High level antibodies to TORCH in the IVIG preparation from Taiwanese

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Abstract

Background: Congenital TORCH (toxoplasmosis, other viruses [varicella-zoster virus, VZV, etc.], rubella, cytomegalovirus [CMV], Herpes simplex virus [HSV]) infections are major causes of prenatal, perinatal, and postnatal morbidity and mortality. Although treatment or prevention strategies are available for these pathogens, all drugs may not be safe during the pregnancy. The aim of this study is to measure the antibodies (Abs) concentration in the intravenous immunoglobulin (IVIG) preparation to evaluate the therapeutic potential for TORCH infection.

Methods: We tested the only one commercial IVIG preparation from Taiwanese for the presence of Abs against *Toxoplasma gondii*, VZV, Epstein–Barr virus (EBV), measles, mumps, rubella, CMV, HSV type 1 (HSV-1), and HSV type 2 (HSV-2) by using enzyme-linked immunosorbent assay or chemiluminescent microparticle immunoassay.

Results: In our study, the median level (range) of anti-CMV immunoglobulin G (IgG) is > 250 (All > 250) (arbitrary unit, AU)/mL, anti-EBV > 200 (All > 200) (relative unit, RU)/mL, anti-HSV > 200 (152.75 to >200) RU/mL, anti-VZV > 5000 (All > 5000) IU/L, anti-measles > 5000 (All > 5000) IU/L, anti-mumps > 200 (156.5 to > 200) RU/mL, anti-rubella 209.8 IU/mL (192.7 to 238.5), and anti-*Toxoplasma* is 14.05 (12.3 to 16) IU/mL. There was not any immunoglobulin M (IgM) against HSV, VZV, mumps, measles, rubella, CMV, EBV, and *Toxoplasma* in the "Taiwan Blood Services Foundation" IVIG preparations.

Conclusion: There was high activity against *T. gondii*, VZV, EBV, measles, mumps, rubella, CMV, HSV-1, and HSV-2 in all IVIG batches. Further investigation is warranted to confirm the efficacy of IVIG from Taiwanese for congenital TORCH infections.

Keywords: Antibodies; Intravenous immunoglobulins; TORCH infections

1. INTRODUCTION

Infections acquired in utero or during the birth process are major causes of maternal and fetal morbidity and mortality because fetuses and neonates have not acquired protective antibodies (Abs).[1,2] Congenital TORCH (toxoplasmosis, other viruses [varicella-zoster virus, VZV, etc.], rubella, cytomegalovirus [CMV], Herpes simplex virus [HSV]) infections are associated with fetal anomalies and demise and with postnatal infection of variable manifestations from no symptoms to multiorgan involvement, such as hearing, visional, liver, neurologic and cardiac systems, hyperbilirubinemia, thrombocytopenia, abnormal growth, and developmental anomalies.[3] Transmission of the pathogens may occur prenatally, perinatally, and postnatally through transplacental passage of organisms, contact with blood and vaginal secretions, or breast milk.[3] TORCH infections in pregnant women are easily overlooked because it is often asymptomatic and maternal physical findings are not specific. Limited treatment or prevention strategies are available for fetal TORCH infections; however, some of the medications may not be safe during pregnancy. Because a therapeutic abortion is often suggested in the case of antenatal TORCH infection,[4] any means to preserve the fetus in women with TORCH infection warrants an effort.

