Recognized and Unrecognized Sensitization

Assessment of pre-transplant immunologic memory

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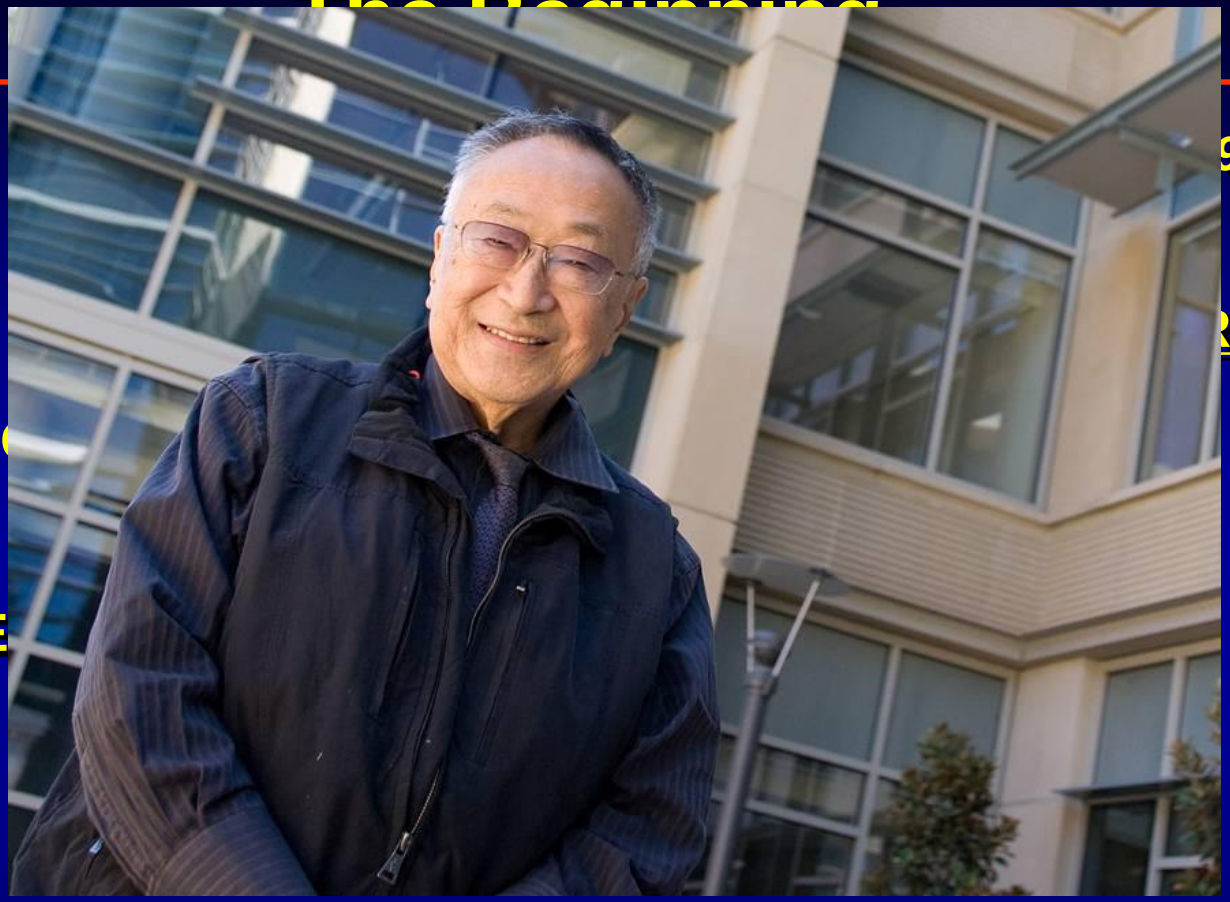
No financial relationships related to this presentation

AND

The presentation *does not* include discussion of "off-label" or "investigational" use.

The Beginning



9

REJECTION

POSITIVE 6

NEGATIVE 187

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

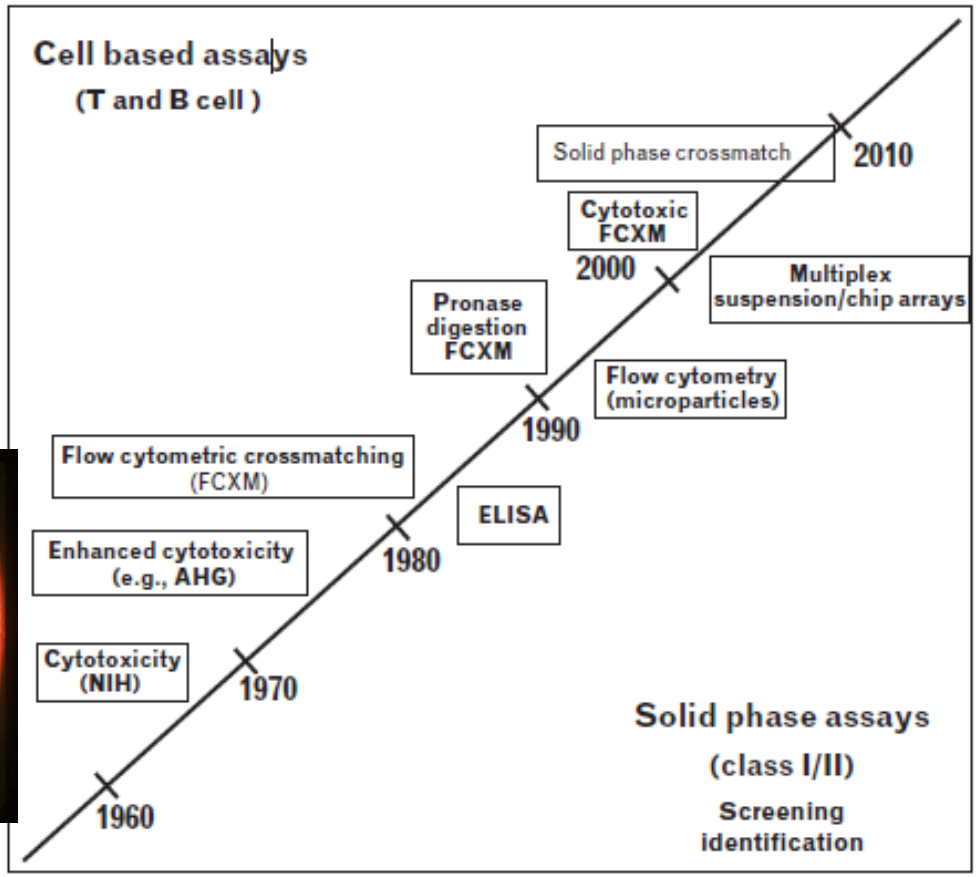
"..the ethics of transplanting kidneys without the prior knowledge of the results of the lymphocyte crossmatch test…
can reasonably be expected to be questioned."

The evolution and clinical impact of Human Leukocyte Antigen technology

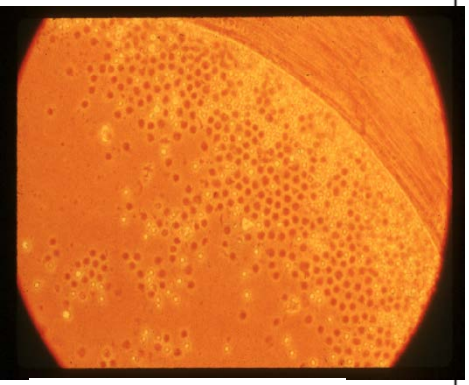
Howard M. Gebel and Robert A. Bray

Current Opinion in Nephrology and Hypertension 2010, 19:598–602

Figure 1 Evolution of human leukocyte antigen antibody testing

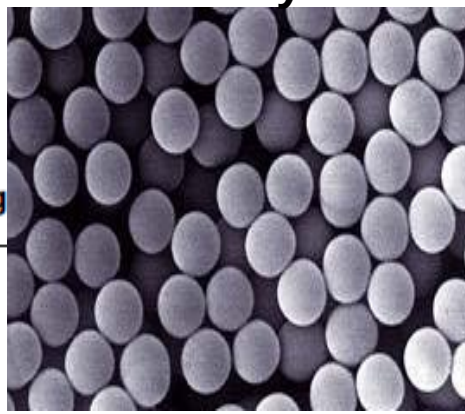


- Cumbersome cell-based assays for XM, anibody ID and HLA typing.



