PORTLAND
PRESS

Review Article

Extracellular DNA in blood products and its potential effects on transfusion

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Blood transfusions are sometimes necessary after a high loss of blood due to injury or surgery. Some people need regular transfusions due to medical conditions such as haemophilia or cancer. Studies have suggested that extracellular DNA including mitochondrial DNA present in the extracellular milieu of transfused blood products has biological actions that are capable of activating the innate immune systems and potentially contribute to some adverse reactions in transfusion. From the present work, it becomes increasingly clear that extracellular DNA encompassed mitochondrial DNA is far from being biologically inert in blood products. It has been demonstrated to be present in eligible blood products and thus can be transfused to blood recipients. Although the presence of extracellular DNA in human plasma was initially detected in 1948, some aspects have not been fully elucidated. In this review, we summarize the potential origins, clearance mechanisms, relevant structures, and potential role of extracellular DNA in the innate immune responses and its relationship with individual adverse reactions in transfusion.

Introduction

The presence of extracellular DNA (ecDNA) was first reported by Mandel and Métais [1] in 1948. The term ecDNA describes any DNA existing in the extracellular environment, regardless of structure (association with protein complexes and extracellular vesicles) [2,3]. Although another term, cell-free DNA (cfDNA), is widely used presently, the term ecDNA is more accurate in this review because a large proportion of DNA *in vivo* is localized in complexes or is packaged in vesicles rather than being truly free [4,5]. Found in the extracellular milieu including serum, plasma, lymph, bile, milk, urine, saliva, spinal fluid, amniotic fluid, and cerebrospinal fluid, ecDNA can be isolated from individuals in both healthy and various disease states [6–10]. ecDNA comprises mainly nuclear DNA (nucDNA) and mitchondrial DNA (mitDNA). It is also present in the extracellular milieu of blood products (as shown in Table 1) [11–20]. However, a novel and significant role of ecDNA has emerged, involving its ability to trigger innate immune system responses and drive inflammation when released from mechanically injured cells [21]. ecDNA, along with other host molecules released upon cell damage, falls into the category of damage-associated molecular patterns (DAMPs) [22,23].

ecDNA in blood products

Blood can be transfused without prior modification (whole-blood transfusion) or divided into red blood cell units (RBCUs), fresh frozen plasma (FFP), platelet concentrates (PCs) and sometimes granulocytes. These blood products for therapeutic use usually contain donors' plasma, platelets, residual leukocytes and erythrocytes. The DNA of a donor can, therefore, be transferred to a recipient via ecDNA in the plasma fluid, ecDNA bound to the surfaces of blood components (such as erythrocytes and platelets) or via the DNA localized in complexes or packaged in vesicles. According to the report of Garcia-Olmo et al. [24], ecDNA from human plasma can pass through the 0.4 micron filters of Corning Transwell plates and

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Table 1 Extracellular DNA in blood products and their concentration

Reference	Blood products	DNA concentration	Sample number	DNA type	Quantitative method
Duxbury et al., 1995 [11]	Whole blood	Range 260–1474 ng/ml	7	total DNA	Threshold total DNA Assay system
Dijkstra-Tiekstra et al., 2004 [12]	PCs (before filtration)	1.7 ± 0.8 leucocyte-eq/µl	5	nucDNA	Quantitative real-time polymerase chain reaction
	PCs (after filtration)	1.5± 0.8 leucocyte-eq/µl	5		
Ivancic-Jelecki et al., 2009 [13]	Plasma	Range 0.06–22.5 ng/ml	10	nucDNA	Quantitative real-time polymerase chain reaction
Lee et al., 2014 [14]	RBCUs (LR)	Range 0.8–87-fold	11	mitDNA	Quantitative real-time polymerase chain reaction
	PCs	Range 0.4–235.6-fold	5		
	FFP	Range 0.7–46.5-fold	16		
Cognasse et al., 2016 [15]	AE-associated PCs	484 ± 313.45 ng/ml (peak concentration)	42	mitDNA	Quantitative real-time polymerase chain reaction
	Control PCs	122.55 ± 52.64 ng/ml (peak concentration)	59		
Shih et al., 2016 [16]	RBCUs (WBF)	1.08 ± 0.90 ng/ml	44	total DNA	PicoGreen assay
	RBCUs (WBF)	3.57 ± 1.99 µg/ml	47		Spectrophotometry
	RBCUs (RCF)	0.50 ± 0.77 ng/ml	73		PicoGreen assay
	RBCUs (RCF)	3.28 ± 1.28 µg/ml	73		Spectrophotometry
Bakkour et al., 2016 [17]	RBCUs (non-LR)	5.3×10^5 copies/µl (mean concentration)	12	mitDNA	Quantitative real-time polymerase chain reaction
	RBCUs (MCS+ apheresis)	1.3×10^5 copies/µl (mean concentration)	12		
	RBCUs (Trima apheresis)	1.2×10^5 copies/µl (mean concentration)	12		
Yashui et al., 2016 [18]	NHTR-associated PCs	Range $1.4–110 \times 10^4$ copies/ml	17	mitDNA	Quantitative real-time polymerase chain reaction
	NHTR-associated RBCUs	Range $0.3–23 \times 10^4$ copies/ml	20		
	NHTR-associated FFP	Range $2.3–18 \times 10^4$ copies/ml	11		
	Control PCs	Range $0.1–3.8 \times 10^4$ copies/ml	320		
Simmons et al., 2017 [19]	RBCUs	3 ± 0.4 ng/ml	114	mitDNA	Quantitative polymerase chain reaction
	PCs	94.8 ± 69.2 ng/ml	17		
	FFP	213.7 ± 65 ng/ml	81		
Waldvogel Abramowski et al., 2018 [20]	RBCUs	290 ± 120 ng/ml	23	total DNA	Qubit Fluorometer
	PCs	339.6 ± 114 ng/ml	23		
	FFP	2.875 ± 0.996 ng/ml	23		