Intravenous immunoglobulin (IVIG) comprises pooled Ab collections from at least 3000 to 60 000 healthy blood and plasma donors.[5] The initial IVIG application is administered as Ab replacement therapy in patients with primary immunodeficiency and IVIG preparations contain variable quantities of Abs to support patients against other infections, such as hepatitis A virus,[6,7] West Nile virus,[8] and enterovirus,[9] suggesting that IVIG has broad antimicrobial activity. If a virus or microbe is common in an endemic area, Abs induced by exposure of the formerly naïve population to this pathogen are the main content of IVIG in a given endemic area. Investigations have revealed the following seroprevalence rates: for *Toxoplasma*, 6.1% to 14.7% in the USA[10] and 9.3% in Taiwan;[11] for VZV, 99.6% in the USA[12] and 91.1% in Taiwan;[13] for CMV, 1.8% of 12- to 49-year-old women in the USA[14] and 91.1% of women of childbearing age

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in Taiwan;[15] and for HSV type 1 (HSV-1) and HSV type 2 (HSV-2), 51.0% and 5.3%, respectively, in the USA,[16] and 63.2% and 7.7%, respectively, in Taiwan.[17] These data reflect a high prevalence rate of TORCH in Taiwan.

There are several brands of IVIG available in Taiwan; however, the cost of Taiwan Blood Services Foundation (TBSF) human immunoglobulin has only been reimbursed since 2008 because it is prepared in cooperation with the "self-sufficiency" recommendation set forth by the Taiwan Department of Health, from pooled human plasma donated by Taiwan's voluntary and nonremunerated donors. Supposedly, local IVIG preparations may contain higher titers of Abs for the specific diseases and there is no Taiwanese data for levels of Abs to TORCH pathogens; then, it is essential to ensure that the quantities of specific Abs against TORCH in IVIG is adequate. Because maternal intravenous human immunoglobulin has been successfully and safely used in the treatment of several fetal diseases,[18] we conducted a study to determine the neutralizing Ab titers in IVIG preparations for each of the TORCH pathogens.

2. METHODS

2.1. Quantify subspecific antibodies

We measured the levels of anti-TORCH Abs in one commercially used IVIG preparations that were obtained from "TBSF" Human Immunoglobulin for Intravenous Use (LOT B3740501018, CSL Limited, Australia). Four batches from different periods of time were checked. Ab levels were measured in these IVIG preparations at 60 mg/mL.

2.2. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was used for the detection of Abs against VZV, HSV, measles, mumps, and Epstein–Barr virus (EBV). The following assays were determined by using commercial kits: Anti-VZV enzyme-linked immunosorbent immunoglobulin M (IgM) assay (EUROIMMUN Medizinische Labordiagnostika AG, EI 2650–9601-2 M, Luebeck, Germany); anti-VZV enzyme-linked immunosorbent immunoglobulin G (IgG) assay (EUROIMMUN Medizinische Labordiagnostika AG, EI 2650-9601 G, Luebeck, Germany); anti-HSV-1/2 pool ELISA IgM (EUROIMMUN Medizinische Labordiagnostika AG, EI 2531-9601-1 M, Luebeck, Germany); anti-HSV-1/2 pool ELISA IgG (EUROIMMUN Medizinische Labordiagnostika AG, EI 2531-9601-1 G, Luebeck, Germany); anti-Measles virus ELISA IgM (EUROIMMUN Medizinische Labordiagnostika AG, EI 2610–9601 M, Luebeck, Germany); anti-Measles virus ELISA IgG (EUROIMMUN Medizinische Labordiagnostika AG, EI 2610–9601 G, Luebeck, Germany); anti-Mumps virus ELISA IgM (EUROIMMUN Medizinische Labordiagnostika AG, EI 2630–9601 M, Luebeck, Germany); anti-Mumps virus ELISA IgG (EUROIMMUN Medizinische Labordiagnostika AG, EI 2630–9601 G, Luebeck, Germany); anti-EBV-CA ELISA IgM (EUROIMMUN Medizinische Labordiagnostika AG, EI 2791–9601 M, Luebeck, Germany); and anti-EBV-CA ELISA IgG (EUROIMMUN Medizinische Labordiagnostika AG, EI 2791–9601 G, Luebeck, Germany). Briefly, these assays used highly purified glycoproteins of the VZV, HSV-1, HSV-2, measles, mumps, and EBV-CA reference strains as antigens, which represent the main target antigens, respectively. These glycoproteins were bound to the surface of microtitration wells. When the IVIG sample was added into microtitration wells, specific Abs in the sample attached to the glycoproteins. In the next step, peroxidase-labelled anti-human Abs were added to detect the attached Abs. Then, using chromogen/substrate solution, the bound Abs were made visible and they promote a color reaction. The intensity of color produced is directly proportional to the concentration of Abs in IVIG samples. It is a quantitative in-vitro assay for Abs detection and was performed automatically using the BEP III system (Siemens Healthcare Diagnostics).

