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CORRESPONDENCE

Eosinophil accumulation and airway hyperreactivity

To the Editor:

On the basis of correlation studies, UNDERWOOD *et al*. [1] have concluded that accumulation and activation of eosinophils determines the hyperreactivity that results from an allergic reaction in guinea-pig airways. In justifying their policy of correlating responses at various intervals following an allergic reaction, they suggested that previous studies had used insufficient time points to permit definitive conclusion. In our case, this is a misrepresentation since our analysis did not depend solely upon correlation data. We followed the precepts of DALE [2], who was alert to the problem posed by physiological processes for which there were many candidate mediators. He listed a series of criteria by which to judge candidates. These included a requirement that the appearance of a causative agent must coincide with, or antecede, the effect for which the agent is held responsible.

When studying accumulation of eosinophils during allergic reactions, we were surprised to observe near-maximal accumulation of eosinophils in the airway lumen following exposure to doses of antigen that were only weakly effective, or even ineffective, in causing increased reactivity to intravenous injections of histamine [3]. Our finding of a lack of correlation between hyperreactivity and accumulation of eosinophils in the airways questioned the dogma that allergic airway hyperreactivity was determined by materials released from activated eosinophils. To address this issue experimentally, we elected to study the initial stages of an allergic reaction, which antecede accumulation and activation of eosinophils.

When sensitized guinea-pigs received an intravenous bolus of antigen at a low dose level, there was transient bronchospasm. On resolution of this bronchospasm, increased reactivity to intravenous histamine was already manifest and had comparable amplitude to hyperreactivity that was evident several hours after exposure to antigen [4]. Similar results were obtained following infusion of a low dose of antigen in passively sensitized guinea-pigs, when airway reactivity was increased disproportionately for different spasmogens (*i.e*. in rank order: acetylcholine, serotonin, peptidoleukotriene E_4, bradykinin, prostaglandin $F_{2\alpha}$, histamine and peptidoleukotriene C_4) [5]. By way of contrast, accumulation of eosinophils within the airway lumen was only detected after an interval of some hours [3] and, as UNDERWOOD *et al*. [1] have demonstrated, there is an even greater delay before products of eosinophil activation are released in significant amounts. We contend, therefore, that it is highly unlikely that accumulation of eosinophils during the initial phase of an allergic reaction determines the changed behaviour of the airways. This inference was reinforced by demonstration that allergic hyperreactivity is wholly suppressed by SDZ PCO 400, an opener of potassium channels [4]. Since accumulation of eosinophils in the lung during an allergic reaction was unaffected by SDZ PCO 400, and since the capacity of this drug to suppress allergic bronchospasm is not demonstrable following bilateral vagal section, we incline to the opinion that increased reactivity during allergic reactions in the guinea-pig is determined by modified behaviour of nerves (*e.g*. facilitated transmission across synapses in parasympathetic ganglia).

Activated eosinophils secrete peptidoleukotrienes and cytotoxic proteins, materials that have been shown to induce hyperreactivity in the guinea-pig. Hence, it must be acknowledged that accumulation and activation of eosinophils may contribute to allergic hyperreactivity. However, the data presented by UNDERWOOD *et al*. [1] do not exclude the possibility that increased reactivity which is manifested acutely might persist into the phase of eosinophil recruitment. It is suggested, therefore, that additional experimental data will be needed to justify their assertion that eosinophil accumulation and activation determines hyperreactivity during allergic reactions.

Use of multiple test spasmogens will provide an incisive test of the hypothesis. With this technique, it has been possible to distinguish hyperreactivity in which eosinophil involvement is not suspected (*e.g*. in response to intravenous endotoxin) or unlikely (*e.g*. in response to subcutaneous infusion of racemic salbutamol) from hyperreactivity that is known to be associated with eosinophil accumulation within the airways (*e.g*. in response to intravenous infusion of platelet-activating factor (PAF)) [6], and to differentiate these forms of hyperreactivity from that which is manifest acutely following an allergic reaction [5]. It is already established that there are marked differences between patterns of increased airway reactivity to various spasmogens following exposure of sensitized animals to allergen, where histamine>acetylcholine>0, and that which follows intravenous infusion of PAF, where histamine>0>acetylcholine [5, 6]. From these observations, it seems unlikely that activation of eosinophils will prove pivotal in the genesis of allergic hyperreactivity. Nevertheless, we endeavoured to demonstrate hyperreactivity following intravenous infusion (when cells are entrapped within pulmonary capillaries) or intratracheal instillation of activated eosinophils, but without success (unpublished observations). Should others succeed, they might establish a rank order of spasmogen reactivity and compare this order with that already established for an allergic reaction in this species [6]. Such evidence will either support eosinophil involvement unequivocally or provide conclusive grounds for rejecting this hypothesis.

