Inhibition of CINC-1 Decreases Right Ventricular Damage Caused by Experimental Pulmonary Embolism in Rats

John Zagorski, Michael A. Gellar, Maria Obraztsova, Jeffrey A. Kline and John A. Watts

*J Immunol* 2007; 179:7820-7826; doi: 10.4049/jimmunol.179.11.7820
http://www.jimmunol.org/content/179/11/7820

References
This article cites 52 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/179/11/7820.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Right ventricular (RV) dysfunction is a strong risk factor for poor clinical outcome following pulmonary embolism (PE), the third most prevalent cardiovascular disease. Previous studies in our laboratory demonstrated that RV failure during PE is mediated, in part, by neutrophil-dependant cardiac inflammation. In this study we use DNA microarray analysis of gene expression to demonstrate that PE results in increased expression of the CXC chemokines CINC-1 and CINC-2 between 6 and 18 h after the start of PE in a rat model of PE. Neutrophils accumulate in RV tissue by 18 h, and this inflammation is associated with decreased right heart function. Treatment of rats with Abs to CINC-1 significantly suppressed neutrophil accumulation in RVs during PE (52% reduction in tissue myeloperoxidase) and ameliorated RV failure. In addition, plasma concentration of cardiac troponin I, an established diagnostic marker for cardiac damage, was reduced by 90%. These results suggest that selective anti-inflammatory therapies targeted at neutrophil chemoattractants will reduce cardiac inflammation and reduce RV damage in the setting of PE.

Pulmonary vascular obstruction in humans can result from multiple disease processes, including aggregated carcinoma cells (1), erythrocyte blockage secondary to sickle cell disease (2, 3), massive blood transfusion (4), surgical repair of fractures (5, 6), and fibrin deposition in the setting of acute respiratory distress syndrome (3, 7, 8). However, in the clinical setting the most common cause of acute pulmonary vascular occlusion is pulmonary embolism (PE) from blood clots formed in the deep venous system. Acute PE is a major cardiopulmonary disease with an incidence of one per 1000 individuals in a year and a 30-day mortality rate that exceeds 15% (9–11). Furthermore, the occurrence of right ventricular (RV) dysfunction with pulmonary embolism is associated with a significant increase in morbidity and mortality (12–17). Approximately 40% of survivors of pulmonary embolism have symptomatic RV damage for at least 6 mo after diagnosis (15, 18–20). These data strongly suggest that new therapies aimed at reducing RV damage following PE would be beneficial in the clinical setting. Studies of progressive pulmonary artery occlusion in canines indicate that increased RV work load due to PE-induced pulmonary hypertension can exceed the capacity for increased coronary flow, resulting in functional ischemia (21, 22). It has also been predicted that severe PE increases RV wall tension, causing both shear force injury to myocytes and compression of the right coronary artery (23, 24). Although case reports have associated massive PE with frank RV infarction, and patients with RV dysfunction do liberate contractile proteins such as troponins into the blood, no study has shown evidence to support RV tissue necrosis as the primary cause of RV contractile dysfunction observed after PE (25, 26). Indeed, postmortem histological analyses of RV muscle from humans with fatal PE demonstrated the accumulation of neutrophils and monocyte/macrophages in the RV but without histological evidence of infarction (27, 28).

We have previously shown that pulmonary embolism induces a profound neutrophil-mediated inflammatory response in a rat model of PE (29). Treatment with anti-neutrophil antiserum reduced neutrophil accumulation in RV tissue and reduced RV damage. These data support our hypothesis that anti-inflammatory therapies directed to reduce RV damage following PE will be beneficial. However, the specific neutrophil chemoattractants mediating acute inflammation in our previous study were not identified. In this current study we now demonstrate that in vivo inhibition of the rat CXC chemokine CINC-1 (cytokine-induced neutrophil chemoattractant) reduces neutrophil recruitment into RV tissue following PE, attenuates RV failure, and lowers the concentration of cardiac troponin I released into plasma. Targeted therapies directed against related human CXC chemokines such as the three growth-related oncogene (GRO) proteins or IL-8 may be efficacious in treating PE in the clinical setting.

