Transepidermal Induction of Contact Hypersensitivity in Mice with a Water-Soluble Hapten

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An experimental analysis has been conducted on the capacity of a highly-reactive, water soluble hapten, trinitrobenzene sulfonic acid, to induce contact hypersensitivity when applied epicutaneously to body wall skin of normal mice. Application of plastic chambers containing the hapten to murine skin for as little as 1 h produced readily detectable sensitization in several genetically disparate inbred strains. Moreover, the efficiency of sensitization was found to be similar to that following epicutaneous application of this hapten's lipid-soluble cogen, trinitrochlorobenzene. Using this approach, it has been determined that UVB radiation, intradermally injected tumor necrosis factor-alpha, and epicutaneously applied cis-urocanic acid can impair contact hypersensitivity induction by transepidermally delivered trinitrobenzene sulfonic acid, and that animals that fail to become sensitized proceed to acquire hapten-specific unresponsiveness. It is concluded that epicutaneous sensitization to chemically reactive, water-soluble molecules is experimentally attainable if precautions are taken to insure that contact between the hapten solution and the cutaneous surface is maintained for at least 1 h. Understanding the cellular and molecular mechanisms of sensitization and tolerance induction by epicutaneously applied water soluble hapten may prove to be important in understanding the pathogenesis of allergic contact dermatitis that develops to chemicals in the industrial setting, in the environment, and in the clinic. J Invest Dermatol 101:749–753, 1993

Contact hypersensitivity (CH) is a cell-mediated immune response that is elicited by applying relevant antigenic material to an epicutaneous surface of a previously sensitized individual. Because this inflammatory response can be elicited by environmental, industrial, or pharmaceutical agents, CH is a significant human affliction, and is an important dermatologic problem. For these reasons, experimentalists have studied the immunologic basis of CH in laboratory animals for almost 100 years [1,2]. Within the past 25 years, experimental analysis of CH has been particularly rewarding because it has been possible to induce and elicit the phenomenon in mice—a species highly favorable to immunologic analysis. Among the hapten that have been most commonly studied in this manner (such as trinitrochlorobenzene, dinitrofluorobenzene, oxazolone, and fluorescein isothiocyanate), virtually all are lipophilic, rather than water soluble. This property is thought to be important because lipophilic agents can more readily penetrate the lipid barrier of the stratum corneum [3]. In the induction of contact hypersensitivity, it is widely believed that epicutaneously applied hapten penetrates the stratum corneum, then proceed to derivatize epidermal proteins that are present in soluble form in the extracellular fluid of the intraepidermal space, and/or are expressed on the surfaces of Langerhans cells, keratinocytes, and any other cells within this space. Derivatized epidermal proteins are then endocyotized by epidermal Langerhans cells, processed intracellularly into hapten-peptide conjugates, and re-presented on the cell surface in association with class II MHC molecules encoded by the major histocompatibility complex (MHC). Eventually, the hapten-peptide conjugates complexed with class I or II MHC molecules are carried by mobile Langerhans cells so that the immunogenic moiety is presented to naive, hapten-specific, MHC-restricted T lymphocytes in draining regional lymph node(s). Subsequently, the T-cell effectors of CH that emerge from this activation are systemically disseminated [4].

In clinical medicine, the list of agents known to be capable of eliciting CH in human beings is large and diverse. Although agents that are lipophilic are well represented, some of the offending agents are water soluble (for example, formaldehyde). It is important therefore to understand the cellular and molecular mechanisms responsible for induction of CH with water soluble agents. Experimental studies of CH induced epicutaneously in mice and other laboratory animals by water soluble hapten has been very frequently reported. Perhaps because of the paucity of literature in this field, there is a general belief that water-soluble hapten do not usually sensitize via the skin, presumably because they can penetrate the stratum corneum only with great difficulty, and therefore do not achieve an immunizing concentration within the epidermal compartment.