there were no significant differences between the effects of human plasma administered to cell cultures indirectly through the Transwell plates and plasma administered directly to the cells, indicating that no DNA was lost due to the filter.

As early as in 1995, detectable DNA has been shown to be present in stored human donor blood at levels in the range of 250–1500 ng/ml with a Threhold Total DNA Assay Kit, and the total amount of DNA administered to a patient during the transfusion of a single unit of whole blood can be as much as 450 µg (based on 1.5 µg DNA/ml of CPD plasma and assuming a plasma volume of 60% in a 500 ml unit of blood) [11]. According to studies in the subsequent years, ecDNA was also observed in the plasma of transfusion blood components including RBCUs, PCs, and FFP [12–20]. The studies on ecDNA in transfusion products and their concentration are summarized in Table 1.

As can be seen from the table, in the report of Waldvogel Abramowski et al. [20], cellular products including RBCUs (290 ± 120 ng/ml) and PCs (339.6 ± 114 ng/ml) contained more ecDNA than did FFP (2.875 ± 0.996 ng/ml), suggesting a potential link with the number of cells found in the blood bag. Simmons et al. [19] found detectable levels of extracellular mitDNA in FFP (213.7 ± 65 ng/ml), PCs (94.8 ± 69.2 ng/ml), and RBCUs (3 ± 0.4 ng/ml). In the present study, the concentration of extracellular mitDNA detected in RBCUs was lowest among the three tested, perhaps because RBCs do not contain mitochondria and are subjected to leukocyte reduction [19]. Of note, the generation or release of ecDNA present in blood products could be influenced by the details of the processing and

manufacturing methods of blood components [16,17]. Shih et al. [16] have shown that that the levels of ecDNA are affected by RBCUs processing method as well as product age: whole blood filtered (WBF), short-term storage RBCUs had more ecDNA than red cell filtered (RCF), aged RBCUs. However, according to the study of Dijkstra-Tiekstra et al. [12], the amount of ecDNA was not influenced by filtration of the PCs (1.7 ± 0.8 vs. 1.5 ± 0.8 leucocyte-eq/μl).

Unfortunately, many studies with respect to ecDNA in blood products were performed with dissimilar analytical procedures, rendering comparison with their results infeasible. Some of these research results do not differentiate between nucDNA and mitDNA that are thought to be of separate evolutionary origins [25,26]. MitDNA consists of a high number of unmethylated CpG islands, as typically found in bacteria [27].

Potential origins and clearance of ecDNA in blood products
Potential origins

To date, no consensus has yet been reached with regard to the main origin of ecDNA. However, there are two contenders for the main origin of ecDNA: (i) cellular breakdown mechanisms and (ii) active release mechanisms [28]. Cellular breakdown mechanisms such as apoptosis and necrosis are considered to be the main processes for producing ecDNA by some researchers [29,30]. It may be of interest to note that other forms of cell death such as NETosis can also serve as sources for ecDNA [31]. Numerous studies have demonstrated that ecDNA can also be derived from active release mechanisms [32–35]. However, it is still unclear which mechanism accounts for the ecDNA observed in blood products.

