Involvement of Human Leukocyte Antigen Class II Antibody in Pathogenesis of Transfusion-Related Acute Lung Injury (TRALI): Vascular Permeability Enhancement

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Abstract: Vascular endothelial cells regulate the passage of fluids, solutes, and cells from the vascular space to the tissues. Disruption of vascular integrity is involved in the pathogenesis of inflammatory diseases including transfusion-related acute lung injury (TRALI), a most severe nonhemolytic transfusion reaction with symptoms such as dyspnea and/or hypotension and fever. Pulmonary edema, due to increased vascular permeability for macromolecules and plasma, is a hallmark of TRALI. The mortality rate of TRALI ranges from 5 to 10%. While donor antibodies (Abs) against human leukocyte antigen (HLA) class I and granulocytes are regarded as causative factors, various clinical studies have demonstrated the roles of anti-HLA class II-Ab on the etiology of TRALI, although the detailed mechanisms have not been clarified. Over several years we have investigated to clarify the underlying mechanism by which anti-HLA class II Abs cause an increase in endothelial permeability. In this review, we show that anti-HLA class II Ab generates proinflammatory cytokines and chemokines from HLA class II positive mononuclear cells of peripheral blood in an FcγR-dependent manner. As a result, the produced interleukin-1β and tumor necrosis factor-α lead to increased endothelial permeability *via* the nuclear factor-κB pathway but not apoptosis of endothelial cells. These findings provide a better understanding of the roles of anti-HLA class II Ab in the etiology of TRALI.

Keywords: Transfusion-related acute lung injury (TRALI), antibody, HLA class II, cytokine, chemokine, vascular permeability, endothelial cells.

INTRODUCTION

Vascular endothelial cells form a continuous cell layer between the walls of blood vessels and regulate the passage of fluids, solutes, and cells from the vascular space to the tissues [1]. Disruption of vascular integrity is involved in the pathogenesis of inflammatory diseases. Transfusion-related acute lung injury (TRALI) is one of the most severe nonhemolytic transfusion reactions of blood transfusion [2]. Typically, the patient develops symptoms such as dyspnea and/or hypotension and fever, and the chest radiograph shows bilateral infiltrates composed of pulmonary edema, due to increased vascular permeability for macromolecules and plasma, which is a hallmark of TRALI [3-6]. The symptoms of TRALI are recognized as being indistinguishable from acute lung injury (ALI) / acute respiratory distress syndrome (ARDS) [7]; however, the mortality rate of TRALI, ranging from 5 to 10%, is less than that of ARDS, suggesting that there may be certain differences in the pathologic mechanisms which play roles in TRALI and ARDS.

In the literature dating back to the first TRALI description by Popovsky and Moore [3] in 1985, antibodies (Abs) to granulocytes or lymphocytes were shown in the blood of 89% of implicated donors. Furthermore, a recent systematic review revealed that leukocyte Abs contributed to 80% of all TRALI cases [8]. Among leukocyte Abs, anti-HLA class I Ab is the most frequently associated with 58 fatal cases of TRALI [4]. Meanwhile, it was reported that Abs to HLA class II might also be associated with TRALI [9]. Kopko *et al.* [9] showed anti-HLA class II Abs in 7 of 11 TRALI cases. In 5 patients, anti-HLA class II Abs were detected in combination with anti-HLA class I Abs, and in two cases anti-HLA class II Abs only were detected. Since then, reports of TRALI cases in which anti-HLA class II Abs were the sole Abs detected have been accumulating [10-15]; however, the detailed mechanisms by which anti-HLA class II Abs play roles in the pathogenesis of TRALI have not been clarified. In this review, based upon our results along with the literature, we will present the underlying mechanism by which anti-HLA class II Abs cause an increase in endothelial permeability and the implication for TRALI.