Cytotoxicity

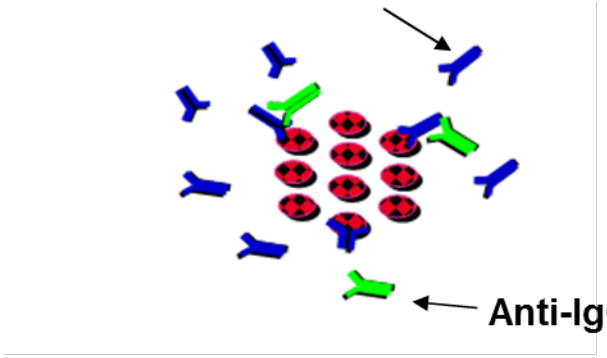
Solid Phase Assays

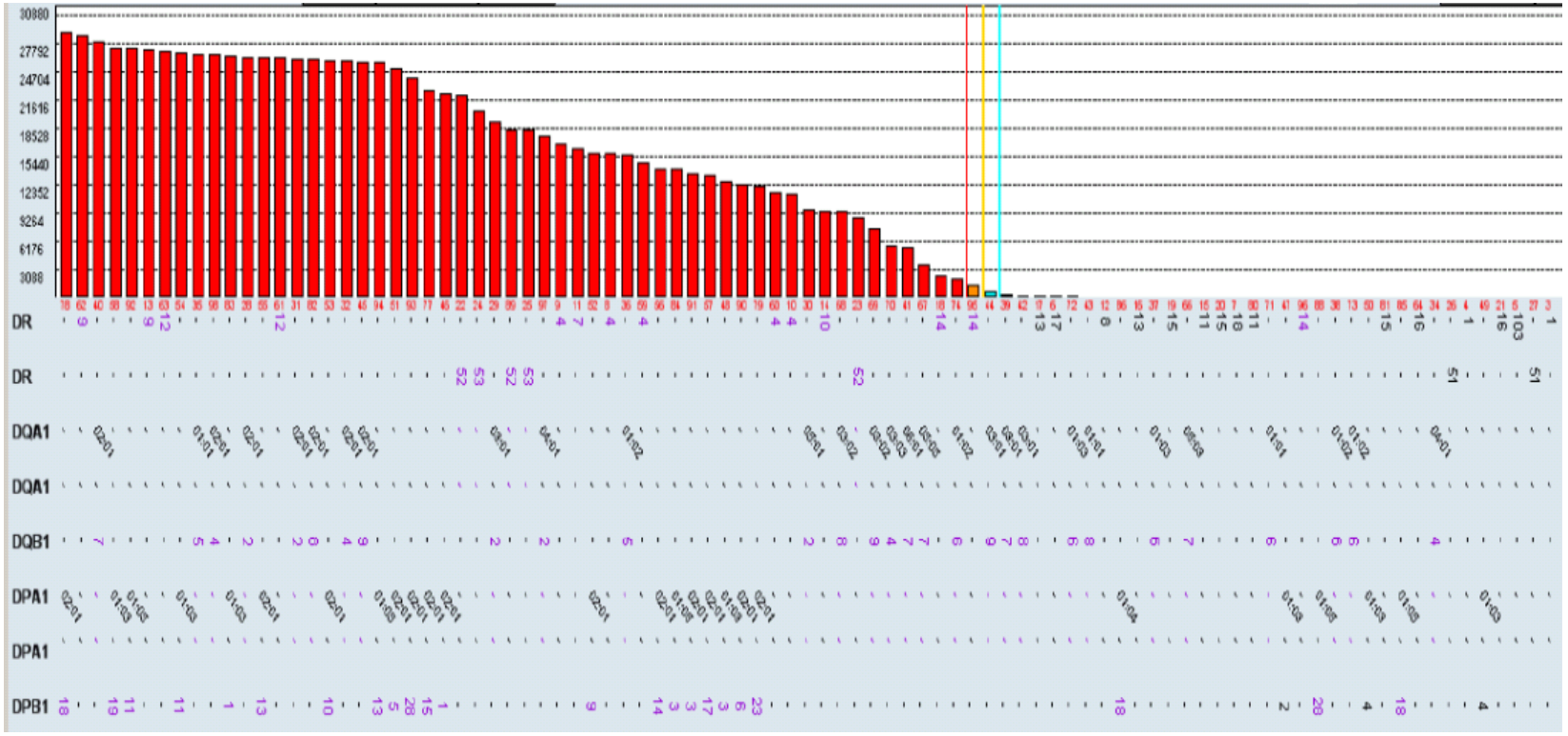


- Highly sensitive and specific bead-based assays for antibody ID and Molecular-based HLA typing.

Solid Phase HLA antibody detection

HLA alloantibody



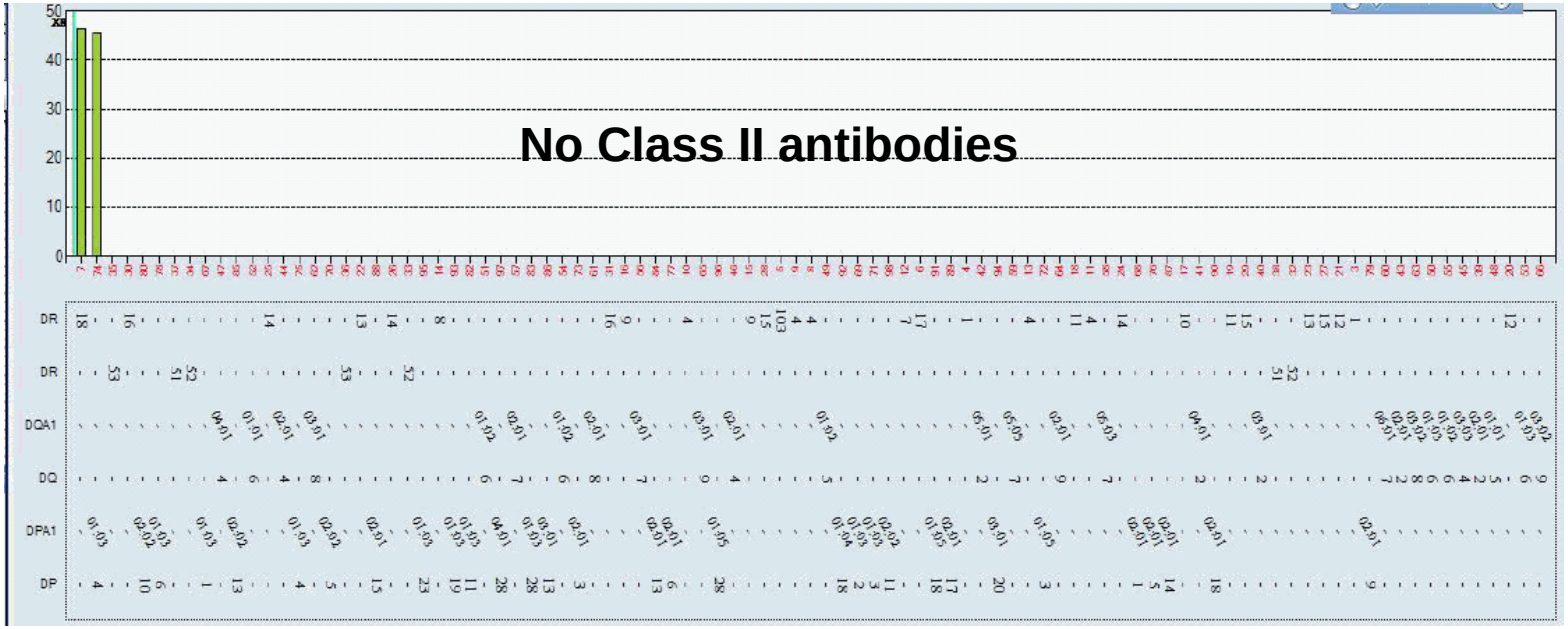


Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

Antibody profile of three potential transplant candidates

Class I

Class II



The Details

(The Devil is in them…)

