10th International
Summer School on Immunogenetics

Stintino | Sardinia | Italy

15th - 18th September 2013

Minor Histocompatibility Antigens in Transplantation

Marco Andreani
Laboratory of Immunogenetics and Transplant Biology (LIBT) - IME Foundation

Minor Histocompatibility Antigens in Transplantation

Background

HSCT and acute GvHD

Minor Histocompatibility Antigens

Characteristics

Role in Transplantation

Our data in Thalassemia

Minor Histocompatibility Antigens in Transplantation

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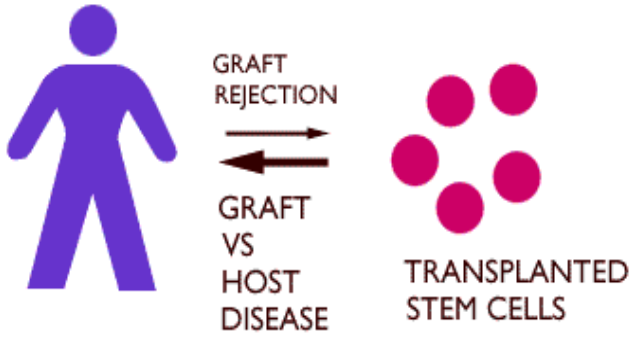
Minor Histocompatibility Antigens in Transplantation

Success following HCT is ultimately determined by the ability to achieve sustained engraftment and eradication of abnormal or malignant host cells



avoiding reactions influenced by the nature and extent of the genetic disparity between donor and recipient such as:

if possible/necessary to obtain a GvL effect

Clinical features of Acute GVHD

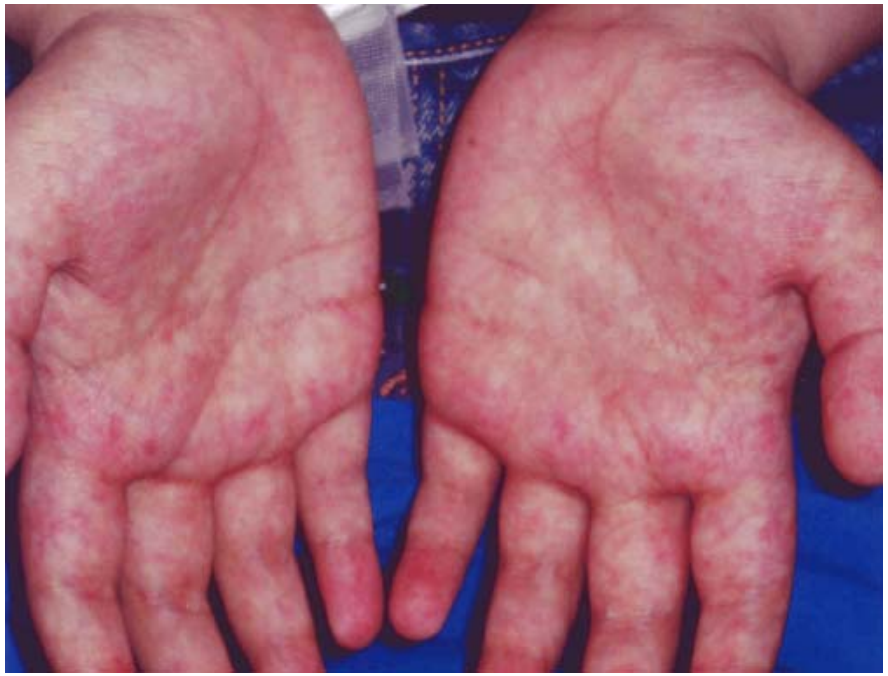
Acute GVHD was defined to occur prior to day 100, whereas chronic GVHD occurred after that time.

The clinical manifestations of acute GVHD occur in the skin, gastrointestinal tract and liver.

81% skin involvement

54% GI involvement

50% liver involvement



James L.M. Ferrara, Lancet. 2009 May 2; 373(9674): 1550–1561

Pathophysiology of Acute GVHD

Two principles are important to consider regarding the pathophysiology of acute GVHD:

Acute GVHD reflects **exaggerated but normal inflammatory mechanisms mediated by donor lymphocytes** infused into the recipient

Second, the **recipient tissues** that stimulate donor lymphocytes **have usually been damaged** by underlying disease, prior infections, and the transplant conditioning regimen.

Pathophysiology of Acute GVHD

The development of acute GVHD can be summarized in three sequential steps or phases:

1 - activation of the APCs;

2 - donor T cell activation, proliferation, differentiation and migration;

