ERYTHROCYTE ANTIGEN LOSS DEFINES ANTIBODY-MEDIATED IMMUNE SUPPRESSION: STUDIES WITH POLYCLONAL AND MONOCLONAL ANTIBODIES

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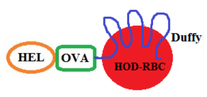
INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is an alloimmune condition that occurs when maternal antibodies specific to fetal red blood cell (RBC) antigens cross the placenta and cause fetal RBC destruction or fetal bone marrow suppression of RBC progenitors [1, 2]. Polyclonal anti-D has been used to prevent HDFN related to the RhD antigen [3, 4] and this mechanism has been referred to as antibody-mediated immune suppression (AMIS) [5]. Although this therapy has been highly successful, the mechanisms of anti-D remain poorly understood. In addition, none of the anti-D monoclonal antibodies that have been assessed have been as effective as polyclonal anti-D for AMIS [4]. Unfortunately, the RhD on RBC is not immunogenic in standard laboratory mice [6], for this reason several alternative mouse models have been developed. The transgenic HOD mouse model, with RBC-specific expression of the HOD (hen egg lysozyme [HEL], in sequence with ovalbumin peptide [OVA] and the human Duffy transmembrane protein)-antigen has provided useful information about AMIS [7, 8, 9, 10]. The major theories behind AMIS are based upon erythrocyte clearance, epitope masking, and immunological deviation [11]. Recently antigen (Ag) modulation (also called Ag-loss) has been proposed as a potential mechanism of anti-KEL immunoprophylaxis [12]. However, the relevance of Ag-modulation as a predictor of AMIS effect has not been assessed. In the present work, we studied the ability of polyclonal and monoclonal antibodies specific to different portions of HOD-Ag to induce *i)* AMIS activity, *ii)* RBC clearance and *iii)* erythrocyte-Ag loss. The correlation between AMIS effect induced by each antibody with their ability to promote RBC clearance and epitope-specific loss was also evaluated. The results obtained with two antibodies studied are showed.

MATERIALS AND METHODS

HOD-Red blood cells (HOD-RBC)



Ability of HOD-specific Ab to induce:

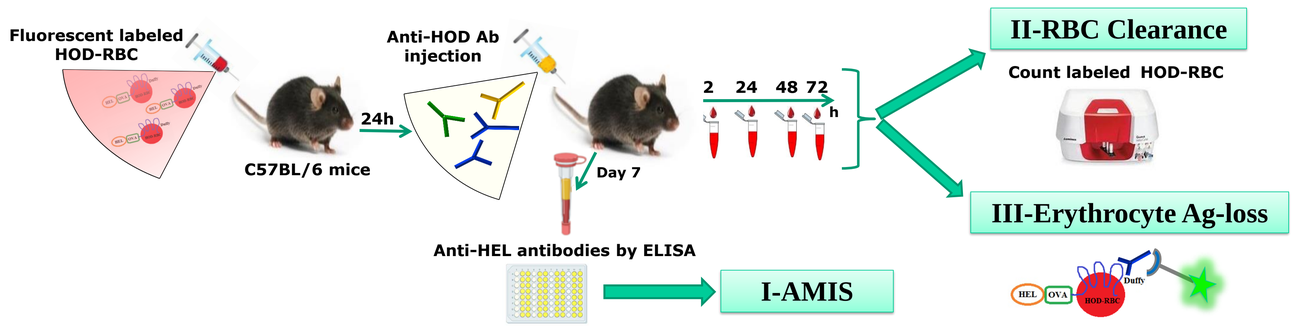
I. Antibody-mediated immune suppression (AMIS)

