

Review article

Animal models of allergic and inflammatory conjunctivitis

Allergic eye diseases are complex inflammatory conditions of the conjunctiva with an increasing prevalence and incidence. The diseases are often concomitant with other allergic diseases such as allergic rhinitis, atopic dermatitis and allergic asthma. Despite the disabling and prominent symptoms of ocular allergies, they are less well studied and further insights into the molecular basics are still required. To establish new therapeutic approaches and assess immunological mechanisms, animal models of ocular allergies have been developed in the past years. The major forms of allergic ocular diseases, seasonal and perennial allergic conjunctivitis, vernal and atopic keratoconjunctivitis and giant papillary conjunctivitis, each have different pathophysiological and immunological components. In contrast to these distinct entities, the current animal models are based on the sensitization against a small number of allergens such as ovalbumin, ragweed pollen or major cat allergens and consecutive challenge. Different animal species have been used so far. Starting with guinea-pig models of allergic conjunctivitis to assess pharmacological aspects, new models including rats and mice have been developed which mimic major features of ocular allergy. The presently preferred species for the investigation of the immunological basis of the disease is represented by murine models of allergic conjunctivitis. In the future, combined ocular, nasal and aerosolic challenges with allergens may provide a model of allergy that encompasses simultaneously the target organs eye, nose and airways with conjunctivitis, rhinitis and asthma.

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Numerous recent reports have demonstrated an increase in the prevalence and incidence of allergic conditions during the past years which affect approximately 25% of the general population (1) and about US\$ 6 billion alone are estimated as direct costs of allergic rhinoconjunctivitis (2). Whereas a large amount of studies have focused on the pathophysiology of allergic rhinitis and asthma over the past years, ocular allergies are less well studied. However, allergic diseases affecting the eye are very common (3) and pediatric studies have shown that within distinct cohorts, up to 32% of allergic children have an ocular allergy as the primary manifestation of allergy (4). Allergic eye diseases such as allergic conjunctivitis commonly present with the symptoms of itchy, watery, red, sore and swollen eyes, but not pain (5).

In contrast to the large amount of experimental studies on allergic asthma and the detailed knowledge that exists on mediators of allergic airway inflammation (6–8), there are few experimental studies using models of allergic eye diseases. Detailed reviews to assess the pathophysiology and immunology of ocular allergies have been limited.

This review hopefully fills this void to provide the clinical and translational researcher in the area of ocular allergy.

Pathophysiology of ocular allergies

Before characterizing the different animal models of ocular allergy which have been established so far, it is crucial to reflect that within the area of ocular allergies, different disease entities exist and that only some of them may be mimicked by animal models. Formally, conjunctivitis may be divided into allergic and nonallergic conjunctivitis and the allergic form may also be subdivided into IgE-mediated and non-IgE-mediated allergic conjunctivitis (Fig. 1) (9). Clinically, the spectrum of conjunctivitis types can be subdivided into allergic conjunctivitis, atopic keratoconjunctivitis, vernal keratoconjunctivitis and giant papillary conjunctivitis.

In contrast to the other forms of conjunctivitis, allergic conjunctivitis is commonly based on an IgE-mediated hypersensitivity reaction. Furthermore, allergic

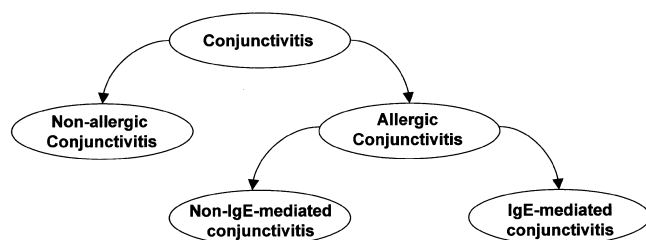


Figure 1. Nomenclature of conjunctivitis.

conjunctivitis may be divided into subgroups depending on the duration of the symptoms. Basing on the WHO document 'Allergic Rhinitis and its Impact on Asthma' (ARIA) and guidelines of the EAACI (9), allergic conjunctivitis should be divided into intermittent allergic conjunctivitis (IAC), formerly termed seasonal allergic conjunctivitis and persistent allergic conjunctivitis (PAC) formerly termed perennial allergic conjunctivitis. They display the most common forms of ocular allergic diseases caused by direct exposure of the conjunctiva to allergens. The old terms 'seasonal' and 'perennial' are not useful in zones where climatic seasons are perennial and therefore, intermittent or persistent should be used to describe these forms of conjunctivitis (9). Both forms of allergic conjunctivitis are IgE mast cell-mediated hypersensitivity reactions to aeroallergens such as ragweed or grass pollen after they come into contact with the conjunctival surface. Next to the clinical symptoms of allergic conjunctivitis that often includes a milky or pink conjunctiva with vascular congestion, elevated tear fluid and serum levels for IgE are often found. In contrast to intermittent allergic conjunctivitis, which is more common and seasonal and mainly evoked by allergens such as grass pollens or ragweed, the persistent form is considered to be a variant of IgE-mediated allergic conjunctivitis which persists through the seasons. It is often caused by dust mites or animal dander and more likely than intermittent allergic conjunctivitis associated with other forms of persistent allergies such as persistent allergic rhinitis. Further causes of persistent allergic conjunctivitis are exposures to work-related allergens that make this condition an important occupational disease.

Differences between the two forms can be found by analysis of specific IgE (10). In this respect, patients suffering from PAC in contrast to IAC have been reported to have elevated house dust-specific IgE serum levels, 89% vs 43% respectively. In addition, house dust-specific IgE levels in the tear fluid were found in 78% of PAC patients versus none of the IAC patients. Further differences can be found in the cellular infiltrate in the conjunctiva exemplified by the presence of eosinophils in 25–84% of patients with PAC and in 43% of IAC patients. A large variety of mast cell and eosinophil mediators such as major basic protein or adhesion molecules have also been identified in tear fluid and conjunctival tissues (11).

Atopic keratoconjunctivitis (AKC), in contrast to IAC and PAC, has not been clearly associated with allergens. AKC may lead to blindness and is a chronic process, usually related to a history of atopic dermatitis or allergic asthma, which commonly involves the lower tarsal conjunctiva and can involve the cornea. Clinical symptoms such as itching eyelid eczema, burning and tearing tend to be more severe than that in IAC or PAC. Histopathologically, a conjunctival inflammatory cell influx containing mast cells, eosinophils and lymphocytes can be detected and increased tissue levels of interleukin (IL)-2, IL-3, IL-4 and IL-5 were found (12, 13). IL-5 tear fluid levels in particular may be a marker for the more chronic proliferative forms of ocular allergy (14).

Another chronic form of ocular allergy is represented by the vernal keratoconjunctivitis (VKC) which has also not been clearly associated with allergens. This is a severe recurrent chronic inflammatory process with a seasonal incidence that primarily affects the upper tarsal conjunctiva. It most often occurs in prepubertal patients with a history of seasonal rhinitis, atopy or asthma (15). Clinical symptoms are severe and include an intense pruritus, photophobia and foreign-body sensations. Morphologically, VKC is characterized by Charcot–Leyden crystals within the secretions, corneal shield ulcers and transient conjunctival or limbal yellow-white 'Tantras–Horner' dots (15). In the conjunctiva, an infiltration consisting of mast cells, eosinophils, macrophages, basophils and lymphocytes can be found (16). The disease may progress to a superficial keratitis and then after degeneration of the epithelial layer into a shield ulcer. Pathophysiologically, the disease does not depend solely upon IgE-mediated processes as multiple other inflammatory mediators have been found (17, 18). Both the VKC and the AKC are in part explained by IgE-mediated reactions and may be classified as subgroups of IgE-mediated conjunctivitis (9).

The giant papillary conjunctivitis (GPC) which can be related to chronic exposure to foreign bodies such as contact lenses, has an increased incidence during the spring pollen season but has not been related to distinct allergens. The disease is clinically characterized by pruritus, tearing, ocular discomfort and hypersecretion. Prominent giant papillae can be seen commonly on upper eyelid eversion and histopathologically, an infiltration consisting of mast cells, basophils, eosinophils and lymphocytes can be found. Pathophysiologically, increased levels of IL-3, IL-4 and IL-5 (19) and IgG, IgE and IgM have been found in tear fluids of patients with GPC (20). It is generally hypothesized that GPC represents a type of reversible contact allergy to allergens which adhere to a foreign body such as a contact lens (21).

In general, ocular allergies encompass a broad spectrum starting from seasonal allergic conjunctivitis to severe types such as vernal keratoconjunctivitis. These different types share common pathophysiological findings such as mast cell infiltration but also differ to a major extend concerning clinical symptoms, prognosis and

immunological background. In contrast to this diversity, the presently known models of allergic eye diseases are limited to a selected number of species and antigens and cellular mechanisms.

