

The role of memory B cells in Kidney Transplantation: Relevance, Risk and Research

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Transplantation Immunology, UMCG



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- Relevance
 - Memory B cells = immune memory
 - Produce donor-specific Abs faster
 - Especially important in sensitized patients
- Risk
 - Accelerated ABMR
 - Hard to control with standard immunosuppression
 - Leads to worse graft survival
- Research
- Detection
 - Methods: ELISPOT, flow cytometry, luminex
 - Limited clinical application
 - Can we predict rejection risk before transplantation?

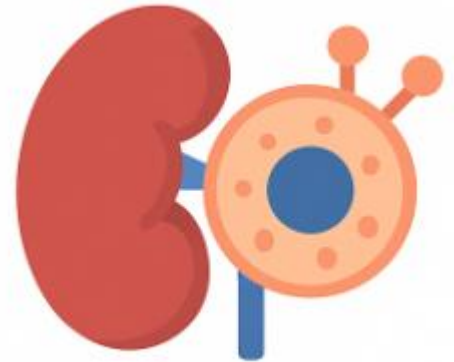
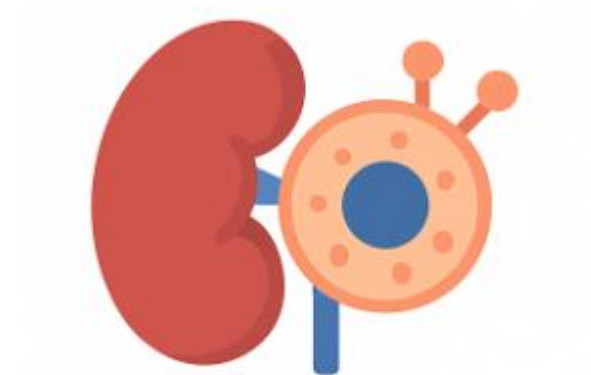


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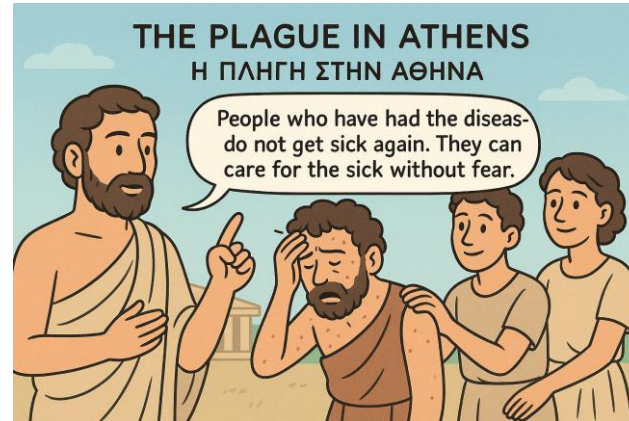
What is immunological memory?

- Adaptive immune response
- Immunological memory is the adaptive ability of the immune system to recognize pathogens encountered previously and respond effectively upon re-exposure



Thucydides
The Peloponnesian War
A new translation by Martin Hammond

OXFORD WORLD'S CLASSICS



430 BC



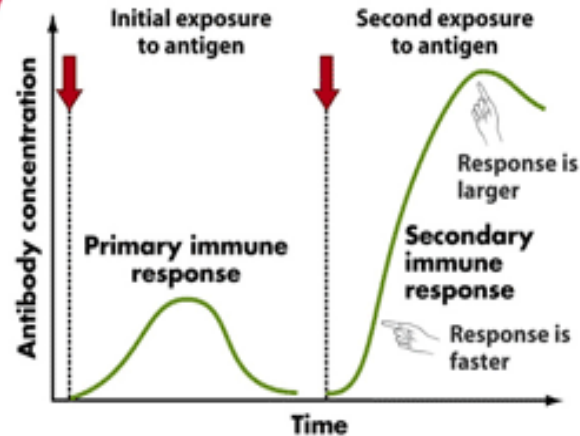
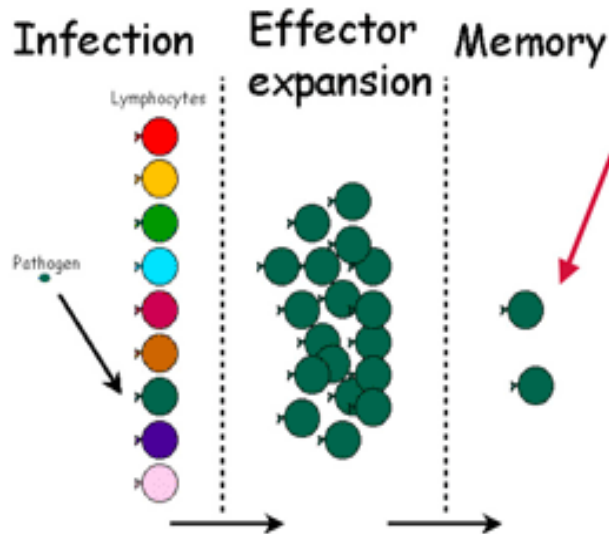
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IMMUNE MEMORY



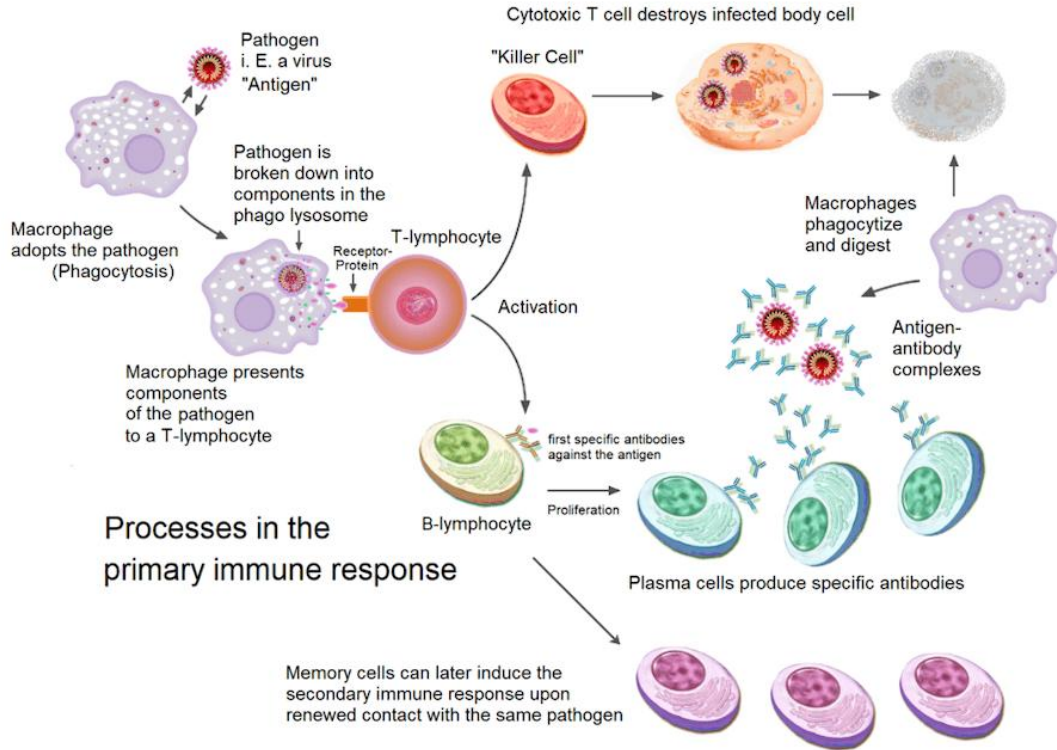
Once a lymphocyte has recognised antigen a foreign antigen it expands to eliminate the infection

