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An HIV elite controller patient carrying the homozygous H63D variant in the homeostatic iron regulator gene
A case report

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Abstract

Rationale: HIV elite controllers represent a rare subset of persons living with HIV, able to spontaneously control viral replication without antiviral therapy. HLA-B*57 and HLA-B*27 alleles are associated to efficient polyfunctional CD8^{+} T-cell response and are overrepresented in elite controllers but these alleles alone incompletely explain spontaneous HIV replication control in these subjects. Further mechanisms involved in innate and adaptive immune response and host genetics may contribute to this control. In this context, the homeostatic iron regulator (HFE) gene encodes a major histocompatibility complex-class-I-like molecule involved in both innate immunity, acting also through autophagy regulation, and iron homeostasis, strictly related to immune functions and susceptibility to infections.

Patient concerns: Homozygousity for the p.His63Asp (H63D) variant in the HFE gene was identified in an 80-year-old HIV-infected woman with spontaneous control of viral replication.

Diagnosis: HIV-1 RNA was undetectable in patient's serum with a routine assay and an ultra-sensitive assay (<1 copy/mL) during the 30 years follow-up. CD4^{+} and CD8^{+} T cell counts were stable and normal during all this period.

Interventions: The patient had a history of absence of any physical ailment and no antiviral therapy has been prescribed during the 30 years of follow-up. The subject did not harbor HLA-B*57 and HLA-B*27 alleles. HFE gene was sequenced by Sanger, as part of a larger study on a cohort of HIV infected patients, aged >65 years and screened for polymorphisms in genes belonging to several pathways involved in neuroinflammation.

Outcomes: The woman had CD4^{+} and CD8^{+} T cell normal values and spontaneously controlled serum HIV-1 RNA levels for 30 years.

Lessons: We assume that the interplay between the HFE H63D variant in homozygosity and innate immunity, perhaps through autophagy regulation, could play a role in HIV-1 replication control in our patient. This hypothesis needs to be explored in in vitro and in vivo studies. Understanding mechanisms involved in spontaneous control of HIV-1 replication remains indeed a challenge due to its possible implications for HIV cure research.

Abbreviations: ART = antiviral therapy, EC = elite controller, HFE = homeostatic iron regulator, HH = hereditary hemochromatosis, HLA = Human Leukocyte Antigen, IFN = interferon, MHC = major histocompatibility complex, mTORC1 = mammalian target of rapamycin complex 1, PBMC = peripheral blood mononuclear cell, Tf = transferrin.

Keywords: case report, elite controller, H63D, HFE variant, HIV

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1. Introduction

A little subset of HIV infected people spontaneously controls viral replication in the absence of antiviral therapy (ART). These individuals, named elite controllers (ECs), represent less than 1% of all HIV-infected patients and are characterized by viral loads undetectable by standard assays, strong HIV specific CD8+ T-cell responses and normal CD4+ T-cell counts. Great heterogeneity has, however, been described in these individuals regarding residual plasma viremia, extent of viral replication control, duration of stability of CD4+ T-cell counts and clinical outcome.[1] Several host factors and virus characteristics seem associated with spontaneous HIV control. In some cases, control of viremia is due to infection with attenuated HIV variants, although host factors may be more relevant even in these cases.[2] Integration sites of proviral sequences seem to be preferentially located in chromosome regions with heterochromatin features in ECs in contrast to individuals treated with long-term ART.[3] The strong HIV-specific CD8+ T cells response, the main immunological feature of ECs, is partly due to host genetics.

