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COMMENTARY

FROM LUNG HYPERSENSITIVITY TO BRONCHIAL HYPERREACTIVITY

WHAT CAN WE LEARN FROM STUDIES ON ANIMAL MODELS?

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Major developments in the field of acute hypersensitivity and allergy tend to blunt some of the differences between immediate (type I) and delayed (type III) hypersensitivity reactions, which were considered essential in the past. These differences concern on one side the recognition of the role of non-histamine-containing effector cells, basically eosinophils and, on the other side, that of antigen-presenting cells and T- and B-lymphocytes.

The concept that allergy is an immunological disorder due to the production of specific IgE antibodies which bind to mast cells and support the release of bronchoconstrictor mediators (histamine) following exposure to allergen is presently replaced by a broader one, according to which asthma is "a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils" [1]. Since excellent reviews are available [2, 3], our purpose is not to add more references to the subject, but rather to provide a critical overview of some of the presently available models used for studying asthma, particularly in the guinea-pig, with emphasis on its inflammatory components.

MODELS OF LUNG HYPERSENSITIVITY: WHY THE GUINEA-PIG?

Anaphylactic bronchoconstriction in sensitized guinea-pigs is the most used model for testing anti-allergic agents, despite wide differences between this species and humans. Guinea-pigs possess a developed respiratory smooth muscle, which contracts intensively and rapidly in response to *in vivo* or *in vitro* exposure to antigen. This anatomical prerequisite is required for both the expression of bronchoconstriction of the immediate hypersensitivity reaction, which evolves in minutes, and for its late component, which evolves in hours, but is far from being sufficient. Animals that have poorly developed airway smooth muscle, as reported for mice, may not be the first choice to study straightforward antigen-induced bronchoconstriction, even if their upstream components, particularly the immune and inflammatory loops, are well studied and are in many aspects comparable to those of humans.

The prevailing antibody involved in human allergy belongs to the IgE subclass, identified as the reagin of allergy by Ishizaka *et al.* [4]. Nevertheless, there is evidence that IgG may confer antigen-specific anaphylactic sensitivity to the human skin [5, 6]. Guinea-pig anaphylactic responses usually involved IgG_1 antibodies [7, 8], even though the model can be tailored for the production of IgE [9–11]. The difference between humans and guinea-pigs in the subclass of antibodies accounting for the allergic reactions should thus be kept in mind, particularly in drug studies [7, 10, 11].

Another important difference between guinea-pigs and humans is the route of sensitization. The s.c. route is used in most experimental studies, the amounts of antigen varying between 1 and 100 mg/kg. Frequently, but not always, antigen is used in the presence of adjuvants, such as $Al(OH)_3$, *Bordetella pertussis* toxin or Freund's complete or incomplete. Large doses of antigen tend to support earlier and more intense bronchopulmonary hypersensitivity [7, 10, 11] and favor IgG [12], whereas smaller doses or cyclophosphamide treatment favor mixed IgE and IgG production [9]. Clearly, the inflammatory response varies according to the protocol, and antigen persistence and constant stimulation, in particular, may lead to important differences. It is generally accepted that $Al(OH)_3$ induces the synthesis of IgE, even though transfer of serum from sensitized to naive animals demonstrated an important role for thermoresistant IgG_1 antibodies [8]. Sensitization can also be performed by aerosol [13–15]. This allows for marked responses to antigen, whether delivered by the intravenous or intrapulmonary route, in the absence of a significant increase of the amounts of specific circulating IgG [13]. Lung hypersensitivity can be monitored easily *in vivo* or *in vitro*, by following smooth muscle contraction and/or the formation/release of mediators from whole lungs or isolated tissues in the presence of antigen. In contrast, lung hyperresponsiveness, which does not depend on antigen specificity but rather on the repetition of antigen

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provocation, has not been correlated to a selective route of administration of antigen for the initial sensitization. This important point demands clarification, since it involves the distinction between the local (pulmonary) and the systemic components of the immune system which participate in the protracted inflammatory reaction accompanying (and possibly determining) the perpetuation of asthma as a chronic inflammatory disease (see below).