During storage, cellular breakdown mechanisms, such as necrosis, apoptosis and NETosis, could theoretically lead to ecDNA release in blood products. Neutrophils can release both nucDNA and mitDNA in structures known as neutrophil extracellular traps (NETs), which are composed of decondensed chromatin decorated with granular proteins [36–38]. The formation of NETs requires the activation of neutrophils and the release of their DNA in a process that may or may not result in neutrophil death. Additionally, ecDNA could be encapsulated in extracellular vesicles (EVs), such as microparticles (MPs) [39], which have also been detected in blood products [40,41]. This ecDNA, present in membrane-bound EVs, can be protected from nuclease mediated degradation and can be released through the breakdown of EVs [4].

What's more, activated platelets have been reported to release intact mitochondria, which can be hydrolysed by group IIA secretory phospholipase A2 (sPLA2-IIA), and thereby release extracellular mitDNA [42]. In their study, Boudreau et al. [42] demonstrated that inactivated platelets contain an average of ∼4 mitochondria using fluorescence and transmission electron microscopy.

Clearance mechanisms

Since 1966, work on autoimmune pathologies has permitted the first characterization of ecDNA [43–46], it is resistant to RNase and proteinase K [47], but can be hydrolyzed by DNase. Studies on ecDNA clearance revealed that extracellular DNases such as DNase1 and DNase1L3 could degrade ecDNA associated with a variety of structures [48–52]. DNase1 and DNase1l3, which have close structural and functional resemblance, may substitute or cooperate with each other during DNA degradation [50]. The enzyme DNase1 plays a role in the clearance of chromatin during necrosis [49]. DNase1L3 is uniquely capable of digesting chromatin in microparticles released from apoptotic cells [51]. DNase1 along with DNase1L3 are essential for disassembly of NETs [50,52].

The subsequent fate of such ecDNA in recipients' blood is still unknown. DNase activity is one possible pathway of degradation of ecDNA, but other mechanisms of clearance cannot be discounted. The clearance of ecDNA could also be achieved by renal excretion into the urine [53] or uptake by the liver and spleen followed by macrophagic degradation [54]. Y-chromosome-specific sequences were detected in the urine of women who had been transfused with blood from male donors [55]. Detailed information on the mechanisms associated with these processes is lacking and remains somewhat controversial.

A combination of DNase degradation, renal clearance, and uptake by the liver and spleen are likely to play a role in the clearance of ecDNA in transfusion recipients. Whether the ecDNA is complexed with lipid/proteins or nucleosomes, or is encapsulated within membrane-enclosed particles may influence the ability of DNases to clear ecDNA [4,56–58]. It was shown that DNA bound to nucleosomes can be protected from nuclease cleavage during apoptosis [56,57]. The ecDNA contained in EVs was also shown to be protected from degradation [4,58]. And the clearance of ecDNA may vary with the physiological state of the recipients [59]. Moreover, ecDNA can be recognized by various cell-surface DNA-binding proteins and can be transported into cells for possible degradation to mononucleotides or for transportation into the nucleus [60]. Therefore, the rate of ecDNA uptake by different cells may also affect the rate
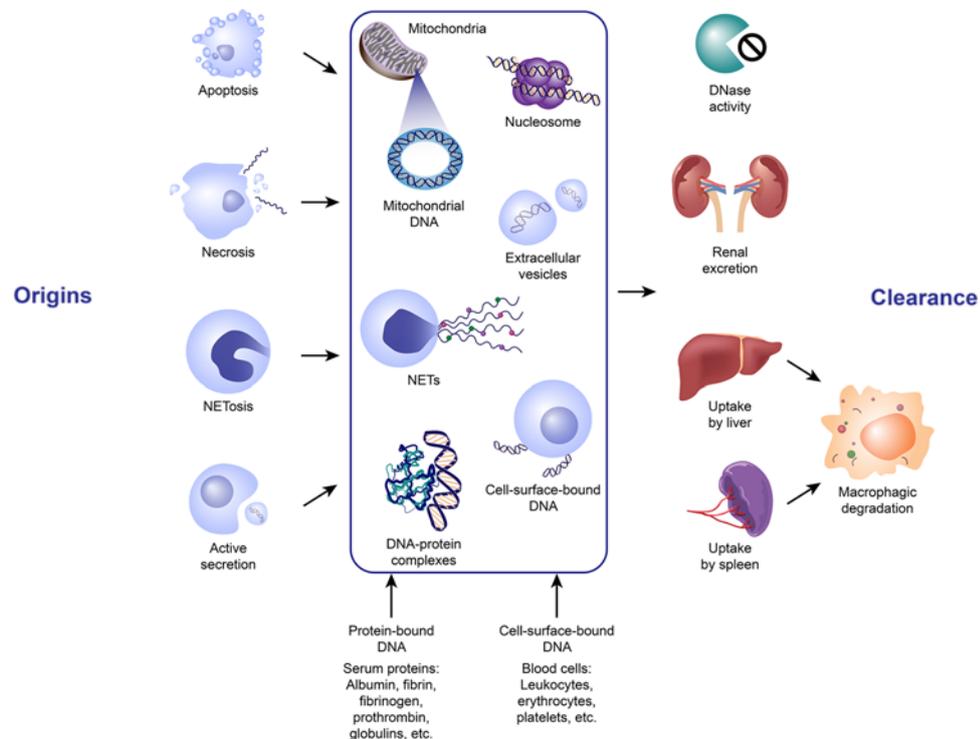
Figure 1. The potential origins and clearance of extracellular DNA in blood products
ecDNA in blood exists in a number of forms, namely histone/DNA complexes or nucleosomes, extracellular vesicles packed DNA, cell-surface-bund DNA, DNA-protein complex, NETs and etc. It can be liberated from the blood cells via different mechanisms, most prominently apoptosis, necrosis, and active secretion, although other forms of cell death and clearance may contribute. Mitochondrial DNA can also be released by these mechanisms. Elimination of ecDNA could be achieved by DNase degradation, renal excretion into the urine or uptake by the liver and spleen followed by macrophagic degradation. Abbreviations: NETs, neutrophil extracellular traps.