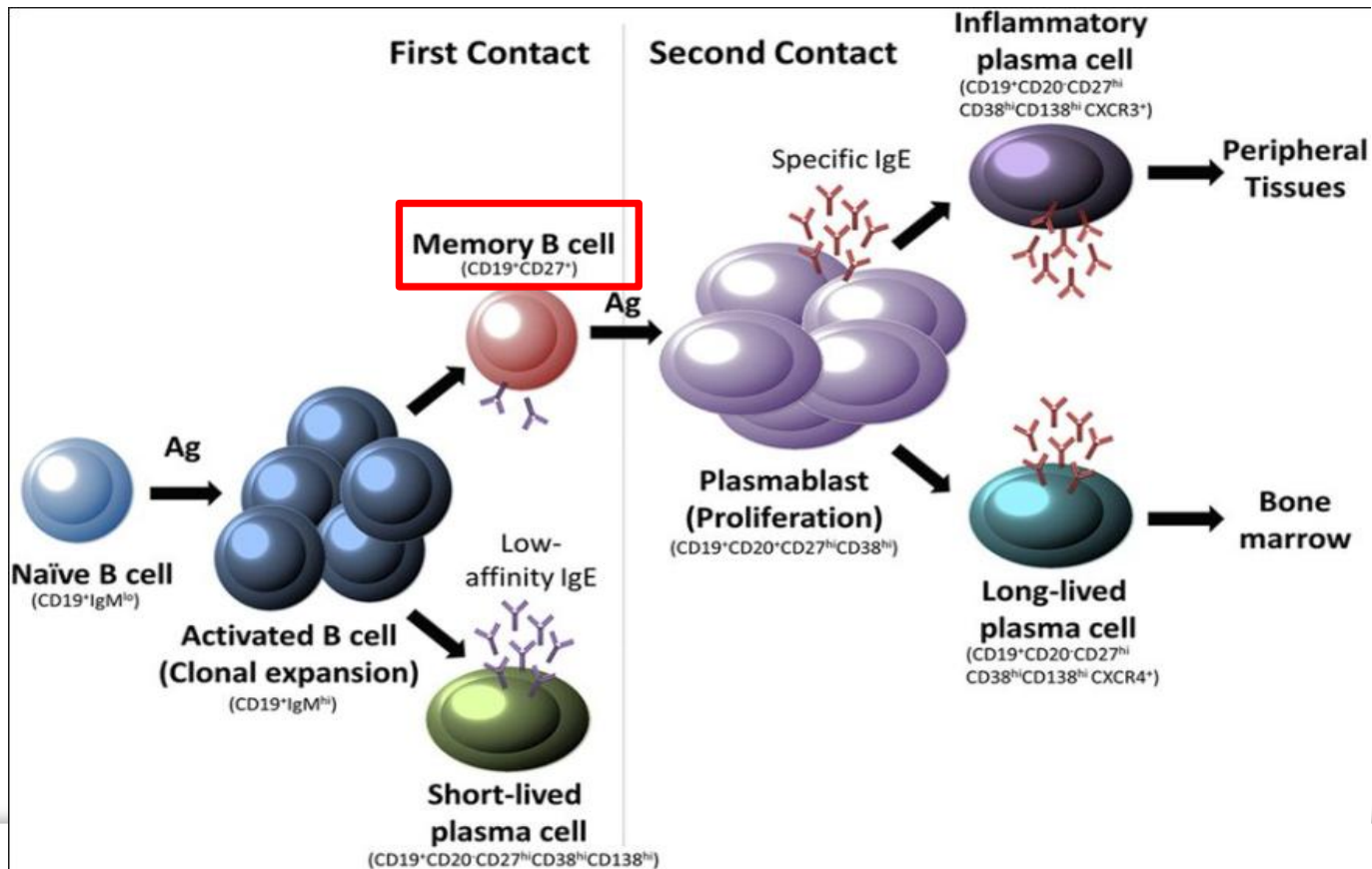
Some cells then become long lasting >20 years 'memory' cells.



Memory cells respond very quickly to subsequent exposure to antigen







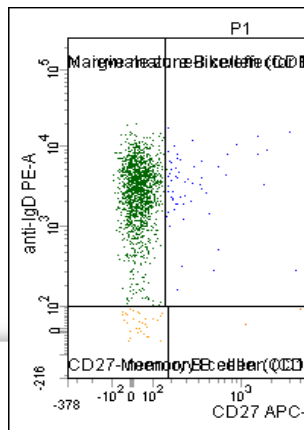
What if y

response?

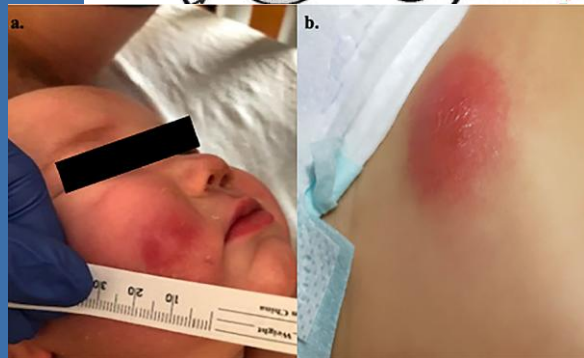
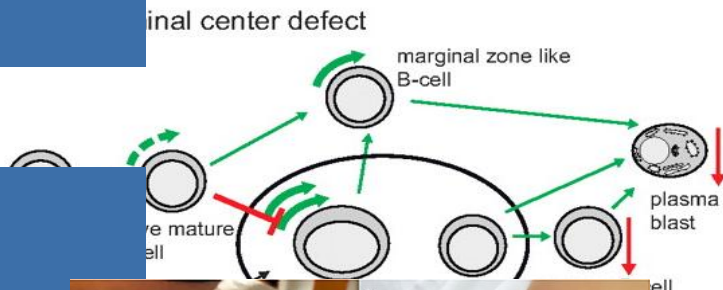
Consequences?

- Primary
- CVID (XLA) → no memory B cells

patient



Decreased immunoglobulins
(IgG, IgM, IgA)
Infections
Auto-immunity
Reduced vaccination responses

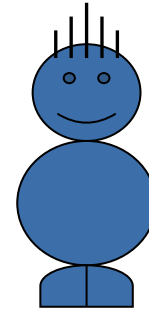


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The immune system responds to foreign material

Pathogens

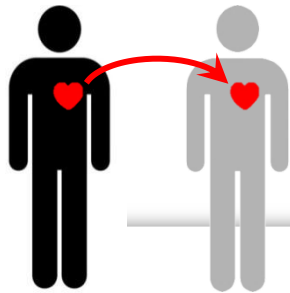
- bacteria
- viruses
- tumors



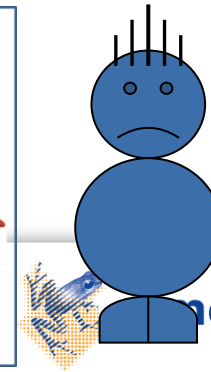
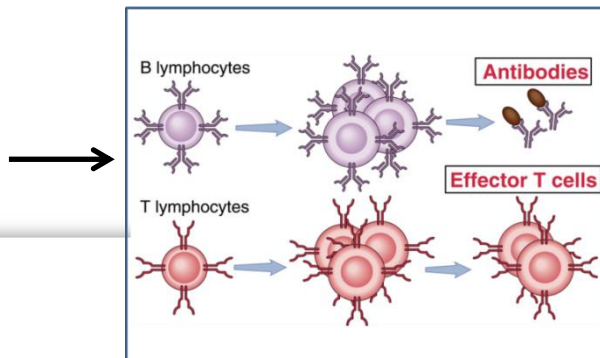
Transplanted organ