Pharmacological control of anaphylactic bronchoconstriction

Acute antigen-induced bronchoconstriction is suppressed by small doses of antihistamine agents, which have no major indications in human asthma. The anaphylactic bronchoconstriction in the guinea-pig is mostly histamine dependent, a major drawback for using this model to mimic the human condition. For this reason, models have been developed in which antihistamines are used to uncover the role of non-histamine mediators of bronchoconstriction. For example, platelet-activating factor (PAF)* antagonists inhibit anaphylactic bronchoconstriction in passively sensitized guinea-pigs, but are inactive in actively and antigen-boosted animals [16–18], unless associated with small doses of mepyramine [16, 19]. Cyclooxygenase is easily activated in the guinea-pig lung and most of the arachidonic acid that becomes available is transformed into thromboxane (TX) A_2, a powerful bronchoconstrictor agent. Thus, the administration of indomethacin is used to suppress the cyclooxygenase components and favor the transformation of arachidonate into peptidoleukotrienes [20, 21]. Accordingly, to demonstrate the effectiveness of 5-lipoxygenase inhibitors or leukotriene antagonists against anaphylactic bronchoconstriction requires suppression of the effects of endogenous histamine and rechannelling of the arachidonate metabolism towards the lipoxygenase pathway. Thus, animals and isolated preparations are exposed to a histamine antagonist and to a cyclooxygenase inhibitor in order to tailor a model for studying 5-lipoxygenase inhibitors. These different and rather arbitrary maneuvers will be justified if and when the respective antagonists or inhibitors demonstrate clinical effectiveness. It is nevertheless clear that if histamine accounts for practically the whole bronchoconstrictor response to antigen challenge in guinea-pigs, agents which suppress histamine release should show acute protective activity in the non-pharmacologically manipulated animal. This may be the case for sodium cromoglycate, which reduced the anaphylactic response in guinea-pigs sensitized with low amounts of ovalbumin in $Al(OH)_3$ [10], even though nedocromil sodium failed to suppress antigen-induced histamine release and the accompanying bronchoconstriction when perfused directly into isolated lungs of actively sensitized and boosted guinea-pigs [22].

Immediate hypersensitivity is also characterized by increased vascular permeability in the airways, which is probably due, largely, but not exclusively, to the release of endogenous histamine. It has been stated that the presence of exuded plasma in the lower airways may be involved in the pathology of asthma, particularly because tracheobronchial exudation is followed by formation in the bronchoalveolar lavage (BAL) fluid of potent bronchoconstrictive and inflammatory mediators (kinins, complement and clotting factors, fibrinolysis). Cromoglycate may also be partially effective against exudation [23] by inhibiting mast cell degranulation, but other explanations are available [22, 24 and see below].

Phosphodiesterase inhibitors and β_2-adrenergic agonists counteract the acute effects of antigen. The importance of β_2 receptors is highlighted by the reduction of their presence on lungs from guinea-pigs sensitized by the i.p. route and further challenged for 4 weeks with an aerosol of antigen [25]. In agreement, propranolol was shown to induce bronchoconstriction [26] and lung hyperresponsiveness [27], but since both its isomers were equieffective, even though they show distinct potencies as β_2-adrenergic inhibitors, it is likely that other effects, such as the endogenous formation of leukotrienes, may be involved [27, 28].

Overall, the guinea-pig model for acute bronchoconstriction and vasopermeation of asthma thus shows advantages and drawbacks: the advantages consist in the developed bronchial smooth muscle structures, in the ease of pharmacological manipulations and in the effectiveness of most anti-allergic drugs (glucocorticosteroids, which may be an exception to this rule, are not discussed here). The drawbacks relate to the comparatively important role of histamine, as compared to humans and possibly to the specific characteristics of the guinea-pig mast cell as well.