of its clearance. The potential origins and clearance of ecDNA in blood products are explained and summarized in Figure 1.

Particular structures relevant to ecDNA in blood products

Depending upon different mechanisms of release, ecDNA is relevant to different complex structures; particulate structures such as EVs or macromolecular structures such as NETs and other less relevant structures that will not be detailed here [3].

EVs

The first discovery of EVs was in 1964 when Chargaff and West, identified ´subcellular factors' in cell-free plasma and showed that these factors played a role in blood clotting [61,62]. In 1967, Wolf [63] confirmed the presence of these subcellular factors using electron microscopy when he was studying the ´platelet dust' that was known to be shed by platelets during storage [64]. Activated or apoptotic cells and platelets in blood products can shed small membrane vesicles, called EVs which are generally identified as MPs in transfusion research [62]. In particular, MPs derived from stored RBCs have been shown to contribute to neutrophil priming and activation, thereby enhancing the inflammatory response observed in patients who receive older RBCUs during transfusion [65].

Recently, the immunomodulatory potential of EVs in blood products has emerged as an important focus of studies in transfusion medicine. Based on the current knowledge, it has been suggested that EVs in stored blood are associated with a number of adverse outcomes such as neutrophil activation and the promotion of an inflammatory response in the recipients [41,65–67]. A study showed that EVs accumulating in RBC products during storage contribute to a strong inflammatory host response in recipients, which depends both on the number of EVs as well as on changes

in the EVs related to storage [41]. Some studies suggested that platelet-derived EVs, such as those that convey mito-chondrial DAMPs, may be a useful biomarker for the prediction of the potential risk of adverse transfusion reactions [68].

NETs

NET formation, or ´NETosis', was first described by Brinkmann et al. [36] in 2004. It occurs when neutrophils are activated by pathogen agents or under particular conditions: NETosis leads to chromatin decondensation, lysis of cell and nuclear membranes, and finally the release of NETs. The principal function of the NETs is believed to be to entrap and kill circulating pathogens.

The composition of NETs was initially widely believed to be predominantly nucDNA; however, under specific stim-ulatory conditions, NETs composed exclusively of mitDNA were demonstrated [69]. The emerging body of evidence suggests that NETs can indeed be composed exclusively or predominantly of mitDNA, which means that NETosis may represent a significant source of extracellular mitDNA in certain inflammatory conditions. In addition to the role of intracellular mitDNA in NET composition, mitDNA may also trigger NET formation as a DAMP after major trauma and with signaling mediated through a TLR9 dependent pathway [70]. Of note, Caudrillier et al. [71] reported that activated platelets induce the formation of NETs in transfusion-related acute lung injury (TRALI), which is the leading cause of death after transfusion therapy.

Potential effects of ecDNA on transfusion

Over the past several decades, the effects of ecDNA on transfusion have rarely been investigated. A report published in 2018 pointed out that cell-free nucleic acids in blood products contained mainly double-stranded DNA (dsDNA) , which has been shown to regulate genes of innate immune response [20]. The total ecDNA encompasses nucDNA and mitDNA. It was found that only mitDNA and bacterial DNA (bacDNA), increased neutrophil viability as a conse-quence of their activation [72]. In another report, mitDNA induced neutrophil matrix metalloproteinase 8 (MMP-8) and MMP-9 release, while nucDNA did not [73]. This evidence suggested that the extracellular mitDNA encom-passed in ecDNA in transfusion may be immunological or proinflammatory. Here, we review the role of extracellular mitDNA in innate immune responses and its relationship with individual adverse reactions in transfusion. This sec-tion will also describe the potential role of transfusion in horizontal gene transfer (HGT).

