

Relevance of Animal Models for Studies of Immune Regulation of Atopic Allergy

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A number of studies, primarily in animals, corroborate clinical experience that the allergic inflammatory reaction is more complicated from an immunological point of view than previously appreciated. Allergic inflammation is a consequence of an interplay between various components of the immune system, including IgE antibody formation and regulation thereof, IgG antibodies and complement, cell-mediated immune reactions and lymphokines as well as immune regulation of the proliferation and differentiation of mast cells, basophils and mucous cells. In addition, histamine and other mediators of the inflammatory reaction exert a feed-back regulation on the immune system (28, 31). Eosinophils play a yet not fully understood role, possibly as scavengers of mediators that produce tissue lesions (92) or, as indicated by recent findings, as producers of tissue damaging substances (20).

Much of the new knowledge concerning the allergic inflammatory process has been gathered from animal studies. Such studies have obvious advantages, including a possibility to compare the effects of experimental manipulations of single parameters in genetically similar individuals. Such data, however, cannot automatically be assumed to be relevant for the situation in humans. The purpose of this review is to discuss the clinical relevance of some immunological data generated in animal models cur-

rently used in allergy research, relating the experimental findings to observations in allergic humans. The discussion will be limited to immune regulation of conceivable relevance for the development and regulation of atopic disease, by which is meant a genetically influenced hyperreactivity to minute amounts of antigens most often accompanied by high levels of IgE antibodies. The involvement of the autonomous system and the importance of mediator substances like prostaglandins and leukotrienes in the regulation of the cellular events are outside the scope of the presentation.

Characteristics of atopic allergy in humans

The atopic diseases include allergic rhinitis, conjunctivitis, asthma, anaphylactic shock and some forms of urticaria and eczema. Traditionally, high levels of reagins of the IgE isotype are thought to represent the only or the dominating immunological feature of importance for the diseases. In addition to this immunological abnormality, eosinophilia is commonly seen in atopic patients. They also have an unspecific hyperreactivity in the shock organs.

A fulminant attack of asthma, whether it is elicited by immune or non-immune mechanisms, is associated with extensive degranulation of mast cells and basophils as well as with bron-

chospasm and an enormous production of mucus. The lungs of patients who have died in status asthmaticus show congestion, contracted bronchi, increase in number and size of mucus-producing cells and glands, increased secretions and acute inflammation with infiltration of polymorphonuclear leukocytes, eosinophils and lymphocytes (21). In patients with allergic rhinitis increased numbers of eosinophils, mast cells and basophils are found in the mucosa and in the secretions (80). These cell types can also be found in some eczematous lesions (23). Appreciation of the interplay between factors regulating mast cells, basophils and mucus-producing goblet cells, chemotactic factors for eosinophils and neutrophils and the regulation of IgE synthesis are therefore necessary for an understanding of the allergic inflammatory reaction.

Features of IgE antibody formation

An elevated IgE level is a hallmark of atopic disease. Although the IgE levels' conditions are usually determined in the serum of patients, the antibodies probably express their most important biological functions in secretions and mucous membranes, but there is, at least in rats, a relation between levels of cell-fixed and systemic IgE antibodies (85). In man, serum IgE appears to be largely derived from local production (76). The clinical importance of circulating IgE antibodies not associated with the cell membrane of mast cells or basophilic granulocytes is unknown. Local factors apparently play an important role, since patients, even with high levels of circulating IgE antibodies, usually only have regional symptoms, e.g. from part of the respiratory tract or the skin. It is also well established that patients with IgE antibody levels to a particular allergen of similar magnitude in the serum have widely varying clinical sensitivity and may show reactions from different shock organs. This could indicate that the clinically most relevant IgE antibodies are produced locally in regional lymph nodes and that they primarily exert their functions in mucous membranes drained by these nodes.

