Anti-HLA Abs

- ✓ 非自己HLA抗原の移入により産生
  輸血、妊娠、移植歴など、
- ✓ 抗体関連型拒絶反応のハイリスク群
- ✓ グラフト廃絶の主要な原因

Methods for HLA-specific Ab screening and donor cross-matching testing

Cell-Based Assays

- Complement-dependent cytotoxicity (CDC)
  - =Lymphocyte cytotoxicity test (LCT)
    - →Anti-human immunoblobulin-LCT (AHG-LCT)
- Flow cytometry crossmatch (FCXM)

Solid-Phase Immunoassays

- Enzyme-linked immunosorbent assay (ELISA)
- Bead-based array assays
  - (Flow cytometry or Luminex)

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)

The New England
Journal of Medicine

Volume 280 APRIL 3, 1969 Number 14

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

Abstract Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)

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SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

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Kidneys transplanted across a positive crossmatch failed immediately.

Higher risk: multiparous females

patients reciving secondary transplants

Immediate failures occurred among transplants from unrelated than among those from related donors.

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)

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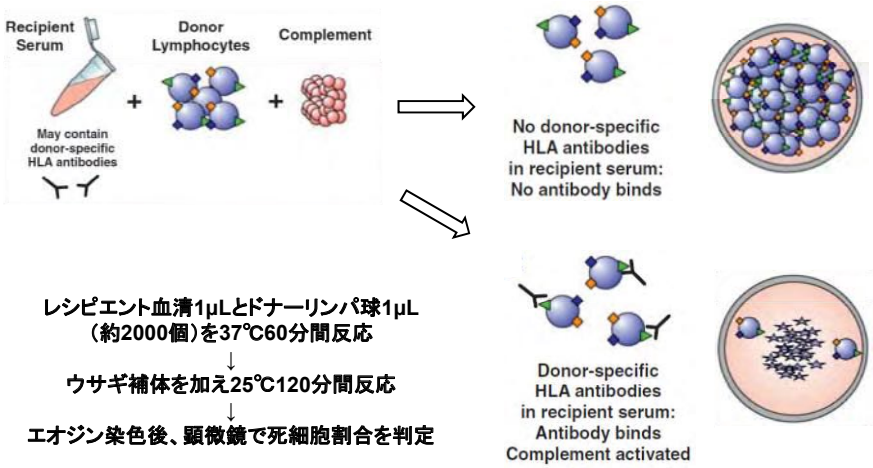
SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

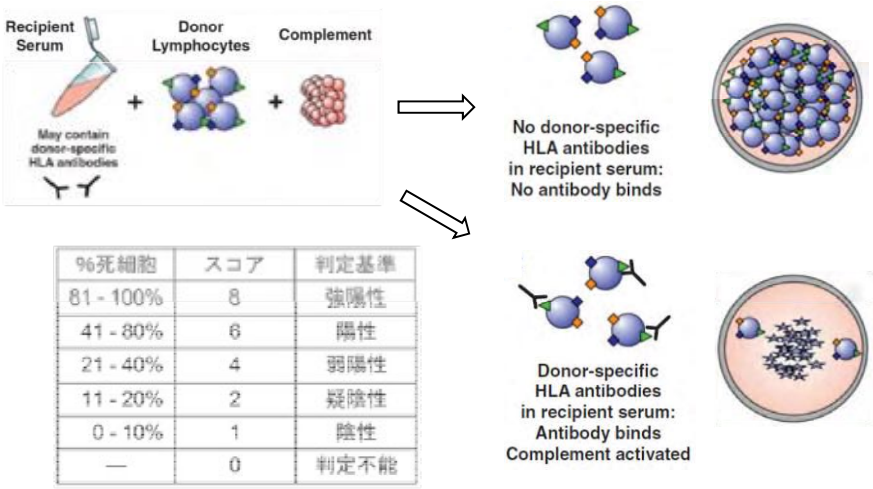
The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)



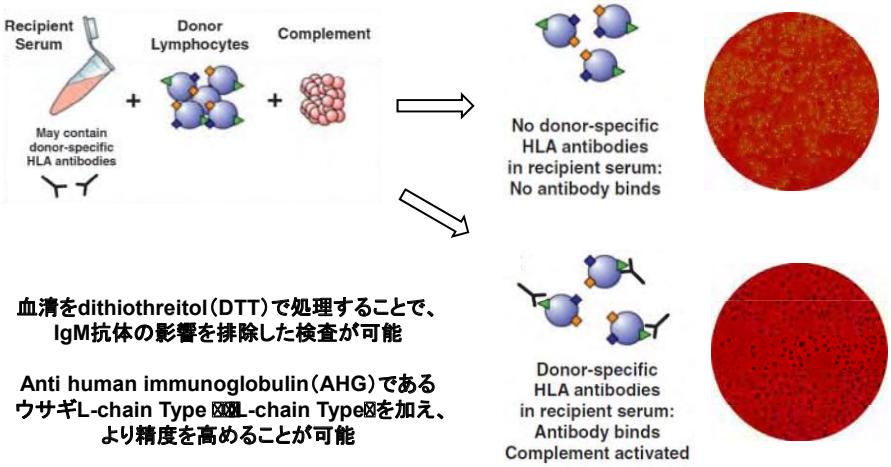
レシピエント血清1μLとドナーリンパ球1μL
（約2000個）を37℃60分間反応
↓
ウサギ補体を加え25℃120分間反応
↓
エオジン染色後、顕微鏡で死細胞割合を判定

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)



%死細胞	スコア	判定基準
81 - 100%	8	強陽性
41 - 80%	6	陽性
21 - 40%	4	弱陽性
11 - 20%	2	疑陰性
0 - 10%	1	陰性
—	0	判定不能

Complement-dependent cytotoxicity (CDC) =Lymphocyte cytotoxicity test (LCT)



血清をdithiothreitol（DTT）で処理することで、IgM抗体の影響を排除した検査が可能

Anti human immunoglobulin（AHG）であるウサギL-chain Type □L-chain Type□を加え、より精度を高めることが可能

Interpretation of crossmatch result

T-Cell XM	B-Cell XM	Interpretation
–ve	–ve	No DSAb to HLA class I or II OR DSAb titre too low to cause positive reaction OR (DSAb that is not complement-fixing – relevance unclear)
+ve	+ve	DSAb/s to HLA class I OR Multiple DSAbs to HLA class I +/– II
–ve	+ve	DSAb/s to HLA class II OR Low level DSAb/s to HLA class I
+ve	–ve	Technical error (possibly related to B-cell viability). The test should be repeated

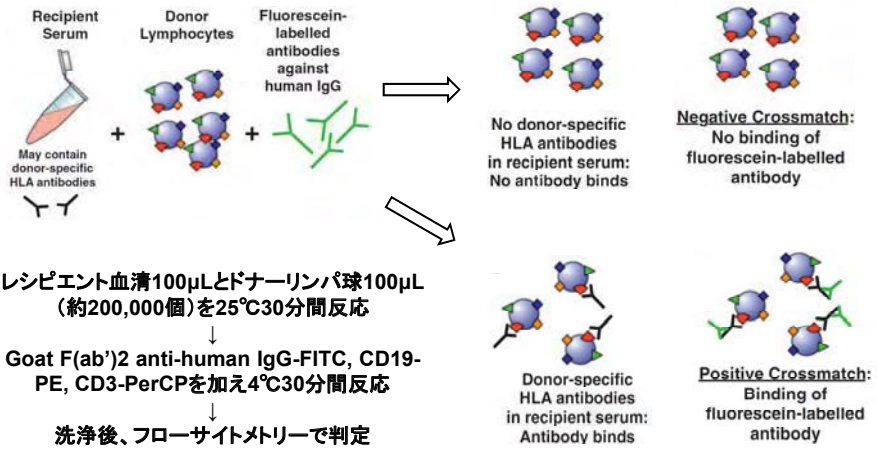
+ve, positive; –ve, negative, DSAb: donor-specific anti-HLA antibody; HLA, human leucocyte antigen, XM: crossmatch.