PAF AND BRONCHOPULMONARY RESPONSE

Role of PAF in lung hypersensitivity

Over the past years, two families of lipid mediators have been proposed as alternatives to histamine in mediating acute allergic bronchoconstriction: peptidoleukotrienes and PAF. Reviews are available for both [29, 30], and accordingly our comments will only focus on recent developments on PAF. The implication of PAF in bronchial asthma results from its ability to mimic its characteristic features, namely the increase in bronchial resistance to inflation, protein extravasation and accumulation of inflammatory cells at the sites of challenge [30]. After an initial enthusiasm brought about by the description of its original properties unshared by other putative mediators (cyclooxygenase-independent increase in bronchial resistance to inflation, induction of hyperresponsiveness in animals [31–33] and in humans [34; see Ref. 35 for contradictory results]), limited but negative clinical results became available with respect to provocation tests in human asthmatic subjects [36–38]. We have expressed reservations

* Abbreviations: PAF, platelet-activating factor; TX, thromboxane; BAL, bronchoalveolar lavage; MBP, major basic protein; EPO, eosinophil peroxidase; LT, leukotriene; IL, interleukin; and GM-CSF, granulocyte-macrophage colony-stimulating factor; 5-HT, serotonin; TNF, tumor necrosis factor.

concerning the effectiveness of the available PAF antagonists against active anaphylactic shock in guinea-pigs and indeed the pretreatment with the antihistamine drug mepyramine was required to inhibit bronchoconstriction by antigen [16–19]. In addition, we demonstrated that three PAF antagonists (WEB 2086, CV 6209 and BN 52021), which suppress bronchoconstriction and mediator release induced by PAF in lungs from non-sensitized animals [16, 19, 39–41], lose their effectiveness when applied to those from sensitized and boosted guinea-pigs [42]. This allows the speculation that the sites of interaction for PAF present in "normal" and in "allergic" airways may differ, and that the PAF antagonists screened on platelets and developed *in vivo* using non-immunized animals do not interact with those sites recruited into the lungs following allergen exposures. It is noteworthy that the situation seems to differ in mice, in which the booster injection during the immunization process shifts the anaphylactic paw edema from a PAF-independent to a PAF-dependent reaction [43].

Even though a role for PAF in acute bronchoconstriction of asthma seems unlikely at this stage, its involvement in experimental models of asthma in the guinea-pig may be related to chronic components, such as the recruitment and/or activation of inflammatory cells in the airways. The possibility that those components include overlooked basophils is raised by the work of Golden *et al.* [24].

Bronchopulmonary hyperreactivity and PAF

The administration of PAF by aerosol to the guinea-pig triggers a marked bronchial hyperreactivity to the subsequent administration of serotonin (5-HT) [31], histamine or acetylcholine [32, 44] and bombesin [45]. This phenomenon peaks 24 hr after PAF admainistration, at the time of the plateau of eosinophil accumulation in the airways. PAF also induces a non-specific bronchial hyperreactivity in the rabbit [33]. Interest in this field increased markedly after the first demonstration of the ability of PAF to elicit bronchial hyperreactivity to methacholine in non-asthmatic subjects [34], a finding further confirmed by other investigators [46–49]. However, the failure of PAF, even in large doses, to induce bronchial hyperreactivity to methacholine in normal [35, 50, 51], and in asthmatic subjects [52], has been reported recently. The reason for these discrepancies is not known, but because the effects of PAF are highly variable and, when present, are expressed only as one doubling dilution of methacholine, bronchial challenge with PAF in humans seems not to be a model of bronchial hyperresponsiveness [53]. By contrast, since the ability of PAF to trigger airway inflammation in humans and animals has been widely described, its possible role in allergic disorders is an open possibility. Thus, studies on the role of PAF in animal models of hypersensitivity and bronchial hyperreactivity remain relevant.

