AABB Annual Meeting Education Program 2014

October 25-28, 2014 | Pennsylvania Convention Center | Philadelphia, PA

Presentation Handouts

(9117-TC) Sally Frank Memorial Award and Lectureship: The Monocyte Monolayer Assay (MMA) Past and Present and Other Tests That Help Predict An Antibody's Potential Clinical Significance

October 25, 2014 ✧ 2:00 PM - 5:30 PM

Advancing Transfusion and Cellular Therapies Worldwide

Event Faculty List

Event Title: **(9117-TC)** Sally Frank Memorial Award and Lectureship: The Monocyte Monolayer Assay (MMA) Past and Present and Other Tests That Help Predict An Antibody's Potential Clinical Significance
Event Date: October 25, 2014
Event Time: 2:00 PM - 5:30 PM

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Disclosure: Did not disclose

Advancing Transfusion Medicine

More than 30 years' experience with the monocyte monolayer assay (MMA)

Donald R. Branch, MT(ASCP)SBB, PhD
Centre for Innovation
Canadian Blood Services

2014 AABB Sally Frank Memorial Award and Lectureship
Philadelphia, Pennsylvania

Canadian Blood Services
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I dedicate this talk to one of the *GIANTS* of transfusion medicine

George Garratty
1935-2014

Sir John Dacie

Patrick Mollison

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Early Beginnings

- 1978/79 – Dr. Lawrence D. Petz recruits me to City of Hope National Medical Center
  - MT(ASCP)
  - CA licensed MT

- Dr. Petz asks me to develop an *in vitro* assay using monocytes to test alloantibody significance

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Early Beginnings

- **1980– Dr. Moses Schanfield publishes first paper on MMA (calls it the mononuclear phagocyte assay; MPA; uses human peritoneal macrophages)**

Prog Clin Biol Res. 1980;43:305-23

New approaches to detecting clinically significant antibodies in the laboratory.

Schanfield MS, Schoeppner SL, Stevens JO

- **1981 – Schanfield again; in *Transfusion***

Transfusion
September-October 1981

The Detection of Clinically Significant Erythrocyte Alloantibodies Using a Human Mononuclear Phagocyte Assay

M. S. SCHANFIELD, J. O. STEVENS, AND D. BAUMAN

From the Veterans Administration Center, Wood, Wisconsin, and the Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

Early Beginnings (Con't)

- **1980 – Michael T. Gallagher, PhD recruited by Dr. Petz to COH**

- **Work begins on the monocyte-macrophage assay (MMA)**

Early Beginnings (Con't)

- **1980: Assay developed and validated: PBMCs onto glass cover slips, monocytes adhere, overlay R1R2 RBCs sensitized with different amounts of anti-D to work out conditions and test reproducibility of assay**
- **Conditions: 37C, CO2 incubator (tissue culture conditions to maintain physiologic pH) and extra buffer (HEPES) to maintain pH at RT.**
- **Readout: Hematological stain and look for RBC phagocytosis alone (PRBC) or adhered RBC (ARBC)**
- **Validate assay: DAT positive and negative patients with and without documented hemolytic anemia**

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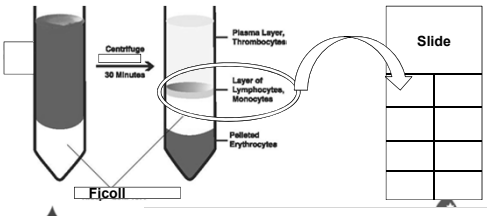
1) Isolate Peripheral Blood Mononuclear cells (PBMCs), wash and let adhere to slide in incubator for 1 hour



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Method Design

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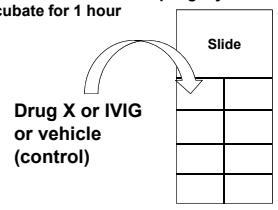
2) Can test potential inhibitors of phagocytosis as an intermediate step and incubate for 1 hour



Drug X or IVIG or vehicle (control)

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Method Design

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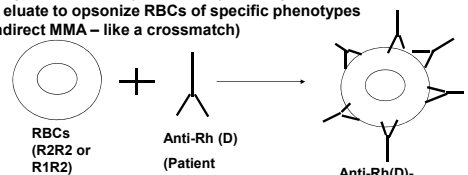
3) Opsonize RBCs to serve as positive phagocytosis control (prepare ahead of time or day of assay) and can use patients' RBCs (direct MMA) and/or patient serum or eluate to opsonize RBCs of specific phenotypes (indirect MMA – like a crossmatch)



