SUPPLEMENT ARTICLE

Properties and Mechanisms of Immunoglobulins for Congenital Cytomegalovirus Disease

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Immunoglobulins are one major component of adaptive immunity to external and resident microorganisms, evolving very early in phylogenesis. They help eukaryotes in controlling infections, mainly through their neutralizing activity, which quenches both the cytopathic and inflammatory potential of invading microorganisms. Cytomegalovirus (CMV)–related disease is generally blunted in seropositive subjects with conserved specific humoral responses. CMV-seropositive pregnant women, in accordance with such evidence, suffer little or no fetal damage when reexposed to CMV. Several seminal experiences and early experimental models confirmed that repeated infusions of immunoglobulins, either with hyperimmune or standard preparations, may help to reduce maternal-fetal CMV transmission, as well as to quench fetal disease upon transmission. This review focused on experimental evidence supporting the potential role of immunoglobulins as a tool to control fetal CMV-related disease in pregnant women.

Keywords. congenital cytomegalovirus disease; immunoglobulins; IgG avidity; immunomodulation.

After primary cytomegalovirus (CMV) infection, innate response primarily involves natural killer (NK) cells and type I interferons (IFN-α/ß) produced by dendritic cells (DCs) through Toll-like receptor (TLR) 7 or TLR9-dependent pathways, endorsing both direct antiviral and immunoregulatory effects, including the regulation of cytolytic activity of NK cells [1]. CMV-induced innate cytokines promote upregulation of major histocompatibility complex (MHC) and cosignaling ligands on antigen-presenting cells. In this interplay between innate and adaptive immunity, which is conserved against most pathogens, the key role of immunoglobulins will take place [2]. In fact, adaptive immunity, including polyclonal generation of B and T cells that establish both cell-mediated immunity and humoral specific responses [2], is needed to efficiently control primary CMV infection and maintain long-term control of CMV reactivation [1]. CMV, however, can display a multitude of strategies to modulate host immune responses, ultimately facilitating its own persistence, even in the face of robust innate and adaptive immunity [1]. As most of the key cellular immune players are not productively infected by CMV (NK, T, and B cells), CMV modulates their responses by altering the function of cells of myeloid or stromal lineage directly infected, promoting the secretion of both host and viral cytokines acting upon these cells [3]. By these weapons, CMV will reactivate in hosts with less efficient immunity [1].

IMMUNOGLOBULIN PRODUCTION

After antigen presentation, naive B cells are activated, proliferating, and differentiating into effector cells that will actively secrete immunoglobulins [4]. Immunoglobulins are one major component of adaptive immunity, being the fruit of the complex evolution of 1 multigene family expression very early in phylogenesis [4]. Upon presentation of a variety of epitopes from viruses, bacteria, fungi, and other metazoans, polyclonal antibodies are produced, all sharing the same basic structure [4]. The antibodies confer to the immune system a greater probability of effectively controlling pathogens by their multifaceted action [2, 4]. Epitopes for each pathogen are presented in association to MHC; B-cell clones

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generated from immunodominant epitopes, best fitting the MHC pocket, get a proliferative advantage and establish clonal memory for their moiety [4]. Some epitopes, targeting functionally relevant sites on pathogen structures, result in neutralizing (NT) activity, that is, the ability to prevent their pathogenic action, mainly by pathogen opsonization and phagocytosis [2, 4]. Immunoglobulins, however, are extremely versatile molecules with simultaneous biological activities upon binding of target sites, including blockage of toxin activity and reduction of inflammatory reactions to cytopathic insults [2, 4]. Polyclonal immunoglobulins undergo quite a complex maturation process, including heavy chain class switching and affinity maturation [2, 4]. Upon persisting or repeated exposure, production of antibodies with increasing affinity for their target antigens is achieved, involving the region V of the gene for immunoglobulin generation [4]. This region undergoes additional genetic modifications, through a process known as somatic hypermutation. This process consists of a high rate of point mutations, resulting in additional diversity to each specific epitope [2, 4]. This ultimately leads to an increase in the affinity of immunoglobulins for their target epitope, enhanced neutralizing activity, and other effects [2, 4]. All of the above-mentioned properties have been demonstrated for CMV, both in human and animal specific experimental models [1]. Immunoglobulins react with a broad spectrum of viral proteins from either purified virus or CMV-infected cells [4].

