

Commentary

A New Controversial Pathophysiology Confirms that HIV does not Kill the CD4+ T-Cell But Mutates Its Physiological Behavior Becoming an Unaccountable CD8+ T-Cell

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Abstract

HIV pathogenesis is known to cause a progressive depilation of CD4+ T-cell cell population in close association with progressive impairment of cellular immunity and increase the susceptibility to infection. This new study is giving a new explanation about the mode of action of this virus. We assume that there is an alteration in the physiological behavior of CD4+ T-cells causing it to mutate to CD8+ T-cells and that CD4+ T-cell are neither destroyed nor lost during the infection. Twelve consenting adults took part in a randomized control trail, six were tested positive for HIV and had never received any antiretroviral therapy while the other six were tested negative. Blood samples withdrawn from participants were tested for total CD4+ T-cells and CD8+ T-cells .Infected cells from HIV positive patients were stimulated with a purified recombinant HIV-1 p17 matrix as a viral protein along with other immunological assays. The collected data showed that the sum of both CD4+ T-cells and CD8+ T-cells did not change in HIV positive patients, although there were a decrease in CD4+ T-cells and an increase in CD8+ T-cell count. Our study confirms that CD8+ T-cells is responsible for the increase in scope of HIV and the susceptibility to (OI), we assumed that this resulted from the duplication in cell signals of both newly formed (mutant) and originally found CD8+ T-cells causing a complete cellular discrepancy. According to our findings a new area of medications could arise to be a promising therapeutical modality for treating HIV-1 infection.

Keywords: HIV; CD4+ T-cell; CD8+ T-cell; Infection

Abbreviations: IFN- γ : Gamma Interferon; OI: Opportunistic Infections; CAF: Cell Antiviral Factor; STRA: Simplified Thermo Resistant Assay

Introduction

Viruses and the immune system are linked; viruses infect and replicate in an environment where cells are devoted to destroying the infection, and viruses respond by evolving mechanisms to elude the immune response; conversely, viruses have impacted the survival of their hosts, hence the genomes of survivors are imprinted by former battles. CD4+ and CD8+ T-cells are the main effectors in the adaptive cellular immune responses, and are responsible for the clearance of the viral infections. During Human immunodeficiency viral infection, a marked CD4+ T-cell depletion and CD8+ T-cell expansion takes place and a chronic immune activation transpires leading to an immune dysfunction, a syndrome subjecting the immune system to opportunistic infection [1,2].

The study of how the immune system copes with virus infection is key to understanding virus replication strategies and their overall structure along with why certain populations of cells are targeted for infection and the selection of virus mutants with growth advantage. Consequently, deciphering how the immune system functions assists in understanding how viruses evolve to survive and propagate in hosts. CD4+ T-cells impact antiviral immunity at multiple stages of the immune response. CD4+ T-cells influence antiviral cellular and humoral immunity. CD4+ T-cells enhance early expansion of virus-specific primary CTL, their subsequent differentiation into memory cells, guide their localization to sites of infection, and drive secondary expansion of memory CD8+ T-cells during re-infection [3,4]. The mechanism(s) of the HIV-induced immune activation are not fully understood; however it is established that CD4+ (the primary HIV receptor) and CD8+ T-cells are altered in many ways during HIV infection [5].

All previous studies reported that during HIV infection, CD4+ T-cells are rapidly destroyed and depleted. Loss in the antiviral IFN-y produced by CD8+ T-cells and the direct cytotoxic activity against infected cells contribute in the immunodeficiency [6], but what's important is the loss of CD4+ T-cell helper activity. The importance of CD4+ T-cells in human health and immunity was dramatically displayed early in the AIDS epidemic as patients presenting with reduced CD4+ T-cell counts developed opportunistic infections. CD4+ T-cell help is necessary for long-term CD8+ T-cell memory and the development of high-avidity antibody responses. CD8+ cytotoxic T-cells appear to produce a cytokine called CD8+ antiviral factor (CAF) that inhibits HIV replication and may or may not directly kill CD4+ T-cells [7]. To explain the role of CD8+ T-cells in HIV infection, some Researchers compared CD8+ cytotoxic T-cells from HIV-infected asymptomatic individuals with those from symptomatic AIDS patients and found that CD8+ T-cells from asymptomatic individuals could

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recognize and kill both types of target cells. In contrast, the CD8+ T-cells from symptomatic patients, while still able to recognize and eliminate the laboratory strain targets, no longer killed target cells infected with their own virus.

