

Acute Pulmonary Embolism and Immunity in Animal Models

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Abstract

Venous thromboembolism, encompassing acute pulmonary embolism (APE) and deep vein thrombosis (DVT), is a potentially fatal disease with complex pathophysiology. Traditionally, the Virchow triad provided a framework for understanding the pathogenic contributors to thrombus formation, which include endothelial dysfunction, alterations in blood flow and blood hypercoagulability. In the last years, it has become apparent that immunity plays a central role in thrombosis, interacting with classical prothrombotic mechanisms, oxidative stress and vascular factors. Thrombosis amplifies inflammation, and exaggerated inflammatory processes can trigger thrombosis mainly due to the activation of leukocytes, platelets, and endothelial cells. APE-related endothelium injury is a major trigger for immune system activation. Endothelium is also a key component mediating inflammatory reaction and it is relevant to maintain vascular permeability. Exaggerated right ventricular wall stress and overload, with coexisting systemic hypotension and hypoxemia, result in myocardial injury and necrosis. Hypoxia, tissue factor activation and cytokine storm are engaged in the thrombo-inflammatory processes. Thrombus development is characterized by inflammatory state vascular wall caused mainly by an early extravasation of leukocytes and intense selectins and cytokines production. Nevertheless, immunity of DVT is well described, little is known about potential chemokine and cellular differences between thrombus that develops in the vein and thrombus that detaches and lodges in the pulmonary circulation being a cause of APE. There is a paucity of data considering inflammatory state in the pulmonary artery wall during an acute episode of pulmonary embolism. The main aim of this review is to summarize the knowledge of immunity in acute phase of pulmonary embolism in experimental models.

Keywords

inflammation · pulmonary embolism · cytokine · chemokine · platelet aggregation · endothelium

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Abbreviations

APE	Acute pulmonary embolism
BAL	Bronchoalveolar lavage
CCR	CC-chemokine receptor
CINC-1	Cytokine-induced neutrophil chemoattractant 1
CXCL	Chemokine (C-X-C motif) ligand
DVT	Deep vein thrombosis
IL	Interleukin
M1	Macrophage type 1
M2	Macrophage type 2
MCP-1	Monocyte-chemoattractant protein 1
MIP-1	Macrophage inflammatory protein 1
MMP	Matrix metalloproteinase
MPO	Myeloperoxidase
NLRP3	NLR family pyrin domain containing 3
NO	Nitric oxide
PA	Pulmonary artery
PAP	Pulmonary arterial pressure
PAR	Protease activated receptor
PE	Pulmonary embolism
PH	Pulmonary hypertension
PMN	Polymorphonuclear cell
RANTES	Regulated on activation, normal T cell expressed and secreted
RV	Right ventricle
RVOT	Right ventricular outflow track
RVSP	Right ventricle systolic pressure
SMC	Smooth muscle cell
TNF- α	Tumor necrosis factor α
VTE	Venous thromboembolism

1. Introduction

Venous thromboembolism (VTE), including acute pulmonary embolism (APE) and deep vein thrombosis (DVT), is a potentially fatal disease with complex etiology and pathophysiology (Huisman et al., 2018; Klok et al., 2022). Except from myocardial infarction and stroke, VTE is a considered to be the third most acute cardiovascular disease worldwide. APE is occurring globally in 600,000 to 1 million individuals each year and causing almost 15% one-year mortality (Konstantinides et al., 2020; Klok et al. 2022). Traditionally, the Virchow's triad described the pathological factors responsible for thrombus formation, such as endothelial dysfunction, turbulence alterations in blood flow and blood hypercoagulability (Bagot and Arya 2008).

In the last years, it has become evident that immunity plays a crucial role in thrombosis (Bagot and Arya 2008; Jackson et al., 2019; Takahashi, 2022). Thrombosis amplifies inflammation, and exaggerated inflammatory processes can trigger thrombosis mainly due to the activation of platelets, leukocytes and endothelial cells (Jackson et al., 2019). It has been demonstrated that during thrombus formation, an activation of platelets takes place (Ding et al., 2022), leading to release of vasoactive agents such as thromboxane A₂, adenosine diphosphate, prostaglandins, and serotonin. It was suggested that APE-related

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endothelium injury is a major trigger for immune system activation. Endothelial cells compose a mechanical barrier between the smooth muscle cells (SMC) and circulating blood and are a key component mediating inflammatory reaction and it is relevant to maintain the vascular permeability (Tang et al., 2016). Endothelial cells are damaged by a thrombus trapping into the pulmonary vessels which results in the release of pro-inflammatory cytokines (Wood, 2002; Battistini, 2003). As a result, the clot occlusion is triggering the rise in pulmonary arterial resistance, leading to a rapid increase in right ventricle (RV) systolic pressure (RVSP) and, consequently, to acute myocardial damage. Exaggerated RV wall tension and work with coexisting systemic hypotension and hypoxemia result finally in myocardial injury and necrosis. Lysed monocytes presents a pro-inflammatory phenotype (Watts et al., 2008). Recently, SARS-CoV-2 virus pandemic demonstrated a close link between inflammation and thrombotic events in clinical setting. COVID-19 patients have a higher risk of VTE events, which increases among females and with aging (Brauninger et al., 2022; Nishijima et al., 2022; Kapten et al., 2023). Moreover, it seems that complications associated with clotting contributed to up to 70% of deaths related to

COVID-19 (Task Force for the management of COVID-19 of the European Society of Cardiology et al., 2022). It could be hypothesized that hypoxia, tissue factor activation and cytokine storm could be potentially engaged in the thrombo-inflammatory processes (Springall et al., 2022).

Numerous studies revealed that thrombus formation is accompanied by inflammatory state in vein wall, caused mainly by an early extravasation of leukocytes and intense selectins and cytokines production (Galeano-Valle et al., 2021). Nevertheless, immunity of DVT is well described; there is a limited amount of data on chemokine and cellular differences between the thrombus that develops in the vein and thrombus that detaches and lodges in the pulmonary circulation being a cause of APE.