3 - target tissue destruction

Pathophysiology of Acute GVHD

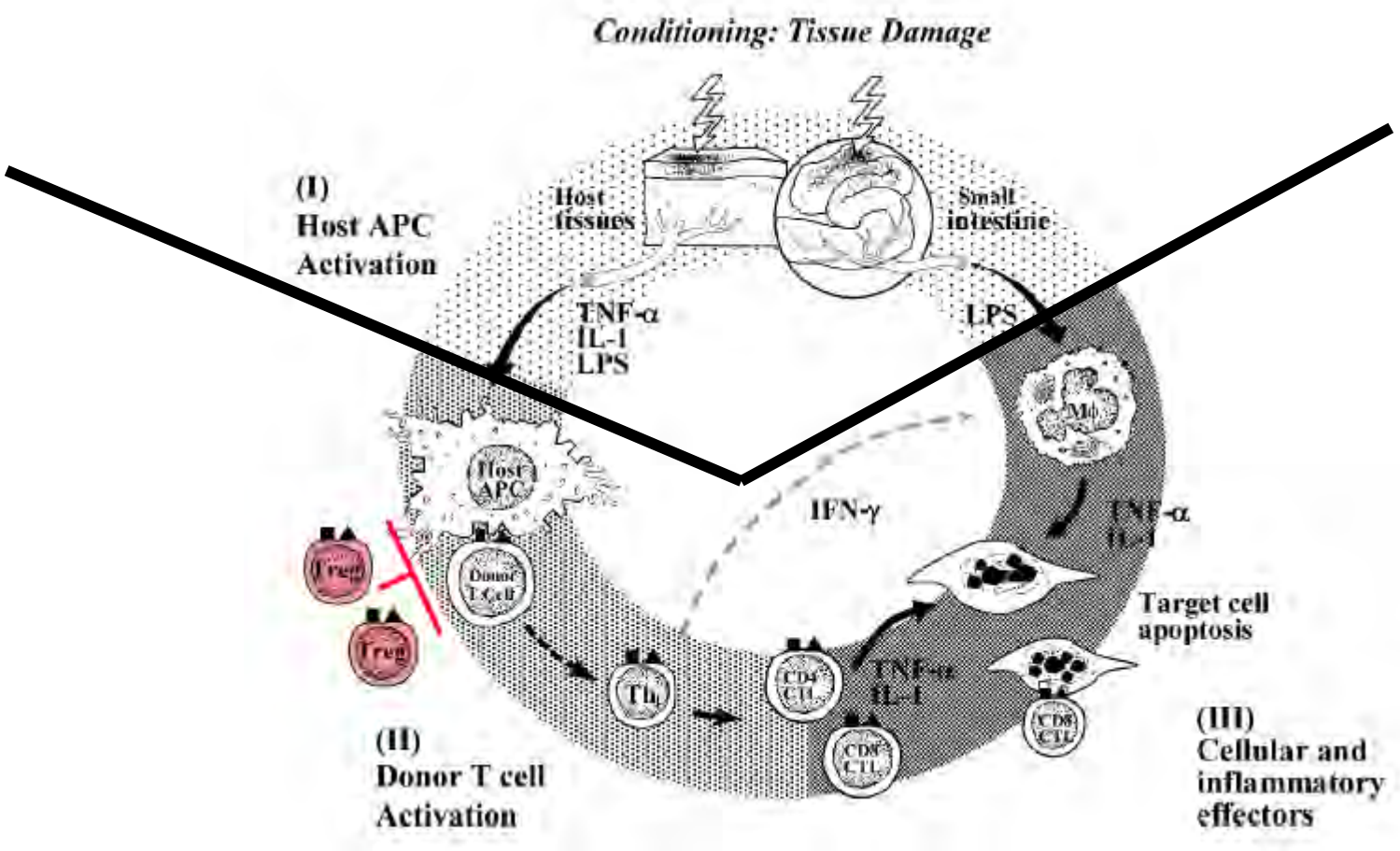
Based largely on experimental models, the development of acute GVHD can be conceptualized in three sequential steps or phases:

1 - activation of the APCs;

2 - donor T cell activation, proliferation, differentiation and migration;

3 - target tissue destruction.

Pathophysiology of Acute GVHD



In Phase I the **recipient conditioning regimen damages** host tissues and causes **release of inflammatory cytokines** such as TNFα, IL-1 and IL-6. Increased levels of these cytokines leads to activation of host antigen presenting cells (APCs)

James L.M. Ferrara, Lancet. 2009 May 2; 373(9674): 1550–1561

Pathophysiology of Acute GVHD

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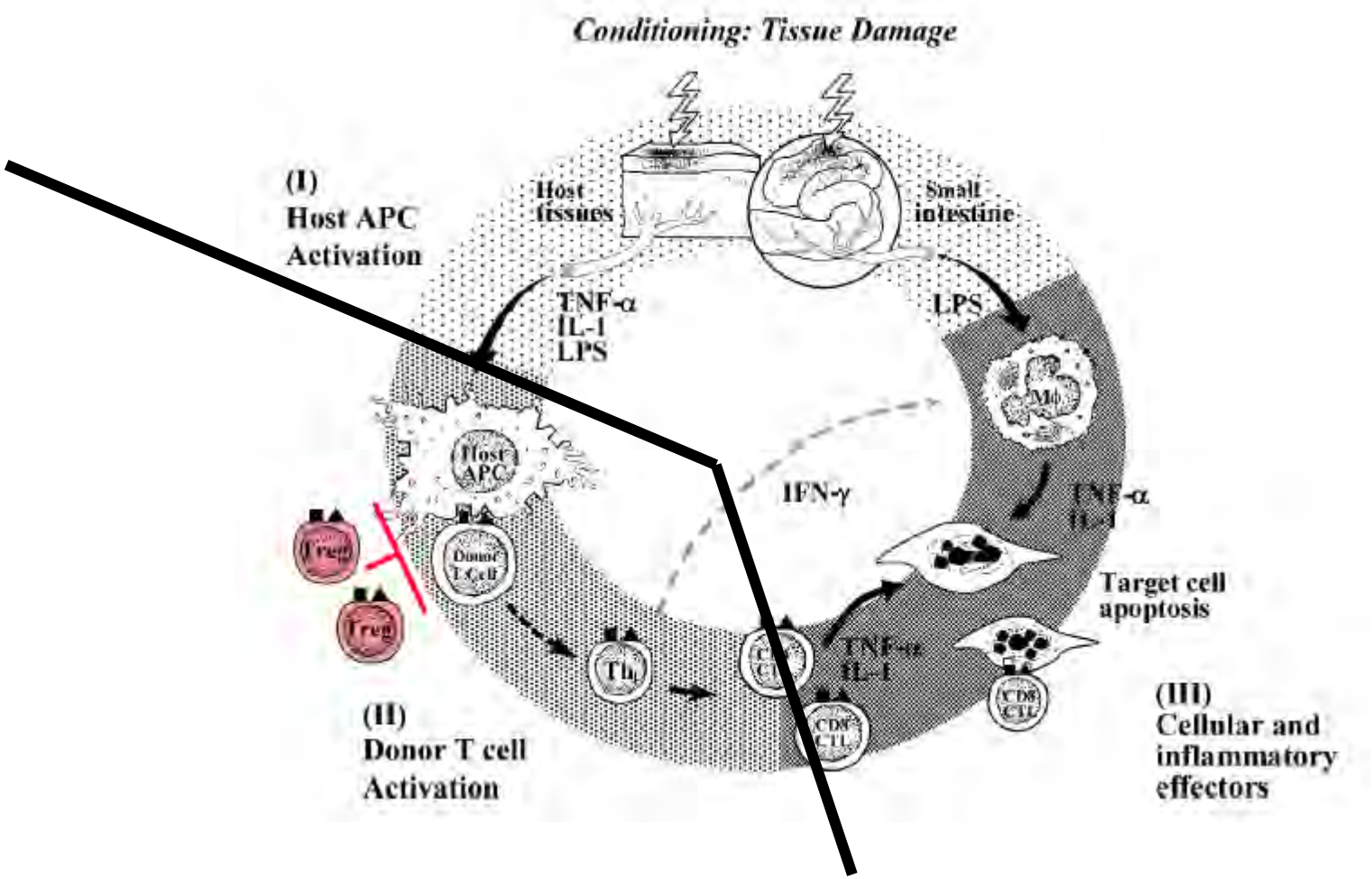
Pathophysiology of Acute GVHD



