Allogeneic haematopoietic stem cell donation and transplantation across the MHC class I barrier: "Faster is better than more. More is better than less".

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Summary and General Discussion

"*The only thing that one really knows about human nature*
is that it changes.
Change is the one quality we can predicate of it.
The systems that fail
are those that rely on the permanency of human nature,
and not on its growth and development."

From *The soul of man under socialism* by Oscar Wilde

Summary

Many patients in need of haematopoietic stem cell transplantation do not reach transplantation, because they lack a suitable donor. Suitable donors are Major Histocompatibility complex (MHC) identical siblings or members of the extended family or unrelated donors if donor and recipient are MHC matched or have minor MHC differences that do not preclude successful transplantation. However, as the worldwide unrelated donor pool nowadays contains more than 10 million donors, it is highly likely that other causes for not reaching transplantation have become more important.

An analysis on 549 unrelated donor searches for Dutch patients performed between 1987 and 2000 showed a significant decrease of the percentage of patients for whom no donor was available **(Chapter 2)**. Between 1996 and 2000, 59 percent of the patients of Northwest European origin received a graft from an unrelated donor. Compared to 30 percent between 1987 and 1990, only eleven percent of the patients lacked a compatible donor. On the other hand the proportion of patients who became medically unfit for transplantation during the process remained 30 percent during the whole period. This was not due to a lack of donors, but rather to the duration of the donor search and the preparations for transplantation. Thus between 1996 and 2000, the efficiency of the donor search and transplantation process was the biggest constraint in unrelated stem cell transplantation for patients of Northwest European origin. For patients of non-Northwest European origin however, unrelated donor shortage was still the most important impediment. Between 1995 and 2000, 50 percent of these patients did not reach transplantation because they lacked a compatible donor.

One method to increase the unrelated donor search efficiency and effectiveness is to identify a back-up donor once the primary suitable unrelated stem cell donor has been requested **(Chapter 3)**. This is important because one cannot completely rely on the unrelated volunteer donors to be willing, available, and medical fit for actual donation. Of the 502 unrelated donor work up procedures performed between 1987 and 2002, one out of eleven donor work-ups ended in failure of the primary requested donor to donate, either due to medical or personal reasons. It is remarkable that more female than male donors failed to donate due to medical reasons. In cases where a back-up donor was already identified patients were transplanted with a minimal delay, while in cases without a back-up donor there was an extensive delay of on average more than 4 months. This is a good example of how small changes in the search process can be highly effective.

In the absence of a completely matched donor it is more effective to search for a donor who has an acceptable MHC mismatch than to wait for a MHC matched donor to appear. The main problem here is how to identify an acceptable mismatch. In stem cell transplantation CD8+ T cell-mediated alloreactivity is perceived as the key player hampering successful transplantation outcome. CD8+ T cell-mediated alloreactivity is generally believed to involve recognition of the $\alpha 1/\alpha 2$ domains of donor-type class I MHC molecules as well as

the peptides they present. Using the Cytotoxic T cell precursor (CTLp) assay outcome as a parameter for the induction of alloreactivity, we surveyed haematopoietic stem cell donor/patient pairs that feature a range of allelic differences at single HLA-A, -B, and -C loci in an attempt to probe the prognostic value of several analytical tools.

The first two, HLAMatchmaker and HistoCheck, are algorithms presumed to be able to estimate the degree of HLA dissimilarity between donor and recipient. The HLAMatchmaker algorithm is based on theories on antibody recognition of allogeneic MHC. It converts each MHC class I allele into a linear string of amino acid triplets, which are accessible to alloantibodies and then determines by intralocus (within e.g. HLA-A) and interlocus (between HLA-A, -B, and -C) comparison which allogeneic amino acid triplets on the mismatched MHC molecules are not shared with the recipient. A high number of mismatched triplets correlated with alloantibody production. It is however not possible to predict in vitro Cytotoxic T cell (CTL) alloreactivity with HLAMatchmaker **(Chapter 4)**. The second algorithm, HistoCheck was developed to predict T cell alloreactivity. It uses structural data on MHC molecules and functional similarity of amino acids to calculate a sequence similarity matching (SSM) score. The emphasis lies on amino acid positions involved in MHC peptide binding or those important for recognition by T cell receptors. High SSM scores correspond to high dissimilarity between MHC molecules and should correlate with strong T cell alloreactivity. However, SSM scores did not correlate with CTL alloreactivity measured with the CTLp assay, nor was there an obvious benefit for patients receiving an HLA mismatched graft with a low SSM score **(Chapter 6)**.

The assumption that highly diverged MHC class I molecules lead to more T cell alloreactivity was challenged in the analysis described in **Chapter 5**. An MHC class I difference with more than five amino acid sequence differences in the α helices and more than five in the β sheet ($\geq 5\alpha 5\beta$) did not elicit an immune response by allogeneic CTL. We propose that in generating a T cell repertoire with a sufficiently narrow responsive for self-MHC, positive thymic selection limits the capacity to recognize allogeneic MHC molecules whose structure and sequence have diverged extensively.