In addition, there is a paucity of data concerning inflammatory state in the pulmonary artery wall during an acute episode of pulmonary embolism (PE). Although APE pathogenesis is multifactorial, inflammation is one of the major components in the crosstalk between endothelium injury, hypoxia, platelets activation, hemodynamic disturbances, and genetic background (**Figure 1**). The main aim of this review is to summarize the current knowledge of immunity in the acute phase of PE in experimental models.

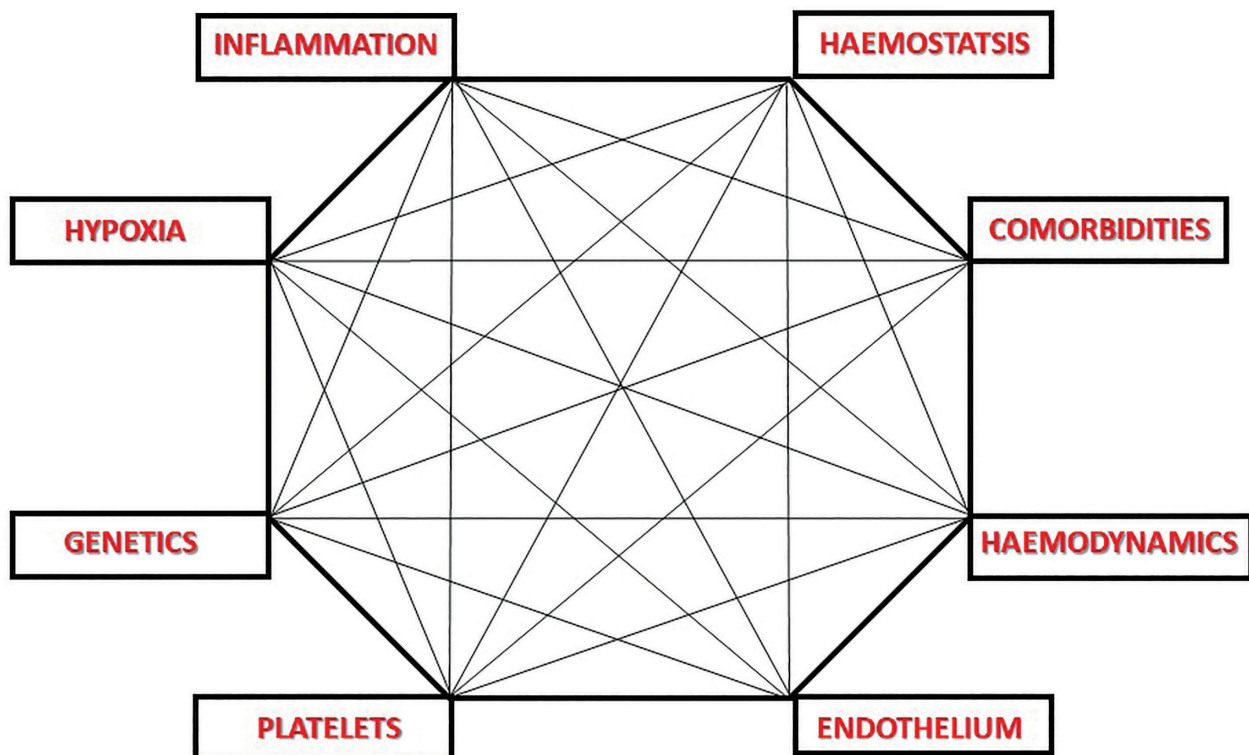


Fig 1. A mosaic theory of acute pulmonary embolism – an interplay between inflammation and other essential factors in the pathophysiology of pulmonary embolism.

2. Evidence of Inflammation in Lung Parenchyma

2.1. Altered expression of chemokines and inflammatory cell influx

There is scarcely any data regarding the role of immunity in experimental models of APE. One of the first studies by Eagleton et al. (2022) showed an early influx of polymorphonuclear cells (PMNs) and higher monocyte chemoattractant protein (MCP)-1 concentration within the pulmonary artery (PA) wall. APE experimental model was initiated by inferior vena cava thrombosis in 70 Sprague-Dawley rats while other 70 rats underwent sham operation only (Eagleton et al., 2002). The rise in neutrophils level began after 3 h of APE episode, peaked after 2 days and it was marked mainly

in PA wall (Figure 2). The macrophage infiltration was the highest at 1 day after APE and returned to the baseline after 4 days of the episode and predominated lung parenchyma (Eagleton et al., 2002). Of note, MCP-1 concentration was higher in experimental model of APE, with a peak level at 1 day, than in sham animals. It is well-known that MCP-1 is a strong activator of monocytes which is produced by the numerous types of cells (Colotta et al., 1994; Conti et al., 1997). After thrombus formation, the peak of MCP-1 in the vessel wall, blood and thrombus was observed (Humphries et al., 1999). Monocytes produce not only fibrinolysis inhibitors but also urokinase and tissue plasminogen activators. It seems that MCP-1 promotes thrombus resolution via macrophages fibrinolytic functions. In the experimental model of APE, exaggerated MCP-production was also related to intimal vessel hyperplasia (Eagleton et al., 2002). Shortly after

