Anti-hapten antibodies in response to skin sensitization

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Summary

Whereas T lymphocyte (T cell) activation is the key event in the acquisition of skin sensitization and subsequent elicitation of allergic contact dermatitis, the humoral component of immune responses to organic contact allergens has received little consideration. There is evidence that, in experimental animals, topical exposure to potent contact allergens is associated with B cell activation and proliferation, and hapten-specific antibody production. However, there is very limited evidence available for anti-hapten antibody responses being induced following topical exposure of humans to contact allergens. Nevertheless, it is important to appreciate that there are almost no negative studies in which evidence for antibody production as the result of skin sensitization has been sought and not found. That is, there is absence of evidence rather than evidence of absence. Furthermore, exposure to chemical respiratory allergens, in which the skin has been implicated as a potential route of sensitization, results in anti-hapten antibody responses. It is proposed that skin sensitization to contact allergens will normally be accompanied by antibody production. The phenomenon is worthy of investigation, as anti-hapten antibodies could potentially influence and/or regulate the induction of skin sensitization. Moreover, such antibodies may provide an informative correlate of the extent to which sensitization has been acquired.

Key words: allergic contact dermatitis; antibody response; B cell; hapten; IgG; IgM; skin sensitization.

From an immunological perspective, the acquisition of skin sensitization and the elicitation of allergic contact dermatitis are immune and inflammatory responses mediated by antigen (hapten)-specific T cells (1–3). For this reason, it is the activation, differentiation and regulation of T cells upon which attention has been focused, and there are available excellent recent reviews that describe the roles played by T cells in skin sensitization and allergic contact dermatitis (4–7).

However, at least in animals, contact allergens provoke both humoral and cell-mediated immune responses. Thus, topical encounter with a contact allergen will induce anti-hapten antibody formation in addition to the activation and expansion of responsive T cells (8–10). Given the predominant role played by T cells in skin sensitization, it is perhaps not surprising that anti-hapten antibody responses have received comparatively little attention. This is unfortunate because, in theory, a hapten-specific antibody has the ability to influence both the acquisition of skin sensitization and the subsequent elicitation of allergic reactions. Moreover, it is possible that the vigour and isotype distribution of anti-hapten antibodies induced by exposure to contact allergens...
may provide correlates of the quality of T lymphocyte responses and the extent of skin sensitization.

Here, we review the available evidence from experimental animals and humans that topical exposure to contact allergens will elicit antibody responses. We consider also whether such antibodies have the ability to influence the development of sensitization and/or the elicitation of allergic contact dermatitis.

**T cell responses in allergic contact dermatitis**

Although a detailed review of T cell responses in allergic contact dermatitis is outside the scope of this article, it is necessary to briefly outline what is known of the role of T cells in the acquisition of skin sensitization. It is generally accepted that, in humans and in relevant experimental rodent models of skin sensitization, both hapten-specific CD8+ T cytotoxic (Tc) and CD4+ T helper (Th) cell subsets play important roles (11, 12). Tc and Th cell populations have been shown to polarize into characterisic subsets with distinctive cytokine expression profiles and functions. The most well studied of these subsets are type 1 (Tc1 and Th1) cells, which express interferon-γ, and type 2 (Tc2 and Th2) cells, which preferentially secrete interleukin (IL)-4, IL-5, and IL-13 (13–15). Thus, following challenge of sensitized individuals with allergen, there is rapid recruitment of skin-homing Tc1 cells that cause apoptosis of keratinocytes, and CD4+ Th1 cells that release proinflammatory cytokines, activating keratinocytes, and other skin cells, contributing to the inflammatory reaction (2, 7, 11). Other subsets of CD4+ T cells with regulatory properties appear to restrict the magnitude of the response to hapten, particularly potent sensitizers (2, 16–18). Whereas Th1 cells are associated with cell-mediated reactions such as allergic contact dermatitis, the primary function of Th2 cells is to help B lymphocytes (B cells) make antibodies (19, 20). However, Th1 cells can also provide help for B cells and antibody production; thus, experiments with protein antigens conducted in mice showed that Th1 and Th2 cells could support B cell responses to a similar extent in vivo (21), with both subsets sustaining IgM production, whereas Th1 and Th2 cells preferentially induced IgG2a and IgG1 responses, respectively (22). This phenomenon is paralleled by human T cell subsets, with the IgG1 subclass being driven by Th1 cells and the IgG4 subclass being associated with Th2-dominant responses (23, 24).