PROPOSED MECHANISMS OF PULMONARY EDEMA IN TRALI

It is well recognized that neutrophils are central to the pathogenesis of ALI/ARDS. Similarly, neutrophils and endothelial cells of the lung capillaries are considered to play key roles in the pathogenesis of Ab-mediated TRALI in the following scenario. In the early stage of TRALI, Abs against human neutrophil antigen (HNA) [16-18] or HLA class I [3, 17] in either donor or recipient plasma trigger or potentiate the activation of neutrophils. The activated neutrophils come into close contact with the pulmonary microvasculature,

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which results in firm neutrophil adhesion and retention. The neutrophils are immobilized in the alveolar capillaries, and release their microbicidal arsenal, thereby inducing endothelial damage and capillary leak. Proteinaceous fluid moves from the vessels into the air spaces, culminating in acute pulmonary edema.

Several reports have described the relationship between neutrophil activation *via* anti-HNA Abs and the development of TRALI [19, 20]. Perfusion of anti-HNA-3a Ab, HNA-3a-positive human neutrophils, and rabbit plasma (as a complement source) into *ex vivo* rabbit lung caused severe lung edema [19]. In this *ex vivo* rabbit lung model of TRALI, neutrophils stimulated with an anti-HNA-3a Ab produced arachidonic acid metabolites, reactive oxygen species, and elastase, and these mediators are suggested to play key roles in the induction of lung microvascular disturbances [19]. Similarly, vascular leakage was shown in an *ex vivo* rat lung model with perfusion of anti-HNA-2a Ab and HNA-2a-positive human neutrophils [20]. In this case, Sachs *et al.* [20] suggested that the production of reactive oxygen species from neutrophils activated by the Ab is a central process in endothelial damage and capillary leakage.

On the other hand, Silliman and colleagues [21] proposed a two-event pathogenesis for TRALI. According to this theory, the first event is activation of the patient's endothelial cells and neutrophils by underlying clinical conditions such as surgery, infection, or other inflammatory responses. The second event is the infusion of anti-HNA Abs or biologic response modifiers, which augments the activation of neutrophils, resulting in endothelial damage and capillary leakage. Coculture of bacterial lipopolysaccharide (LPS)-activated human neutrophils and LPS-activated pulmonary endothelial cells in the presence of possible causative factors of TRALI in blood components (biologically active lipid [22], soluble CD40 ligand [23], or anti-HNA-3a Ab [24]) induced cytotoxicity in endothelial cells. From these aspects, it is convincing that production of reactive oxygen species from neutrophils plays a causative role in vascular leakage in TRALI [5, 21].

Although the Abs against HLA class I and class II have been shown to be associated with TRALI, only a few reports have described the mechanism of anti-HLA class I or II Ab-related TRALI. To manifest the pulmonary symptoms in TRALI, intrapulmonary macrophages *via* anti-HLA-class II Ab might be required. The immunohistochemical study of a TRALI case having both anti-HLA-class I and class II Abs demonstrated that HLA class II antigen exposure was not observed at the vascular endothelium or intravascular white blood cells, whereas strong expression was detected on alveolar macrophages, suggesting that these cells might contribute to magnify the pathogenesis of this symptom [25]. However, this possible activation of pulmonary macrophages may be a secondary phenomenon, because Abs do not usually pass through an intact endothelial barrier in the pulmonary wall. Therefore, Kao *et al.* [25] proposed that the initial damage by another mechanism (e.g. sequestration of neutrophils by anti-HLA-class I Abs) would be required for the entry of anti-HLA-class II Abs. On the other hand, Flesch and Neppert [10] showed that pulmonary edema developed in a healthy volunteer followed by complete disappearance of monocytes from the peripheral blood after infusion of plasma containing HLA alloantibody alone. This finding implies that monocytes in circulation may be an initial target for alloantibody against HLA class II.

