Lecture «Mimmo Adorno»

RIGETTO E TOLLERANZA NEI TRAPIANTI

Antonina Piazza

AIBT – Summer School 2015

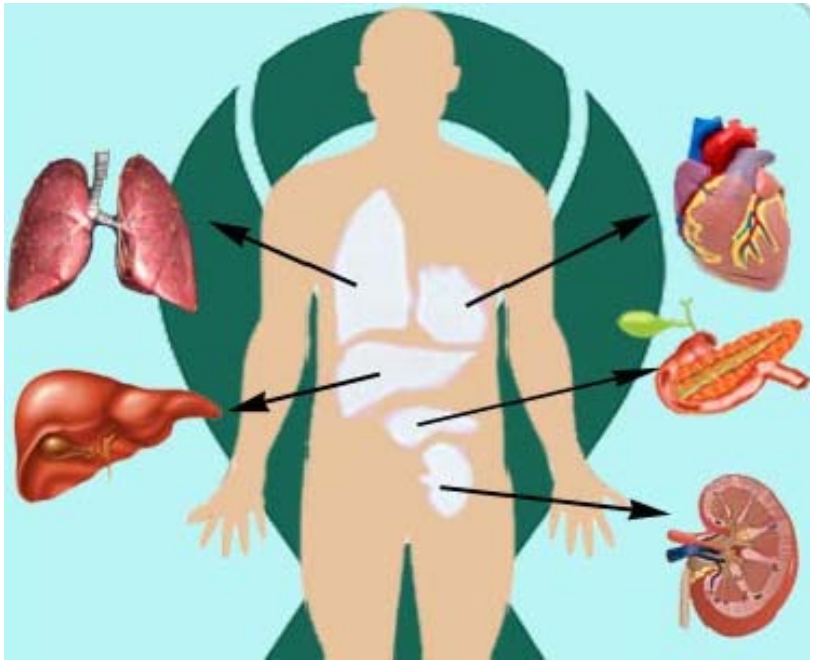
04-06 Giugno, Favignana

C.N.R., IFT UOS di Roma S. Camillo - Centro Regionale Trapianti Lazio

antonina.piazza@cnr.it

Allotrapianto di Organi Solidi

Il trapianto d'organo rappresenta la terapia d'elezione per pazienti con patologie d'organo allo stadio finale.

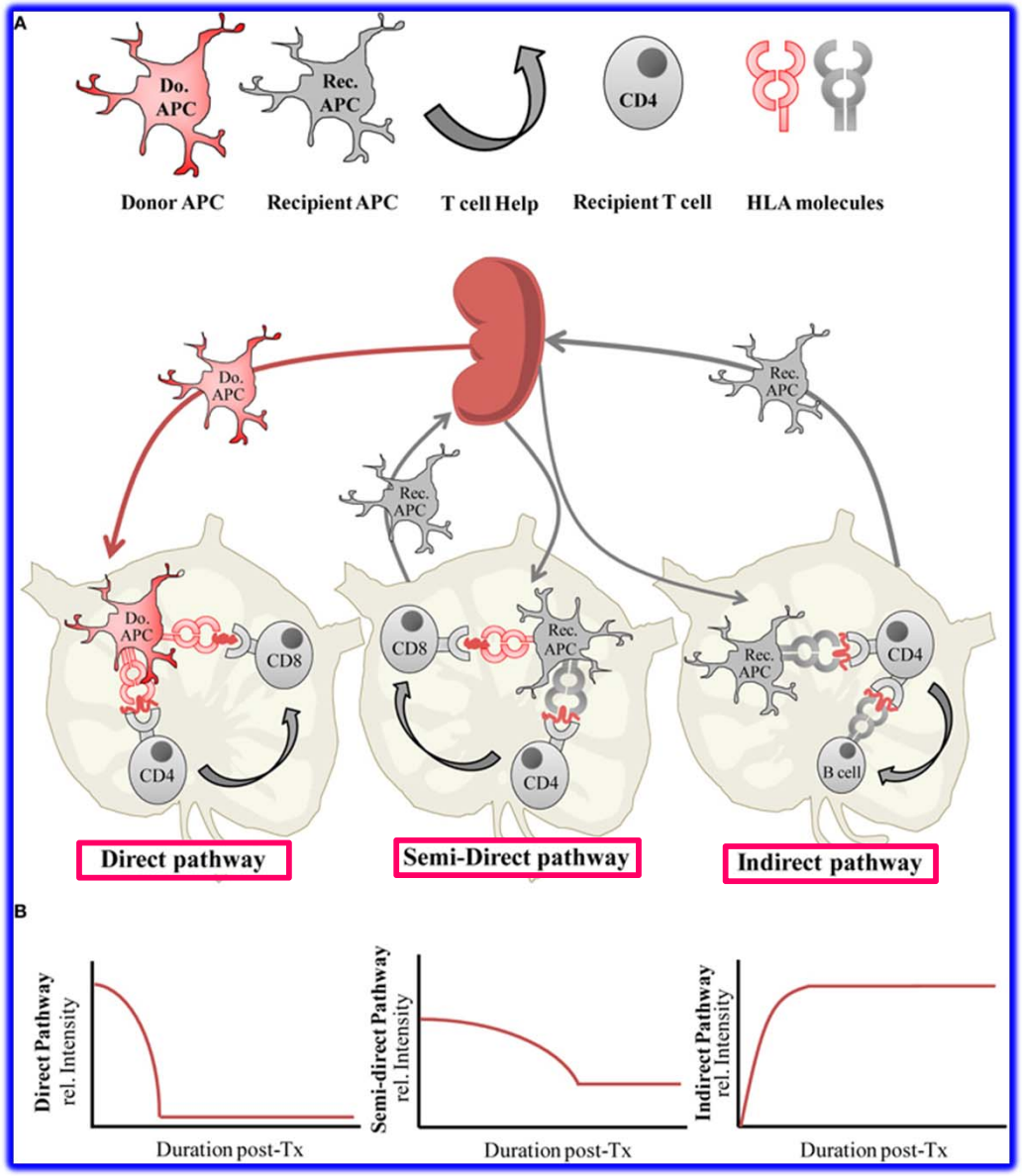


Risposta Immune al Trapianto

Riconoscimento di alloantigeni (molecole HLA mismatched)

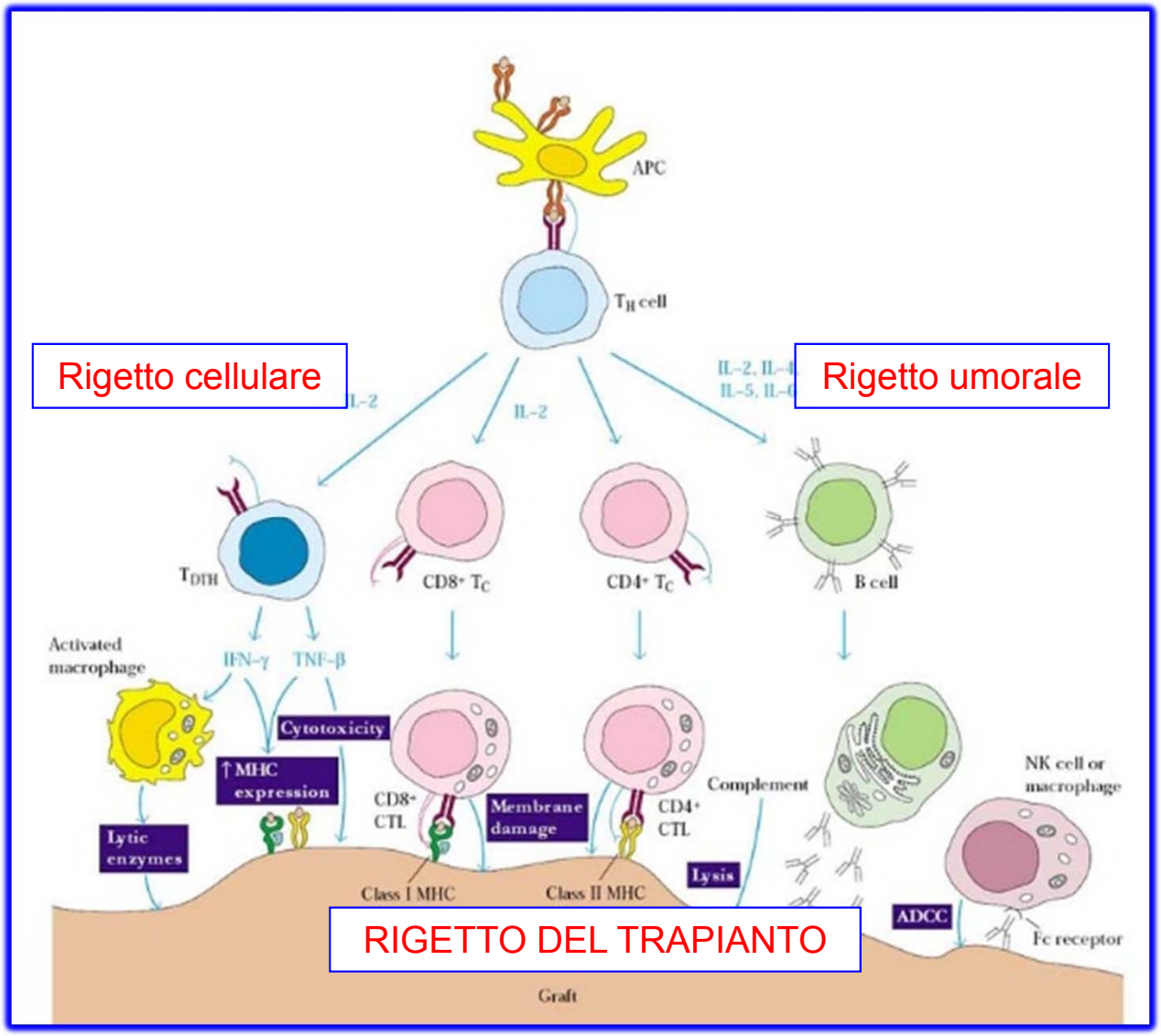
Riconoscimento di alloantigeni del donatore, soprattutto molecole HLA di classe I e II «mismatched», da parte dei linfociti del ricevente attraverso tre possibili meccanismi:

- **Riconoscimento diretto;**
- **Riconoscimento semi-diretto;**
- **Riconoscimento indiretto**



Risposta Immune al Trapianto)

Rigetto Cellulare ed Umorale dell'allotrapianto di organi

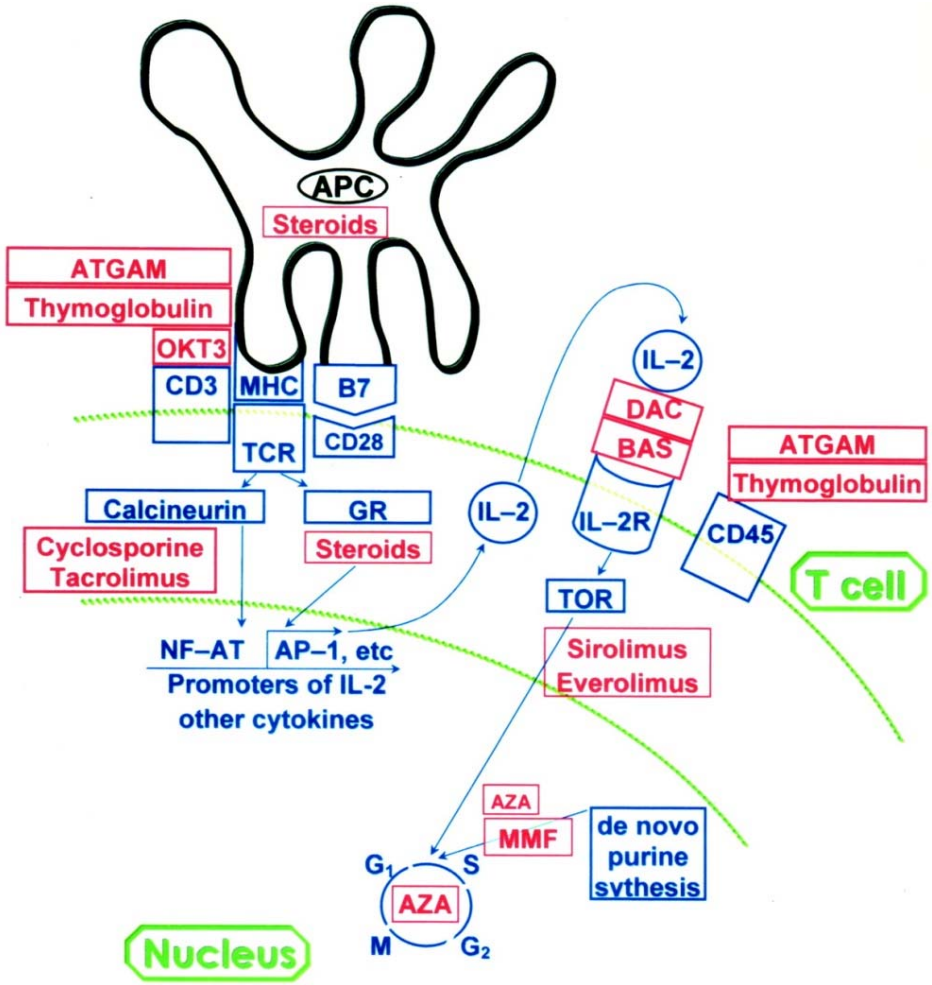


Risposta Immune al Trapianto

Immunosoppressione *(Tolleranza indotta farmacologicamente)* *(I)*

Immunologic mechanisms leading to graft rejection and sites of action of immunosuppressive drugs

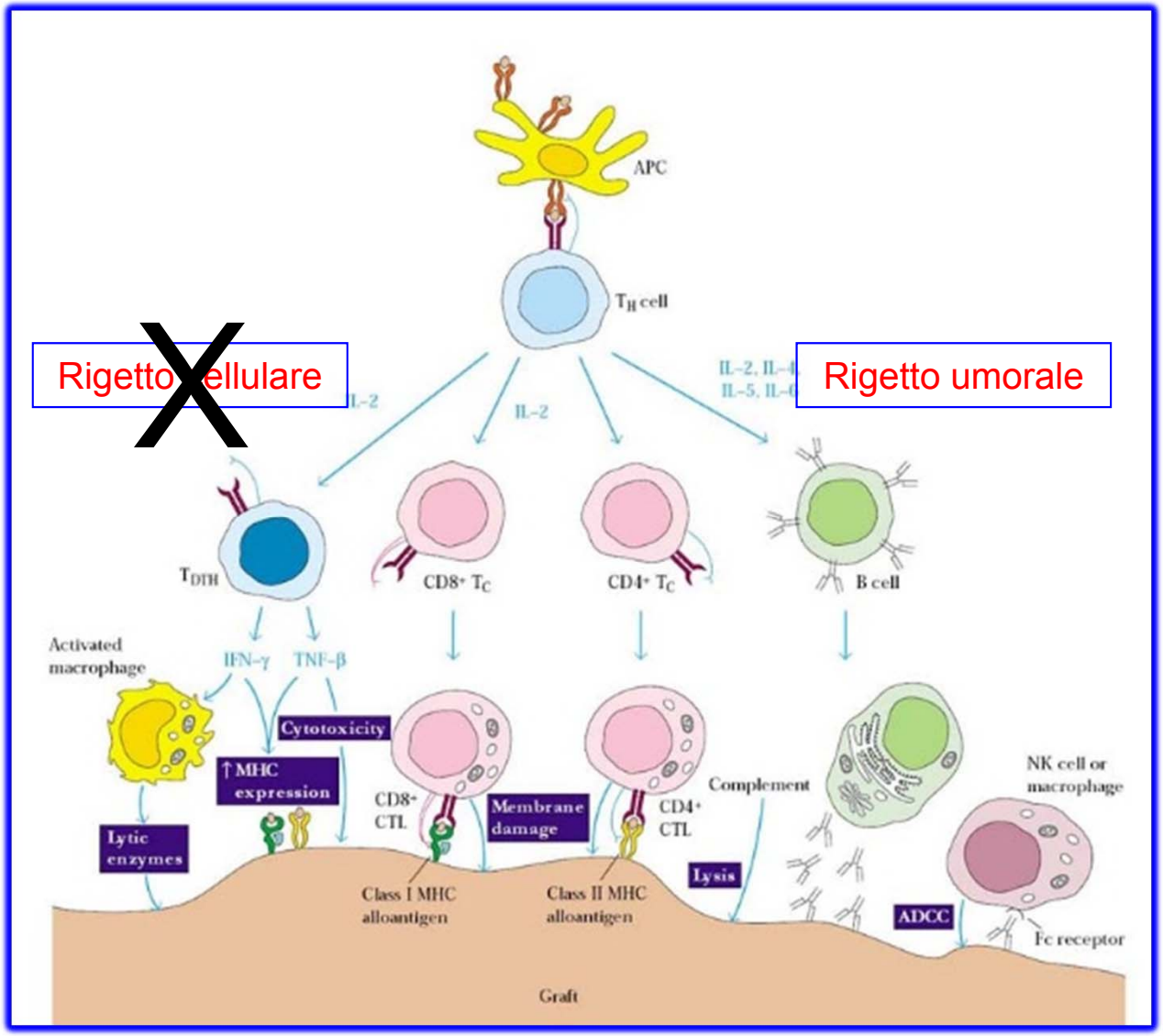
JoAnn Lindenfeld et al. Circulation. 2004;110:3734-3740



- ✓ **Corticosteroidi:** proprietà antiinfiammatorie e immunosoppressive;
- ✓ **Inibitori della calcineurina**: blocco della trascrizione dei geni delle citochine nei linfociti T;
- ✓ **Inibitori mTOR** (mammalian target of rapamycin): protein-chinasi che fosforila serina e treonina che regola crescita, proliferazione, motilità, sopravvivenza delle cellule nonché sintesi proteica e trascrizione;
- ✓ **Anti-proliferativi**: Azatioprina = antimetabolita purinico con effetti soppressivi su linfociti attivati; attiva su tutti i tipi cellulari con elevato indice mitotico; Micofenolato Mofetile = inibitore della sintesi *de novo* delle purine, inibisce la proliferazione e produzione di linfociti B e T, blocca la produzione di anticorpi e l'adesione dei linfociti all'endotelio vascolare;
- ✓ **Anticorpi policlonali anti-T**: diminuiscono il numero dei linfociti circolanti;
- ✓ **Anticorpi monoclonali anti-IL2Rec:** legame con la catena α del recettore (CD25) dell'IL2.