As no international reference serum exists for IgM Abs against VZV, HSV-1, HSV-2, measles, mumps, and EBV-CA, these results are provided in the form of ratios, which are a relative measure for the concentration of Abs. For anti-VZV IgM assay, anti-HSV-1/2 IgM assay, anti-measles virus IgM, anti-mumps virus IgM, and anti-EBV-CA IgM assays, recommended positive results were equivalent to ≥1.1, equivocal results were between ≥0.8 and <1.1, and results of <0.8 were interpreted as negative. There is also no international reference serum for IgG Abs against HSV-1, HSV-2, mumps virus, and EBV-CA. Therefore, the calibration is performed in relative units (RU) and the recommended positive results were equivalent to ≥22 RU/mL, equivocal results were between ≥16 and <22 RU/mL, and results of <16 RU/mL were interpreted as negative for HSV-1, HSV-2, mumps virus, and EBV-CA IgG assays.

In the anti-VZV enzyme-linked immunosorbent IgG assay, positive results were equivalent to ≥110 IU/L anti-VZV IgG, equivocal results were between ≥80 and <110 IU/L, and results of <80 IU/L were interpreted as negative. The controls of the ELISA have been calibrated with the international anti-VZV serum W1044 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands). In the anti-Measles virus ELISA IgG assay, positive results were equivalent to ≥275 IU/L anti-measles IgG, equivocal results were between ≥200 and <275 IU/L, and results of <200 IU/L were interpreted as negative. The controls of the ELISA have been calibrated using the third international standard serum NIBSC 97/648 (anti-measles and anti-polio virus serum, National Institute for Biological Standards and Control, Hertfordshire, England).

2.3. Chemiluminescent microparticle immunoassay

Chemiluminescent microparticle immunoassay (CMIA) was used for detection of Abs against *Toxoplasma*, rubella, and CMV. The following assays were determined by using commercial kits: ARCHITECT Toxo IgM assay (ABBOTT ARCHITECT Toxo IgM, G3-0550/R02, VA, USA); ARCHITECT Toxo IgG assay (ABBOTT ARCHITECT Toxo IgG, G4-7734/R05, VA, USA); ARCHITECT Rubella IgM assay (ABBOTT ARCHITECT rubella IgM, 34–8771/R3, VA, USA); ARCHITECT Rubella IgG assay (ABBOTT ARCHITECT rubella IgG, 840627/R3, VA, USA); ARCHITECT CMV IgM assay (ABBOTT ARCHITECT, G2-6298/R02, VA, USA); and ARCHITECT CMV IgG assay (ABBOTT ARCHITECT, G2-3325/R05, VA, USA).

Briefly, the ARCHITECT Toxo IgM, Rubella IgM, and CMV IgM assays are CMIA for the qualitative detection of IgM Abs to *T. gondii*, rubella virus, and CMV in human serum and plasma, respectively. The IVIG sample and pretreatment reagent are combined. An aliquot of the pretreated sample, assay diluent, and one of these specific pathogens, including native *T. gondii* lysate, rubella whole virus (strain HPV 77) or CMV virus lysate (strain AD169), and recombinant CMV antigen-coated paramagnetic microparticles are combined. Anti-Toxo, Rubella, or CMV IgM present in the IVIG binds to the microparticles. After washing, murine acridinium-labeled anti-human IgM conjugate is added to create a reaction mixture in the second step. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of specific IgM in the IVIG and the RLUs detected by the ARCHITECT i System optics. For IgM Abs to *T. gondii*, reactive results were defined as index values ≥0.35, gray zone results ranged from 0.29 to 0.34, and nonreactive results were defined as index values <0.29. For IgM Abs to rubella virus, reactive results were defined as index values ≥1.6, gray zone

results ranged from 1.2 to 1.6, and nonreactive results were defined as index values <1.2. For IgM Abs to CMV, reactive results were defined as index values ≥1.0, gray zone results ranged from 0.85 to 1.0, and nonreactive results were defined as index values <0.85.

