VALUES OF T-LYMPHOCYTES IN PATIENTS WITH KIDNEY TRANSPLANTATION ACCORDING TO THE PRESENCE OF HCV

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Keywords: lymphocytes, kidney transplant, flow cytometry, HCV (C Hepatitis), graft rejection.
Abstract: T-lymphocyte is a cell with a complex functional activity, involved in the development of humoral effectors (limfokine) and in the building of the immune response "cell mediated".
Our study aims to investigate a potential immunological mechanism after kidney transplantation; a mechanism immunoregulated by CD4 and CD8. After our study we found that hepatitis C is a risk factor for kidney transplantation, CD3 and CD4 values have significant increase up to 40 times higher compared with the uninfected patients, CD8 average increase is only three, four times higher compared to normal values.

INTRODUCTION

T-lymphocyte is a cell with a complex functional activity, involved in the development of humoral effectors (limfokine) and in the building of the immune response "cell mediated". (fig 1.)

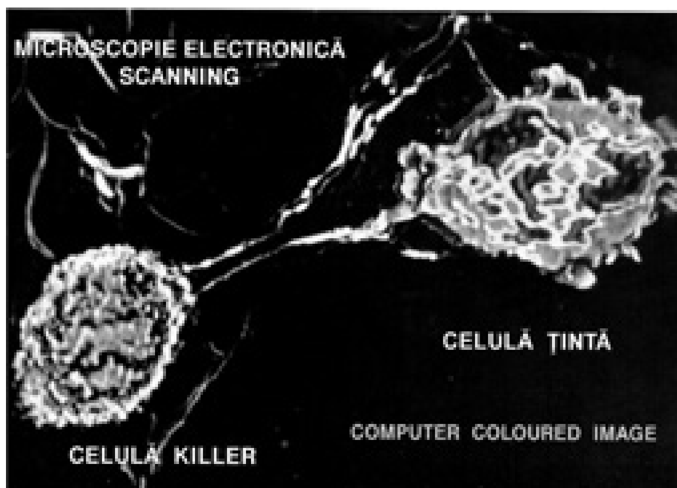


Fig.1. Cell-mediated cytotoxicity, as demonstrated by exploration electron microscopy (scanning) (L.M. Popescu) (1)

The development of T lymphocyte takes place in three successive stages: **prethymic** (bone marrow), **thymic** (in the external cortex if the thymus) and **mature**: T lymphocytes migrate from the medullar part of thymus to periphery and they populate the lymphoid tissues where they oversee any antigenic invasion. In the presence of the antigen mature resting T lymphocytes proliferate, then these cells will become lymphoblast and finally T cells will be differentiated in effector T cells and memory T cells.

T cells population is divided into two major subpopulations: T helper lymphocytes (CD4+) and cytotoxic T lymphocytes (CD8+).

Common immunological markers for both subpopulations of T cells are:

- TCR type αβ or γδ antigen receptor
- CD3 molecule associated with the antigen receptor from the functional complex which ensure specific recognition of antigen
- CD2 molecule specific to T cell
- CD5
- CD45- common leukocyte antigen

- Adhesion molecules LFA-1 (CD1/CD18) and ICAM-1(CD54) with a role in the interaction with B lymphocytes and with antigen presenting cells
- MHC class 1 molecules

Narrowly markers for subpopulations of T lymphocytes:

- CD4 for T helper subpopulation
- CD8 for T cytotoxic subpopulation

T cells functions. T cells play an important role in regulating the specific immune response (T helper cells) and also in the antigen specific cell mediated cytotoxicity (cytotoxic T cells).

Activated T helper cells have the role to secrete cytokines which helps the development of different types of immune response. T helper cells also have two major subpopulations: Th1 subtype induced by IL-2 and the cytokine profile secreted by these cells includes IL-2 and IFNγ. These cells have a key role mediation of the immune response. Th2 subtype is induced by IL-4 and the cytokine secretion profile consists in secretion of IL-4, IL-5, IL-6, IL-10, IL-13 and TGFβ, intervening in mediation of immune response.(1)

Differentiation to Th1 line simultaneously inhibits the differentiation to Th2 profile and appropriate cytokines, reciprocal is valid.

There is an intermediate form marked with Th0 secreting both types of cytokines.

The main surface structure of T cells, the antigen receptor is involved in specific recognition of nonself peptides presented in association with MHC molecules.

