TRANSFUSION-RELATED IMMUNOMODULATION MECHANISMS: A SCOPING REVIEW

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ABSTRACT

Introduction: Red blood cell (RBC) transfusions are a common life-saving treatment. However, studies suggest they can raise the risk of hospital-acquired infections (HAI). This scoping review aims to map out described RBC transfusion-related immunomodulation (TRIM) mechanisms that might increase HAI incidence.

Methods: We performed a systematic search of Ovid/MEDLINE, Ovid/EMBASE, and Cochrane/CENTRAL from their inception to June 13, 2019, for original clinical or laboratory-based studies that described RBC TRIM. Using PubMed's Similar articles feature on February 21, 2020, we identified and screened additional eligible articles related to the primary search. Two trained researchers screened the citations and independently collected data.

Results: Of the 8,179 articles screened, 82 studies met eligibility and were included (54 were laboratory-based, and 28 were clinical studies). Cytokine concentrations varied after blood transfusions due to the effect of transfusions on T cells. We grouped TRIM mechanisms into four categories: 1) effects related to the presence of allogeneic white blood cells (WBCs) in transfused RBC units, 2) release of bioactive substances due to WBC apoptosis, 3) effects of allogeneic RBCs on T helper and T regulatory cells, and 4) release of bioactive substances by hemolytic allogeneic RBCs. Various TRIM mechanisms were identified related to the length of storage time of RBC units that occur primarily in either fresh or stored blood. The transfusion of stored RBCs was associated with 1) the absence of the costimulatory signal required for T cell activation, with consequent T cell anergy, and 2) the release of bioactive soluble factors,

including histamine, eosinophil cationic protein, and soluble Fas ligand. The transfusion of fresh RBCs was associated with microchimerism.

Conclusions: Several different TRIM mechanisms may result in an increased risk of developing HAIs. Importantly, some of these mechanisms appear to be linked to the length of storage of transfused RBC units. Understanding these mechanisms will inform future studies and the development of blood bank strategies to mitigate HAI risks in transfused patients to improve their clinical outcomes.

KEYWORDS

Red blood cell transfusion; transfusion-related immunomodulation; TRIM; immunosuppression; scoping review

INTRODUCTION

Each year around 117 million blood donations are collected worldwide, and 85 million red blood cell (RBC) units are transfused.[1, 2] In the United States alone, approximately 11 million transfusions were given in 2017, equivalent to one unit of blood transfused every 4 seconds.[3] In 2019, the Canadian Blood Services reported 801,281 donations in Canada.[4]

Blood cells have many different functions, including transporting oxygen and nutrients, immunological functions, and coagulation to prevent excess bleeding.[5] In critically ill patients, the main reasons for transfusing RBCs include low hemoglobin levels and the need to increase oxygen delivery to better support dysfunctional organs.[6-8] Despite the positive aspects associated with RBC transfusions, there are also risks linked to them, such as transfusion-transmitted infectious diseases like hepatitis and transfusion-related adverse events like transfusion-associated circulatory overload (TACO).[9, 10] In addition, some studies suggest an increased risk for hospital-acquired infections (HAI) with RBC transfusion.[11-18]

Blood service agencies have implemented rigorous screening strategies worldwide to avoid the direct transmission of infectious diseases by contaminated RBC units.[19, 20] However, despite transmissible blood-borne infection rates being rare, studies still show a trend of increased rates of HAI after patients receive RBC transfusions. The mechanisms behind the increased observed risk are relatively unknown. One hypothesis is that RBC transfusions can lead to infections through transfusion-related immunomodulation (TRIM).[17, 21]

TRIM refers to the changes in the immune system that follow the transfusion of blood products, which includes pro-inflammatory and immunosuppressive effects.[22] Most known TRIM mechanisms are related to the presence of white blood cells (WBCs) in transfused RBC units.[21] This scoping review aimed to map out TRIM mechanisms that might increase the risk

of HAI. A better knowledge of such mechanisms may help develop strategies to modulate the potential HAI risk associated with RBC transfusions.

MATERIALS AND METHODS

We developed the study protocol using the Joanna Briggs Institute approach to conducting scoping reviews.[23, 24] We performed the study in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines.[23, 25]

Information Sources and Search Strategy

We developed an electronic search strategy (Appendix A) in collaboration with a liaison Health Sciences librarian (GG). We searched Ovid/MEDLINE, Ovid/EMBASE, and Cochrane/CENTRAL databases for eligible studies published from their inception to June 13, 2019. Based on a selection of included studies from the primary search, we used PubMed's Similar articles feature on February 21, 2020, to find additional eligible articles related to the original citations' titles, abstracts, and MeSH terms. We screened the first 300 highest ranked records. Additionally, we searched published abstracts and looked at the reference list of included papers, reviews, and editorials. We also identified other publications using personal libraries and contacting content experts in the field.

Study Selection

Two independent and trained reviewers (LKF and KCN) screened titles and abstracts (first screen) and full-text reports (second screen). Discrepancies were resolved through

consensus or consultation with an arbitrator (PSF). Articles that passed the two stages of screening were charted for relevant data. We included published abstracts or full reports of original studies that were either clinical or laboratory-based and described potential biological mechanisms that could lead to an increased risk of HAI after RBC transfusion. Inclusion was limited to articles published in English. For clinical studies, we included papers that compared two groups of patients and described potential TRIM mechanisms. Comparisons included patients who received RBC transfusions vs. non-transfused patients or patient groups that received RBCs with differing storage lengths (fresh vs. stored blood). We described potential TRIM mechanisms associated with the age of blood, as studies suggest that varying lengths of RBC storage lead to HAIs[26-32]. For laboratory-based studies, we included studies that utilized animal models or blood cell models to test the hypothesis of TRIM mechanisms.

Data Collection Process

Two reviewers (LKF and KCN) extracted data from each included study. Disagreements among reviewers were resolved by consensus. Data extracted included first author, publication year and country, study design, type of model (clinical, animal, or cell), intervention group (fresh/stored, transfused/not transfused, leukoreduced/non-leukoreduced, other), outcomes, results, and if the author's proposed or studied a mechanism.

Analysis

Included studies were grouped based on their characteristics, including study design, country of origin, and publication year. Next, studies were analyzed together in broad categories based on the proposed mechanisms: 1) effects related to the presence of allogeneic WBCs in

transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs) and charted in respective tables. Finally, the proposed mechanisms were mapped in a figure along with information gathered on the expression of cytokines.