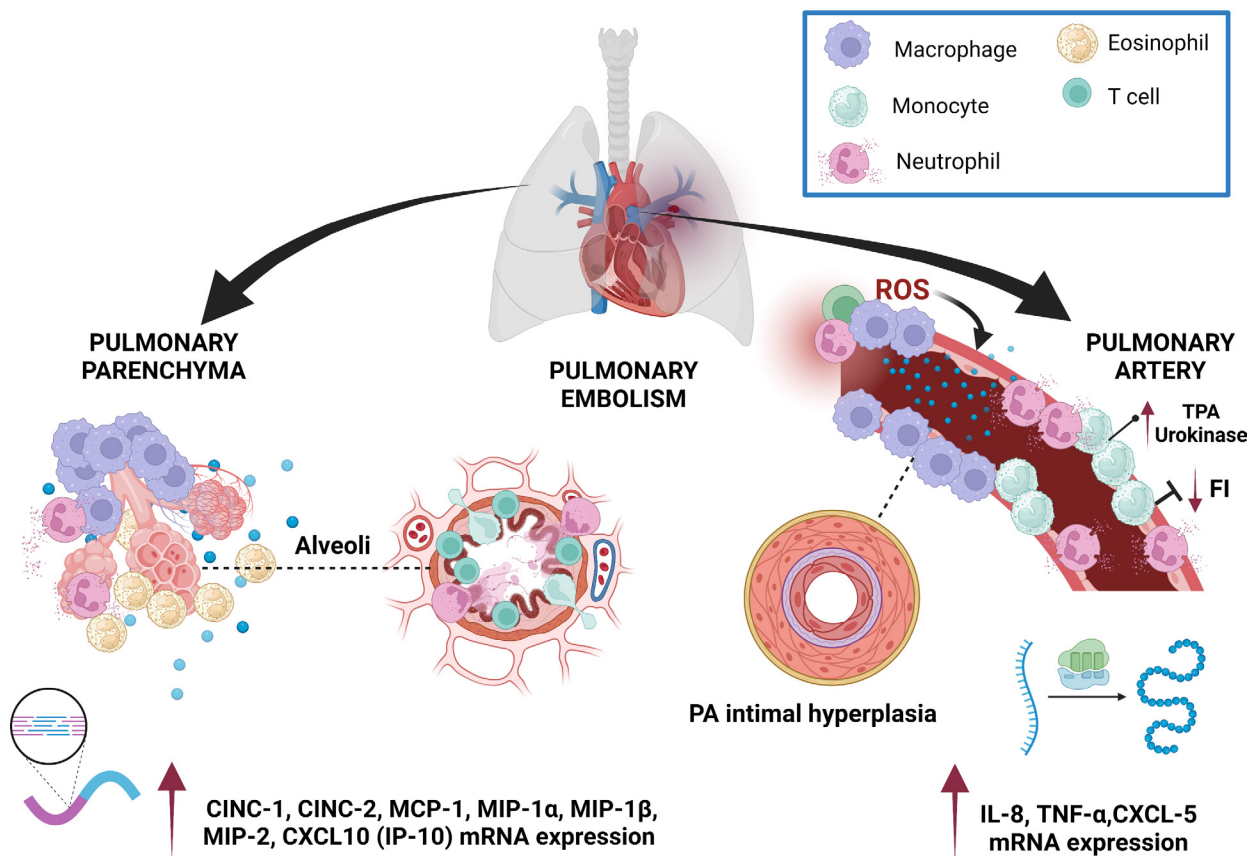


Fig 2. Chemokine accumulation and pro-inflammatory cell influx in the lung parenchyma as well as in the pulmonary artery wall during acute pulmonary embolism episode. Recruitment of inflammatory cells into lung parenchyma, bronchoalveolar lavage (BAL) and pulmonary artery (PA) wall. Shortly after acute pulmonary episode starts, a rapid and robust influx of macrophages occurs. In lung parenchyma the inflammatory cell response is caused mainly by macrophages, while in PA wall the inflammatory response is related to neutrophil with simultaneous rise in macrophages. Higher accumulation of proteins, eosinophils and macrophages is present in BAL. Increased chemokines and cytokines expression is observed under hypoxic conditions in lung parenchyma and PA wall. This figure has been created with BioRender.com. Abbreviations: CINC, Cytokine-induced neutrophil chemoattractant; CXCL, Chemokine (C-X-C motif) ligand; FI, Fibrinolysis inhibitor; IL, Interleukin; MCP, Monocyte-chemoattractant protein; MIP, Macrophage inflammatory protein; ROS, Reactive oxygen species; TNF, Tumor necrosis factor; TPA, Tissue plasminogen activator.

the peak MCP-1 concentration, the PA intimal region became thickened (Eagleton et al., 2002). Moreover, an increased intima-to-media thickness was observed in study groups compared with controls (Eagleton et al., 2002). This study indicated that APE is associated with leukocyte influx, mainly in the early phase (1 day) after acute PE episode. The infiltration of pro-inflammatory cells was present not only in the pulmonary artery wall but also in lung parenchyma (Eagleton et al., 2002). Neutrophils infiltration in the PA wall peaked at 2 days in experimentally induced PE mouse model in compare with controls (sham operation) however almost 6-fold increase in neutrophil content was observed, at the early stage (18 h after APE) in severe APE mouse model compared with moderate APE (**Figure 2**). Neutrophils were present mainly in the PA wall, while macrophages were dominant in lung parenchyma (Eagleton et al., 2002). In parallel to DVT, there was a similar cellular response including neutrophils activation in the PA wall during APE episode (Wakefield et al., 1995; Downing et al., 1996; Eagleton et al., 2002). However, on the contrary, macrophages peak activation takes place later in DVT experimental model (after 3 days) than in APE animal study (1 day) (Wakefield et al., 1995; Eagleton et al., 2002). However, no significant changes in the terms of tumor necrosis factor (TNF)- α , nitric oxide (NO), interleukin (IL)-10, keratinocyte derived chemokine and E and P-selectin levels were observed between rats with experimentally induced PE compared with control rats (sham operations) (Eagleton et al., 2002). The lack of change in plasma selectins level differentiate APE from DVT (Walcheck et al., 1996; Konstantopoulos et al., 1998; Eagleton et al., 2002). In DVT, P-selectins are of key importance for mediating interactions between leukocytes, platelets, and endothelial cells (Walcheck et al., 1996; Konstantopoulos et al., 1998). In addition, the blockade of P-selectin with antibody is associated with a decrease in thrombosis propagation and inflammation intensity; however, it is not related to the leukocyte vein wall influx (Wakefield et al., 1996; Downing et al., 1997). It can be postulated that the inflammatory cells in PA wall might originate from the lung parenchyma, and that selectins might be responsible for thrombogenesis than extravasation of pro-inflammatory cells. Next, Zagorski et al. (2003) have observed chemokine accumulation in lungs of rats with APE induced by polystyrene microspheres (Jones et al., 2003; Zagorski et al., 2003). Severe APE was defined by a significant increase in RVSP (RVSP~50 mmHg, arterial hypoxemia PaO_2 =70mmHg), while moderate APE (RVSP~35 mmHg, and no arterial hypoxemia) were compared to vehicle rats (RVSP<30 mmHg). In the model of APE, the inflammatory changes were presented after the pulmonary vascular occlusion (Zagorski et al., 2003). Massive protein and eosinophilic accumulation was found in the alveoli in the severe APE group comparing with moderate APE and controls (**Figure 2**). Interestingly, bronchoalveolar lavage (BAL) samples showed a 6-fold increase

