Complement-dependent immune complex-induced bronchial inflammation and hyperreactivity

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Received 15 July 1999; accepted in final form 16 October 2000

Lučaks, Nicholas W., M. Michael Glovsky, and Peter A. Ward. Complement-dependent immune complex-induced bronchial inflammation and hyperreactivity. Am J Physiol Lung Cell Mol Physiol 280: L512–L518, 2001.—Bronchoconstriction responses in the airways are caused by multiple insults and are the hallmark symptom in asthma. In an acute lung injury model in mice, IgG immune complex deposition elicited severe airway hyperreactivity that peaked by 1 h, was maintained at 4 h, and was resolved by 24 h. The depletion of complement with cobra venom factor (CVF) markedly reduced the hyperreactive airway responses, suggesting that complement played an important role in the response. Blockade of C5a with specific antisera also significantly reduced airway hyperreactivity in this acute lung model. Complement depletion by CVF treatment significantly reduced tumor necrosis factor and histamine levels in bronchoalveolar lavage fluids, correlating with reductions in airway hyperreactivity. To further examine the role of specific complement requirement, we initiated the immune complex response in C5-sufficient and C5-deficient congenic animals. The airway hyperreactivity response was partially reduced in the C5-deficient mice. Complement depletion with CVF attenuated airway hyperreactivity in the C5-sufficient mice but had a lesser effect on the airway hyperreactive response and histamine release in bronchoalveolar lavage fluids in C5-deficient mice. These data indicate that acute lung injury in mice after deposition of IgG immune complexes induced airway hyperreactivity that is C5 and C5a dependent.

Histamine; tumor necrosis factor; lung

MULTIPLE PATHWAYS AND MECHANISMS can mediate the induction of airway hyperreactivity (7, 8, 18, 47, 52). Classic investigations have described IgE-mediated mast cell activation followed by an intense late-phase inflammatory response that includes the presence of lymphocytes, monocytes, and eosinophils. Although the mediators for acute and chronic airway responses have not been completely identified, it has been established that the severity of the airway response is a function of the intensity of the inflammation (7, 8, 18, 47, 52). Several specific and nonspecific events are likely involved and contribute to the induction of airway hyperreactivity.

Acute lung injury that is induced by IgG immune complex deposition in the lung includes a vascular leak syndrome, significant recruitment and activation of leukocytes, and damage of vascular endothelial cells and alveolar epithelial cells (27, 49, 62). These types of events are observed in many diseases including autoimmune diseases and specific types of immune-mediated diseases such as allergic aspergillosis. In these diseases, deposition of immune complexes is often associated with a severe hemorrhagic alveolitis in association with intense infiltration of neutrophils into the interstitial and intra-alveolar compartments within 1–4 h (26, 37, 50, 67). Subsequently, the inflammatory response results in the loss of integrity of the alveolar capillary wall. Neutrophils recruited to the site are activated and release a number of mediators, further damaging the inflamed tissue. The response is dependent on the activation of a number of complement proteins (45, 46). Inhibition of complement components or complement activation products attenuates tissue damage. Although the response in animal models of IgG immune complex-induced inflammation in the lung has been extensively studied, the ability of immune complex-induced responses to alter lung function has not been well analyzed. In the present study, we demonstrate that IgG immune complex-induced pulmonary inflammation induces airway hyperreactivity in murine lungs in a manner that is complement dependent.

MATERIALS AND METHODS

Animals. Female CBA/J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained under standard pathogen-free conditions. C5-deficient mice (B10.D2 “old line”) and C5-sufficient mice (B10.D2 “new line”) were also obtained from the Jackson Laboratory and are congenic mouse strains.

Induction of pulmonary immune complex inflammation. Polyclonal rabbit anti-BSA IgG antibody (50 μg/mouse in 50 μl) was injected intratracheally into CBA/J mice followed by an injection of BSA (1 mg) via the tail vein. The mice were euthanized at various time points post-BSA injection (1, 4, and 24 h). The parameters examined included bronchoalveolar lavage (BAL) fluid content such as leukocytes, tumor necrosis factor (TNF), and histamine and quantitation of airway physiological changes.

