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Research Article

Approach to Homozygous Mutation Δ32 in the CCR5 Gene and its Protectionagainst HIV Infection

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Abstract

Introduction: The human immunodeficiency virus (HIV) has two glycoproteins exposed on its viral membrane, gp41 and gp120, essential molecules for the mechanism of viral infection when it interacts with the CD4 molecule and chemokine coreceptors CCR5 and/or CXCR4 located on the host cell membrane. In 1996, a homozygous mutation was discovered in the gene encoding the CCR5 coreceptor protein, called Δ32. This study aimed to understand the mechanisms that provide the CCR5Δ32 coreceptor with protection against HIV-1 and its evidence.

Methodology: A study was carried out based on bibliographical research, with 16 Basic references and 16 complementary references.

Results: All studies consulted confirm that the genetic coordinates of the CCR5 gene in the human karyotype are 3p21.31; The mutation is characterized by the deletion of 32 pairs of nitrogenous bases, making the CCR5Δ32 coreceptor a non-functional protein, preventing the virus from carrying out adsorption/fusion with the host cell membrane, making its penetration impossible; There was 5 evidence of patients with HIV-1 undergoing bone marrow transplantation from a donor carrying the CCR5Δ32 mutation in their stem cells. After stopping antiretroviral therapy, the viral load remained undetectable. The common factor among these patients is that they all had cancer as a comorbidity (4 Acute Myeloid Leukemia and 1 Hodgin Lymphoma).

Conclusion: The CCR5Δ32 mutation renders the CCR5 coreceptor nonfunctional, preventing HIV-1 from penetrating the cell. Investigations confirm the existence of cured cases. There are no studies that prove the presence of the CCR5Δ32 mutation in individuals from Luanda. We propose an intersectoral investigation between healthcare institutions to screen individuals with this mutation and carry out a line of experimental research in patients with HIV-1.

Keywords: HIV-1; CCR5 Receptor; CCR5Δ32 Mutation

Introduction

AIDS is caused by retroviruses, specifically the Human Immunodeficiency Vírus (HIV). HIV is a retrovirus belonging to the lentivirus family, its genome is 9 kb and contains 9 genes that code for 15 proteins. HIV-1 is more prevalent and more pathogenic, moreover, this strain is responsible for the vast global pandemic. The introduction of HIV into the human species possibly occurred between 1920 and 1940. Phylogenetic and epidemiological analyses suggest that HIV-1 may have evolved from the simian immunodeficiency virus (SIVcpz) of chimpanzees,

which is closely related to HIV-1 [1]. The transmission of the virus between species likely occurred through people who had contact with primates, specifically in the context of hunting, when humans consumed chimpanzee meat, thus coming into contact with blood contaminated by the virus. It is an infection of human lymphocytes and other organs. It is transmitted from person to person through sexual relations, transfusions, placental or transvaginal routes, breast milk, or any other direct contact with infected human fluids [1]. Two types of HIV have been identified: HIV-1 and HIV-2 [2].

HIV can directly affect the brain, gonads, kidneys, and heart, causing cognitive impairment, hypogonadism, renal failure, and cardiomyopathy [3].

HIV Epidemiology

HIV continues to be a significant global public health issue, having claimed 40,4 million [32,9-51,3 million] lives so far, with ongoing transmission in all countries around the world; some countries are reporting increasing trends in new infections when they were previously on the decline. It is estimated that there were 39,0 million [33,1-45,7 million] people living with HIV at the end of 2022, two-thirds of whom (25,6 million) are in the African Region of the World Health Organization (OMS). In 2022, 630.000 [480.000-880.000] people died from HIV-related causes, and 1,3 million [1,0-1,7 million] people acquired HIV [4].

There is no cure for HIV infection.However, with access to prevention, diagnosis, treatment, and effective care for HIV, including for opportunistic infections, HIV infection has become a manageable chronic health condition, allowing people living with HIV to lead long and healthy lives [4].

Characteristics of the HIV virus

The morphological structures of HIV-1 and HIV-2 include: structural and functional proteins; an RNA genome; and the enzymes (reverse transcriptase or p64, integrase or p32, and protease p10).

There is a viral envelope that consists of a lipid bilayer that was inherited from the host cell during the budding process. On its outer layer, there are two distinct proteins exposed: the transmembrane glycoprotein gp41 and gp120. These two play a significant role in the viral infection mechanism. The gp120 is the outermost part, responsible for the virus's binding to host cells. She is linked to gp41, which is a transmembrane protein, meaning it crosses the viral envelope [5].