of neutrophils in severe APE rats when compared with moderate APE and control groups. Under physiologic conditions, neutrophils compose only a small percentage of total alveolar cellular fraction (Zagorski et al., 2003). *In vitro* neutrophil chemotaxis assays on BAL after 18 h of APE episode demonstrated almost 100-fold higher neutrophil chemotactic activity among severe APE rats comparing with controls (Zagorski, Debelak et al. 2003). While cytokine-induced neutrophil chemoattractant (CINC)-1 and macrophage inflammatory protein (MIP)-1 were found in remarkably higher concentration in BAL of severe APE rats, they were not detected in the moderate APE group (**Figure 2**). Use of both anti-CINC and anti-MIP-1 blocking antibodies revealed that CINC is substantially responsible for a neutrophil chemotactic activity in BAL. Consequently, the chemokine genes expression was evaluated also in lung parenchyma demonstrating upregulation of chemokines exerting chemotactic function for neutrophils such as CINC-1, CINC-2, and MIP-2 genes as well as T lymphocytes – MCP-1, MIP-1 α , MIP-1 β and IP-10 (Zagorski et al. 2003). It was found that an elevated number of neutrophils and platelet activating factors as well as alterations in the lung surfactant were present in humans with APE (Zagorski et al., 2003; Sreejit et al., 2022).

In summary, the very first studies illustrated the robust influx of macrophages and neutrophils in pulmonary artery wall in APE rat experimental models. The main chemokine responsible for inflammation in the early APE phase is MCP-1, which has a chemotactic function, and it is associated with intimal hyperplasia and thrombus resolution. Further, the inflammatory state is observed also in the BAL, which contains more chemokine proteins such as cytokine-induced neutrophil chemoattractant and macrophage-inflammatory protein-2. Lastly, an increased gene expression of chemokines was indicated in lung parenchyma.

3. The Vicious Circle Between Inflammation and Right Ventricle

3.1. The inflammatory changes in right ventricular damage following APE

Acute RV pressure overload and RV failure caused by an episode of APE is one of the most severe APE consequences and it is related to the higher rate of mortality (Ribeiro et al., 1997, 1999; Kreit 2004; Schoepf et al., 2004; Harjola et al., 2016; Konstantinides et al., 2020). Autopsy studies revealed monocyte and macrophages infiltration in myocardial tissue (Iwadate et al., 2003). To establish the role of potential inflammatory state in RV as a response to APE, series of experimental studies to clarify this issue were performed.

In the rat models of polystyrene-induced APE resulting in acute RV dysfunction, animals and subsequent with severe hypertension RVSP~50 mmHg were characterized by 95-fold

higher myeloperoxidase activity (MPO) and higher MCP-1 protein plasma levels in RV when compared with mild hypertension rats (RVSP~40 mmHg) or controls (2 h RVSP~30 mmHg) (Watts et al., 2006). Moreover, MCP-1, MIP-1 α , MIP-2, CINC-1 and -2 mRNA expression was increased in RV myocardium of rats with severe APE, as compared to mild APE group and controls (**Figure 3**). An exaggerated accumulation of macrophages and neutrophils in RV myocardium was revealed by histological data of note; it was found in the necrotic tissue of rats with severe pulmonary hypertension (PH) (Watts et al., 2006). Moreover, treatment with antibody depleting granulocytes/PMNs (anti-PMN antibody) was connected with a notable reduction of MPO in RV myocardium and resulted in amelioration of myocardium damage (Watts et al., 2006). Interestingly, the changes mentioned above were not demonstrated in left ventricle.

Accordingly, another study was performed to identify a specific molecule responsible for neutrophils recruitment. The aggravated CINC-1 and CINC-2 expression between 6 and 18 h after the onset of APE in experimental model was observed (Zagorski et al., 2007). The intense neutrophil accumulation after 18 h was associated with RV failure. In addition, administration of anti-CINC-1 antibody to rats resulted in a decreased neutrophil accumulation and was associated with improved RV function (Zagorski et al., 2007).

Zagorski et al. (2008) continued research revealing the alterations in gene expression of chemokines in RV myocardium during acute APE in rats with the usage of DNA microarrays. Chemokines are the main factors contributing to the infiltration of leukocytes to the sites of inflammation (Mikolajczyk et al., 2021; Sreejit et al., 2022). After 18 h of moderate PE, nine C-C chemokine ligands genes, five CXC-chemokine