Animal experiments give evidence for a re-

gional production of IgE at sites where also mast cells may be coated with antibodies. Several studies have demonstrated regional production of IgE antibodies in lymph nodes draining mucous membranes (5, 10, 11, 16, 65). Furthermore, studies in rats infected with *Nippostrongylus brasiliensis* show that IgE antibodies can be demonstrated regionally in the draining lymph nodes before a systemic response can be detected (11). Tracheal sensitivity to worm allergens developed prior to intestinal sensitivity and correlated with the early local synthesis of reaginic antibody in the mediastinal lymph nodes, while sensitivity to the allergens in the skin and gastrointestinal tract correlated more closely with serum reaginic antibody. Thus local reagin synthesis influences local tissue sensitization and responsiveness to allergens. These data were recently supported by a study demonstrating locally produced immunoglobulin E in tear fluid from patients with allergic conjunctivitis (60).

The formation of IgE antibodies is strongly genetically regulated and only minute amounts of antigen may be sufficient to stimulate the synthesis. For humans it has been calculated that the amount of allergenic material to which a sensitive patient is exposed during the season may be as low as a microgram (64). Studies in breast fed infants lend further support to the hypothesis of low dose sensitization since it has been convincingly shown that specific IgE antibodies to cow's milk may occur in fully breast-fed infants sensitized by extremely small amounts of antigen present in human milk (30, 78). Similarly, administration of low doses of antigen to experimental animals preferentially stimulate IgE antibody production. In rats injected with *Bordetella pertussis* as adjuvant parenteral administration of antigen doses in the picogram-range for booster injection was sufficient to stimulate IgE antibody formation (47). In rats, mice and rabbits administration of antigen in ng to µg doses without using any adjuvant has been shown to induce IgE antibody formation (1, 4, 44, 38, 57, 63). Using protocols in which the antigen is injected in 10-day periods without the use of any adjuvant,

even such low doses as 4 ng per s.c. injection induce IgE antibody formation in certain strains of mice (32, 57).

Inhalation of allergen is the most likely common route of sensitization in humans with the exception of infants. In mice, rats, guinea pigs and rabbits this route has been difficult to use for stimulation of IgE antibody synthesis. We and others have reported IgE antibody production (1, 2), whereas other investigators have reported suppression of IgE antibody synthesis after inhalation of antigen (26, 37, 39). The demonstration of IgE antibodies may be complicated by the fact that most of the inhaled antigens are swallowed (93). Although oral immunization has been shown by some investigators to induce IgE antibody formation, particularly in combination with *B. pertussis* as adjuvant (9), we have not succeeded in sensitizing mice and rats by feeding the animals for 10-day periods with antigen without adjuvant (1). There are also reports demonstrating inhibition of IgE antibody responses by previous feeding of the antigen (89). The fact that the antigen in most experimental models is injected rather than inhaled should be remembered and may limit the clinical relevance of certain results. This source of criticism may be particularly relevant in light of the recent suggestion that abnormal permeability of antigens through mucous membranes may be part of the anomaly in atopy (46).

Effects of external factors on the IgE antibody formation

It is well established in animals that some adjuvants, such as alum (90) and *B. pertussis* bacteria (69), or certain parasites such as *Ascaris* (67) or *Nippostrongylus brasiliensis* (56), enhance IgE antibody responses (Table 1). Lipopolysaccharides have also been shown to stimulate IgE antibody production, whereas other strong adjuvants, e.g. Freund's complete adjuvant (FCA), are unfavourable to the generation of IgE antibodies (35, 45). The fact that immunizations of experimental animals usually are done parenterally, with relatively high doses of antigen in the presence of adjuvants, is a major

source of criticism of the use of rodent models for studies of IgE antibody regulation. In addition to increased IgE antibody levels the use of adjuvants may result in antibodies with characteristics partially different from those obtained by immunization without adjuvants, e.g. with different antibody avidity. Some support for this suggestion was recently given in a study in which IgE antibody responses in mice were measured by passive cutaneous anaphylaxis (PCA) and by RAST (32). The two methods for IgE determination gave similar results in animals immunized with low doses of antigen without any adjuvant. However, there was less agreement between RAST and PCA in animals immunized using *B. pertussis* bacteria as adjuvant, the latter method giving higher values in these animals.

