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Local Blockade of Allergic Airway Hyperreactivity and Inflammation by the Poxvirus-Derived Pan-CC-Chemokine Inhibitor vCCI

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Allergen-induced asthma is characterized by chronic pulmonary inflammation, reversible bronchoconstriction, and airway hyperreactivity to provocative stimuli. Multiple CC-chemokines, which are produced by pulmonary tissue in response to local allergen challenge of asthmatic patients or experimentally sensitized rodents, chemoattract leukocytes from the circulation into the lung parenchyma and airway, and may also modify nonchymotactic function. To determine the therapeutic potential of local intrapulmonary CC-chemokine blockade to modify asthma, a recombinant poxvirus-derived viral CC-chemokine inhibitor protein (vCCI), which binds with high affinity to rodent and human CC-chemokines in vitro and neutralizes their biological activity, was administered by the intranasal route. Administration of vCCI to the respiratory tract resulted in dramatically improved pulmonary physiological function and decreased inflammation of the airway and the lung parenchyma. In contrast, vCCI had no significant effect on the circulating levels of total or allergen-specific IgE, allergen-specific cytokine production by peripheral lymph node T cells, or peritoneal inflammation after local allergen challenge, indicating that vCCI did not alter systemic Ag-specific immunity or chemotraction at extrapulmonary sites. Together, these findings emphasize the importance of intrapulmonary CC-chemokines in the pathogenesis of asthma, and the therapeutic potential of generic and local CC-chemokine blockade for this and other chronic diseases in which CC-chemokines are locally produced. The Journal of Immunology, 2000, 165: 3418–3422.

Allergen-induced asthma is characterized by allergen-specific IgE production by B cells and plasma cells (1) and inflammation and hyperreactivity of the airway following allergen re-exposure (2, 3). The bronchoconstrictive and inflammatory events of allergen-induced asthma can be recapitulated by administering allergen to the airways of sensitized asthmatics, resulting in a stereotyped early phase response characterized by acute bronchoconstriction, due to the IgE-dependent release of mediators (2). This is typically followed ~6–12 h later by a late phase response, in which there is the onset of progressive inflammation of the lung parenchyma and airway with eosinophils and mononuclear leukocytes (4) and the recurrence of bronchoconstriction. The recruitment of leukocytes to the lungs of allergic individuals is believed to be primarily mediated by CC-chemokines, a number of which, including macrophage inflammatory protein (MIP)-1α, monocyte chemoattractant protein (MCP)-1, MCP-3, MCP-4, eotaxin, and RANTES, are expressed in increased amounts in the lungs of asthmatic patients (2) and in rodents with experimentally induced asthma (3, 5).

The pleiotropic functions of CC-chemokines suggest that they do not only play a critical role in the recruitment of inflammatory cells during the late phase response (6), but potentially may also directly enhance Ag-driven T cell activation, cytokine production and differentiation (7, 8). IgE synthesis by B cells (9), and dendritic cell Ag presentation (6). In addition to their role in inflammation and adaptive immune responses, CC-chemokines are also important in the normal homeostasis of lymphocytes via their effects on their circulation and homing between lymphoid and nonlymphoid organs (6, 10). The presence of CCRs on nonhematopoietic cells, such as smooth muscle cells (11, 12), also raises the possibility that CC-chemokines by exert effect in tissues that are not directly related to their role in innate and adaptive immune responses.

The functional importance of CC-chemokines in eliciting inflammation and airway hyperreactivity in rodent asthma models was demonstrated in studies in which CC-chemokine function was systemically blocked using neutralizing Abs, amino-terminal modified CC-chemokines that act as receptor antagonists (5) or by selective gene targeting (13, 14). Although there is growing evidence for the biological importance of locally elaborated CC-chemokines in the lung in allergen-induced asthma, it remains unclear whether selective blockade of CC-chemokines in the lung compartment has potential as a therapy for the inflammatory and physiologic abnormalities in this disease. Broad spectrum chemokine/CCR antagonists have been proposed to be more advantageous than selective antagonists (15). This view (6, 16) is supported by a recent study that demonstrated a distinct temporal sequence of the pulmonary expression and role of the individual chemokines MCP-1, eotaxin and MCP-1α following repeated aeroallergen challenge of sensitized mice (5). It is also plausible that the relative importance of a
particular CC-chemokine in asthma may be influenced by the context of provocation of acute attacks. For example, because respiratory syncytial virus infection of airway epithelial cells induces their production of CC-chemokines, such as RANTES (17, 18), it is possible that RANTES might play a more important role in asthmatic airway hyperreactivity and inflammation triggered by respiratory viral infection than by allergen alone.