Additionally, HIV- specific CD8+ T-cells may fail to produce the cytotoxic molecule and perforin [8], which CD8+ T-cells use to kill virus-infected cells. Few Studies suggested that the molecular pathways that drive immune activation during chronic HIV infection are influenced by differences in the homeostatic regulation of the CD4+ and CD8+ T-cell pools [9,10]. Multiple theories have attempted to account for the gradual failure of CD8+ T-cells to control HIV replication in the absence of CD4+ T-cells [11,12]. The 'viral escape' theory states that the cells begin to lose the ability to recognize HIV's genetic sequences due to the high level of viral turnover and mutation. One study found that CD8+ T-cells lose their ability to recognize and kill viral variants, even though they may be responsive to normal 'wild type' viruses.

Pertaining to previously discussed points, our current study suggests a novel pathophysiology which describes that throughout the period of chronic HIV-1 infection, CD4+ T-cells are not killed but the pathogenesis of HIV-1 cause a mutation in all the helper cells to become a modulate version of CD8+ T-cells. The mutated CD4+ T-cells is now considered to be CD8+ T-cells but with the absence of the same physiological behavior, functions and totally dissimilar from the original CD4+ T-cells. The loss of CD4+ T-cells cause a complete cellular discrepancy and a duplication in the cell signals, as that the newly formed CD8+ T-cells inhibits these signals. This imbalance leaves the immune system in a state of confusion with two contradictory responses that directly cause deregulation and the ultimate failure of the host cellular immune networks.

On-going studies prove that the HIV strategy depends on mutation of all CD4+ T-cells in the body into CD8+ T-cells and newly formed CD8+ T-cells become increasingly irrelevant. These studies and their results introduce the vital role for the CD8+ T-cells in inducing the HIV infection and that interferes with the process CD4+ and CD8+ T-cells conjugate eventually leading to CD4+ T-cell apoptosis.

Materials

Participants

This study was applied in Cairo (R&D center of HIV researches) between October 2015 and March. Subjects were excluded whether they have any chronic diseases (Diabetes, renal and liver affection, hypertension, cancer). Twelve adult blood donors participated in the study after giving informed consent. Six donors were carefully screened for the presence of HIV (infected test group); while another six healthy individuals (control test group) were tested negative for HIV, hepatitis B surface antigen, and venereal infections.

Methods

Blood sample collection and preparation

10 cc's of blood was withdrawn and collected twice on the same day of examination. Blood specimens from all donors were collected by venipuncture followed by the anti-coagulation technique using Ethylene diamine tetra acetic acid (2 mg/ml).

Flow cytometry

Enumerations of T-cell subsets (CD4+ T-cells and CD8+ T-cells)

were done in all subjects with Coulter flow cytometer (STRA assay) [13,14] to enumerate the count of [CD4+ T-cells, CD8+ T-cells and the total number for both cells] in all tested donor's samples. 50 μ l of whole blood was added to the test tube that contains anti-CD4+ T-cells and anti-CD8+ T-cells monoclonal antibodies (STRA employs), After 15 min of incubation, blood samples were diluted 1:10 using a phosphate buffer and analyzed on a flow cytometer. The resulted CD4+ T-cells were expressed by cell/ μ l (normal range 87–1856 cells/ μ l) and for CD8+ T-cells (normal range 150-1000 cells/ μ l). These results would be compared with the results of the next assay.

Cell stimulation with a purified recombinant HIV-1 p17 matrix protein

A purified recombinant HIV-1 p17 matrix protein is one of the viral proteins that can enhance HIV-1 replication in infected cells and facilitate infection of other cells, or interfere with immune functions. A 100 microliters of whole blood was added to the test tube that contains anti-CD4+ T-cells and anti-CD8+ T-cells monoclonal antibodies (STRA employs), a 20 μ l ng/ml purified recombinant p17 (PROSPEC) [15] were added to the mix. The samples were then incubated and analyzed (1, 2, 3, 4 h) on a flow cytometer. Another counting for T-cell subsets (CD4+ T-cells, CD8+ T-cells) were done for all samples to evaluate the changes in numbers of CD4+ T-cells, CD8+ T-cells at different time intervals (1, 2, 3, 4 h) in order to be compared with the results of the first assay.