Exposure of animals to *B. pertussis* bacteria or other adjuvants could possibly correspond to an influence of external factors on IgE antibody formation in allergic patients. The association between respiratory tract infections and onset of allergic disease (91) could thus in part be a consequence of microorganisms functioning as adjuvants for inhalant allergens. Supporting this possibility, infections of dogs with SV virus has been shown to significantly enhance IgE antibody formation to simultaneously administered antigen (27). In mice such effects of infections are less obvious and depend on virus and animal strain (6). In addition to the adjuvant effects on IgE antibody formation, viral infections and irritating substances could trigger allergic symptoms by affecting the autonomic nervous system, but this discussion is beyond the scope of the present review.

Various irritating agents could, besides their effects on the adrenergic-cholinergic system, increase the risk for sensitization. Several recent studies have documented an association between smoking and elevated S-IgE antibody levels and increased risk for development of occupational allergy (29, 94). This has been supported by findings in animal studies with air pollutants like SO₂ and ozone (74). We have recently shown that exposure of rats to tobacco smoke and to acrolein resulted in increased

Table 1
Biological effects of certain adjuvants

	Humans		Rodents	
	IgE synthesis	Organ sensitivity	IgE synthesis	Organ sensitivity
<i>Ascaris</i>	potentiates	?	potentiates	?
<i>N. brasiliensis</i>	potentiates	?	potentiates	?
<i>B. pertussis</i>	potentiates	?	potentiates	increases
Alum	?	no effect	potentiates	?
Freund's complete adjuvant	?	?	suppresses	suppresses
Lipopolysaccharide	?	?	potentiates	?

Table 2
Regulatory factors selectively active on the IgE antibody system

Species	Stimulatory agent	Binding to	Activity	Mw	Reference
Mouse	spontaneously in CFA primed animals		antigen non-specific suppression	150,000	50
Mouse		ConA	antigen non-specific enhancement	?	50
Human	MLC		antigen non-specific suppression, species unrestricted	?	95
Rat	parasitic infection BCG	peanut agglutinin, IgE	antigen non-specific suppression	10–20,000	35, 41, 43
Rat	parasitic infection BCG	IgE, ConA lentil, lectin	antigen non-specific enhancement	10–20,000	34, 41, 43, 84, 86
Human	MLC, lectin, antigen	IgE			44
Mouse	DNP-primed T-cells cultured with antigen	?	antigen specific suppression	55–60,000	55
Human	hyper IgE state	IgE	enhancement	?	79

levels of total serum IgE, as well as in higher levels of specific IgE antibodies after aerosol but not after subcutaneous immunization (submitted). The mechanism for the increased IgE antibody formation is thus most likely to be found locally on the respiratory tract mucous membrane (25).

Regulation of IgE antibody formation

The immunoregulation of the IgE antibody formation has been excellently reviewed recently (41, 43, 50, 55) and will only be briefly

mentioned here. In animals there is an interplay of components enhancing and suppressing the IgE antibody response. Factors include T-lymphocyte derived lymphokines as well as B-cell derived anti-idiotypic antibodies (13, 55). Three research groups have been particularly involved in the animal studies of IgE regulation by T-lymphocyte derived factors, i.e. the laboratories of Ishizaka, Katz and Kishimoto. All factors have one striking feature in common: their biological effects concern only the synthesis of IgE antibody molecules. The three research groups have focused their interest on IgE regu-

latory factors, particularly in rodents. The factors have affinity for IgE and various lectins (41, 43, 50, 55, 86) (Table 2). Although findings in experimental animals should not be automatically extrapolated to what may be taking place in man, it must be borne in mind that so far there has been an encouraging concordance between mouse and man as to immunoregulation of cell-mediated immunity and humoral responses of other antibody classes. Indeed, factors with similar binding activities have been demonstrated to be released from human peripheral blood cells after appropriate stimulation (44). Thus, albeit very little is known about the regulation of IgE formation in the human system, T-lymphocytes seem to be involved, possibly by producing IgE binding factors similar to those identified in the rat system (79).