In Phase II, host APCs activate mature donor cells

James L.M. Ferrara, Lancet. 2009 May 2; 373(9674): 1550–1561

Pathophysiology of Acute GVHD

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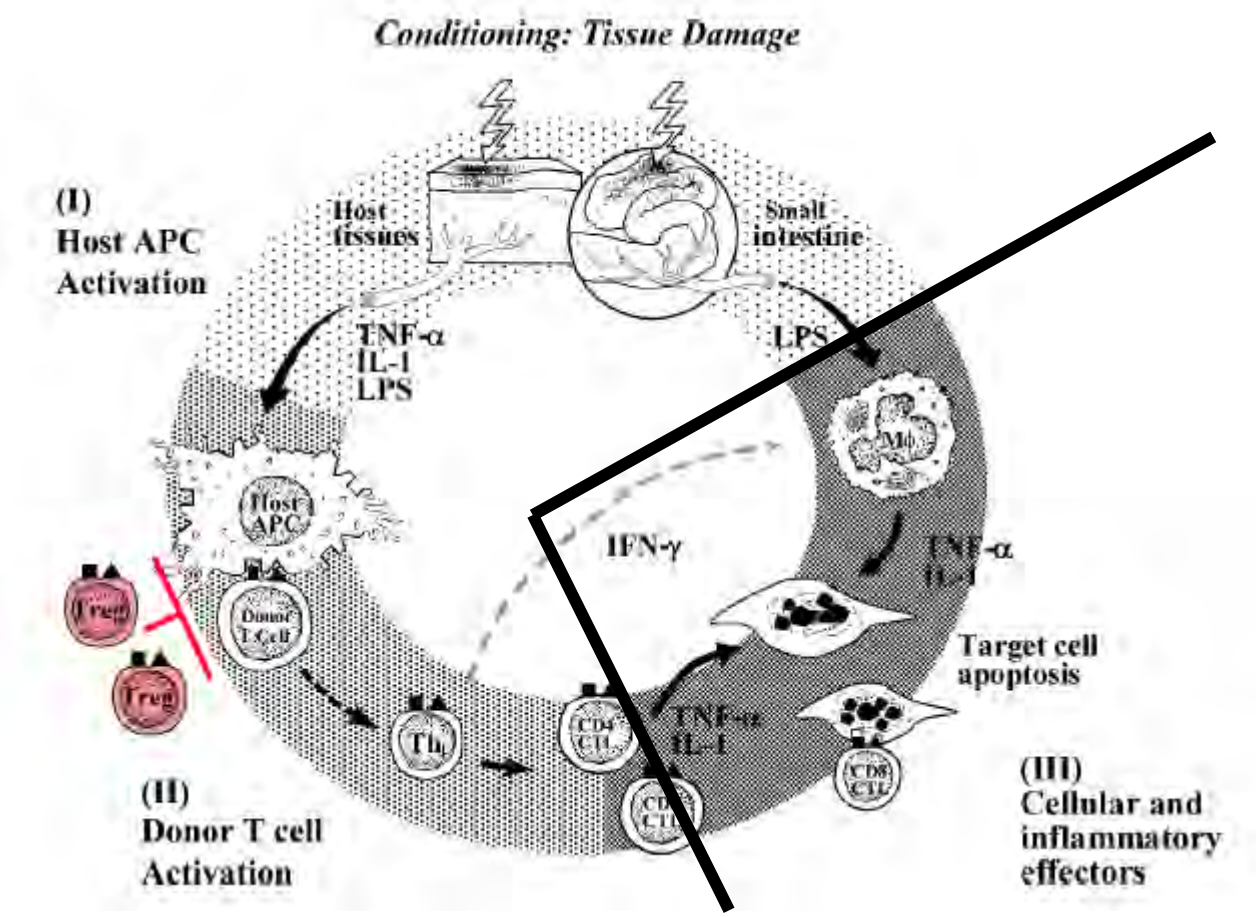
Pathophysiology of Acute GVHD



The subsequent **proliferation and differentiation of activated donor T cells** produces additional effectors that mediate the tissue damage, including Cytotoxic T Lymphocytes, Natural Killer (NK) cells, TNFα and IL-1

James L.M. Ferrara, Lancet. 2009 May 2; 373(9674): 1550–1561

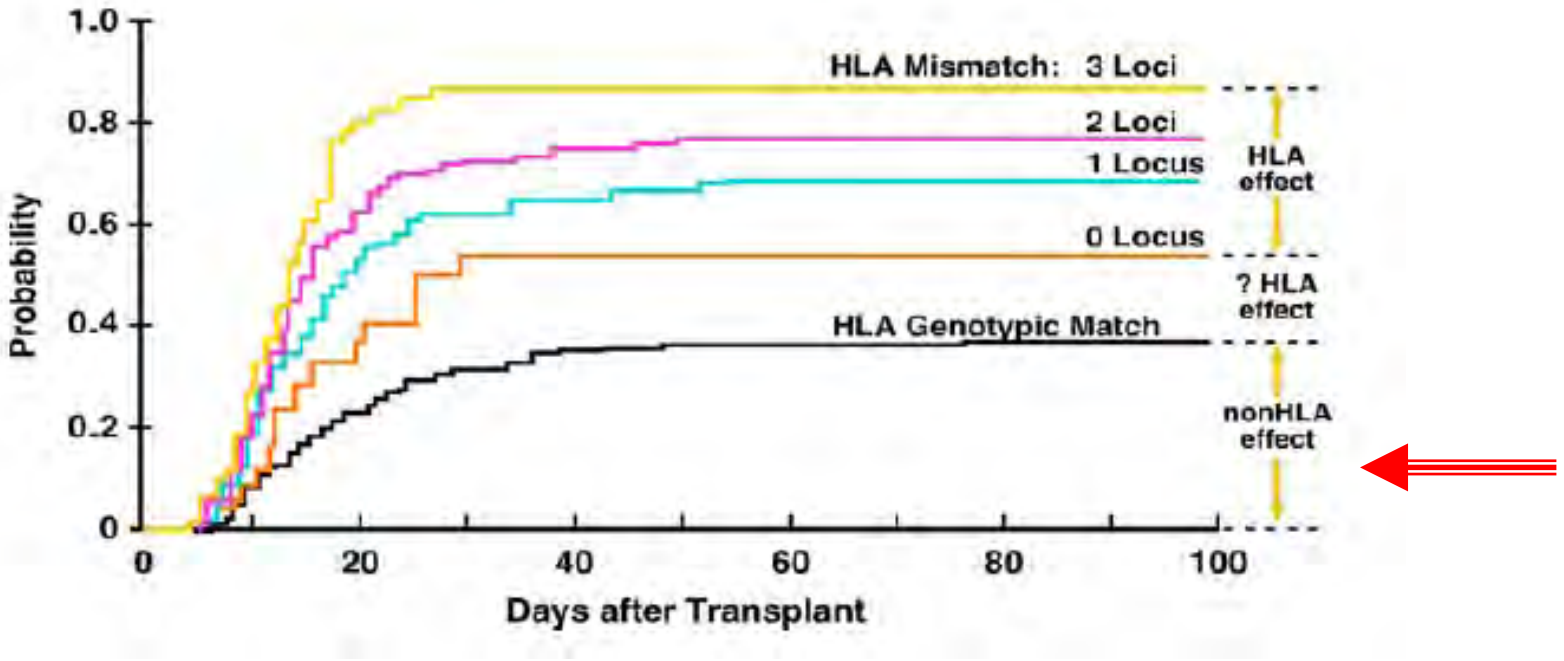
HLA disparities between donor and recipient are risk factors associated with GvHD