RESULTS

Study Selection

Our systematic search yielded 7,879 potentially relevant publications (Figure 1). After removing 1,678 duplicates, we excluded a further 5,951 publications during the title and abstract screening and examined the full text of the remaining 250 articles. A total of 47 articles met our inclusion criteria. Using the Similar Articles feature in PubMed, within the highest ranked 300 records (out of a total of 1,881), we identified 5 additional publications that met our inclusion criteria. A further 30 articles were identified by screening personal libraries and the reference list of relevant review articles. Overall, a total of 82 articles were included in the scoping review (Appendix Table B.1).[33-114]

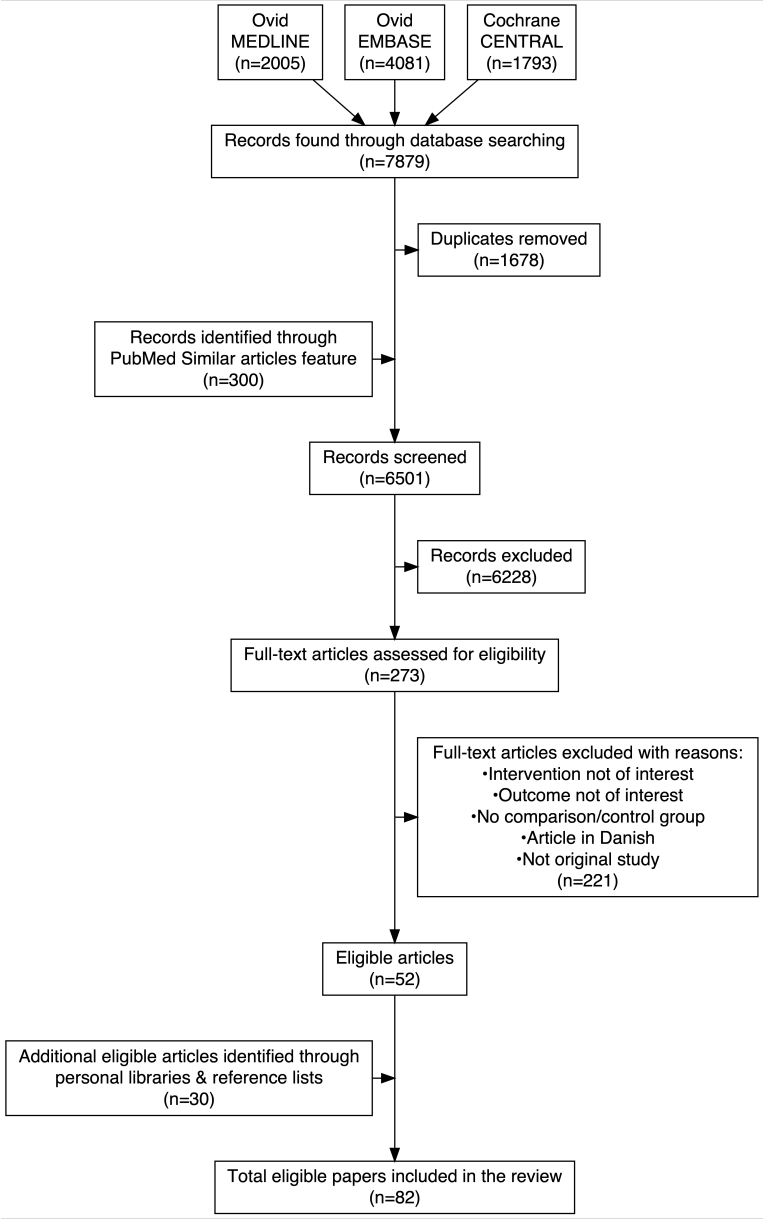

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of systematic literature search for studies related to TRIM.

Study Characteristics

Forty-one studies (50%) were conducted in North and South America (Table 1). Two-thirds of the studies (54 of 82) were laboratory-based, with cell models (38; 70%) being the most

common model for hypothesis testing. Among clinical studies, cohort studies (22; 79%) were the most common. The number of papers included from periods 1980-1989, 1990-1999, 2000-2009, and 2010-2019 were 5, 25, 20, and 32, respectively.

Table 1 Characteristics of articles on TRIM mechanisms after RBC transfusions

Characteristics	Number (%) of 82 articles
Type of article	
Laboratory-based	54 (66%)
Cell model	38
Murine model	14
Canine model	1
Porcine model	1
Clinical study	28 (34%)
Cohort study	22
Randomized controlled trial	6
Country of origin [a]	
North and South America	41 (50%)
Eastern Mediterranean	1 (1%)
Europe	33 (40%)
South-East Asia	1 (1%)
Western Pacific	6 (7%)
Year of publication	
1980 – 1989	5 (6%)
1990 – 1999	25 (30%)
2000 – 2009	20 (24%)
2010 – 2019	32 (39%)

[a] based on World Health Organization country groupings[115]
Percentages may not sum to 100 because of rounding.

Change in cytokines concentrations associated with blood transfusion

Of the initial 47 articles identified through the primary systematic search, 33 describe the effect of blood transfusions on cytokine concentrations. Fifteen articles compared the concentration of cytokines between transfused and non-transfused patients (Table 2);[44, 50, 53,

56, 57, 66, 67, 71, 79, 92, 102, 107, 108, 110, 113] no overall trend in the difference of pro- and anti-inflammatory cytokine levels were observed, whichever the model (cell, animal, or human). The blood concentration of interleukin-6 (IL-6), which is a systemic pro-inflammatory cytokine, was higher in transfused patients; however, in laboratory-based models, there was no difference in IL-6 levels between transfused and non-transfused animals, while the level of IL-6 was greater in cell models not exposed to blood.

Table 2 Comparison of cytokine concentrations between transfused and non-transfused groups

Model	Cytokine Concentration in Transfusion Recipients					
	Transfused > Non-Transfused		No difference between groups		Non-Transfused > Transfused	
	Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory
Cell			IL-2 [102] IFN-γ [102]	IL-10 [102]	IL-6 [102]	
Animal			IL-1 [44] IL-6 [44] IL-21 [44]	IL-10 [44]		
Human	sIL-2-R [113] IL-6 [53, 56, 67, 110, 113] IL-8 [53] IFN-γ [107]	IL-4* [57] IL-10 [53, 57, 110] TGF-β [57]	IL-1β [50, 71] IL-2 [50, 71] IL-6 [50, 71] IL-8 [50, 71] IL-12 [71] IL-17A [50] TNFα [50, 66, 71, 79] IFN-γ [50, 71, 108]	IL-4* [50, 71, 108] IL-5* [50, 71] IL-10 [50, 71, 79] TGF-β [50, 66]	IL-8 [92] IL-9 [107] TNFα [53] MIP-1α [107]	

Abbreviations: IFN-γ, interferon-gamma; IL, interleukin; MIP-α, macrophage inflammatory protein 1 alpha; sIL-2-R, soluble interleukin 2 receptor; TGF-β, transforming growth factor-beta; TNF-α, tumour necrosis factor-alpha. * Type 2 cytokines which modulate allergic/parasitic immune response and decrease type 1 response against bacteria.