Structural TCR (T-cell receptor) is a αβ or γδ dimmer with a role in coupling of the antigen and it is associated with the CD3 macromolecular complex with a role in transduction of the received signal.

Most of the T cells express αβ type of TCR, known as TCR2. In the glycoproteic heterodimmer αβ, both types of chains have an extracellular segment, a transmembrane segment and a short intracytoplasmic segment.

The extracellular segment has two immunoglobulin-type domains, both the chain and the β chain. Immunoglobulin-type domains of α and β chains located N-terminal are variable (V), followed by constant (C) domains. α and β chains are richly glycosylated.

Structural organization as well as the organization of the genetic material are very similar if TCR or immunoglobulin, which suggests that these molecules have a same origin (superfamilly of immunoglobulin) and same functions in coupling the antigen.

The transmembrane segment (20-24 AA) is made of hydrophobic amino acids which participate at the physical association between the αβ heterodimmer and CD3 complex.

The intracytoplasmic segment is short (5-12), unable to interfere directly in the intracellular transduction of the priming signal produced by the interaction of TCR with the specific ligant.

Another T lymphocyte subset (5%) has a type TCR1 receptor composed of a γδ glycoproteic heterodimmer. Structural organization of this receptor is similar with the one presented at the αβ receptor. Chain γ have three isoforms which differ by molecular weight and by the type of association with δ chain.(2)

First part in activation of cytotoxic T cells is represented by the coupling of T cell's antigenic receptor with the antigenic peptide presented in association with MHC class I molecules.

This is how TCR simultaneously recognize α1 and α2 domains of MHC molecule and the peptide inserted between the two domains, in binding situ.

In the same time CD8 coreceptor recognize nonpolymorphic MHC domain (α3), taking part at the increase of intracellular adhesion and at the activation of T cell.

Variable region diversity of TCR is emphasized, as is Ig, by the intervention of exonucleases and of TdT enzyme (terminal dezoxinucleotidil transferase) which induce the modification of nucleotides from N-termination of genes V, D and J. Unlike genetic mechanisms which ensures immunoglobulin's diversity, in case of TCR reordering somatic mutations are not efficacious which ensures a big stability for reorder process and removes the possibility of autoimmune phenomena by failing to recognize MHC self peptides.(3)

Immunoglobulin class switching mechanism is also absent.

Allele exclusion, fact by which successful rearrangement of a chain on a chromosome prevents initiation of rearrangement on homologous chromosome occurs also in the case of TCR recombination.

As in immunoglobulin where first is arranged the heavy chain and after that the light chain, in case of TCR first to be rearranged is β chain. Non-productive rearrangement causes a new attempt on homologous β locus. If this rearrangement also fails the cell dies by apoptosis. Productive rearrangement of β chains triggers the same process in α locus. Allele exclusion is less strict than in immunoglobulinic rearrangement, proven by the identification of some T cells with two different α chains or even β.

Despite the similarity of genetic rearrangement mechanisms in B and T lymphocytes, in B lymphocytes only the immunoglobulin-type genes are rearranged while in T lymphocytes only the genes for TCR are rearranged. This fact is the result of the intervention of some regulating factors specific for every cell type.

TCR expression control only in T cells may be the result of some regulating proteins specific to this cell line which bound with nucleotide's sequences from promoters and enhancers.(4)

Narrowly markers for T cells subpopulations are:

