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Clinical impact of H-Y alloimmunity

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Abstract

H-Y antigens are a group of minor histocompatibility antigens encoded on the Y-chromosome with homologous H-X antigens on the X-chromosome. The disparate regions of the H-Y antigens are highly immunogenic and play an important role in understanding human alloimmunity. In this review, we investigate the history of H-Y antigen discovery along with their critical contributions in transplantation and pregnancy. In hematopoietic cell transplantation, male recipients with female donors who become seropositive for B-cell responses as H-Y antibodies following transplantation have increased rates of chronic graft-versus-host disease and decreased rates of relapse. Conversely, female patients who receive male kidney allografts are more likely than other gender combinations to develop H-Y antibodies and reject their allografts. Finally, in the setting of pregnancy, mothers who initially gave birth to boys are more likely to have subsequent pregnancy complications, including miscarriages, in association with H-Y antibody development. H-Y antigens continue to serve as a model for alloimmunity in new clinical scenarios. Our development of more sensitive antibody detection and next-generation DNA sequencing promises to further advance our understanding and better predict the clinical consequences of alloimmunity.

Keywords

H-Y antigen; Graft-versus-host disease; Graft rejection; Pregnancy complications; Alloimmunity; Kidney transplantation

Introduction

Human alloimmunity has significant consequences in a variety of transplantation settings. For human leukocyte antigen (HLA)-matched transplants, minor histocompatibility antigens (mHAs) are important targets for alloimmunity. mHAs are peptides which, when presented in HLA class I and class II proteins, are able to elicit an adaptive immune response [1]. H-Y antigens are a class of well-characterized mHAs encoded on the Y-chromosome. H-Y proteins tend to be highly expressed throughout the body and show a great degree of similarity to the homologous H-X proteins located on the X-chromosome, but with distinct

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regions of disparity which are generally immunogenic [2]. H-Y antigens provide an important model for alloimmunity because they serve as significant immunogenic targets with clinical consequences in either the donor graft or the recipient in sex-mismatched transplantation.

In hematopoietic cell transplantation (HCT), for example, grafts from female donors to male recipients (F → M) lead to increased rates of graft-versus-host disease (GVHD), a common complication of HCT which affects the skin, GI tract, liver and other organs. In sex-mismatched transplantation, GVHD is associated with alloimmunity, which occurs when naïve donor lymphocytes target mHAs such as H-Y antigens on normal host tissues in order to produce a combined humoral and cellular responses leading to significant morbidity and mortality [3–5].

Conversely, in kidney transplantation, kidney grafts from male donors to female recipients (M → F) experience increased rates of graft rejection [6]. The rationale behind this increase in graft rejection is that the recipient's lymphocytes develop an alloimmune response against the H-Y antigens present on the donor graft [7]. Finally, pregnant women with male fetuses may develop alloimmune response against these H-Y antigens. This is particularly important in the context of secondary recurrent miscarriage (SRM), defined as having three or more recurrent miscarriages after a successful birth [8].

In this review, we aim to explore the historical discovery of H-Y antigens as T- and B-cell alloimmune targets. We elucidate the clinical impact of H-Y alloimmunity in sex-mismatched HCT, organ transplantation and pregnancy.

H-Y alloimmunity in hematopoietic cell transplantation (HCT)

The first biological model of sex-mismatched transplantation tested skin graft rejection in mice. In the 1950s, Eichwald et al. first described that skin grafts from male donors to female recipients (M → F) had the highest rate of skin graft rejection among all gender combinations [9–12]. Eichwald et al. predicted that the female mice became sensitized to antigens encoded on the Y-chromosome, thus leading to graft rejection [12]. Further studies by Billingham showed that this effect could be prevented by tolerizing the females with injections of male donor cells into newborn females [13, 14]. These studies were the first to identify the importance of sex-mismatched transplants and led to the coining of the term “H-Y factor.”

Clinical studies of patients following HCT have found that F → M patients are between 1.5 and 4 times as likely to develop chronic graft-versus-host disease (cGVHD) in comparison to male recipients with male donors (M → M) [15–20]. Additionally, further studies have shown that male patients who receive allografts from female donors with high parity (more than two pregnancies) are more likely to develop cGVHD than male patients who receive allografts from nulliparous female donors (Table 1) [21].