The remaining 18 articles focused on the difference in cytokine levels between recipients of fresh blood compared to stored blood (Table 3).[35-39, 47, 48, 51, 60, 73, 81, 85-87, 91, 94, 103, 111] No major trend in the difference of cytokine levels was observed, independently of the model (cell, animal, or human) or use of pre-storage leukoreduction. However, when specifically

looking at cell models, there is an increase in pro-inflammatory cytokines in both fresh and stored blood.

Table 3 Comparison of cytokine concentrations in transfusion recipients between fresh and stored blood, separated by pro-inflammatory and anti-inflammatory, model, and blood processing.

Model	Blood Processing	Cytokine Concentration in Transfusion Recipients					
		Fresh > Stored		No difference between groups		Stored > Fresh	
		Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory
Cell	Leukoreduced (pre-storage)	IL-1β [37] IL-2 [37] IL-6 [51] IL-8 [51] IL-17 [81] IFN-γ [37] TNF-α [37, 51, 81] MIP-1α [51]	IL-4* [37] IL-10 [37, 81]	IL-8 [35, 36, 48] IL-12 [51] MCP-1 [51] MIP-1β [51] TNF-α [39] IFN-α [51] IFN-γ [35, 81]	IL-10 [36] IL-13 [35] TGF-β [37]	IL-17 [35] MCP-1 [39]	
	Not pre-stored leukoreduced		IL-22 [35]	IL-8 [35, 36, 48] TNF-α [39, 111] IFN-γ [35] IL-6 [47, 94] IL-8 [47] MCP-1 [47]	IL-10 [36] IL-13 [35] TGF-β [94]	IL-1β [36] IL-6 [36] IL-8 [111] TNF-α [36] MCP-1[39]	TGF-β [35]
Animal	Leukoreduced (pre-storage)						
	Not pre-stored leukoreduced			IL-6 [38, 47] IL-8 [47] IFN-γ [38] TNF-α [38] MCP-1 [38]	IL-10 [38]	IL-6 [86, 103] MIP-1α [38] MIP-2 [38] MCP-1 [47]	IL-10 [85]
Human	Leukoreduced (pre-storage)			IL-1β [91, 103] IL-2 [86, 87, 91, 103] IL-6 [87, 91] IL-7 [91, 103] IL-8 [73, 91, 103] IL-12 [91, 103] IL-17 [91, 103] IL-21 [91, 103] IL-23 [91, 103] IFN-γ [86, 91]	IL-4* [86] IL-10 [86, 103]		

		TNF-α [73, 86, 87, 91] MCP-1 [87] MIP-1α [91, 103] MIP-1β [91, 103]
Not pre-stored leukoreduced		TGF-β [60]

Abbreviations: IFN-α, interferon-alpha; IFN-γ, interferon-gamma; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; MIP-2, macrophage inflammatory protein 2; TGF-β, transforming growth factor-beta; TNFα, tumor necrosis factor-alpha. * Type 2 cytokines which modulate allergic/parasitic immune response and decrease type 1 response against bacteria.

TRIM Mechanisms

The TRIM mechanisms proposed by the 33 articles deemed eligible by our search were grouped into four major categories: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs (Figure 2).

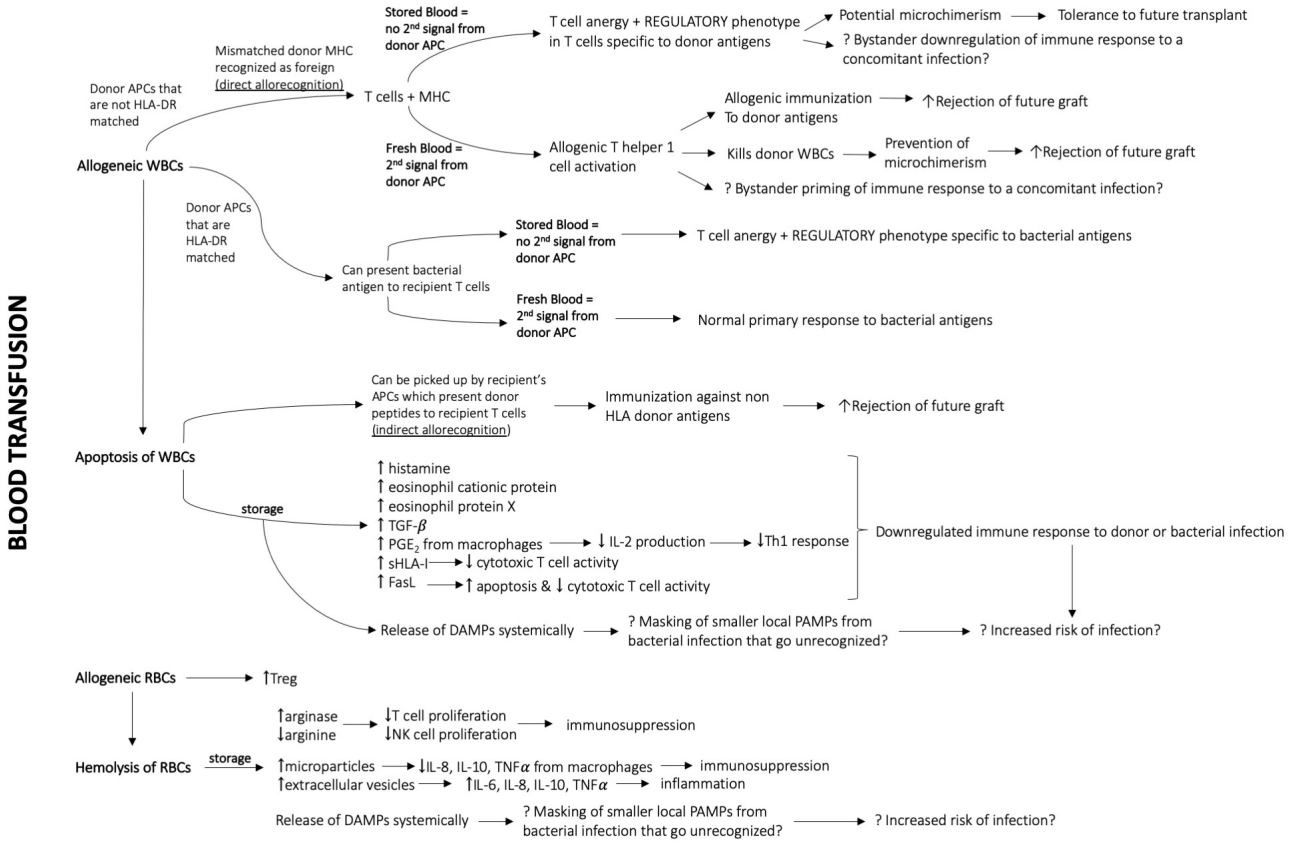

Figure 2 Described TRIM mechanisms separated by WBCs and RBCs

Abbreviations: APC, antigen-presenting cell; DAMPs, damage-associated molecular patterns; FasL, Fas ligand; HLA-DR, human leukocyte antigen – DR isotype; IFN-γ, interferon-gamma; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; PAMPS, pathogen-associated molecular patterns;PGE_2, prostaglandin E2; sHLA-1, soluble human leukocyte antigen 1; RBCs, red blood cells; TGF-β, transforming growth factor-beta; Th1, T helper 1; TNFα, tumour necrosis factor-alpha; Treg, T regulatory cells; TRIM, transfusion-related immunomodulation; WBCs, white blood cells.