recipient

donor



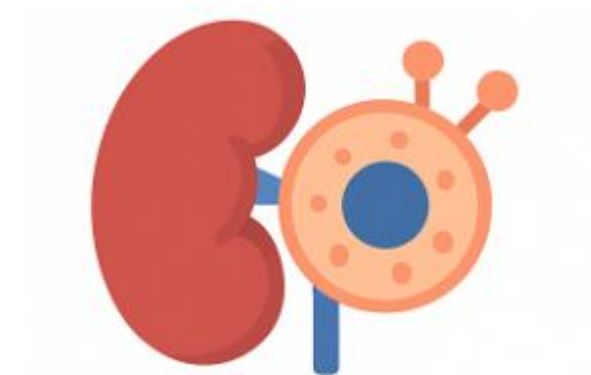
allogeneic
transplantation



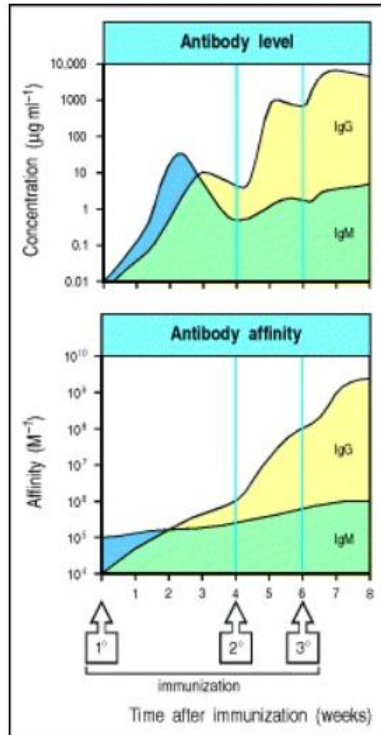
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Secondary antibody responses from memory B cells



	Source of B cells	
	Unimmunized donor Primary response	Immunized donor Secondary response
Frequency of specific B cells	$1:10^4 - 1:10^5$	$1:10^3$
Isotype of antibody produced	IgM > IgG	IgG, IgA
Affinity of antibody	Low	High
Somatic hypermutation	Low	High

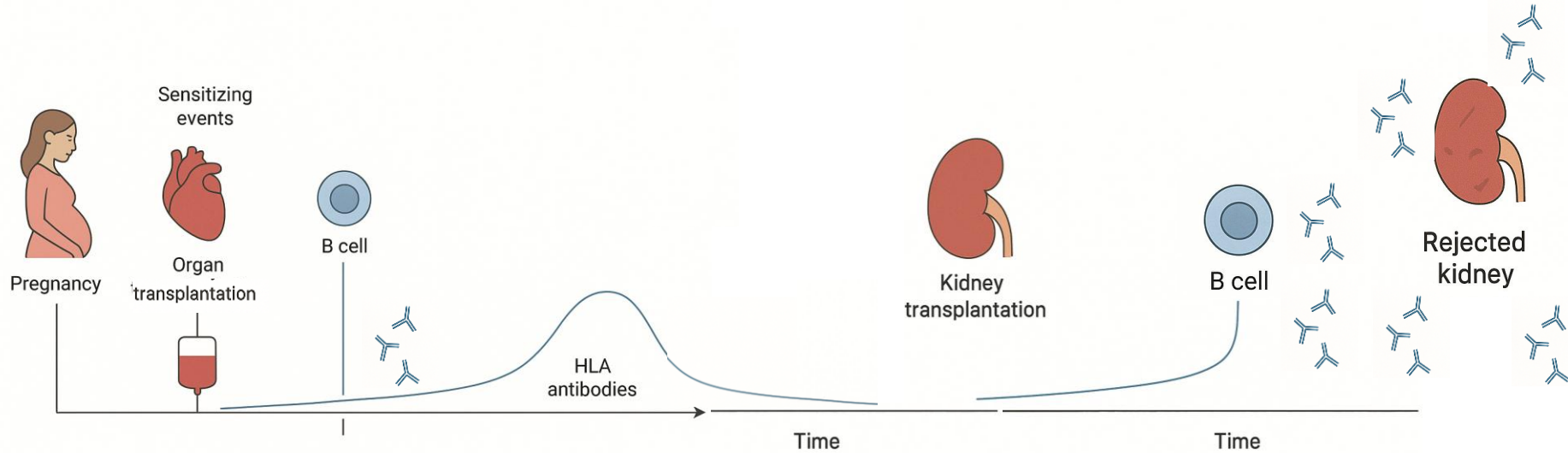


Immunobiology, 5th edition
 Charles A Janeway



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Memory B cell response



Long-Lived Plasma Cells (LLPCs) vs. Memory B Cells in ABMR

Long-Lived Plasma Cells (LLPCs):

- Reside mainly in bone marrow niches (*Radbruch et al. 2006*)
- Continuously secrete antibodies, including donor-specific antibodies (DSA) (*Halliley et al. 2015*)
- Do not proliferate or replenish after depletion (*Nutt et al. 2015*)
- Directly contribute to **persistent DSA production** and chronic ABMR (*Kwun et al. 2019*)

Memory B Cells:

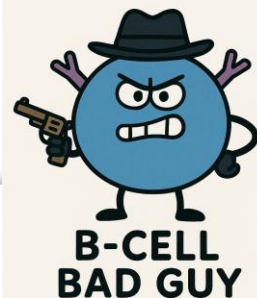
- Circulate in blood or secondary lymphoid organs (*Tangye & Tarlinton 2009*)
- Do not secrete antibodies until re-activated (*McHeyzer-Williams et al. 2012*)
- Upon antigen re-exposure, rapidly proliferate and differentiate into plasma cells (*Good-Jacobson & Shlomchik 2010*)
- Act as a **reservoir for new DSA production**, fueling acute ABMR episodes (*Angeletti et al. 2021*)

•Key Difference in ABMR:

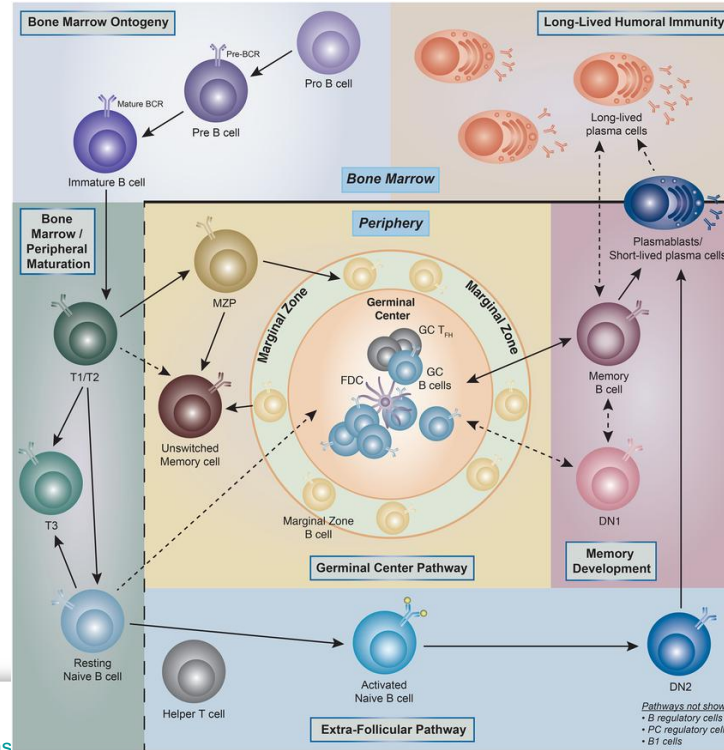
- LLPCs → maintain **ongoing baseline antibody production** (*Lightman et al. 2019*)
- Memory B Cells → enable **rapid recall responses**, amplifying rejection (*Angeletti et al. 2021*)



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The diverse world of B-cells



<https://www.clinicalleader.com/doc/diverse-patient-populations>

Challenges and Opportunities for Consistent Classification of Human B Cell and Plasma Cell Populations

DOI:10.3389/fimmu.2019.02458



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Human B-cell subsets and their markers

Hematopoietic / pro-/pre-B

Development in bone marrow
Markers: CD34+, CD19 (later),
CD10, CD38, CD93

Transitional B-cells

Immature; just out of bone marrow
Markers: CD24+, CD38hi, IgD+,
CD10+, CD27-

Naive B-cells

Antigen-naive
Markers: IgD+, IgM+, CD19+, CD27-,
CD21+

Memory B-cells

Rapid reactivation upon re-exposure
Markers: CD19+ CD27+, IgG+/IgA+
(switched), sometimes IgM+

