

A REVIEW ON FACTS OF CD4 CELLS AND THEIR FUNCTIONS AND DIFFERENTIATION

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ABSTRACT

CD4 cells are crucial in achieving a regulated effective immune response to pathogens. CD4 effector T cells, also called helper T (Th) cells, are the functional cells for executing immune functions. Balanced immune responses can only be achieved by proper regulation of the differentiation and function of Th cells. Dysregulated Th cell function often leads to inefficient clearance of pathogens and causes inflammatory diseases and autoimmunity. CD4 cells along with the CD8 cells make up the majority of T-lymphocytes. The cells after being activated and differentiated into distinct effectors' subtypes play a major role in mediating immune response through the secretion of specific cytokines. Unlike TH1 and TH2 cells which are considered to

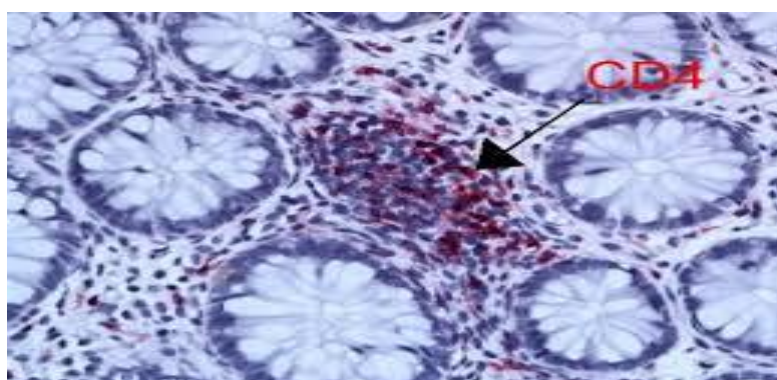
be terminally differentiated, TH17 and Treg have shown plasticity, thereby suggesting that they are not terminally differentiated. The CD4 cells represent a unique branch of the adaptive immune system that is crucial in achieving a regulated effective immune response to pathogens and their proper functioning is vital for survival.

KEYWORDS: CD4 Cell, Helper T cell, Autoimmunity, Immunization, Inflammatory disease.

INTRODUCTION

In molecular biology, CD4 (cluster of differentiation) is a glycoprotein found on surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. It was discovered in the late 1970 and was originally known as leu-3 and T4 before being named

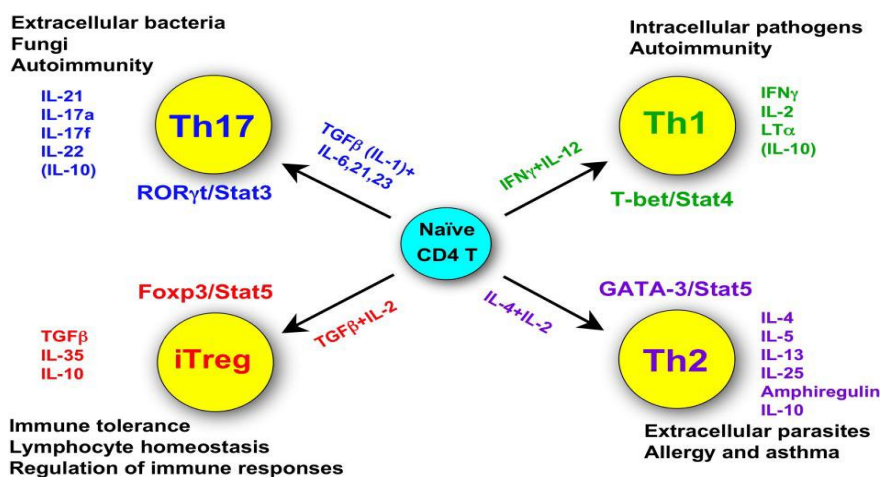
CD4 in 1984. In humans it is encoded by CD4 gene. Later in 1986, Mosmann and Coffman identified 2 subsets of activated CD4 cells, TH1 and TH2 from each other in the pattern of cytokine production and their functions. CD4 helper cells are White blood cells that are an essential part of human immune system. They are also often referred as CD4 cells, T-helper cells, T4 cells. They are called as helper cells because one of their main roles is to send signals to other types of immune cells including CD8 killer cells, which then destroy the infectious particle. If CD4 cells become depleted, for example in untreated HIV infection or following immune suppression prior to a transplant, the body is left vulnerable to a wide range of infections that it would otherwise have been able to fight.^[1-3]