The fact that Th1 cells, which constitute one of the main T cell subpopulations activated in contact sensitization, can provide B cell help supports the assertion that antibodies may be produced in individuals with contact allergy. There is reason to assume, therefore, that contact allergens will induce antibody responses, in addition to T lymphocyte responses. This should come as no real surprise, because the vast majority of immunogens are known to stimulate both T cell and antibody responses.

**Anti-hapten antibody responses induced following exposure to contact allergens in experimental animals**

Consistent with the ability of Th1 cells to support antibody responses, there is clear evidence from studies conducted in experimental animals that detectable antibody production is induced following exposure to skin-sensitizing chemicals. Very early work in the 1940s using guinea-pigs showed that skin hypersensitivity reactions to the known human contact allergen 2,4-dinitrochlorobenzene (DNCB) could be transferred with immune serum to naive guinea-pigs, indicating a humoral (antibody) component (25). However, in these experiments, animals were immunized by repeated intradermal injection of free chemical, which is not directly relevant to the human experience. Some decades later, it was shown that topical treatment of guinea-pigs with DNCB resulted in a marked increase in the number of germinal centres in skin-draining lymph nodes, indicative of B cell activation and the production of antibodies (26).

Furthermore, topical application to mice of the experimental skin sensitizer oxazolone or picryl chloride [trinitrochlorobenzene (TNCB)] was shown to stimulate antibody production, detected as a function of the ability of serum to agglutinate haptenated red blood cells (RBCs) (27, 28), although this effect was somewhat mouse strain-dependent (28). However, interpretation of the latter results was hampered by the detection of antibodies in non-immune sera, suggesting that there was considerable background in the assay, or that natural antibodies are present that are reactive with some haptons (29–31).

More sophisticated techniques for antibody detection, particularly enzyme-linked immunosorbent assays (ELISAs), have permitted the improved characterization of anti-hapten antibody responses, and have provided a more robust body of evidence that such antibodies are produced following skin sensitization. These studies encompass a relatively narrow range of skin sensitizers, being confined to the experimental contact allergens oxazolone and TNCB, and the human allergen DNCB and the chemically related dinitrofluorobenzene (DNFB). A number of reports have shown that specific IgM production is induced in mice treated topically with oxazolone or TNCB (9, 32–34). This antibody class can be detected within 24 hr of sensitization with hapten (32, 33).
is also good evidence that simple epicutaneous exposure of rodents to contact allergens can induce the production of hapten-specific IgG antibodies. Thus, topical application of DNCB or DNFB induces relatively vigorous IgG responses in mice, and somewhat more variable responses in rats (10, 35–40). In mice, anti-DNCB IgA has also been detected (10), and kinetics studies have shown that measurable levels of IgG are not observed until some 8 days after the initiation of exposure (10, 36), or after repeat exposure to allergen some 10 days later (38). Consistent with the early reports of haemagglutinating antibodies (27, 28), skin sensitization with oxazolone also induces a hapten-specific IgG response in mice, as measured by ELISA (37, 41–43). In those murine studies where the isotype of the IgG response has been characterized, detectable anti-hapten IgG1, IgG2a and IgG2b production has been recorded (10, 44), with several reports of a preferential IgG2a response to both DNBC and oxazolone (36, 41, 45), consistent with selective Th1 cell activation. Interestingly, there are also reports that IgE may also be detected following topical exposure of mice to contact allergens, particularly when there is repeated skin exposure and through abraded or damaged skin (46, 47). In general, however, it appears that, when less vigorous exposure protocols are used, contact allergens are not associated with increases in serum IgE, usually measured as a function of the increase in the total serum concentration of this isotype (48–51).