1. **Non-transfused male-Unsensitized**
2. **Multiparous female, 3 children**

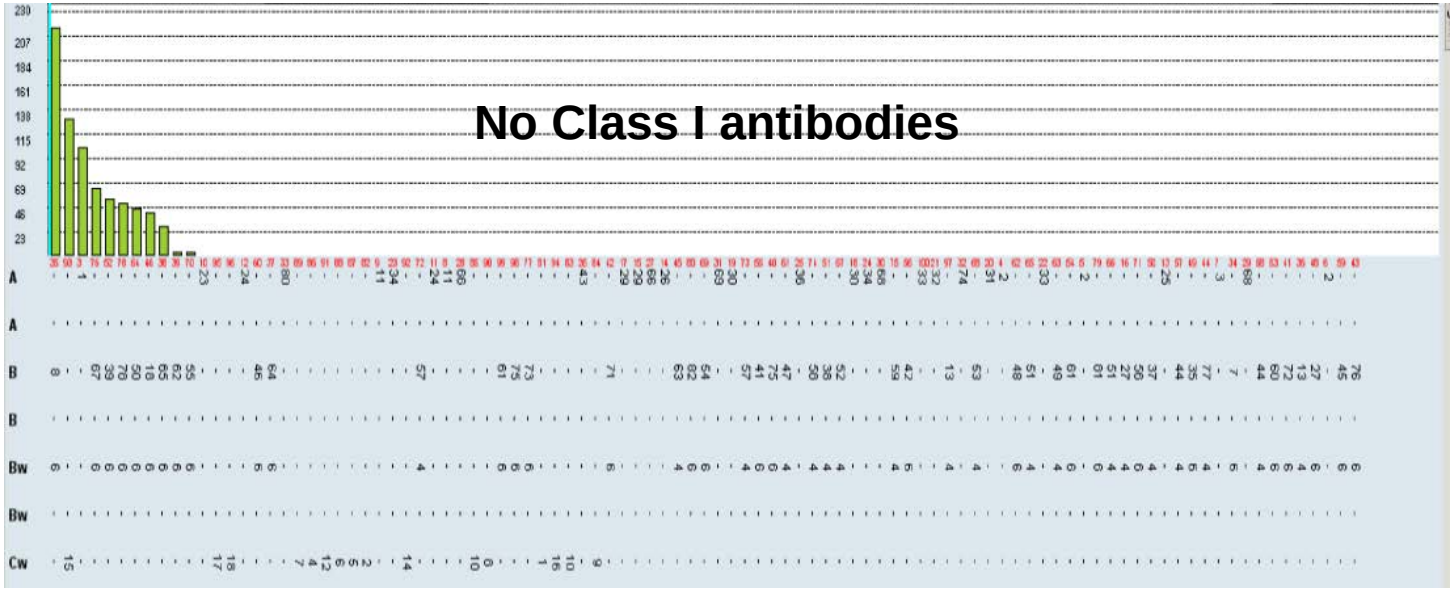
   <u>Exposed</u> to mismatched paternal ags

   but

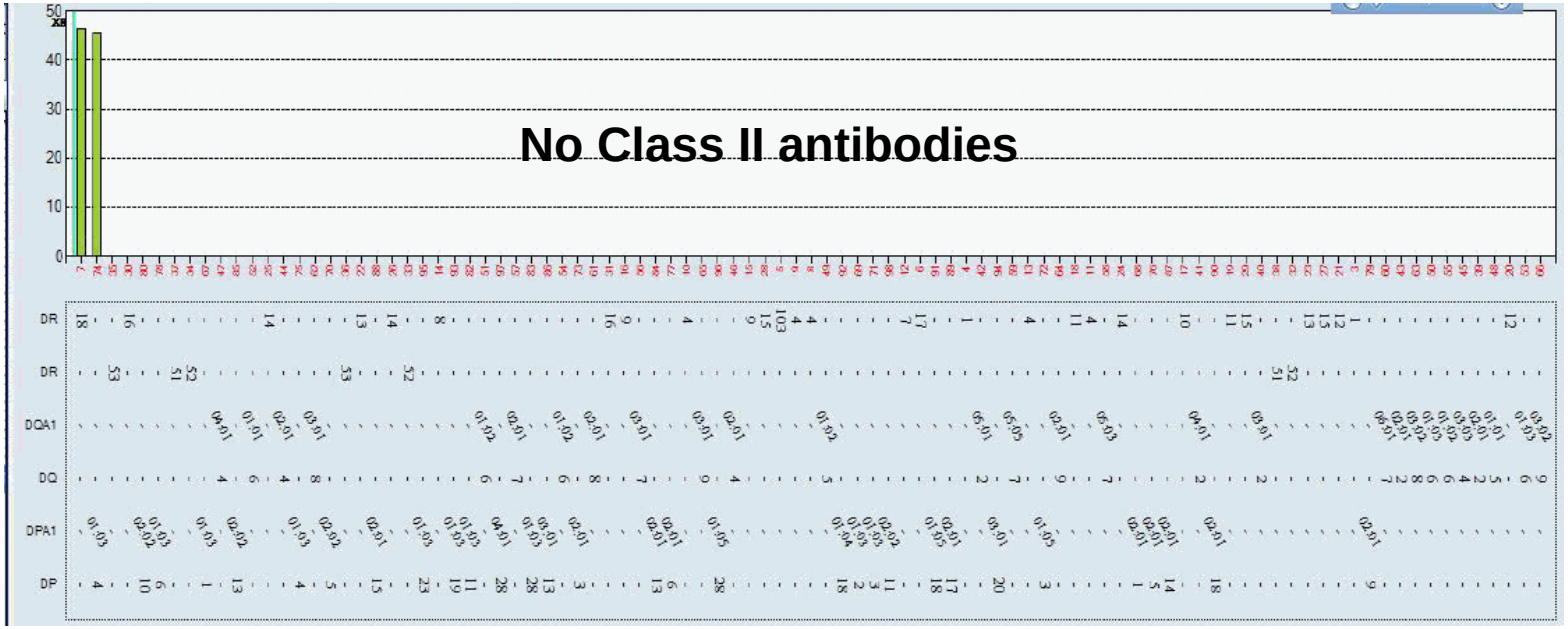
   Unsensitized or Sensitized?

