Sinusitis and asthma: an animal model

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We have developed an animal model in which nonspecific lower airways hyperresponsiveness to inhaled histamine was elicited in rabbits after complement-induced maxillary sinusitis. The most likely mechanism to explain this occurrence is the direct passage ("postnasal drip") of inflammatory mediators from the upper to the lower respiratory tract. The contribution of other potential mechanisms, such as the blood-borne delivery of inflammatory mediators, nasobronchial reflexes, and passage of cells with the induction of a secondary inflammatory process, could not be demonstrated. Rather, the most likely explanation for the current finding is the passage of mediators elaborated from activated inflammatory cells into the lower airways. Whether these findings explain the common clinical association of upper airways disease to lower airways dysfunction in sinusitis and asthma remains to be determined. These results suggest that even small numbers of granulocytes, when activated, can exert significant effect on lower airways function. It is perhaps appropriate to speculate at this point about the anecdotal but dramatic improvement in the asthma of patients with sinusitis who undergo surgery. The current results cause us to suggest that this success is due to the removal of the source of inflammatory products that drip into the lung. More important, these current results may have an important implication in the diagnosis of asthma. Finally, there is the clear conclusion that airways dysfunction can be caused by a mechanism that is associated with inflammation but without evidence of cell migration into the airways. (J ALLERGY CLIN IMMUNOL 1992;90:521-33.)

Key words: *Sinusitis, asthma*

Sinusitis and rhinitis and other disorders of the upper respiratory tract have long been associated with asthma. Little definitive information, however, is available regarding whether inflammation of the upper respiratory tract actually contributes to the pathogenesis of asthma. If sinusitis does play a causal role in asthma, the exact mechanisms that might explain this common clinical association remain uncertain.[1, 2]

Numerous studies have observed a high coincidence of asthma with either rhinitis or sinusitis. In the beginning of this century, Bullen,[3] Gottlieb,[4] and Weille[5] reported on large numbers of asthmatic adults in whom 20% to 70% had coexistent sinusitis. Chobot[6] found a similar proportion of children whose asthma was associated with sinusitis. More recent studies have corroborated these observations and reported on the high frequency of radiographic evidence of sinusitis in approximately 50%[7, 8] of children and adults[9] with respiratory allergy and asthma. Despite the clear association between sinusitis or rhinitis and asthma, there continues to be a poor appreciation and indeed some doubt about upper airways disease as a *cause* of asthma.

Numerous clinical investigations have suggested that specific medical treatment of sinusitis, including oral antibiotics, nasal decongestants, and nasal steroids, results in significant improvement of asth-

Abbreviations used

LAR:	Late asthmatic response
IAR:	Immediate asthmatic response
Vtg:	Thoracic gas volume
RL:	Total pulmonary resistance
CL:	Compliance of the lung
SaL:	Specific pulmonary conductance
EC_{50}:	Effective concentration of histamine
WBCs:	White blood cells
PMN:	Polymorphonuclear
BAL:	Bronchoalveolar lavage

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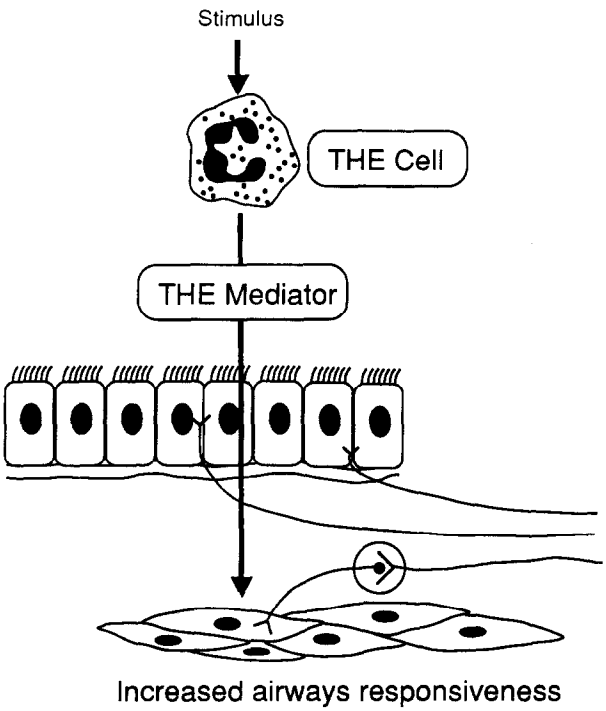

FIG. 1. Simplified scheme of the relationship between inflammation and airways dysfunction. Current research efforts in the field focus on identification of a unique cell or mediator responsible for airways dysfunction.

ma.[10-16] In refractory cases, surgical intervention has been found to affect a remission of asthma.[10, 16, 17] These later observations will be of particular interest in view of the results and mechanism suggested by the results of our animal studies. A matter of some debate is whether significant upper respiratory tract disease is merely another manifestation of global airways disease.[1, 18] Evidence of lower airways hyperresponsiveness to a number of provocative agents can be found in about 15% to 56% of individuals who have only allergic rhinitis.[19-21] On the other hand, several investigators have shown no alteration in lower airways function in patients with allergic rhinitis and asthma when they perform nasal provocation with antigen or histamine.[22-24] Thus the actual causal role of the upper respiratory tract in precipitating or enhancing disease in the lungs remains controversial.

