Targeting HLA class II in allogeneic stem cell transplantation

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CHAPTER 4

Mismatched HLA-DRB3 can induce a potent immune response after HLA 10/10 matched stem cell transplantation

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ABSTRACT

BACKGROUND

Donors for allogeneic stem cell transplantation (alloSCT) are preferentially matched with patients for HLA-A, B, C and DRB1. Mismatches between donor and patient in these alleles are associated with an increased risk of graft-versus-host disease (GVHD). In contrast, HLA-DRB3, 4 and 5, HLA-DQ and HLA-DP are usually assumed to be low expression loci with limited relevance, although mismatches in HLA-DQ and HLA-DP can result in allo-immune responses. Mismatches in HLA-DRB3, 4 and 5 are usually not taken into account in donor selection.

METHODS

Conversion of chimerism in the presence of GVHD after CD4 donor lymphocyte infusion (DLI) was observed in a patient, HLA 10/10 matched, but mismatched for HLA-DRB3 and HLA-DPB1 compared to the donor. Alloreactive CD4 T cells were isolated from peripheral blood after CD4 DLI and recognition of donor derived target cells transduced with the mismatched patient variant HLA-DRB3 and HLA-DPB1 molecule was tested.

RESULTS

A dominant polyclonal CD4 T cell response against patient's mismatched HLA-DRB3 molecule was found in addition to an immune response against patient's mismatched HLA-DPB1 molecule**.** CD4 T cells specific for these HLA class II molecules recognized both hematopoietic target cells as well as GVHD target cells.

CONCLUSION

In contrast to the assumption that mismatches in HLA-DRB3, 4 and 5 are not of immunogenic significance after HLA 10/10 matched alloSCT, we show that in this matched setting not only mismatches in HLA-DPB1, but also mismatches in HLA-DRB3 may induce a polyclonal allo-immune response associated with conversion of chimerism and severe GVHD.

INTRODUCTION

Both the therapeutic effect and the most severe complications of allogeneic stem cell transplantation (alloSCT) are the result of immune responses of donor T cells against the malignancy and normal tissues of the patient, respectively. Although unrelated donors are preferentially matched for HLA alleles with the patient, mainly the HLA loci classified as high expression loci (HEL) HLA-A, B, C and DRB1 are taken into account, since mismatches in HLA-HEL are strongly associated with an increased incidence of severe Graft-versus-Host Disease (GVHD).[1-3] 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[4,5] HLA-LEL mismatches are considered not to be associated with any adverse outcome in HLA 8/8 matched alloSCT and therefore are frequently not taken into account in donor selection.[4] However, we and others have illustrated that immune responses against mismatched HLA-DQ and HLA-DP can contribute to the development of both GVHD and Graft-versus-Leukemia reactivity.[6-12] Besides this, in the setting of 7/8 HLA matched alloSCT, multiple mismatches in HLA-LEL do have negative impact on occurrence of GVHD and mortality illustrating HLA-LEL can be of immunogenic significance in this setting.[4] Recently, it has been shown that also mismatches in HLA-DRB4 are not irrelevant but are associated with an increased transplant related mortality.[13] Here, we show that also a mismatch in HLA-DRB3, not considered to be relevant during donor selection, can result in the development of a strong polyclonal HLA-DRB3 specific immune response associated with severe GVHD mediated by CD4 T cells.

MATERIAL AND METHODS

INFUSION OF POSITIVELY SELECTED DONOR CD4 T CELLS

At the Leiden University Medical Center we are conducting a clinical trial (EudraCT Number: 2008-001447-19) in which we treat patients 3 months after T cell depleted (TCD) alloSCT from HLA-A, B, C, DRB1 and DQB1 (10/10) matched unrelated donors (MUDs) with purified donor CD4 T cells to promote immune reconstitution.[8] Purified CD4 T cell products were manufactured by positive selection using CD4 Reagent and the CliniMACS System (Miltenyi Biotec, Bergisch Gladbach, Germany).