(Mulley WR, Kanellis J. Nephrology. 2011)

Points at LCT-XM, AHG-LCT-XM

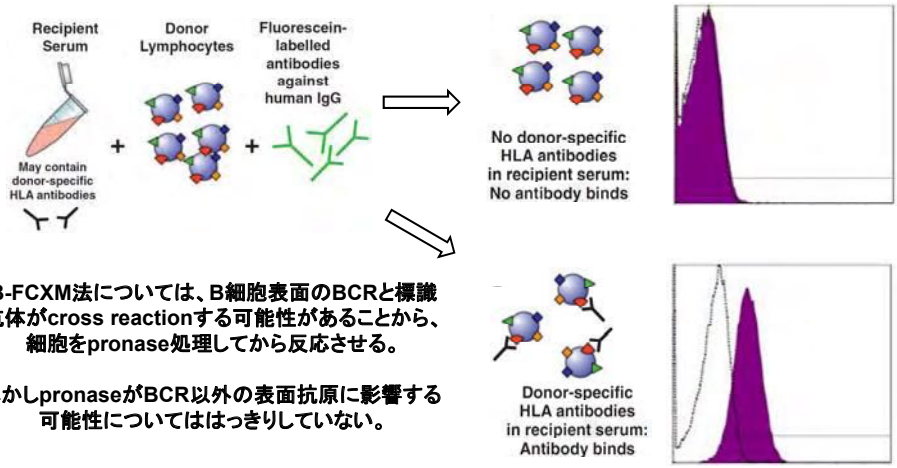
- 判定は検査技術者の主観、熟練度に左右
- 高抗体価でないと検出できない
- リンパ球のViabilityによっても大きく左右
- 非特異的抗体によっても陽性となることがある
- 血清中の抗体量が少量の場合、補体制御蛋白の機能に影。
- IgGのisotypeによって補体結合能が異なるため検査結果に影響

Flow cytometry crossmatch (FCXM)



レシピエント血清100μLとドナーリンパ球100μL
（約200,000個）を25℃30分間反応
↓
Goat F(ab')2 anti-human IgG-FITC, CD19-PE, CD3-PerCPを加え4℃30分間反応
↓
洗浄後、フローサイトメトリーで判定

Flow cytometry crossmatch (FCXM)



B-FCXM法については、B細胞表面のBCRと標識抗体がcross reactionする可能性があることから、細胞をpronase処理してから反応させる。

しかしpronaseがBCR以外の表面抗原に影響する可能性についてははっきりしていない。

Points at FCXM

- フローサイトメーターが必要
- 偽陽性→HLA 抗体の有無、感作歴等の総合的な判定が必要
- 操作方法、判定基準、報告形式が各施設によって異なる

陰性コントロール（ドナー血清？AB型男性のプール血清？）

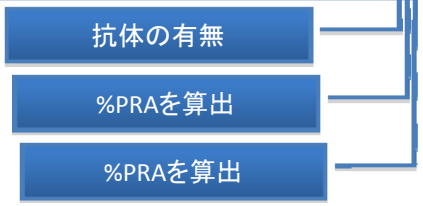
判定方法

- MFI絶対値が陰性コントロールから10以上のシフトで陽性？
  20以上あるいは40以上を陽性？
- 患者血清のMFIが陰性コントロールのMFIの2倍以上を陽性？
- 限られたドナー採血では、FCXM 解析用のB細胞数が不足

Solid-Phase Immunoassays

- **Enzyme-linked immunosorbent assay (ELISA)**
- **Bead-based array assays**

Flow cytometry	Luminex
Flow PRA Screening Test	LABScreen Mixed
Flow PRA Specific	LABScreen PRA
Flow PRA Single Antigen	LABScreen Single Antigen



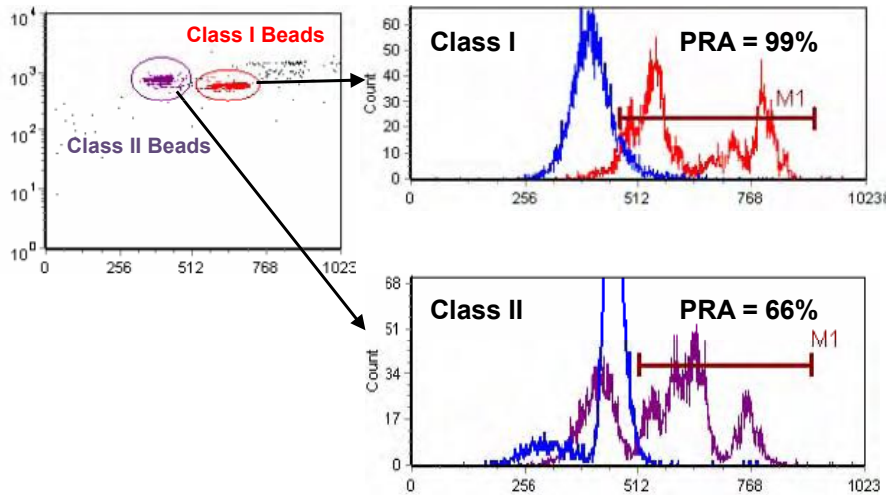
Differences between Flow PRA & LABScreen