About 25% to 40% of ECs harbor protective Human Leukocyte Antigen (HLA) class I alleles, like HLA-B*52, HLA-B*57, HLA- B*14, and HLA-B*27, that have been associated to efficient polyfunctional CD8+ T-cell response. However, not all ECs have those protective HLA alleles nevertheless exhibit a strong HIV-specific CD8+ T cell response. CD4+ T cells, humoral immune response, antibody-dependent functions, and innate immune cells may further contribute to the spontaneous control of HIV-1 infection and the role of sex, ethnicity, and host genetics is still debated.[4–6]

2. Case presentation

Here we present the case of an 80-year-old woman, first tested for HIV in 1990 when she was 50 years old and regularly followed until July 2021. Western-blot showed reactivity for gp160, gp120, p65, p55, gp41, p40, p32, p24, and p18 of HIV-1 and no reactivity for HIV-2 proteins. Plasmatic HIV-1 RNA was undetectable with our routine assay (<20 copies/mL) during all the follow-up. At her last visit, hypertension, non-insulin-dependent diabetes, and mild kidney disease were present comorbidities while both neurocognitive and motorial test were normal according to her age. HIV-1 RNA was not detected in serum with an ultra-sensitive assay (<1 copy/mL) in 1999, 2003, and 2015, while total HIV-1 DNA was detected in peripheral blood mononuclear cells (PBMCs) collected in 2010. Patient's CD4+ and CD8+ T-cells and CD4+/CD8+ remained normal throughout the follow-up (Fig. 1). HLA testing revealed HLA-B*44 and B*51 positivity. No ART was prescribed during the follow-up.

This patient belongs to a cohort of HIV infected patients, aged >65 years and screened in our department for neurocognitive impairment, including search for gene polymorphisms belonging to several pathways involved in neuroinflammation like iron metabolism and lipid homeostasis. The study was approved by the Ethics Committee of ASST-Spedali Civili di Brescia and informed consent for genetic screening was obtained. Genomic DNA was extracted from a peripheral blood sample using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI). Extracted DNA was quantified by the use of Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA), with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Genomic DNA was amplified by polymerase chain reaction, with previously described primers and conditions.[7] Amplicons were analyzed by direct Sanger sequencing on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). Sequencing results were analyzed using Chromas software. Within the cohort, we found that the only EC also was the only patient homozygous for the p.His63Asp (H63D) variant in the homeostatic iron regulator (HFE) gene, one of the main gene involved in human hereditary hemochromatosis (HH). Iron homeostasis biomarkers of patient were however normal.

3. Discussion

The HFE gene encodes a major histocompatibility complex (MHC)-I-like molecule involved in both iron homeostasis and immunity. HFE is a transmembrane protein, exposed with β-2 microglobulin on the cell surface of many cells, particularly
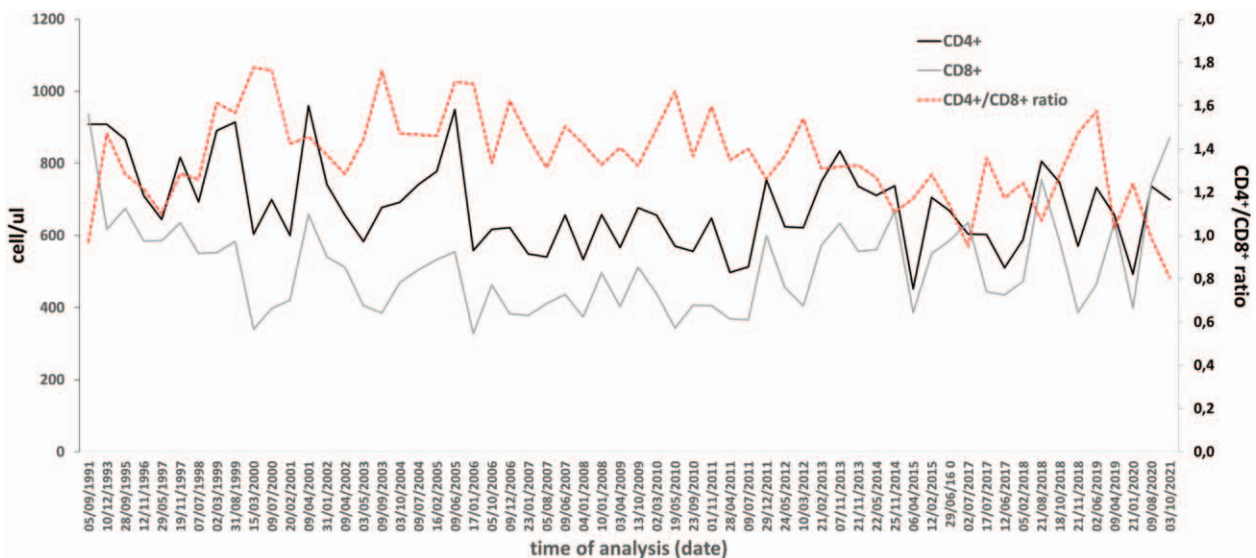