We have attempted to circumvent experimentally the problem of hapten penetration through stratum corneum of murine skin by placing the water-soluble hapten trinitrobenzene sulfonic acid (TNBS) in a plastic chamber, which is then affixed directly to clean-shaved murine epidermal surfaces for one or more hours. Using this artifice we have been able to induce typical trinitrophenyl-specific CH in several different strains of mice. In addition, we have shown that acute low-dose ultraviolet (UV) B radiation prior to epicutaneous application of water-soluble hapten in an occlusive chamber prevents CH induction in UVB-susceptible strains

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of mice, and that these mice develop hapten-specific unresponsive­ness. Moreover, intradermal injection of tumor necrosis factor-alpha (TNFα) or UV-irradiated (cis) urocanic acid (UCA) prior to epicutaneous application of TNBS impaired CH induction. Finally, dose-response experiments have revealed that the amount of water­soluble TNBS required to induce CH via an occlusive chamber is very similar to the amount of lipophilic TNBC needed to induce CH by conventional epicutaneous application.

MATERIALS AND METHODS

Mice  Adult mice (8–16 weeks of age) of the following inbred strains—BALB/c, C57BL/6, C3H/HeN—were obtained from our domestic breeding facility, or purchased from Jackson Laboratories (Bar Harbor, ME). BALB/c has been designated a UVB-resistant strain, whereas C57BL/6 and C3H/HeN have been found to be UVB susceptible [5].

Reagents  TNBS was purchased from Sigma Co. (St. Louis, MO), and dissolved in phosphate-buffered saline (PBS); the pH of this solution was adjusted to 6.3 prior to epicutaneous application. Trinitrochlorobenzene (TNBC) was also purchased from Sigma, and dissolved in acetone as described elsewhere [6]. Urocanic acid (UCA), purchased in the trans isomorph from Sigma, was dissolved in PBS and DMSO, then irradiated with UVB to create the cis isomorph as described elsewhere [7]. Approximately 50–60% of the UCA was determined to be in the cis configuration after UVB exposure. Recombinant murine tumor necrosis factor-alpha (TNFα) was purchased from Genzyme Inc (Boston, MA); specific activity 4 × 10^7 U/mg, and diluted in PBS at 50 ng/0.4 ml for intracutaneous injection.

Methods of Epicutaneous Sensitization  Mice were anesthetized with pentobarbital; the fur was removed from abdominal wall skin with a dry razor blade. For sensitization with water-soluble TNBS, appropriate concentrations of TNBS in PBS were applied to the prepared area in an occlusive chamber (19 mm), Hill Top Research, Inc. (Cincinnati, Ohio). The chamber was held in place for one to six hours with a circumferential dressing, after which it was removed. For sensitization with TNBC, appropriate concentrations of TNBC in acetone were applied to the prepared area and allowed to air dry as previously described [6].

Elicitation of CH  Five days after abdominal application of hapten for sensitization, the dorsal surface of one ear of each mouse was challenged with 20 µl of 1% TNBC (200 µg). Ear thickness was measured with an engineer’s micrometer immediately before, and at 24 and 48 h after challenge with hapten. Negative control mice received an identical ear challenge without prior exposure to TNBS or TNBC. All experiments were repeated at least twice with similar results.

Ultraviolet B Radiation  Shaved abdominal skin was exposed to ultraviolet B radiation (UVB) from a bank of four FS-20 fluorescent lamps with a tube to target distance of 46 cm, as described previously [8]. Shaved abdominal wall skin was exposed to UVB on four consecutive days (40 J/m²/d). Hapten was applied epicutaneously within 1 h of the last exposure to UVB.

Assay for Unresponsiveness  Some mice that received epicutaneous hapten immediately following four daily doses of UVB were tested for hapten-specific unresponsiveness as follows. Fourteen days after initial exposure to hapten, a sensitizing dose of TNBC (3500 µg/50 µl) was painted on unirradiated, normal body wall skin. Five days thereafter their ears were challenged with dilute TNBC as described above.

Figure 1. Induction of contact hypersensitivity with TNBS in plastic chamber. Panels (five each) of BALB/c (a), C3H/HeN (b), and C57BL/6 (c) mice received epicutaneous application of 3500 µg TNBC directly (paint), 3500 µg TNBC via plastic chamber, or 3500 µg TNBS via chamber. All panels, plus negative controls, were ear challenged 5 d later with 200 µg TNBS in 20 µl acetone. Ear-swelling responses at 24 h are presented as mean ± SEM (µm). * and ** indicate means significantly greater than negative control with p < 0.0005 or 0.005, respectively.