ANIMAL MODELS OF BRONCHIAL HYPERREACTIVITY

Models have been developed, particularly using the guinea-pig, to investigate the mechanisms of the late phase reaction of asthma and of the accompanying bronchial hyperreactivity. Other species have been proposed as well, such as rats, rabbits, dogs, sheep and monkeys, but the guinea-pig continues to be used, basically because of the marked reactivity of its respiratory system. Those models are based either on the immunization and subsequent challenge with the allergen, on the exposure to irritants such as ozone [54], vanadate [55], toluene iisocyanate [56], or on the inoculation of influenza virus [57]. A single inhalation of antigen to guinea-pigs sensitized by the s.c. route to ovalbumin in the absence of adjuvant is reported to induce, 24 hr later, bronchial hyperreactivity to i.v. histamine and acetylcholine [44], accompanied by eosinophil infiltration in the BAL fluid. However, we failed to confirm these results, since in our recent experiments intense BAL eosinophilia was unaccompanied by bronchial hyperreactivity.* A careful examination of the literature shows that in fact the models of allergic bronchial hyperreactivity are mostly based on the repeated exposures of the animals to the allergen [14]. In some cases, 12–15 challenges were required to induce a significant increase in the bronchoconstrictor response to acetylcholine [58]. In one report, allergen inhalation in guinea-pigs sensitized by aerosol was followed by early and late phase bronchoconstriction accompanied by neutrophil and eosinophil infiltration in the BAL [15]. In view of its similarity to the human situation, this model seems relevant, particularly since the same authors showed more recently an increased proportion of hypodense eosinophils in the BAL from antigen-challenged animals, as compared to controls [59]. These results are consistent with the observation that the majority of the eosinophils of asthmatic patients are hypodense [60]. However, no evidence of bronchial hyperreactivity developing during the late phase reaction following antigen challenge was reported in this model.

Is the eosinophil infiltration in the airways required for bronchial hyperreactivity?

From the results discussed above, it becomes apparent that eosinophil infiltration in the airways following exposure to antigen may not account *per se* for the development of non-specific bronchial hyperreactivity. It is indeed likely that several investigators, including us, have assumed that airway inflammation is based essentially on the accumulation of cells in the BAL, or in the lung parenchyma and bronchial submucosa, as we have shown in passively sensitized guinea-pigs challenged with the specific antigen [61]. It is clear that the presence of eosinophils in the airways, even if recruited by the "natural" antigenic challenge, is not sufficient to support bronchial hyperreactivity. An additional component, such as further activation and receptor expression on the eosinophil surface brought by secreted cytokines, or the active participation of other cell types, such as the basophil [24], seems to be required. In addition, bronchial hyperreactivity may also be associated with other signs of airway inflammation, including the increase in vascular

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permeability and epithelium injury, and not only with the cellular infiltration in the airways.

Airways eosinophilia as a sufficient requisite for the expression of bronchial hyperreactivity has, however, been described in rats [62], in which i.v. injections of Sephadex particles trigger a marked blood and BAL eosinophilia. This phenomenon, which peaks 7–8 days after the administration of Sephadex particles, is accompanied by a significant enhancement of the bronchoconstrictor response to 5-HT. Blood and lung eosinophilia and bronchial hyperreactivity were inhibited by dexamethasone and β-agonists, but not by sodium cromoglycate [63]. Since sodium cromoglycate and, more recently, nedocromil sodium have been shown to antagonize airway eosinophilia and the accompanying bronchial hyperreactivity induced by antigen provocations in various models [22, 64, 65], one may speculate that the mechanisms underlying both phenomena vary depending on the triggering stimulus, antigen or other, leading to different pharmacological modulations.