TRIM Mechanisms Associated with the Presence of Allogeneic WBCs

1) Absence of a secondary costimulatory signal triggered by the donor's WBCs

Nine studies described a mechanism where the presence of donor WBCs leads to the absence of the secondary costimulatory signal necessary to release IL-2 and fully activate T cells

(Table 4; see Appendix C for a description of the T cell activation process).[52, 61, 64, 69, 70, 74, 80, 83, 84] Jenkins & Schwartz were the first to show that the absence of this secondary costimulatory signal may cause T cells to enter an unresponsive state, including a decrease in IL-2 production.[52, 69, 83] They also discovered that the IL-2 receptor is not inhibited during the unresponsive state, as T cells were able to respond to exogenous IL-2.[69] Therefore, the T cell's state of unresponsiveness results in a decrease of IL-2 production but does not affect the IL-2 receptor function. Further analyzing the mechanism behind T cell unresponsiveness, Mueller et al. showed that the binding of an antigen to the T cell receptor increases intracellular calcium and protein kinase C activation. However, without the costimulatory signal, these two secondary messengers are insufficient to induce T cell proliferation, causing an unresponsiveness state.[84]

Mincheff et al. described a mechanism where the presence of donor WBCs leads to the absence of the secondary costimulatory signal.[83] In this study, donor antigen-presenting cells (APCs) lost the ability to induce either alloreactive T cell response or T cell response to a new exogenous antigen by day 13 of storage.[83] However, T cell proliferation could be rescued by the exogenous addition of IL-2, which indicated T cell anergy. The authors hypothesized that such inability to activate T cells could be explained by the loss of the costimulatory signal on donor APCs from stored blood units.[83] This would lead to tolerance against the antigen presented.[83] Therefore, when patients are transfused with stored blood, and the secondary signal is absent, T cells would not proliferate or release IL-2. This jeopardizes patients' ability to trigger an inflammatory reaction, making them more susceptible to bacterial infections.

2) Microchimerism and HLA antigens

Eight studies described a mechanism involving the sharing, or sometimes mismatch, of HLA antigens between the blood donor and transfusion recipient with the potential to lead to microchimerism (Table 4; see Appendix C for a definition of microchimerism).[55, 75, 76, 78, 82, 97, 99, 109] Studies have demonstrated that microchimerism is 1) caused by donor WBCs in the recipient and 2) not dependent on the number of WBCs present.[55, 78] Lagaaij et al. observed an increase in ex vivo measurements of cell-mediated cytotoxicity against donor lymphocytes when the donor WBCs and the recipient did not share an HLA-DR antigen ("mismatch"), which peaked 2 weeks after transfusion.[76]

Presentation of donor antigens can occur via two different pathways: 1) direct allorecognition and 2) indirect allorecognition.[116] Direct allorecognition occurs when donor APCs present antigens directly to the recipient's T helper cells.[116] Indirect allorecognition happens when the recipient's APCs process and present an antigen from the recipient's cells to the T helper cells.[116]

Lagaaij et al. observed that the chance of sensitization against donor cells decreased when blood donors and transfusion recipients shared HLA-DR antigens, hypothesizing that direct allorecognition of a matched HLA-DR leads to the downregulation of the transfusion recipient's T cells.[75] Contrary to what Lagaaij et al. reported, Middleton et al. found no difference in sensitization between those patients awaiting a renal transplant and who received one HLA-DR antigen-matched blood transfusion compared to those who received random blood.[82] However, they observed a reduction in the incidence of renal transplant rejection episodes for those patients receiving matched blood.[82] Additionally, van Twuyver et al. found that when the blood donor and recipient share one HLA haplotype, there is an absence of T cell response against donor alloantigens.[109]

Regarding the indirect allorecognition pathway, Roelen et al. demonstrated that in vitro-generated regulatory T cells can recognize synthetic peptides that are presented in self-HLA class II molecules by autologous T cells or dendritic cells.[104] The regulatory T cells downregulate the response of these autologous T cells by killing them.[99]

TRIM mechanisms involving direct allorecognition may only be relevant for the transfusion of fresh blood since donor dendritic cells are only functional for 7 days after blood is donated.[117] Reed et al. further showed that the freshness of stored blood influenced the survival and function of WBCs, as microchimerism could not be detected in patients who received blood stored for more than 12 days.[97] The clinical consequences of microchimerism for transfused patients is unknown, but it could include graft-vs.-host or auto-immune effects. Importantly, when the donor and recipient are mismatched for HLA-DR antigens, alloreactive T lymphocyte activity increases, which may have immunomodulatory effects.

3) Release of bioactive substances during WBC apoptosis

Sixteen studies described mechanisms involving the release of bioactive substances from transfused WBCs when they undergo apoptosis (Table 4).[33, 43, 46, 57-60, 63, 65, 68, 85, 88-90, 95, 100] The release of eosinophil cationic protein and eosinophil protein X was observed to inhibit lymphocyte proliferation through the formation of enhanced suppressor activity leading to immunosuppression.[89, 95]

Furthermore, Bury et al. showed that released histamine inhibits T cells proliferation and human neutrophil chemotaxis via H2 receptors.[46] Studies showed that pre-storage leukoreduction reduces the amount of histamine present in stored RBC units.[88, 90]

Three studies reported that RBC transfusions increase prostaglandin E2 (PGE2) levels.[43, 57, 100] They showed that PGE2 inhibits IL-2 and interferon-gamma (IFN-γ) production from T helper 1 cells but does not inhibit T helper 2 cells from producing IL-4 and IL-5, thus causing further suppression of Th1 response.[43, 57, 100] It has also been shown that PGE2 is generated during the storage of blood and that there is a positive correlation between the number of WBCs and PGE2 levels.[68, 100]

Soluble Fas ligand (FasL) is a transmembrane protein that induces apoptosis when cells bind to its cell-surface receptor Fas.[33, 63, 65] Soluble HLA class I (sHLA-I) has been shown to induce FasL expression, specifically in cytotoxic T cells.[33, 58, 59, 63, 65] The upregulation in the expression of Fas and FasL on T cells is due to the increase in sHLA-I.[65] Studies demonstrated that the concentrations of soluble FasL and sHLA-I molecules were higher in RBC units containing residual WBCs, which is expected with fresher RBC units.[59, 65] It was further shown that pre-storage leukoreduction prevented the accumulation of these bioactive substances from increasing in a storage-dependent manner.[65] This decrease in bioactive substances would prevent changes in the cytotoxic T cells membrane during storage.[58-60, 65] Several studies showed that the development of immune tolerance depends on the apoptosis of cytotoxic T cells.[33, 58, 59, 63, 65] Ghio et al. found that sHLA-I, sFasL, and transforming growth factor-beta (TGF-β) are involved in the downregulation of NK cell-mediated cytolysis.[60] As a result, in the downregulation of cytotoxic T cell activity due to the release of these bioactive substances, patients would be limited in their ability to develop an immune response against bacterial infections.