Further evidence for B cell activation and proliferation in murine models of contact sensitization comes from examination of alternative endpoints to antibody production. Autoradiographic analyses of oxazolone-activated lymph node tissue showed an increased frequency of proliferating cells in the lymphoid follicles (B cell areas) (37). More sophisticated flow cytometric analyses using a number of skin sensitizers [DNCB, hexyl cinnamal (α-hexyl cinnamaldehyde), TNCB, iso- eugenol, and eugenol] showed that epicutaneous application of these chemicals to mice, unlike application of skin irritants, was associated with increases in the number of B220+ lymphocytes (B cells) and their activation status, measured as a function of upregulation of expression of the costimulatory marker CD86 and major histocompatibility complex class II, 5 days after the initiation of exposure (52–55).

All together, there is very convincing evidence that, in experimental animals (primarily rodents), topical exposure to a number of contact sensitizers is associated with B cell activation and proliferation and the production of hapten-specific antibodies, particularly of the IgM and IgG subtypes.

Anti-hapten antibody responses induced following exposure to contact allergens in humans

There is limited evidence available for anti-hapten antibody responses being detected following topical exposure of humans to chemical contact allergens. That is, there are very few studies that have reported analysis of antibody responses following contact sensitization; rather, there are many negative studies. An early study, reported in 1969, investigated the presence of anti-hapten antibodies in actively sensitized human volunteers hospitalized for diverse diseases unrelated to immune function (56). Here, 17 volunteers were contact-sensitized by open topical application of TNCB, and a further 8 patients were sensitized to DNFB, with between four and six biweekly treatments with the same chemical. All individuals developed delayed-type hypersensitivity responses in the skin, but, in serum derived from serial (weekly) bleeds, there was no detectable antibody, measured as a function of haemagglutinating activity. In parallel experiments, immunization with TNCB conjugated with human serum albumin carrier protein by subcutaneous injection resulted in delayed-type hypersensitivity reactions within 7 days of immunization, and the production of agglutinating antibodies within 2 weeks of exposure. This antibody response was relatively transient, peaking after 3–4 weeks and, in the absence of further antigen stimulation, being undetectable after 90–110 days. It should be noted that these analyses were conducted prior to the development of more sensitive and sophisticated techniques such as ELISAs to measure specific antibody production, and that the same authors found that very high doses of contact allergen were required to stimulate detectable anti-hapten antibody production in guinea-pigs with the same techniques (56).

Since that time, few studies have measured antibody production following exposure to contact allergens. There are some reports of antibody responses to selected contact allergens; however, these have been observed following exposure via non-topical routes. For example, following long-term exposure to contact-sensitizing metals such as nickel and cobalt through metallic orthopaedic devices, a marked increase in metal-specific antibodies was detected (57). Thus, in the 10 patients studied, pre-implant and post-implant serum analyses showed that the majority showed an increase in the level of antibody (IgG, IgA, or IgE) directed against at least one metal–protein carrier complex. Similarly, IgG against the contact allergen formaldehyde has been detected, but only in individuals who have been exposed to this material either intravenously (through sterilization of medical devices) or by inhalation (occupationally as arc welders) (58, 59).
both cases, anti-formaldehyde IgG was detected in some of the individuals (6/19 and 5/6, respectively), but it was of relatively low titre.

Intriguingly, Vojdani et al. recently described the detection of both hapten-specific IgG and IgM against a number of chemicals in a proportion of serum samples derived from 400 healthy individuals (60). Although information regarding the amount and route of chemical exposure is unavailable, hapten-specific antibody responses to some contact allergens, including formaldehyde, parabens, bisphenol-A, and dinitrophenyl (DNP) (the leaving group of the hapten DNCB and DNFB) have been reported. It is of interest that much earlier studies also reported anti-DNP IgM and IgG activity in human serum drawn from non-sensitized donors, with the suggestion that these antibodies may represent ‘natural’ antibodies or antibodies that are cross-reactive with a number of different antigens (29–31), and with some evidence of association with bacterial infection (61). However, this does not explain the findings of antibody responses to the other haptens described by Vojdani et al. (60).