Species

So far, mainly three different laboratory animal species have been used for the establishment of animal models of ocular allergy using a variety of antigens. To distinguish between the different methodological approaches, it is useful to characterize the models as IgE-mediated and non-IgE-mediated models of conjunctivitis (Table 1).

Guinea-pigs

The first major laboratory animal species to be used for the assessment of ocular allergies was the guinea-pig (Fig. 2). As the first experimental studies were largely focused on the investigation of drugs effects, this species was chosen because of its beneficial pharmacological properties. As with guinea-pig models of other allergic diseases such as allergic asthma or rhinitis, the guinea-pig model of allergic conjunctivitis proved to be a valid model to assess the anti-allergic effects of various compounds.

IgE-mediated models. Most of the published studies using animal models of allergic eye disease used active immunization protocols. In this respect, studies used the sensitization and topical challenge of guinea-pigs to normal rabbit serum to test the efficacy of compound in inhibiting conjunctival hypersensitivity (22). Using primarily ovalbumin-based protocols, a large number of well-established anti-allergic drugs were investigated: starting from compounds such as antihistamines (23), salbutamol, theophylline (24), nedocromil, cromolyn sodium (25), levocabastine (26), chlorpheniramine, ketotifen (27), benzodiazepine (28), later also compounds

such as novel prostaglandin receptor antagonists (29), leucotriene D₄ receptor antagonists (30, 31) or 5-lipoxygenase inhibitors (32) were examined.

Also, other compounds such as 2-deoxy-D-glucose, a combination of isoproterenol and diethylcarbamazine (22), or immunotherapy (33, 34) were examined.

With regard to the efficacy and its frequent use for the characterization of pharmacological effects, the guinea-pig model was also adapted to study basis mechanisms underlying allergic conjunctivitis. In this respect, insights into the role of platelet-activating factor (PAF) and PAF acetylhydrolase (AH) in regulating the conjunctival vascular permeability in allergic conjunctivitis were gained (35) and the relationship between the release of histamine and the production of prostaglandin I₂ (PGD₂) was examined (36).

Next to the various guinea-pig pharmacological studies (37–39) that targeted mast cell mediators, the role of eosinophils in allergic conjunctivitis was also assessed using the same animal model: to determine the contribution of the very late activation antigen-4 (VLA-4) molecule to eosinophil infiltration within allergic conjunctivitis, effects of a monoclonal antibody against VLA-4 were examined and it was shown that VLA-4 plays a critical role in eosinophilic infiltration (40). Cell profile studies also underlined the importance of eosinophils and basophils (41).

With regard to numerous studies from the field of neuroimmunology of allergic airway inflammation, which demonstrated that guinea-pigs are currently the best small laboratory animal species to study the contribution of the autonomous nervous system to the allergic reaction (42, 43), guinea-pig models were also used to study the role of neuromediators such as substance P and it was shown that substance P is a mediator of allergic conjunctivitis, and that the conjunctival hyperpermeability induced by substance P occurs through NK1 receptors on the blood vessels, rather than by the direct action on the conjunctival mast cells during allergic conjunctival

Table 1. Experimental models of inflammatory and allergic conjunctivitis

Species	IgE/Non-IgE	Antigen	Research target	Examples
Guinea-pig	IgE	Ovalbumin	Pharmacology, pathophysiology, neurophysiology	(37)
		Ragweed	Pharmacology, pathophysiology, neurophysiology	(50)
	Non-IgE	Japanese cedar pollen	Pharmacology, pathophysiology, neurophysiology	(51, 52)
		Haptens	Pharmacology, pathophysiology, neurophysiology	(53)
		Compound 48/80	Pharmacology, pathophysiology, neurophysiology	(47)
Rat	IgE	Ovalbumin	Pharmacology, pathophysiology, immunology	(61)
		Ragweed	Pharmacology, pathophysiology, immunology	(78, 79)
	Non-IgE	Haptens	Pharmacology, pathophysiology, immunology	(70)
		Compound 48/80	Pharmacology, pathophysiology, immunology	(85)
Mouse	IgE	Ovalbumin	Immunology, genetics	(110)
		Ragweed	Immunology, genetics	(99)
		Cat dander antigens	Immunology, genetics	(106, 107)
	Non-IgE	–	–	–

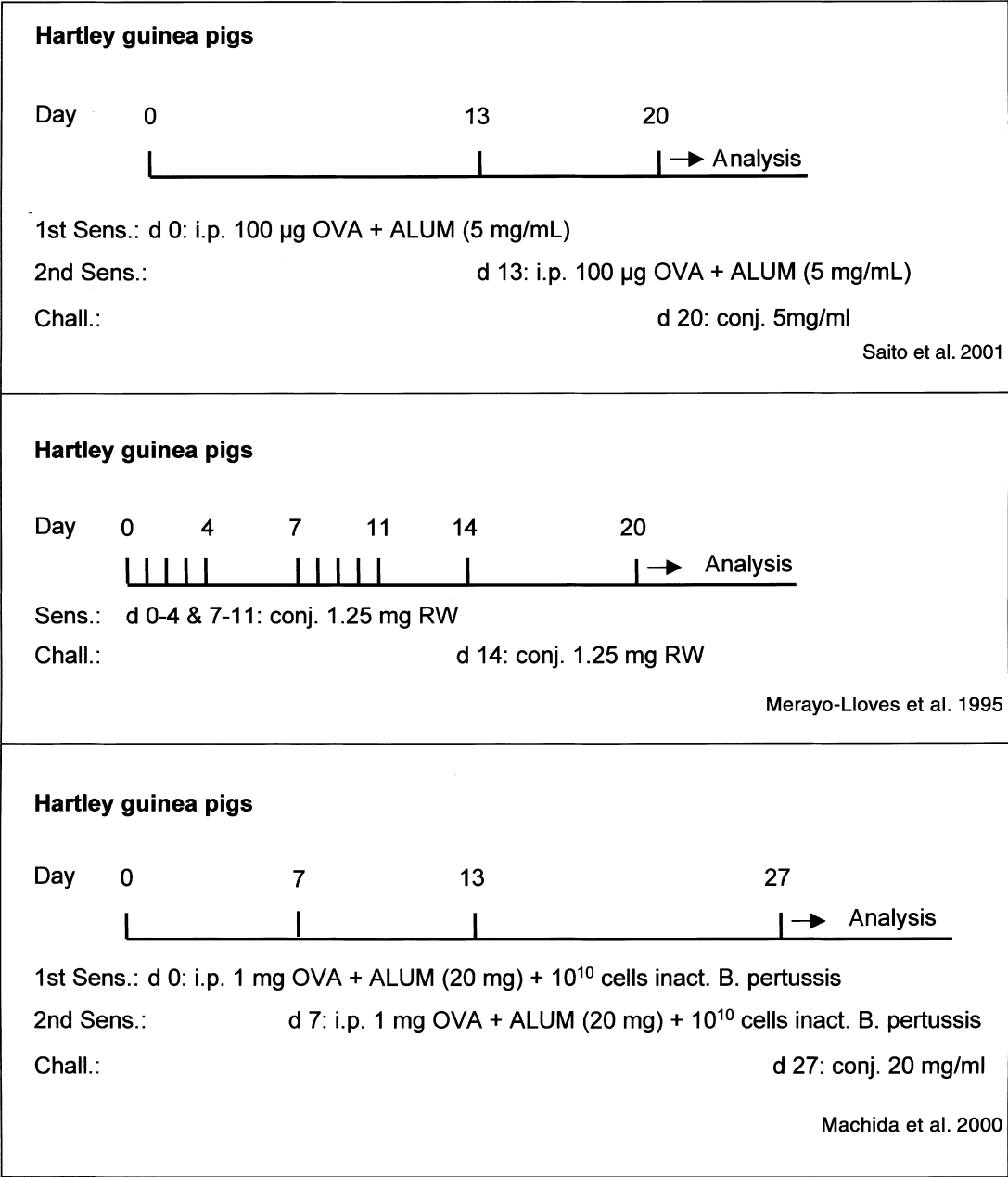


Figure 2. Guinea-pig models of allergic conjunctivitis. Different guinea-pig models of allergic conjunctivitis have been established so far using different antigens such as ovalbumin (OVA) or ragweed (RW) and different time periods of allergen sensitization and challenge. ALUM, aluminium hydroxide; chall., allergen challenge; conj., topical administration to the conjunctivae; i.p., intraperitoneal; sens., sensitization.

reactions (44). Also, the role of nitric oxide, a previously characterized mediator of allergic airway inflammation (45, 46) was studied in a guinea-pig of allergic conjunctivitis and a series of experiments demonstrated a major role of this gaseous molecule in regulating the vascular permeability and stimulating prostaglandin E2 production (47, 48).

