Blood First Edition Paper, prepublished online July 8, 2014; DOI 10.1182/blood-2014-05-538181

Spotlight on pathogenesis of TRALI: HNA-3a (CTL2) antibodies

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Abbreviated title: TRALI: human neutrophil antigens and antibodies

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Abstract

Human neutrophil antigen-3a (HNA-3a) antibodies contained in donor plasma can result in severe, sometimes fatal transfusion related acute lung injury (TRALI). Recent developments in TRALI secondary to antibodies to HNA-3a antigen span diagnosis, pathophysiology, treatment and prevention resulting in improved understanding, potential treatments and mitigation strategies. First, on the molecular level, characterization of HNA-3 antigen has allowed for genotyping methods that clarify population prevalence. Related work has led to generation of multiple antibody detection assays. These assays aid in determining potential population at risk and potential mitigation strategies. Second, the development of TRALI requires a hit from the patient and from the product. Anti-HNA-3a is one of the product derived factors, and appears to result in TRALI by binding directly to pulmonary endothelium as well as to neutrophils expressing the corresponding antigen. Finally, potential mitigation strategies include red blood cell product filtration to remove anti-HNA-3a as well as other antibodies.

Introduction

Transfusion-Related Acute Lung Injury (TRALI) remains the leading cause of transfusion related fatalities as indicated both by the United States Food and Drug Administration (FDA) and by international hemovigilance systems such as the UK Serious Hazards of Transfusion (SHOT) and the French Agency for the Safety of Health Products (AFSSAPS). TRALI accounted for 38% of transfusion-related fatalities reported to the FDA during the period 2008-2013. [1] Decreases in the incidence of TRALI have occurred through mitigation strategies, particularly with male, never pregnant female, or solvent detergent plasma. The use of male only plasma decreased TRALI risk of all transfused units by 68% from 2006 to 2009 [2], and solvent detergent plasma has reportedly eliminated risk. [3] TRALI usually arises secondary to transfused donor white blood cell antibodies (human leukocyte antigen [HLA] class I or class II, or human neutrophil antigen [HNA] antibodies), but patient factors also play a role. Antibodies to HNAs, in particular anti-HNA-3a, are associated with severe cases of TRALI although why these antibodies cause more severe disease than HLA class I or class II antibodies remains unknown. While antibodies to HNA-1a, -1b, and -2a are also implicated in TRALI, given the particular association of anti-HNA-3a with severe and fatal cases, this review will highlight HNA-3a and its corresponding antibodies, including their role in TRALI development, incidence, and potential testing and mitigation strategies. Please refer to other recent reviews that more broadly discuss the clinical entity, epidemiology, and pathogenesis of TRALI. [4,5]

TRANSFUSION RELATED ACUTE LUNG INJURY

TRALI is a clinical syndrome defined by hypoxia and pulmonary edema, occurring within 6 hours of transfusion in the absence of alternate causes of pulmonary injury.[6] HNA antibodies are implicated in a significant proportion of cases. A prospective case-controlled study of TRALI demonstrated the presence of HNA antibodies in 42% TRALI cases, of which 12% were exclusively HNA. [2] Two large case series of TRALI showed HNA antibodies in 33% and 23% of cases, respectively. [7,8] Similarly, FDA data from 2007-2008 and UK hemovigilance data from 1996-2006 show HNA antibodies in 22% and 25% of cases with demonstrated donor antibodies, although not all cases were tested for recipient cognate antigens. [9,10]

TRALI develops in a two-hit model; the first hit from the recipient and the second hit from the blood product. The product can result in TRALI either from an antibody-mediated or non-antibody-mediated mechanism. Then patient characteristics, such as higher IL-8 levels, together with the product determine if TRALI is none, mild or severe. [2,4] The antibody-mediated mechanism is that TRALI is caused by the transfusion of blood products containing HLA Class I and II, or HNA alloantibodies directed against the cognate antigen of the recipient. Non-antibody-mediated mechanisms involve infusion of biologically activated lipids or cytokines that stimulate neutrophil activation. Both mechanisms then result in lung damage through capillary leakage and subsequent pulmonary edema.

