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Lymph Node, Spleen and Peripheral Blood Lymphocytes as Stimulators of Alloreactivity

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A B S T R A C T

Before and after kidney transplantations, in vitro tests that measure the level of reactivity between donor and recipient lymphocytes are performed for better organ selection and as indicator of possible organ rejection. In these tests, donor's and recipient's lymphocytes are stimulated for proliferation, which intensity is measured and accordingly organ recipient reactivity towards graft is determined. Lymph node, spleen and peripheral blood lymphocytes are used for those purposes. For better interpretation of these in vitro tests it should be important to determine mitogenic ability of lymphocytes of different origin and to choose the most adequate cells. To compare mitogenic ability of deceased donor lymph node, spleen and peripheral blood lymphocytes one-way mixed lymphocyte culture (MLC) was used. As stimulators irradiated lymphocytes from spleen, lymph node and peripheral blood samples of 12 deceased donors were used while as responders lymphocytes from peripheral blood of healthy individuals, chosen according HLA-DRB1 alleles (stimulators and responders were HLA-DRB1 identical, semi-identical or different), were used. Spleen lymphocyte activity was the best with different cells and the weakest with identical cells. Impact of polyclonal mitogens (PHA – phytohemagglutinin, Con A – concanavalin A and PWM – pokeweed mitogen) on lymphocyte proliferation was tested on lymphocytes from spleen and lymph node of deceased donors. Results abtained in culture in vitro showed that spleen cells had exerted the best mitogenic potential and PHA had the greatest impact upon lymphocyte proliferation. This investigation is of importance for establishing the best model to reflect in vivo situation in transplanted patient.

Key words: *alloreactivity, HLA, lymphocyte proliferation, phytomitogens, transplantation*

Introduction

Kidney transplantation has become a fairly common and effective modality for the treatment of end-stage renal failure. The transplant may be derived from living related or unrelated donors or a recently deceased individual (deceased). All allograft recipients are at risk of graft rejection because the recipient's immune system recognizes the graft as foreign and seeks to destroy it[1–7]. The success rate of kidney transplantation depends on the degree of the compatibility in histocompatibility alleles (HLA alleles) between donor and recipient[3,8–11]. The greater the compatibility the higher probability of survival and kidney transplant function. Immune reactions developing after transplantation of genetically non-identical transplants result in rejection.

In our laboratory, in The Tissue Typing Centre, University Hospital Zagreb, after kidney transplantation, *in vitro* tests (MLC – mixed lymphocyte culture, CML – cell mediated lympholysis) that measure the level of reactivity between donor and recipient lymphocytes are performed in order to predict and follow the outcome of kidney transplantation. Lymph node, spleen or peripheral blood lymphocytes are used for those purposes[12].

Mitogens, as substances which cause DNA synthesis, blast transformation and ultimately division of lymphocytes are used in CML *in vitro* tests. For studying and analyzing mechanisms of lymphocyte activation, division, proliferation and maturation, phytomitogens such as phytohemagglutinin (PHA), concanavalin A (Con A) or

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pokeweed mitogen (PWM) can be used[12]. PHA, Con A and PWM are polyclonal activators that can stimulate lymphocytes in a nonspecific manner, without involving antigen-specific receptors, and they cause lymphocyte transformation into lymphoblasts[5,13–18].

For better interpretation of results it is necessary to choose most adequate cells (lymphocytes) as the best *in vitro* model for presenting and monitoring posttransplantation immunological events *in vivo*. Therefore we used spleen, lymph node and peripheral blood lymphocytes in one-way mixed lymphocyte culture (MLC) for determination and comparison of their mitogenic ability[19–22]. As control experiment mitogenic impact of various phytomitogens (PHA, Con A and PWM) on spleen, lymph node and peripheral blood lymphocytes was compared.

Materials and Methods

Lymphocyte samples

In The Tissue Typing Centre spleen, lymph node and peripheral blood samples from deceased organ donor are delivered for regular pre- and post-transplant testing. These samples are used for tissue typing (determination of HLA alleles) and crossmatch before transplantation and for numerous tests in post-transplantation monitoring of recipients. We used lymphocytes from spleen, lymph node and peripheral blood for the one-way MLC test. Irradiated lymphocytes from spleen, lymph node and peripheral blood samples of 12 deceased donors were used as stimulators while as responders, lymphocytes from peripheral blood of healthy individuals, volunteers from laboratory personnel were used. Lymphocyte responders and stimulators were used in three combinations: 1) stimulators and responders were HLA-DRB1 identical, 2) stimulators and responders were HLA-DRB1 semi-identical, 3) stimulators and responders were HLA--DRB1 different (Table 1). Impact of polyclonal mitogens on lymphocyte proliferation was tested on lymphocytes from spleen, lymph node and peripheral blood from deceased donors.