Donor T-cell activation occurs when **donor T cells respond to genetically defined proteins** on host cells

The most important proteins are Human Leukocyte Antigens (HLA) which are highly polymorphic and are encoded by the major histocompatibility complex (MHC)

It is well known that HLA matching reduces, but does not prevent the development of graft versus host disease

HLA disparities between donor and recipient are risk factors associated with GvHD



J. A. Hansen et al. Immunol Res 2008

Despite HLA identity between a patient and donor, approximately 30% of patients receiving HLA-identical grafts develop acute GVHD due to genetic differences that lie outside the HLA loci.

Non-HLA Genetics as risk factors associated with GvHD

Many other genetic factors, besides HLA, are involved in GvHD occurrence.

Polymorphisms in both donors and recipients for cytokines

- Tumor Necrosis Factor (TNF)-α,
- Interleukin 10 (IL-10),
- Interferon-γ (IFNγ)

Genetic polymorphisms of proteins involved in innate immunity

Nucleotide oligomerization domain 2

Keratin 18 receptors

HLA-G 14 bp transcript presence

KIR receptors and ligands

Minor histocompatibility antigens (mHAgs)

Minor Histocompatibility Antigens in Transplantation

Background

- HSCT and acute GvHD

Minor Histocompatibility Antigens

- Characteristics
- Role in Transplantation
- Our data in Thalassemia

Minor Histocompatibility Antigens in Transplantation

mHAg are peptides derived from allelic variants of normal cellular proteins

mHAg are generally HLA restricted

some allelic variants has **high affinity** for specific HLA loci

some allelic variants has **low affinity** for specific HLA loci

Minor Histocompatibility Antigens in Transplantation

These protein/peptide variants most often arise due to single nucleotide polymorphisms (SNPs) or deletions

example:

HA-1 exists in 2 allelic form:

R: no immunogenic – **H**: immunogenic

HA-1^{R}

GTG TTG C**G**T GAC GAC CTC CTT GAG GCC

*V L **R** D D L L E A*

HA-1^{H}

GTG CTG C**A**T GAC GAC CTC CTT GAG GCC

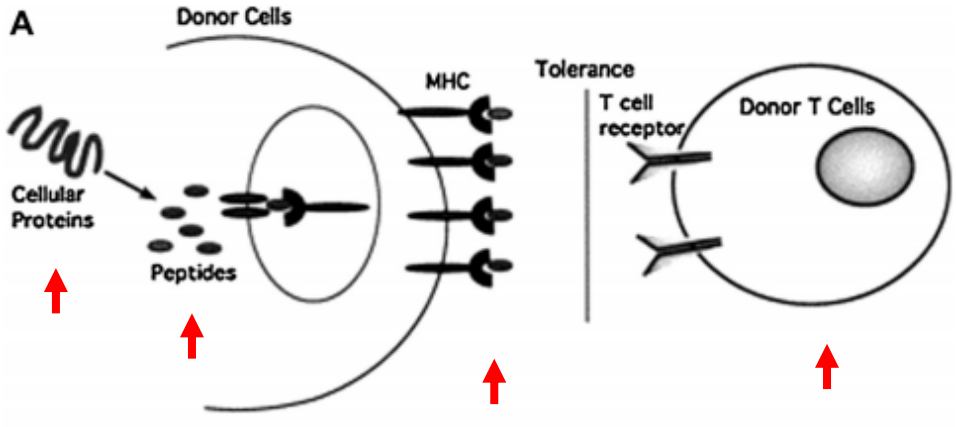
*V L **H** D D L L E A*

(M.Wilke et al. Tissue Antigens 1998)

Minor Histocompatibility Antigens in Transplantation

When presented by self class I or II MHC antigens, the immunogenic variant induces cellular immune responses in HLA-matched individuals lacking the same allelic variant

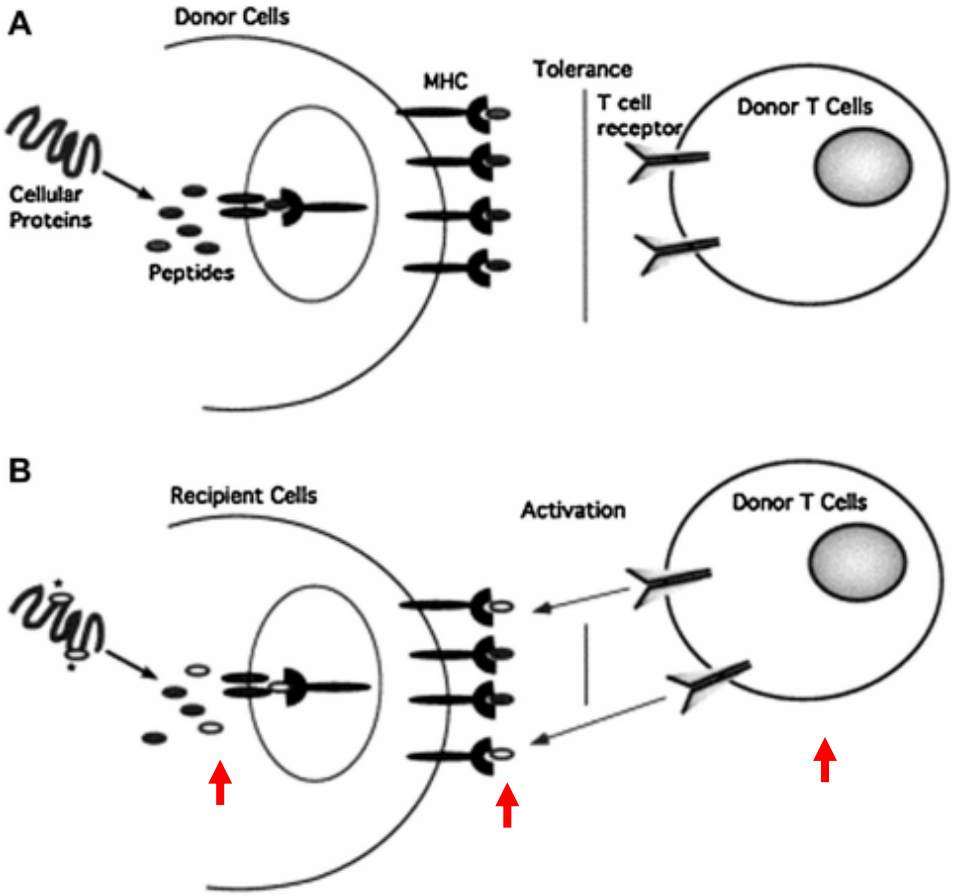
Minor Histocompatibility Antigens in Transplantation



