1 General Introduction

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Principles of allogeneic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a successful treatment for patients suffering from a variety of hematological malignancies.[1] Prior to SCT patients are treated with high dose chemotherapy combined with total body irradiation in order to eradicate malignant cells and to suppress the hematopoietic system of the patient to allow engraftment of donor hematopoietic cells. Initially the purpose of the SCT was to replace the lethally damaged patient hematopoietic cells by donor hematopoietic cells to reconstitute the hematopoietic system.[2] In this view autologous stem cells or stem cell from a syngeneic twin were supposed to be the best source of hematopoietic stem cells, since allo-SCT was associated with immunological complications such as graft versus host disease (GVHD) or graft rejection. However, clinical studies demonstrated that the occurrence of GVHD was associated with a decreased likelihood of disease relapse after transplantation, resulting in a better overall survival.[3] Donor derived T-cells appeared not only to be responsible for the detrimental GVHD but also for a reduced risk of disease relapse. Depletion of T-cells from the graft to prevent GVHD resulted in an increased risk of disease relapse illustrating that donor derived T-cells were capable of mediating a graft versus leukemia (GVL) effect.[4-6] Since transplantation between homozygous twins did not result in a GVL-effect it was concluded that the presence of T-cells in the graft was not sufficient to mediate GVL-reactivity but that alloreactive T-cells were essential for the antitumor effect.[7;8] Direct evidence that GVL-reactivity was mediated by donor derived T-cells was provided by the observation that donor lymphocyte infusion (DLI) could induce durable remissions in patients with persistent or relapsed leukemia. DLI as a treatment for recurrence of the malignant disease after allo-SCT has resulted in 20% to 90% complete remissions depending on the malignancy. In chronic myelogenous leukemia (CML) in chronic phase the best results were found. Although response rates in other hematological malignancies were much lower, evident clinical responses have been found in patients treated for relapsed acute myelogenous leukemia (AML), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma (NHL) and acute lymphoblastic leukemia (ALL).[9-16]

The potential advantage of allo-SCT over autologous SCT appears to be the possibility to exploit the immune system to eradicate residual malignant cells after transplantation. Following allo-SCT normal hematopoietic and immune cells are of donor origin, whereas residual leukemic cells are of patient origin. In this setting where the hematopoietic system is of donor origin, donor derived T-cells can be infused to eliminate residual patient normal hematopoietic cells and leukemic cells without a risk for rejection. In other words, allo-SCT provides a platform for adoptive immunotherapy with DLI to eradicate residual leukemic

cells. In this approach conditioning regimens prior to transplantation may not be aimed at fully eradicating malignant cells but can be limited to doses sufficient to permit donor stem cell engraftment. Therefore toxicity of the conditioning regimen prior to SCT can be reduced.[17] However, the major complication following adoptive immunotherapy with DLI is GVHD. The main focus in hematopoietic SCT research is to develop strategies for immunotherapy of hematopoietic malignancies resulting in reduced GVHD while preserving GVL-reactivity.

GVL-reactivity and GVHD following HLA-matched allo-SCT

Since alloreactive T-cells recognizing HLA-mismatched alleles are present in high frequencies in peripheral blood, HLA-mismatched SCT may result in strong allo-immunity.[18] To reduce the risk of GVHD and allograft rejection, allo-SCT is preferably performed using an HLA-matched donor.[19;20] The optimal donor for an allo-SCT is a fully matched sibling of the patient. However, GVHD and GVL-reactivity coincide in the majority of patients following a fully HLA-matched allo-SCT with a T-cell repleted graft or after DLI. This illustrates that both GVL-reactivity and GVHD are triggered by genetic disparities other than HLA molecules. Single nucleotide polymorphisms present in the coding region of a gene may result in amino acid substitutions in a protein. In most cases this will not influence the biological activity of the protein, however processing of these polymorphic proteins may result in presentation of polymorphic peptides in the context of HLA-molecules. Differences between patient and donor in these polymorphic peptides presented on the cell-surface by HLA-class I or class II molecules are called minor histocompatibility antigens (MiHA) and may be recognized by CD8+ or CD4+ T-cells respectively. MiHA specific T-cell responses have been identified both in patients with selective beneficial GVL-reactivity as well as in patients suffering from GVHD.[10;17;21]