	Flow PRA	LABScreen
検体処理数	少ない	多い
機器調整	要	不要
測定方法	手動	自動
解析方法	手動	自動
熟練度	要	不要

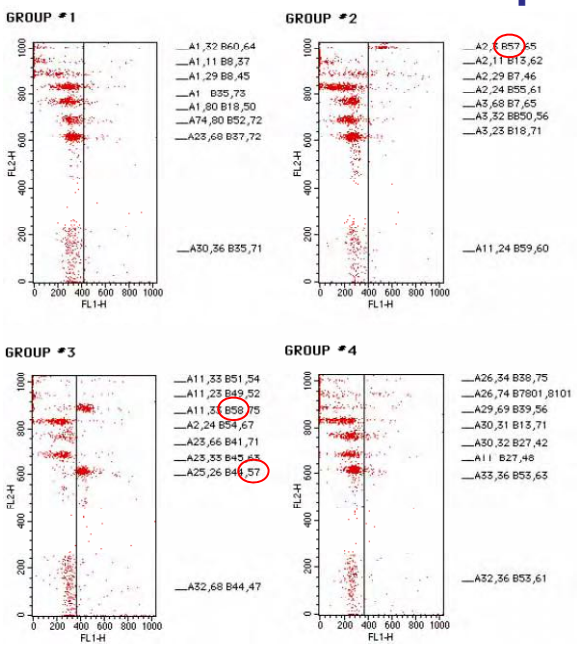
いずれも高額機器が必要、試薬も高価であることが問題点

またnon-HLA抗体は検出できない

Flow PRA Screening Test



Flow PRA Specific

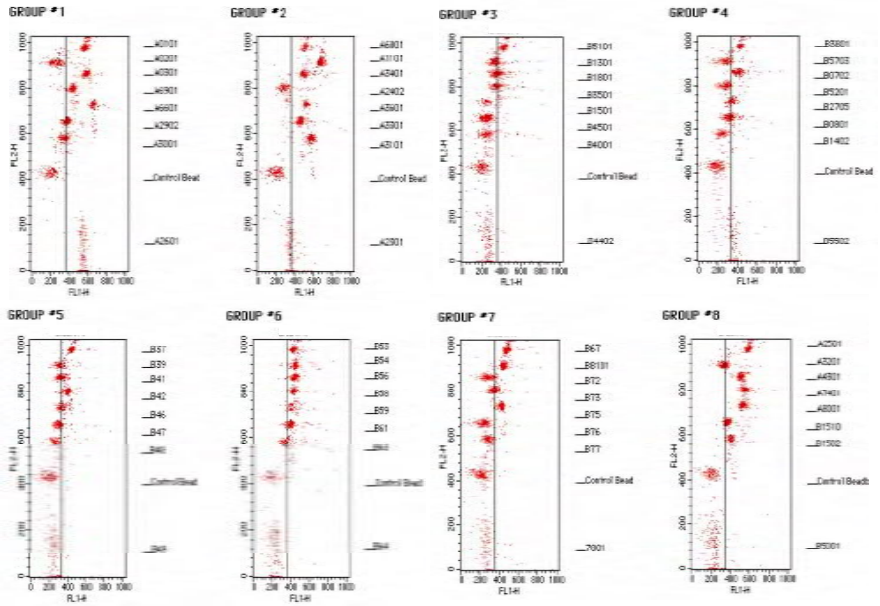


Specificity: B57, 58
PRA: 9.4%

Flow PRA Specific

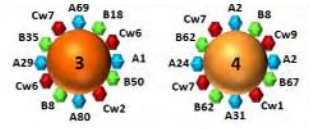
Flow PRA Single Antigen

Flow PRA Single Antigen

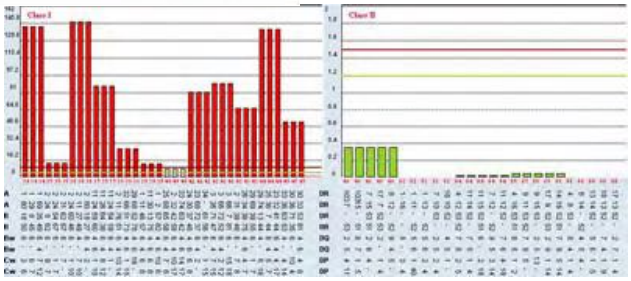


LABScreen Mixed

- HLA抗体・MICA抗体の有無をスクリーニング
- Luminexビーズ1種類あたり、3～5種類の**パネル細胞**（HLA抗原が分かっている細胞株）のHLA抗原、MICA抗原がコーティング



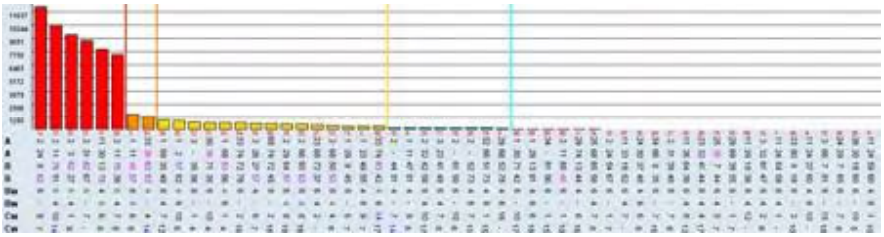
Class I: 12beads (3 panels/bead)
Class II: 5beads (5panels/bead)
MICA-Ag: 2beads (5panels/bead)



Class I: 陽性　　**Class II: 陰性**

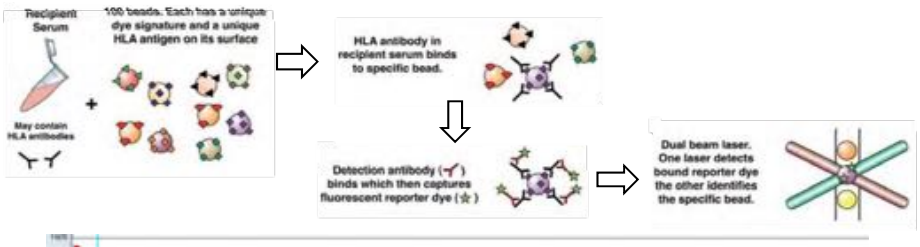
LABScreen PRA

- HLA抗体の有無と%PRAを判定
- Luminexビーズ1種類あたり、1種類のパネル細胞のHLA抗原がコーティング
  （Class Iは55種類のビーズ、Class IIは35種類のビーズに、精製HLA抗原がコーティングされている）



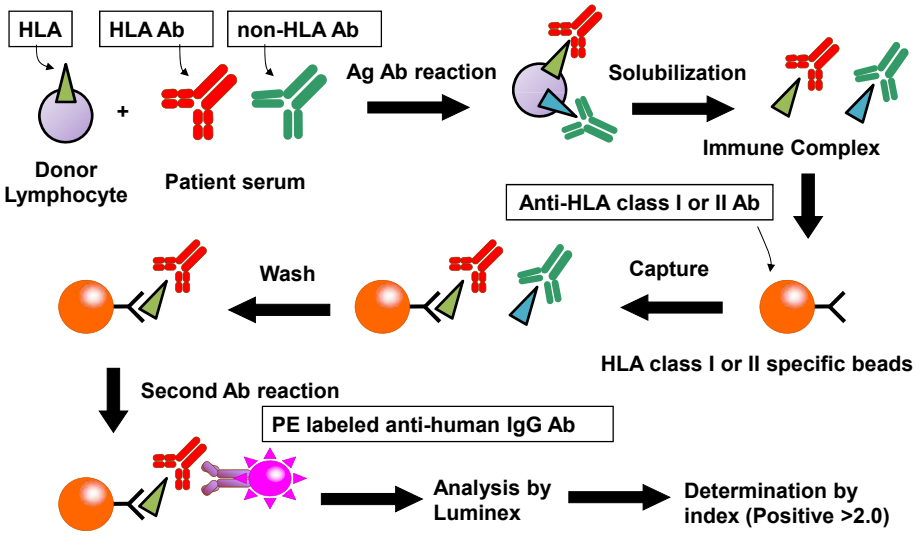
LABScreen Single Antigen

- HLA抗体の特異性を同定
- ビーズ1種類あたり、1種類の精製HLA抗原がコーティング



ICFA

(Immunocomplex Capture Fluorescence Analysis)