Viral-derived CC-chemokine inhibitor (vCCI) is a 35-kDa secreted virulence protein encoded in the genome of pox viruses that enhances their evasion of host immune responses. It is the only CC-chemokine inhibitor known to bind specifically and generically to CC-chemokines (human and rodent) with high affinity and to completely inhibit their biological activity; the mechanism is one of competitive inhibition of CC-chemokine receptors (19). The dissociation constant of vCCI is in the subnanomolar range for all 15 different CC-chemokines that have been tested, and the affinity of CC-chemokines for vCCI is often higher than that for their native CCRs (19), arguing for an unusually effective inhibition. Although vCCI has no apparent amino acid or structural homology to known mammalian or other eukaryotic proteins, including CCRs (20), its broad high-affinity CC-chemokine binding suggested that it might be particularly useful dissecting the biological role of CC-chemokines in inflammatory diseases and as a clinical anti-inflammatory agent. To examine the potential for local and generic blockade of CC-chemokines to treat asthma, we determined the effect of the intranasal (i.n.) administration of a vCCI to the respiratory tract in a murine model of allergen-induced asthma.

Materials and Methods
vCCI protein
The entire coding region of the 32-kDa vCCI protein from the cowpoxvirus genome was fused to a segment encoding the Fc region of human IgG1, expressed and purified as previously reported (19).

Allergen-induced pulmonary and peritoneal disease
Female BALB/cJ mice, 8–12 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained under specific pathogen-free conditions. The dose of crystalline OVA (Pierce, Rockland, IL) was given 100 μg unless specified. In the standard protocol, OVA-treated mice received single i.p. injections of alum-precipitated OVA on days 1 and 14, and i.n. OVA in normal saline on days 14, 24, and 25 (21). Two hours before each i.n. dose of OVA, mice were briefly anesthetized by i.p. injection with a mixture of OVA and KLH (50 μg) in 50 μl of normal saline and given was 100 μg/kg of acetylcholine (Mch; Sigma, St. Louis, MO) in normal saline. This dose results in an ~40–60% reduction in dynamic compliance and a 300–450% increase in resistance in OVA-sensitized/challenged BALB/cJ mice (21). The methods used were similar to those previously described (21), except that the chest was not opened and a commercial plethysmograph, transducer, and amplifier system was used for data collection (Buxco, Sharon, CT). Enhanced pause (Penh) was assessed by whole body plethysmography (Buxco) of conscious, unrestrained mice (22).

OVA-specific IgE, KLH-specific IgG1 and total IgE ELISAs
OVA-specific and total IgE determinations were performed as previously described using TMB substrate (Kierkegaard & Perry, Gaithersburg, MD) for development (23). Pooled plasma from BALB/c mice immunized twice with alum-precipitated OVA was used as an internal standard, and was arbitrarily assigned an OVA-specific IgE titer of 10 U/ml. KLH-specific IgG1 ELISAs were performed as for OVA-specific IgE ELISA, except that KLH (Pierce) was used to coat wells and the IgE mAb was replaced by peroxidase-conjugated goat anti-mouse IgG1 Ab (Southern Biotechnology Associates, Birmingham, AL).

OVA-specific T cell responses in vitro
Bronchial lymph node cells were isolated and cultured as previously described (24) with medium alone or with OVA (100 μg) for 96 h (24). IL-4, IL-5, and IFN-γ content in cell culture supernatants was determined using ELISA (PharMingen, San Diego, CA).

Statistical analysis
Statistical significance was determined using either the one-way ANOVA or the two-tailed, unpaired Student’s t-test as appropriate. Differences between means were considered significant when p values were less than 0.05.

