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Case report

CMV-Specific Immune Reconstitution after Allogenic Stem Cell Transplantation: Central Role of Specific IgG in Immunodefense Against CMV Reactivation as a Leading Parameter during Profound and Long-Lasting Immune Deficiency

Przemyslaw Zdziarski

[1] **Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences**

[2] **Department of Clinical Immunology and Hospital Infection Control Team, Lower Silesian Center Wroclaw**

[3] **Military Institute WITI Wroclaw; 136 Obornicka Str Wroclaw Poland**

***** For correspondence P.O.Box 1818 50-385 Wrocław-46, Poland

Przemyslaw Zdziarski (ORCID 0000-0002-5692-4734) zdziarski@oil.org.pl priony@mp.pl

A b s t r a c t :

Although the existing paradigm states that CMV reactivation is under control of cellular immune response, the role of humoral counterpart is underestimated.

Anti-CMV positive woman after conditioning with Bu-Flu-ATG underwent stem cell transplantation from fully matched, seronegative sibling donor. In immunodeficient recipient fast and significant decrease of specific immune response was observed but reconstitution of marrow-derived B and NK cells was prior thymic origin T cells. The lowest CMV-IgG(89 U/ml) was observed just before CMV viremia. Noteworthy, the sole and exclusive factor of CMV-specific immune response is a residual recipient antibody class IgG. The CMV-quantiferon increase was observed later, but in the first phase immune reconstitution of the PHA-induced IFNγ release was significantly lower than that CMV-induced. It corresponds with NK cells increase at the top of lymphocyte reconstitution and undetected CMV-specific CD8 cells by pentamer technique. Most of NK cells are CD16+, thus are stimulated by residual IgG. In immunocompetent donor the cellular and humoral immune response increases in parallel manner but symptoms of CMV mononucleosis were observed till the increase of specific IgG.

Our observations shed light on a unique host-CMV interaction: indicate that significant decrease of CMV-IgG is a good predictor for CMV reactivation during secondary immunodeficiency.

Keywords: cytomegalovirus (CMV); infection, reactivation, epidemiology, host-virus interaction, CMV-specific IgG; protective IgG level, avidity, adoptive/acquired immune response, hematopoietic stem cell transplantation (HSCT); secondary immunodeficiency, Quantiferon, pentamer, β2-microglobulin

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1. INTRODUCTION

Although cytomegalovirus (CMV) is most common cause of viral infection in human virus-host interaction (named here host-CMV balance) it appears to be an important problem in clinical practice of infectious disease immunology. Noteworthy, CMV plays underestimated role in co-morbidity in neonatal, pediatric, adult as well as geriatric medicine. It may be strictly local (CMV retinitis, hearing loss [1], mental retardation, cognitive defects and neurodevelopmental abnormalities [2]), multiorgan or systemic infectious process in patients undergoing bone marrow transplantation (the most common infectious cause of morbidity and mortality). CMV infection is most common in the world, but CMV produces usually mild or asymptomatic infections in immunocompetent individuals. Clinical manifestations include also: suppression of myelopoiesis, a mononucleosis-like syndrome, hepatosplenomegaly, lymphadenopathy, thrombocytopenia, hemolytic anemia and oral mucositis. The distinguishing symptoms are not observed, common symptoms are headaches, myalgia (53%), profuse sweat (50%), abdominal pain, diarrhea, recent loss of weight, dry cough (51%) and splenomegaly in 24% of the cases[3]. Unique conditions of the affected patients and diversity of clinical manifestation of cytomegalovirus infection do not allow formation of a real comparable cohort of patients. This might be due to the presence of several confounding factors (e.g. immunoparameters, immunosuppressive therapy, immunogenetic background) that may bias the final results, including site-specific classification of transplant-related morbidities in multicenter EBMT/NMDP studies [4], use of different serologic techniques and antiviral prophylaxis/preemptive regiments in transplant centers[5]. Differential diagnosis and classification of CD8 T cells infiltration in histological examination were quite difficult: whether it was GVH- or virus-related cellular cytotoxicity. The isolation of this virus from blood does not necessarily mean pathogenicity. In immunocompetent patients CMV mononucleosis with prolonged fever and weight loss and lymphocytosis were observed [6].