Statistical Analysis of Data  The statistical significance of differences in the means of each experimental group was evaluated by a Student t test. Mean differences were considered to be significant when p < 0.05 or less.

RESULTS

Induction of Contact Hypersensitivity with Trinitrobenzenesulfonic Acid in an Occlusive Chamber  The conventional dose of TNBC used to sensitize adult mice epicutaneously is in the range of 3000 to 7000 µg [6]. We decided to begin our studies of the sensitizing capacity of the water-soluble hapten, TNBS, by placing 3500 µg TNBS dissolved in 50 µl PBS at pH 6.3, directly on cleanshaved abdominal skin of normal adult mice. Unfortunately, the aqueous solution ran off the cutaneous surface within less than 15 min, in part because the mice began to awaken from anesthesia and moved about. To circumvent this problem, we elected to incorporate the TNBS-PBS solution into an occlusive chamber of 2 cm diameter. The chamber was then applied directly to the dry shaved abdominal skin. We observed that the cutaneous surface to which an occlusive chamber containing TNBS had been applied for 60 or more min acquired a yellow stain that could not be removed by rinsing the skin with saline. This finding implies that a chemical reaction had taken place between the hapten and the epidermis, suggesting that hapten-protein conjugates may have formed in the epidermis. Accordingly, TNBS (3500 µg) in an occlusive chamber was applied to shaved abdominal skins of panels (five each) of BALB/c, C3H/HeN, and C57BL/6 mice. The chambers were held in place with adhesive dressings for 6 h. Positive control panels of the same three inbred strains of mice were sensitized in the conventional manner, i.e., each animal received an epicutaneous

Figure 2. Role of plastic chamber in sensitization with TNBS. Panels of BALB/c mice received epicutaneous application of 3500 µg TNBS in a plastic chamber, or without chamber (direct), for 1 h. Five days later the ears of these mice, and negative controls, were challenged and assayed as described in the legend to Fig. 1. * and ** indicate means significantly greater than negative controls with p < 0.005 and 0.005, respectively.
application of 3500 μg TNCB in 50 μl of acetone which was allowed to air dry. Separate panels of mice received 3500 μg TNCB in acetone that was incorporated into occlusive chambers and held in place for 6 h. The ears of all mice, including negative controls, were challenged with dilute TNCB (200 μg in 20 μl acetone) 5 d later. Results of representative experiments from this series are displayed in Fig 1a, b, c. In each strain of mice, 3500 μg TNBS applied with the aid of an occlusive chamber induced vigorous CH. The intensity of these ear-swelling responses was comparable to that evoked by TNCB applied epicutaneously—with or without the aid of a chamber. In separate experiments, it was possible to demonstrate that CH could be induced with 3500 μg TNBS in PBS, which was incorporated in a much smaller (1 cm diameter) occlusive chamber (data not shown). Subsequent experiments using chamber application times ranging from 10 min to 24 h indicated that at least 1 h was sufficient, and 6 h was optimal at achieving sensitization (data not shown).

Next, we attempted to sensitize BALB/c mice by applying TNBS in PBS (50 μl) directly to shaved abdominal skin without the aid of a plastic chamber. The mice were deeply anesthetized, placed supine, and immobilized. On one group of mice, 50 μl of TNBS in PBS was placed directly on the abdominal skin, and remained on the surface without the aid of a chamber for 1 h. In another group of mice, 50 μl of TNBS in PBS was incorporated into a chamber and placed on the skin surface for 1 h. When the ears of these mice were challenged with TNCB 5 d later, they displayed significant swelling responses, indicating that CH had been induced (see Fig 2). In fact, the intensity of the ear swelling of mice exposed to hapten without a chamber was similar to that of mice that received their initial exposure to TNBS via a chamber. This outcome indicates that the chamber itself is not essential for sensitization with a water-soluble hapten. Its utility appears to stem from its ability to maintain the liquid on the epicutaneous surface for an extended period of time. For the sake of convenience, in all subsequent experiments, plastic chambers were routinely used for epicutaneous application of TNBS in PBS.

