Targeting HLA class II in allogeneic stem cell transplantation

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CHAPTER 1

General introduction

ALLOGENEIC STEM CELL TRANSPLANTATION

Allogeneic stem cell transplantation (alloSCT) provides a potentially curative therapy for patients with a variety of hematologic malignancies. Besides the therapeutic effect of the pre-transplant condition regimen with chemotherapy or radiation therapy, the most important therapeutic effect of alloSCT is due to an immune response of donor immune cells against the malignancy of the patient. In alloSCT not only stem cells of the donor are transfused into the patient, but also donor T cells. The aim is that these donor T cells induce an immune response against patient hematopoietic cells. Since the malignancy the patient is treated for, is a malignancy of hematopoietic origin, an immune response against patient hematopoiesis is an immune response against the disease of the patient. The immune response of donor T cells directed against the malignant hematopoietic cells, which usually include an immune response to other hematopoietic cells of the patient, is called graft-versus-leukemia (GVL) reactivity. However, donor T cells can also be directed against non-hematopoietic healthy tissues of the patient, causing graft-versus-host disease (GVHD). GVHD and treatment of GVHD with immunosuppressive agents remain major causes of transplant related morbidity and mortality. The ultimate aim in treatment with alloSCT is to find the optimal balance between the beneficial GVL reactivity and the detrimental GVHD. The most efficient method to prevent GVHD is T-cell depletion (TCD) of the graft. AlloSCT regimens using infusion of positively selected CD34 cells, grafts depleted of alpha-beta T cells or CD3 T cells, or using the CD52 antibody Alemtuzumab for TCD have demonstrated efficient engraftment and reduced acute GVHD.[1-10] However, TCD substantially impairs post-transplant anti-viral and anti-tumor immunity.[4,11,12] Due to the reduced antitumor effect of TCD grafts, post-transplant donor lymphocyte infusion (DLI) containing donor T cells, may be needed for treatment persistent disease or for minimizing the relapse risk by eliminating residual patient hematopoiesis after alloSCT. TCD alloSCT with postponed DLI is indeed associated with GVL reactivity and with reduced incidence of GVHD.[4,13-17] Since the ultimate aim in alloSCT is to induce GVL without GVHD, it is important to explore whether the postponed DLI can be manipulated to better achieve this goal.

ANTIGEN PRESENTATION ON CELL SURFACE AND RECOGNITION BY T CELLS

Under normal circumstances in a healthy individual, T cells can detect the presence of pathogens because infected cells display peptide fragments derived from pathogen specific proteins on their surface. These foreign peptides are presented to T cells by major histocompatibility complex (MHC) molecules, also called human leucocyte antigen (HLA) molecules. There are two classes of these highly polymorphic HLA molecules, HLA class I and HLA class II. Both molecules consists of two domains that fold together to create a cleft or binding groove, where the peptide binds to the HLA molecule. Peptides presented in HLA class I molecules can be recognized by CD8 T cells and after recognition these CD8 T cells can exert direct cytotoxicity towards the target cell presenting the peptide. Peptides presented in HLA class II can be recognized by CD4 T cells and after recognition these CD4

T cells can exert direct cytotoxicity on target cells, can provide help to the development and persistence of CD8 T-cell responses or can activate macrophages or B cells to help fighting the pathogen. Under normal circumstances, T cells will only recognize foreign peptides presented in self-HLA molecules of an individual. Due to positive and negative thymic selection, T cells are able to recognize a multitude of foreign molecules without attacking the body itself. Positive selection involves the selection of developing T cells that can recognize self-HLA molecules expressed in the thymus. Negative selection prevents the survival of T cells that interact with self-peptides presented in the thymus. The result is that only T cells recognizing non-self-peptides in self-HLA remain present.