In considering the evidence that contact allergens induce anti-hapten antibody responses in humans, the data relating to metal allergens must be treated with some caution. Whether metals are able to provoke humoral immune responses is uncertain, and, in those reports in which anti-metal antibodies have been described, the specificity of the analytical methods is not known.

Although the evidence is lacking with respect to detection of antibodies following topical exposure of humans to chemical contact allergens, there is very clear evidence that exposure to other types of chemical hapten does indeed provoke strong antibody responses. Thus, exposure to chemicals that cause respiratory sensitization, such as the acid anhydrides and the diisocyanates, is often associated with the development of specific IgG, and, considerably less frequently, the production of specific IgE (62–67). There are many commonalities between chemical contact and respiratory allergens, as both types of allergen are: protein-reactive or metabolized to a protein-reactive species, form hapten–protein conjugates that are recognized by the immune system, induce the expression of proinflammatory cytokines, and are immunogenic (68–71). The differences between these classes of chemical allergen apparently reside in the quality of the immune response that is stimulated and the route of elicitation, namely Th1/Tc1 for contact sensitizers, and Th2 for chemical respiratory sensitizers (69, 70), which results in the divergent clinical manifestations of delayed-type hypersensitivity skin reactions, and asthma and rhinitis, respectively. One further commonality is that, despite the clinical manifestations of chemical respiratory allergy being confined to the lung, there is an increasing consideration that skin exposure may well be an important route in the induction and subsequent acquisition of respiratory sensitization (72–76). Therefore, these observations represent a precedent for the ability of the skin to provide a route for the development of antibody responses.

Technical difficulties in detecting anti-hapten antibody responses

Although it seems likely that the paucity of studies examining antibody responses to contact allergens is at least in part attributable to the fact that the appropriate experiments have not been conducted, it is important to emphasize that there are technical difficulties in measuring anti-hapten serum antibody responses in humans. The nature of the hapten–protein conjugates that represent the most relevant form for detecting antibodies remains uncertain, and, in human studies, it is often difficult to control the timing of serum isolation relative to the last exposure (77, 78). In addition, studies in mice using crude reagents (haptenated RBCs) have suggested that the anti-hapten antibodies produced following topical exposure are of relatively low affinity and require highly substituted substrates for detection (27).

A role for anti-hapten antibodies and B cells in the induction and regulation of skin sensitization

Given that there is reason to believe that the elicitation of hapten-specific antibodies will result from topical exposure to contact allergens, at least in experimental animals, the potential significance of such antibodies and/or B cell activation for the development of contact sensitization should be acknowledged. Necessarily, the experiments that support a role for antibody and B cells in the acquisition of skin sensitization have been conducted exclusively in rodents, and there have been sporadic reports that antibodies may play a variety of roles in the induction of skin sensitization or elicitation of allergic contact dermatitis. Thus, in gene-targeted JH−/− mice, in which the disruption in IgH rearrangement specifically results in the absence of B cells, impaired challenge-induced increases in ear thickness were observed in TNCB-sensitized mice, suggesting that B cells are necessary for optimal contact sensitization in mice (33). It was shown that TNCB-specific IgM was detected in the serum within 1 day of immunization, and it was hypothesized that this antibody was important in the local recruitment of effector T cells (32, 33), through mast cell and platelet
activation, resulting in increased vascular permeability via release of serotonin and tumour necrosis factor-α (79, 80). Indeed, the differential sensitivity of BALB/c strain mice and C57BL/6 strain mice to oxazolone-induced contact sensitization was suggested to be attributable to increased numbers of IgM-producing B cells and higher levels of hapten-specific IgM in the more sensitive BALB/c strain mouse (8).

There have also been some reports that B cells may play a regulatory role, serving to constrain elicitation reactions. For instance, Katz et al. described B lymphocyte suppressor cells in guinea-pigs that act to reduce elicitation reactions (81). Furthermore, polyclonal B cell activators, such as lipopolysaccharides, inhibited contact sensitivity to oxazolone in mice when administered 24 hr prior to sensitization (82). There are also other, more recent, reports. Thus, in mice depleted of B cells 7 days prior to or 2 days after sensitization with oxazolone, enhanced challenge-induced ear-swelling responses were recorded (83). Similar results were observed in CD19-deficient mice with the chemically unrelated hapten DNB, indicating that it is this B cell-specific surface molecule that is important in this regulatory activity. In these mice, delayed-type hypersensitivity reactions were exacerbated as compared with wild-type controls, with respect to both the duration of the response and the degree of inflammation (84). Other examples of potential regulatory B cell function in experimental (mouse) models of contact hypersensitivity have also been described (85, 86).