Consistent with the mouse models above, the rationale is that lymphocytes from the female donor graft recognize several mHAs found on the Y-chromosome as foreign and mount an

adaptive immune response against these targets. H-Y antigens present on normal host tissue are attacked leading to detrimental GVHD.

However, H-Y antigens present on malignant cells are also attacked, leading to a graft-versus-leukemia (GVL) benefit, which explains why high H-Y alloimmunity is associated with both high levels of GVHD and low levels of relapse.

Discovery of H-Y antigens as T-cell targets

Considering that F → M patients were more likely to develop GVHD, scientists predicted that there were proteins encoded by genes on the Y-chromosome which triggered an immune response from the female-derived allograft. However, it was not until the 1970s when sex-mismatched models began to be applied to human HCT. In 1977, Goulmy et al. [22, 23] reported a case in which blood from an HLA-matched F → M patient who had experienced acute GVHD possessed HLA-A2-restricted cytotoxic T lymphocytes (CTLs) which only attacked male cells. Therefore, she demonstrated that GVHD occurred in sex-mismatched HLA-matched patients and discovered HLA-restricted CTLs against an unidentified mHA presumed to be encoded on the Y-chromosome [24, 25]. Furthermore, she isolated T-cell clones specific to five autosomal mHAs and used these to score for the presence and absence of mHA expression in HCT patients and donors. Her first clinical application showed that GVHD associates with mHA disparity, meaning that the mHA is expressed in the recipient, but was absent in the donor. Therefore, naïve donor T lymphocytes would react to mHA expression following HCT. As such, Goulmy was the first to demonstrate mHA alloimmunity in humans in association with GVHD [26, 27]. In 1995, the first H-Y antigen was biochemically identified from the protein lysine (K)-specific demethylase 5D (SMCY) [28]. The identification of this H-Y peptide sequence facilitated subsequent soluble presentation of the mHA in HLA, called tetramers. These fluorescently conjugated tetramers stain mHA-specific T cells, thereby quantifying them and facilitating their isolation. Clinical studies utilizing tetramers showed H-Y-specific CTLs develop following sex-mismatched transplantation in association with GVHD [29].

In the late 1990s, the completion of the human genome project provided the complete Y-chromosome sequence and identified nine mHA candidate genes that Page postulated may play a role in graft rejection, paving the way for the molecular characterization of H-Y antigens [30–32]. Thus far, six of these H-Y candidate genes have been shown to encode T-cell-detected antigens and six encode B-cell antigens (Table 2) [5, 28, 33–46].

Detection of H-Y antibodies and H-Y-specific allogeneic B cells

While H-Y antigens were first described as T-cell-specific targets, humoral H-Y immune responses have developed as a clinically useful measurement of alloimmunity. In fact, H-Y antigens have been shown to elicit a coordinated B-cell and T-cell response [5, 47, 48]. The utility of H-Y antibodies was first established in sex-mismatched HCT.

Alloantibodies were first detected by Miklos et al. studying F → M HCT targeting H-Y antigen DEAD box helicase 3, Y-linked (DBY) using enzyme-linked immunosorbent assay (ELISA). His study of 150 patients using H-Y ELISA found that H-Y antibodies are ten

times more frequent in F → M patients compared to M → M patients (50 % of F → M patients as opposed to only 5 % in M → M patients) [2].

Subsequently, clinical studies have shown that H-Y antibodies detected a year post-HCT associate with cGVHD development and long-term disease remission (Table 1) [49–51]. Characterizing 75 F → M patients, seropositivity against any of five H-Y antigens was significantly associated with cGVHD development (OR 56.5, $p < 0.0001$). Furthermore, none of the H-Y seropositive patients relapsed compared to 48 % relapse in H-Y seronegative patients [46].