ISOLATION, EXPANSION AND SELECTION OF T-CELL CLONES

To characterize the immune response, in vivo activated T cells were isolated from peripheral blood mononuclear cells using HLA-DR as a marker of T cell activation. HLA-DR positive CD4 and CD8 T cells were sorted single cell and expanded. Proliferating T cell clones were tested for reactivity against patient and donor derived target cells by measuring Interferon gamma (IFNγ production using IFNγ ELISA (Sanquin Reagents, Amsterdam, Netherlands). T cell receptor-β variable chain (TCRBV) usage was analyzed using a TCRBV repertoire kit (Beckman Coulter, Woerden, Netherlands).

HLA RESTRICTION OF ALLOREACTIVE T CELLS

To determine whether HLA-DR, HLA-DQ or HLA-DP was the HLA restriction molecule for recognition by alloreactive CD4 T cells, patient derived EBV-LCL were pre-incubated with saturating concentrations of monoclonal antibodies against HLA class II (PdV5.2), HLA-DR (B8.11.2), HLA-DQ (SPVL3) or HLA-DP (B7.21) for 30 minutes before addition of the T cells, and inhibition of IFNγ production was determined.

TRANSDUCTION OF PATIENT VARIANT HLA CLASS II MOLECULE IN DONOR DERIVED EBV-LCL

To investigate whether the mismatched HLA class II molecules were targeted by alloreactive T-cell clones, donor derived EBV-LCL were transduced with each of the patient variant mismatched HLA class II molecules. HLA-DRB3*02:02 molecule was cloned into a MP71.60 retroviral vector and HLA-DPB1*19:01 molecule was cloned into a pLZRS retroviral vector. Constructs were verified by sequencing. For retroviral transduction of EBV-LCL recombinant human fibronectin fragments (BioWhittaker, Verviers, Belgium) were used.

RECOGNITION OF HEMATOPOIETIC AND GVHD TARGET TISSUES

To investigate the recognition of hematopoietic as well as GVHD target tissues by CD4 T cells, T cells were overnight incubated with monocyte derived dendritic cells (DC), T cells treated with phytohemagglutinin (PHA blasts) and skin derived fibroblasts from the patient. In addition, cholangiocarcinoma cell line EGI-1 (DSMZ, Braunschweig, Germany) and colon

carcinoma cell lines Sw480 (ATCC CCL-228, Wesel, Germany), WiDr (ATCC CCL-218, Wesel, Germany) and HCT116 (ATCC CCL-247, Wesel, Germany) were transduced with patient variant of mismatched HLA class II molecules. To force expression of HLA class II molecules on non-hematopoietic target cells, they were incubated with 200 IU/ml IFNγ (Boehringer Ingelheim, Rijnland-Palts, Germany) during five days before recognition was tested.

RESULTS

PATIENT CHARACTERISTICS

A 62-year old male with T-lymphoblastic leukemia in complete remission after chemotherapy was treated with a non-myeloablative TCD alloSCT.[14] Patient and donor were HLA 10/10 matched, but mismatched for HLA-DRB3 and HLA-DPB1 (Table 1). No immunosuppressive medication was given. Three months after transplantation mixed chimerism was measured in the bone marrow with percentages of patient cells of 2, 13 and 95% for all leukocytes, CD4 and CD8 T cells, respectively. At this time point a mild EBV reactivation and infection with respiratory syncytial virus was present. After infusion of 0.25 x 10^6 /kg donor CD4 T cells without GVHD prophylaxis, conversion from mixed to full donor chimerism occurred, accompanied with the development of GVHD of liver and skin, overall grade III, which required immunosuppressive treatment resulting in complete response of GVHD after several weeks.

ISOLATION OF ALLOREACTIVE T CELLS

At the onset of GVHD 4 weeks after CD4 donor lymphocyte infusion (DLI), 22% of CD4 and 6% of CD8 T cells expressed HLA-DR as a marker of in vivo activation. HLA-DR positive T cells were clonally isolated and expanded resulting in 380 CD4 T cell clones and 192 CD8 T cell clones. 4.5% of the CD4 T-cell clones and none of the CD8 T-cell clones were alloreactive as determined by recognition of patient derived EBV-LCL and patient derived PHA blasts and absence of recognition of donor derived EBV-LCL. Analysis of TCRVB usage showed that the 17 isolated alloreactive CD4 T-cell clones consisted of at least 11 different clones (Table 2), illustrating that the observed conversion of chimerism and occurrence of GVHD after CD4 DLI was associated with the development of a polyclonal alloreactive CD4 T-cell response.