Results and Discussion
Selective blockade of airway inflammation by respiratory tract administration of vCCI
A murine asthma model was used in which the combination of OVA administered twice i.p. and three times i.n. results in disease that faithfully mimics cardinal features of human allergen-induced asthma, with intense eosinophilic and mononuclear inflammation of the lung, marked airway hyperreactivity, high levels of circulating IgE, and expression of Th2 cytokines by lung-associated lymph node cells (21, 23). A similar murine OVA-induced asthma model induces the pulmonary expression of a number of CC-chemokines, including eotaxin, RANTES MCP-1, MCP-5, and MIP-1α (5). To evaluate the effect of the blockade of local intrapulmonary chemokines on experimental allergen-induced asthma, vCCI in the form of a dimeric fusion protein with the human IgG1 Fc domain, or an equivalent amount of purified human IgG1 (hIgG1), was administered i.n. 2 h before each of three i.n. doses of OVA on days 14, 24, and 25. vCCI treatment significantly reduced the number of total leukocytes and eosinophils in BAL fluid obtained on day 26 compared with mice that received an equivalent amount of hIgG1 (Fig. 1A). The mononuclear leukocytes of the vCCI-treated and hIgG1-treated groups were >95% lymphocytes and mononuclear phagocytes (data not shown), in agreement with previous results (21). In contrast, OVA sensitization/challenge resulted in intense inflammation of the lung parenchyma in mice that received hIgG1, including in the perivascular and peribronchial regions (Fig. 2B), in agreement with previous results (21). vCCI administration dramatically reduced this inflammation, particularly in the peribronchial region (Fig. 2A), which had a histological appearance similar to that of tissue from unsensitized mice (Fig. 2C).
A single dose of vCCI given i.n. was highly effective at blocking the recruitment of inflammatory cells into the lung (Fig. 1B). In contrast, it did not affect the recruitment of lymphocytes, eosinophils and monocytes into the peritoneal cavity of mice subsequently challenged with OVA i.p. (Fig. 1C). Together, these results indicated that the vCCI-mediated inhibition of CC-chemokine function by i.n. administration was
highly effective for the local blockade of eosinophil and mononuclear phagocyte lung inflammation, while sparing effects on inflammatory reactions in the peritoneum.

Blockade of airway hyperreactivity by vCCI

Asthma is characterized by airway hyperreactivity, in which there is an exaggerated increase in airway resistance (R), a measure of the amount of pressure to achieve a given airflow, and a decrease in dynamic compliance (Cdyn), a measure of how distensible the lungs are at end-expiration, in response to provocative stimuli, such as Mch (21, 25). We found that local intrapulmonary administration of vCCI was strikingly effective in preventing the increase in R and the decrease in Cdyn elicited by OVA challenge. Results are shown as mean ± SEM, and are representative of three independent experiments. *p < 0.05 vs other two groups by ANOVA.

may have a minimal effect on normal pulmonary physiological function, at least in the short term. Finally, vCCI abrogated by ~50% the increases in Penh, a physiological measurement that correlates with airway obstruction and increased R (22), in sensitized mice in response to Mch challenge compared with mice treated with hlgG1 (data not shown). This indicated that these beneficial effects on pulmonary function applied to conscious, spontaneously breathing animals.

The cellular and molecular mechanisms underlying late phase pathology in allergen-induced asthma and its relationship with the early phase response are complex and only partly understood (4). It is likely that decreased airway hyperreactivity resulting from vCCI treatment is due, at least in part, to blockade of the influx of inflammatory cells, such as eosinophils, which produce a number of potent bronchoconstrictive mediators (2). vCCI might also locally inhibit the function of pulmonary inflammatory cells, because in vitro and in vivo studies have shown that CC chemokines can directly activate leukocytes, such as eosinophils (26) and mast cells (14). However, our results do not exclude the possibility that CC-chemokines may, at least in part, directly regulate airway caliber and function independently of effects on leukocytes. For example, functional CCRs are expressed by a number of nonhematopoietic cell types that are involved in bronchoconstriction, such as smooth muscle cells (11), but the importance of such expression for bronchoconstriction remains to be established. The fact that vCCI treatment resulted in nearly normal pulmonary function, whereas only partially inhibiting airway inflammation, also suggests that CC-chemokine-dependent, leukocyte-independent mechanisms regulating airway hyperreactivity are plausible.

