Immunological and virological findings in a patient with exceptional post-treatment control: a case report

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Summary

Background **Although antiretroviral therapy (ART) is effective in suppressing viral replication, HIV-1 persists in reservoirs and rebounds after ART has been stopped. However, a very few people (eg, elite and post-treatment controllers) are able to maintain viral loads below detection limits without ART, constituting a realistic model for long-term HIV remission. Here, we describe the HIV control mechanisms of an individual who showed exceptional post-treatment control for longer than 15 years.**

Methods **We report the case of a Hispanic woman aged 59 years with sexually acquired acute HIV infection, who was included in an immune-mediated primary HIV infection trial involving a short course of ciclosporine A, interleukin-2, granulocyte macrophage colony-stimulating factor, and pegylated interferon alfa, followed by analytical treatment interruption. We did the following viral assays: total and integrated HIV-1 DNA in CD4 T cells and rectal tissue, quantitative viral outgrowth assay, HIV-1 infectivity in peripheral blood mononuclear cells and CD4 T-cell cultures and viral inhibitory activity by natural killer (NK) and CD8 T cells. NK and T-cell phenotypes were determined by flow cytometry. HLA, killer cell immunoglobulin-like receptors, Δ32*CCR5*, and *NKG2C* alleles were genotyped.**

Findings **After ART and immunomodulatory treatment, the person maintained undetectable plasma viral load for 15 years. HIV-1 subtype was CFR_02AG, *CCR5*-tropic. We found progressive reductions in viral reservoir during the 15-year treatment interruption: total HIV DNA (from 4573·50 copies per 10^6 CD4 T cells to 95·33 copies per 10^6 CD4 T cells) and integrated DNA (from 85·37 copies per 10^6 CD4 T cells to 5·25 copies per 10^6 CD4 T cells). Viral inhibition assays showed strong inhibition of in vitro HIV replication in co-cultures of CD4 T cells with autologous NK or CD8 T cells at 1:2 ratio (75% and 62%, respectively). Co-cultures with NK and CD8 T cells resulted in 93% inhibition. We detected higher-than-reference levels of both NKG2C-memory-like NK cells (46·2%) and NKG2C γδ T cells (64·9%) associated with HIV-1 control.**

Interpretation **We described long-term remission in a woman aged 59 years who was treated during primary HIV infection and has maintained undetectable viral load for 15 years without ART. Replication-competent HIV-1 was isolated. NKG2C-memory-like NK cells and γδ T cells were associated with the control viral replication. Strategies promoting these cells could bring about long-term HIV remission.**

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Introduction

Despite its efficacy, current antiretroviral therapy (ART) does not cure HIV-1 infection. The virus persists in reservoirs and rebounds can occur following treatment interruptions. Rare cases of sterilising cure have been achieved in people treated with allogeneic stem-cell transplantation for severe oncological diseases. As this procedure is associated with high morbidity,[1] the clinical scenario more accurately serves as a proof of concept, to show that HIV can be eliminated from the body, than an actual scale-up model. A functional cure has been described in elite controllers—a small proportion (<0·5%) of people living with HIV who control viral replication spontaneously, even though the body does not eliminate the virus.[2] The case of elite controllers, and particularly the so-called exceptional elite controllers, who maintain the control for more than 25 years, presents a more achievable scenario than that of a sterilising cure. However, a substantial proportion of these individuals will eventually lose spontaneous control of HIV replication. Even elite controllers sometimes undergo ART since inflammatory markers and co-morbidities in these people have shown to be higher than in the general population.[2] Different immunological studies point to the main mechanism involved in HIV control as being the mediation of cellular immune responses by

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For the Spanish translation of the abstract see Online for appendix 1

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Research in context

Evidence before this study

We searched PubMed for randomised trials published in English from inception to Aug 31, 2022, on functional cure in people with HIV-1 without antiretroviral treatment (ART), using the search terms "HIV" or "human immunodeficiency virus" or "AIDS", "functional cure", "post-treatment control (post-treatment controllers)" and "remission". We also searched PubMed for bibliographies published in English in the same period on "HIV prevention" or "HIV control" without ART mediated by memory-like natural killer (NK) cells or γδ T cells and candidate HLA and killer cell immunoglobulin-like receptor (KIR) genes associated with this type of innate response, using the search terms: "memory-like NK cells HIV control", "adaptive NK cells", "NKG2C HIV", "HLA-E", "γδ T cells", "NKG2C γδ T cells", "CD8 γδ T cells", "CMV NK cells", "CMV γδ T cells", "Cyclosporine A CMV NK cells", "HLA B*4403", "HLA Bw4", "HLA E*01:03", "NKG2C deletion", "KIR3DL1", "KIR2DL3", "KIR2DL1" and "KIR2DS4". Long-term remission was described in 2015 in elite controllers, who spontaneously control viral replication. In contrast, post-treatment controllers are a subset of people living with HIV who briefly receive ART and who control replication over variable periods of time after treatment is stopped. There is very little published research, however, on virological control beyond 15 years after stopping ART (exceptional post-treatment controllers). The virological control mechanisms are poorly understood, although some studies have shown the role of memory-like NK cells and the γδ T cell in inducing cytotoxicity in HIV-infected cells and in HIV protection or control.

Added value of this study

We describe a woman who exhibits exceptional HIV-1 post-treatment control, mediated by memory-like NKG2C NK cells and γδ CD8 T cells. We observed that NKG2C-memory-like NK cells and γδ CD8 T cells could contribute to the control of viral replication and long-term remission.

Implications of all the available evidence

Our findings could pave the way for the development of therapeutic strategies based on the promotion of these cell subsets in natural immunity and, from there, for the achievement of long-term HIV remission in more people. Functional cure can probably be achieved, particularly in individuals possessing genetic features associated with the protective *HLA* genes and certain NK receptors (eg, specific *KIR* and *NKG2C* alleles).