Table 1. HLA alleles of patient and donor

Patient	Donor
A*02:01 A*02:01	A*02:01 A*02:01
B*15:01:01 B*51:01:01	B*15:01:01 B*51:01:01
C*03:03:01 C*15:02:01	C*03:03:01 C*15:02:01
DRB1*04:08:01 DRB1*13:01:01	DRB1*04:08:01 DRB1*13:01:01
DRB3*02:02:01 DRB4*01:03:01	**DRB3*01:01:02** DRB4*01:03:01
DQB1*03:01 DQB1*06:03	DQB1*03:01 DQB1*06:03
DPB1*02:01 **DPB1*19:01**	DPB1*02:01 **DPB1*04:02**

Patient and donor are matched for HLA-A, B, C, DRB1 and DQB1 loci and mismatched for HLA-DRB3 and HLA-DPB1.

Table 2. TCRBV usage of alloreactive T-cell clones

TCRBV usage	Number of T cell clones	Target of CD4 T-cell clone
1	1	DRB3*02:02
2	1	DRB3*02:02
13.2	2	DRB3*02:02
14	1	DPB1*19:01
17	1	DPB1*19:01
18	2	DRB3*02:02
20	1	DRB3*02:02
23	1	DRB3*02:02
unknown	4	DRB3*02:02
unknown	1	DPB1*19:01
unknown	2	MiHA in HLA class II

An unknown TCRBV means no staining with one of the antibodies in the TCRBV kit (Beckman Coulter). MiHA: minor histocompatibility antigen.

MISMATCHED HLA CLASS II MOLECULES ARE TARGETS OF RECOGNITION

To determine the HLA restriction molecule for CD4 T cell recognition, blocking experiments were performed with antibodies against HLA class II, HLA-DR, HLA-DQ or HLA-DP. Inhibition of recognition of patient EBV-LCL using the pan HLA class II antibodies could be demonstrated for all T-cell clones (Figure 1). Recognition by 6 clones was inhibited using HLA-DR antibodies, no inhibition of recognition was observed using anti HLA-DQ antibodies and inhibition of recognition was demonstrated for 4 clones using anti HLA-DP antibodies, whereas the HLA restriction molecule could not clearly be determined for 7 clones.

To investigate whether the mismatched HLA class II molecules, being HLA-DRB3 and HLA-DPB1, were the target of recognition, we retrovirally transduced donor derived EBV-LCL with the patient variant of HLA-DRB3, being HLA-DRB3*02:02 or the patient variant of HLA-DPB1, being HLA-DPB1*19:01. After transduction 12 alloreactive CD4 T-cell clones recognized the HLA-DRB3*02:02 expressing target cells and 3 alloreactive CD4 T-cell clones recognized the HLA-DPB1*19:01 expressing target cells (Figure 1). 2 alloreactive CD4 T-cell clones did not recognize transduced donor EBV-LCL and therefore these CD4 T-cell clones most probably recognized an HLA class II restricted minor histocompatibility antigen.

Analysis of TCRBV usage showed that within the 12 HLA-DRB3*02:02 directed CD4 T-cell clones there were at least 7 different clones. All 3 HLA-DPB1*19:01 directed CD4 T cell clones had different TCRBV usage (Table 2).

These results illustrate the development of a dominant polyclonal CD4 T-cell response directed against mismatched HLA-DRB3*02:02 besides an immune response against mismatched HLA-DPB1*19:01.