The presentation of peptides by HLA molecules is the result of a series of reactions that are different between HLA class I and class II molecules. For presentation in HLA class I, proteins are degraded by the proteasome and the resulting peptides are translocated via transporter associated with antigen presentation (TAP) into the endoplasmic reticulum (ER) lumen and loaded onto HLA class I molecules. Peptide–HLA class I complexes are released from the ER and transported via the Golgi apparatus to the plasma membrane for antigen presentation to CD8 T cells.[18] The process of peptide presentation in HLA class II is different. HLA class II α- and β-chains assemble in the ER and form a complex with the invariant chain (Ii). The Ii–HLA class II heterotrimer is transported through the Golgi apparatus to the HLA class II compartment. Endocytosed proteins and Ii are degraded by resident proteases in this compartment. The HLA class II associated Ii peptide (CLIP) fragment of Ii remains in the peptide-binding groove of the HLA class II dimer and is exchanged for an antigenic peptide with the help of the dedicated chaperone HLA-DM. HLA class II molecules are then transported to the plasma membrane to present antigenic peptides to CD4 T cells.[18] HLA-DO is another molecule playing a role in peptide presentation in HLA class II. HLA-DO is an HLA class II mimic and functions as a competitive and essentially irreversible inhibitor of HLA-DM activity.[19] Presence of HLA-DO in a cell expands the repertoire of peptides presented by HLA class II molecules.[20,21] HLA-DO is mainly expressed in hematopoietic cells, especially B cells, and not in non-hematopoietic cells.[22] Therefore, it is possible that some particular peptides are only presented if HLA-DM activity is inhibited by the presence of HLA-DO.

HLA molecules present either peptides derived from pathogens or peptides derived from self-proteins. In the setting of alloSCT donor T cells will recognize patient derived peptides as foreign and attack patient cells if the presented peptide is polymorphic and different between patient and donor. The recognized cell is targeted because of the presence of a foreign peptide in HLA and depending on which cells the peptide is presented, different tissues will be attacked. If the recognized peptide is expressed on hematopoietic cells, including the malignant hematopoietic cells of the patient, GVL will be the result. However, if the recognized peptide is expressed in normal tissues other than hematopoietic tissue, GVHD will occur.

EXPRESSION OF HLA CLASS I AND II MOLECULES

HLA class I molecules are expressed on all nucleated human cells.[18] In contrast to HLA class I, constitutive expression of HLA class II molecules is predominantly restricted to normal and malignant hematopoietic cells.[23-26] This hematopoiesis restricted expression makes targeting of HLA class II molecules by donor alloreactive CD4 T cells interesting in the setting of alloSCT, where an hematopoiesis restricted immune response is aimed for. This is one of the reasons that in the Leiden University Medical Center a clinical trial was started in which patients are being treated with purified donor CD4 T cells three months after TCD alloSCT. Besides the primary endpoint of immune reconstitution, conversion of mixed chimerism after alloSCT due to infusion of donor CD4 T cells is being investigated as secondary endpoint. Since CD4 T cells target peptides in HLA class II and HLA class II under non-inflammatory circumstances is predominantly expressed on hematopoietic cells, including malignant hematopoietic cells, GVL without GVHD could be the result of infusion of donor CD4 T cells. Previously it has been demonstrated that GVL reactivity can indeed be caused by donor CD4 T cells recognizing peptides presented in HLA class II.[27,28] However, the period after alloSCT is often complicated by inflammatory conditions in the patient and it is known that expression of HLA class II molecule can be upregulated under inflammatory conditions like CMV reactivations.[18,29] The result of this upregulation of HLA class II on non-hematopoietic cells makes GVHD target tissues vulnerable for recognition after infusion of CD4 donor T cells. Therefore, under specific clinical circumstances, both GVL and GVHD can be expected after CD4 T cell infusion after alloSCT.

