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T-Cell Responses to HSV-1 in Persons Who Have Survived Childhood Herpes Simplex Encephalitis

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Abstract

Background—Herpes simplex encephalitis (HSE) after primary HSV-1 infection can occur in children due to inborn errors of cell-intrinsic immunity in the central nervous system (CNS). Paradoxically, symptomatic mucocutaneous HSV-1 recurrences are rare survivors of childhood HSE. T-cell acquired immunity is thought to be involved in control of recurrent mucocutaneous HSV infection. We thus tested HSV-1-specific immunity in HSE survivors.

Methods—We obtained serum and peripheral blood mononuclear cells (PBMC) a median of 13.5 years after HSE. HSV-1 and HSV-2 IgG was detected by type-specific immunoblot. PBMC from subjects passing quality control criteria were tested using ELISPOT for CD4 IFN-γ responses with an HSV-1 lysate, and for CD8 responses using pooled synthetic HSV-1 peptide CD8 T-cell epitopes. Healthy adult PBMC were used to standardize assays and as comparators.

Results—All participants were HSV-1 seropositive. Most (23/24) HSE survivors has HLA class I types matching the HLA restriction of the pooled peptides. We detected HSV-specific CD8 T-cell responses in 14/24 (58%) HSE survivors and in 9/9 healthy HSV-1 seropositive adults. HSV-specific CD4 T-cell responses were present in all 5 HSE subjects tested and in 8/9 healthy adults. Response magnitudes were overlapping between subject groups.

Conclusions—The defects in cell-intrinsic immunity leading to failure to control primary CNS HSV-1 infection do not preclude the acquisition of specific immunity or the control of recurrent

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mucocutaneous HSV infections. The rarity and lack of severe or recurrent mucocutaneous HSV infection in survivors of childhood HSE corresponds with intact adaptive T-cell immunity.

Keywords

CD8 T-cell; herpes simplex virus; encephalitis

Introduction

Herpes Simplex Virus 1 (HSV-1) is a prevalent virus that typically causes herpes labialis (cold sores) and is usually acquired during childhood. In very rare post-neonatal children, primary HSV-1 infection is not limited to epithelia and innervating ganglia, but spreads to cause HSV encephalitis (HSE). This condition may affect adolescents and, more rarely, young adults (1). These patients do not display mucocutaneous lesions during HSE, and the virus does not infect other organs than the CNS. Recently, a series of lesions in genes involved in Toll like receptor 3 (TLR3)- and type I IFN-mediated cell-intrinsic antiviral responses operating in the central nervous system (CNS) have been identified in collectively about 5% of tested HSE children (2). It is currently unknown if the remaining individuals harbor as-yet unknown genetic lesions. Regardless, childhood HSE is a devastating condition, with high mortality in the absence of treatment with acyclovir and high proportion of neurologic sequelae in acyclovir-treated survivors.

Neonatal HSE is a distinct clinical entity linked to inoculation of mucocutaneous surfaces with large amounts of HSV, typically HSV-2, with the highest risk per exposure in the setting of the absence of placental transferred antibody during primary maternal HSV-2. Cases also occur during established, recurrent genital herpes at a much lower per-exposure rate (3). It has not been studied for germline genetic lesions. These patients can have disseminated disease caused by HSV-2 involving the CNS, visceral organs, skin and mucosae, or with overlap between these three classic syndromes. Neonatal HSE survivors are prone to both CNS and peripheral relapses, with 40–80% having mucocutaneous lesions by 6–8 months after primary treatment depending on receipt of suppressive antivirals (3). In contrast, clinical CNS relapses after childhood HSE are rare (around 10%), and survivors of childhood HSE are paradoxically not prone to herpes labialis, prior to, during, and after HSE (1).

Acquired immunity seems not to be involved in host defense against childhood HSE, as children with the prevalent forms of severe combined immune deficiency (SCID) lacking both T- and B-cell responses are not prone to HSE (4). However, T-cells participate in control of recurrent mucocutaneous HSV, such that antiviral prophylaxis is recommended for HSV-seropositive children with SCID (4) to prevent episodic epithelial infection. CD8 T-cells can kill HSV-infected cells or secrete antiviral cytokines, and in animals directly protect against HSV re-infection. CD4 T-cells also function in animal models and their decrease is implicated in poor HSV control during HIV co-infection (5). HLA-restricted CD8 and CD4 T-cell traffic from blood to HSV lesions and reside as tissue resident memory (T_{RM}) cells in skin, mucosae, and ganglia (5, 6). Most of these findings apply to both viral types, albeit not all systems have been explored for both HSV-1 and HSV-2.