As with other species, some guinea-pig strains may be more subtle to allergen sensitization and challenge. An

important study investigated inbred guinea-pigs selected for high (IMM/S) and low (IMM/R) responsiveness to ovalbumin for atopic immune responses of their conjunctival mucosa. The high-response IMM/S animals sensitized either by inhalation of ovalbumin, or by instillation of antigen into the conjunctival sac, developed an acute allergic ocular inflammatory response to conjunctival challenge with a minimum of 1 µg of ovalbumin dropped repeatedly into the conjunctival

sac being sufficient for both ocular and systemic sensitization of the animals.

In low-responding IMM/R guinea-pigs, ocular hypersensitivity was not achieved by ovalbumin-inhalation, but only after topical administration of the antigen did some of the low-respond strains develop ocular anaphylactic responses of the same intensity as observed in high-responding IMM/S guinea-pigs. It was also shown that both IMM/S and IMM/R animals could be sensitized passively by intraperitoneal injection of guinea-pig sera containing homocytotropic ovalbumin antibodies, but topical conjunctival administration of such ovalbumin antibody sera did not lead to sensitization (49).

While most of the guinea-pig studies were performed using ovalbumin as antigen, two other allergens have evolved recently as effective sensitizers for guinea-pig models: Next to topical conjunctival and nasal sensitization and challenge to ragweed which proved to mimic human hay fever conjunctivitis more naturally than other animal models (50), Japanese cedar pollen has recently been described as a sensitizing allergen in the guinea-pig model of allergic conjunctivitis (51, 52).

Non-IgE-mediated models. Next to IgE-mediated models of allergic conjunctivitis, also non-IgE-mediated guinea-pig models of conjunctivitis have been used. The most common molecules used were haptens. In one study, conjunctivae of guinea-pigs were sensitized with IgG1 antisera to dinitrophenol carrier and challenged topically with hapten. Assessing periorbital swelling, conjunctival oedema and redness, and tear cytology, an early-phase reaction (EPR) peaking at 0.5 h and a late-phase reaction (LPR) peaking at 5–7 h were found. The periorbital swelling was minimal 4 h after appearance of the EPR and conjunctival oedema and redness increased in both the early and late phases. Tear cytology displayed an abnormal increase in neutrophils and eosinophils in the later time periods (53). Similar protocols with non-IgE-mediated active immunizations were also reported in other studies (39, 54). Other non-IgE-mediated protocols use compound 48/80 to induce conjunctivitis (44, 47).

Inflammatory models. In contrast to these sensitization and challenge protocols, contact lens-associated GPC was mimicked in guinea-pigs by insertion of a sterile needle directly into the conjunctiva of unsensitized animals resulting in high numbers of neutrophils in the traumatized tissues (41). However, these morphological changes do clearly not represent the true histopathological picture found in human GPC (55, 56).

In summary, the guinea-pig has proved to be a valid species for the assessment of anti-allergic effects of established and newly developed compounds. This species also seems to be helpful when studying the role of the nervous system inside the development and progression of allergic conjunctivitis. However, with regard to the

investigation of immunological mechanisms underlying the allergic eye disease, the guinea-pig models appear to be clearly limited as there are only restricted numbers of genetic and immunological data of the existing species and different strains. In addition, techniques such as gene depletion (57), gene arrays (58) or analysis of mRNA on a single cell level (59) are currently not possible because of missing genomic data.

Rats

The second major species used to study ocular allergies is displayed by laboratory rats (Fig. 3). All major laboratory rat strains have been used to establish allergic conjunctivitis models. Next to Brown–Norway rats (60, 61), which are also the preferred strain for the induction of allergic airway inflammation (45, 46), Lewis rats (62, 63), Wistar rats (64, 65) and Sprague–Dawley rats (66, 67) have been assessed for the development of allergic conjunctivitis. The different models all based upon the use of either ovalbumin (68), compound 48/80 (69), dinitrophenyl (DNP)-*Ascaris* immunization and di-DNP-lysine challenge (70) or passive sensitization protocols (71).

IgE-mediated models. IgE-mediated models of rat conjunctivitis used either direct or indirect sensitization and a large number of questions were answered with this species. Using ovalbumin-based protocols, it was shown that the rat models of allergic eye disease exhibit similar pharmacological properties as guinea-pig models concerning the effects of drugs such as lodoxamide, nedocromil, sodium cromoglycate, levocabastine, cimetidine, diclofenac sodium: (65, 72), or lodoxamide (73).

Further studies characterized the effects of immunomodulation in the rat model of allergic conjunctivitis. The role of interferon (IFN)- γ treatment was assessed in a Brown–Norway model of ovalbumin-induced allergic conjunctivitis (61). To investigate if the inhibitory effects of IFN- γ are exerted during the induction or effector phase of allergic conjunctivitis, IFN- γ (10 μ g per time, total 70 μ g) injected intraperitoneally every other day from the day of immunization was compared with ovalbumin-specific T cells transferred into syngeneic rats or to IFN- γ (10 μ g per time, total 50 μ g) or phosphate-buffered saline (PBS) was injected each day after the transfer until the induction of conjunctivitis 4 days later. Also, the role of IFN- γ in the effector phase was assessed by the injection of an anti-IFN γ monoclonal antibody (3 mg per time) on days 3 and 4. It was demonstrated that IFN- γ is a suppressive cytokine for the development of allergic conjunctivitis and that it exerts its suppressive effect during the induction phase (61).

Other studies focused on the effects of immunosuppressive compounds in rat models of allergic conjunctivitis. The possible inhibition of allergic conjunctivitis by topical application of FK506, which is an immunosuppressive agent that mainly targets T cells, was studied