Marginal Zone (MZ)

Rapid blood response (spleen)
Markers: CD19+ IgMhi, IgDlow/-,
CD27+, CD21+

Follicular B-cells / Germinal Center

Affinity maturation in GC
Markers: CD19+ IgD+/IgM+, CD21+,
CD23+, BCL-6 (GC)

Atypical / Exhausted Memory

Chronic infection / dysfunction
Markers: CD19+ CD11c+, CD21low,
often CD27-

Plasmablasts / Plasma cells

Antibody-producing
Markers: CD19+/lo (plasmablast),
CD38hi, CD27+, CD138+

Regulatory B-cells (Bregs)

Immunoregulation via IL-10
Markers: CD19+ CD24hi, CD38hi
and/or CD24hi CD27+, IL-10+



Transplantation-relevant



Other B-cell subsets

Based on data from Tijmensen *et al.*, *Front. Immunol.* 2020; Sanz *et al.*, *Semin. Immunol.* 2019; Palanichamy *et al.*, *J. Immunol.* 2009; Blair *et al.*, *J. Immunol.* 2010; Mauri & Bosma, *Annu. Rev. Immunol.* 2012; Klein & Dalla-Favera, *Nat. Rev. Immunol.* 2008; Maecker *et al.*, *Cytometry A* 2012.



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Functional roles of B-cell subsets in Kidney transplantation

Memory B cells (CD27+)
plasmablasts and plasma cells

Donor-specific antibody
production → complement
activation and graft injury

Antibody-mediated rejection

Transitional B cells (CD24+
CD38^{hi}) regulatory B cells
(Bregs)

IL-10 secretion → suppression
of T-cell activation and reduced
inflammation

Regulation & Graft tolerance



Balance between effector and regulatory B cells

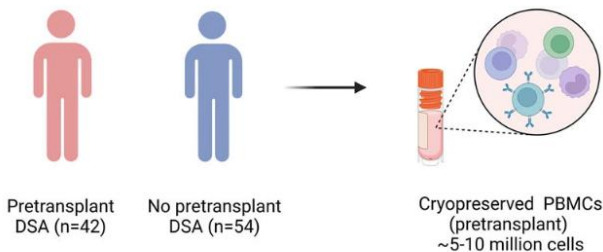
Outcome depends on B-cell subset dominance



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Phenotyping and cytokine production of circulating memory B cells

Part 1: Subset phenotyping



Group characteristics	Pretransplant DSA	No pretransplant DSA	p value
Sex (% female)	24 (57%)	26 (48%)	NA
Age (mean; range)	48 (18-69)	45 (18-74)	NA
Rejection (%)	20 (48%)	11 (20%)	0.008



Spectral flow cytometry (Aurora)

Purpose	Markers
Cleanup	Viability, CD3, CD14
B cell lineage	CD19, CD20
B cell maturation/differentiation	CD38, CD27, CD24, CD138, IgM, IgD, IgG, IgA, CD21, CD10, CD43
Stimulatory checkpoints	CD40, ICOS, BAFFR, IL-21R, IL-6R, TACI
Inhibitory checkpoints	BTLA(CD272), PD1, FcγRIIb, CD22, FcRL5
Migration	CXCR5, CXCR3
Misc.	CD84, CD39, CD5, CD11c



Data analysis with OMIQ

Part 2: Cytokine production



Classical flow cytometry (BD FACSymphony)

Purpose	Markers
Cleanup	Viability, CD3
B cell lineage	CD19, CD22
B cell Cytokines	IL-10, TNF-α, IL-6

Flow cytometry

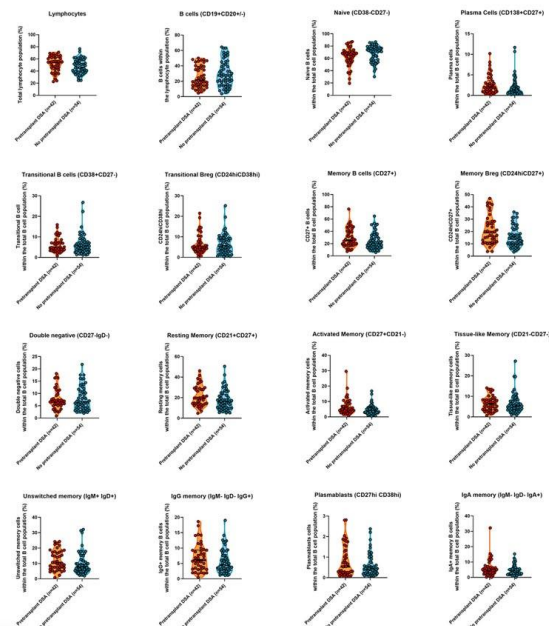
- 33-color B-cell spectral flow cytometry panel
- B cell subsets
- Cytokines
- Immune regulatory proteins

		Marker and color	Concentration (per sample)	Clone and brand
Viability		Live/Dead Blue	Diluted 1:40; 5 µl added	2339921; Invitrogen
Washing step				
1 st round of staining	1	CD126 BV421	1.25 µl added directly	M5; BD Horizon
	2	CD275 BB790	2.5 µl added directly	2D31B7-H2; BD OptiBuild
	3	CD11c BV650	5 µl added directly	3.9; BioLegend
	4	CD267 PE-Dazzle 594	Diluted 1:8; 5 µl added	1A1; BioLegend
Washing step				
2 nd round of staining	5	CD43 BUV615	Diluted 1:32; 5 µl added	1G10; BD OptiBuild
	6	CD185 eFluor 450	Diluted 1:16; 5 µl added	MU5UBEE; Invitrogen
	7	FcRL5 BB700	Diluted 1:16; 5 µl added	509F6; BD OptiBuild
	8	IgA VioBright FITC	Diluted 1:16; 5 µl added	IS11-8E10; Miltenyi Biotec
	9	CD279 (PD-1) PE-Fire 640	Diluted 1:8; 5 µl added	EH12.2H7; BioLegend
	10	CD27 BV480	Diluted 1:8; 5 µl added	L128; BD Horizon
	11	IgD BUV563	Diluted 1:8; 5 µl added	IA6-2; BD OptiBuild
	12	CD38 PE-Fire 810	Diluted 1:8; 5 µl added	S17015F; BioLegend
	13	CD32 BV750	Diluted 1:8; 5 µl added	3D3; BD OptiBuild
	14	CD5 BUV661	Diluted 1:8; 5 µl added	UCHT2; BD OptiBuild
	15	CD84 PE Vio 770	Diluted 1:8; 5 µl added	REA1102; Miltenyi Biotec
	16	IgM BV570	1.25 µl added directly	MHM-88; BioLegend
	17	CD20 BV786	1.25 µl added directly	2H7; BD OptiBuild
	18	CD14 BV510	1.25 µl added directly	MMP9; BD Horizon
	19	CD21 APC-Vio 770	1.25 µl added directly	HB5; Miltenyi Biotec
	20	CD19 cFluor BYG710	1.25 µl added directly	HIB19; Cytek Biosciences
	21	CD10 APC-R700	1.25 µl added directly	HI10a; BD Biosciences
	22	CD86 BUV737	1.25 µl added directly	2331 (FUN-1); BD Horizon
	23	CD39 BV711	2.5 µl added directly	TU66; BD Horizon
	24	CD138 BUV496	2.5 µl added directly	MI15; BD OptiBuild
	25	CD40 PerCP-eFluor 710	2.5 µl added directly	5C3; Invitrogen
	26	CD22 BUV805	2.5 µl added directly	HIB22; BD OptiBuild
	27	IgG BB515	2.5 µl added directly	G18-145; BD Horizon
	28	CD268 BV605	2.5 µl added directly	11C1; BD OptiBuild
	29	CD24 BUV 395	2.5 µl added directly	ML5; BD Horizon
	30	CD272 APC	2.5 µl added directly	MIH26; BioLegend
	31	CD3 BV510	2.5 µl added directly	UCHT1; BioLegend
	32	CD183 APC-Fire	5 µl added directly	G025H7; BioLegend
	33	IL-21R BB600	1 µl added directly	7A12; BD Horizon



