HIV-1 seroreversion in HIV-1-infected children: do genetic determinants play a role?

Corinna Asang[a], Hans-J. Laws[a], Ortwin Adams[b], Jürgen Enczmann[c], Cornelia Feiterna-Sperling[d], Gundula Notheis[e], Bernd Buchholz[f], Arndt Borkhardt[a] and Jennifer Neubert[a]

Background: HIV-1 seroreversion in infants with vertically transmitted HIV-1 infection who started ART in the first months of life has been reported in only a subset of patients. However, the reason why most infants remain seropositive despite similar treatment response is not understood. Here, we assessed whether HIV-1 seroreversion in maternally infected infants is associated with genetic determinants.

Methods: HIV-1-infected infants with a history of documented HIV-1 seroreversion were identified throughout Germany using a standardized questionnaire. At study entry immune reconstitution and anti-HIV-1 antibody expression were monitored as clinical parameters. To search for genetic determinants high-resolution HLA genotyping was performed. In addition, the coding sequence of the chemokine receptor *CCR5* was analyzed by Sanger sequencing regarding potential mutations.

Results: Patients showed normal numbers and frequencies of lymphocyte subpopulations. Five out of eight patients still had seronegative HIV-1 antibody status at study entry. HLA genotyping revealed the enrichment of *HLA-DQB1*03* and *DQB1*06* alleles within the patient cohort. Only one patient was found to carry a 32 bp-deletion within the *CCR5* gene.

Conclusion: Our results indicate that the phenotype of HIV-1 seroreversion in infants might correlate with the presence of HLA class II alleles *DQB1*03* and *DQB1*06*. This finding supports the idea of genetic predisposition determining HIV-1 seroreversion in vertically infected infants effectively treated with ART.

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Introduction

The introduction of combined ART has dramatically changed the course of infections with the human immunodeficiency virus type 1 (HIV-1) in adults and children [1]. In current guidelines ART is recommended for all HIV-1–infected infants in the first year of life regardless of the HIV clinical, immunological and virological category, because infants starting ART in the first year of life have less progression to AIDS compared with those starting later [2]. Treatment of HIV-1–infected children under 2 years of age is challenging due to the

[a]Department of Pediatric Oncology, Hematology and Clinical Immunology, Center for Child and Adolescent Health, [b]Institute for Virology, Medical Faculty, [c]Bone Marrow Donor Center with Eurocord Bank and Transplantation Immunology, Heinrich-Heine-University Düsseldorf, Düsseldorf, [d]Department of Pediatric Pneumology and Immunology, Charité University Medicine Berlin, Berlin, [e]Children Hospital, Ludwig Maximilians University, Munich, and [f]University Medical Center Mannheim, Pediatric Clinic, Mannheim, Germany.

Correspondence to Jennifer Neubert, Heinrich-Heine-University Düsseldorf, Medical Faculty, Department of Pediatric Oncology, Hematology and Clinical Immunology, Moorenstrasse 5, 40225 Düsseldorf, Germany.

Tel: +492118118297; e-mail: Jennifer.neubert@uni-duesseldorf.de

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limited availability of appropriate drugs, poor palatability of liquid formulations, difficulties in therapy adherence and long-term ART-toxicity making full viral suppression difficult and a rebound of viral replication more frequent than in adults [3].

In infants starting ART in the first months of life and achieving effective virus suppression HIV-1 seroreversion has been documented in a subset of patients [4,5]. We reported on a child initially presenting with AIDS who achieved HIV-1 seroreversion following effective ART [6]. It has been postulated that HIV-1 seroreversion might not be an exception when infants are treated early and efficiently [7]. However, in clinical practice seroreversion is observed only in few infants, whereas most infants develop and maintain a robust antibody response to the infection despite early therapy, effective virus suppression and good therapy adherence. The physiological requirements for seroreversion in infants receiving ART are not clearly understood. Besides external factors for example maternal antibodies and virus subtype, the difference in antibody response between seroreverted and nonseroreverted infants might be caused by genetic determinants.