In aggregate, these experiments permit us to conclude that mice can be sensitized through normal body wall skin with a water-soluble hapten if precautions are taken to ensure that the aqueous solution remains in contact with the skin surface for a sufficient period of time—at least 1 h, and preferably 6 h.

**Table I. Effect of UVB on Induction of Contact Hypersensitivity with Conventional Doses of TNBS (3500 μg)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Panel</th>
<th>UVB</th>
<th>TNBS</th>
<th>Ear Swelling (X 10⁻³ mm ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>1</td>
<td>−</td>
<td>+</td>
<td>91.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>54.4 ± 7.3</td>
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<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>18.8 ± 5.9</td>
</tr>
<tr>
<td>BALB/c</td>
<td>1</td>
<td>−</td>
<td>+</td>
<td>134.2 ± 12.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>131.0 ± 18.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>30.8 ± 4.0</td>
</tr>
</tbody>
</table>

* Four consecutive daily exposures to UVB (400 J/m²/d).

* Trinitrobenzene sulfonic acid (3500 μg in PBS) applied to epidermal surface, 5 mice per panel.

* Mean ear-swelling responses 24 h after challenge with 20 μl 1% TNCB.

* Panel 2 significantly less than panel 1, p < 0.0005.

**Table II. Effect of UVB on Induction of Contact Hypersensitivity with Optimal Sensitizing Doses of TNBS (35 μg)**

<table>
<thead>
<tr>
<th>Strain</th>
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<th>UVB</th>
<th>TNBS</th>
<th>Ear Swelling (X 10⁻³ mm ± SEM)</th>
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</thead>
<tbody>
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<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>16.4 ± 2.2</td>
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<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>18.5 ± 3.2</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>1</td>
<td>−</td>
<td>+</td>
<td>54.4 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>21.8 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>19.0 ± 1.4</td>
</tr>
<tr>
<td>BALB/c</td>
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<td>−</td>
<td>+</td>
<td>65.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>14.8 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
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<td></td>
<td>3</td>
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<td>30.8 ± 4.0</td>
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* Four consecutive daily exposures to UVB (400 J/m²/d).

* Trinitrobenzene sulfonic acid (3500 μg in PBS) applied to epidermal surface, 5 mice per panel.

* Mean ear-swelling responses 24 h after challenge with 20 μl 1% TNCB.

* Panel 2 significantly less than panel 1, p < 0.0005.
epicutaneous sensitization with a water soluble hapten is not only possible, but is similar in efficiency to that achieved with a lipid-soluble form of the same hapten. The following experiments were undertaken to explore other parameters of epicutaneous sensitization with water-soluble hapten leading to CH and its regulation.

**Effects of Acute, Low-Dose UVB Radiation on Induction of Contact Hypersensitivity with TNBS**

Development of an acute, low-dose regimen of UVB exposure prior to epicutaneous application of hapten has proved to be an informative means of studying the cellular basis of CH induction in mice [8], and other species including humans [9]. Using this approach, it has been discovered that the ability of UVB to impair CH induction in mice is genetically determined, with certain strains being UVB susceptible (C57BL/6, C3H/HeN) and others being UVB resistant (BALB/c) [5,10]. We next determined whether UVB radiation has a similar effect on CH induction when the sensitizing hapten is TNBS in aqueous solution, applied epicutaneously via plastic chamber. Shaved abdominal skin of panels of C57BL/6 and BALB/c mice was treated with four daily exposures of UVB (400 J/m²). Immediately thereafter chambers containing either 3500 or 35 µg TNBS were applied to the irradiated surface for 6 h. The animals were challenged 5 d later with TNCB to measure CH induction. Results of representative experiments from this series are summarized in Tables I and II. When 3500 µg TNBS was used in plastic chambers, UVB-exposed BALB/c mice developed intense CH, whereas UVB-exposed C57BL/6 mice mounted only feeble CH responses. These response patterns mimic the responses reported for UVB-treated mice of the same inbred strains that were painted on irradiated sites with 3500 µg of lipophilic TNCB. However, when only 35 µg TNBS was placed in the plastic chamber, UVB impaired the induction of CH in both UVB-resistant (BALB/c) and UVB-susceptible (C57BL/6) mice (Table II). These results are essentially identical to those recently obtained by Kurimoto and Stirewalt [11], who have determined that CH induction is impaired by UVB treatment in both UVB-susceptible and UVB-resistant strains of mice if an optimal (rather than excessive) sensitizing dose of dinitorfluorobenzene (DNFB) is used for epicutaneous application. Thus, the capacity of TNBS to sensitize (or not) through UVB-treated skin resembles that of lipophilic hapten, implying that epicutaneously applied hapten with widely differing solubility properties utilize similar cellular mechanisms for achieving CH induction.