Figure 1. CD4+ and CD8+ T-cell counts over time during the follow-up of the elite controller case here reported.

hepatocytes, Kupffer cells, monocytes, macrophages, granulocytes, and intestinal cells. It is composed of 3 extracellular α domains, a transmembrane domain, and a cytoplasmatic tail. Binding with β-2 microglobulin occurs through the α3 domain and is required for cell surface expression, like for classical MHC-I molecules. α1/α2 domains are mainly responsible for HFE interaction with the transferrin receptor 1, the main cellular receptor involved in iron uptake from the extracellular milieu through the binding with transferrin (Tf), a blood protein that binds circulating iron.

Since the discovery of the involvement of HFE gene mutations in HH, the role of HFE in iron homeostasis has been thoroughly characterized.[8] HFE regulates cellular iron uptake but also senses blood iron levels and regulates expression of the major regulator of systemic iron homeostasis, the peptide hepcidin. The molecular mechanisms involved in this regulation are not yet completely understood. Hepcidin, released in the circulation, acts inducing the degradation of the cellular iron exporter ferroportin both in the basolateral surface of enterocytes, reducing intestinal iron absorption, and in macrophages, reducing iron recycling and resulting in intracellular iron accumulation, in turn reducing blood iron level and Tf saturation.[8]

In all cell types, classical MHC-I molecules are involved in adaptive immunity through the displaying of antigens that are processed within the cells, like invading or intracellular pathogens, to CD8+ cytotoxic T-cells. MHC-I molecules present antigens to these cells through the interaction of T-cell receptor with the open groove in their α1/α2 domains, while the α3 domain engages the CD8 molecule. Non-classical MHC-I molecules (i.e., HFE) are both involved in adaptive and innate immunity and engaged in the regulation of T- and natural killer cells. HFE, although does not bind peptides, is recognized by the T-cell receptor of a specific subset of CD8+ T-cells in mice and is able of shaping the T-cell repertoire.[9–11] Moreover, HFE, through its α1/α2 domains, seems to be a negative regulator of CD8+ T-cell activation, reducing the release of several soluble factors like macrophage inflammatory protein-1β and interferon (IFN)-ɣ and reducing the expression of markers of T-cell activation: this could have clear implications in the immune response to pathogens and autoimmune diseases.[12]

HFE-related HH is characterized by low hepcidin production, elevated serum Tf saturation, serum ferritin levels, and iron overload in tissue and organs like liver, skin, pancreas, and heart. HH is mainly associated with homozygosity for the p.Cys282Tyr (C282Y) variant, present in 0.4% of Caucasian people. Homozygosity for the C282Y variant has however a low penetrance. C282Y/H63D compound heterozygotes have lower risk of developing iron overload than C282Y homozygotes and only some individuals who are heterozygous for either C282Y or H63D have elevated serum Tf saturation and ferritin levels, but they do not develop complications of iron overload. People homozygous for the H63D allele, like the EC subject here reported, are relatively rare (1%–2% of the general population) and are considered at very low risk of HH.