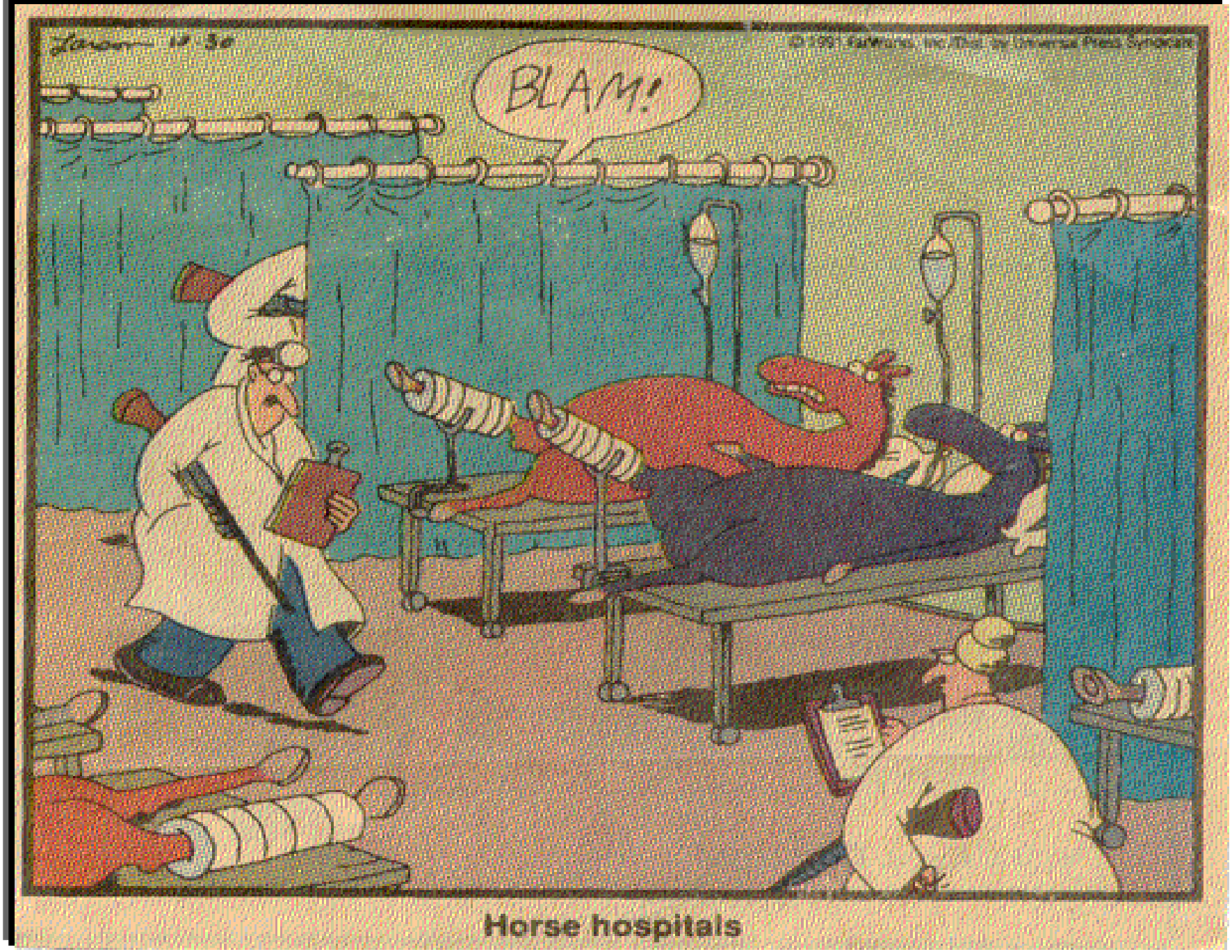
3 **Previous allograft recipient**

Class I



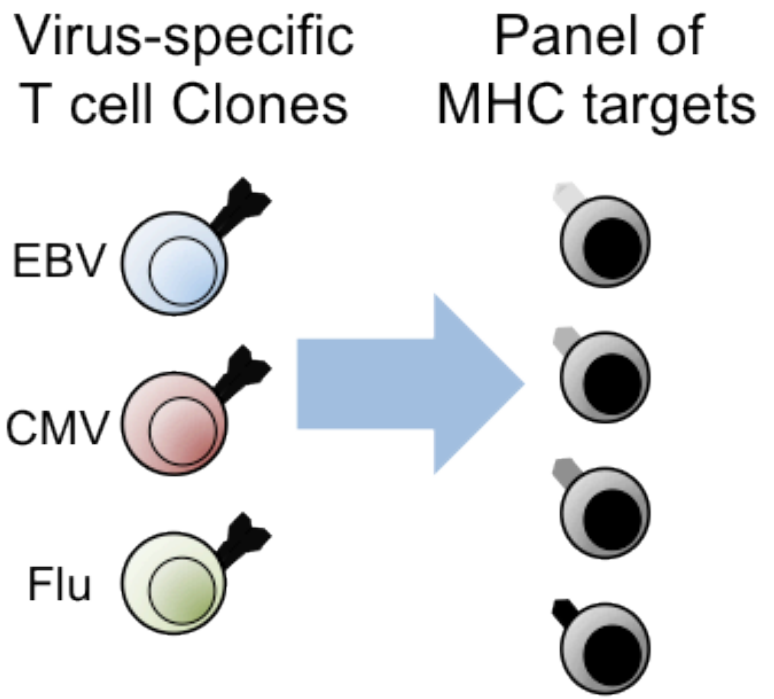
Class II

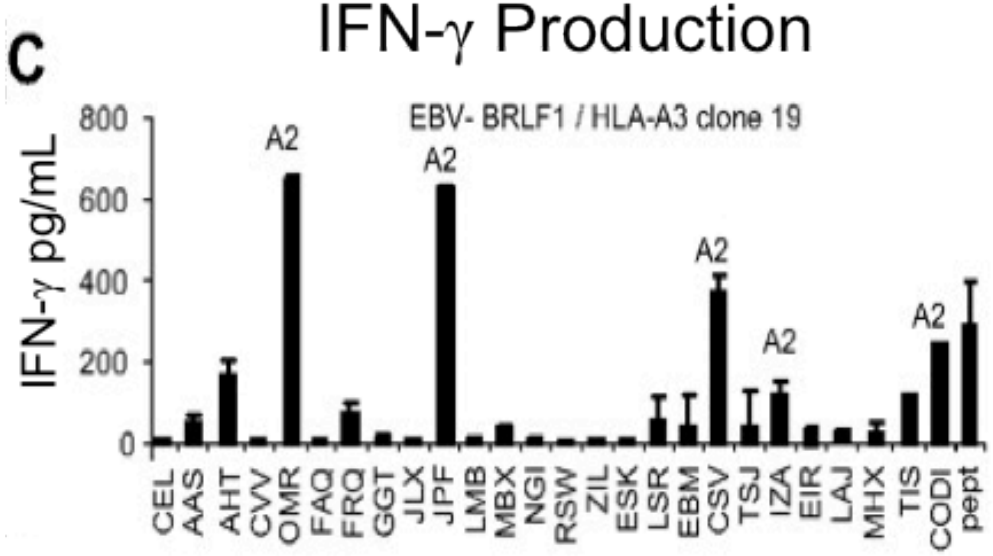




Horse hospitals

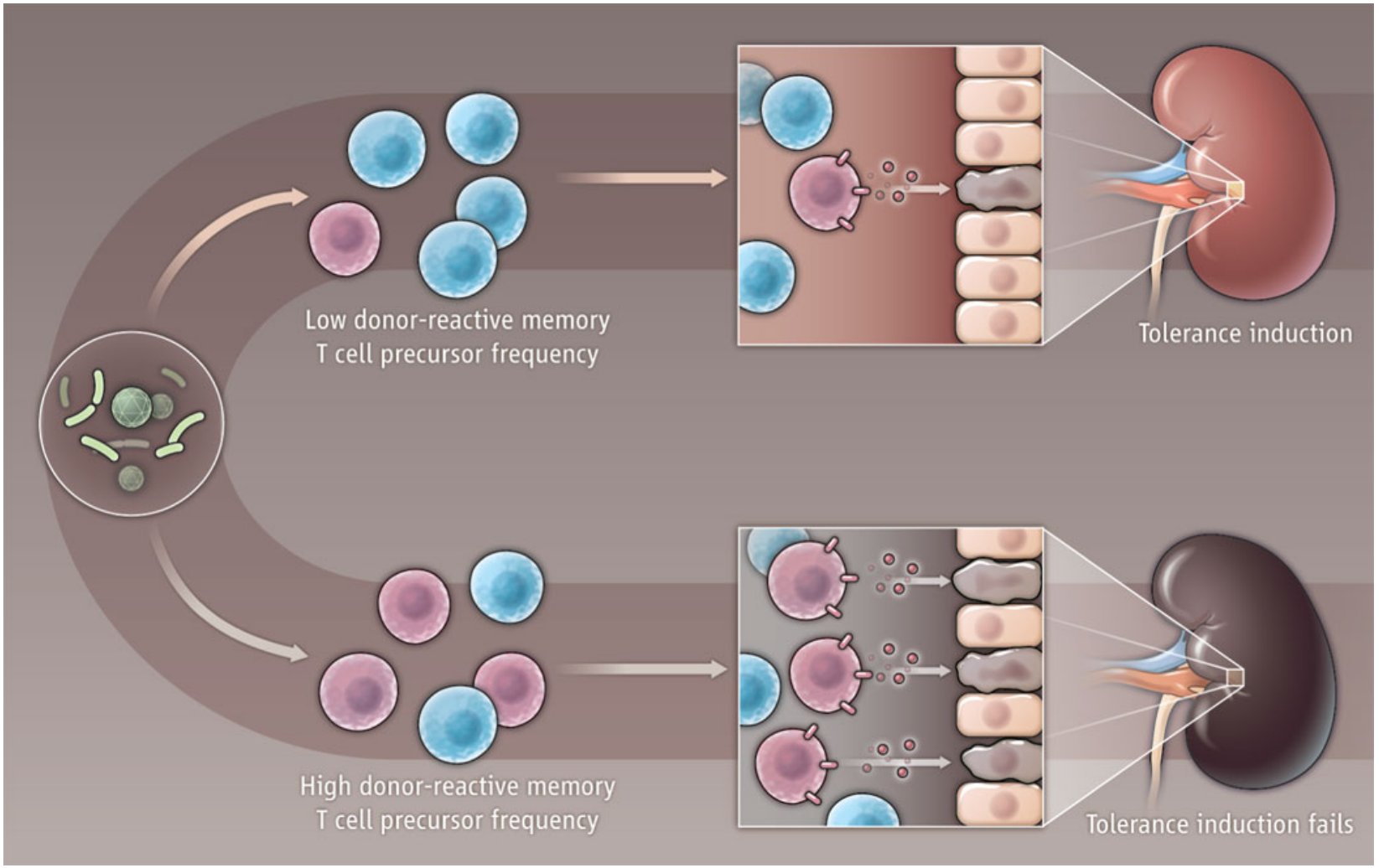
Alloreactive memory can arise not just from prior HLA sensitization, but also from pathogen exposure