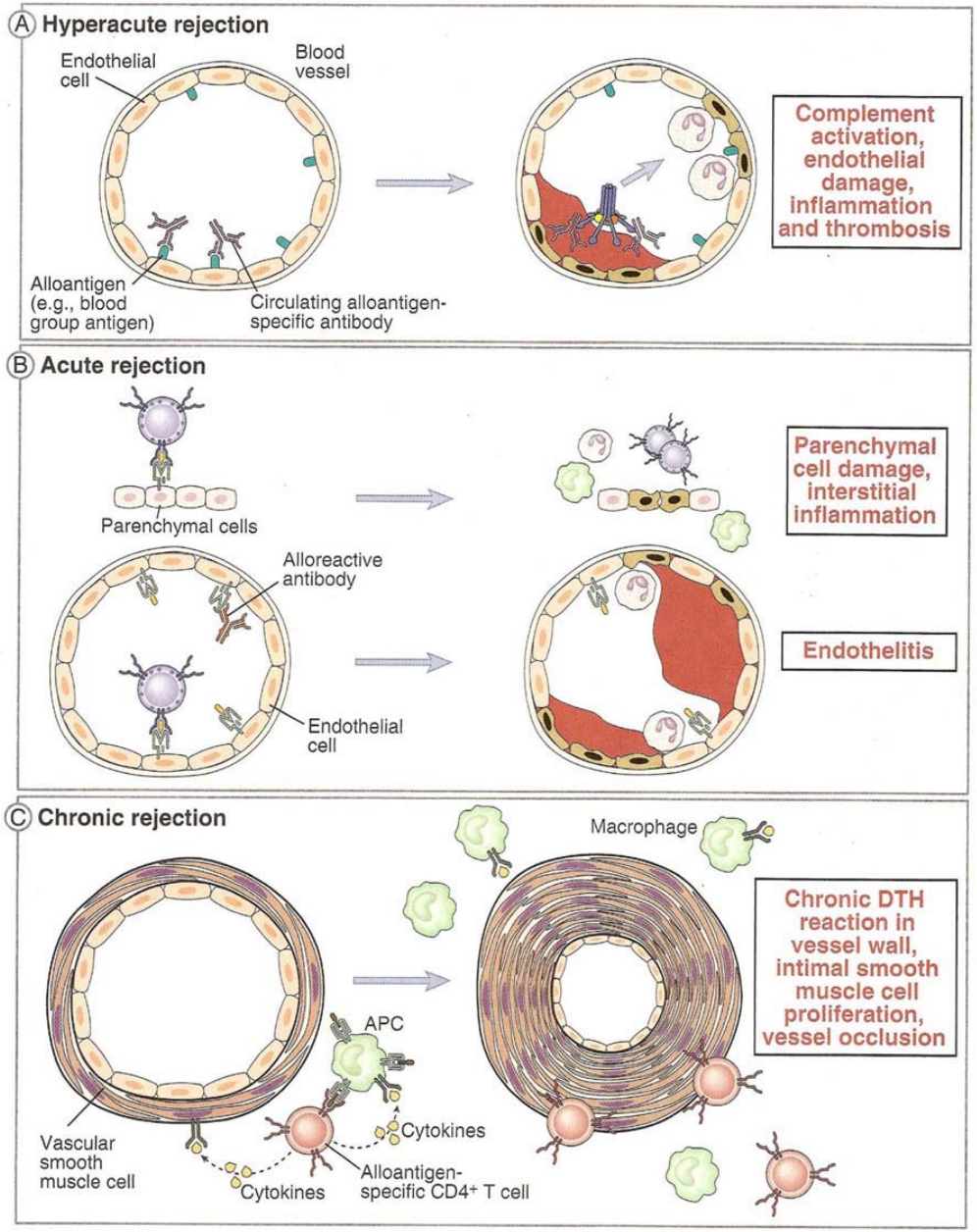
Risposta Immune al Trapianto

*Tolleranza indotta farmacologicamente (*Immunosoppressione*) (II)*



Risposta Immune al Trapianto

Rigetto Anticorpo Mediato *(I)*



ABMR phenotype 1:
- Pre-existing complement-fixing DSA
- Early occurence in posttransplant period

ABMR phenotype 2:
- Development of de novo DSA (complement-fixing or no complement-fixing)
- Late occurence in posttransplant period

Comprehensive Review

Diagnosis and Management of Antibody-Mediated Rejection: Current Status and Novel Approaches

American Journal of Transplantation 2014; 14: 255–271

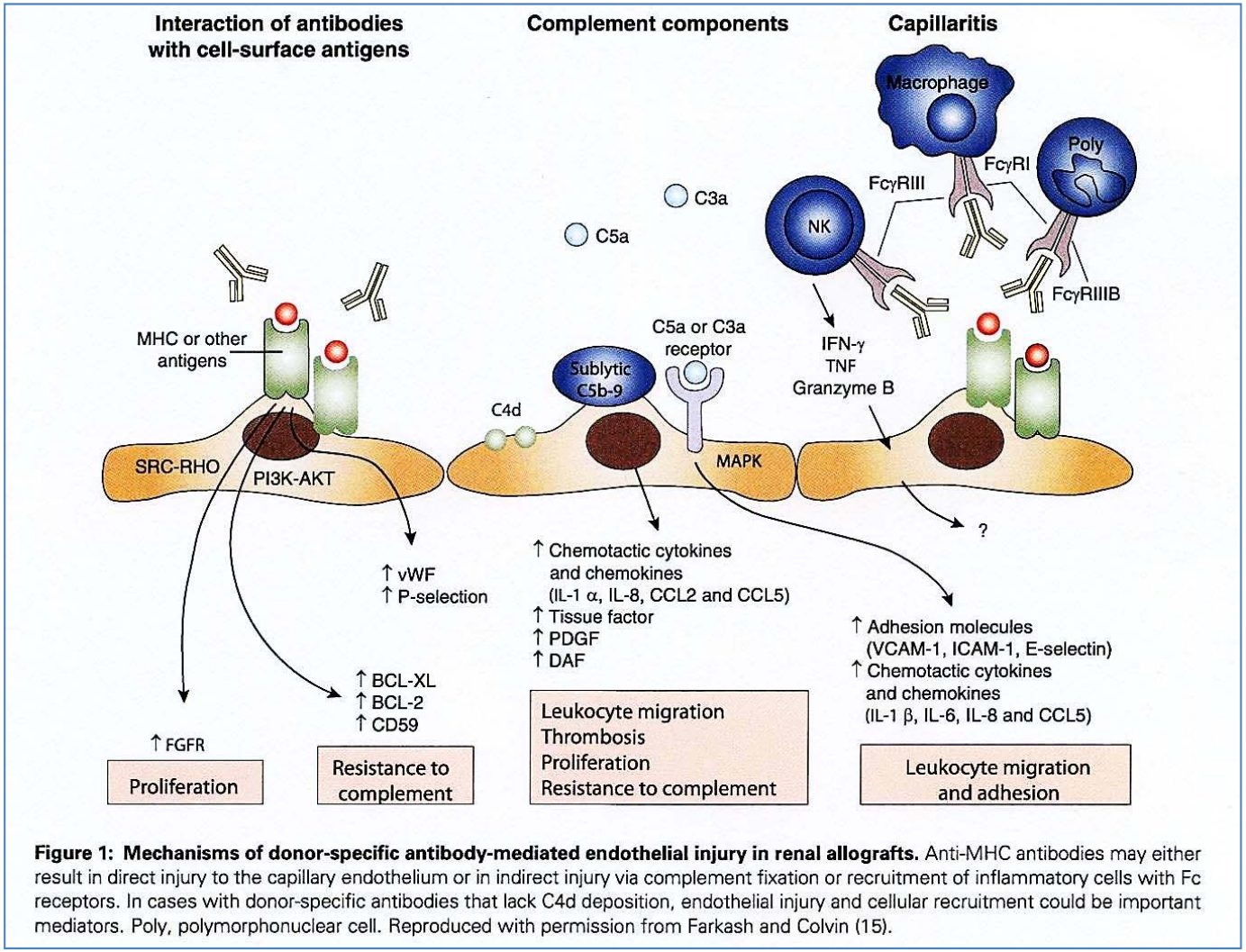


Figure 1: Mechanisms of donor-specific antibody-mediated endothelial injury in renal allografts. Anti-MHC antibodies may either result in direct injury to the capillary endothelium or in indirect injury via complement fixation or recruitment of inflammatory cells with Fc receptors. In cases with donor-specific antibodies that lack C4d deposition, endothelial injury and cellular recruitment could be important mediators. Poly, polymorphonuclear cell. Reproduced with permission from Farkash and Colvin (15).

Current Status of Antibody-Mediated Rejection

C4d-Positive ABMR

Serologic Evidence
- DSA present

+

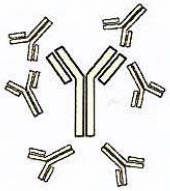
Immunopathologic Evidence
- IF: Diffuse-positive C4d in PTC
- IHC: Diffuse- or focal-positive C4d in PTC

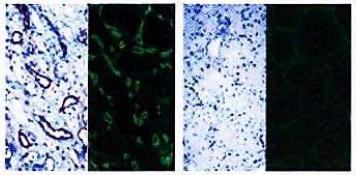
+

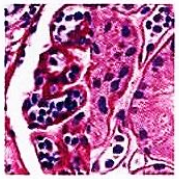
Histopathologic Evidence ACUTE
- ATN like changes, and/or
- Peritubular capillaritis, and/or
- Glomerulitis, and/or
- Arterial fibroid necrosis, and,
- No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement membranes)

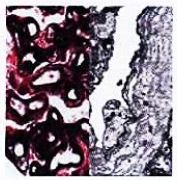
+

Histopathologic Evidence CHRONIC
- Transplant glomerulopathy, and/or
- PTC basement membrance multilamination, and/or
- IFTA, and/or
- Fibrous intimal thickening of arteries
- May be accompanied by glomerulitis and/or capillaritis









C4d-Negative ABMR

Banff 2013 Meeting Report: Inclusion of C4d-Negative Antibody-Mediated Rejection and Antibody-Associated Arterial Lesions
American Journal of Transplantation 2014; 14: 272–283

Serologic Evidence
- DSA present

+

Immunopathologic Evidence
- Negative C4d staining; and
- Endothelial activation, detected by increased mRNA expression of endothelial genes, such as W/F, PECAM, SELE, etc; and/or
- Evidence for glomerular and/or capillary endothelial cycling (CD31+Ki67+ cells lining the microcirculation)

+

Histopathologic Evidence ACUTE
- Peritubular capillaritis, and/or
- Glomerulitis, and/or
- Thrombotic microangiopathy, and/or
- Arterial fibroid necrosis, and
- No evidence for chronic capillary injury (reduction and/or multilayering of glomerular and peritubular capillary basement)

+

Histopathologic Evidence CHRONIC
- Transplant glomerulopathy, and/or
- PTC basement membrane multilamination, and/or
- Fibrous intimal thickening of arteries
- May be accompanied by glomerulitis and/or capillaritis

Stadi del Rigetto Anticorpo-Mediato

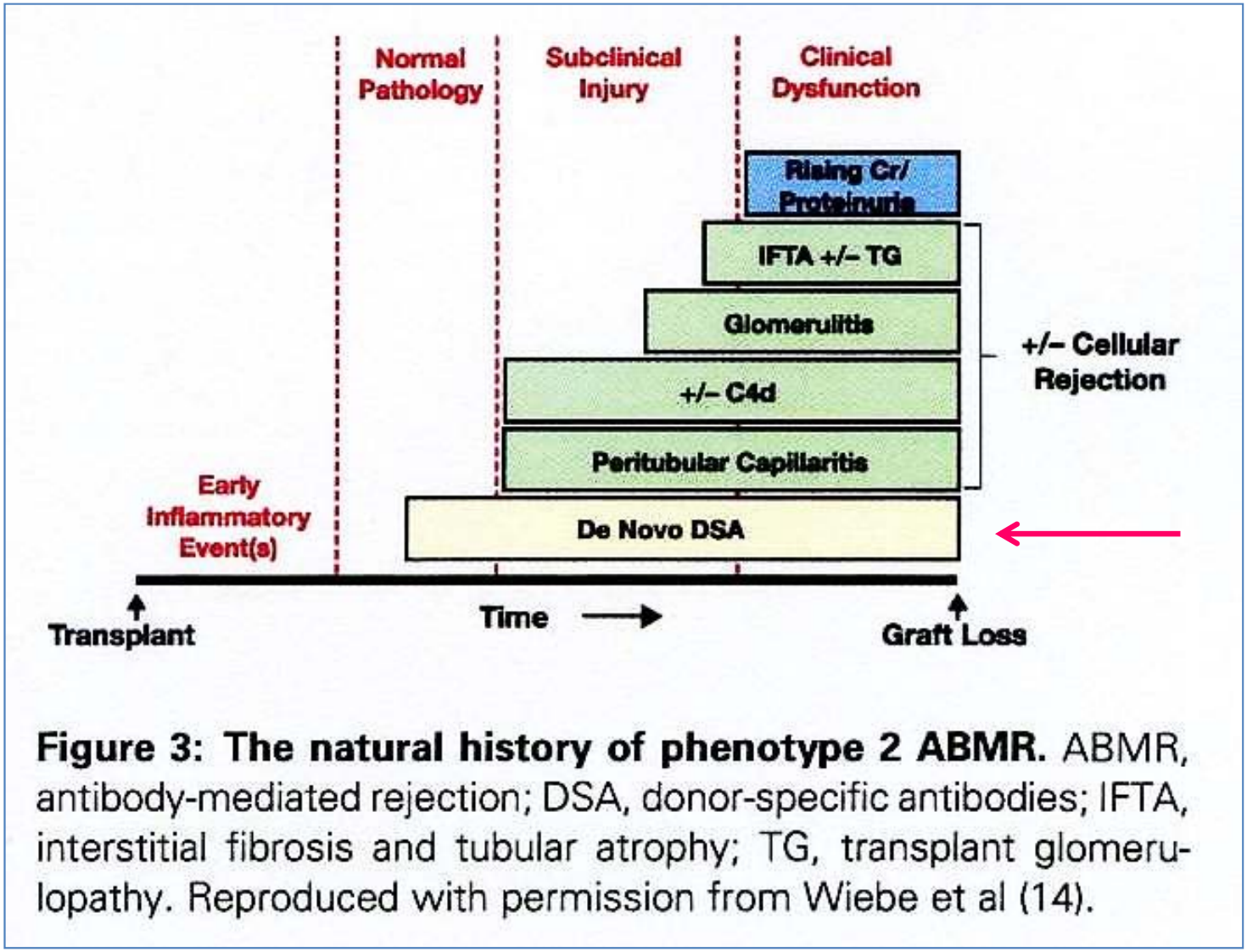


Figure 3: The natural history of phenotype 2 ABMR. ABMR, antibody-mediated rejection; DSA, donor-specific antibodies; IFTA, interstitial fibrosis and tubular atrophy; TG, transplant glomerulopathy. Reproduced with permission from Wiebe et al (14).

American Journal of Transplantation 2014; 14: 255–271

Risposta Immune al Trapianto

Rigetto Anticorpo Mediato

Humoral Theory of Transplantation: Mechanism, Prevention, and Treatment

Junchao Cai and Paul I. Terasaki

Human Immunology 66, 334–342 (2005)

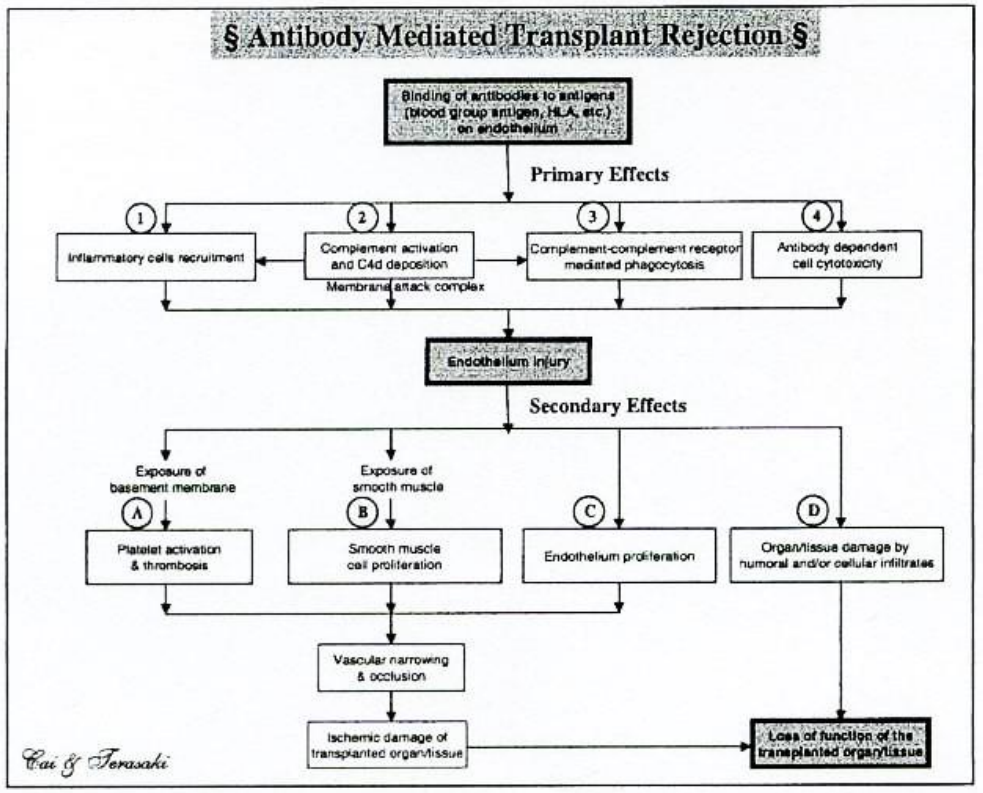


FIGURE 1 Mechanisms of antibody-mediated transplant rejection.

Human Immunology 66, 334–342 (2005)

PREVENTION IS BETTER THAN CURE—IMPORTANCE OF ANTIBODY MONITORING

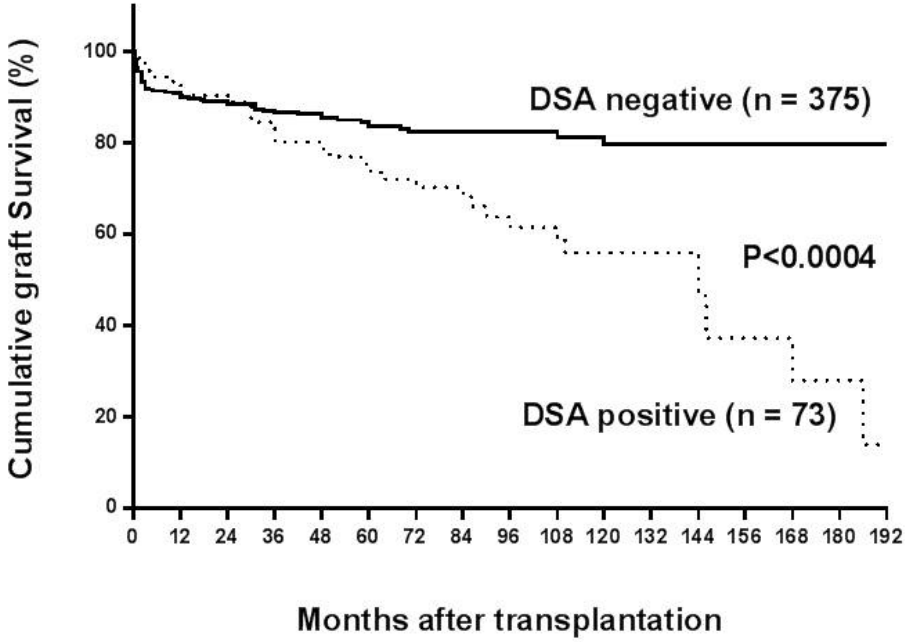
In current transplant clinics, the principal universal method of monitoring is periodical laboratory examination and/or protocol biopsy. However, in light of the recent evidence in renal transplantation that HLA antibodies appear before rise in serum creatinine [3, 4, 102], we can now suggest that testing for HLA antibodies is added to the routine monitoring of patients. At any given time after transplantation, approximately 20% of patients can be expected to have HLA antibodies [3]. Evidence from the prospective study [3] suggests that it is these patients with HLA antibodies who will eventually have grafts that fail as a result of humoral rejection. Ongoing humoral rejection is not apparent by laboratory indexes until the organ parenchyma is injured to some critical level, after which no amount of immunosuppression can reverse the changes.