The ARCHITECT Toxo IgG, Rubella IgG, and CMV IgG assays are automated and two-step CMIAs are used for the detection of IgG Abs to *T. gondii*, rubella virus, and CMV, respectively. Basically, *T. gondii*-specific Abs present in the IVIG sample bind to the *T. gondii* recombinant antigen P30 (SAG1) and P35 (GRA8)-coated microparticles, rubella-specific Abs bind to the rubella virus-coated microparticles, and CMV-specific Abs bind to the CMV virus (strain AD169)-coated microparticles, forming an antigen–antibody complex. After several washes, murine anti-human IgG acridinium-labeled conjugate is added in a second step to create a reaction mixture with *T. gondii*, Rubella virus, and CMV-specific IgG bound to the microparticles. After another wash, pretrigger and trigger solutions are added to the reaction mixture. The final chemiluminescent reaction is measured as RLUs. The ARCHITECT optical system can detect and analyze the relationship between the amount of IgG in the IVIG and the RLUs. Results are calculated automatically based on a previously established calibration curve. Specimens with concentration values ≥3.0 IU/mL are considered reactive for IgG Abs to *T. gondii*, concentration values from 1.6 to 2.9 IU/mL are considered gray zone, and concentration values <1.6 IU/mL are considered nonreactive. Specimens with concentration values ≥10.0 IU/mL are considered reactive for IgG Abs to rubella virus, concentration values from 5.0 to 9.9 IU/mL are considered gray zone, and concentration values <4.9 IU/mL are considered nonreactive. Specimens with concentration values ≥6.0 (arbitrary unit, AU)/mL are considered reactive for IgG Abs to CMV, and concentration values <6.0 AU/mL are considered nonreactive.

3. RESULTS

In our study, the median level (range) of anti-CMV IgG is > 250 (All > 250) AU/mL, anti-EBV > 200 (All > 200) RU/mL, anti-HSV > 200 (152.75 to >200) RU/mL, anti-VZV > 5000 (All > 5000) IU/L, anti-measles > 5000 (All > 5000) IU/L, anti-mumps > 200 (156.5 to >200) RU/mL, anti-rubella 209.8 IU/mL (192.7 to 238.5), anti-*Toxoplasma* is 14.05 (12.3 to 16) IU/mL. The result of anti-TORCH IgG Abs is presented in Table. All IVIG batches had high activity (compared with the assay's cut-off value) against HSV, VZV, EBV, CMV, measles, mumps, rubella, and *Toxoplasma*.

When compared with some of the IVIG preparations from other countries, the only one commercial IVIG preparation from Taiwanese owned high activity against CMV and *Toxoplasma*, which were too low to be detectable in some of IVIG preparations from other countries. The IVIG preparations were further tested for IgM, and there was not any IgM against HSV, VZV, mumps, measles, rubella, CMV, EBV, and *Toxoplasma* in the "TBSF" IVIG preparations.

4. DISCUSSION

In this study, our results showed that a significant amount of the anti-TORCH Abs were detected in this Taiwanese IVIG preparation. Additionally, we used four batches of IVIG product to reduce the batch-to-batch variations. A study confirmed that the mean immune Ab titers in different IVIG preparations were fairly constant,[19] and the effects of batch-to-batch variations might be minor, suggesting that the significant amount of these Abs were a common phenomenon, not a random result.