Purifying a mix of CD4 and CD8+ T-cells

10 cc of blood samples were obtained from the twelve donors, Centrifugation of the samples were performed to separate a 1 cc of buffy coat and 4-5 cc of plasma, the buffy coat samples were then treated with ammonium chloride lysis solution for 5 min to lyse any residues of erythrocytes. Samples were washed with phosphate buffer saline (PBS) containing 1% bovine serum albumin (BSA), subsequently CD4+ T-cells and CD8+ T-cells were purified from washed buffy coat, Dyna bead magnetic particles coated with antibody to CD4+ T-cells and CD8+ T-cells antigens were added to capture CD4+ T-cells and CD8+ T-cells lymphocytes (Dynal T4 Quant Kit) (Invitrogen) [16] from the buffy coat .The system we used has three types of beads, the first bead coated with CD14 antibodies to deplete monocytes in 10 min from washed sample because a significant fraction of human monocytes express low CD4+ T-cells could bind with CD4+ T-cells monocytes, thus producing artificially high CD4+ T-cell counts, the second bead coated with Anti CD4+ T-cells and the last bead coated with anti CD8+ T-cell antibodies. The purified CD4+ T-cells and CD8+ T-cells were suspended in separate test tubes with 1 cc of enriched plasma. 40 µl of purified recombinant p17 were added to every suspended T-cell. We classified them as follows: tube A containing (1 cc of suspended CD4+ T-cells enriched plasma, 40 µl of purified recombinant p17 and 20 µl Red colored anti CD4+ T-cells), tube B had (1 cc of suspended CD8+ T-cells, enriched plasma, 40 µl of purified recombinant p17 and 20 µl green colored anti CD8+ T-cells) and tube C had (1 cc of suspended mix of CD4+ T-cells, CD8+ T-cells enriched plasma, 40 µl of purified recombinant p17, 40 µl green and red colored anti CD 4+ CD8+ T-cells (Becton Dickinson). The contents of the three tubes were mixed slowly and incubated for different time intervals; 1, 2 and 3 h at room temperature, then examined under immunofluorescence microscope.

ELISPOT assay used to identify the difference in responses between the healthy CD8+ T-cells and the newly formed, mutated CD8+ T-cells and peptides

A 40 μl were obtained from contents of Test tube C and tested

again for the activity of the mutated CD4+ T-cells that acquired the two Anti CD4+ T-cells and CD8+ T-cells in their surface of the infected sample and CD8+ T-cells of non-infected sample, A Dyna bead magnetic used again to separate the cells, supernatants were removed and the cells washed again with PBS to remove others Abs, the cells were diluted with 2 cc normal saline and centrifuged at 500 rpm for 5 min at room temperature the supernatants were removed again, leaving the precipitated cells. IFN-y secretion by virus-specific CD8+ T-cells were quantitated by ELISPOT assay and IFN-enzyme linked immunospot assay (Bio-Rad laboratories Inc., Hercules, California, USA) [17,18]. Appropriate purified recombinants of p17 were used at a final concentration of 1 g/mL as a stimulator for CD8+ T-cells interferon production, Colored spots in counting wells of the test were counted using a stereomicroscope. By counting the number of spots in stimulated cultures and controls. The Positive controls consisted of 6 wells containing 300-1,000 cells with 50 ng/mL while negative controls yielded 0-1 spot per well.

