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Predictive testing for respiratory sensitization in the mouse

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Abstract

Attempts to develop predictive test methods for the identification of chemical respiratory allergens have to date focused almost exclusively on the guinea pig. In recent years there has, however, been a growing interest in the mouse as a model for examination of sensitization potential. In this article two alternative approaches to the toxicological investigation of respiratory sensitization are described. Both are based on an understanding of the nature of immune responses induced in mice by chemical allergens. The mouse IgE test seeks to identify chemicals capable of causing allergic sensitization of the respiratory tract as a function of induced increases in the serum concentration of IgE. The second approach, cytokine fingerprinting, makes use of the observation that chemical allergens of different types provoke in mice qualitatively divergent immune responses characterized by discrete cytokine secretion profiles.

Keywords: Respiratory sensitization; Chemical allergy; Serum IgE; Mouse IgE test; Cytokines

1. Introduction

There is a wide variety of methods for the toxicological evaluation of skin sensitization potential. In contrast, there are currently no definitive or well validated assays available for the prospective identification of chemicals that have the ability to cause sensitization of the respiratory tract [1]. Most approaches have favoured the guinea pig, a species in which allergic pulmonary reactions can be elicited that share some features with those observed in man. The emphasis here has been to measure challenge-induced changes in respiratory rate, breathing pattern or other aspects of pulmonary function following inhalation exposure of previously sensitized guinea pigs to the test allergen [2–11]. Progress achieved to date with such models is described elsewhere in this issue (Kimber). However, although progress has been made there is no doubt that the available guinea pig methods have certain important limitations, not least of which are expense and the requirement for inhalation facilities. There is interest therefore in seeking alternative approaches for the predictive assessment of respiratory sensitization potential. In this article the development of methods in the mouse is described.

2. The mouse IgE test

The mouse IgE test currently represents the only systematic approach to the development of predictive methods in the mouse. The assay grew from an understanding of the nature of immune responses

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elicited in mice by chemical allergens and of the qualitative differences in immune responses provoked by contact and respiratory sensitizers.

2.1. Immunobiological basis

It has been reported previously that different classes of chemical allergen induce in mice variable immune responses. Contact allergens such as 2,4-dinitrochlorobenzene (DNCB), which are considered not to cause sensitization of the respiratory tract, stimulate in mice immune responses consistent with the preferential activation of T helper 1 (Thl) cells. Such responses are associated with the production by draining lymph node cells (LNC) of interferon γ (IFN-γ). The converse picture is seen with chemicals that have been shown to cause allergic respiratory hypersensitivity and occupational asthma in humans. Thus, chemical respiratory allergens such as trimellitic anhydride (TMA) and toluene diisocyanate (TDI) elicit in mice Th2-type immune responses, associated with the production by draining LNC of high levels of interleukin 4 (IL-4) and of other cytokine products of Th2 cells [12–19]. IgE antibody responses are regulated by cytokines. In mice the induction and maintenance of IgE responses are dependent on the availability of IL-4, but are inhibited by IFN-γ [20,21]. In humans also the stimulation of IgE antibody production is subject to the reciprocal antagonistic effects of IL-4 and IFN-γ [22,23]. As a consequence it has been found that exposure of mice to TMA, but not to DNCB, results in the appearance of specific IgE antibody.

In the same series of investigations it was found that topical administration to mice of chemical respiratory allergens stimulated a substantial increase in the serum concentration of total IgE, a response not seen with contact allergens considered to lack the ability to cause sensitization of the respiratory tract [13,14]. These observations suggested that it might be possible to identify chemical respiratory sensitizers as a function of induced changes in serum IgE concentration, the advantage of this approach being that measurement of a serum protein, rather than of hapten-specific antibody, is required. Such forms the basis of the mouse IgE test.

2.2. Test development

In original studies a series of chemical allergens was examined in the mouse IgE test at single concentrations that were known on the basis of parallel experiments to stimulate positive responses in the local lymph node assay and therefore to be immunogenic following epicutaneous administration. The test was performed as follows. Groups of BALB/c strain mice were exposed topically to the test material, or to vehicle alone, bilaterally on the shaved flanks. Seven days later the same material diluted 1:1 with vehicle (or vehicle alone) was applied to the dorsum of both ears. At various periods following the initiation of exposure mice were exsanguinated and serum prepared. The concentration of serum IgE was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) [24]. The results of these analyses confirmed that only those chemicals known to cause respiratory sensitization and occupational asthma provoked in mice a substantial increase in serum IgE levels. On the basis of those investigations it was determined that, of the time points examined, 14 days following the initiation of exposure was the most appropriate for routine use in the assay. In a subsequent series of experiments dose–response relationships were examined in the mouse IgE test and here again a clear discrimination between contact and respiratory chemical allergens was observed [25]. A representative experiment is illustrated in Fig. 1, where responses to hexahydrophthalic anhydride, a known human respiratory allergen [26,27], are examined and compared with responses elicited by 1% DNCB and 25% TMA. This chemical was found to elicit a dose-dependent increase in the serum concentration of IgE relative to control values derived from mice treated concurrently with the relevant vehicle alone.