A - Peptides derived from cellular proteins are displayed on the surface of cells complexed to MHC molecules and autologous T cells are tolerant to these self-peptides

Rezvani and Storb, J Autoimmunity 2008

Minor Histocompatibility Antigens in Transplantation



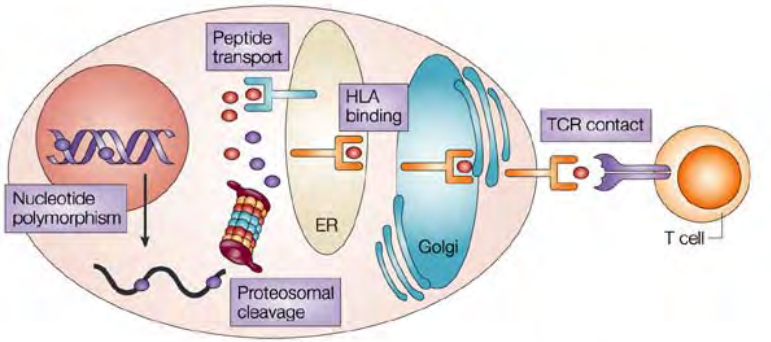
A - Peptides derived from cellular proteins are displayed on the surface of cells complexed to MHC molecules and autologous T cells are tolerant to these self-peptides

B - After processing T cells of the donor will recognize the unique peptides on recipient cells as foreigner

Rezvani and Storb, J Autoimmunity 2008

Minor Histocompatibility Antigens in Transplantation

Cytotoxic T lymphocytes directed againts mHAgs have been isolated from recipients of HLA-matched transplants with aGVHD….



Marie Bleakley and Stanley R. Riddell - NATURE REVIEWS | CANCER VOLUME 4 | MAY 2004 | **371**

….and cytotoxic T cell clones from such patients have been used to identify and characterize mHAgs

Minor Histocompatibility Antigens in Transplantation

There likely exist thousands of protein variants with the potential of functioning as mHAgs

However, only about 2 dozen human mHAg have been identified and mostly extensive studied

Some mHAg are ubiquitously expressed:

- HA-3, HA-8

Most of the mHAg have more restricted tissue expression

- HA-1, HA-2 in hematopoietic tissue
- CD31 in platelets and endothelial cells
- HB-1 in B lymphoblastoid cells

May be present both in autosomal or sex chromosomes

List of mHAgs located in autosomal chromosomes

mHGgs	HLA restriction	Peptide sequence	Tissue distribution
HA-1	HLA-A *02 HLA-B *60	**VLHDDLLEA**	haematopoietic
HA-2	HLA-A *02	**YIGEVLVSV**	haematopoietic
HA-3	HLA-A *01	**VTEPGTAQY**	broad
HA-8	HLA-A *02	**RTLDKVLEV**	broad
HB-1	HLA-B *44	**EEKRGSLHVW**	haematopoietic
ADIR	HLA-A *02	**SVAPALALFPA**	broad
ACC-1	HLA-A *24	**DYLQYVLQI**	haematopoietic
ACC-2	HLA-B *44	**KEFEDDIINW**	haematopoietic
CTSH	HLA-A *31	**ATLPLLCAR**	broad
ECGF1	HLA-B *07	**RPHAIRRPLAL**	haematopoietic
LRH-1	HLA-B *07	**TPNQRQNVC**	haematopoietic
PANE-1	HLA-A *03	**RVWDLPGVLK**	haematopoietic
SP1 10	HLA-A *03	**SLPRGTSTPK**	haematopoietic
UGT2B17	HLA-A *29 HLA-B *44	**AELLNIPFLY**	broad

List of mHAgs located in sex chromosomes

mHGgs	HLA restriction	Peptide sequence	Tissue distribution
DRBY	HLA-DQ5	**HIENFSDIDMGE**	haematopoietic
	HLA-DRB1 *1501	**ASTASKGRYIPPHLRN KEA**	
	HLA-B *2705	**SRDSRGKPGY**	
DFFRY	HLA-A*01:01	**IVDCLTEMY**	broad
RPS4Y	HLA-DRB3 *0301	**VIKVNDTVQI**	broad
	HLA-B *5201	**TIRYPDPVI**	
SMCY	HLA-B *0702	**SPSVDKARAEL**	broad
	HLA-A *0201	**FIDSYICQV**	
TMSB4Y	HLA-A *3303	**EVLLRPGLHFR**	broad
UTY	HLA-B *08	**LPHNHTDL**	broad
	HLA-B *60	**RESEEESVSL**	

blood

2009 113: 5041-5048
Prepublished online September 22, 2008;
doi:10.1182/blood-2008-07-171678

HapMap scanning of novel human minor histocompatibility antigens

Michi Kamei, Yasuhito Nannya, Hiroki Torikai, Takakazu Kawase, Kenjiro Taura, Yoshihiro Inamoto, Taro Takahashi, Makoto Yazaki, Satoko Morishima, Kunio Tsujimura, Koichi Miyamura, Tetsuya Ito, Hajime Togari, Stanley R. Riddell, Yoshihisa Kodera, Yasuo Morishima, Toshitada Takahashi, Kiyotaka Kuzushima, Seishi Ogawa and Yoshiki Akatsuka

Kamei introduced an innovative approach for identifying the genes that encode novel T cell-defined human minor histocompatibility antigens (mHags)