MiHAs in GVL-reactivity and GVHD

Several studies have demonstrated the presence of both CD4+ and CD8+ MiHA specific T-cells in patients suffering from GVHD following HLA-identical SCT. However, MiHA specific CD4+ and CD8+ T-cells have also been isolated from patients with selective GVL-reactivity. The appearance of both MiHA specific CD4+ T-cells and CD8+ T-cells in patients following allo-SCT and DLI who showed long term complete remissions of their malignancies illustrated their clinical relevance for GVL-reactivity. Detailed studies analyzing kinetics of MiHA specific immune responses showed that the appearance of MiHA specific T cells coincided with the disappearance of malignant cells from peripheral blood, further illustrating a GVL-effect of these T-cells.[22-27] Clonal isolation of these MiHA specific CD4+ and CD8+ T-cells from patients responding to DLI after HLA-matched allo-SCT demonstrated polyclonal immune responses directed against a significant number of distinct MiHAs.[22;23;27;28]

T-cell responses directed against MiHAs expressed on both hematopoietic cells and non-hematopoietic cells are likely to induce GVL-reactivity as well as GVHD. Since HLA-class I molecules are expressed on most nucleated cells, expression of HLA-class I restricted MiHAs on target cells is mainly determined by expression, translation and processing of genes encoding the MiHA in different target tissues. CD8+ T-cells recognizing broadly expressed MiHAs in the context of HLA-class I may mediate GVL-reactivity as well as cause damage in different organs resulting in profound GVHD. However, CD8+ T-cell responses directed to hematopoiesis restricted antigens may more selectively induce GVL-reactivity without GVHD.[24;29-31] Not only differential expression of MiHAs but also differential expression of HLA-molecules may contribute to hematopoiesis restricted allogeneic T-cell responses. In contrast to ubiquitous expression of HLA-class I molecules, expression of HLA-class II molecules is mainly restricted to hematopoietic cells. In patients with HLA-class II positive hematological malignancies, an HLA-class II restricted MiHA specific immune response may therefore more selectively induce GVL-reactivity. Several HLA-class II restricted MiHAs encoded by broadly expressed genes have been identified from immune responses in patients without severe GVHD.[28;32]

Identification of MiHAs

To gain more insight into the biology of MiHA specific T-cell responses in GVHD and GVL-reactivity, MiHAs have been molecularly characterized. Laborious techniques such as biochemical identification of peptides eluted from HLA-molecules and the use of cDNA libraries to express MiHAs in target cells were initially used to identify MiHA encoding gene transcripts. These strategies resulted in successful discovery of several MiHAs, however low efficiency of the techniques hampered identification of the majority of MiHAs recognized by T-cell clones.[25-28;33-41] To better understand the development of GVL-reactivity and GVHD, characterization of large numbers of MiHAs is essential. High-throughput identification and expression analysis of MiHAs in patients with various clinical responses after allo-SCT may provide insight into differences and allow manipulation of the balance between GVL-reactivity and GVHD. We developed an efficient strategy for MiHA identification using whole genome association scanning (WGAS). Multiple MiHA specific T-cell clones were isolated from clinical immune responses in patients who received an HLA-matched allograft followed by DLI. Using the WGAS-based strategy we identified multiple novel MiHAs.[27] This strategy may allow characterization of the biological relevance of immune responses directed against various MiHAs in different clinical immune responses. Analysis of different immune responses in patients after allo-SCT and DLI using the WGAS based strategy have demonstrated that immune responses with high amplitudo directed against broadly expressed antigens resulted in GVHD, whereas smaller immune responses directed against hematological restricted antigens resulted in more selective GVL-reactivity.[42;43]