Conjugate formation of CD8-CD4 T-cells Explain the role of CD8+ in HIV infection

Ammonium chloride solution used for 5 min to lysis the buffy coat of the blood samples taken from infected and non-infected volunteer's. Then the samples were washed with PBS containing 1% BSA. CD4+ T-cells and CD8+ T-cells were purified from washed buffy coat sample with Dyna bead magnetic particles coated with antibody to CD4+ T-cells and CD8+ T-cells antigens to capture CD4+ T-cells and CD8+ T lymphocytes in a pure form (Dynal T4 Quant Kit) (Invitrogen). Every purified CD8+ T-cells isolated from infected and non-infected blood samples and labeled with fluorescent dye calcein (green florescence) 1-2 µm (Molecular Probes #C-1430; Molecular Probes, Eugene) in PBS for 10 min at 37° and allowed to mix with isolated CD4+ T-cells of non-infected samples and infected sample in a separate tubes: (A) and (B). 1 ml PBS-fetal calf serum was added, the suspension was allowed to stand for 10 min after which the cells were co-centrifuged at 185 g for 10 min at room temperature, re-suspended and placed on ice. We allowed a Conjugate formation for CD4+ T-cells and CD8+ T-cells by co-centrifugation followed by re-suspension. Conjugates were counted in a hemocytometer chamber under fluorescence microscopic magnification × 20 at different time intervals; 1, 2, 3 and 4 h [19]. Then, we identified the conjugate formation for the CD8+ T-cells of the infected sample and the autologous CD4+ T-cells in the non-infected blood and in non-autologous CD8+ T-cells of non-infected blood sample by CD4+ T-cells obtained from infected donors blood samples.

Results

Data display of the final CD4+ and CD8+ count

The collected data were coded, tabulated and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean \pm SD (standard deviation) for quantitative normally distributed data, while it was done for qualitative data as number and percentage.

Flow cytometric enumeration

Flow cytometric enumeration of T-cell subsets (CD4+ T-cells and CD8+ T-cells) was done in all subjects with Coulter flow cytometer. Where the infected donors blood samples expressed as infected group from (1 to 6) and the healthy samples as control group from (7-12). The results obtained showed that the CD4+ T-cells count in donors number

(1, 4, 6) were ranged from [50-240 cells/ μ], CD8+ T-cells count [550-900 cells/ μ], for donors number (2, 3, 5) ranged from [120-340 cells/ μ], CD8+ T-cells count [400-750 cells/ μ] while the results obtained from the control group samples showed that the CD4+ T-cells count in donors number (7, 8, 10, 12) were ranged from [480-700 cells/ μ], CD8+ T-cells count [120-450 cells/ μ] and for donors number (9,11) ranged from [600-900 cells/ μ], CD8+ T-cells count [150-700 cells/ μ]. The results of these experiments were showed in Table 1.

To further characterize the changes in CD4+ and CD8+ count of infected group

A second Flow cytometric enumeration of T-cell CD4+ T-cells and CD8+ T-cells showed that at different incubation time intervals the CD4+ T-cells count in all infected group start to be depleted and their counts decreased but, on the other hand their CD8+ T-cells counts start to increase at end of the 4 h incubation time. In the control groups no remarkable change has been detected in CD4+ T-cell, CD8+ T-cell counts. these results obtained support our idea that CD4+ T-cells of infected donor's blood samples start to mutate under the long time stimulation with the Purified recombinant HIV-1 p17 matrix protein to CD8+ T-cells and explained the increase in CD8+ T-cells counts of infected samples while no changes had happened to the control samples that also were stimulated with the same viral protein. The results of this assay were showed in Figures 1-3.

Patient Group	CD4 count	CD8 count	Total T cells	
Infected group				
Pt. 1	50	900	950	
Pt. 2	120	780	900	
Pt. 3	275	700	975	
Pt. 4	140	780	920	
Pt. 5	340	400	740	
Pt. 6	240	650	890	
Control group				
Pt. 7	480	440	920	
Pt. 8	570	300	870	
Pt. 9	600	200	800	
Pt. 10	700	240	940	
Pt. 11	705	310	1015	
Pt. 12	680	230	910	

NB: The normal range for CD8 is 150-800 cells/cell/µl

Normal CD4 count is in the range 460 to 1600 cells/cell/µl

Table 1: Summary for immunological results of all donors' blood samples.





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These results obtained support our idea that CD4+ T cells of infected donor's blood samples start to mutate under the long time stimulation with the Purified recombinant HIV-1 p17 matrix protein to becoming CD8+ T-cells and explained why the increase in CD8+ counts for the infected samples and no changes had happened to the control samples that also were stimulated with the same viral protein. The results of this assay were showed in Table 2 and expressed in Figure 4.