Lymphocyte separation

Lymphocytes were isolated from the spleen, mesenteric lymph nodes and heparinised blood. Fresh heparinised blood (2 tubes of 8 mL) was mixed with an equal volume of physiological solution and placed into 50 mL sterile conical centrifuge tubes. 10 mL of Lymphoprep (Ficoll-Triosill solution, density 1,077 g/cm^3) was slowly layered underneath the blood/physiological solution mixture by placing the tip of the pipette containing the

TABLE 1
LYMPHOCYTE STIMULATORS AND RESPONDERS IN MIXED LYMPHOCYTE CULTURE

	Stimulators	Responders			
Sample	HLA classes I and II	HLA-DRB1 identical	HLA-DRB1 semi-identical	HLA-DRB1 semi-identical	HLA-DRB1 different
K1	A24, 25, B17, 18, DRB1*07, *15	R1 (*07, *15)	R2 (*03, *07)	R3 (*11, *15)	R4 (*03, *11)
K2	A23, 24, B40, 44, DRB1*07, *11	R5 (*07, *11)	R2 (*03, *07)	R3 (*11, *15)	R6 (*13, *16)
K3	A2, –, B13, 15, DRB1*13, *15	R7 (*13, *15)	R3 (*11, *15)	R8 (*07, *13)	R2 (*03, *07)
K4	A2, 3, B7,40, DRB1*11, *15	R3 (*11, *15)	R1 (*07, *15)	R4 (*03, *11)	R2 (*03, *07)
K5	A1, 24, B8, 44, DRB1*03, *11	R4 (*03, *11)	R2 (*03, *07)	R9 (*01, *11)	R6 (*13, *16)
K6	A2, 11, B18, 51, DRB1*01, *11	R9 (*01, *11)	R10 (*01, *12)	R4 (*03, *11)	R6 (*13, *16)
K7	A2, 11, B61, 35, DRB1*11, *12	–	R10 (*01, *12)	R4 (*03, *11)	R8 (*07, *13)
K8	A24, 11, B27, 35, DRB1*01, –	–	R9 (*01, *11)	–	R4 (*03, *11)
K9	A1, –, B8, –, DRB1*03, –	R11 (*03, –)	R2 (*03, *07)	–	R12 (*11, *16)
K10	A2, –, B51, 44, DRB1*16, –	R13 (*16, –)	R6 (*13, *16)	–	R2 (*03, *07)
K11	A2, –, B51, 27, DRB1*11, *16	R12 (*11, *16)	R6 (*13, *16)	R4 (*03, *11)	R2 (*03, *07)
K12	A2, 24, B17, 51, DRB1*03, *14	R14 (*03, *14)	R2 (*03, *07)	R15 (*14, *16)	R6 (*13, *16)

K1-12 – deceased organ donor cells (stimulators), R1-15 – healthy individuals cells (responders)

Lymphoprep at the bottom of the sample tube. Tube was centrifuged at 2500 rpm at room temperature (18° to 20°C) for 20 minutes. Centrifugation sedimented erythrocytes and polynuclear leukocytes to the bottom of the tube. With a sterile pipette, mononuclear lymphocyte layer, which formed above the Lymphoprep, was transferred to a 15 mL centrifuge tube and centrifuged for 15 minutes at room temperature and 1800 rpm. After removal of the supernatant, cells were resuspended in physiological solution, centrifuged for 15 minutes at room temperature and 1800 rpm. Supernatant was removed again and mononuclear cells were resuspended in RPMI 1640 (Institute of Immunology, Zagreb, Croatia), counted and viability was determined by trypan blue exclusion.

Spleen and lymph nodes were placed in Petri dishes and thoroughly syringed with physiological solution. Cell suspension from Petri dish was carefully layered over 10 mL of Lymphoprep in a sterile 50 mL centrifuge tube to create a sharp cell suspension-Lymphoprep interface and centrifuged at 2500 rpm at room temperature for 20 minutes. Mononuclear lymphocyte layer was aspirated and transferred to a 15 mL centrifuge tube and centrifuged for 15 minutes at room temperature at 1800 rpm. Cells were washed with physiological solution, resuspended in RPMI 1640, counted and viability was determined. Cells were ready for immediate use or cryopreservation for future use.