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To deplete animals of complement, cobra venom factor (CVF; 200 U/kg) was injected intraperitoneally the night before surgery. This dose has previously been shown to reduce C3 levels in plasma to <3% of normal levels (17, 34).

Measurement of airway hyperreactivity. Airway hyperreactivity was measured with a mouse plethysmograph specifically designed for the low tidal volumes (Buxco, Troy, NY) as previously described (8–11). Briefly, the mouse to be tested was anesthetized with pentobarbital sodium and intubated via cannulation of the trachea with an 18-gauge metal tube. The mouse was subsequently ventilated with a Harvard pump ventilator (tidal volume = 0.4 ml, frequency = 120 breaths/min, positive end-expiratory pressure = 2.5–3.0 cmH₂O), and the tail vein was cannulated with a 27-gauge needle for injection of the methacholine challenge. The plethysmograph was sealed, and readings were monitored by computer. Because the box was a closed system, a change in lung volume was represented by a change in box pressure (Pbox) that was measured by a differential transducer. The system was calibrated with a syringe that delivered a known volume of 2 ml. A second transducer was used to measure the pressure swings at the opening of the tracheal tube (Paw) referenced to the body box (i.e., pleural pressure) and to provide a measure of transpulmonary pressure (Ptp = Paw − Pbox). The tracheal transducer was calibrated at a constant pressure of 20 cmH₂O. Resistance was calculated with Buxco software by dividing the change in pressure (ΔPtp) by the change in flow (ΔF; box pressure; ΔPtp/ΔF in cmH₂O·ml⁻¹·s⁻¹) at two time points from the volume curve based on a percentage of the inspiratory volume. Once the mouse was hooked up to the box, it was ventilated for 5 min before readings were acquired. Once baseline levels were stabilized and initial readings were taken, a methacholine challenge was given via the cannulated tail vein. After the determination of a dose-response curve (0.001–0.5 mg), an optimal dose of 0.1 mg of methacholine was chosen. This dose was used throughout the rest of the experiments in this study. After the methacholine challenge, the response was monitored, and the peak airway resistance was recorded as a measure of airway hyperreactivity.

Collection of BAL fluid. Lungs from mice were perfused with 1 ml of PBS via intratracheal injection with a 1-ml syringe and a 26-gauge needle. After 30–40 s, the PBS was collected by aspiration with the same syringe and needle. Between 700 and 800 μl could routinely be re-collected from the perfused lung. The cells were then collected by centrifugation, resuspended in fresh PBS, and cytocentrifuge onto a glass slide. The cytopsin were then differentially stained with eosin and hematoxylin. The percentage of cells was then determined by counting the number of eosinophils per 200 total cells. Histamine levels were measured in cell-free BAL fluid by ELISA with commercially available kits (Amac, Westbrook, MA). Leukotrienes (LTs) from the BAL fluid were assessed with enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI).

Cytokine analysis by ELISA. The levels of TNF-α in BAL fluid were measured by specific ELISA with a modification of a double-ligand method as previously described (6). Briefly, lung tissue was homogenized on ice with a tissue tearor (Biospec Products, Racine, WI) for 30 s in 1 ml of PBS containing 0.05% Triton X-100. The resulting supernatant was isolated after centrifugation (10,000 g). Flat-bottom, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 50 μl/well of rabbit polyclonal antibodies specific for the cytokine or chemokine in question for 16 h at 4°C and then washed with PBS and 0.05% Tween 20. Nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer, and cell-free supernatants were added (heat and diluted 1:10) followed by incubation for 1 h at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were washed again, and chromogen substrate (Bio-Rad) was added and incubated at room temperature to the desired extinction. The reaction was terminated with 50 μl/well of 3 M H₂SO₄ solution, and the plates were read at 490 nm in an ELISA reader. Standards were 0.5 log dilutions of recombinant protein from 1 pg/ml to 100 ng/ml.