Table 4 Studies that validated TRIM mechanisms associated with WBCs

Mechanism	Studies that validated or mentioned mechanism

Secondary signal: expression of costimulatory molecules on donor's WBCs	
Transfusion of blood independent of the length of storage	DeSilva et al. (1991) [52] Gimmi et al. (1991) [61] Harlan et al. (1994) [64] Jenkins & Schwartz (1987) [69] Jenkins et al. (1991) [70] Koulova et al. (1991) [74] Linsley et al. (1991) [80] Mueller et al. (1989) [84]
Transfusion of stored blood	Mincheff et al. (1993) [83]
Microchimerism and HLA Antigens	
Transfusion of blood independent of the length of storage	Flesland et al. (2004) [55] Lagaaij et al. (1989) [75] Lagaaij et al. (1991) [76] Lapierre et al. (2007) [78] Middleton et al. (1994) [82] Roelen et al. (2002) [99] van Twuyver et al. (1991) [109]
Transfusion of fresh blood	Reed et al. (2007) [97]
Release of Bioactive Substances	
Transfusion of blood independent of the length of storage	Arase et al. (1995) [33] Betz & Fox (1991) [43] Bury et al. (1992) [46] Gafter et al. (1996) [57] Ghio et al. (2001) [59] Griffith et al. (1996) [63] Hashimoto et al. (2004) [65] Peterson et al. (1986) [95] Zavazava & Kronke (1996) [112]
Transfusion of stored blood	Ghio et al. (1999) [58] Ghio et al. (2001) [59] Ghio et al. (2011) [60] Jacobi et al. (2000) [68] Mukherjee et al. (2014) [85] Nielsen et al. (1996) [88] Nielsen et al. (1996) [89] Nielsen et al. (1997) [90] Ross et al. (1990) [100]

TRIM Mechanisms Associated with Allogeneic Red Blood Cells:

1) Effects of RBC transfusions on T regulatory cells

Three studies described a mechanism where the generation of T regulatory cells was induced by RBC transfusion (Table 5).[37, 54, 114] Baumgartner et al. showed that RBC

supernatant induces T regulatory cell phenotype and that this effect was unaltered by pre-storage leukoreduction or prolonged storage.[37] Alternatively, Efron et al. found that T regulatory cells' generation depends on the age of transfused allogeneic RBCs.[54] T regulator cells induced by RBC supernatant were found to suppress the proliferation of effector T cells, including cytotoxic cells, leading to immunosuppression.[37] Zou et al. discovered that patients who received a transfusion had a lower number of NK cells than those who did not receive a transfusion and hypothesized this could be due to regulatory T cells' effect.[114] In the event of a bacterial infection, the T helper 1 response could be downregulated by T regulatory cells' inhibitor activity. Therefore, other immune cells, including NK cells, would not be activated, and cytokine production would be affected, increasing the patient's susceptibility to bacterial infections.

2) Release of bioactive substances during RBC hemolysis

Sixteen studies described a mechanism involving the release of bioactive substances from hemolytic RBCs (Table 5).[34, 40-42, 45, 49, 62, 72, 77, 93, 96, 98, 101, 104-106] The release of the enzyme arginase I from RBCs depletes L-arginine, leading to T cells having reduced expression of the T cell receptor zeta chain (CD3 zeta). This results in decreased T cell proliferation.[40-42, 72, 96, 98] Additional studies showed that arginase I also impairs NK cells' function and proliferation and IFN-γ secretion.[77, 93] The secondary decrease in T cell and NK cell proliferation could impair the immune response in the event of a bacterial infection.

In addition, several studies showed an increase in bioactive RBC-derived microparticle and microvesicle formation as storage time increases.[45, 49, 62, 101, 104-106] Their membranes consist primarily of lipids, which increase after 2 to 3 weeks of storage.[118] Such microparticles have been shown to inhibit IL-8, IL-10, and tumour necrosis factor-alpha (TNFα)

being released from macrophages.[101] Importantly, it was observed that there was a higher concentration of RBC extracellular vesicles during the storage of RBC units.[104] Such vesicles modify T cell responses and have dose-dependent pro-inflammatory capabilities by increasing IL-6, IL-8, IL-10, and TNFα.[49, 104-106] Barkkour et al. found that damage-associated molecular patterns (DAMPs) were also associated with stored blood.[34] They showed that the concentration of mitochondrial DNA accumulated early during RBC storage, within 7 to 14 days.[34] DAMPs are associated with inflammation as they are known immune mediators.[119] Based on these studies, RBC-derived microparticles, extracellular vesicles, and DAMPs can result in immunosuppression or increased inflammation, thus altering the response to bacterial pathogens depending on the bioactive substance released.

Table 5 Studies that validated TRIM mechanisms associated with RBCs

Mechanism	Studies that validated or mentioned mechanism
Effects due to T Regulator Cells	
Transfusion of blood independent of the length of storage	Baumgartner et al. (2009) [37] Efron et al. (2010) [54] Zou et al. (2016) [114]
Release of Bioactive Substances	
Transfusion of blood independent of the length of storage	Bernard et al. (2007) [41] Bernard et al. (2010) [42] Kim et al. (2002) [72] Lamas et al. (2012) [77] Oberlies et al. (2009) [93] Rodriguez et al. (2002) [98]
Transfusion of stored blood	Bakkour et al. (2016) [34] Bernard et al. (2008) [40] Bosman et al. (2008) [45] Danesh et al. (2014) [49] Greenwalt et al. (1980) [62] Prins et al. (2001) [96] Sadallah et al. (2008) [101] Straat et al. (2014) [105] Straat et al. (2015) [104] Straat et al. (2015) [106]

DISCUSSION

This scoping review identified and described several RBC TRIM mechanisms that may lead to an increase in HAIs. We classified TRIM mechanisms into four groups: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs. In addition, some of these mechanisms seem to be related to the length of storage of RBC units, with the transfusion of both fresh and stored blood being able to lead to immunosuppression.