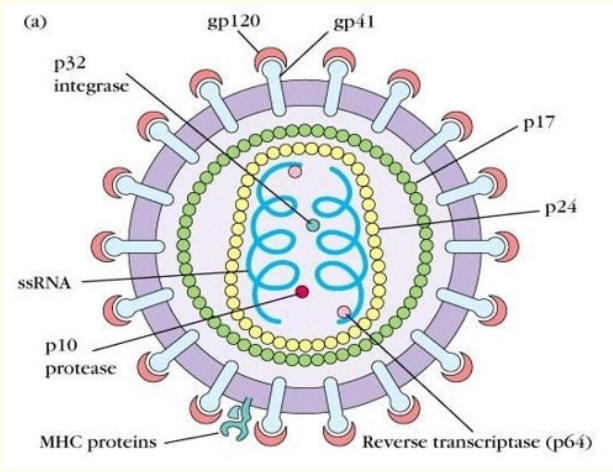

Figure a

The HIV inside contains the viral matrix protein of the virus, which is located between the envelope and the capsid, called p17, enclosed by this protein, is the capsid composed of p24.

In the innermost part, there are two simple RNA filaments, the p17 protein (matrix), and three essential enzymes: p51/p64 (reverse transcriptase), p10 (protease), and p32 (integrase). The reverse transcriptase enzyme is responsible for transcribing viral RNA into a double-stranded DNA, thus creating a copy of double-stranded DNA (cDNA). The integrase enzyme is responsible for incorporating the viral cDNA into the host cell's genome.

Characteristics of the viral genome

The genetic material of HIV is approximately 9.8 Kb and consists mainly of three structural genes (gag, pol and env), and six regulatory genes (tat, rev, nef, vif, vpr, vpu). The gag and env genes encode structural proteins, the pol gene encodes viral enzymes, and the other regulatory genes are important in regulating the viral cycle and pathogenesis of the virus, and the env gene is one of the most variable regions of the HIV genome, being responsible for encoding transmembrane and surface glycoproteins, whose main function is to mediate the entry of HIV into the host cell.

In the case of HIV-1, the gag gene encodes for the precursor protein (p55) which, after cleavage, gives rise to the capsid protein (p24), genome-associated proteins (p6 and p9), and the matrix protein (p17); the pol gene produces a precursor protein (p68) that, when cleaved, generates enzymes such as reverse transcriptase (p51), integrase (INp31), and protease (p10); and the env gene produces a precursor polypeptide (gp160) of the envelope glycoprotein that, when cleaved, results in the external or surface glycoprotein (gp120) and the transmembrane glycoprotein (gp41) [6].

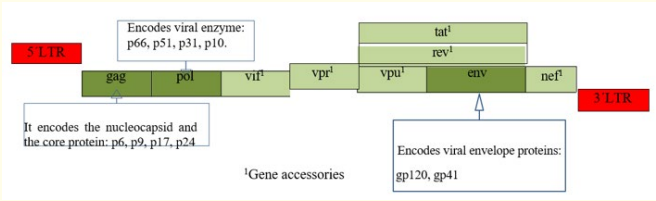

Figure 1: HIV genome, with a description of its genes and the proteins that each gene encodes.

Common characteristics of the host cells of the HIV virus

The term viral tropism refers to the type of cells that a specific virus infects. HIV can infect various immune cells, such as helper T cells or CD4+ cells, macrophages, and microglia [7].

Helper T lymphocytes or CD4 cells are cells that organize and command the immune response to antigens. Produced in the thymus gland, they learn to memorize, recognize, and thus coordinate activities aimed at destroying foreign microorganisms that enter the human body. Macrophages are phagocytic cells, being the differentiated form of blood monocytes, which are strategically distributed in various tissues to give rise to the mononuclear phagocyte system, just as microglia are cells that are part of this system and are located in the central nervous system.

In 1996, several research groups discovered that receptors for chemotactic cytokines in cells, known as chemokines, act as co-receptors for the penetration of HIV into the target cell and the initiation of its replicative cycle. Chemokines are classified into families according to the position of their cysteine residues in the amino terminal region. There are at least four families of chemokines, two of which are well characterized: the α and β chemokines [8]. α chemokines have an amino acid separating the two terminal cysteines (CXC). In ß chemokines the first two cysteine residues are adjacent to each other (CC). In the family of ά chemokines (CXC) there are four receptors described: from CXCR-1 to CXCR-4 and these bind to the CXC chemokines. In the ß chemokine (CC) family there are eight receptors: CCR-1 to CCR-8, which interact with CC chemokines. Chemokine receptors are expressed on different types of leukocytes. The interaction of receptors and their chemokines activates multiple intracellular signaling pathways, as the receptors traverse the cell membrane seven times and are associated with G proteins. This interaction culminates in cell activation and migration. [9].