Balance between GVHD and GVL-reactivity

Before HLA-matched allo-SCT, donor derived T-cells most likely have not been exposed to patient specific antigens and must be primed by professional antigen presenting cells (APC) in order to exert an efficient anti-tumor reactivity.[44-46] Residual patient derived dendritic cells, B-cells or macrophages which co-express the same antigens as patient leukemic cells can serve as APC for the induction of GVL-reactivity but also GVHD responses. Since APCs are hematopoietic cells, it may be expected that preferentially hematopoiesis specific antigens are presented, although also ubiquitously expressed antigens will be presented.[22;23] In the early post-transplant period, residual patient derived APCs may directly prime donor T-cells resulting in GVL-reactivity if the antigens are co-expressed by patient leukemic cells.[44;46] Leukemic cells themselves may also acquire an APC phenotype in vivo and function as APC to directly prime anti-leukemic T-cell responses.[47-49] However, in the context of a pro-inflammatory environment as a consequence of tissue damage caused by conditioning regimens or ongoing infections, APCs may not only express endogenous antigens, but can cross-present antigens from different cell types as well, resulting in the induction or amplification of anti-host responses which may induce GVHD.[50] Various conditions of the local microenvironment will determine whether T-cells are capable of recognizing different antigens and cause damage to target organs. This includes activation status of local environment, homing of T-cells and expression of adhesion molecules on target organs.[21;51] In contrast to the early post-transplant period, late after transplantation patient APCs will gradually be replaced by APCs of donor origin. In the absence of pro-inflammatory stimuli there might be a lower chance to develop a broad immune response with high amplitudo resulting in GVHD. In this period, leukemic cells may acquire an APC phenotype and directly trigger an immune response resulting in selective GVL-reactivity. However, if leukemic cells do not acquire an APC phenotype in this period, GVL-reactivity has to be induced by either limited number of residual patient APCs or by donor derived APCs cross-presenting patient derived antigens.[50] Although mice studies have demonstrated that this indirect antigen presentation does occur, the question remains how effective this is in humans.[10;17;50;52] The efficacy of DLI in this period may therefore either depend on the presence of residual patient APCs and their activation status or on the capacity of leukemic cells to acquire an APC phenotype.

Traditionally CD8+ T-cells are considered to be the primary effector cells in GVL-reactivity, whereas CD4+ T-cells are known to be required as helper cells for the induction and maintenance of CD8+ T-cell mediated immunity.[53] However, CD4+ T-cells with direct cytolytic activity against leukemic cells have also been isolated from patients with GVL-responses.[23;28;32;54;55] These data suggested that CD4+ T-cells may also serve as effector cells in GVL-reactivity. Since constitutive expression of HLA-class II molecules is predominantly restricted to hematopoietic cells, CD4+ T-cells may more selectively mediate GVL-reactivity. Indeed several clinical studies have shown that depletion of CD8+ T-cells from the graft or

DLI indeed resulted in a reduced incidence and severity of GVHD without compromising GVL-reactivity.[56;57] However, HLA-class II expression on non-hematopoietic cells can be upregulated after exposure to pro-inflammatory cytokines. An HLA-class II restricted immune response may therefore also result in GVHD if HLA-class II molecules on non-hematopoietic tissues are upregulated.

Unrelated donor stem cell transplantation

Allo-SCT is preferably performed using an HLA-matched donor to reduce the risk of GVHD and allograft rejection. The optimal donor for allo-SCT is a fully matched sibling of the patient. However, identical sibling donors are only available in about 30% of the patients.[20] In the absence of a family donor a good alternative is a matched unrelated donor (URD). In a recent large study it was shown that the highest survival in URD-transplantation was associated with high resolution DNA matching for HLA-A, -B, -C, and -DRB1. However, a perfectly matched donor is frequently not found. A single mismatch at one of these loci (7/8 match) was associated with lower survival, lower disease free survival, higher treatment related mortality and more acute GVHD (aGVHD) compared to 8/8 HLA matched pairs. This applied both high resolution (allele) and low resolution (antigen) mismatches. Remarkably, no differences for relapse and engraftment were found. Single mismatches at HLA-B or -C appeared to be better tolerated than mismatches for HLA-A or -DRB1. Mismatching for an additional allele was associated with even lower survival. A single HLA-DQB1 mismatch was not associated with adverse outcome. However, an HLA-DQB1 mismatch in a 7/8 matched pair was associated with a small but not statistically significant adverse effect on survival. Mismatching for HLA-DPB1 was not associated with a difference in survival.[19;20;58]

In URD-SCT preferably a fully matched donor for HLA-A, -B, -C, -DRB1 and HLA-DQB1 is used (10/10 match). However, in the absence of a full match, a donor matched at 9/10 alleles may be selected. A single mismatch at HLA-DQB1 may be preferred over another locus mismatch, since mismatching for a single HLA-DQB1 was not associated with adverse outcome, whereas a mismatch for HLA-A, -B, -C or -DRB1 resulted in lower overall survival.[19] Since mismatching for HLA-DPB1 did not result in a difference in transplant outcome in most studies, HLA-DPB1 is not taken into consideration in donor selection in most centers. Although the role of HLA-DP in GVL-reactivity and GVHD has been unclear for a long time, several studies have now demonstrated that HLA-DP does function as a classical transplantation antigen.[19;59;60]