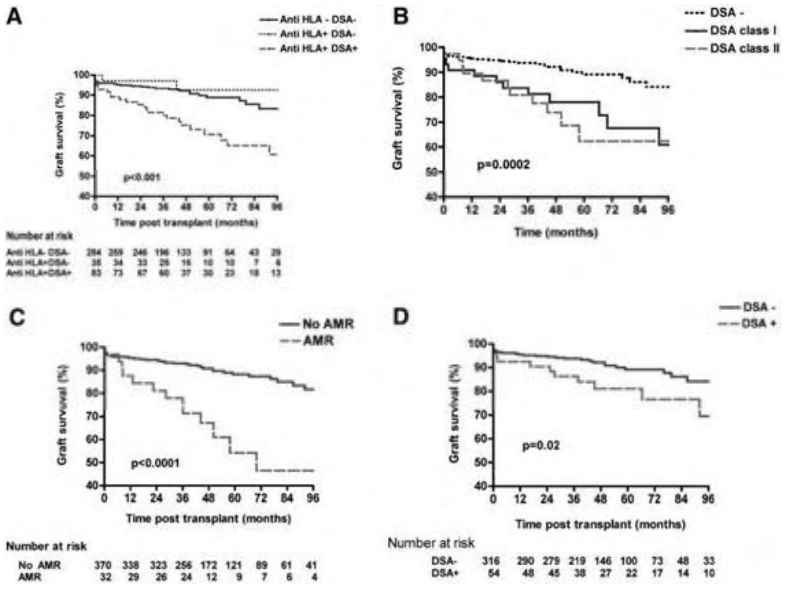
Variation in Results by Choice of Anti-HLA Antibody Detection Technique

Method	Positive	Negative
CDC	102	162
AHG-CDC	116 (+13%)	148
ELISA	127 (+10%)	137
Flow PRA	139 (+10%)	125

(Gebel, HM & Bray, RA. Transplantation. 2000)

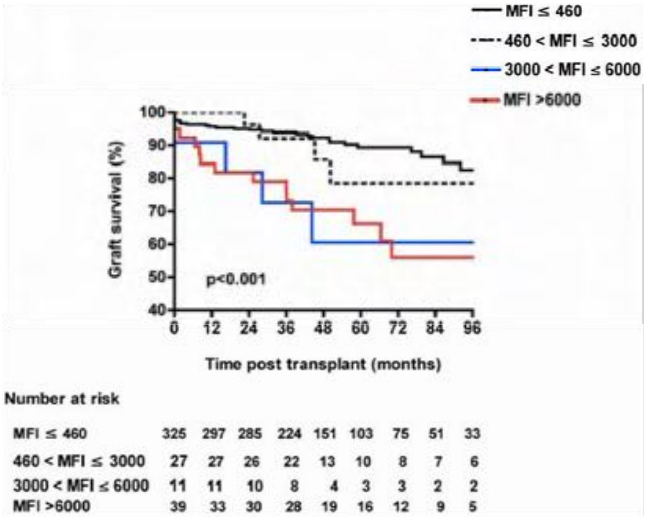
Preformed DSA

Preformed DSAはclass I/IIに関わらず廃絶の危険因子



Lefaucheur C, et al. *J Am Soc Nephrol* 2010; 21: 1398

ハイタイターpreformed抗HLA-DSA を持つ患者は移植腎廃絶のリスクが高い

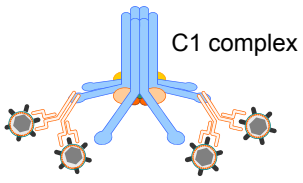


Lefaucheur C, et al. *J Am Soc Nephrol* 2010; 21: 1398

IgG サブクラスの機能特性

Complement fixing subclass

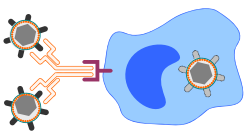
IgG3 >> IgG1 > IgG2 > IgG4



Complement activation

Fc-receptor binding properties

IgG3 > IgG1 >> IgG2 > IgG4



Cell mediated cytotoxicity

Pretransplant IgG subclasses of donor-specific HLA Abs and development of AMR

subclass pattern	‚biological' group	N
1	strong C-activating	32
1+3	strong C-activating	14
3	strong C-activating	2
2	weak/no C-activating	6
2+4	weak/no C-activating	2
4	weak/no C-activating	1
1+2	mixture	21
1+2+3	mixture	19
1+2+3+4	mixture	14
1+4	mixture	6
1+2+4	mixture	5
2+3	mixture	2
2+3+4	mixture	-
1+3+4	mixture	-
3+4	mixture	-
no IgG1-4	all negative	17

→

strong C-activating
(n=48; 34%)

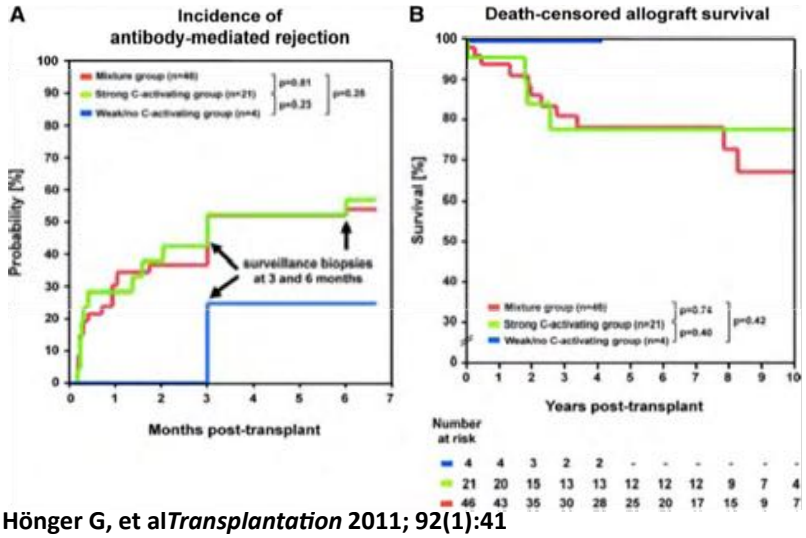
weak/no C-activating
(n=9; 6%)

mixture
(n=67; 48%)

all negative
(n=17; 12%)

Hönger G, et al. (University Hospital Basel, Switzerlan)
Transplantation 2011; 92(1):41