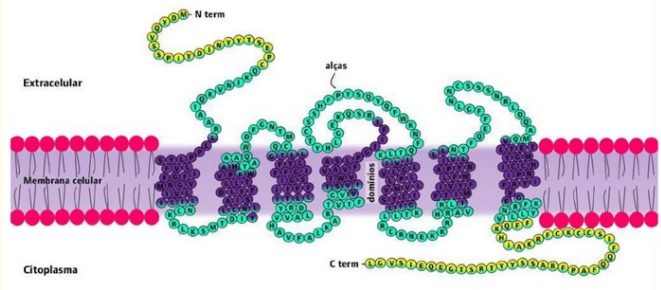

Figure 2: Predicted structure of the CCR5 protein. The protein encoded by the CCR5 gene is a transmembrane protein, featuring seven domains connected by loops, three of which face the extracellular side and the other three face the intracellular side. The N-terminal portion (the upper end of the protein, highlighted in yellow) and the second extracellular loop are binding sites for the CD4 cell to HIV receptors.

Source: Oliveira., *et al*. 2021.

Mechanism of HIV infection

The HIV virus primarily infects cells that have the CD4 molecule on their surfaces.CD4 is found in immune cells, mainly in T-helper lymphocytes, which are responsible for the functioning of the immune system, and also in macrophages, cells that travel throughout the body fighting bacteria and other germs [10]. The viral replication mechanism that occurs inside the host cell takes place according to the following steps: adsorption, fusion and/or penetration, uncoating, reverse transcription, import of proviral DNA into the nucleus, integration, assembly and budding.

Adsorption (binding)

The active complex of the surface glycoproteins gp120 and the transmembrane gp41 controls the entry of the virus components into the cell, together with the surface receptors and co-receptors CCR5 and CXCR4 of the CD4 cell.The entry of the HIV virus begins with the binding of the gp120 protein to the CD4 cell surface receptor.This binding results in a rearrangement of the gp120 protein, allowing it to bind to the co-receptor on the surface of the cell.The interactions between the gp120 protein and the co-receptors CCR5/CXCR4 are established [11].

Fusion/Penetration

After the virus binds to the cell, the activation of the gp41 protein occurs, altering the conformation of gp41, which exposes the fusion peptide that inserts into the T cell membrane, leading to the fusionof the viral envelope with the cell membrane, allowing the virus to penetrate [12]. The fusion of viral and cellular membranes results in the creation of a pore that connects the interior of the virion with the cytoplasm of the target cell, facilitating the entry of the viral capsid into the cytoplasm of the host cell [13].

Undressing

It consists of the loss of the viral capsid, which involves cellular factors and the viral proteins MA, Nef, and Vif, facilitating the release of the capsid's contents (genomic RNA and the proteins MA, TR, IN, and Vpr) into thecytoplasm of the target cell [14].

Reverse transcription

At this stage, viral replication requires that reverse transcriptase (an RNA-dependent DNA polymerase) copies the RNA of HIV, producing proviral DNA from the transfer RNA (tRNA) primer that is attached to the genomic RNA at the primer binding site (PBS), located just after the end of the 5' LTR [15]. This copying mechanism is prone to errors, resulting in frequent mutations and, therefore, new genotypes of HIV. Once in the cytoplasm, the viral RNA genome is retrotranscribed into a double-stranded proviral DNA by viral reverse transcriptase [16].

Import of proviral DNA into the nucleus

After reverse transcription, the proviral DNA is associated with viral and cellular proteins in a large pre-integration nucleoprotein complex (PIC) that is transported to the cell nucleus through the nuclear pore. In addition to important cellular factors, three HIV proteins associated with the PIC (MA, Vpr, and IN) play a crucial role in this transport [17].

Integration

At this stage, the double-stranded linear DNA of the PIC is inserted into the host chromosome by the integrase enzyme (IN), which integrates the viral DNA into the host cell's DNA (17). The inserted viral DNA is called proviral DNA. After the integration phase, the processes that make up the Central Dogma of Cellular and Molecular Biology occur, where with each cell division, the integrated proviral DNA is duplicated along the host's DNA. Subsequently, the proviral DNA of HIV can be transcribed into viral RNA of HIV. In the cytoplasm, the viral mRNA strands are translated, producing the polyproteins that will give rise to the viral proteins (3) [17].