If this concept of antibody caused humoral injury is correct, then antibody testing will be the key to monitoring before irreversible damage has occurred.

Clinical Transplants 2006, Terasaki Foundation Laboratory, Los Angeles, California

Post-Transplant Donor-Specific Antibody Production and Graft Outcome in Kidney Transplantation: Results of Sixteen-Year Monitoring by Flow Cytometry

Antonina Piazza,[1] Elvira Poggi,[1] Giuseppina Ozzella,[1] Laura Borrelli,[1] Alessandra Scornajenghi,[1] Giuseppe Iaria,[2] Giuseppe Tisone,[2] and Domenico Adorno[1]

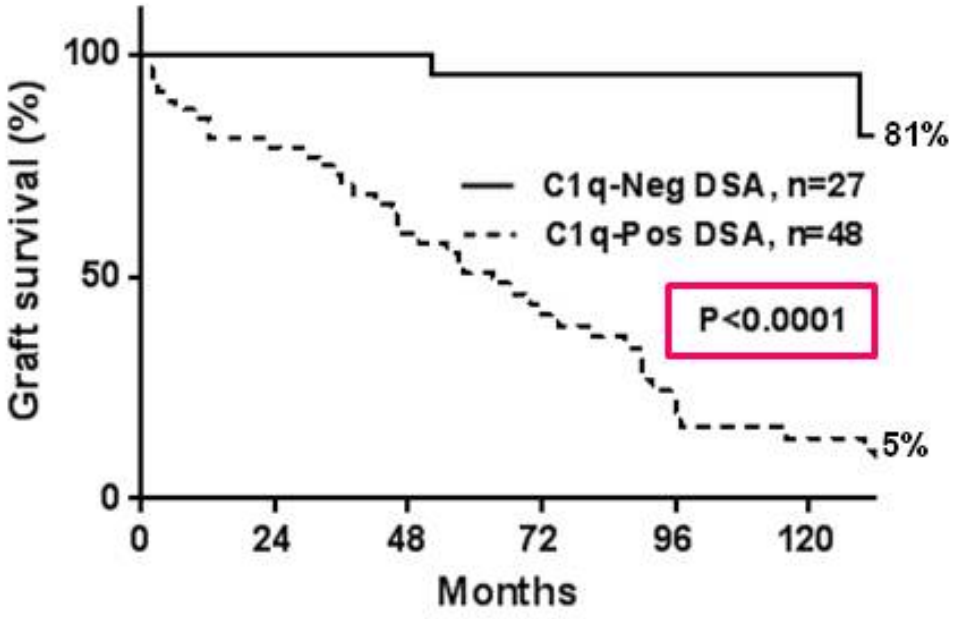


Graft Survival according to "de novo" DSA Production

Clinical Transplants 2013, Terasaki Foundation Laboratory, Los Angeles, California

Post-transplant Development of C1q-Positive HLA Antibodies and Kidney Graft Survival

Antonina Piazza[1], Elvira Poggi[1], Giuseppina Ozzella[1], and Domenico Adorno[1, 2]



Graft Survival according to C1q-fixing Capability of "de novo" DSA

Humoral theory of transplantation: some hot topics

Junchao Cai[1], **Xin Qing**[2], **Jianming Tan**[3], and **Paul I. Terasaki**[1*]

British Medical Bulletin 2013; **105**: 139–155

Introduction: Antibody is a major cause of allograft injury. However, it has not been routinely tested post-transplant.

Sources of data: A literature search was performed using PubMed on the topics of 'antibody monitoring', 'autoantibody and allograft dysfunction' and 'prevention and treatment of antibody-mediated rejection (AMR)'.

Areas of agreement: Donor-specific antibody (DSA) monitoring not only helps to identify patients at risk of AMR, but also serves as a biomarker to personalize patient's maintenance immunosuppression. Development of autoantibody is a secondary response following primary tissue injury. Some autoantibodies are directly involved in allograft injury, while others only serve as biomarkers of tissue injury.

Areas of controversy: It remains controversial whether DSA-positive patients without symptoms need to be treated. In addition, given the variation in study designs and patient's characteristics, there is discrepancy regarding which treatment regimens provide optimal clinical outcome in preventing/treating AMR.

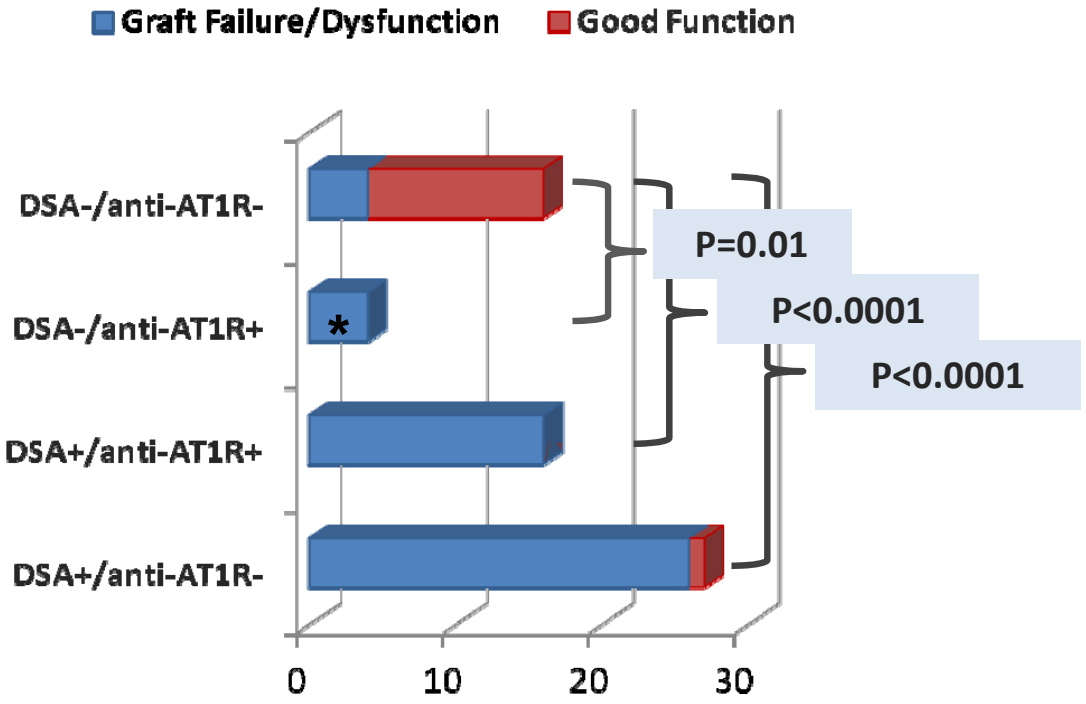
Growing points: Efficacy of B-cell and/or antibody-targeted therapies in treating or preventing AMR would be better measured by the incorporation of antibody monitoring into current functional and pathological assays.

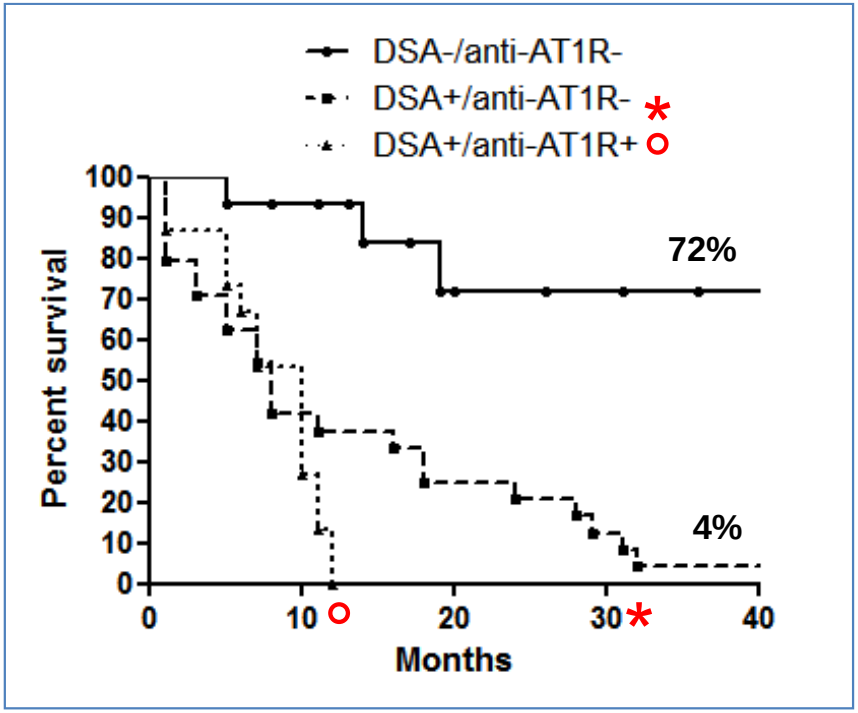
Areas timely for developing research: Research in B-cell targeted therapies to prevent and treat AMR is rapidly growing, which includes monoclonal antibodies against B-cell markers CD20, CD40, CD19, BlyS, etc. It requires extensive clinical research to determine the best approach to inhibit or delete antibody and how to balance the drug efficacy with safety.

ANTICORPI ANTI-AT_1R E TRAPIANTO D'ORGANO

Antonina Piazza, Elvira Poggi, Giuseppina Ozzella, Maurizio Valeri, Nicola Torlone, Domenico Adorno

• *38° Congresso SITO, Siena 24-26 Settembre 2014*





* All had graft dysfunction

Terapia (prevenzione/trattamento) del Rigetto Anticorpo-Mediato

American Journal of Transplantation 2014; 14: 255–271

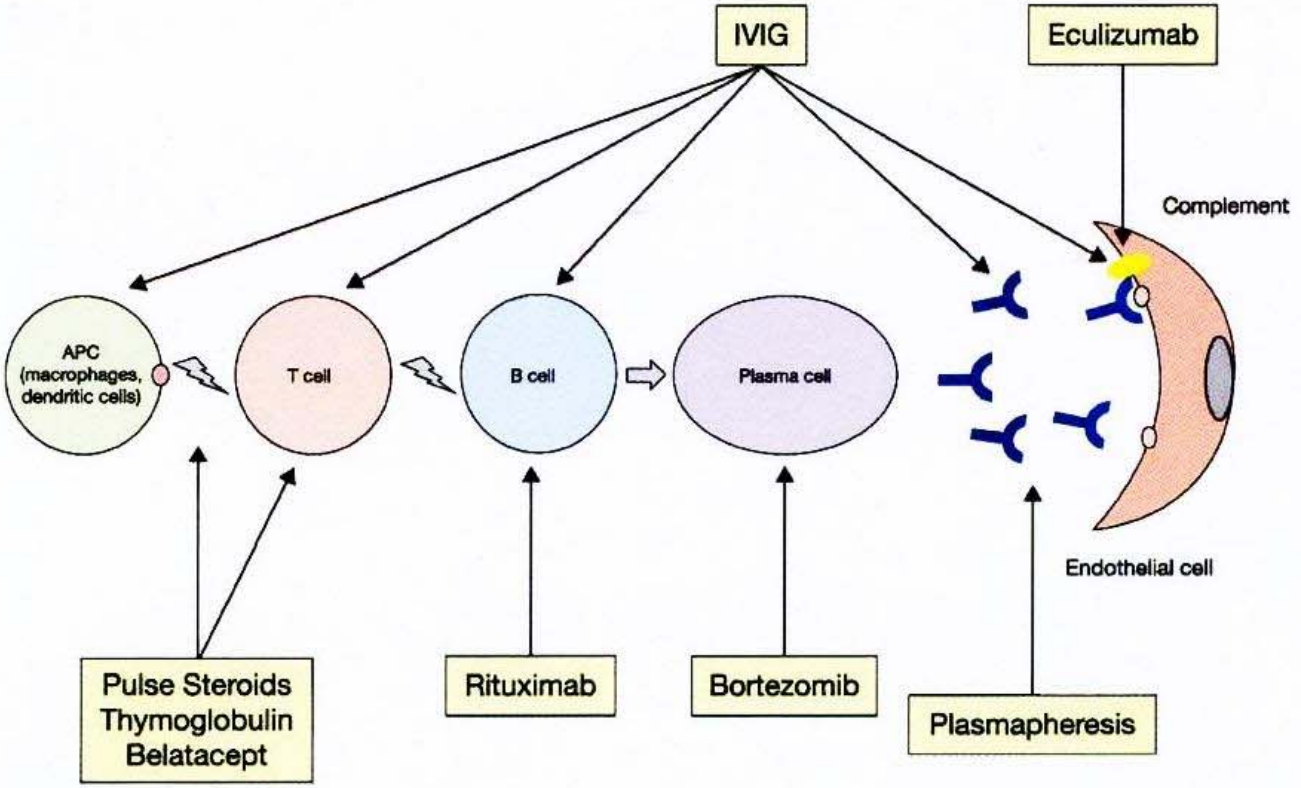


Figure 4: Therapeutic modalities for ABMR. ABMR, antibody-mediated rejection; APC, antigen-presenting cell; IVIG, intravenous immunoglobulins.

Reducing De Novo Donor-Specific Antibody Levels during Acute Rejection Diminishes Renal Allograft Loss

American Journal of Transplantation 2009; 9: 1063–1071

The effect of de novo DSA detected at the time of acute cellular rejection (ACR) and the response of DSA levels to rejection therapy on renal allograft survival were analyzed. Kidney transplant patients with acute rejection underwent DSA testing at rejection diagnosis with DSA levels quantified using Luminex single-antigen beads. Fifty-two patients experienced acute rejection with 16 (31%) testing positive for de novo DSA. Median follow-up was 27.0 ± 17.4 months postacute rejection. Univariate analysis of factors influencing allograft survival demonstrated significance for African American race, DGF, cytotoxic PRA >20% (current) and/or >50% (peak), de novo DSA, C4d and repeat transplantation. Multivariate analysis showed only de novo DSA (6.6-fold increased allograft loss risk, $p = 0.017$) to be significant. Four-year allograft survival was higher with ACR (without DSA) (100%) than mixed acute rejection (ACR with DSA/ C4d) (65%) or antibody-mediated rejection (35%) ($p < 0.001$). Patients with >50% reduction in DSA within 14 days experienced higher allograft survival ($p = 0.039$). De novo DSAs detected at rejection are associated with reduced allograft survival, but prompt DSA reduction was associated with improved allograft survival. DSA should be considered a potential new end point for rejection therapy.

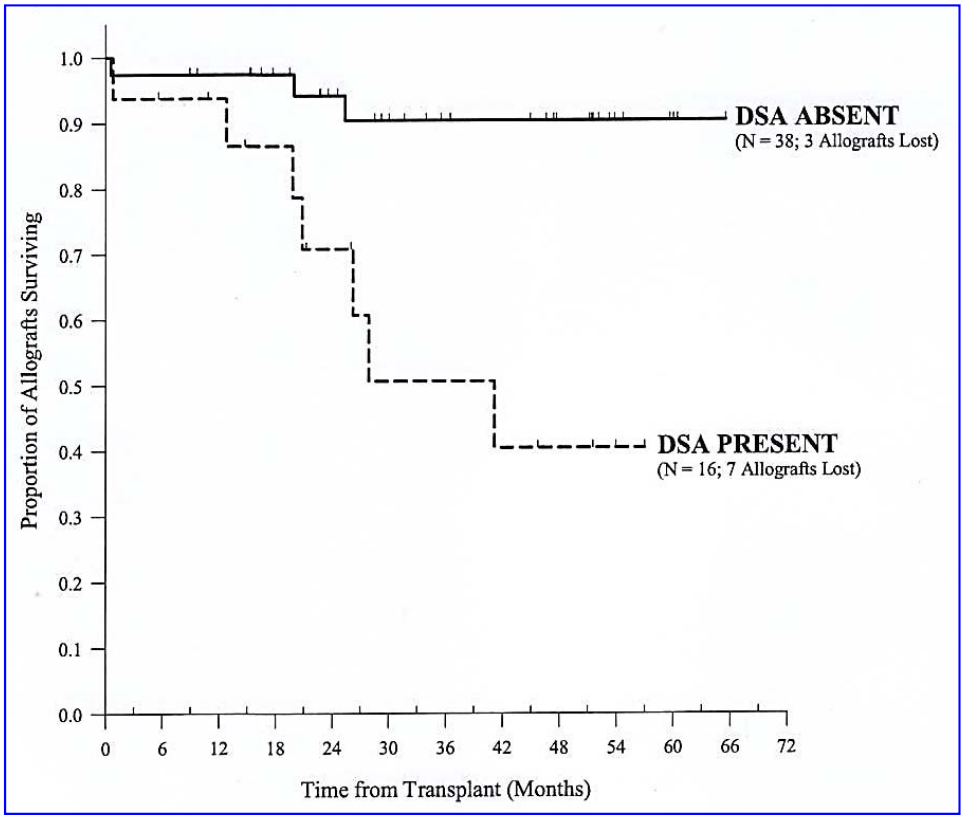


Figure 1: Kaplan–Meier survival in patients with or without DSA at rejection diagnosis (p = 0.001; log-rank).

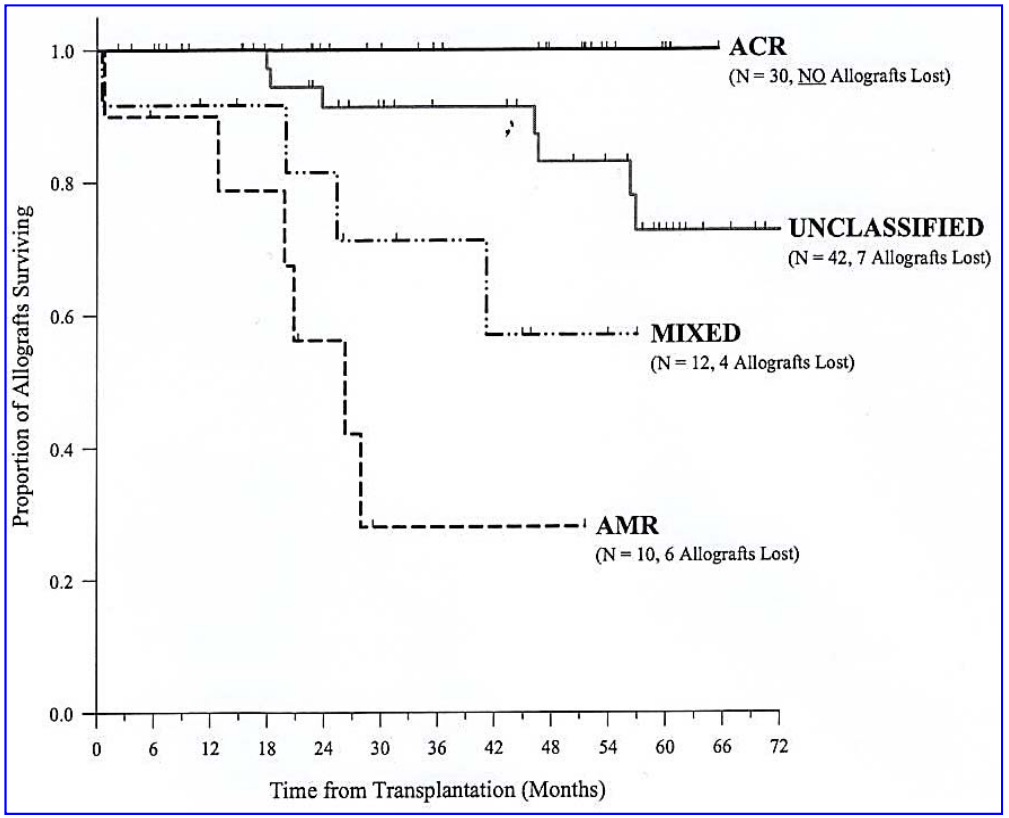


Figure 2: Death-censored allograft survival stratified by rejection type (ACR vs. mixed ($p < 0.001$; log-rank), ACR vs. AMR ($p < 0.001$; log-rank) and unclassified vs. AMR ($p < 0.001$; log-rank)).