TARGETING MINOR HISTOCOMPATIBILITY ANTIGENS PRESENTED IN HLA CLASS II

The immune response of donor T cells directed against patients cells is based on recognition of a non-self-peptide-HLA complex in the recipient. This complex can be non-self due to a non-self-peptide, to a non-self HLA molecule or both. Searching for a suitable alloSCT donor starts with identifying potential HLA identical siblings. In the setting of an HLA identical transplantation, the immune response of donor T cells will be directed to a non-self-peptide presented in self HLA since all HLA alleles are shared between donor and recipient. The non-self-peptides that can be recognized by alloreactive donor T cells are called minor histocompatibility antigens (MiHA). MiHA are polymorphic peptides derived from genes containing genetic differences between patient and donor. These polymorphic peptides can be encoded by the male-specific Y-chromosome (H-Y antigens) or other chromosomes (autosomal MiHA). The molecular mechanisms by which genetic variants can create autosomal MiHA include several ways. First, MiHA can be created by single nucleotide polymorphisms (SNP) between donor and recipient in primary gene transcripts in the normal or an alternative reading frame. Second, MiHA that are derived from polymorphic proteins as created by frameshift insertions or deletions in primary gene transcripts. And third, MiHA that are encoded by polymorphic genes (gene deletion).[30] All these molecular mechanisms can result in the presentation of a recipient derived peptide

that is foreign to donor T cells.

Following HLA identical alloSCT, infusion of donor CD8 T cells recognizing HLA class I restricted MiHA that are expressed on all nucleated cells can result in GVL and GVHD. Donor CD8 T cells recognizing MiHA selectively expressed in hematopoietic cells may result in destruction of patient hematopoietic cells including the malignant cells, without harming other normal tissues.[30-35] Since HLA class II is predominantly expressed on hematopoietic cells only, donor CD4 T cells recognizing HLA class II restricted MiHA may result in selective recognition of recipient normal and malignant hematopoietic cells, thereby inducing GVL without GVHD, even if MiHA are targeted that are encoded by genes that are broadly expressed in recipient tissues.[36-40] However, under inflammatory circumstances, expression of HLA class II is significantly upregulated on non-hematopoietic cells, making these tissues susceptible to recognition by CD4 T cells. It was previously demonstrated that, unlike presentation of MiHA in HLA class I, not all MiHA encoded by broadly expressed genes are adequately presented in HLA class II on non-hematopoietic cells due to differences in peptide processing between different cell types, based on presence or absence of HLA-DO.[20] Thus, even under inflammatory conditions, GVHD target tissues may not always be damaged by CD4 T cells recognizing MiHA encoded by broadly expressed genes. This combination of relatively hematopoiesis restricted expression of HLA class II molecules and limited presentation of several broadly expressed MiHA in HLA class II, makes infusion of donor CD4 T cells after alloSCT an attractive strategy to separate GVL from GVHD.

HLA MATCHING BETWEEN PATIENT AND DONOR

HLA molecules are highly polymorphic. Probably the presence of many different HLA molecules makes it possible to present many different peptides from pathogens and thereby protecting a population against being eliminated by infectious diseases.[41]

One of the factors that influences the balance between GVL and GVHD after alloSCT is the degree of HLA matching between patient and donor. In case more HLA alleles are mismatched, the risk of developing GVHD is higher.[42-45] The reason is that mismatched HLA molecules can be targets for immune responses by donor T cells after alloSCT.[46] The T-cell repertoire of an healthy individual contains T cells interacting with peptides in the context of non-self-HLA molecules. Mixed lymphocyte reactions showed that roughly 1-10% of all T cells in an individual may respond to stimulation by cells from allogeneic HLA mismatched individuals.[47-50] The frequency of these T cells recognizing mismatched HLA molecules is much higher than the precursor frequency of known MiHA specific T cells recognizing the MiHA in self-HLA.[30] Therefore, an HLA mismatch between patient and donor is more likely to induce an immune response by donor T cells and the risk of developing GVHD will be higher. The high frequencies of allo-HLA reactive T cells is the reason for preferring HLA matched donors. If an HLA identical sibling donor is not available, a matched unrelated

