Specific T-cell immunity against HHV-6 in healthy children and adolescents

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Background: Primary infection with **human herpes virus 6 (mainly HHV-6B)** commonly occurs in the first two years of life leading to persistence and the possibility of virus reactivation later in life. Consequently, a **specific cellular immune response** is essential for effective control of virus reactivation. **Virus-specific CD4+ and CD8+ T-cell** populations secrete **IFN- γ , TNF- α and IL-2** and have various cytolytic functions. In this study, we measured cell-mediated immune response to HHV-6 in healthy children and adolescents.

Materials and methods: By **flow cytometry** (Figure 1), the amount of cytokine (interferon gamma – IFN- γ , interleukin 2 – IL-2, tumour necrosis factor alpha – TNF- α) secreting T-cells after **10 days of pre-sensitization**, expansion with **proliferative cytokines IL-2 and IL-7**, and 6 hours of re-stimulation were measured with mixtures of pooled overlapping peptides from **HHV-6 specific protein U54, staphylococcal enterotoxin B (SEB, positive control)** or **Actin (negative control)** in **healthy children and adolescents** without any underlying immune disorder or infectious disease. To define the HHV-6 (U54) specific T- cell response, the following 2 criteria had to be fulfilled:
(I) The frequency of cytokine-secreting cells after U54 stimulation in the total population (CD4+ or CD8+ T-cells) was at least 0.1 percentage points higher than in unstimulated cells of the negative control.
(II) The frequency of cytokine-secreting cells after U54 stimulation was at least two times higher than in the negative control.

Results: All **25 tested individuals** (age 3.1-18.3, median 8.2 years, 28% female) showed a virus-specific response for at least one cytokine in either CD4+ or CD8+ cells. Percentages of individuals with HHV-6-specific TNF- α response in **CD4+ (48% of individuals)** as well as **CD8+ T-cells (56% of individuals)** were almost the highest, and TNF- α secreting T-cells were the most prominent HHV-6-specific cells (Table 1). Our data show significantly **higher frequencies of HHV-6-specific TNF- α producing CD8+ T-cells in individuals older than 10 years of life** (n=12, p=0.033, Figure 2). Additionally, the frequency of **TNF- α producing CD8+ T-cells positively correlated with the age of the individuals** (Spearman-Rho, r=0.465) on a two-sided significance level of p=0.019.

Conclusions: HHV-6 specific T-cells are commonly present, expandable, and detectable in healthy children and adolescents with higher frequencies of antigen-specific T-cells in older children and adolescents possibly reflecting repeated stimulation by viral persistence and subclinical reactivation.

Table 1: Number (percentages) of individuals with U54-specific T-cell response, frequencies of U54-specific T-cells and correlation coefficients for age and frequency of HHV-6 specific T-cells.

		Positive/tested individuals (%)	Frequency (%) of U54-specific CD4+ and CD8+ T-cells, respectively median (range)	Correlation coefficient (between age and frequency of HHV-6 specific T-cells) *p< 0.05
CD4+	Positive INF- γ response	8/25 (32%)	1.31 (0.63-10.54)	-0.037
	Positive IL-2 response	3/25 (12%)	1.38 (0.10-11.48)	-0.002
	Positive TNF- α response	12/25 (48%)	2.29 (0.50-24.91)	0.145
	Positive response (any cytokine)	14/25 (56%)	1.48 (0.12-8.26)	0.168
	Positive IFN- γ /IL-2 response	2/25 (8%)	1 (0.25-17)	0.112
	Positive IFN- γ /TNF- α response	8/25 (32%)	1.36 (0.38-19)	0.077
	Positive IL-2/TNF- α response	3/25 (12%)	1.29 (0-14.50)	-0.079
	Positive IL-2/TNF- α /IFN- γ response	2/25 (8%)	1 (0.25-14)	-0.018
CD8+	Positive INF- γ response	10/25 (40%)	1.69 (0.80-10.21)	0.088
	Positive IL-2 response	3/25 (12%)	1.01 (0.60-4.67)	0.225
	Positive TNF- α response	14/25 (56%)	2.10 (0.72-9.83)	0.465*
	Positive response (any cytokine)	17/25 (68%)	1.86 (0.69-8.04)	0.286
	Positive IFN- γ /IL-2 response	2/25 (8%)	1.40 (0.60-6)	0.146
	Positive IFN- γ /TNF- α response	11/25 (44%)	1.93 (0.33-19.67)	0.142
	Positive IL-2/TNF- α response	1/25 (4%)	1.23 (0.60-6)	0.141
	Positive IL-2/TNF- α /IFN- γ	1/25 (4%)	1.29 (0.60-7)	0.083

Figure 2: Box plots show significantly higher percentages of TNF- α producing CD8+ T-cells in individuals over 10 years after stimulation with HHV-6 specific antigen U54. *p = 0.033. For the other cytokine patterns and CD4+ cells, differences between age groups were not statistically significant.

Figure 1: FACS analysis of cytokine-producing CD8+ T-cells of a 17-year-old adolescent after stimulation and 10 days of expansion in cell culture. The upper two images show the negative controls (ACTS) compared to the U54 stimulated cells (left: TNF- α , right: IFN- γ)