A recent large prospective case-controlled study of TRALI identified significant factors associated with TRALI. [2] First, amount of anti-HNA determined by granulocyte immunofluorescence test and unit volume was a product risk factor. Second, other product risk factors included volume of HLA Class II antibody with normalized background ration >27.5, and plasma or whole blood from female donors. Third, recipient risk factors were higher IL-8 levels, liver surgery, chronic alcohol abuse, shock, higher peak airway pressure during mechanical ventilation, current smoking, and positive fluid balance. Lastly, older red blood cell units, noncognate or weak cognate Class II antibody, and Class I antibody were not found to be associated with TRALI occurrence. Overall, antibodies were only found in 52% of the 89 TRALI patients in this study.

Current Mitigation Strategies

Current mitigation strategies focus on reducing the risk from antibody-mediated TRALI, because the majority of TRALI cases are due to large volume plasma containing products associated with HLA and/or HNA antibodies. Accordingly such measures target high plasma volume products, designated as whole blood, plasma products (fresh frozen plasma, plasma frozen within 24 hours and cryoprecipitate reduced plasma), apheresis platelets (PLTs) and buffy coat PLTs resuspended in plasma; the efficacy of applied mitigation measures regarding TRALI risk from platelet products however is uncertain at this time. Mitigation interventions for plasma products have included using plasma from exclusively male donors, never

pregnant females, resuspending pooled buffy coat platelets in male only plasma, HLA antibody screening of female donors based on parity, and using solvent detergent plasma. National and international data (UK, Netherlands, Canada) show all TRALI cases from plasma transfusions in the US were reduced by 23 to 10 cases (57%) from 2006 to 2009, [2] and by 32 to 7 cases (80%) from 2006 to 2007 [11]; by 36 to 10 (72%) in the UK in 2003 vs 2006, [10] by 36 to 8 (77%) in the Netherlands from 2002 to 2009, [12] and by 57 to 34 cases (40%) in Canada from 2007 to 2008 [13] since the adoption of strategies utilizing predominantly male plasma. [2,10,12,13]

Human Neutrophil Antigen-3/CTL2

Recent advances have provided information on the structure of the HNA-3 antigen. Although HNA-3 was discovered 45 years ago, only recently the antigen system was characterized molecularly. [14] Two alleles designated HNA-3a and -3b result from a single nucleotide polymorphism (SNP) at position 154 in the first extracellular loop of CTL2, with arginine in HNA-3a and glutamine in HNA-3b. [14,15] The HNA-3 antigen is located on the choline transporter-like protein 2 (CTL2) which is a 70-95 kD glycoprotein encoded by the *SLC44A2* gene. [14,16] In addition to neutrophils, HNA-3 is expressed on monocytes, lymphocytes, and platelets, as well as pulmonary endothelium. [17,18] CTL2 is a membrane-bound protein comprising 10 transmembrane domains and 5 extracellular loops.[19,16] Two different isoforms exist, designated CTL2-P1 and CTL2-P2, both equally able to bind HNA-3a antibodies, but with choline transporter activity limited to CTL2-P2. [16,18] It was

recently demonstrated that neutrophils, mononuclear cells and platelets express only CTL2-P1, implying that choline transport is not instrumental for neutrophil activation. Both transcript variants however are expressed in lung, suggesting a potential role of choline transport in anti-HNA-3a binding directly to endothelium resulting in TRALI. [17]

HNA-3a antibodies and TRALI

HNA-3a antibodies are the most frequently detected of the HNA antibodies in TRALI cases, seen in up to 85% of cases, [7] can induce TRALI in the context of minimal residual plasma volume, such as from red cell transfusion, and account for a disproportionate share of TRALI fatalities compared to other mediators. [7,20] The higher fatality association was demonstrated in a study of Reil et al, who reported 36 TRALI cases, 12 of which were due to HNA antibodies. Of these, 10 were due to HNA-3a antibodies and 6 of the 10 cases were fatal.[7] In contrast, of the remaining 24 cases due to non-HNA antibodies, only 4 cases were fatal.

Lookback studies have also provided important information about the severity of TRALI cases involving HNA-3a antibodies. Kopko et al evaluated 36 patients who had received transfusion from a donor with HNA-3a antibodies associated with a fatality, and found 8 additional cases of severe reactions consistent with TRALI, only two of which had been reported.[21] In contrast a lookback performed by Davoren et al did not find any instance of adverse reaction in 25 previous donations from an

implicated donor with HNA-3a antibodies, however this information was only obtained from communication with the involved blood banks and did not include recipient chart reviews.