On the other hand, CMV may cause a serious disease in severe immunosuppressive patients for example with highly developed AIDS, after HSCT, when CD4+ T helper cells are below 100/µl. In solid organ transplant and in stem cell transplant, the impact of the cytomegalovirus (CMV) serologic status can play a significant role in the outcome [7]. However, the issue on the effect of the donors anti-cytomegalovirus immunoglobulin G (CMV-IgG) seronegativity on the immune system and overall survival is controversial. Sometimes CMV plays a crucial role in immune response and inflammatory stimulation; it prompts lymphoproliferative disease [8] or cross-reactive immune response and graft versus host reaction [9].

Interestingly, the level of recovery of thymopoiesis has been directly linked to CMV clearance and survival [10]. In recent years there has been renewed interest in the role of bone

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marrow-derived NK and B cells in context of haplo-HSCT and immunosurveillance against CMV. The case report sheds light on the host-CMV balance during severe lymphopenia and shows a crucial role of CMV-specific IgG level.

2. Case report

A CMV-positive 58-year old woman with acute myeloblastic leukemia (AML) was admitted to Lower Silesian Center for Cellular Transplantation in order to receive hematopoietic stem cell transplantation from haploidentical family donor after first complete remission. After reduced-intensity conditioning with Busulfan-Fludarabine and anti-thymocyte globulin (Bu-Flu-ATG [11] i.e. 8 mg/kg BU, 180 mg/m2 FLU and 40 mg/kg ATG 30 (Fresenius) she received peripheral blood stem cell transplantation (PBSCT). Graft-versus-host disease (GVHD) prophylaxis consisted of CSA alone. The HLA-matched sibling donor was CMV IgG negative. Therefore, the evolution of adaptive immune response to CMV was observed during immune system switching using CMV serology (humoral immune response), CMV-Quantiferon, pentamer analysis(cellular immune response) and CMV-viremia(RT-PCR)(fig. 1).

Mild aGvHD was observed in the recipient but because of CMV reactivation, lymphopenia and ATG regimen, the narrow immunosuppressive regimen was used with higher CsA dose till 250µg/ml level. The regimen showed acceptable toxicity profile. Engraftment of hematopoietic stem cells and hematologic recovery were observed within the second week: neutrophil count over the first 14 days of treatment was below 1000/µl and red blood cells with donor blood group antigens were found at +25 day. Full chimerism was observed in the second month after HSCT but prolonged lymphopenia was observed with low B and T cells expression (<50 and<100cell/µl, respectively) over four months. It corresponds with weak T cell response to phytohemagglutinin and low level of immunoglobulins as the result of the decrease of antibody synthesis. Unfortunately, the interferon immune response after CMV-peptide stimulation showed higher level than PHA-induced in the first wave of reconstitution. Furthermore, the increase of CMV-specific IgG as result of de-novo synthesis (patient did not receive immunoglobulins) preceded CMV-specific IgM as well as CMV-quantiferon increase (Fig.1). Routine monitoring of immunologic parameters (total cell counts and T-cell and B-cell phenotyping) was performed and presented in Figure 1. The flow cytometry with pentamer analysis showed substantial disadvantage. During severe lymphopenia: when CD8-postive T cells were low, the flow cytometric analysis showed discrepancy and low reproductivity: within 1 week the difference was 30-50%. Those two significant results are presented in Table 1.

 doi:10.20944/preprints201810.0491.v1

Unexpectedly, during the follow-up visit of the donor, mild lymphopenia, lymphadenopathy and splenomegaly were observed. Because of the donor's and the recipient's close place of residence and the risk of opportunistic infection transmission CMV viremia and serostatus were controlled for potential risk of the infectious process. Neither EBV viremia nor bacterial infection was observed. Stool, urine and throat culture were negative but in the donor primary CMV infection on day 55 after GCSF mobilization CMV-DNA was detected temporally with low viral load during peak of CMV-viremia (data not shown). It corresponds with low CMV-specific pentamer and intermediate β2-microglobulin level. The symptoms of CMV mononucleosis were observed till the increase of specific IgG. Details of demographic and clinical parameters of the donor and the recipient are summarized in Table 1.