Patients with HH due to homozygosity for C282Y variant are also affected by several immune defects like low number of CD8+ T-lymphocytes affecting CD4+/CD8+ ratios, lower levels of MHC-I expression in PBMCs due to an increased rate of MHC-I endocytosis and increased occurrence of autoimmune diseases.[9] Nonetheless, HFE C282Y mutation may offer an advantage to asymptomatic patients, interfering with the inflammatory response of macrophages. Reduced iron levels in macrophages of

C282Y allele carriers make them less susceptible to bacteria that depend on macrophage iron like *Salmonella Typhimurium*, *Mycobacterium tuberculosis*, *Leishmania amazonensis*, *Chlamydia* or *Legionella* species. This advantage against some infectious diseases may explain the high frequency of HFE mutations in the Caucasian population.[9]

People homozygous for the H63D allele, like the EC subject here reported, are relatively rare (1%–2% of the general population) and are considered at very low risk of HH.[13]

Little is known about the effects of the HFE H63D variant on the immune system but some interesting findings are described in the literature. The H63D variant, located in the α1 domain of HFE protein, retains only partially the negative regulation of CD8+ T-cell activation observed for wild type HFE.[12] Further, mutated transcripts predominate on the wild-type transcripts and mutated protein is increased in PBMCs from H63D heterozygotes.[14] Iron is strictly involved in macrophage functions and C282Y homozygotes have limited iron storage in macrophages despite iron overload in several tissues. Wild-type HFE leads to iron accumulation in the human macrophage THP-1 cell line through the inhibition of iron release, while H63D mutated HFE abolish this inhibitory effect.[15] Murine macrophages harboring the H67D variant, corresponding to the human H63D, show lower cellular proliferation under iron exposure, higher L- ferritin expression suggestive of higher iron storage capability, reduced cell migration, higher phagocytic activity, and altered cytokine release profile suggestive of decreased inflammatory response compared with the wild-type counterpart.[16] H67D mice have increased survival over wild-type mice after experimental cerebral malaria due to the infection with Plasmodium falciparum.[17] Finally, the H63D mutation has been associated with long-term virus suppression in chronic hepatitis C patients treated with IFN-based therapy in a large meta-analysis.[18] All together these findings suggest that H63D variant may have an impact and, in some cases, also positively influence the immune response against some pathogens.

Furthermore, autophagy has recently been described as increased in cells carrying the H63D variant, probably through the inhibition of the mammalian target of rapamycin complex 1 (mTORC1), a negative regulator of autophagy.[19] Autophagy is a process of degradation and recycling of cellular components and invading pathogens, involved in cellular homeostasis and activation of innate and adaptive immunity, through the regulation of inflammatory responses and antigen presentation. Interestingly, HFE has been recently identified as a negative regulator of antiviral innate immunity related to type I IFNs, acting on the degradation of antiviral signaling proteins through selective autophagy.[20] Type I IFNs are important mediators in the control of HIV infection.[21] These findings also evidence the strict cross-talk among iron balance, autophagy, and innate immune response.[20] Autophagy also plays an important role in HIV-1 replication,[22] especially in ECs.[23,24] The treatment of PBMCs isolated from ECs with rapamycin, an inhibitor of mTORC1, promotes autophagy, resulting in the impairment of viral production.[23] Further, in humanized mouse models pan-inhibitors of mTORC1 inhibit HIV-1 infection by interfering with virus entry, through the reduction of C-C chemokine receptor type 5 levels, and with the transcription of HIV genes.[25]

Considering all the above findings, our hypothesis is that the interplay between H63D mutant HFE and innate immunity, perhaps through autophagy regulation, could play a role in HIV-1 replication control in our patient. Certainly, it will be necessary

to screen more ECs patients for HFE mutations and explore our hypothesis with in vitro and in vivo studies, but we think that it may be an interesting hypothesis for future research. Understanding mechanisms involved in spontaneous control of HIV-1 replication remains indeed a challenge due to its possible implications for HIV cure research.

Author contributions

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