Extracellular mitDNA in innate immune responses

The particular DNA double-helix structure, the particular motifs of certain sequences and the molecular interac-tions are three factors at the origin of the stimulation of the immune response [74]. In effect, the exposure of cells of the innate immune system to dsDNA could provoke the activation of the genes of the innate immune response [20,74]. This stimulation is at the origin of a strong inflammatory response mediated by the secretion of cytokines. The abundant nucleic acid receptors in the cells play an important role in the innate immune system, which employs them in response to DNA within the hosts [75,76]. How exactly mitDNA may mediate their immunological role in transfusion is unknown, but different studies in some other areas of medicine rather than transfusion provided mech-anistic insights. MitDNA has been shown to bind to Toll-like receptors (TLRs) or nucleotide oligomerization domain (NOD)-like receptors (NLRs) and more recently it has been shown to be linked with the stimulator of interferon genes (STING) pathway, thus providing distinct mechanisms potentially leading to immunological and inflammatory re-sponses [77,78].

TLRs

Members of the TLR family are major pattern recognition receptors (PRRs) in cells [79], which are implicated in the innate immune response and are present in immune cells such as dendritic cells (DCs), neutrophils (PMNs), and macrophages (M∅) [80–83]. Zhang et al. [73,84] found that mitDNA activates neutrophil p38 mitogen-activated protein kinase (MAPK) through TLR9 with release of MMP-8, a proinflammatory cytokine, leading to severe in-flammation in mouse lungs. Gu et al. [85] also found that intratracheal administration of mitDNA provokes lung inflammation through TLR9-p38 MAPK. In addition to MMP-8, mitDNA has been reported to trigger the activation of the nuclear factor kappa B (NFκB) pathway via TLR9 [86], resulting in up-regulation of proinflammatory cytokine production including TNF-α [87], IL-1β [88], and IL-6 [77].

NLRs

The NLRs are another major PRRs in the innate immune system. Of the NLRs, the NLR pyrin domain 3 (NLRP3) inflammasome is the most widely studied mainly due to its affinity for a wide variety of ligands [78]. The NLRP3 inflammasomes are targets of mitDNA, leading to the activation of caspase-1 in the inflammasome complex. Caspase-1 cleaves pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18 [78], which is a potent pyrogen that elicits a strong proinflammatory response [89]. Shimada et al. [90] showed that it is the oxidized form of mitDNA that confers the inflammatogenic potential to mitDNA, which could directly bind NLRP3 to activate the inflammasome. Interestingly, the genetic deletion of NLRP3 and caspase-1 results in less mitDNA release [91]. Conversely, NLRP3 inflammasome formation releases mitDNA [91]. This suggests a positive feedback loop, in which activation of the NLRP3 inflammasome by oxidized mitDNA further promotes mitDNA release.

STING pathway

What's more, mitDNA has the ability to stimulate the innate immune system through stimulation of the interferon genes (STING) pathway, resulting in interferon (IFN) release. The STING pathway was recently mechanistically dissected to reveal an intricate relationship demonstrating how mitDNA triggers interferon release [92]. The study showed that through the depletion of mitochondrial transcription factor A (TFAM), mitDNA stability was disturbed, causing enlargement of the mitochondrial nucleoid. Subsequently, fragmented mitDNA was released, activating peri-mitochondrial cyclic GMP-AMP synthase (cGAS) causing increased cGAMP formation. The second messenger cGAMP then activates the endoplasmic-reticulum-bound STING pathway which ultimately activates TANK-binding kinase 1 (TBK1) and results in IFN I and other interferon-stimulated genes [92].

To conclude, mitDNA participates in a variety of innate immune pathways, including the mitDNA–TLR9–NFκB axis, mitDNA–NLRP3–caspase1 pathway and mitDNA–cGAS–cGAMP–STING signaling (as summarized in Figure 2). The extracellular mitDNA appears to be a potent danger signal that could be recognized by the innate immune system and modulate the inflammatory response [93].

Extracellular mitDNA and adverse transfusion reactions

The establishment of strict procedures to avoid the transfusion of microbial components has greatly reduced the transmission of infections in recipients. However, sterile inflammation and organ injury in transfused recipients still occur in the absence of any apparent infectious agents [94]. TRALI is the leading cause of transfusion-related death and is initiated by soluble mediators in plasma [95]. Nonhemolytic transfusion reactions (NHTRs) are more frequent [96,97]. In addition, the transfusion of the plasma fraction (without cells or platelets) is sufficient to trigger these reactions [98].

