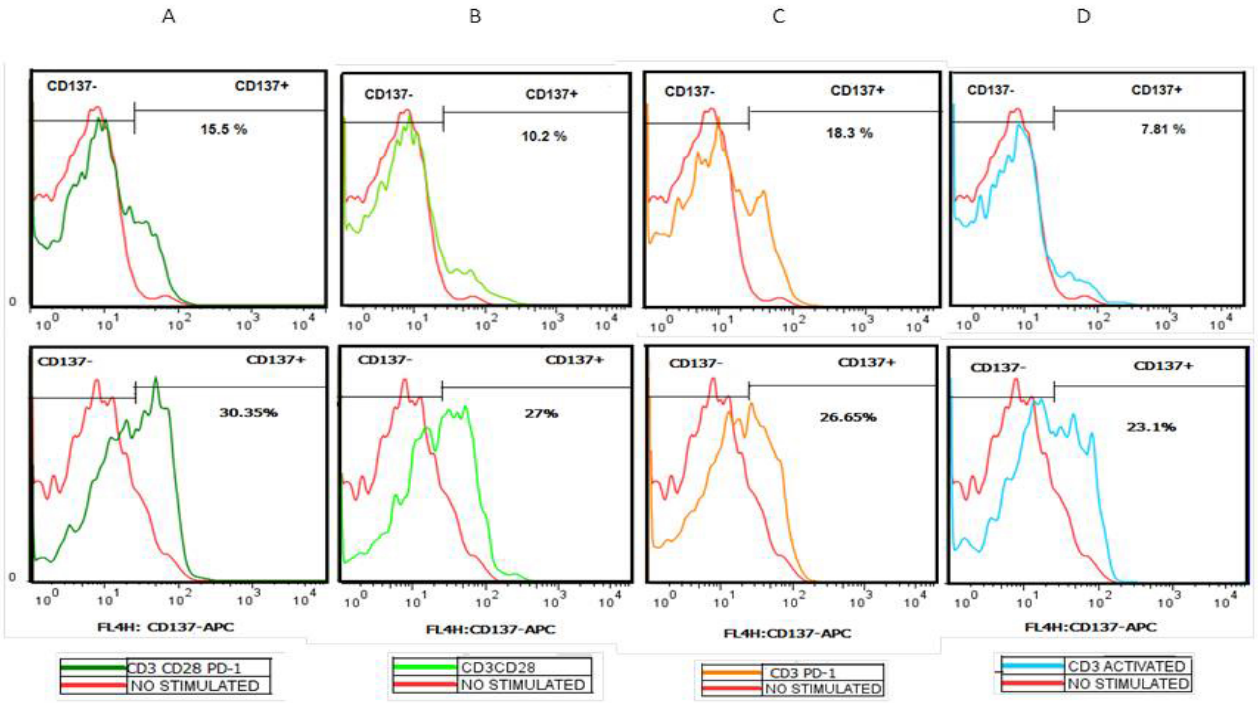


**Figure 6. Histograms showing CD137 expression in different stimulation conditions**. The highest increase in the CD137 expression of CD3+CD8+ T cells was observed in the presence of CD28 co-simulation and PD-1 blockade in the two patients analyzed. Peripheral blood mononuclear cells were stimulated with plate coated anti-CD3 (1 µg/µl) with and without 1 µg/µl soluble anti-CD28 where indicated. PD-1 signaling was blocked at the time of stimulation using 1 µg/µl blocking anti-PD-1 antibody. After 16 hrs incubation at 37°C in 5% CO2 atmosphere the cells were stained for CD3, CD8 and CD137 expression.

which express high levels of PD-1 (12, 36) . *Varicella zoster* virus is another member of alpha herpesviridae that after childhood chicken pox disease becomes latent in neurons (13) . It is shown that several VZV antigens continue to be expressed during latency and there are epitopes in these proteins that can be recognized by CD8+ T cells (37) . IE62 and IE63 are among the short-list of such antigens to be named (37, 38) . The information on the PD-1 expression on the VZV-specific CD8+ T cells is scarce and our finding shows that at least there is an accumulation of CD137- PD-1+ CD8+ T cells in this population. CD137 (4-1BB) is known as a T cell co-stimulatory receptor and is induced on recently activated T cells (39) . It has a crucial role in survival and function of activated CD8+ T cells, such as production of type-1 cytokines (IL-2, IFN-γ, TNF-α), effector and memory T cell formation, activation of TRAF- dependent NF-κB and expression of anti-apoptotic BCL-$x_2$ , BCL-$x_l$ molecules (40) . CD137 enhances CD8+ T cell proliferation by inducing β-catenin/TCF1 signaling pathways (41) . The CD137+ CD8+ T cells are considered as recently activated cells and can be functional in the absence of PD-1 molecule.