Although ELISA could effectively characterize H-Y antibody responses, more sensitive technologies have been developed. Protein microarray technology, for example, facilitates high-throughput, ultra-sensitive, multiplexed antibody detection which is considered to be more sensitive than ELISA [52, 53]. As an example of serologic utility, Fig. 1 shows H-Y microarray detected DBY antibodies in 136 F → M HCT patients measured prospectively over 3 years. In this study, 51 % of F → M patients are DBY seropositive at some point post-HCT (Fig. 1a). DBY antibody is absent 2 months post-HCT and detected in 19 % of F → M patients at 3 months post-HCT and further develops in association with cGVHD onset (Fig. 1b) (Nakasone Unpublished Data).

Microarray technology has also led to the discovery of immune-dominant epitopes through the multiplexed testing of “tiled” overlapping peptides across multiple H-Y antigens. This led to the discovery of the 18 amino acid DBY-2 peptide as an immune-dominant epitope. Using fluorescence-activated cell sorting (FACS), this epitope was used to identify H-Y-specific B cells. Identifying H-Y-specific B cells should provide earlier alloimmunity detection relative to H-Y antibodies. For example, a prospective analysis of DBY-2-specific B cells in a study of 28 F → M patients confirmed that allogeneic B cells preceded cGVHD development [54].

The identification of allogeneic B-cell responses in association with cGVHD supported the use of in vivo B-cell depletion for cGVHD prevention and treatment. In vivo B-cell depletion has been safely accomplished with rituximab, a humanized monoclonal antibody against B-surface antigen CD20. Clinical trials using rituximab have confirmed that B-cell depletion therapy is both effective cGVHD therapy and prophylaxis [55–59].

In the field of HCT, measured alloimmune response to H-Y antigens has been an important biomarker associated with significant clinical outcomes including cGVHD and disease relapse. While there are a variety of autosomal mHAs which have been shown to be targeted by lymphocytes, their low disparity rates limit their clinical usefulness [60, 61].

From T cells to antibodies and now B cells, the targets of H-Y alloimmunity have been molecularly identified allowing for their disease impact assessment (Table 3). Therefore, H-Y antigens remain the most powerful model to better understand the effect of alloimmunity in HCT.

Parity in HCT donors

Mothers with previous male births may become sensitized to the male fetus and develop H-Y alloimmunity leading to subsequent miscarriages. Studies have shown the persistence of H-Y-specific T cells in parous female donors who had given birth to boys many years previously [62, 63]. For example, a study found H-Y-specific CTLs in 50 % of female donors with multiple male pregnancies [64]. These studies support the notion that multiparous females are likely to induce an alloimmune H-Y response with an impact on pregnancy and transplantation. Thus, female donors with high parity may put male recipients at higher risk of GVHD due to H-Y alloimmunity (Table 1).

Consistent with the H-Y hypothesis, studies have shown that F → M patients with multiparous female donors have a higher rate of cGVHD and a lower rate of relapse relative to nulliparous female donors [20, 21]. However, it is important to note that there is still a much larger difference in outcomes between F → M patients with nulliparous female donors and M → M patients than F → M patients with nulliparous female donors and F → M patients with multiparous female donors, thus suggesting that the large naïve repertoire due to gender disparity plays a larger role in GVHD development than H-Y sensitization from pregnancy. Ultimately, studies measuring H-Y antibodies and T cells found within multiparous female donors are necessary to characterize adoptive H-Y alloimmunity and its clinical impact.

H-Y alloimmunity in kidney transplantation

HLA-matched kidney transplants survive longer on average than mismatched ones [65]. Studies have shown that donor-specific antibodies (DSA) including anti-HLA antibodies associate with both acute and chronic kidney rejection [66–70]. When HLA-matched organs are transplanted, significant levels of kidney graft rejection still occur [71]. For example, in an analysis of the UNOS Renal Transplant Registry, Terasaki estimated that 38 % of the kidney failures in the transplant registry were due to non-HLA immunogenic factors [72, 73]. This analysis supports the conclusion that kidney rejections in the presence of HLA-matched donors most likely represented other immunologic factors, including mHAs. Furthermore, biopsies of kidneys undergoing rejection show high levels of C4d, a component of the complement cascade, thus implicating pathogenic B cells and antibodies in kidney graft rejection [74–76]. Not surprisingly, H-Y alloimmunity is believed to contribute to kidney graft rejection in HLA-matched, sex-mismatched patients.