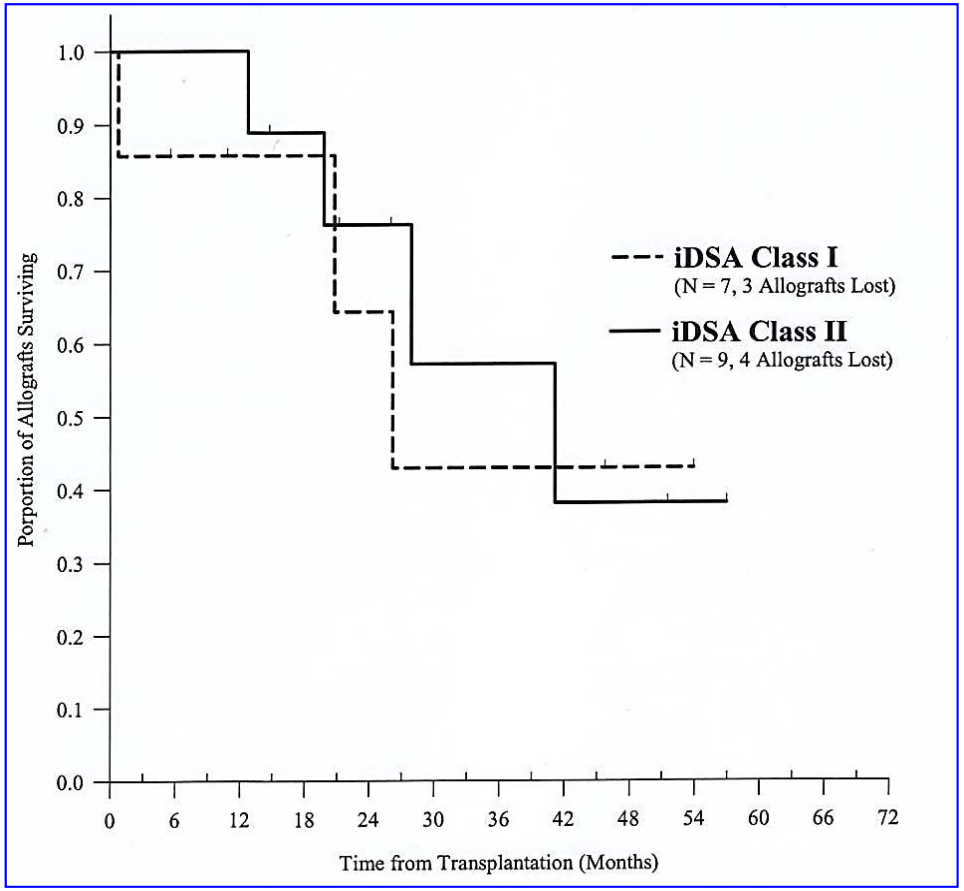


Figure 4: Death-censored allograft survival stratified by iDSA class (p = 0.797; log-rank).

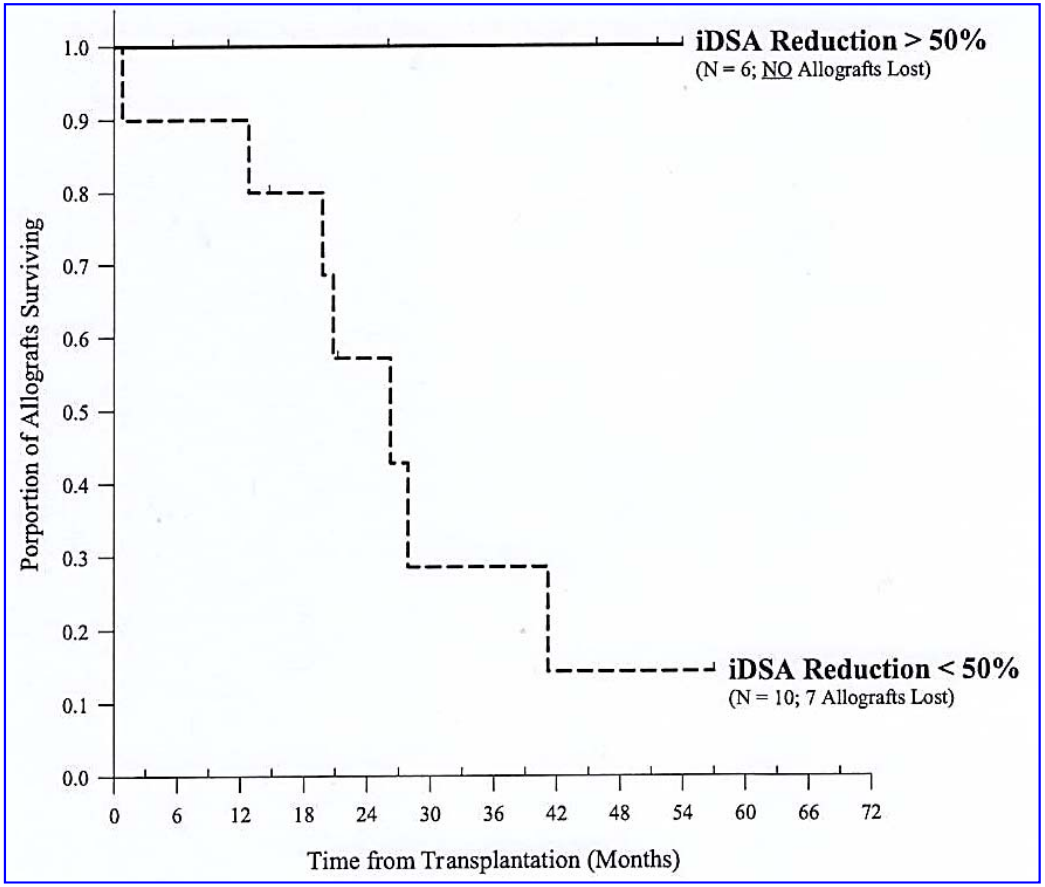


Figure 5: Death-censored allograft survival stratified by % reduction in iDSA at 14 days postbiopsy (p = 0.021; log-rank).

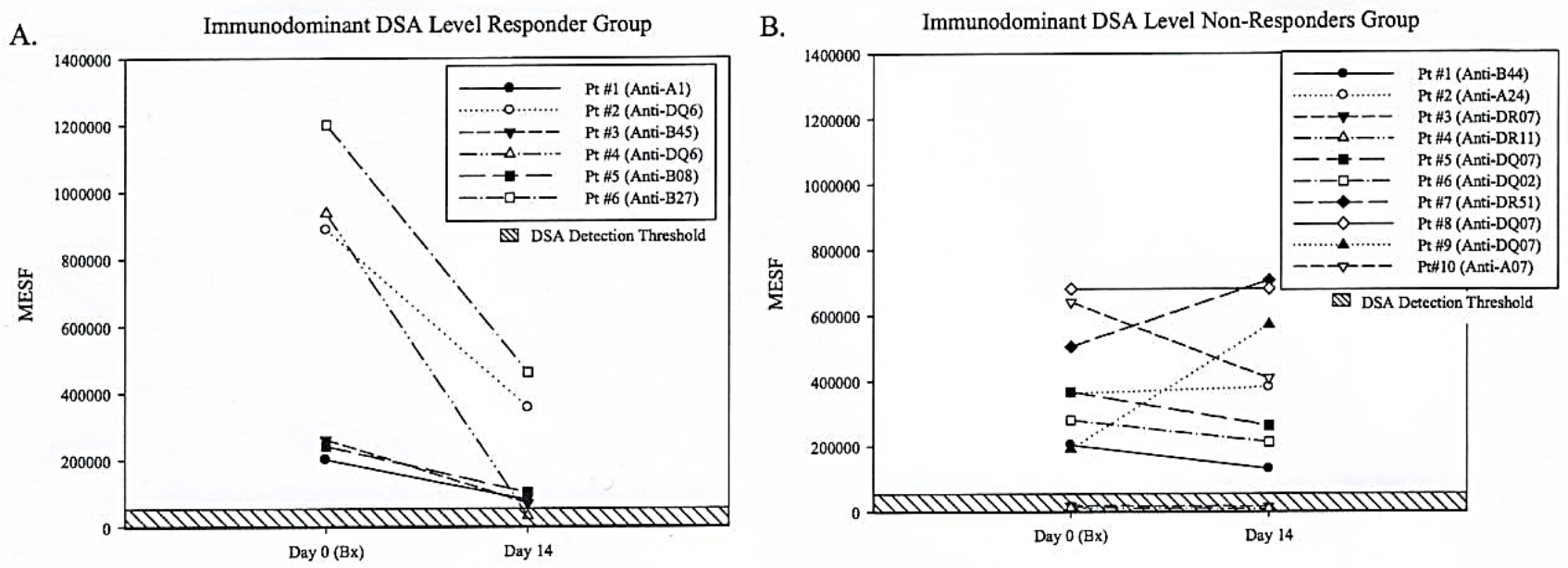


Figure 6: iDSA levels at time of biopsy and 14 days after initiation of rejection therapy. (A) iDSA responders were primarily class I specific DSA and (B) iDSA nonresponders were predominantly class II specific DSA.

Conclusions: De novo DSAs detected at rejection are associated with reduced allograft survival, but prompt DSA reduction was associated with improved allograft survival. DSA should be considered a potential new end point for rejection therapy.

Tolleranza Immunitaria del Trapianto d'Organo

Definizione

Assenza di una risposta immune del ricevente, a carattere «distruttivo», specifica per i tessuti dell'organo trapiantato.

Tale definizione non è utilizzabile per i trapianti nell'uomo, dove si può parlare di «operational tolerance»

Criteri per valutare l'operatività della tolleranza del trapianto:

- ✓ Completa interruzione di terapie immunosoppressive;
- ✓ Nessuna evidenza di rigetto del trapianto per un anno dalla sospensione della terapia.

Possibilità di indurre tolleranza strettamente dipendente dall'organo trapiantato (>20% per il fegato, <1‰ nel rene).

Operational Tolerance: Past Lessons and Future Prospects

Josh Levitsky
Division of Hepatology and Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL

LIVER TRANSPLANTATION 17:222-232, 2011

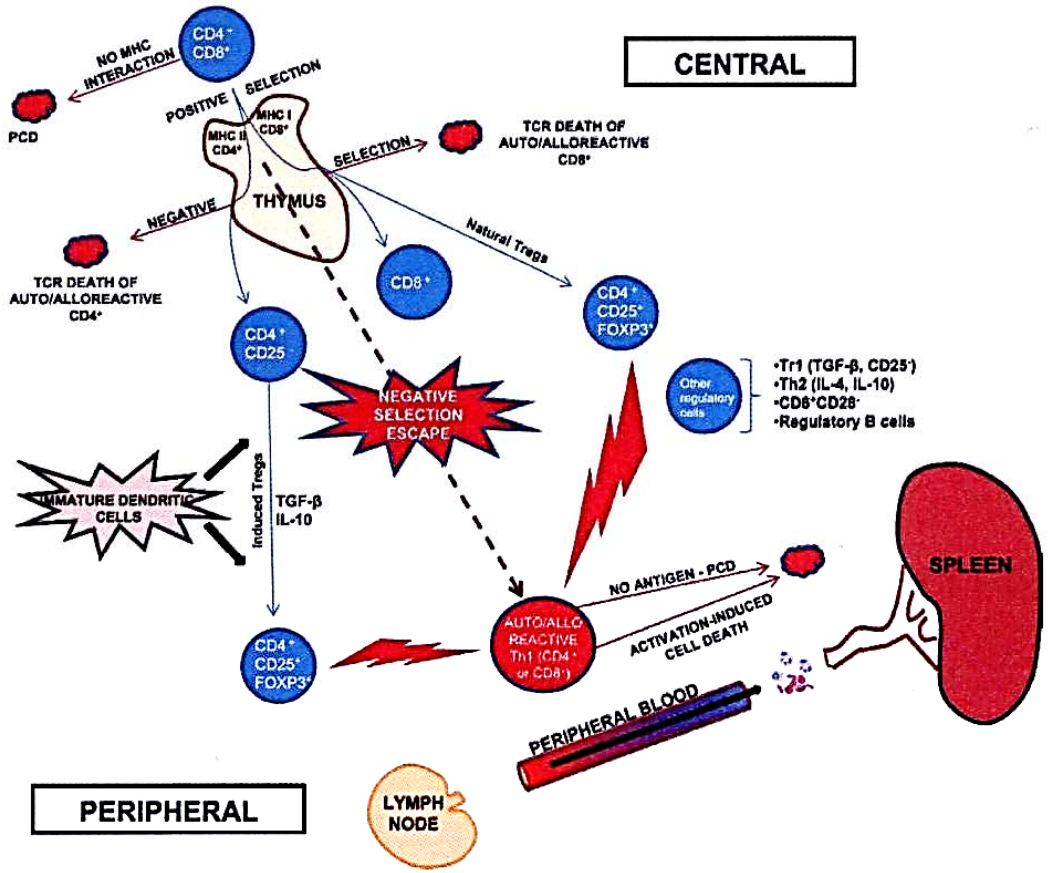


Figure 1. Basic mechanisms of tolerance. This figure displays the central and peripheral mechanisms for regulation of autoimmune/alloimmune responses. $CD3^+$ cells that are initially double positive for CD4 and CD8 first undergo positive selection to become either $CD4^+$ (MHC class 2 interaction) or $CD8^+$ (MHC class 1 interaction) followed by deletion or negative selection if autoreactive/alloreactive. However, some $CD4^+$ and $CD8^+$ cells can escape negative selection and become autoreactive/alloreactive Th1 cells in the periphery (lymph nodes, blood, spleen). Mechanisms to regulate these peripherally reactive T cells that can cause autoimmune diseases and transplant rejection are displayed: (1) Immunoregulation from natural (thymic-derived) or induced (in the context of proregulatory cytokines/dendritic cells) Tregs or other regulatory cells; (2) activation-induced cell death; and (3) no activation resulting in programmed cell death.

THE LIVER AS A TOLEROGENIC ORGAN

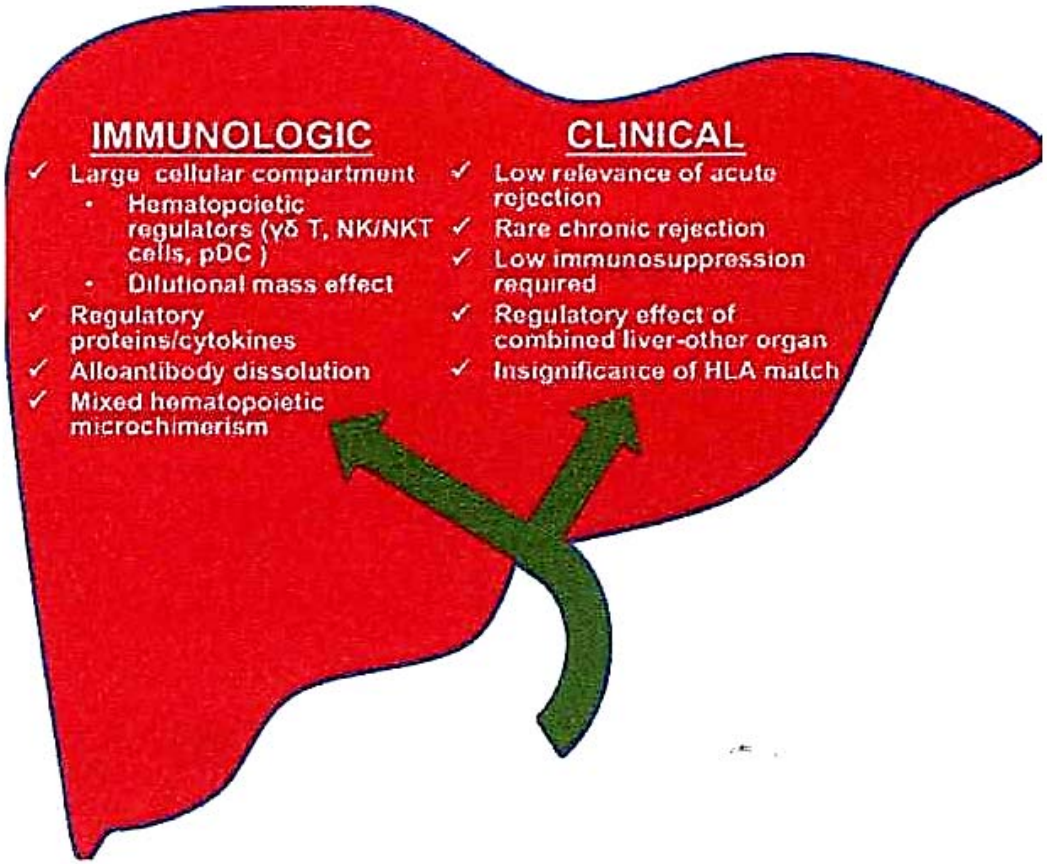


Figure 2. Putative components of liver immunoregulation. This figure illustrates clinical and immunologic evidence supporting liver transplant immunoregulation. The significant contribution from resident regulatory cells and cytokines is likely a major factor leading to low rejection rates and IS requirements.

TABLE 3. Candidate Liver Transplant Tolerance Assays

More Definitive

- Peripheral blood immunophenotyping
  - $CD4^+CD25^{high}FOXP3^+$ cells
  - DC2:DC1 ratio
  - γδ T cells (Vδ1/Vδ2 ratio)
- Genomic signatures
  - NK cell, γδ T cell, $CD8^+$ cell receptors
  - Cytokine gene polymorphisms (TNF-α, IL-10)
- Soluble HLA-G

Less Definitive

- Allograft immunophenotyping
  - Immunohistochemical staining and in vitro culture for Treg:Teff ratio
  - FOXP3 mRNA
- Donor-specific approaches
  - Mixed lymphocyte reaction (proliferation, CFSE labeling, Treg MLR)
  - Cell-mediated lymphotoxicity
  - ELISPOT (Th1 and Th2 cytokines)
  - Delayed type hypersensitivity (Trans-vivo)
  - Donor-specific antibodies and HLA typing
- Detection of hematopoietic chimerism

The more definitive assays have been validated in operationally tolerant liver transplant recipients. The less definitive assays are either inconclusive or inadequately studied in clinical tolerance protocols.