ANIMAL MODELS OF AIRWAYS HYPERRESPONSIVENESS

The exponential increase in medical knowledge and most advances in medical science can be linked to the use of animals in research.[25] Indeed, a conservative estimate is that animal studies have contributed to half of such research and during certain periods the contribution is even higher. In fact animal research contributed to 74% of the research conducted between 1901 and 1975, that subsequently lead to important advances.[26] Of the 82 Nobel prizes in Medicine or Physiology between 1901 and 1982, 71% depended on studies in which animals were used.[25] Animal models have been developed for the purpose of better defining the mechanisms of pathogenesis of human diseases. Such models have addressed important questions that otherwise would be difficult or impossible to pursue through clinical investigation. Although all animal models have certain real limitations in their application to humans, information obtained from these models can provide important mechanistic insights.

Any relevant animal model of asthma should mimic all or some of the features of this disease as found in humans. A principal feature of asthma is airway hyperresponsiveness, which is defined as an exaggerated bronchoconstrictor response to various provocative agents including histamine or methacholine and physical stimuli, such as exercise, hyperventilation, or cold air.[28] Airways hyperresponsiveness is the hallmark of asthma in subjects who have symptoms and is used to establish the diagnosis. The importance of this feature of the disease is underscored by the direct relationship of the level of airway hyperresponsiveness to the overall severity of asthma, as reflected in the severity of symptoms and need for medical therapy.[29, 30]

Pathologically, the airways of a patient who dies as a result of their asthma are characterized by infiltration of the airways by inflammatory cells (particularly eosinophils), denudation of the epithelium, and plugging by mucous secretions and cellular debris.[31, 32] These findings are typical of most pathologic assessments at death, and although some pathologic heterogeneity has been noted,[33] the pathologic picture of milder cases of asthma are only now appearing in the literature.[34] Because of this limitation on the availability of lung tissue of humans, it is difficult to correlate structure (pathology) to function (physiology), making animal studies essential. The ideal animal model of asthma would exhibit a respiratory disease that mimics in clinical signs, as well as laboratory and pathologic findings, the major features of asthma found in humans. Unfortunately, such an animal model does not exist,[35, 37] but several animal systems develop an increase in airways responsiveness after a laboratory-induced insult to the airways.[35, 36, 37]

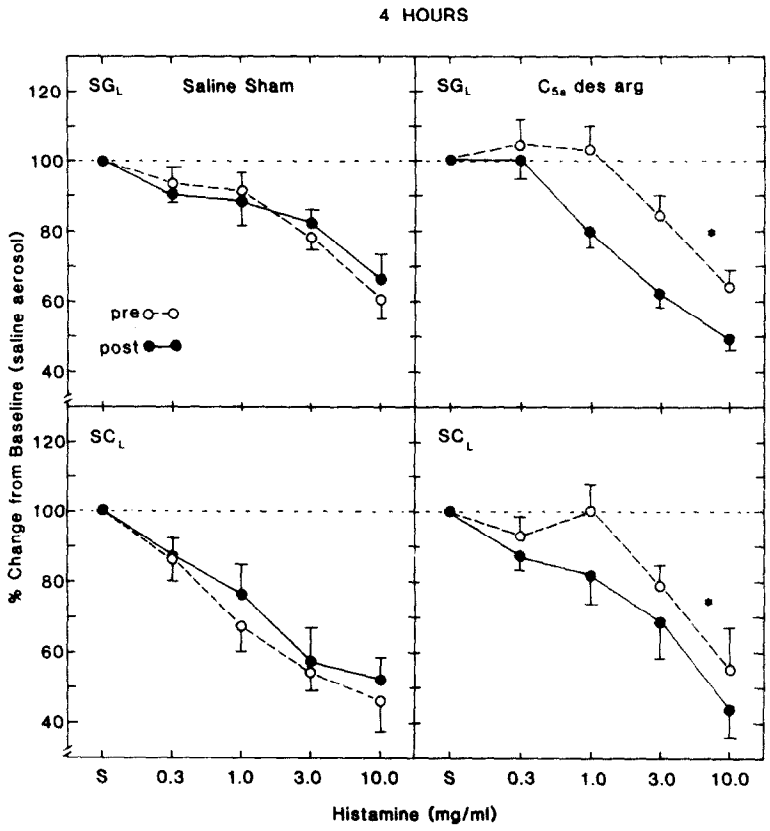

FIG. 2. Effects of saline exposure *(left panels)* and C5a des arg exposure *(right panels)* on the histamine response. Data are expessed as a percent change from baseline versus concentration of histamine (mg/ml) for preexposure *(open circles, dashed line)* and postexposure *(closed circles, solid line)* at 4 hours. *Horizontal dashed lines* indicate a 0% change. (*S,* Saline; *SCL,* specific pulmonary compliance). Data points are mean ± SEM. (From Irvin CG, et al. Am Rev Respir Dis 1986;134:777-83.)

RABBIT MODEL OF ASTHMA

The rabbit model of the late asthmatic response was initially developed to investigate the immunopathogenesis of the response to the airways to antigen. Animals were rendered immune by injections of adjuvant and antigen (*Alternaria* or ragweed) and then challenged with an aerosol of antigen. Subsequent to this antigen challenge, the physiologic and pathologic findings were shown to be qualitatively similar to the responses that occur in patients with atopic asthma after bronchial challenge with antigen. As in humans, the rabbit develops a delayed airflow limitation or LAR that is usually greater than the airflow limitation observed during the initial time points or IAR.[38] That the mechanisms involved might be similar to those involved in humans is suggested by the reaction of this model to common antiasthmatic drugs; for example, cromolyn will block the IAR and LAR, whereas corticosteroids inhibit only the LAR.[39] In addition, after the LAR has started, β-adrenergic agents are ineffective in reversing airflow limitation.[38, 40]