HIV-1-specific cytotoxic T cells associated with specific HLA haplotypes.[3]

In contrast with elite controllers, post-treatment controllers are a subset of adults[4] and children[5] living with HIV who briefly receive ART and control replication for a varying period following the discontinuation of the aforementioned therapy. Unfortunately, no markers have been reported to identify which people will effectively control viral replication after the cessation of ART. Moreover, like elite controllers, some post-treatment controllers will either lose viral replication control after some time or present with intermittent viraemia.[6] Immune mechanisms involved in HIV control have not been fully described in post-treatment controllers; nonetheless, post-treatment controllers do not carry the HLA genotypes associated with the control of HIV replication, which is the case for elite controllers.[7]

The mechanisms of viral replication control could include the development of broad neutralising antibodies and a robust cytotoxic cellular response against HIV.[8] Apart from adaptive immunity, natural killer (NK) cells provide quick early responses to HIV infection and substantially contribute to disease modulation. However, much like other lymphocyte populations, NK cells can become functionally exhausted in chronic HIV infection, resulting in impaired cytotoxic function, altered cytokine production, and impaired antibody-dependent cell-mediated cytotoxicity.[9] Nonetheless, recent studies have found a special subset of NK cells—so-called memory-like NKG2C CD57 NK cells with memory features[10]—as well as a subset of innate T cells, NKG2C γδ T cells.[11] Both types of cells have high cytotoxic activity and have a key role in controlling HIV-1 replication.[12,13] These cells were also expanded by clinical or subclinical cytomegalovirus reactivation.[12]

Overall, it is extremely unusual to find cases of people living with HIV without ART and with prolonged aviraemia; however, these cases are important in both helping to understand the immune mechanisms involved in HIV control and designing strategies for functional cure. We describe the case of a women aged 59 years who underwent ART and several immunological interventions during primary HIV infection, but who has now maintained undetectable viral load following 15 years of ART discontinuation. Following virological and immunological studies, we propose a mechanism for viral control, which includes mediation via memory-like NK cells and γδ T cells. Furthermore, we hypothesise about strategies that could induce such a state.

Methods

Case report

In 2006, a Hispanic woman aged 59 years with sexually acquired primary HIV infection was included in an immune-mediated primary HIV infection trial[14] that involved several immunological interventions (a short, low-dose course of ciclosporine A, interleukin-2, granulocyte macrophage colony-stimulating factor (GM-CSF), and pegylated interferon alfa, as well as structured interruptions in ART), at Hospital Clinic-IDIBAPS in Barcelona, Spain. This woman was the only person in the study who was identified with long-term

HIV remission after treatment interruption. As part of an observational study, follow-ups continued after the trial. Routine laboratory tests included T-cell counts for nadir CD4, CD4, and CD8; CD4 to CD8 ratio; and HIV-1 RNA load (measured using COBAS Ampliprep or COBAS Taq-Man HIV-1 test [Roche Molecular Systems, Basel, Switzerland]). The detection limit for this assay was 50 copies per mL. Additionally, we quantified HIV RNA using an ultrasensitive protocol that concentrated viral particles from large-volume plasma at a high speed (detection threshold at 2 copies per mL). The study received approval by the Ethics Committee of Hospital Clinic of Barcelona, Spain (HCB/2015/0775). The participant gave informed consent to be included in the trial and for publication of this case report. The study protocol is registered at ClinicalTrials.gov (NCT00979706).

Total and integrated HIV DNA

Total and integrated HIV-1 DNA in highly enriched CD4 T cells by negative selection (STEMCELL Technologies, Vancouver, Canada) was quantified in blood and rectal samples as described elsewhere.[15] We determined the CD3 gene copy number in each tube. The HIV to CD3 copy ratio showed the frequency of T cells harbouring HIV DNA. A standard curve included serial dilutions from 3×10^5 to 3 ACH2 cells, each carrying one single HIV provirus. We then optimised HIV primers and probes to amplify and detect HIV-1 in the A, B, C, D, and A/E (CRF01) clades.

Quantitative viral outgrowth assay

CD4 T cells isolated by negative magnetic selection from previously cryopreserved peripheral blood mononuclear cells (PBMCs) were seeded in duplicate on 24-well plates and later stimulated with anti-CD3 and anti-CD28 magnetic beads in 10% fetal bovine serum supplemented Roswell Park Memorial Institute (RPMI) medium (culture media). We started with 10^6 cells 5-fold serial dilutions of these lymphocytes until reaching 320 CD4 T cells per well in the final dilution. Two days after stimulation, CD4 T cells were co-cultured with CEM-CCR5 (National Institutes of Health HIV reagent programme, Manassas, VA USA) cells in fresh media containing 100 international units [IU]/mL of IL-2. We collected supernatants and did HIV-1 RNA real-time-quantitative PCR for viral detection at 7 and 14 days after stimulation. We used a maximum likelihood method to determine the frequency of HIV-1-infected cells.

In vitro infection and viral inhibition assays by CD8 T cells and NK cells

PBMC and CD4 T cells from both an HIV-1-uninfected donor and the participant were seeded at a concentration of 10^5 cells per well in culture media with 100 IU/mL of human interleukin-2. We activated cells for three days with either phytohaemagglutinin (5 ug/mL) or anti-CD3 and anti-CD28 microbeads (Invitrogen Dynal AS, Oslo, Norway). Then, we infected cultures with HIV-1 NL4·3-Renilla (AIDS Immunopathology Laboratory, Instituto de Salud Carlos III, Madrid, Spain; 100 000 Renilla activity [RLU] per well) a replicative-competent HIV-1 clone. RLU was quantified in cell lysates using the Renilla Luciferase Assay System (Promega, Madison, WI, USA) in an OrionII luminometer (Berthold Detection Systems, Oak Ridge, TN, USA).