HNA-3a antibodies result in TRALI both through neutrophil activation and direct pulmonary endothelial damage. It has been suggested that HNA-3a antibodies serve as the second of a 2-hit pathogenesis, following the first hit of a clinical insult such as sepsis or shock. Silliman et al developed an analogous in vitro model showing that HNA-3a antibodies primed the fMLP respiratory burst, and that endotoxin-activated human microvascular endothelial cells were able to prime PMNs through increased ICAM-1 and chemokine synthesis. Then the subsequent addition of HNA-3a antibodies was able to activate the primed PMNs leading to release of reactive oxygen species causing neutrophil-mediated destruction of pulmonary endothelial cells. [22]

The direct binding of HNA-3a antibodies to HNA-3a+ neutrophils results in neutrophil agglutination, priming and activation of the PMN oxidase, and subsequent neutrophil-mediated destruction of pulmonary endothelial cells. [4] An early study using an ex-vivo lung model showed that infusing explanted rabbit lungs with human anti-HNA-3a induced significant alterations in synthetic arachidonic acid metabolites, endothelial permeability, and lung edema after cotransfusion of HNA-3a positive but not HNA-3a negative human neutrophils. [23,24]

The two-hit pathogenesis was also recently demonstrated in a mouse model showing the formation of neutrophil extracellular traps (NETs) in TRALI. NETs, defined as decondensed chromatin fibers decorated with neutrophil granular proteins, are released from activated neutrophils and trap extracellular proteins.[25,26] NET formation, while potentially beneficial in capturing pathogens and killing bacteria, can also lead to pathological tissue injury, such as when excessive activation of neutrophils leads to TRALI.[27,25,28] Thomas et al showed that NETs were produced in vitro by a 2-hit mechanism of neutrophils primed with TNF-alpha and then exposed to HNA-3a antibodies, which were previously implicated in TRALI. [25] No significant NET formation was seen from TNF-alpha primed PMN's without HNA-3a antibody. Importantly, further experiments showed improved oxygenation from administration of intranasal DNAse1, which destroys NETs, suggesting that NETs play a role in TRALI and that DNAse1 is a potential treatment option.

In contrast to anti-HNA-3a mediating TRALI through PMN binding, recent work suggests that anti-HNA-3a mediates endothelial destruction by direct binding. Bayat et al developed an in vitro model showing that treatment of HNA-3a positive endothelial cells with anti-HNA-3a can induce production of reactive oxygen species (ROS) by endothelial cells, although treatment of HNA-3a positive neutrophils with anti-HNA-3a did not induce ROS production, consistent with previous work. [17,22] Additional effects of anti-HNA-3a on endothelium included a decrease in endothelial resistance and increased endothelial permeability leading to impaired barrier integrity.[17] Further, recent mouse models have demonstrated that HNA3 (choline

transporter) antibodies can bind to the analogous mouse choline transporter epitope, [29] and these antibodies can induce TRALI-like symptoms when injected into mice. [17]

Most recently, experimental filters have shown efficacy in mitigating TRALI due to HNA antibodies. Silliman et al [30] describe a filtration system based on Pall BPF4 leukoreduction filters that depletes both antibodies and biological response modifiers from the red blood cell products. In a murine model of HNA-3a antibody induced TRALI these filters resulted in reduced neutrophil activation and prevention of lung injury. [30] By showing that prevention of neutrophil priming with filtration reduces acute lung injury, the study provides further support to a PMN-dependent model.

HNA Genotyping

Genotyping methods recently were developed based on the molecular characterization of HNA-3 [31,32] that could allow for screening of donors at risk of developing HNA-3a antibodies (e.g. previously pregnant women). Reil et al recently developed such a method using PCR-SSP (sequence specific primers) to genotype HNA-3a and -3b of 398 blood donors. They concurrently phenotyped 40 individuals by GAT (granulocyte agglutination test), GIFT (granulocyte immunofluorescence test), and LIFT (lymphocyte immunofluorescence test), and found the results concordant with the genotypes obtained by their PCR-SSP method.

Genotyping methods now exist for HNA-1, -3, -4, and -5, although a method is still unavailable for HNA-2a as it represents a defect in transcription of the HNA-2 gene.

Population studies demonstrate variation in the frequencies of the genes encoding the HNA alleles 3a and 3b. The above study by Reil et al found 22 out of 398 (5.5%) donors in Germany homozygous for the HNA-3b allele and thus at risk for alloimmunization to HNA-3a. Other studies similarly show approximately 5-6% of both the European and American Caucasian population homozygous for HNA-3b. [14,31] In contrast, 16% of the Han Chinese population is homozygous for HNA-3b, while no African Americans were HNA-3b homozygous. [31] These findings suggest a potential value in stratifying donors for screening based on race/ethnicity.