We evaluated the clinical relevance of this novel finding **(Chapter 7)**. We could subdivide the donor-recipient pairs with a negative CTLp assay into a prognostic favourable and unfavourable group based on the $\geq 5\alpha 5\beta$ MHC class I mismatch category. In multivariate analysis, recipients of a $\geq 5\alpha 5\beta$ mismatched graft with negative CTLp frequencies in vitro prior to transplantation demonstrated a superior chance on survival; four-year survival was 80 percent. The $\geq 5\alpha 5\beta$ category however can not replace the CTLp assay as both have the same discriminative power. These findings are important for donor and patient MHC matching strategies as it generates a larger pool of potentially compatible stem cell donors for patients lacking a fully MHC matched donor.

General Discussion

Merits and pitfalls of research on databases

This thesis primarily covers database analyses. The most apparent advantage is the large amount of available data. Especially if a database has already been used for a long time, it is suitable for analyses on progress made over time. However, changes over time can also hamper a good analysis. For instance, the definition of a suitable donor for transplantation changed over time due to introduction of more elaborate histocompatibility tests, i.e. the introduction of new cellular histocompatibility assays and the switch from serological HLA typing to HLA typing at the allele level. This could complicate comparison between transplantations performed in different periods. Fortunately, most of the patients and their donors in the Europdonor database were retrospectively HLA typed at the allele level, which made comparison between periods more reliable.

A second advantage was that we could link data on molecular HLA typing and HLA matching, and cellular histocompatibility assays with data on patient survival after stem cell transplantation.

Database research itself has some tricky pitfalls, especially when registration databases are used. First, useful data have to be extracted from the bulk of data. Secondly, registration databases do not necessarily contain the right variables needed for analysis. The main parameters discussed in chapter 2, 'a suitable donor found' and 'the time on which this donor was identified' were not included, but were deduced from patients' charts. Furthermore, not all parameters are reliable due to their practical unimportance in the donor search itself. Most illustrative are the entries on the donor search results. From the search coordinators point of view a donor search ends with or without a donor. However, in case of the stop code 'no donor was found' the primary reason for ending the search could actually be I) a patient becomes medically unfit for transplantation before a donor is chosen, II) a donor search seemed to last forever due to inefficient logistics and indecisiveness, or III) the actual lack of a donor. Just using the original stop codes from the original registration database without further examination would have given a completely different result. Namely, there still is a lack of unrelated donors for a significant number of patients.

There can also be a selection bias within the database. Most of the CTLp tests used in the described analyses were not performed for research purposes, but had been performed for clinical purposes. The distribution of the HLA differences in these studies is not random. HLA-B mismatched pairs were underrepresented in the study and included mismatches with only one or few amino acid sequence differences. The reason is that most HLA-B mismatches are associated with an HLA-C mismatch due to linkage disequilibrium. HLA-A mismatches were also underrepresented, although less than HLA-B mismatches. HLA-C mismatches however, were well-represented (both in number and diversity) and showed far less selection

bias. Clinicians have viewed HLA-C as less immunogenic than HLA-A and HLA-B and thus less important for donor selection. If a choice had to made between a HLA-A, -B or -C mismatched donor a HLA-C mismatch was chosen in most cases, irrespectively of the kind of HLA-C mismatch. Recently the theory on HLA-C being less immunogenic has been challenged.[1] This observation made it possible to analyse HLA-A, –B and –C mismatches together in the patient survival study described in chapter 7.
On the other hand, because the patients were paired with donors from the large worldwide donor pool or from the extended family, the number of single MHC class I mismatched pairs that were matched for HLA-DRB1 and -DQB1 is much higher than the number available in our random panel. This made it possible to include enough pairs with the single MHC class I mismatch criterion.
The last criticism is that this kind of research is retrospective. However, this is not true if the analysed variables were unknown or thought to be irrelevant at that time. In this case there is neither selection bias nor the possibility of clinical intervention due to the studied variable. Of course correlating factors have to be taken into consideration before a selection bias can be excluded.