NK cells were purified by positive selection with antibody-coated magnetic beads (EasySep Human CD56 Positive Selection Kit II, STEMCELL Technologies, Vancouver, Canada). The CD56-depleted PBMCs eluent was used to purify CD4 T cells by positive selection (EasySep Human CD4 Positive Selection Kit II, STEMCELL Technologies). CD8 T cells were then enriched from this second eluent by negative selection (EasySep Human CD8 T Cell Enrichment Kit, STEMCELL Technologies). Every cell population was phenotypically characterised by flow cytometry using fluorescently labelled monoclonal antibodies. We activated purified CD4 T cells for three days with anti-CD3 and anti-CD28 microbeads and 100 IU/mL of IL-2. Simultaneously, CD8 T cells and NK cells were cultured in culture media with IL-2; however, the latter was supplemented with 18 ng/mL of IL-15 (CellGenix, Freiburg, Germany). Once activated, 10^5 CD4 T cells per mL were seeded on a 96-U-well plate, either alone or in the presence of CD8 T cells alone or with NK cells at 1 to 2 cell ratios, before being infected with HIV-1 NL4·3-Renilla (100 000 RLU per well) by spinoculation. Cells were cultured for three more days with IL-2. RLU was quantified as previously mentioned.

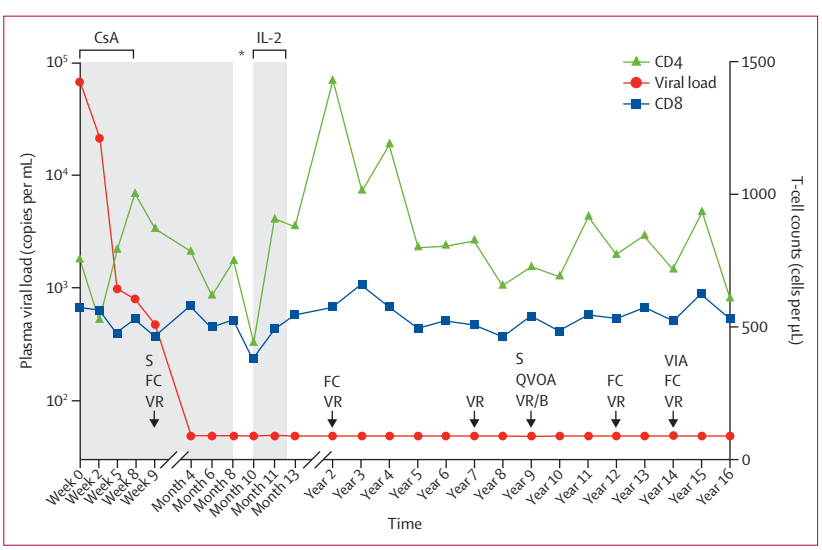

Figure 1: **Clinical course of a woman with an HIV-1 infection**
Participant's CD4 and CD8 T-cell counts were sampled at the referred post-infection times and viral plasma load was quantified simultaneously. T-cell counts are graphed in linear scale on the right y-axis scale, and viral plasma loads are plotted on the logarithmic left y-axis scale. Shaded grey areas correspond to periods when the patient was receiving antiretroviral therapy. The black arrows point to post-infection times when additional analyses were done. CsA=ciclosporine A. S=sequencing. FC=flow cytometry. VR=viral reservoirs in CD4 T cells. QVOA=quantitative viral outgrowth assay. B=viral reservoirs in rectal tissue-derived biopsies. VIA=viral inhibition assays. *Granulocyte macrophage colony-stimulating factor and pegylated interferon alfa treatment.

Characterisation of NK cell, γδ T cell, and resting or activated conventional T cells subpopulations in PBMCs via flow cytometry

PBMCs were stained with a combination of anti-human antibodies against CD4, CD8, NK, and γδ T-cell markers to evaluate purity after the implementation of purification steps (appendix 2 p 5). Isotype control antibodies were used as controls. Acquisition was done in either a BD LSRFortessa or a BD FACSCanto II 3L Flow Cytometer, and later analysed by FlowJo version 10 software (all BD Bioscience, San Diego, CA, USA). Additional methods are described in appendix 2 (pp 1–3).

See Online for appendix 2

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report or decision to submit for publication.

Results

The individual received an early-stage diagnosis, presenting with a severe primary HIV infection that included oral thrush and herpetic oesophagitis. Hospitalisation was necessary, and the individual underwent treatment with fluconazole and intravenous acyclovir. She reported having sexual intercourse only with her husband, who stated having multiple partners and condomless sex. HIV-1 subtype was CFR_02AG, R5 tropic; no transmitted drug resistance was detected. ART (which included ritonavir-boosted lopinavir, emtricitabine, and tenofovir disoproxil fumarate per guideline recommendations at the time) was initiated. An 8-week course of ciclosporine A was also initiated during the first 8 weeks. At month 4, viral load became undetectable. After 8 months on ART, the patient did an analytical treatment interruption including GM-CSF and pegylated interferon alfa for 2 months, but without any rebound. At month 10, ART was started again and interleukin-2 for 2 months. Finally, following a second analytical treatment interruption, viral replication was controlled and was undetectable for the following 15 years without ART. The retrospective determination of plasma levels of protease (lopinavir amd darunavir) and integrase inhibitors (raltegravir) at 7, 9, 12 and 14 years after primary HIV infection were always undetectable. The individual presented a complete, quick clinical recovery without any medical complications and, by the end of this clinical study, was aged 75 years (figure 1).