DIFFERENTIATION AND FUNCTIONS

CD4 cells along with the CD8 cells make up the majority of T-lymphocytes. The cells after being activated and differentiated into distinct effectors' subtypes play a major role in mediating immune response through the secretion of specific cytokines. The CD4 cells carry out multiple functions, ranging from activation of the cells of the innate immune system. New subsets of CD4 cells besides the T-helper 1 and T-helper 2. These include T-helper 17, follicular cells (Tfh), induced T-regulatory cells (iTreg) and also T helper 9. The differentiation of the different lineages depends on the complex network of specific cytokine signaling and transcription factors followed by epigenetic modifications. The initial step of differentiation of the naïve cells is the antigenic stimulation as a result of interaction of TCR and CD4 as co-receptor with antigen-MHC II complex, presented by professional antigen presenting cells (APCs). TCR coupled with CD3 activation consequently induces a network of downstream signaling pathways that eventually lead to naïve cell proliferation and differentiation into specific effectors cells. Lineage-specific differentiation depends on the cytokine milieu of the microenvironment, as well as on the concentration of antigens, type of APCs and costimulatory molecules. Among the APCs, the dendritic cells (DCs) are

considered to be most important due to their enhanced ability to stimulate naïve T cells. Dendritic cells are activated through the recognition of pathogenic antigens by cell surface pattern recognition receptors, such as toll-like receptor and intracellular pathogen sensing receptors such as the nucleotide oligomerization domain (NOD)-like receptors. DCs consist of different subsets which interfere with the differentiation lineage. In mice, CD8 α^+ DC were involved with Th1 lineage, while the CD8 α^- subsets were linked to Th2 differentiation, through the secretion of IL-12 and IL-6, respectively. Costimulatory signals augment TCR signals, thereby promoting proliferation and differentiation. The main co-stimulatory receptor is CD28, which is expressed in all naïve T cells. The ligands of CD28 on the DC are the CD80 (B7-1) and CD86 (B7-2), which are up regulated upon activation of DC. Other less potent co-stimulatory molecules include CD28 homolog inducible co-stimulator (ICOS), members of TNF receptor family (CD27, 4-1BB and OX-40). These receptors have their ligands expressed on DC. The initial source of cytokines are from the APCs as well as other members of the innate immune cells. Subsequently, some of the cytokines produced by the differentiating cells can create a positive feedback loop, whereby the differentiation and response are marginally enhanced.^[3]



- CD4 lymphocytes play a central role in regulation of immune response.
- They have capacity to help B cells for generating the antibodies to recruit neutrophils, eosinophils and basophils to sites of infection and inflammation.
- The immune phenol-typing of the lymphocytes especially CD 4 cells from blood is being used to assess the extent of immune dysfunction in the primary and secondary immunodeficiency.
- Also the chronic infections and diseases such as Hodgkin's disease, lymphoma.

- The clinical applications of immune phenotyping of it include monitoring of disease, progression in HIV infection diagnosis of immunodeficiency disorders, evaluation of immune mediated diseases and the assessment of immune reconstitution following stem cell transplantation.
- CD4 is a co receptor that assists the T cell receptor in communicating with the antigen presenting cell.

PLASTICITY OF CD4 CELLS

Unlike TH1 and TH2 cells which are considered to be terminally differentiated, TH17 and Treg have shown plasticity, there by suggesting that they are not terminally differentiated. However recent studies found that even TH2 cells exhibit plasticity. TGF- β caused TH2 cells to switch their characteristic cytokine profile into a predominating one, suggesting the conversion in to TH9 cells. TH17 in the presence of IL12 switched to TH1 phenotype and interaction with IL4 lead to the differentiation of TH2 cells. Treg showed tendency to convert to TH17 and Tfh. In the presence of IL6, CD4⁺CD25⁺FOXP3⁺ cells up on activation reprogrammed in to TH17.

CD4 CELLS COUNT AND NEED OF IT

Flow cytometry is the gold standard for the estimation of CD4⁺ T cell counts due to its accuracy, precision and reproducibility and hence is used widely. This technology is capable of high sample throughput and offers great versatility in its applications. However, flow cytometry based CD4⁺ T cell estimation is relatively complex and technically demanding. The equipment is costly and needs regular maintenance. Additionally, it is essential that operators of the flow cytometer be sufficiently trained in the technical and biological aspects of CD4⁺ T cells measurement. The introduction of simpler and portable instruments working on the modified flow cytometry principles are now available and are being increasingly used throughout the world.