The role of HLA-DPB1 in allo-SCT

HLA-DP is the sixth classic HLA-molecule. It consists of an alpha and a beta chain encoded by the HLA-DPA1 and HLA-DPB1 genes respectively. The genes are located centromeric to HLA-DR and HLA-DQ in the class II region of chromosome 6p21.3. HLA-DP shares structural similarities with the HLA-DR and HLA–DQ. For the HLA-DP alpha chain 34 alleles are known, whereas for the highly polymorphic beta chain 155 alleles are know to date.[62] Population studies have shown strong linkage disequilibrium between HLA-DPA1 and HLA-DPB1, but weak disequilibrium between HLA-DP and the other HLA-class II loci.[63;64] This may result from a recombination 'hotspot' in chromosome 6 between HLA-DPA1 and HLA-DQB1 genes. In sibling donors HLA-DPB1 mismatch has been reported up to 10.9%.[65] In HLA-A, -B, -C, -DRB1 and HLA-DQB1 matched URD hematopoietic SCT 70-89% HLA-DPB1 mismatch rates are reported.[60;66-70]

The relevance of matching for HLA-DPB1 in URD-SCT has been inconclusive for a long time. Several clinical studies failed to show a statistical significant difference in the incidence of severe GVHD or patient survival between HLA-DPB1 matched and mismatched patient-donor pairs.[71;72] As a result, HLA-DPB1 has not been taken into consideration in donor selection. Large clinical studies performed between 1999 and 2008 provided evidence for an immunogenic role of HLA-DPB1 in allo-SCT. In T-cell repleted allo-SCT mismatching for HLA-DPB1 was associated with an increased risk of severe GVHD and a decreased risk of disease relapse.[19;60;68;73;74] The overall effect on survival of patients who received an HLA-DPB1 matched or mismatched graft did not statistically differ, possibly due to a balanced effect of an increased risk of GVHD and reduced relapse rate. In contrast, in T-cell depleted allo-SCT mismatching for HLA-DPB1 was not associated with an increased risk of GVHD, whereas a significant decreased risk of disease relapse was still observed. This was particularly evident in patients transplanted for ALL, where HLA-DPB1 mismatched transplantation resulted in a significantly better overall survival.[59]

Further support that HLA-DPB1 mismatched transplantation can result in strong T-cell responses in vivo was provided by several studies demonstrating CD4+ T-cell responses directed against mismatched HLA-DPB1 molecules. HLA-DP specific CD4+ T-cells were isolated from skin biopsies in patients who developed GVHD following HLA-DPB1 mismatched SCT.[75;76] HLA-DP specific CD4+ T-cells were also isolated from an allograft rejection where patient and donor differed for a single HLA-DPB1 mismatch in the rejection direction.[77] It was speculated that HLA-DP specific CD4+ T-cells might also be able to induce a selective GVL-effect in the context of a T-cell depleted allo-SCT. To exploit this concept, HLA-DP expression on leukemic blasts was analysed. HLA-DP expression was found on the vast majority of leukemic cells analyzed. However considerable variability was found and expression was lower on AML than on B-ALL or B-CLL cells. Most leukemic

blasts were also susceptible to direct lysis by allogeneic HLA-DP specific T-cells.[61] However, thus far direct evidence that HLA-DP specific CD4+ T-cells were involved in GVL-reactivity had not been shown.

Permissive and non-permissive mismatches

Since different studies resulted in conflicting outcomes concerning the role of HLA-DPB1 in allo-SCT, it was suggested that there might be a difference in immunogenicity between different HLA-DPB1 mismatches. In primary mixed lymphocyte reaction (MLR) a variety in response values to different HLA-DPB1 mismatches was found. This observation resulted in the hypothesis that not all HLA-DPB1 incompatibilities would elicit measurable T-cell responses in MLR. In vitro studies showed that specific amino acid sequence differences between donor and recipient were associated with high T-cell responses.[78-80] From these studies it was suggested that patient and donor needed to be matched only for specific regions in the hypervariable regions of the HLA-DPB1 allele to prevent GVHD. In 2008 a study was performed in which it was indeed shown that incompatibilities at distinct positions in the hypervariable regions of HLA-DPB1 were risk factors for developing acute GVHD. A specific amino acid mismatch at position 69 of the HLA-DPB1 molecules was associated with a higher rate of treatment related mortality.[73] In two large studies around 5 000 patients who underwent an allo-SCT through the Japan Marrow Donor Program were analyzed for specific mismatches associated with an increased risk of GVHD or a decreased risk of disease relapse. Two specific HLA-DPB1 mismatch combinations were found to be statistically significantly correlated with severe aGVHD and 6 different specific HLA-DPB1 mismatch combinations were associated with a decreased risk of disease relapse and a better overall survival.[81;82] It was suggested that these observations should be taken into consideration in donor selection.