Overall, inflammation and microbial translocation markers remained low (appendix 2 p 6). Some, however, were slightly higher than the upper reference limit. Cytomegalovirus viraemia has always been undetectable, but serology does show an infection by the cytomegalovirus (appendix 2 p 6).

Full-genome deep sequencing by bulk sequencing in both primary HIV infection plasma samples (n=2) and PBMC cells samples from year 9 and later (n=2) showed no major insertions or deletions when compared with CRF_02AG consensus and between both sequences. The virus was R5 tropic. Sequence differences were related to either low coverage regions in one of the datasets or, more importantly, a high editing signal by APOBEC3G/F in the GGX mutation hotspots from PBMC samples taken at year 9 and later, generating non-majority stop codons in the protease and retro transcriptase.

Viral reservoir kinetics in CD4 T cells showed a pronounced and progressive reduction in the viral reservoir, occurring for years after treatment interruption (figure 2; appendix 2 p 6). Over a 14-year consecutive period after infection and in the absence of ART, this reduction ranged from 4573·50 total HIV-1 DNA copies per 10^6 CD4 T cells and 85·37 integrated HIV-1 DNA

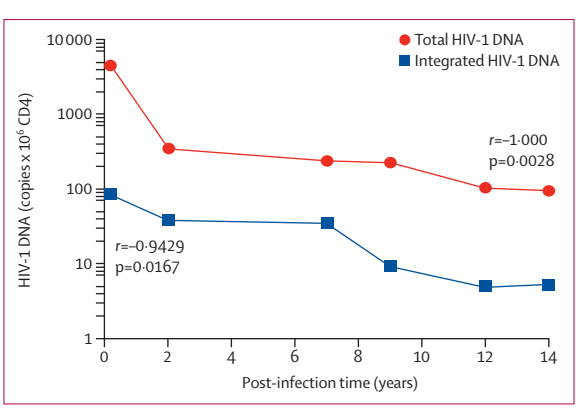

Figure 2: **Evidence of a detectable HIV-1 reservoir during post-treatment control**
Spearman correlation test was done; n=6 timepoints.

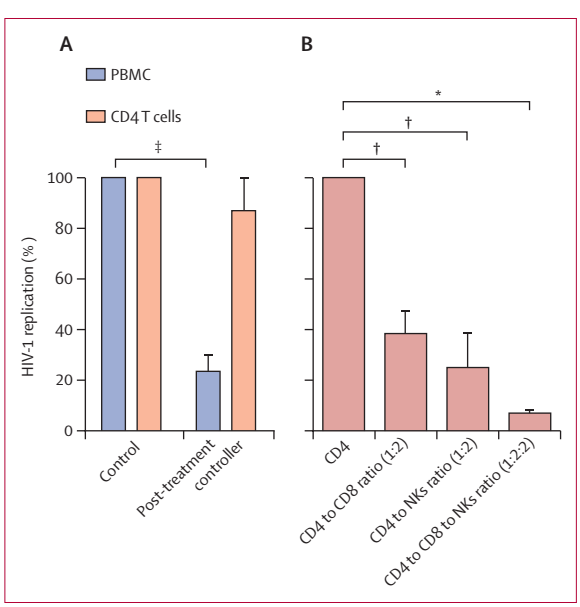

Figure 3: **HIV-1 infectivity assays in vitro (A) and the ex vivo ability of the participant's CD8 T cells and NK cells to inhibit superinfected autologous CD4 T cells at a different ratio (1:2 or 1:2:2; B)**
Student *t* test, n=3. The mean and standard error of the mean bars are shown in the graphs. NK=natural killer. PBMC=peripheral blood mononuclear cells. *p<0·01. †p<0·0001. ‡p<0·05.

copies per 10^6 CD4 T cells at infection onset to 95·33 total HIV-1 DNA copies per 10^6 CD4 T cells and 5·25 integrated HIV-1 DNA copies per 10^6 CD4 T cells at 14 years after infection. We found a significantly inverse association when comparing integrated and total HIV-1 DNA with post-infection time (figure 2). Viral reservoir in a rectal biopsy sampled at post-infection at year 12 showed three total HIV-1 DNA copies per 10^6 total cells (appendix 2 p 6). These data were accompanied by undetectable viraemia, determined by an ultrasensitive assay (<2 HIV-1 copies per mL). Finally, as assessed by quantitative viral outgrowth assay, the frequency of latently infected CD4 T cells yielded 1·61 infectious units per million of cells after 9 years of infection.

We evaluated in vitro infection with an infectious HIV-1 clone carrying the Renilla-luciferase gene as reporter (NL4·3 Renilla) in both PBMCs and purified CD4 T cells obtained from an HIV-1 seronegative donor and the studied participant (figure 3A). The rate of infection in the seronegative donor was considered 100%. No significant differences in the replication of HIV-1 in CD4 T cells were found between the participant (87%) and seronegative donor. In contrast, when the infection was introduced into all PBMCs, luciferase expression decreased to 23% in the participant when compared with the donor. These data suggest that some cell subpopulations in PBMCs inhibited viral replication in the participant's CD4 T cells.

To further investigate this inhibitory effect, we used a viral infectivity assay and measured the ex vivo viral replication capacity in the participant's CD4 T cells when in the presence of either CD8 T cells, NK cells at a 1:2 ratio, or both cell types at a 1:2:2 ratio (figure 3B). HIV replication in CD4 T cells significantly decreased when CD4 T lymphocytes were co-cultured with either autologous CD8 T lymphocytes (62% inhibition rate of HIV replication *vs* control; p=0·022) or NK cells (75% inhibition rate of HIV replication *vs* control; p=0·030). An additive effect was found when both cell subpopulations (CD8 and NK) were added to culture and resulted in a 93% inhibition rate of HIV replication compared with infection of CD4 T lymphocytes alone (p<0·0001).