Amir et al, *Blood*, 2010

Donor-Reactive Memory T Cells are a Barrier to Success in Transplantation

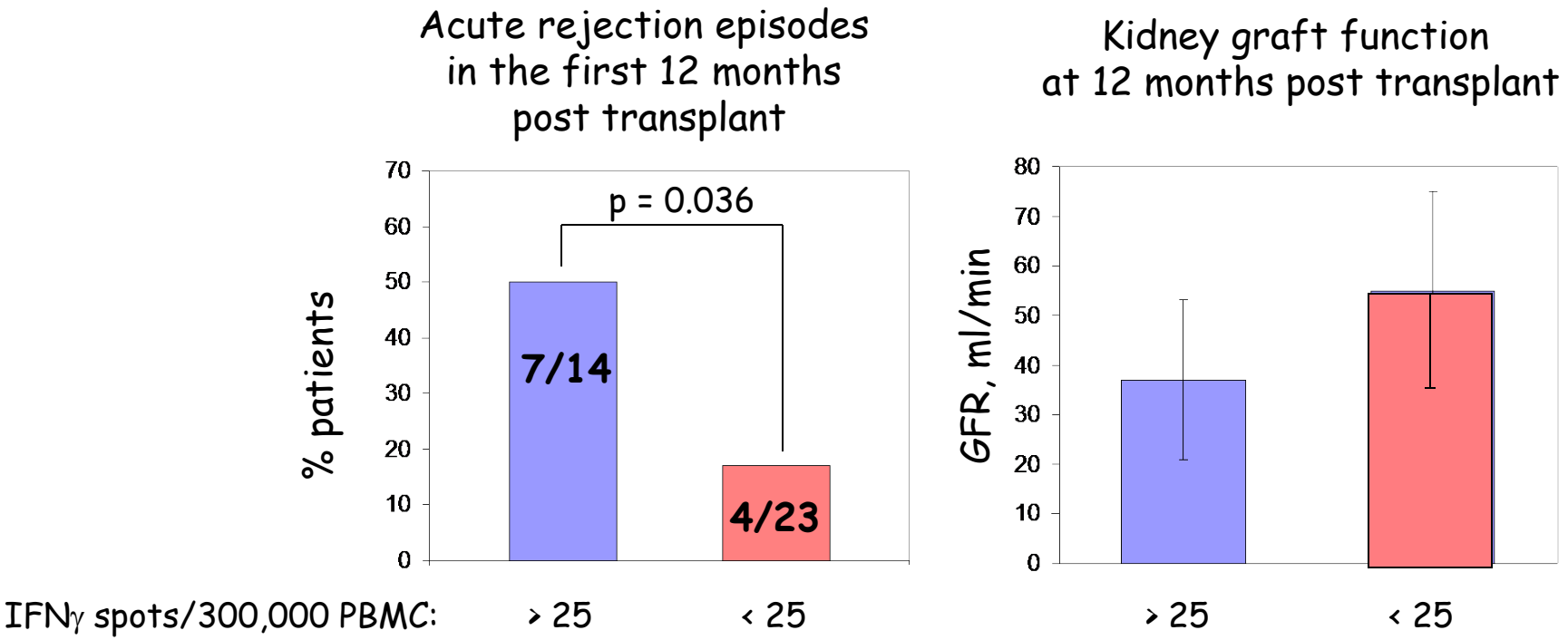


M. L. Ford and C. P. Larsen *Sci Transl Med* 2011;3:86ps22

Science Translational Medicine
AAAS

How do we identify and quantify alloreactive memory T cells?

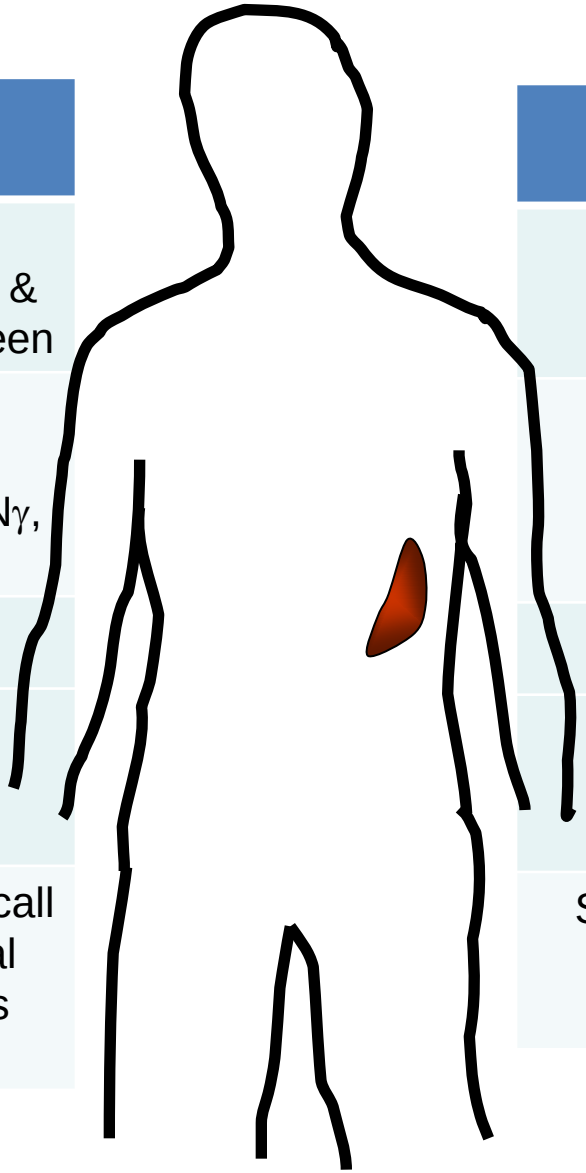
Clinical transplantation: presence of donor-specific memory T cells before transplantation correlates with the risk of post transplant acute rejection episodes and decreased graft function



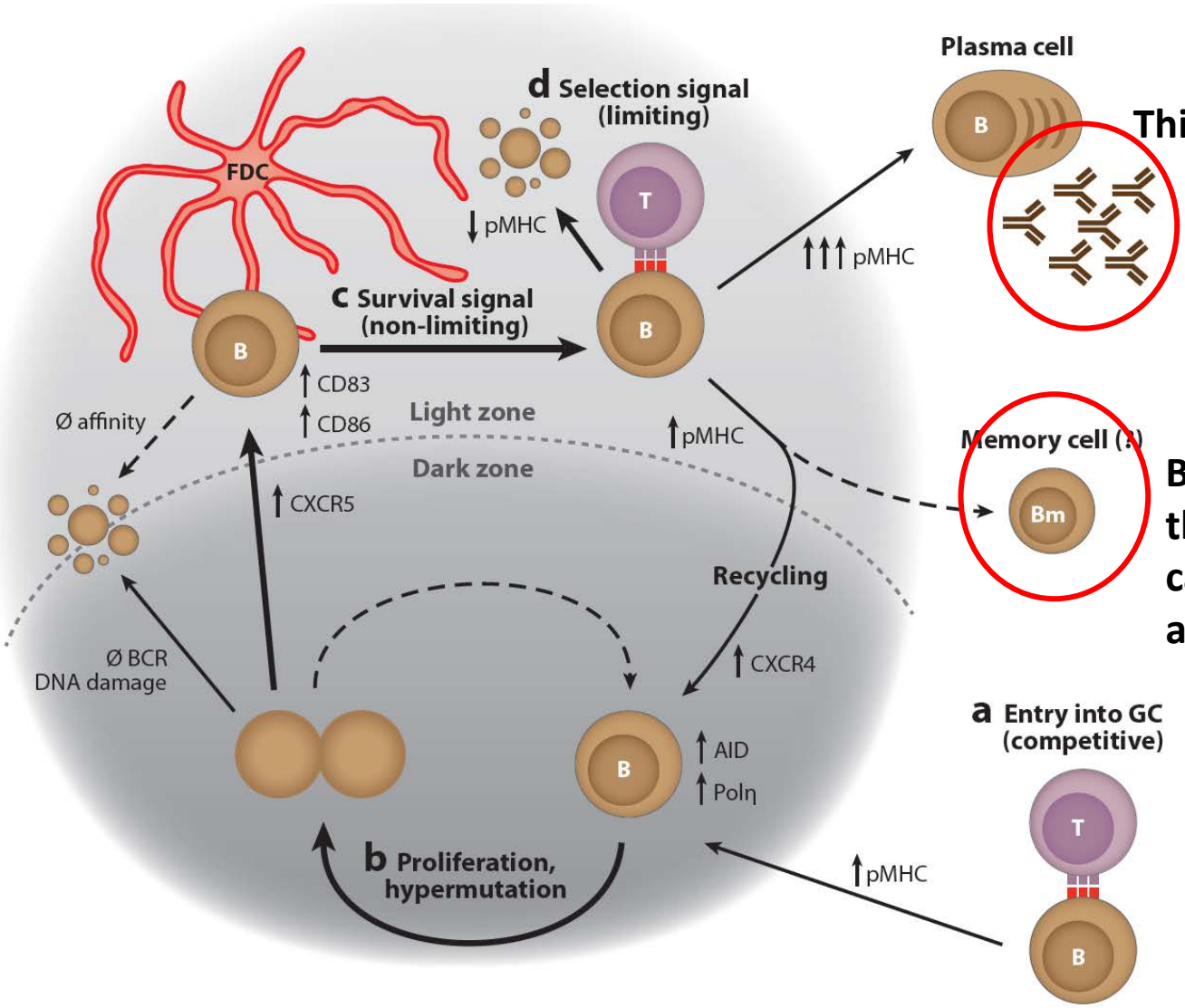
Augustine et al., AJT 2005

Specialized memory subsets:
regionalization of immune surveillance

Feature	Tissue Resident	Effector	Central
Distribution	Non-Lymphoid Tissues	Peripheral tissues (lung & liver) and spleen	Lymphoid tissues Lymph node and spleen
Cytokine	Immediate effector cytokines IFNγ, TNF	Immediate effector cytokines IFNγ, TNF	IL-2
Killing	immediate	immediate	inducible
Homing molecule	Sessile, non-circulating $CD69^+$ $CD103^+$	Peripheral homing CCR5 & 6	Lymphoid homing CCR7, CD62L
Putative function	Immuno-surveillance in non-lymphoid tissues	Immediate recall at peripheral barrier sites	Sustained memory response