Identification the CD4+ cell mutation

To examine the process of CD4+ T-cells to CD8+ T-cells cell



Figure 2: CD8+ T-cells were significantly higher among infected than noninfected cases at basal and the following follow ups. CD8+ T-cells significantly increase from basal beginning from hour¹ in infected group, while notsignificantly changed from basal in non-infected group.



Figure 3: Total CD4+ and CD+ 8 T-cells were non-significantly lower among infected than non-infected cases at basal and the following follow ups. CD4+ and CD+ 8 T-cells did not significantly change from basal in non-infected group.

mutation we observed a smeared films of the three classified Wells A, B and C at different duration times a three representative films were taken and shown in Figure 5 (1, 2, 3).

These results have explained the process of CD4+ T-cells mutation in infected samples. the smear sample for Well A showed a precipitation of a red color of the Anti CD4+ T-cells in the cell surface receptors of CD4+ T-cells, the smear sample of Well B showed a precipitated of a green color of the Anti CD8+ T-cells in the cell surface receptors for



Figure 4: In infected group changes of CD4 and CD8 were significantly different at follow ups; reductions in CD4 and elevations in CD8. The absolute changes of CD4 were higher than those of CD8, but the differences were significant only at hours 3 and 4; the reductions in CD4 were more than the elevations in CD8+ T-cells.



Figure 5: Dyes Immunolocalization on the cell surface receptors of the examined CD4+ and CD8+ showed a precipitation of a red color of the anti CD4+ in the cell surface receptors of CD4+ representative in film [1], the film obtained from sample of well B showed a precipitated of a green color of the Anti CD8+ in film [2] while the smear film obtained from Well C showed a formation of green and red colors of Anti CD8+ and Anti CD8+ on the surface receptors of CD4+ T-cells as representative in film [3].

	CD4+ count				CD8+ count			
Incubation times	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Infected group								
Pt. 1	46	30	21	7	902	913	921	937
Pt. 2	112	90	71	40	783	791	819	851
Pt. 3	256	192	123	77	714	756	836	889
Pt. 4	123	98	64	34	797	817	851	882
Pt. 5	332	331	279	235	402	420	449	452
Pt. 6	211	183	149	89	673	699	741	793
Control group								
Pt. 7	478	479	467	471	437	443	436	440
Pt. 8	572	568	560	563	310	302	317	305
Pt.9	592	589	585	590	213	204	207	214
Pt. 10	689	684	704	700	244	238	232	228
Pt. 11	707	700	711	696	322	314	308	300
Pt. 12	678	674	676	670	234	227	231	230

Table 2: The result of immunological data for the two group after stimulation with a purified recombinant HIV-1 p17 matrix protein at different incubation times and the marked changes in numbers of the CD4+ and CD8+ T-cells for all infected donors blood samples in compare with non-infected donors blood samples.

CD8+ T-cells while the smear film obtained from Well C showed a formation of green and red colors of the Anti CD4+ T-cells and anti CD8+ T-cells on the surface receptors of CD4+ T-cells. The maximum mutated CD4+ T-cells cells that acquired the both dyes starting to appear clearly between (3 to 4 h) Incubation time, also in the same field we observed a numbers of CD8+ T-cells with green color and a little few cells had red color on their surface receptors at the end of the incubation times. These results may carry a landmark for a process of CD4+ T-cells cell mutation which is clarified in the acceptance of CD4+ T-cells to both Anti CD4+ T-cells, CD8+ T-cells in their surface receptors only at the end of the incubation times and this explains the effect of time required for the process of conjugation to happen between the two cells.

To further characterization of the difference in CD8+ T-cells activity in both examined groups a comparison study for detecting the activity of CD8+ T-cells for IFN-y production under stimulation with one of the viral proteins structure, between CD8+ obtained from a healthy samples and the mutated CD8+ from Well C using negative and positive control of ELISPOT assay IFN-y enzyme-linked immunospot assay (ELISPOT) a remarkably difference in spot numbers formed in both examined sources of CD8+ were recorded, a definite high spot numbers were formed in non-infected CD8+ T-cells at the end of incubation in correlation with the positive control, a significant loss of detectable spots examined in CD8+ of the infected samples by comparing it with the negative control. The results of this assay showed in Figure 6.

