Journal of Clinical Virology 90 (2017) 40–45

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Comparison of cytomegalovirus (CMV)-specific neutralization capacity of hyperimmunoglobulin (HIG) versus standard intravenous immunoglobulin (IVIG) preparations: Impact of CMV IgG normalization

Matthias Stefan Schampera[a], Katrin Schweinzer[a,1], Harald Abele[b], Karl Oliver Kagan[b], Reinhild Klein[c], Ingo Rettig[d], Gerhard Jahn[a], Klaus Hamprecht[a,*]

[a] Institute of Medical Virology, University Hospital of Tuebingen, D-72076 Tuebingen, Germany
[b] Department of Obstetrics and Gynaecology, University Hospital of Tuebingen, Germany
[c] Department of Internal Medicine II, Immunopathological Laboratory; University Hospital of Tuebingen, Germany
[d] Department of Internal Medicine, Central Laboratory, University Hospital of Tuebingen, Germany

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form 30 January 2017
Accepted 6 March 2017

Keywords:
CMV primary infection
Neutralizing antibodies
Hyperimmunogloblulin (HIG)
Standard immunoglobulin
Recombinant immunoblot
Pregnancy

ABSTRACT

Background: Based on a non-randomized study of Nigro et al. (2005) the intravenous administration of hyperimmunoglobulins (HIGs) is applied frequently to women with primary CMV-infection as "off-label use" in Germany.
Objectives: In order to describe their CMV-specific neutralization-capacity in vitro, we analyzed the HIG preparations Cytotect®, and Cytogam® as well as the standard intravenous immunoglobulins (IVIG) Octagam®, Gamunex®, Kiovig®.
Study design: We performed short-term cell-free CMV neutralization assays (CFNT) and long-term cell-adapted neutralization-plaque-reduction assays (PRANT). Human retinal epithelial cells (ARPE-19) were used as target cells. A clinical CMV primary-isolate from amnion fluid propagated in epithelial cells without any initial fibroblast adaption was used. For calibration we previously generated serum-pools ($N = 100$) from two cohorts of mothers at birth: seronegative and latently CMV-infected mothers. Biochemical analysis included total protein, albumin, Ig-class, and IgG-subclasses. Additionally, CMV antibody-reactivity was checked using recombinant immunoblotting.
Results: HIG and IVIG preparations showed differences in levels and patterns of protein, Ig-class and CMV-specific antibody concentrations. All IgG-preparations showed high in vitro NT-capacity and high IgG-avidity. The NT_{90}-values for HIGs and IVIGs and our seropositive reference-pool showed similar NT-capacity at a dilution of (1:100) which corresponded well to 4.1 PEI-Units/ml.
Conclusion: All HIG- and IVIG-preparations showed similar NT-capacity following CMV IgG-normalization. Our in vitro results are in strong contrast to former findings suggesting higher functional CMV NT titers in IVIG-preparations compared to HIGs.

1. Background

The maternofetal CMV-transmission is the most common intrauterine-infection worldwide with a prevalence of 0.2–2% of all live births, potentially causing permanent CNS disorders. Particularly, the transmission-rate rises up to about 40% during a CMV primary infection in the first trimester of pregnancy and results in a higher percentage of fetal damage than later in pregnancy [1].

The current CMV-prevention strategies during pregnancy are not satisfying up-to-now. There are no effective vaccines to generate a sufficient immunogenic prophylaxis against CMV [2]. An initial antiviral treatment-study with valaciclovir (VACV) did not show to be a treatment-option for pregnant women [3]. However, recent results from a phase-II-study with high-dosage of VACV indicated efficiency for improving the outcome of moderately

* Corresponding author at: Institute of Medical Virology, University Hospital of Tuebingen, Elfriede-Aulhorn-Str. 6, 72076 Tuebingen, Germany.
E-mail address: klaus.hamprecht@med.uni-tuebingen.de (K. Hamprecht).
[1] Present address: Department of Dermatology, University Hospital of Tuebingen, Germany.