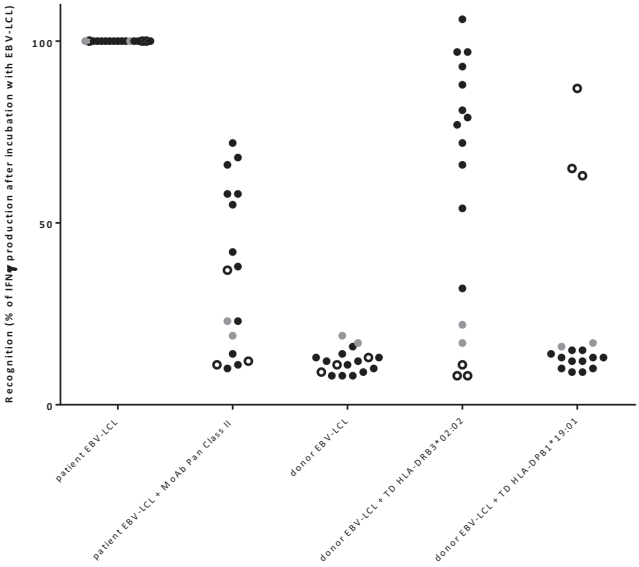

Figure 1. Interferon gamma (IFNγ) production by different T-cell clones. IFNγ production of T cells after overnight incubation with target cells with and without blocking monoclonal antibody PdV5.2 against HLA class II molecules or TD with an HLA class II molecule. Closed black dots represent T-cell clones recognizing the mismatched HLA-DRB3*02:02 molecule. Open black dots represent T-cell clones recognizing the mismatched HLA-DPB1*19:01 molecule. Grey dots represent T-cell clones recognizing no mismatched HLA class II molecule, but an HLA class II restricted minor histocompatibility antigen. TD, transduction.

HLA-DRB3*02:02 AND HLA-DPB1*19:01 DIRECTED ALLOREACTIVE T CELLS RECOGNIZE BOTH HEMATOPOIETIC CELLS AS WELL AS GVHD TARGET TISSUE

To investigate which target cells could be recognized by the isolated alloreactive donor CD4 T cells, these T cells were incubated with hematopoietic and non-hematopoietic target cells. All HLA-DRB3*02:02 and HLA-DPB1*19:01 directed CD4 T cells recognized EBV-LCL, dendritic cells and PHA blasts derived from the patient. Furthermore, patient skin derived fibroblasts, forced to express HLA class II molecules by treatment with IFNγ, were also recognized by both HLA-DRB3*02:02 and HLA-DPB1*19:01 directed CD4 T cells. HLA-DRB3*02:02 directed CD4 T cells recognized HLA-DRB3*02:02 expressing cholangio- and colon carcinoma cells and HLA-DPB1*19:01 directed CD4 T cells recognized HLA-DPB1*19:01 expressing cholangio- and colon carcinoma cells (Figure 2). These results show that the isolated alloreactive HLA-DRB3*02:02 and HLA-DPB1*19:01 directed CD4 T cells were capable of recognizing both hematopoietic patient derived target cells and patient derived and third party GVHD target tissue expressing the mismatched patient variant HLA class II molecules.

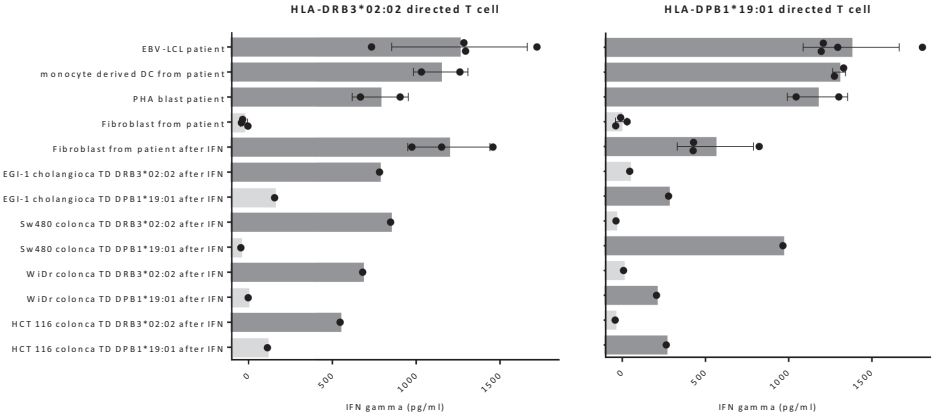

Figure 2. Recognition of target tissues by HLA class II directed CD4 T cells. HLA-DRB3*02:02 **(A)** and HLA-DPB1*19:01 **(B)** directed CD4 T cells isolated from the patient recognized patient monocyte-derived DCs and PHA blasts, but also GVHD target tissues like skin-derived fibroblasts and cholangiocarcinoma and colon carcinoma cells transduced (TD) with HLA-DRB3*02:02 and HLADPB1* 19:01, respectively. Nonhematopoietic targets cells were pretreated with IFNγ to upregulate HLA class II expression. Dark grey bars indicate that the target cell expressed the recognized HLA class II molecule. Light grey bars indicate that the target cell did not express the recognized HLA class II molecule and therefore are negative controls.