In fact, it has been demonstrated that the anti-HLA-class I and anti-HLA-class II Abs implicated in TRALI activated monocytes expressing the corresponding antigens and increased intracellular concentrations of tumor necrosis factor (TNF-α), interleukin (IL)-1β and extracellular levels of tissue factor *in vitro* [26]. Coculture of an anti-HLA class II Ab that was implicated in TRALI, monocytes with corresponding antigens, and pulmonary endothelial cells has also been shown to induce the apoptosis of endothelial cells [27]. Leukotriene B_4 and TNF-α released into the supernatant of the coculture were speculated to cause endothelial apoptosis [27, 28].

Both TNF-α and IL-1β induce an increase in endothelial permeability [29-31]; however, direct evidence that such cytokines generated from monocytes stimulated with anti-HLA class II Abs actually cause an increase in endothelial permeability to large molecules is missing so far. Thus, the investigation of whether anti-HLA class II Ab-induced leukocyte activation and the resulting mediator release affect endothelial permeability to large molecules may lead to better understanding of the mechanism of pulmonary edema in TRALI.

EXPRESSION OF INFLAMMATORY CYTOKINES AND CHEMOKINES BY PBMNCS TREATED WITH ANTI-HLA-DR AB-CONTAINING PLASMA *IN VITRO*

We experienced a case of severe nonhemolytic transfusion reactions in a patient who had received a platelet apheresis unit because of thrombocytopenia after chemotherapy [32]. The symptoms consisted of chills, tachycardia, dyspnea, lumbar and abdominal pain, fever and audible coarse crackles. SaO_2 dropped to 70% and transfusion was immediately discontinued. The symptoms disappeared 5 hr after oxygen treatment and intravenous administration of corticosteroid and aminophyrine [32].

The recipient had HLA-DR4 and DR13, while the donor was homozygous for HLA-DR15. Screening test of the patient and donor sera revealed the presence of multi-specific Abs against HLA-DR antigen, including the recipient's HLA-DR antigen (i.e. DR13) in the donor blood. There were no Abs against HLA-class I antigen [32]. The cross-match between the recipient's lymphocytes (DR13, DR15) and the donor serum was also positive. However, the donor serum did not react with either granulocytes or platelets of the patient. In addition, it did not react with the plasma proteins tested. The donor serum did not react with healthy volunteers with HLA- DR (1, 15) [32]. Based on these laboratory tests, this donor plasma was referred to as "anti-HLA-DR Ab-containing plasma" [32].

The observation that anti-HLA-DR Abs, detected in donor blood, have the ability to activate blood cells expressing the corresponding HLA-DR antigen even *in vitro* supports the association of anti-HLA-DR Abs with the transfusion reaction. In fact, incubation of this anti-HLA-DR Ab-

containing plasma with peripheral blood mononuclear cells (PBMNCs) expressing HLA-DR13 for 3 hr significantly upregulated the mRNA expression of inflammatory cytokines in comparison to pooled AB plasma which have no Abs against HLA antigens and plasma proteins [33]. As shown in Table **1**, the upregulated cytokines and chemokines were IL-1β, IL-6, TNF-α growth-related gene product-α(GRO-α)/CXCL1, IL-8/CXCL8, inducible protein-10/CXCL-10, monocyte chemoattractant protein-1 (MCP-1)/CCL2 and monocyte inflammatory protein-1α /CCL3 [33].

PRODUCTION OF INFLAMMATORY CYTOKINES AND CHEMOKINES BY PBMNCS TREATED WITH ANTI-HLA-DR AB-CONTAINING PLASMA *IN VITRO*

In accordance with the results of mRNA expression, the donor plasma implicated in this transfusion reaction had the ability to activate PBMNCs derived from two healthy subjects who possessed the relevant HLA-DR antigens, including DR13, leading to the *in vitro* production of inflammatory cytokines (i.e. IL-1β, IL-6, and TNF-α), and neutrophil attractant chemokines (i.e. IL-8 and GRO-α) (Fig. **1**) [32].