Results similar to those obtained by Ishizaka et al. were reported by Katz, who forwarded the concept of allergic breakthrough in a series of papers (reviewed in 50). Studies in mice had identified suppressive molecules for IgE synthesis produced *in vivo* in animals immunized with Freund's complete adjuvant (FCA). The factors appeared to play important operational roles in regulating IgE antibody synthesis under physiological conditions. The relevance of the findings for the human situation remains to be established, although Katz's group has claimed that similar factors are operative (95).

Several of the described factors have been claimed to possess regulating activity on IgE antibody formation by human cells *in vitro* (50, 51, 95) but the clinical relevance of studies of IgE antibody synthesis *in vitro* is controversial. It should be pointed out that although IgE regulatory factors in humans have been described, at least in some studies the reagents used were not specific for human IgE (48), thus invalidating the results. Studies of human IgE regulation are further complicated by the fact that IgE production and regulation most likely is a regional phenomenon. Cells and immune regulatory activity demonstrable in the blood, therefore, do not necessarily reflect the situation on the site of action. Recently, however, irradiation *in vitro* was reported to eliminate cells in the peripheral blood of normal per-

sons with the capacity to suppress mitogen-induced IgE production (73). This may indicate that studies of IgE regulation by human cells are possible using cells isolated from peripheral blood.

Immunotherapy

Although hyposensitization has been used for more than 70 years and in several recent controlled studies has been proved effective, the underlying mechanisms explaining the clinical effectiveness of hyposensitization with extracts of good quality remain an enigma. So far no proper animal models have been described which would allow studies of the effects of immunotherapy. Possibly the therapy is active in the formation of IgE regulating factors, particularly as regards modified allergens. Various such preparations have been produced with the hope that modified allergens possess lower allergenicity than native allergens and that they could suppress the IgE antibody formation and enhance the IgG antibody formation. Several allergen modification procedures have been suggested, including antigen denaturation with urea (42) and coupling of allergens to Poly: D-glutamic acid: D-lysine (D-GL) (61), polyvinyl alcohol (PVA) (40) and monomethoxy polyethylene glycol (mPEG) (59). Clinical trials have been performed with denaturated allergens (71) and with D-GL (15) and mPEG (49, 54, 72) modified preparations. The extracts modified with D-GL and mPEG induced no or very little suppression of the IgE antibody responses and significantly increased IgG antibody responses. This was in contrast to the initial animal studies with allergens modified by conjugation with mPEG showing an almost complete suppression of the IgE antibody production. In these animal studies IgE antibodies were induced by an injection of antigen in adjuvant, and the effects of modified allergens on the primary immune response were studied. However, in mice with an established IgE response induced by daily administration of low doses of antigen without any adjuvant, the subsequent administration of mPEG modified allergens only slightly suppressed the IgE anti-

body formation and enhanced the IgG antibody responses (3). The findings in this animal model corroborate those seen in human clinical trials with mPEG modified allergens with ragweed (49) and honey bee venom (72). Similar results were obtained in dogs who had been immunized from birth at weekly intervals for 8 weeks and thereafter at bi-weekly intervals (81). In the sensitized dogs the circulating IgE levels were not affected by treatment with mPEG modified ragweed extract, although the treated dogs showed less broncho-constriction than controls when challenged by inhalation of allergen. The results indicate that immunization protocol is of great importance for the results of animal studies and underline the importance of proper models for analysis of immune responses to allergens. The findings also stress the danger of extrapolating the results of studies in less well-characterized models to the clinical situation.

