Impact of C1-INH and sCR-1 on Respiratory Failure After Complement Activation

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Summary

Admixture of 35% human serum to the perfusion fluid of isolated rabbit lungs provoked immediate activation of the complement cascade with production of C5b-9, subsequent tissue damage and release of thromboxane (TX) A_2. Within 20 min overwhelming pulmonary artery pressures and pulmonary edema occurred in the control group. The complement regulators C1-Inh and sCR1 significantly reduced complement-induced lung edema and vasoconstriction due to reduced complement activation and subsequent decreased TXA_2 levels.

Introduction

Activation of the complement system plays a major role in the pathogenesis of acute respiratory distress and downregulation of the complement cascade has demonstrated beneficial effects in a variety of human and animal studies [1]. Depletion or selective inhibition of the complement system can preserve endothelial function and limit the degree of tissue injury [2]. C1-Inh inhibits activated C1s and C1r of the classical pathway of complement [3] but also serves as a major regulator of both factor XIIa and kallikrein of the contact system. Besides this, the extramembraneous part of complement receptor 1 (CR1, CD35), serves as potent regulator of C3/ C5 convertases of both the classical and the alternate activation pathway [4].We investigated the impact of blockade of the complement cascade with C1-Inh or sCR1 on non-granulocyte dependent complement effects in isolated blood free perfused rabbit lungs [5].

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Materials and Methods

The techniques of preparing and perfusing isolated rabbit lungs have been previously described in detail [5, 6]. Briefly, anesthetized rabbits were mechanically ventilated by means of a respirator and the pulmonary artery was cannulated. The lung organ-preparation was isolated and suspended from a weight transducer in a temperature-controlled and humidified chamber. The lungs were perfused with 200 ml Krebs Henseleit hydroxy-ethyl-starch buffer solution (KHHB) by a roller pump at a constant flow of 150 ml/min in a recirculating system. Pulmonary arterial (PAP) and airway pressures (AP) were continuously recorded via Statham strain gauge transducers. And the weight gain of the lungs was continuously monitored. Intermittently samples of perfusate were taken for measurements of TXB_2, and C5b-9 concentrations.

A cell- and plasma-free perfusion medium was used in the present study in order to avoid the complex interactions with different circulating cells, which may mask direct effects on vascular tone and mediator release. C1-Inh (Berinert® HS) Lot 0736211 was a generous gift from Dr. Henkel, Centeon Pharma GmbH, Marburg, FRG. Human rsCR-1 was kindly provided by Dr. Levin, T-Cell Sciences Inc., Needham MA, USA. Doses were chosen according to previous studies and on the basis of our own dose response experiments.

In situ complement activation was achieved by admixture of normal human serum (NHS) to the perfusate of isolated rabbit lungs (final concentration 35%). Following a 30 min equilibration period, NHS was applied in absence of inhibitors (controls; n=6) or in the presence of C1-Inh (1.0 U/ml; n=6) or sCR-1 (2.0μg/ml; n=6). TXB_2 was assayed from 100 μl of recirculating KHHB as stable hydrolysis product of TXA_2 by an enzyme immunoassay according to the manufacturers specifications. C5b-9 analysis was performed by ELISA [7]. Tissue bound C3c and C5b-9 (Dako, Hamburg, Germany) was evaluated semiquantitatively. All data are presented as means ± standard error of means (SEM). Differences between groups were tested by one-way analysis of variance (ANOVA) followed by a Student Newman Keuls multiple comparison procedure. Significance was accepted at $p<0.05$. This study was approved by the Animal Subject Protection Committee of the local government.

Results

Baseline values of PAP (7-9 mmHg) and airway pressure between 10 and 12 mbar were similar in all groups and in agreement with previous studies reported by our group [5]. NHS administration induced an acute pressure increase up to 42 ± 6 mmHg within 20 min (Fig. 1). The pressure increase in the lungs was significantly ($p<0.05$) reduced after pretreatment

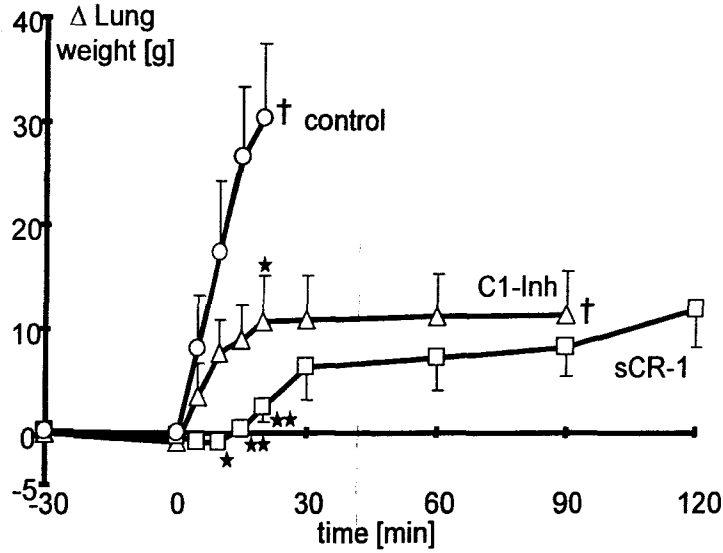

*FIGURE 1: Time course of pulmonary edema formation as assessed by lung weight gain after administration of human serum (35%) in control lungs (n=6), and under pretreatment with C1-Inh (1 U/ml; n=6) or with sCR-1 (2 μg/ml; n=6). Severe lung edema (controls) was reduced by both C1-Inh (p<0.05) and sCR1 (p<0.01). *p<0.05; **p<0.01; vs. control.*