This animal model also parallels the physiologic events in humans, because the LAR is associated with an increase in airways responsiveness to inhaled histamine.[41] Histopathologically, granulocytes can be recovered from bronchoalveolar lavage and observed within the large and small airways.[42] At the time of the IAR, antigen-challenged but nonimmune (control) and immune rabbits have approximately the same numbers of granulocytes within their airways, whereas most of these inflammatory cells can be identified as neutrophils. However, at the time of the LAR (6 hours), only the immune and antigen-challenged rabbits with late responses still have significantly increased numbers of granulocytes, of which approximately 40% are eosinophils. Within the bronchioles,

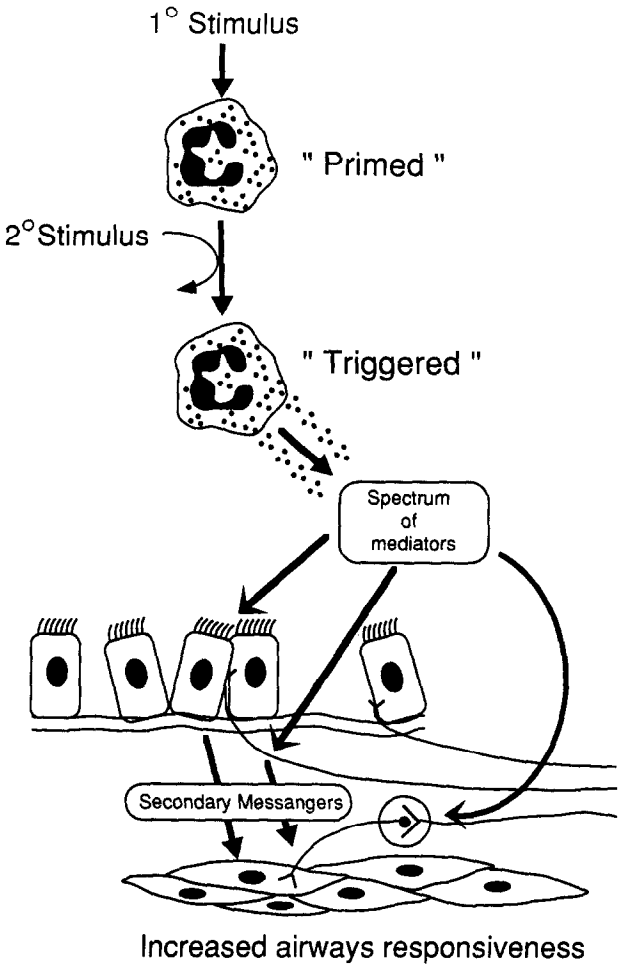

FIG. 3. A current working hypothesis of the relationship between inflammatory cells and mediator release in affecting increased airway responsiveness. Cells likely pass through a two-step process where first "priming" occurs by exposure to the primary stimulus. The secondary stimulus then "triggers" the release (or greater amounts) of a spectrum of mediators. This spectrum of mediators targets a variety of tissues that may include the generation of secondary messengers.

80% of the granulocytes were eosinophils. Thus in this animal model, the LAR has been associated with significant increases in granulocytes within both bronchi and bronchioles and a heightened responsiveness to an inhaled agonist.

However, these findings do not necessarily mean that with the accumulation of inflammatory cells there is a release of mediators, which are then responsible for these changes in airway function. This can only be established through experiments in which cells or products of these cells are removed and then reconstituted. This is initially accomplished by first depleting the animals of circulating granulocytes and then repleting previously depleted animals with purified populations of granulocytes. In immune rabbits made neutropenic with the administration of nitrogen mustard, subsequent antigen challenge led to an IAR but no LAR or increase in responsiveness.[43] To determine if this effect was truly from granulocyte depletion and not an unrelated effect of nitrogen mustard, granulocytopenic ragweed-immune and granulocytopenic ragweed nonimmune rabbits were transfused with a neutrophil-rich population of white cells at the time of ragweed exposure. Control (nonragweed immune) rabbits had neither asthmatic responses nor changes in airways responsiveness after exposure to this antigen, whereas ragweed-immune rabbits had early and late decreases in lung function, as well as marked increases in airways responsiveness. It appears that in this rabbit model, both the LAR and the subsequent IAR in airways responsiveness is dependent on the presence of granulocytes within the circulation at the time of antigen exposure.

Although the response of an animal rendered immune through various immunization schemes probably represents the most realistic approach to the study of asthma pathogenesis,[36, 37] it is not without problems. The most important problems are the time, costs, and energy required to manipulate such a model. To speed the work and render the stimulus more specific, researchers have investigated several other inflammatory stimuli (Fig. 1). Cellular inflammation has been firmly established to cause airways dysfunction in ozone-induced hyperresponsiveness in dogs[44-46] but has not been established for either guinea pigs[47] or rats.[48] Toluene diisocyanate will cause inflammation and airways hyperresponsiveness in guinea pigs,[49] but whether these effects are granulocyte dependent is unclear.[50] Endotoxin produces airways hyperresponsiveness in sheep[51, 52] and certain inbred strains of rats[53]; this has been associated with increased airways responses to inhaled agonists.