The correlation between the eosinophil distribution in the BAL fluid and/or in the lung tissue and the evaluation of the release of eosinophil-derived products is now widely accepted as determining the degree of activation of eosinophils in pathological conditions. Among these products, interest has focused mostly on major basic protein (MBP), a protein cytotoxic for the respiratory epithelium of various species, including humans and guinea-pigs [66, 67]. The production of MBP following eosinophil activation may thus contribute to the epithelium shedding and lysis, leading to the exposure of the submucosal structures, essentially the nerve terminals, otherwise protected from the environment. This phenomenon may play a critical role in the increased sensitivity of the smooth muscle from asthmatics to non-specific contracting agents. In a very interesting work, Sedwick *et al.* [68] recently demonstrated the presence of elevated amounts of MBP and of other eosinophil granular proteins, such as the eosinophil-derived neurotoxin, the eosinophil cationic protein and the eosinophil peroxidase (EPO), together with high levels of leukotriene (LT) C_4 and of interleukin (IL) 5, in the BAL fluid from allergic rhinitis patients, as compared to normal individuals. Interestingly, the mediator concentrations correlated well with the number of eosinophils present in the lavage, suggesting that these cells, once attracted to the airways, may be activated by IL-5 to release granular proteins responsible for the epithelium injury. Work is in progress to characterize pro-inflammatory mediators and cytotoxic substances in the BAL fluid of antigen-challenged animals. Indeed, the amounts of albumin and of β-glucuronidase, histamine, prostaglandin D_2 and TXB_2 were evaluated in the BAL from antigen-challenged guinea-pigs and compared to those of control animals [69]. An assay for EPO was described using guinea-pig peritoneal eosinophils stimulated by a large variety of agonists [70, 71]. More recently, an immunoenzymoassay specific for detecting MBP in the BAL fluid was also developed in the guinea-pig [72].

Role of lymphocytes in bronchial hyperreactivity

In the last few years, increasing evidence suggests that the events of late phase reactions, including local eosinophilia, mast cell activation and IgE production are orchestrated by T-lymphocytes. Following the specific recognition of the antigen, these cells elaborate cytokines, such as IL-4, which are responsible for the synthesis of IgE by B-lymphocytes, or IL-5, which account for the proliferation and differentiation of the eosinophils from their precursors. Numerous results indicate the presence of activated T helper (Th) lymphocytes in BAL fluid, bronchial biopsies and peripheral blood from atopic subjects, their number correlating with that of circulating eosinophils. More recently, the observation in BAL fluid from asthmatics of increased numbers of cells expressing mRNA for IL-2, IL-3, IL-4 and IL-5, but not for interferon-γ, suggests the presence of a Th2-like pattern of cytokine gene expression in allergic asthma [73]. Even though the presence and characterization of T-lymphocytes and of the genes expressing their derived cytokines is now well described in humans, the situation is not clear in animals. Indeed, Frew *et al.* [74] described increased numbers of T cells with a CD8 negative phenotype in the lung parenchyma and bronchial submucosa of sensitized guinea-pigs following exposure to antigen delivered by aerosol. These observations suggest that $CD4^+$ cells predominate in the bronchial wall. Recently, using specific monoclonal antibodies, we found evidence that guinea-pigs actively sensitized to ovalbumin exhibit an increased number of $CD4^+$ lymphocytes infiltrating the bronchial mucosa [75]. The expansion of the $CD4^+$ population parallels the development of a non-specific bronchial hyperresponsiveness, that we have demonstrated previously in isolated lungs from guinea-pigs sensitized by this same procedure [76].