B cell subsets in patient groups with and without pretransplant DSAs

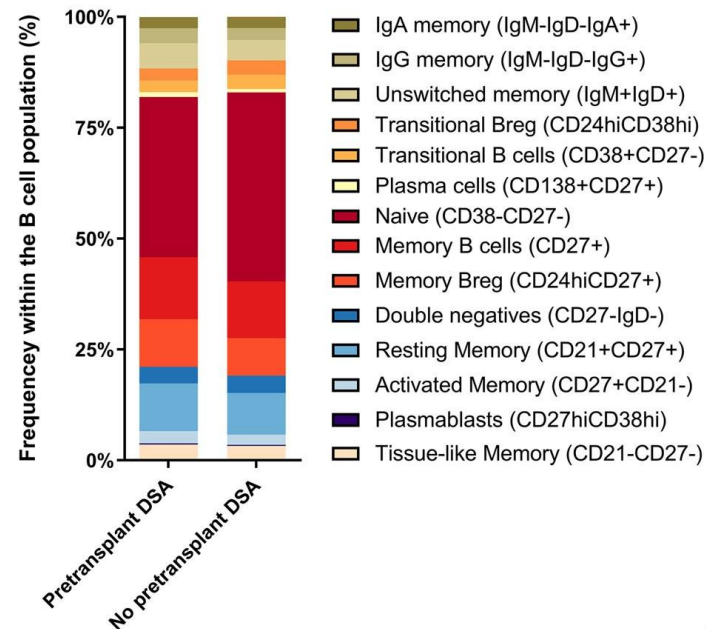
A



B

Lymphocytes, B cells, and subsets within the CD19 ⁺ CD20 ⁺ B cells gate	Median % PreTX DSA, n=42 No preTX DSA, n=54	P value	Corrected P value
Lymphocytes	54.9 45.7	0.06	NA
B cells (CD19 ⁺ CD20 ⁺)	21.3 27.5	0.25	NA
Naive (CD38 ⁻ CD27 ⁻)	65.9 71.9	0.05	0.14
Plasma Cells (CD138 ⁺ CD27 ⁺)	1.96 1.37	0.08	0.16
Transitional B cells (CD38 ⁺ CD27 ⁻)	4.77 5.39	0.91	0.99
Transitional Breg (CD24 ^{hi} CD38 ^{hi})	5.01 5.43	0.99	0.99
Memory B cells (CD27 ⁺)	25.4 21.5	0.08	0.16
Memory Breg (CD24 ^{hi} CD27 ⁺)	19.5 14.3	0.01	0.14
Double negative (CD27 ⁻ IgD ⁻)	6.84 6.52	0.86	0.99
Resting Memory (CD21 ⁺ CD27 ⁻)	19.6 15.9	0.04	0.14
Activated Memory (CD27 ⁺ CD21 ⁺)	4.94 3.86	0.051	0.14
Tissue-like Memory (CD21 ⁻ CD27 ⁻)	6.44 5.45	0.54	0.69
Unswitched memory (IgM ⁺ IgD ⁻)	10.4 7.84	0.05	0.14
IgG memory (IgM ⁺ IgD ⁻ IgG ⁺)	6.08 4.47	0.14	0.26
Plasmablasts (CD27 ^{hi} CD38 ^{hi})	0.55 0.42	0.24	0.38
IgA memory (IgM ⁺ IgD ⁻ IgA ⁺)	4.68 4.25	0.32	0.45

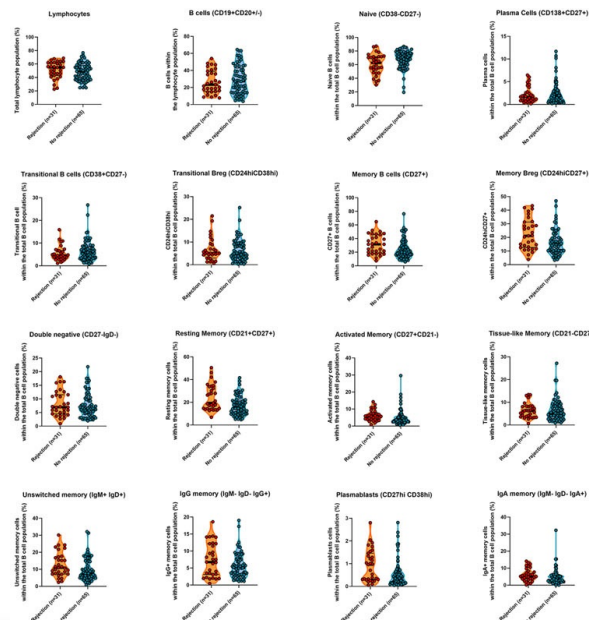
C



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B cell subsets in patient groups with and without rejection

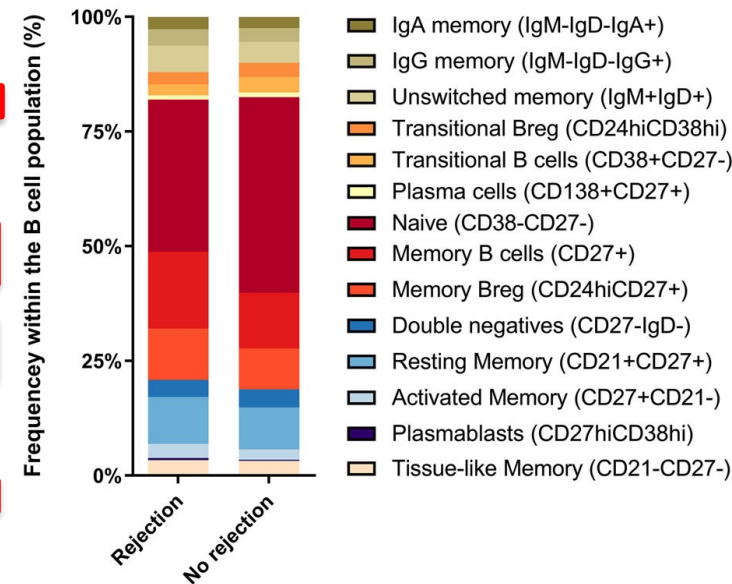
A



B

Lymphocytes, B cells, and subsets within the CD19 ⁺ CD20 ⁺ B cells gate	Median % Rejection, n=31 No rejection, n=65	P value	Corrected P value
Lymphocytes	54.8 48.9	0.19	NA
B cells (CD19 ⁺ CD20 ⁺)	23.3 27.3	0.64	NA
Naive (CD38 ⁻ CD27 ⁻)	62.7 74.0	0.01	0.039
Plasma Cells (CD138 ⁺ CD27 ⁺)	1.67 1.73	0.94	0.95
Transitional B cells (CD38 ⁺ CD27 ⁻)	4.53 5.59	0.25	0.34
Transitional Breg (CD24 ^{hi} CD38 ^{hi})	5.10 5.13	0.81	0.88
Memory B cells (CD27 ⁺)	31.5 20.1	0.01	0.039
Memory Breg (CD24 ^{hi} CD27 ⁺)	21.1 14.5	0.01	0.037
Double negative (CD27 ⁻ IgD ⁺)	6.94 6.55	0.52	0.67
Resting Memory (CD21 ⁺ CD27 ⁻)	19.4 15.3	0.003	0.037
Activated Memory (CD27 ⁺ CD21 ⁺)	5.76 2.69	0.008	0.037
Tissue-like Memory (CD21 ⁻ CD27 ⁻)	6.26 5.33	0.64	0.76
Unswitched memory (IgM ⁺ IgD ⁻)	10.9 7.58	0.03	0.057
IgG memory (IgM ⁺ IgD ⁺ IgA ⁺)	6.70 5.01	0.15	0.22
Plasmablasts (CD27 ^{hi} CD38 ^{hi})	0.95 0.49	0.01	0.039
IgA memory (IgM ⁺ IgD ⁺ IgA ⁺)	5.14 4.08	0.03	0.057