These experiments did confirm the inability of mutated CD8+ to produce IFN-y secretions by virus-specific CD8+ T-cells when stimulated with purified recombinant p17 ng/ml. In conclusion to these findings we found that due to the increased number of IFN- γ secretions the number of spots were subsequently increased, which support our hypothesis that describes the effect of HIV infection on the formation of a newly copy of CD8+ T-cells but with major difference in physiological functions than the original CD8+ T-cells.

Also we study the ability of CD8+ T-cells procured from HIV-1-infected group to form a conjugate with CD4+ T-cells of infected



Figure 6: IFN-y ELISpot analyses of reactive T cells. The mutated CD8+ and the healthy CD8+ T cells were stimulated with purified recombinant p17 ng/ml peptide for 16 h incubation time. The first column showed high IFN- γ by the healthy CD8+T-cells similar to the cells of Positive control of the forth column while the mutated CD8+ T-Cells in column three showed no stimuli like the result of a negative control described on column 2.

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Figure 7: Conjugate formation: A conjugate formation between CD8+ T-cells and their target cells were identified in a formation of two green fluorescence cells in contact to each other in B while in the films obtained from (B) mix of suspended cells the CD8+ did not acquire the green color surface receptors of CD4+ T-cells.

and non-infected individuals and the inability of CD8+ T-cells from non-infected group to form a conjugate with CD4+ T-cells of infected individuals. In a comparison study between the suspended mix of noninfected CD4+ T-cells, labeled CD4+ T-cells of infected samples (A) and the suspended mix cells of CD4+ T-cells of infected sample, labeled CD8+ T-cells for non-infected samples (B), we recorded a number of conjugates formation in suspended mix cells (A) and no any conjugates have been observed in suspended mix (B) under fluorescence microscopy, the Conjugate formation between CD8+ T-cells and their target cells were identified in a formation of two green fluorescence cells in contact to each other, while in the films obtained from (B) mix suspended cells the CD8+ T-cells acquired the green fluorescence dye but the CD4+ T-cells did not acquire the green color surface receptors of CD4+ T-cells. The results obtained by this assay support our assumes that the infected CD8 has a role in the transmission of infection to the non-infected CD4+ T-cells, which oppose the previous studies that denied any involvement of the CD8 in transmission HIV infection . Two representative films thereof are shown in Figure 7 illustrates conjugation of CD4-CD8+ T-cells.

Discussion

HIV is considered one of the most dangerous epidemics in our present time. Till now there is still no obvious sign of any precise effective treatment. The mechanism that the virus adopts in causing the disease is not yet fully understood but all the previous attempts inform us that the main cause of immunodeficiency is due to a depletion of the CD4+ T-cells, expansion of CD8+ T-cells, and chronic activation of the immune system which leads eventually to an immune dysfunction. Our new hypothesis suggests a completely different mode of action. All previous studies informed us that the CD8+ T-cells kill the infected CD4+ T-cells, but this assumption raised an important question, if these cells have the ability to kill the infected CD4+ T-cells why they do not kill the virus itself? And why asymptomatic people living with HIV may have small numbers of CD4+ T-cells that reach to a count of 50 cell/µl and yet can cope without any life threatening illness or death? In an attempt to understand what could possibly be the cause, we assumed that the virus tricks the immune system and changes its defensive strategy, causing a mutation in the CD4+ T-helper cells and change it to be similar to the CD8+ T-cells, i.e., changing its identity, leaving it intractable, fooling us by thinking that the CD4+ T-cells were destroyed by the CD8+ T-cells. Although the cells had lost all their characteristics but actually they still exist. The concurrent increase in CD8+ T-cell numbers lead to the assumption that the CD8+ T-cells are the one that is responsible for causing the illness, our new Trails introduce a novel pathophysiology to explain HIV infection. we believe that this

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dramatic alteration (in CD4+ T-cells) cause a discrepancy within the immune system, as that the newly formed (mutant) CD8+ T-cells will permit an activating signals while the original CD8+ T-cells inhibits these orders, Leaving the immune system in a state of imbalance and confusion with two contradictory responses, which cause deregulation and ultimate failure to the host cellular immune networks. To prove our hypothesis We collected blood samples from infected and non-infected individuals and analyzed them on a Flow cytometer, the obtained results of CD4+ T-cells and CD8+ T-cells counting (First experiment) were compared with the results of second experiment, which used a Purified recombinant HIV-1 p17 matrix protein as a viral protein on the donors blood samples of both groups to enhance HIV-1 replication in the infected cells, then we compared them with the results of the first trail but with different incubation times, we noticed that in the second experiment the CD4+ T-cell count in the infected group started to deplete, their counts decrease and their CD8+ T-cells counts started to increase in the last 3 to 4 h. Of Incubation time, while in the control group no changes. These results explain why there is an increase in the CD8+ T-cells numbers due to mutation of the CD4+ T-cells of the infected donor's blood.