Traditionally, TRIM has been studied in the context of immunization against organ donor antigens and how it affects future graft transplantation. TRIM has not been studied explicitly in the context of the immediate immune response to inflammation, although it has been hypothesized to contribute in a number of papers.[17, 21]

The most well-known TRIM mechanisms are associated with the presence of WBCs in the transfused blood and their apoptosis. It has been hypothesized that these TRIM mechanisms could be partly prevented by pre-storage leukoreduction and transfusing fresh instead of stored blood.[120] However, leukoreduction does not entirely remove all WBCs. In each leukoreduced RBC unit, up to $5X10^6$ WBCs can still be present.[121] These remaining WBCs are immunologically active in leukoreduced RBC units during the first 10 days of storage.[122, 123] Furthermore, the exact number of WBCs needed to trigger a TRIM mechanism is unknown. Thus, the small number of WBCs remaining in leukoreduced blood could still be enough to trigger TRIM.

RBC-related TRIM mechanisms could also be significant, but they were less frequently studied. Such mechanisms may potentially explain why studies have demonstrated an association between the transfusion of leukoreduced RBCs and HAIs.[11-18] A double-blinded, randomized

controlled trial (RCT) by Titlestad et al. comparing infection rates in leukocyte-depleted RBC transfusions and non-leukocyte-depleted RBCs found that there was no significant difference between the two transfusion groups (leukoreduced vs. non-leukoreduced) in terms of rates of infection.[14] The infection rates were 38% in the leukoreduced RBC group and 45% in the non-leukoreduced RBC group (P = 0.5250).[14] Despite the evidence behind RBC mechanisms presented in this review, we still need to understand better how the presence of donor RBCs can lead to the development of T regulatory cells and why this does not happen to all patients who receive a transfusion. Additionally, we need to clarify the clinical relevance of these RBC-induced T regulatory T cells.

Some of the mechanisms described in this scoping review, specifically the apoptosis of WBCs, the expression of costimulatory signals, and the hemolysis of RBCs, may be linked to blood transfusions' product storage time. As storage time increases, the concentration of bioactive substances related to these mechanisms also increases. This was the rationale for hypothesizing that the transfusion of fresh blood would lead to better clinical outcomes. Numerous RCTs have evaluated the effect of length of storage of RBCs on HAIs in adult, pediatric and neonatal critical patients.[26-32] However, no individual RCT proved that fresh blood was superior to stored blood with respect to patient outcomes.

Furthermore, some of the aforementioned RCTs showed a trend towards higher HAI incidence when patients received fresh blood. Fergusson et al. showed that the relative risk (RR) of HAIs in neonatal intensive care unit (ICU) patients receiving RBC transfusions was 2% higher; however, this was not statistically significant (RR 1.02, 95% confidence interval [CI] 0.88, 1.19).[27] Similarly, Spinella et al. identified a HAI relative risk of 1.1 (95% CI 0.6, 1.8) for pediatric ICU patients receiving fresh RBC transfusions.[31] Lacroix et al. reported a higher

incidence rate of nosocomial infections in critically ill adults who received fresh vs. older RBC units (34.1% vs. 31.3%; absolute risk reduction in the standard delivery group = 2.8, 95%CI -0.9, 6.5).[28] Finally, Schrieber et al. demonstrated that 30% of adult trauma patients transfused with fresh leukoreduced packed RBCs had an infection.[29] In comparison, only 26% of patients receiving old blood (P = 0.77), but this result did not reach statistical significance.[29]

Such results may be partially explained by the fact that microchimerism, due to direct allorecognition, is linked to the presence of donor's dendritic cells in fresh blood. A study by Markowicz & Engleman showed that dendritic cells are only viable for up to one week after blood is donated, which may explain why Reed et al. found that microchimerism was associated with fresh blood.[97, 117] Thus, based on the data presented in our scoping review, it is plausible that the transfusion of both fresh blood and stored blood may lead to immunosuppression by different mechanisms.

Knowing the effects of length of storage time on T cells, and subsequently, cytokines is important for understanding how blood transfusions can lead to immunosuppression or inflammation, ultimately leading to an increased risk for developing a HAI. Understanding these mechanisms will inform future studies in the transfusion community resulting in the development of blood bank strategies to mitigate HAI risks in transfused patients to improve their clinical outcomes. Additionally, as most of these studies were performed in laboratory-based models instead of in vivo, even if we demonstrate relative differences in cytokine and bioactive substances concentrations in units of blood stored for different lengths of time, it would be more relevant to study the absolute concentrations of these substances. This would allow us to determine whether the change in concentrations is significant, considering the volume transfused and the distribution volume in the patient.

Regarding post-transfusion cytokine expression, we found no real pattern when comparing transfused to non-transfused groups, neither when comparing fresh to stored blood transfusions. This may be since RBC transfusions can trigger both pro-inflammatory and anti-inflammatory reactions. In addition, the lack of a pattern might be because we have included clinical studies and laboratory experiments of varied quality in this review, which poses a challenge for the comparison of study results. Finally, most clinical studies included sick patients in their study population, most of whom have diseases caused by different conditions and are in varying stages of their disease evolution. Since these patients were previously ill before receiving a blood transfusion, their comorbidities may have primed their immune system; therefore, when they received a blood transfusion, this acted as a second insult activating an inflammatory response. Depending on the patient, this insult could exacerbate a pro-inflammatory reaction or trigger an anti-inflammatory response.[124]

Our study has limitations. Although we have performed a comprehensive search, we may have missed studies published in other languages and possibly in grey literature as we focused on published English literature. There is also a potential for publication bias; despite having searched for articles that did not validate the TRIM mechanisms presented in this scoping review, we could not find any. Furthermore, scoping reviews do not evaluate the quality of evidence. Therefore, the conclusions of this review are based on the studies' existence instead of their quality. Finally, most of TRIM literature studies T cell related mechanisms because the term is also used to describe the perspective of RBC effect on future graft rejection in transplant patients. This may explain the lack of studies focusing on the effect of RBC transfusion on innate immunity.

Nevertheless, our scoping review has several strengths. We have used a robust methodology to perform this study. We have also reviewed the literature using a comprehensive approach that allowed for the inclusion of evidence from the bench and clinical studies. In this review, the clinical studies included were all, except for one study, performed on sick hospitalized patients instead of healthy volunteers. Thus, the mechanisms reported herein are probably applicable only in hospitalized patients who are at higher risk of acquiring HAIs.

CONCLUSION

Despite the significant reduction in the incidence of transmissible blood-borne infections transmitted by the transfusion of blood products, studies still show an association between RBC transfusions and increased risk of HAI potentially due to TRIM. Our scoping review described four main categories of TRIM mechanisms that are due to the presence of both WBCs and RBCs in blood transfusions and the breaking down of these cells over time. Such mechanisms can partly explain the variability in cytokine levels after RBC transfusions, independently of the RBC unit storage time. Understanding the immunological mechanisms that occur after blood transfusions is crucial to inform the development of strategies that can mitigate HAI risks associated with blood transfusions to improve hospitalized patients' outcomes worldwide.