T. gondii is an opportunistic, zoonotic pathogen with a worldwide distribution. This obligate intracellular parasite can infect humans as well as virtually all warm-blooded animals, including mammals and birds. Congenital toxoplasmosis may present anomalies in the brain, eyes, ears, skin, and digestive tract.[20] Krause et al.[21] found that no anti-*Toxoplasma* Abs were present in IVIG batches, such as Isiven from Italy, Omr-IgG-am from Israel, Sandoglobulin from Switzerland, and Gamimune-N and Pentaglobin from the USA. The rate of seropositive IgG against *Toxoplasma* in pregnant women was 19.9% in Israel,[22] 46.1% in Switzerland,[23] 15.8% to 35.4% in Italy,[24] 6.1% to 14.7% in the USA,[10] and 9.3% in Taiwan.[11] The level of anti-*Toxoplasma* IgG was significantly higher in this study. We suspected that the local seroprevalence of *Toxoplasma* infection in plasma donors was largely responsible for this discrepancy. A combination treatment with pyrimethamine-sulfadiazine and folinic acid can reduce the severity of congenital *Toxoplasma* infection of infants,[25] but teratogenicity, acute renal failure, bone marrow suppression, and hepatotoxicity deserve careful investigation before their use.[26] Spiramycin can prevent *Toxoplasma* transmission to the fetus;[27] however, the safety and efficacy of these drugs for treating in utero toxoplasmosis infection are unknown.[28] Because a therapeutic abortion is often suggested and IVIG contains a high level of anti-*Toxoplasma* IgG, and anti-*Toxoplasma* IgG could be detected in vivo after IVIG infusion,[29] the use of IVIG in the treatment of *Toxoplasma* infection warrants investigation.

Chickenpox infection in early pregnancy is associated with serious adverse sequelae, such as fetal demise, skin lesions, limb anomalies, abnormalities of the eyes, brain, cardiovascular system, gastrointestinal and genitourinary tracts, and sensorineural hearing loss.[30] Acyclovir may inhibit the transplacental

Table.

Antiviral antibodies in commercial IVIG preparations from Taiwanese

		IVIG Preparations			
	Cut-Off Value	1	2	3	4
Cytomegalovirus	<6 AU/mL	>250	>250	>250	>250
Epstein–Barr virus	<20 RU/mL	>200	>200	>200	>200
Herpes simplex type 1 and 2	<20 RU/mL	152.75	>200	>200	>200
Varicella zoster virus	<100 IU/L	>5000	>5000	>5000	>5000
Measles	<250 IU/L	>5000	>5000	>5000	>5000
Mumps	<20 RU/mL	156.5	>200	>200	>200
Rubella	<10 IU/mL	238.5	192.7	225.1	194.5
Toxoplasma	<3 IU/mL	16	12.3	14	14.1

IVIG concentration is 60 mg/mL

AU = arbitrary unit; IU = international unit; IVIG = intravenous immunoglobulin; RU = relative unit.

transmission of VZV,[31] but potential risks exist with its use in the first trimester.[32] It is unknown whether VZIG can prevent viremia and the occurrence of congenital varicella syndrome and reduce the severity of neonatal infection, but it is of no benefit once the signs of chickenpox become evident.[33] IVIG infusion has been reported to increase the levels of VZV Abs.[34] Immunocompromised adults, adolescents, and children, who are susceptible to VZV, and exposed seronegative pregnant women are candidates for VZIG. IVIG may be an alternative to VZIG if VZIG is not available. In a case report,[35] we observed a boy with acute lymphoblastic leukemia who was in an immune-compromised condition while receiving induction chemotherapy. He presented with fulminant hepatitis, disseminated intravascular coagulation, and myocarditis due to varicella infection. His condition was aggravated despite acyclovir administration and a combined therapy with IVIG and intravenous acyclovir successfully cured his fulminant varicella, suggesting that more IgG was needed to control disseminated varicella. Therefore, IVIG preparations may replenish varicella-specific IgG and synergize the antiviral effects of acyclovir. The very high levels of anti-varicella Abs that we found in our IVIG batches may provide further support for the potential role of IVIG as an alternative option for preventing varicella in immune-compromised patients.