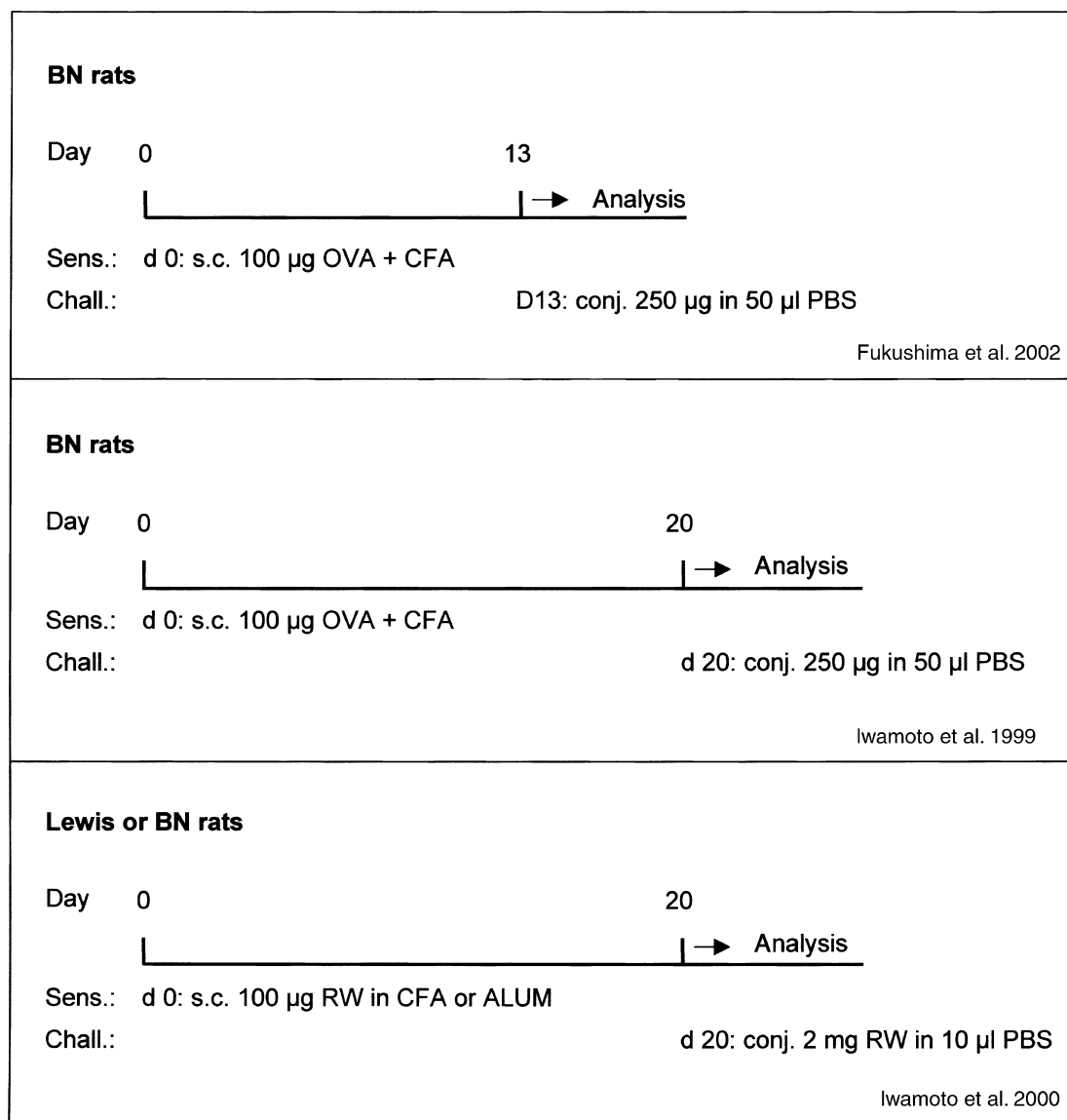


Figure 3. Rat models of ocular allergy. A variety of rat models of allergic conjunctivitis have been described using different modes of exposition and antigens. ALUM, aluminium hydroxide; CFA, complete Freund's adjuvant; chall., allergen challenge; conj., topical administration to the conjunctivae; OVA, ovalbumin; PBS, phosphate-buffered saline; RW, ragweed; s.c., subcutaneously; sens., sensitization.

in a Brown-Norway ovalbumin model, and it was shown that FK506 treatment suppressed infiltration of both lymphocytes and eosinophils in the conjunctiva after both active and passive immunization. Regarding changes in the conjunctival cytokine expression, a significant difference was noted for IL-4, and it was suggested that topical FK506 treatment may suppress allergic conjunctivitis by inhibition of conjunctival IL-4 production (74). Other studies on the effects of this compound demonstrated that within a Lewis rat model of ovalbumin-induced conjunctivitis, systemic treatment with 200 µg of FK506 inhibits the development of conjunctivitis both in the induction and effector phase.

whereas topical administration was not as effective as systemic administration (75).

As the early phase reaction of conjunctivitis has been studied extensively both in humans and animals, other studies in rat models focused on the role of the cellular infiltration which is the main feature of the late phase reaction and indicated, that the cellular immunity plays a major role in the development of allergic conjunctivitis, both in the induction and effector phases (62, 63).

As the genetic background determines the nature of immune responses within rat models of allergic conjunctivitis, and the choice of susceptible strains is of major importance for the biological significance of the experi-

mental model, studies were conducted to identify differences in rat strains towards allergen susceptibility. In one study, the development of allergic conjunctivitis in Lewis rats was compared with Brown–Norway rats, which were previously characterized as a suitable model for the study of allergic airway inflammation (45, 46). In this study, also the influence of complete Freund's adjuvant (CFA) or aluminum hydroxide [Al(OH)₃] was tested. Lewis and Brown–Norway rats were immunized once with 100 µg of ovalbumin in CFA or Al(OH)₃ and 3 weeks later animals were challenged topically with ovalbumin. Conjunctivitis, ovalbumin-specific IgG, and cellular immunity were induced in Lewis rats by using CFA or [Al(OH)₃], whereas IgE was not produced by either adjuvant. In contrast, IgE was produced in the Brown–Norway rats using CFA or [Al(OH)₃], whereas cellular immunity was evoked only when CFA was used. Less cellular infiltration as well as cellular proliferation was detected in Brown–Norway rats immunized with Al(OH)₃. In both strains, Al(OH)₃ induced a higher IgG1/IgG2a ratio than CFA. IL-4 was only detected in Brown–Norway rats. It was concluded that the severity of allergic conjunctivitis as evaluated by cellular infiltration is dependent on ovalbumin-specific cellular immunity in both models and that the genetic background is more important than adjuvants (76). In a second study, Fischer rats which were reported to be less susceptible to experimental autoimmune uveoretinitis than Lewis rats, were compared with each other concerning the development of allergic conjunctivitis (77). It was shown that Fischer rats developed a milder allergic conjunctivitis when compared with Lewis rats. However, cellular proliferative responses, IFN-γ production of culture supernatant and serum IgG specific for ovalbumin did not differ largely between the both strains. These studies suggested that factors unrelated to the examined immunological parameters may be related to the susceptibility and clinical picture of allergic conjunctivitis (77).

Next to ovalbumin, other allergens such as ragweed pollen were used to induce experimental allergic conjunctivitis (78, 79).

Non-IgE-mediated models. Other studies used haptens such as DNP as a model compound to induce ocular anaphylaxis. Rats immunized with DNP-*Ascaris* were skin-tested with DNP-bovine serum albumin (DNP-BSA) and Evans blue and challenged topically with varying amounts of di-DNP-lysine. The degree of clinical conjunctival edema was assessed, and ocular tissues were evaluated histologically for neutrophil, eosinophil, lymphocyte and atypical epithelial cell levels, which were increased in antigen-treated animals (70). After its establishment, this model was repeatedly used (80–82) and also modified using other species as well as other sensitization and challenge protocols (83, 84). Other non-IgE-mediated protocols to induce conjunctivitis use compound 48/80 (85, 86). Basing on the topical application of compound

48/80 to the surface of the rat eye in doses ranging from 50 to 1000 µg, graded oedema of the conjunctiva and swelling of the lid was reproducibly observed. On histological examination, 50 µg compound 48/80 produced no changes distinguishable from those in PBS-treated controls, while 150 µg produced mild alterations, and 250, 500 and 1000 µg of compound 48/80 produced a marked increase in degranulated mast cell numbers and a mild influx of neutrophils (69). Evaluating the time course of the response to 250 and 1000 µg of compound 48/80 over a 72-h period it was reported that both doses elicited epithelial damage and a mild reduction in the number of mast cell was seen at 6 h in rats receiving 250 or 1000 µg. This reduction persisted to 72 h in rats receiving 1000 µg. The number of neutrophils was increased at 1 and 6 h, and at 1, 6 and 24 h in eyes treated with 250 and 1000 µg compound 48/80, respectively (69). It was summarized that the clinical and histological changes induced by application of 250 µg compound 48/80 resemble those seen human allergic conjunctivitis suggesting that a model of ocular anaphylaxis based on the topical application compound 48/80 can be clinically relevant and experimentally practical (69).

In summary, rat models of experimental allergic conjunctivitis display a bridge between the classical guinea-pig models which were used to assess the effects of pharmacological active compounds and recently developed murine models. Future studies involving modern techniques such as microdissection-assisted single-cell reverse transcriptase polymerase chain reaction (RT-PCR) (59), RNA interference (RNAi) (87) or cloning techniques (88) and classical methods such as immunohistochemistry (89, 90), *in situ* hybridization (91–93), Northern blotting (94, 95), Western blotting (96) or electron microscopy (97) will help to further elucidate the pathophysiology of allergic conjunctivitis within experimental rat models.

Mice

Murine models of allergic conjunctivitis were established only after the development, characterization and acceptance of guinea-pig and rat models (Fig. 4). The great advantage of the murine model is based on the availability of genomic sequences and techniques such as RT-PCR were used to examine the murine model (98) and proved its pharmacological integrity by assessing the effects of common anti-allergic drugs (98).

IgE-mediated models. The first major murine model of allergic conjunctivitis to airborne allergens was established in 1996 using SWR/J mice which were exposed to ragweed by topical contact with the conjunctival and nasal mucosae (99). The development of allergic conjunctivitis was assessed by examination of clinical signs, serum IgE levels, and histological findings and it was shown that SWR/J mice exposed to ragweed developed clinical signs