RBCs (R2R2 or R1R2)

Anti-Rh (D)

(Patient serum or eluate)

Anti-Rh(D)-coated RBCs

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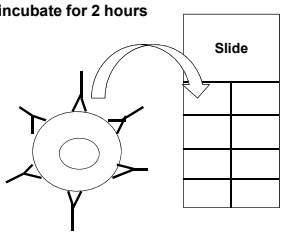
4) Aspirate inhibitors if used from the PBMCs, add the opsonized RBCs, and incubate for 2 hours



Fix slides in methanol; Readout

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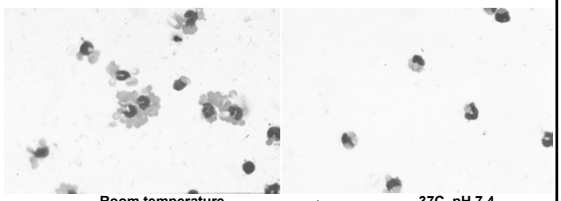
Method Design

Readout for MMA

Using Hematological Stain and Light Microscopy

Adherent RBCs (ARBC) Rosetting

Phagocytosed RBCs (PRBC) Eaten



Room temperature → **37C, pH 7.4**

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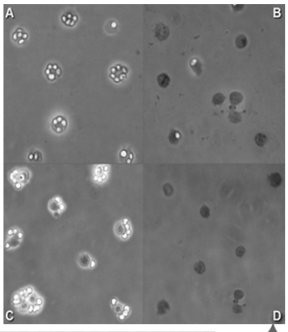
Using Phase Contrast Microscopy

Phagocytosed RBCs (PRBC) (Eaten)

37C, pH 7.4

Adherent RBCs (ARBC) (Rosetting)

Room Temperature



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Phagocytosis and/or Adherence?

- **Adhered + Phagocytosed (total associated RBCs)**
  - **<5% - Non-significant**
  - **≥5% ≤ 20% - Borderline**
  - **>20% - clinically significant**
- **Phagocytosed only**
  - **> 2 phagocytic index (≥3 RBC phagocytosed per 100 monoctyes)**

Early Beginnings (Con't)

- **Gallagher MT, Branch DR, Mison A, Petz LD. A short term in vitro assay of monocyte-macrophage (MØ) interaction with erythrocytes (RBC's). Fed. Proc. 40:1046, 1981 (Poster Presentation)**
- **Branch DR, Gallagher MT, Mison A, Petz LD. Evaluation of reticuloendothelial function and erythrocyte antibody significance using an in vitro monocyte macrophage assay. Blood 58 (Suppl 1): 88a, 1981 (Oral Presentation)**

1982: Our first publication using our MMA - to study phagocytosis of younger vs. older RBCs

MEDICAL INTELLIGENCE

AUTOIMMUNE HEMOLYTIC ANEMIA WITH RETICULOCYTOPENIA AND ERYTHROID MARROW

C. Lockard Conley, M.D.,
Scott M. Lippman, M.D.,
Paul M. Ness, M.D.,
Lawrence D. Petz, M.D.,
Donald R. Branch, B.S.,
and Michael T. Gallagher, Ph.D.

N Engl J Med. 1982 Feb 4;306(5):281-6

Interaction of Monocyte-Macrophages with Reticulocyte-Rich and Reticulocyte-Poor Preparations of Normal Red Cells Sensitized with Antibody.

Experiment *	Sensitized Erythrocytes †	Reticulocytes (Percentage)	Monocyte-Macrophage Interaction: Total-association index ‡	Monocyte-Macrophage Interaction: Phagocytic index §
1A	Reticulocyte-rich	27	12	2
	Reticulocyte-poor	0.4	19	7
2A	Reticulocyte-rich	27	12	6
	Reticulocyte-poor	0.4	19	10
3B	Reticulocyte-rich	22.5	6	2
	Reticulocyte-poor	0.2	30	11
4B	Reticulocyte-rich	22.5	2	0
	Reticulocyte-poor	0.2	17	7
5C	Reticulocyte-rich	19	10	9
	Reticulocyte-poor	0	24	17
6C	Reticulocyte-rich	19	8	7
	Reticulocyte-poor	0	24	12
Mean ±S.D.	Reticulocyte-rich		8.3±3.9	4.33±3.7
	Reticulocyte-poor		22.2±4.8 ¶	10.67±3.72 ‖