Mixed lymphocyte culture (MLC)

One-way MLCs between lymphocytes isolated from peripheral blood, lymph node or spleen of 12 deceased (stimulators) against peripheral blood lymphocytes (PBL) of 15 healthy individuals chosen according their HLA--DRB1 alleles (responders) were performed (Table 1). Irradiated lymphocytes from peripheral blood, lymph node or spleen of each deceased (stimulators) were cultured with HLA-DRB1 identical, semi-identical or different PBL (responders) of healthy individuals.

Lymphocytes responders and stimulators were suspended to 5×10^5/mL in RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Institute of Immunology, Zagreb, Croatia). MLCs were performed in triplicate in 96-well round-bottom microtiter plates (Costar, Cambridge, MA, USA).100 μL of lymphocyte responders were cultured with an equal amount of irradiated lymphocyte (2000Gy) stimulators. MLCs were incubated for 5 days at 37°C, in 5% CO_2/95% air humidified atmosphere. On the fifth day of culture, 1% methyl tritiated thymidine (Amersham [6-^{3}H] Thymidine, Aqueous solution, GE Healthcare, UK; 0,05 mCi per well) in RPMI solution was added (50 μL/well) to the cultures. Incorporation of methyl tritiated thymidine was measured on the sixth day of culture. Cultures were harvested into deepwell LumaPlates 96 (PerkinElmer). The filter plates were counted in β-counter (Hidex Chameleon). Data are given as the average of triplicate samples.

Mitogens

The content of one bottle freeze dried PHA (PHA HA16, Murex Diagnostics Ltd, Dartford, England) was diluted in 5 mL of sterile RPMI, and stored frozen in aliquots at –20°C until use. Con A (Sigma-Aldrich Inc, Saint Louis, Missouri, USA) was applied (5 mg) to a 10 mL amber serum vial. PWM (Biochrom KG, Berlin, Germany) was applied (0.1 mL) in a 1.9 mL RPMI (Institute of Immunology, Zagreb, Croatia).

Stimulation with mitogens

Lymphocytes from spleen, lymph node or PBL of deceased donors were stimulated with different mitogens (Con A, PHA and PWM). Lymphocytes were suspended to 1×10^6/mL in RPMI 1640 supplemented with 10% heat--inactivated human AB serum (Institute of Immunology, Zagreb, Croatia), 20 μL/mL human recombinant IL-2 (Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany) and 5 μL/mL one of the aforementioned mitogens. In experimental groups, 1–2 mL of cells was cultured in 24-well microtiter plates (Costar, Cambridge, MA USA). Cultures were incubated for 3 days at 37°C, in 5% CO_2/95% air humidified atmosphere. After that, 200 μL of each cell culture suspension was placed in 96-well deepwell LumaPlates 96 (PerkinElmer), 50 μL 1% methyl tritiated thymidine in RPMI solution was added (50 μL/well) and culture was incubated for another 18 hours in the same way. Radioactivity was measured on the next day as described before.

Results

The mitogenic capability of lymph node, spleen or peripheral blood lymphocytes was tested by MLC. Peripheral blood, lymph node and spleen lymphocytes from 12 deceased donors were used as stimulators and peripheral blood lymphocytes from healthy individuals were used as responders. There were three combinations of lymphocyte responders and stimulators: 1) stimulators and responders that were HLA-DRB1 identical, 2) stimulators and responders that were HLA-DRB1 semi-identical, 3) stimulators and responders that were HLA-DRB1 different, except for the autolog control where stimulators and responders were lymphocytes from the same person. Lymphocyte stimulators were irradiated and thus only lymphocyte responders were capable of proliferation. Proliferation of responders was detected by methyl tritiated thymidine incorporation 18 hours before harvesting. Results were given as mean counts per minute in triplicate wells (cpm) (Table 2).