In contrast, markedly low levels of cytokines and chemokines were produced in response to anti-HLA-DR Ab-containing plasma in PBMNCs from two subjects with irrelevant HLA-DR antigens [32]. In addition, the pooled AB plasma did not affect the production of these inflammatory cytokines in any subjects. LPS is a well-known activator of monocytes/macrophages, causing production of inflammatory cytokines and chemokines [34]. PBMNCs from all subjects, including two with irrelevant HLA-DR antigens, responded to LPS similarly, producing a high level of each cytokine or chemokine [32]. From these results, we can conclude that anti-HLA-DR Ab-containing plasma has the ability to produce inflammatory cytokines and chemokines by PBMNCs in a cognate antigen-antibody interaction [32].

According to reports of the pathology of TRALI as described above, neutrophils are sequestered in the lung by an unknown mechanism. Thus, the findings that donor plasma containing anti-HLA-DR Abs induced the production of chemokines, IL-8, and GRO-α is very interesting, because these chemokines are powerful activators of human neutrophils, inducing chemotaxis, exocytosis, and respiratory burst *in vitro* and neutrophil sequestration *in vivo* [35].

REQUIREMENT OF FCγR FOR PRODUCTION OF INFLAMMATORY CYTOKINES BY PBMNCS TREATED WITH ANTI-HLA-DR AB-CONTAINING PLASMA *IN VITRO*

The requirement of FcγR for the production of inflammatory cytokines by PBMNCs in response to anti-HLA-DR Ab-containing plasma is an important aspect to understand the activation mechanism of PBMNCs by anti-HLA-DR Abs. Studies using monoclonal Abs (mAbs) against human FcγRs show that FcγRII (CD32) mAb (clone 7.3) and anti-FcγRIII (CD16) mAb (clone 3G8) substantially reduced the production of IL-1β and IL-6 by PBMNCs in response to anti-HLA-DR Ab-containing plasma, while anti-FcγRI (CD64) mAb (clone 32.2) had no significant inhibitory effect on these two cytokines [32]. These results suggest that FcγRII (CD32) and/or FcγRIII (CD16) are involved in the anti-HLA-DR Ab-containing plasma–triggered production of inflammatory cytokines by relevant PBMNCs [32].

FcγRII is expressed on monocytes/macrophages, and FcγRIII is expressed on macrophages and a subpopulation of monocytes [36, 37]. FcγRIII-positive monocytes are reported to produce inflammatory cytokines [36, 37]; therefore, a subpopulation of FcγRIII-positive monocytes seems to be involved in the production of inflammatory cytokines in response to the anti-HLA-DR Ab-containing plasma. The precise role of the two FcγRs in this process remains to be solved.

ENHANCED PERMEABILITY OF ENDOTHELIAL MONOLAYERS BY THE SUPERNATANT OF PBMNCS STIMULATED WITH ANTI-HLA-DR AB-CONTAINING PLASMA *IN VITRO*

Confluent endothelial cells, such as human umbilical vein endothelial cells (HUVECs), are widely used as an *in vitro* model for the study of permeability changes to macromolecules [38-41]. The supernatants from cross-match-positive PBMNCs treated with anti-HLA-DR Ab-containing

Table 1. Upregulation of mRNA Expression of PBMNCs Stimulated by the Anti-HLADR Ab-Containing Plasma

	mRNA	Fold Increase (vs. Control Plasma)
Cytokine	IL-1β	2.58
	IL-6	7.45
	TNF-α	4.62
Chemokine	GRO-α(CXCL1)	3.84
	IL-8 (CXCL8)	2.35
	IP-10 (CXCL10)	8.01
	MCP-1 (CCL2)	5.28
	MIP-1α(CCL3)	4.01