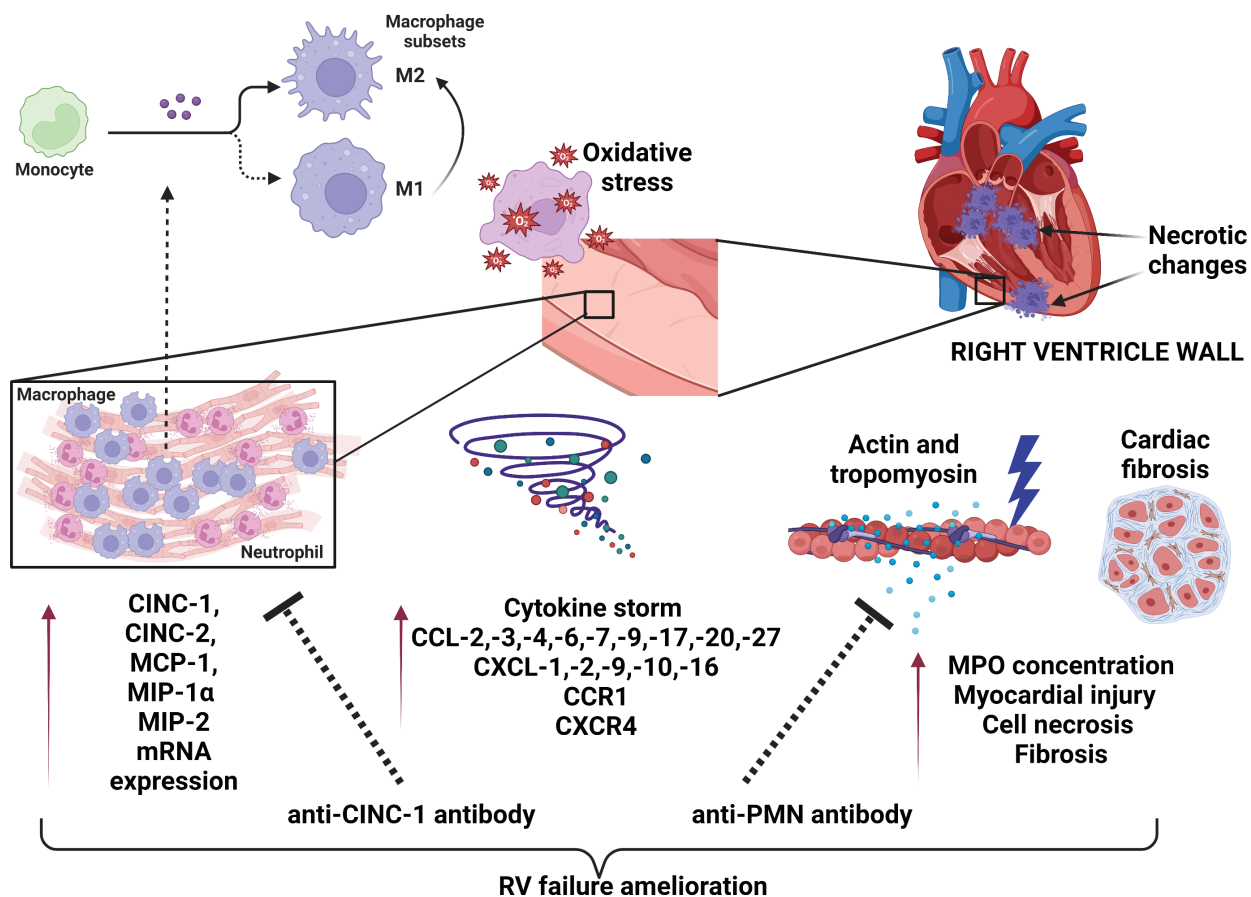


Fig 3. Cardiac inflammatory processes in right ventricle during acute pulmonary embolism episode. Schematic overview of the contribution of pro-inflammatory cells in the pathogenesis of right ventricular (RV) failure during acute pulmonary embolism (APE) episode. During APE, neutrophils, and macrophage infiltrate myocardium. Mononuclear cells with M1 phenotype occur during early phase of APE episode and convert into M2 phenotype in the later phase. RV inflammation is characterized by exaggerated chemokines accumulation, cytokine storm and oxidative stress. Chronic inflammation and hypoxia results in myocardial damage, necrosis and fibrosis. Treatment with selective antibodies such as anti-CINC and anti-PMN resulted in RV failure amelioration. This figure has been created with BioRender.com. Abbreviations: CCL, C-C motif chemokine ligand; CCR, CC Chemokine receptor; CINC, cytokine-induced neutrophil chemoattractant; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C chemokine receptor; M, macrophage; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MPO, Myeloperoxidase; PMN, polymorphonuclear cell antibody; RV, right ventricle.

ligands genes and the receptors CCR1 and CXCR4 were upregulated. By contrast, C-X-C motif chemokine ligand (CXCL)-12 and X-C motif chemokine ligand (XCL)-1 were downregulated (Zagorski et al., 2008). It was also observed that notable changes occurred in the gene expression profile of PA that was impacted by an embolus (Gromadzinski et al., 2023). Moreover, the observed changes in the transcriptome were dependent on the severity of PH and the time after APE episode. A plenty of transcriptional pathways regarding inflammation pathways and hypoxia inducible transcription factors were upregulated; however, oxidative enzymes and fatty acids transporters were downregulated after 18 hours of APE episode in comparison to controls after 18 h (**Figure 3**). Microarray data showed that selective chemokines could be responsible for monocytes recruitment into cardiac tissue. Monocyte accumulation was present even up to 6 weeks after APE episodes in opposition to neutrophils, which had a tendency to vanish after one week following APE episode (Zagorski et al., 2008).

In line with these findings, the same study group demonstrated the late consequences of APE in rat hearts (Watts et al., 2008). The study demonstrated the presence of neutrophils not only in the early stage after APE episode but also showed the progression of inflammation over 6 weeks. The M1 macrophage phenotype and thin right ventricular outflow tract (RVOT) wall was observed in the day 1 after APE episode (**Figure 3**). Later, deposition of collagen, accumulation of myofibroblasts and M2 macrophages were present in RVOT, forming a fibrotic scar (Watts et al., 2008). Neutrophils infiltration resolves between 1 and 4 week after APE episode; however, monocyte influx lasts until 6 weeks after APE (Watts, Gellar et al. 2008).