TABLE 2. Elective Withdrawal Studies

Center (No. of Patients)	Adult or Pediatric	DDLT or LDLT	Baseline IS	Years from LT to Tapering	Tolerant	Failure*
Pittsburgh (n = 95)	Both	DDLT	TAC or CyA + AZA	Mean, 8.4 ± 4.7	18 (18.9%)	40 (42.1%)
London (n = 18)	Adult	DDLT	CyA, AZA, prednisolone	Median, 7 (5-11)	5 (27.7%)	13 (72.2%)
Kyoto (n = 115)	Pediatric	LDLT	TAC	>2	49 (42.6%)	20 (17.4%)
Murcia (n= 9)	Adult	DDLT	CyA	Median, 5.1 (2-9)	3 (33.3%)	6 (66.6%)
Rome (n = 34, only HCV)	Adult	DDLT	CyA	Mean, 5.3 ± 1.7	8 (23.5%)	26 (76.5%)
New Orleans (n = 18)	Adult	DDLT	TAC	>0.5	1 (5.6%)	17 (94.4%)
Winnipeg (n = 26)†	Adult	DDLT	CyA + AZA or prednisolone	Mean, 4.3 ± 1.1	8 (30.8%)	18 (69.2%)
Miami (n = 104)‡	Adult	DDLT	TAC or CyA	Median, 4 (3.6-4.6)	23 (22.1%)	81 (61.5%)
Barcelona (n = 102)	Adult	DDLT	TAC or CyA	Median, 7.9	40 (77.9%)	62 (60.0%)

*Either due to rejection, immune-mediated hepatitis, noncompliance, resumption of immunosuppression, disease recurrence, or other. The remaining patients were deemed "weaning in progress" in all studies.

†Randomized controlled trial of ursodeoxycholic acid given at 15 mg/kg/day versus placebo in withdrawing patients; 3 patients developed autoimmune hepatitis recurrence after withdrawal.

‡45 received donor bone marrow cell infusions; 59 did not.

frontiers in
IMMUNOLOGY

July 2012 | Volume 3 | Article 198 |

Tolerogenic therapies in transplantation

Eugenia K. Page, Wasim A. Dar and Stuart J. Knechtle*

Department of Surgery, Emory University Hospital, Atlanta, GA, USA

Since the concept of immunologic tolerance was discovered in the 1940s, the pursuit of tolerance induction in human transplantation has led to a rapid development of pharmacologic and biologic agents. Short-term graft survival remains an all-time high, but successful withdrawal of immunosuppression to achieve operational tolerance rarely occurs outside of liver transplantation. Collaborative efforts through the NIH sponsored Immune Tolerance Network and the European Commission sponsored Reprogramming the Immune System for Establishment of Tolerance consortia have afforded researchers opportunity to evaluate the safety and efficacy of tolerogenic strategies, investigate mechanisms of tolerance, and identify molecular and genetic markers that distinguish the tolerance phenotype. In this article, we review traditional and novel approaches to inducing tolerance for organ transplantation, with an emphasis on their translation into clinical trials.

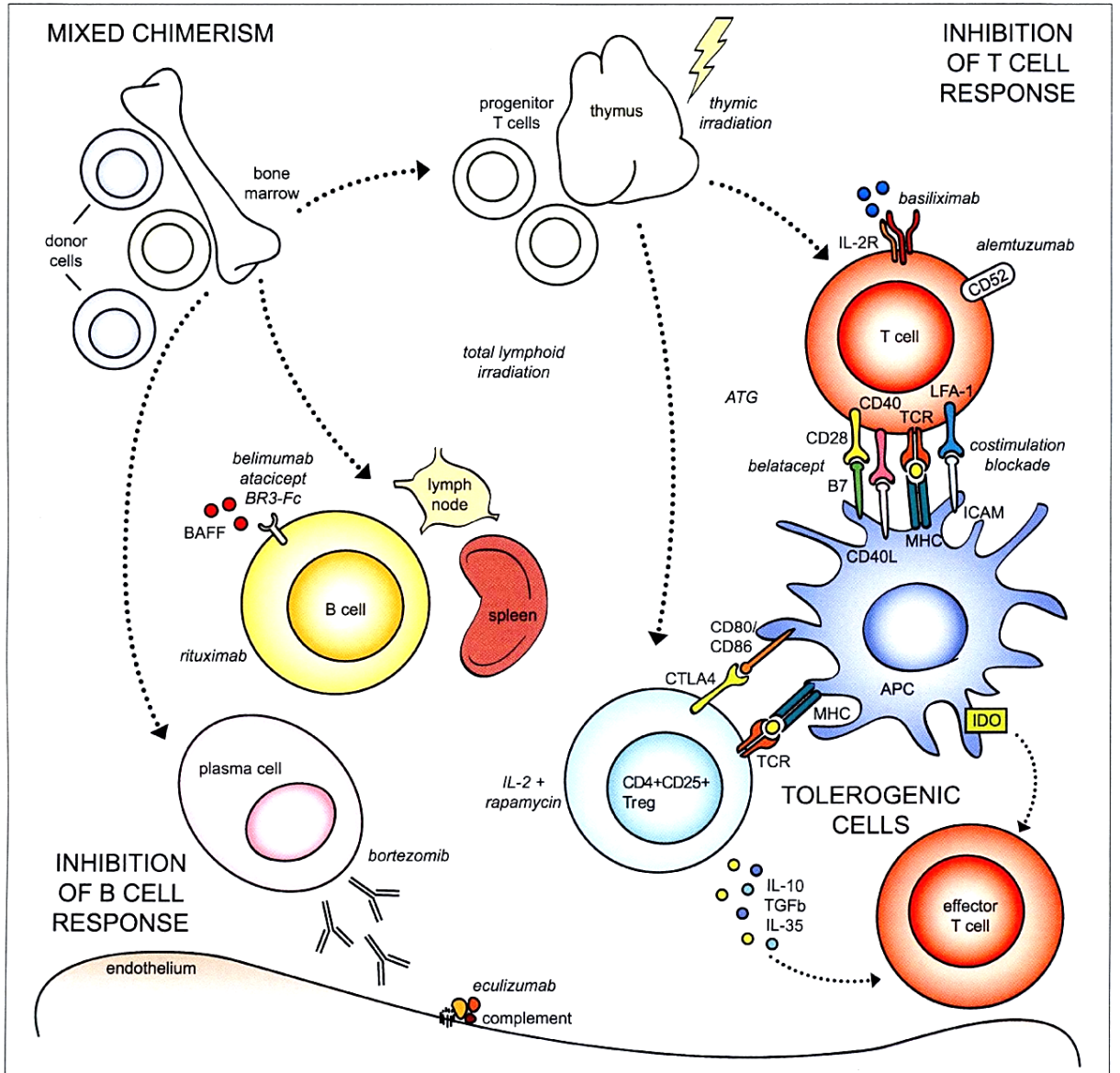


FIGURE 1 | Approaches to transplant tolerance induction. (Top left) Mixed chimerism is achieved by infusing donor bone marrow into myelo-conditioned recipients, to establish co-existence of donor and recipient cells in the setting of organ transplantation. The dotted arrows indicate cell types originating from the bone marrow, unrelated to mixed chimerism. (Top right) Allospecific T cell responses can be abrogated through a number of mechanisms, including irradiation, pharmacologic lymphodepletion by ATG or alemtuzumab, suppression of activation by costimulation blockade or IL-2 receptor blockade. (Bottom right) Tolerogenic cell types, including regulatory T cells, macrophages, and mesenchymal stromal cells, can inhibit effector T cells through direct ligation or inhibitory cytokine production. (Bottom left) The humoral response can be suppressed through B cell depletion, and blockade of survival factors (BAFF), plasma cells, and complement.

Table 1 | Strategies for tolerance induction. This table outlines the pharmacologic, biologic, and cellular therapies discussed in this article, categorized by T cell agents, B cell agents, and cellular therapies (including mixed chimerism).

Category	Therapeutic	Mechanism
T cell depletion	Anti-thymocyte globulin (ATG)	Depleting polyclonal antibodies to thymocytes that express multiple target antigens; possible induction of regulatory T cells
	Alemtuzumab	Depleting mAb to CD52, on T, B, NK cells, some monocytes
Costimulation blockade	Abatacept	CTLA-4 Ig, blockade of CD28:CD80/86 costimulatory pathway
	Belatacept	CTLA-4 Ig, blockade of CD28:CD80/86 costimulatory pathway
	Efalizumab	Blockade of LFA-1:ICAM-1 costimulatory pathway
Other T cell therapies	Basiliximab	Blockade of CD25 (interleukin 2 receptor α chain)
	Aldesleukin + rapamycin	Interleukin 2 + rapamycin, to increase regulatory T cell proliferation and survival, and stabilize the expression of Forkhead box P3 (FoxP3)
B cell therapeutics	Rituximab	Depleting mAb to CD20
	Belimumab	Blockade of B cell activating factor (BAFF), causing depletion of follicular and alloreactive B cells, decrease in alloantibody response, and promotion of immature/transitional B cell phenotype and a regulatory cytokine environment
	Atacicept	Blockade of BAFF and APRIL
	BR3-Fc	Blockade of BAFF, causing decrease in peripheral, marginal zone, and follicular B cells
	Bortezomib	Proteosome inhibitor, causing apoptosis of mature plasma cells
	Eculizumab	Blockade of complement protein C5, to prevent complement mediated injury due to circulating alloantibody
Cellular therapy	Mixed chimerism	Infusion of donor bone marrow into myoablated/immune-conditioned recipient, to produce co-existence of donor and recipient cells
	Regulatory T cells	Infusion of expanded regulatory T cells, to inhibit inflammatory cytokine production, down-regulate costimulatory and adhesion molecules, promote anergy and cell death, convert effector T cells to a regulatory phenotype, and produce suppressive cytokines IL-10, TGFβ, and IL35
	Regulatory T cells + IL-2	As above, plus the addition of IL-2 to promote Treg survival, development, and expansion
	Dendritic cells	Immunomodulatory effects include their ability to acquire and present antigen, expand and respond to antigen-specific Tregs, constitutively express low levels of MHC and costimulatory molecules, produce high IL-10 and TGFβ and low IL-12, resist activation by danger signals and CD40 ligation, resist killing by natural killer or T cells, and promote apoptosis of effector T cells
	Macrophages	Immune suppression mediated through the enrichment of $CD4^+$ $CD25^+$ Foxp3 cells and cell contact- and caspase-dependent depletion of activated T cells
	Mesenchymal stromal cells	Inhibition of T cell activation and proliferation, potentially due to production of IL-10, NO, and IDO, and suppression of IFNγ and IL-17

HLA-Mismatched Renal Transplantation without Maintenance Immunosuppression

Tatsuo Kawai, M.D., A. Benedict Cosimi, M.D., Thomas R. Spitzer, M.D., Nina Tolkoff-Rubin, M.D., Manikkam Suthanthiran, M.D., Susan L. Saidman, Ph.D., Juanita Shaffer, B.S., Frederic I. Preffer, Ph.D., Ruchuang Ding, M.D., Vijay Sharma, Ph.D., Jay A. Fishman, M.D., Bimalangshu Dey, M.D., Dicken S.C. Ko, M.D., Martin Hertl, M.D., Nelson B. Goes, M.D., Waichi Wong, M.D., Winfred W. Williams, Jr., M.D., Robert B. Colvin, M.D., Megan Sykes, M.D., and David H. Sachs, M.D.

N ENGL J MED 358;4 WWW.NEJM.ORG JANUARY 24, 2008

SUMMARY

Five patients with end-stage renal disease received combined bone marrow and kidney transplants from HLA single-haplotype mismatched living related donors, with the use of a nonmyeloablative preparative regimen. Transient chimerism and reversible capillary leak syndrome developed in all recipients. Irreversible humoral rejection occurred in one patient. In the other four recipients, it was possible to discontinue all immunosuppressive therapy 9 to 14 months after the transplantation, and renal function has remained stable for 2.0 to 5.3 years since transplantation. The T cells from these four recipients, tested in vitro, showed donor-specific unresponsiveness and in specimens from allograft biopsies, obtained after withdrawal of immunosuppressive therapy, there were high levels of P3 (FOXP3) messenger RNA (mRNA) but not granzyme B mRNA.

Table 1. Patient Characteristics and Results of Laboratory Tests.*

Patient No.	Sex	Age	Original Disease	Pretransplantation PRA	Chimerism†			Discontinuation of Immunosuppressive Therapy	Kidney Survival	Current Serum Creatinine Level	Creatinine Clearance	Antidonor Alloantibody	Banff Score‡
					Day 7	Day 14	Day 21						
		yr		%					*days*	*mg/dl*	*ml/min*		
1	F	22	Alport's syndrome	4§	T cell 5.2%, B cell 4.6%, monocytes 44.5%, GRN 90.6%	Undetectable		Day 240	>1932	1.2	61	None	i0t0v0g0; ci1ct1cv1cg0
2	M	22	MPGN type 1	0	T cell 3%, GRN 60%	Undetectable		Day 422	>1666	1.5	75	None	i0t0v0g0; ci1ct0cv0cg0
3	M	39	Polycystic kidney disease	52¶	T cell 25.6%, monocytes 25.6%, GRN 46%	Undetectable		Not discontinued	10	Underwent second kidney transplantation			
4	M	25	Alport's syndrome	0	T cell 1.8%, monocytes 40%, GRN 98.1%	Undetectable		Day 244	>1050	1.5	60	New antidonor HLA class II antibody	i0t0v0 g1; ci0ct0cv0cg1
5	M	46	Polycystic kidney disease	0	T cell 15%, monocytes 19%, GRN 86.5%	T cell 0.1%, B cell 46%, monocytes 19%, GRN 9.9%	T cell 0%, B cell 0%, monocytes 0%, GRN 3.5%	Day 272	>707	1.8	71	None	i0t0v0 g0; ci0ct0cv0cg0

* GRN denotes granulocytes, MPGN membranoproliferative glomerulonephritis, and PRA panel-reactive antibodies.
† The percentages of donor chimerism were measured by means of flow-cytometric analysis. To verify the results, polymerase-chain-reaction (PCR) assays for variable short tandem repeats were used. For these latter assays, T-cell and myeloid-cell subpopulations were sorted by means of magnetic beads before PCR assays.
‡ The Banff scoring system is used for the grading and classification of short- and long-term changes that occur in the interstitium, tubules, vessels, and glomeruli of a kidney transplant. Scores range from 0 to 3, with higher scores indicating more severe changes. Banff scores are given for the most recent biopsy specimens obtained.
§ This result is class II, not donor-specific.
¶ This result is class I, not donor-specific.

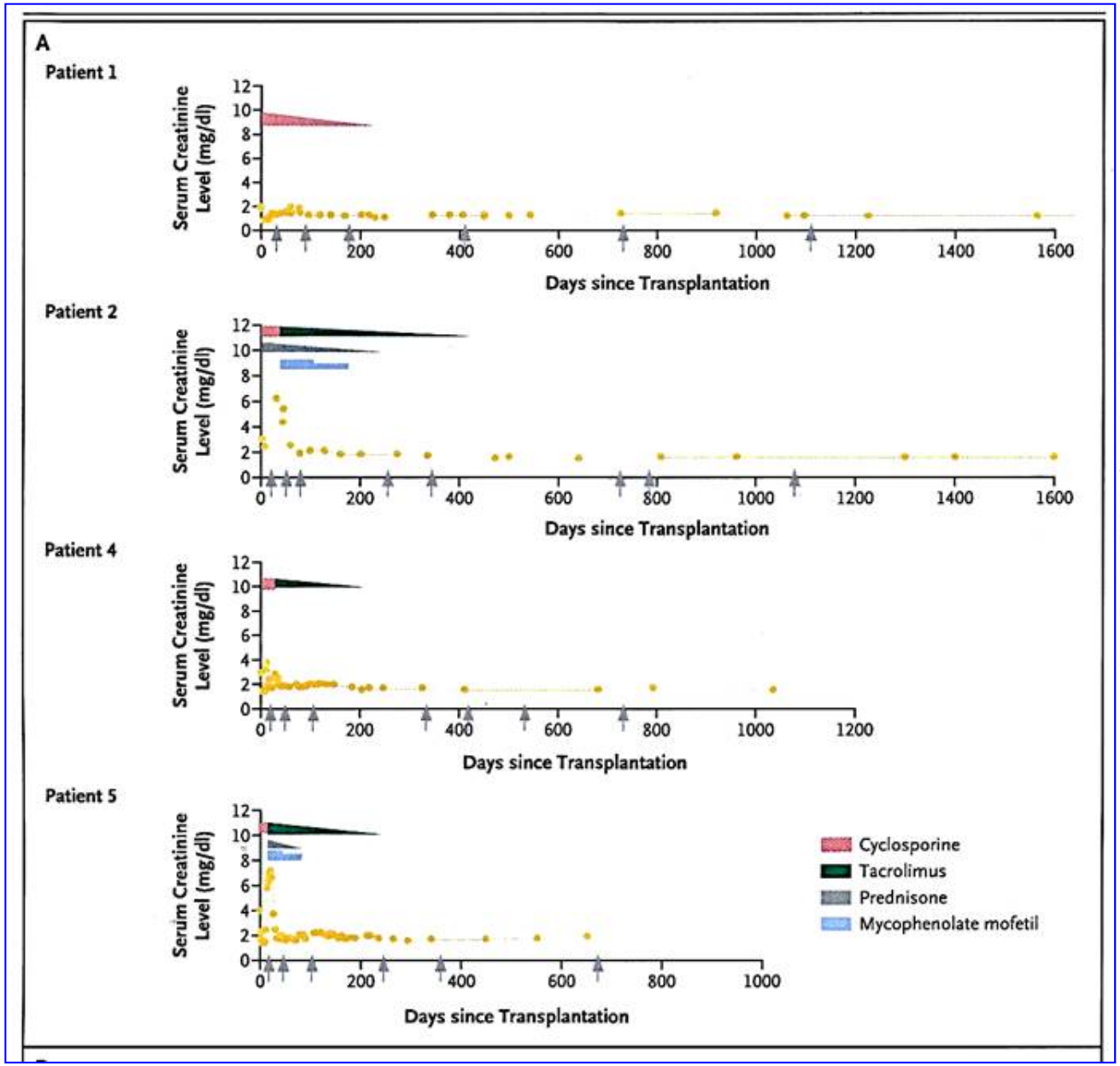


Figure 1 (facing page). Clinical Course of Patients with Tolerance of Kidney Transplants.

In Panel A, the serial creatinine levels in Patients 1, 2, 4, and 5 are indicated in yellow, and the withdrawal of immunosuppressive medications is shown as tapering bars above each creatinine curve. Arrows indicate the time at which allograft-biopsy specimens were obtained from these four patients. To convert the values for creatinine to micromoles per liter, multiply by 88.4.

