Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency

In this study, we describe four patients from two unrelated families of different ethnicities with a primary immunodeficiency, predominantly manifesting as susceptibility to Epstein-Barr virus (EBV)-related diseases. Three patients presented with EBV-associated Hodgkin’s lymphoma and hypogammaglobulinemia; one also had severe varicella infection. The fourth had viral encephalitis during infancy. Homozygous frameshift or in-frame deletions in CD70 in these patients abolished either CD70 surface expression or binding to its cognate receptor CD27. Blood lymphocyte numbers were normal, but the proportions of memory B cells and EBV-specific effector memory CD8+ T cells were reduced. Furthermore, although T cell proliferation was normal, in vitro-generated EBV-specific cytotoxic T cell activity was reduced because of CD70 deficiency. This reflected impaired activation by, rather than effects during killing of, EBV-transformed B cells. Notably, expression of 2B4 and NKG2D, receptors implicated in controlling EBV infection, on memory CD8+ T cells from CD70-deficient individuals was reduced, consistent with their impaired killing of EBV-infected cells. Thus, autosomal recessive CD70 deficiency is a novel cause of combined immunodeficiency and EBV-associated diseases, reminiscent of inherited CD27 deficiency. Overall, human CD70-CD27 interactions therefore play a nonredundant role in T and B cell-mediated immunity, especially for protection against EBV and humoral immunity.

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Modifications of TIGIT expression contribute to CD8 T cell exhaustion in chronic virus infection

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Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals

HIV-specific CD8+ T cells demonstrate an exhausted phenotype associated with increased expression of inhibitory receptors, decreased functional capacity, and a skewed transcriptional profile, which are only partially restored by antiretroviral treatment (ART). Expression levels of the inhibitory receptor, T cell immunoglobulin and ITIM domain (TIGIT), the co-stimulatory receptor CD226 and their ligand PVR are altered in viral infections and cancer. However, the extent to which the TIGIT/CD226/PVR-axis is affected by HIV-infection has not been characterized. Here, we report that TIGIT expression increased over time despite early initiation of ART. HIV-specific CD8+ T cells were almost exclusively TIGIT+, had an inverse expression of the transcription factors T-bet and Eomes and co-expressed PD-1, CD160 and 2B4. HIV-specific TIGIThi cells were negatively correlated with polyfunctionality and displayed a diminished expression of CD226. Furthermore, expression of PVR was increased on CD4+ T cells, especially T follicular helper (Tfh) cells, in HIV-infected lymph nodes. These results depict a skewing of the TIGIT/CD226 axis from CD226 co-stimulation towards TIGIT-mediated inhibition of CD8+ T cells, despite early ART. These findings highlight the importance of the TIGIT/CD226/PVR axis as an immune checkpoint barrier that could hinder future "cure" strategies requiring potent HIV-specific CD8+ T cells.
CD4+ T cells with an activated and exhausted phenotype distinguish immunodeficiency during aviremic HIV-2 infection

OBJECTIVE: HIV-2 represents an attenuated form of HIV, where many infected individuals remain “aviremic” without antiretroviral therapy (ART). However, aviremic HIV-2 disease progression exits, and in the current study we therefore aimed to examine if specific pathological characteristics of CD4+ T cells are linked to such outcome. DESIGN: HIV-seronegative (n=25), HIV-1 (n=33), HIV-2 (n=39, of whom 26 were aviremic), and HIV-1/2 dually (HIV-D) (n=13) infected subjects were enrolled from an occupational cohort in Guinea-Bissau. METHODS: CD4+ T cell differentiation, activation, exhaustion, senescence, and transcription factors were assessed by polychromatic flow cytometry. Multidimensional clustering bioinformatic tools were used to identify CD4+ T cell subpopulations linked to infection type and disease stage. RESULTS: HIV-2-infected individuals had early- and late-differentiated CD4+ T cell clusters with lower activation (CD38+HLA-DR+) and exhaustion (PD-1) than HIV-1 and HIV-D-infected subjects. We also noted that aviremic HIV-2-infected individuals possessed fewer CD4+ T cells with pathological signs compared to other HIV-infected groups. Still, compared to HIV-seronegatives, aviremic HIV-2-infected subjects had T-bet+ CD4+ T cells that showed elevated immune activation/exhaustion, and particularly the frequencies of PD-1+ cells were associated with suboptimal percentage of CD4+ T cells. CONCLUSIONS: Increased frequencies of CD4+ T cells with an activated/exhausted phenotype correlate with exacerbated immunodeficiency in aviremic HIV-2-infected individuals. Thus, these findings encourage studies on the introduction of ART also to individuals with aviremic HIV-2 infection.
PD-1 Blockade Expands Intra-tumoral Memory T Cells