Assembly and Sprouting

As the Env proteins migrate and insert themselves into the plasma membrane. The Gag and Gag-Pol polyproteins also move to the cell membrane and begin the assembly of the virion directed by the Gag polyprotein. Furthermore, viral enzymes, genomic RNA, and cellular compounds associate in the immature nucleocapsid. Later, this complex emerges through the plasma membrane, producing an immature virion [181].

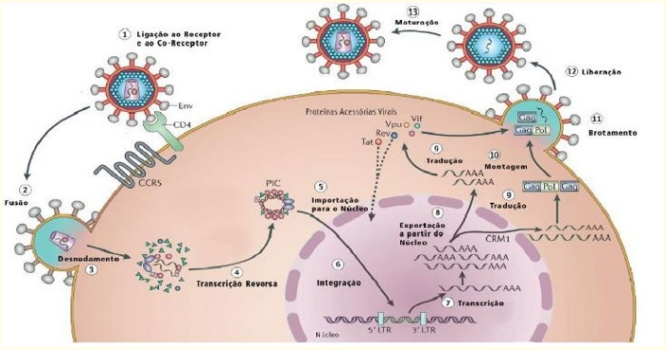

Figure 3: HIV-1 replicative cycle.

Source: Technical Manual for the Diagnosis of HIV Infection; 2016.

Bone marrow transplant

Bone marrow is located inside the bones and is composed of hematopoietic stem cells; it is responsible for producing the cells that make up the blood (red blood cells, white blood cells, and platelets). For the transplant to take place, it is necessary to conduct tests that prove compatibility between the individuals. From there, the donor undergoes a procedure performed in a surgical setting, where needles will be used to puncture the long bones, specifically those located behind the pelvis (preferably the femur). For the recipient, treatment is necessary to destroy their bone marrow cells, which involves conditioning through chemotherapy and, in some cases, total body radiation. The transplant consists of a quick procedure, similar to a blood transfusion, in which a bag of cryopreserved material rich in bone marrow progenitor cells is infused into the recipient. They circulate and settle in the area where the patient's bone marrow used to be, and there they develop, generating a new immune memory. Individuals infected with HIV, when untreated, have a predisposition to the development of cancers such as non-Hodgkin lymphomas, Kaposi's sarcoma, and invasive cervical cancer. These neoplasms are potentially influenced by AIDS and most often occur due to the individual's involvement and co-infection with certain oncogenic viruses (Epstein-Barr, herpes virus, and HPV) related to immunosuppression, which do not affect individuals with competent immune systems. With the development of antiretroviral therapy and the increase in the life expectancy of these individuals, other cancers not influenced by AIDS began to rise in occurrence, among them, Hodgkin lymphoma. From this, tumors that affect the functioning of the spinal cord may make the patient a candidate for transplant, in an attempt to restore its normalcy. When HIV-infected patients receive a bone marrow transplant with wild-type CCR5 gene, a phenomenon known as viral rebound occurs, as the preparation for the transplant does not recognize a complete elimination of the virus. In these patients, the hypothesis of a transfusion with a sample from a homozygous Δ32/Δ32 donor has been considered as a therapeutic measure since the last decade of the 20th century.

A significant impact on HIV research occurred in 1996 when scientists discovered that the CCR5 delta 32 mutation (CCR5Δ32), present in both alleles of the CCR5 gene (CCR-5-Δ32/Δ32), provided protection to CD4 cells against HIV-1 infection. This mutation, according to the HGVS (Human Genome Variation Society), is referred to as g.8315_8346del [19].

This mutation is present in about 1% of the global population. It is mainly found in Europe among Caucasian populations, with a frequency of about 10% that varies in its geographical area. The allele shows a decline in frequency from the north to the southeast of Eurasia, with rare or nonexistent occurrences in Asians and in native populations of Africa, the Americas, and Oceania.

Justification

Since the discovery of HIV infection in humans, various researchers have been engaged in finding a more effective treatment. Clinical and scientific discoveries regarding the pathogenesis of HIV have been achieved through the understanding of the CCR5 gene. The CCR5 coreceptors present on the membrane of agranulated cells such as helper T lymphocytes (CD4 cells) act as chemokine coreceptors, essential for the binding and entry of HIV into the cell and other pathogenic viruses.

It has been discovered that there are individuals who have a homozygous mutation for the gene that encodes the co-receptor protein CCR5, known as CCR5/Δ32. This genetic mutation provides protection against HIV infection.