Extracellular mitDNA falls into the category of DAMPs, which are known immune mediators associated with inflammation [22,23]. Studies have shown the potential contribution of extracellular mitDNA to adverse transfusion reactions [14,18,19,42]. Boudreau et al. [42] quantified mitDNA in the extracellular milieu of PCs that has induced adverse reactions and compared the levels to those within samples that were transfused without incidents. Interestingly, they confirmed that significantly higher levels of extracellular mitDNA correlated with adverse reactions [42]. Yasui et al. [18] further confirmed that elevated levels of mitDNA were present in PCs that induced NHTRs in platelet transfusion. Cognasse et al. [15] found that extracellular mitDNA did not correlate with cytokine levels and might be an independent risk factor in PC transfusion-linked inflammation.

TRALI is defined as new acute lung injury that develops during or within 6 h of receiving a transfusion of any blood product and progresses to a non-resolving severe respiratory failure such as acute respiratory distress syndrome (ARDS) [99]. Simmons et al. [19] found that the levels of mitDNA present in FFP and PCs correlate well with the levels of mitDNA in the serum of post-transfusion patients and are associated with a higher risk of ARDS. Mitochondrial DAMPs, including mitDNA, have been shown to potentiate inflammatory lung injury when introduced into healthy rats in a landmark paper by Zhang et al. [84]. In addition, mitDNA DAMPs could increase the EC permeability that was observed in acute lung injury and ARDS [100].

Extracellular mitDNA DAMPs present in transfusion products may act as a potential effector of TRALI, as hypothesized by Lee et al. [14]. According to current evidence as mentioned before, the working hypothesis of the involvement of extracellular mitDNA in the adverse transfusion reaction is summarized in Figure 3.

The potential role of transfusion in HGT

During the 1950s to 1970s, blood transfusion experiments were found to alter the hereditary traits of the offspring in poultry [101–103]. As early as 1871, Galton [104] carried out intervarietal blood transfusion experiments among rabbits, but failed to induce heritable changes. Later, Sopikov [103] re-established the use of this method to analyse
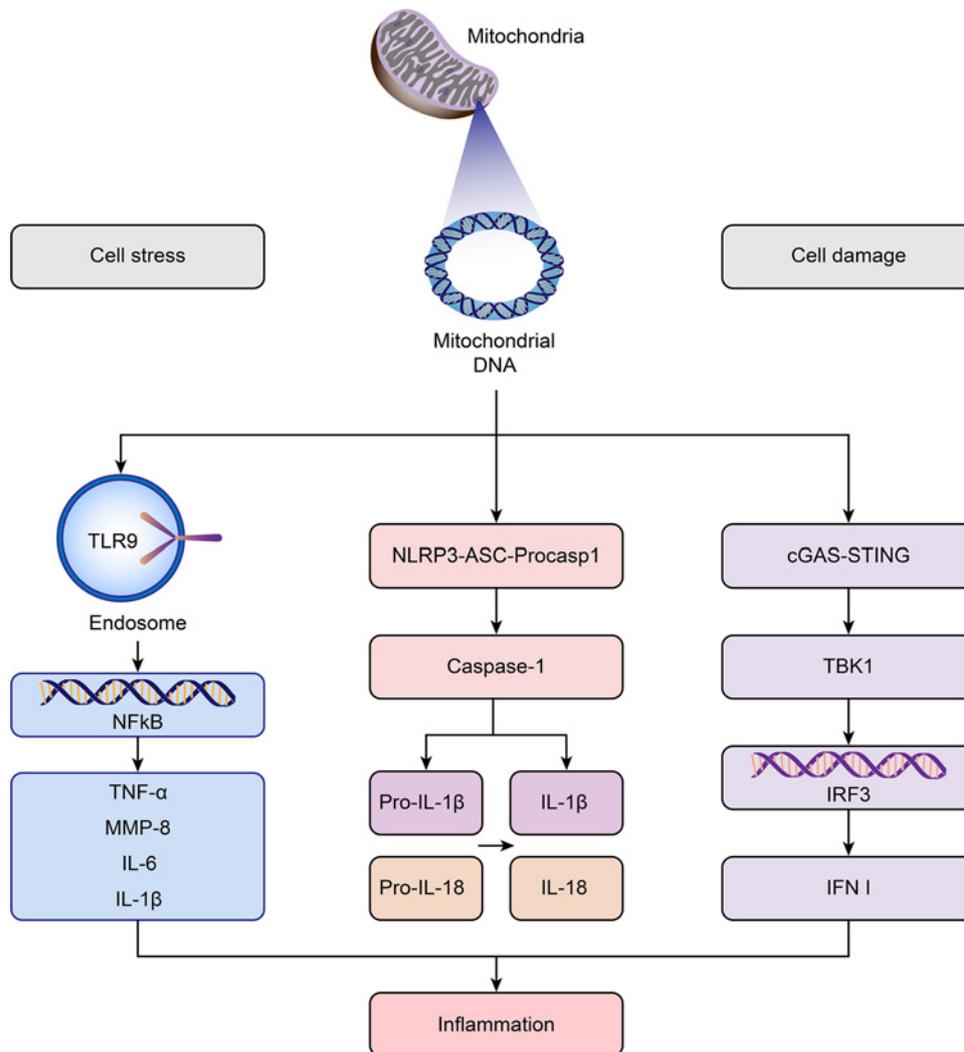
Figure 2. Innate immune pathways activated by mitochondrial DNA
Upon cell stress or cell damage, mtDNA escaped from mitochondria engages in the activation of multiple innate immune pathways, including mitDNA–TLR9–NFκB axis, mitDNA–NLRP3–caspase1 pathway and mitDNA–cGAS–cGAMP–STING signaling, thereby igniting inflammation. Abbreviations: IFN, interferon; IRF, interferon regulatory factor; MMP, matrix metalloproteinase; NF-κB, nuclear transcription factor kappa B; NLRP3, Nod-like receptor pyrin domain containing 3; TBK1, TANK-binding kinase 1; TLR9, Toll-like receptor 9; TNF, tumor necrosis factor.