**Induction of Hapten-Specific Unresponsiveness with TNBS Applied to UVB-Exposed Skin**

Another way to compare the immunologic characteristics of water-soluble and lipid-soluble hapten applied epicutaneously is to determine whether mice that are UVB susceptible are rendered tolerant if they first encounter hapten via UVB-exposed skin [8]. Accordingly, abdominal skin of panels of C57BL/6 mice was exposed to four consecutive daily treatments with UVB, and shortly thereafter 3500 µg TNBS in PBS was applied via plastic chamber to the exposed site. Positive control panels of mice received similar doses of TNBS via occlusive chamber on unirradiated body wall skin. Two weeks later, all panels of mice received 3500 µg TNBS in PBS on unirradiated dorsal body wall skin to test for unresponsiveness. When their ears were challenged 5 d later, mice that were first exposed to TNBS via UVB-treated skin mounted significantly lower ear-swelling responses than did positive controls (see Fig 4). Thus, water-soluble TNBS resembles lipophilic TNCB in its capacity to induce unresponsiveness when applied via plastic chamber to UVB-exposed skin.

### Figure 4

Effect of acute, low-dose UVB radiation on induction of unresponsiveness by TNBS. Panels of C57BL/6 mice received four consecutive daily exposures to UVB (400 J/m²/d). Shortly thereafter TNBS (3500 µg) was applied to the site via plastic chamber. Positive control mice received TNBS on unirradiated skin. Both sets of mice, plus a second "positive control" group, received an epicutaneous application of TNCB in acetone to dorsal body wall skin 14 d later. After 5 d, the ears of all mice, plus negative controls, were challenged with TNCB (200 µg). Ear-swelling responses presented as mean ± SEM (µm). * and ** indicate responses significantly greater than mean of mice exposed to UVB, p < 0.005 and <0.025, respectively.

### Figure 5

Effect of intradermal injections of TNFα or UV-UCA on CH induction by TNBS. Panels of BALB/c mice received intradermal injections of UV-UCA (200 µg) (A) or TNFα (50 ng) (B). Positive control mice received intradermal injections of PBS instead. Five and one hour later, respectively, TNBS (35 µg) in a plastic chamber was applied to these sites. For comparison, TNCB (35 µg) was applied epicutaneously to other recipients of intradermal injected TNFα. Five days later the ears of these mice, and negative controls, were challenged and assayed as described in the legend to Fig 1. Asterisks in A indicate responses significantly less than PBS + TNBS control, p < 0.05. In B, (b) is significantly less than (a), and (d) is significantly less than (c), p < 0.05.
Effects of Intradermal Injections of Tumor Necrosis Factor-Alpha or cis-Urocanic Acid on CH Induction by TNBS

We have reported that an intradermal injection of 50 ng recombinant murine tumor necrosis factor-alpha (TNFα) prior to epicutaneous painting of the site with a conventional sensitizing dose of DNFB significantly impairs CH generation [10]. Similarly, an intradermal injection of 200 μg UV-irradiated UCA (which contained approximately 50–60% UCA in the cis isomer) potently inhibited the induction of CH if DNFB was applied to the injected site [7]. It was of interest to determine whether either of these maneuvers would interfere similarly with CH induction by water-soluble TNBS. TNFα (50 ng) or UV-UCA (200 μg) was injected intradermally into abdominal wall skin of normal BALB/c mice. Positive controls received intradermal injections of PBS alone. Within 5 h after UV-UCA or 1 h after TNFα, 35 μg TNBS in PBS was placed in a plastic chamber and applied to the injected site for 6 h. CH was assayed by challenging the ears of these mice with TNCB 5 d later. The results, displayed in Fig 5, indicate that pretreatment of skin with TNFα or cis-UCA profoundly impaired the ability of that surface to support CH induction by water-soluble hapten applied epicutaneously.