In Luanda, there are few studies on the subject, while at the global level, there is an abundance of evidence reporting cases of individuals with these particularities. This leads us to conduct this bibliographic research supported by a brainstorming session among researchers to outline more effective methods for obtaining cells with this type of mutation and to propose a more advanced investigation on the subject in the future.

Objectives

1 General Objective

- Investigate the mechanisms that confer the CCR5Δ32 co-receptor protection against the HIV virus.

Specific Objectives

- Locate the genetic coordinates of the CCR5 gene on the human karyotype;
- Describe how mutation can confer protection;
- Identify the evidence of CCR5/Δ32 cell therapy and its results;
- To address in a brainstorm among researchers methods for the experimental use of this technique in patients with HIV-1, in order to develop a research line in Luanda.

Metodology

A study will be conducted based on bibliographic references, related studies, and evidence from cases of patients infected with the HIV virus who underwent treatment with genetically modified cells or material and/or with mutations in the CCR5Δ32 coreceptor. In this context, specialized literature and scientific and technical reports on the subject were consulted.

To contextualize the reality of the province of Luanda, we will conduct a brainstorming session to evaluate which methodology to use to obtain cells with these characteristics for therapy in patients infected with the HIV virus.

Results

Location of the genetic coordinates of the CCR5 gene in the human genome

The CCR5 gene (C-C chemokine receptor type 5) is located on the short arm of chromosome 3, band 21.31 (3p21.31), has a length of 6kb, containing 3 exons, 2 introns, and 2 promoter regions. The promoter upstream of exon 1 is called P2, while the distal promoter is referred to as P1 and encompasses regions of intron 1 and exon 2. The transcription of the CCR5 gene is initiated at multiple start sites found in exons 1 or 2; the result of using the P1 or P2 promoter influences the generation of different transcripts, or isoforms [20].

How mutation can provide protection to patients with HIV

During the adsorption process, there will be a binding between the surface glycoprotein of HIV and the CD4 protein of the cytoplasmic membrane of helper T lymphocytes, monocytes, and macrophages. The co-receptors most commonly used by HIV for entry into the cell are CCR5 and CXCR4. However, while CCR5 is used more frequently in interactions, CXCR4 is more utilized in the later stages of the infection.

The CCR5Δ32 mutation is characterized by the deletion of 32 base pairs that spans from nucleotide 794 to 825 on the short arm of chromosome 3, band 21.31. (3p21.31). This mutation is a structural chromosomal mutation by deletion and results in a change in the reading of mRNA during translation, and it also leads to a nonsense mutation due to a premature termination caused by the formation of a stop codon. Due to this fact, the mutated allele encodes a truncated protein that contains 215 amino acids, whereas the normal protein (wild-type protein) contains 352 amino acids.

The affected region corresponds to the second extracellular loop of the protein, which loses the last three transmembrane domains, as well as important interaction regions with the G protein, responsible for extracellular signal transduction, rendering the CCR5 co-receptor non-functional. Anatomically, these coreceptors are located in the plasma membrane, crossing it 7 times; however, due to a mutation, it will be found in the cytoplasm. The phenotypic expression of this mutation will provide protection against infection caused by HIV, as this virus will not be able to carry out the actions of adsorption or fusion to penetrate the cell.

This mutation has an autosomal recessive characteristic, and therefore, individuals who are heterozygous (CCR5/CCR5Δ32) express the receptors in a reduced manner, showing decreased susceptibility and a delay in the progression of the infection and evolution to AIDS. Individuals who are homozygous for the mutation exhibit an absence of the CCR5 receptor, which leads to a high resistance to HIV infection. Based on this knowledge, scientists inferred that the transplantation of bone marrow, stem cells, and umbilical cord from a donor carrying the CCR5Δ32/Δ32 mutation in homozygosity could be an effective treatment for curing patients with HIV. However, it ceased to be a hypothesis and became a reality due to evidence of curing HIV infection in people who underwent this procedure.

Evidence of CCR5/Δ32 cell therapy and its results

During the last four decades of the HIV/AIDS epidemic, it has only been possible to achieve a cure in five cases, with the pioneering patients being from Berlin and London, through experimental procedures involving stem cell transplants from donors with a genetic mutation.

First case (Berlin Patient) according to Dr. Hutter, 2009 [21].