IMMUNOGLOBULINS FOR CMV DISEASE

Humoral response to many viruses is effective in quenching viral replication through neutralizing antibodies, as for rubella, measles, and hepatitis A virus. At variance, immunoglobulins do not confer protection against highly variable viruses, including hepatitis C virus, dengue, respiratory syncytial virus, influenza, and human immunodeficiency virus [5]. Their presence, however, is able to determine a measurable and significant reduction of the cytopathic effect of the respective viruses on infected cells [5]. During primary CMV infection, NT immunoglobulins contribute only marginally to viral clearance and latency establishment, for which cell-mediated immunity is crucial [6]. Neutralizing antibodies, however, will provide the host with lifelong protection from most cytopathic effects of CMV during early and relapsing replication cycles, with the exception of hidden sites, such as photoreceptor layers in the retina, poorly accessible to immunoglobulins. When assayed on fibroblasts in vitro, CMV glycoproteins B (gB) and H (gH) were considered the major targets of NT immunoglobulins. Recently, a protein complex including gene products of the CMV UL128-131 locus, assembled with gH and gL to form a 5-protein (pentamer) complex, gH/gL/pUL128-130-131, was found to be indispensable for virus growth in endothelial cells and virus transfer to leukocytes [7]. NT immunoglobulins to this pentamer compared to immunoglobulins toward gB were a thousand-fold more potent in neutralizing cytopathic activity [7]. Evidence of the inhibitory potency of antipentamer antibodies support the hypothesis that the CMV pentamer glycoprotein complex may be a major target for NT immunoglobulins in congenital CMV infection, interfering with viral spread in vivo and virus transmission to the placenta and fetus [7]. Data obtained from pregnant women with primary CMV infection indicated a significant correlation between titers of serum pentamer immunoglobulins and NT activity in epithelial cells. Furthermore, early detection of antipentamer immunoglobulins was associated with lower rates of CMV transmission from mother to fetus, suggesting that early administration of immunoglobulins to infected pregnant women could both prevent and control congenital CMV infection [7]. Different immunoglobulin preparations were used in clinical studies, mostly hyperimmune preparations; standard immunoglobulin preparations, however, were also tested for their good NT activity in vitro [8].

EVIDENCE FROM ANIMAL MODELS

Investigation of CMV pathogenesis in humans and in human fetuses was hampered by host-specific evolution of CMV strains, complicating the extrapolation of the results of studies on animal models [9–12]. The guinea pig CMV (GPCMV) model was particularly useful, as GPCMV may cause infection in utero [10]. GPCMV models were used to test passive transfer of immunoglobulins to prevent intrauterine transmission and/or protect the infected fetus [9, 11]. Preexposed pregnant animals had lower GPCMV viremia and fewer maternal and fetal infections [10]. Anti-gB passive immunization or immune sera to purified GPgB decreased maternal viremia, placental and fetal infections, pregnancy losses, and intrauterine growth retardation [9, 11]. High-titer immune immunoglobulins given either before or after maternal challenge with GPCMV reduced fetal infections from 39% to 0% [11]. Murine CMV (MCMV) models could not shed direct light on fetal infection, as MCMV is unable to cross the placental barrier. When congenital infections were artificially obtained through injection of MCMV into the peritoneal cavity of fetuses, however, passive transfer of anti MCMV-gB decreased viral replication in the brain, as well as number and severity of inflammatory brain lesions [12]. In conclusion, in animal models immunoglobulins protected against CMV vertical transmission, but also improved fetal disease-free survival.