DISCUSSION

In the setting of an ongoing clinical trial, the patient received purified CD4 donor lymphocytes 3 months after TCD alloSCT resulting in conversion from mixed to full donor chimerism in the presence of skin and liver GVHD. Patient and donor were HLA 10/10 matched, but mismatched for HLA-DRB3 and HLA-DPB1. Characterization of the immune response showed a dominant polyclonal CD4 T-cell response directed against patient HLA-DRB3*02:02 together with a CD4 T-cell response directed against patient HLA-DPB1*19:01. The CD4 T cells recognized both hematopoietic cells as well as GVHD target tissue. Although mismatches in HLA-DRB3 are considered being of limited relevance and are not taken into account in donor selection[4,15], our results illustrate that not only CD4 T cells directed against HLA-DPB1, but also CD4 T cells directed against HLA-DRB3 can cause a polyclonal immune response associated with conversion of chimerism and development of GVHD. We previously showed that T cells recognizing a peptide in the context of HLA-DRB3 can be generated in vitro.[16] Our results illustrate that also in vivo profound polyclonal immune response against mismatched HLA-DRB3 associated with conversion of chimerism and development of GVHD can develop.

The chance of developing GVHD after alloSCT and DLI is depending on multiple factors In a cohort study of 3853 patients with unrelated donors, multiple mismatches in HLA-LEL had negative impact on occurrence of GVHD and mortality in HLA 7/8 matched alloSCT. However, there was no increased risk of GVHD in patients with a matched donor for HLA-A, B, C and DRB1 (8/8 match), but mismatches in HLA-DRB3, 4 or 5.[4] It is possible that other factors are overwhelming the influence of a mismatched HLA-DRB3 allele, depending on the transplant strategy and the type of DLI.[17] Our study does not allow to make conclusions about the chance of developing GVHD caused by donor T cells directed against a mismatched HLA-DRB3 molecule, but illustrates that such an immune response can occur with the same clinical sequelae as other major HLA mismatches.

After the CD4 DLI only alloreactive CD4 T cells and no alloreactive CD8 T cells were isolated and therefore these CD4 T cells most probably caused both conversion of chimerism as well as GVHD by interaction of the CD4 T cells with their target cells resulting in a direct effect of cytotoxicity and in indirect effects like production of cytokines, making the target cells more susceptible for attack by T cells. The polyclonal alloreactive CD4 T-cell response was predominantly directed against mismatched HLA-DRB3 and to a lesser extent against mismatched HLA-DPB1. We hypothesize that the immune response was initiated by professional antigen presenting cells of the patient. Figure 2 shows that patient derived dendritic cells were recognized by these CD4 T cells. The CD4 T cells recognized also activated patient T cells indicating that the conversion of chimerism in the T-cell compartment could also be caused by the alloreactive CD4 T cells. The patient developed GVHD of

skin and liver and alloreactive CD4 T cells recognized patient skin derived HLA class II expressing fibroblasts as well as biliary epithelial cells (EGI-1) expressing the mismatched HLA class II molecule (Figure 2). GVHD target tissues do not constitutively express HLA class II, but under inflammatory conditions HLA class II expression is upregulated making these cell susceptible for recognition by allo-HLA class II directed CD4 T cells.[8] In summary, we hypothesize that patient dendritic cells initiated the HLA-DRB3 and HLA-DPB1 directed immune response and that the HLA-DRB3 and HLA-DPB1 directed CD4 T cells are responsible for both the conversion of chimerism as well as the occurrence of GVHD.

In contrast to the assumption that mismatches in HLA-DRB3, 4 and 5 are not of immunogenic significance after HLA 10/10 matched alloSCT and are usually not taken into account in donor selection, we show that in this matched setting not only mismatches in HLA-DPB1, but also mismatches in HLA-DRB3 may induce a polyclonal allo-immune response associated with conversion of chimerism and severe GVHD.

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