- **Patient data:** male patient, sexual orientation homosexual, white, American citizen residing in Germany, 44 years old "at the time."
- **Team leader of the investigation:** Dr. Gero Hütter
- **Presentation:** 15th Conference on Retroviruses and Opportunistic Infections (CROI), held in Boston, United States.

The hypotheses of Dr. Hutter and his team were as follows

- If all the immune cells of a patient with HIV were eliminated and then replaced with immune cells that could not be infected by the virus, then HIV could be eradicated from the body (although some strains of HIV can bind to CD4 and another co-receptor, CXCR4, the strain in question was using CCR5 to infect its cells).

- Thus, instead of simply injecting normal stem cells for the treatment of leukemia, he could look for a donor who had the CCR5Δ32 mutation.
- The intention was to replace all the patient's immune system cells with others that would cure them of leukemia and HIV.
- In addition to the complex process of finding a compatible donor (tissue type between donor and patient), the compatible donor would also need to have two copies of the CCR5 Δ32 mutation, one inherited from his father and the other from his mother.

According to the available scientific literature, the mutation would be more common in Northern Europeans, and Germany would be the ideal place to locate the ideal donor, as it has a large and centralized registry of stem cell donors. From blood samples, Hütter found 232 donors worldwide who matched the patient in the stem cell donor bank. If the odds were confirmed, two would have the double delta 32.

As is standard procedure for a stem cell transplant, the patient underwent a "conditioning regimen," an intense process of chemotherapy and radiation that destroys the immune system in order to make room for the development of the transplanted stem cells. During this procedure, the patient is completely vulnerable to different microorganisms, which can lead to new infections. There was also the possibility of what is known as "graft-versus-host disease," meaning that the cells of the new immune system do not recognize the body and begin to attack the patient's cells, causing infection. Other risks were also part of the procedure, in addition to the transplant itself. Even if the transplant prevented infection by the most common strain, it was possible for another strain of the virus to emerge and reinfect him. And, finally, the procedure might simply not work.

The patient received the transplant on February 6, 2007, at the Charité Hospital in Berlin. In an agreement with the doctors, antiretroviral therapy for the treatment of HIV has been discontinued at this time. This was considered an important action to ensure that drugs did not harm the ability of transplanted cells to survive in the new body and for a potential cure to emerge. At any sign of the virus in the blood, the medications would be resumed. In addition to the rounds of chemotherapy conducted before the transplant, immunosuppressive medications were also used to prevent the rejection of the stem cells post-transplant.

He survived the operation and the "graft" was achieved 13 days after the procedure. The recovery process was good, and the patient was able to return to work and physical activities. In the months that followed, HIV was not found in his blood until the beginning of 2008, when leukemia returned. The return of leukemia led the doctors to decide on a second transplant using stem cells from the same donor. Returning to the same donor is the conventional procedure, as the patient would now be "used to" that immune system. So, in February 2008, the second transplant took place, 13 months after the first.

The patient's second transplant cured his leukemia, but it was much more difficult for him than the first. Neurological problems can be a side effect of chemotherapy and radiation used in ablation. In your case, the doctors suspected that leukemia might have spread to the brain and requested a biopsy. The result was negative, but it brought new problems. Due to the intervention, he temporarily lost the ability to walk and speak, as well as having his vision affected.

The patient continued to receive immunosuppressive treatment to prevent the rejection of the transplanted cells for 38 months. At 5, 24, and 29 months after the transplant, colon biopsies were performed to investigate possible graft-versus-host disease in the intestine. In each search, between 10 and 13 additional samples were collected to check for signs of HIV infection in the immune cells of the intestinal wall. During the 38-month follow-up period, the donor cells repopulated the mucosal immune system of the intestine to such an extent that the frequency of CD4+ cells became almost twice as high as in healthy HIV-negative controls, and this phenomenon was also observed in a control group of ten HIV-negative individuals who received stem cell transfer. The repopulation of CD4+ cells was accompanied by the complete

disappearance of the host's CD4+ cells. After two years, the patient had a CD4+ count comparable to that of a healthy adult of the same age. Macrophages carrying CCR5 could not be detected after 38 months, suggesting that chemotherapy had destroyed these long-lived cells and that they had also been replaced by donor cells with the CCR5Δ32 mutation.

However, at the end of September 2020, after a five-month battle against relapsed leukemia, the Berlin patient passed away, 13 years after the first bone marrow transplant, during which the viral load remained undetectable.

Second case (Patient from London) according to Dr. Gupta, 2019. [22].