B Cell Differentiation Pathways



Detecting the Humoral Alloimmune Response: We Need More Than Serum Antibody Screening

Gonca E. Karahan,[1] Frans H. J. Claas,[1] and Sebastiaan Heidt[1]

Abstract: Whereas many techniques exist to detect HLA antibodies in the sera of immunized individuals, assays to detect and quantify HLA-specific B cells are only just emerging. The need for such assays is becoming clear, as in some patients, HLA-specific memory B cells have been shown to be present in the absence of the accompanying serum HLA antibodies. Because HLA-specific B cells in the peripheral blood of immunized individuals are present at only a very low frequency, assays with high sensitivity are required. In this review, we discuss the currently available methods to detect and/or quantify HLA-specific B cells, as well as their promises and limitations. We also discuss scenarios in which quantification of HLA-specific B cells may be of additional value, besides classical serum HLA antibody detection.

(*Transplantation* 2015;99: 908–915)

HLA-Specific B Cells

I. A Method for Their Detection, Quantification, and Isolation Using HLA Tetramers

Andrea A. Zachary,[1,3] Dessislava Kopchaliiska,[1] Robert A. Montgomery,[2] and Mary S. Leffell[1]

(*Transplantation* 2007;83: 982–988)

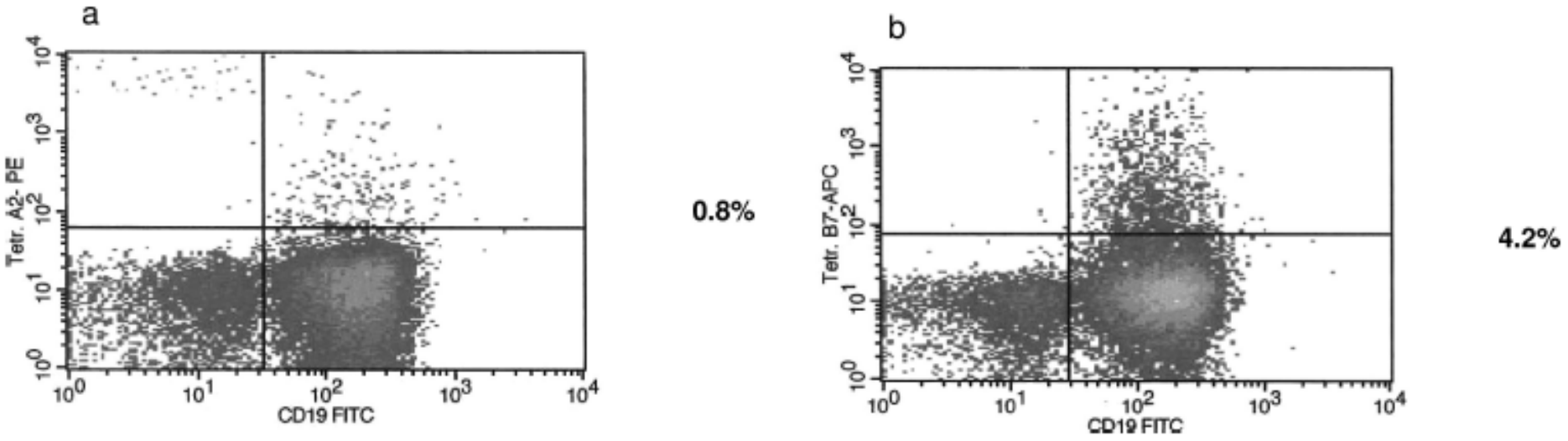


TABLE 1. Frequency of tetramer-positive B lymphocytes: subjects with antibody vs. those without

	Percent tetramer + cells among CD19+ cells				
Tetramer	**N[a]**	**Antibody positive[b]**	**N[a]**	**Antibody negative[c]**	***P***
A2	27	4.07 (1.34)[d]	19	1.55 (0.86)	1×10^{-9}
A24	17	4.43 (1.39)	16	2.19 (1.39)	4.4×10^{-6}
B7	23	5.46 (1.90)	22	3.25 (1.50)	5.0×10^{-5}

[a] Number of subjects.
[b] Subjects with current or historic antibody specific for the tetramer HLA antigen.
[c] Subjects with no history of antibody to the tetramer HLA antigen.
[d] Frequencies are given as the percent of CD19+ cells. Values in parentheses are standard deviations.

HLA-Specific B Cells

II. Application to Transplantation

Andrea A. Zachary,[1,3] Dessislava Kopchaliiska,[1] Robert A. Montgomery,[2] Joseph K. Melancon,[2] and Mary S. Leffell[1]

(*Transplantation* 2007;83: 989–994)

TABLE 2. Frequency of tetramer positive B lymphocytes among patients categorized by transplant history

	Antibody negative[a]				Antibody positive[b]			
		Previous transplant				Previous transplant		
Tetramer	N	No	Yes	*P*	N	No	Yes	*P*
A2	19	1.18[c] (0.66)	1.83 (0.92)	0.05	23	4.03 (1.46)	4.18 (1.45)	0.47
A24	16	1.98 (1.15)	2.47 (0.63)	0.15	17	4.34 (1.08)	4.47 (1.55)	0.44
B7	22	2.35 (1.09)	4.14 (1.38)	0.002	23	5.24 (1.83)	5.47 (1.93)	0.41

[a] Patients lacking antibody specific for the tetramer.
[b] Patients with antibody specific for the tetramer.
[c] Frequency is the percentage of tetramer positive cells among CD19+ cells. Numbers in parentheses are standard deviations.

American Journal of Transplantation 2012; 12: 1469–1479
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2011.03982.x

A Novel ELISPOT Assay to Quantify HLA-Specific B Cells in HLA-Immunized Individuals

S. Heidt[a,*], D. L. Roelen[a], Y. J. H. de Vaal[a], M. G. D. Kester[b], C. Eijsink[a], S. Thomas[c], N. M. van Besouw[d], H. D. Volk[c,e], W. Weimar[d], F. H. J. Claas[a] and A. Mulder[a]

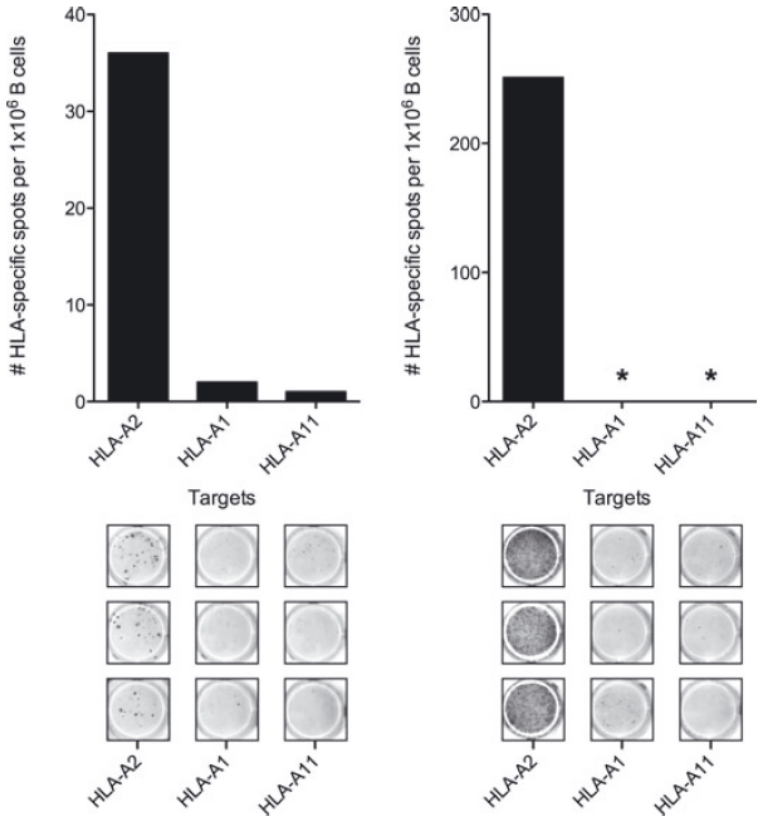


Figure 3: B cells from two healthy individuals (left panel HLA class I typed A3, B7, B15, Cw3, Cw7 and right panel A3, A26, B44, B56, Cw1, Cw5, respectively), who were immunized against HLA-A2, but not HLA-A1 or HLA-A11, formed spots against HLA-A2 and no spots against HLA-A1 or HLA-A11. *: no HLA-specific spots detected.