the effect of blood transfusion on hereditary traits: repeated transfusion of the blood of Black Australorp roosters to White Leghorn hens, and subsequent mating of these hens with White Leghorn roosters yielded progeny with modified inheritance. In exchanged donor and recipient roles, similar results were obtained [103]. Afterward, Sopikov's observations were confirmed not only by many Soviet researchers [105,106], but also by investigators in other countries [107,108].

Subsequently, increasing evidence that DNA injection could induce heritable changes emerged [109,110]. For instance, DNA extracted from the Khaki Campbell was exclusively used to induce heritable changes in the Pekin duck in the study of Benoit et al. [111] Hereditary modifications of morphological characteristics in ducks as a result of the injection of DNA and RNA from other breeds of ducks were also reported. It is clearly possible that when DNA-rich avian blood cells are transfused to other members of the same species, the transferred DNA can be expressed. Currently, many researchers have detected ecDNA in the plasma of donor-derived blood products, as listed before in Table 1.
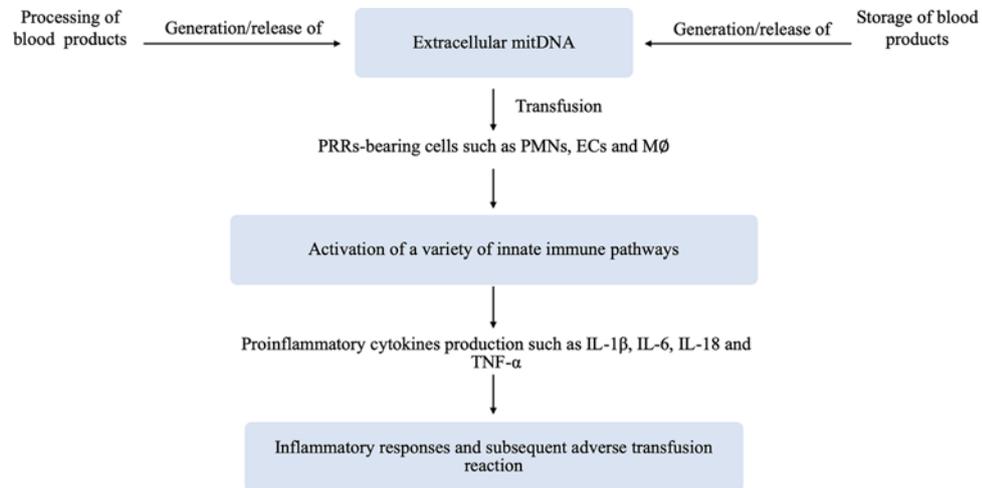
Figure 3. Working hypothesis of the involvement of extracellular mitDNA in the adverse transfusion reaction
Briefly, the released/generated extracellular mitDNA in blood products during processing and storage time, which can be sensed by PRR-bearing cells of recipients (i.e. PMNs, ECs and M), is capable of activating a variety of innate immune pathways. Subsequent production of cytokines (i.e. IL-1β, IL-6, IL-18, and TNF-α) together with other proinflammatory mediator substances, thereby inducing inflammatory responses leading to adverse transfusion reactions in recipients. Abbreviations: EC, endothelial cell; INF, interferon; M, macrophages; PMN, neutrophil; PRR, pattern recognition receptor; TNF, tumor necrosis factor.