Tumor responses to programmed cell death protein 1 (PD-1) blockade therapy are mediated by T cells, which we characterized in 102 tumor biopsies obtained from 53 patients treated with pembrolizumab, an antibody to PD-1. Biopsies were dissociated, and single-cell infiltrates were analyzed by multicolor flow cytometry using two computational approaches to resolve the leukocyte phenotypes at the single-cell level. There was a statistically significant increase in the frequency of T cells in patients who responded to therapy. The frequency of intra-tumoral B cells and monocytic myeloid-derived suppressor cells significantly increased in patients' biopsies taken on treatment. The percentage of cells with a regulatory T-cell phenotype, monocytes, and natural killer cells did not change while on PD-1 blockade therapy. CD8+ memory T cells were the most prominent phenotype that expanded intra-tumorally on therapy. However, the frequency of CD4+ effector memory T cells significantly decreased on treatment, whereas CD4+ effector T cells significantly increased in non-responding tumors on therapy. In peripheral blood, an unusual population of blood cells expressing CD56 was detected in two patients with regressing melanoma. In conclusion, PD-1 blockade increases the frequency of T cells, B cells, and myeloid-derived suppressor cells in tumors, with the CD8+ effector memory T-cell subset being the major T-cell phenotype expanded in patients with a response to therapy.
Multidimensional Clusters of CD4+ T Cell Dysfunction Are Primarily Associated with the CD4/CD8 Ratio in Chronic HIV Infection

HIV infection provokes a myriad of pathological effects on the immune system where many markers of CD4+ T cell dysfunction have been identified. However, most studies to date have focused on single/double measurements of immune dysfunction, while the identification of pathological CD4+ T cell clusters that is highly associated to a specific biomarker for HIV disease remain less studied. Here, multi-parametric flow cytometry was used to investigate immune activation, exhaustion, and senescence of diverse maturation phenotypes of CD4+ T cells. The traditional method of manual data analysis was compared to a multidimensional clustering tool, FLOW Clustering with K (FLOCK) in two cohorts of 47 untreated HIV-infected individuals and 21 age and sex matched healthy controls. In order to reduce the subjectivity of FLOCK, we developed an "artificial reference", using 2% of all CD4+ gated T cells from each of the HIV-infected individuals. Principle component analyses demonstrated that using an artificial reference lead to a better separation of the HIV-infected individuals from the healthy controls as compared to using a single HIV-infected subject as a reference or analyzing data manually. Multiple correlation analyses between laboratory parameters and pathological CD4+ clusters revealed that the CD4/CD8 ratio was the preeminent surrogate marker of CD4+ T cells dysfunction using all three methods. Increased frequencies of an early-differentiated CD4+ T cell cluster with high CD38, HLA-DR and PD-1 expression were best correlated (Rho = -0.80, P value = 1.96x10^{-11}) with HIV disease progression as measured by the CD4/CD8 ratio. The novel approach described here can be used to identify cell clusters that distinguish healthy from HIV infected subjects and is biologically relevant for HIV disease progression. These results further emphasize that a simple measurement of the CD4/CD8 ratio is a useful biomarker for assessment of combined CD4+ T cell dysfunction in chronic HIV disease.
HIV disease progression is characterized by numerous pathological changes of the cellular immune system. Still, the CD4 cell count and viral load represent the laboratory parameters that are most commonly used in the clinic to determine the disease progression. In this study, we conducted an interdisciplinary investigation to determine which laboratory parameters (viral load, CD4 count, CD8 count, CD4 %, CD8 %, CD4/CD8) are most strongly associated with pathological changes of the immune system. Multiparametric flow cytometry was used to assess markers of CD4+ and CD8+ T cell activation (CD38, HLA-DR), exhaustion (PD-1, Tim-3), senescence (CD28, CD57), and memory differentiation (CD45RO, CD27) in a cohort of 47 untreated HIV-infected individuals. Using bioinformatical methods, we identified 139 unique populations, representing the “combined T cell pathogenesis,” which significantly differed between the HIV-infected individuals and healthy control subjects. CD38, HLA-DR, and PD-1 were particularly expressed within these unique T cell populations. The CD4/CD8 ratio was correlated with more pathological T cell populations (n = 10) and had a significantly higher average correlation coefficient than any other laboratory parameters. We also reduced the dimensionalities of the 139-unique populations by Z-transformations and principal component analysis, which still identified the CD4/CD8 ratio as
the preeminent surrogate of combined T cell pathogenesis. Importantly, the CD4/CD8 ratio at baseline was shown to be significantly associated with CD4 recovery 2 y after therapy initiation. These results indicate that the CD4/CD8 ratio would be a suitable laboratory predictor in future clinical and therapeutic settings to monitor pathological T cell events in HIV infection.