Materials and Methods

Animals

Experiments were performed using male Sprague–Dawley rats weighing between 325 and 375 g. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the Carolinas Medical Center in accordance with the Guide for the Care and Use of Laboratory Animals (30). Animals were cared for in the Cannon Research Center Vivarium of the Carolinas Medical Center. All animals were allowed ad libitum access to food and water.
Preparation of anti-CINC-1 polyclonal antibodies

The production and purification of polyclonal goat anti-rat CINC-1 Abs was reproduced precisely as previously published (31). Briefly, we expressed recombinant CINC-1 using a previously described CINC-1 expression vector (pIZ240) that fused the N terminus of CINC-1 to the C terminus of the gene for Schistosoma japonicum GST in the expression vector pGEX-3X (Pharmacia/GE Health Sciences). A factor Xa cleavage site was retained in the vector multiple cloning site between GST and CINC-1. Recombinant CINC-1 was made by expressing the fusion protein in Escherichia coli BL21 and separating it from bacterial whole cell lysates by glutathione-agarose affinity chromatography (Sigma-Aldrich catalog no. G4510). After cleavage of GST-CINC-1 with factor Xa, (New England Biolabs; 1 U of enzyme per 50 μg of fusion protein overnight at room temperature), the CINC moiety was purified to homogeneity using C18 reverse phase HPLC by standard procedures. Purification of CINC-1 protein was verified by SDS-PAGE and silver staining.

Recombinant CINC-1 was supplied to a custom Ab vendor (Pocono Rabbit Farm and Laboratory) for immunization of a goat following previously published protocols (31). Anti-CINC-1 Abs were separated from vendor-supplied whole goat serum using CINC-1 specific affinity chromatography. GST-CINC-1 fusion protein was coupled to cyanogen bromide-activated Sepharose 4 Fast Flow using a standard technique recommended by the vendor (Pharmacia/GE Healthcare catalog no.17-0981-01). Goat antiserum was applied to the GST-CINC-Sepharose column under gravity flow, which was subsequently washed extensively with PBS, CINC-1 specific Abs were eluted with 100 mM glycine (pH 3.0), dialyzed against saline, and concentrated using 30 kDa molecular mass cut-off ultrafiltration cartridges (Amicon Centricon YM-30; Millipore). Diluted Ab was filter sterilized though 0.45-μm nylon filters and quantitated by the bicinchoninic acid (BCA) method using a commercially available kit (Pierce). Purified Ab was homogeneous for the large and small Ig protein subunits in reducing SDS-PAGE.

PE model

Poly styrene microspheres were used to create pulmonary emboli as previously described (29, 32). Microspheres (25 ± 1 μm; Duke Scientific catalog no. 7525A) were sterilized with 70% ethanol, washed with sterile saline, and concentrated using 0.5% Tween 20, and resuspended in 0.01% sterile Tween 20 to produce a 10% suspension (13 million beads/ml). Animals were anesthetized using an i.p. injection of ketamine (70 mg/kg) and xylazine (3 mg/kg) and placed on a warming pad. Microspheres were then injected via the right jugular vein at a dose of 0.16 ml per 100 g of rat body weight to produce PE. Sham animals received i.v. vehicle alone (0.01% Tween 20 at 0.16 ml per 100 g of body wt). Surgical incisions were sealed with staples.

Treatment of PE

For treatment of PE with anti-CINC Abs, a sterile solution of Ab in saline was injected at a dose of 10 mg/kg 15 min before the injection of microspheres. Three groups were then compared: vehicle (no PE), PE, and PE plus anti-CINC Abs.

Physiological measurements

Following 20 h of PE, animals were anesthetized with i.p. xylazine (3 mg/kg) and ketamine (100 mg/kg) and placed on a warming pad. Breathing rates were counted by visual observation. A 2-French catheter (SPR-249-A; Millar Instruments) was placed in the left carotid artery to monitor systemic arterial blood pressure. Pressures were recorded using Acknowledge software (BIOPAC Systems) The arterial Miller catheter was removed and PE-50 tubing was inserted to draw arterial blood samples (0.4 ml) for blood chemistry (1-Stat blood analyzer; Abbott Point of Care). An additional sample of blood was drawn into EDTA-containing tubes for the preparation of plasma. Chelated blood was centrifuged for 10 min at 2,500 rpm in a table top centrifuge and plasma supernatants were stored at −70°C until needed. Plasma concentrations of cardiac troponin I were measured using an ELISA (Life Diagnostics).