Comparison between spleen, lymph node and peripheral blood lymphocytes as stimulators of alloimmune reaction

For identical HLA-DRB1 lymphocytes results of MLC tests have shown that spleen lymphocytes were the best stimulators in 60% of cases, while in 20% of cases each,

TABLE 2
MIXED LYMPHOCYTE CULTURE TEST RESULTS

	Stimulators				Stimulators		
	Sample 1				Sample 2		
Responders (Peripheral blood)	Spleen	Lymph node	Peripheral blood	Responders	Spleen	Lymph node	Peripheral blood
	cpm	cpm	cpm		cpm	cpm	cpm
Autolog control	335	292	N.C.	Autolog control	181	142	144
HLA-DRB1 identical R1	246	164	212	HLA-DRB1 identical R5	240	141	386
HLA-DRB1 semi-identical R2	360	301	204	HLA-DRB1 semi-identical R2	302	3242	667
HLA-DRB1 semi-identical R3	449	258	389	HLA-DRB1 semi-identical R3	289	4746	1319
HLA-DRB1 different R4	903	684	1352	HLA-DRB1 different R6	312	2161	1287
	Sample 3				Sample 4		
Autolog control	191	149	210	Autolog control	N.C.	N.C.	N.C.
HLA-DRB1 identical R7	146	119	229	HLA-DRB1 identical R3	433	218	372
HLA-DRB1 semi-identical R3	646	1616	584	HLA-DRB1 semi-identical R1	2429	1471	2700
HLA-DRB1 semi-identical R8	394	246	287	HLA-DRB1 semi-identical R4	3064	3053	2616
HLA-DRB1 different R2	2508	2992	752	HLA-DRB1 different R2	7144	9173	3034
	Sample 5				Sample 6		
Autolog control	260	N.C.	192	Autolog control	385	147	218
HLA-DRB1 identical R4	384	535	414	HLA-DRB1 identical R9	233	113	208
HLA-DRB1 semi-identical R2	1072	2438	2352	HLA-DRB1 semi-identical R10	302	249	204
HLA-DRB1 semi-identical R9	959	2756	2385	HLA-DRB1 semi-identical R4	2620	495	1385
HLA-DRB1 different R6	1931	1441	1258	HLA-DRB1 different R6	1150	4288	4974
	Sample 7				Sample 8		
Autolog control	223	257	716	Autolog control	2514	3102	N.C.
HLA-DRB1 semi-identical R10	348	135	124	HLA-DRB1 semi-identical R9	470	424	N.C.
HLA-DRB1 semi-identical R4	3756	2508	694	HLA-DRB1 different R4	2042	1843	N.C.
HLA-DRB1 different R8	1885	184	211				
	Sample 9				Sample 10		
Autolog control	370	137	N.C.	Autolog control	198	N.C.	N.C.
HLA-DRB1 identical R11	202	174	199	HLA-DRB1 identical R13	256	236	N.C.
HLA-DRB1 semi-identical R2	459	149	192	HLA-DRB1 semi-identical R6	352	159	N.C.
HLA-DRB1 different R12	1175	424	851	HLA-DRB1 different R2	3398	2107	N.C.
	Sample 11				Sample 12		
Autolog control	567	N.C.	288	Autolog control	226	172	N.C.
HLA-DRB1 identical R12	1917	974	379	HLA-DRB1 identical R14	362	158	187
HLA-DRB1 semi-identical R6	3139	1178	738	HLA-DRB1 semi-identical R2	1143	2468	1083
HLA-DRB1 semi-identical R4	3916	1093	934	HLA-DRB1 semi-identical R15	1106	1053	343
HLA-DRB1 different R2	9612	7734	3545	HLA-DRB1 different R6	1005	1085	323

cpm = counts per minute, R1-15 – lymphocyte responders, N.C. – not enough cells for test

lymph node and peripheral blood lymphocytes were the best stimulators (Figure 1).

Results of MLC tests for semi-identical HLA-DRB1 lymphocytes are shown in Figure 2 and Figure 3. In 61,9% samples of spleen lymphocytes, in 28,6% of lymph node lymphocytes and in 9,5% peripheral blood lymphocytes were shown to be the best stimulators.

For HLA-DRB1 different lymphocytes spleen lymphocytes were the best stimulators in 50% of cases, lymph node in 33,3% and peripheral blood lymphocytes in 16,7% of cases (Figure 4).

Analyzes of all MLC tests imply that spleen lymphocytes are the best proliferative promoters for HLA-DRB1 different cells and the weakest proliferative promoters for HLA-DRB1 identical cells (Figure 5).