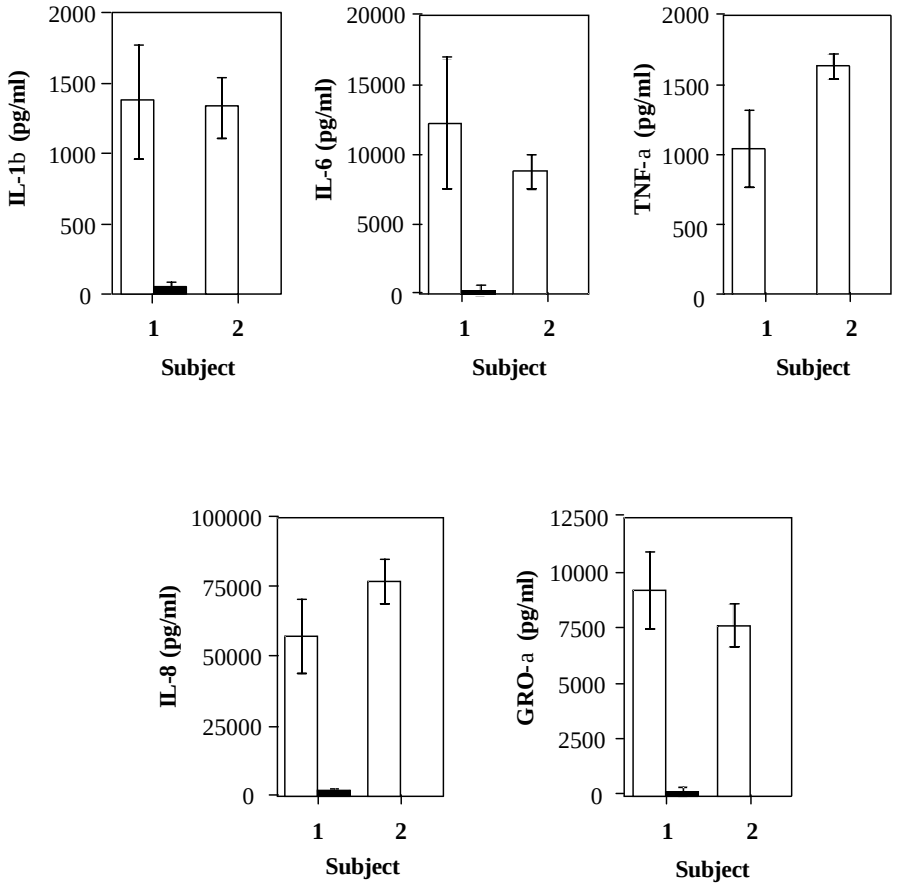

Fig. (1). Production of IL-1β, IL-6, TNF-α, IL-8, and GRO-α by PBMNCs of two healthy subjects having the HLA-DR13 antigen in response to anti-HLA-DR Ab-containing plasma. PBMNCs derived from two subjects having HLA-DR13 antigen were incubated with 20% (v/v) of anti-HLA-DR Ab-containing plasma (open column) or pooled AB plasma (filled column) for 20 hr. Reproduced from ref. [32] with the permission of the copyright holder of the Wiley-Blackwell.

plasma for 20 hr caused a significant enhancement of HUVEC permeability to dextran when compared to PBMNC supernatants obtained by incubation with AB plasma, whereas supernatants from cross-match-negative PBMNCs treated with anti-HLA-DR Ab-containing plasma had no significant effect on HUVEC permeability [42] (Fig. **2**). It has been shown that the addition of plasma obtained from LPS-treated whole blood to monolayers of cultured HUVEC increases its permeability [30]. In accordance with this report, the supernatant from PBMNCs stimulated with LPS showed similar increases in HUVEC permeability in all subjects, including two with irrelevant HLA-DR antigens [42] (Fig. **2**).

The increased permeability of HUVEC monolayers by activated PBMNC supernatants was also observed in human lung microvascular endothelial cells (HMVECs) [42]. From these results, it can be concluded that the supernatants of PBMNCs stimulated with anti-HLA-DR Ab-containing plasma have the ability to increase endothelial permeability in the corresponding antigen-antibody combinations [42].