Heart perfusions

Hearts were removed from anesthetized rats via midline thoracotomy and coronary vasculature and ventricular chambers and manually dissected to isolate the RV free walls, which were weighed and then frozen using liquid nitrogen-cooled Wallenburger tongs. Frozen RVs were crushed using a custom-built liquid nitrogen-cooled stainless steel mortar and pestle and stored as frozen tissue powders at −70°C until needed.

Myeloperoxidase (MPO) assays

MPO activity was measured in RV tissue extracts that were prepared in lysis buffer (100 mM potassium phosphate (pH 6.0), 5 mM EDTA, 0.5% hexadecyltrimethylammonium bromide) using a Fisher Power Gen 125 tissue homogenizer followed by 10 s of sonication and three freeze-thaw cycles. The homogenates were centrifuged (10,000 rpm at room temperature) for 5 min to remove tissue debris and supernatants were stored at −70°C. Supernatants were diluted 5-fold with lysis buffer and assayed for MPO activity in 96-well microtiter plates by adding lysis buffer containing 0.01% o-dianisidine-HCl and 0.01% hydrogen peroxide (29, 34). Plates were read at 460 nm in a UV Max microplate reader (Molecular Devices). Protein was quantitated in each MPO extract using the BCA method and MPO activity was normalized to protein concentration. MPO standard curves were constructed using purified human myeloperoxidase (Sigma-Aldrich catalog no. M6908).

Gelatinate assays

Matrix metalloproteinase (MMP) activity was detected by SDS-PAGE zymography using Tris-glycine gels containing gelatin as substrate (Invitrogen/Novex 10% Zymogram (gelatin) gels). Protein extracts were prepared by adding −25 μg of frozen RV powder to 600 μl of PBS and homogenizing. Cell debris was removed by centrifugation (10,000 rpm for 5 min at room temperature) and supernatants were stored frozen at −70°C until needed. Protein concentrations were determined by the BCA method. Pooled protein extracts were made by combining equal volumes of 8–10 individual RV tissue extracts from vehicle-treated control rats, PE rats, and rats treated with PE plus anti-CINC Ab. Zymogram gels were loaded with 100 μg of pooled protein per lane and electrophoresed with Tris-glucine SDS sample buffer in the absence of a reducing agent or heat denaturation. Gels were renatured after electrophoresis for 30–60 min and then developed overnight at 37°C using Invitrogen Life Technologies buffers. Proteins were visualized by staining with 0.1% naphthol blue black dye (Sigma-Aldrich catalog no.19,524-3) in 25% isopropanol and 10% acetic acid.

Chemokine expression

Chemokine gene expression in RVs was assessed by DNA microarray analysis. RNA was isolated from frozen RV tissue powder by the TRIzol method (Invitrogen Life Technologies). The extracted RNAs were purified on Qiagen RNeasy columns, converted to double-stranded cDNA with a SuperScript double-stranded cDNA synthesis kit (Invitrogen Life Technologies), and transcribed into biotin-labeled cRNA by in vitro transcription (IVT) with the Affymetrix IVT labeling kit. The biotin-labeled cRNA was fragmented nonenzymatically according to the manufacturer’s procedures (Affymetrix). Each fragmented sample was spiked with bioB, bioC, bioD, and cre, which served as hybridization controls. The fragmented cRNAs were then hybridized to Affymetrix rat genome 230 version 2.0 microarrays in Affymetrix hybridization buffer for 16 h at 45°C. Five arrays were used for each of the four treatment conditions examined (2 h after treatment with vehicle; 2, 6 and 18 h after start of PE). The hybridized arrays were then washed and fluorescently stained in an Affymetrix Fluidics Station 400 following Affymetrix procedures. Each array was scanned twice by the Agilent gene array scanner G2500A (Agilent Technologies).
Microarray data were analyzed with GeneSifter web-based software (vizXlab). Affymetrix ".cel" files were up-loaded to the GeneSifter web site using GeneChip robust multiarray analysis (GC-RMA) normalization. PE/vehicle gene expression ratios were compared with the GeneSifter “Project Analysis” function with ANOVA statistics and Benjinni and Hochberg adjustment for false discovery rates. A full description of our transcriptional analysis of RV tissue in response to PE is in preparation for publication elsewhere. All microarray data have been deposited in the National Institutes of Health/National Center for Biotechnology Information Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/projects/geo; GEO accession no. GSE6104).