A complementary approach would be to ascertain whether cyclosporin A suppressed allergic airway hyperreactivity. We were greatly impressed with the capacity of nonimmunosuppressive doses of cyclosporin A to suppress eosinophil accumulation during active or passive

allergic reactions in the guinea-pig. As well as providing a lead for new chemical entities which might inhibit activation of eosinophils selectively, this finding was fortuitous, for it allowed evaluation of allergic hyperreactivity in the absence of eosinophil accumulation. The outcome of our studies was quite conclusive: doses of cyclosporin A which suppressed eosinophil accumulation and activation did not diminish acute allergic bronchospasm and did not influence acute allergic hyperreactivity in the guinea-pig [7, 8] an observation reported in rats by others [9]. We did not extend our studies to include later time-points, but we suggest that this would be more decisive than correlation studies.

Observations in laboratory animals are consistent with clinical findings and indicate that, despite close association in many circumstances, accumulation and activation of eosinophils within the airways and exacerbation of airway reactivity are effectively independent processes. Our assessment of the clinical and laboratory evidence has led us to conclude that pharmacologists should consider hyperreactivity of the airways and accumulation and activation of eosinophils as distinct and separate entities, with differing susceptibilities to inhibition by drugs [9]. The report by UNDERWOOD *et al*. [1] has not caused us to modify our opinion.

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REPLY

From the authors:

We are encouraged by the fact that our paper on airway inflammation and bronchial hyperresponsiveness (BHR), as intended, stimulated discussion on this important, but controversial subject. In our study, we demonstrated a relationship between eosinophils and their cytotoxic products and BHR, which has now been contested by Chapman and Morley (see above), who argue that eosinophil accumulation and BHR are two separate entities. We believe that this view does not embrace all available information and that there is now important evidence to support a role for activated eosinophils and their products (cytotoxic proteins, cytokines and other mediators) in the development of airway hyperresponsiveness.

Chapman and Morley cite a number of their earlier guinea-pig studies, in which they found either bronchoalveolar lavage (BAL) eosinophilia with no or little increase in airway reactivity to bronchoconstrictors or, conversely, BHR before the accumulation of significant numbers of eosinophils. However, these observations fail to take into account the generation of eosinophil mediators from cells resident in the submucosa at the time of challenge and occurrence of BHR. Even at baseline, guinea-pigs are known to have a small number of eosinophils residing in airway tissue and only by careful tissue studies (immunohistochemistry or similar techniques) would it be possible to assess cell accumulation and mediator release. Unfortunately, the above studies did not include such measures.

Chapman and Morley, further argue that their experiments with the potassium channel opener SDZ PCO 400 conclusively demonstrates the separation between eosinophilia and airway reactivity. Reportedly, they found an inhibition of BHR despite no change in the number of cells in BAL fluid. However, the appropriate correlation would have been between eosinophil mediators and airway reactivity. Likewise, in a separate study, cyclosporin A reduced the number of eosinophils in BAL fluid at a dose that did not alter airway responsiveness. As already discussed, the number of eosinophils in BAL does not necessarily correlate with cells in the bronchial wall (the relevant site of tissue damage), so these observations remain inconclusive.

The third argument raised relates to changes in airway reactivity by nonantigenic stimuli and the use of different spasmogens. The authors used platelet-activating factor, isoprenaline and endotoxin to alter the sensitivity of guinea-pig airways to various spasmogens. These different approaches have little in common with antigen challenges, as also acknowledged, and are, therefore, unlikely to progress the debate about the role of the eosinophil in asthma and BHR.

Numerous studies in guinea-pigs and other species have demonstrated a relationship between eosinophilia and airway reactivity. Particularly interesting are studies with anti-interleukin 5 (anti-IL-5) antibodies, which diminish the antigen-induced eosinophilia and significantly reduce BHR [1, 2]. Interestingly, IL-5 knock-out mice are unable to mount an eosinophilic response, and airway responsiveness is not altered after sensitization and antigen challenge [3]. Reconstitution with IL-5-producing virus, restored the mice's capability to generate eosinophils and caused a concomitant increase in BHR [3].

The postulate by DALE [4] was originally developed to define criteria for neurotransmitter candidates but can be applied to mediators of a wide range of autonomic physiological processes. Our data are consistent with a role for eosinophil-derived cytotoxic proteins in enhancing allergic airway hyperresponsiveness, and are in agreement with a previous study reporting that eosinophil activation, rather than accumulation, is required for development of BHR in the guinea-pig [5]. Obviously, as discussed in our paper, other cells and proinflammatory mediators and cytokines contribute to the complex series of events leading to the development of bronchial hyperresponsiveness. A relationship between eosinophil mediators, tissue damage and BHR has also been reported in asthmatic subjects [6]. Inferentially, understanding the cellular and molecular mechanisms behind these and other symptoms in asthma pathophysiology may translate into improvements in current therapy and in the quality of life of asthma patients:

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