Sex-mismatch kidney transplantation and kidney graft rejection

Although the mechanism for increased graft rejection is not fully understood, the H-Y model suggests that host lymphocytes from the female recognize several mHAs such as H-Y proteins on the male graft as foreign, thus leading to decreased engraftment and increased rates of kidney graft rejection. As opposed to F → M HCT in which a functional immune system from the female donor is transferred into the male recipient, M → F solid organ transplantation involves the transfer of an immune system target (the kidney graft) from the male host to the female recipient, which explains why M → F patients were found to have higher rates of kidney graft rejection.

Unlike HCT where most studies agree that F → M transplants result in more cGVHD, the impact of sex mismatch in organ transplantation is less clear. While some studies have shown that M → F patients have increased rates of both acute and chronic graft failure [6], others have only shown significantly increased rates of acute graft failure [77–79] or no difference at all [80]. Although there are many risk factors responsible for kidney failure, these studies suggest that alloimmunity against mHAs such as H-Y antigens may be a major cause of rejection.

H-Y antibodies and acute kidney rejection

Some researchers have investigated H-Y antigens as a potential risk factor for the development of acute kidney failure post-transplant. In a study of 26 M → F kidney transplant patients, it was shown that 54 % of these patients developed H-Y antibodies, higher than any other gender combination ($p < 0.001$). Furthermore, 92 % of these M → F patients developed acute rejection compared to 21 % of those who were H-Y seronegative. The study also showed that those who developed H-Y antibodies were found to have higher amount of plasma cell infiltrates on renal biopsy, thus further implicating B-cell pathogenesis [7].

Similar to studies in kidney transplantation, sex mismatch in other solid organ transplantation has shown mixed clinical outcomes. However, there does tend to be an increased risk of graft rejection in cardiac transplantation in particular [81–84] and limited evidence for increases in chronic liver rejection in sex-mismatched patients [85].

H-Y alloimmunity in secondary recurrent miscarriage

Pregnancy is a natural phenomenon which has very interesting qualities in regard to immunology. Considering that a fetus is only half identical to the mother, it is reasonable to interpret pregnancy as a time when a haploidentical fetus resides in the mother's body [86, 87]. Consequentially, pregnancy has become an intriguing field for the study of H-Y alloimmunity. In particular, H-Y alloimmunity has provided one potential explanation for secondary recurrent miscarriage (SRM).

SRM is defined as three or more recurrent miscarriages after a successful birth. It is important to contrast SRM from primary recurrent miscarriage (PRM), in which three or more miscarriages occur without a preceding successful birth. PRMs are believed to be caused primarily by chromosomal abnormalities along with problems with proper implantation of the embryo, while SRMs are more likely to be the result of immunological causes [8].

Interestingly, most SRM patients initially give birth to a boy prior to the recurrent miscarriages [88]. Furthermore, mothers who initially gave birth to boys are also more likely to experience infertility and future miscarriages [89]. In fact, studies have shown that the sex ratio [defined as the ratio of male/female (M/F) births] in SRM patients is shifted toward a significantly higher M/F sex ratio prior to SRM and then shifted to a lower M/F sex ratio for successful births subsequent to SRM [90]. For example, in a 20-year cohort study of

unexplained SRMs, the sex ratio for SRM was 1.49 previous to miscarriages and 0.76 in births subsequent to miscarriages [91].

The H-Y hypothesis suggests that, during pregnancy with a male fetus, the mother's immune system develops an alloimmune response against these H-Y antigens, which would harm future male fetuses and potentially contribute to future miscarriages [8, 92, 93].

H-Y antibodies, along with the presence of H-Y restricted HLA alleles, have been associated with the development of SRM along with other pregnancy complications [90, 94–96]. One study of 84 SRM patients found that H-Y-specific antibodies were present in a significantly higher percentage of SRM patients (46 %) compared to female controls (19 %). This study also found that H-Y seropositive patients only delivered boys 12 % of the time compared to 44 % of the time in H-Y seronegative patients [97]. Thus, measured H-Y seropositivity may be preventing implantation or successful gestation of the male fetus.