Host genetic factors were reported to fundamentally determine HIV-1 susceptibility and disease progression [8,9]. During immune response against HIV-1 many of them directly or indirectly participate in virus recognition (chemokine receptors, human leukocyte antigen (HLA), T-cell receptor (TCR), killer immunoglobulin-like receptors (KIRs), and toll-like receptors (TLRs)) [8,9]. A homozygous 32 bp deletion in the chemokine receptor *CCR5* has long been identified as protective variant against HIV-1 infection [10]. As another determinant, heterozygosity at HLA class I regions appears to provide an advantage for the immune system against the development of AIDS, because it expands the hosts ability to present viral antigens and thereby broadens the immune response. In addition, various individual HLA class I and II alleles have been associated with either slow (e.g. *HLA-B*57*, *B*3502, B*3503, B*3504*, and *DRB1*13*) or fast disease progression (e.g. *HLA-B*3501* and *B*3508*) following HIV-1 infection [8,9].

The aim of this study was to follow-up patients with initially documented HIV-1 seroreversion receiving early ART and to examine whether, in addition to early and efficient treatment, HIV-1 seroreversion in HIV-1-infected children might also depend on genetic determinants.

Methods

Patients with HIV-1 seroreversion were identified using a standardized questionnaire that was sent to clinicians caring for children with HIV-1 infection in Germany. Patients were enrolled in the study, if they had at least one negative HIV-1 antibody test result following effective ART in their medical history. Eight seronegative patients were enrolled from five sites between 2011 and 2012. HIV-1-infected children who started therapy before 5 months of age but seroconverted despite effective viral suppression served as controls for genetic analysis. Research was conducted in accordance with the Declaration of Helsinki, revised in 2008. The study was approved by the ethics committee of the Medical Faculty of the Heinrich Heine University Düsseldorf. All parents gave written informed consent prior to enrollment.

From each patient 3–12 ml EDTA-anticoagulated peripheral blood was obtained. Lymphocyte subsets were characterized on whole blood by standard flow cytometry. Cells were stained with monoclonal antibodies to CD3 [SK7, Becton Dickinson GmbH (BD), Germany], CD4 (SK3, BD), CD8 (SK1, BD), CD25 (B149.9, Beckmann Coulter GmbH [BC], Germany), CD45RA (L48, BD), CD45RO (UCLH1, BD) and HLA-DR (L243, BD) for T-cell subsets, CD19 (SJ25C1, BD), CD20 (L27, BD), CD27 (1A4, BC) and IgD (IA62, BD) for B-cell subsets and, additionally, CD56 (My31, BD) for NK cell identification.

From each sample 400 μl plasma was obtained by standard procedures. Expression of antibodies targeting HIV-1 proteins was monitored at study entry using INNO-LIA HIV I/II Score (Innogenetics N.V., Belgium). PBMC were isolated form peripheral blood by standard Ficoll-Paque density gradient centrifugation. DNA from the naive $CD4^+$ and $CD8^+$ subsets was isolated by anion exchange chromatography according to the manufacturer (AllPrep DNA/RNA/Protein Mini Kit; Qiagen GmbH, Germany).

Genetic analyses were performed on DNA pooled from naive CD4 and CD8 fractions. *CCR5* genotypes were initially characterized by PCR using published primers [11] and confirmed by direct sequencing (BigDye Terminator v1.1 Cycle Sequencing Kit; Life Technologies GmbH, Germany). Genotypes were further validated using allele-specific PCR primers (CCR5 wt: 5′-TCATTTTCCATACAGTCAGT, CCR5 Δ32: 5′-TCATTTTCCATACATTAAAG; Eurofins MWG Operon, Germany).