3. DISCUSSION

Delayed immune reconstitution has also been a major limitation in some haplo-HSCT platforms, especially now with the development of innovative strategies such as cord blood or transplants from haplo-identical family donors. The advantages of this alternative of donor type include immediate donor availability, motivation of family donors, simplicity of use and low cost. Despite fast hematologic recovery, prolonged immune reconstitution is still a clinical problem. It is a clinical paradigm that anti-CMV-IgG positive donors facilitate post-transplant immunological recovery and chronic CMV infection has an effect on the immune system [7]: a seropositive donor is associated with higher proportions of CD4+ as well as regulatory T cells in the blood in hematologic recovery. On the contrary, a seronegative donor and conditioning with T cell depleting agents (anti-thymocyte globin or alemtuzumab) are associated with reduced GVHD and the delay in immune reconstitution shows a severe drawback. Last finding in kidney transplantation meta-analysis showed that the effects of alemtuzumab therapy were not significantly different from those of ATG therapy, including granulocytopenia with GCSF use, CMV infection as well as overall mortality [13], but the effects were not statistically significant because of the limited number of trials. Unfortunately, weak CD4 increase, preferentially CD8+ expansion and CD8CD57+ increase were observed (Fig.1) It corresponds with previous data: ratios of CD4+ to CD8+ cells were significantly and persistently lower in the ATG-treated patients within prolonged period (years) and this inversion was due to a persistent depletion of the CD4+ cells and an increased regeneration of the CD8+ cells, in particular of the CD8+brightCD57+ subpopulation [14]. Following the manufacturer's (Fresenius) data, ATG shows high reactivity to immune response antigen: (CD2,CD3,CD4/8, TCR,CD5,CD7, HLA class I and II, β2-microglobulin), cell adhesion molecule/integrins (CD11a/CD18, ICAM, LFA-1,3), B cell (CD19,20,CD40,)

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and plasma cells (CD38). Therefore, the serum IgG level during first months after transplantation reflect the IgG half-life and decrease of recipient's humoral immune response as the lymphocyte B and CD4-helper role as well as antigen presentation were blocked. Interestingly, because of a seronegative donor, a residual recipient's IgG-class antibody is the sole and exclusive factor of CMV-specific immune response (Figure 1). Contrary to our observation, most of the literature, especially data from European Group for Blood and Marrow Transplantation (EBMT) or US National Marrow Donor Program (NMDP) database are devoted to the influence of the donor CMV serostatus on overall survival with conflicting results carefully and accurately summed up by Snydman DR [4]. That may bias the final results, including site-specific classification of transplant-related morbidities [7], rough and inadequate GvHD description (sometimes it is getting out of control), classification of adverse drug reactions as GvHD or within WHO toxicity scale - not adequate for immunomodulatory, not bioequivalent drugs such as ATG and in a broad sense - immunoglobulin[15].

For example, in EBMT analysis of a seronegative recipient the estimated 5-year survival after HLA-identical sibling HSCT was 54% for patients with CMV-seropositive donors and 59% for patients with CMV-seronegative donors: multivariate Cox regression model, donor CMV serological status had a borderline significant effect on overall survival after HLA-identical sibling HSCT. Unfortunately: seropositive patients receiving grafts from seropositive unrelated donors had improved overall survival (HR, 0.92; 95% CI, .86–.98; $P < .01$) compared with seronegative donors, if they received myeloablative conditioning but reduced intensity conditioning abrogate the significance [16]. The analysis did not include *in vivo* T cell depletion therapy. Interestingly, in a similar to our CMV constellation i.e. R+ (recipient CMV-positive), the estimated 5-year overall survival after HLA-identical sibling HSCT was 52% both for CMV-seropositive and -seronegative donors.

It is a clinical paradigm, that IgM/IgG titer increased in sequential samples may indicate active CMV infection [17,18,19]. The prediction with the test results and preemptive therapy was quite difficult when IgM and CMV viremia increased pararelly (Fig.1). In our observation, the ganciclovir regimen based on CMV-specific IgM was too late if the antiviral therapy did not prevent further CMV reactivation: CMV-viremia increase was still observed (Fig.1). Thus, specific IgM testing is not useful for clinical expectation and preemptive therapy in secondary post-transplant immunodeficiency as well as in a healthy donor. This is because CMV IgM may persist for 6–9 months following primary infection [20,21] or may be detected during latent reactivation [18] but false negative results are underestimated [22]. CMV IgM detection is a sensitive marker for primary CMV infection but its specificity is