http://dx.doi.org/10.1016/j.jcv.2017.03.005
1386-6532/

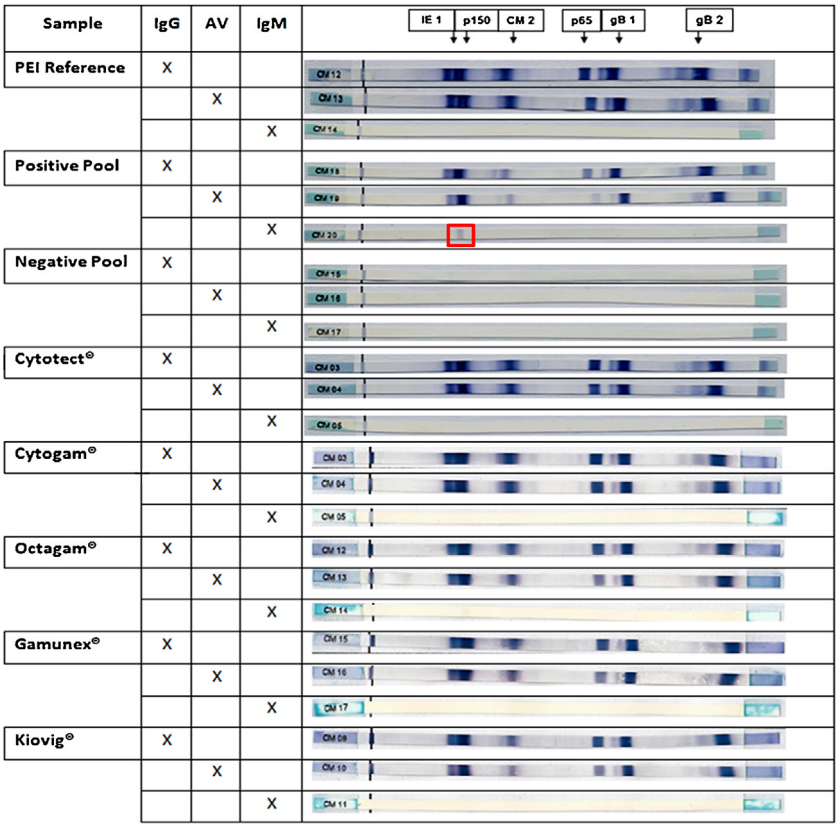

Fig. 1. Recombinant CMV immunoblot. All IgG-preparations showed high concentrations of CMV-specific IgG-antibodies with high IgG-avidity and lack of CMV-specific IgM antibodies. However, the pool of latently-infected women showed a weak unspecific anti-CMV rec-p150 IgM-reactivity (red square). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

symptomatically infected fetuses [4]. Nevertheless, for routine-practice, hygiene-counseling of pregnant mothers is very efficient for prevention of CMV-transmission [5,6].

Several studies evaluated prevention of CMV-transmission via CMV-specific hyperimmunoglobulin-treatment (HIG) of pregnant women with primary CMV-infection. A first relevant non-randomized study could demonstrate an effective reduction of maternofatal CMV-transmission of about 24% of congenital CMV (cCMV)-infected infants in the prevention-group compared to the untreated control-group [7]. Thereafter, many case-reports and observational studies showed a trend toward reduction of CMV-transmission and fewer cases of symptomatically infected infants [8]. But the first RCT-study (using the design of the Nigro study [7]) could not confirm the assumed effectivity of HIG for prevention of maternofetal CMV-transmission and showed a reduction of CMV-transmission of only about 14% in the prevention-group [9].

Based on the treatment of a volunteer-pregnant woman with a proven CMV primary infection who received intravenous HIG (Cytotect®) monthly using the study design of Nigro et al. [7] and Revello et al. [9], periodic decreases were found in CMV-IgG-levels with a half-life time of about 11 days, together with fluctuations in epithelial-cell-specific neutralization-capacity. These findings may have an important effect on clinical-outcome [10].

2. Objectives

Therefore, we analyzed biochemically the antibody composition of two HIG-preparations together with the standard intravenous immunoglobulin-preparations (IVIG) as well as their CMV-specific neutralization-capacity in vitro. In order to standardize neutralization-assays, we generated large serum pools of 100 CMV-seropositive latently-infected and 100 CMV-seronegative mothers at birth. In the absence of a WHO-certified CMV-IgG unit standard we used the PEI-CMV-IgG-reference-preparation to calibrate our CMV serum-pools for neutralization-experiments.

3. Study design

3.1. Native reference serum-pools

Two reference serum-pools were created, containing a CMV-IgG positive/IgM-negative serum-pool of latently-infected mothers and a CMV-IgG-negative/IgM-negative serum pool. The sera of these pools were derived of each 100 healthy pregnant mothers at birth from our Tuebingen cCMV study.

3.2. Virus strain

A cell-free CMV isolate from amniotic-fluid (H2497-11) was primary isolated on retinal-pigment-epithelial-cells (ARPE-19; ATCC LOT 59814960) without any adaption to human foreskin-fibroblasts (HFF). After serial propagation of the virus-isolate, the quantity of cell-free infectious virus was determined by tissue-culture infectious dose 50% ($TCID_{50}$) determination. ARPE-19 cells were used as target cells.

3.3. Immunoglobulin preparations

Two hyperimmunoglobulin-preparations (HIG) and three standard immunoglobulin preparations (IVIG) were used for evaluation of the neutralization-capacity. The HIG included Cytotect®