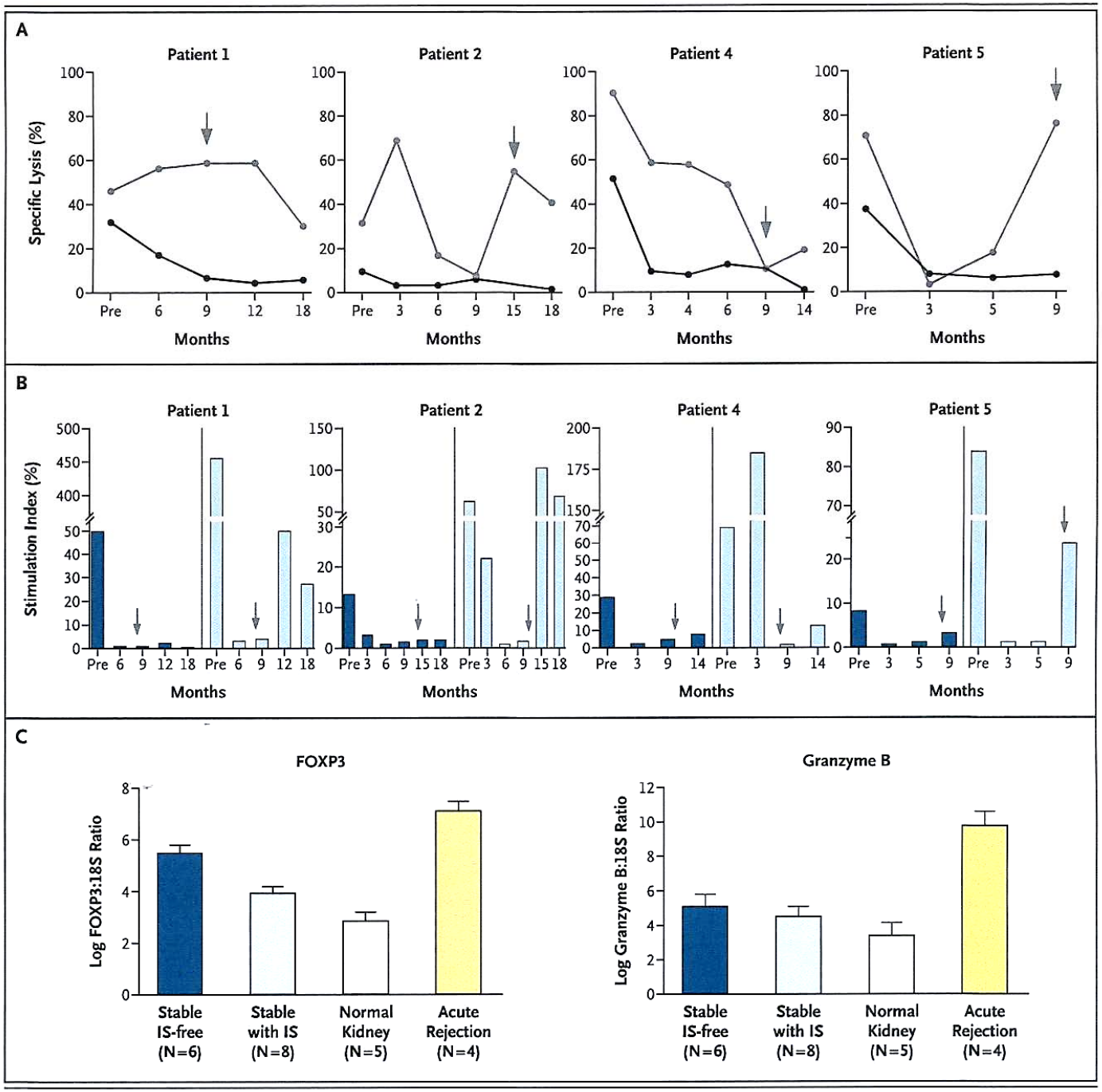


Figure 2 (facing page). In Vitro Assays for Tolerance of Kidney Transplantation.

Sequential cell-mediated lympholysis assays in each patient against the donor (black circles) and third-party persons (gray circles) showed specific unresponsiveness to the donor at most post-transplantation time points tested (Panel A). Sequential assays of mixed-lymphocyte reactions in each patient against the donor (dark-blue bars) and third-party persons (light-blue bars) showed specific unresponsiveness to the donor, as indicated by return of anti–third-party, but not anti-donor, responses after discontinuation of immunosuppressive therapy (Panel B). Arrows indicate the time of complete discontinuation of immunosuppressive therapy. Panel C shows intragraft levels of mRNA in renal allografts. Total RNA was isolated from renal-allograft biopsy specimens and reverse transcribed to cDNA, and levels of mRNA were measured with the use of preamplification enhanced real-time quantitative polymerase-chain-reaction assays. A total of 23 biopsy specimens were examined for intragraft levels of FOXP3 mRNA, granzyme B mRNA, and housekeeping gene 18S ribosomal RNA (18S rRNA). Of the 23 biopsy specimens, 6 were obtained from four patients with stable renal-allograft function who were not receiving immunosuppressive therapy (IS) (stable IS-free group). Eight biopsy specimens were obtained from eight patients with stable renal-allograft function and normal protocol biopsy results; these patients were receiving maintenance immunosuppressive drug therapy comprising tacrolimus and mycophenolate mofetil (stable-with-IS group). Five biopsy specimens were obtained from five kidney donors (normal-kidney group), and four biopsy specimens were obtained from four patients with biopsy-confirmed acute rejection (acute-rejection group). The mRNA copies were normalized with the use of 18S rRNA copies and log-transformed. The log-transformed mean (±SE) ratio of FOXP3 mRNA copies to 18S rRNA copies was 5.48±0.31 in the stable IS-free group, 3.90 ±0.27 in the stable-with-IS group, 2.85±0.34 in the normal-kidney group, and 7.09±0.38 in the acute-rejection group. The log-transformed mean ratio of granzyme B to 18S rRNA was 5.12±0.69 in the stable IS-free group, 4.53±0.59 in the stable-with-IS group, 3.41±0.75 in the normal-kidney group, and 9.76±0.84 in the acute-rejection group. Pre denotes pretransplantation.

Acute Renal Endothelial Injury During Marrow Recovery in a Cohort of Combined Kidney and Bone Marrow Allografts

A.B. Farris[a,b,c,*], D. Taheri[a,c], T. Kawai[c,d], L. Fazlollahi[a,c], W. Wong[c,e], N. Tolkoff-Rubin[c,e], T. R. Spitzer[c,e], A. J. Iafrate[a,c], F. I. Preffer[a,c], S. A. LoCascio[f,g], B. Sprangers[g], S. Saidman[a,c], R. N. Smith[a,c], A. B. Cosimi[c,d], M. Sykes[c,f,g], D. H. Sachs[c,f] and R. B. Colvin[a,c]

American Journal of Transplantation 2011; 11: 1464–1477

An idiopathic capillary leak syndrome ('engraftment syndrome') often occurs in recipients of hematopoietic cells, manifested clinically by transient azotemia and sometimes fever and fluid retention. Here, we report the renal pathology in 10 recipients of combined bone marrow and kidney allografts. Nine developed graft dysfunction on day 10–16 and renal biopsies showed marked acute tubular injury, with interstitial edema, hemorrhage and capillary congestion, with little or no interstitial infiltrate (<10%) and marked glomeru-

Conditioning regimen: cyclophosphamide, anti-CD2 monoclonal antibody, rituximab, thymic irradiation and post-transplant cyclosporine

tion. The cells in capillaries were primarily CD68$^+$ MPO$^+$ mononuclear cells and CD3$^+$CD8$^+$ T cells, the latter with a high proliferative index (Ki67$^+$). B cells (CD20$^+$) and CD4$^+$ T cells were not detectable, and NK cells were rare. XY FISH showed that CD45$^+$ cells in PTCs were of recipient origin. Optimal treatment remains to be defined; two recovered without additional therapy, six were treated with anti-rejection regimens. Except for one patient, who later developed thrombotic microangiopathy and one with acute humoral rejection, all fully recovered within 2–4 weeks. Graft endothelium is the primary target of this process, attributable to as yet obscure mechanisms, arising during leukocyte recovery.

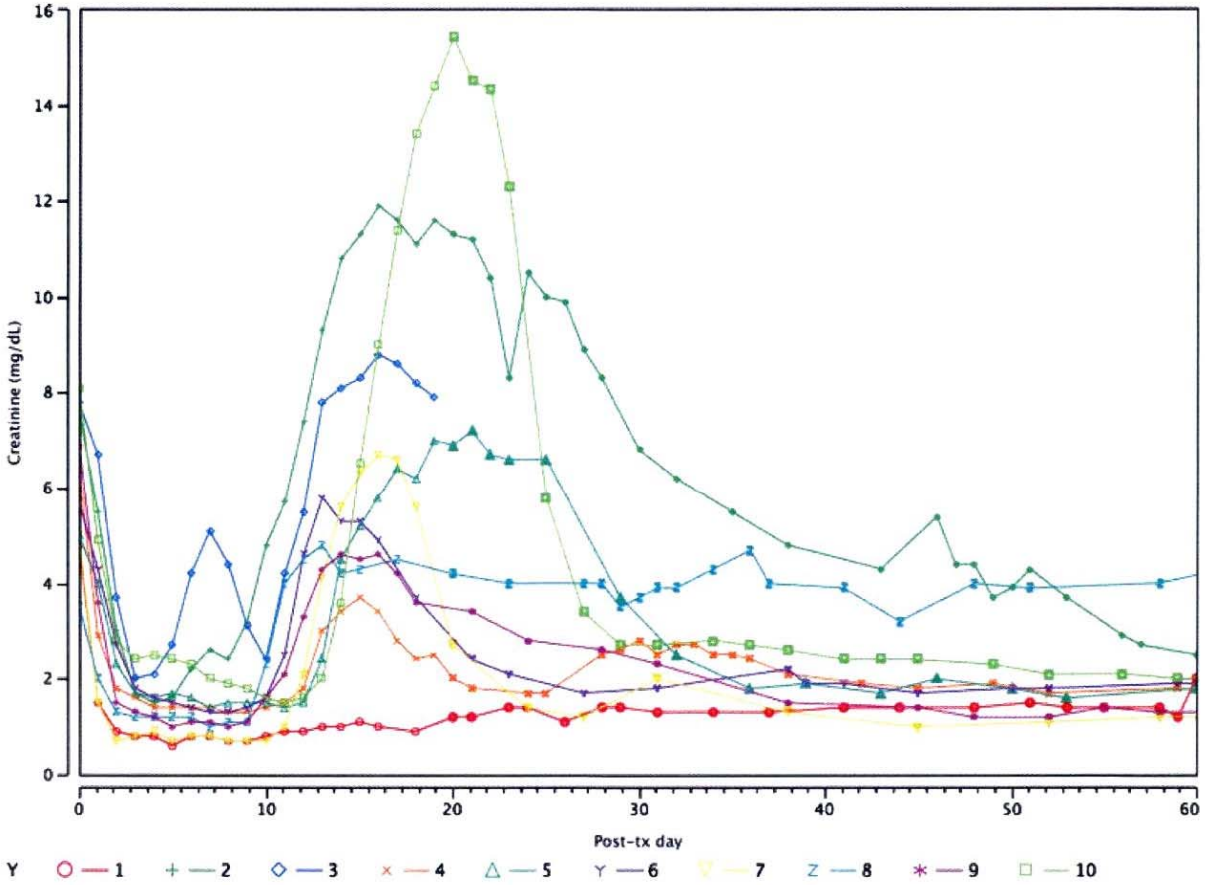


Figure 1: Longitudinal plot of serum Cr in the 10 recipients of combined kidney/bone marrow transplantation. Each one except #1 experienced a transient episode of renal dysfunction in the second to third week, here termed the engraftment syndrome (ES). Patient #3 lost the graft due to acute humoral rejection and #8 developed thrombotic microangiopathy.

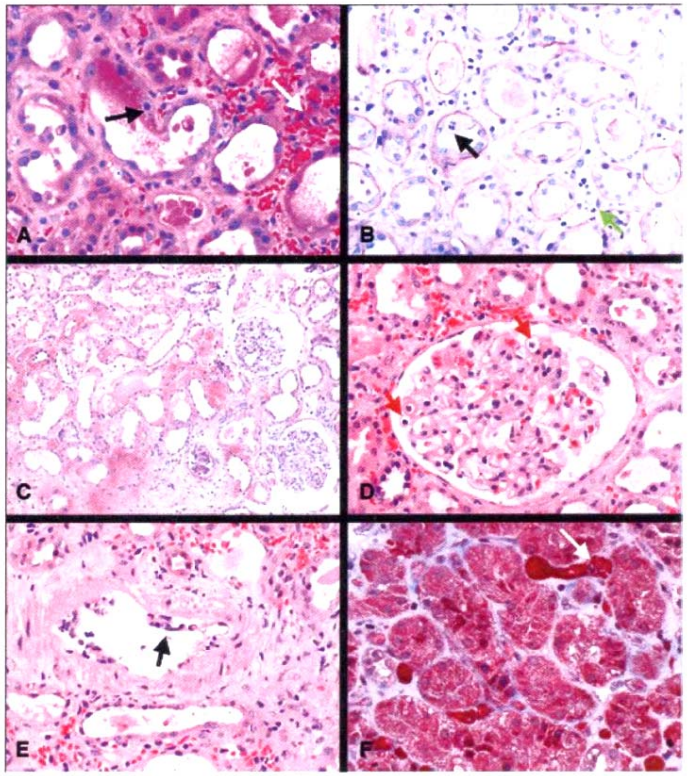


Figure 3: Light microscopy of representative engraftment syndrome cases: Interstitial hemorrhage and congestion are prominent in these cases, as illustrated in A, C and F.

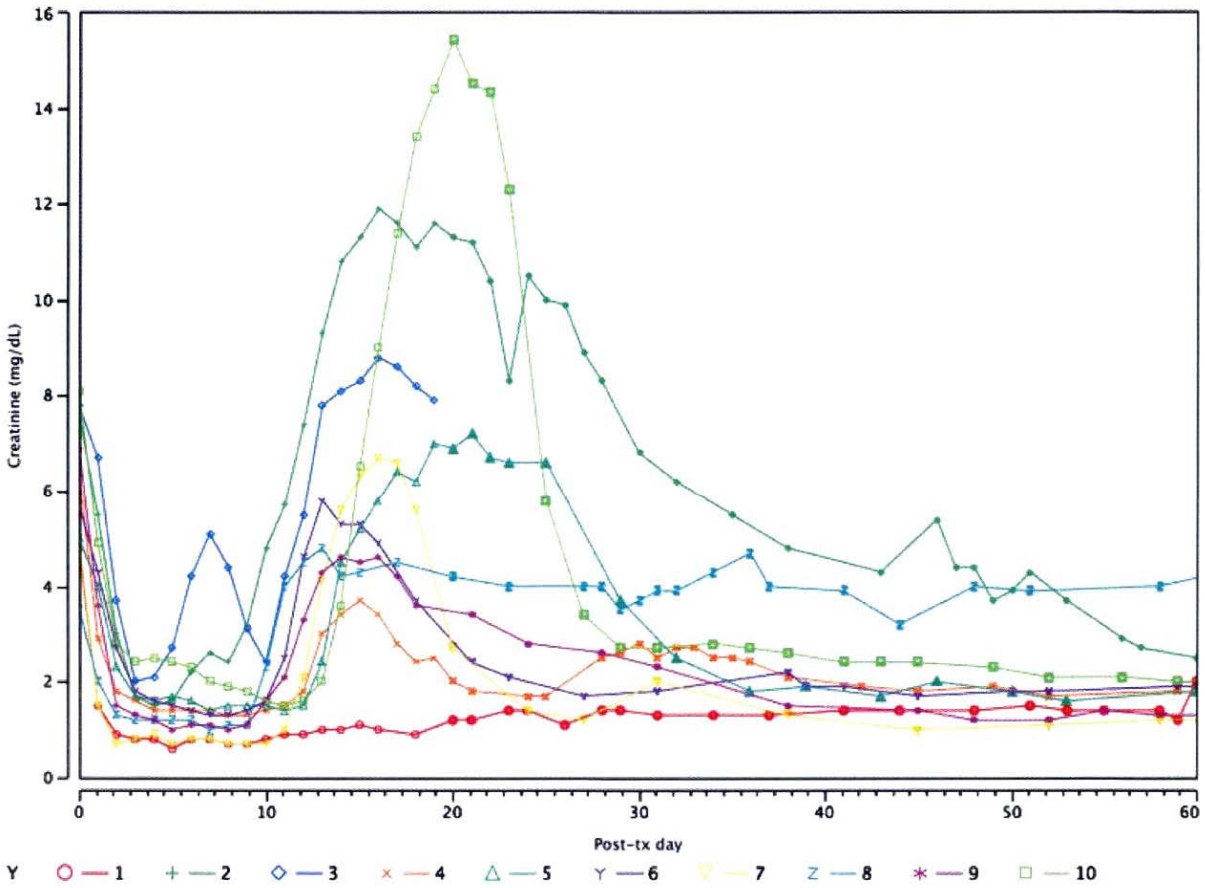


Figure 1: Longitudinal plot of serum Cr in the 10 recipients of combined kidney/bone marrow transplantation. Each one except #1 experienced a transient episode of renal dysfunction in the second to third week, here termed the engraftment syndrome (ES). Patient #3 lost the graft due to acute humoral rejection and #8 developed thrombotic microangiopathy.

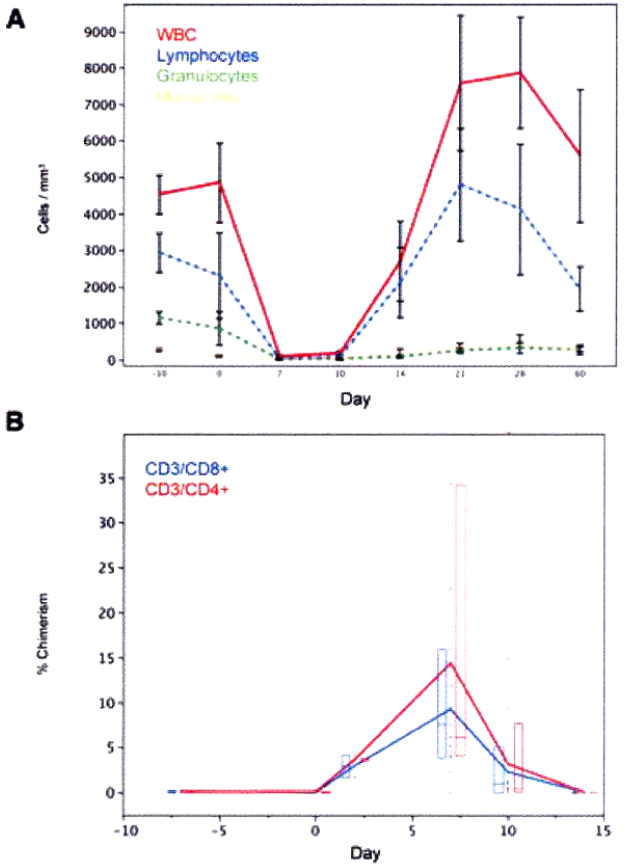


Figure 2: (A) A plot of the mean levels of circulating blood leukocytes (overall white blood cells [WBC], lymphocytes, granulocytes, and monocytes) after combined bone marrow and kidney transplantation shows that a nadir of the circulating cells occurs at 7–10 days, just at the onset the engraftment syndrome.

REVIEW Transpl Int. 2015 Apr 8. doi: 10.1111/tri.12578. [Epub ahead of print]

Reconsidering the detection of tolerance to individualize immunosuppression minimization and to improve long-term kidney graft outcomes

Daniel Baron,[1,2,3] Magali Giral[1,2,3] and Sophie Brouard[1,2,3]

Summary

In kidney transplantation, minimizing the side effects of the immunosuppressive regimen and inducing tolerance to allograft are the two main objectives to improve outcome. At present, these objectives are far from being achieved and remain elusive for the majority of transplant recipients. Rejection rate and mortality on the long term are still unacceptable. There is thus a pressing need to improve this situation. Therefore, some spontaneously tolerant kidney recipients are described in clinics, and recent advances in immunological and molecular techniques have led to a resurgence of interest in studying those rare transplanted recipients through coordinated efforts from international consortia. Indeed, they offer, on the one hand, the possibility to develop specific biomarkers indicative of this state that would constitute a major advantage in the care of the patients allowing personalized minimization of drugs, so reducing related costs and side effects. On the other hand, they represent a unique model of study to understand the mechanisms of regulation implicated in this state that may help the development of inducing therapies. Recent efforts, concentrated on noninvasive analyses of peripheral blood, identified a predominance of several B-cell subsets, some of which harbouring regulatory functions, and related marker genes. These findings, validated in independent multicentric cohorts, led credence to an unsuspected role for the B-cell compartment in tolerance to kidney allograft. The identification of patients, harbouring these markers, among immunosuppressed recipients with stable graft function and the existence of drugs with selective effect on B cell pave the way for the possibility to improve long-term graft outcomes. Therefore, before routine application, these findings need to be confirmed in large prospective studies in the context of planned reduced immunosuppression.

