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Intrafamilial transmission of human cytomegalovirus (HCMV): Long-term dynamics of epitope-specific antibody response in context of avidity maturation

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ABSTRACT

Background: The role of a special early family formation (PEKiP®), which is popular in Germany, as a potential origin of HCMV-transmission to seronegative mothers is not documented.
Objectives: To describe the clinical courses, to identify the virological origin and to evaluate a new tool for diagnosis of a cascade of intrafamilial HCMV primary infections.
Study design: This prospectively analyzed long-term course of HCMV primary infection leading to hospitalization of two family members, included the evaluation of different IgG/IgM/IgG avidity-assays with an epitope-specific recombinant immunoblot-assay. Additionally, neutralization (NT) assays using fibroblast-and epithelial-target cells were performed to correlate NT_{50} values to avidity maturation. HCMV gN/gO/gB-RFLP-genotyping and phylogenetic analyses were performed using urine viral isolates.
Results: The clinical courses of the sequentially occurring intrafamilial HCMV primary infections were unusual, leading to hospitalization. Long-term-serology of the mother revealed concordant results for an unimodal IgG-course and a rapid decrease of IgM-indices from week 7 to week 21 p.i. Interestingly, the cut-off definitions for low and high avidity ranged discordantly from 15 to 25 weeks, and from 18 to 42 weeks p.i., respectively. A good correlation was found between the increase of fibroblast-adapted NT_{50} values and the appearance of high avidity using the epitope-specific immunoblot (>18 weeks p.i.). RFLP-genotyping and sequencing could identify the index patient as member of PEKiP®-meetings.
Conclusions: PEKiP®-meetings with naked babies may be an important source of horizontal HCMV-transmission to seronegative pregnant mothers in Germany. Using epitope-specific immunoblots, persisting HCMVp150-IgM-reactivities and good concordance between high IgG-avidity and increase of fibroblast adapted neutralization capacity were found.

1. Background

The overall HCMV-seroprevalence in pregnant women in Germany was estimated recently at 42% [1], while the rate of HCMV IgG-seroconversion is about 0.5% [2], similar to data from France with 0.46% [3]. No data are available on HCMV-shedding rates of infants in German day care centers. A recent meta-analysis reported on HCMV shedding-prevalence of healthy infants in US day care centers of about 23% [4].

Breastfeeding has a major impact on global HCMV-epidemiology and vertical virus transmission, since nearly every seropositive mother reactivates the virus and the mother-to-infant-transmission rate is about 40% [5]. Vertical transmission of

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HCMV during pregnancy in low seroprevalence-settings [6] results in rates of congenital HCMV infection of 0.7 of live births [7]. However, beside vertical transmission also horizontal transmission, notably during group day care, plays an important epidemiological role in virus spread [8–11]. A special form of interaction between babies and their parents in Germany is the 'Prague-Programme-for-Parents-and-Children' (PEKiP®) [12] consisting of group-work of parents and their infants from 4 weeks to 12 months post partum, in which up to 65 000 families in Germany, Switzerland and Austria, participate weekly.

2. Objectives

The primary aim of this study was to identify the origin of an unusual case series of intrafamilial HCMV-transmissions of a seronegative mother who visited a (PEKiP®)-group weekly with her daughter. Comprehensive long-term-HCMV-monitoring included avidity maturation, epitope-specific recombinant immunoblotting, and neutralization using fibroblasts- and epithelial cells was performed.

3. Study cohort and methods

3.1. Study cohort

The term and healthy female *infant* was born in July 2007. The 9 months old healthy girl was still breastfed from her seronegative mother in 2008, and both visited a PEKiP®-group weekly until early May 2008. The mother planned her second pregnancy in summer 2008. The 53 years old healthy *maternal grandmother-I*, was also involved in child-care, like the 54 years old *paternal grandmother-II*.

3.2. Methods

3.2.1. Direct HCMV detection

Virus isolation using fibroblast microculture was performed as described previously [13]. DNA was purified by spin columns using QIAmp DNA Blood Mini Kit (Qiagen, Germany) and used for qualitative nested-PCR of the IE1Ex4-target region [13].