Flow cytometry can be used to estimate CD4⁺ T cell count using either dual platform or single platform approach. The dual platform flow cytometry approach uses haematology analyzer to measure absolute lymphocyte count and flow cytometer to estimate the relative percentages of CD4⁺ T cells; the absolute CD4⁺ T cell number is calculated by multiplying absolute lymphocyte number with percentage of CD4⁺ cells and dividing the product by 100. The variations in absolute lymphocyte count add to the variations in the output of

flowcytometer In the single platform approach, the flow cytometer is equipped to give both relative percentages and absolute counts of lymphocyte subsets using single observation eliminating the variations introduced due to haematology analyzer. Hence a single platform approach is now being used widely for estimation of absolute CD4⁺ T cell count. To date, single-platform technologies have two options, microbead-based technologies and the volumetric technologies³. There are a number of alternative technologies for CD4⁺ T cells estimation reported in literature; however, the flow cytometry is still the method of choice.

CD4 PERCENT IN HEALTHY ADULT POPULATION

As against absolute CD4⁺ T cell count, CD4 per cent is found to be more stable with respect to time of the day, reagents, equipment used, gating strategies and biological factors that influence the CD4 counts. Though CD4 per cent is not considered a good predictor of HIV disease progression, it indicates whether the rise or drop in CD4⁺ T cell count is a real change or just a fluctuation. It has been observed that patients with relatively high absolute CD4⁺ T cells count but low CD4⁺ T cell percentages experienced faster disease progression than subjects with low CD4⁺ T cell counts but high CD4 percentages Hence, CD4⁺ T cell percentage may be used as an additional indicator for monitoring the success of antiretroviral therapy (ART). CD4⁺ T cell percentage is less variable than absolute count; within-subject coefficient of variation is 18 per cent for CD4 per cent vs. 25 per cent for CD4 count³⁵. An assessment of the effects of instrumentation, monoclonal antibody and fluorochrome on flow-cytometric immune phenotyping has suggested that by controlling these parameters the inter-laboratory agreement on CD4⁺ T cell percentages will greatly improve²¹.

It has been observed that CD4 per cent or CD8 per cent values are also less variable in pregnant women as compared to absolute counts. The absolute CD4⁺ or CD8 T cell counts are significantly lower in pregnant women as compared to non pregnant women³⁶. This could be because of haemodilution which occurs in pregnancy and hence percentages would be more useful indicator of immune function in pregnant women also.

CD4⁺ T CELLS IN HEALTHY CHILDREN

The CD4 cell counts are normally higher in children compared to adult population. With the increasing age the CD4⁺ T cell counts decrease to attain adult levels at about three to six years of age. A study conducted by Kotylo has shown that the relative and absolute numbers of CD4⁺ T cells are high at birth, decrease The CD4⁺ T cell counts are normally higher in children compared to adult population. With increasing during early childhood and closely

approximate adult reference values after the age of 3 yr. It was shown that the CD4+ T cell count declined with age until the age of 18 yr in Ugandan and Turkish population and until the age of 10 yr in children from Kenya³ The reference ranges for both CD4+ T cell percentages and absolute counts in African children differ from those reported in Europe and North America. These age related variations in absolute CD4+ T cell counts in children present a challenge while monitoring disease in HIV infected children. The studies conducted in paediatric population have shown relatively stable CD4 per cent in different age groups in children and hence, usually CD4+ T cell percentages are referred for monitoring children with HIV infection and management.

NEED FOR ESTIMATION OF REFERENCE RANGE IN CD4 CELL

The information on the reference ranges of CD4+ T cell counts in a population is required for the application in the clinical settings such as various immune deficiencies. Reference values help in proper assessment of the degree of immunodeficiency in various conditions including HIV infection. The lower normal limits of CD4+ T cell counts are important in the routine diagnosis for interpreting putative HIV-associated changes.

EXAMPLE

HIV infected individuals with CD4 counts of 200-350 cells/ μ l had higher viral load than that suggested by the International AIDS society⁷⁵ and a cut-off CD4+ T cell count of 243 cells/ μ l reported in this study distinguished asymptomatic (CDC clinical category A) from symptomatic (CDC clinical category B) individuals⁷⁶. This observation highlights the need of validation of the western cut-off values for the target population. Recently a study conducted by Kitahata suggested that the early initiation of the treatment might be important for better prognosis of the HIV infection. The study showed that among patients with a 351-500 CD4+ count, the deferral of antiretroviral therapy was associated with an increase in the risk of death of 69 per cent, as compared with the early initiation of therapy. In the light of this finding, the reference ranges might be of great importance in making decisions on the initiation of the ART.