**Discussion**

Taken together, the data reviewed here suggest that chemical allergens have a clear potential to induce the production of hapten-specific antibodies, the exceptions to this rule probably being metal contact allergens. The argument is that the lack of evidence for detectable antibody responses in humans sensitized to contact allergens is attributable to there being available too few relevant investigations; that is, absence of evidence rather than evidence of absence. The ability of contact allergens to elicit antibody responses should not be unexpected from an immunological perspective. There is no reason why a chemical allergen, in the form of a hapten–protein complex formed in vivo, should not induce an immune response capable of initiating and sustaining antibody production. In fact, haptens linked to carrier proteins constituted one of the important tools used in some of the pioneering investigations that first showed the requirement for T cell help in most antibody responses (87–89).

It could be argued, of course, that, under normal circumstances, exposure via the skin does not favour the elicitation of an antibody response, the priority being cell-mediated immunity. However, it is well established that skin exposure of mice to proteins will elicit an antibody response (90), and it is now postulated that skin exposure to peanut proteins, and possibly other food allergens, can induce IgE-mediated allergic sensitization (91). Furthermore, there is a growing appreciation that sensitization of the respiratory tract to chemical respiratory allergens, associated with IgG and, in some cases, IgE responses can be acquired via skin contact (72–76).

If, on the basis of the above arguments, it is accepted that antibody production is a common, if not invariable, consequence of the development of skin sensitization to organic contact allergens (as indicated above, metal allergens possibly representing a special case), then it is appropriate to consider what the relevance and potential utility of this may be.

It could be argued that the simultaneous, or nearly simultaneous, induction of an antibody response during the acquisition of T cell-mediated skin sensitization may have some immunoregulatory consequences. That is, the presence of hapten-specific antibody could, in theory, augment or downregulate specific T cell responses to the same allergen. This possibility is supported by a variety of observations derived from animal studies. However, the possible regulatory role of antibodies in influencing the development or vigour of skin sensitization has not been investigated in a systematic way in experimental animals, and not at all in humans. One interesting speculation is that antibodies may serve to remove or mask haptens and thereby inhibit or limit T cell responses, but this, to our knowledge, has not been addressed experimentally.

Even if the possible immunological and biological consequences of anti-hapten antibodies are unproven with regard to skin sensitization, they may have some potential utility in toxicological considerations of allergic contact dermatitis. For instance, it is now well established that contact allergens vary significantly, and by up to five orders of magnitude, with respect to their relative skin-sensitizing potency. Although there is no reason to suppose that antibody responses as such contribute to relative potency, it may be that they serve as quantitative biomarkers of skin sensitization. If that proved to be the case, then serum levels of hapten-specific antibody might provide an interesting correlate of the extent to which sensitization has been acquired.

In line with the results of animal studies, it has been shown that sensitization to a contact allergen in humans may also be influenced by the quality of induced T cell responses, and, in particular, by the balance of discrete function subpopulations of T cells (subpopulations of
Th and Tc T cells) (92). These same T cell subsets also influence the nature and isotype distribution of antibody responses, and it could be that the spectrum of antibodies produced following exposure to a contact allergen might correlate with the extent of sensitization achieved.

The conclusion drawn is that, although some evidence is available to suggest that the acquisition of skin sensitization to organic contact allergens will normally be accompanied by the development of an antibody response, this has not yet been formally proved.

It is proposed that contact allergen-induced antibody production warrants further investigation, particularly in humans. Irrespective of whether anti-hapten antibodies influence the induction of skin sensitization, such antibody responses could provide informative correlates of the quality and vigour of the cell-mediated immune responses induced by contact allergens.

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