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relatively poor: only about 50% of CMV IgM-positive individuals have primary infection [21]. Profound and long-term immunodeficiency and immunological switching which follows HSCT procedure is serious especially now with the development of haploidentical family donors. The current therapy for CMV disease with either ganciclovir or valganciclovir is associated with significant toxicity, therefore it shows unacceptable side effect after HSCT for preventive use: myelotoxicity. In clinical practice a fast and accurate diagnostic test before the decision for preemptive therapy is precious: the above mentioned CMV- or ganciclovir-induced suppression of myelopoiesis appears to be crucial for hematological recovery dilemma between two courses of medical action, sometimes both undesirable. Therefore, assessing immune parameter, especially specific response to cytomegalovirus, represents an appealing strategy for identifying immunodeficient patients and transplant recipients at risk of CMV opportunistic infection. Contrary to IgM and disadvantages of pentamer analysis (significant decrease below detection limit was observed) CMV-specific IgG level, CMV-quantiferon analysis and significant decrease of such specific immunity may be used in clinical practice. The intensive and significant decrease of CMV-quantiferon and specific IgG precedes virus reactivation. During severe lymphopenia, before the second wave of immunoreconstitution, the specific IgG is a “big player”. In the first phase after HSCT, after fast myeloid cell line recovery IgG acts by:

1) classical complement activation
    a) membrane attack complex (MAC) formation
    b) after hematologic recover CMV-unspecific cells bind CMV-IgG-complement immune complexes via complement receptors
2) receptors for the constant regions of their H chain (Fc receptors) and “transfer” this antigen CMV specificity to antigen-nonspecific cells (so-called opsonisation, specific phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC)):
    a) CD64 (high affinity FcγRI) on monocytes and activated granulocytes after hematological recovery
    b) CD32 (FcγRII) granulocytes, B cells, monocytes, subpopulation of macrophages, eosinophils
    c) CD16 (low affinity IgG receptor. FcγRIII) on neutrophils
3) CD16 on NK or Tγδ lymphocytes to activate effector function (e.g. IFNγ release) (see below)
4) FcγRIIb (CD32B) and activation of follicular dendritic cells with IFNα secretion as well as follicle (germinal center) formation [23]

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Such matters (antibody-mediated and specific immune response) are translated into antiviral effector mechanism [23]:

1. blocking glycoprotein H (gH) and viral entry
2. aggregation of viral particles blocking virus absorption by inducing conformational changes in the attachment site
3. lysis of virion or virus-infected cells by MAC
4. inhibition of the release of virus particles
5. intracellular inhibition of virus replication

Interestingly, during crucial for CMV reactivation and immune response period the CMV-induced IFNγ release was higher than phitohemagglutinin (PHA)-induced. PHA – pan-T-cell mitogen and stimulator does not act when severe T-cell lymphopenia is observed (Fig.1 –first months after HSCT). On the contrary, CD16+56+ NK cells increase was observed before T cell reconstitution. It is therefore clear that in the first phase of immune reconstitution NK cells cause IFNγ release. Noteworthy: previous studies demonstrated that the Quantiferon-CMV assay was highly precise and strongly correlated with CMV-specific antibodies [24]. The statistical correlation is clear in our observation in severe T cells lymphopenia, when the CMV-specific IFN, analyzed by CMV-quantiferon technique, release from CD16+56+ NK-cells was mediated by FCγRIII (CD16) (Fig.1).

Additionally, serum CMV IgG measurements showed three intervals (negative, ≤230; borderline, 230–240; and positive, ≥240), but most of the results were significantly higher with mean 12 433 (1290–176 632) [25]. The serum titers of CMV IgG were not significantly different between the two groups –with and without congenital CMV, contrary to IgG avidity. CMV avidity was initially high (residual recipient-derived), but its decrease is difficult in clinical interpretation because parallel increase of IgG synthesis by naive B cells from graft (table 1). Furthermore, none of ELISA kits and the immunoblot kit are cleared by the U.S. Food and Drug Administration, even purchased in the United States [21].

A pool from 3 human plasmapheresis units (citrate plasma) proposed as a standard WHO candidate showed highly positive for anti-CMV IgG (reactivity to all CMV proteins with endpoint titers 37.5 to 118; mean=68) and high IgG avidity (81%), contrary to weakly positive probe with following profile: low mean level (14.1) and antibody pattern indicating long past infection [26]. In the collaborative study the highest CMV-specific IgG titer was 93 and 111 representing primary and past infection, respectively. Noteworthy, our patient showed persistent high IgG level, in spite of immunodeficiency, lymphopenia and virus

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reactivation.