In a consecutive project conducted by Zagorski et al. (2009), the differences in the inflammatory response were demonstrated depending on the heart anatomy (Watts et al., 2008). With the usage of DNA microarrays key differences were observed. This study revealed numerous transcriptional changes including pro-inflammatory and profibrotic remodeling in RV outflow tract in comparison to RV apex during chronic APE in rats (Zagorski et al., 2009). RV outflow tract was more severely damaged than the RV apical region. As compared to RV apex, a significant upregulation of the gene expression for collagen synthesis, fibroblast growth factor and CCN proteins (cysteine-rich protein 61, connective tissue growth factor, and nephroblastoma overexpressed gene) was observed in RV outflow tract (Zagorski et al., 2009). It has to be pointed out that apex and RVOT are characterized by different transcriptional mechanisms (Haddad et al., 2008). It was reported that RV outflow tract is a subject of strong profibrotic remodeling and pro-inflammatory responses during late phase of APE. The discrepancies might result from specifics in RV anatomy – the thin wall of RV outflow tract is probably more prone to mechanical stress than more muscular apex. RVOT

wall is also thin and smooth, in opposition to thicker and trabeculated apex part. The mechanical stress mainly results from an increased RV pressure overload and pulmonary artery resistance. Moreover, RV outflow tract contracts later than apical part of RV and it can generate greater stretch forces (Haddad et al., 2008; Zagorski et al., 2009).

In addition, a shift in the cell metabolism after 6 weeks of APE episode was also reported. During the late phase of APE, a decrease in crucial metabolic pathways were observed including amino acid, carbohydrate and fatty acid metabolisms (Zagorski et al., 2009). Downregulation of metabolic pathways might be related to the myocyte damage during chronic APE and replacement with cells with lower metabolic demand (Zagorski et al., 2009). Interestingly, matrix metalloproteinases (MMPs) in RVOT were activated, which can indicate an intense healing pathways due to the cardiac remodeling.

Generally, RVOT seems to present a significant pro-inflammatory response under chronic APE condition, resulting in myocyte damage and loss, followed by replacement with cells with lower metabolic rate such as monocytes and fibroblasts, extracellular matrix remodeling and upregulation of wound healing. All these adaptive changes are a consequence of RV damage and enable thin and vulnerable RVOT to form a scar and convert into the stable but non-contractile structure. Correspondingly, all the above-mentioned changes in RVOT histology and metabolomics are consistent with RV contractility disorders – hypokinesis in the basal and mid-free wall and normal motion of the RV apex known as “McConnell sign” (McConnell et al., 1996). In the clinical setting, “McConnell sign” is a valuable echocardiographic marker forcing rapid patient management and it is associated with higher mortality rate (Pruszczyk et al., 2014; Kurnicka et al., 2016).

Tang et al. published data concerning changes in transcriptome in rabbits. In a rabbit model of autologous blood clot-induced APE, almost 1343 genes were upregulated mainly in the genes related to the immune disease, inflammation, pulmonary disease, and cardiovascular disease in genome-wide gene expression profiling (Tang et al., 2016). Eighty-seven genes were upregulated in the inflammatory genes including: chemokine signaling pathways, T and B cell receptor signaling pathway, nucleotide-binding and oligomerization domain-like signaling pathway, Toll-like receptor signaling pathway, retinoic acid-inducible gene I like receptor and FcεRI signaling pathway (Tang et al., 2016). Importantly, the higher expression of TNF-α, IL-8 and CXCL-5 was shown (Tang et al., 2016).

Chemokines and cytokines are important cell mediators taking part in cellular and adaptive immune response and in acute phase of inflammation as well (Griffith et al., 2014). In the previously cited studies (Zagorski et al., 2003, 2007, 2008, 2009; Zagorski and Kline 2016), the presence of pro-inflammatory mediators in the lung parenchyma was described. TNF-α is

produced by activated macrophages, which were detected in the highest content after 1 day in the PA wall after APE episode (Eagleton et al., 2002). Importantly, TNF- α is a marker of systemic inflammation among patients with APE (Halici et al., 2014). IL-8 is produced by activated macrophages during innate immune responses. IL-8 is a strong neutrophil chemoattractant. Higher IL-8 concentration was also found in patients with idiopathic venous thrombosis as well as with acute coronary syndromes (Jezovnik and Poredos 2010; Li et al., 2015). CXCL-5 is a crucial chemoattractant protein enabling the neutrophil activation and their recruitment into the lung parenchyma (Gibbs et al., 2014).

Subsequently, another study performed by Zagorski and co-workers in 2016 considered the changes in rat lung transcriptome with the usage of DNA microarrays, depending on APE severity (Zagorski and Kline 2016). This data indicated also that lungs are even more prone to APE induced damage than myocardial cells. However, mild-APE (defined as 2 h RVSP normal, 18-h RVSP 44 mmHg) showed no significant transcriptional changes in RV and very few changes after 18 h. In contrast, at the same time serious changes were observed in the lung parenchyma. Two-hour mild APE with minimal changes in PH caused a plenty of changes in the pro-inflammatory gene expression in lungs (Zagorski and Kline 2016). However, after 18 h of severe-APE (defined as 2-h RVSP > 50 mmHg; 18-h RVSP 44 mmHg), significant alterations in the expression of genes related to lipid, steroid and cholesterol synthesis were noticed (Zhao et al., 2014; Zagorski and Kline 2016).

In summary, the study revealed that mild APE produces severe and profound alterations in gene transcription in lungs, especially genes encoding pro-inflammatory cytokines, chemokines, and cholesterol synthesis (Zagorski and Kline 2016). It has to be pointed out that even mild APE without PH, can have a deleterious, inflammatory effect on the lung parenchyma (Zagorski and Kline 2016).

Wang et al. (2020) conducted a study to assess the interplay of inflammatory mediators and blood flow in a rabbit model of autologous-induced APE leading to a shock. In this rabbit model of massive PE, they demonstrated higher levels of pro-inflammatory cytokines IL-1 β , TNF- α as well as inflammatory infiltration of neutrophils and macrophages in both pulmonary and non-pulmonary tissues. What is more, in comparison to controls, a notable upregulation of the expression of inducible NO synthase and Arginase-1 was observed in pulmonary vascular endothelium and alveolar space (Wang et al., 2020).