It is remarkable that after childhood HSE, clinical CNS relapses are rare, and herpes labialis happens with lower prevalence than that of the general population, and almost never in severe or recurrent forms ((1) and Casanova *et al.* unpublished data)). Large population-based studies have estimated that the lifetime prevalence of herpes labialis amongst HSV-1 seropositive adults is 50.1.% (7) and that the annual prevalence of herpes labialis amongst HSV-1 seropositives is 24.1% (8). Our preliminary data suggest that herpes labialis strikes less than 10% of survivors of HSE, and typically runs a mild clinical course (Casanova et al. unpublished data). We tested the hypotheses that adaptive immunity is present after childhood HSE that could contribute to the control of mucocutaneous HSV-1 infection. We used recently available collections of proven HSV-1 CD8 T-cell peptide epitopes (9). CD4 responses were similarly evaluated directly *ex vivo* whenever possible.

Materials and Methods

Specimens

Standard diagnostic criteria were used to diagnose childhood HSE (10). The criteria included clinical signs of meningoencephalitis, lesions on cerebral computed tomography scan and/or magnetic resonance imaging, and cerebrospinal fluid PCR positive for HSV-1 DNA in CSF and/or detectable HSV-1-specific antibodies in serum. Data were collected by review of pertinent medical records including structured interviews (1). Anticoagulated venous blood was obtained at Université Paris Descartes – INSERM. Protocols were approved by INSERM (Institut National de Santé et Recherche Médicale) (subjects) and University of Washington (controls) Institutional Review Boards. PBMC were cryopreserved with 10% DMSO using standard methods.

Laboratory Studies

Modified published IFNγ ELISPOT methods were used (11). Thawed PBMC were rested overnight, viability checked (Guava, EMD Millipore, Billerica, MA), and 250,000 cells/well plated. Antigens included 0.3% DMSO, three pools of 37–40 known CD8 T-cell HSV-1 peptide epitopes (including (9, 11) and unpublished data) at 1 μg/ml each in 0.3% DMSO final, UV-HSV-1 or -mock antigen, or PHA positive control. The HLA restriction alleles proven to restrict these peptides included A:*0101, *0201, *0301, *2402, *2902, *3101, *3103, *6801, and B:*1501, *1502, *1801, *1802, *3501, *3801, *3906, *4001, and *5701. Pools 1–3 were combined at 1/3 strength if cell availability was low, resulting in 0.33 μg/ml final of each peptide. While there is no generally accepted cutoff for positive responses, we used a level of 20 spot forming units (SFU)/10^6 PBMC (12). An automated analyzer acquired images (CTL, Cleveland, OH). HSV IgG serology used type-specific Western blot (13). HLA typing used standard molecular methods.

Results

Subjects and Specimens

Patients providing specimens were either recruited from a previously described cohort (1) or seen subsequently. Patients were 3 months to 15 years of age at their initial HSE episode. Among specimens from 43 persons without known genetic lesions, 34 had adequate cell

number after overnight rest for IFNγ ELISPOT. Amongst these, 24 with > 200 SFU/10^6 PBMC to PHA positive control were analyzed for HSV-1 responses. For these 24 patients, the median age at HSE was 18.5 months (range 1–156), median age at phlebotomy was 16 years (range, 5–36), and median interval between HSE and phlebotomy was 13.5 years (range, 4–33). All 22 subjects with serum available were HSV-1 IgG seropositive; 3 were also HSV-2 seropositive (Table 1). HLA typing, available on 23 subjects, showed all but one was HLA matched to the CD8 peptide set at HLA A and/or B. Two of 24 (8.3%), neither of whom were in our previous cohort study (1), had recurrent herpes labialis after HSE. Two had HSE recurrences either at age 2, 1 year after initial presentation (subject 01353), or 14 months and 14 years after an initial presentation at 10 months (subject 01983). Two were born to consanguineous parents. Five had moderate/severe sequelae, five had mild neurologic sequelae, and fourteen had favorable recovery. Amongst 9 HSV-1 seropositive adult healthy donors (median age 43, range 29–65), five had a history of oral herpetic lesions and two were HSV-2 seropositive.