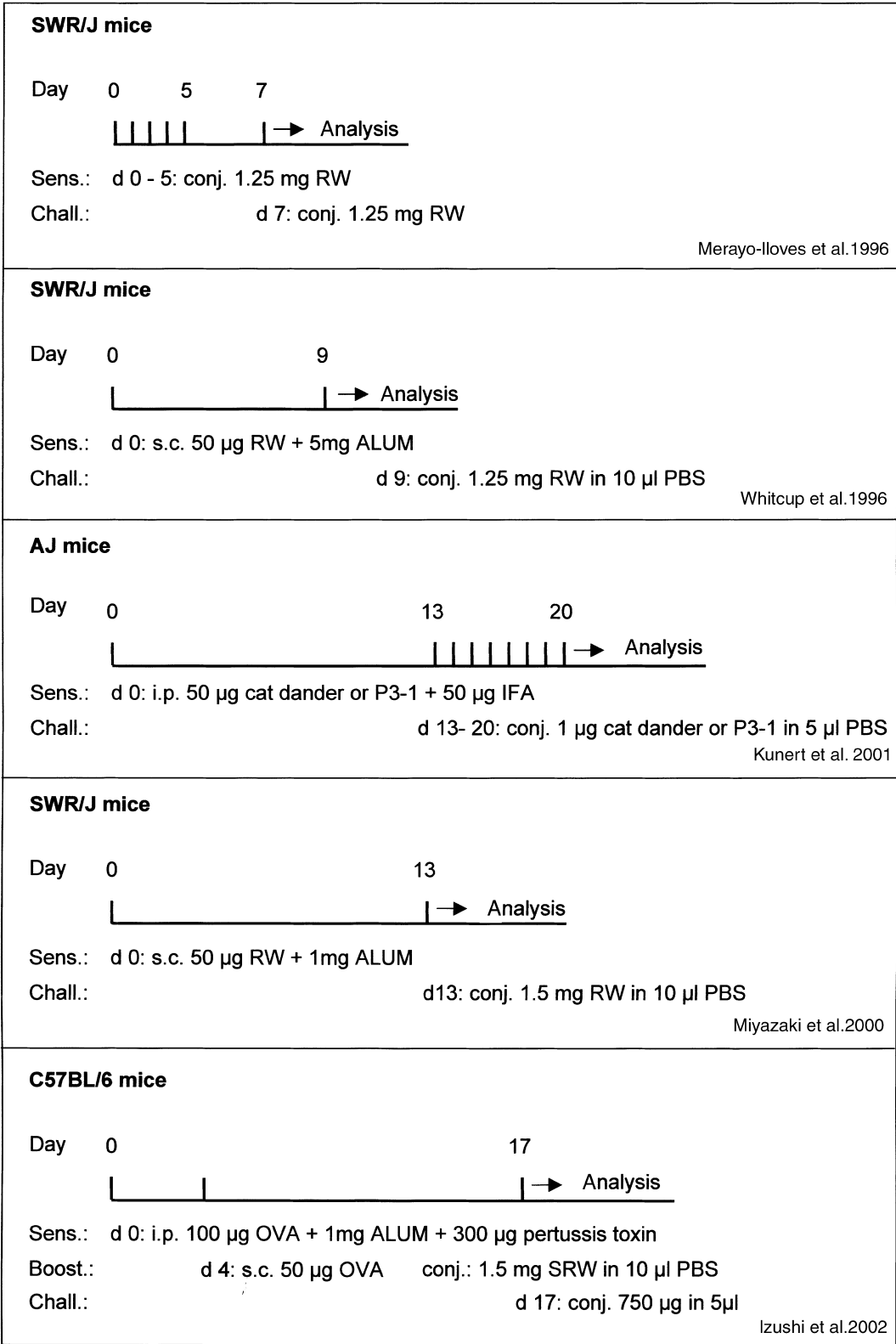


Figure 4. Murine models of experimental allergic conjunctivitis. Different strains, antigens and sensitization – challenge protocols have been used to establish murine models of allergic conjunctivitis recently. ALUM, aluminium hydroxide; CFA, complete Freund’s adjuvant; chall., allergen challenge; conj., topical administration to the conjunctivae; i.p., intraperitoneal; OVA, ovalbumin; PBS, phosphate-buffered saline; RW, ragweed; s.c., subcutaneously; sens., sensitization.

of allergic conjunctivitis and exhibited specific anti-ragweed IgE levels. Nedocromil sodium was efficient in diminishing signs of allergic conjunctivitis and treated animals showed decreased levels of ragweed-specific IgE, as well as a reduction in conjunctival eosinophil infiltration, mast cells numbers, cytokine release and fewer clinical signs of allergy (99).

Other centres also established modified murine models of allergic conjunctivitis using ragweed as allergen (100) and used the model to assess immunological mechanisms underlying ocular allergy. In this respect, the ability of immunostimulatory DNA sequences (ISS or CpG motifs) to modulate allergic responses was examined. It was shown that both the systemic and mucosal administration of ISS oligonucleotide (ISS-ODN) after ragweed sensitization inhibited both the immediate hypersensitivity response and the late-phase cellular infiltration and induced a ragweed-specific Th1 response (101, 102). Also, gene-depleted mice were screened for the participation of different cytokines in the development of ragweed-induced allergic conjunctivitis: the role of IL-4, IL-12 and IFN- γ in the early and late phases of ocular allergy was assessed using gene-depleted mice and neutralizing antibodies. In these studies, it was shown that IL-12, although described as Th1-inducing cytokine, is crucial for the development of the late phase of allergic conjunctivitis and that IFN- γ is a limiting factor in the late phase of ocular allergy (103).

Other studies have focused on the anti-inflammatory role of the soluble extracellular form of P-selectin glycoprotein ligand 1 (sPSGL-1) and demonstrated that the antagonistic intervention of cellular interactions through the blockade of selectin-dependent leucocyte adhesion may lead to new therapeutic options to modulate inflammatory responses (104). Also, the cell adhesion molecules ICAM-1 and LFA-1 were shown to play a major role in the development of ragweed-induced murine allergic conjunctivitis, since antibody treatment against ICAM-1 and LFA-1 significantly inhibited the development of clinical and histological conjunctivitis signs (105).

A recently developed new model of murine allergic conjunctivitis used molecular distinct cat dander antigens (106, 107). Apart from demonstrating the significance of IL-1 for inflammatory changes in allergic conjunctivitis by treatment with an IL-1receptor antagonist (106), this model was also used to enlarge the panel of functional parameters to characterize allergic conjunctivitis on a molecular level. As it was known that mucin proteins do not only play a role in the pathophysiology of airway and gastrointestinal diseases (108, 109) but also in ocular allergy, an assay was developed to investigate MUC4 and MUC5AC mRNA levels in experimental allergic conjunctivitis. Next to ragweed or cat dander antigens, also the conventional ovalbumin was used as antigen to induce murine allergic conjunctivitis (110).

Non-IgE-mediated models. In contrast to the widespread use of non-IgE-mediated models of airway inflammation (111) or dermatitis (112), similar murine models have not been established to simulate conjunctivitis so far. However, as non-IgE-mediated conjunctivitis types are of major clinical importance because of their severity, future studies should establish murine models of non-IgE-mediated conjunctivitis to target the molecular basis of these conjunctivitis forms.

As modern molecular biology techniques enable us to study disease mechanisms on the transcriptional, translational and functional level using genomics, proteomics, morphological and classical physiological approaches, the few presently established murine models of allergic allergy apparently represent a promising tool to assess the molecular mechanisms of ocular allergies. However, in some instances such as defining the role of certain neuromodulators, classical guinea-pig models of allergic conjunctivitis may offer a closer replica to the human condition.

Conclusions

In contrast to different forms of allergic ocular diseases such as intermittent and persistent allergic conjunctivitis, vernal and atopic keratoconjunctivitis and giant papillary conjunctivitis, which represent pathophysiological distinct entities, currently available animal models of allergic eye diseases are based on active or passive immunization against a small number of allergens including ovalbumin, ragweed pollen, cat allergens or haptens. Therefore, all of these models more or less represent experimental approaches to mimic acute forms of allergic conjunctivitis and are therefore limited concerning clinical significance. However, the pathophysiology of experimental conjunctivitis with regard to the induction and effector phases of allergic conjunctivitis can be studied efficiently and underlying molecular mechanisms and potential therapeutic approaches can be revealed. Whereas guinea-pig models of allergic eye disease were the first to be established because of their advantages of the assessment of drug effects, rat and murine models were later developed. With regard to the present focus on the investigation of molecular and genomic mechanisms of allergy, mice are the presently preferred species for models of ocular allergy. In future, simultaneous ocular, nasal and aerosolic challenges with allergens may provide a combined model of allergy which encompasses the primary target organs: eye, nose and airways.