Decision on initiation of ART or prophylaxis for opportunistic infections (OIs) is a critical issue in the management of HIV infected persons. It has been observed that most of the OIs like cryptosporidiosis, toxoplasmosis, herpes zoster, cryptococcal meningitis, *Pneumocystis jirovecii* pneumonia, penicilliosis and CMV retinitis were seen in patients having CD4+ T cells <200 cells/ μ l. On the contrary, tuberculosis and candidiasis may be seen below the

count of 400 cells / μ l as observed in one of the Indian studies⁷¹. This study showed that all patients with CD4+ T cell counts <200 cells/ μ l were symptomatic. Of the patients with CD4+ T cells count of 201-300 cells/ μ l, 70 per cent males and 30 per cent females were symptomatic. The presence of oral candidiasis and weight loss were highly predictive of low CD4 counts as reported by Ghatge *et al*⁷⁸ and these can be considered as markers of HIV disease progression. A study conducted in south Indian population⁷⁹ reported that tuberculosis is more common in patients with CD4 counts <300 cells/ μ l, however, it can occur over a wide range of CD4 counts, which may be indirectly influenced by wider range of basal pre-infection CD4 counts in a population indicating importance of information.

FACTORS INFLUENCING CD4 COUNT

- The absolute CD4 count is a calculated value based on the total white blood cell (WBC) count and the percentages of total and CD4+ T lymphocytes.
- This absolute number may fluctuate in individuals or may be influenced by factors that may affect the total WBC count and lymphocyte percentage such as use of bone marrow-suppressive medications or the presence of acute infections.
- Splenectomy or co-infection with human T-lymphotropic virus type I (HTLV-1)⁸ may cause misleadingly elevated absolute CD4 counts. Alpha-interferon, on the other hand, may reduce the absolute CD4 count without changing the CD4 percentage. ⁹ In all these cases, CD4 percentage remains stable and may be a more appropriate parameter to assess the patient's immune function.
- The CD4+ T cell count has been shown to be influenced by sex, age, race, time of specimen collection (diurnal rhythms), physical and psychological stress, pregnancy, drug administration zidovudine, cephalosporin, cancer chemotherapy, nicotine and steroids), tuberculosis, viral infections, presence of anti-lymphocyte auto antibodies and procedures like splenectomy Females tend to have higher CD4+ T cell counts than males; on the contrary males have higher CD8+ T cell counts than females Although in adults, age does not have influence on CD4+ T cell counts significantly²⁰, decrease in CD4+ T cell counts may be observed in geriatric population.
- Apart from physiologic or pathologic conditions, factors that cause variations in the CD4+ T cell counts include instrument used, time of collection and methodologies used for collection, processing and analyzing the whole blood samples.
- The factors such as integrity of the blood sample, staining reagents and fluorochromes, equipment calibration and performance, gating strategies used for the analysis also add to

the variation in the CD4⁺ T cell counts Experience and proficiency in gating and use of lyse-no-wash procedure markedly controlled the inter-laboratory variation in CD4⁺ T cell counts The standardized procedures are now also available in terms of CDC/WHO guidelines strict adherence to guidelines can reduce the variation further.

CLINICAL APPROACH

The role of CD4⁺ T cells in antitumor immunity is a lot more controversial. It was suggested that CD4⁺ T cells can have a direct role in antitumor immunity through direct recognition of tumor antigens presented on the surface of tumor cells in association with MHC class II molecules.^[1] Of note, results from recent reports suggest that direct recognition of tumors from tumor-antigen specific CD4⁺ T cells might not be always beneficial. For example, it was recently shown that CD4⁺ T cells primarily produce TNF after recognition of tumor-antigens in melanoma. TNF may in turn increase local immuno suppression and impair the effector functions of CD8 T cells.