The suggestion that matching at an epitope level might be clinically more relevant in terms of transplant outcome than matching at allele level was translated into a clinical algorithm by Zino et al.[83] In this algorithm permissive and non-permissive mismatches were defined. HLA-DPB1 molecules were classified in different immunogenicity groups based on T-cell recognition patterns by HLA-DPB1*0901 specific CD4+ T-cell clones. Individuals were not supposed to elicit strong anti-HLA-DP responses to HLA-DPB1 molecules classified within the same immunogenicity group. This was based on the hypothesis that T-cells should not respond to foreign HLA-DPB1 molecules sharing specific amino acids with the 'self' HLA-DPB1 allele. These mismatches were called permissive mismatches. In contrast, strong T-cell responses were expected to be generated in response to HLA-DPB1 molecules classified in higher immunogenic groups, representing non-permissive mismatches. Retrospective analysis of a patient cohort showed a significantly higher probability of 2-year survival in permissive compared to non-permissive mismatches. However, no significant

effect on aGVHD or disease relapse was observed in 10/10 matched patients.[84;85] These different observations have not been confirmed in other studies.[73;85]

Mismatched HLA-DPB1 as a specific target for GVL-reactivity

As discussed before, immune responses directed against hematopoiesis restricted antigens can be expected to result in selective GVL-reactivity, whereas immune responses directed against broadly expressed antigens may result in both GVL-reactivity and GVHD. We demonstrated in patients with selective GVL-reactivity low frequencies of MiHA specific T-cells, whereas in patients with both GVL-reactivity and GVHD higher frequencies of MiHA specific T-cells directed against multiple MiHAs were found.[42;43] Since constitutive expression of HLA-class II molecules may be expected to be predominantly restricted to hematopoietic cells, an immune response directed against a single HLA-class II restricted antigen can be hypothesized to induce selective GVL-reactivity. In case of an immune response directed against an HLA-class II restricted MiHAs, a relatively restricted immune response inducing selective GVL-reactivity may be expected to occur. However, alloreactive T-cells recognizing mismatched HLA-molecules are present in high frequencies in peripheral blood.[18] A single HLA-class-II locus mismatched SCT may therefore result in a broad allo-immune response, thereby increasing the risk for GVHD. Mismatching for HLA-DPB1 has indeed been shown to induce strong HLA-DPB1 specific CD4+ T-cell responses in vivo.[75-77] In T-cell repleted SCT mismatching for HLA-DPB1 was associated with both a decreased risk of disease relapse and an increased risk in GVHD.[19;60] In contrast, in T-cell depleted SCT, mismatching for HLA-DPB1 was associated with a decreased risk of disease relapse without an increased risk in GVHD.[59] These data suggest that in HLA-DPB1 mismatched allo-SCT the transplant regimen used may be important for the balance of GVL-reactivity and GVHD.

HLA-class II expression can be upregulated on non-hematopoietic tissues by pro-inflammatory cytokines released as a consequence of tissue damage caused by the conditioning regimen, ongoing infections, or other immune responses. In T-cell repleted SCT, infusion of T-cells derived from an HLA-DPB1 mismatched donor in an inflammatory environment may therefore result in a strong immune response resulting in GVHD. In contrast, HLA-DPB1 mismatched allo-SCT followed by donor derived T-cells at a later time point may more selectively induce GVL-reactivity when tissue damage is largely restored and HLA-class II expression may be anticipated to be restricted to hematopoietic cells. In HLA-DPB1 mismatched allo-SCT local circumstances in the host at the time of T-cell infusion may therefore determine whether GVL-reactivity develops in the presence or absence of GVHD.

Aim of this study

The overall survival between patients transplanted with an HLA-DPB1 matched or mismatched SCT did not statistically differ in most studies.[19;60] However, it has been shown that HLA-DP can function as a classical transplantation antigen and can induce strong allo-immune responses in vivo.[75;76;86;87] In T-cell repleted SCT mismatching for HLA-DPB1 is associated with an increased risk of GVHD. In contrast, in T-cell depleted SCT no association with an increased risk of GVHD was found, whereas a decreased risk of disease relapse was still observed, suggesting that in T-cell depleted SCT mismatching for HLA-DPB1 may induce a selective GVL-effect.[59] Furthermore, it has been suggested that specific HLA-DPB1 mismatches were associated with an increased risk of GVHD or treatment related mortality.[73;81;83;84] Together these data suggest that different outcomes can be expected following HLA-DPB1 mismatched allo-SCT depending on the transplant regimen used and possibly also influenced by specific circumstances present in the host at the time of transplantation.