The characterization of lymphocyte subsets in the bronchial wall has been described in the past in mice, a species in which most of the immunological tools have been developed thus far. Curtis and Kaltreider [77] characterized by flow cytometry the numbers and phenotypes of lymphocyte subpopulations recovered in the BAL fluid from antigen-challenged mice, demonstrating that Th-cells predominate over suppressor T-cells and over B-cells. The increment in those cells in the lung after antigen challenge, however, was not correlated with changes in the lung function. Furthermore, the presence of T-cells at the site of antigen challenge is not a proof that they participate actively in airway inflammation. This demonstration came partially from more recent studies showing that the increased contraction to carbachol of isolated trachea from antigen-challenged Balb/c mice is abolished in athymic animals [78]. Accordingly, transfusion of non-immunized mice with lymphoid cells purified from immunized donors restored the *in vitro* airway hyperreactivity, suggesting a participation of T cells in this phenomenon. Similarly, *in vivo* airway hyperresponsiveness to methacholine and 5-HT in naive mice challenged with the antigen after being

transfused with spleen and lymph node cells from sensitized animals has been reported recently [79].

Evidence for the participation of cytokines in animal models of airway inflammation and bronchial hyperreactivity

It has been suggested recently that cytokines produced by Th lymphocytes in response to antigen stimulation may play a role in airway inflammation and in the consequent hyperresponsiveness. These cytokines include IL-5, IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which share the ability to support eosinophil proliferation and differentiation from their bone marrow precursors and prime target cells for increased responses to stimuli. Since those synergisms also involve an increased release of arachidonic acid metabolites, including LTC_4 [80], the link between the regulatory role of hematopoietic growth factors and lipid mediator has been suggested. Accordingly, the interaction between PAF and Th2 lymphocyte-derived cytokines was investigated both with isolated eosinophils and *in vivo* in the guinea-pig. Among the various lipid mediators, PAF was selected because it is a strong eosinophil activator and because the effects of most cytokines produced by activated Th2 lymphocytes are directed against this cell type or its precursor. Thus, the abilities of recombinant human (rh) IL-5, PAF and LTB_4 to promote guinea-pig eosinophil migration, superoxide anion production and calcium influx were compared in our laboratory [81]. rhIL-5 stimulated eosinophil chemotaxis dose dependently, a peak being reached at 500 ng/mL. Eosinophil preincubation for 5 min with rhIL-5 increased significantly the migration, the superoxide anion production and the calcium fluxes induced by PAF, but not by LTB_4 [81]. The mechanism by which this interaction takes place was not elucidated. One possibility may involve an enhanced phospholipase C activity, leading to an increased leukotriene production, as reported [82]. Interestingly enough, the incubation of an undifferentiated human eosinophilic cell line with IL-5 increased markedly the mRNA encoding for PAF receptor [83], an hypothesis which may parallel our findings.

We further demonstrated that priming by rhIL-5 of the effects of PAF on purified eosinophils may be relevant for the lung function, since the intratracheal administration of rhIL-5 into isolated lungs from actively sensitized guinea-pigs triggered a marked time-dependent hyperresponsiveness to PAF [84]. This phenomenon, which is expressed as an enhanced bronchoconstriction and an increased release of TXB_2 and histamine into the effluent, was intense in lungs from immunized and boosted animals and was present, even though to a much lower extent, in those from guinea-pigs actively sensitized but not boosted. The key role of active sensitization was confirmed by the failure of rhIL-5 to enhance bronchoconstriction and mediator release in response to PAF in lungs from non-immunized or passively sensitized animals [84].

The previous observation that actively sensitized and boosted guinea-pigs exhibit an increased number of eosinophils in the BAL fluid, as compared to non-immunized animals [22], and the evidence that IL-5 stimulates selectively mature eosinophils [85], may explain the increase in lung response to PAF brought by this cytokine. Indeed, the number of eosinophils was increased markedly in BAL obtained from guinea-pigs killed after the booster injection of antigen, suggesting that the development of lung hyperresponsiveness to PAF induced by rhIL-5 follows a profile similar to that of eosinophil invasion into the airways. On the basis of these results, one can speculate that repeated allergen exposures of asthmatic subjects is followed by the production of PAF and of IL-5 in the airways. The interaction between both substances on the eosinophils leads to their activation, characterized by the release of arachidonate derivatives, free radicals, proteolytic enzymes and cytotoxic basic proteins, as discussed above. Various cell types may produce PAF and IL-5 in the airways of asthmatic patients or of sensitized animals after antigen exposure. An additional interesting hypothesis involves the mast cell, since it has been shown that murine mast cells differentiated from bone marrow precursors in the presence of T-cell growth factors produce PAF upon antigen challenge [86]. More recently, the release of PAF from human lung mast cells challenged with anti-IgE, concomitantly with that of histamine and LTC_4, has been reported [87]. Furthermore, the mast cell has been shown to produce cytokines, particularly IL-5 [88].

