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Review

Chemical reactivity measurements: Potential for characterization of respiratory chemical allergens

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ABSTRACT

Allergic diseases of the skin and respiratory tract resulting from exposure to low molecular weight chemicals remain important issues for consumer product development and occupational/environmental health. Widespread opportunities for exposure to chemical allergens require that there are available effective methods for hazard identification and risk assessment. In the search for new tools for hazard identification/characterization there has been interest in developing alternative methods that will reduce, refine or replace the need for animals. One approach that shows promise is based on the measurement of the peptide reactivity of chemicals; the potential to form stable associations with protein/peptide being a key requirement for the induction of sensitization. Recent investigations using these systems have focused primarily on skin sensitizing chemicals. However, there is interest in the possibility of exploiting these same experimental approaches to distinguish between different forms of chemical allergens – as individual materials are primarily associated with one or the other form of sensitization in humans. These investigations may also provide insight into why chemical sensitizers can differ in the form of allergic disease they will preferentially induce. These opportunities are surveyed here against a background of the immunobiology of allergic sensitization and current state-of-the-art approaches to measurement of peptide/protein reactivity.

Contents

Abbreviations: ACD, allergic contact dermatitis; AHCP, ammonium hexachloroplatinate; APC, antigen presenting cells; Da, Dalton; DAD, diode array detector; DC, dendritic cell; DNCB, 2,4-dinitrochlorobenzene; DNFB, 2,4-dinitrofluorobenzene; DTT, d,l-dithiothreitol; EC50, effective concentration 50; EU, European Union; FIA, flow-injection analysis; FITC, fluorescein isothiocyanate; FR, factor of reactivity; GSH, glutathione; HHPA, hexahydrophthalic anhydride; HSA, human serum albumin; HDI, hexamethylene diisocyanate; HPLC, high-performance liquid chromatography; HSAB, hard and soft acid base theory; IFN-γ, interferon-gamma; Ig, immunoglobulin; IL, interleukin; LC, Langerhans' cell; LLNA, local lymph node assay; MDI, diphenylmethane diisocyanate; MHC, major histocompatibility complex; MS, mass spectroscopy; NIH, National Institutes of Health; NMR, nuclear magnetic resonance; OECD, Organization for Economic Co-Operation and Development; PA, phthalic anhydride; QSAR, quantitative structure activity relationship; REACH, registration, evaluation, authorization and restriction of chemicals; RIFM, Research Institute for Fragrance Materials; TDI, toluene diisocyanate; TMA, trimellitic anhydride; TNF, tumor necrosis factor; TNP, trinitrophenyl; UV, ultra violet.

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1. Introduction

Allergic hypersensitivity can be defined as an inappropriate immune response to a normally innocuous antigen resulting in tissue injury and adverse health effects (Janeway et al., 1997). Many classes of materials encountered in the environment may provoke such an immune response. Broadly, these materials may be divided into two categories: high molecular weight compounds, greater than 1000 daltons (Da) and low molecular weight chemicals, less than 1000 Da. High molecular weight compounds are able to interact with the immune system directly to provoke an immune response; these include proteins encountered in the environment or occupationally; such as pollen, house dust mite excreta, animal dander and enzymes (Chapman et al., 2007). In contrast, chemicals of low molecular weight are too small to be recognized by the immune system directly and act as haptens, which must first react directly or indirectly with a protein in order to provoke an immune response. Of particular importance in the contexts of occupational health, predictive toxicology and ensuring the safety of manufactured products is the ability of some chemicals to cause allergic sensitization of the skin and respiratory tract.