In addition to constant antigenic stimulation, an individual's age is another factor that may affect PD-1 expression. Recent evidence shows that PD-$1^{hi}$ memory T cells are disabled and cannot give rise to effector memory T cells due to PD-1 inhibition of cell function (42) . The fact that our control group had already a history of VZV infection but did not have a recurrence of zoster symptoms may be explained by their lower age average. Interestingly, the implicit dysfunctional CD137-PD-1+ cells were enriched in the CD8+ T cell population of patients but not controls. It is logical to assume that both age and repeated antigenic stimulation (also related phenomenon) have played a role in the activation of T cells.

In our study, peptide stimulation could only increase the frequency of PD-1 and CD137 expressing cells in 2-4 patients. It is possible that the competition between overlapping peptides for MHC binding has hampered an overt induction of the response as detected by CD137 expression. However, prolonged VZV antigen exposure of CD8+ T cells may have resulted in a repertoire of exhausted VZV-specific T cells that are prone to apoptosis. In this regard, PMA/Ionomycin activation of CD8+ T cells from patients induced both PD-1 and CD137 expression while the frequency of PD-1+CD137-CD8+ T cells decreased (figure 3). This is notable in the view of high baseline frequencies of PD-1+CD137-CD3+CD8+ T cells in both patients and controls. Interestingly, non-specific PMA/Ionomycin activation could highly induce PD-1-CD137+CD3+CD8+ T cell population in patients (figure 3). This may further highlight the unresponsiveness of T cells to the TCR-mediated signals in patients.

Our most interesting finding in this study was that the lower telomerase activity of the patients compared to controls could be overcome by blocking PD-1 signaling pathway at the time of TCR stimulation. The highest accelerating effect of blocking anti-PD-1 was observed in the presence of CD28 co-stimulatory signals. Blockade of PD-1/PD-L pathway in CD8+T cells has recently become a promising approach for novel therapeutic programs in chronic infections and cancer (43) . In HIV infection, PD-1/PDL inhibition is shown to improve cell proliferation and IFN-γ secretion (30) . Inhibition of PD-1 signaling by anti-PDL-1 at the time of CD8+ T cells stimulation with cognate HIV-peptides is shown to increase telomerase activity while telomerase activity remained unchanged in the absence of peptide stimulation (30) . In our experiments, telomerase activity was measured in non-senescent memory CD57-CD8+ T cells of patients with reactivated herpes zoster infection. The hallmark of senescent T cells is decreased telomerase activity and lack of proliferative capacity. Previous studies have shown that forced expression of telomerase in senescent cells results in cytokine secretion, stabilizing telomere length and proliferation (31) . We showed that non-senescent CD8+ T cells have lower telomerase activity, which can be rescued after PD-1 blockade. Interestingly, CD28 co-stimulation at the time of TCR signaling could enhance telomerase activity and PD-1 blockade augmented this effect. It is shown that PD-1 signaling inhibits T cell activation through phosphatases that prevent CD28-mediated activation of phosphatidylinositol 3-kinase (PI3K) upstream of Akt molecule (23) . Both SHP-1 and SHP-2 phosphatases are reported to dock at the immunoreceptor tyrosine-based switch motif (ITSM) domain of PD-1 cytoplasmic tail (44) . However, it is suggested that in the exhausted T cells the relative contribution of SHP2 in the PD-1 inhibitory signal increases (45) . Moreover, it is known that CD28 co-stimulatory signals can up-regulate telomerase activity which is well associated with the Akt phosphorylation (46) . Phosphorylated Akt in turn phosphorylates human telomerase reverse transcriptase and induces telomerase activity.

Therefore, it is likely that by blocking PD-1 at the time of memory T cell stimulation, we could relieve the inhibitory signal on the Akt phosphorylation and telomerase activation in CD57- CD8+ T cells, however, this assumption needs further investigation. Considering the fact that memory T cells are not dependent on co-stimulation for their activation our observation that PD-1 blocking could also improve the telomerase activity in CD3 mediated stimulation is noteworthy. Previous studies have shown that expression of chimeric molecules of co-stimulatory molecules other than CD28 can also increase the telomerase activity of CD8+ T cells. It is therefore interesting to find the pathway(s) through which telomerase activity can be restored in $CD28^-$ memory T cells. In conclusion, targeting PD-1 may be useful to recover effector function of PD-1+CD8+T cells in VZV infection as well as other chronic infections and cancer. Increased telomerase activity that can be induced by PD-1 blockade can further rescue the proliferative capacity of CD8+ T cells.

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