Antibody detection

Current testing for HNA-3a antibodies includes the GAT and the GIFT, as recommended by the ISBT working party on granulocyte immunobiology. [33] These classical methods however, are cumbersome because they require fresh granulocytes that must be prepared daily and used immediately. Consequently screening for HNA-3a antibodies is not commonly performed. A recent national survey demonstrated that none of 47 participating US blood centers were screening for anti-HNA-3a. [34] While a combined Luminex test is available that detects HLA class I and class II and HNA 1a, 1b, and 1c antibodies (LABScreen MULTI, One

Lambda, Inc., Canoga Park, CA), it does not currently include HNA-3a antigens as until recently HNA-3 had not been molecularly defined.[35]

The recent characterization of HNA-3a could potentially allow for the development of solid phase assays for future high-throughput donor screening tests, with recombinant antigens targeting the first extracellular loop of CTL2, however such efforts have proved difficult. For example, cyclic and linear synthetic peptides corresponding to HNA-3a do not consistently bind HNA-3a antibodies. [36] Greinacher et al attempted to characterize HNA-3a antibody binding by developing an enzyme immunoassay to screen blood donors for HNA-3a antibodies using a library of synthetic peptides covering the first extracellular loop of CTL2. However they found that at most 9 out of 21 plasma samples containing anti-HNA-3a recognized CTL2 peptides corresponding to segments of the first extracellular loop containing the R154 peptide. [37] Other studies have found similarly that approximately half of HNA-3a antibodies from blood donors implicated in TRALI recognize such peptides. [35,36]

Due to the inability of synthetic peptides to recognize all clinically relevant HNA-3a antibodies, efforts have been made to express the CTL2 protein in transfected HEK293 cells. [35,38,39] Several studies found that the full-length CTL2 protein was recognized by all clinically relevant anti-HNA-3a sera as detected by flow cytometry.[38] Despite the relative success of these studies, a cell-based assay is not ideal for large scale screening of blood donors.

Future directions

In summary, recent advances in TRALI research focus on the role of HNA-3a antigens and antibodies. Typing of HNA-3a antigens allows for identification of donors at risk of developing HNA-3a antibodies, and progress in antibody detection will potentially lead to widespread screening of HNA-3a antibodies, both of which will enhance the safety of the blood supply. New understanding of the mechanism of HNA-3a antibodies through NET formation introduces the possibility of novel treatment options, such as intranasal DNAse/pulmozyme, while PMN independent mechanisms pose remaining treatment challenges. While important preventative measures have been proposed in the form of experimental filtration, work remains to determine if the concept will prove clinically relevant.

Authorship: All authors contributed to the writing of this manuscript.

Conflict of Interest Disclosure: All authors have no relevant conflict of interest to this manuscript.

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Table 1. HNA antibodies in published TRALI reports

Authors, year	Cases (#)	Antibody	Donors (#)	Donor gender	Mean pregnancies per donor (range)	Fatal cases	Products transfused	Type of study
Kopko et al, 2002 [14]	15	HNA-3a	1	Female	3	0	Plasma	Lookback
Davoren et al, 2003 [13]	2	HNA-3a	2	Female	2	2	Platelets, Plasma	Case review
Fadeyi et al, 2006 [40]	9	HNA-2a	1	Male	NA	0	Platelets	Lookback
Reil et al, 2008 [7]	12	1 HNA-1a, 1 HNA-2a, 10 HNA-3a	-	Female	(1-6)	6	Plasma, Red blood cells	Interventional
Keller-Stanislawski, 2010 [8]	8	1 HNA-2a, 7 HNA-3a	2	Female	2.5	2	Plasma	Observational cohort

Figure 1a. Anti-HNA-3a induced TRALI. Antibodies to HNA-3a are postulated to bind to primed neutrophils expressing HNA-3a, leading to activation, agglutination and subsequent pulmonary injury resulting in TRALI. First anti-HNA-3a in the blood component binds to human neutrophils expressing HNA-3a/CTL2-P1. Next antibody-antigen interactions lead to agglutination of neutrophils and activation of the PMN oxidase. Then activated PMNs release ROS and enzymes that mediate endothelial damage, vascular compromise, and edema, subsequently leading to TRALI. Neutrophil extracellular traps (NETs) are postulated to form as a pathological bioproduct of neutrophil activation, leading to further endothelial injury.