Early Beginnings (Con't)

- **1983 – Our full publication validating the MMA**

Exp. Hematol., January 1983, Vol. 11, No. 1, pp. 82-89
0301-472X/83/101-1183$02.00/0 Printed in USA
© 1983 International Society for Experimental Hematology

Evaluation of Reticuloendothelial Function in Autoimmune Hemolytic Anemia Using an In Vitro Assay of Monocyte-Macrophage Interaction with Erythrocytes

MICHAEL T. GALLAGHER, DONALD R. BRANCH, ANGELES MISON & LAWRENCE D. PETZ

Department of Clinical and Experimental Immunology, Division of Medicine, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA

Received 14 June, accepted 5 October 1982

Correlation of MMA with DAT and AIHA

- **MMA distinguished between hemolysing and non-hemolysing patients independent of the degree of red cell sensitization as determined by the DAT when using phagocytosis as readout**
- **16/16 patients with a positive DAT (0.5+ to 4+) and clinical evidence of hemolysis showed elevated phagocytic index (PRBC) (>2)**
- **6/6 patients with a positive DAT (0.5+ to 4+) without clinical evidence of hemolysis did not show an elevated PRBC**
- **2/6 patients with a positive DAT following alpha-methyldopa therapy were hemolysing, 4 were not; the MMA correlated 100% with *in vivo* findings**
- **7/11 patients with DAT-negative acquired hemolytic anemia showed elevated PRBC index, indicating a possible immune etiology involving extravascular lysis**
- **3/7 of these MMA positive DAT-negative patients only showed a significantly elevated PRBC when autologous monocytes were used**

Conclusions

- **MMA correlates 100% with *in vivo* hemolysis if using phagocytosis (phagocytic index (or PRBC) >2)**
- **It may be useful to use autologous monocytes**

MMA correlates with hemolysis independent from DAT

Vox Sang. 45: 278–287 (1983)

Reticuloendothelial Cell Function in α-Methyldopa-Induced Hemolytic Anemia

Donald R. Branch[a], Michael T. Gallagher[a], Ira A. Shulman[b], Angeles P. Mison[a], Anita L. Sy Siok Hian[a], Lawrence D. Petz[a]

[a] Department of Clinical and Experimental Immunology, Division of Medicine, City of Hope National Medical Center, Duarte, Calif., [b] Blood Bank and Transfusion Service, Los Angeles County-University of Southern California Medical Center, Los Angeles, Calif., USA

MMA can distinguish between hemolyzing and non-hemolyzing antibody

Measured indices in 2 hemolyzing patients having similar levels of red-cell-bound IgG during hemolysis and following discontinuance of α-methyldopa

Patient	Weeks after stopping drug	Remission	Hb g/dl	Reti-culocytes %	DAT titration		
					DAT	score	MØ PRBC
1	3	no	13.7	5.5	3+	46	43
1	38	yes	15.7	2.0	3+	46	1
2	2	no	9.7	15.3	4+	55	15
2	29	yes	14.0	2.0	4+	50	2

MØ PRBC = Monocyte-macrophage PRBC using patient MØ and autologous red cells. Normal ≤2.

MMA useful to study clinical significance of RBC alloantibodies

British Journal of Haematology, 1984, 56, 19–29

In vitro determination of red cell alloantibody significance using an assay of monocyte–macrophage interaction with sensitized erythrocytes

DONALD R. BRANCH, MICHAEL T. GALLAGHER, ANGELES P. MISON, ANITA L. SY SIOK HIAN AND LAWRENCE D. PETZ *Department of Clinical and Experimental Immunology, Division of Medicine, City of Hope National Medical Center, Duarte, California*

Received 15 March 1983; accepted for publication 24 May 1983

Four Critical Points from Study

- **Not all alloantibodies cause significant phagocytosis.**

53% of the alloantibodies studied mediated significant phagocytosis *in vitro.* Significant phagocytosis was independent of the strength of the indirect antiglobulin test