A time-course study showed differences between HUVECs and HMVECs in the permeability response of endothelial cells to the activated PBMNC supernatants. At least 3 hr of exposure of PBMNCs to anti-HLA-DR Ab-containing plasma was required to produce a supernatant that exerts an increase in HUVEC permeability, while in HMVECs, significant increases of permeability were induced by supernatants of PBMNCs treated with anti-HLA-DR Ab-containing plasma for 20 hr [42]. In general, endothelial cells have heterogeneity based on the organ or tissue type and the size or type of blood vessels from which the endothelial cells are isolated [1, 43-46]. Comparison of the permeability between HUVEC and HMVEC monolayers under stimulation with a mix of cytokines (TNF-α, IL-1β, and interferon-γ) showed a slightly earlier increase in HUVEC permeability to albumin than in HMVEC [47]. The tissue-specific gene expression [48] and differences in sensitivity to cytokines might explain the different permeability between HUVEC and HMVEC in response to the supernatant of PBMNCs stimulated with anti-HLA-DR Ab-containing plasma.

MECHANISM OF THE PERMEABILITY ENHANCEMENT INDUCED BY THE SUPERNATANT OF PBMNCS STIMULATED WITH ANTI-HLA-DR AB-CONTAINING PLASMA

Inflammatory cytokines, including IL-1β, TNF-α, IL-6, and IL-8 have been shown to be mediators of the increased permeability of endothelial cells [29-31, 49-51]. In fact, as described above, the anti-HLA-DR Ab-containing plasma can produce all of these cytokines from PBMNCs in corre-

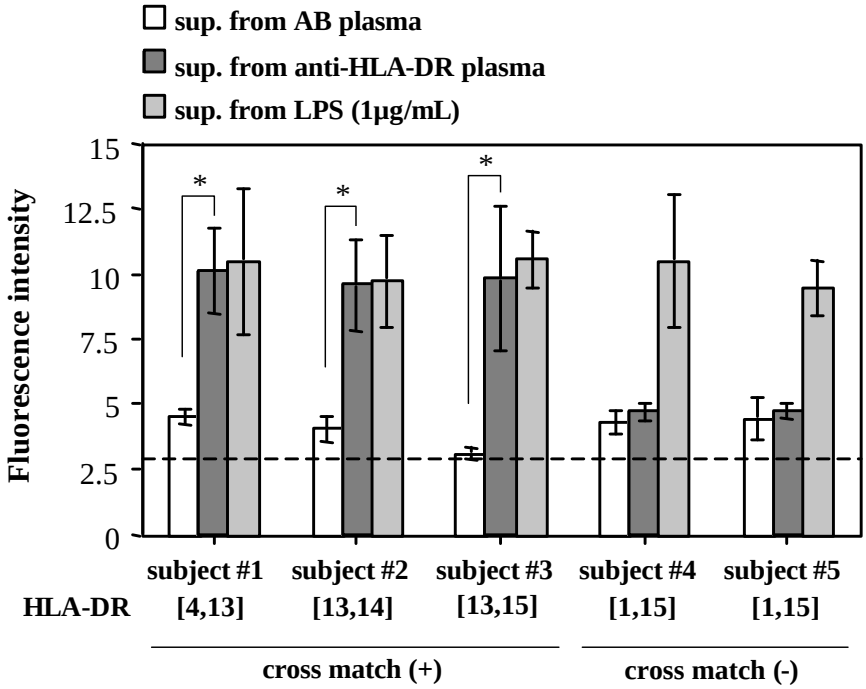

Fig. (2). Effect of activated PBMNC supernatant on the permeability of HUVEC monolayers. Cross-match-positive (n=3) or -negative (n=2) PBMNCs were incubated with AB plasma or anti-HLA-DR plasma for 20 hr. HUVECs in each transwell were exposed to PBMNC supernatant (sup) for 6 hr in order to measure the induction of endothelial permeability. The dotted line shows fluorescence intensity, which represents the spontaneous permeability of HUVEC monolayers incubated with culture medium (2.84 ± 0.85: mean ± SD, 7 separate experiments). The results of activated PBMNC supernatants are expressed as mean ± SD of duplicate determinations of three separate experiments for each subject. *p<0.05. Reproduced from ref. [42] with the permission of the copyright holder of the Wiley-Blackwell.

