Monitoring indirect allorecognition in renal transplant recipients;(INDIRECT)

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2. OBJECTIVESPrimary Objective: To test the proof of concept whether recombinant HLA molecules (A1, A2, B7, B8) can be used for the monitoring of the indirect allorecognition pathway in renal transplant recipients.Secondary Objective(s): To...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Renal disorders (excl nephropathies)
Study type	Observational non invasive

Summary

ID

NL-OMON37042

Source
ToetsingOnline

Brief title
Indirect allorecognition in renal transplantation

Condition

- Renal disorders (excl nephropathies)

Synonym
renal transplantation

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: onderzoeksbeurs/ afdeling nierziekten

Intervention

Keyword: allorecognition, indirect, renal transplantation

Outcome measures

Primary outcome

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

Test whether recombinant HLA molecules (A1, A2, B7, B8) can be used for the monitoring of the indirect allorecognition pathway in renal transplant recipients (as described in study procedures)

5.1.3 Other study parameters (if applicable)

Not applicable

5.2 Randomisation, blinding and treatment allocation

Not applicable

5.3 Study procedures

For all patients 50ml (5x 10ml) of heparinized blood will be obtained during one standard visit for blood and urine samples, for the measurement of indirectly reactive T-cells. We will determine in the same blood samples presence of anti-HLA antibodies.

Mononuclear cells will be isolated from the 40ml of obtained blood using Ficoll-Hypaque (Pharmacie LUMC, Leiden, the Netherlands). Based on normal values for PBMCs at least 36*10^6 cells will be obtained. 1*10^6 PBMCs/well will be plated in 96-well plate with/without 25µg/ml HLA-I monomer (the mismatched monomer will be added). As a control, cultures will be performed

with/without 2µg/ml MHC-II antibodies to inhibit the indirect T-cell response. Remaining activity represent the semi-direct allorecognition and should be considered as the background reactivity level. The PBMCs will be incubated with the HLA-I monomer for 4 hours, after which they will be washed. HLA-II antibodies will then be added for at least 24h at which time supernatants will be collected and cytokines will be measured. Moreover, cultures will be supplemented with 3H thymidine, and after overnight culture, proliferation will be measured. A total volume of 200µl will be used throughout the assay. All tests will be performed at least in triplo and if required culture conditions will be further optimized.

Secondary outcome

5.1.2 Secondary study parameters/endpoints (if applicable)

Investigate the correlation between frequencies of T cells with indirect reactivity T-cells and simultaneous presence alloreactive antibodies.

Study description

Background summary

1. INTRODUCTION AND RATIONALE

Kidney transplantation has improved survival and quality of life for patients with end-stage renal failure. Despite dramatic improvements in short-term survival, long-term survival of transplanted renal allografts has changed little during the past decades. The improvements in short-term survival is mainly due to superior immunosuppressive drugs that are directly aimed at T-cells and very efficiently inhibit their activation. However, since these drugs are non-specific, this is a double edged sword as it will compromise the recipient in its defense against different pathogens or against developing tumors.
In transplantation recognition by T cells can occur through two separate

pathways named the direct and indirect pathway of allorecognition. The direct pathway involves the direct recognition of donor non-self HLA (class I or II) by recipient T cells. The direct pathway of allorecognition has an important role in the first months after transplantation, but decreases over time. The indirect pathway of allorecognition involves the recognition of a non-self HLA-derived peptide presented in the context of recipient (self) HLA-II on recipient APC. This pathway has been shown to be correlated with chronic rejection. Importantly, T cells with indirect specificity are required to function as helper cells for B cells producing alloreactive antibodies. It is therefore of great importance to monitor indirect presentation, which can be used as a tool to monitor the immune response against the graft. This will be instrumental in improving the current treatment regimes.
Indirect allorecognition has been proven to be difficult to measure and as of yet there is no clinical applicable test that can be used. We have recently developed an in vitro model system in which a mismatched HLA monomer is used and given to recipient APCs which in turn process and present the non-self HLA and present it to T-cells in the context of their own HLA. We propose to investigate whether this method could potentially be applied in the monitoring of T cells with indirect specificity in renal transplant recipients. For this proof of concept study we want to collect blood of 40 renal transplants recipients during a regular visit at least 6 months after transplantation.
time progresses, donor APCs will disappear and the contribution of the direct pathway to the rejection processes diminishes.
The indirect pathway of allorecognition involves the recognition of a non-self HLA derived peptide processed and presented in the context of recipient HLA-II on recipient APC, resembling the normal route of antigen presentation. Although this pathway starts with low precursor frequencies, it increases in time. This pathway has been shown to be correlated with chronic rejection. Importantly, T cells with indirect specificity are required to function as helper cells for B cells producing alloreactive antibodies.

Currently used immunosuppressive agents are very efficient in inhibiting T cell activation, however they do this in an antigen nonspecific manner (6). Even more, part of the long term problems in the transplant population are associated with over-immunosuppression, including infectious problems, malignancies and nephrotoxicity. Therefore, there is a medical need for reduced dosing of immunosuppressive drugs, however without increasing the risk of rejection. This requires an accurate monitoring of the alloimmune response (7). Methods to monitor alloreactive T-cells with direct specificity are available and have been used extensively in the transplantation field. However, a reliable method to monitor indirectly alloreactive T-cells is currently not available (8).
Previous studies have exploited different strategies to provide donor alloantigen and to monitor T cell reactivity, ranging from loading with synthetic peptides (9) or with freeze-thawed donor cells (10;11), till a trans-vivo DTH reaction in mice (12). Synthetic peptides offer the advantage that the exact antigen is known resulting in a highly reproducible assay.