Minor Histocompatibility Antigens in Transplantation

Introduction

Acute GvHD: clinical aspects and physiopathology

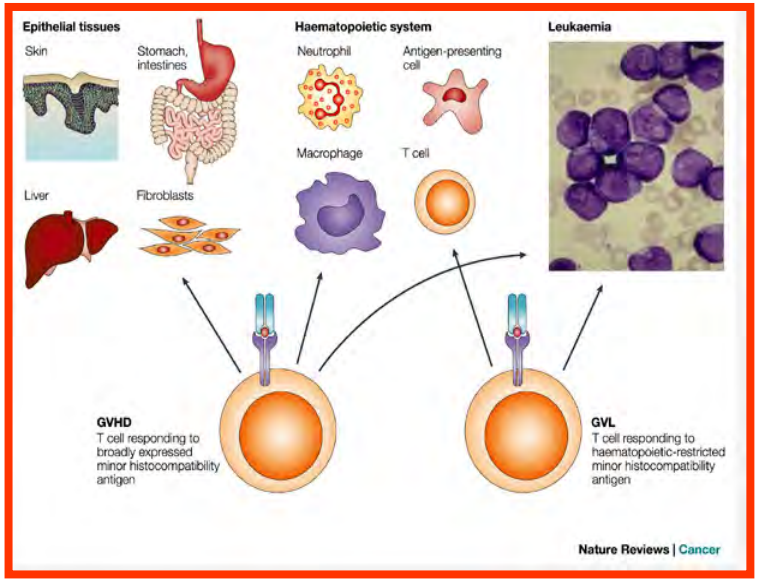
Minor Histocompatibility Antigens

Characteristics

Role in Transplantation

Our data in Thalassemia

Minor Histocompatibility Antigens in Transplantation



Potential role in GvHD:

Presence of **Donor T cells** specific for mHAgs broadly **expressed by both haematopoietic and epithelial cells** of the recipient

Potential role in GvL:

Presence of **Donor T cells** specific also to the graft-versus leukaemia effect if **leukaemic cells express** the minor histocompatibility antigens

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Minor Histocompatibility Antigens in Transplantation

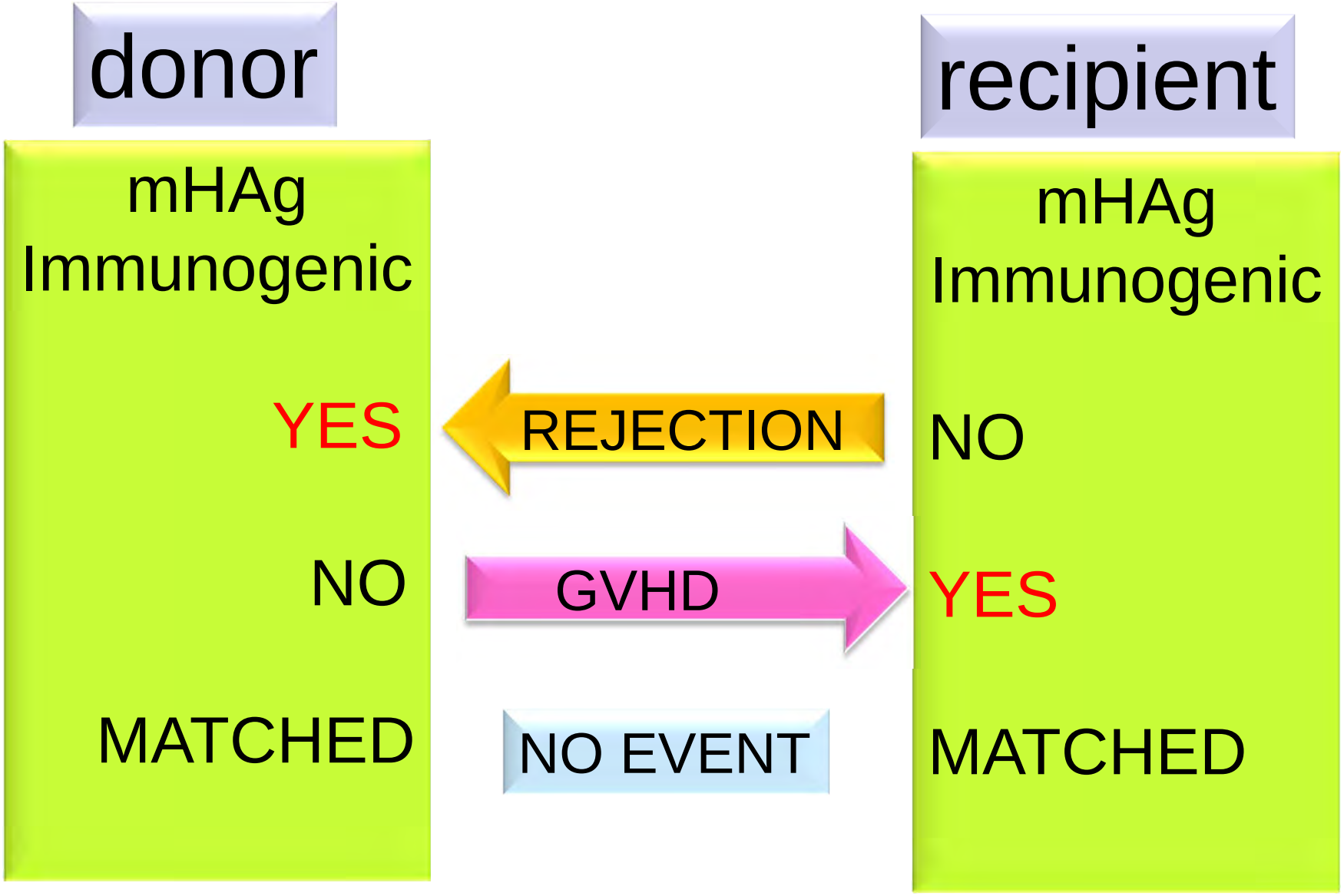
To be considered in the analysis:

HLA restriction

the direction of the mHAg mismatch

the distribution of mHAg : broad or tissue restricted

Minor Histocompatibility Antigens in Transplantation



Perform a specific Software mHAg analysis

Assessment of the theoretical potential for mismatches mHAgs, through the analysis with a specific software made available online by the University of Leiden, in the clinical course post-transplant

DdMinor: http://www.lumc.nl/dbminor

Minor Histocompatibility Antigens in Transplantation

The New England
Journal of Medicine

Volume 334 FEBRUARY 1, 1996 Number 5

MISMATCHES OF MINOR HISTOCOMPATIBILITY ANTIGENS BETWEEN HLA-IDENTICAL DONORS AND RECIPIENTS AND THE DEVELOPMENT OF GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION

ELS GOULMY, PH.D., RONALD SCHIPPER, M.SC., JOS POOL, ELS BLOKLAND, J.H. FREDERIK FALKENBURG, M.D., PH.D., JAAK VOSSEN, M.D., PH.D., ALOIS GRATWOHL, M.D., PH.D., GEORGIA B. VOGELSANG, M.D., PH.D., HANS C. VAN HOUWELINGEN, PH.D., AND JON J. VAN ROOD, M.D., PH.D.