低オプソニンIgG2/IgG4 subclass DSAのみを有する レシピエントはAMR発症率は低くグラフト生着は良好である



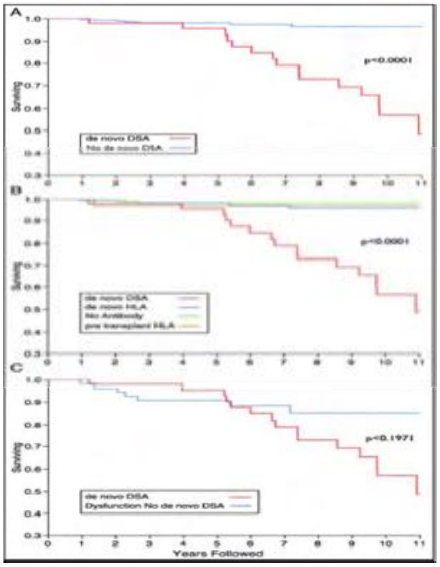
Hönger G, et al*Transplantation* 2011; 92(1):41

Preformed DSAに関するコンセンサス

- Preformed DSAはclass I/IIに関わらず廃絶の危険因子
- Cross Reactive Epitope Group (CREG) Absの存在もAMRの危険 (Nainani N, et al. *Transpl Immunol* 2009; 20:113)
- ハイタイターpreformed DSAを持つ患者は、廃絶のリスクが高い
- IgG2/IgG4 subclass DSAのみを有する場合、グラフト生着は良好

De novo DSA

DSA発生率15% -- グラフトロスのハイリスク



De novo DSAは
AMRの危険因子

Non-DSAは
危険因子ではない

De novo DSAは他の
機能障害因子に比べ
予後不良

Wiebe C, et al. *Am J Transplant* 2012;12(5):1157

Significant association between chronic AMR and DR-DSA in renal transplantation

1. Iniotaki-Theodoraki AG, et al. *Transplantation* 2003; 75(9): 1601.
2. Zhu L, et al. *Clin Transpl* 2008: 171.
3. Kobayashi T, et al. *Hum Immunol* 2011; 72(1): 11.
4. Wiebe C, et al. *Am J Transplant* 2012;12(5):1157.

De novo DQ-DSA are associated with a significant risk of AMR and transplant glomerulopathy

1. Worthington JE, et al. *Transplantation* 2003; 75(7): 1034.
2. Willicombe M, et al. *Transplantation*. 2012;94(2):172-7.
3. Devos JM, et al. *Kidney Int* 2012;82(5):598-604.

De novo DSAに関するコンセンサス

- De novo DSAはAMR/グラフトロスの危険因子
- Non-DSA HLA抗体は危険因子ではない
- 標的はHLA class II >> class I
  DR? DQ? DP?
- De novo DSAの発生は組織傷害に先行する?

De novo DSA対策

**Antibody-mediated rejection—
an ounce of prevention
is worth a pound of cure**

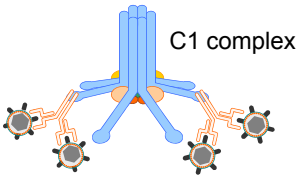
1ozの予防は1lbの治療に値する

Bradley JA, Baldwin WM, Bingaman A, Ellenrieder C, Gebel HM, Glotz D, Kirk AD. *Am J Transplant* 2011;11(6):1131

Functional properties of IgG isotypes

Complement fixing subclass

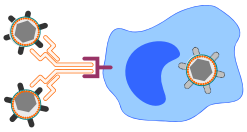
IgG3 >> IgG1 > IgG2 > IgG4



Complement activation

Fc-receptor binding properties

IgG3 > IgG1 >> IgG2 > IgG4



Cell mediated cytotoxicity

IgG3 subclass DSAを

いかに抑制するか？

T_H細胞によるIgGクラススイッチの誘導パターン

T_H1-biased isotype (IFN-γ, TGF-β)
→ IgG2, IgG3

T_H2-biased isotype (IL-4)
→ IgG1, IgG4

**MMF/mTORiは、STAT1のリン酸化を抑制し、
STAT5のリン酸化を維持する**

↓

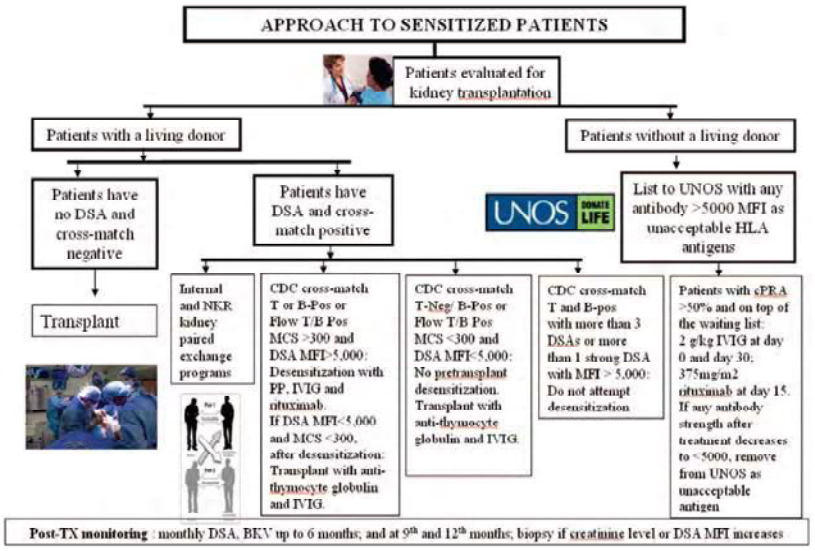
T_H1応答を抑制するがTreg応答は抑制しない

↓

IgG3 subclass DSAの出現を抑制する？

**CNIのminimizationに伴うIgG3-DSAの出現を、
MMF/mTORiが予防できる可能性**

Algorithmic approach to sensitized patients



(Marfo K, et al. Clin J Am Soc Nephrol. 2011)

Basic Concepts in Desensitization

Removal of existing antibodies

- Plasmapherersis
- Immunoadsorption

Depletion of antibody producing cells

- Naïve and memory B cell – Rituximab (anti-CD20)
- Plasma cells- Bortezomib (proteasome inhibitor)

Inhibition of residual antibody and Complement system cascade

- Intravenous immunoglobulin (IVIG)
- Eculizumab (C5 inhibitor)

Suppression of the T cell response

- Induction agents
- Triple immunosuppression with CNI, MMF and steroids

J Am Soc Nephrol 15: 3256–3262, 2004

Evaluation of Intravenous Immunoglobulin as an Agent to Lower Allosensitization and Improve Transplantation in Highly Sensitized Adult Patients with End-Stage Renal Disease: Report of the NIH IG02 Trial

STANLEY C. JORDAN,*† DOLLY TYAN,‡ DON STABLEIN,§ MATTHEW MCINTOSH,§ STEVE ROSE,‖ ASHLEY VO,* MIEKO TOYODA,† CONNIE DAVIS,¶ RON SHAPIRO,# DEBORAH ADEY,** DAWN MILLINER,†† RALPH GRAFF,‡‡ ROBERT STEINER,§§ GAETANO CIANCIO,‖‖ SHOBAH SAHNEY,¶¶ and JIMMY LIGHT##

高感作献腎移植待機患者48 例
月1回2g/BWの免疫グロブリン投与を4ヵ月間施行
→ placebo群に比べてPRA値は有意に減少
移植率が改善（placebo群は17%、IVIG群は35%）
待機期間が短縮（placebo群は10.3年、IVIG群では4.8年）
2年生着率はplacebo群75%, IVIG群80%（有意差なし）

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Rituximab and Intravenous Immune Globulin for Desensitization during Renal Transplantation

Ashley A. Vo, Pharm.D., Marina Lukovsky, Pharm.D., Mieko Toyoda, Ph.D., Jennifer Wang, M.D., Nancy L. Reinsmoen, Ph.D., Chih-Hung Lai, Ph.D., Alice Peng, M.D., Rafael Villicana, M.D., and Stanley C. Jordan, M.D.