Cells of the allergic inflammation

Although eosinophilia in blood, secretions and tissues are observed in atopic patients, the precise role of the eosinophils in atopic disease has yet to be defined. Eosinophils may have regulatory functions that could modulate allergic reactions and they act as scavengers eliminating toxic mediators released by mast cells and basophils (reviewed in 92). However, recently they were reported also to be involved in the induction of tissue lesions by releasing the so-called major basic protein (MBP) into inflammatory foci (20). Eosinophils may appear in tissues in response to chemotactic stimuli released by mast cells or basophils (52). In guinea pigs eosinophils and basophils seem to be part of the defence system against ticks (14). Eosinophils are also recruited in experimental systems (33) and probably also in man (75), by factors released from immune-stimulated T-lymphocytes (17). This is a feature which they have in common with the basophils and mast cells. On the other hand, eosinophils rapidly disappear in patients under stress, including pronounced allergic reactions (92). The clinical significance of this characteristic feature of

eosinophil kinetics is unknown as is the precise regulation of eosinophils.

Blood basophils and tissue mast cells represent reactor cells of primary importance for the allergic reaction. New data indicate that they might have a common stem cell in the bone marrow (19). The number of blood basophils in atopic patients seems to be influenced by exposure to allergen. Thus, Hirsch & Kalbfleisch reported elevated basophil counts in patients with hay fever during season (36). The cell counts varied closely with the symptom scores in the patients. Increased numbers of circulating basophils have also been reported to precede an asthmatic attack in patients (53). After skin testing with the appropriate allergen in sensitive individuals, basophils accumulate, both as part of the IgE-mediated allergy (82) and of the so-called cutaneous basophilic hypersensitivity (CBH) of a contact dermatitis nature (7, 87). This latter condition is a delayed reaction mediated by immune T-lymphocytes and is characterized by basophil infiltrates in the tissue lesions. The participation of basophils in delayed reactions is also, at least in mice, associated with release of mediators traditionally associated with immediate hypersensitivity (77). It has been suggested that a subclass of T-lymphocytes, or particular chemotactic factors released by T-lymphocytes, may be responsible for the striking basophil infiltrates (87). Repeated skin testing of sensitive individuals was recently shown to induce a transient appearance of basophils and later on of mast cells, ending in the histological inflammatory pattern of chronic eczema (68). Numerous mast cells and basophils are also evident in secretions of allergic subjects exposed to antigen.

Dvorak et al. have shown that guinea pigs locally sensitized with antigen develop basophilia (22). The histological findings in biopsies from skin lesions of patients with allergic contact dermatitis showed similar infiltration of basophil leukocytes, different from biopsy findings in tuberculin reaction sites. Serial biopsies from one individual after patch testing revealed early perivascular accumulation of granulocytes followed by an influx of basophils and subsequently of eosinophils (23). This baso-

philia is linked to an increased number of precursor cells which mature to basophils *in vitro* after stimulation with culture supernatants from antigen-stimulated T-lymphocytes (18), indicating that the maturation is immune-driven. The similarity between the guinea pig model and humans in other respects of the basophil kinetics makes it reasonable to hypothesize that human basophil maturation may be influenced by T-lymphocyte-derived factors.

Mast cells, particularly of the mucous type with particular histochemical, biological and pharmacological characteristics (12), are found in increased numbers in patients with gut inflammations like coeliac disease (83). Studies in rats have demonstrated that the regulation of the local mast cell hyperplasia is done by T-lymphocytes (66). We recently showed a similar pattern in lungs of rats and mice sensitized with aerosolized protein antigen (2, 5), although in these studies unspecific irritation could also have partly contributed to the increased mast cell numbers in the mucosa. The increased mast cell numbers in the lungs after exposure to aerosolized antigen was most likely due to immune factors, since the mast cells in these animal species were predominantly associated with mononuclear cell accumulation (2). Furthermore, we have shown that mice with contact hypersensitivity to PiCl exposed to antigen in the airways rapidly developed mast cells in the lung (24). The findings from studies in mice, rats, guinea pigs and humans indicate that immune T-lymphocyte-driven mast cell accumulation, as well as degranulation (77), could occur in allergic disorders in which no IgE antibodies have been demonstrated.