donor (MUD) is searched. Mainly the HLA loci classified as high expression loci (HEL) HLA-A, B, C and DRB1 are taken into account in the donor selection, since mismatches in HLA-HEL are strongly associated with an increased incidence of severe GVHD.[44,51,52] If patient and donor are matched for these HLA alleles, it is called an 8/8 HLA match. In contrast, HLA-DRB3, 4 and 5, HLA-DQ and HLA-DP are usually assumed to be low expression loci (LEL) based on their surface expression.[53,54] HLA-LEL mismatches were considered not to be associated with adverse outcome in HLA 8/8 matched alloSCT and therefore are frequently not taken into account in donor selection.[53] However, it has been demonstrated that immune responses against mismatched HLA-DQ can contribute to the development of both GVHD and GVL reactivity.[42,55,56] Therefore, in many centers a matched unrelated donor is searched for that is HLA 10/10 matched, meaning matched for HLA-A, B, C, DRB1 and DQB1. In the setting of HLA 10/10 matched transplantations, besides potential GVL and GVHD due to recognition of MiHA in a shared HLA molecule, there is additional potential GVL and GVHD due to recognition of allo-HLA molecules by donor T cells, since the HLA class II alleles HLA-DRB3, -DRB4, -DRB5 and -DPB1 can be mismatched. After infusion of CD4 donor T cells, also these alleles can be targeted.

HLA-DP MISMATCHES IN alloSCT

Unrelated donors for alloSCT who are HLA 10/10 matched with the recipient are mismatched for HLA-DP in 71-88% of cases.[57-60] Although not taken into account in donor selection, immune responses by donor CD4 T cells against this mismatched HLA allele can result in both GVL and GVHD.[27,29,61] Not all mismatches in HLA-DP between donor and patient turned out to be equally immunogenic. HLA-DPB1 alleles have been categorized into T-cell epitope (TCE) groups based on in vitro experiments using recognition patterns of anti-HLA-DP directed T cells and amino acid sequences of the binding groove defining functional distance among the different HLA-DPB1 alleles.[62-64] Based on this classification, HLA-DP mismatches have been classified as permissive (mismatch within the same TCE group) or non-permissive (mismatch across different TCE groups) with predictive value for the outcome of transplantation.[62,65-68] Others conclude that the difference in immunogenicity of HLA-DP mismatches is based on differences in expression of HLA-DP alleles due to the absence or presence of SNP rs9277534.[69] It has been shown that polymorphisms within the peptide binding groove of HLA-DP molecules are more important for HLA-DP restricted alloreactivity than polymorphisms outside the peptide binding groove.[63,70] Therefore, it is possible that differences in composition of peptides bound to the various HLA-DP molecules determine the potency to induce immune responses between mismatched HLA-DP molecules. Mismatched HLA-DP alleles that are more similar to each other and present similar peptides will then be more permissive than mismatched HLA-DP alleles with large structural differences presenting different peptides.

AIM OF THIS STUDY

Donor CD4 T cell infusion may be an attractive strategy for separating GVL and GVHD because of the relatively hematopoiesis restricted expression of HLA class II molecules. Therefore, the aim of this thesis is to investigate in detail the immunological effects of donor CD4 T cell infusion after TCD alloSCT and to explore whether this strategy indeed can separate GVL from GVHD.

Since CD4 T cells target HLA class II expressing cells, the first aim is to answer the question which patient derived cells express HLA class II after alloSCT. HLA class II expression is upregulated under inflammatory conditions. Thus tissue damage caused by the conditioning regimen before alloSCT may influence the expression of HLA class II alleles on GVHD target tissue.