sponding antigen-antibody pairs [42]. Among neutralizing Abs against these cytokines, simultaneous addition of neutralizing Abs against both TNF-α and IL-1β to the supernatant from PBMNCs stimulated by the anti-HLA-DR Ab-containing plasma almost completely abrogated the enhanced permeability of both HUVECs and HMVECs, showing that both TNF-α and IL-1β produced by PBMNCs are primarily responsible for the enhancement of permeability of both endothelial cell types [42].

Because both TNF-α and IL-1β are major determinants of the increased permeability of HUVECs and HMVECs induced by the supernatant of PBMNCs stimulated with the anti-HLA-DR Ab-containing plasma, the following two aspects can be considered: (1) TNF-α and IL-1β increase permeability *via* activation of their downstream target nuclear factor-κB (NF-κB), a cytokine-inducible transcription factor that regulates various proinflammatory gene products [31] and (2) cytokine-mediated endothelial apoptosis contributes to the disruption of vascular integrity as has been suggested in investigations of various cardiovascular diseases [52]. Pharmacological studies using inhibitors show that incubation of HUVECs with pyrrolidine dithiocarbamate [53], an inhibitor of the NF-κB pathway, almost completely inhibited the increase in endothelial permeability induced by the supernatants stimulated by the anti-HLA-DR-Ab-containing plasma [42]. In contrast, two different types of apoptosis inhibitors (N-acetylcysteine [54], antioxidant, and Z-VAD-fmk [55], a caspase inhibitor) had no effect [42]. These findings indicate that NF-κB activation by TNF-α and IL-1β exerts a key mechanism in the activated PBMNC supernatant–induced permeability increase in HUVECs and possibly HMVECs [42]. One of the NF-κB-induced events *via* TNF-α is the upregulation of adhesion molecules ICAM-1 and E-selectin [56-59]. This promotes neutrophil adhesion to endothelial cells and production of reactive oxygen species [59-61], resulting in enhanced endothelial permeability; however, the *in vitro* model used in our study [42] did not contain neutrophils; therefore, other mechanisms must be operating. An increase in cytosolic Ca^{2+} is the pivotal signal that precedes endothelial cell shape change and the opening of interendothelial junctions that causes barrier dysfunction [62-64]. Proteins of the transient receptor potential channel family are nonselective cation channels present in endothelial cells that increase intracellular Ca^{2+} [64-66]. TNF-α has been shown to express this transient receptor potential channel-1 through an NF-κB-dependent pathway in endothelial cells, thereby augmenting the increase in endothelial permeability [66-68]. Thus, whether the upregulation of transient receptor potential channel-1on HUVEC in our condition might have occurred remains to be elucidated.

TNF-α exerts proapoptotic effects on numerous tumor cells or virally infected cells; however, most normal cells are resistant to killing by TNF-α *via* anti-apoptotic TNF-α-inducible signals [69, 70] unless mRNA or protein synthesis is blocked [71-73]. IL-1β has also been shown to exert anti-apoptotic effects on HUVECs [70]; thus, these reports support our findings [42] implying that HUVEC apoptosis is not involved in the enhancement of permeability induced by the supernatants stimulated by the anti-HLA-DR Ab-containing plasma.