Immunohistochemistry

Hearts were briefly perfused as described above to remove blood from the coronary vasculature and ventricles, after which the RV walls were dissected from the LV and septum. RV walls were fixed overnight in 10% neutral buffered formalin, embedded in paraffin, cut in cross-sections, deparaffinized, and rehydrated. Neutrophils were visualized in RV tissue sections by staining for neutrophil-specific esterase using a commercially available kit (napthol AS-D chloroacetate; Sigma-Aldrich catalog no. 90C2). RV sections were counterstained with Meyer’s hematoxylin.

Statistical analysis of biochemical and physiological measurements

Data are presented as mean ± SE. Comparisons of two independent groups were made by t test. Comparisons of more than two independent groups were made by one-way ANOVA with Tukey’s post hoc testing. Significance was determined as p < 0.05 using two-tailed testing. Comparison of CINC-1 and CINC-2 microarray gene expression in the four data sets (vehicle and 2, 6 and 18 h after start of PE) were determined by ANOVA of log2 GeneSifter fluorescence data with Dunnett’s post hoc analysis to compare PE values with vehicle control. Data were then converted to anti-log2 for graphical presentation. Investigators were blinded in the microarray sample processing and data uploading, histology blocks, and slide preparation, but were aware of treatment groups for all other analyses.

Results

CINC-1 and CINC-2 gene expression precedes neutrophil recruitment into RVs

To assess the time course of inflammation in RVs in rats with PE, we harvested rat hearts 2, 6, and 18 h after the initiation of PE and stained RV tissue sections for neutrophil-specific esterase. As shown in Fig. 1, A and B, few neutrophils were observed 2 and 6 h after the start of PE. However, at 18 h of PE there was a massive accumulation of neutrophils in the RVs and the RV morphology exhibited obvious signs of cardiomyocyte cell-cell separation and tissue damage. These data indicate that PE induces a severe but delayed neutrophil infiltration of RV tissue.

We next sought to identify neutrophil chemoattractants that might mediate neutrophil recruitment during PE. Examination of transcriptional changes in RVs during PE using Affymetrix DNA microarrays revealed that the genes for the CXC chemokines CINC-1 and CINC-2 were increased in expression starting at 6 h after PE and were highly expressed by 18 h, implicating these two chemoattractants in the recruitment of neutrophils into RV tissue (Fig. 2). Further mining of our expression data base using GeneSifter software was done to identify other genes in the “neutrophil chemotaxis” ontology that were overexpressed during PE. The CC chemokine CCR3 (MCP-3), which is usually associated with monocyte chemotaxis, was induced by PE as was TGFβ2, IL-1β, integrin β2, integrin αM, and several genes involved in intracellular signaling (a full genomics analysis of our microarray study of PE gene expression is in preparation for a separate article) The increased expression of the neutrophil chemoattractants CINC-1 and CINC-2 led us to focus on their role(s) in neutrophil recruitment into RVs during PE.

Anti-CINC-1 treatment reduces neutrophil accumulation in RVs

Neutrophil accumulation during PE was assessed by measuring MPO activity in protein extracts made from powdered frozen RVs (Fig. 3). MPO activity was undetectable in RV extracts from control vehicle rats (detection limit ~10 mU/mg protein). In contrast, RVs from rats with PE contained a mean of 267 ± 99 mU/mg MPO activity (n = 16), which was reduced to 128 ± 87 mU/mg (n = 17) by treatment with anti-CINC, a 52% decrease that was statistically significant (t test, p < 0.05). These data indicate that CINC-1 is a major mediator of neutrophil chemotactic activity in RVs with PE.