Not all "clinically significant" alloantibodies cause phagoctyosis

In vitro interaction of alloantibody sensitized red blood cells with allogeneic monocyte-macrophage monolayers

Antibody specificity	No. of alloantibodies evaluated	Range of antiglobulin test strength	No. showing significantly elevated phagocytic indices (PRBC)
Anti-Jk^a	14	Micro‡–4+	11 (32%)
Anti-Jk^b	3	Micro–2+	2 (67%)
Anti-Fy^a	26	Micro–4+	16 (62%)
Anti-Kell	22	Micro–4+	16 (73%)
Anti-Rh$_0$(D)	16	‡+–4+	12 (75%)
Anti-E	5	‡+–4+	3 (60%)
Other Rh [C, c, e]	3	‡+–4+	2 (67%)*
Anti-Vel	8	Micro–4+	2 (25%)
Anti-Yt^a (Cartwright)	8	Micro–4+	2 (25%)
Anti-Ge (Gerbich)	9	Micro–2‡+	2 (22%)
Others [M, S, s, Fy^b, Kp^b Lu^a, Lu^b, Di^b, Co^a]	14	Micro–4+	10 (71%)†
Totals	148	Micro–4+	78 (53%)

* Significantly elevated PRBC with 1/1 C, 1/1 c, 0/1 e.
† Significantly elevated PRBC with 1/2 M, 2/2 S, 1/1 s, 1/2 Fy^b, 1/1 Kp^b, 0/1 Lu^a, 1/2 Lu^b, 2/2 Di^b, 1/1 Co^a.
‡ Microscopically positive reactions.

Four Critical Points from Study

- **Use of autologous monocytes may be necessary to obtain correct significance**

Autologous monocytes were more reliable than random allogeneic monocytes in that phagocytosis was increased over that obtained using allogeneic monocyte-macrophages with two of four alloantibodies having documented clinical significance

Four Critical Points from Study

- **Addition of fresh complement and homozygous target cells is important for certain alloantibodies**

The percentage of anti-Jka and anti-Fya mediating significant phagocytosis was increased when fresh complement was added during the sensitization procedure and/or red cells homozygous for the antigen in question were used

Four Critical Points from Study

- **Where clinical data was available, the MMA would have predicted hemolysis or no hemolysis prior to transfusion**

The *in vivo* clinical significance or lack of significance was documented for nine alloantibodies; five caused hemolysis and four did not. Those causing *in vivo* hemolysis mediated *in vitro* phagocytosis by monocyte-macrophages whereas the antibodies that did not result in hemolysis showed no increased *in vitro* phagocytosis.

MMA correlates 100% with *in vivo* clinical data

Results using an *in vitro* monocyte-macrophage assay with alloantibodies having documentation of their *in vivo* clinical significance or insignificance

Antibody specificity	IAT*	Documented clinical significance or insignificance	Phagocytic index† (PRBC)
Anti-M	3+	Delayed haemolytic transfusion reaction; ^{51}Cr-survival 1·4% at 24 h (Alperin *et al.* 1981)	41.§.*
Anti-Ge	2+	5 units of Ge-positive blood transfused; no untoward response	0
Anti-Ge	2+	Newborn with 1+ DAT, no clinical haemolytic disease	1
Anti-Ge	4+	Haemolytic transfusion reaction; ^{51}Cr-survival <4% at 24 h	68
Anti-Vel	Micro**	3 units Vel-positive blood transfused; no untoward response	0
Anti-Vel	4+	Nine pregnancies, five miscarriages, three live-born infants requiring exchange transfusion	49
Anti-Jk^a	2+	Delayed haemolytic transfusion reaction; ↓ HCT; only Jk(a−b+) blood transfusions tolerated	46
Anti-Jk^b	1+	Jk^b-positive blood transfused: no untoward response	0
Anti-Co^a	2+	Developed during pregnancy; amniocentesis showed ΔOD_{450nm} = 0·061, Zone 2; caused mild HDN	202.§

* Indirect antiglobulin test using anti-IgG.
† Normal PRBC ≤2.
‡ PRBC values were higher when using autologous monocyte–macrophages.
§ PRBC values were highest when using sensitized homozygous antigen positive test red blood cells.
¶ Using autologous monocyte–macrophages; results with allogeneic monocyte–macrophages were less conclusive (Alperin *et al.* 1983).
** Microscopically positive reaction.