Statistical analysis. Significance was determined by ANOVA, with P values < 0.05.

RESULTS

IgG immune complex-induced, complement-dependent airway hyperreactivity. Intrapulmonary deposition of IgG immune complexes can result in injury, including a vascular leak syndrome, significant recruitment and activation of neutrophils, and damage to lung tissue. To determine whether this complement-dependent inflammatory response had a pathophysiological consequence, we examined lung function parameters in mice with IgG immune complex-induced injury in the lung. Mice were injected intratracheally with anti-BSA IgG followed by the intravenous injection of BSA (1 mg). As shown in Fig. 1, the induction of airway hyperreactivity to a methacholine challenge was most severe by 1 h, was still apparent but diminishing by 4 h, and had resolved by 24 h postimmune complex deposition. Control mice given intratracheal antibody with saline challenge instead of BSA showed no significant increase in airway resistance. These data demonstrated an early response to IgG immune complexes that was characterized by increased airway resistance. Histological changes in the lungs indicated interstitial and intra-alveolar accumulations of neutrophils and were associated with the airway hyperreactivity changes observed (Fig. 2). Because the immune complex-induced inflammation has been shown to be a complement-mediated pathway, we examined mice that had been complement depleted. This was accomplished with an injection of CVF 18 h before induction.
of the immune complex-induced response. The histo-
logical results indicated reduced inflammation in the
complement-depleted animals after immune complex
deposition (Fig. 2C), whereas in complement-intact
mice, a marked neutrophil infiltration was detected in
the bronchial wall (Fig. 2, B and D). The data pre-
sented in Fig. 3 clearly demonstrate that when com-
plement was inhibited by pretreatment with CVF, the
airway hyperreactivity response was nearly abolished.
These results are consistent with previous data (21, 43,
57) that indicated that the immune complex-induced
airway responses require complement-mediated acti-
vation events and suggest that airway hyperreactivity
requires at least C3.

To further define the complement products that may
be responsible for the responses during intrapulmo-
nary immune complex deposition, we treated the chal-
lenged mice with specific anti-C5a antibody given in-
tratracheally. The data in Fig. 4 indicate that the
blockade of C5a significantly attenuated airway hyper-
reactivity responses. We examined the neutrophil re-
cruitment responses and found a decrease in the anti-
C5a-treated mice compared with the control mice at 4 h
postdeposition (12.6 ± 2.0 × 10^4 and 18.8 ± 1.8 ×
10^3/ml, respectively; n = 5/group). Thus the immune
complex complex induces an airway hyperreactivity is dependent
on the activity of the complement, specifically, C5a.

Fig. 2. Histological examination of im-
mune complex-induced inflammation
inhibited by cobra venom factor (CVF).
Animals were given CVF (200 U/kg)
and challenged with immune complex-
deposition the next morning. Normal animals (A) were used for
comparison, and complement-depleted
animals (C) demonstrated substantially less inflammation compared with
control immune complex-challenged mice (B and D). Arrow, neutrophils;
arrowhead, edema or leak from the inflam-
matory response.

Fig. 3. Complement depletion blocked the immune complex induc-
tion of airway hyperreactivity. Animals were given CVF (200 U/kg)
and challenged with immune complex-induced inflammation the next morning. The mice were subsequently examined for airway
hyperreactivity responses after an intravenous injection of metha-
choline (100 μg/kg) 4 h after immune complex challenge. Bkgd, background. Data are means ± SE from 5–6 mice/group and repre-
sent the change in airway resistance over background levels. *P <
0.05 compared with BSA/anti-BSA positive control.
Association of airway hyperreactivity with TNF-α and histamine. Because the early time point (1 h) showed peak airway hyperreactivity, we examined common cytokine pathways. TNF was significantly reduced after CVF-mediated complement depletion 1 h postchallenge (Fig. 5). Measurement of histamine levels in the BAL fluid demonstrated that the mediator was released into the airways 1 and 4 h postchallenge (Fig. 6), producing levels of 8–10 and 25–30 nM, respectively. When animals were complement depleted, significantly less histamine was detected in the BAL fluid; at 1 and 4 h, the level was <1 nM. When LTE4 was examined, there was a significant increase in its levels in BAL fluid 1 h after the immune complex response compared with that in control animals (412 ± 112 and <10 pg/ml, respectively). However, no significant decrease in LTE4 was observed after CVF treatment (422 ± 116 pg/ml). Thus at least two important mediators were significantly affected by depletion of complement before immune complex-induced inflammation, whereas levels of LTE4 were unaffected.