- **Patient data:** Known as the "London patient," a 40-year-old homosexual man, Venezuelan and residing in London. In addition to HIV, I was undergoing therapy for the treatment of stage 4 Hodgkin's lymphoma.
- **Team leader of the investigation:** microbiologist Ravindra K. Gupta, from the University of Cambridge and Imperial College London, used a procedure similar to that applied to the Berlin patient.
- **Case presentation:** 26th Conference on Retroviruses and Opportunistic Infections (CROI), held in Seattle, USA, and published in the scientific journal Nature.

In 2019, a new wave of enthusiasm was sparked by what could be the second case of a cure. A group of researchers from the University of Cambridge and Imperial College London used a procedure similar to that applied in the Berlin case. The "London patient," however, the therapy would have been less intense for the treatment of stage 4 Hodgkin lymphoma, a type of cancer in the lymphatic system, and the attempt to cure HIV. About sixteen months after the suspension of antiretroviral therapy, tests were unable to detect the presence of HIV in the patient's body. The case was presented at the 26th Conference on Retroviruses and Opportunistic Infections (CROI), held in Seattle, USA, and published in the scientific journal Nature.

Still in 2020, after further sensitive tests and examinations with tissues and substances similar to those in the Berlin case, doctors and scientists presented the "evidence for the cure of HIV" at the CROI and published an extensive article in the scientific journal The Lancet. The results now confirmed that a cure for HIV infection could be reproduced, although it was difficult, the researchers said. Just as happened in the case of Berlin, the article sought to put an end to the controversies surrounding the cure. To this end, the researchers presented long-term data for this patient (up to 30 months after the interruption of antiretroviral therapy), including sampling from various HIV reservoir sites.

Adam was included in a larger clinical trial of IciStem - International Collaboration to guide and investigate the potential for HIV cure by Stem Cell Transplantation, which maintains a database of about 22,000 donors. IciStem is a consortium of European scientists studying stem cell transplants to treat HIV infection with the aim of finding a cure. The consortium is supported by amfAR, the foundation U.S. research on AIDS. In 2019, their scientists were monitoring 38 people living with HIV who had received bone marrow transplants, including six from donors without the mutation. The patient from London was number 36 on this list. There was another in remission, number 19 on the list, referred to as the "Düsseldorf patient [23].

Third case (Patient from Düsseldorf) according to Dr. Jensen (2019) [24]

- **Patient data:** Known as the "Düsseldorf Patient," a 49-year-old German male homosexual, underwent a procedure to treat his acute myeloid leukemia.

- **Team leader of the research:** Björn Jensen, at the University of Düsseldorf, Germany. Case presentation: The initial results of the case were presented at CROI 2016 in Boston, Massachusetts.

Just like the patient from Berlin, this patient underwent the procedure to treat his acute myeloid leukemia. He interrupted antiretroviral therapy in November 2018, and since then, HIV has remained undetectable in his body. Three years after the interruption of antiretroviral therapy and without the return of the virus infection, your doctor began to refer to you as cured.

Fourth case (patient from City of Hope) (2019) [25]

- **Patient data:** Known as the "City of Hope patient," male, 66 years old, from Southern California. He has been living with HIV since 1988 and was diagnosed with acute myeloid leukemia in 2018.

- **Team leader of the research team:** Center for Health and Hematologic Cancer Research, California, USA.

- **Case presentation:** 24th International AIDS Conference, in Montreal, CanadaIn early 2019, he received a stem cell transplant from an unrelated donor with a double CCR5/Δ32 mutation. Before the procedure, he underwent reduced-intensity conditioning chemotherapy. After the transplant, he developed only a mild case of graft-versus-host disease. The clinical tests showed that the patient achieved "100% chimerism," which means that all of their immune cells originated from the donor. He continued antiretroviral therapy for two years after the transplant. At this point, with a stable undetectable viral load, in agreement with their doctors, they decided to interrupt the treatment in a carefully monitored manner.

Three years after transplantation, and 17 months after discontinuing antiretrovirals, the patient has no evidence of rebound HIV RNA viral load and no detectable HIV DNA in peripheral blood cells, a marker of the latent viral reservoir. Intestinal tissue biopsies also found no evidence of viruses capable of replication. Laboratory studies showed that his new blood cells were immune to strains of HIV that use the CCR5 receptor. However, as in other cases, it may remain susceptible to infection by the virus that uses the alternative CXCR4 receptor. His leukemia also remains in remission.