Rubella is an infectious viral disease that often presents a mild febrile rash illness in adults and children. Rubella infections during the first trimester of pregnancy have high teratogenic potential affecting the skin, ears, eyes, heart, and neurologic and endocrinologic system.[36] There is no specific therapy for maternal and congenital rubella syndrome. In this study, we detected high level of Abs against Rubella in the commercial IVIG batches. Further investigation is warranted to test the protective ability of IVIG against Rubella.

CMV, a member of the Herpesviridae family, is characterized by a worldwide distribution. Infants with congenital CMV are at risk of developing hearing, vision, and brain anomalies.[37] Once the diagnosis of congenital CMV infection is confirmed, a proposed option is the use of antiviral agents, such as ganciclovir, foscarnet, and cidofovir; the other option is pregnancy termination.[38] Ours and other studies[19] detected a significantly higher level of anti-CMV IgG. However, Krause et al.[21] could not detect anti-CMV Abs in any commercial batches of IVIG. As the seroprevalence of CMV varies geographically, with rates of 42.3% of pregnant women in Israel,[39] 37% of pregnant women in Switzerland,[40] 65.9% of pregnant women in Italy,[41] 1.8% of 12- to 49-year-old women in the USA,[14] and 91.1% of women of childbearing age in Taiwan,[15] IVIG products from these areas with variable CMV infection rates might yield different results. Whether these differences were responsible for the negative results obtained by Krause et al.[21] is unknown. Anti-CMV Abs might provide host protection against CMV because, according to several reports, CMVIG, like VZIG or IVIG, could prevent symptomatic CMV disease in solid-organ and bone marrow transplant recipients.[42–44] Moreover, observational clinical studies indicate that administration of CMV hyperimmune globulin to pregnant woman with primary CMV infection may be effective in treating and preventing fetal infection,[45] needing further studies to evaluate the efficacy of IVIG against CMV infection.

HSVs are categorized into two types, HSV-1 and HSV-2. Primary maternal infections may cause a serious risk of spontaneous abortion and fetal growth retardation, while infection during delivery may present temperature instability, respiratory distress, poor feeding, lethargy, hypotension, jaundice, disseminated intravascular coagulation, apnea, and shock.[46] However, as acyclovir and valaciclovir are officially approved for the treatment of pregnant women, abortion may often be suggested in early pregnancy. IVIG has been shown to have fewer recurrences, less severe lesions, and reduced duration of lesions in patients with recurrent genital HSV infection when compared with patients treated with acyclovir.[47] In our study, we found high levels of Abs directed against both HSV-1 and HSV-2, indicating that IVIG may be considered a reasonable treatment for HSV infection in selected populations.

In utero TORCH infections in early pregnancy can lead to significant morbidity and mortality in the newborn. However, as the safety and efficacy of the treatments for in utero TORCH infection are uncertain and therapeutic abortion is often suggested, any means to preserve the fetus in women with TORCH infection warrant an effort. IVIG has broad antimicrobial activity because it contains numerous specific Abs to neutralize invading pathogens. Moreover, IVIG may enhance the proliferation of natural killer cells to eradicate invading viruses. IVIG has the ability to impair the entrance of viruses into the human being. IVIG may have direct antimicrobial effects through the enhancement of polymorphonuclear neutrophils (PMN) activation and delaying of PMN apoptosis.[48]

In conclusion, we found high level of Abs in this Taiwanese IVIG preparation against *Toxoplasma*, VZV, rubella, CMV, HSV type 1, and HSV type 2. Our findings may offer further support for clinical trials of IVIG in the TORCH infections in the future. Further investigation is warranted to confirm the efficacy of IVIG for congenital TORCH infections.

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