MMA useful for drug-induced clinical significance

American Journal of Hematology 18:213–219 (1985)

Extravascular Hemolysis Following the Administration of Cefamandole

Donald R. Branch, Lee R. Berkowitz, Robert L. Becker, Judy Robinson, Marsha Martin, Michael T. Gallagher, and Lawrence D. Petz

Department of Clinical and Experimental Immunology, City of Hope National Medical Center, Duarte, California (D.R.B., M.T.G., L.D.P.), North Carolina Memorial Hospital, Chapel Hill (L.R.B., R.L.B., M.M.), and The American Red Cross Blood Services, Carolina Region, Charlotte, North Carolina (J.R.)

Interaction of Monocyte-Macrophages with Cefamandole-Coated Red Cells Sensitized With Anticefamandole

	Monocyte-macrophage interaction					
	Using untreated red cells sensitized with patient's serum		Using cefamandole-coated red cells sensitized with normal serum		Using cefamandole-coated red cells sensitized with patient's serum	
Monocyte-macrophage source[c]	AGT[a]	PRBC[b]	AGT	PRBC	AGT	PRBC
Normal 1	0	0	0	0	3+	17
Normal 2	0	0	0	0	3+	31

[a]Antiglobulin test using a standard technique employing anti-IgG.
[b]Phagocytic index, ie, the average number of phagocytosed RBC per 100 monocyte-macrophages. Normal PRBC $\leqslant$ 2 [10].
[c]Monocyte-macrophage function was determined to be normal by testing against known IgG-positive control red blood cells [10].

Series of Important Abstracts

- **BRANCH DR**, Gallagher MT, Mison AP. In vitro blockade of monocyte-macrophage (MØ) phagocytosis using I.V. gamma globulin (γG) preparations. Fed. Proc. 44:1703, 1985
- Gallagher MT, Beatty BG, Beatty JD, Mison AP, **BRANCH DR.** The effect of pretreatment with liposomes on in vitro phagocytosis of sensitized red blood cells by human macrophages. Fed. Proc. 44:1703, 1985
- Shulman IA, Thompson JC, Nelson JM, Vengelen-Tyler V, **BRANCH DR.** Autoanti-Gerbich causing severe autoimmune hemolytic anemia. Transfusion 25:447, 1985
- **BRANCH DR**, Branch CA, Shulman IA, Nitsun M, Mison AP, Gallagher MT. Participation of cellular thiol/disulfide groups in Fc-receptor (FcR) mediated attachment and phagocytosis of IgG-coated target red cells. Fed. Proc. 45:1110, 1986.

Branch DR, Gallahger MT.
Br J Haematol. 1986 Apr;62(4):783-5.

Summary of all results of MMA where good clinical data was available
-complete correlation with clinical findings-

Results using an *in vitro* monocyte-macrophage phagocytosis assay with alloantibodies having documentation of their *in vivo* clinical significance or insignificance

Antibody specificity	IAT*	Documented clinical significance or insignificance	Phagocytic index†
Anti-M	3+	Significant (Alperin *et al*, 1983)	4
Anti-M	2½+	Significant, caused HDN (Cohen, 1985)	61
Anti-Ge	2+	Insignificant (Branch *et al*, 1984)	0
Anti-Ge	2+	Insignificant (Branch *et al*, 1984)	1
Anti-Ge	4+	Significant (Branch *et al*, 1984)	68
Anti-Ge‡	2½+	Significant (Shulman *et al*, 1985)	73
Anti-Vel	Micro§	Insignificant (Branch *et al*, 1984)	0
Anti-Vel	4+	Significant (Branch *et al*, 1984)	49
Anti-Lan	2½+	Significant (Judd *et al*, 1984)	5
Anti-Co^a	2+	Significant (Branch *et al*, 1984)	20
Anti-D	2½+	Insignificant: anti-D present in i.v. gammaglobulin: 1 litre infused; patient became DAT+ (2+); no clinical evidence of haemolysis (Judd, 1983)	0
Anti-Jk^a	2+	Significant (Branch *et al*, 1984)	4
Anti-Jk^a	3½+	Significant: caused DHTR; falling Hct 40·7–24·5%); increased bilirubin. LDH; Hct stable after infusion of Jk^a neg RBC	26
Anti-Jk^b	1+	Insignificant (Branch *et al*, 1984)	0
Anti-P	3+	Significant (Shirey *et al*, 1984)	14
Anti-Yt^a	2½+	Insignificant: no HDN (Cohen, 1985)	0
Anti-Yt^a	Micro	Insignificant: haemodialysis patient: Hct stable; $^{51}Cr\ T_{\frac{1}{2}} = 21$ d (Garratty *et al*, 1985)	0