with both sCR1 (20 ± 3 mmHg) and C1-Inh (25 ± 5 mmHg). Following primary PAP elevation, sCR1 was more potent in reducing pulmonary artery pressure until the end of observation period. Levels of pulmonary artery pressure regained approximately baseline values (12 ± 1 mmHg). In parallel to the observed increase in pulmonary artery pressure, edema formation occurred in control lungs. From baseline values of 30 to 40 g control lungs showed a weight gain of 30 ± 7 g within 20 min. Both C1-Inh and sCR1 significantly reduced edema formation to a weight gain of 11 ± 4 ($p<0.05$) and 2 ± 1 g ($p<0.01$) respectively (Fig. 1). C5b-9 levels at timepoint 0 min ranged between 4.1 and 5.9 μg/ml. In control lungs, C5b-9 levels increased to 873 ± 233% at 20 min, while C1-Inh (414 ± 104%) and sCR1 (258 ± 58%; $p<0.05$) reduced terminal complement complex formation. Compared to controls, lungs pretreated with either C1-Inh or sCR1 showed significantly decreased C3c and C5b-9 immunohistologic precipitation in the pulmonary vasculature

Baseline TXB_2 ranged between 6.8 and 10.0 nM. Immediately after complement activation TXB_2 release rates increased to 3000- 3700 pmol/min. 30 min later no further TXB_2 release was detectable. C1-Inh and sCR1 significantly suppressed TXB_2 release rates five min after serum administration ($p<0.001$) and sCR1 additionally after 10 min ($p<0.01$).

Discussion

Systemic activation of the complement system with generation of anaphylatoxins and pulmonary granulocytosis has been suggested as a general concept for the development of acute respiratory distress in patients suffering from bacterial sepsis or multiple trauma [8]. Modulation of the complement cascade reduced tissue injury in a number of animal models of severe complement dependent inflammation.

In the current study we investigated the possible protective effect of complement inhibition by C1-Inh and sCR1 in states of massive complement system activation. Experiments were chosen to be performed in isolated rabbit lungs which allow to analyze the pulmonary vascular response and mediator release involved in such complex reactions. In the current study the complement inhibitors C1-Inh and sCR1 significantly decreased the pressure response to NHS and reduced pulmonary edema formation (Fig. 1). The reduced vasoconstriction and edema formation was paralleled by decreased levels of both, soluble terminal complement complex (C5b-9) in the circulating perfusate and tissue bound complement C3c and C5b-9. Correspondingly, TXB_2 release rates into the perfusion fluid of the isolated rabbit lungs were reduced. In the present experiments, local complement activation was initiated in the pulmonary circulation at the surface of pulmonary cells. Under these conditions, acute alterations in the pulmonary circulation including TXB_2 mediated pressure response and vascular leakage were evoked, which may be caused by in situ generation of membrane bound C5b-9 complexes. The formation of C5b-9 complexes obviously triggeres prostanoid generation in lung tissue, since C8 requirement has been reported for TXB_2 and PGI_2 generation [9]. In isolated rabbit lungs, TXB_2 release by endothelial cells was found to be the crucial mechanism of complement induced pulmonary injury [10]. Complement receptor 1 is a potent regulator of C3 and C5 activation [4]. Thus, sCR1 develops its inhibitory activity at the common sequence of the complement cascade, regardless which pathway caused activation [4]. In the present study, sCR1 attenuated C5b-9 generation and protected lungs from subsequent tissue damage as assessed by edema formation and vasoconstriction. Since C1-Inh is an inhibitor of activated C1s and C1r [3] it modulates the activation sequence at an early step of the classical pathway, but does not interfere with activation via the alternate pathway. While the current study utilizes a pathomechanism which predominantly triggers the alternate pathway, a somewhat lower capability of C1-Inh to reduce tissue injury may have been expected, as compared to sCR1. The protective effects of C1-Inh, however, may be due to inhibitory effects on other mediator generating cascade systems such as the kinin system. Second, activation of the classical pathways by pro- inflammatory mediators, which are released in response to sublytic doses of C5b-9 may

account for the impact of early classical pathway blockade by C1-Inh as demonstrated in the current study. Third, additional activation of the classical pathway, caused by anti-rabbit antibodies in the utilized NHS cannot be excluded, which would also explain the beneficial effects of C1-Inh in our experimental set-up.

Conclusions

The complement regulators C1-Inh and sCR1 significantly reduced complement-induced lung edema and vasoconstriction due to reduced complement activation and subsequent decreased TXA_2 levels.

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