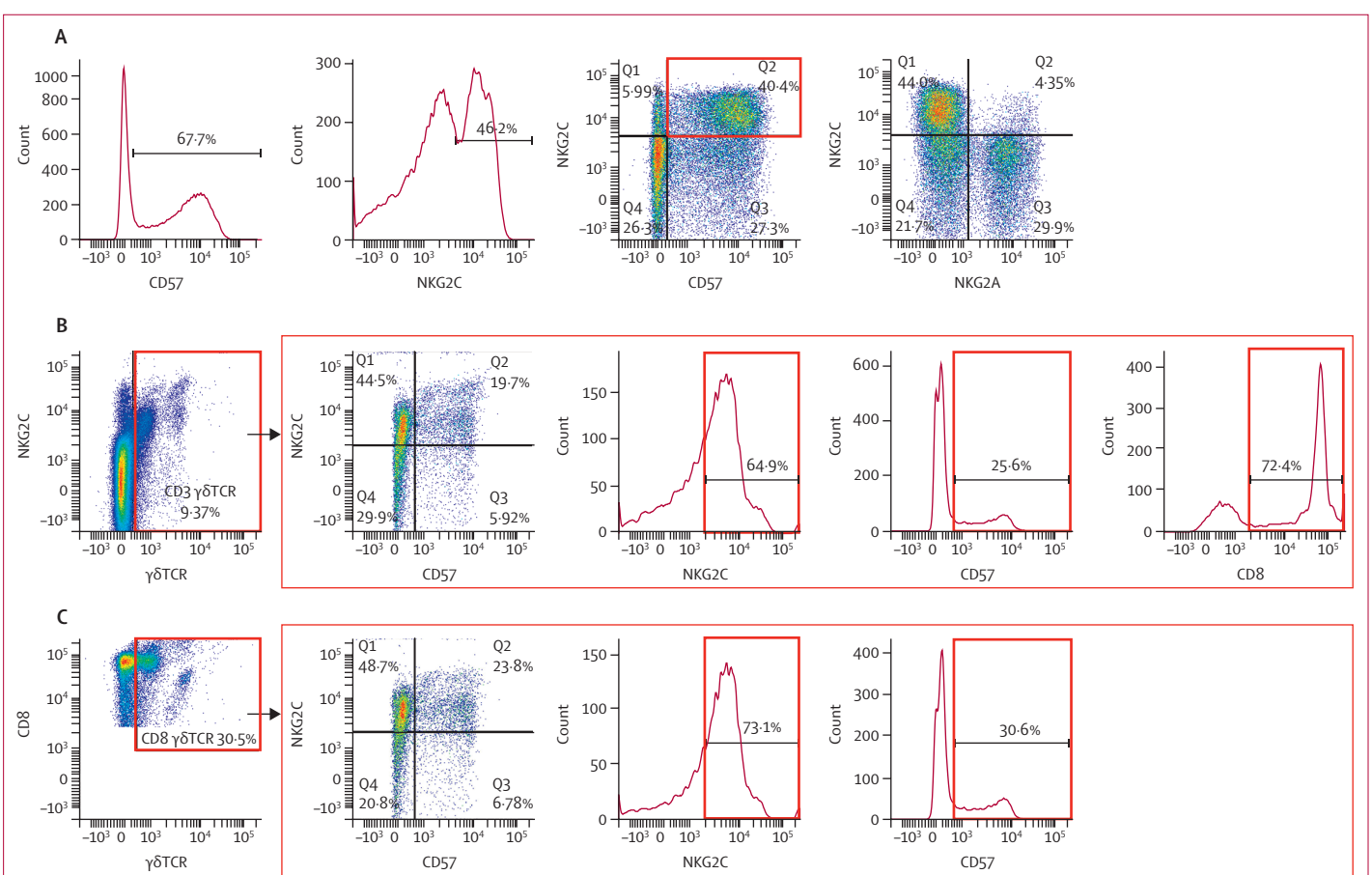

Figure 4: **Frequency of memory-like NKG2C CD57 NK cells and γδT cells, gating from NK cells (A), frequency of NKG2C, CD57, and CD8 in unconventional CD3 γδ T cell gating from CD3 cells (B) and unconventional γδ CD3 CD8 T cells gating from CD8 T cells (C)**

In the NK cell population, frequency of memory-like or adaptive NK cell subpopulations were determined by CD57, NKG2C, and NKG2A expression. In A, frequency of adaptive NKG2C CD57 NK cell double positive is shown in the red box. Q=quartile. TCR=T-cell receptor. NK=natural killer.