Consistent with the idea that H-Y antibodies might be responsible for SRM, intravenous immunoglobulin (IVIG) infusion, a treatment used normally to neutralize circulating antibodies, has been shown to increase the birth rate in SRM patients [98–100] while others have failed to show a significant difference [101]. Nevertheless, immunosuppressive therapies continue to be investigated in this patient population.

Many investigations aimed to understand why only some mothers with previous male births develop H-Y antibodies and others do not [93]. Clinical trials implicate both regulatory T cells and CTLs to be involved in developing tolerance to mHA [62, 64, 102–104]. Murine studies have shown that depletion of H-Y-specific regulatory T cells in pregnant mice resulted in rejection of male fetuses [104].

Future of H-Y alloimmunity

Although H-Y antigens were first discovered in the 1970s, ongoing studies continue to identify clinically important T-and B-cell epitopes. A contemporary challenge in understanding H-Y alloimmunity is determining the progression and coordination of T- and B-cell alloimmunity. These adaptive immune responses are now being directly measured using next-generation high-throughput sequencing of the B- and T-cell receptors.

The recent identification of immune-dominant B-cell epitopes now makes it possible to use FACS to sort H-Y antigen-specific B cells for functional studies. This is analogous to the use of mHA tetramers to isolate and characterize H-Y-specific T cells in the late 1990s. The combination of antigen-specific cell sorting and immune receptor high-throughput sequencing is going to allow us to detail the evolution of adaptive alloimmunity (Fig. 2).

With the advent of these new technologies, there is potential to advance our understanding of alloimmunity and improve our ability to predict alloimmune clinical phenomenon in transplantation and pregnancy, thus allowing for more effective immune modulation therapies. Furthermore, these same technologies hold the promise to help us understand more about the various other mHAs implicated in alloimmunity in both sex-matched and sex-mismatched transplantation.

Since the study of murine skin graft rejection in the 1950s, H-Y antigens have provided an essential model for studying alloimmunity in a variety of clinical settings. As discussed in this review, our understanding of H-Y alloimmunity has progressed significantly from their initial discovery as T-cell-specific targets. Furthermore, H-Y alloimmunity has expanded beyond HCT to solid organ transplantation and pregnancy and will continue to be the driving model of clinical alloimmunity.

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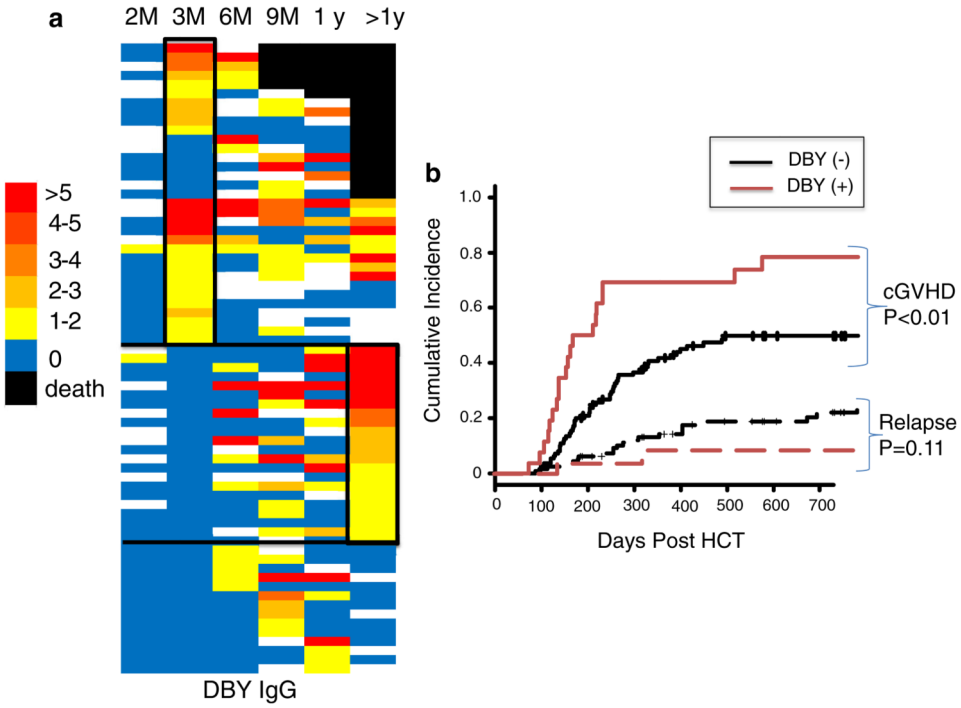