HLA-genotyping of HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA0-DQB1 was performed using SBT excellarator HLA-kits (GenDX, Netherlands). Genomic DNA was amplified using primers flanking exons 2–4 (HLA-A, HLA-B,HLA-C) or exon 2 (HLA-DRB1, HLA-DQB1). PCR products were purified by ExoSAP-IT (Affymetrix, USA). Remaining dyes of the sequencing reactions were removed using the BigDye XTerminator Purification Kit (Applied Biosystems, USA). Sequencing reactions were analyzed on a 3730 DNA Analyzer (Applied Biosystems).

Table 1. Patient characteristics and clinical parameters at study entry.

Patient	Age	Gender	Age at ART start	ART at study entry	anti-HIV-1 antibodies						Lymphocytes					
					gp120	gp41	p31	p24	p17	Test result	Total rel. [%]	$CD3^+$ rel. [%]	$CD3^+$ abs. [μl^{-1}]	$CD4^+/CD3^+$ rel. [%]	$CD4^+/CD3^+$ abs. [μl^{-1}]	$CD8^+/CD3^+$ rel. [%]	$CD8^+/CD3^+$ abs. [μl^{-1}]
P1	2 years	Female	Immediately	ZDV, 3TC, NVP	–	–	–	+/–	–	–	59	74	3253	59	2593	10	440
P2	2 years	Female	5 months	ABC, 3TC, NVP	–	+	–	+	+/–	+	56	75	3969	41	2170	30	1588
P3	13 years	Male	3 months	ABC, 3TC, NVP	+++	+++	+++	+++	+++	+	32	71	1363	44	845	26	499
P4	12 years	Female	3.5 months	ABC, 3TC, LPV/r	–	–	–	+/–	–	–	24	68	1240	41	748	25	456
P5	5 years	Female	1 day	ZDV, 3TC, NVP	–	–	–	–	–	–	n.d.						
P6	8 years	Male	1 months	ABC, 3TC, LPV/r	–	+++	–	+++	+/–	+	43	69	1620	38	892	26	611
P7	6 years	Male	4 months	ZDV, 3TC, NVP	–	–	–	+	–	+/–	55	67	2764	36	1485	25	1031
P8	5 years	Male	4 months	ABC, 3TC, NVP	–	–	–	+	–	+/–	41	71	1659	44	1028	21	491

3TC, lamivudine; ABC, abacavir; LPV/r, lopinavir; n.d., not determined; NVP, nevirapine; ZDV, zidovudine.

Results

Thirty-seven children who had started therapy before 5 months of age and achieved effective virus suppression following ART were reported from the participating centres. Eleven of these children had documented HIV-1 seroreversion and eight were recruited for our study. The patient characteristics are shown in Table 1. Mean age at follow-up was 6.6 ± 3.9 years. Effective prenatal screening and prophylaxis to prevent mother to child HIV-1 transmission especially antiretroviral treatment of mothers was missing in all eight patients.

Five out of eight children still had negative HIV-1 antibody tests at study entry (Table 1). Although two patients showed partial reactivity against p24, this is interpreted as negative assay result in standard diagnostic testing [12]. Three out of eight children had developed an antibody response against HIV-1 resulting in positive antibody tests at the time point of follow-up. Only one of these patients had a complete reactivity against all tested HIV epitopes (P3). This child had experienced treatment failure due to adherence problems and therapy interruption but returned to undetectable viral load after switching ART. The remaining two patients with a positive HIV-1 antibody test (P2 and P2) showed only partial reactivity. Except for the patient who interrupted ART, neither of the patients evolved antibodies against gp120 and only two generated antibodies against gp41.

All patients had normal numbers and frequencies of overall lymphocytes as well as T cells, B cells and NK cells (Table 1) with only negligible deviations from standard values of age-matched healthy children [13]. With regard to the vulnerable T-cell compartment, no decrease of naive $CD4^+$ T cells was observed. Two patients (P1 and P3) showed an increase in the naive CD4 pool. One patient (P2) had an expansion in the memory $CD8^+$ T-cell pool; however, this patient also presented increased numbers of naive $CD8^+$ cells.