On the other hand, the recipient had immunosuppressive conditioning, ATG showed prolonged action (months/years) but the scale of CMV infection is inestimable in the recipient during latency. The delicate balance is disturbed when one and the sole immunosurveillance mechanism is passive (i.e. specific IgG from recipients), significantly decreased. The protective IgG level against the virus (assessed for example in vaccinology) is the much lower during primary than during secondary infectious process (i.e. reactivation) (table 2) as observed in the case report CMV-IgG>93,2U/ml (Fig.1). The primary infection occurs through the local process i.e. physiological barrier destruction at the portal of entry.

Therefore, immunological escape after HSCT and the decrease of maternal IgG after birth[27] show a good model for assessing protective IgG level against CMV reactivation (table 2). These are unique situations with different populations at risk. MSL-109 -human monoclonal IgG isolated from seropositive individual binds to the essential CMV entry glycoprotein H (gH). Previous data correspond with our observation that MSL-109 confers protection in subsets of the HSCT patients i.e. improved survival rate in D+/R-. On the contrary, in CMV-seropositive recipients overall mortality increased compared to that of the placebo group [28]. Wrongly and ultimately, MSL-109 has not been developed further [29]. The phenomena -survival advantage in CMV D+/R- pairs and the negative effect on survival in a seropositive recipient may be explained by our observation. In our patient, when the recipient was CMV seropositive, the decrease of initial high IgG level was observed, up to the point of immune reconstitution and IgG synthesis i.e. within 2-4 months (Figure 1). The CMV infectious process was delayed as a result of passive immunization, although initial viral load was high (Fig. 1). The benefit was observed in D+/R- group, when the initial anti-CMV IgG was zero and viral load –low. The first situation (virus reactivation and secondary infection) required higher IgG level, contrary to D+R- (primary infection, low viral load by the portal of entry) (Table 2). Unfortunately, the study protocol was not adapted to distinct situations (patients ultimately received MSL-109 intravenously 15 or 60 mg/kg every 2 weeks from day -1 until day 84 after transplantation regardless of anti-CMV IgG level and long-lasting immunodeficiency which followed HSCT procedure). Noteworthy: CMV viremia in our situation was observed within the period of 2-4months after HSCT, when the CMV-specific IgG seemed to be “big player”, but in the study protocol the immunoglobulin therapy was stopped (Fig. 1). In seronegative recipient the benefit was observed because the point of start was accurate.

Such anti-CMV glycoprotein entry complex (MSL-109) antibodies prevent infection of cells and portal of entry but do not block the egress of cells and viremia as well as portal of exit.

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Interestingly, as described for MSL-109, a low, suboptimal concentration of antibody to gH protein during multiplicity of infection induces resistant CMV strain with gH C511T mutation.

The last concept of human health assumes existence of host-microbial symbiosis defined as microbiome. The discovery of viral populations in biosphere and within the human microbiome has shed light on considerable host-virus interaction and virome genetic complexity [30]. The latest observation shows beneficial role of CMV: overall effect of early CMV reactivation in the combined cohort was a 53% decrease in the risk of AML relapse by day 100 [31], but interestingly the effect appeared to be independent of CMV viral load, acute graft-versus-host disease or ganciclovir-associated neutropenia. Such observations as well as the analysis from our center show that CMV-positive donors facilitated immunological post-transplant recovery [7]. The case report with CMV-positive recipient and CMV-negative donor indicates that chronic CMV infection affects the immune system. Sterile conditions and HSCT procedures with prophylactic antibiotic regiments cause that endogenous latent viral antigens are predominant: the proportion of virus-specific T cells including CMV-specific T cells was high and in some cases CMV-specific T cells make up about 10% of the circulating T cells after HSCT [32]. Such dysfunctional CMV-specific CD8 T cells are observed in our case report: fast and significant CD8+CD57+ increase after full chimerism is not translated into CMV-specific IFN release as well virus replication (Fig.1) Furthermore, high CD8CD57+ lymphocytes indicate prolonged proliferation (Fig.1). Mild sign of GvHD, high quantiferon release as well as high pentamer analysis confirm these data. CD57+ lymphocytes consist of about one third of CMV-pp65+CD8+ cells (table 1). In such cells CMV-specific population in humoral primary immunodeficiency and the frequency of late effector cells correlate inversely with the frequency of cells expressing programmed death. Supernatants from proliferating CMV-specific CD8(+) cells from patients with inflammatory disease can confer proliferative potential on cells from healthy subjects mediated in part by IFN-γ and TNF-α [12] . Therefore CD8-induced apoptosis of target cells, IFNγ release prompt inflammatory, non-specific process but do not undergo basic immunoregulatory process i.e. apoptosis. Such cellular cytotoxicity can be a sign of GvHD. On the contrary: high IgG level and avidity prompt death of target cells by classical complement activation, membrane attack complex, cytolysis and cellular burst, therefore necrosis. Furthermore, CD64 and CD32 prompt high and effective opsonization. Furthermore last findings indicate that viral dsRNA may initiate fast Immunoglobulin class switching, frontline IgG and IgA responses through an innate TLR3-dependent pathway without Th/B cooperation in follicular niche [33]. CMV is strong TLR3 ligand [34] therefore fast immunoglobulin class switching, serological evolution

