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Letter to the Editor

Association of Human Leukocyte Antigen and Cytomegalovirus disease after Kidney Transplantation

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Dear Editor,

Cytomegalovirus (CMV) is the most important infectious cause of morbidity and mortality in transplantation. More and more patients are transplanted and because of the increasing immune-modulating agents, the risk for developing CMV disease in those patients is increasing. CMV is a DNA virus from human herpesviruses class. Human cytomegalovirus-human herpesvirus 5 belongs to order *Herpesvirales*, family *Herpesviridae*, subfamily *Betaherpesvirinae*, genus *Cytomegalovirus*, species *Human herpesvirus* [1]. There are three important mechanisms of CMV infection acquisition in patients with solid organ transplants [2,3]: primary infection (D+/R-), reactivation infection (D-/R+) and superinfection (D+/R+). a) Primary infection with CMV occurs in case of transplantation of a CMV negative recipient (R-) from a positive donor (D+). This D+/R- serologic mismatch is estimated to occur in 15% to 25% of all solid organ transplantation [4]. In the absence of antiviral prophylaxis, a D+/R- serologic mismatch will almost always result in the transmission of CMV to the susceptible transplant recipient where it can cause clinically severe primary CMV disease. Primary CMV infection may occur in R- when CMV is transmitted through blood transfusion from D+ or through natural transmission routes in community [5]; b) The CMV infection can reactivates in an CMV positive recipient during the periods of decreased immunity after kidney/pancreas transplantation from a CMV negative donor (D-/R+). The degree of CMV reactivation and replication in R+ patients is relatively lower in comparison with the primary CMV infection in D+/R- patients because patients have preexisting CMV specific cell-mediated and humoral immunity, so they have a relatively lower risk of developing symptomatic CMV disease [6]. c) Superinfection/reinfection occurs when an CMV positive recipient is infected from a positive donor (or other exogenous source). In this way, the exogenous CMV transmitted through allograft and reactivated endogenous CMV can cause clinical disease after kidney/pancreas transplantation [6]. The predominant virus that reactivates in CMV D+/R+ patients is, in the majority of cases, donor-derived CMV [7,8] suggesting a potentially incomplete degree of cross-protection against other viral strains. There are important differences between "CMV infection" and "CMV invasive disease":

a. CMV infection assumes detection of CMV pp65 antigen in blood leukocytes in the absence of clinical manifestations or organ function abnormalities.
b. CMV disease was defined as the association of documented CMV infection with clinical symptoms, such as unexplained fever and leucopenia combined with the presence of the virus and/or histopathologic or immunohistochemical diagnosis of CMV in tissue samples.

In immunosuppressed seropositive patients, the presence of CMV into urine is common in the absence of invasive disease. The CMV disease diagnosis should be made only in the presence of microbiologic data, even if clinically would be important to initiate therapy on the moment of identification of CMV in urine [9].

The HLA system is known to play an important role in susceptibility and resistance to many infectious diseases. The HLA system plays a significant role in acceptance or rejection of a transplant [10, 11]. Polymorphisms of the HLA will impact susceptibility to CMV infection, disease progression and treatment. The influence of HLA allele on CMV disease may be protective, predisposing or neutral. The impact of HLA on CMV disease is examined by analysis of allelic influences for each HLA-A, HLA-B, and HLA-DRB1. The relationship of each allele to CMV disease is expressed through an OR calculated of all alleles at each locus. We have analysed the association of particular HLA alleles and CMV disease after transplantation. This study included 674 kidney transplantations between January 2009 and December 2014 in Clinical Institute of Urology and Renal Transplantation Cluj-Napoca. The patients were divided into two groups according to the presence or absence of CMV disease. All recipients were positive CMV IgG and negative CMV IgM.