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Multi-parametric flow cytometry (FCM) represents an invaluable instrument to conduct single cell analysis and has significantly increased our understanding of the immune system. However, due to new techniques allowing us to measure an increased number of phenotypes within the immune system, FCM data analysis has become more complex and labor-intensive than previously. We have therefore developed a semi-automatic gating strategy (NetFCM) that uses clustering and principal component analysis (PCA) together with other statistical methods to mimic manual gating approaches. NetFCM is an online tool both for subset identification as well as for quantification of differences between samples. Additionally, NetFCM can classify and cluster samples based on multidimensional data. We tested the method using a data set of peripheral blood mononuclear cells collected from 23 HIV-infected individuals, which were stimulated with overlapping HIV Gag-p55 and CMV-pp65 peptides or medium alone (negative control). NetFCM clustered the virus-specific CD8+ T cells based on IFN and TNF responses into distinct compartments. Additionally, NetFCM was capable of identifying HIV- and CMV-specific responses corresponding to those obtained by manual gating strategies. These data demonstrate that NetFCM has the potential to identify relevant T cell populations by mimicking classical FCM data analysis and reduce the subjectivity and amount of time associated with such analysis. (c) 2014 International Society for Advancement of Cytometry
T-bet and Eomes Are Differentially Linked to the Exhausted Phenotype of CD8+ T Cells in HIV Infection

CD8+ T cell exhaustion represents a major hallmark of chronic HIV infection. Two key transcription factors governing CD8+ T cell differentiation, T-bet and Eomesodermin (Eomes), have previously been shown in mice to differentially regulate T cell exhaustion in part through direct modulation of PD-1. Here, we examined the relationship between these transcription factors and the expression of several inhibitory receptors (PD-1, CD160, and 2B4), functional characteristics and memory differentiation of CD8+ T cells in chronic and treated HIV infection. The expression of PD-1, CD160, and 2B4 on total CD8+ T cells was elevated in chronically infected individuals and highly associated with a T-bet^{dim}\text{Eomes}^{hi} expression profile. Interestingly, both resting and activated HIV-specific CD8+ T cells in chronic infection were almost exclusively T-bet^{dim}\text{Eomes}^{hi} cells, while CMV-specific CD8+ T cells displayed a balanced expression pattern of T-bet and Eomes. The T-bet^{dim}\text{Eomes}^{hi} virus-specific CD8+ T cells did not show features of terminal differentiation, but rather a transitional memory phenotype with poor polyfunctional (effector) characteristics. The transitional and exhausted phenotype of HIV-specific CD8+ T cells was longitudinally related to persistent Eomes expression after antiretroviral therapy (ART) initiation. Strikingly, these characteristics remained stable up to 10 years after ART initiation. This study supports the concept that poor human viral-specific CD8+ T cell functionality is due to an inverse expression balance between T-bet and Eomes, which is not reversed despite long-term viral control through ART. These results aid to explain the inability of HIV-specific CD8+ T cells to control the viral replication post-ART cessation.

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Projects:

**Analysis of the T cell immune response in Yellow fever virus and HIV infections**

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