Minor Histocompatibility Antigens in Transplantation

Spaapen R, Mutis T. Best Pract Res Clin Haematol. 2008. Targeting haematopoietic-specific minor histocompatibility antigens to distinguish *graft-versus-tumour* effects from *graft-versus-host disease*.

Feng X, Hui KM, Younes HM, Brickner AG. Trends Immunol 2008. Targeting minor histocompatibility antigens in *graft versus tumor* or *graft versus leukemia responses*.

Goulmy E. Hum Immunol. 2006. Minor histocompatibility antigens: from transplantation problems to therapy of cancer. Targeting minor histocompatibility antigens in *graft versus tumor* or *graft versus leukemia responses*.

Hambach L, Vermeij M, Buser A, Aghai Z, van der Kwast T, Goulmy E. Blood. 2008. Targeting a single mismatched minor histocompatibility antigen with tumor-restricted expression eradicates human solid tumors.

Published in final edited form as:
Biol Blood Marrow Transplant. 2009 July ; 15(7): 856–863. doi:10.1016/j.bbmt.2009.03.018.

Effects of mismatching for Minor Histocompatibility Antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants:

Minor antigen mismatching in unrelated donors

Stephen Spellman[1], **Melissa B. Warden**[2], **Michael Haagenson**[3], **Bradley C. Pietz**[2], **Els Goulmy, Ph.D.**[4], **Edus H. Warren, M.D., Ph.D.**[5], **Tao Wang, Ph.D.**[6], and **Thomas M. Ellis, Ph.D.**[2]

[1]National Marrow Donor Program, Minneapolis, MN [2]BloodCenter of Wisconsin, Milwaukee, WI [3]Center for International Blood and Marrow Transplant Research, Minneapolis, MN [4]Leiden University Medical Center, Leiden, The Netherlands [5]Fred Hutchinson Cancer Research Center, Seattle, WA [6]Medical College of Wisconsin, Milwaukee, WI

Minor Histocompatibility Antigens in Transplantation

Recipient/donor pairs from **730 unrelated** HLA-A, B, C, DRB1, and DQB1 allele-matched transplants facilitated by the National Marrow Donor Program (NMDP) were studied

The majority (86%) of the pairs were mismatched at HLA-DP

Transplants were performed between 1996 and 2003

Patients had different disease characteristics

Biol Blood Marrow Transplant. 2009

Minor Histocompatibility Antigens in Transplantation

Spellman: **Results for Single mHAg mismatches**

Results for Multiple mHAg mismatches

Biol Blood Marrow Transplant. 2009

Minor Histocompatibility Antigens in Transplantation

Spellman: Results for Single mHAg mismatches (UNRELATED HSCT)

No significant association with any GvH or HvG for single mismatch: HA-1, HA-2, HA-3, HA-8, and HB-1

Reduced risk of grades III–IV GvHD when pairs were **mismatched for CD31(563)** in the host versus graft direction (p=0.001) in HLA-A2 positive pairs

No effect of HY mismatching was observed for any outcome

Biol Blood Marrow Transplant. 2009

Minor Histocompatibility Antigens in Transplantation

Spellman: Results for Multiple mHAg mismatches (UNRELATED HSCT)

Reduced risk of acute GvHD in HLA-A2 positive pairs mismatched for 2 or more mHAg for HA-1, HA-2, HA-8 and/or $CD31_{(563)}$ in the HvG direction (perhaps reflecting the influence of CD31(563) mismatching on this group)

Lower survival in HLA-A2 positive pairs mismatched for 2 or more mHAg (HA-1, HA-2, HA-8, and/or $CD31_{(563)}$ in the GvH direction (p=0.01).

Decreased survival and increased TRM in HLA-A1 positive pairs mismatched for both CD31 and HA-3 in the GvH direction (p=0.02)

Minor Histocompatibility Antigens in Transplantation

Biol Blood Marrow Tranplant 2013 Aug

Multicenter Analyses Demonstrate Significant Clinical Effects of Minor Histocompatibility Antigens on GvHD and GvL after HLA-Matched Related and Unrelated Hematopoietic Stem Cell Transplantation

Spierings E, Kim YH,Hendriks M, Borst E, Sergeant R, Canossi A, Oudshoorn M, Loiseau P, Dolstra H, Markiewicz M, Leffell MS,Pereira N, Kircher B, Turpeinen H, Eliaou JF, Gervais T, Laurin D, Enczmann J, Martinetti M, Thomson J, Oguz F, Santarone S,Partanen J, Siekiera U, Alessandrino EP, Kalayoglu S, Brand R, Goulmy E.

Minor Histocompatibility Antigens in Transplantation

Spierings E et al. Biol Blood Marrow Tranplant 2013 Aug

The **International Histocompatibility and Immunogenetics Workshops** (IHIW)

In collaboration with **20 laboratories of the IHIW**, the roles of 10 autosomal and 10 Y chromosome-encoded minor H antigens were investigated on GvHD and relapse incidence in:

639 HLA-identical related donor (IRD)
210 HLA-matched unrelated donor (MUD)

Donor and recipient DNA samples were genotyped for the minor H antigens:

HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, SP110, PANE1, UGT2B17 and HY.

The correlations with the primary outcomes GvHD (acute or chronic GvHD), survival, and relapse were statistically analyzed.