FIGURE 1. Time course of neutrophil accumulation in RVs. Samples were stained with a neutrophil-specific esterase stain in RV sections isolated from rats 2 h (A), 6 h (B), and 18 h after the start of PE. Arrows indicate positive staining neutrophils. Bar, 20 μm.

FIGURE 2. Time course of CINC-1 and CINC-2 gene expression assessed by DNA microarray analysis. RV samples were isolated from vehicle-treated rats or 2, 6, and 18 h after the start of PE. Value are means ± SE, n = 5 separate hearts per group. Asterisk (*) Indicates values significantly different from those of vehicle hearts.
Anti-CINC-1 treatment ameliorates RV damage caused by PE/PH

Hearts were isolated from animals 18 h after treatment with microparticles and tested for contractile function ex vivo on a Langendorff heart perfusion apparatus. Pressure-transducing balloons were inserted into RVs, hearts were electrically paced, and balloon volumes were set to establish end diastolic pressure of 0 mm Hg, allowing direct comparisons of right ventricular peak systolic pressure (RVPSP) and $+dP/dt$ (maximum rate of pressure development during contraction, an index of cardiac contractility). Hearts isolated from animals receiving PE treatment showed significantly lower RVPSP (Fig. 4A) and $+dP/dt$ (Fig. 4B) values than hearts that were isolated from vehicle treated animals, indicating RV damage. Treatment with anti-CINC Ab resulted in elevated RVSP and $+dP/dt$ to near normal levels, indicating that anti-CINC treatment reduced RV damage.

A plasma biomarker of cardiac damage was also improved by the treatment of rats with anti-CINC Ab. Plasma cardiac troponin I was elevated above normal levels during PE, but this elevation was significantly reduced by anti-CINC (90% reduction; Fig. 5), indicating reduced RV tissue damage with the Ab treatment. Arterial lactic acid increased during PE compared with vehicle-treated rats (1.9 ± 0.2 vs 0.7 ± 0.1 mM, respectively; $p < 0.05$) but was significantly reduced in Ab-treated rats (1.2 ± 0.1 mM; $p < 0.05$), indicating that tissue oxygen delivery was improved in the animals receiving anti-CINC compared with PE alone. Taken together, these data demonstrate that the reduction of neutrophil accumulation in RVs by anti-CINC suppressed RV damage during PE.

Anti-CINC-1 treatment reduces MMP9 expression in RVs

The observed benefit to RV function with anti-CINC treatment led us to examine the effect of this treatment on the expression of some

FIGURE 3. MPO activity in hearts isolated 18 h after treatment of rats with PE (n = 16) or PE plus anti-CINC (n = 17). Values are mean ± SE. Asterisk (*) indicates values significantly different from those of the other groups. n.d., Not detected.

FIGURE 4. RV peak systolic pressure (A) and $+dP/dt$ (B) in hearts isolated 18 h after treatment of rats with vehicle, PE, or PE plus anti-CINC Ab. Values are mean ± SE, n = 10–11 per group. Asterisk (*) indicates values that are significantly different.

FIGURE 5. Cardiac troponin I protein concentrations in plasma samples isolated 18 h after treatment of rats with vehicle, PE, or PE plus anti-CINC Ab. Values are mean ± SE, n = 8–9 per group. Asterisk (*) indicates a significant difference between PE and PE plus anti-CINC groups. n.d., Not detected.

