Future Webinars

Optimisation of HLA Antibody Testing

Featuring
Dr Rob Liwski
Dalhousie University and Queen Elizabeth II Health Sciences Centre
Halifax, Canada

28 March 2018

Post-Haematopoietic Stem Cell Transplant Chimerism Testing and Engraftment Monitoring

Featuring
Dr Anil Handoo
BLK Super Speciality Hospital,
New Delhi, India

12 April 2018

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Histocompatibility testing in clinical transplantation
from HLA antigens towards epitopes

Frans Claas
Leiden University Medical Center
Eurotransplant Reference Laboratory
Leiden, the Netherlands.
1954, antibodies can cause leukocyte agglutination

HLA: Human Leukocyte Antigens
Jean Dausset  Jon van Rood  Rose Payne

Reactivity of HLA antisera was very complex
Use of a computer enabled the detection of HLA alleles.

(J. Clin. Invest. 42, 1382, 1963)

Van Rood et al. 1963
HLA matching enhances skin graft survival

Van Rood et al. 1964
Trip around the world to collect cells from renal transplant recipients and donors
HLA matching benefits patient survival

HLA typings of 39 sib-sib combinations

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Already many different HLA antigens: difficult to find an HLA identical unrelated donor

Only possible if a large donor pool is available
That was the reason why Eurotransplant was founded.

A Proposal for International Cooperation in Organ Transplantation:
EUROTRANSPLANT

J. J. VAN ROOD
Department of Immunohematology, University Hospital, Leiden

HISTOCOMPATIBILITY TESTING 1967
After 50 years Eurotransplant is still going strong!
Beneficial effect of matching for HLA-A and -B

Figure 1. Effect of HLA-A and HLA-B Matching on Graft Survival in 2522 Recipients of a First Cadaveric Renal Allograft.
Improved renal allograft survival during the years by better HLA matching and more potent immunosuppressive drugs.

![Graph showing graft survival percentages over months for different time periods. The graph includes data from 1966-1970 (447) to 1996-2000 (16916).]
HLA matching is still beneficial for kidney graft survival even in the presence of efficient immunosuppression.
Especially, production of donor specific HLA antibodies (DSA) after transplantation is associated with poor graft survival.
HLA matching prevents that a patient develops HLA antibodies after graft rejection

More HLA mismatches: more antibody formation

Sensitization status of patients on the waiting list

Legend:
- □ T [<6% PRA]
- ☢ I [6-84 % PRA]
- ▼ HS [>84% PRA]
HLA matching prevents the induction of DSA and will improve graft survival but...

- Many patients will be transplanted with an HLA mismatched donor.
- However…. not every HLA mismatch leads to an antibody response and graft rejection.

**Challenge:**

Identification of non-immunogenic HLA mismatches, which do not lead to antibody formation in an individual patient, and use this knowledge for donor selection.
How to distinguish immunogenic and non-immunogenic HLA mismatches?

One needs a reliable parameter for immune reactivity

In soccer, the color of the shirt is a reliable parameter
At a first glance: prediction of immunogenicity of an HLA antigen seems a "mission impossible".

Every HLA allele has many polymorphic positions.

All yellow amino acids configurations are potential targets for antibodies.
HLA antigens share antibody epitopes

Immunization by pregnancy: antibodies induced by HLA-A2 react also with HLA-B17
Polymorphic structures are often shared between different HLA alleles

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Duquesnoy et al. 2010
Every HLA antigen carries an unique set of epitopes but the individual epitopes can also be present on other HLA antigens.
HLAMatchmaker algorithm predicts the immunogenicity of an HLA alloantigen.

Principles of HLAMatchmaker:

1. Antigen has many potentially immunogenic epitopes (triplets/eplets) but some of these are shared with the patients’ own HLA molecules.

2. Patient will not make antibodies to epitopes present on the own HLA antigens and therefore:

3. Polymorphism of an HLA mismatch should be considered in the context of the HLA type of the potential antibody producer.

Duquesnoy, Human Immunol. 2002
HLAMatchmaker principle

Donor mm

B18

Patient: B7

Consequence: immunogenicity of a specific HLA mismatch is different for individual patients.

Number of foreign "epitopes" on the same HLA-B51 mismatch for:

- **Patient A**: many
- **Patient B**: quiet some
- **Patient C**: few
- **Patient D**: few
- **Patients E**: no
The number of foreign epitopes on an HLA mismatch predicts antibody production after renal allograft rejection.