Minor Histocompatibility Antigens in Transplantation

Spierings E et al. Biol Blood Marrow Tranplant 2013 Aug

Results - of mHAgs disparities in sex chromosome

none of the HLA class I-restricted HY antigens were found to be associated with any of the primary outcomes

Analysis of the overall gender effect:

increased GvHD incidence in the female-to-male transplantations ($P<0.005$)

decreased GvHD-free survival in the female-to-male transplantations ($P< .001$)

Minor Histocompatibility Antigens in Transplantation

Spierings E et al. Biol Blood Marrow Tranplant 2013 Aug

Results - of mHAgs autosomally encoded

I**ncreased GvHD incidence in IRD** HSCT - **but not in MUD** - only when mismatching for the broadly expressed mHAgs **HA-8** P<0.005)

In recipients with GvHD - but not in those without GvHD - mismatching for hematopoietic mHAgs correlated with

- lower relapse rates (P=0.078)
- higher relapse-free survival (P=0.029)
- higher overall survival (P=0.032)

The GvHD-GvL association - demonstrating a significant lower relapse in hematopoietic mHAgs mismatched patient/donor pairs - underlines their clinical applicability for adoptive immunotherapy, enhancing the GvL effect in a GvHD controllable manner

Minor Histocompatibility Antigens in Transplantation

Blood. 2013 Aug 1

Human regulatory T cells against minor histocompatibility antigens: ex vivo expansion for prevention of graft-versus-host disease.

Veerapathran A, Pidala J, Beato F, Betts B, Kim J, Turner JG, Hellerstein MK, Yu XZ, Janssen W, Anasetti C. Department of Blood and Marrow Transplantation, Moffitt Cancer Center,Tampa, FL, USA

They identified and expanded regulatory CD4 T cells (Treg) **specific for human mHAs**

Cultured Treg produced allospecific suppression, maintained demethylation of the Treg-specific Foxp3 gene promoter, Foxp3 expression and TGF-β production

This is the first report of detection and expansion of potent mHA-specific Treg from HLA-matched siblings in sufficient numbers for application in human transplant trials

Minor Histocompatibility Antigens in Transplantation

Background

- HSCT and acute GvHD

Minor Histocompatibility Antigens

- Characteristics
- Role in Transplantation
- **Our data in Thalassemia**

Aim of the study

We investigate the impact of **mHAg matching** on the outcome of HSCT for Thalassemia in a retrospective study of 146 **12/12 HLA identical related** donor-recipient

Thalassemic HSCT Patients	**146**
aGvHD (III-IV)	**8** (5,4%)
Rejection	**18** (12,3%)

Obtain donor and patients mHAg data

	Donor			Recipient		
HA-1	☐ H	☑ R	RR	☑ H	☑ R	HR
HA-2	☑ V	☐ M	VV	☑ V	☑ M	VM
HA-3	☑ T	☐ M	TT	☑ T	☐ M	TT
HA-8	☐ R	☑ P	PP	☐ R	☑ P	PP
HB-1	☑ H	☐ Y	HH	☑ H	☐ Y	HH
ACC-1	☐ Y	☑ C	CC	☐ Y	☑ C	CC
ACC-2	☐ D	☑ G	GG	☐ D	☑ G	GG
SP110	☑ R	☐ G	RR	☑ R	☐ G	RR
PANE-1	☑ R	☐ *	RR	☑ R	☐ *	RR
UGT2B17	☑ +	☐ -	+	☑ +	☐ -	+
LRH-1	☐ 4C	☐ 5C		☐ 4C	☐ 5C	
ECGF-1	☐ H	☐ R		☐ H	☐ R	
CTSH	☐ R	☐ G		☐ R	☐ G	
LB-ADIR	☐ F	☐ S		☐ F	☐ S	
HY	☐ +	☑ -	-	☑ +	☐ -	+

HLA typing

HLA-A	A*01 A*02	HLA-B	B*44 B*55	HLA-C	Cw*01 Cw*07
HLA-DRB1	DRB1*07 DRB1*10	HLA-DRB3	Select Select	HLA-DRB4	Select Select
HLA-DRB5	Select Select	HLA-DPA1	Select Select	HLA-DPB1	DPB1*02 DPB1*09
HLA-DQA1	Select Select	HLA-DQB1	DQB1*03 DQB1*05		

Correlation between donor / patient mHAg disparities and aGvHD (III-IV)

No statistical significant influence on aGvHD due to donor – recipient disparities for mHAgs in Thalassemic transplanted patients was observed

Influence of mHAgs on rejection after HSCT in Thalassemia

Total number of patients = 49	HA-8 with A*02 restriction				
	HA-8 disparities = 5		HA-8 NO disparities = 44		Fisher
Rejection YES – 7 pts	2	40%	5	11,3%	
Rejection NO – 42 pts	3	60%	39	88,7%	p=0,14

Total number of patients = 146	HA-8 NO A*02 restriction				
	HA-8 disparities = 15		HA-8 NO disparities = 131		Fisher
Rejection YES – 18 pts	5	33,3%	13	9,9%	
Rejection NO – 128 pts	10	66,3%	118	90,1%	p=0,02

CONCLUSION

The analysis of the minor histocompatibility antigens differences between donor and recipient represents an important element in the occurrence of complications after HLA-identical related donor HSCT, although up to date many contrasting results are still reported in different studies.

Acknowledgments

Centro Trapianti IME - Roma

Guido Lucarelli
Javid Gaziev
Pietro Sodani
Katia Paciaroni
Gioia De Angelis
Cecilia Alfieri
Cristiano Gallucci
Luisa Cardarelli
Marco Marziali
Antonella Isgrò
Michela Ribersani

HSR TIGET – Milano

Katharina Fleischhauer
Maria Grazia Roncarolo
Federico Sizzano

LIBT Fondazione IME

Manuela Testi
Maria Troiano
Mariarosa Battarra
Tiziana Galluccio
Rossella Condello
Annalisa Guagnano
Giuseppe Testa
Chiara Stellitano
Renata Rosati
Andrea Di Luzio
Simona De Petris
Eleonora Palladini
Martina Mangione

Minor Histocompatibility Antigens in Transplantation

The role for mHAg disparities in HSCT outcomes has been supported by studies showing

higher rates of acute GvH and lower survival in HLA-identical sibling transplant recipients who are mHAg disparate.

Goulmy E et al N Engl J Med 1996
Marijt WA et al. Proc Natl Acad Sci U S A 2003
Grumet FC et al. Biol Blood Marrow Transplant 2001

increased rates of GvHD and lower rates of leukemia recurrence observed in pairs who are disparate at HA-1 or HA-2

Goulmy E et al N Engl J Med 1996
Tseng et al Blood 1999

….although this last is disputed by other studies

Cavanagh G et al. Transplantation 2005

Minor Histocompatibility Antigens in Transplantation

Additionally, disparities in HA-8 and CD31 were associated with decreased patient survival

Goulmy E et al N Engl J Med 1996
Tseng et al Blood 1999
Marijt WA et al. Proc Natl Acad Sci U S A 2003
Grumet FC et al. Biol Blood Marrow Transplant 2001