- CD4 for T helper lymphocytes
- CD8 for cytotoxic T lymphocytes

Cell subtypes

1. **T helper lymphocytes** help effector cells in development of appropriate reactivity against antigen. They can help both B cell systems which synthesize and then release Ig, and cytotoxic cell systems (Tc). Beside these main actions, it was proved that helper cells can activate suppressor cell systems (Ts), and also some cells which is consider that act nonspecific (ex. by lymphokines activate some NK cells, PMN cells and also some macrophages). Antenatal thymectomy causes important disturbances in functioning of all cells that helper lymphocytes activates'.
2. **Ta lymphocytes** are part of helper cells. They appear in experimental systems through the thymus-dependence of Ig synthesizers B lymphocytes. In these systems B cells can synthesize and release Ig, but adding Ta lymphocytes the Ig synthesize is accelerated and augmented.
3. **Tcs lymphocytes** were recently highlighted in experimental models in which the tissue used, in most of the cases, was the peptic one. In these models it was observed that Ag activates simultaneously two cell categories: on one hand the suppressor ones and on the other hand some cells which have the ability to inhibit local suppressor cells in gastrointestinal mucosa. Tcs lymphocytes, which ain't helper, don't help any effector cell and don't have a large migration. It is important the fact that:
   - Tcs cells allow helper cells "to become" non-reactive at the suppressor action of Tcs cells, within local immunological reactivity triggered by the action of antigens permeated through the digestive tract or bronchial tree.
   - Unlike contra-suppressor cells, suppressor cells (Ts), simultaneously activated by antigens that enter the gastrointestinal tract or the bronchial one, have the capacity to migrate and to cross their own lamina and also they can enter the big circulation. Here suppressor cells act on secondary lymphoid organs inhibiting the immunological reactivity at the action of antigens which entered through gastrointestinal tract. In this way a local immune response is triggered in a " big suppression".
4. **Ts lymphocytes** are in balance with helper ones. Their action is to limit the immune response at the action of nonself antigens, and also to maintain an adequate ratio between the self antigens' effectors, not allowing the destruction of their organism.
5. **Tc lymphocytes** are activated in the presence of antigens and of helper cells and they have the ability to act on antigens (in general a cell or infected cells) through direct ignition; classic these cells react at the antigenic stimulation and can be inhibit by specific Ts cells.
6. **Td lymphocytes** have the ability to release factors with local effects on a large amount of cells involved in inflammatory mechanisms. These cells are similar to T helper lymphocytes both phenotypic and functional, but Td cells don't have the ability to help cells. (2,4).
   Our study aims to investigate a potential immunological mechanism after kidney transplantation; a mechanism immune-regulated by CD4 and CD8.

MATERIAL AND METHOD

We worked on a group of 27 patients with kidney transplant from the Nephrology Clinic of Oradea City Hospital during 2006-2008. From these 27 patients, 22,2% (6 cases) were HCV positive. By the presence of HCV + we have studied the changes in the proportion of T-lymphocyte, these changes were compared between patients with HCV + and HCV-. Thus we divided the results in two subgroups:

- HCV + group - 6 cases, 5 women (83.3%)
- HCV - group - 21 cases, of which 10 women (47.6%).

We assessed at the two groups: CD3, CD3 + CD4 CD8 and CD3 + T helper/suppressor ratio.

The investigation method used was flow cytometry. Flow cytometry allows simultaneous determination of several physical parameters and indirectly chemical parameters characteristic for a single cell. With the help of flow cytometry we were able to assess simultaneously several parameters (size, granularity, fluorescence), characteristic features for each cell analyzed.(5)

By flow cytometry it can be determined both qualitative and quantitative presence of markers and receptors in the membrane, cytoplasm or nucleus of cells. This method allows us to characterize the DNA cycle of cancer cells compared with the cycle of normal diploid cells. Flow cytomery tests can pe used also to assess the functional capacity of different types of cells (WBC, PMN, lymphocytes, monocytes/macrophages etc.).

Flow cytometry offers us the possibility of complex investigations in cellular and molecular medicine like: cell activation studies, cell divisibility, apoptosis, phagocytosis, aging processes.

SIMULTEST IMK-TM LYMPHOCYTE BECTON-DICKINSON (the mixture and the method uses Control LeucoGATE Y1/Y2, and the phenotype reagent is published by US Patent Nos. 4,987,086 and 5,064,6169) is immunofluorescence reagent kit in two colors used to set the percentage and to watch WBC subsets in whole lysis blood:

- T lymphocytes (CD3+)
- B lymphocytes (CD19+)
- T helper/stimulating lymphocytes (CD3+CD4+)
- Suppressor/cytotoxic T lymphocytes (CD3+CD8+)
- NK lymphocytes (natural killer)(CD3+CD16+ and/or CD56+)
- T helper/T suppressor lymphocytes ratio (CD3+CD4+/CD3+CD8+)

Human lymphocytes are divided in three major populations based on their biological function and the surface antigen expression:

- T lymphocytes
- B lymphocytes
- NK lymphocytes

Principles of procedure in flow cytometry. When the reagents with monoclonal antigens are added to whole blood, fluorescent labeled antibodies specifically bind to antigens on the surface of leucocytes. Marked samples are treated with solution to lysis erythrocytes from cells G*. the samples are then washed and fixed before the flow cytometric assay.(5,6)

Reagents. The purchased reagents are enough for 50 tests. Used reagents: control reagent, CD3/CD4/CD8 reagent, lysis reagent.