Plant mitogen effect on lymphocyte proliferation

In the second part of this study the impact of different plant mitogens (PHA, Con A or PWM) upon spleen,

lymph node and peripheral blood lymphocyte transformation was analyzed. After 3 days of treatment with mitogens (PHA, Con A or PWM), transformation of lymphocytes was noted. Typical big blast cells forming clusters at the bottom of wells were present. PHA, Con A and PWM stimulation of lymphocyte proliferation was measured by methyl tritiated thymidine uptake. Results were given as mean counts per minute per culture (cpm) (Table 3).

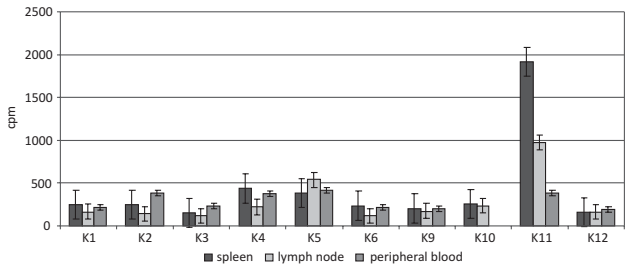

Fig. 1. Comparison of alloimmune reactions of spleen, lymph node and peripheral blood lymphocytes from different deceased organ donors (samples K1-K12) with HLA-DRB1 identical persons. cpm – counts per minute.

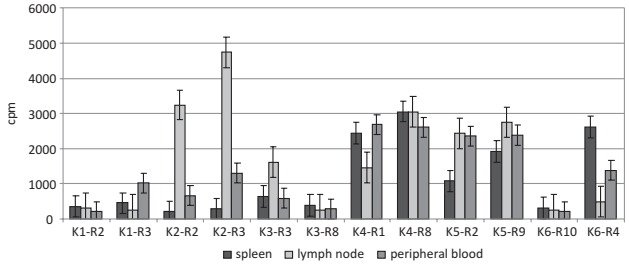

Fig. 2. Comparison of alloimmune reactions of spleen, lymph node and peripheral blood lymphocytes from different deceased organ donors (samples K1-K6) with HLA-DRB1 semi-identical persons (samples R1-R10). cpm – counts per minute.

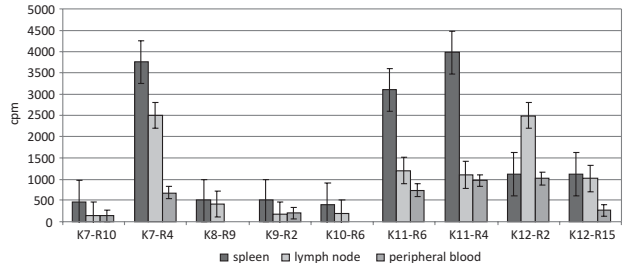

Fig. 3. Comparison of alloimmune reactions of spleen, lymph node and peripheral blood lymphocytes from different deceased organ donors (samples K7-K12) with HLA-DRB1 semi-identical persons (samples R2-R15). cpm – counts per minute.

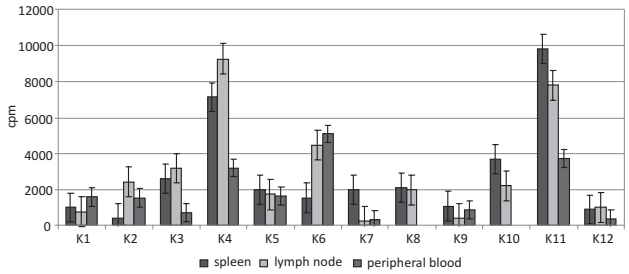

Fig. 4. Comparison of alloimmune reactions of spleen, lymph node and peripheral blood lymphocytes from different deceased organ donors (samples K1-K12) with HLA-DRB1 different persons. cpm – counts per minute.

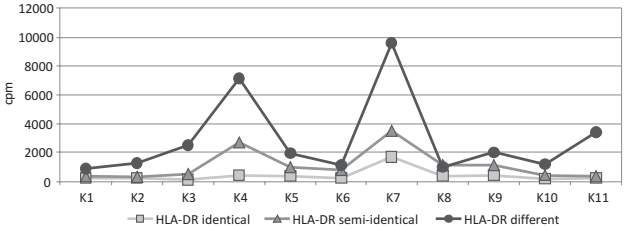

Fig. 5. Comparison of alloimmune reactions of spleen lymphocytes from different deceased organ donors (samples K1-K11) with HLA-DRB1 identical, semi-identical and different persons. cpm – counts per minute.

