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EDITORIAL COMMENTARY

Beyond the Absence of CD4 T-Cell Count: A Novel Genetic CD4 T-Cell Deficiency Disorder With a Contingency Plan

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In healthy individuals, the role of CD4 helper T (Th) cells is to protect the host from pathogens while preventing excessive inflammation to avoid collateral damage [1]. However, their action against invading pathogens, commensal microbes, or self-antigens can still be inappropriate, leading to chronic inflammation and autoimmunity [2]. CD4 T cells are key players in the immune response as they orchestrate CD8 cytotoxic T-cell responses and activate germinal centers, leading to B-cell activation and antibody production, while also exhibiting cytotoxic activity themselves. Five major Th subsets, Th1, Th2, Th17, regulatory T (Treg), and follicular T helper (Tfh) CD4 T cells are distinguished by their specific functions such as expression of lineage markers and transcription factors as well as interleukin production. Among these CD4 T-cell subsets, metabolic heterogeneity, stemness, and plasticity have been observed [3].

Acquired or primary CD4 T-cell immunodeficiencies are characterized by low CD4 T-cell number or dysfunction and lead to recurrent infections, increased risk for virus-induced tumor, and lymphoma. Acquired CD4 T-cell deficiency is the hallmark of HIV infection, where type and severity of opportunistic infections are related to patient CD4 T-cell count decay [4]. In 1993, during the dark age of HIV infections, CD4 T-cell count measurement was common in clinical practice, and an idiopathic nonHIV-related CD4 lymphocytopenia syndrome (ICL) was identified [5–7]. The clinical presentation ranged from asymptomatic to opportunistic infections. This syndrome, which only affects adults, is characterized by CD4 T-cell count below 300 cells/mm^3 in the absence of other known immunodeficiencies. The reason underlying the inability to maintain CD4 T-cell homeostasis in ICL remains unclear and perhaps arises from a combination of decreased production, increased destruction, and altered tissue distribution.

Primary T-cell immunodeficiencies represent only 5% of primary immunodeficiency cases [8]. Due to their specific and regulatory function, inborn CD4 and CD8 T-cell immunodeficiencies are disorders characterized by alteration of both T- and B-cell function, with fewer critical clinical features than severe combined immunodeficiencies (SCID) [9]. These primary CD4 and CD8 T-cell deficiencies are further characterized by the presence or absence of major histocompatibility complex (MHC) class II or I expression on T cells, respectively, and by the absence of hypogammaglobulinemia. The clinical presentations range from asymptomatic to persistent skin warts and molluscum contagiosum, and recurrent respiratory and/or gastrointestinal infections. The onset of symptoms varies from early childhood to middle age in both men and women. In contrast to SCID, CD4 or CD8 T-cell deficiencies do not present with lymphopenia (defined by total T cells below 300 cells/mm^3). The absence of lymphopenia in such T-cell deficiencies immediately raises the question of what lymphocyte subsets replace or compensate for the absence of CD4 or CD8 T cells.

Fernandes et al in 2019 reported a case of an adult woman born from consanguineous parents with recurrent

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treatment-refractory skin warts since childhood, in the absence of either recurrent infections or chronic viral infections [10]. Flow cytometry analyses revealed the absence of any CD4 T cells and lack of CD4 expression at the cell surface or intracellular compartment of T cells. The absence of CD4 expression was also observed on myeloid cells, including monocytes and dendritic cells. This patient's selective CD4 molecule deficiency was caused by a homozygous autosomal recessive mutation in the *CD4* gene, which was reflected in both lymphoid and myeloid cell lineages, leading to an unexpectedly nonsevere clinical presentation. Increased counts of B-cell and naive CD8 T-cell counts were noted along with elevated frequency of $CD4^{-}CD8^{-}$ double-negative (DN) T cells ($CD3^{+}$, $TCR\alpha\beta^{+}$, $TCR\gamma\delta^{-}$). These odd DN T cells showed some CD4 phenotypic markers and functions. This case mimics a *CD4* knockout mouse model, indicating that DN helper T cells and Treg can be produced in the absence of CD4 and that these cells are able, in part, to replace the functional roles usually played by CD4 T cells [11].

In this issue of *The Journal of Infectious Diseases*, Lisco et al [12] confirm and expand existing knowledge on this rare inborn-error immunodeficiency, identifying a family with a novel primary immunodeficiency mutation that prevents the translation-initiation of the mRNA encoding the CD4 protein, thus abrogating the presence of membrane and plasma soluble CD4 in blood. In this case, a 22-year-old woman with homozygous disruption of the gene encoding the CD4 molecule was characterized by a complete loss of CD4 expression in lymphoid and myeloid lineages with distinctive clinical and immunological features. The mother, father, and brother were heterozygous for the same variant.