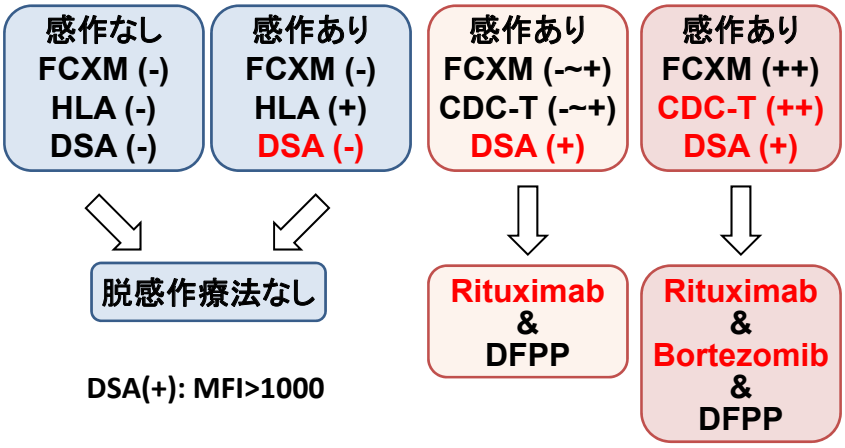
N ENGL J MED 359;3 WWW.NEJM.ORG JULY 17, 2008

高感作献腎移植待機患者20例
IVIG (2g/kg×2 dose) + Rituximab (1g×2 dose)を施行
→平均PRAレベルは減少（77±19%から 44±30%）
移植までの平均透析期間は短縮（144±89 ヵ月から5±6 ヵ月）
移植率は80%と上昇（ IVIGのみでは35% ）
1年生存率は100%, 1年生着率は94%

Outcomes in Kidney Recipients Receiving Desensitization Treatment 2000-2010

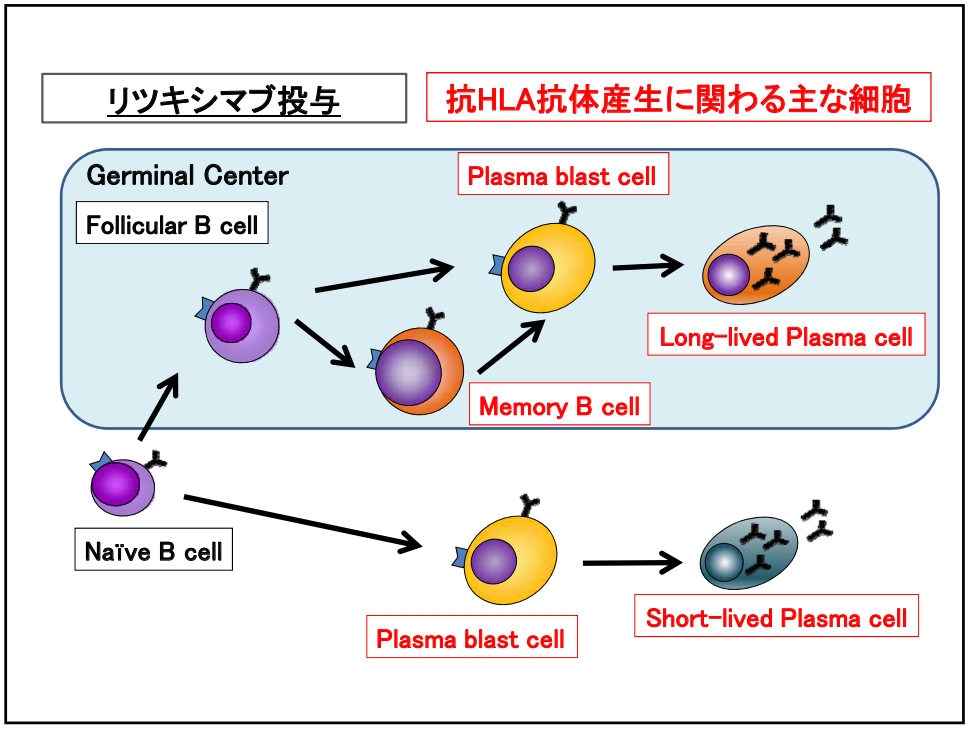
Author/Year PP/ Low-Dose IVIG	N	Follow up (Months)	Patient Survival	Graft Survival	Acute Rejection	AMR
Schweitzer 2000	11	13	100%	100%	36%	27%
Montgomery 2000	4	14	100%	100%	100%	100%
Gloor 2003	14	15	86%	78%	43%	43%
Magee 2008	28	22	93%	89%	42%	39%
Thielke 2009	51	23	95%	93%	43%	33%
High Dose IVIG						
Jordan 2003	43	24	98%	89%	31%	31%
Akalin 2003; 2005	17	15	100%	88%	18%	18%
Vo 2006	58 39	24 24	96% 100%	84% 90%	36% 31%	22% 21%
Vo 2008	54 16	14 12	98% 100%	96% 94%	35% 50%	20% 31%
Mai 2009	20	36	94%	89%	50%	30%
Bachler 2010	37	24	95%	87%	38%	38%
Vo 2010	76	24	95%	84%	37%	29%