On the contrary, two studies conducted among pigs have shown only minor changes in IL-1 β , IL-8, TNF- α (Tsang et al., 2002) and no changes in IL-6 (Dolci, Fuentes et al. 2010) circulatory level. In the study by Dolci et al. (2010) considering microspheres-induced APE in pigs, a higher central temperature and a trend towards higher protein content in BAL were

noticed. However, these studies were performed in pigs, not in rats – as previously described – and did not analyze the local inflammation in lung parenchyma, which seems play a pivotal role in pathogenesis of APE.

In general, in experimental models of APE leading to PH and resulting finally in RV dysfunction, the exaggerated inflammation in cardiac myocardium was observed. In the molecular pathogenesis of RV failure, a pivotal role is played by macrophages and neutrophils. CINC is the primary chemokine involved in neutrophils recruitment. Treatment with anti-CINC or anti-PMN antibodies caused a reduction in cardiac troponin concentration and ameliorated the RV failure pointing out that neutrophils might be of key importance in the pathophysiology of RV dysfunction (Watts, Zagorski et al. 2006). Originally, the higher activation of MPO, MMP-9 and presence of M1 macrophages phenotype in RVOT myocardium was observed. Over the weeks, RVOT is characterized by poor contractile function, myocardium necrosis, collagen deposition and neovascularization. M1 macrophages in the early phase transform into M2 phenotype in the healing stage. Genome-wide expression profiling of PA revealed crucial changes in gene expression mainly associated to inflammation and cardiomyopathy among APE rabbits. Similarly, higher IL-8, TNF- α and CXCL-5 mRNA level was suggested. APE without PH might generate inflammatory changes in lungs resulting in systemic procoagulant state and inflammation.

4. Inflammation a Potential Therapeutic Target in APE

4.1. Anti-inflammatory effect of non-vitamin K antagonist oral anticoagulants

Substantial data support the hypothesis about crosstalk between inflammation and coagulation, due to the mutual relationship between tissue factor, coagulation proteases such thrombin and factor Xa, platelets as well as endothelial cells, leukocytes adhesion molecules and pro-inflammatory cytokines. Vascular endothelial cells play a crucial role in inflammatory state enhancement. Molecularly, the connection between inflammation and thrombosis takes place with the usage of protease activated receptors (PAR) (Ellinghaus et al., 2016).

Thrombin exerts a plethora of non-hemostatic functions such as modulating endothelial cell function, stimulating cytokine production, or mobilizing adhesive molecules. PAR-1 is supposed to be responsible for pro-inflammatory thrombin effect. Next, factor Xa is recognized to stimulate tissue fibrosis, vascular remodeling, and inflammatory processes due to the PAR-1 and PAR-2 receptor interactions (Ellinghaus et al., 2016; Joseph et al., 2022a). Data from previous studies illustrated that transcriptional changes endeavored by thrombin in

in vitro studies with the human umbilical vein endothelial cells are mediated by PAR-1 activation (Ellinghaus et al., 2016). Furthermore, the expression of adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule 1, ELAM-1, IL-8, CXCL-1, CXCL-2 and MCP-1 is stimulated by thrombin (Coughlin 2000). Laurent et al. (2014) have studied the effect of rivaroxaban on cytokine release and procoagulant activity with the usage of monocytes as well as THP-1 cells, that is human leukemia monocytic cell line. After stimulation of THP-1 cells with lipopolysaccharide, great amount of IL-8, moderate amount of TNF- α and IL-10 and minor elevation in MCP-1, RANTES, epidermal growth factor and MIP-1 α was found (Laurent et al., 2014). Rivaroxaban completely blocked thrombin generation and caused a significant increase in IL-10 secretion. IL-10 is an anti-inflammatory cytokine, while TNF- α and IL-8 promotes angiogenesis, RANTES induces plaque destabilization due to the MMPs secretion.

In the SMCs stimulated by clot, rivaroxaban decreased the mRNA expression of IL-6 and TNF- α (Rosenkranz et al., 2011). In a series of study considering atherosclerosis, in apolipoprotein E-deficient mice, rivaroxaban reduced macrophages accumulation, plaque progression, pro-inflammatory TNF- α , IL-6 and MMP-9 expression (Zhou et al., 2011; Hara et al., 2015; Hettwer et al., 2022). Of note, it was observed in a rat model of ischemia/reperfusion brain injury that pretreatment with rivaroxaban led to reduced stroke severity (Dittmeier et al., 2016). In experimental model of diabetes mellitus type 2, rivaroxaban ameliorated thrombus formation and abolished leukocyte adhesion in the microvascular bed (Iba et al., 2014). In summary, data from literature confirm the immunomodulatory effect of factor Xa inhibitor-rivaroxaban in *in vitro* studies (Ma et al., 2021).

4.2. Statins

A growing body of evidence indicated the association between VTE and atherothrombosis (Becattini et al., 2005; Bova et al., 2006; Sorensen et al., 2007; Rodriguez et al., 2012). In the series of observational and intervention studies, a possible role of statins in VTE management was described (Ray et al., 2001; Doggen et al., 2004; Lacut et al., 2004; Ramcharan et al., 2009; Sorensen et al., 2009; Kunutsor et al., 2017). In the meta-analysis by Squizzato et al. (2010) statins were described to decrease the risk of the first episode of VTE. In a placebo-controlled, randomized trial in humans with elevated high sensitivity C-reactive protein level, rosuvastatin treatment reduced the occurrence of first VTE episode by 43% (Glynn et al., 2009). In accordance with data from HOPE-3 and JUPITER trials, rosuvastatin use was associated with 47% reduction in the risk of VTE; this was independent of the occurrence of VTE-related clinical risk factors (Joseph et al., 2022b).