* Indirect antiglobulin test using anti-IgG; † normal values ⩽2 (Gallagher *et al*. 1983).
‡ Autoantibody which caused an immediate haemolytic transfusion reaction when Ge-positive RBC were transfused.
§ Microscopically positive.

"Our current experience, although still limited, is encouraging. Thus far, we have correlated 100% using our in vitro monocyte-macrophage phagocytosis assay system with the in vivo clinical significance or insignificance of 17 antibodies (four anti-Ge; two each anti-M, -Vel and –Jka; and one each anti-Lan, -Coa, -Jkb, -P, -Yta and –Ytb). These results suggest that use of an optimal monocyte-macrophage phagocytosis assay system can accurately reflect the clinical relevance of a given antibody-containing serum in vitro…"

Branch DR, Gallahger MT.
Br J Haematol. 1986 Apr;62(4):783-5

MMA can indicate suppressed macrophage function

Vox Sang. *51:* 341–343 (1986)

Rhesus D^u Incompatibility in a Newborn without Hemolytic Disease: a Possible Role for the Mononuclear Phagocyte System in the Benign Clinical Course

D. Pérez[a], *D.W. Cohen*[b], *I.A. Shulman*[c], *D.R. Branch*[d]

[a]Blood Bank, Kaiser Permanente, Fontana; [b]Blood Bank of San Bernardino and Riverside Counties, San Bernardino; [c]Los Angeles County-University of Southern California Medical Center, Los Angeles, Calif., USA; [d]Department of Immunology, University of Alberta, and Canadian Red Cross Blood Transfusion Service, Edmonton, Alta, Canada

Newborn's MMA activity greatly reduced compared to maternal and normal

Results of normal, maternal, and infant monocyte-macrophage interaction with sensitized red cells

Monocyte-macrophages	Target red cells[1]							
	control red cells[2]			Rh phenotypes[3]				
	high	medium	low	R_2R_2	R_1R_1	R_1r	R_1r''[4]	rr
Normal	124	43	12	111	68	44	11	0
Maternal	105	52	4	100	NT	70	NT	0
Infant	47	17	4	44	NT	24	NT	0

Anti-D titer: 2048

Results are expressed as the phagocytic index (PRBC); normal values are ≤ 2 [6]. NT = Not tested.
1 All sensitized target red cells were 3+ to 4+ positive using IAT.
2 Group O, R_1R_2 target red cells sensitized with high, medium, and low levels of commercial, single-lot anti-D [6].
3 Group O red cells of various Rh phenotype having decreasing Rh_o (D) antigen site density sensitized with maternal anti-D.
4 Infant's red cells sensitized with maternal anti-D after adsorption of the maternal serum with group A_1, Rh-negative red cells to remove anti-A.

MMA of normal newborns is similar to adult controls

Results of normal cord blood monocyte-macrophage interaction with sensitized red cells

Cord blood monocyte-macrophages	Control target red cells[1]		
	high	medium	low
1	2.3	3.1	2.9
2	1.5	0.6	0.4
3	1.5	2.4	1.6
4	1.7	1.9	1.3
5	1.8	2.1	1.0

Results are expressed as the ratio of cord blood phagocytic index (PRBC) to normal control PRBC.
1 Group O, R_1R_2 target red cells were sensitized with high, medium, and low levels of commercial, single-lot anti-D [6].

University of Alberta

- Left COH in Dec/1985 – all work done on MMA while a Medical Technologist
- 1986 - Started PhD program in immunology at the University of Alberta
- Study of macrophages and cytokines
- Used mouse bone marrow-derived macrophages
- Published a number of papers on regulation of mouse macrophges by CSF-1 and TNFα
- Did not publish anything more on MMA until 2005

Others begin to use MMA

TRANSFUSION 1987;27:449–452.

Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay

S. J. NANCE, P. ARNDT, AND G. GARRATTY

Acta Haematol 1991;85:199–201

Monocyte Phagocytic Activity in Sickle Cell Disease

E. Mendoza[a], N. Gutgsell[b], J.D. Temple[a], P. Issitt[c]

[a] William J. Harrington Center for Blood Diseases, University of Miami School of Medicine, Miami, Fla.; [b] Research Laboratory, American Red Cross Blood Services of South Florida, Miami, Fla.; [c] Transfusion Service, Duke University Medical Center, Durham, N.C., USA

TRANSFUSION 1993;33:484–487.

Predicting hemolytic disease of the newborn: a comparison of the monocyte monolayer assay and the chemiluminescence test

G.F. LUCAS, A.G. HADLEY, S.J. NANCE, AND G. GARRATTY

After about 20 years – *he's baack!*

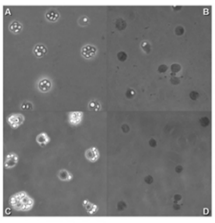
IMMUNOHEMATOLOGY

Chemical compounds that target thiol-disulfide groups on mononuclear phagocytes inhibit immune mediated phagocytosis of red blood cells

Gregory C. Rampersad, Garnet Suck, Darinka Sakac, Soad Fahim, Alison Foo, Gregory A. Denomme, Richard F. Langler, and Donald R. Branch

Transfusion. 2005 Mar;45(3):384-93

TRANSFUSION



MMA useful to identify phagocytosis inhibitors

IMMUNOHEMATOLOGY Transfusion. 2007 Feb;47(2):290-8

Structure-function studies for in vitro chemical inhibition of Fcγ receptor–mediated phagocytosis

Alison H. Foo, Stephen P. Fletcher, Richard F. Langler, Charlotte H. Porter, and Donald R. Branch

BLOOD COMPONENTS Transfusion. 2007 Dec;47(12):2250-9

Chemical treatment of anti-D results in improved efficacy for the inhibition of Fcγ receptor–mediated phagocytosis

Alison H. Foo, Stephanie Ramkumar, Simone Helke, and Donald R. Branch

IMMUNOHEMATOLOGY Transfusion. 2009 May;49(5):928-36

Reduction of disulfide bonds within anti-D results in enhanced Fcγ receptor blockade

Andrew S. Kruspe, Yulia Katsman, Darinka Sakac, Cécile Chagneau, Anna Glistvain, Richard F. Langler, and Donald R. Branch

Drug discovery for inhbitiors of phagocytosis

Bioorganic & Medicinal Chemistry Letters 23 (2013) 2324–2327

Disulfide linked pyrazole derivatives inhibit phagocytosis of opsonized blood cells

Meena K. Purohit [a,b], Iain Scovell [c], Anton Neschadim [c], Yulia Katsman [c], Donald R. Branch [c], Lakshmi P. Kotra [a,d,e,*]

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Bioorganic & Medicinal Chemistry 22 (2014) 2739–2752

Structure–activity relationships of pyrazole derivatives as potential therapeutics for immune thrombocytopenias

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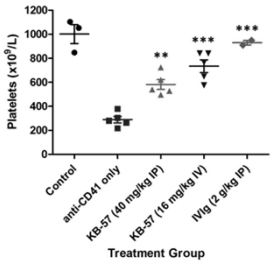
2nd Generation drugs



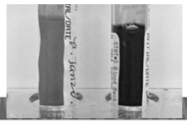
Second Generation Drug: KB-57

MMA can be used to study IVIg-associated hemolysis

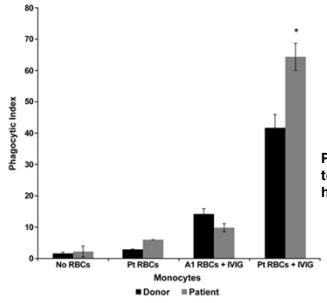
TRANSFUSION COMPLICATIONS

Acute hemolysis after intravenous immunoglobulin amid host factors of ABO-mismatched bone marrow transplantation, inflammation, and activated mononuclear phagocytes

Fotios V. Michelis, Donald R. Branch, Iain Scovell, Evgenia Bloch, Jacob Pendergrast, Jeffrey H. Lipton, and Christine M. Cserti-Gazdewich