II. HOD-RBC clearance

III. Antigen-loss

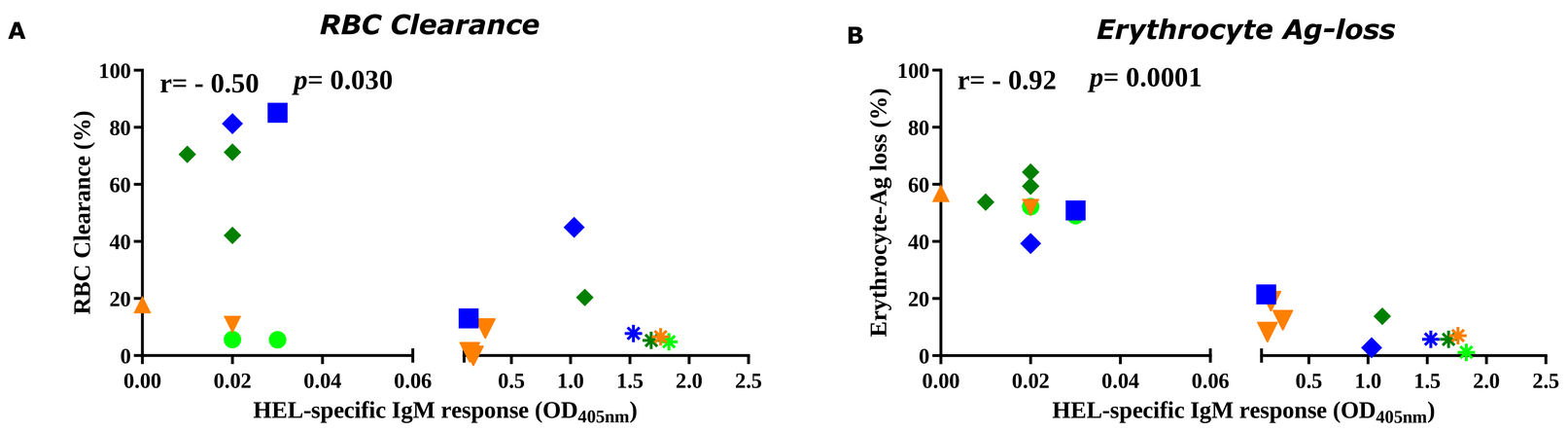
Table 1. HOD-specific antibodies assessed

#	Antibodies	Specificity	IgG isotype	Ab quantity/mouse (µg)
1	anti-HEL	HEL	Polyclonal	
2	4B7	HEL	IgG1	
3	2F4	HEL	IgG1	5
4	GD7	HEL	IgG2b	
5	4B7+2F4	HEL	IgG1	2.5 each/ antibody
6	Anti-OVA rabbit IgG	OVA	Polyclonal	10, 25, 50, and 75
7	Anti-OVA mouse IgG	OVA	Polyclonal	25 and 50
8	MIMA 29	Fy3	IgG2a	
9	deMIMA29	Fy3	IgG2a	
10	CBC-512	Fy3	IgG1	5
11	deCBC-512	Fy3	IgG1	



RESULTS

AMIS induced by HOD-specific Abs correlate better with their ability to cause Ag-specific loss than RBC Clearance



HOD-specific antibodies induced AMIS independently of their ability to induce RBC Clearance, but they caused a remarkable erythrocyte-Ag loss

Fig.1 AMIS-inducer antibody ability correlate better with their capacity to induce Ag loss than erythrocyte clearance.
C57BL/6 mice were transfused with PKH26+HOD-RBC, and 24h later mice received HEL-, OVA-, or Duffy-specific IgG. A group of mice was injected PBS and used as a negative control of erythrocyte clearance and positive control alloinmmunization. All mice were bled before and 24h after antibody injection. The percentage of PKH26+HOD-RBC in circulation as well as the loss of the epitope targeted on HOD-RBC recovery from each mouse was measured by flow cytometry. In addition, anti-specific IgM response was measured at day 7 after erythrocyte transfusion. The correlation of HEL-specific IgM response with (**A**) RBC Clearance and (**B**) epitope-specific modulation at 24h after Ab injection was measured by Spearman.