EVIDENCE FROM IMMUNOSUPPRESSED PATIENTS

Also supporting the use of immunoglobulins in pregnancy is the overwhelming evidence of their effectiveness in reducing CMV disease and mortality in patients with immune

suppression, including those temporarily or permanently deprived of cell-mediated immunity [8, 13, 14]. Two different preparations of immunoglobulins have been so far used: hyperimmunoglobulins, purified exclusively from the plasma of CMV-seropositive subjects with high CMV immunoglobulin G (IgG) titers; and standard immunoglobulins, derived from donor pools with no selection according to CMV serostatus and therefore including a varying proportion of subjects unexposed to CMV. It has been recently reported that standard preparations had significantly higher titers of neutralizing IgG3 antibodies for CMV in comparison with hyperimmune preparations, suggesting that standard products could be a valuable therapeutic alternative [8]. The efficacy of immunoglobulin prophylaxis in bone marrow transplant recipients was first demonstrated, both in reducing all-cause mortality and CMV infection [13]. More recently, additional investigations confirmed only a reduced incidence of interstitial pneumonia [15]. Similarly, trials in renal transplant patients showed that both standard and hyperimmune immunoglobulins attenuated CMV disease [13]. Immunoglobulin infusions in patients undergoing liver transplant in pediatric settings reduced the incidence of posttransplant CMV disease from 40% to 4% in low-dose immunosuppression recipients [16]. Immunoglobulin infusions and antiviral therapy after cardiac transplants had a well-established protective role [14]. Interestingly, equal protection was reported for standard and hyperimmune immunoglobulins [8, 16].

SAFETY ISSUES

Immunoglobulins are the most purified blood derivative, being the only one pasteurized; in recent years no case of viral transmission has been reported upon infusion [17]. Immunoglobulins were long and safely used in pregnancy to treat blood group incompatibilities, as well as for passive immunization of fetuses against rubella, hepatitis A virus, measles and varicella. Only anecdotal and minor side effects were reported [17].

ONGOING RESEARCH

In 2009, the Abruzzo regional government funded a prospective study to investigate the use of standard immunoglobulin infusions in an uncontrolled series of pregnant women with confirmed primary CMV infection [18]. Women and newborns are followed in the study up to 5 years after delivery. Two large batches of standard immunoglobulins with mean CMV IgG titers of 187 ± 15 U/mL and 164 ± 11 U/mL, and mean IgG avidity indexes of 85.3% ± 4.2% and 82.3% ± 7.2%, respectively, were alternatively used for monthly infusions, at a dose of 0.5 g/kg. The research project is ongoing, and enrollment is expected to continue through 2014, recruiting approximately 400 pregnant women; to date, 205 women have consented to participate. Mean CMV antibody titers and avidity indexes at diagnosis were 39.7 ± 48.8 U/mL and 25.4% ± 15.9%, respectively. All women received at least 1 infusion, 45% ≥3 infusions. Mean avidity indexes rose from 44.8% ± 16.5% to 63.4% ± 13.4% soon after the first infusion and similarly after each subsequent infusion (all $P < .001$, paired t test); women with lower preinfusion (≤30%) indexes had larger increases ($P < .001$). Postnatal follow-up is ongoing for 130 neonates, 39% of whom were CMV infected at birth; transmission rate was 11% in women treated in their first gestational trimester. Thus far, 4 neonates demonstrated mild growth retardation at birth and all recovered during follow-up. Two children were hypoacusic at birth, one fully recovered. These preliminary results provide evidence that immunoglobulin infusions significantly increase CMV IgG titers and avidity indexes in pregnant women, transferring protective humoral immunity as indicated by the lack of significant postnatal events. One major limitation is the lack of randomization in the experimental design [18]. However, many experts in the field found it ethically unacceptable to randomize patients to mock treatments [19]. Uncontrolled prospective series, including a number of patients large enough to have the statistical power to prove immunoglobulin protection in comparison with historical controls, will be both ethical and valid [20].

Notes

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