Ingathering and preparing of samples. Ingathering of sample is done intravenous . In a sterile vacutainer K3 EDTA (with purple cap). We have to ingather minimum of 1 ml of whole blood for this sample. The blood can stay 6 hours after ingathering in optimum conditions. The blood with preservative can be stored at 20 -25 degrees Celsius more than 6 hours. Frozen blood can give false results. The best results are obtained from patients with samples which are stored less than 6 hours. Samples obtained from patients who are submitted to immunosuppressive therapy can have a poor resolution . The presence of blast cells or red nucleated cells may interfere the results.

Hemolyzed sample is rejected.

The ingathering guide of the sample and the minimum quantity are closely followed.(7)

Statistic method

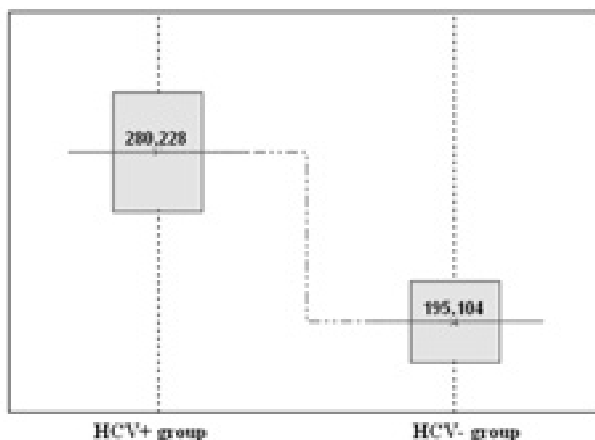
Statistic analysis was made with the help of EPIINFO application, version 6.0, this program belongs to CDC (Center of Disease and Prevention) from Atlanta, this program was adapted for medical use. There were calculated average parameters, frequency, standard deviations, statistic tests by Student method (t test) and $\chi 2$ (8).

RESULTS AND DISCUSSIONS

Table 1. Mean values for studied parameters according to the presence of HCV

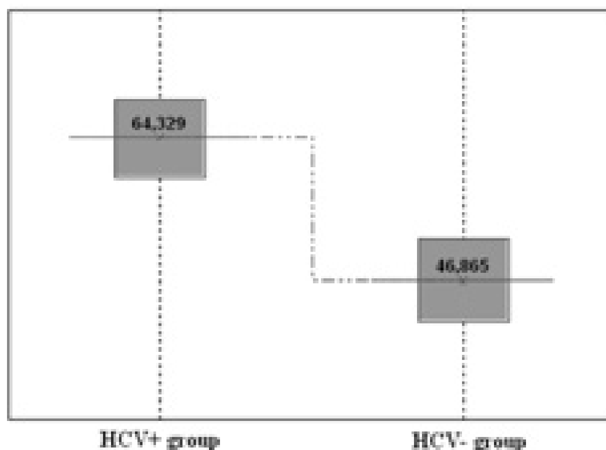
Parameter	HCV+ Group	HCV- Group	P
CD3	280228±30123	195104±20180	P=0,0192
CD3+CD4	64329±6718	46865±5018	P=0,0452
CD3+CD8	3876±400	5326±590	P=0,0553
CD3+CD4+CD8	3500±339	4356±492	P=0,0585
T helper	21,3±4,4	17,7±3,1	P=0,0024

In HCV+ group we haven't found any normal values for CD3, and in HCV- group we found normal values at 2 patients (9,5%). Mean values are 110, respectively 77 times higher compared with the upper limit of normal value. The difference between the two groups is significant (p=0,0192).