Table 1: Concurrent determination of HLA-A2-specific B cells (by spot formation) and their secreted HLA antibody (by Luminex assay)

Individual	Immunization status	Spot number[1]	MFI supernatant[2]	MFI neg. control
70a	A2 immunized	99	59.79	0.98
71a	A2 immunized	0	0.00	0.76
71b	A2 immunized	6	61.80	0.75
73a	A2 immunized	8	112.18	1.48
73b	A2 immunized	24	140.22	1.00
70b	Nonimmunized	0	0.00	1.14
71c	Nonimmunized	1	0.00	2.91
71d	Nonimmunized	0	0.00	1.10
73d	Nonimmunized	0	3.90	0.90

[1]Per million total B cells.
[2]MFI positive control serum: 3012.75.

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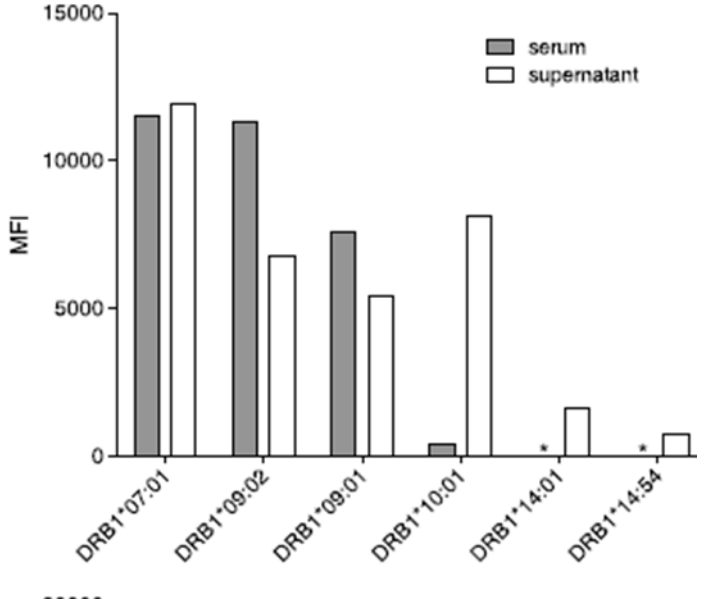
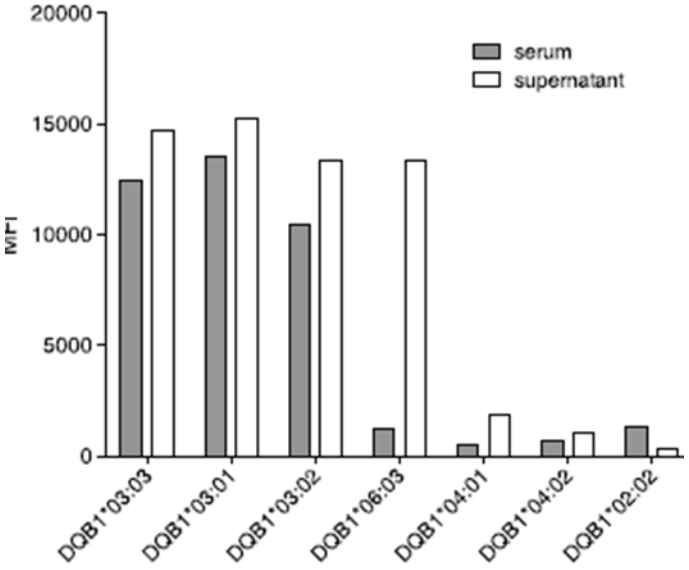
(*Transplantation* 2015;99: 908–915)





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Original Article

A memory B cell crossmatch assay for quantification of donor-specific memory B cells in the peripheral blood of HLA-immunized individuals

G.E. Karahan, Y.J.H. de Vaal, J. Krop, C. Wehmeier, D.L. Roelen, F.H.J. Claas, S. Heidt

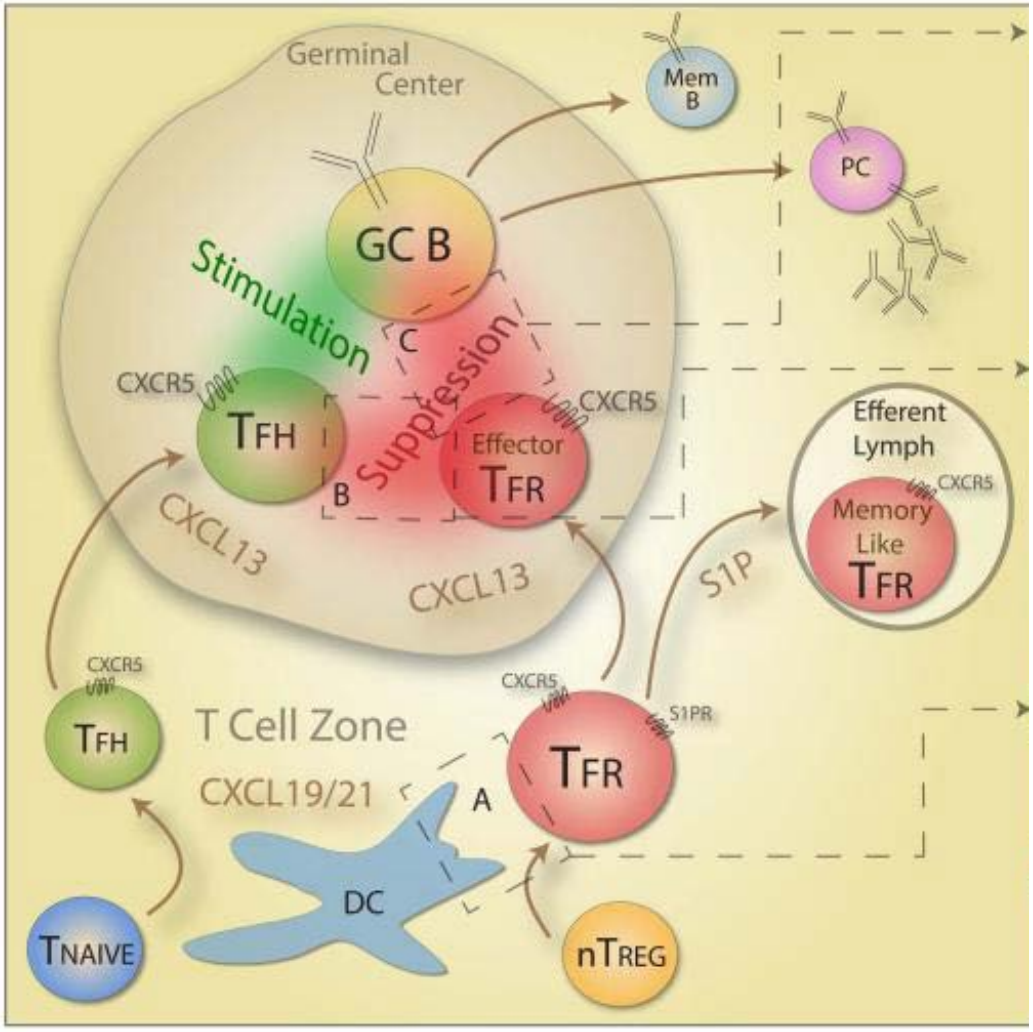
Accepted manuscript online: 30 March 2017 Full publication history

It’s not as easy as it looks!

1) Peripheral blood (30 mL)
2) Ficoll-Hypaque isolation
3) B cell enrichment/T cell depletion
4) 7 day cell culture/stimulation-L-CD40L cells as stimulators plus IL-2, IL-10, IL-21 TLR-9 Ligand
5) Collection, freezing and storage of culture supernatant for ab testing.