3.2.1.1. HCMV gB-, gN-, gO-genotyping. The variable regions from the viral glycoprotein gene sequences that encode for the proteins UL55, UL73 and UL74, were amplified and sequenced as previously reported [14–16]. Multiple alignments of the sequences using clustal W2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) were generated for the clinical isolates from the child, the mother and the grandmother I, as well as for reference strains AD169, Towne, Merlin, Toledo and Davis (Genebank access code: X17403, FJ616285, Y4468942, GU937742 and X99845, respectively) as well as for representative strains of each genotype. The pairwise identities between the isolates were calculated using the Smith Waterman algorithm using Jalview Version 2. The gO-variants were also identified using restriction-fragment-length-polymorphism (RFLP) using HpaII [16]. Additionally, the RFLP-genotyping of HCMV glycoprotein N was performed as described previously using SacI and SalI [15].

3.2.2. HCMV serology

The following commercial tests were used for diagnosis of the HCMV antibody status: "ELISA"-CMV IgG ELISA PKS/CMV IgM-ELA-Test PKS (Medac, Germany); "CLIA"-Liaison CMV IgG/IgG avidity/IgM (DiaSorin, Italy); "ECLIA" Elecsys CMV IgG/IgM/IgG avidity (Roche Diagnostics, Switzerland); "CMIA" Architect CMV IgG/IgM/IgG avidity assay (Abbott, Germany); recomblot CMV IgG/IgM/IgG avidity (Mikrogen, Germany). The recomblot assay (recIB) detected HCMV-specific antibodies to the following HCMV recombinant proteins: IE1, p150, CM2, p65, gB1, gB2. RecIB-scoring reached from +/− weaker than IgG/IgM-control (negative), to +++, very strong reactivity.

3.2.3. HCMV neutralization

Target cells were both, human foreskin-fibroblasts (HFF) and human retinal pigment-epithelial cells (ARPE-19; ATCC-CRL-2302/LGC-Standards, Germany). The viral target strain originated from urine of a congenitally infected newborn. The reference sera included an HCMV-specific hyperimmunoglobulin (HIG) (Cytotect®) and serum-pools ($N = 100$) from healthy seropositive and seronegative mothers at birth. Each 200 μl of the cell-free target virus-passage ($50 \times TCID_{50}$/ml) were preincubated for 90 min at 37 °C and 5%CO_2 with 200 μl of heat inactivated (30 min 56 °C) serum dilutions from 1:50 to 1:3200 of the maternal serum. Thereafter, each 100 μl of the virion-antibody mixtures were added to the different microcultures. After 18 h of incubation, monolayers were fixed and IE1 immunoperoxidase staining followed [13]. The neutralization-titers 50% (NT_{50}) were calculated referring to 100% of viral infectivity in seronegative serum pool replica.

4. Results

4.1. Clinical courses and routine diagnostics

4.1.1. Index-patient

In April 2008, the 9 month old girl was presented at the pediatrician with fever, flu-like symptoms and increasing hidrosis. Symptoms of an infection of the upper-respiratory tract dominated (Fig. 1a and b) and persisted for about 3 months. Serological testing was performed in July, detecting the presence of HCMV-IgG without IgM (Fig. 1a:1). Semiquantitative viruria was detectable up to November 2009, and DNAuria still 3 months longer. In total, viral DNAuria and viruria were analyzed at 8 different time points. At the end of viruria the infant was 28 months old. Viruria-levels were continuously decreasing. Peak-level of viruria (111 infected fibroblasts/ml of urine) under observation was during October 2008 at the age of 15 months. The viral isolate from observed peak-level of viruria was propagated in vitro and used for sequencing analysis (Fig. 1a:2). Viral DNAload in October 2008 was 1360 copies/ml, and in December 2008 15,000 copies/ml. Interestingly, the infant did not shed HCMV into saliva during the observation.

4.1.2. Mother of the index-patient

On 24th June 2008, maternal symptoms started with fever (>39 °C), flu like-symptoms with shivering, lymphadenopathy and symptoms of a pulmonary infection leading to hospitalization after ineffective empiric antibiotic treatment (doxycyclin for 6 days) (Fig. 1a and b). Computed tomography in July 2008 revealed atypical pulmonary infiltration, but no pathogen was detectable in bronchoalveolar lavage (BAL). Ultrasonography revealed hepatosplenomegaly. Laboratory findings presented elevated LDH and CRP, slightly elevated transaminases and slight lymphocytosis in the differential blood count (Fig. 1b). Routine serology revealed detection of HCMV-IgM and -IgG, but negative HCMV-PCR from blood and BAL (Fig. 1a:3–6). Interestingly, in week 25 after onset of symptoms, viral DNAuria was still detectable with >2000 copies/ml (Table 1). Since the onset of symptoms was exactly defined, it was possible to refer all later findings to that date. We assume an incubation time of about 4 weeks, thus exposition might have been in May, which fits well with the potential onset of viral shedding of the index patient.