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observed here i.e. increase IgG before CD4 reconstitution may be TLR3-dependent (Figure 1).

4. Materials and Methods

Microplate ELISA (Euroimmun product EL 2570-9601 G) was used with native CMV antigens and linear calibration 2/20/200 RU/ml VIDAS CMV IgG 30204. The assays were mostly based on viral lysate or antigens derived from CMV strain AD169. For avidity the Euroimmun ELISA test was applied with cut-off Low- and High-avidity score <40 and >60, respectively. Peptides representing HLA-A*0201–restricted viral epitopes (i.e. tegument protein pp65) were used in pentamer analysis as described elsewhere [12]. All data were acquired from FACSCalibur 2 (Becton Dickinson). Flow cytometry analysis and evaluation of B/T/NK cells population were carried out in accordance with the guidelines in the EBMT Handbook 2012 Edition. IFNγ release under the influence of CMV epitopic peptides - quantiFERON-CMV assay (named here as quantiferon) was used for cellular immune response monitoring [24]

5. Conclusions:

Marrow-derived lymphocytes rather than those of thymic origin i.e. with Nk and B cells predominance are an early phase of immune reconstitution. The presence of several confounding factors, very differential, sometimes impenetrable immunological parameters in dynamic clinical situation cause that strict clinical situation, for example presented here, gets out of control and simple classification, therefore statistical analysis: these calculations contain hundreds of factors, but one of them is specific IgG level before transplantation, since it is the one and exclusive specific element of immune response within 2-4 months. Recipient CMV-specific IgG level and corresponding CMV quantiferon release is better for clinical decision than IgM/CMV pentamer analysis. Contrary to avidity invariant chain of HLA class I (human leukocyte antigens) - β2microglobulin level shows good accuracy and reflect differencies between primary and secondary infectious process (i.e. correlation with viremia). Furthermore, our data support the possible effectiveness of hyperimmunoglobulin infusions for the prevention of congenital or posttransplant CMV disease. On the other hand, the knowledge about the potential efficacy of therapeutic administration is controversial, should be enlarged by multi-center randomized studies, which may be favored by the implementation of CMV screening.

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Description of figures and tables:

Table 1 Recipient and donor demographic data and crucial immunoparameter

[1]- Avidity index (AI), calculated using the formula AI = (OD of the urea-washed well/OD of the well washed with regular buffer) × 100, expressed as a percentage. The AI cutoff point for defining low avidity is <40%, whereas the AI cutoff point for defining intermediate and high avidity is 40-60% and >60% respectively as described in Euroimmun test.

[2]-Flow cytometry analysis and evaluation of naïve and memory T cells population were carried out in accordance with the guidelines in the EBMT Handbook 2012 Edition

[3] -Peptides representing the HLA-A*0201–restricted viral epitopes, which is derived from the CMV tegument protein (pp65)

[3] –before the reconstitution and the +110 day after HSCT significant decrease below detection limit (0,01%) was observed or with high discrepancy in pentamer analysis.

[4] invariant chain of HLA class I (human leukocyte antigens) i.e. β2microglobulin level show good accuracy and reflect differencies between primary and secondary infectious process. The good correlation of β2microglobulin and viremia level was observed.