Airway hyperreactivity in C5-deficient mice. We examined immune complex-induced inflammation in C5-deficient mice compared with congenic C5-sufficient mice. As shown in Fig. 7, airway hyperreactivity in C5-deficient mice was similar to that in the C5-sufficient mice. The data indicated that the responses in the B10 strain of mice shown in Fig. 7 were lower compared with those observed in the CBA/J strain (Fig. 1), making comparisons between strains somewhat problematic. In these same studies, complement depletion was carried out with an overnight treatment with CVF. Induced complement depletion treatment significantly lowered airway hyperreactivity responses in C5-sufficient mice, whereas the C5-deficient mice pretreated with CVF showed no significant decrease (Fig. 7). These data suggested that mechanisms other than CVF-sensitive products were operative in the C5-deficient mice for the induction of airway hyperreactivity. To correlate these results with previous observations in Association of airway hyperreactivity with TNF-α and histamine, we also examined histamine levels in the BAL fluids of these mice (Fig. 8). The C5-deficient mice demonstrated a lower level of histamine in the
BAL fluid compared with the C5-sufficient mice after immune complex responses. CVF treatment of C5-sufficient animals virtually abrogated the increase in histamine content in the BAL fluid, whereas CVF treatment only partially attenuated histamine release in the BAL fluid of C5-deficient mice. These results partially correlate with the airway hyperreactivity data. Collectively, the data suggest that IgG immune complex-induced airway hyperreactivity is C3, C5, and C5a dependent. Increases in BAL fluid levels of TNF and histamine (but not of LTE4) are complement dependent. In C5-deficient mice, there appears to be some C3 dependency, but other mediator pathways, such as Fcγ, have been engaged.

**DISCUSSION**

Bronchial asthma is a disorder associated with airway inflammation and characterized pathophysiologically by airway hyperreactivity that is induced by a variety of stimuli (5, 15, 32, 46, 59, 64). Classically, asthmatic responses have been related to IgE-mediated pathways that induce mast cell and basophil activation and degranulation that lead to mediator release. In these present studies, we have demonstrated that IgG immune complex deposition in the lung can induce severe airway hyperreactivity in the mouse, a finding that has not been previously reported. The extensive body of information related to the pathophysiological mechanisms involved in this model of lung injury has allowed us to obtain further information on events that are triggered by tissue deposition of IgG immune complexes. These reactions are accompanied by TNF-α production, histamine release, and neutrophil accumulation. The hyperreactivity response peaked early (by 1 h) and had resolved by 24 h. Interestingly, the airway permeability observed in this model had previously been shown to peak at 4 h (42, 43, 63), suggesting that the hyperreactivity may be a related but separate event. Previous studies (21, 43, 57) have clearly demonstrated that this inflammatory response is dependent on complement activation products. Depletion of complement by CVF inhibited the development of airway hyperreactivity.