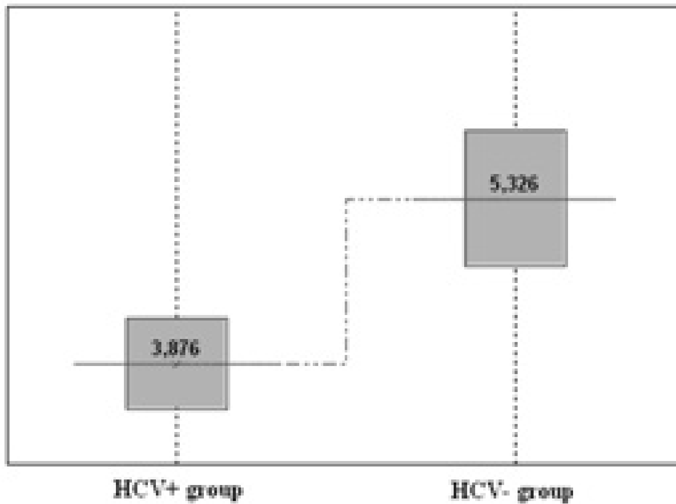
Graphic 1. Mean values of CD3 according to HCV+ presence at patients with kidney transplant

In HCV+ group we haven’t found normal values for CD3+CD4 at any patient, and in HCV- we found normal values at 2 patients (9,5%). Mean values were between 40 and 29 times higher than the upper limit of normal value. The difference between the two groups is significant (p=0,0452).



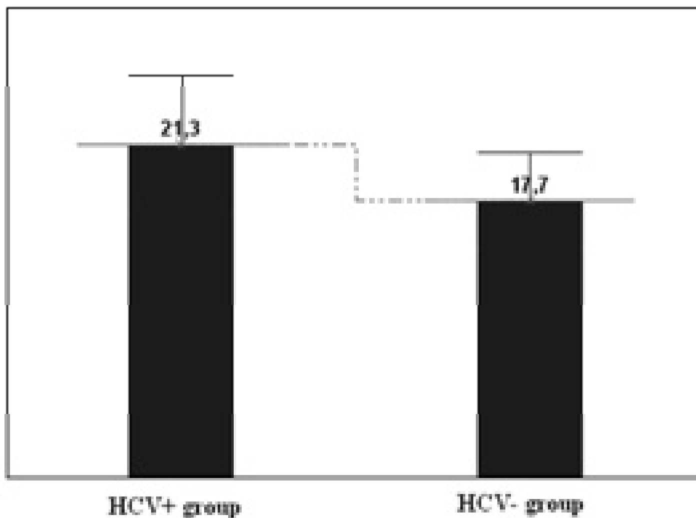
Graphic 2. Mean values of CD3+CD4 according to HCV+ presence at patients with kidney transplant

Just like in the case of CD3 also for CD3+CD4 we haven’t found any normal value in HCV+ group but we found 2 normal values (9,5%) for CD3+CD4 in HCV- group. Mean values are between 3,4 and 4,7 higher than the upper limit of normal value. The difference between the two groups is significant (p=0,0553).



Graphic 3. Mean values for CD3+CD8 according to HCV+ presence at patients with kidney transplant

Regarding T helper, most of the patients from both groups had increased values, normal values were found only at one patient from HCV+ group (16,7%) and at six patients from HCV- group (28,6%). The difference between the two groups was significant (p=0,0024).



Graphic 4. Mean values of T helper according to the presence of HCV+ at patients with kidney transplant

Discussions:

One of the greatest achievements of twentieth century medicine is the organ transplantation. A major problem with solid organ transplantation is graft rejection reactions,

estimated from 10 to 15%.
Flow cytometric analysis of cell subsets using the intracellular fluorescence intensity of carboxifluoresceine succinilester diacetate (CFSE) is a more sensitive method to measure T cell proliferation than mixed lymphocytic reaction.(9,10).

Flow cytometric analysis gives us info-details and a direct view of lymphocyte subpopulations and proliferation process in the immune response to donor graft and it is a non-invasive method which allows us to assess the status of renal graft.(9)
Codarri and others study in which they tried to discover the specificity of cellular markers for CD4 T cells being known the fact that in healthy population CD4 has poor representation, and in transplant recipients these markers can play a role in allograft rejection or induction of immune tolerance.(12)

After our study we found that hepatitis C is a risk factor for kidney transplantation, CD3 and CD4 values have significant increase up to 40 times higher compared with the uninfected patients, CD8 average increase is only three, four times higher compared to normal values. Functional and phenotypic analysis of regulatory T cells in circulation and suppressor T cells in patients with kidney transplantation may be useful to detect the operational tolerance in these patients.
Trying to handle these cells in vitro in a way to elaborate a therapy which is able to induce the clinical tolerance in transplantation is a purpose for the future.