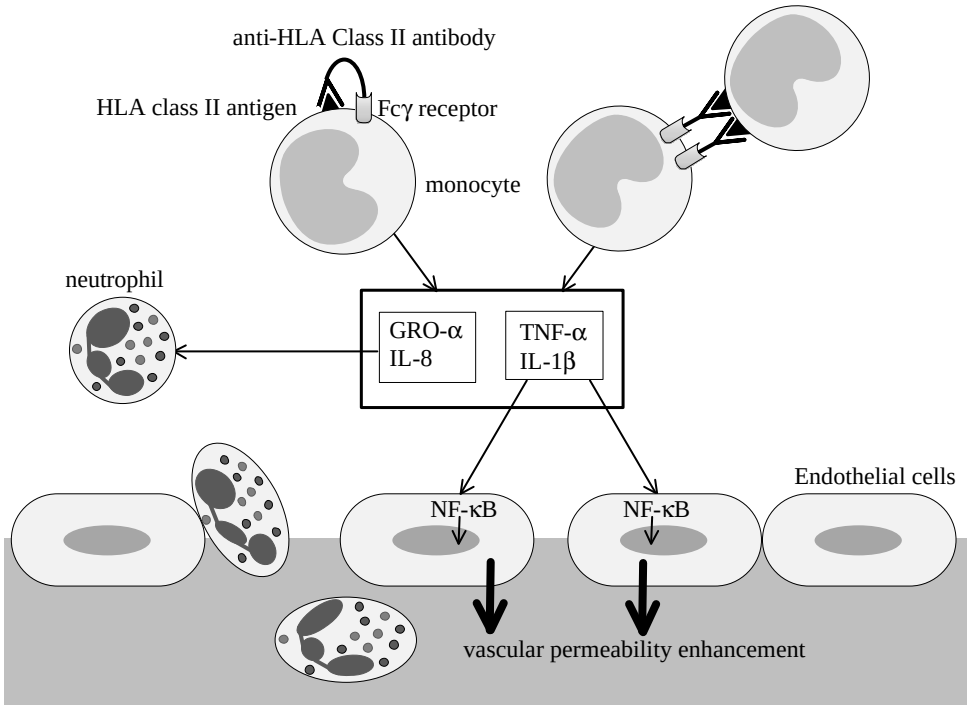

Fig. (3). The possible mechanism by which anti-HLA class II Ab may contribute to the pathology of TRALI.

CONCLUSION AND PERSPECTIVE

Based upon the findings obtained in our *in vitro* experiments [32, 33, 42], we propose a mechanism by which anti-HLA class II Ab may contribute to the pathology of TRALI, as illustrated in Fig. (**3**).

In this model, anti-HLA-DR Abs bind HLA-class II expressing cells, particularly monocytes, and activate these cells, resulting in the expression and generation of inflammatory cytokines and neutrophil-activating chemokines. Among cytokines, both TNF-α and IL-1β are mainly active in the enhancement of endothelial permeability through NF-κB-dependent pathways. Simultaneously, neutrophil-activating chemokines, such as IL-8 and GRO-α, attract neutrophils to endothelial cells and help to activate neutrophils, playing an important role in the pathogenesis of TRALI. There are limitations of *in vitro* studies, however, that cannot reproduce the complexity of the *in vivo* condition of TRALI. One of the most significant limitations is that the *in vitro* process requires a relatively longer incubation period to cause a permeability increase in both HUVEC and HMVEC; therefore, this step may not be fast enough to explain the rapid onset of TRALI (within 6hr). In clinical cases, in addition to TNF-α and IL-1β, PBMNC-derived mediators with short half-lives, including leukotrienes, prostaglandins, platelet activating factor or reactive oxygen species, may also rapidly affect endothelial cells. To elucidate this point, coculture of PBMNCs and endothelial cells in the presence of anti-HLA-DR Abs is required. Additional priming events for PBMNCs or endothelial cells, which may reflect the clinical as well as genetic background of patients who receive transfusion, should be considered. The autonomic nervous system and the immune system demonstrate cross-talk during inflammation [74]. In this regard, phagocytes are capable of de novo production of catecholamines and of enhancing acute inflammatory injury such as ALI [75]. Further examinations investigating the effect of priming agents that mimic the condition of recipients before transfusion would supplement the limitations of the *in vitro* study to reflect the *in vivo* situation.

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