Comparison between PHA, Con A and PWM impact on spleen lymphocytes from deceased donors showed that PHA stimulation induced the most significant proliferative response while lymph node lymphocytes also showed a significant proliferative response when induced with PHA. By simultaneous culture of spleen and lymph node lymphocytes with PHA, spleen lymphocytes exerted a better proliferative response to PHA stimulation.

Discussion and Conclusion

The MLC (mixed lymphocyte culture) used in this study together with CML (cell-mediated lympholysis)

TABLE 3
STIMULATING EFFECT MITOGENS ON SPLEEN, LYMPH NODE AND PERIPHERAL BLOOD LYMPHOCYTES PROLIFERATION

Cells	Samples	Phytomitogens		
		PHA	Con A	PWM
		cpm	cpm	cpm
Spleen lymphocytes	K1	193	6 130	114
	K4	17 971	10 953	N.C.
	K5	10 619	8 554	896
	K6	2 161	N.C.	N.C.
	K8	279	N.C.	N.C.
	K10	13 011	12 071	4 747
	K11	15 039	8 808	5 554
Lymph node lymphocytes	K1	11 347	N.C.	N.C.
	K6	227	N.C.	N.C.
	K7	9 527	N.C.	N.C.
	K12	15 910	584	6452
Peripheral blood lymphocytes	K10	313	116	164

PHA – phytohemagglutinin, Con A – concanavalin A, PWM – pokeweed mitogen, cpm – counts per minute, K1-12 – cell deceased organ donors, N.C. – not enough cells for test

represent traditionally used tests for pretransplantation evaluation of adequate donor-recipient pairs[19,23–25]. In these tests, lymphocytes from donor (stimulators) and lymphocytes from recipient (responders) are mixed together in tissue culture. While CML test measures the cytotoxic T-cell reactivity to mismatched HLA class I antigens of donor, the MLC test measures the degree of proliferation recipient leukocytes to respond to HLA class II differences expressed by donor leukocytes. Although some of the common and newly developed cellular, humoral, genomic and proteomic tests to assess the immune status of the transplant recipient have been developed[19,23,26–30], the MLC test has been used in the most recent study of human stem cells to establish allorecognition of human neural stem cells by peripheral blood lymphocytes[30]. Therefore, the MLC and CML tests may be regarded as *in vitro* versions of *in vivo* transplantation and have been also used for transplant monitoring after organ transplantation[20,31–33]. Monitoring the immune response to the allograft permits the early identification of patients at risk for rejection and graft loss, monitoring responses to the therapy after intervention, and guides the development of new immunosuppressive therapies[26,34,35]. Immune monitoring might aid in differentiating rejection from other forms of graft dysfunction and drug toxicity.

Most recently, these tests have been used in the post-transplantation setting to identify patients who are hyporesponsive, who display decreased alloresponses to donor and to predict of the optimal immunosuppressive dose for individual patient[36–39] in order to personalize immunosuppressive therapy for the each patient. Several studies report that donor antigen-specific hyporesponsiveness, defined as a significantly lower MLC or CML between the recipient and donor after transplantation, is associated with lower incidence of chronic rejection and improved graft outcome[36,40–42].

In our laboratory, before and after kidney transplantation, *in vitro* tests (MLC and CML/AD CML -cell-mediated lympholysis/antibody depended cell-mediated lympholysis) for better organ (kidney) selection and as indicator of possible organ rejection are routinely performed. For these tests blood samples of patients and blood samples, lymph node or spleen samples of donors are used. Present study using the MLC test has shown that spleen lymphocytes were in most cases better stimulators then lymph node and peripheral blood lymphocytes. As expected, spleen lymphocyte activity was the best with HLA-DRB1 different cells and the weakest with HLA--DRB1 identical cells, HLA-DRB1 being HLA class II antigens with more than 321 known alleles which makes them the most polymorphic protein-encoding regions of the human genome[43]. These results support the clinical benefit of HLA-DRB1 matching and previous findings that HLA matching correlates with improved graft (kidney, heart) survival[44]. There are a numerous studies with results which confirm that degree of HLA-DR mismatch in particular, correlates with freedom from early acute rejection and long-term graft survival[45,46]. Accordingly, HLA-matching can clearly reduce the number of targets for alloantigen-specific antibodies and both direct and indirect pathway T effector cell response. HLA-class II matching might also favour the activity of the natural T regulatory (Treg) cells restricted by self class II, and the generation of adoptive T regulatory (Treg) cells that inhibit alloreactive B and T cells. Even partial HLA-DRB1 match is better than none. Some papers show how to improve graft (kidney, pancreas-kidney, heart) outcome with patient (recipient) pretransplant blood transfusion sharing an HLD-DR antigen with blood/organ donor. Both clinical and *in vitro* models suggest that these HLA-DR shared transfusions may lead to down-regulation of the immune response against allogeneic HLA antigens presented by transplantated organs. These down-regulation of alloreactive cells is the result of the induction of $CD4^+$Treg cells that recognize an allopeptide in the context of a self HLA class II molecule[47,48].