Skin sensitization resulting in allergic contact dermatitis (ACD) is by far the most frequent manifestation of immunotoxicity in humans and a common occupational disease. Several hundred chemicals have been implicated as skin sensitizers based on human experience and many more have been identified as potential allergens based on experimental models. Extensive databases have been published including over 200 representative examples of skin sensitizing chemicals (Gerberick et al., 2005; Kern et al., 2010). There are a number of experimental models available to assess the potential for a chemical to act as a skin sensitizer. Historically, guinea pigs were the species most commonly used for skin sensitization hazard identification (Buehler, 1965; Magnusson and Kligman, 1969). In these assays, the potential of a material to act as a skin sensitizer is based on visual scoring of skin responses (erythema/edema) following challenge of previously sensitized animals. More recently, the murine local lymph node assay (LLNA) has been increasingly used for hazard assessment (Kimber and Basketter, 1992; Kimber et al., 1994, 2002a). In the LLNA, the sensitizing potential of a chemical is assessed quantitatively by measurement of cell proliferation in the lymph nodes draining the site of topical exposure to the test material. The LLNA has been validated as an alternative to the more traditional guinea pig models for the identification of potential skin sensitizers (Gerberick et al., 2000; National Institutes of Health [NIH], 1999; Organization for Economic Co-Operation and Development [OECD], 2002). In addition to its application as a method for hazard identification, the LLNA provides a basis for the objective and quantitative measurement of relative skin sensitizing potency (Basketter et al., 2000). In the context of human health risk assessments, the ability to determine potency is of particular importance since the skin sensitizing potency of chemical allergens can vary by several (and probably up to 5) orders of magnitude.

In contrast to skin sensitizers, there are far fewer chemicals that are known to cause respiratory allergy. Though less numerous, respiratory sensitizers are of concern because respiratory allergy is commonly associated with high levels of morbidity, and occasionally mortality, and has significant socio-economic consequences (Bernstein et al., 2006; Kimber and Dearman, 1997). In the context of this review sensitization of the respiratory tract refers to immunologic priming resulting from the initiation of an immune response by a chemical allergen. The chemicals that can cause an asthmatic response via these immunologic mechanisms are considered to be true 'respiratory allergens'. This is distinct from similar physiological changes that can occur via non-immunological mechanisms, in which case the offending chemicals and agents have been termed 'asthmagens' (Kimber et al., 2007).

Published reviews, that have sought to identify potential chemical respiratory allergens based on clinical evidence, have reported numbers in the range of 40–80 materials (Graham et al., 1997). Chemicals generally agreed to cause allergic sensitization of the respiratory tract resulting in occupational asthma include: diisocyanates (such as diphenylmethane diisocyanate [MDI], hexamethylene diisocyanate [HDI] and toluene diisocyanate [TDI]); acid anhydrides (such as trimellitic anhydride [TMA] and phthalic anhydride [PA]); metals (such as certain platinum salts), pharmaceuticals and their intermediates (such as penicillin and phenylglycine acid chloride) and other industrial agents that have applications in painting, dye making, plastics and electronics manufacturing (ECETOC, 1999; Graham et al., 1997; Kimber and Dearman, 1997). Discrepancies between the numbers of allergens identified reflect the individual authors' criteria for classifying a chemical as a respiratory sensitizer. These criteria often ascribe the highest weighting to clinical reports of varying quality to identify potential allergens and may therefore include asthmagens as well as respiratory sensitizers. The default to clinical indications for identification of potentially hazardous materials has been driven by the fact that there are no validated or generally accepted models to identify respiratory sensitization hazards (reviewed by Kimber et al. (2007)). The lack of an agreed methodology is the direct result of controversy and lack of understanding of the underlying mechanisms leading to sensitization of the respiratory tract.

Given the extent of human exposure to chemicals that may have the potential to cause allergic disease, there is a need to identify hazards and to conduct accurate risk assessments to protect health. The requirement for a high degree of confidence with regards to the accuracy of risk assessments is an important consideration. The aim is to safeguard public health, while not overly restricting the use of materials that have important potential benefits. The quality of assessments is reliant on the tools, methodologies and information available. Current risk assessment practices rely almost exclusively on *in vivo* models. There is significant social, scientific and economic pressure to replace animal testing where possible. The most pressing example is that of the European Union (EU) ban on *in vivo* testing of cosmetic and toiletry ingredients, which came into general force in 2009 (EU Directive 2003/15/EC). Registration, evaluation, authorization and restriction of chemicals (REACH) legislation has also mandated that *in vivo* testing be conducted only when appropriate alternatives are not available (EU, 2002). Irrespective of legislative pressures, there is a significant public desire, and indeed an ethical responsibility, to reduce, refine or replace (3Rs) animal testing wherever this is possible. In recent years, progress has been made in the design of *in vitro* tools for the identification of skin sensitizers. One important development has been the application of chemical reactivity measurements to the identification of skin sensitizing chemicals (Gerberick et al., 2004, 2008). Given their apparent utility with respect to the identification of skin sensitizers, there has been