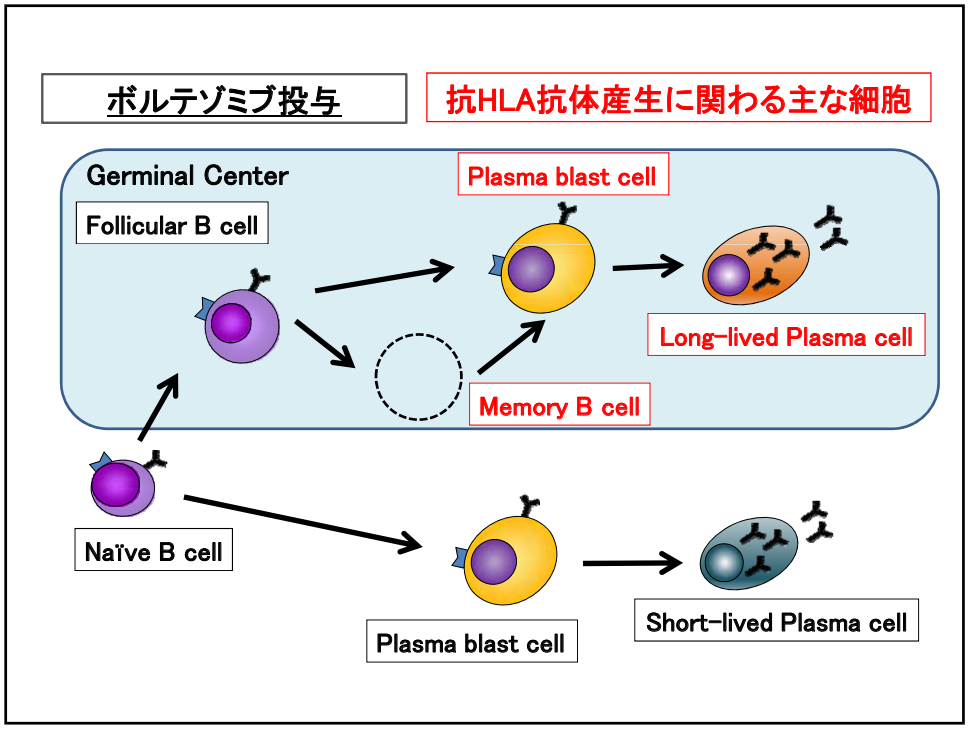
広島大学病院における術前処置



DSA(+): MFI>1000

Rituximab（リツキサン®）およびBortezomib（ベルケイド®）の使用については学内倫理委員会の承認済


リツキシマブ投与
抗HLA抗体産生に関わる主な細胞
Germinal Center
Follicular B cell
Plasma blast cell
Long-lived Plasma cell
Memory B cell
Naïve B cell
Plasma blast cell
Short-lived Plasma cell


ボルテゾミブ投与
抗HLA抗体産生に関わる主な細胞
Germinal Center
Follicular B cell
Plasma blast cell
Long-lived Plasma cell
Memory B cell
Naïve B cell
Plasma blast cell
Short-lived Plasma cell

症例①　42才女性　（血液型一致、輸血により感作）

HLA typing: Full mismatch

	A	A	B	B	DRB1	DRB1
Recipient	0206	2603	4002	4002	0410	1401
Donor	3101	3101	1501	3901	0406	0802

【CDC】 T-warm 弱陽性、B-warm 陽性、B-cold強 陽性

【T-cell FCXM】 陽性

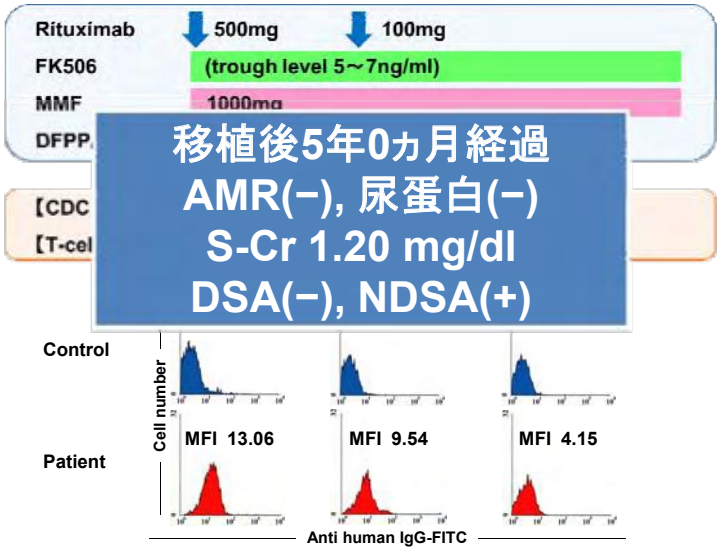
LABScreen® Single Antigen

Class I 抗体：HLA-B*1501 (MFI: 12966)を含めて計34種

Class II 抗体：計3種

CFSE-MLR: 脱感作療法前の抗ドナー応答は亢進

血液型不適合移植に準じた脱感作療法を行い クロスマッチは陰性化した



症例②　58才男性　（血液型不適合、二次移植症例）

HLA typing: Full mismatch

	A	A	B	B	DRB1	DRB1
Recipient	2402	3101	4002	5401	0405	0802
Donor	0201	0206	1301	4801	1202	1501
First Donor	0206	3101	4002	4801	0410	0802

【CDC】 T-warm 強陽性、B-warm 強陽性、B-cold強陽性

【T-cell FCXM】 陽性

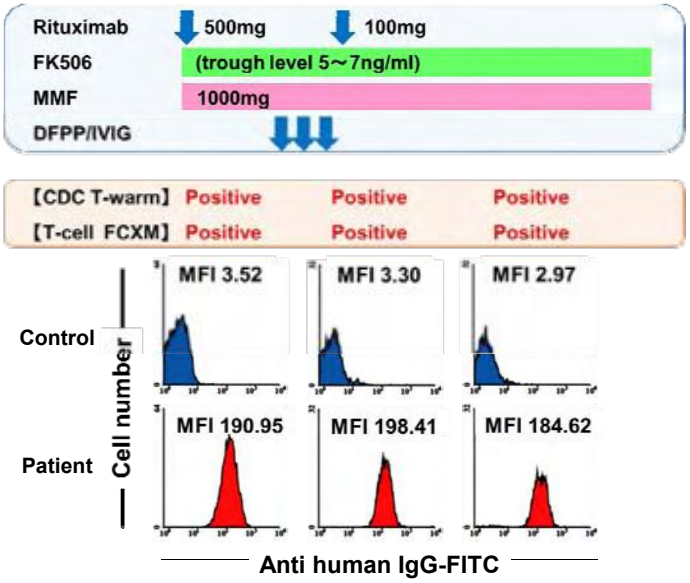
LABScreen® Single Antigen

Class I 抗体 : HLA-A2 (MFI: 14958)を含めて計11種

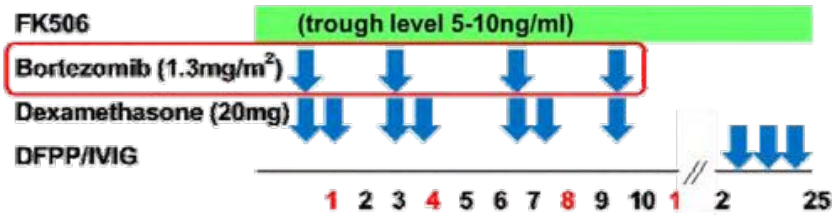
Class II 抗体 : 計6種

CFSE-MLR: 脱感作療法前の抗ドナー応答は亢進

血液型不適合移植に準じた脱感作療法を行ったが、クロスマッチは陰性化しなかった

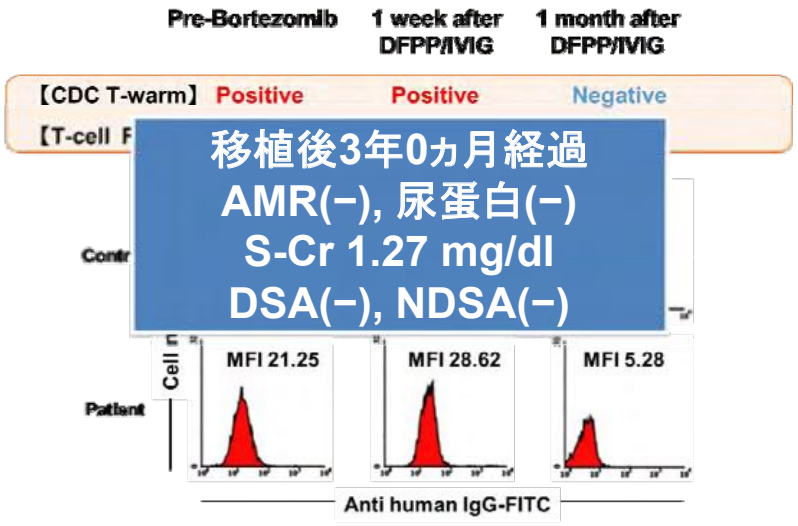


末梢血中に成熟B細胞が存在し、Memory B細胞が存在していないことを確認後、ボルテゾミブを投与した



ボルテゾミブは1サイクル（1.3mg/m^2（体表面積）を週2回、2週間（1, 4, 8, 11日目））投与（デキサメタゾン併用）し、2週間後にDFPPを行った。