We have identified the HLA alleles association of 639 transplanted patients without CMV disease and 35 renal transplanted patients with CMV disease. HLA-A, HLA-B typing was performed by the standard microlymphocytotoxicity method.HLA-DR antigens were determined by the DNA-based PCR-SSP and PCR-SSO techniques. We have used *innuPREP Blood DNA Mini kit* (Analytik Jena) to extract DNA from whole blood. We have tested CMV antigenemia in circulating peripheral blood leukocytes using immunofluorescence assay for detection of CMV pp65 antigen (Merck). In our center, we use the prophylaxis for CMV disease in all kidney transplants. All the transplanted patients are receiving oral Valganciclovir for 3 months. We perform weekly monitoring of CMV by pp65 antigenemia in patients with CMV disease. In case of serious CMV disease (including most patients with tissue invasion), all patients are treated with intravenous Ganciclovir. The significance of difference between variables was assessed by Fischer's exact test. P values less than 0.05 were considered significant. Odds Ratio (OR) were

Table 1a. HLA - A alleles and CMV disease in kidney transplantation patients

Allele HLA-A	Alleles from patients with CMV disease 70	Alleles from patients without CMV disease 1278	OR	p value
1	4 (5.71%)	182 (14.24%)	0.365	0.7126
2	27 (38.57%)	389 (30.43%)	1.435	0.9816
3	7 (10%)	120 (9.39%)	1.072	0.9479
11	2 (2.85%)	96 (7.51%)	0.362	0.7052
23(9)	3 (4.28%)	38 (2.97%)	1.461	0.9221
24(9)	10 (14.28%)	164 (12.82%)	1.140	0.9673
25(10)	1 (1.42%)	46 (3.59%)	0.460	0.7361
26(10)	3 (4.28%)	56 (4.38%)	0.977	0.9172
29(19)	1 (1.42%)	13 (1.01%)	1.410	0.9346
30(19)	0	10 (0.78%)	0	0.5748
31(19)	4 (5.71%)	46 (3.6%)	1.623	0.8733
32(19)	2 (2.85%)	51 (3.99%)	0.707	0.8241
33(19)	3 (4.28%)	19 (1.48%)	2.967	0.5061
34(10)	0	1 (0.07%)	0	0.5748
36	1 (1.42%)	1 (0.07%)	18.507	0.0008
66(10)	0	8 (0.62%)	0	0.5748
68(28)	2 (2.85%)	36 (2.81%)	1.014	0.9300
69(28)	0	2 (0.15%)	0	0.5748

calculated and used to appreciate the association of CMV disease and HLA alleles in the transplanted patients. OR<1 means protective allele; OR>1 means predisposing allele; OR=1, neutral allele.

From a total of 674 patients transplanted in CIUTR Cluj Napoca between 2009 and 2014, 416 patients were males and 258 patients were females. The age of patients was 4-74 years. Thirty-five recipients (5.2%) with positive CMV pp65 antigenemia developed CMV disease.

The results of HLA typing in patients with/ without CMV disease are presented in Tables 1a, 1b and 1c. Relation of each allele to CMV disease was expressed through an OR calculated in the context of all allele at each locus. The HLA alleles frequencies were determined in patients with CMV disease and recipients without

Table 1b. HLA - B alleles and CMV disease in kidney transplantation patients

Allele HLA-B	Alleles from patients with CMV disease 70	Alleles from patients without CMV disease 1249	OR	p value
7	5 (7.14%)	73 (5.84%)	1.269	0.7776
8	3 (4.28%)	108 (8.65%)	0.485	0.7184
13	2 (2.85%)	47 (3.76%)	0.770	0.9032
17	0	2 (0.16%)	0	0.4086
18	8 (11.42%)	145 (11.6%)	1.008	0.9382
27	5 (7.14%)	72 (5.76%)	1.288	0.7666
35	7 (10%)	195 (15.61%)	0.617	0.8193
37	0	14 (1.12%)	0	0.4086
38(16)	4 (5.71%)	49 (3.92%)	1.520	0.6318
39(16)	1 (1.42 %)	25 (2%)	0.726	0.8710
40	1 (1.42%)	17 (1.36%)	1.075	0.8932
41	1 (1.42%)	27 (2.16%)	0.671	0.8336
42	0	1 (0.08%)	0	0.4086
44(12)	10 (14.28%)	112 (8.97%)	1.735	0.5544
45(12)	0	4 (0.32%)	0	0.4086
46	0	2 (0.16%)	0	0.4086
47	1 (1.42%)	9 (0.72%)	2.043	0.3677
48	**1 (1.42%)**	**3 (0.24%)**	**6.159**	**0.0011**
49(21)	1 (1.42%)	33 (2.64%)	0.546	0.7495
50(21)	1 (1.42%)	14 (1.12%)	1.308	0.7438
51(5)	8 (11.42%)	119 (9.52%)	1.256	0.7924
52(5)	0	18 (1.44%)	0	0.4086
53	1 (1.42%)	7 (0.56%)	2.631	0.1854
55(22)	1 (1.42%)	18 (1.44%)	1.014	0.9335
56(22)	0	11 (0.88%)	0	0.4086
57(17)	**3 (4.28%)**	**12 (0.96%)**	**4.723**	**0.0113**
58(17)	1 (1.42%)	10 (0.8%)	1.837	0.4562
60(40)	1 (1.42%)	14 (1.12%)	1.308	0.7438
61(40)	1 (1.42%)	20 (1.6%)	0.911	0.9972
62(15)	0	8 (0.64%)	0	0.4086
63(15)	1 (1.42%)	7 (0.56%)	2.631	0.1854
64(14)	2 (2.85%)	14 (1.12%)	2.655	0.1854
65(14)	0	36 (2.88%)	0	0.4086
70	0	1 (0.08%)	0	0.4086
73	0	1 (0.08%)	0	0.4086
75(15)	0	1 (0.08%)	0	0.4086