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AUTHOR CONTRIBUTIONS

Leah K. Flatman: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – Original draft, Writing - Reviewing & Editing. **Kim C. Noël:** Investigation, Writing – Reviewing & Editing, Approval of final manuscript. **Genevieve Gore:** Methodology, Writing – Reviewing & Editing, Approval of final manuscript. **Catherine Goudie:** Formal analysis, Writing – Reviewing & Editing, Approval of final manuscript. **Philippe Bégin:** Writing – Reviewing & Editing, Approval of final manuscript. **Jacques Lacroix:** Writing – Reviewing & Editing, Approval of final manuscript. **Jesse Papenburg:** Writing – Reviewing & Editing, Approval of final manuscript. **Dean A. Fergusson:** Formal analysis, Writing – Reviewing & Editing, Approval of final manuscript, Supervision. **Patricia S. Fontela**: Conceptualization, Methodology, Formal analysis, Writing – Reviewing & Editing, Approval of final manuscript, Project administration, Supervision.

CONFLICTS OF INTEREST

Authors have no conflicts of interest to report.

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Appendix A: Search Strategies

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily <1946 to Present> Search Strategy:

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1 Erythrocyte Transfusion/ or *blood transfusion/ae, im (8437)
2 (((Transfus* or stored or unit?) adj3 (red blood cell* or red cell* or erythrocyte* or RBC*)) or allogen?ic transfusion* or allogen?ic blood transfusion*).mp. (19928)
3 1 or 2 (19935)
4 exp Immunomodulation/ (304404)
5 (immunomodulat* or immunosuppress* or (immun* adj3 (mediat* or modulat* or suppress*))).mp. or immun* response*.ti. (386570)
6 exp chimerism/ or exp cytokines/bl, an, im, me or eosinophil granule proteins/ or eosinophils/me or exp HLA antigens/an, im, me or exp interferons/ or exp interleukins/an, bl, me or exp leukocytes/an, im, me or peroxidase/an or plasminogen activator inhibitor/an or exp prostaglandins/ or exp transforming growth factor beta/me or exp transforming growth factors/ or exp tumor necrosis factors/ or (((4-1BB or cd27 or cd30 or cd40 or OX40 or RANK) adj ligand*) or antigen presenting or b-cell activating factor* or basophil* or chimerism* or dendritic cell* or ectodysplasin* or eosinophil* or fas ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen activator inhibitor* or prostaglandin* or shla* or t helper* or th1 or th2 or th 1 or th 2 or tnf* or transforming growth factor* or tumo?r necrosis factor* or wbc or white blood cell?).ti,kf. (1262225)
7 4 or 5 or 6 (1689636)
8 3 and 7 (2195)
9 ((transfusion related or transfusion associated) adj2 (immunomodulat* or immun* modulat* or immunosuppress* or immun* suppress*)).mp. (142)
10 8 or 9 (2258)
11 10 and english.lg. (2005)

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Database: Embase Classic+Embase <1947 to 2019 June 12> Search Strategy:

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1 Erythrocyte Transfusion/ (25001)
2 (((Transfus* or stored or unit?) adj3 (red blood cell* or red cell* or erythrocyte* or RBC*)) or allogen?ic transfusion* or allogen?ic blood transfusion*).mp. (43255)
3 1 or 2 (43255)
4 exp Immunomodulation/ (75393)
5 (immunomodulat* or immunosuppress* or (immun* adj3 (mediat* or modulat* or suppress*))).mp. or immun* response*.ti. (585135)
6 exp *antigen presenting cell/ or exp *cytokine/ec or cytotoxic t lymphocyte/ or exp dendritic cell/ or *eosinophil cationic protein/ or eosinophil cationic protein/ec or *eosinophil granule protein/ or eosinophil/ or exp *HLA antigen/ec or HLA system/ or exp *interferon/ or exp *interleukin/ec or interleukin 2/ or interleukin 2 receptor/ or interleukin 4/ or interleukin 10/ or

interleukin 12/ or exp *leukocyte antigen/ or exp *leukocyte/ or *microchimerism/ or *myeloperoxidase/ec or *plasminogen activator inhibitor/ or exp *prostaglandin/ or t lymphocyte/ or TH1 cell/ or TH2 cell/ or exp *transforming growth factor beta/ or exp transforming growth factor beta/ec or exp *transforming growth factor/ec or exp *tumor necrosis factor/ (1430788)
7 (((4-1BB or cd27 or cd30 or cd40 or OX40 or RANK) adj ligand*) or antigen presenting or b-cell activating factor* or basophil* or chimerism* or dendritic cell* or ectodysplasin* or eosinophil* or fas ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen activator inhibitor* or prostaglandin* or shla* or t helper* or th1 or th2 or th 1 or th 2 or tnf* or transforming growth factor* or tumo?r necrosis factor* or wbc or white blood cell?).ti,kw. (933532)
8 4 or 5 or 6 or 7 (2131538)
9 3 and 8 (4214)
10 red blood cell transfusion related immunomodulation/ or ((transfusion related or transfusion associated) adj2 (immunomodulat* or immun* modulat* or immunosuppress* or immun* suppress*)).mp. (242)
11 9 or 10 (4316)
12 11 and english.lg. (4081)

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Search Name: CENTRAL
Date Run: 13/06/2019 17:05:02
Comment:

ID Search Hits
#1 (((Transfus* or stored or unit?) near/3 (red next blood next cell* or red next cell* or erythrocyte* or RBC*)) or allogen?ic next transfusion* or allogen?ic next blood next transfusion*) 8083
#2 (immunomodulat* or immunosuppress* or (immun* near/3 (mediat* or modulat* or suppress*))) or immun* next response*:ti 24272
#3 ((("4 1BB" or cd27 or cd30 or cd40 or OX40 or RANK) near/1 ligand*) or antigen next presenting or b next cell next activating next factor* or basophil* or chimerism* or dendritic next cell* or ectodysplasin* or eosinophil* or fas next ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer next cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen next activator next inhibitor* or prostaglandin* or shla* or t next helper* or th1 or th2 or th next 1 or th next 2 or tnf* or transforming next growth next factor* or tumo?r next necrosis next factor* or wbc or white next blood next cell?):ti,kw 63275
#4 #2 OR #3 80973
#5 #1 AND #4 1871
#6 ((transfusion next related or transfusion next associated) near/2 (immunomodulat* or immun* next modulat* or immunosuppress* or immun* next suppress*)) 31
#7 #5 OR #6 in Trials 1793