C



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Pretransplant Cytokine producing B cells and immune regulatory molecules

- No differences in the frequencies of cytokine-producing B Cells
 - IL-6, IL-10, and TNF- α
- No differences in the expression of immune regulatory molecules on circulating B cells and subsets
 - BAFF-R, FcR γ IIB (CD32), CD40, BTLA, CD22, TACI, ICOSL, FcRL5, PD-1, IL-6R, IL21R, CD86



General overview of some key studies

First Author	Study type / population	Measurement	Main Outcomes
Luque AJT 2019	Observational cohort, kidney Tx n=175 (sensitized & non-sensitized)	Donor-reactive memory B cells (HLA B-cell ELISpot)	Higher donor-reactive mBC associated with acute/chronic AMR; worse graft outcome
Fischman Transplantation direct 2019	Prospective, pediatric kidney Tx (n=10 DSA, n=11 control)	Memory + antibody-secreting B-cell subsets (mass cytometry)	Increased before DSA and AMR; predictive potential
Wehmeier Transplantation 2020	Pilot n=20, alloimmunized kidney recipients with pre-Tx DSA	Memory B-cell derived DSA via SAB assay, comparison with plasma DSA	Detected in ~45% DSA+ patients; possible AMR role, limited graft survival data
Chong Transplantation 2015	Review	Memory B cells and plasma cells overview	Central role in AMR and chronic graft injury
Altuleia Front Immunol 2024	Retrospective, sensitized kidney recipients n=60	Pre-transplant DSAm via SAB	No significant association with ABMR or early graft survival

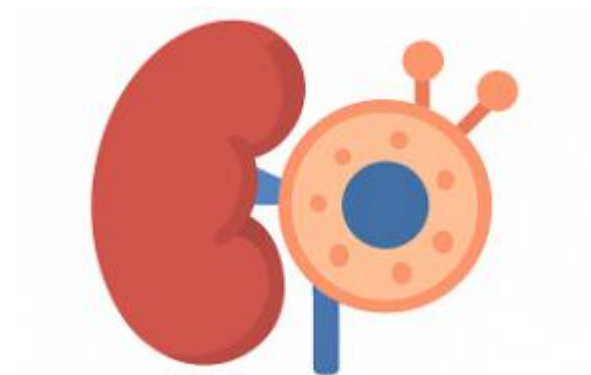


Comparative findings

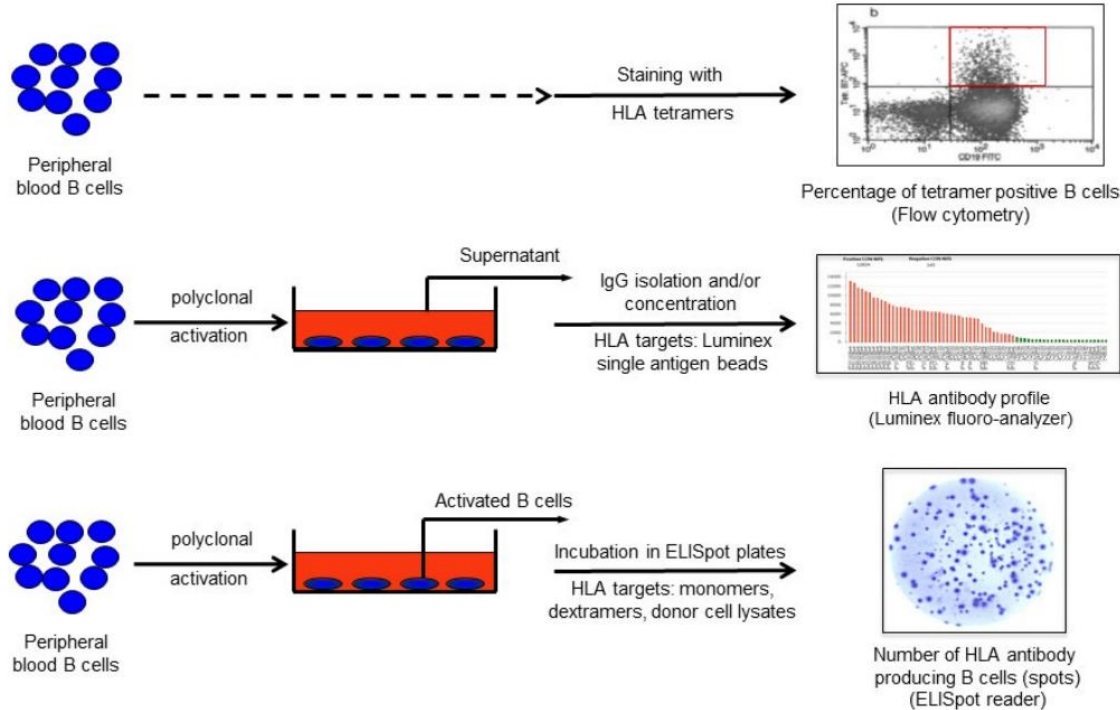
Study	Patient Sensitization	Memory B-cell presence	Association with AMR	Effect on Graft Survival	Notes
Luque AJT 2019	Both sensitized and non-sensitized	Detected via ELISpot; higher in AMR patients	Strongly associated with acute (100%) & chronic AMR (80%)	Negative effect (poorer function, more damage)	Observational, cut-offs not standardized
Fischman Transplantation direct 2019	Mostly non-sensitized (pediatric)	Memory & antibody-secreting B-cells increased pre-DSA ~50%	Increased AMR risk later	Potential clinical relevance; graft survival not directly shown	Small cohort; limited biopsies
Wehmeier Transplantation 2020	Sensitized with pre-Tx DSA	DSA-M present in ~45%	Descriptive only, possible AMR link	No clear graft survival data	Pilot, short follow-up
Chong Transplantation 2015	Mixed	Overview	Frequently linked to chronic AMR	Likely worse long-term survival	Indirect evidence
Altulea Front Immunol 2024	Sensitized	Pre-Tx DSA _m measured ~43%	No significant association with ABMR	No worse graft survival at 1 year	Follow-up limited, assay variation

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HLA specific memory B cell assays



Pro's	Con's
No B cell culture phase	Antibody secretion? Sorting necessary
Direct comparison Familiar readout	No quantification
Quantification	Standardization is difficult Labor intensive

Adapted from Wehmeier, Karahan and Heidt, Int J Immunogenetics 2020



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Polyclonal B cell activation