MMA Results on hemolyzing patient



Patient monocytes tested 6 days post hemolytic episode

Other important studies

- **1987 – Nance et al. showed that MMA predicted clinical outcome of transfusion for all 12 patients' antibodies to high frequency antigens examined**
  Transfusion 1987;27(6):449-52
- **1989 – Nance et al. showed in 16 women that MMA was more predictive than amniotic fluid analysis in HDFN requiring transfusion**
  Am J Clin Pathol 1989;92(1):89-92
- **2004 – Arndt and Garratty found with 46 patients that the MMA was 100% accurate in predicting a non-significant antibody**
  Transfusion 2004;44(9):1273-81

MMA track record

- **Results using MMA have been published since 1981**
  [I myself have17 pubs;12 abstrs]
- **Early work with AIHA and DAT+ indicated MMA predictive for clinically significant and insignificant antibodies**
- **Subsequent work with alloantibodies indicates MMA useful for predicting clinical significance**
- **MMA has been used occasionally over the years in donor selection for certain patients**

Antibodies	Clinical Data?	Significant?	MMA concordant?
HIGH FREQUENCY			
Anti-Do[a]	Yes	Yes	Yes
Anti-Tc[a]	Yes	Yes	Yes
Anti-Ge2	Yes	No	Yes
Anti-MAM (x3)	Yes (2/3)	1/2	Yes
Anti-Lu8 (x3)	Yes	1/3	Yes
Anti-U	Yes	No	Yes
Anti-Jr[a] (x2)	Yes	1/2	Yes
Anti-Cr[a]/anti-M	Yes	Cr[a] no/M yes	Yes
Anti-Yt[b]	Yes	No	Yes
Anti-K11	Yes	No	Yes
Anti-Di[b] (x2)	Yes (1/2)	1/2	Yes
Anti-Holley	Yes	Yes	Yes
Anti-GP.Mur	Yes	Yes	Yes
Anti-At[a]	Yes	Yes	Yes
Anti-At[a]	Yes	Yes	Yes
Anti-Uz	Yes	No	Yes
Anti-Yt[a]	Yes	No	No
Anti-Ge	Yes	Yes	Yes
Anti-Lu8	Yes	Yes	Yes
Anti-Jr[a]	Yes	No	No
Anti-Di[a]/-E	Yes	Yes	Yes
Anti-Ant(t)	Yes	Yes	Yes
Anti-MER2	Yes	Yes	Yes
Anti-Ku	Yes	Yes	Yes
Anti-Jd[b]	Yes	No	No - ?
Anti-Ge3	Yes	No – initially?	Yes – initially?
OTHER WITH KNOWN SIGNIFICANCE			
Anti-P[1]	Yes	Yes	Yes
Anti-D	Yes	No	No
Anti-i	Yes	Yes	Yes
DRUG RELATED			
Anti-cefotetan	Yes	Yes	Yes
Anti-oxaliplatin (x2)	Yes	Yes	Yes
OTHER WITHOUT KNOWN SIGNIFICANCE			
Anti-M[a] (x26)	No	Unknown	18/26 significant
Anti-Yt[a] (x79)	Yes	Uncertain	18.2% significant
Anti-JMH	No	Unknown	significant
Anti-Ge	No	Unknown	significant
Anti-Ea[a]	No	Unknown	significant

Since 1988, 33/37 antibodies with documented clinical significance or insignificance show concordance with MMA

Does not include the 17 alloantibodies we reported in 1986 (50/54)

Summary

- **MMA use going on 4 decades**
- **Early and subsequent work showed efficacy of MMA to accurately predict *in vivo* antibody clinical significance or insignificance**
- **Not many reference laboratories using MMA – more should set it up**
- **MMA useful for:**
  - **Deciding on clinical significance of autoantibodies irrespective of strength of DAT**
  - **Deciding on clinical significance of red cell alloantibodies**
  - **Selection of blood for transfusion to individuals having antibodies to high frequency antigens**
  - **Deciding on clinical significance of drug-related antibodies**
  - **Investigation of mononuclear phagocyte function**
    - **Resolving problems where suspected clinically significant antibodies do not cause hemolysis**
    - **Investigation of hemolysis where serologic findings are negative**
    - **Identification of phagocytosis inhibitors**

Thank You