Dankers et al., Transplantation 2005
Different parameters are available to predict antibody formation after graft rejection

Amino acid differences           Eplet differences

Degree of sensitization
vPRA
- 85-100%
- 51-84%
- 10-50%
- 0-10%

Kosmoliaptis et al. AJT, 2016
Epitope load is more predictive for DSA free graft survival than HLA antigen matching
It is not all about B cell epitopes.
T cell help is crucial for the production of IgG antibodies.

Tambur & Claas AJT 2015
The number of T cell epitopes and B cell epitopes independently affect the induction of Donor-Specific HLA Antibodies and graft survival.

**B cell epitopes:**
Number of eplets

**T cell epitopes:**
Predictable number of peptides able to bind to HLA class II of the recipient. (PIRCH II)

Lachmann et al. AJT, 2017
Preformed donor specific HLA antibodies lead to hyperacute rejection

The introduction of a serological crossmatch and exclusion of donors toward which the patient has preformed antibodies, will prevent hyperacute rejection.

Problem for the hyperimmunized patient.

Broadly reactive HLA antibodies due to previous sensitizing events

- Often a positive serological crossmatch or donor specific HLA antibodies: contra-indication for transplantation.
- Therefore, these patients accumulate on the waiting list.
Waiting time in the UK in relation to sensitization

Fuggle et al. 2015
Options for highly sensitized patients

- Transplant with HLA identical or compatible donor.

- Do not accept that the patient is sensitized and try to remove antibodies.

- Accept that the patient is sensitized and try to facilitate the allocation of crossmatch negative donor kidneys to these patients.
The Dutch paired kidney donor exchange program for sensitized patients with a living donor

All 8 transplantation centers in the Netherlands participate and register their donor/recipient combinations with positive crossmatches and/or ABO incompatibility in a central data base.

The Dutch Transplantation Foundation performs a transparent match procedure, every 3 months.
The acceptable mismatch (AM) program for patients waiting for a deceased donor within Eurotransplant

**Principle:**
Identification of those HLA antigens toward which the patient did not form antibodies and use this knowledge for donor selection.

**Procedure:**
- Search for acceptable antigens by a variety of cell based and solid phase assays: CDC, Flow, Single antigen beads.
- Acceptable antigens are added to the HLA phenotype of the patient in order to identify compatible donors.
- Mandatory shipment of kidney to AM patient if a compatible organ becomes available.
Combination of patient HLA and AM predicts negative crossmatch

Patient HLA: A24 A31; B27 B51; DR4

Suitable kidney donors:
A24, A31; B27, B51; DR4
Increased chance to be transplanted

% patients transplanted vs. waiting time (months)

- ET-KAS
- AM
Graft survival in re-transplant recipients

Selection:
- ≥ 1996
- Renal only
- Deceased donor
- ≥ 1 HLA antigen mm
- Re-transplant

[Graph showing graft survival data with different PRA categories: 0-5% PRA (n=2232), 6-85% PRA (n=4100), >85% PRA (n=1038), AM (n=619).]

P< 0.0001

Heidt et al, Kidney Int, 2017
Positive identification of acceptable mismatches leads to a better graft survival than avoidance of unacceptable mismatches.

Selection:
- ≥ 1996
- Renal only
- Deceased donor
- ≥ 1 HLA antigen mismatches
- Re-transplant

Heidt et al. Kidney Int. 2017
Need for more extended European collaboration

- The acceptable mismatch (AM) program has proven to be an efficient tool to enhance transplantation of highly sensitized patients (within Eurotransplant >1000 patients transplanted).

- However, a proportion of the patients, especially those with rare HLA phenotypes in relation to the donor population, remain on the waiting list.

- Options for these patients:
  
  Desensitization: removal of donor specific antibodies.
  
  A larger donor pool: a Europe wide acceptable mismatch program
Knowledge of acceptable and unacceptable epitopes enables prediction of antibody reactivity with almost every HLA antigen.
Future HLA matching strategies will be based on epitope rather than antigen matching.

1. **Less complicated + epitope matching prevents sensitization**
   - More than 10,000 HLA class I alleles.
   - Polymorphism can be explained by about 180-200 crucial epitopes.

2. **Enables prediction of acceptable mismatches.**
   - Knowledge of the actual epitopes recognized by existing antibodies enables prediction of acceptable HLA mismatches for (highly) immunized patients (virtual cross matching)
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Rene Duquesnoy

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Tissue typing and transplantation centers
affiliated to Eurotransplant
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