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Persistent rhinovirus infection in pediatric hematopoietic stem cell transplant recipients with impaired cellular immunity

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ABSTRACT

Background: HRV infections are generally self-limiting in healthy subjects, whereas in immunocompromised hosts HRV infections can lead to severe complications and persistent infections. The persistence of HRV shedding could be due to the inefficient immunological control of a single infectious episode.
Objectives: To investigate the clinical, virologic and immunologic characteristics of pediatric HSCT recipients with HRV-PI infection.
Study design: During the period 2006–2012, eight hematopoietic stem cell transplant (HSCT) recipients presented with persistent rhinovirus infection (HRV-PI, ≥30 days). Viral load and T-CD4$^+$, T-CD8$^+$, B and NK lymphocyte counts at the onset of infection were compared with those of fourteen HSCT recipients with acute HRV infection (HRV-AI, ≤15 days).
Results: The median duration of HRV positivity in patients with HRV-PI was 61 days (range 30–174 days) and phylogenetic analysis showed the persistence of a single HRV type in all patients (100%). In HSCT recipients with HRV-PI, T-CD4$^+$, T-CD8$^+$ and NK cell counts at the onset of infection were significantly lower than those observed in recipients with HRV-AI ($p < 0.01$), while B cell counts were similar in the two groups ($p = 0.25$). A decrease in HRV load was associated with a significant increase in T-CD4$^+$, T-CD8$^+$ and NK lymphocyte counts in HRV-PI patients ($p < 0.01$).
Conclusions: This study suggests a role for cellular immunity in HRV clearance and highlights the importance of its recovery for the control of HRV infection in HSCT recipients.

1. Background

Human rhinoviruses (HRVs) have been previously classified into two species, HRV-A and HRV-B, while a distinct rhinovirus group (HRV-C) was recently identified in patients with acute respiratory infections (ARIs) [1–6]. HRV-C and HRV-A are more frequently associated with ARIs in hospitalized patients and often co-circulate in near-equivalent proportions, while HRV-Bs are often underrepresented [4,6,7].

Even though ARIs caused by HRVs are generally self-limiting in healthy subjects, in hematopoietic stem cell transplant (HSCT) recipients [8,9] and patients with hematological diseases [10] HRV infection may lead to severe complications such as pneumonia [9,11,12]. In addition, in HSCT [13–15] and lung transplant recipients, persistent HRV infections have been reported [16]. The persistence of HRV shedding could be due to the inefficient immunological control of a single infectious episode [16,17].

Schiffer et al. reported that respiratory virus infections are more frequent and severe in the first 100 days following transplant in patients receiving myeloablative *vs* non-myeloablative HSCT, despite an overall similar infection rate [18]. These data would suggest an important role for T-cell immunity in the control of HRV infection. However, correlates of severe or persistent HRV infections (HRV-PI) after HSCT are still largely obscure.

2. Objectives

This study investigated the clinical, virologic and immunologic characteristics of pediatric HSCT recipients with HRV-PI infection.

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Table 1
Characteristics of the pediatric HSCT recipients with HRV infection.

Patient category	Patient ID	Gender/age (years)	Underlying disease	Type of transplant	Conditioning regimen	Time post-HSCT (days)	HRV duration (days)	Nearest HRV type	Presentation
Patients with HRV-PI ($n=8$)	#1	M/6	AML	PMFD + TCD	BU + CY + MEL	−8	106	A18	URTI
	#2	F/2	ALL	MUD	BU + CY + MEL	−14	35	A54	URTI
	#3	M/3	AML	PMFD + TCD	BU + CY + MEL	65	174	C23	URTI
	#4	M/11	ALL	MUD	BU + CY + MEL	14	31	A32	URTI
	#7	M/1	SCID	MUD	THIO + TREO	−36	135	C16	URTI
	#8	F/7	JMML	MUD	BU + CY + MEL	19	52	A102	URTI
	#9	M/4	ALL	PMFD + TCD	TBI + THIO + FLU	−13	30	A34	URTI
	#11	M/7	ALL	MUD	TBI + THIO + CY	−24	70	B97	URTI
Patients with HRV-AI ($n=14$)	#12	F/1	ALL	MFD	BU + CY + MEL	272	10	C19	URTI
	#13	M/2	Thal Major	MUD	THIO + TREO	97	8	A94	URTI
	#14	M/4	Thal Major	MFD	THIO + TREO	52	13	B100	URTI
	#15	F/12	AML	MUD	THIO + TREO	47	10	C15	LRTI
	#16	F/14	LH	PMFD	THIO + TREO	33	10	C42	URTI
	#17	M/11	HLH	MUD	BU + THIO + FLU	60	9	C	URTI
	#18	M/2	HLH	MUD	BU + THIO + FLU	136	10	A88	URTI
	#19	F/7	ALL	MFD	TBI + THIO + CY	90	7	B69	LRTI
	#20	M/5	ALL	PMFD + TCD	TBI + THIO + MEL	56	12	C16	URTI
	#21	F/3	ALL	PMFD + TCD	TBI + THIO + FLU	0	6	A38	URTI
	#22	M/4	CML	MUD	BU + THIO + FLU	91	9	C36	URTI
	#23	F/2	ALL	PMFD + TCD	BU + THIO + FLU	88	5	A59	URTI
	#24	F/14	LH	PFMD	THIO + TREO + FLU	33	10	B42	URTI
	#25	M/2	SCA	MFD	THIO + TREO + FLU	8	6	C25	URTI