FIGURE 6. MMP9 expression in RV tissue isolated 18 h after treatment with vehicle, PE, or PE plus anti-CINC Ab. Pooled protein extracts were made by combining equal volumes of 8–10 individual RV tissue extracts from vehicle-treated control rats, PE rats, and rats treated with PE plus anti-CINC Ab. These extracts were electrophoresed through gelatin-containing zymogram SDS-polyacrylamide gels.
neutrophil products in RV tissue. In particular, we were interested in the expression of neutrophil-derived MMPs. PBS protein homogenates were made from PE, Ab-treated, and control rat RVs and tested for gelatinase activity on gelatin-containing SDS-PAGE zymogram gels. These results are shown in Fig. 6. RV tissue from vehicle control rats showed expression of a series of gelatinases in the size range of ~50–60 kDa, consistent in size with several MMPs including collagens I, II, and III and stromelysins I and 3. RV tissue from rats with PE also expressed these activities as well as a unique gelatinase of ~90 kDa, which can be attributed to MMP9 (92 kDa gelatinase B). RVs from rats treated with anti-CINC showed no change in the 50–60 kDa activities but reduced expression of MMP9.

Discussion

Current treatment of pulmonary embolism focuses on resolving the acute cause of the vascular obstruction, either passively by the use of anticoagulant heparins to prevent additional clot formation while waiting for endogenous lysis or, in extreme cases, with thrombolytics such as tissue plasminogen activator (tPA). Acute RV hypertension is detrimental to RV function in an inflammation-dependent manner and may need to be treated more aggressively than with the current therapies to minimize damage to the heart. This hypothesis is supported by studies demonstrating that RV dysfunction following PE results in a dramatic increase in mortality in studies lasting 30 days, 1 year, or 5 years (9, 10, 12, 15, 16, 19, 20). Thus, it is essential to identify the pathways contributing to the development of RV dysfunction during PE.

Cardiac inflammation contributes to myocyte damage in LV ischemia and reperfusion (35–37). Multiple factors initiate LV inflammation, including complement activation (38), reactive oxygen species generation (39), cytokines (40), and chemokines (41, 42). Neutrophils contribute to cardiac myocyte damage in LV ischemia by releasing reactive oxygen species, proteases, cytokines, vasoconstricting lipids, and up-regulating adhesion molecules (35). Multiple anti-inflammatory interventions reduce infarct size in acute models of LV ischemia and reperfusion, supporting this concept. These studies underscore the critical significance of the inflammatory response in aggravating LV damage after acute heart trauma such as myocardial infarction. Curiously, clinical studies also indicate that prolonged anti-inflammatory therapy prevents cardiac healing (43), leading to a dilemma in patient care. Optimal treatment of cardiac inflammation may require a delicate manipulation of the inflammatory response to mitigate early detrimental inflammatory mediators while not interfering with the beneficial wound-healing processes.

In contrast with what is known about inflammatory damage during LV ischemia and reperfusion, virtually nothing is known about the contribution of inflammation to RV damage following PE. Histological studies of human RV tissue taken postmortem demonstrated the presence of neutrophil and monocyte/macrophage cells in a small series of human PE cases (27, 28), but the contribution of those inflammatory cells to cardiac injury has not been defined in the clinical setting. To study mechanisms of cardiac injury, we have successfully developed a rat model of PE that employs polystyrene microspheres as emboli (29, 32, 34). Jones et al. (32) demonstrated significant improvement in survival, mean arterial blood pressure, and heart function in rats with experimental PE treated with the anti-inflammatory agent ketorolac sodium. These observations strongly indicated that PE places the RV in a proinflammatory state. A subsequent study has validated this hypothesis and showed that moderately severe pulmonary embolism with an associated pulmonary hypertension results in massive neutrophil-mediated inflammation in RV tissues (29). Treatment of rats with a commercially available anti-neutrophil Ab significantly reduced neutrophil accumulation into RV tissue, protected RVs from hypertension-induced damage, and tended to lower the plasma concentration of cardiac troponin I. These results demonstrated that neutrophil-mediated inflammation is detrimental to RV function during PE and strongly suggested that targeted anti-inflammatory therapies would be beneficial in the treatment of PE.