Attempts have been made recently to investigate the stability of the method by analyses in 15 independent experiments of responses induced by TMA and DNCB. It was found that in each instance exposure of mice to TMA resulted in a significant increase in serum IgE concentration, whereas in no case was IgE elevated significantly after treatment with DNCB [28]. It was concluded

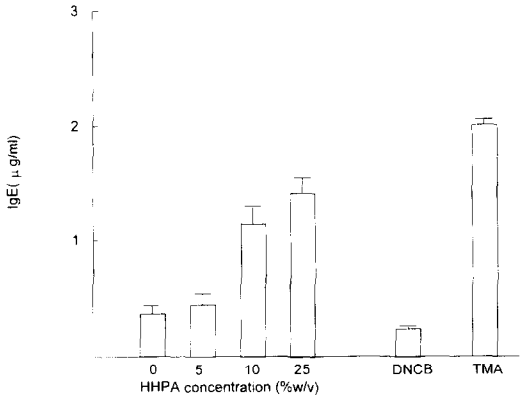

Fig. 1. Serum IgE concentration following topical exposure of BALB/c strain mice to various concentrations of hexahydrophthalic anhydride (HHPA). Groups of mice ($n = 6$) received 50 μl of various concentrations (5%, 10% and 25% w/v) of HHPA in vehicle (4:1 acetone:olive oil; AOO), or an equal volume of vehicle alone, on each shaved flank. Other groups of mice were treated in an identical manner with 1% DNCB or 25% TMA in AOO. Seven days later, 25 μl of test chemical in AOO at half the concentration used previously was applied to the dorsum of both ears. Fourteen days following the initation of exposure, mice were exsanguinated, serum was prepared and the concentration of IgE measured by enzyme-linked immunosorbent assay. Results are expressed as mean (±SE) serum IgE concentration (μg/ml) for each experimental group.

that for routine use in the predictive evaluation of the respiratory sensitization potential of chemicals, the following experimental design should be employed. Three concentrations of the test material, together with the appropriate vehicle alone, are examined in the standard assay described above. Induced changes in serum IgE concentration are measured by ELISA in serum samples drawn from each group 14 days following the initiation of treatment. The significance of any alterations in the concentration of serum IgE is evaluated by reference to values obtained with concurrent vehicle-treated controls. The integrity of each analysis is monitored by inclusion in the experimental design of TMA and DNCB which serve, respectively, as positive and negative controls. Presently chemical respiratory allergens are defined as materials that, at one or more test concentration, elicit a significant increase in the concentration of serum IgE relative to values recorded with control mice treated concurrently with the relevant vehicle alone. It has yet to be established whether in practice this criterion for positivity will be of appropriate sensitivity and/or selectivity for the routine identification of all potential chemical respiratory allergens. Finally, it is our policy at present, where possible, to select test concentrations on the basis of prior local lymph node assays results. Analyses performed with concentrations of the test material that are known to induce positive responses in the local lymph node assay permit one to conclude that the failure of a chemical to provoke an increase in serum IgE concentration does not result from a lack of immunogenicity when applied topically.

Investigations to date suggest that the mouse IgE test may provide a useful method for the prospective identification of chemical respiratory allergens, a conclusion that is supported by recent studies in an independent laboratory [29]. It is of interest also that preliminary analysis of a rat variant of the mouse IgE test has revealed that TMA, but not DNCB, will elicit an increase in IgE concentrations in Brown Norway strain animals [30].

It must be emphasized, however, that to date the assay has been evaluated only with a limited number of chemicals and that most of the analyses have been performed in a single laboratory. It is necessary now to examine the utility of the test with a wider range of chemicals and in independent laboratories. To this end an international, inter-laboratory evaluation of the mouse IgE test has recently been initiated.