Our laboratory has investigated the ability of a modified complement fragment, C5a des arg, to induce inflammation and subsequent airways of dysfunction.[54] We chose to study the effects of C5a des arg, the fifth component of complement, which has had the terminal arginine removed. The molecule C5a is a potent phlogistic factor, but with the terminal arginine residue removed, there is a loss of a direct spasmogenic effect on smooth muscle. When aerosolized into the airways of rabbits, we showed that this molecule caused an inflammatory lesion of the airways that was associated with bronchospasm and an increased responsiveness to inhaled histamine (Fig. 2). Granulocyte depletion abrogated the effects of C5a

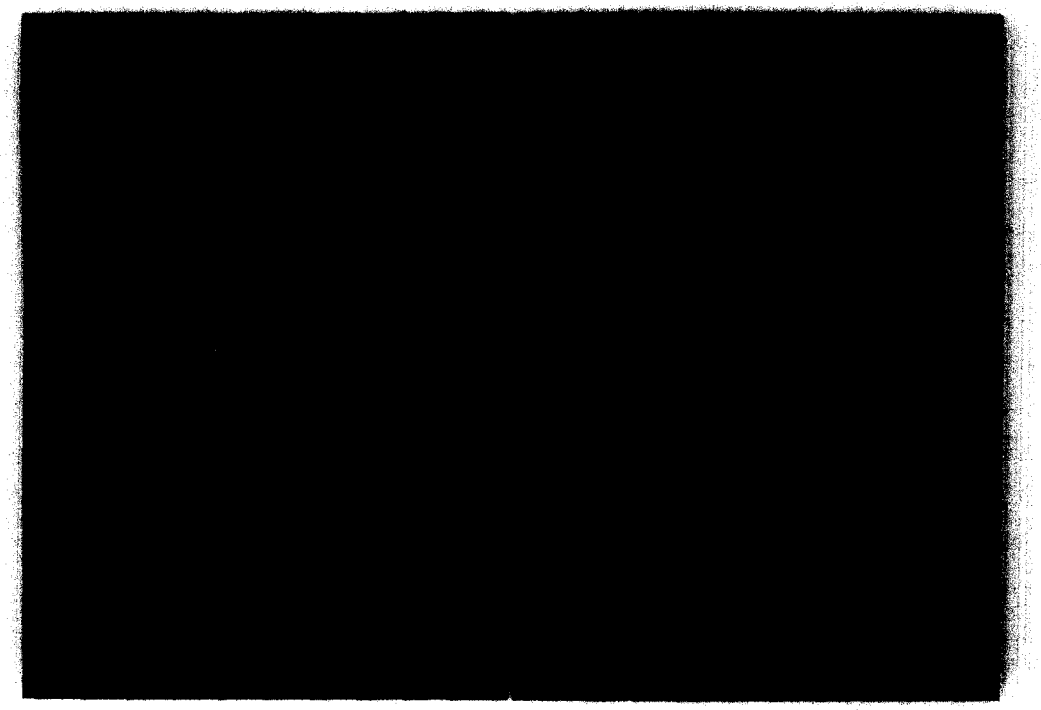

FIG. 4. Radiographs of a rabbit head. **A,** Needle is passed into the paransal sinus. **B,** Radiocontrast has been injected. Note passage of material to the contralateral side.

des arg. It is particularly interesting that when the lungs from sham-challenged experiments were examined, significant cellular infiltration occurred. It was subsequently shown that this was from trauma; however, the important point is that this inflammatory lesion was not associated with airways hyperresponsiveness. This demonstrates that the mere presence of inflammatory cells does not automatically translate to physiologic dysfunction. Our current operational scheme shows a much more involved process (Fig. 3). In addition to the importance of cellular activation, some of the additional features include: (1) The notion that granulocyte activation probably involves a two step process of "priming" and then "triggering." (2) The exact spectrum of mediators involved is probably critical to the specificity of the response. (3) These mediators probably target structures other than the smooth muscle itself, and the mechanisms that control the occurrence of airways narrowing are, in all likelihood, different from those mechanisms that determine heightened airways responsiveness.

ANIMAL MODEL OF SINUSITIS AND AIRWAYS HYPERRESPONSIVENESS

We have developed an animal model of sinusitis[55, 56] for two immediate purposes: (1) to develop a model in which sinusitis and airways hyperresponsiveness are associated and (2) to have a system in which to investigate some of the postulated mechanisms that might explain this association. We chose to use C5a des arg, because this molecule might in fact be involved in the natural development of inflammatory disorders[57] and because of our previous experience with this molecule in the lower airways.[54]

New Zealand white rabbits were used for these experiments. Rabbits underwent pulmonary function and airways responsiveness testing with the methods previously described.[41, 43, 54] Briefly, animals were anesthetized and intubated. An esophageal balloon catheter was then passed into the lower esophagus to measure pleural pressure. Airflow was obtained as the pressure drop across a pneumotachygraph. The animals were placed supine in a volume-displacement plethysmograph and Vtg was obtained by Boyle's law technique.[54] RL and CL were determined breath-by-breath during spontaneous respiration by means of the electrical subtraction technique.

Airways responsiveness was assessed as previously described.[41, 43, 54] Briefly, recordings of RL, CL, and Vtg were initially obtained to serve as a baseline determination. An aerosol of 0.9% saline solution was then administered for 2 minutes. Tripling concentrations of histamine sulfate (0.1 to 50 mg/ml of histamine) were then nebulized. Using the value of SGL