Table 2. The unique host-virus constellations and populations at risk in transplantology and obstetrics

The unique host-virus constellation after hematopoietic stem cell transplantation (HSCT) or in neonatal herpes virus infection was presented contrary to typical constellation i.e. primary immune response during primary infection.

[1] - increase of specific IgG (within 1-2 weeks) shows primary immune response manner (but without IgM increase) after virus reactivation (endogenous infection, not by portal of entry).

*Noteworthy: primary immune response is induced in lymphoid tissue of many places and organs at the same time. Therefore the antigenic stimulation is more intensive than in physiological manner (route of transmission → the portal of entry: the site of infection is localized. It correspond with much more higher β2-microglobulin level in recipient than donor (table 1).

**Because of fast TLR3-mediated immunoglobulin class switching [33] the IgM may be absent or later than IgG increase (Figure 1)

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[2] –after Donor Lymphocyte Infusion (DLI), that contains CMV-specific T and B lymphocytes (the known high prevalence in general population), the secondary immune response is observed after primary infection. The same phenomenon is observed in neonatal after MFT (maternal-fetal transplantation) or breastfeeding (milk contain 10-20% lymphocytes). In D-/R+ constellation (as our case report) DLI do not contain CMV-specific lymphocytes, therefore it is not option to treat, contrary to CMV hyperimmune globulin.

[3] patients ultimately developed CMV disease with negative Quantiferon-CMV responses and CMV-positive donors [24]

Figure 1

Host-CMV balance: CMV immune reconstitution during lymphocyte recovery after HSCT. CMV-specific immune reconstitution during lymphocyte recovery after HSCT was tested by CMV-IgG and –IgM as well as Quantiferon analysis. Noteworthy, during severe lymphopenia (i.e. between 2-11 and 19-01) the sole and exclusive factor of CMV-specific immune response is a residual recipient CMV-IgG, but in the first phase immune reconstitution of the PHA-induced IFNγ release was significantly lower than that CMV-induced. It corresponded with Nk cells increase. Furthermore, increase of CMV-IgG precedes CMV-IgM, probably as a result of CMV-TLR3 interaction [33] .

Figure 2.

CMV specific reconstitution as a hallmark of immunorecovery after HSCT.

Residual CMV-specific recipient T and B cells are blocked by lymphocyte depleting conditioning but donor memory and effector lymphocytes from the graft are the first wave of immunoreconstitution. It is very narrow because of lymphocyte depletion, small amount of Nk, B lymphocytes and *(*to a lesser extent*)* T/NKT cells have survived the conditioning. In our report the first wave was without CMV-specific T and B cells because the donor was seronegative. Noteworthy, the sole and exclusive factor of CMV-specific immune response is residual recipient antibody class IgG (Figure 1). DLI in our case do not contain CMV-specific cells (D-) (see table 2)

Author Contributions

P.Z. collected and analyzed data, wrote, reviewed, and revised the manuscript.

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Conflicts of Interest

The author declares no conflict of interest.

Ethics approval and consent to participate

Not applicable: causative and retrospective nature of the publication.

Consent for publication

Written informed consent for publication of clinical details was obtained from the patient in accordance with the 5 Declaration of Helsinki.. A copy of the consent form is available for review by the Editor of this journal.

Notes

Abbreviations

hematopoietic stem cell transplantation (HSCT)

US National Marrow Donor Program (NMDP)

European Group for Blood and Marrow Transplantation (EBMT)

peripheral blood stem cell transplantation (PBSCT)

cytomegalovirus (CMV)

anti-cytomegalovirus immunoglobulin G (CMV-IgG)

Graft-versus-host disease (GVHD)

Donor Lymphocyte Infusion (DLI)

phitohemagglutinin (PHA)

CMV glycoprotein H (gH)

antibody-dependent cell-mediated cytotoxicity (ADCC)

receptors for the constant regions of their H chain (Fc receptors)

membrane attack complex (MAC)

CMV serostatut constelation:

D+/R-(donor positive/recipient negative) D-/R+ (donor negative /recipient positive) etc

avidity index (AI)

Cyclosporine A (CsA)

human leukocyte antigens (HLA)

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7. Table 1 Table 1 Recipient and donor demographic data and crucial immunoparameter