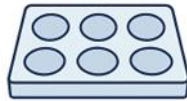
Activation cocktail

- Toll-like receptor 7/8 agonist (R848)
- IL-2

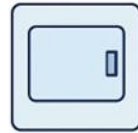


Activation cocktail

2×10^6 cells per well



37 °C



5% CO₂ incubator



Supernatant collected

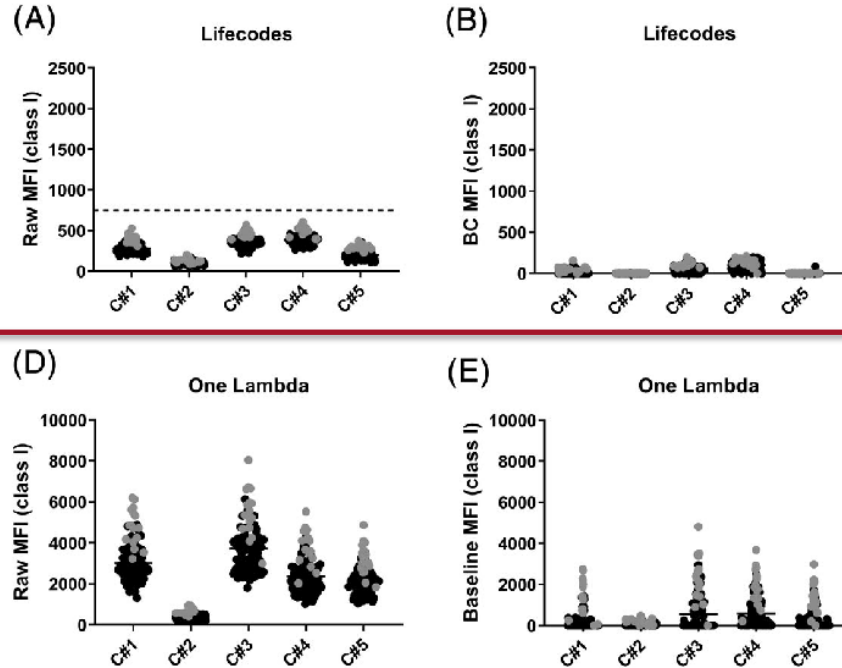
D6, d10, d20, DX



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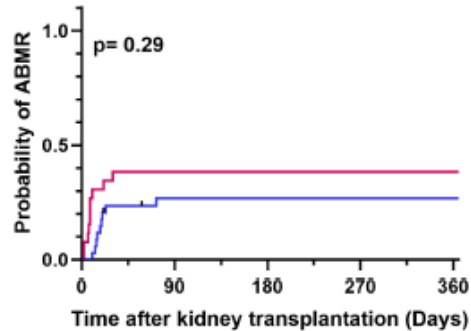
Comparison of different luminex single antigen bead kits for memory B cell-derived HLA antibody detection

Gonca E. Karahan  | Yvonne de Vaal | Kim Bakker | Dave Roelen |
Frans H. J. Claas | Sebastiaan Heidt

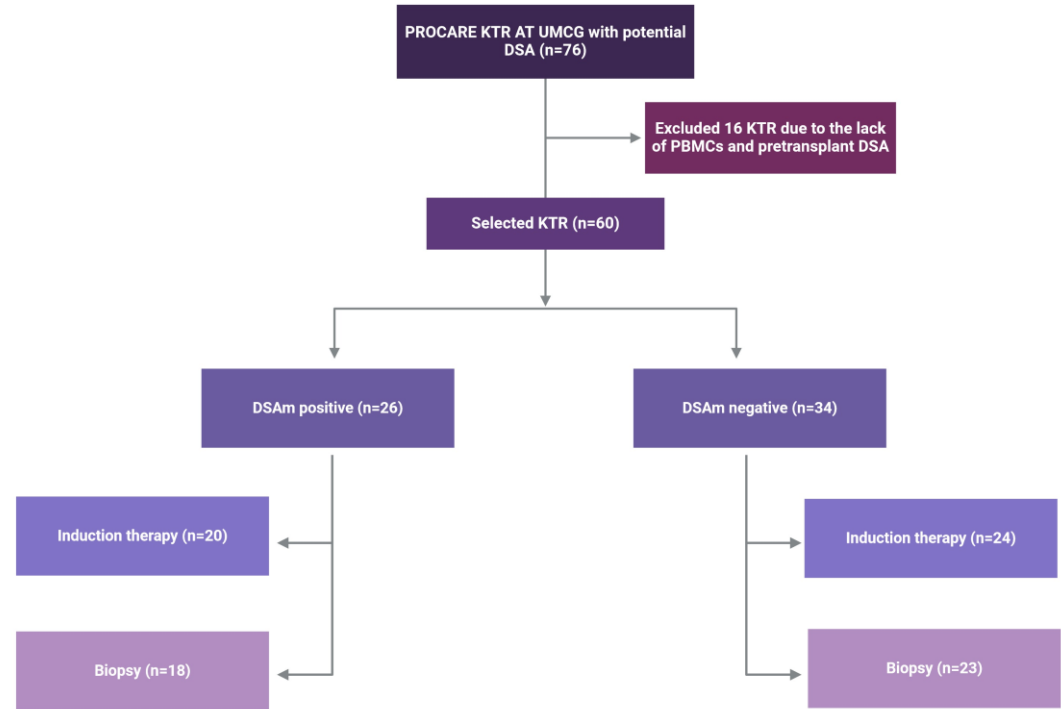


Retrospective single-center study

- Banff 2019
- 78% detectable HLAm
- 43% DSAm
 - 58% class I
 - 38% class II
 - 4% both



		Number of KTRs at risk				
—	DSAm positive	26	19	18	17	16
—	DSAm negative	34	33	27	25	23



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Single-center prospective explorative study

Donor-specific B Cell Memory in Alloimmunized Kidney Transplant Recipients: First Clinical Application of a Novel Method

Caroline Wehmeier, MD,¹ Gonca E. Karahan, PhD,¹ Juliette Krop, BSc,¹ Yvonne de Vaal, BSc,¹ Janneke Langerak-Langerak, BSc,¹ Isabelle Binet, MD,² Stefan Schaub, MD,³ Dave L. Roelen, PhD,¹ Frans H.J. Claas, PhD,¹ Sebastiaan Heidt, PhD,¹ and the Swiss Transplant Cohort Study*

- 20 pt with Luminex DSA
- Banff 2017

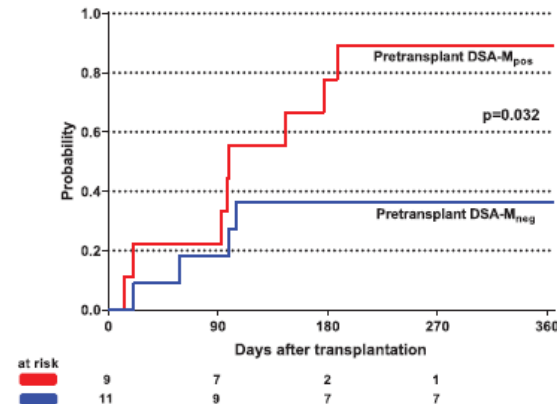


FIGURE 2. Incidence of (sub)clinical AMR in patients with (DSA-M_{pos}) and without (DSA-M_{neg}) pretransplant memory B cell-derived donor-specific HLA antibodies. AMR, antibody-mediated rejection; DSA-M, donor-specific memory B cell-derived HLA antibodies.

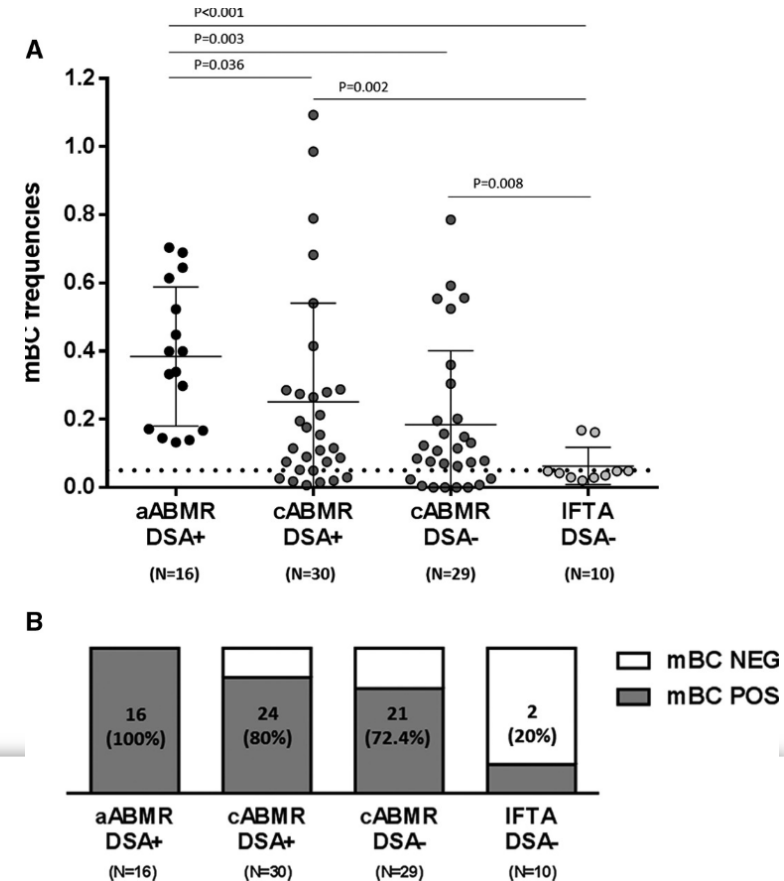


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Value of monitoring circulating donor-reactive memory B cells to characterize antibody-mediated rejection after kidney transplantation