REFERENCE RANGE OF CD4 COUNTS

Studies have been carried out worldwide to establish reference ranges for CD4⁺ T cell counts. Variations in the reference ranges for CD4⁺ T cell counts have been observed in different populations. In United Kingdom, the mean CD4⁺ T cell count in normal healthy persons was 830 ± 290 cells/ μ l⁴⁷. The mean CD4⁺ T cell counts varied from 868 to 1036 cells/ μ l in healthy adult non-smoker Caucasian populations in different studies in Western populations^{19,49}. Mean/median CD4⁺ T cell counts reported from other parts of world also fall approximately in the same range such as 910 ± 310 cells/ μ l for Thai population⁵⁰, 863.9 ± 234.8 for African populations⁵¹, 727 ± 255 cells/ μ l for Chinese population and 869 ± 310 for Saudi men^{52,53}. A study conducted on 232 healthy Asian individuals reported a wide range of CD4 cell counts, (401 to 1451 cells/ μ l) with a mean and median of 838 and 814 cells/ μ l respectively⁵⁴. Earlier, a Study conducted in China reported a reference range of 330 to 1508 cells/ μ l from 208 healthy volunteers (mean 785 and median 730 cells/ μ l) ⁵⁵. Similarly, the CD4⁺ T cell reference range in healthy adults from Turkey was from 437 to 2072 (mean 1095 and median 1055 cells/ μ l) ⁵⁷. Comparison of mean CD4 T cell count between Ethiopian and Dutch population showed considerably lower CD4 T cell counts in Ethiopian population (mean=775 cells/ μ l) as compared to Dutch populations (mean=993 cells/ μ l) The CD4⁺ T cell reference range in 102 study participants from Tanzania was reported as 312 to 1368 cells/ μ l (mean 746 and median 723 cells/ μ l) ⁵⁹. Reference ranges of

CD4+ T cell counts in HIV negative blood donors of Botswana was found to be 171-1652 cells/ μ l (Mean 759 and median 726 cells/ μ l) 60. Such a varied difference in CD4 ranges reported in various studies could be because of racial and ethnic differences in the populations studied.^[6-10]

Geographical location	No. of subjects	Absolute, CD4 count (cells/microltrs) Range	Absolute CD4 counts Mean and/or median	CD4 Range	CD4% Mean and/or median
East(E)	14, 44	- M:379-1128 FM:547-1181	848% M:711/651 FM:766/745	- -	36/- -
West(W)	94 30 252 65	430-1740 539-1627 M:374-1398 FM:380-1493 M:379-1800 FM:321-1265	865% 965% M:727/705 FM:845/839 M:743.4/690 FM:790.4/741	30.75-49.60 48-68 M:24.2-55.1 FM:27.5-65 -	40.2/- 55.27/- M:36.89/36.60 FM:41.38/41.60 -
North(N)	84 200 40 125	M:365-1328 FM:415-1257 304-1864 - M:640-734 FM:656-824	M:763.6/- FM:797.9/- 666/- 818.4/- M:687/- FM:740/-	- 17.5-50.6 - - -	- 35/- - -
South(S)	44 99 30 213	- 753.3-844.7 - M:383-1347 FM:448-1593	1048/- 799/- 834.6/- M:865/845 FM:1021/954	- - - -	- 33.0/- - 40.2/40.1
Multicentric study	1027	-	-	E:.... W:15-65 N:15-60 S:14-51	E:... W:39.46/38.75 N:37.38/37.26 S:32.43/33.0

CONCLUSION

The CD4 cells represent a unique branch of the adaptive immune system that is crucial in achieving a regulated effective immune response to pathogens and their proper functioning is vital for survival. Through their distinct phenotypes with their respective cytokine profile, they modulate the functions of the innate immune cells as well as the members of the adaptive immune system. During the recent years, subsets with more specialized and more defined properties have been identified, such as the Tfh and Th9, thereby reinforcing their control over the immune system. The epigenetic modifications that occur during the differentiation process and hence we will gain more insights in their development, which will prove useful for later clinical use. Once considered terminally differentiated after antigen-

mediated activation, recent studies have been showing the plasticity of the different subsets, particularly the Treg and Th17 cells. This plasticity makes the potential use of Treg risky in autoimmune diseases and organ transplant, since the Treg cells can reprogram into proinflammatory phenotypes in the presence of relevant cytokine milieu and cause more harm. Moreover, aberrantly functioning CD4 cells are associated with the development of multiple autoimmune and allergic pathologies. More research will bring new insights about the epigenetic program of the current and probably novel subsets of CD4⁺T cells and their mechanism and means of functioning, thus subsequently becoming a valuable asset, which clinicians can use against immune-mediated diseases.

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