	Recipient		Donor
Age	56y		64y
Gender	Female		Male
Blood group	A Rh-positive		B Rh-positive
Serostatus (IgG)	CMV, EBV, VZV, HSV-positive		CMV, VZV –negative, HSV,EBV (+)
Body weight [kg]	58		86
Lowest WBC	250 (+10 DAY)		2800
Lowest lymphocyte count	190 (+31 DAY)		650
IgG AWIDITY [%][1]	High (till +60 day) Intermediate (60-84 day) Low (84-150 day) High (>180 day)		Low (till 60day after IgG detection) Intermediate (>70 day)
	19-01[4]	30-01	During CMV viremia
FLOW CYTOMETRY[2]			
CD4+CD27+CCR7+CD45RA+ Naive	0,05	0,23	NT
CD4+CD27+CCR7+CD45RA- Central memory	0,69	2,22	NT
CD4+CD27-CCR7-CD45RA- Effector memory	5,30	4,16	NT
CD4+CD27-CCR7-CD45RA+ TEMRA	0,41	0,15	NT
PENTAMER ANALYSIS[3,4] [% of CD8 lymphocytes]			
$CD8^{high}$+CMV pp65+	2,32	6,91	0,08
$CD8^{high}$+CD57+CMV pp65+	0,9	2,32	0,00
β2-microoglobulin[4] [mg/L]	6,4	9,2	3,0

[1]- Avidity index (AI), calculated using the formula AI = (OD of the urea-washed well/OD of the well washed with regular buffer) × 100, expressed as a percentage. The AI cutoff point for defining low avidity is <40%, whereas the AI cutoff point for defining intermediate and high avidity is 40-60% and >60% respectively as described in Euroimmun test.

[2]-Flow cytometry analysis and evaluation of naïve and memory T cells population were carried out in accordance with the guidelines in the EBMT Handbook 2012 Edition

[3] -Peptides representing the HLA-A*0201–restricted viral epitopes, which is derived from the CMV tegument protein (pp65)

[3] –before the reconstitution and the +110 day after HSCT significant decrease below detection limit (0,01%) was observed or with high discrepancy in pentamer analysis.

[4] Contrary to avidity invariant chain of HLA class I (human leukocyte antigens) i.e. β2microglobulin level show good accuracy and reflect differencies between primary and secondary infectious process. The good correlation of β2microglobulin and viremia level was observed.

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8. Table 2. The unique host-virus constellations and populations at risk in transplantology and obstetrics

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Immune response / Infection	Primary	Secondary (days)
Primary (by mucosa, skin)	Typical IgM→IgG	**DLI, MFT[2] Breastfeeding,**
Secondary (i.e. reactivation*)	**HSCT[1], neonatal CMV (without IgM**)** solid organ transplantation[3]	Typical (IgG only)

The unique host-virus constellation after hematopoietic stem cell transplantation (HSCT) or in neonatal herpes virus infection was presented contrary to typical constellation i.e. primary immune response during primary infection.

[1] –In HSCT with D-R+ constellation: increase of specific IgG (within 1-2 weeks) shows primary immune response manner (but sometimes with not detectable IgM increase) after virus reactivation (endogenous infection, not by portal of entry). Under influence of viral particles (dsRNA) fast, TLR-mediated class switching occur [33] without Th and CD40L. On the contrary such situation was observed in D+/R- constealtion in solid organ (especially liver, pulmonary) transplantation.
*Noteworthy: primary immune response is induced in lymphoid tissue of many places and organs at the same time, therefore the antigenic stimulation is more intensive than in physiological manner (route of transmission → the portal of entry: the site of infection is localized). It correspond with much more higher β2-microglobulin level in recipient than donor (table 1).
**Because of fast TLR3-mediated immunoglobulin class switching [33] the IgM may be absent or later than IgG increase (Figure 1)
[2] –after Donor Lymphocyte Infusion (DLI), that contains CMV-specific T and B lymphocytes (the known high prevalence in general population), the secondary immune response is observed after primary infection (D+/R- constellation). The same phenomenon is observed in neonatal after MFT (maternal-fetal transplantation),breastfeeding (milk contain 10-20% lymphocytes) and solid organ transplantation .
[3] patients ultimately developed CMV disease with negative Quantiferon-CMV responses and CMV-positive donors