T-cell responses

CD8 IFNγ responses were evaluated using pools of HSV-1 peptide epitopes in 16 subjects. For the other 8, limited PBMC required testing one inclusive peptide pool (Table 1). We observed that 14 of 24 (58%) HSE survivors had a positive response to HSV-1 peptides (>20 SFU/10^6 PBMC). This included 9 of 16 tested with three peptide subpools and 5 of 8 tested with a single inclusive pools. Amongst the two subjects with recurrent herpes labialis, one each was positive and negative for CD8 T-cell responses. IFNγ responses were observed for 9/9 (100%) control HSV-1 seropositive adults. HSE survivors generally had lower magnitudes than controls, but the responses overlapped. Many HSE survivors reacted to >1 peptide pool, implying a poly-epitope CD8 response. We did note some subjects such as 00240, 00378, and 01806 with absent CD8 responses to HSV-1 peptides, despite maximal PHA control responses.

Amongst 5 subjects with sufficient PBMC, all showed CD4 IFNγ responses to whole HSV-1 antigen, including one (01806) without detectable CD8 responses. CD4 responses in adult controls were variable but present in 8/9 (89%), with one showing <20 SFU/10^6 PBMC despite brisk CD8 responses.

Discussion

The observations that childhood HSE is dissociated from simultaneous infection at other sites, and that mucocutaneous lesions (orolabial herpes) are rare and mild in survivors, suggested HSE pathogenic mechanism(s) specific to CNS cells. Indeed, *in vitro* experiments indicated that mutations in genes of the TLR3-IFN pathway genes, such as *TLR3* or *UNC93B1*, increased HSV-1 susceptibility intrinsically in neurons (2). In this report, we investigated acquired immunity to HSV-1 in HSE survivors without a currently known genetic lesion, hoping to gain clues to the general lack of mucocutaneous HSV-1 infection in these persons. Overall, our data showed that CD8 T-cell responses to HSV-1 are similar in HSV-1 seropositive healthy donors and survivors of childhood HSE, including children with

recurrent HSE and one of two with herpes labialis. The unknown host factors responsible for these cases of childhood HSE do not preclude the development of CD8 T-cell responses.

We observed heterogeneity amongst childhood HSE survivors for CD8 responses. In general, the magnitude of both CD4 and CD8 responses was lower in HSE survivors than healthy adults, but PBMC viability was also lower, such that dichotomous rather than quantitative results are most appropriate when evaluating responses in these specimens. Overall, most patients had a positive response in CD8 assays. For these individuals, while the pathogenesis of their original HSE remains obscure, innate-acquired immune cooperation to prime and maintain HSV-1-specific T-cell responses is intact. This is consistent with the finding that the known genetic disorders for childhood HSE are CNS-intrinsic (2), and SCID is not associated with childhood HSE (14). One subject with recurrent herpes labialis after HSE showed detectable (10 SFU/10^6 PBMC) CD8 responses below our positivity threshold. The low peptide concentrations tested for this subject may have reduced assay sensitivity. Given variability between HLA-compatible persons for peptide-specific responses, further tests are required to definitively assign any subject as CD8 T-cell non-responders. An alternative test of CD8 responses to whole HSV-1 using dendritic cell cross-presentation, overcoming HLA and TCR repertoire issues, requires more blood than was available for this study (11). We also did not have adequate PBMC to test whole virus CD4 responses in most persons, but each HSE survivor tested was positive. It cannot be excluded that some HSE survivors, despite intact B cell responses as measured by serum IgG, have a somewhat impaired T-cell response, as suggested in this report several years after HSE. Studies to address this would similarly require additional specimens for thorough investigation. Regardless, they do not display any clinical signs of T cell deficit, including mucocutaneous HSV-1 lesions.