Figure 1b. Effect of filtration on TRALI. Pall BPF4 leukoreduction filters deplete blood products of anti-HNA-3a among other antibodies. Reduction of TRALI-inducing substances in the blood component can result in decreased subsequent lung injury, as a potential preventative mechanism.

Figure 1a. Anti-HNA-3a induced TRALI.

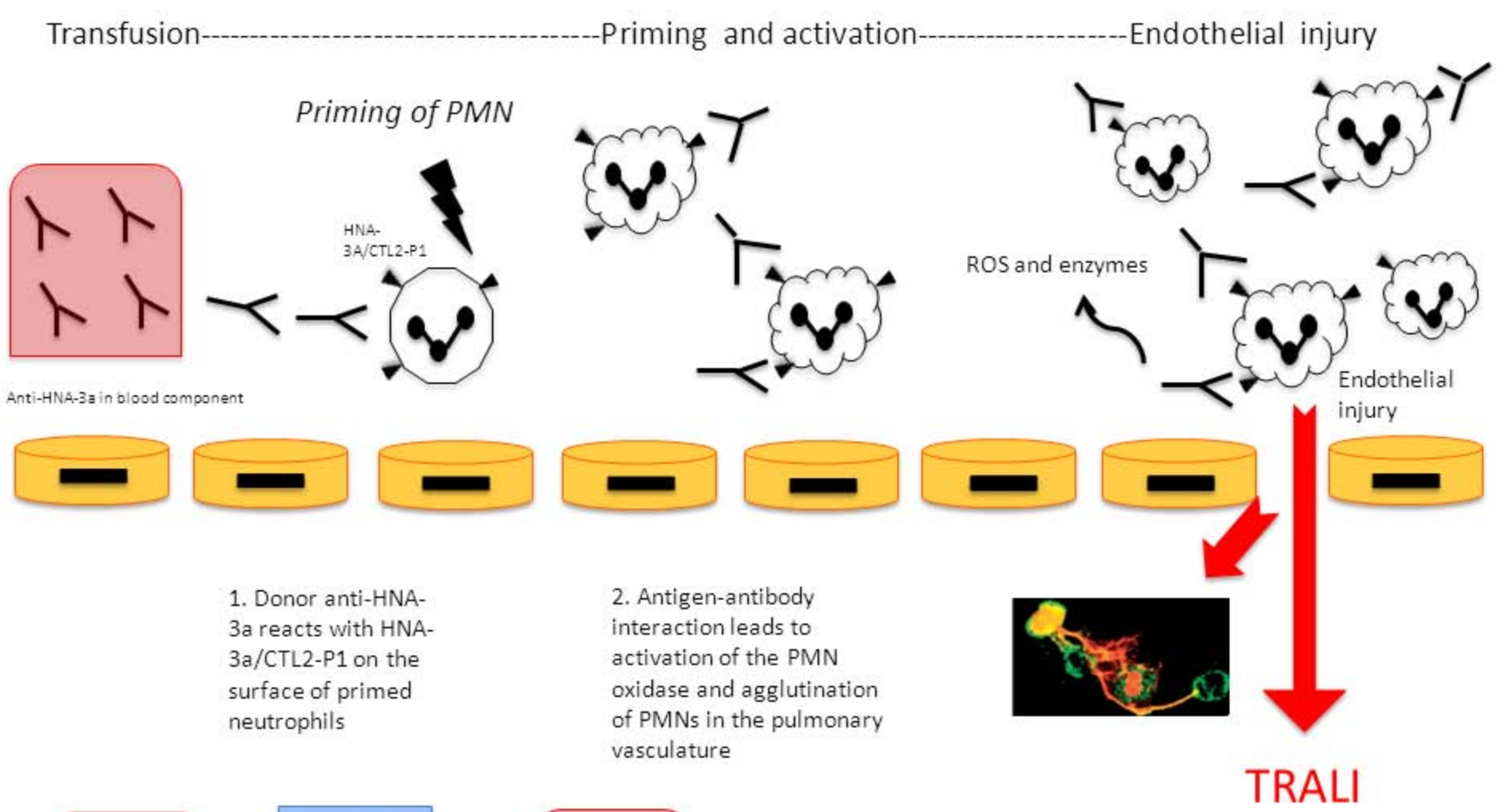

Figure 1b. Effect of filtration on TRALI.

Prepublished online July 8, 2014;
doi:10.1182/blood-2014-05-538181

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.