Study cases

1. OVA-specific Polyclonal Antibody

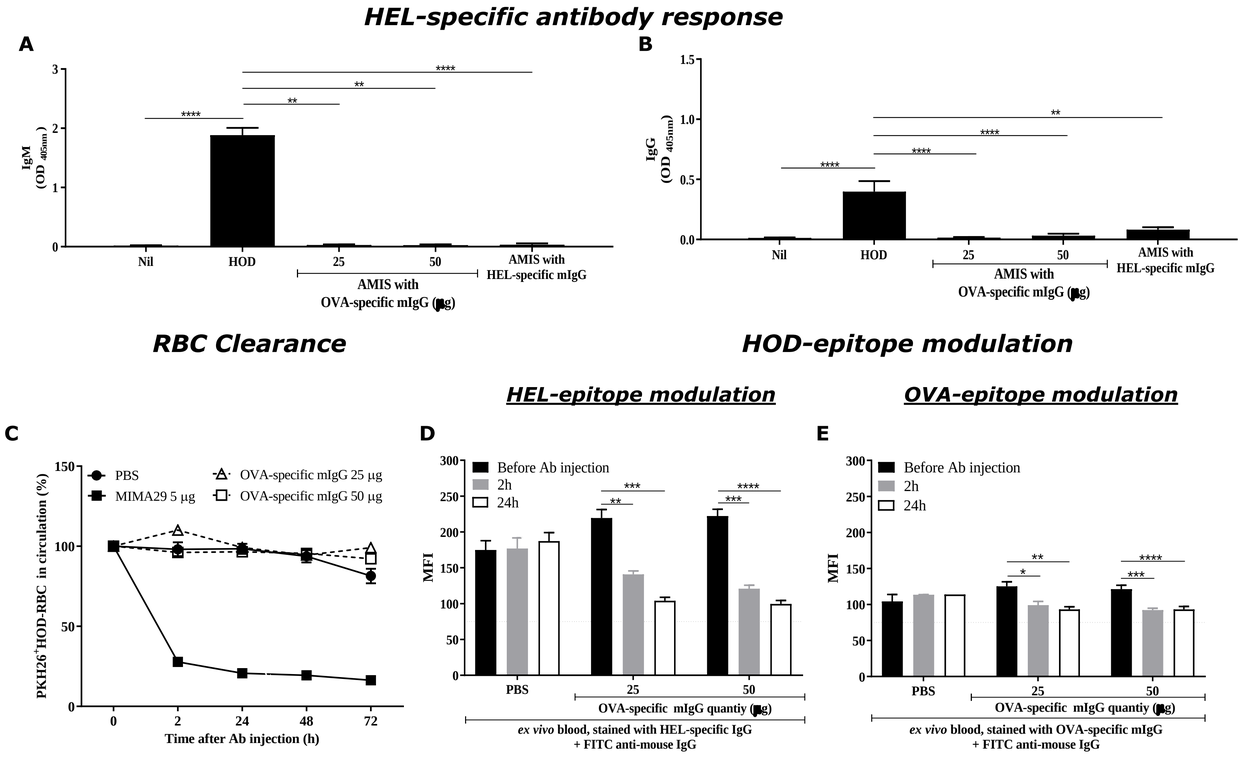


Figure 2. OVA-specific mouse IgG (mIgG) induced AMIS without RBC clearance but promoted erythrocyte Ag modulation.
RBCs were isolated from HOD mice and labeled with the fluorescent dye PKH26. C57BL/6 mice were challenged with 10^8 PKH26+ HOD-RBCs per mouse. Twenty-four hours after HOD-RBC transfusion, mice were injected with the indicated quantities of OVA-specific mIgG, PBS, or 5 µg of the HEL-specific mIgG and anti-Duffy mAb (MIMA29) as a positive control for AMIS and RBC clearance respectively. Mice were bled for serum on day 7. HEL-specific (**A**) IgM and (**B**) IgG induced were evaluated by ELISA. (**C**) The percentage of PKH26+ HOD-RBCs 2 h before and 2, 24, 48 and 72h after Ab injection were examined. Detection of (**D**) HEL, and (**E**) OVA epitopes on recovered PKH26+ HOD-RBCs from C57BL/6 recipient mice, transfused in the presence or absence of the OVA-specific mIgG, were performed by FACS. Data represent the mean± SEM from three different experiments. Statistical analyses were performed by Kruskal–Wallis test with Dunn's posttest (* p=0.05, ** p =0.01, *** p=0.001, **** p= 0.0001).