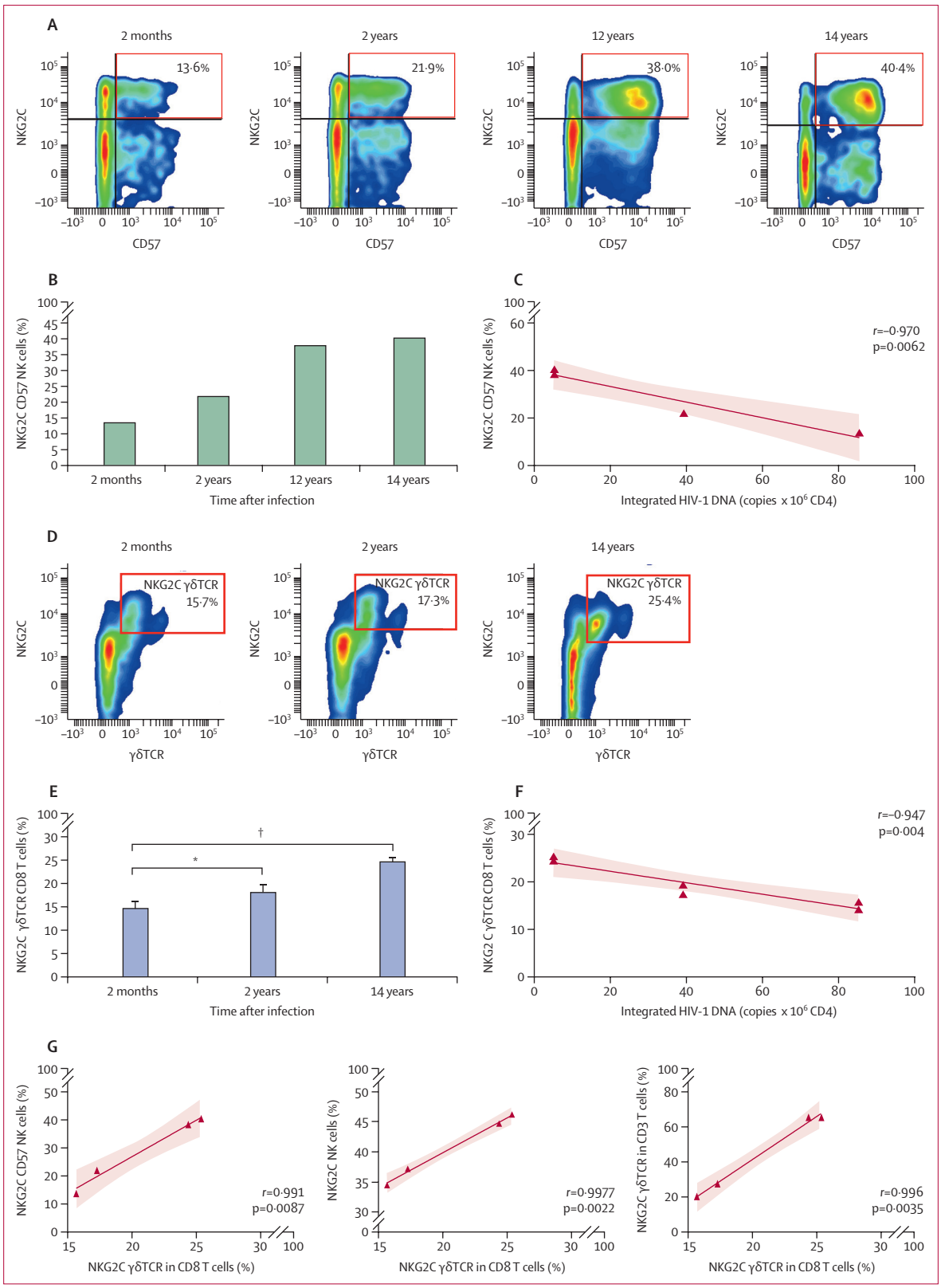

Figure 5: **Frequency of memory-like NK cell subpopulations at month 2 and years 2, 12, and 14 by density plot, gating from NK cells (A) and bar graph (B), correlation of NKG2C CD57 NK cells with integrated HIV-1 DNA (C), the frequency of unconventional NKG2C γδ CD8 T cells during the course of the clinical infection at month 2 and years 2, 12, and 14 by density plot, gating from CD3 CD8 T cells (D) and bar graph (E), the correlation of unconventional NKG2C γδ CD8 T cells and integrated HIV-1 DNA (F), and the correlation of NKG2C γδ CD8 T cells and NKG2C CD57 NK cells (G)**

In C, F, and G, shaded areas represent 95% CIs and the continuous line is the line of best fit from Pearson correlation test. In E, one-way Anova with Dunnett's multiple comparisons test (n=2), mean with standard error of the mean is shown. NK=natural killer. TCR=T-cell receptor. *p<0·05. †p<0·01.

Given that NK cells and CD8 γδ T cells could be involved in the HIV control of this participant, we used flow cytometry to analyse these subpopulations. The gating strategy to characterise NK and γδ T cells is shown in appendix 2 (p 4). In the NK cell population, frequency of memory-like NK cell subpopulations were determined by CD57, NKG2C, and NKG2A expression. The percentage of CD57 NK cells (as measured by CD3 CD56 CD57) was 67·7% in this participant. The frequency of NKG2C NK cells was 46·2%, memory-like NKG2C CD57 NK cells was 40·4%, and NKG2C+NKG2A- NK cells was 44·0% (figure 4A). Regarding γδ T cells, this subset represented 9·37% of CD3 T cells. Most γδ T cells expressed CD8 (72·4%; figure 4B). Nearly a third (30·5%) of CD8 T cells were γδ CD8 T cells, and NKG2C expression was present in 73·1% of γδ CD8 T cells (figure 4C).

Overall, this participant had an unusually large number of memory-like NK cells and γδ CD8 T cells; higher than described in the seronegative population and people living with HIV who present typical progression (appendix 2 p 7).

We analysed the expansion of memory-like NKG2C CD57 NK cells and cytotoxic γδ CD8 T-cell subpopulations expressing NKG2C to characterise any possible association with post-treatment control. We determined the frequency of memory-like NK cell subpopulations at month 2 and years 2, 12, and 14, over which time we found a 3-fold expansion of this subpopulation (figure 5A, B). Expansion of NKG2C CD57 NK cells inversely correlated with the amount of integrated HIV-1 DNA (p=0·0062; figure 5C). NKG2C NK cells and CD57 NK cells also correlated inversely with the amount of integrated HIV-1 DNA (appendix 2 p 4).

Concerning the frequency of unconventional NKG2C γδ CD8 T cells, this subpopulation significantly increased during the course of the clinical infection (figure 5D, E) and inversely correlated with the amount of integrated DNA HIV-1 as well (figure 5F). Indeed, the frequencies of NKG2C γδ CD8 T cells positively correlated with those of NKG2C CD57 NK cells (figure 5G). These results suggest an association between the increase in those subpopulations and the reduction observed in this individual's viral reservoirs.