Transpl Int. 2015 Apr 8. doi: 10.1111/tri.12578. [Epub ahead of print]

Operational tolerance as a unique model of research

Definition of the clinical status

The original definition of Medawar in the 1950s referred to nonresponsiveness to antigens [117]. In animal studies, tolerance may be defined as good long-standing graft function in the presence of a competent immune system, with no signs of graft immune injury. The latter definition is obviously not useful in human transplantation, and therefore, 'operational tolerance' is the term most widely used. Given that no biopsy can be performed, kidney transplant recipients who have been successfully weaned from IS and have maintained stable graft function for 1 year or more are referred to as functionally or operationally tolerant [118–120].

Spontaneously tolerant patients stop their immunosuppressive treatment for two major reasons: noncompliance and the occurrence of deleterious side effects of the IS drugs (drug toxicity or malignancy) [118–120].

Tolerant patients do not differ from other transplant recipients as to whether they received a kidney from a deceased or living donor, and the number of HLA incompatibilities is at the same level as in other transplant recipients [72,118,121,138–141]. If the individual parameters and history of these patients are extremely variable [118–120], several interesting features could emerge from their careful follow-up. First, operational tolerance can develop even in the presence of either HLA mismatches at baseline or anti-HLA antibodies during follow-up, as well as in patients having experienced acute rejection [118]. Second, tolerant cases had not been nonspecifically immunosuppressed, as they did not present any significantly increased risk for either opportunistic/severe infections or cancers, but showed responses to vaccination comparable to the general population [142]. Third, operational tolerance process has been shown to be metastable over time, as demonstrated by a non-negligible proportion of patients who lose their graft for immunological reasons or simply due to physiological age defects [120]. Finally, operational tolerance corresponds to an immunocompetent situation [142] associated with immune regulation as shown by a decrease of the donor-reactive delayed-type hypersensitivity (DTH) response specific to the donor [140,143].

The Natural History of Clinical Operational Tolerance After Kidney Transplantation Through Twenty-Seven Cases

American Journal of Transplantation 2012; 12: 3296–3307

We report here on a European cohort of 27 kidney transplant recipients displaying operational tolerance, compared to two cohorts of matched kidney transplant recipients under immunosuppression and patients who stopped immunosuppressive drugs and presented with rejection. We report that a lower proportion of operationally tolerant patients received induction therapy (52% without induction therapy vs. 78.3% [$p = 0.0455$] and 96.7% [$p = 0.0001$], respectively), a difference likely due to the higher proportion (18.5%) of HLA matched recipients in the tolerant cohort. These patients were also significantly older at the time of transplantation ($p = 0.0211$) and immunosuppression withdrawal ($p = 0.0002$) than recipients who rejected their graft after weaning. Finally, these patients were at lower risk of infectious disease. Among the 27 patients defined as operationally tolerant at the time of inclusion, 19 still display stable graft function (mean 9 ± 4 years after transplantation) whereas 30% presented slow deterioration of graft function. Six of these patients tested positive for pre-graft anti-HLA antibodies. Biopsy histology studies revealed an active immunologically driven mechanism for half of them, associated with DSA in the absence of C4d. This study suggests that operational tolerance can persist as a robust phenomenon, although eventual graft loss does occur in some patients, particularly in the setting of donor-specific alloantibody.

Table 2: Statistical analysis of qualitative and quantitative clinical parameters of the operationally tolerant patients compared to a matched cohort of stable patients under standard immunosuppression

A.		Global population			Op-Tol		STA-Controls		
		NA	n	%	n	%	n	%	p-Value
Qualitative parameters	Male	0	34	65.4	18	69.2	16	61.5	0.7237
	Transfusion> 10	3	8	16.3	5	20.8	3	12.0	0.4795
	PRA class I	4	15	31.2	7	31.8	8	30.8	1.0000
	>4 HLA-A-B-DR incompatibilities	1	4	7.8	1	3.8	3	12.0	0.4795
	>1 HLA-DR incompatibilities	3	31	63.3	13	54.2	18	72.0	0.2888
	Acute rejection episodes	1	16	31.4	10	40	6	23.1	0.3428
	First graft	0	45	86.5	22	84.6	23	88.5	1.0000
	No Induction therapy	4	17	35.4	12	48.0	5	21.7	0.0455*
Quantitative parameters	Donor age (years)	2	28.8	13.7	27.0	15.1	30.5	12.3	0.5294
	Cold ischemia (hours)	2	23.9	13.4	19.9	13.4	28.2	12.1	0.0741

B.		Global population			Op-Tol		REJ-Controls		
		NA	n	%	n	%	n	%	p-Value
Qualitative parameters	Male	0	29	54.7	18	69.2	11	40.7	0.0513
	Transfusion> 10	10	6	14.0	5	20.8	1	5.3	0.2060
	PRA class I/II	5/4	14/10	29.2/20.4	7/8	31.8/34.8	7/2	26.9/7.7	0.030
	>4 HLA-A-B-DR incompatibilities	0	4	7.5	1	3.8	3	11.1	0.6237
	>1 HLA-DR incompatibilities	2	31	60.8	13	54.2	18	66.7	0.4073
	Acute rejection episodes	2	28	54.9	10	40	18	69.2	0.0468
	First graft	0	42	79.2	22	84.6	20	7401	0.4977
	No Induction therapy	1	13	25.0	12	48.0	1	3.7	0.0001
Quantitative parameters	Donor age (years)	2	29.3	14.4	27.0	15.1	31.2	13.8	0.2530
	Recipient age (years) At transplantation time	0	29.5	13.7	34.7	16.3	24.6	8.4	0.0211
	Recipient age (years) At the time of arrest of IS	3	36.6	15.5	44.8	16.7	28.4	9.0	0.0002
	Cold ischemia (hours)	1	20.4	13.3	19.9	13.4	21.0	13.3	0.8764

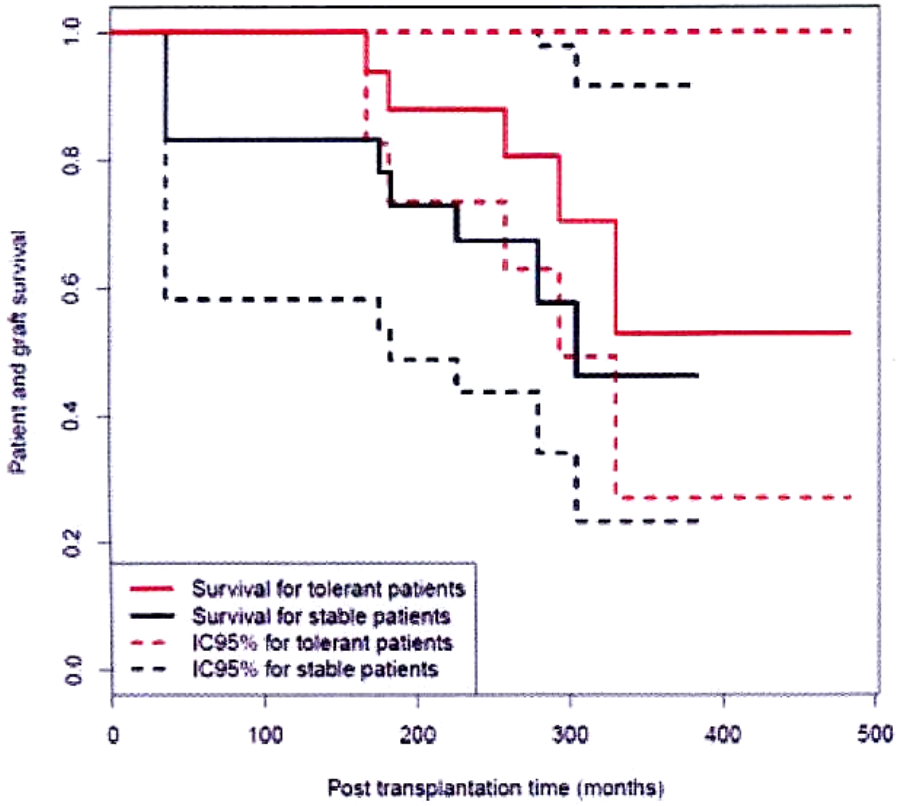


Figure 1: Graft and recipient survival probability according to time posttransplant and status of operational tolerance or stable under immunosuppression.

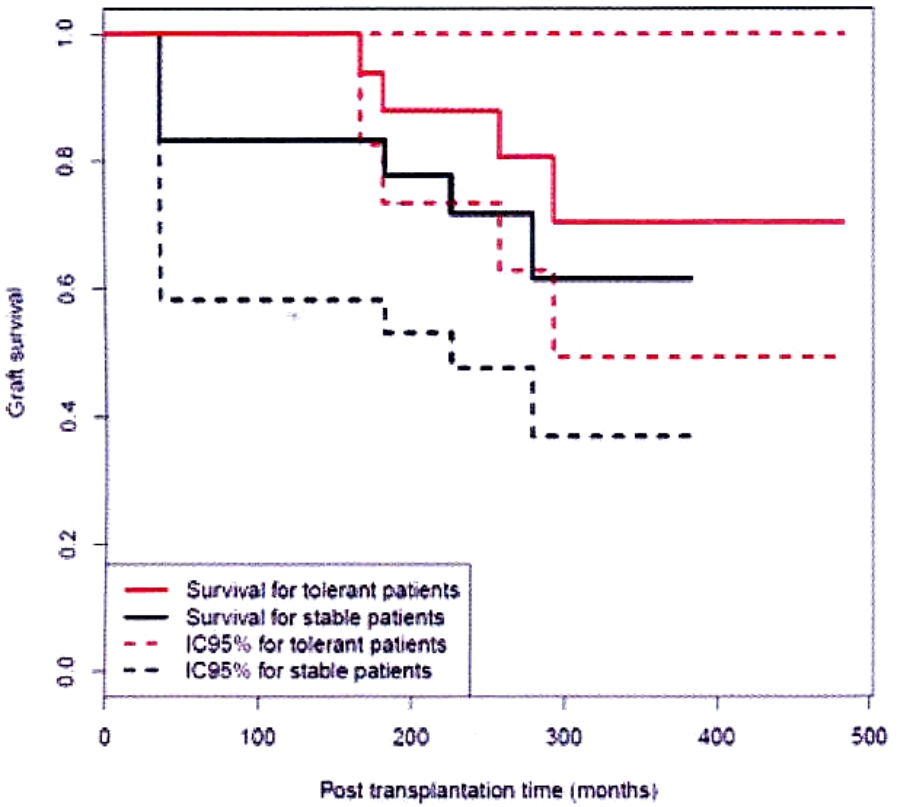


Figure 2: Graft survival probability according to time post-transplant and status of operational tolerance or stable under immunosuppression.

Table 4: Diagnostic classification assay from operationally tolerant patients

27 Operationally tolerant patients		
13 patients	**6 patients**	**8 patients**
• Functional graft • Without HLA Abs Operational tolerance	• Functional graft • With HLA Abs (± DSA) Operational tolerance (Lack of a destructive immune response toward the graft Partial operational tolerance)	• Graft failure • With or without HLA Abs (± DSA) Lose of operational tolerance

Transpl Int. 2015 Apr 8. doi: 10.1111/tri.12578. [Epub ahead of print]

Technical considerations on the biodetection of tolerance

However, these studies on operationally tolerant kidney patients are very heterogeneous, either due to the techniques, controls used or to the various clinical profiles of the tolerant recipients [118–120,140,143–159]. Moreover, the cohorts studied are small, which prevent a robust statistical approach. These considerations markedly contribute to the difficulty for generalization and standardization of the results [157]. Finally, the lack of biopsies is a problem in these patients, as some indications of graft deterioration may not be detected [120]. In the search for new biomarkers and more specifically tolerance biomarkers, there are three major dilemmas: the first concerns the technology to be used, the second is the origin of the samples, and the third is the control population.

Choice of the technology: In humans, many immunological assays have been used as surrogate tests to monitor the immune response after transplantation [97,98].

Benefiting from advances in genomic science [105,162], very sensitive molecular techniques have become available to quantify relevant gene expression patterns and protein signatures in biological samples [163,164]. They have been of added value [165] to characterize spontaneously tolerant transplant recipients [166] and establish a tolerance gene signature [167,168].

Choice of the compartment: Regarding the origin of the samples, peripheral blood, graft biopsy [145] and urine have all been used for analysis [157].

Analysing the peripheral blood [111,170] has the advantage of being less invasive and less expensive than biopsy, which makes it the main method used to analyse gene profiles [111].

Choice of the control: The other significant dilemma is choosing the right control population, which is not easy for tolerant patients [104,176].
have been grafted and have good graft function, so we might suppose that stable patients under immunosuppressant treatment would be the best control, but the absence of immunosuppressive drugs in the tolerant patients could influence the results. On the other hand, the absence of treatment makes tolerant patients similar to healthy individuals, but we cannot ignore the absence of transplantation in the latter group. Comparison of tolerant patients with patients undergoing chronic transplant rejection has also been used, but these patients are clinically very different.

Faced with the lack of the perfect control population, the use of multiple controls may be the best alternative.

Transpl Int. 2015 Apr 8. doi: 10.1111/tri.12578. [Epub ahead of print]

Tolerant recipient display a b-cell gene signature

Our group and others have analysed the transcriptome of peripheral blood mononuclear cells. These five analyses revealed an increased expression of B-cell-related genes in tolerant patients compared with stable patients [146,154,157,159,177].

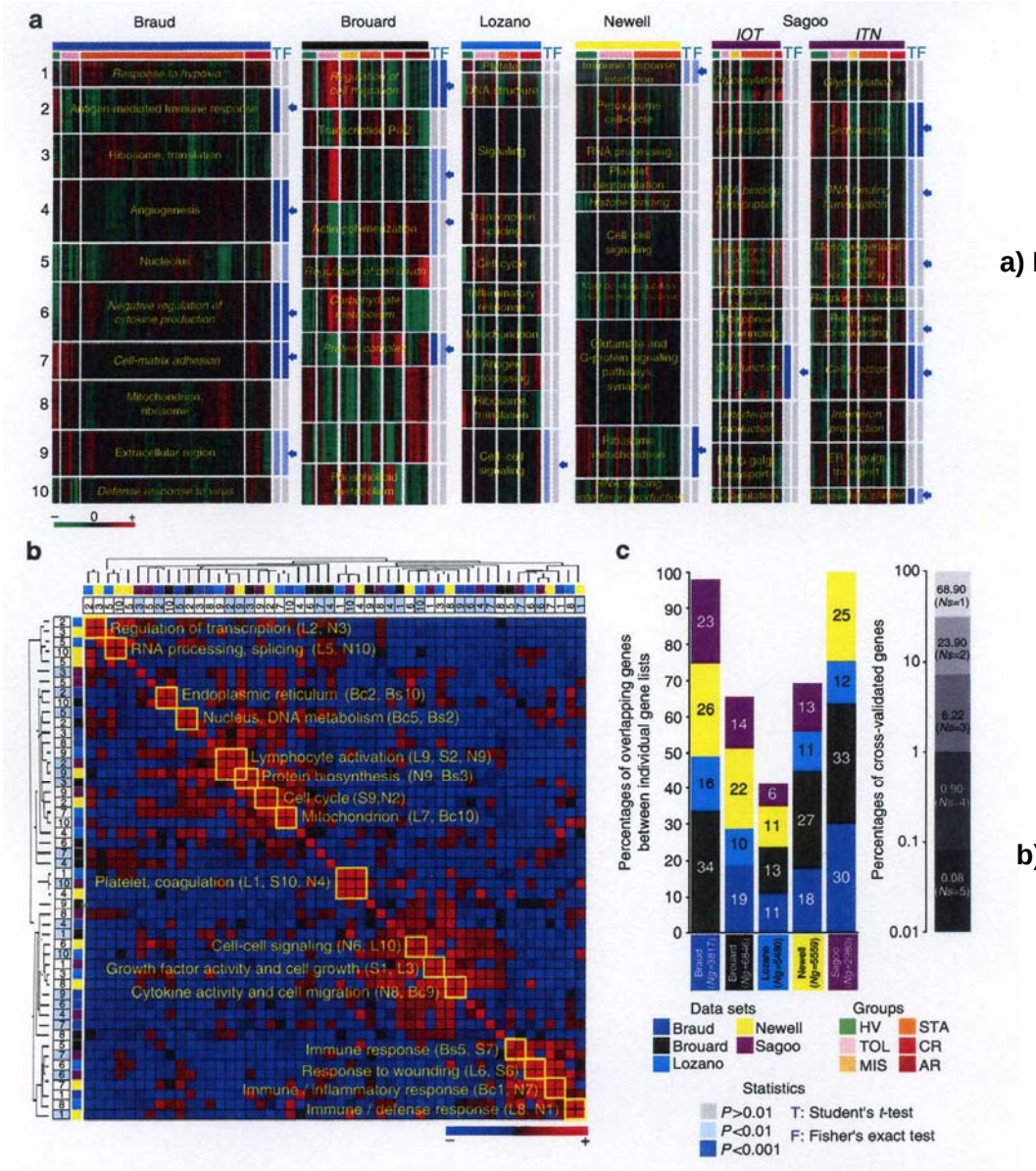
As the number of samples in each of the studies was relatively low, the significance of these results was assessed through a meta-analysis [179]. The high number of samples analysed (96 samples from 50 tolerant recipients from three independent multicentric cohort: French, UK and USA) led to the identification, for the first time, of a specific gene signature. This signature could unequivocally distinguish tolerant from other recipients (>90% accuracy) through cross-validation and was remarkably enriched in B-cell-related genes.

These data provide proof of principle that tolerance can be identified among transplanted recipients by the use of a 20-gene predictor, mostly centred on B cells [179]. Hence, these biomarkers could be used to detect tolerance and stratify kidney recipients in clinics. First, they may help for a better follow-up of the tolerant recipients. Several lines of evidences indicate that tolerance is likely not a stable situation for 'entire life' [177]. In such situation, these biomarkers could predict future graft loss and immunotherapy could be reinstated before the first clinical symptoms appear. Second, these biomarkers may help to monitor recipients under IS regimens. Among stable cases, those detected as having a low risk of rejection would be highly eligible for progressive IS weaning.