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References

1. NIMMAGADDA SR, EVANS R, III. Allergy: etiology and epidemiology. *Pediatr Rev* 1999;**20**:111–115.
2. RAY NF, BARANIUK JN, THAMER M, RINEHART CS, GERGEN PJ, KALINER M et al. Direct expenditures for the treatment of allergic rhinoconjunctivitis in 1996, including the contributions of related airway illnesses. *J Allergy Clin Immunol* 1999;**103**:401–407.
3. DINOWITZ M, RESCIGNO R, BIELORY L. Ocular allergic diseases: differential diagnosis, examination techniques, and testing. *Clin Allergy Immunol* 2000;**15**:127–150.
4. BIELORY L. Allergic and immunologic disorders of the eye. Part II: ocular allergy. *J Allergy Clin Immunol* 2000;**106**:1019–1032.
5. JUNIPER EF, GUYATT GH, DOLOVICH J. Assessment of quality of life in adolescents with allergic rhinoconjunctivitis: development and testing of a questionnaire for clinical trials. *J Allergy Clin Immunol* 1994;**93**:413–423.
6. FISCHER A, FOLKERTS G, GEPPETTI P, GRONEBERG DA. Mediators of asthma: nitric oxide. *Pulm Pharmacol Ther* 2002;**15**:73–81.
7. BARNES PJ. Histamine and serotonin. *Pulm Pharmacol Ther* 2001;**14**:329–339.
8. SPRINGER J, GEPPETTI P, FISCHER A, GRONEBERG DA. Calcitonin gene-related peptide as inflammatory mediator. *Pulm Pharmacol Ther* 2003;**16**:121–130.
9. JOHANSSON SG, HOURIHANE JO, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
10. BOURCIER T, MOLDOVAN M, GOLDSCHILD M, BECQUET F, BRIGNOLE F, GOGUEL A et al. Value of lacrymal IgE determination and conjunctival cytology in the diagnosis of chronic conjunctivitis. *J Fr Ophtalmol* 1998;**21**:209–213.
11. CHURCH MK, MCGILL JI. Human ocular mast cells. *Curr Opin Allergy Clin Immunol* 2002;**2**:419–422.
12. AVUNDUK AM, AVUNDUK MC, DAYANIR V, TEKELIOGLU Y, DAYIOGLU YS. A flow cytometric study about the immunopathology of vernal keratoconjunctivitis. *J Allergy Clin Immunol* 1998;**101**:821–824.
13. SRIVASTAVA A, SUR S, TROCME SD. The role of eosinophils in ocular allergy. *Int Ophthalmol Clin* 2003;**43**:9–25.
14. UCHIO E, ONO SY, IKEZAWA Z, OHNO S. Tear levels of interferon-gamma, interleukin (IL)-2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis. *Clin Exp Allergy* 2000;**30**:103–109.
15. LEONARDI A, SECCHI AG. Vernal keratoconjunctivitis. *Int Ophthalmol Clin* 2003;**43**:41–58.
16. IRANI AM, BUTRUS SI, TABBARA KF, SCHWARTZ LB. Human conjunctival mast cells: distribution of MCT and MCTC in vernal conjunctivitis and giant papillary conjunctivitis. *J Allergy Clin Immunol* 1990;**86**:34–40.
17. ABU EL-ASRAR AM, STRUYF S, VAN DAMME J, GEBOES K. Role of chemokines in vernal keratoconjunctivitis. *Int Ophthalmol Clin* 2003;**43**:33–39.
18. TABBARA KF. Immunopathogenesis of chronic allergic conjunctivitis. *Int Ophthalmol Clin* 2003;**43**:1–7.
19. METZ DP, HINGORANI M, CALDER VL, BUCKLEY RJ, LIGHTMAN SL. T-cell cytokines in chronic allergic eye disease. *J Allergy Clin Immunol* 1997;**100**:817–824.
20. DONSHIK PC, BALLOW M. Tear immunoglobulins in giant papillary conjunctivitis induced by contact lenses. *Am J Ophthalmol* 1983;**96**:460–466.
21. FONACIER L, LUCHS J, UDELL I. Ocular allergies. *Curr Allergy Asthma Rep* 2001;**1**:389–396.
22. STOCK EL, DWYER RS, JONES BR, WATERS JA. Pharmacologic inhibition of immediate hypersensitivity in the guinea pig conjunctiva. *J Ocul Pharmacol* 1985;**1**:183–187.
23. YANNI JM, STEPHENS DJ, MILLER ST, WEIMER LK, GRAFF G, PARNELL D et al. The in vitro and in vivo ocular pharmacology of olopatadine (AL-4943A), an effective anti-allergic/antihistaminic agent. *J Ocul Pharmacol Ther* 1996;**12**:389–400.
24. GAUTHERON P, SUGRUE MF. The ability of salbutamol and theophylline to suppress immediate allergic conjunctivitis in the guinea pig. *Graefes Arch Clin Exp Ophthalmol* 1987;**225**:331–334.
25. CALONGE M, MONTERO JA, HERRERAS JM, RAMON JJ, PASTOR JC. Efficacy of nedocromil sodium and cromolyn sodium in an experimental model of ocular allergy. *Ann Allergy Asthma Immunol* 1996;**77**:124–130.
26. KAMEI C, IZUSHI K, TASAKA K. Inhibitory effect of levocabastine on experimental allergic conjunctivitis in guinea pigs. *J Pharmacobiodyn* 1991;**14**:467–473.
27. KAMEI C, IZUSHI K, NAKAMURA S. Effects of certain antiallergic drugs on experimental conjunctivitis in guinea pigs. *Biol Pharm Bull* 1995;**18**:1518–1521.
28. KAWATA E, MORI J, ISHIZAKI M. Effect of benzodiazepine on antigen-induced eosinophil infiltration into guinea pig conjunctiva. *Arerugi* 1996;**45**:478–484.
29. ARIMURA A, YASUI K, KISHINO J, ASANUMA F, HASEGAWA H, KAKUDO S et al. Prevention of allergic inflammation by a novel prostaglandin receptor antagonist, S-5751. *J Pharmacol Exp Ther* 2001;**298**:411–419.
30. CHAN CC, DAGENAIS F, FIRBY P, FOSTER A, FORD-HUTCHINSON AW. Immediate hypersensitivity reactions in the guinea-pig conjunctiva: studies with a second-generation leukotriene D4 receptor antagonist, MK-571. *Can J Physiol Pharmacol* 1989;**67**:845–850.
31. GARCEAU D, FORD-HUTCHINSON AW. The role of leukotriene D4 as a mediator of allergic conjunctivitis in the guinea-pig. *Eur J Pharmacol* 1987;**134**:285–292.
32. GARCEAU D, FORD-HUTCHINSON AW, CHARLSON S. 5-Lipoxygenase inhibitors and allergic conjunctivitis reactions in the guinea-pig. *Eur J Pharmacol* 1987;**143**:1–7.
33. SAITO K, SHOJI J, INADA N, IWASAKI Y, SAWA M. Immunosuppressive effect of cholera toxin B on allergic conjunctivitis model in the guinea pig. *Jpn J Ophthalmol* 2000;**44**:189–190.
34. MACHIDA H, NAKAGAMI T, WATANABE I. Local ocular immunotherapy for experimental allergic conjunctivitis. *Jpn J Ophthalmol* 2000;**44**:634–638.
35. KATO M, MANO H, OTA A, KONOMI K, NAKATA K. Platelet activating factor degradation in tear fluid from guinea pigs with allergic conjunctivitis. *J Ocul Pharmacol Ther* 2001;**17**:83–91.
36. HELLEBOID L, KHATAMI M, WEI ZG, ROCKEY JH. Histamine and prostacyclin. Primary and secondary release in allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 1991;**32**:2281–2289.
37. COOK EB, STAHL JL, BARNEY NP, GRAZIANO FM. Ocular mast cells. Characterization in normal and disease states. *Clin Rev Allergy Immunol* 2001;**20**:243–268.
38. GRAZIANO FM, STAHL JL, COOK EB, BARNEY NP. Conjunctival mast cells in ocular allergic disease. *Allergy Asthma Proc* 2001;**22**:121–126.
39. LEONARDI A, SECCHI AG, BRIGGS R, ALLANSMITH MR. Conjunctival mast cells and the allergic late phase reaction. *Ophthalmic Res* 1992;**24**:234–242.