Sequencing of the *CCR5* coding region revealed no deviation from the reference sequence with exception of patient P3 featuring a heterozygous form of the Δ32 variant (Table 2). The results of the high-resolution HLA genotyping are shown in Table 2. The most intriguing association was found for HLA class II locus *DQB1*. We found seven out of eight patients carrying a *03 allele and four of those patients additionally harbored a *06 allele. Additionally, three of the patients positive for *DQB1*03 and DQB1*06* also carried *DRB1*13*, an allele which had already been implicated in slow disease progression [14].

Discussion

Although it is quite challenging to regularly administer antiretroviral treatment to infants, our data show that, in a

Table 2. *HLA* and *CCR5* genotypes of the study cohort.

Patient	Ethnicity	CCR5 CDS mut	HLA alleles							
			A*		B*		C*		DRB1*	DQB1*
P1	African	–	30:01	74:AB	15:03	18:01	02:02	–	13:03	15:03	03:SAK	06:02
P2	Caucasian	–	02:01	–	50:01	51:01	03:03	07:02	04:02	13:01	03:02	06:03
P3	Caucasian	het Δ32	11:01	29:02	35:08	44:03	04:KBG	16:01	07:01	11:04	02:MS	03:SAK
P4	African	–	01:01	23:01	15:16	35:01	04:KBG	14:02	03:02	11:01	03:SAK	04:02
P5	African	–	02:ANGA	23:01	08:01	58:01	03:04	07:WTR	13:02	13:04	03:SAK	06:09
P6	Caucasian	–	02:ANGA	11:01	49:01	51:01	07:WTR	07:02	12:CVT	16:01	03:SAK	05:02
P7	African	–	03:01	33:03	15:03	57:03	02:02	18:AB	09:01	16:02	02:MS	05:02
P8	Caucasian	–	02:ANGA	02:05	18:01	58:01	07:WTR	12:03	11:03	15:01	03:SAK	06:02
C1	African	–	23:01	68:02	15:18	50:01	03:04	04:KBG	11:01	14:BCAD	06:02	05:03
C2	African	–	02:02	68:02	45:01	53:01	04:KBG	–	08:04	12:CVT	03:SAK	05:01
C3	African	–	02:ANGA	33:01	53:01	57:03	04:KBG	18:AB	13:03	16:02	02:MS	05:02

C, control; het Δ32, heterozygous 32 bp deletion in the *CCR5* gene; P, patient. Both alleles of the HLA loci class I (A, B, and C) and class II (DRB1 and DQB1) were characterized by Sanger sequencing on four-digit resolution. The first two digits indicate the allele group, which is identical to the serologic specificity of the protein encoded by the allele, whereas the last two digits represent the allele number within the allele group. Alleles which are identical in exons 2 and 3 have identical immunological properties and are not distinguished in standard HLA high resolution typing. These allele groups are represented by letters: A*02:ANGA – 02:01, 02:01L; A*74:AB – 74:01,74:02; C*04:KBG – 04:01, 04:09N; C*07:WTR – 07:01, 07:06, 07:18; C*18:AB – 18:01, 18:02; DRB1*12:CVT – 12:01, 12:06, 12:10; DQB1*02:MS – 02:01, 02:02, 02:04; DQB1*03:SAK – 03:01, 03:09, 03:19.

subset of patients, HIV-1 replication can be suppressed to an extent that the mounted humoral immune response does not result in antibody generation against HIV proteins (HIV seroreversion). Maintenance of HIV-1 seroreversion following ART is not limited to infancy, because the oldest patient demonstrating continuous seronegativity following ART in our cohort was 12 years old. However, since a large proportion of HIV-1-infected infants experience seroconversion despite early and efficient reduction of viral RNA loads, the seroreversion phenotype appears to depend on crucial factors.