MHC class I, B cells and T cells.
The highly diverged ≥5α5β MHC class I molecules that do not lead to CTL alloreactivity are in many cases B cell epitopes, which is shown by the studies on HLAMatchmaker and antibody production. In other words, B cell alloreactivity against MHC class I sequence differences does not correlate with CTL alloreactivity. In itself, this is not surprising as CTL have a different way of interacting with allogeneic MHC class I than B cells. The T cell receptors of CTL need strong binding with the whole quaternary structure of MHC class I and peptide before an immune reaction can be induced. Allogeneic MHC class I need to fulfil its function as antigen presenting molecule in order to lead to alloreactivity. Thus besides the classic “self MHC” and “foreign MHC” there is also “undetectable MHC” for CTL. The primary function of MHC is not important for B cells as HLA-alloantibodies are in most cases directed against an epitope of a few accessible amino acid positions. It is therefore erroneous to assume that B cells and T cells or other cell types for that matter will react similar against specific MHC mismatches.
At the start of this study however, the expectation was that strong CTL alloreactivity would correlate with high amounts of MHC class I amino acid differences. This idea probably originates from the fact that this is the case for B cells, CTL alloreactivity correlates with the number of MHC allele differences, and that some allogeneic MHC class I with only a few amino acid differences did not lead to CTL alloreactivity or poor transplant outcome.[1,2]
Another cause, which I think is very important but is often underestimated, is the association people have when MHC is mentioned. If one considers the names major histocompatibility complex and human leukocyte antigen, one is almost forced to primarily think of them as

epitopes for alloreactivity. This was the case at first because they were discovered as antigens that induce lymphocyte-mediated alloreactivity. The fact that MHC is a peptide-presenting complex was not known at that time. Because their actual function, antigen presentation to lymphocytes, cannot be deduced from their name, it will not be the first thing to come in mind. I therefore have to disagree with Shakespeare's phrase "What's in a name? That which we call a rose by any other word would smell as sweet." A name may not change the object itself, but can certainly change the expectations of the spectator and cloud his or hers judgement. I wonder what would have happened to the Aztec empire if its king Montezuma had referred to the Spanish conquistador Hernando Cortez with his Christian name instead of the god Quetzalquatl (**General Introduction**). In conclusion, molecules are epitopes if they are recognised as such by immune cells, but if they are not recognised as such they do not classify as possible epitopes nor should it be named as such. Therefore, I think another name for MHC molecules, as 'peptide presenting complex' or 'human peptide presenting complex' for HLA is more appropriate.

Donor patient matching strategies

Transplantation over a single ≥5α5β MHC mismatch led to successful SCT outcome in many cases where there was a negative CTLp assay result in the graft versus host direction. However, can this be implemented in the allogeneic donor search and donor choice? There are many highly diverged MHC alleles for each MHC allele, which makes these combinations less rare than MHC mismatches with only one or two amino acid differences. This does increases the possible donor pool for patients in need of an unrelated donor and thus makes it possible to find a suitable donor in time. However, new matching algorithms have to be made for practical use in the clinic. Furthermore, the ≥5α5β categorisation of these too diverged MHC class I molecules has to be sophisticated, as there are still some MHC differences falling into this category that lead to a CTL response or poor transplant outcome. The main reason is that not al amino acid differences will change the actual quaternary structure of MHC and thus influence T cell receptor MHC interaction. Until that time, cellular histocompatibility tests will be mandatory for the use of this category.

Besides MHC matching, donor patient gender matching is also important. It is advisable to search a male donor for a male patient. First of all, male patients who received stem cells from a MHC class I mismatched female donor have poor survival chances as shown in **chapter 7**. Secondly, from a logistics point of view most of the donors deferred for medical reasons were female male donors (**chapter 3)**.

The use of ≥5α5β MHC class I mismatched donors if no matched donor is available would also decrease the search time span. This is advantageous as time is a major limitation for the number of patient receiving a transplant. A recent analysis of the donor searches performed by the Europdonor Foundation between 2001 and 2004 showed the results of a decreased

search time span (to be submitted). The median time needed to find a donor for patients of Northwest European origin decreased from 2.5 months to 1.5 months and the median time span between the start of the search transplantation decreased from 4.4 months to 3.9 months. This has resulted in a decrease of the percentage of patients for whom the donor search had to be stopped because the patient was no longer fit for transplantation (from 30 percent in 1995-2000 to 24 percent in 2001-2004). As a result, 63 percent of patients received a graft compared to 59 percent in the previous period. This indicates that if the search time span can be brought down to 6 weeks this could lead to a significant increases of the proportion of patients reaching transplantation and therefore heighten the chances of survival for these patients.

However it should be noted that a ≥5α5β MHC mismatched donor is not more suitable for transplantation than a fully matched donor. As a general rule it can be stated that more MHC mismatches lead to more transplantation related complications and thus clinical intervention and a less rapid stem cell engraftment leading to more chance on relapse. The last point is nicely shown by the fact that single MHC class I mismatches that lead to a positive CTLp assay not only lead to more transplant related mortality but also to more relapses of the treated disease. A graft versus leukaemia effect would have been expected in these cases. However, this was probably not the case due to the extra interventions, graft versus host disease treatment or slow engraftment.

In conclusion, the ≥5α5β MHC mismatches generate more possibilities for patients who lack a fully matched donor. Choosing such a donor instead of waiting for the fully matched one can save a lot of precious time when time is short. In my opinion, an increase of efficiency in the whole donation and transplantation process will result in a considerably higher chance of patient survival.

Reference List

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(2) Petersdorf EW, Hansen JA, Martin PJ et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001;345:1794-1800.