The patient presented with recurrent respiratory infections and large warts on her trunk and extremities, in the absence of any chronic viral infections. At first glance, the presence of warts may suggest a warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) disorder, a condition where neutrophils are being trapped in the bone marrow [13, 14]. However, the patient had normal immunoglobulin values and isotype distribution, making the diagnosis of this combined immunodeficiency unlikely.

Despite total absence of CD4 T cells in the blood, the patient presented with a normal total lymphocyte and CD8 T-cell counts. Sequential flow cytometry staining during and months after hospitalization confirmed the absence of extracellular and intracellular CD4 expression on both T cells and monocytes. The lack of CD4 T-cell expression was further confirmed by the inability of the patient's cells to be infected by HIV. Investigators achieved a tour de force by assessing CD4 expression in tissue. Immunohistochemical evaluation of CD4 expression was performed on an inguinal lymph node biopsy, confirming the total absence of CD4 expression, while the lymph node architecture and the B-cell and CD8 T-cell distributions were partially preserved in follicular and parafollicular zones, respectively. Furthermore, other biopsies performed in the ileum, cecum, skin warts, and in bone marrow were all negative for CD4 expression.

In contrast to HIV infection where a CD4 T-cell count below 200 cells/mm^3 is associated with life-threatening opportunistic infections, the complete multilineage loss of CD4 in the patient did not translate into immunosuppression earlier in life. To address this issue, the investigators identified a 10-fold expansion of DN $TCR\alpha\beta^{+}$ T cells in blood compared to control subjects, with preserved TCRγδ and mucosal-associated invariant T (MAIT) cells, rare subsets of lymphocytes bridging innate and adaptative immunity. Surprisingly, these DN T cells were able to mimic several phenotypic and functional characteristics of CD4 T cells with a normal naive/memory subset ratio and Treg frequency, as well as an intact interleukin-7 (IL-7) stimulation pathway, a signature cytokine for T-cell homeostasis [15].

DN T cells can act as surrogates for CD4 T-cell specialized functions, as previously reported in a CD4 knockout mouse model and in the proband reported by Fernandes et al [10]. Lisco et al demonstrated that both CD8 and DN T cells were able to exert an MHC-II–restricted CMV-specific activity [12], illustrating a novel aspect of T-cell plasticity in a context of a primary immunodeficiency. However, plasticity had its own limits, as the lack of CD4-mediated B-cell helper functions led to partially regressed germinal centers. Indeed, the low frequency of Tfh cells, which are important for the promotion of germinal center formation and antibody maturation, contributed to the relative expansion of naive B cells with low frequency of plasmablasts or plasma cells in the biopsied lymph node. As expected from other noncombined T-cell deficiencies, levels of all immunoglobulins were normal and long-lived plasma cells were present in the bone marrow. However, a global functional immune evaluation was conducted by assessing vaccine responses for neoantigens (hepatitis A and B, *Meningococcus*, and *Haemophilus influenzae)* on antibody plasma titers, which were found to be short-lived. Moreover, as antigen-specific CD4 T cells contribute to natural killer (NK) activation, their cytotoxic function was found to be impaired and did not recover upon exogenous IL-2 stimulation. Finally, as CD4 engagement by MHC-II also contributes to differentiation and function of monocytes, lipopolysaccharide or endotoxin-stimulated monocytes were found to be dysfunctional as determined by their blunted ability to secrete inflammatory cytokines.

This well documented case report indicates for the first time that loss of CD4 expression in blood, lymphoid tissues, and bone marrow induces defects in humoral and innate antiviral immunity. Despite total absence of CD4-expressing cells, the patient presented with a modest infection history compared to other primary immunodeficiencies or HIV infection. This case and the one reported by Fernandes et al [10] indicate

that functional MHC-II–restricted DN and Treg cells can be generated independently of CD4. Cell plasticity allowed a contingency plan for CD8 and DN T cells to act as "CD4 T-like" cells, including the induction of MHC-II–restricted proliferative response. However, defects in B-cell, NK, and monocyte functions were observed. Such Mother Nature's experiments of CD4 deficiency contribute to a better understanding of CD4 T-cell function in mucosal and skin protection from bacterial and viral infections. Nevertheless, the compensatory expansion of DN T cells present in those with primary CD4 deficiencies was not observed in HIV infection or in ICL [7]. Evaluation of bone marrow and thymic determinants of such T-cell plasticity will be needed for these rare patients, as well as in the context of HIV infection, as such DN cells are noninfectable, and for COVID-19, where CD4 T-cell decay is a predictor for undesirable clinical outcomes [16].

Notes

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