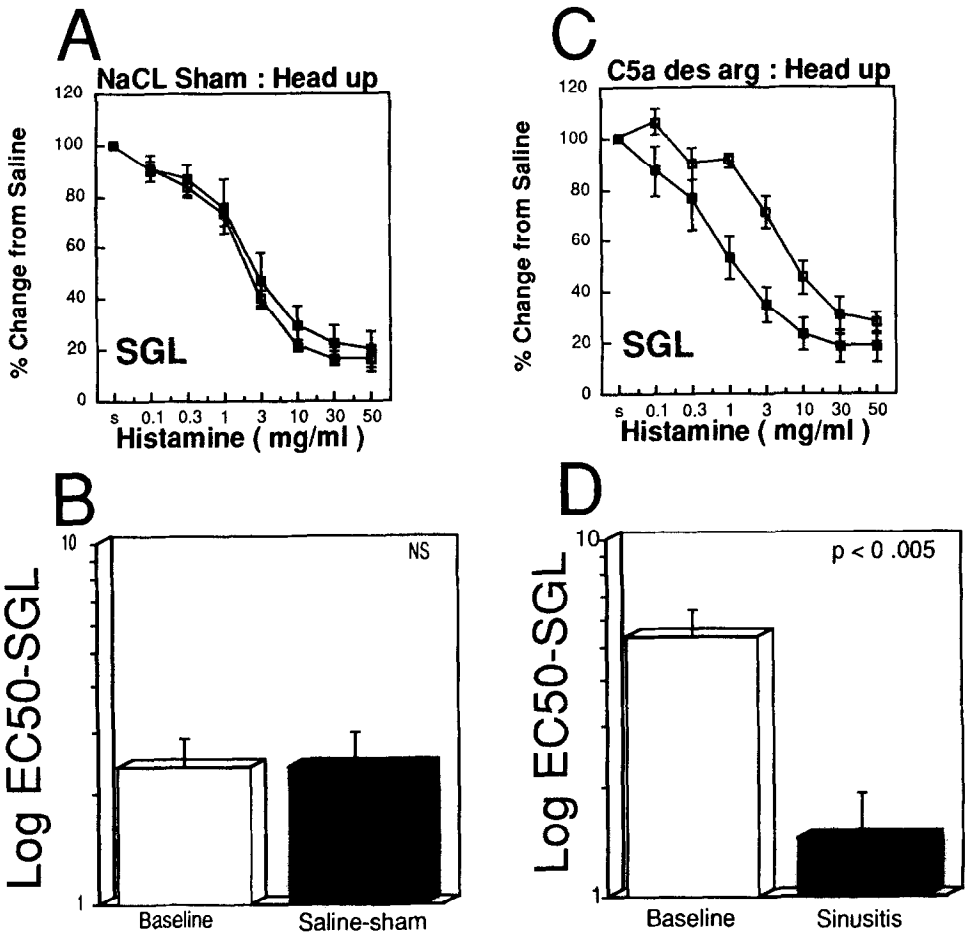

FIG. 5. After the injection of saline solution (NaCl sham) or 30 μg of human C5a des arg into each paranasal sinus, the animals have airways responsiveness to inhaled histamine determined. After injection of saline solution, there is no shift in the dose-response curves *(panels A and B).* After C5a des arg, the dose-response curve shifts left and the change is significant. *Open symbols* and *bars* are prechallenge, and *closed symbols* and *bars* are postchallenge. EC_{50} *(panels B and D)* is the dose of inhaled histamine that causes 50% of the maximal fall in SGL. Data points are mean ± SEM.

after saline nebulization as a baseline, a plot was drawn on semilog paper of the percentage change in SGL versus the log dose of histamine. The point representing 50% of the maximum change in SGL from saline was plotted and expressed as EC_{50}.

Animals underwent baseline pulmonary function testing and establishment of a histamine dose-response relationship. They were then allowed to recover for 1 to 2 weeks. Animals were randomly assigned to the various groups.

As a control group, animals were injected in each maxillary sinus (Fig. 4) with 0.3 ml of a sterile saline control solution and then positioned prone with the head elevated (induction period). Sixteen hours after treatment, animals were anesthetized again and underwent, in order, baseline pulmonary function tests, histamine bronchial challenge, sinus or knee lavage, and bronchoalveolar lavage, and were killed for histologic evaluation. In contrast to animals injected with C5a des arg, rabbits who received sinus injections of the saline protein diluent had no change in airways responsiveness (Fig. 5, *A* and *B*).

In a second group of rabbits each maxillary sinus was injected transcutaneously with 0.3 ml (30 μg) of human recombinant C5a des arg. These animals were also positioned head up, and airway function was determined 16 hours later. Tests of baseline lung function in both groups demonstrated no change in Vtg, RL, or CL after either sinus injection of C5a des arg or saline solution. Compliance of the lung, however, de-

TABLE I. Mechanisms as suggested by Gottlieb in 1925 that link nasal disease to asthma

1. Postnasal drip of mucus, mediators, or chemotactic factors into the lower airways that either directly alters airways reactivity or causes airways inflammation
2. Hyperresponsiveness of the airways is caused by reabsorption of mediators or chemotactic factors from inflammatory process in the nose or sinuses
3. Mouth breathing of cold and/or dry air caused by nasal obstruction that elicits asthma by increasing the heat and water loss in the lower airways
4. Activation of nasopharyngeal-bronchial reflexes caused by stimulation of the nose, sinuses, or pharynx
5. Diminished β-adrenergic responsiveness

Modified from Gottlieb MA. JAMA 1925;85:105-9. Copyright 1925, American Medical Association.

creased significantly after C5a des arg–induced sinusitis. Rabbits receiving C5a des arg, positioned head up and tested 16 hours later, demonstrated a marked increase in airways responsiveness to histamine (Fig. 5, *B*) after treatment.

Inflammation of the maxillary sinuses with C5a des arg was demonstrated in animals by a marked increase in WBCs recovered from the sinus lavage fluid with a predominance of PMN cells. Saline-injected rabbits had significantly fewer total WBCs and a smaller absolute number of PMN. Saline-injected sinus lavage did not differ significantly from that obtained from normal, unmanipulated rabbits in either total WBC or numbers of PMN.