Appendix B: Included Studies

Table B.1 Individual study characteristics of included studies

Author	Country	Year of Publication	Study Design	Study Model
Arase et al.	Japan	1995	Animal research study	Mouse
Bal et al.	Turkey	2018	Test-tube lab research	Cell
Baumgartner et al.	USA	2009	Test-tube lab research	Cell
Baumgartner et al.	USA	2009	Test-tube lab research	Cell
Bakkour et al.	USA	2016	Test-tube lab research	Cell
Belizaire et al.	USA	2012	Animal research study	Mouse
Benson et al.	USA	2012	Test-tube lab research	Cell
Bernard et al.	USA	2007	Test-tube lab research	Cell
Bernard et al.	USA	2008	Test-tube lab research	Cell
Bernard et al.	USA	2010	Test-tube lab research	Cell
Betz &Fox	USA	1991	Animal research study	Mouse
Biagini et al.	Brazil	2017	Animal research study	Pig
Bosman et al.	Netherlands	2008	Test-tube lab research	Cell
Bury et al.	Belgium	1992	Prospective cohort study	Human
Callan et al.	USA	2013	Animal research study	Dog
Chin-Yee et al.	Canada	1997	Test-tube lab research	Cell
Danesh et al.	USA	2014	Test-tube lab research	Cell
De Andrade Pereira et al.	Brazil	2012	Prospective cohort study	Human
Dean et al.	Australia	2011	Test-tube lab research	Cell
DeSilva et al.	USA	1991	Animal research study	Mouse
Drosos et al.	Greece	2012	Prospective cohort study	Human
Efron et al.	USA	2010	Animal research study	Mouse
Flesland et al.	Norway	2004	Prospective cohort study	Human

Fransen et al.	Netherlands	1999	Prospective cohort study	Human
Gafter et al.	Israel	1996	Prospective cohort study	Human
Ghio et al.	Italy	1999	Test-tube lab research	Cell
Ghio et al.	Italy	2001	Test-tube lab research	Cell
Ghio et al.	Italy	2011	Prospective cohort study	Human
Gimmi et al.	USA	1991	Test-tube lab research	Cell
Greenwalt et al.	USA	1980	Test-tube lab research	Cell
Griffith et al.	USA	1996	Animal research study	Mouse
Harlan et al.	USA	1994	Animal research study	Mouse
Hashimoto et al.	Brazil	2004	Animal research study	Mouse
Hassani et al.	Iran	2017	Prospective cohort study	Human
Ishijima & Suzuki	Japan	1998	Prospective cohort study	Human
Jacobi et al.	Germany	2000	Test-tube lab research	Cell
Jenkins & Schwartz	USA	1987	Animal research study	Mouse
Jenkins et al.	USA	1991	Test-tube lab research	Cell
Jiwaji et al.	United Kingdom	2014	Randomized controlled trial	Human
Kim et al.	USA	2002	Test-tube lab research	Cell
Kor et al.	USA	2011	Randomized controlled trial	Human
Koulova et al.	USA	1991	Test-tube lab research	Cell
Lagaaij et al.	Netherlands	1989	Prospective cohort study	Human
Lagaaij et al.	Netherlands	1991	Prospective cohort study	Human
Lamas et al.	France	2012	Test-tube lab research	Cell

Lapierre et al.	France	2007	Randomized controlled trial	Human
Leal-Noval et al.	Spain	2010	Prospective cohort study	Human
Linsley et al.	USA	1991	Test-tube lab research	Cell
Long et al.	USA	2014	Test-tube lab research	Cell
Middleton et al.	Ireland	1994	Prospective cohort study	Human
Mincheff et al.	USA	1993	Test-tube lab research	Cell
Mueller et al.	USA	1989	Animal research study	Mouse
Mukherjee et al.	USA	2014	Animal research study	Mouse
Muszynski et al.	USA	2015	Prospective cohort study	Human
Neuman et al.	USA	2015	Randomized controlled trial	Human
Nielsen et al.	Denmark	1996	Test-tube lab research	Cell
Nielsen et al.	Denmark	1996	Test-tube lab research	Cell
Nielsen et al.	Denmark	1997	Test-tube lab research	Cell
Norris et al.	USA	2019	Prospective cohort study	Human
Nunn et al.	United Kingdom	2013	Randomized controlled trial	Human
Oberlies et al.	United Kingdom	2009	Test-tube lab research	Cell
Osei-Hwedieh et al.	USA	2015	Animal research study	Mouse
Peterson et al.	Sweden	1986	Test-tube lab research	Cell
Prins et al.	Netherlands	2001	Test-tube lab research	Cell
Reed et al.	USA	2007	Prospective cohort study	Human
Rodriguez et al.	USA	2002	Test-tube lab research	Cell
Roelen et al.	Netherlands	2002	Test-tube lab research	Cell

Ross et al.	United Kingdom	1990	Animal research study	Rat
Sadallah et al.	Switzerland	2008	Test-tube lab research	Cell
Shao et al.	USA	1998	Animal research study	Mouse
Spinella et al.	USA	2019	Prospective cohort study	Human
Straat et al.	Netherlands	2014	Test-tube lab research	Cell
Straat et al.	Netherlands	2015	Test-tube lab research	Cell
Straat et al.	Netherlands	2015	Test-tube lab research	Cell
Suksompong et al.	Thailand	2019	Prospective cohort study	Human
Sun et al.	Taiwan	2001	Prospective cohort study	Human
van Twuyver et al.	Netherlands	1991	Prospective cohort study	Human
Ydy et al.	Brazil	2007	Prospective cohort study	Human
Zallen et al.	USA	2000	Test-tube lab research	Cell
Zavazava & Kronke	Germany	1996	Test-tube lab research	Cell
Zhao et al.	China	2018	Randomized controlled trial	Human
Zou et al.	China	2016	Prospective cohort study	Human

Appendix C: Immunologic concepts/definitions used in this scoping review

1. T cells activation process

The first step involved in T cell activation is the interaction between T cell receptors and major histocompatibility complexes (MHC) located on the antigen-presenting cell (APC).[1-5] This step occurs when MHC binds an antigen peptide, and together, this MHC-peptide complex is recognized by the T cell receptor.[1-5] The occupancy of the T cell receptor by an MHC-peptide complex results in the expression of IL-2 receptors.[1-5]

A secondary costimulatory signal is simultaneously required to activate T cells fully, leading IL-2 production, which in turn acts in an autocrine fashion on the cells own IL-2 receptors to induce proliferation.[6] High expression of IL-2 is therefore indicative of T cell proliferation, and the expression of the IL-2 receptor on T cell is used as a marker of T cell activation. In the absence of a costimulatory signal, TCR activation will result in T cell anergy or the induction of a T regulatory phenotype characterized by persistently high expression of the IL-2 receptor and anti-inflammatory properties.

2. Microchimerism

Microchimerism is the persistence of donor cells in recipients years after blood transfusions that occur in a state of reciprocal tolerance.[7] For this to happen, the recipient must mount a sufficiently low immune response to accommodate these cells allowing for their survival.[7]

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