In our previous studies, we did not identify the specific molecules responsible for recruiting neutrophils into RV tissue during PE. We now demonstrate that the CXC chemokine CINC-1 is at least partially responsible for the neutrophil recruitment into RVs, because the treatment of PE rats with goat anti-CINC-1 Abs inhibited neutrophil accumulation in RVs during PE and reduced subsequent RV damage. These results identify at least one specific protein responsible for the deleterious inflammation in RV tissue during PE. The use of a polyclonal anti-CINC-1 Ab for these studies does raise the possibility of cross-reactivity with CINC-2 or CINC-3/ MIP-2 because the three CINC proteins are closely related, sharing ~85% sequence identity. Redundancy is a common theme in chemokine biology and it is difficult to be precise in delineating the contributions of closely related proteins. However, a previous preparation of anti-CINC-1 Ab, prepared by the same protocol as the current reagent, was extremely successful in delineating neutrophil recruitment mechanisms in other models of inflammation (31, 44–53).

Additional aspects of the inflammatory response to PE warrant specific comment. Although we cannot rule out the direct activation of complement or cytokine/chemokine secretion by the presence of the polystyrene microspheres, several observations argue against this interpretation. First, the microspheres lodge within the lung vasculature (34) and there are no polystyrene microspheres observed by histology within the right ventricle or left ventricle or the heart muscle of the rats following 2, 6, or 18 h of pulmonary embolism (29). Thus, it is unlikely that there should be a direct inflammatory response to the microspheres in the heart. Second, there was no evidence of up-regulation of CINC-1, CINC-2, MIP-2, and MCP-1 message when polystyrene microspheres were added to rat pulmonary artery endothelial cells in culture (34). Third, there is no evidence of inflammation or damage occurring in the left ventricle of the heart as the injury is confined to the right ventricle in this model (29), suggesting that the inflammation is localized rather than systemic. Fourth, while the hypertensive dose of polystyrene microspheres (2.0 million per 100 g of body weight) causes the response, a 35% lower dose of polystyrene microspheres (1.3 million per 100 g of body wt) does not cause right ventricular inflammation or dysfunction of the RV (29). Thus, the dysfunction is associated with hypertension and not just the presence of beads.

The present studies evaluated CINC message in the RV tissue in response to PE but did not evaluate chemokine proteins. Although the microarray fluorescence intensity of the message increases 73- and 16-fold, respectively (present studies), we have previously shown, in contrast with a 21-fold increase in MCP-1 protein, that CINC-1 and CINC-2 proteins are decreased (37 and 38%, respectively) 18 h after PE (29). CINC protein storage is not described in cardiac tissues; however we have proposed that this decrease may be due to the release of previously stored CINC or to the binding and removal of this
chemokine by the accumulating inflammatory cells within the RV tissue (29).

We believe that our results have profound implications for the treatment of RV dysfunction in human patients with PE. A key concept that we have been developing in our laboratory is the “bridge hypothesis,” namely, that if RV tissue can be protected from acute inflammatory damage during the time required for resolution of the pulmonary emboli in the lung vasculature, long-term patient morbidity and mortality can be reduced. Treatment modalities might focus on blocking neutrophil influx into RV tissue, perhaps by using chemokine antagonists or by inhibiting downstream neutrophil-secreted factors. Therapies might need to be focused on ameliorating the damaging acute inflammation caused by neutrophils without retarding the likely wound-healing benefit from monocyte recruitment.

We conclude that experimental PE leads to RV dysfunction with a marked cardiac inflammatory response that is mediated, at least in part, by CINC-1 in the rat. Inhibition of the CINC/CXCR2 pathway in vivo provides improvement in cardiac function, suggesting that modulation of inflammatory responses may provide the “bridge” needed to protect the heart from inflammatory damage during clot dissolution in human patients with PE.

Acknowledgments

Samples for microarray analyses were prepared by Nina Sanaparredy (current address: Bio-Informatics Program, University of North Carolina, Chapel Hill, NC). Microarray hybridizations and scanning were done by the staff of the CMC-Cannon Research Center Microarray Core Facility. The histology samples were prepared by Jane Ingram, Histology Core Facility, Cannon Research Center, Carolinas Medical Center.

Disclosures

The authors have no financial conflict of interest.

References

ROLE OF CINC-1 IN HYPERTENSIVE RIGHT VENTRICULAR FAILURE