HRV-PI, rhinovirus persistent infection; HRV-AI, rhinovirus acute infection; mos, months; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; JMML, juvenile myelomonocytic leukemia; SCID, severe combined immunodeficiency; Thal Major, thalassemia major; LH, Hodgkin's disease; HLH, hemophagocytic lymphohistiocytosis; SCA, sickle cell anemia; PMFD, partially matched family donor (haploidentical); MUD, matched unrelated donor; MFD, matched family donor; TCD, T-cell depletion; BU, busulfan; CY, cyclophosphamide; MEL, melphalan; THIO, thiotepa; TREO, treosulfan; TBI, total body irradiation; FLU, fludarabin; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection

3. Study design

3.1. Patients and samples

From January 2006 through December 2012, 1687 respiratory samples (936 nasal swabs, 713 nasopharyngeal aspirates, 25 bronchoalveolar lavages and 13 pharyngeal swabs) from 536 pediatric HSCT recipients with acute respiratory syndromes admitted to the Pediatric Hematology–Oncology Unit, Fondazione IRCCS Policlinico San Matteo were collected both at admission and discharge. Follow-up samples were prospectively collected 7–10 days apart from positive patients to monitor viral load until the clearance of HRV or hospital discharge. In the latter case, follow-up samples were collected at subsequent outpatient clinical evaluations. Respiratory samples were routinely tested using a panel of RT-PCR and real-rime RT-PCR assays [13,19] for human influenza virus types A and B, human metapneumovirus A and B, human respiratory syncytial virus A and B, four human coronaviruses (OC43, 229E, NL63, HKU1), human parainfluenza types 3, human adenovirus, rhinoviruses and enteroviruses.

A further characterization of HRV infection was retrospectively performed on the samples collected prospectively for acute respiratory infection screening. HRV-PI was defined as HRV shedding $\geq$30 days, and all patients fulfilling the criteria were included in the study. A cohort of pediatric HSCT recipients with HRV-AI (defined as virus shedding $\leq$15 days), matched for clinical characteristics, was selected as a control group for virologic and immunologic analysis. This retrospective study was performed according to the guidelines of the Institutional Review Board on the use of biological specimens for scientific purposes in keeping with Italian law (art.13 D.Lgs 196/2003).

3.2. Definition of clinical respiratory syndromes

Patients with rhinitis, pharyngitis, and laryngitis were classified as upper respiratory tract infection (URTI), while patients with bronchitis, bronchiolitis, and pneumonia (characterized by cough, wheezing, and/or dyspnea as well as suggestive X-ray findings) were classified as affected by lower RTI (LRTI).

3.3. Rhinovirus quantification and typing

Following RNA extraction (Nuclisens® easy MAG™ automatic extractor; BioMérieux, Lyon, France), HRV RNA was quantified by real-time RT-PCR [20]. Results were reported as HRV RNA copies/ml respiratory sample. HRV molecular typing was performed as reported [21] by sequencing a 549 nt fragment spanning part of the 5′ NCR and the VP2 gene (nt 534–1083) or using an alternative protocol that amplifies the same genomic region with different primer sets (nt 547–1087) [6]. Sequencing reactions were carried out using primer sets utilized for amplification with the BigDye Terminator Cycle-Sequencing kit (Applied Biosystems, Foster City, USA) and run in an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, USA). Sequences were assembled using the Sequencher software, version 4.6 (Gene Codes Corporation, Ann Arbor, USA).

3.4. Phylogenetic analysis

Nucleotide sequences were aligned using the ClustalW method and a phylogenetic tree was constructed using the neighbor-joining method and the kimura-2-parameter for simultaneously estimating distance among sequences with MEGA software (version 5.05) [22]. Bootstrap values included 1000 replicates. HRV type assignment was defined by the nearest HRV reference strains observed in the phylogenetic tree. The nucleotide sequences used in this study were deposited into GenBank under accession numbers KJ603464–KJ603520.

3.5. Immunological data

Serial T-CD4$^+$, T-CD8$^+$, B and NK lymphocyte counts were routinely performed in HSCT recipients (both with HRV-PI and HRV-AI), before and after transplant, while patients with hema-