In ***chapter 2*** the hypothesis was investigated that activated patient originated HLA class II positive cells are present for several months in skin due to tissue damage caused by the conditioning regimen given before alloSCT and that these HLA class II positive cells are able to initiate an immune response by donor T cells. To investigate this, the quantitative presence of HLA class II expressing cells and T cells in the dermal layer was substantiated by dermal area count calculation in skin biopsies taken from patients at several time points from transplantation, and compared to normal skin biopsies. To investigate whether the HLA class II positive cells and T cells were of patient or donor origin, XY-FISH combined with staining of HLA class II was performed after alloSCT from patients with a gender mismatched donor.

The second aim of this thesis is to investigate whether donor CD4 T cells from HLA matched sibling donors can induce GVL without GVHD after alloSCT by targeting HLA class II restricted MiHA. In ***chapter 3*** we investigated in detail the observed hematopoiesis restricted immune responses after CD4 DLI, as illustrated by conversion from mixed to full donor chimerism, without GVHD in four patients transplanted with an HLA identical sibling donor. Alloreactive CD4 T cells were isolated from blood and bone marrow after CD4 DLI and using whole genome association scanning, the MiHA that were recognized by alloreactive CD4 T cells were identified. Some of the identified MiHA turned out to be encoded by genes with broad expression in both hematopoietic cells as well as non-hematopoietic cells. Since an hematopoietic restricted immune response was observed in patients, we aimed to identify factors responsible for this hematopoietic restricted immune response besides hematopoiesis restricted expression of HLA class II. We investigated whether differences in peptide processing between hematopoietic and non-hematopoietic cells in the presence or absence of HLA-DO was of influence in separating GVL from GVHD.

Donor CD4 T cells can target the HLA class II molecules HLA-DQ and HLA-DP in case of a mismatch in these alleles between patient and donor. However, also the HLA class II molecules HLA-DRB3, -DRB4 and -DRB5 can be mismatched between patient and donor in case of an HLA 10/10 MUD. These HLA-LEL are not taken into account in donor selection since they are regarded as not relevant in alloSCT. We aimed to investigate whether donor CD4 T cells from HLA 10/10 MUD can induce an immune response targeting mismatched HLA-DRB3. In ***chapter 4***, we analysed the immune response in a patient who received purified CD4 donor lymphocytes 3 months after TCD alloSCT. Afterwards conversion from mixed to full donor chimerism occurred in the presence of skin and liver GVHD. Patient and donor were HLA 10/10 matched, but mismatched for HLA-DRB3 and HLA-DPB1. We investigated whether these mismatched HLA-LEL class II molecules were the target of recognition and whether CD4 T cells targeting mismatched HLA class II molecule are likely to have caused the immune response consisting of GVL and GVHD.

In the setting of alloSCT with HLA 10/10 matched, but HLA-DP mismatched donors, some HLA-DP mismatches are more permissive and less immunogenic than other mismatches. HLA-DPB1 alleles have been categorized into TCE groups and based on this classification, HLA-DP mismatches have been classified as permissive (mismatch within the same TCE group) or non-permissive (mismatch across different TCE groups) with predictive value for the outcome of transplantation. The functional groups of TCE-1 alleles (HLA-DPB1*09:01, 10:01 and 17:01) and TCE-2 alleles (HLA-DPB1*03:01, 14:01 and 45:01) were clearly defined, but TCE-3 included any HLA-DPB1 allele not belonging to either group 1 or 2, and represents a relatively heterogeneous group. In ***chapter 5***, we aimed to redefine the current classification into TCE groups and to unravel potential new functional hierarchies of different HLA-DP mismatches in the setting of alloSCT. The hypothesis was that HLA-DP molecules that are structurally more similar to each other and present more similar peptides, are less immunogenic when mismatched and that HLA-DP molecules that are structurally more different and present more different peptides are more immunogenic when mismatched. To investigate whether permissiveness and non-permissiveness with respect to alloreactivity in the context of alloSCT could be defined based on the similarity or differences in their respective immunopeptidomes, we analysed the peptidome of the 12 common HLA-DP molecules.

In ***chapter 6*** the most important results of the performed analyses are summarized and the relevance of the results in the light of the current knowledge are discussed.

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