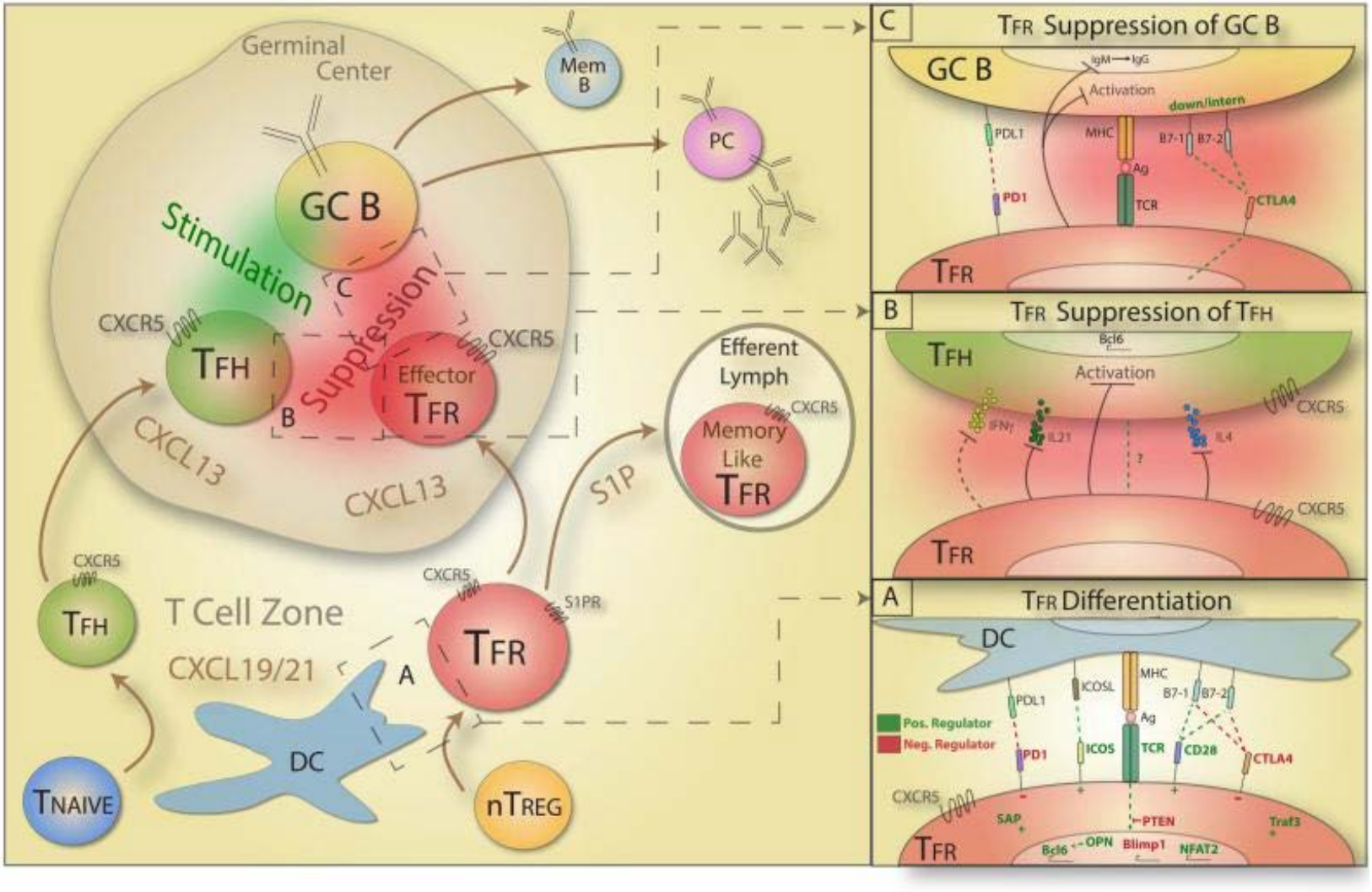
Labor intensive, extensive QC, proficiency testing, maintenance of cell cultures, etc

Antibody Production is Controlled by the Balance of T_{FH} and T_{FR}



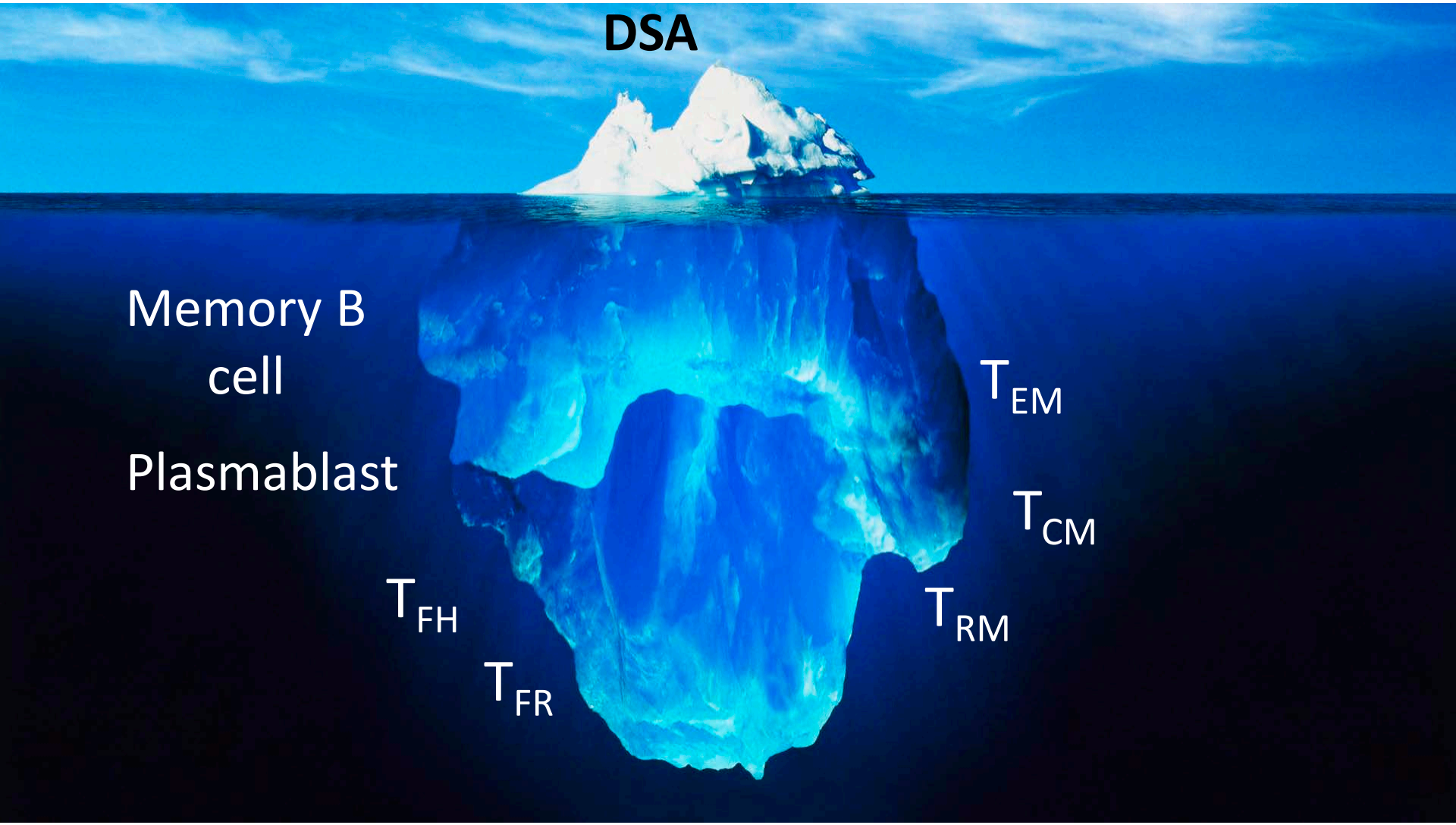
Sage and Sharpe.
Trends in Immunol. 2016.

Antibody Production is Controlled by the Balance of T_{FH} and T_{FR}



Summary

- One test for all issues related to antibodies
- Not quantifiable
- Not uniform
- The tip of the iceberg


DSA
Memory B cell
Plasmablast
T_{EM}
T_{CM}
T_{RM}
T_{FH}
T_{FR}

Conclusions

- Current tools are better than anything we've had before. But they remain rudimentary.
- Antibodies are surrogates for sensitization/memory. They tell only one part of a story.
- Risk assessment by antibody alone is at best incomplete, at most misleading.
- Need to transition to cellular assays for additional (better?) information
- Current testing for T and B cell memory still in early stages of development. Not yet quantifiable, labor intensive, clinical application still speculative.
- Moving forward-AUTOMATION. VETTING. CLINICAL UTILITY.
- Cannot reliably implement cell based assays without this consideration