Table 1c. HLA - DRB1 alleles and CMV disease in kidney transplantation patients

Allele	Alleles from patients with CMV disease 70	Alleles from patients without CMV disease 1278	OR	p value
*01	3 (4.28%)	117 (9.15%)	0.44	0.2885
*03	5 (7.14%)	174 (13.61%)	0.49	0.3494
*04	5 (7.14%)	128 (10.01%)	0.69	0.6106
*07	7 (10%)	104 (8.13%)	1.25	0.5181
*08	1 (1.42%)	21 (1.64%)	0.87	0.8787
***09**	**0**	**8 (0.62%)**	**0**	**0.0419**
*10	1 (1.42%)	14 (1.09%)	1.31	0.4205
*11	19 (27.14%)	293 (22.92%)	1.25	0.826
*12	1 (1.42%)	19 (1.48%)	0.96	0.9561
*13	7 (10%)	113 (8.84%)	1.14	0.6641
*14	4 (5.71%)	72 (5.63%)	1.01	0.8609
*15	8 (11.42%)	99 (7.74%)	1.58	0.2488
*16	9 (12.85%)	116 (9.07%)	1.49	0.3001

CMV disease. The difference in HLA frequencies between these two groups was statistically significant. Association analysis of HLA-A reveals the rare A36 allele that was excluded. HLA-B with greater allelic polymorphism has two alleles predispose to CMV disease: B48 and B57(Table 1b). Concerning HLA-DRB1, our results reveals one protective allele against developing CMV disease: DRB1*09 and no alleles for this locus which give susceptibility to CMV disease (Table 1c). We focused our study on patients CMV disease after kidney transplantation. Many investigators showed the importance of the HLA system in the anti-viral responses, especially against the CMV pathogen. Several reports showed that HLA-A2 and HLA-DR11 increase and HLA-B16 reduces the risk for CMV infection [12]. The other studies have shown that HLA–DR7 have increased risk for CMV infection [13], while Retierre *et al*. [14] reported a different HLA class I gene A11, A32 and HLA class II gene DR11 with prevalence in the viral infection among solid organ recipients. However, other investigators found that HLA-A11 increases the risk for CMV infection in kidney graft recipients [15]. Kekik *et al.* have demonstrated a higher incidence of HLA-A30, HLA-B40, and HLA-DRB1*15 CMV infection [16]. We found that HLA-B48 and HLA-B57 have a significant influence in CMV disease after transplantation and HLA-DRB1*09 indicates the opposite results. In the literature, HLA alleles are recognized as risk factors for CMV disease, but some of them can have a protective role [16-19], and this situation might be useful pretransplant in estimating the risk of CMV disease after transplantation and designing individualized therapy. A better understanding of the different HLA associated immune mechanism within CMV disease may lead to improved management strategies in kidney transplantation. The identification of the genes which are involved in the pathogenesis of an infectious disease is very important for the development of new therapeutic strategies or even new therapies for that infectious disease.

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Conflicts of interest

The authors declare no conflicts of interest.

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