Landman [112] suggested that the genes of organisms could be divided into two groups: most were inherited 'vertically' from ancestors, but some were acquired 'horizontally'. HGT is defined as the transfer of genes between organisms in a fashion other than that, which is found through traditional reproduction [113]. The transfer of biological sources, including blood transfusion products, from donor to recipient will likely result in the presence of donor ecDNA in the recipient's blood circulation. This raises concern over the potential of ecDNA to transfer the genetic or epigenetic information of clinical risk factors (genetically related illness, mutations or pharmaceutically induced adverse effects) from donors to recipients.

Stroun and Anker [114] have been suggested that nucleic acids are released by living cells and circulate throughout the whole organism. Newly synthesized, actively released ecDNA can translocate to neighbouring and remote parts of the body, enter cells and alter their biology [115,116]. Genometastasis experiments have shown that ecDNA in the plasma of cancer patients can indeed transfer oncogenic information to susceptible cells [24]. Thierry et al. [3] illustrated that many structures carrying ecDNA could be involved in this genometastasis. However, this implies that ecDNA in the plasma of blood transfusion products might be capable of transferring an activated human oncogene to recipients during blood transfusions. In addition, many pharmaceutical and botanical compounds have been found to induce epigenetic alterations [117–119]. It is, therefore, possible that the ecDNA in donors using medication can contain these pharmaceutically induced epigenetic alterations and that these alterations can be transferred to recipients during blood transfusions.

Moreover, it was shown that microchimerism might be related to real or potential health implications in autoimmune diseases, graft-versus-host reactions, and transfusion complications [120]. Microchimerism refers to a small number of cells or DNA in one individual that is derived from another genetically distinct individual [121]. Regarding blood transfusion, microchimerism from non-leukoreduced cellular blood products has been found to persist from months to years after transfusion in traumatically injured patients [122]. Transfusion-associated microchimerism appears to be an identified complication of blood transfusion [123,124]. Whether patients with transfusion-associated microchimerism have consequent adverse health effects, such as transfusion associated graft-versus-host disease, is still under investigation.

Conclusion

Blood transfusion is the intravascular transfer of blood products into a recipient. A certain amount of DNA encompassed mitDNA was demonstrated to be present in the extracellular milieu of blood products for transfusion. This raises the question of whether such ecDNA carries a risk. We provide a comprehensive review of the potential origins, clearance mechanisms and relevant structures of ecDNA in blood products in order to familiarize researchers

with previous works and to show that there are still many unanswered questions relating to the nature and biological functions of ecDNA in blood products. Our review shows that ecDNA especially mitDNA participates in a variety of innate immune pathways and relevant to some adverse transfusion reactions. In addition, it should be concerned that ecDNA might be capable of transferring some genetic or epigenetic information of clinical risk factors from donors to recipients during blood transfusion. Notably, methods of isolation and quantification of ecDNA are crucial when analysing data from different reports. At this time, there is a lack of uniformity in the methods of ecDNA extraction and quantification, which need to be standardized. What's more, the detailed clearance mechanisms of these ecDNA in recipients administrated via transfusion also require further research, including (i) the activity of DNase, (ii) the rate of renal excretion into urine, and (iii) the rate of uptake by the liver and spleen. However, this remains an under-explored field in transfusion, and more insights will likely emerge in the near future. Hopefully, the articles presented herein will stimulate researchers to give serious consideration to the potential harmful effects of ecDNA, especially extracellular mitDNA, on transfusion.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

Abbreviations

ARDS, acute respiratory distress syndrome; bacDNA, bacterial DNA; cfDNA, cell-free DNA; cGAS, cyclic GMP-AMP synthase; DAMP, damage-associated molecular pattern; DC, dendritic cell; dsDNA, double-stranded DNA; ecDNA, extracellular DNA; EV, extracellular vehicle; FFP, fresh frozen plasma; HGT, horizontal gene transfer; IFN, interferon; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-18, interleukin-18; M$\varnothing$, macrophages; MAPK, mitogen-activated protein kinase; mitDNA, mitochondrial DNA; MMP-8/9, metalloproteinase-8/9; MP, microparticle; NET, neutrophil extracellular trap; NF-κB, nuclear factor kappa B; NHTR, nonhemolytic transfusion reaction; NLRP3, NLR pyrin domain 3; NLR, nucleotide oligomerization domain (NOD)-like receptor; nucDNA, nuclear DNA; PC, platelet concentrate; PMN, neutrophil; PRR, pattern recognition receptor; RBC, red blood cell; RBCU, red blood cell unit; sPLA2-IIA, secretory phospholipase A2 IIA; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TFAM, mitochondrial transcription factor A; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; TRALI, transfusion-related acute lung injury.

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