The complement activation products C3a and C5a (anaphylatoxins) are known to produce airway smooth muscle constriction in guinea pigs and humans (48, 54, 55, 60). C3a and C5a receptors have been found on eosinophils, basophils, mast cells, monocytes, neutrophils, activated lymphocytes, and numerous nonmyeloid cell populations (21, 22). Anaphylatoxins can cause histamine and mediator release from basophils and mast cells (23, 25, 28, 31, 53, 65, 66). In addition, C5a is a chemotactic and activating factor for effector cells such as neutrophils and eosinophils (29, 36, 45, 57, 61). Finally, C3a and C5a have been shown to upregulate the expression of selectins and β3-integrin molecules (2, 30, 35, 41, 42). Endothelial cells respond directly to C5a with increased expression of P-selectin on their surfaces. In the present studies, it appears that complement activation products lead to stimulation of mast cell/basophil populations, resulting in the release of histamine, LTE4, and other acute mediators. TNF-α, which can be readily released from mast cells, was also affected by depletion of the complement. Because histamine levels were more elevated at 4 h compared with 1 h, it is possible that multiple factors and cell populations are involved in the progression of the airway hyperreactivity responses. Taken together, these studies suggest a significant role for complement activation in the lung that leads to alteration of lung function.

CVF (cobra C3b) is known to bind to factor B of the alternative complement pathway, forming a stable C3bBb complex and a markedly depleted C3, resulting in blockade of all three complement pathways (3, 12, 13, 44). Pretreatment of mice with CVF to inhibit complement-mediated pathways attenuates the immune complex-induced response within the lung. After CVF injection, the hyperreactivity response to the bronchospastic mediator methacholine was nearly completely attenuated. This was further supported by the histopathology in mice treated with CVF, which demonstrated decreased injury, inflammation, and reduced airway changes. In addition, the depletion of C5a with specific antisera further suggests that this complement component is one of the key factors involved in the development of airway hyperreactivity with this model. The data in this report demonstrate that complement may be an essential pathway in the hyperreactive airway response.

In an attempt to examine the requirements for specific complement components, we used congenic mice that were either deficient or sufficient in C5. Although there were some differences between the two groups in their hyperreactive airway responses, the differences were not significant. However, when these animals were complement depleted with CVF, a distinct difference was observed. As noted above, treatment with CVF attenuated the hyperreactivity response and reduced histamine levels in C5-sufficient but, surprisingly, not in C5-deficient mice. These results suggest that although C3 and C5 play an important role in the
responses to immune complexes in complement-intact mice, other pathways appear to compensate for the absence of C5. It remains to be determined how this compensation occurs, but it may involve several other mediator pathways (1, 33, 58). Anti-C5a treatment significantly blocked the airway hyperreactivity in C5-intact mice, suggesting that this component plays a significant role in the development of the responses. This is in keeping with recently published results of a study (16) that used anti-C5a antibody for the treatment of acute septic responses. The connection between mast cell/basophil activation and airway hyperreactivity is well accepted. Taken together, these studies connect the interacting effects of complement activation, mast cell/basophil degranulation, and early-phase airway hyperreactivity, at least in complement-intact mice.

The bronchoconstrictive responses observed in asthma are multifactorial and are triggered by numerous factors including allergens, cold air, exercise, pol lutants, and viral infections (19, 38–40). Thus it is not surprising that multiple mediators are involved in pathophysiological responses during the evolution of bronchospastic disease. The results from these studies indicate that bronchospastic mediators that may be common to allergen-induced responses are also produced during an immune complex-induced response. Histamine and leukotrienes (LTC4, LTE4) as well as TNF-α are produced at significant levels within the airway during the immune complex response. Although the exact source of these mediators was not identified in these studies, all of these can be produced by mast cell/basophil populations within the lung. Other cell populations, such as epithelial cells and recruited inflammatory cell populations, have the ability to produce leukotrienes (4, 20, 51). Interestingly, the C5-deficient mouse studies indicated that immune complex-induced airway hyperreactivity is not completely dependent on this pathway. Fcy receptors are also relevant and may be important in the airway hyperreactive response. Several studies (14, 24, 56) have indicated that local lung populations may be a source of complement proteins relevant in this injury. In a global sense, this model demonstrates that both complement-dependent and complement-independent pathways may be relevant in acute bronchial hyperactivity responses induced by IgG immune complexes. Whether these responses translate directly to human asthma needs to be considered and is worthy of further investigation.

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