We further demonstrated that lung hyperresponsiveness to PAF induced by rhIL-5 can be inhibited by anti-allergic drugs. Thus, the administration to the guinea-pigs, during the interval between the booster injection of the antigen and the day of lung removal, of nedocromil sodium, an anti-allergic drug which counteracts airways inflammation, suppressed rhIL-5-induced hyperresponsiveness to PAF and the increased numbers of eosinophils in the BAL [89]. The observation that incubation with nedocromil sodium of normal density human eosinophils inhibits IL-5-, but not interferon γ-induced increased survival [90], leads to the hypothesis that this drug may interfere with the direct effects of IL-5 on the eosinophil. These results suggest that protection by nedocromil sodium against the late phase reaction of asthma may derive from its ability to inhibit IL-5-induced up-regulation of the inflammatory activity of eosinophils. In recent experiments from our laboratory [91], eosinophils purified from BAL of antigen-challenged guinea-pigs exhibited a marked increase of migration induced by different stimuli, supporting that they had been exposed continuously to potentiating factors present in their microenvironment [91]. This eosinophil hyperresponsiveness was not reduced when the cells were collected from the nedocromil-treated animals, even though their number was decreased significantly [91]. In this case, it is clear that the reduction of the number of recruited inflammatory cells is the target of nedocromil sodium, but that the up-regulated eosinophil function is not modified.

Sanjar *et al.* [92] showed that the administration to guinea-pigs of 1 μg/kg rhIL-5 induces a marked blood eosinophilia which peaks between 1 and 7 hr,

depending on the route of administration, and persists for at least 24 hr. Also in the guinea-pig, these authors studied the relationship between the effects of PAF and of eosinophilotactic cytokines [93] and showed that 2-day i.p. administration of rhGM-CSF increased the percentage of bone marrow and airway eosinophils. PAF aerosolization was followed by a rise in the number of eosinophils in the BAL fluid, which was increased significantly in rhGM-CSF-treated animals. These results suggest that exposure of airway eosinophils to GM-CSF released upon antigen stimulation may contribute to their enhanced response to chemotactic stimuli such as PAF.

The involvement of IL-5 in the antigen-induced airway eosinophil recruitment in the guinea-pig was also suggested by Chand *et al.* [94], who reported that a single i.p. administration of a specific antibody directed against IL-5 markedly reduced the increase in the number of eosinophils in the BAL following antigen inhalation. Mauser *et al* [95] demonstrated that the same anti-IL-5 antibody impaired the bronchial hyperreactivity to substance P observed 24 hr after antigen challenge in sensitized guinea-pigs. However, the dose effective against eosinophil infiltration was below the one needed to suppress bronchial hyperreactivity, suggesting again a dissociation between both phenomena. Furthermore, the amounts of antibody used in these experiments were very high (10–30 mg/kg), as compared to those effective in the work by Chand [94] (30–100 μg/kg). Since the protocols of immunization, antigen challenge and timing of the experiments were similar in these studies, a discrepancy between both results remains to be clarified.