DISCUSSION

The results of our experiments reveal that sensitization with a water-soluble hapten, leading to the acquisition of typical contact hypersensitivity, is readily achievable in mice—if the hapten is applied to the epidermal surface for a sufficient period of time. Using trinitrobenzene sulfonic acid as hapten applied via plastic chamber, we have been able to induce CH in several different inbred strains of mice. Moreover, we have determined in BALB/c mice that the minimum sensitizing dose of TNBS applied epicutaneously in this manner is approximately 35 μg, an amount very similar to that described by Sullivan et al [6] as the minimum sensitizing dose of the lipophilic form of the same hapten, TNCB, applied epicutaneously to mice of the same inbred strain. Thus, the efficiency of sensitization achieved with water-soluble TNBS applied via plastic chamber appears to be comparable to that displayed by the lipid-soluble hapten TNCB.

Our demonstration that mice can be sensitized to a water-soluble hapten leading to typical CH is not the first description of the phenomenon. Enander et al [12] in 1983 reported that BALB/c mice acquired CH when picroxyl sulfonic acid diluted in 70% ethanol was painted epicutaneously on the abdomen. Ethanol is known to alter the barrier properties of the stratum corneum [13]. Therefore, in the Enander experiments alcohol may have promoted entry of the hapten into the intraepidermal space. Because our hapten preparations do not contain ethanol, alteration of the stratum corneum by this agent cannot be invoked to explain our results. However, it is possible that, by applying soluble hapten in an aqueous solution to the epidermal surface, the barrier properties of the stratum corneum are nonetheless modified. It has been reported that constant exposure of the stratum corneum to an aqueous solution causes progressive hydration, thereby opening intercellular spaces through which hapten in aqueous phase can diffuse [14]. In our experiments, maintaining the aqueous solution containing hapten on the epidermal surface for 1 h with or without a chamber proved to be sufficient to induce CH. These results do not inform us concerning whether the epidermal barrier was altered by hapten in aqueous solution, but they do emphasize that the length of time that hapten in aqueous form is applied to the epidermal surface is important. Whatever the mechanism, our data imply that once hapten gains access to the intra-epidermal space, derivatization of soluble and cell-surface proteins proceeds—just as derivatization takes place in vitro when cell suspensions are exposed to TNBS [15]. It is worth pointing out that

Enander et al [12] also demonstrated that picroxyl sulfonic acid placed directly into the nares of mice induced CH. Moreover, Stein-Streilein [16] has instilled TNBS in PBS intraocularly into hamsters as a means to achieving systemic CH. The surfaces of the upper and lower respiratory tracts are wettable, and readily permit (or perhaps even promote) the transfer of water-soluble molecules from the lumen into the living epithelium.

Our findings indicate that once the barrier of the skin is breached, soluble hapten, such as TNBS, not only induce CH, but that the process by which CH is induced is susceptible to modification by UVB radiation and local injections of TNFα and cis-UCA. The responses of UVB-susceptible and UVB-resistant mice to chamber-applied TNBS after UVB radiation are indistinguishable from the responses of mice exposed to TNCB after UVB exposure. Intradermal injections of TNFα or cis-UCA impair CH induction, whether the applied hapten is TNCB or TNBS. The ability to sensitize mice to water-soluble haptenes may have relevance to contact dermatitis in humans. If our findings can be generalized to other water-soluble compounds that have the potential to function as hapten, then there may be situations in the work place, in the environment, and in the clinic where individuals whose skin is exposed sufficiently may become sensitized to water-soluble haptenes.

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REFERENCES