Respiratory tract administration of vCCI does not influence systemic immune responses

Previous studies have found that CC-chemokines, in addition to their chemotactic effects, can augment in vitro lymphocyte functions that are critical for allergic disease, such as the production of IgE by B cells (9) and the production of IL-4 by T cells (8). To determine whether vCCI administration by the i.n. route altered in vivo Ag-specific responses involved in allergic disease, we analyzed the circulating levels of total and OVA-specific IgE at day 26.

FIGURE 3. Allergen-induced airway hyperreactivity is significantly reduced by vCCI treatment. Mice (n = 6 per group) were sensitized and challenged with OVA by the standard protocol and treated with three i.n. doses of vCCI or human IgG1. Unsensitized mice (n = 4) received alum alone i.p. and normal saline alone i.n. in lieu of OVA and served as a negative control. Mice were analyzed on day 26, 24 h after their last i.n. dose of OVA or of PBS. A, R was determined before (basal) and after challenge with i.v. Mch, and in B, the value obtained after Mch is expressed as the percentage relative to the basal value. C, Cdyn was similarly determined before and after challenge with i.v. Mch, and in D, the value obtained after Mch is expressed as the percentage relative to the basal value. vCCI treatment dramatically blocked the increase in R and the decrease in Cdyn elicited by OVA challenge. Results are shown as mean ± SEM, and are representative of three independent experiments. *p < 0.05 vs other two groups by ANOVA.

FIGURE 4. OVA-specific IgE levels and cytokine production by bronchial lymph nodes in mice sensitized and challenged with OVA by the standard protocol and treated with either vCCI (n = 6) or with hlgG1 (n = 6). B, In vitro production of IL-4, IL-5, and IFN-γ by bronchial lymph node T cells isolated from mice treated with vCCI (n = 5) or with hlgG1 (n = 5) after stimulation with OVA (100 μg/well) for 96 h. Results are shown as mean ± SEM.
of the standard protocol. Mice that received alum-precipitated OVA by the i.p. route had an ~25-fold increase in total IgE levels compared with sham immunized mice in agreement with previous results (21, 23) and vCCI administration had no influence on this increase (Fig. 4A). In addition, the levels of KLH-specific IgG1 in hlgG1-treated mice (43 ± 11 U/ml; mean ± SEM, n = 6) were not significantly different (p = 0.1 by Student’s t test) from those in vCCI-treated mice (125 ± 42 U/ml, n = 6) following KLH given with OVA i.p. on day 14. Because >90% of protein allergen-specific IgE production by B cells and their plasma cell derivatives is dependent on IL-4 production by CD4 T cells (27), these results also suggest that vCCI treatment did not inhibit CD4 T cell activation, expansion, or Th2 differentiation in vivo. This was supported by studies demonstrating that OVA-stimulated bronchial lymph node T cells from vCCI-treated mice produced similar amounts of IL-4, IL-5, and IFN-γ (Fig. 4B) and proliferated equally, based on the incorporation of [3H]thymidine (data not shown), as cells from hlgG1-treated mice. Thus, vCCI treatment did not alter T cell-dependent immune responses in vivo, including those associated with the local lymphoid tissue draining the lung, and appeared to act locally within the airway and adjacent parenchymal tissue to inhibit asthmatic inflammation and airway hyperreactivity. Taken together, these results indicate that local intrapulmonary inhibition of CC-chemokines by vCCI appears to be highly effective in blocking both the inflammatory and airway physiological consequences of allergen-induced asthma in vivo, without significantly altering systemic Ag-specific immunity. Such a therapeutic approach for asthma would be expected to have certain major advantages over local or systemic glucocorticoid therapy, such as avoiding inhibition of neutrophil migration to the lung and airways, and the systemic negative effects on bone homeostasis (2). The results of our study, taken with the recent reports of the clinical efficacy of systemic administration of a soluble TNF receptor protein for advanced rheumatoid arthritis (28), suggest that high-affinity binding proteins that disrupt specific cytokine/receptor interactions have general promise for the treatment of immunologically mediated diseases. Our results also raise the intriguing possibility that the vCCI protein, or nonimmunogenic small molecule mimetics of vCCI, might also be useful for the treatment of other disease states in which excessive, local elaboration of CC-chemokines and tissue-specific influx of eosinophils and/or mononuclear leukocytes are hallmarks. Chronic autoimmune diseases and allograft rejection are clear examples.

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