Donor-recipient HLA compatibility has been also the leading predictor of outcome after allogeneic blood or marrow transplantation (BMT). Increasing degrees of HLA mismatch at either the antigen or allele level have been repeatedly associated with worse overall outcomes in series of myeloablative, related or unrelated donor BMT for hematologic malignancies. The reported effect of HLA disparity on relapse risk has been variable. Although some studies have found a lower relapse risk with increasing degrees of HLA mismatch or specific mismatches, suggesting a graft-versus-tumor effect, this has generally been offset by higher rates of graft-versus-host disease (GVHD), graft failure, and nonrelapse mortality (NRM). Thus, although one or more partially-HLA mismatched (HLA-haploidentical) first-degree relatives can be readily identified in most patients who lack a histocompatible donor, the toxicity of such transplants has prevented many centers from pursuing this approach. Recent study suggests that with nonmyeloablative, partially-HLA mismatched, T-cell replete BMT that combines high-dose cyclophosphamide and standard post-grafting immunosuppression, greater HLA disparity does not appear to worsen overall outcomes[49] and new approach of allogeneic stem-cell transplantation (allo-SCT) as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation (ASCT) is a promising therapeutic modality for patients with a long remission after ASCT and with sensitive disease at allo-SCT[50].

In a control experiment of this study, all types of donor cells could proliferate under different mitogens but in all combinations tested, PHA impact on spleen lymphocytes was the best which might be of importance also for blast transformation necessary for CML, AD/CML.

Our results of comparison of spleen, lymph node and peripheral blood lymphocytes as stimulators of alloreactivity according to HLA-DRB1 matching showed that spleen lymphocytes were the most adequate and suitable *in vitro* model for immunological events *in vivo*.

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LIMFOCITI LIMFNOG ČVORA, SLEZENE I PERIFERNE KRVI KAO STIMULATORI ALOREAKTIVNOSTI

SAŽETAK

Prije i nakon transplantacije bubrega rade se *in vitro* testovi kojima se mjeri razina reaktivnosti između limfocita primatelja i davatelja radi što boljeg odabira davatelja organa te radi utvrđivanja mogućeg odbacivanja organa. U tim testovima limfociti primatelja i davatelja se potiču na proliferaciju, intenzitet proliferacije se mjeri te se na temelju toga određuje reaktivnost primatelja organa prema presatku. Pritom se koriste limfociti slezene, limfnog čvora ili periferne krvi. Za bolju interpretaciju tih *in vitro* testova važno je odrediti mitogenu sposobnost limfocita iz različitih izvora (periferna krv, slezena, limfni čvor) te izabrati najprikladnije stanice za upotrebu što boljeg *in vitro* modela. Kako bismo

usporedili mitogenu sposobnost limfocita limfnog čvora, slezene i periferne krvi koristili smo jednosmjerni test miješane kulture limfocita (MLC). Kao stimulatore smo koristili ozračene limfocite iz uzoraka limfnog čvora, slezene i periferne krvi 12 mrtvih davatelja, dok su nam responderi bili limfociti periferne krvi zdravih osoba izabranih prema HLA-DRB1 alelima (tako da su stimulatori i responderi bili HLA-DRB1 identični, poluidentični i različiti). Utjecaj poliklonskih mitogena (PHA – fitohemaglutinin, Con A – konkanavalin, PWM – korovski mitogen) na proliferaciju limfocita smo testirali na limfocitima slezene i limfnog čvora mrtvih davatelja. Rezultati dobiveni u kulturi *in vitro* pokazali su da stanice slezene imaju najsnažniji mitogeni potencijal. Od svih testiranih poliklonskih mitogena, PHA potiče najobilniju proliferaciju. Ovo istraživanje je važno za uspostavu najboljeg *in vitro* modela koji prikazuje *in vivo* situaciju u transplantiranih bolesnika.