Statins can exert pleiotropic effects such as attenuating inflammatory state, oxidative stress, preventing thrombosis and improving endothelial state (Girotra et al., 2012; Owens et al., 2014; Krata et al., 2021; Bergami et al., 2022). Most importantly, statins are responsible for lowering LDL-cholesterol level, tissue factor expression, reducing thrombin generation, as well as supporting fibrinogen cleavage (Biere-Rafi et al., 2013). According to the literature, simvastatin was capable of mitigating the risk of VTE in animal models (Patterson et al., 2013; Feng et al., 2016) and improving neurological status after ischemic stroke disease (Shabanzadeh et al., 2005). However, there are barely any data concerning the statin role in APE animal models. Souza-Costa et al. (2007) have performed a study in Wistar rats lung perfusion model which illustrated that pretreatment with atorvastatin was related to 27% reduction in mean artery pulmonary pressure (mPAP) and 49% higher circulation plasma nitrite/nitrate after APE episode comparing with controls. Additionally, statin therapy increased the 24 h survival rate – 48% in embolized rats versus 64% in rats treated with atorvastatin, mainly by lowering the MMP-9 concentration in the lung parenchyma (Souza-Costa et al., 2007). MMP's function is to degrade the extracellular matrix. Higher MMP activation is associated to the vasoconstriction by endothelin generation and PH development after APE episode (Fernandez-Patron et al., 1999). Moreover, MMP's generated vasoconstriction might be due to the MMP-2 cleavage of vasodilators such as adrenomedullin and calcitonin gene-related peptide (Fernandez-Patron et al., 2000; Martinez et al., 2004). Subsequently, Sun et al. (2011) investigated the effect of atorvastatin on the serum levels of IL-1 β and TNF- α in a New Zealand rabbits with autologous blood clot-related APE episode. Despite the fact that both groups were characterized by increase in serum IL-1 β and TNF- α concentration, pretreatment with atorvastatin diminished the increase in these cytokines (Sun et al., 2011).

Next, the impact of combined therapy-statins and sildenafil was evaluated by Neto-Neves et al. (2011). In this elegant study, male lambs were divided into subgroups- pretreated with atorvastatin (10 mg/kg/day, subcutaneously, 1 week) and vehicle (10% dimethyl sulfoxide, subcutaneously). Next, saline or sildenafil infusion were performed after 1 h after APE episode. Non-embolized controls received saline. It turned out that atorvastatin pretreatment reduced the mPAP and pulmonary vascular resistance index (Neto-Neves et al., 2011). However, the combination of sildenafil with atorvastatin was associated with even greater mPAP decrease in comparison with atorvastatin group alone. APE caused a significant increase in MMP-2 and MMP-9 level in BAL and this increase was diminished by atorvastatin and sildenafil treatment. The MMPs role in the pathophysiology of APE was described (Souza-Costa et al., 2005, 2007; Dias-Junior et al., 2009). Atorvastatin treatment alone was related to decrease in MMP-2 concentration

only (Neto-Neves et al., 2011). Moreover, atorvastatin and sildenafil treatment ameliorated neutrophil accumulation in BAL after APE episode and the effect was vanished after atorvastatin treatment only.

5. Conclusions and Future Perspectives

Acute pulmonary embolism is undoubtedly an inflammatory state condition as shown by marked increase in chemokines in lung parenchyma and BAL, in experimental models of APE. The lung's response to inflammation is rapid and robust. Increased number of macrophages and neutrophils in pulmonary artery wall was also reported after clot embolization. Furthermore, APE-related acute HP initiates inflammatory changes in RV myocardium defined as increased influx of neutrophils and monocytes/macrophages as well as exaggerated chemokine secretion. Magnitude of these alterations correlates with severity of APE-related acute HP. Treatment with anti-PMN antibodies abolished RV dysfunction suggesting the main pathophysiological role of neutrophils. By contrast, mild APE resulted in no significant transcriptional changes in RV, whereas the same "mild APE model" illustrated dramatic pro-inflammatory gene expression in the lung parenchyma. Genes encoding chemokine signaling pathways were identified among rabbits with APE and chemokines were widely studied in lung parenchyma and RV as a key mediator of inflammation.

The findings presented above constitute very strong evidence demonstrating that inflammatory dysregulation occurs during APE and that the circulating biomarkers can identify it. Exaggerated immune response was also observed during COVID-19 pandemic. Almost one third of all hospitalized patients in acute intensive care units were diagnosed with APE in spite of using standard prophylactic anticoagulation (Sakr et al., 2020). Activation of coagulation pathways mediated by immune system acts as a "defense mechanism" but ameliorated immunothrombosis can pathologically lead to multiorgan failure (Potere et al., 2023). Studies in preclinical models demonstrated that NLRP3 inflammasome activation promotes the thrombotic response by endothelial cell and platelets as well as the release of tissue factor and neutrophil extracellular traps (Li et al., 2023; Potere et al., 2023). Higher NLRP3 inflammasome activation was observed among patients with COVID-19 and NLRP3 (NLR family pyrin domain containing 3) inflammasome blockade inhibits pathological immune response in murine mouse models (Veras et al., 2023). Treating not only with glucocorticosteroids but also with tocilizumab (anti-IL-6 monoclonal antibody) and anakinra (recombinant human IL-1 receptor antagonist) were approved among patients diagnosed with COVID-19 and signs of hyperinflammation (Potere et al., 2023). Yet, despite a wealth of evidence, there is a general reluctance

to use immune-targeting approaches either in APE (without COVID-19 infection) or in its late consequences.

Inflammatory injury, especially in lung parenchyma, begin early after the APE episode. Currently, the widely used therapies with anticoagulants are focused mainly on clot resolution, which might take weeks or months. So why is there such inertia to introduce immune-targeting therapies as a strategy to treat APE and its side effects? It has to be emphasized that more aggressive treatment to prevent rapid pro-inflammatory genes expression and improve clot resolution is of great importance.

To conclude, the data presented in this paper indicate that APE, even in mild forms without HP, is related to the systemic inflammation and higher risk of clot recurrence and hypercoagulability. Similarly to other cardiac acute episodes, unresolved inflammation might be an important mechanism involved in late APE consequences.

Conflict of interest statement

None declared.

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