Given that combinations of some NK receptors and specific HLA genotypes are associated with resistance to, or control of, HIV infection, we studied the participant's genome to determine the presence of NKG2C, HLA class I A, B, C, and E, HLA class II, killer cell immunoglobulin-like receptors (KIR), and *CCR5* alleles.

Accordingly, with the high expression of the *NKG2C* marker in NK cells and γδ T cells, the participant did not have deletion of the *NKG2C* gene; she displayed a *NKG2C*$^{wt/wt}$ genotype. As it relates to HLA-E, the participant had an *HLA-E*01:03* genotype. This genotype has been found to be correlated with HIV protection.[16] The participant was homozygous at several HLA-I loci (figure 6). Additionally, she was homozygous at nine KIR alleles (two KIR A haplotypes), including genes *KIR2DL1, KIR2DL3, KIR3DL1,* and full length for activating *KIR2DS4* (figure 6). Finally, we analysed genetic deletion Δ32 on the *CCR5* gene and found that the participant expressed wild-type alleles (data not shown).

Discussion

Herein, we have presented a case study of a patient who has had exceptional HIV-1 post-treatment control for more than 15 years associated with strong memory-like NK and γδ cytotoxic T cells.

The patient was included in a clinical trial involving ART for nine months. Her treatment comprised several immune modulatory interventions (ie, ciclosporine A, GM-CSF, pegylated interferon alfa, interleukin-2, and two analytical treatment interruptions). It is not possible to make conclusive statements about the role of this treatment on the outcome. This participant was the only person in the trial with controlled viraemia after analytical treatment interruption. Her clinical profile during seroconversion was not typical of elite controllers: she was extremely symptomatic, with a viral load that reached 70 000 copies per mL and no classical genetic factors associated with control.

Furthermore, as it was isolated and grown in culture, the virus was replication-competent. This participant presented a frequency of latently infected CD4 T cells of 1·61 infectious units per million. This frequency is more than 10 times that found in elite controller cohorts,

A

	HLA class I				HLA class II					
	A	B	C	E	*DRB1*	*DRB3*	*DRB4*	*DQB1*	*DQA1*	*DPB1*
Allele 1	29:02P	44:03P	16:01P	01:03P	07:01P	02:02P	01:01P	02:01P	01:01P	04:02P
Allele 2	29:02P	44:03P	16:01P	01:03P	14:01P			05:03P	02:01P	11:01P

B

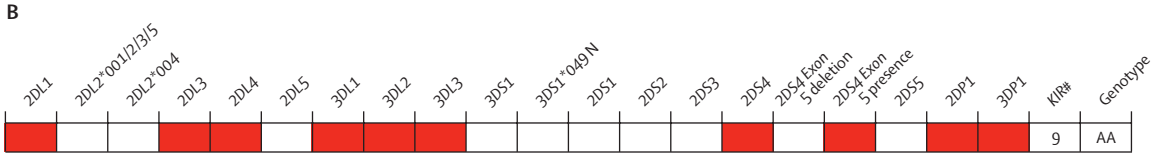

Figure 6: **HLA class I and II alleles (A) and KIR genotypes (B)**
In B, red boxes indicate the presence of 9 KIR genes. The genotype is AA. KIR=killer cell immunoglobulin-like receptor.

and is similar to rates observed in chronic progressors on suppressive ART regimens.[17] In our study, we measured viral reservoirs for both total and integrated HIV-1 DNA on six occasions and found a pronounced and progressive decline during the follow-up.

Interestingly, PBMCs were highly resistant to infection in vitro; however, purified CD4 T cells were fully susceptible to HIV-1 infection, suggesting that non-CD4 T-cell subpopulations could contribute to HIV-1 control. Indeed, when using viral inhibition assays, we showed strong inhibition of HIV infection via mediation by NK cells and CD8 T cells. Due to both the high percentage of γδ T cells in the CD8 subpopulation (30·5%) and weak CD8 T-cell responses against gag peptide pools,[18] we hypothesise that γδ T cells—and not classical cytotoxic CD8 αβ T cells—are involved in viral control.

Binding receptor NKG2C to ligand HLA-E[19] can activate NK cells, thereby having a key role in antiviral and anticancer activity. The participant is seropositive for cytomegalovirus and had a high frequency of NKG2C CD57 memory-like NK cells and CD8 γδ T cells expressing NKG2C, typical of subclinical cytomegalovirus reactivation.[12] In fact, the amount of NKG2C NK cells was twice that reported in referenced data from patients with untreated, typically progressing HIV infection (46·2% *vs* approximately 24·0%;[11] appendix 2 p 7). Furthermore, NKG2C CD57 NK cell levels were higher than those of individuals with and without an HIV infection (40·5% *vs* approximately 8·0 and *vs* approximately 25·0%, respectively; appendix 2 p 7[10,20]). NKG2C NK cell levels increase in people who are seropositive for cytomegalovirus, irrespective of HIV status (approximately 29·5% in people with HIV and approximately 25·6% in donors without HIV; appendix 2 p 7[21]). However, such expansion was at lower rates when compared with the case herein reported (46·2%).