3. Cytokine fingerprinting

As described above, contact and respiratory chemical allergens provoke in mice qualitatively different immune responses suggestive of divergent T cell activation and characterized by variable patterns of cytokine production. Thus, chronic exposure of mice over a 13 day period to TMA was found to result in the production by draining LNC of high levels of mitogen-inducible IL-4 and another Th-2 cell cytokine interleukin 10 (IL-10), but only low levels of IFN-γ. In contrast, treatment of mice under the same conditions of exposure with

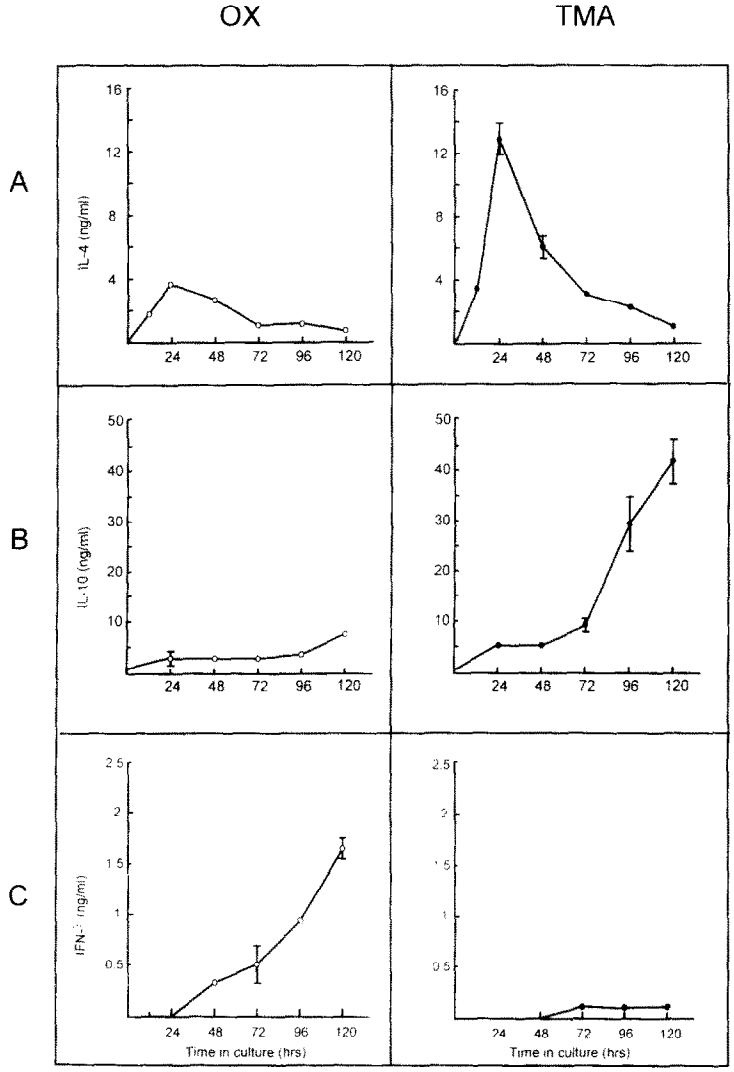

Fig. 2. Production by draining lymph node cells of interleukins 4 and 10 (IL-4 and IL-10) and interferon γ (IFN-γ) following repeated topical exposure of BALB/c strain mice to trimellitic anhydride (TMA) or oxazolone (OX). Groups of mice ($n = 10$) received 50 μl of 0.25% OX or 10% TMA (both in AOO) bilaterally on both shaved flanks. Five days later this treatment was repeated. After a further 5 days, 25 μl of chemical was applied to the dorsum of both ears daily for 3 consecutive days. One day following the final exposure mice were killed and draining auricular lymph nodes excised and pooled for each experimental group. A single cell suspension of lymph node cells was prepared and cultured in the presence (for measurement of mitogen-inducible IL-4 production), or absence (for the measurement of spontaneous IL-10 and IFN-γ secretion) of 2 μg/ml concanavalin A. Culture was terminated after various periods and the concentrations of IL-4, IL-10 and IFN-γ measured in supernatants by enzyme-linked immunosorbent assay. In each case cytokine concentrations are recorded as mean values in ng/ml. Standard errors are shown when greater than 10% of mean: A, IL-4; B, IL-10; C, IFN-γ.

oxazolone (a potent contact allergen) caused the production by draining LNC of comparatively low levels of IL-4 and IL-10, but high concentrations of IFN-γ [19]. A representative experiment is summarized in Fig. 2. Similar selective cytokine secretion profiles have been recorded following exposure of mice to other contact and respiratory chemical allergens [31]. These data raise the ques-

tion of whether it might be possible to monitor the sensitizing properties of chemicals as a function of induced cytokine production profiles. Recent evidence suggests that this is the case, and the value of cytokine fingerprinting in the routine identification and classification of chemical allergens is being explored currently.

4. Conclusions

The approaches described above demonstrate that some important progress has been made in developing procedures for the toxicological evaluation of respiratory sensitization potential in the mouse. Although much has been achieved, much remains to be done. Priorities now are the further evaluation of the mouse IgE test and the refinement and initial validation of cytokine fingerprinting as alternative approaches for predictive testing.

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