However by selecting synthetic peptides, peptides may be selected that do not occur in vivo, because synthetic peptides do not use the normal route of processing and may end up being recognized by T-cells as a new epitope. Another limitation is the choice of peptides, by limiting the response to only one peptide the other possible natural occurring epitopes are ignored. The advantage of the use of cellular fragments derived from donor cells is that the full repertoire of alloantigen is covered. However, donor cells are not always available and also other antigens are introduced in the system, and it has turned out to be difficult to reproduce this method at different laboratories. Furthermore the semi-direct pathway (integrating transmembrane proteins at the surface of recipient APC) may activate T cells with direct specificity thereby leading to false positivity for indirect allorecognizing T-cells (13).
Recently, we have developed a model system making using of recombinant HLA-molecules as a source of donor antigen. This seems to offer an alternative to allow for the normal route of processing by the APC and the presentation of all the possible natural peptides. This method has been developed making use of a CD4+ T cell clone directed against a peptide of HLA-A2, presented in the context of DR1. Using typed PBMC, we confirmed that presentation occurred in a dose dependent manner, resulting in T cell proliferation and IFN-gamma production, thereby theoretically providing an opportunity for monitoring indirect presentation.

Renal transplantation is the preferred treatment for end-stage kidney failure as it delivers superior patient survival and quality of life compared with dialysis. There have been significant improvements in rates of renal transplant rejection over the last two decades, giving 1-year graft survival rates of over 95%. However, this reduction in rejection has not translated into improvements in long-term graft survival. The reasons for the lack of improvement remains unclear and may be multi-factorial including immunological (chronic inflammation, humoral rejection) and non-immunological events (ischemia-reperfusion injury, nephrotoxicity of immuno-suppressive agents, donor risk factors)(1;2).

Transplantation mostly takes place between non-HLA matched individuals which leads to a vigorous immune response as the recipient*s immune-system is confronted with non-self HLA molecules which it recognizes as foreign and leads to rejection of the organ (3). Allograft rejection is an important risk factor in graft survival. In transplantation recognition by T cells can occur through two separate pathways named the direct and indirect pathway of allorecognition (4;5). The direct pathway of allorecognition involves the recognition of donor non-self HLA (class I or II) by recipient T cells, highly expressed on donor APCs. This pathway is unique for the process of transplantation and involves a high frequency of *cross-reactive* allo-specific T cells. The direct pathway was shown to be correlated with acute rejection and is especially important the first months after transplantation. As

Study objective

2. OBJECTIVES

Primary Objective: To test the proof of concept whether recombinant HLA molecules (A1, A2, B7, B8) can be used for the monitoring of the indirect allorecognition pathway in renal transplant recipients.
Secondary Objective(s): To investigate the correlation between frequencies of T cells with indirect reactivity T-cells and simultaneous presence alloreactive antibodies.

Study design

3. STUDY DESIGN
The study is designed as an observational proof of concept study. Eligible patients will be identified a few weeks before their routine follow-up appointed and informed consent will be asked before the hospital visit. Experimental blood samples of participating patients will be combined with the routine blood drawl. Blood samples will be taken at one time point.
The research question of the current study is to obtain proof of concept that recombinant HLA molecules can be used for the detection and quantification of T cells with indirect reactivity. It has been proposed that the frequency of these T cells increases in time. Therefore, we will concentrate on transplant recipients who are at least 6 months after transplantation. We will determine in the same blood samples presence of anti-HLA antibodies. Blood samples will be obtained at regular visits of these patients.

Study burden and risks

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: 50 ml (5x 10ml) of heparinized blood will be obtained during the standard visits for blood and urine samples, for the measurement of indirectly reactive T-cells. When this study has a positive outcome (ie this method is indeed applicable in the clinical transplant setting), there will be several opportunities for follow up: further characterization of T cells with indirect reactivity (cytokine responsiveness, regulatory properties), to perform analysis in patients with signs of (chronic) allograft rejection, and longitudinal samples to predict long term functioning of the graft. These analyses might benefit transplant recipients in general.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Inclusion criteria for renal transplant patients:
• Age: 18 - 80 years
• Female or male
• First kidney transplantation (living and cadaveric)
• Patients must be able to give informed consent and the consent must be obtained prior to any study procedure.
• Patients must be transplanted with an HLA-A1, A2, B7 and/or B8 mismatched kidney. (Recipients must therefore have at least one mismatch with one of the previously mentioned HLA-I)
• At least 6 months after transplantation

Exclusion criteria

Exclusion criteria for renal transplant patients:
• Double organ transplantation.
• Patients with a previous transplantation since the earlier mismatch might complicate interpretation.

• Patients who had a rejection episode less than 2 months before.
• Patients with evidence of active infection or abscesses
• Patients suffering from an active autoimmune disease
• Subjects who currently have an active opportunistic infection (e.g., herpes zoster [shingles], cytomegalovirus (CMV), Pneumocystis carinii (PCP), aspergillosis, histoplasmosis, or mycobacteria other than TB)
• Malignancy (including lymphoproliferative disease) within the past 2-5 years (except for squamous or basal cell carcinoma of the skin that has been treated with no evidence of recurrence).

Study design

Design

Study type: Observational non invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	29-11-2012
Enrollment:	40
Type:	Actual

Ethics review

Approved WMO

Date:	23-08-2012
Application type:	First submission
Review commission:	METC Leids Universitair Medisch Centrum (Leiden)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL40871.058.12