Increased memory-like NK cells contribute to controlling HIV viraemia.[10] Indeed, these cells conferred a protective role against infection in HIV-1-exposed seronegative individuals.[20] An increase in this type of cell has been described in men who have sex with men without HIV infection (approximately 58%;[20] appendix 2 p 7) who were highly exposed to HIV. Additionally, we have found that memory-like NK cells developed in people with chronic myeloid leukaemia in treatment-free remission after the discontinuation of dasatinib treatment (approximately 67%;[13] appendix 2 p 7). This expanded NK cell population protected CD4 T cells from HIV infection ex vivo.[12,13]

Several studies have shown the role of γδ T cells in inducing cytotoxicity in HIV-infected cells, providing HIV control.[11] Indeed, we found that nearly a third (30·5%) of the CD8 T cells from the case study participant were γδ CD8 T cells. This percentage was six times higher than that observed in healthy donors (5%) and similar to that reported in people with chronic myeloid leukaemia in treatment-free remission with high in vitro cytotoxic activity against HIV-infected CD4 T cells (34%;[13] appendix 2 p 7). Additionally, expression of NKG2C in γδ T cells was higher than referenced data from patients with untreated, typically progressing HIV infection (64·9% *vs* approximately 19·7%;[11] appendix 2 p 7). Cytomegalovirus especially expanded adaptive-like γδ1 γδ CD3 T cells expressing CD8[22,23] and NKG2C.[22] In fact, a high frequency of γδ1 T cells expressing *NKG2C* could be degranulated by direct NKG2C receptor and HLA-E interaction, conferring strong cytolytic capacity against HIV-1-infected CD4 T cells.[11] We hypothesise that the CD8 T cells HIV-1 inhibition found in the viral inhibition assays could be mainly done by activating cytotoxic NKG2C γδ CD8 T cells. Consistently, we have found that the increase in the percentage of NKG2C γδ CD8 T cells in blood inversely correlated with the amount of integrated HIV DNA in CD4 T cells from blood, suggesting that these cells could have a direct role in killing HIV-1-infected CD4 T cells by an NKG2C or HLA-E activation. We postulated that NKG2C CD57 memory-like NK cells and γδ CD8 T cells expressing NKG2C subsets could be effective against endogenous cytomegalovirus, leukaemic cells, and, surprisingly, CD4 T cells infected ex vivo with HIV-1.[11–13,24] This assumption might mean that these types of cells, when expanded, could contribute to controlling an HIV infection.[25]

The genetic background of the participant merits some commentary. The participant was homozygous at HLA-B allotype *Bw4* (*B*4403/B*4403, Bw4–80T/Bw4–80T*) and *Bw4* ligand KIR alleles (*KIR3DL1/KIR3DL1*). *Bw4* homozygosis has been associated with HIV control and protection,[20,26–28] lower gag T-cell response,[27] and high NK activity.[28] The participant also had some features associated with protection against HIV progression, especially those related to her non-White race. Those alleles were *KIR2DS4*,[29] *KIR3DL1* combined with *KIR2DL3* in the Indian population,[28] and *KIR2DL1*.[30] Concerning *NKG2C*, the participant had a *NKG2C*$^{wt/wt}$ genotype and concerning its ligand, HLA-E, she had an *E*01:03/E*01:03* genotype. Both alleles have been correlated with HIV protection[16,31] and NK antiviral responses.[31] Overall, these results suggest that the participant had a genetic background that drove potent antiviral innate responses mediated by NK cell and γδ T cells linked to NKG2C-mediated cytotoxic activity.

Moreover, ciclosporine A is an immune suppressor that allows the expansion of memory-like NK cells after cytomegalovirus clinical reactivation in people treated with haematopoietic stem-cell transplantation.[32] Early ART could also preserve the functionality of innate subpopulations such as NK cells.[4] That all stated, we could postulate that the favourable genetic background, early ART, herpes virus infection, and course of ciclosporine A could have contributed to the expansion of innate subpopulations with memory characteristics and

cytotoxic activity against both cytomegalovirus and HIV (eg, memory-like NK and γδ CD8 T cells). This expansion could have been primed by a subclinical and transitory reactivation of cytomegalovirus, allowing the long-term control of the HIV infection in this participant.

There are some limitations in this study, especially because this is a unique case report. More research is needed to find and deeply characterise these innate memory-like NK cell and γδ CD8 T-cell subpopulations in both large post-treatment controller cohorts and in either exceptional or long-term elite controller cohorts.

To our knowledge, despite the harbouring of replication-competent HIV-1, this is the first case of a long-term functional cure in an older individual treated at primary HIV infection. The fact that NKG2C memory-like NK cells and γδ CD8 T cells could control viral replication and could result in the functional cure observed paves the way for therapeutic strategies to be developed on the basis of expansion of such cell subsets of natural immunity. Such approaches could be conducive to functionally curing more people living with HIV.

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Contributors

NC, JAm, JAl, SS-P, and JMM contributed to the study design. NC, SS-P, TG, CX, MC, MN-J, RP, MP, and JG-E did the experiments. JAm enrolled the study participant. JAm and JMM and provided the clinical data. NC, SS-P, MN-J, RP, JAl, and JMM did the analysis and interpreted data. NC, SS-P, JAm, JAl, and JMM drafted the manuscript. NC, SS-P, JM, JAm, JAl, and JMM participated in the study analyses and revised the manuscript for important intellectual content. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

JMM has received consulting honoraria or research grants from Angelini, Contrafect, Genentech, Gilead Sciences, Jansen, Lysovant, Medtronic, MSD, Novartis, Pfizer, and ViiV Healthcare, outside of the submitted work. All other authors declare no competing interests.

Data sharing

The clinical data from this study are available upon request to JMM (jmmiro@ub.edu) or JA (ambrosioni@clinic.cat). The virological and immunological data from this study are available, upon request, to SS-P (ssanchez@recerca.clinic.cat) or NC (ncliment@recerca.clinic.cat). The deep sequencing data from this study are available, upon request, to MC (mcasadella@irsicaixa.es), MN-J (mnogera@irsicaoxa.es), or RP (rparedes@irsicaixa.es).

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