40. EBIHARA N, YOKOYAMA T, KIMURA T, NAKAYASU K, OKUMURA K, KANAI A et al. Anti VLA-4 monoclonal antibody inhibits eosinophil infiltration in allergic conjunctivitis model of guinea pig. *Curr Eye Res* 1999;**19**:20–25.
41. HANN LE, CORNELL-BELL AH, MARTEN-ELLIS C, ALLANSMITH MR. Conjunctival basophil hypersensitivity lesions in guinea pigs. Analysis of upper tarsal epithelium. *Invest Ophthalmol Vis Sci* 1986;**27**:1255–1260.
42. GRONEBERG DA, FISCHER A. Endogenous opioids as mediators of asthma. *Pulm Pharmacol Ther* 2001;**14**:383–389.
43. GRONEBERG DA, SPRINGER J, FISCHER A. Vasoactive intestinal polypeptide as mediator of asthma. *Pulm Pharmacol Ther* 2001;**14**:391–401.
44. YAMAJI M, TAKADA M, FUJIWARA R, OHISHI H, IZUSHI K, SUGIMOTO Y et al. Role of substance P in experimental allergic conjunctivitis in guinea pigs. *Methods Find Exp Clin Pharmacol* 1997;**19**:637–643.
45. EYNOTT PR, GRONEBERG DA, CARAMORI G, ADCOCK IM, DONNELLY LE, KHARITONOV S et al. Role of nitric oxide in allergic inflammation and bronchial hyperresponsiveness. *Eur J Pharmacol* 2002;**452**:123–133.
46. EYNOTT PR, PAAVOLAINEN N, GRONEBERG DA, NOBLE A, SALMON M, NATH P et al. Role of nitric oxide in chronic allergen-induced airway cell proliferation and inflammation. *J Pharmacol Exp Ther* 2003;**304**:22–29.
47. MEIJER F, RUIJTER JM, VAN DELFT JL, VAN HAERINGEN NJ. Nitric oxide induces vascular permeability changes in the guinea pig conjunctiva. *Eur J Pharmacol* 1995;**284**:61–67.
48. MEIJER F, TAK C, van HAERINGEN NJ, KIJLSTRA A. Interaction between nitric oxide and prostaglandin synthesis in the acute phase of allergic conjunctivitis. *Prostaglandins* 1996;**52**:431–446.
49. LUNDBERG L, BERTELSEN C, ZAVARO A, SAMRA Z, SOMPOLINSKY D. Experimental allergic conjunctivitis in inbred guinea pig strains with high respectively low bronchial allergic reactivity. *Allergy* 1987;**42**:262–271.
50. MERAYO-LLOVES J, CALONGE M, FOSTER CS. Experimental model of allergic conjunctivitis to ragweed in guinea pig. *Curr Eye Res* 1995;**14**:487–494.
51. TAKADA M, YAMADA T, NAKAHARA H, SUGIMOTO Y, IZUSHI K, KAMEI C. Experimental allergic conjunctivitis in guinea pigs induced by Japanese cedar pollen. *Biol Pharm Bull* 2000;**23**:566–569.
52. YASUDA M, KATO M, NABE T, NAKATA K, KOHNO S. An experimental allergic conjunctivitis induced by topical and repetitive applications of Japanese cedar pollens in guinea pigs. *Inflamm Res* 1999;**48**:325–336.
53. SAIGA T, BRIGGS RM, ALLANSMITH MR. Clinical and cytologic aspects of ocular late-phase reaction in the guinea pig. *Ophthalmic Res* 1992;**24**:45–50.
54. LEONARDI A, BRIGGS RM, SECCHI AG, ALLANSMITH MR. Double hapten challenge in guinea pigs undergoing an ocular late-phase reaction. *Ophthalmic Res* 1991;**23**:187–195.
55. KATELARIS CH. Giant papillary conjunctivitis – a review. *Acta Ophthalmol Scand Suppl* 1999:17–20.
56. CHOI TH, KO MK, CHOE JK. Increased numbers of Langerhans cell and expression of HLA-Dr antigen in the giant papilla of patients with giant papillary conjunctivitis. *Korean J Ophthalmol* 1996;**10**:18–23.
57. RUBIO-ALIAGA I, FREY I, BOLL M, GRONEBERG DA, EICHINGER HM, BALLING R et al. Targeted disruption of the peptide transporter Pept2 gene in mice defines its physiological role in the kidney. *Mol Cell Biol* 2003;**23**:3247–3252.
58. GRONEBERG DA, WELKER P, FISCHER TC, DINH QT, GRUTZKAU A, PEISER C et al. Down-regulation of vasoactive intestinal polypeptide receptor expression in atopic dermatitis. *J Allergy Clin Immunol* 2003;**111**:1099–1105.
59. PEISER C, SPRINGER J, GRONEBERG DA, MCGREGOR GP, FISCHER A, LANG RE. Leptin receptor expression in nodose ganglion cells projecting to the rat gastric fundus. *Neurosci Lett* 2002;**320**:41–44.
60. MUSOH K, NAKAMURA N, NAGAI H. Effects of an amphoteric antiallergic agent, HSR-609, on antigen-induced late phase nasal eosinophilia in brown Norway rats. *Pharmacology* 2000;**61**:230–237.
61. FUKUSHIMA A, FUKATA K, OZAKI A, TAKATA M, KURODA N, ENZAN H et al. Exertion of the suppressive effects of IFN-gamma on experimental immune mediated blepharoconjunctivitis in Brown Norway rats during the induction phase but not the effector phase. *Br J Ophthalmol* 2002;**86**:1166–1171.
62. FUKUSHIMA A, YOSHIDA H, IWAMOTO H, YOSHIDA O, UENO H. The role of cellular immunity both in the induction and effector phases of experimental allergic blepharoconjunctivitis (EAC) in rats. *Exp Eye Res* 1997;**65**:631–637.
63. FUKUSHIMA A, NISHINO K, YOSHIDA O, UENO H. Characterization of the immunopathogenic responses to ovalbumin peptide 323–339 in experimental immune-mediated blepharoconjunctivitis in Lewis rats. *Curr Eye Res* 1998;**17**:763–769.
64. CALONGE MC, PASTOR JC, HERRERAS JM, GONZALEZ JL. Pharmacologic modulation of vascular permeability in ocular allergy in the rat. *Invest Ophthalmol Vis Sci* 1990;**31**:176–180.
65. BAYER A, ULUDAG HA, SOBACI G, MUTLU FM. Comparison of antiallergic drugs in an experimental model of ocular anaphylaxis. *Ophthalmologica* 2003;**217**:119–123.
66. BONINI S, TROCME SD, BARNEY NP, BRASH PC, BLOCH KJ, ALLANSMITH MR. Late-phase reaction and tear fluid cytology in the rat ocular anaphylaxis. *Curr Eye Res* 1987;**6**:659–665.
67. BONINI S. IgE and non-IgE mechanisms in ocular allergy. *Ann Allergy* 1993;**71**:296–299.
68. CARRERAS I, CARRERAS B, MCGRATH L, RICE A, EASTY DL. Activated T cells in an animal model of allergic conjunctivitis. *Br J Ophthalmol* 1993;**77**:509–514.
69. ALLANSMITH MR, BAIRD RS, ROSS RN, BARNEY NP, BLOCH KJ. Ocular anaphylaxis induced in the rat by topical application of compound 48/80. Dose response and time course study. *Acta Ophthalmol Suppl* 1989;**192**:145–153.
70. TROCME SD, BONINI S, TROCME MC, BARNEY NP, BLOCH KJ, ALLANSMITH MR. A hapten model of topically-induced ocular anaphylaxis in the rat. *Invest Ophthalmol Vis Sci* 1987;**28**:264–269.
71. TANAKA H, UMEMOTO M, MIICHI H, HAYASHI S. Evaluation of an antiallergic agent (disodium cromoglycate) in rat conjunctiva using monoclonal IgE antibody. *Ophthalmic Res* 1987;**19**:240–244.
72. MCGRATH LE, DOHERTY MJ, EASTY DL, NORRIS A. Nedocromil sodium in two models of conjunctival immediate hypersensitivity. *Adv Ther* 2000;**17**:7–13.
73. YANNI JM, WEIMER LK, GLASER RL, LANG LS, ROBERTSON SM, SPELLMAN JM. Effect of lodoxamide on in vitro and in vivo conjunctival immediate hypersensitivity responses in rats. *Int Arch Allergy Immunol* 1993;**101**:102–106.