2. Duffy-specific Monoclonal Antibody

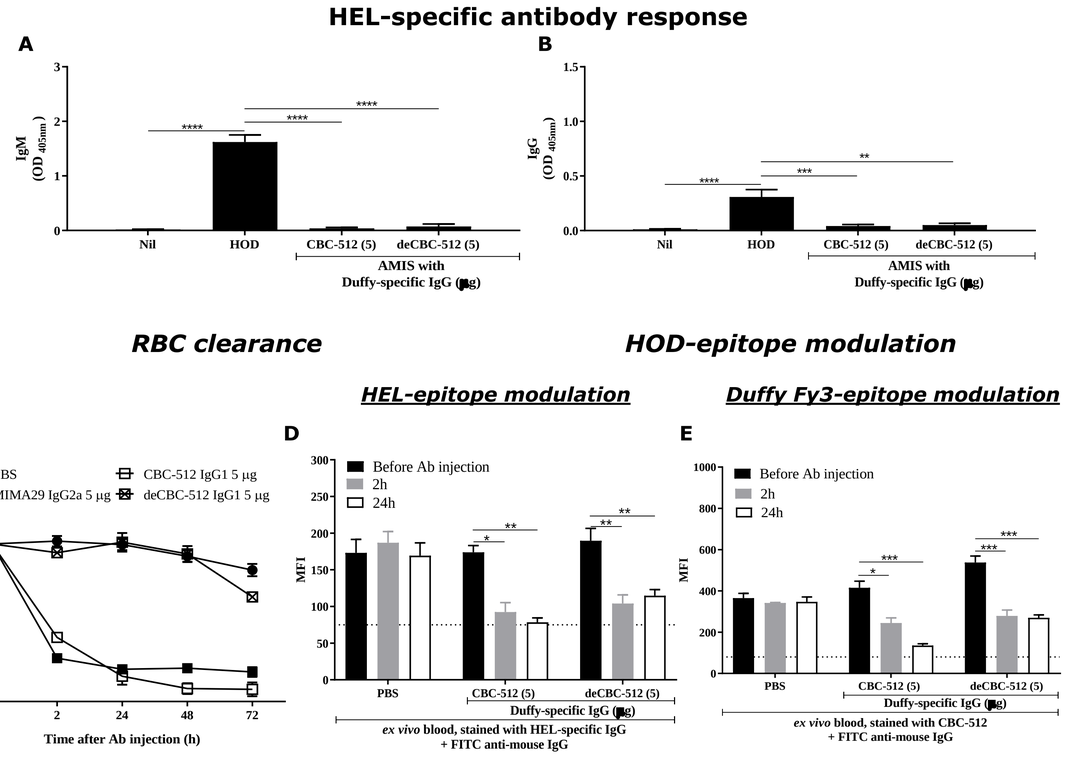


Figure 3. AMIS-inducer Duffy-specific monoclonal antibody is still able to induce AMIS despite losing its RBC clearance ability but keeping Ag loss capacity
RBCs were isolated from HOD mice and labeled with the fluorescent dye PKH26. C57BL/6 mice were challenged with 10^8 PKH26+ HOD-RBCs per mouse. Twenty-four hours after HOD-RBC transfusion, mice were injected with 5 µg of Duffy-specific monoclonal Ab CBC-512 wild type (CBC-512) or deglycosylated (deCBC-512), or PBS. Mice were bled for serum on day 7. HEL-specific (**A**) IgM and (**B**) IgG induced were evaluated by ELISA. (**C**) The percentage of PKH26+ HOD-RBCs 2 h before and 2, 24, 48 and 72h after Ab injection were examined. Detection of (**D**) HEL and (**E**) Duffy epitopes on recovered PKH26+ HOD-RBCs from C57BL/6 recipient mice, transfused in the presence or absence of Duffy-specific mAbs, were performed by FACS. Data represent the mean± SEM from three different experiments. Statistical analyses were performed by Kruskal–Wallis test with Dunn's posttest (* p=0.05, ** p =0.01, *** p=0.001, **** p= 0.0001).

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ACKNOWLEDGEMENTS

We thank Joan Legarda, Peter Norris, Ramsha Khan, Yuexin Shan, Zoya Fatima Tawhidi, Doyoon Won, and the St Michael's Hospital Research Vivarium stuff.

CONCLUSIONS

1. RBC clearance is not an indispensable requirement for AMIS.
2. Ag (epitope) loss is a significant predictor of AMIS activity in HOD mouse model.
3. Complete Ag loss does not seem to be required for successful AMIS induction