In our study, we focused on genetic determinants that could account for the distinct development of seronegative and seropositive phenotypes after early initiation of ART. Analysis of the *CCR5* genotype revealed only one patient harboring a heterozygous *CCR5* Δ*32* allele indicating that the chemokine receptor is not involved in HIV-1 seroreversion in infants. A much more intriguing association was found for the HLA class II region with seven out of eight patients carrying a *DQB1*03* allele. Three out of four seroreverted patients from Africa or Europe carried the allele *DQB1*03:SAK* (allele frequency = 0.375). *The DQB1*03:SAK* frequency is 0.096–0356 in Africa and 0.033–0349 in Europe [15]. As this allele has so far not been correlated with modulation of disease progression, our finding might indicate a new role for *DQB1*03:SAK* in the immune response against HIV-1. Furthermore, four out of eight patients carried a *DQB1*06* allele, three of them in combination with *DRB1*13*. This *DRB1* allele has already been associated with long-term survival among children with vertically transmitted HIV-1 infection [14]. In addition, the *DRB1*13-DQB1*06* haplotype was linked to a trend towards increased AIDS-free time following HIV infection in adults [16] and patients who inherited *HLA-DRB1*13-DQB1*06* had a greater likelihood of controlling HIV-1 replication and maintaining T-cell help activities [17]. Three out of eight (38%) patients in our cohort carried *HLA-DRB1*13-DQB1*06* indicating a potential cooperative effect of both class II loci in generating the HIV-1 seroreversion phenotype. The importance of HLA class II alleles might relate to the central role of $CD4^+$ T cells in sustaining cytotoxic T-cell and B-cell activation, which depends on the recognition of HIV-1 epitopes presented by MHC class II.

Increased heterozygosity at HLA class I regions is thought to be an advantage for the immune system against AIDS, because it broadens the repertoire of presented viral antigens. Twenty-eight to 40% of HIV-1-infected Caucasian patients who were able to delay AIDS for 10 or more years were fully heterozygous at all HLA class I loci [18]. In our cohort, six to eight patients were heterozygous at all loci. However, the majority of control patients was also fully heterozygous at all HLA loci. Therefore, HLA heterozygosity is not decisive for HIV-1 seroreversion. HLA alleles that have been associated with

slow disease progression were found in our cohort, but none of the known dominant protective HLA alleles (e.g. *B*27* and *B*57*) were enriched in our study population.

From our data, it might be suggested that *HLA-DQB1*03* especially *DQB1*03:SAK* and *DQB1*06* could support efficient clearing of viral antigens mediated by early and efficient ART. However, the size of the patient cohort is still limited. As treatment options for infants are improving, the number of patients with HIV-1 seroreversion is expected to increase and thus this correlation might be tested in a larger study cohort.

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Authors contribution: C.A. (biologist): conception and design of the study, sample processing, CCR5 PCR, collection of data, analysis and interpretation of data, drafting of the article.

J.N. (pediatrician): conception and design of the study, coordination of the study, acquisition of pediatric data, analysis and interpretation of data, drafting of the article.

O.A. (virologist): performed the virologic assays at study entry, interpretation of virologic data, critical revision of the article, approved the final version of the article.

H.-J.L. (pediatrician): acquisition of pediatric data, analysis and interpretation of data, critical revision of the article, approved the final version of the article.

C.F.S. (pediatrician): acquisition of pediatric data, interpretation of data, critical revision of the article, approved the final version of the article.

B.B. (pediatrician) acquisition of pediatric data, interpretation of data, critical revision of the article, approved the final version of the article.

G.N. (pediatrician) acquisition of pediatric data, interpretation of data, critical revision of the article, approved the final version of the article.

J.E.: HLA typing, interpretation of genetic data, critical revision of the article, approved the final version of the article.

A.B. (pediatrician, Head of Department): conception and design of the study, analysis and interpretation of data, critical revision of the article, approved the final version.

Conflicts of interest

There are no conflicts of interest.

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