Differentiation of mucous-producing cells seems to be driven by similar factors originating from T-lymphocytes. This is supported by adoptive transfer experiments of immune T-lymphocytes in parasite infected rats which evoke goblet cell hyperplasia (66). Mucous cells also seem to appear in response to aerosolized protein antigen inhalation in animals with contact hypersensitivity (24).

There are very few polymorphonuclear cells seen in the lungs of rats and mice sensitized as described above. In humans, however, the early

stage of allergic inflammatory reactions are characterized by infiltration of pmns (8). This appears to be a result of release of chemotactic factors like neutrophil chemotactic factor (NCF) which are released during the allergic reaction. The NCF is a heat-stable compound of high molecular weight (over 500,000 daltons) which has been demonstrated in patients with urticaria (88) and in the blood of patients after positive bronchial provocation (70). The most likely source of origin for the activity seems to be mast cells (58).

Concluding remarks

From the above it is clear that much of the new knowledge on the induction of the allergic inflammation gained from animal studies is clinically relevant. However, a key issue for human atopic disease, i.e. the type of underlying abnormality which at least in part is genetically determined, has not been possible to approach adequately in animal studies. With the possible exception of dogs and monkeys, animals studied in experimental models are not atopic. Thus, the full sequence of events ending in clinical symptoms cannot yet be properly analysed experimentally. Although this should be borne in mind when data from animal experiments are evaluated, the basic mechanisms of IgE regula-

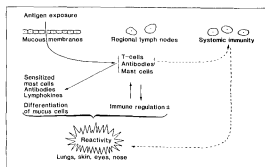


Fig. 1. Suggested steps in the development of the allergic inflammation. Antigen entering through mucous membranes induce a regional immune response, characterized by cell-mediated and humoral immunity. T-cell driven mast cell proliferation is induced. IgE-coated mast cells migrate to the shock organ, where they act as reactor cells in concert with mucous cells proliferating in response to T-cell driven immunity.

tion, particularly when no adjuvants are used for immunization, seem to be similar in man and in mice and rats. Similarly, there is some clinical evidence indicating that the new knowledge on proliferation of mast cells, basophils and mucous cells derived from animal studies is also relevant for the allergic inflammation in humans. In Fig. 1 some of the possible interplay between various components of the process is suggested. To study the complicated interaction between various components of the immune system in the induction and maintenance of the allergic inflammation, proper animal models are essential. One way to develop a relevant animal model would be to use animal strains inbred particularly for the study of respiratory anaphylaxis. Lundberg (62) has succeeded in this and after selective inbreeding for approximately 24 generations obtained two guinea pig strains, one of which has a high and the other a low responsiveness to ovalbumin-induced respiratory anaphylaxis. A comparison of various parameters of immunity in such high and low responder strains should increase our understanding of the development of allergic reactions. An ideal model of respiratory allergy should include typical symptoms like bronchospasm and mucus production after stimulation via the airways with the relevant antigen. To study the immune regulatory process animals should be sensitized in a clinically relevant fashion, i.e. by repeated administration of small doses of antigen, preferably via the airways, and after careful consideration of the possible effects of adjuvants if, for some reason, these are used. An evaluation of the relevance of a particular model should also include a comparison with allergic humans regarding the cell types in the resulting inflammatory lesions as well as the various immunoglobulin isotypes and delayed hypersensitivity reactions involved. Such animal models of documented clinical relevance would contribute enormously to the understanding of the allergic inflammation and thus provide a possibility to develop methods for the precise diagnosis and therapy of allergic diseases.

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