Fig. 1.

DBY seropositivity measured 3 months post-HCT predicts cGVHD development. **a** Heatmap representation of IgG specific for H-Y antigen DBY in 69 seropositive F → M patients. 67 seronegative patients are not shown. Overall, DBY seropositivity at 3 months was 19 and 51 % at any time point following HCT. *Each row* represents the results of a separate patient with the time of serum collection shown on the *x-axis*. The threshold for seropositivity was determined measuring 60 normal male donors. The heat reflects relative DBY antibody level relative to this threshold. Importantly, patient death is denoted by *black*, and missing samples are *white*. **b** The competing incidence of cGVHD development is greater in DBY seropositive patients than in DBY seronegative patients ($p < 0.01$)

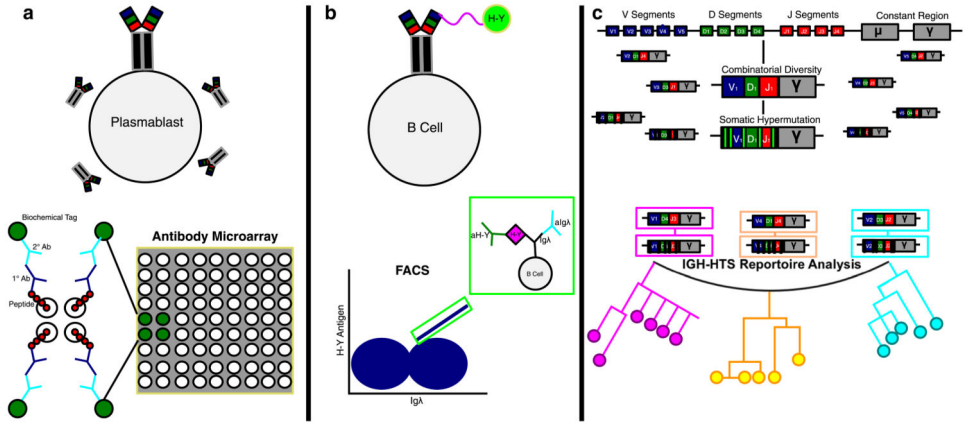

Fig. 2.

Methods for studying H-Y humoral alloimmunity. **a** H-Y-specific alloantibodies are quantified by protein microarray. Their immune-dominant epitopes are identified using overlapping peptides from H-Y proteins. **b** These immune-dominant peptides facilitate the identification of H-Y-specific B cells by fluorescence-activated cell sorting (FACS). **c** The B-cell receptor-binding specificity can be further elucidated by high-throughput sequencing of the immunoglobulin chain which can be used to characterize both heavy- and light-chain repertoire. The evolution of the changes in immunoglobin clonotypes over time is represented by the phylogeny tree

Table 1
Clinical findings in sex-mismatch hematopoietic cell transplantation (HCT)