Several mechanisms could account for our observation that an inflammatory process in the upper airways can also be associated with hyperresponsiveness of the lower airways. Astutely many of these mechanisms were first proposed by Gottlieb in 1925.[4] Based on clinical observations, he postulated several mechanisms that might link upper airways processes to lower airways symptoms (Table I). Of the postulated mechanisms, those most likely to be operational in this model are (1) reabsorption of inflammatory products and subsequent delivery by the circulation to the lower airways, (2) elicitation of a nasobronchial reflex, and (3) passage of cells or mediators of the inflammatory reaction to the lower airways ("postnasal drip"). The subsequent experiments we have conducted were designed to clarify which of these mechanisms were operational in this model.

REABSORPTION OF INFLAMMATORY PRODUCTS

Mediators generated during anaphylactic reactions have been measured in the circulation and provided a compelling argument for pulmonary effects after their blood-borne delivery to the lungs.[58] Likewise, mediators can be found in the circulation after some forms of bronchoprovocation.[59] Hence we first addresed the possibility that absorbed inflammatory mediators might affect lower airways responsiveness by the presence of an inflammatory site distant from and unconnected with the lungs.

Rabbits received injections of C5a des arg in each suprapatellar bursa.[60] Intense joint inflammation was seen after injection of C5a des arg into the knee. This focal inflammatory reaction did not result in a change in airways responsiveness to histamine.

Because products of the inflammatory process may be absorbed into the vascular space, and alter lower airways function after blood-borne delivery, we attempted to study this potential mechanism as distinct from others by the institution of remote inflammation in the knee joints. Our failure to show an increase in airways responsiveness provides circumstantial evidence that reabsorption of inflammatory mediators may not be an important mechanism in this model. However, this negative finding could have been either from the small quantities of inflammatory mediators present or alternatively, from an inadequate amount of mediators that might have been reabsorbed and rapidly metabolized in the vascular space. Thus although this mechanism is undoubtedly operative in some forms of lower airways obstruction, data to support its contribution in the setting of inflammation of the upper respiratory tract could not be demonstrated in the current model.

ELICITATION OF A NASOBRONCHIAL REFLEX

The second mechanism that might potentially link an inflammatory process in the upper airways to a change in lower airways function is elicitation of a nasobronchial reflex arc. As first proposed by Sluder[61] in 1919, such a reflex arc would have a sensory limb

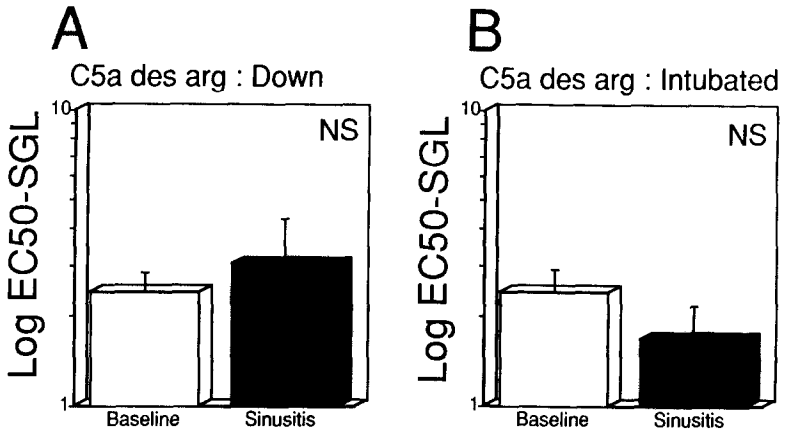

FIG. 6. C5a des arg (30 μg each sinus) was injected, and the determination of the histamine dose-response characteristics was made 16 hours later. Responsiveness was not increased if the animal was positioned head down **(A)** or positioned head up and intubated before C5a des arg injection **(B)**. Prevention of passage of material from the nose and sinuses appears to prevent airways hyperresponsiveness.

consisting of receptors in the nose, sinuses, and pharynx, which send afferent impulses through the trigeminal, facial and glossopharyngeal nerves to the medulla. From there connections are made to the vagal nucleus, from which efferent impulses travel to the lower airways by the vagus nerve, thus resulting in bronchoconstriction.[62] After nasal provocation with irritant agents, it has been clearly demonstrated in both animals[63-65] and humans[66-68] that such nasobronchial reflexes exist. Furthermore, a nasobronchial reflex has been found to be operative in an animal model of viral infections of the upper respiratory tract.[69] However, in humans the role of this reflex has not been established under the conditions of allergen-induced nasal inflammation.[22-24] To indirectly address the postulate that neural reflexes originating in the upper airways caused the observed increase in lower airways responsiveness, we performed the following experiment.

Rabbits underwent injections of C5a des arg in each maxillary sinus while lying in the prone position on a tray with the head positioned down. Florid maxillary sinus inflammation was observed in this group of rabbits and was comparable with that seen in the first study.

However, unlike the first study in which the animal was positioned head up for the induction period, these animals with sinusitis failed to show airways hyperresponsiveness under conditions of maintaining a downward position (Fig. 6, *A*).

We conclude that in this model of experimental sinusitis, there was no evidence either for bronchoconstriction or nonspecific airways hyperresponsiveness from the sinus inflammation alone. Furthermore, although there is a significant inflammatory response in the upper airways that should be more than enough to activate nasobronchial reflexes known to exist in this species,[70, 71] this would appear to be insufficient alone to account for the increase in responsiveness observed in this model.

PASSAGE OF INFLAMMATORY PRODUCTS TO THE LOWER AIRWAYS ("POSTNASAL DRIP")

Passage ("postnasal drip") of the chemotactic factor, inflammatory cells or their products into the lower airways was the third mechanism that we investigated to explain the association between sinusitis and airways hyperresponsiveness.