A common gene signature across multiple studies relate biomarkers and functional regulation in tolerance to renal allograft

Daniel Baron[1,2,3], Gérard Ramstein[4], Mélanie Chesneau[1,2,3], Yann Echasseriau[1,2,3], Annaick Pallier[1,2,3], Chloé Paul[1,2,3], Nicolas Degauque[1,2,3], Maria P. Hernandez-Fuentes[5], Alberto Sanchez-Fueyo[6], Kenneth A. Newell[7], Magali Giral[1,2,3], Jean-Paul Soulillou[1,2,3], Rémi Houlgatte[8,9,10] and Sophie Brouard[1,2,3,10]

Kidney International (2015) **87**, 984–995



a) Individualization of clusters of genes

b) Similarity in gene composition between the clusters

b) Intersection of the 19 differential clusters discriminative of TOL and STA groups when compared altogether

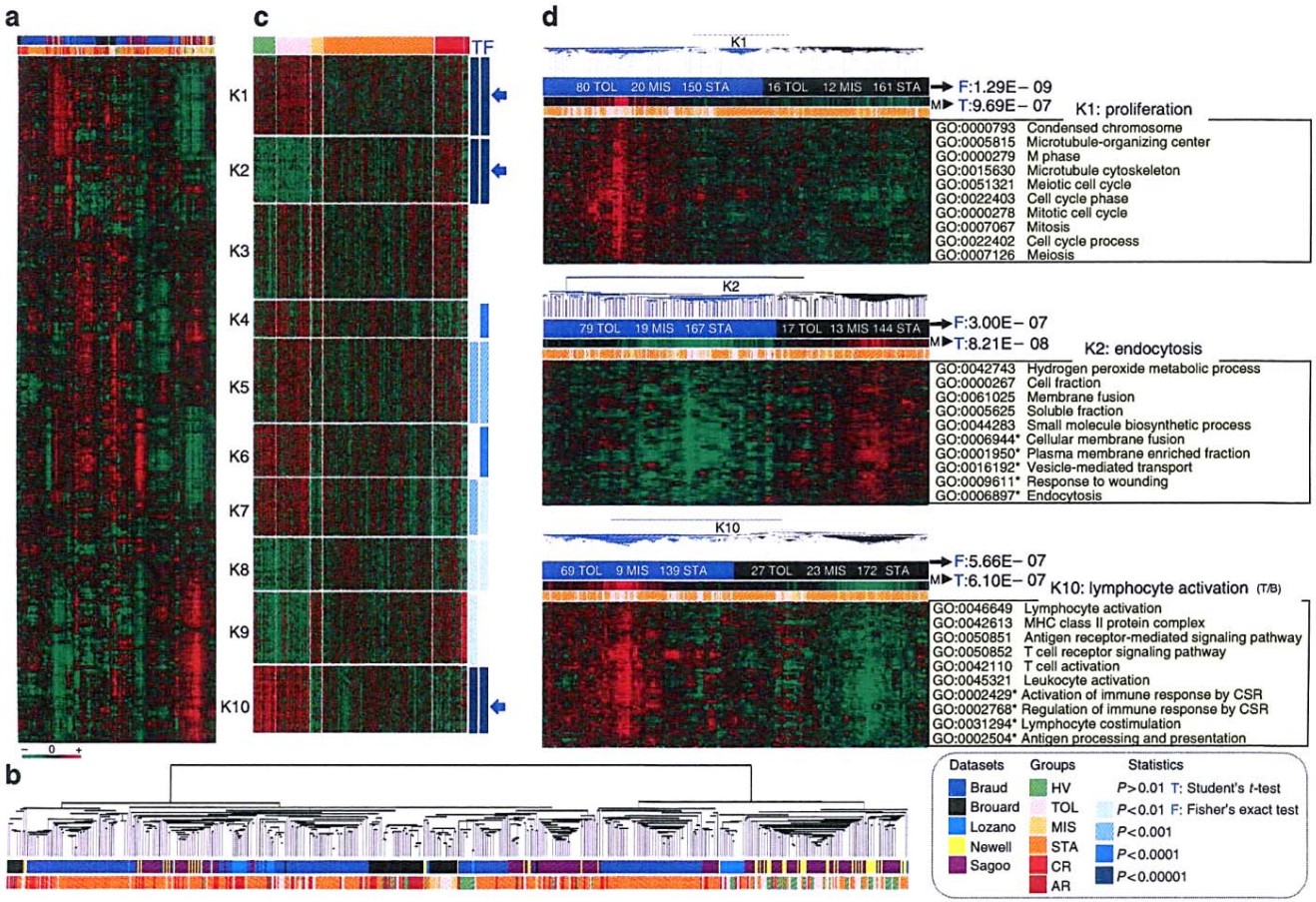


Figure 2 | Meta-analysis of the five studies. (**a**) Reliability of the meta-matrix. Results from a two-way hierarchical clustering are visualized by a heat map (1846 genes in lines, 596 samples in columns) using the same color code: green for gene underexpression and red for gene overexpression (see color scale). (**b**) Sample classification. The zoomed tree (bottom) reflects correlation between samples for which the status and the study of origin are indicated (see color legend). (**c**) Individualization of the meta-clusters. To identify discriminative groups of genes, the meta-data set was partitioned into 10 clusters (from K1 to K10) using a k-means clustering. The results are depicted by a heat map (same color code for gene expression values). Samples are supervised according to their status of origin: healthy volunteer (HV, green), tolerant recipient (TOL, pink), stable recipient under minimum immunosuppression (MIS, light orange) or classical treatment (STA, dark orange), and recipient with chronic (CR, red) or acute (AR, brown) rejection (see legend). The discriminative propensity of each meta-cluster to discriminate tolerant (TOL) from the control group of stable recipients (MIS and STA) was assessed by a Student's *t*-test (T in blue) applied on its median profile and a Fisher's exact test (F in blue) applied on the contingency of its dendrogram (see legend, 'statistics'): resulting *P*-values are indicated on the right of each meta-cluster using shades of blue (see color legend). The three most differential ones (TOL vs. STA/MIS, $P<0.00001$) with the two tests (namely K1, K2, and K10) are denoted by arrows. (**d**) Definition of the meta-signature of tolerance. For each of the three most differential ($P<0.00001$) meta-clusters (K1, K2, and K10) pertaining to the signature, a heat map visualization is depicted. Information provided includes the following: the median profile (M) of the cluster and the *P*-value from the Student's *t*-test (T) applied to it; the contingencies (number of TOL, MIS, and STA samples) from the two main branches of the dendrogram (blue and black, respectively); and the *P*-value from the Fisher's exact test applied to them. For each meta-cluster, the top ten significant Gene Ontology (GO) terms from functional annotation analysis are also given and summarized by a representative term (right side of the panel). Terms with an asterisk indicate significance of the enrichment but sensitivity to multiple testing corrections (FDR-adjusted *P*-values). CSR, class-switch recombination.

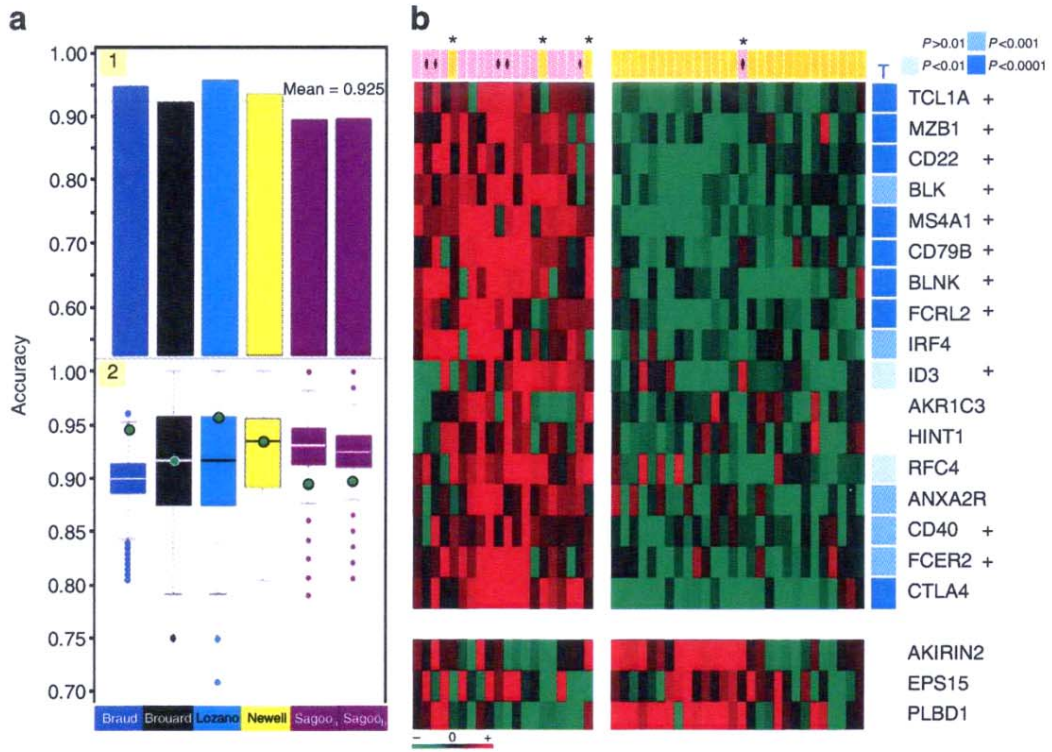


Figure 6 | Reproducibility of the meta-signature of tolerance. (**a**) Cross-validation of the meta-signature. In panel 1, the classification performances through the six cross-validation folds are shown. Results are displayed with a histogram in which each bar (one of the six folds) represents the accuracy obtained on one data set used as test, whereas learning is performed on the five others. From left (first fold) to right (sixth fold), tests were data sets from Braud (dark blue), Brouard (black), Lozano (turquoise blue), Newell (yellow), $Sagoo_a$ (European 'Indices of Tolerance' (IOT) cohort, purple), and $Sagoo_b$ (American 'Immune Tolerance Network' (ITN) cohort, purple). In panel 2, the influence of the origin of a data set on the performances of classification is assessed. The accuracy from the six test data sets (green circle) is compared with the accuracy obtained on comparable test data sets (equal size and same sample composition) constructed by a random selection of tolerant (TOL) and stable (STA) samples from the total pool of samples (whatever the study of origin). Results are depicted by box plots (boxes: interquartile range (IQR); whiskers: 1.5×IQR) corresponding each to the values obtained after 1000 repeated random selections. Values beyond the range are considered outliers and shown as circles. (**b**) Experimental validation of the meta-signature. The expression of the 20 top ranked biomarkers discriminating tolerant (TOL) from stable (STA) recipients is assessed in a new collection of 48 samples. The results from real-time PCR are displayed by an expression heat map (red for gene overexpression and green for gene underexpression) showing the patterns from 18 TOL (pink; dot: new cases) and 30 STA (orange). Misclassified samples are denoted by an asterisk. Significance of the individual markers (17 upregulated and 3 downregulated) is assessed by a Student's *t*-test: resulting *P*-values are depicted by shades of blue on the right side (see color legend). From the 20 genes, those corresponding to B-cell–related markers are quoted by a cross.

Transpl Int. 2015 Apr 8. doi: 10.1111/tri.12578. [Epub ahead of print]

Tolerant recipient display expanded b-cell subsets

Accordingly, cellular analysis by flow cytometry reported an increase in absolute number of B cells in tolerant patients compared with immunosuppressed recipients [153]. This finding has been further replicated and validated by three studies [157–159]. This increase was associated with an enrichment in naive and transitional B-cell subsets in the peripheral blood mononuclear cells of tolerant patients [157,159], and a lack of plasma cells attributed to a default in B-cell differentiation and a higher sensibility to apoptosis in the late stages of differentiation [180]. Phenotypic analysis identifies a global inhibitory profile with a diminution of CD32a/CD32b ratio, increased expression of BANK-1 (which negatively modulates CD40-mediated AKT activation) and augmentation of CD1d CD5-expressing B cells [158], which are considered to be regulatory phenotypes [181].

These studies thus support the fact that operationally tolerant recipients display a strong B-cell signature. This feature is unique to kidney tolerant recipients as not observed in liver tolerant patients [154].

Therefore, it is interesting to note that first recipients with end-stage renal disease have a significant reduction in the peripheral total B-cell count [182–184]. Second, after transplantation, patients are treated with a strong IS therapy which guarantees a stable function of the graft but strongly alters the immune system. But some patients who stopped their treatments managed to tolerate their kidney allograft and are subject to a strong immune reconstitution with an increase of naïve or immature B cells in their blood compared with stable recipients. This phenomenon is observed in kidney transplantation tolerance mediated by mixed chimerism with a repopulation of transitional B cells in tolerant recipients [185]. Accordingly, a recent study suggest that B cells could play a role in the maintenance of tolerance but not in its induction and that their development may be due to the progressive weaning off IS, which may allow regulatory populations to emerge

Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype

Annaick Pallier[1,12], Sophie Hillion[2,3,4,12], Richard Danger[1,12], Magali Giral[1,5], Maud Racapé[1], Nicolas Degauque[1], Emilie Dugast[1], Joanna Ashton-Chess[1], Ségolène Pettré[1], Juan José Lozano[6], Régis Bataille[7,8], Anne Devys[9], Anne Cesbron-Gautier[9], Cécile Braudeau[1,5], Catherine Larrose[10,11], Jean-Paul Soulillou[1,5,10,12] and Sophie Brouard[1,12]

Kidney International (2010) **78**, 503–513

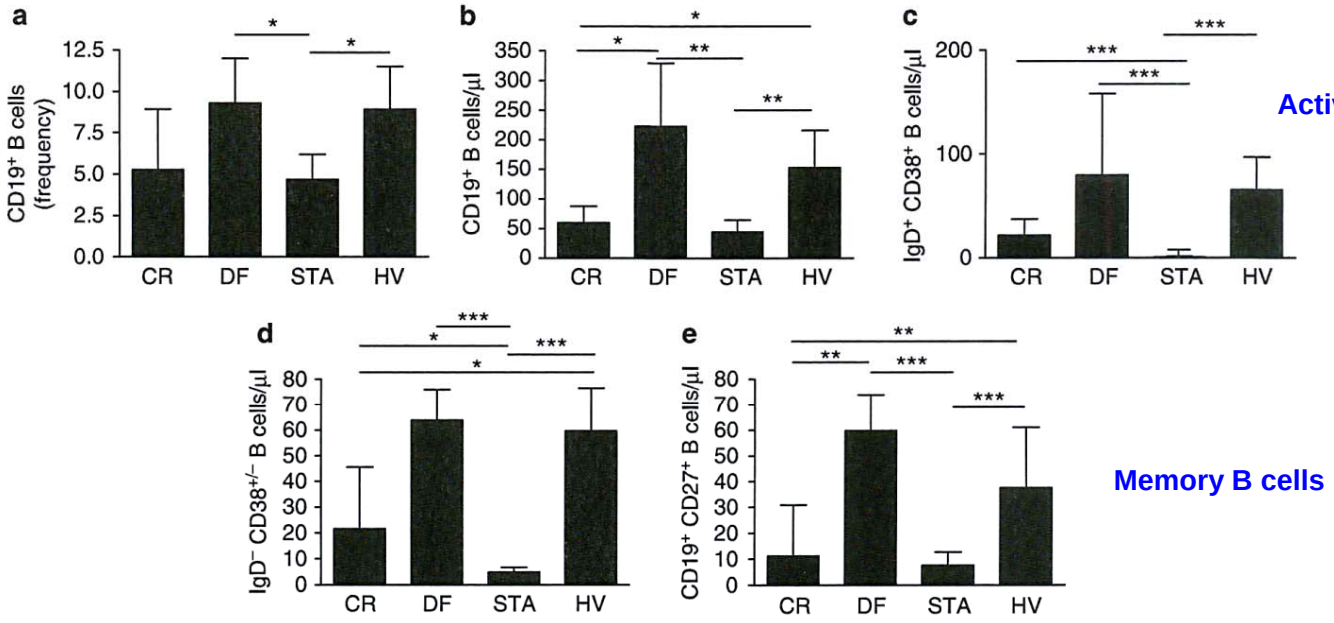


Figure 1 | Drug-free long-term graft function (DF) patients display a higher number of peripheral B cells. DF patients display a significantly higher frequency (**a**) and absolute value (**b**) of total peripheral B cells. The increase of B cells in DF patients is mainly due to a significant increase in $IgD^{+}CD38^{+}$ (activated Bm2 cells) (**c**) and $IgD^{-}CD38^{+/-}$ (EBm5/Bm5 memory B cells) (**c**). This was confirmed by an increase of the number of memory $CD19^{+}CD27^{+}$ B cells in DF patients (**e**). Differences were defined as statistically significant when $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***). CR, patients under standard immunosuppression with deteriorating kidney graft function; HV, healthy volunteers; STA, kidney recipients with stable graft function under standard immunosuppression.

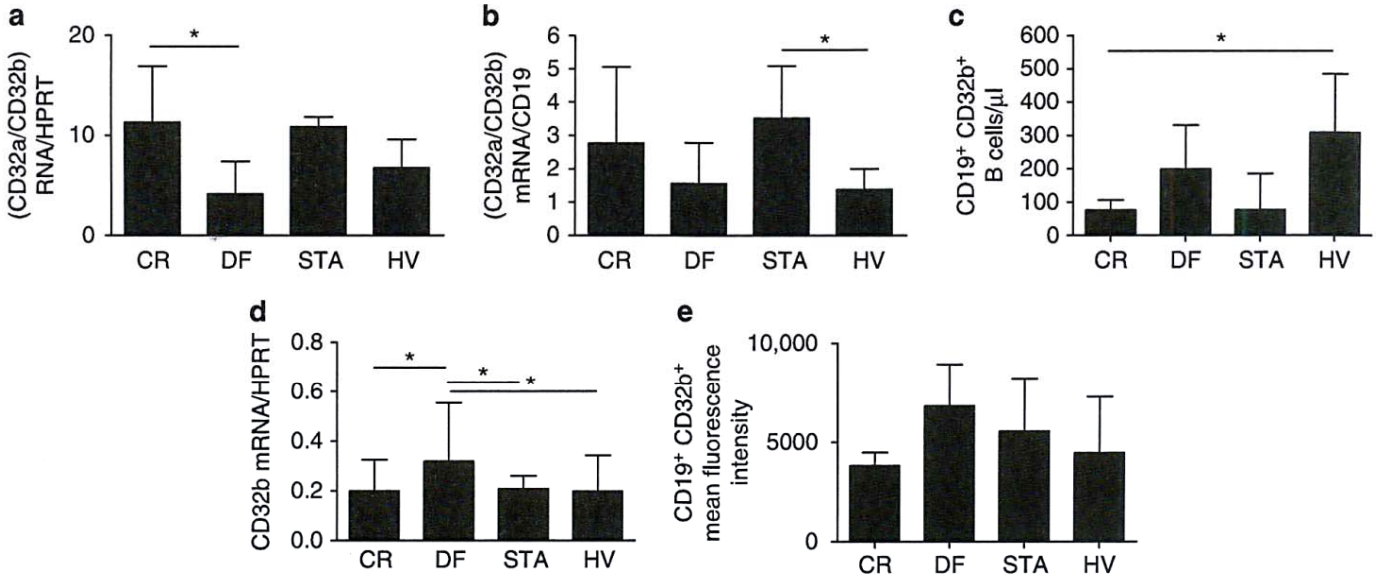


Figure 3 | DF patients display B cells with an inhibitory profile. (**a**) Peripheral blood mononuclear cells (PBMCs) from drug-free long-term graft function (DF) patients display a significantly decreased CD32a/CD32b FcγRIIA/FcγRIIB transcript ratio both at the level of total PBMC (**a**) and at the level of $CD19^+$ B cells (**b**) compared with patients with chronic rejection and healthy volunteers, respectively. Among total PBMC, DF patients express higher number of $CD19^+$ $Fc\gamma RIIB^+$ (CD32b) B cells as shown by flow cytometry (**c**) and a significant increase in FcγRIIB (CD32b) mRNA expression (**d**). Finally, B cells from DF patients tend to express more FcγRIIB (CD32b) at their surface (mean fluorescence intensity) (**e**). Differences were defined as statistically significant when $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***).

Allo-Trapianto di Organi
Rigetto o Tolleranza

Grazie Mimmo

European Organ Donation Day

Roma 11/10/2014