Regulatory T cells have a role in immune suppression, in maintaining immune homeostasis and tolerance of self and nonself antigens and they also have a potentially therapeutic role in patients submitted to transplantation.

The study done by Wang and others have shown that a reduction in regulatory T cell infiltrate is associated with renal graft rejection, also Xia and others have demonstrated the role of regulatory T cells in preventing allograft rejection and in mediation of transplanted allograft tolerance.(11,13).
Our study aim was to discover the high sensitivity and specificity of allospecific CD4 T cells and also to quantify these cells after transplantation and to assess the possible changes according to other diseases present at these patients.

After our study we were able to establish a link between the immune status and the infection with HCV in stable transplant, stable transplant is a good parameter for the maintenance and establishment of immunosuppressive therapy.

CONCLUSIONS

The biological reactions on which the success or failure of a transplantation rely on are linked (connected) to what is called immunological compatibility, namely the connection between the biochemical structure from the donor's tissue and the receiver's, as well as the reactivity of the rearmost's immune system.

In healthy population CD4 has a poor representation, in the transplant recipients we can see a significant increase, which leads us to conclude that cellular markers may play a role as participant in the process of biological rejection or in induction of immune tolerance.

After our study we can observe a link between immune status and the infection with HCV, stable transplant is a good parameter which helps us to establish and maintenance the immune suppression.

REFERENCES

L.M.Popescu,şi colab.,*Dicţionar de imunologie medicală***,**Ed**.**Universitară**"**Carol Davilla**",**Bucureşti**,**2002**.**

Male D.Brostoff J.,Roitt I:*Immunology,*Seventh Edition,Mosby-Elsevier reprinted ,2007.

Drăgurin D.,şi colab."*Imunologie moleculară***",**Ed**.**Mirton Timişoara,1998**.**

Pereţianu D.,şi colab.,*Imunologia în teoria şi practica medicinei,*Ed.All,Bucureşti,1998.

National Committe for clinical Laboratory Standards:*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture(H3-A3),*Villanova,PA:The National Committee for Clinical Laboratory Standards;1991.

National Committee for Clinical Laboratory Standards:*Clinical Application of Flow Cytometry :Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes,Tentative Guidline (H42-T),*Villanova,PA:National Committee for Clinical Laboratory Standards;1992.

Giorgi J:*Lymphocyte subset measurement:Significance in clinical medicine,*In.Rose N.,Friedman H.,Fahey J.,eds.Manual of Clinical Laboratory Immunology 3rd ed.Washington DC:American Society for Microbiology;1986:226-235.

Ţigan Şt.,Achimaş A.,Drugan T.,Gălătuş R.,Gui D.:*Infomed 2000.Informatică şi statistică aplicate în medicină,*Ed.SRIMA 2000,Cluj –Napoca.

Arend SM, Mallat MJ, Westerndorp RJ, van der Woude FJ, and van Es LA.: *Patient survival after renal transplantation ; more than 25 years follow –up,* Nephrol Dial Transplant, 1997; 12:1672-1679

Galante NZ, Câmara NO, Kallas EG, Salamaşo R, Pacheco-Silva A, Medina-Pestana JO.: *Noninvasive immune monitoring assessed by flowcytometry and real time RT-PCR in urine of renal transplantation recipients*, Transplant Immunol 2006; Aug, 16(2):73-80

Wang S, Jiang J, Guan Q, Lan Z, Wang H, Nguan CY, Jevnikar AM, Du C.: *Reduction of Foxp3-expressing regulatory Tcell infiltrates during the progression of renal allograft rejection in mouse model*. Transplant Immunol. 2008, May; 19(2):93-102

Codarri L, Valloton L, Ciuffreda D, Garcia JP, Hadaya K, Buhler L, Rotman s, Panteleo MG. : *Expansion and tissue infiltration of an allospecific $CD4^{+}CD25^{+}CD45RO^{+}IL\text{-}7R\alpha^{high}$ cell population in solid organ transplant recipients*, J Exp Med 2007 July9; 204(7):1533-1541

Xia G, Shah M, Luo X.: *Prevention of allograft rejection by amplification of Foxp3(+) CD4(+) CD25(+) regulatory T cells*. Trasl. Res. 2009 Feb; 153(2):60-70

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28.07.2010