Another experiment was performed to prove that there were changes that happened in the cell surface receptors of infected CD4+ T, We made three suspensions of T cells in tubes A, B and C, then incubated at different times, three representative films were takes to explain what had happened. the smear sample from Tube A showed a red color precipitate of anti CD4+ T-cells on the cell surface receptors of CD4+ T-cells, the smear sample of Tube B showed a green color precipitate of the anti CD8+ T-cells in the cell surface receptors for CD8+ T-cells while the smear film obtained from Tube C showed green and red colors of anti CD4+ T-cells and anti CD8+ T-cells on the surface receptors of CD4+ T-cells. The maximum mutated CD4+ T-cells that acquired both dyes started to appear clearly from 3 to 4 h. Of incubation time, we also noticed a number of CD8+ T-cells with green color and a few cells with red color on their surface receptor at the end of the incubation time. These results support our hypothesis that a process of CD4+ T-cells mutation has occurred and that the CD4+ T-cells accept both anti CD4+ T-cells and anti CD8+ T-cells in their surface receptors by the end of the incubation times. This time interval is a critical factor for the CD8+ cells to transduce its signals to CD4+ in process of cells conjugation. Another trails were performed to Explain that CD8+ T-cells is the one responsible for causing the spread of infection, a CD4+ T-cells and CD8+ T-cells were purified from washed buffy coat sample with Dyna bead magnetic particles coated with antibodies of CD4+ T-cells and CD8+ T-cells antigens to capture CD4+ T-cells and CD8+ T-cells lymphocytes in pure form. Purified CD8+ T-cells were isolated from the infected blood samples and non-infected blood samples then were labeled with fluorescent dye (green florescence), then we mixed them with the isolated CD4+ T-cells cells of the infected and non-infected blood samples in tube A and tube B, We allowed a Conjugate formation of CD4+ T-cells and CD8+ T-cells - by co-centrifugation followed by re-suspension, the number of conjugates that formed in tube (A) were recorded, while no conjugates were observed in the suspended mixture in tube (B), the Conjugate formed between CD8+ T-cells and their target cells were identified in a form of two green fluorescence cells come in contact with each other, while in the films obtained from tube (B) the CD8+ T-cells only acquired the green fluorescence dye while the CD4+ T-cells did not. This is considered as another prove to the role of infected CD8+ T-cells in enhancing the infection by forming a conjugation with only non-infected CD4+ T-cells and not the infected ones.

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Also to have a final prove that the created new version of CD8+ T-cells (mutant CD4+ T-cells) have a new physiological difference than the original cells, we used ELISPOT assay IFN-enzyme-linked immunospot assay (ELISPOT) to study the difference in responses of both the newly formed (mutants) CD8+ T-cells and the normal CD8+ T-cells for IFN-y secretion by virus-specific CD8+ T-cells when stimulated with Appropriate purified recombinants of p17 at different incubation times. We noticed a remarkable difference in spot numbers formed in both examined donors blood of CD8+ T-cells, also we recorded, a definite high spot numbers formed in counting wells of the test in non-infected CD8+ T-cells at the end of incubation similar to the positive control and a significant loss of detectable spots in examined new CD8+ T-cells of the infected samples. The conclusion of this assay confirmed the inability of the newly formed CD8+ T-cells to produce IFN-y secretion by virus-specific CD8+ T-cells after stimulation with purified recombinant p17.

Conclusion

These findings have potential importance for defining immunotherapeutic strategies and establishing the goals for effective vaccination. Also we recommended a Cell line study using CD4+ T-cells of infected, CD4+ T-cells of non-infected, CD8+ T-cells of infected and CD8+ T-cells of non-infected to trace the changes in them.

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