Fifth case (The patient from New York) a new perspective

According to the report, a "middle-aged mixed-race American woman"

In February 2022, a presentation by researchers from the IMPAACT network- International Maternal Pediatric Adolescent AIDS Clinical Trials of the United States, at the CROI. He received a stem cell transplant for the treatment of his acute myeloid leukemia and HIV infection [26]. The transplanted cells came from two sources: stem cells from a healthy adult relative (without HIV or cancer infection) to efficiently and effectively increase and restore the population of blood cells to reduce infectious complications, and the umbilical cord blood of an unrelated newborn CCR5/Δ32 was used to provide long-term blood reconstitution [27].

A "New York patient" had not shown detectable HIV for 14 months since stopping their antiretroviral therapy. At this stage, still cautious with the word "cure," the medical scientists involved considered the patient to be in a state of "HIV remission." Generally, the blood produced by the umbilical cord adapts more easily and requires less matching in the human leukocyte antigen system compared to adult stem cells. On the other hand, umbilical cord blood does not produce large quantities of cells to be effective as a cancer treatment in adults, so transplants of this type have been limited to pediatric oncology. In haplo-cord transplants, the additional transplant of stem cells from an adult donor, which provides a multitude of cells, can help compensate for the shortage of umbilical cord blood cells [28].

Discussion

As for the advantages of the mutation considering CCR5Δ32, 115 studies demonstrate its protective value against HIV-1 infection, hepatitis C, flaviviruses, smallpox, and it facilitates recovery after a stroke.

As for the disadvantages of the mutation, recent studies with other populations have observed that when in homozygous form (Δ32/Δ32), this mutation can have a deleterious effect, resulting in a shorter life expectancy, higher mortality from Influenza-which is a more prevalent disease in global communities compared to HIV- and four times greater susceptibility to certain infectious diseases. (17). Although it is a promising form of protection, it is still not possible to predict all the consequences of this mutation in the individual, and further functional studies are needed to understand the role of CCR5 in human pathologies.

Conclusion

- The CCR5 gene is located on the short arm of chromosome 3, at band 21.31. (3p21.31)
- It is a structural chromosomal mutation due to interstitial deletion, autosomal recessive.
- This mutation occurs due to the deletion of 32 base pairs of nucleotides, ranging from nucleotide 794 to 825, transforming the co-receptor CCR5 into CCR5/Δ32.
- Due to this deletion, the co-receptor becomes obsolete and non-functional, preventing the virus from entering the cell and thereby providing protection to the organism against HIV-1.

- So far, reported data at the CROI (Conference on Retroviruses and Opportunistic Infections) shows us that there have been 5 cases of patients with undetectable viral load 14 months after stopping HIV-1 treatment, due to the use of this treatment method against both HIV-1 and cancer simultaneously.

Recommendations

(Brainstorm among researchers)

Literature informs us that in Africa there is little probability of the existence of individuals homozygous for the CCR5/Δ32 gene, there is no evidence from a study proving that this statement corresponds with the reality in Luanda, this time, during the brainstorm among researchers it led us to following recommendations

For the National Institute for the Fight Against AIDS (INLS)

Create conditions for

- Creation of a database of homozygous individuals for CCR5/Δ32;
- Creation of a genetic material bank for CCR5/Δ32 (umbilical cord, stem cells, blood, etc.).

The actions described above will serve to trigger a collection and storage of cells that have the genetic mutation for the creation of research lines on HIV treatment from cells of individuals homozygous for the CCR5/Δ32 gene, following tests that confirm this mutation.

To the National Institute of Health Research (INIS)

- As an institution that has a focus on diagnostic methods and techniques, in cases of screening and investigation, it can be a great aid in conducting tests for the detection of polymorphism using the PCR (Polymerase Chain Reaction) technique;
- We suggest a partnership with the Genoprimer laboratory in Curitiba, Brazil, as this institution has extensive experience in this field and has already developed a test to detect the Δ32 deletion in the CCR5 gene.

National Blood Institute

- Tracking of the CCR5/Δ32 gene in blood donors in hemotherapy services of the
- sanitary units.

David bernardino pediatric hospital

- Tracking the CCR5/Δ32 gene from the heel prick test performed on newborns.

To the Pediatric Hematology Institute Dra. Victória do Espírito Santo

- Coordinate the research in the clinical forum due to the sensitivity of the methodology applied.
- Creation of a multidisciplinary team that fits the profile of the research.

Suggestion for the Profile of the Multidisciplinary Team: Specialist in applied molecular biology, Specialist in applied molecular biology, Geneticist, Surgeon specializing in bone marrow transplantation, Specialist in the treatment of patients infected with HIV, Project manager, and copywriters.

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