Sergi Luque¹ | Marc Lúcia¹ | Edoardo Melilli² | Carmen Lefaucheur³ | Marta Crespo⁴  | Alex Loupy³ | David Bernal-Casas^{1,5} | Montse Gomà⁶ | Marta Jarque¹ | Elena Crespo¹ | Núria Montero² | Anna Manonelles² | Josep M. Cruzado^{1,2} | Salvador Gil-Vernet² | Josep M. Grinyó^{1,2} | Oriol Bestard^{1,2} 

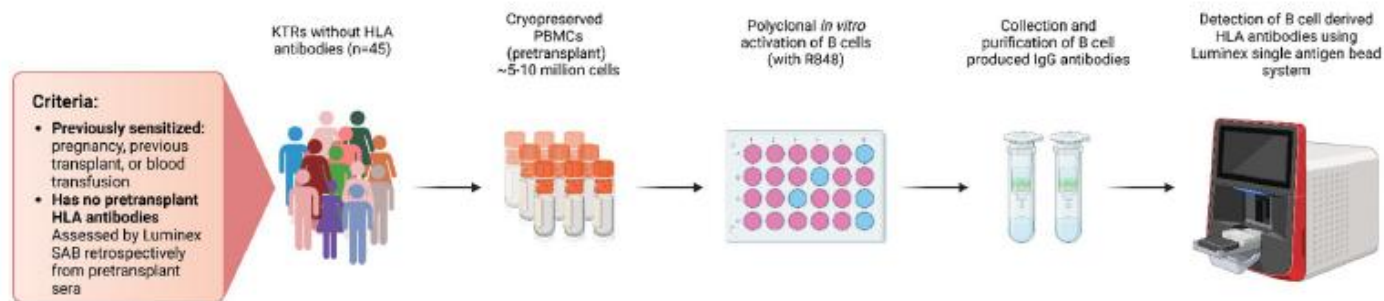
- 175 KTR
- *For-cause* or 6 and 24 months biopsy
- ELISPOT
- Banff 2013



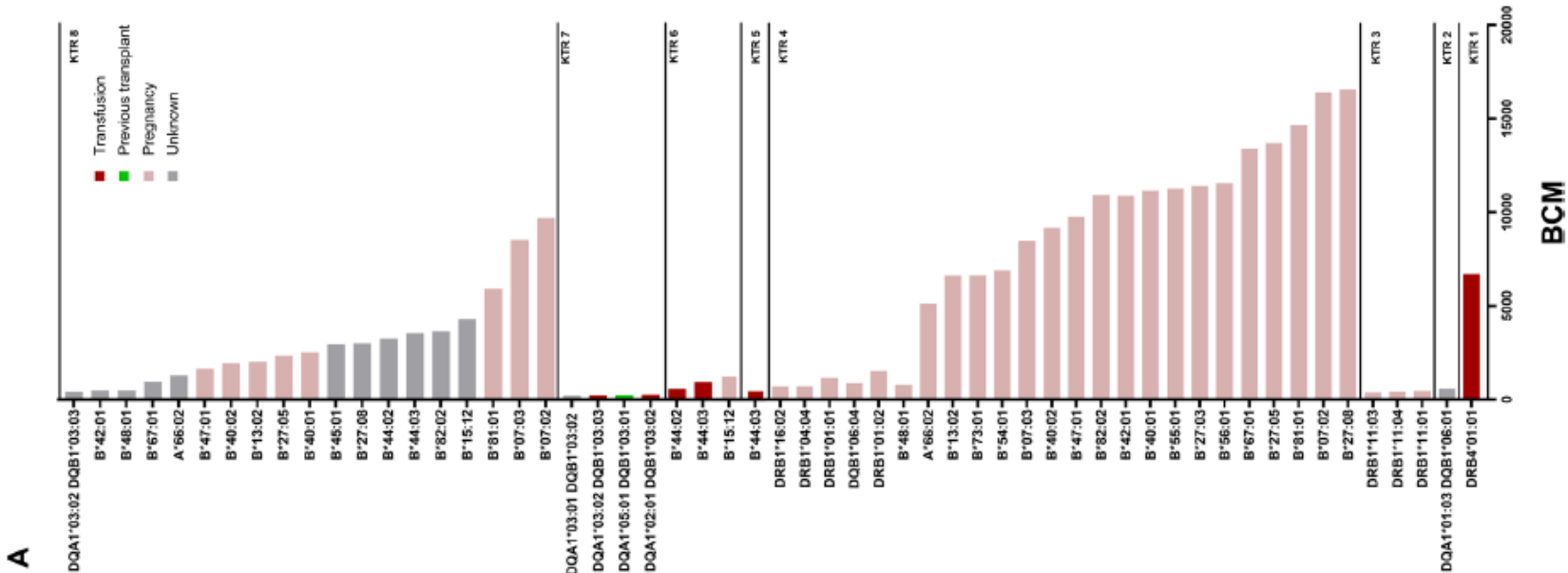
BRIEF COMMUNICATION **OPEN ACCESS**

Memory B-Cell Assay Uncovers Prior HLA Sensitisation in Transplant Candidates With Prior Exposure to Non-Self HLA

Dania Altulea¹  | Rosa G. M. Lammerts²  | Laura Bungener² | Peter Heeringa³ | Stefan P. Berger¹ | Jan-Stephan Sanders¹



HLA memory



Relevance in detection



- No B-cell memory detectable in individuals without a history of alloantigen exposure G. Karahan et al. Transplantation 2019



- B-cell memory detectable in individuals with a history of alloantigen exposure but without serum antibodies D. Altulea et al. HLA 2025



- DSA negative ABMR cases do show B-cell memory S. Luque et al. 2019



Conclusion? Memory B-cells and ABMR

- Several studies and reviews describe that pre-existing or donor-reactive memory B-cells are associated with a higher risk of ABMR and worse graft outcomes
- **BUT!** the evidence is not consistent – some recent studies found no clear association
- Outcomes largely depend on the study population and assay used to detect memory B-cells or donor-specific antibodies
- **Need for improved detection and innovative therapies**
 - Standardization and clinical validation of memory B cell assays

Kwun et al. 2017; Angeletti et al. 2021; Altulea et al. 2024; Wenlong Yue et al. 2022; Karahan et al. 2020



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Treatment



- **Hard to control with standard immunosuppression**
 - Memory B-cells are resistant and persistent
 - Rapid differentiation to plasma cells
- **Classical therapies:**
 - Plasmapheresis and IVIG → temporary decrease of Abs
 - Rituximab (anti-CD20) → naive and some memory B cells, no plasma cells
 - Methylprednisolone (corticosteroid) → no effect on B cell memory



Treatment directed towards plasma cells and memory B cells

- Proteasome inhibitors
(bortezomib, carfilzomib) → elimination of plasma cells
- Anti-CD38
(daratumumab) → plasma cells and a bit memory B cells
- Belatacept
(CTLA4-Ig) → reduces T-cell help to B-cells
- Anti-IL-6
(tocilizumab, clazakizumab) → reduces the formation of plasma- and B-cells



Discussion

- **So...** once you have memory B cell derived DSA it is hard to get rid of them →
- Is it important to determine the absence/presence before tx in order to perform risk stratification? 'Personalized immune profiling'
- **But then:** what do you do? Not accept donors towards whom memory B cell derived antibodies can be detected? Or only when one can explain them by a sensitizing event?



Questions?

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