For this next series of experiments animals were intubated first. The purpose of the endotracheal tube was to physically block the passage of cells or mediators from the nose to the lower airways. Animals were injected with C5a des arg and were placed head up. These animals demonstrated a degree of sinusitis nearly identical to that of a group of animals that were positioned head up but were unintubated. Airways responsiveness was not significantly different (Fig. 6, *B*).

It is important to note that the results of these two experiments in which the animals were placed head down or head up and intubated cross-confirm each other in regard to the unlikely role for either the reabsorption of mediators from the site of inflammation

or a nasobronchial reflex in affecting lower airways hyperresponsiveness in this model. Human studies of allergic rhinitis lend support for these findings. Multiple secretagogues, including potent bronchoconstrictors, are generated within the nose after nasal allergen challenge.[72, 73] Despite this, several studies showed no change in pulmonary function after nasal allergen or histamine provocation in patients with hay fever and allergic asthma.[22-24] Several explanations for the negative results of these clinical studies are suggested by the findings of the current study. One is the possibility that insufficient time was allowed for the development of lower airways dysfunction. In the current study alterations in lung function were observed at 16 hours but not earlier at 4 hours (data not shown). Second, as we have recently shown,[74] a significant result would have been detected in these studies, if the outcome variable had been airways responsiveness.

Presumably, airways hyperresponsiveness after C5a des arg injection in the head-up position was from the passage of some signal from the inflammatory process into the lower airways. Silent pulmonary aspiration of nasopharyngeal secretions occurs frequently in normal humans during sleep[75] or depressed consciousness[76] and has been implicated as a principal cause of chronic cough.[77] The precise mechanism whereby the lower airways are rendered hyperresponsive by dripping material is not entirely clear from our study.

Because we had previously demonstrated that nonspecific histamine responsiveness can be induced in rabbits after pulmonary inhalation of aerosolized complement fragments (Fig. 2), it might be argued that the results of our study only reproduced this finding by slightly altering the delivery method of C5a des arg delivery to the lower airways; that is, the present results are explained by C5a des arg dripping into the lung. The following results show that this is probably not the case. We attempted to address this concern with two different approaches: (1) an examination of the lavage and histopathologic alterations in the lower airways and (2) an experimental design that should result in the specific conveyance of mediators but not the chemotactic signal.

LAVAGE AND HISTOPATHOLOGIC ALTERATIONS IN THE LOWER AIRWAYS

All animals underwent BAL after lung function testing on the experimental day. The lavage technique used for this study has been previously described.[41] Briefly, a catheter was passed into the right or left lower lobe and a lavage was carried out. A total of 25 ml of sterile 0.9% saline solution was instilled and aspirated in 5 ml aliquots. Recovery of significant numbers of inflammatory cells from the lower airways could not be demonstrated in any of the groups. Enumeration of BAL cellularity shows no significant differences in total numbers of WBCs, PMNs, mononuclear cells, or eosinophils. These values are similar to numbers of cells recovered with BAL when performed on previously unmanipulated, untreated rabbits.

After each experimental protocol, animals were euthanized, and the lungs and trachea were carefully excised and inflation fixed. Multiple lung sections were mounted in paraffin and stained with hematoxylin-eosin-azure. Every airway in each slide was examined microscopically and scored for inflammation as 0, 0.5, 1, 2, 3, or 4 depending on the presence and extent of PMN leukocyte infiltration and epithelial damage.

Gross examination of the lungs immediately after death showed areas of atelectasis corresponding to the site of BAL. However, there was no gross evidence of obvious inflammation or hemorrhage. Likewise, histologic study of the airways in all groups failed to show any consistent inflammation. Average scores for each airway level were less than 1.0 in nearly all of the individual cases for both controls and C5a des arg–treated groups.

These findings suggest that the following conclusions are probably warranted. First, that the increase in lower airways responsiveness is not caused by the mere dripping of the chemotactic signal into the lower airways, which by setting up an inflammatory process, leads to the airways hyperresponsiveness. If that had been the case, one should have found a significant pathologic lesion in the airways and recovered significant numbers of granulocytes with lavage. Second, this failure to recover significant numbers of cells in the lavage of the lower airways and the known adhesiveness of stimulated inflammatory cells[57] suggest that the increase in lower airways responsiveness is also not from the passage of inflammatory cells into the lung. Importantly, there is the clear conclusion that airways dysfunction can be caused by a mechanism that is associated with a distal site of inflammation but without evidence of cell migration into the airways that are dysfunctional.

SPECIFIC CONVEYANCE OF INFLAMMATORY MEDIATORS

The results of the above additional measurements suggested that the changes in lower airways function seen in head-up, unintubated animals (Fig. 5) were

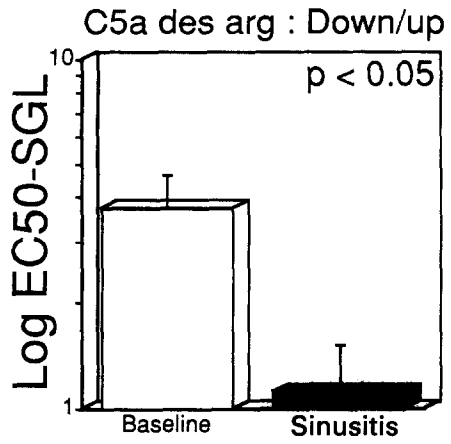

FIG. 7. Responsiveness is increased if the animals are first placed head down (90 minutes) and then head up (40 minutes). Because the C5a des arg is probably dissipated, the effect is likely from the passage of cellular products and not the chemotactic stimulus, to the lower airways.

not from the dripping of either the chemotactic factor or the inflammatory cells into the lower airways. Rather, we suggest that the conveyance of the products of inflammation (i.e., mediators) into the lower airways induced the hyperresponsiveness. To address this particular question by another means, we conducted the following experiment.