ボルテゾミブ投与後、CDC / FCXMは陰転化



症例③ 61才女性 （血液型一致、妊娠による感作）

HLA typing: Full mismatch

	A	A	B	B	DRB1	DRB1
Recipient	0201	0201	3901	1510	0401	0801
Donor	2402	2402	0702	5201	0101	1501

【CDC】 T-warm 陰性、B-warm 陰性、B-cold 陰性

【T-cell FCXM】 陽性

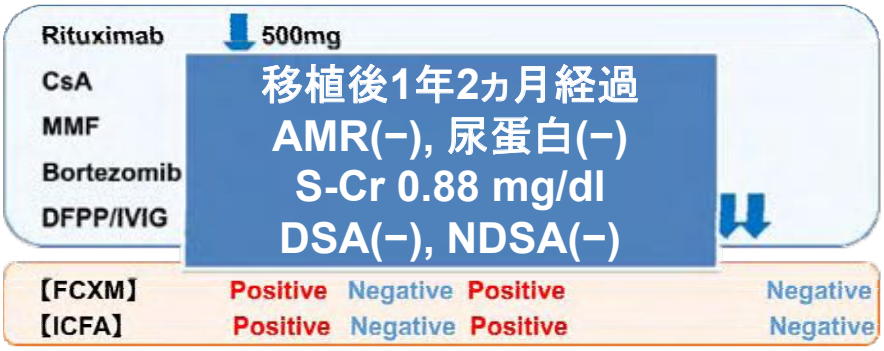
LABScreen® Single Antigen

Class I 抗体：HLA-B7 (MFI: 5317)を含めて計16種

Class II 抗体：なし

CFSE-MLR: 脱感作療法前の抗ドナー応答は亢進

血液型不適合に準じた脱感作療法でクロスマッチは一旦は陰性化したが、再度陽性となった



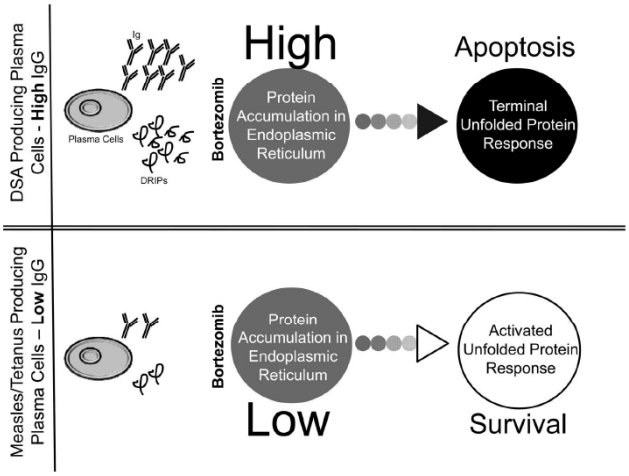
ボルテゾミブ投与後、クロスマッチ陰性が維持し、DSAも消失した。

ボルテゾミブ投与後DSAは低下しても防御免疫は低下しない

	基準値	減感作療法前	移植直前	移植1カ月後	移植6カ月後	移植1年後
単純ヘルペスウイルスIgG	2.0未満陰性	87.9	80.2	120	81.8	78.2
水痘帯状疱疹ウイルスIgG	2.0未満陰性	9.2	67.5	23.3	26.3	26.9
麻疹ウイルスIgG	2.0未満陰性	25.5	8.7	19.6	14.2	9.8
風疹ウイルスIgG	2.0未満陰性	128以上	128以上	128以上	128以上	128以上
ムンプスウイルスIgG	2.0未満陰性	3.7	2.0	3.7	2.5	2.9
サイトメガロウイルスIgG	4.0未満陰性	70	65	53	76	67
EBウイルス抗VCA IgG	10未満	80	20	20	20	40

全例経過中、重篤な感染症は認めていない。

Possible cause of differential depression of antibody DSA vs. protective immunity



Everly MJ, et al. Transplantation 2010; 90:1493

SPECIAL FEATURES

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

Transplantation • Volume 95, Number 1, January 15, 2013

Technical Group

(a) SPI must be used for the detection of pretransplantation HLA antibodies in solid organ transplant recipients and, in particular, the use of the single-antigen bead assay to detect antibodies to HLA loci, such as Cw, DQA, DPA, and DPB, which are not readily detected by other methods.
(b) The use of SPI for antibody detection should be supplemented with cell-based assays to examine the correlations between the two types of assays and to establish the likelihood of a positive crossmatch.
(c) There must be an awareness of the technical factors that can influence the results and their clinical interpretation when using the Luminex bead technology, such as variation in antigen density and the presence of denatured antigen on the beads.

SPECIAL FEATURES

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

Transplantation • Volume 95, Number 1, January 15, 2013

Pretransplantation Group

(a)Risk categories should be established based on the antibody and the XM results obtained.
(b)DSA detected by CDC and a positive XM should be avoided due to their strong association with antibody-mediated rejection and graft loss.
(c)A renal transplantation can be performed in the absence of a prospective XM if singleantigen bead screening for antibodies to all class I and II HLA loci is negative. This decision, however, needs to be taken in agreement with local clinical programs and the relevant regulatory bodies.
(d)The presence of DSA HLA antibodies should be avoided in heart and lung transplantation and considered a risk factor for liver, intestinal, and islet cell transplantation.

SPECIAL FEATURES

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

Transplantation • Volume 95, Number 1, January 15, 2013

Posttransplantation Group

(a)High-risk patients (i.e., desensitized or DSA positive/XM negative) should be monitored by measurement of DSA and protocol biopsies in the first 3 months after transplantation.
(b)Intermediate-risk patients (history of DSA but currently negative) should be monitored for DSA within the first month. If DSA is present, a biopsy should be performed.
(c)Low-risk patients (nonsensitized first transplantation) should be screened for DSA at least once 3 to 12 months after transplantation. If DSA is detected, a biopsy should be performed. In all three categories, the recommendations for subsequent treatment are based on the biopsy results.

まとめ

- DSA陽性例における脱感作療法では、short-lived plasma cellとlong-lived plasma cellの存在状態の把握が重要である。
- RituximabとBortezomibを段階的に投与することにより、重篤な感染症は認めなかった。
- 高感作症例に対しても脱感作は可能である。