74. NISHINO K, FUKUSHIMA A, OKAMOTO S, OHASHI Y, FUKATA K, OZAKI A et al. Suppression of experimental immune-mediated blepharoconjunctivitis in Brown Norway rats by topical application of FK506. *Graefes Arch Clin Exp Ophthalmol* 2002;**240**:137–143.
75. IWAMOTO H, YOSHIDA H, YOSHIDA O, FUKUSHIMA A, UENO H. Inhibitory effects of FK506 on the development of experimental allergic/immune-mediated blepharoconjunctivitis in Lewis rats by systemic but not by topical administration. *Graefes Arch Clin Exp Ophthalmol* 1999;**237**:407–414.
76. YOSHIDA O, YOSHIDA H, IWAMOTO H, NISHINO K, FUKUSHIMA A, UENO H. Genetic background determines the nature of immune responses and experimental immune-mediated blepharoconjunctivitis (EC). *Curr Eye Res* 1999;**18**:117–124.
77. YOSHIDA O, YOSHIDA H, IWAMOTO H, NISHINO K, FUKUSHIMA A, UENO H. Comparison of genetic susceptibility to experimental allergic/immune-mediated blepharoconjunctivitis between Lewis and Fischer rats. *Graefes Arch Clin Exp Ophthalmol* 1998;**236**:859–864.
78. OZAKI A, FUKUSHIMA A, FUKATA K, UENO H. Effects of IL-4 and IL-12 on experimental immune-mediated blepharoconjunctivitis in Brown Norway rats. *Clin Exp Immunol* 2000;**122**:28–34.
79. IWAMOTO H, NISHINO K, MAGONE TM, WHITCUP SM, YOSHIDA O, YOSHIDA H et al. Experimental immune-mediated blepharoconjunctivitis in rats induced by immunization with ragweed pollen. *Graefes Arch Clin Exp Ophthalmol* 2000;**238**:346–351.
80. KAHN M, BARNEY NP, BRIGGS RM, BLOCH KJ, ALLANSMITH MR. Penetrating the conjunctival barrier. The role of molecular weight. *Invest Ophthalmol Vis Sci* 1990;**31**:258–261.
81. BARNEY NP, KLEINMAN RE, TROCME SD, BLOCH KJ, ALLANSMITH MR. Attenuation of rat conjunctival response by repeated hapten applications. *Curr Eye Res* 1988;**7**:843–848.
82. TROCME SD, BONINI S, BARNEY NP, BLOCH KJ, ALLANSMITH MR. Late-phase reaction in topically induced ocular anaphylaxis in the rat. *Curr Eye Res* 1988;**7**:437–443.
83. CALONGE M, BRIGGS RM, LEVENE RB, SAIGA T, BLOCH KJ, ALLANSMITH MR. Early and late phases of ocular anaphylaxis in actively immunized guinea pigs. *Acta Ophthalmol (Copenh)* 1990;**68**:470–476.
84. LEONARDI A, BRIGGS RM, BLOCH KJ, ALLANSMITH MR. Clinical patterns of ocular anaphylaxis in guinea pigs passively sensitized with IgG1 antibody. *Ophthalmic Res* 1990;**22**:95–105.
85. TILIGADA E, ASLANIS D, DELITHEOS A, VARONOS D. Changes in histamine content following pharmacologically-induced mast cell degranulation in the rat conjunctiva. *Pharmacol Res* 2000;**41**:667–670.
86. KHOSRAVI E, ELENA PP, HARITON C. Allergic conjunctivitis and uveitis models: reappraisal with some marketed drugs. *Inflamm Res* 1995;**44**:47–54.
87. HANNON GJ. RNA interference. *Nature* 2002;**418**:244–251.
88. HANZE J, GRONEBERG DA, ROSE F, HANISCH A, DOTSCH J, PEISER C et al. Genomic organization and regulation of a human 7-helix transmembrane receptor which is expressed in pulmonary epithelial cells and induced in hypoxia. *Biochem Biophys Res Commun* 2002;**291**:1160–1165.
89. GRONEBERG DA, PEISER C, DINH QT, SPRINGER J, FISCHER A. Abundant expression of c-Jun in guinea pig sympathetic ganglia under basal conditions and allergen challenge. *Lung* 2002;**180**:221–228.
90. HEPPT W, PEISER C, CRYER A, DINH QT, ZWENG M, WITT C et al. Innervation of human nasal mucosa in environmentally triggered hyperreflexic rhinitis. *J Occup Environ Med* 2002;**44**:924–929.
91. FISCHER TC, HARTMANN P, LOSER C, SPRINGER J, PEISER C, DINH QT et al. Abundant expression of vasoactive intestinal polypeptide receptor VPAC2 mRNA in human skin. *J Invest Dermatol* 2001;**117**:754–756.
92. JACOB P, ROSSMANN H, LAMPRECHT G, KRETZ A, NEFF C, LIN-WU E et al. Down-regulated in adenoma mediates apical Cl⁻/HCO₃⁻ exchange in rabbit, rat, and human duodenum. *Gastroenterology* 2002;**122**:709–724.
93. FISCHER TC, DINH QT, PEISER C, LOSER C, FISCHER A, GRONEBERG DA. Simultaneous detection of receptor mRNA and ligand protein in human skin tissues. *J Cutan Pathol* 2002;**29**:65–71.
94. GRONEBERG DA, HARTMANN P, DINH QT, FISCHER A. Expression and distribution of vasoactive intestinal polypeptide receptor VPAC(2) mRNA in human airways. *Lab Invest* 2001;**81**:749–755.
95. GRONEBERG DA, NICKOLAUS M, SPRINGER J, DORING F, DANIEL H, FISCHER A. Localization of the peptide transporter PEPT2 in the lung: implications for pulmonary oligopeptide uptake. *Am J Pathol* 2001;**158**:707–714.
96. LIM S, GRONEBERG D, FISCHER A, OATES T, CARAMORI G, MATTOS W et al. Expression of heme oxygenase isoenzymes 1 and 2 in normal and asthmatic airways: effect of inhaled corticosteroids. *Am J Respir Crit Care Med* 2000;**162**:1912–1918.
97. GRONEBERG DA, DORING F, THEIS S, NICKOLAUS M, FISCHER A, DANIEL H. Peptide transport in the mammary gland: expression and distribution of PEPT2 mRNA and protein. *Am J Physiol Endocrinol Metab* 2002;**282**:E1172–E1179.
98. HU S, MERAYO-LLOVES J, ZHAO T, FOSTER CS. Comparative effectiveness and molecular pharmacological mechanisms of antiallergic agents on experimental conjunctivitis in mice. *J Ocul Pharmacol Ther* 1998;**14**:67–74.
99. MERAYO-LLOVES J, ZHAO TZ, DUTT JE, FOSTER CS. A new murine model of allergic conjunctivitis and effectiveness of nedocromil sodium. *J Allergy Clin Immunol* 1996;**97**:1129–1140.
100. MAGONE MT, CHAN CC, RIZZO LV, KOZHICH AT, WHITCUP SM. A novel murine model of allergic conjunctivitis. *Clin Immunol Immunopathol* 1998;**87**:75–84.
101. MAGONE MT, CHAN CC, BECK L, WHITCUP SM, RAZ E. Systemic or mucosal administration of immunostimulatory DNA inhibits early and late phases of murine allergic conjunctivitis. *Eur J Immunol* 2000;**30**:1841–1850.
102. MIYAZAKI D, LIU G, CLARK L, ONO SJ. Prevention of acute allergic conjunctivitis and late-phase inflammation with immunostimulatory DNA sequences. *Invest Ophthalmol Vis Sci* 2000;**41**:3850–3855.
103. MAGONE MT, WHITCUP SM, FUKUSHIMA A, CHAN CC, SILVER PB, RIZZO LV. The role of IL-12 in the induction of late-phase cellular infiltration in a murine model of allergic conjunctivitis. *J Allergy Clin Immunol* 2000;**105**:299–308.
104. STRAUSS EC, LARSON KA, BRENNISE I, FOSTER CS, LARSEN GR, LEE NA et al. Soluble P-selectin glycoprotein ligand 1 inhibits ocular inflammation in a murine model of allergy. *Invest Ophthalmol Vis Sci* 1999;**40**:1336–1342.

105. WHITCUP SM, CHAN CC, KOZHICH AT, MAGONE MT. Blocking ICAM-1 (CD54) and LFA-1 (CD11a) inhibits experimental allergic conjunctivitis. *Clin Immunol* 1999;**93**:107–113.
106. KEANE-MYERS AM, MIYAZAKI D, LIU G, DEKARIS I, ONO S, DANA MR. Prevention of allergic eye disease by treatment with IL-1 receptor antagonist. *Invest Ophthalmol Vis Sci* 1999;**40**:3041–3046.
107. KUNERT KS, KEANE-MYERS AM, SPURR-MICHAUD S, TISDALE AS, GIPSON IK. Alteration in goblet cell numbers and mucin gene expression in a mouse model of allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 2001;**42**:2483–2489.
108. GRONEBERG DA, PEISER C, DINH QT, MATTHIAS J, EYNOTT PR, HEPPT W et al. Distribution of respiratory mucin proteins in human nasal mucosa. *Laryngoscope* 2003;**113**: 520–524.
109. GRONEBERG DA, EYNOTT PR, OATES T, LIM S, WU R, CARLSTEDT I et al. Expression of MUC5AC and MUC5B mucins in normal and cystic fibrosis lung. *Respir Med* 2002;**96**: 81–86.
110. IZUSHI K, NAKAHARA H, TAI N, MIO M, WATANABE T, KAMEI C. The role of histamine H(1) receptors in late-phase reaction of allergic conjunctivitis. *Eur J Pharmacol* 2002;**440**:79–82.
111. KRANEVELD AD, VAN DER KLEIJ HP, KOOL M, van HOUWELINGEN AH, WEITENBERG AC, REDEGELD FA et al. Key role for mast cells in nonatopic asthma. *J Immunol* 2002;**169**: 2044–2053.
112. SCOTT AE, KASHON ML, YUCESAY B, LUSTER MI, TINKLE SS. Insights into the quantitative relationship between sensitization and challenge for allergic contact dermatitis reactions. *Toxicol Appl Pharmacol* 2002;**183**: 66–70.