The ability of cytokines to modify the pulmonary function was also examined in rats. The systemic administration of rhIL-2, the major growth factor for T-lymphocytes, was shown to cause bronchial hyperreactivity to methacholine, accompanied by an augmented vascular leakage, by increased numbers of lymphocytes, neutrophils and eosinophils in the BAL fluid and in the bronchial wall and by the detachment of the epithelium from the basement membrane [96, 97]. The mechanisms of these effects have not been explained thus far. One hypothesis involves the structural changes of the bronchial tree observed after the administration of IL-2. Indeed, the thickening of the bronchial wall secondary to edema formation and to the infiltration of inflammatory cells in the airways characterizes asthma and is claimed to be responsible for the development of non-specific bronchial hyperreactivity [98]. On the other hand, the activation of T-lymphocytes by IL-2 is followed by the production of other cytokines acting on eosinophils. These cells may in turn release inflammatory lipid or granule-associated mediators which contribute to the alterations of the bronchial responsiveness. As an example, it has been shown that blood eosinophilia following the administration of IL-2 to mice is antagonized by a specific antibody directed against IL-5 [99]. Concomitantly, an enhancement of IL-5 mRNA expression in spleen cells of IL-2-treated mice has been detected [99]. Taken together, these results suggest that IL-5 can be considered as one of the cytokines responsible for the bronchopulmonary effects of IL-2.

The effect of cytokines other than those implicated with the regulation of the eosinophil functions has been investigated in experimental models of airway inflammation and bronchial hyperreactivity. Kips *et al.* [100] reported that an aerosol of tumor necrosis factor (TNF) induces bronchial hyperreactivity to 5-HT in rats, associated with neutrophil infiltration into the BAL fluid. Since the same authors had reported that the inhalation of endotoxin also induced bronchial hyperreactivity and neutrophil infiltration [101], it was suggested that the release of TNF during endotoxic shock may account for the changes in the airway responsiveness observed. The situation in the guinea-pig is different and the intratracheal instillation of endotoxin was also followed by bronchopulmonary hyperreactivity and neutrophil infiltration in the airways, but both phenomena were dissociated, since neutrophil depletion failed to prevent hyperresponsiveness. In contrast, platelet depletion or inhibition of platelet function by prostacyclin removed endotoxin-induced bronchial hyperresponsiveness [102].

CONCLUSIONS

Experiments with heterologous cytokines indicate that rhIL-2, rhIL-3, rhIL-5, rhGM-CSF and rhTNF may to some extent cross the species barrier. This is of interest, particularly for those groups working with guinea-pigs, a species for which immunological tools have not been developed extensively. Nevertheless, final conclusions will only be reached when adequate homologous tools become available. For this reason, a mouse model, allowing immunological manipulations and cell transfer, is indispensable to unravel the molecular mechanisms of allergic reactions. In this respect, immunohistochemistry and search for gene expression on lungs from sensitized mice as well as the use of transgenic and knock-out animals and of specific anti-cytokine antibodies will be vital for the future trends in research on the mechanisms of allergy. The recent availability of methods for recording the pulmonary functions in anesthetized mice [103], and, accordingly, the possibility to study whether immunization and stimulations with allergen or with xenobiotics in general induce bronchial hyperactivity will deliver exciting information in the near future.

The field of experimental allergy is in profound mutation, evolving from the study of the role and mechanisms of formation and release of small molecular weight mediators which display immediate effect, to that of complex networks involving the secretion of cytokines, colony-stimulating and differentiating factors, and their integrated activity, with positive and negative multicellular loops. This leads to two apparently contradictory demands: on one side, the absolute need to introduce refined tools provided by immunology and by molecular biology and, on the other, to interpret those and other results within the integrated animal. For this reason, the development of *in vivo* models of disease continues to be essential, but not only for practical reasons (drug screening and development) but also

to ensure that *in vitro* discoveries are interpreted in adequate perspective. Thus, tendencies to replace animal studies with test tubes and multi-well plates are as much a reflection of fashion and of reductionist tendencies as the reverse, i.e. the tendency to ignore the potent concepts and tools of molecular biology, reflects resistance to progress.

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