Reference	Significant results	Conclusions
Carlens et al. [4] n = 451 (75 F → M)	cGVHD developed in 61 % of F → M patients compared to 40 % in all others (RR 1.70, p = 0.006)	cGVHD increased in F → M patients
Kollman et al. [20] n = 6,978 (1,543 F → M)	Registry study showing cGVHD in 54 % of patients with multiparous female donors versus 44 % in male donors (HR 1.40, p < 0.0001)	Donor parity increases the risk of cGVHD
Randolph et al. [19] n = 3,238 (858 F → M)	Increased cGVHD in F → M HCT (RR 1.56, p < 0.0001) Decreased relapse in F → M HCT (RR 0.70, p < 0.0003) compared to M → M patients CML-restricted analysis showed F → M benefit in relapse (RR 0.68, p = 0.04)	Large study showing cGVHD risk is specific to F → M HCT GVL benefit confirmed through the use of single-disease analysis (CML)
Loren et al. [21] n = 2,626 (449 F → M)	Donor parity increases cGVHD HR1.00 M → M HR 1.44 Nulliparous F → M HR 1.56 Parous F → M Donor parity decreases relapse: HR 1.00 M → M HR 0.75 Parous F → M	Female donor sensitization by prior pregnancies leads to increased adoptive alloimmunity

cGVHD chronic graft-versus-host disease, *F → M* graft from female donor to male recipient, *RR* relative risk, *HR* hazard ratio, *GVL* graft-versus-leukemia benefit, *CML* chronic myelogenous leukemia

Table 2
Selection of H-Y antigens discovered as T-cell targets

H-Y antigen	HLA restriction	mHA sequence	Reference
DDX3Y (DBY)	DQB1*05	HIENFSDIDMGE	Vogt [38]
	B*2705	SRDSRGKPGY	Rosinski [40]
	DRB1*1501	SKGRYIPPHLR	Porcheray [47]
USP9Y (DFFRY)	A*0101	IVDCLTEMY	Pierce [39]
			Vogt [36]
KDM5D (SMCY)	B*0702	SPSVDKARAEL	Wang [28]
	A*0201	FIDSYICQV	Ofran [43]
RPS4Y	DRB3*0301	VIKVNDTVQI	Spierings [41]
	B*5201	TIRYPDPVI	Ivanov [44]
	DRB1*07	TGKIINFIKFDTGNL	Eljaafari [33]
TMSB4Y UTY	A*3303	EVLLRPGLHFR	Torikai [42]
	B*60	RESEEESVSL	Vogt [37]
	B*8	LPHNHTDL	Warren [34]
	A*2402	YYNAFHWAI	Mortensen [45]

mHA minor histocompatibility antigen

Table 3
Significant studies regarding discovery of T-cell and B-cell response to H-Y antigens

Significant result	Conclusion	References
H-Y factor		
Skin graft rejection found only in M → F mice	Hypothesized "H-Y" factor on Y-chromosome that led to female sensitization	Eichwald et al. [9]
T-cell response		
HLA-matched F → M patient who had experienced acute GVHD possessed HLA A2-restricted CTLs against unknown mHA	First demonstration of sex-mismatched CTL against unknown H-Y antigen	Goulmy et al. [22]
Isolated and sequenced the first H-Y antigen from SMCY using CTL identification	Biochemical identification of first H-Y antigen	Wang et al. [28]
Quantification of HLA-A2-specific T cells in patient with GVHD using SMCY-restricted tetramers	Tetramer complex allowed visualization of H-Y-specific CTL and showed association between H-Y-specific CTL and GVHD	Mutis et al. [29]
B-cell response		
50 % of F → M patients found to be seropositive for the H-Y antigen DBY	F → M patients develop H-Y-specific antibodies post-HCT	Miklos et al. [2]
F → M patient with cGVHD found to have both CTL and antibodies specific for DBY	First demonstration of a coordinated B-cell and T-cell response against H-Y antigens in the context of cGVHD	Zorn et al. [5]
H-Y seropositivity associated with increased cGVHD in a clinical study of 75 F → M patients.	H-Y antibodies in F → M patients demonstrate both increased cGVHD and decreased relapse	Miklos et al. [46]
Quantification and isolation of H-Y-specific B cells using immune-dominant epitope DBY-2	DBY-2-specific B cells are detected in 50 % of F → M patients and nearly all develop cGVHD	Sahaf et al. [54]

M → F graft from male donor to female recipient, *F → M* graft from female donor to male recipient, *GVHD* graft-versus-host disease, *mHA* minor histocompatibility antigen, *CTL* cytotoxic T lymphocyte, *HCT* hematopoietic cell transplantation