Rabbits had sinus injections of C5a des arg in an identical fashion to the initial study (Figs. 4 and 5). However, the induction period was altered by initially placing the rabbit in a head-down posture for 90 minutes. This time point was chosen, assuming that the chemotactic signal would be dissipated.[60]

An increase in airways responsiveness to histamine was observed in this group of rabbits (Fig. 7).

These findings confirm not only the reproducibility of the original model but also suggest that the signals coming from the nasal cavities to the lower airways are most likely products of the inflammatory process.

CONCLUSIONS

Although sinusitis and other inflammatory diseases of the upper respiratory tract have long been associated with asthma, little direct evidence links these processes mechanistically.[1, 2] The purpose of the present study was (1) to establish an animal model wherein lower airways hyperresponsiveness and sinusitis coexist and (2) to examine some of the mechanisms that might account for this association.

Clear, valid reasons for the use of animal models to study human disease processes exist. Yet, limitations exist for any animal model of a human disease.[35, 37] and the model described here is not an exception. Most of all, it must be recalled that important differences in species exist that limit strict application of results to the human condition. In reality, there is not one best model; each model has merit, because relevant questions can be addressed and important conclusions can be derived.[25, 35] Indeed, the current recognition that asthma is largely an inflammatory

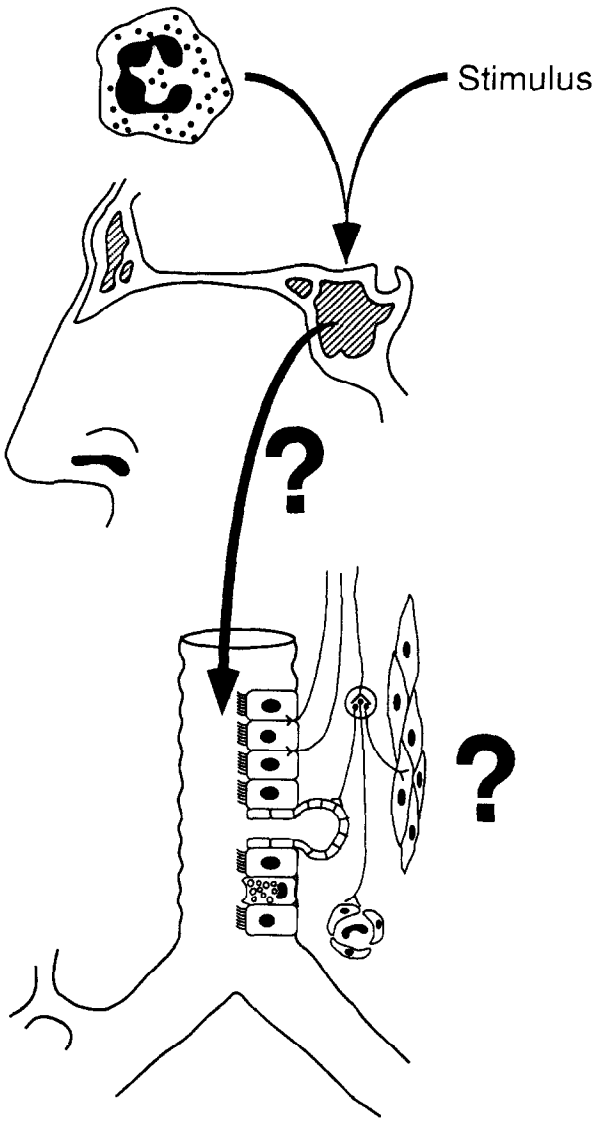

FIG. 8. Unanswered questions about the mechanistic link between upper airways inflammation and lower airways responsiveness are: (1) What is released from the inflammatory process within the nose and sinuses that increases responsiveness? (2) How do those products increase responsiveness?

process came from animal experiments. Despite these limitations, our study shows evidence for a plausible mechanism involved in the association of sinusitis and asthma. Furthermore, it prompted us to perform a clinical study in which nasal challenge with allergen resulted in the alteration in lower airways responsiveness.[74]

With the current rabbit model,[55] we were able to demonstrate significant airways hyperresponsiveness as the consequence of the induction of sinusitis. Mechanistically, this phenomenon appeared to be best explained as the result of the postnasal drip of cell products into the lower airways. This interpretation is based on the finding that despite similar degrees of sinusitis, blocking the spatial relationship between the upper and lower respiratory tracts by either intubating the animals or keeping them in a head-down position prevented the increase in airways responsiveness. The observation that these protocols did not result in airways hyperresponsiveness failed to support a mechanistic role for either a nasobronchial reflex or the absorption of inflammatory mediators from this distal site. The possibility that the chemotactic signal alone might be dripping into the lower airways, which initiated a secondary inflammation with an associated airways hyperresponsiveness, was reasonably eliminated by both a specific experiment and the lavage and histologic findings.

The precise mechanism whereby the lower airways are rendered hyperresponsive by dripping inflammatory products is unclear (Fig. 8). One possible explanation is the activation of a pharyngeal reflex by the inflamamtory process. A further explanation is that dripping mediators might activate a small, sentinal population of resident polymorphonuclear leukocytes within the lower airways. Further questions that we are currently addressing include the following: (1) What are the specific mediators being released in the nose? (2) Are granulocytes required for this response?[56] (3) Are the mechanisms involved similar to those responsible for the changes associated with lower airways inflammation? A system in which the inflammatory process is physically removed from its site of action will allow us to address these questions in a relevant way.

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