Most HSE survivors, including two persons suffering from CNS relapse, have detectable adaptive blood IFNγ responses to HSV-1 when tested approximately a decade later. The responses were not quantitatively different from normal donors, despite the absence of mucocutaneous disease in most of HSE survivors. We are left with the unexplained clinical dichotomy of severe CNS primary yet asymptomatic mucocutaneous chronic-phase infections in most survivors of childhood HSE. The simplest hypothesis to account for this, supported so far by our mechanistic findings covering about 5% of childhood HSE, is that the abnormalities in CNS cell-intrinsic immunity to HSV-1 that underlie HSE (2) do not prevent acquired T-cell responses. The number of circulating CD8 T-cells with IFNγ responses to HSV-1 does not appear to be a key factor differentiating HSV-1 recurrence in most childhood HSE survivors, who rarely have mucocutaneous recurrences, from healthy adults, among whom 25–50% do have recurrences. Further research is needed to detect possible differences in T-cell abundance, effector functions, exhaustion, homing, or tissue residence. While herpes labialis was studied in a structured interview for most of our patients, it is possible that additional cases would be uncovered with a serial, prospective approach. Ultimately, a prospectively followed cohort with disease phenotype and longitudinal specimen collection, host and possibly virus genotyping, and detailed neuronal cell-intrinsic and leukocyte innate and acquired immune studies will be required to explain the benign course of HSV infection after recovery.

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Table 1

IFN-γ ELISPOT responses (SFU/10^6 PBMC) of HSE survivors and controls.

		CD8					CD4		mitogen	serology	
			HSV-1 peptide pools			pools 1–3					
status	**ID**	**DMSO**	**pool 1**	**pool 2**	**pool 3**	**combined**	**UV mock**	**UV HSV-2**	**PHA**	**HSV-1**	**HSV-2**
HSE survivor	**00192**	0	120	34	568	722	0	114	TNTC	+	−
	00240	0	0	0	4	4	ND		TNTC	ND	ND
	00378	0	0	2	2	4	ND	ND	TNTC	+	−
	00577	0				38	ND	ND	1104	+	−
	00585	0				2	ND	ND	1024	+	+
	01065	0				10	ND	ND	716	+	−
	01070	0				144	ND	ND	TNTC	+	−
	01320	0	2	12	16	30	0	444	1852	+	−
	01353	0	148	318	12	478	ND	ND	TNTC	+	−
	01443	0	8	4	0	12	ND	ND	1984	+	+
	01706	0				8	ND	ND	1576	+	−
	01785	0	14	0	0	14	ND	ND	418	+	−
	01794	0				4	ND	ND	1888	+	−
	01797	2	230	0	34	264	ND	ND	1460	+	−
	01806	0	2	0	4	6	0	52	TNTC	+	−
	01813	6	46	70	32	148	0	110	TNTC	+	−
	01979	2	26	10	140	176	ND	ND	TNTC	ND	ND
	01983	0	278	6	18	302	ND	ND	TNTC	+	−
	02029	0	0	0	0	0	ND	ND	212	+	−
	02137	0	344	12	12	368	4	44	TNTC	+	−
	02229	0				92	ND	ND	TNTC	+	−
	02263	0	66	58	46	170	ND	ND	1012	+	−
	02306	0	20	0	0	20	ND	ND	1694	+	−
	21233	0				36	ND	ND	1112	+	+

		CD8					CD4		mitogen	serology	
			HSV-1 peptide pools			pools 1–3					
status	**ID**	**DMSO**	**pool 1**	**pool 2**	**pool 3**	**combined**	**UV mock**	**UV HSV-2**	**PHA**	**HSV-1**	**HSV-2**
healthy adult	**3315**	0	10	136	28	174	0	318	TNTC	+	+
HSV-1 (+)	**10063**	0	128	8	11	147	0	107	TNTC	+	+
	11259	0	275	52	5	332	0	23	TNTC	+	−
	11331	4	31	332	12	375	1	48	TNTC	+	+
	13682	0	54	72	8	134	0	120	TNTC	+	−
	13845	0	64	456	48	568	2	1388	TNTC	+	−
	13850	0	22	210	126	358	8	526	TNTC	+	−
	13916	2	578	10	36	624	8	458	TNTC	+	−
	13990	2	148	304	30	482	4	760	TNTC	+	−
	14578	4	1494	0	0	1494	4	8	TNTC	+	−
healthy HSV (−)	**9270**	0	7	0	4	11	1	25	TNTC	−	−

[1] Sum of pools 1–3 or single inclusive pool of all peptides (see text).

[2] HLA type not available for this HSE survivor.

[3] TNTC = too numerous to count.

[4] ND = not done.

[5] Positive history of orolabial herpes.

[6] Recurrent HSE, studied 17 years (01353) or 1 year (01983) after most recent relapse.

[7] Subject not having an HLA A or B allele with one or more HLA-matched test HSV peptides.