The aim of this thesis is to investigate whether mismatching for HLA-DPB1 can result in selective GVL-reactivity without GVHD. Furthermore, we determined whether all HLA-DPB1 mismatches can be expected to result in an allo-immune response in vivo. Finally, we investigated whether following HLA-DPB1 mismatched SCT HLA-DP specific immune responses are often induced and whether the occurrence of HLA-DP specific immune responses is associated with GVL-reactivity, GVHD or both.

To analyze whether HLA-DP specific CD4+ T could induce a specific GVL-effect without GVHD, we analyzed in ***chapter 2*** the immune response in a patient transplanted with a 10/10 matched, HLA-DPB1 mismatched SCT followed by DLI for relapsed chronic B-cell leukemia. In this patient a profound GVL-effect with minimal skin GVHD resulting in a persistent complete remission of the disease was observed. We analyzed the kinetics of the immune response and clonally isolated leukemia reactive T-cell clones from the peak of the immune response. The specificity of the immune response was unraveled. Furthermore, differential recognition patterns of HLA-DP specific CD4+ T-cells for various hematological malignant cells and non-hematopoietic cells were tested.

Various studies have suggested that some HLA-DPB1 mismatches would result in stronger allo-reactivity than others. It has been suggested that matching at an epitope level would be clinically more relevant in terms of transplant outcome than matching at allele level.[73;81;83;84] An algorithm defining permissive and non-permissive HLA-DPB1 mismatches had been tested in a retrospective analysis of a patient cohort. Higher overall survival in patients transplanted with a permissive mismatch compared to non-permissive mismatches was found, however no significant effect on GVHD or disease relapse was observed.[84] In other retrospective analyses other specific HLA-DPB1 mismatches were suggested to be associated with GVL-reactivity, GVHD or transplant related mortality.[73;81;82]

These different observations from various research groups were not consistent and have not been confirmed in other studies.[85]

In ***chapter 3*** we analyzed whether permissive HLA-DPB1 mismatches could result in HLA-DPB1 specific immune response in vivo. Two patients responding to DLI after HLA-A, -B, -C, -DRB1, -DQB1 matched, HLA-DPB1 mismatched SCT were analyzed for the presence of patient HLA-DPB1 specific CD4+ T-cells. The patients received a permissive or non-permissive HLA-DPB1 mismatched SCT followed by DLI. $CD4^+$ T-cells were isolated from peripheral blood obtained during the clinical immune response to DLI, and tested for specific recognition of stimulator cells transduced with patient and not donor HLA-DP molecules.

To analyze whether different HLA-DPB1 mismatches would result in differential frequencies of immune responses, we analyzed in ***chapter 4*** whether immunogenicity of HLA-DPB1 mismatches could be predicted based on the presence or absence of specific amino acid sequences. We developed a model to generate HLA-DPB1 responses in vitro and tested in total 48 different stimulator/responder combinations by stimulating CD4+ T-cells from 5 HLA-DPB1 homozygous individuals with the same APC transduced with different allo-HLA-DP molecules. Furthermore, we analyzed whether CD4+ T-cell clones with different HLA-DPB1 specificity showed similar cross-recognition patterns as previously demonstrated and we analyzed whether we could identify additional patterns in cross-reactivity of HLA-DPB1 molecules by HLA-DPB1 specific CD4+ T-cells.

To analyze whether HLA-DP specific immune responses are frequently induced after T-cell depleted HLA-DPB1 mismatched SCT and DLI we developed in ***chapter 5*** a method to screen patients for the presence of HLA-DP specific CD4+ T-cells. We analyzed 24 patient-donor combinations. Patients analyzed suffered from various B-cell malignancies, multiple myeloma and myeloid leukemias. Furthermore, we analyzed whether the presence of HLA-DP specific CD4+ T-cells was associated with clinical outcome in terms of GVHD or GVL-reactivity, and whether HLA-DP specific T-cell responses could be found after permissive as well as non-permissive HLA-DPB1 mismatched DLI.

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