

Modulation of Fatty Acid Oxidation Alters Contact Hypersensitivity to Urushiols: Role of Aliphatic Chain β -Oxidation in Processing and Activation of Urushiols

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Lithraea caustica, or litre, a tree of the Anacardiaceae family that is endemic to the central region of Chile, induces a severe contact dermatitis in susceptible human beings. The allergen was previously isolated and characterized as a 3-(pentadecyl-10-enyl) catechol, a molecule belonging to the urushiol group of allergens isolated from poison ivy and poison oak plants. Because urushiols are pro-electrophilic haptens, it is believed that the reactive species are generated intracellularly by skin keratinocytes and Langerhans cells. The active species are presumed to modify self proteins which, after proteolytic processing, would generate immunogenic peptides carrying the hapten. The presence of a 15-carbon-length hydrophobic chain should impair antigen presentation of self-modified peptides by class I MHC molecules, either by steric hindrance or by limiting their sorting to the ER lumen. We have proposed that the short-

ening of the aliphatic chain by β -oxidation within peroxisomes and/or mitochondria should be a requirement for the antigen presentation process. To test this hypothesis we investigated the effect of drugs that modify the fatty acid metabolism on urushiol-induced contact dermatitis in mice. Clofibrate, a peroxisomal proliferator in mice, increased the immune response to the urushiols from litre by 50%. Conversely, tetradecyl glycidic acid, an inhibitor of the uptake of fatty acids by mitochondria, decreased the hypersensitivity to the hapten. An increase in the level in glutathione by treatment of the animals with 2-oxotiazolidin-4-carboxylic acid lowered the response. Those findings strongly support a role for the fatty acid oxidative metabolism in the processing and activation of urushiols *in vivo*. **Key words:** allergy/clofibrate/dermatitis/litre/rhus. *J Invest Dermatol* 108:57-61, 1997

Allergic contact dermatitis induced by contact sensitizers or haptens has been historically classified as a delayed hypersensitivity-type response on the basis of the time course of the reaction (Benezra and Dupuis, 1983) and the involvement of cell-mediated immunity (Meltzer *et al*, 1989; Benezra, 1990). The cognitive phase of these reactions is thought to be initiated by the covalent bonding of the hapten via electrophilic attack on amino acid residues of self-proteins. The modified self-proteins, after antigenic processing by skin Langerhans cells, generate non-self-like immunogenic peptides that trigger the specific response in the context of the major histocompatibility complex (MHC) molecules in the lymph

nodes (Benezra and Dupuis, 1983). At the effector phase of the contact dermatitis keratinocytes, in addition to Langerhans cells, participate by presenting self-modified immunogenic peptides to specific T lymphocytes (Kalish, 1991). The nature of some murine self-modified peptides involved in experimental contact dermatitis has been recently described for electrophilic haptens such as dinitrofluorobenzene and trinitrochlorobenzene (Martin and Weltzien, 1994). From these studies it is clear that, for most of the T-cell clones, the hapten is the immunodominant group, whereas the backbone of the immunogenic peptide participates in the binding to the MHC molecule (Martin *et al*, 1993; Cavani *et al*, 1995).

The role of different populations of T cells in contact dermatitis has been controversial. Earlier studies supported the notion that CD4⁺ cells would be the effectors in contact dermatitis as described for other delayed type hypersensitivity reactions (Ptak *et al*, 1991; Waldorf *et al*, 1991), but *in vivo* experiments using β -2 microglobulin knockout mice (Bour *et al*, 1995) and immunodepleted mice (Gocinski and Tigelaar, 1990) indicate that CD8⁺ lymphocytes are the major effector cells *in vivo* for the response to dinitrofluorobenzene in those animals. Furthermore, CD8⁺ cells can differentiate to effector cells in the absence of CD4⁺, because knockout mice for class II MHC and CD4⁺-depleted mice can develop a stronger specific contact dermatitis response than normal mice (Gocinski and

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Abbreviations: DNCB, dinitrochlorobenzene; OTC, 2-oxotiazolidin-4-carboxylic acid; PDC, 3-pentadecyl (10-enyl) catechol; TDGA, tetradecyl glycidic acid.

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Tigelaar, 1990; Bour *et al*, 1995). In these experiments, CD4⁺ T cells seem to play a dual role, acting as suppressor and contrasuppressor in the system. Currently, the cellular basis of contact dermatitis suggests that the endocytic pathway of antigen processing and presentation is the major determinant in the development of this kind of hypersensitivity (Kalish *et al*, 1994).

Urushiols, the contact sensitizers secreted by plants belonging to the Anacardiaceae family (Gillis, 1971; Dawson, 1973; Gambaro *et al*, 1986), are responsible for severe occupational dermatitis in densely populated areas (Johnson *et al*, 1972; Benezra, 1990). Urushiols are a family of lipophilic molecules consisting of a catechol ring substituted with a C15 or C17 hydrocarbon chain at position 4 (Johnson *et al*, 1972; Gambaro *et al*, 1986). In contrast to electrophilic haptens, urushiols cannot modify self-proteins directly; therefore, they must first be activated to an electrophilic reactive species by the host. The most accepted model for activation of urushiols is their oxidation to a quinone intermediate (Schmidt *et al*, 1990). *In vitro*, these reactive species can attack nucleophilic groups of proteins. The role of oxidative enzymes in the activation of urushiols *in vivo* is not clear. Also, it has been proposed that this activation may involve the participation of free radical intermediates (Schmidt *et al*, 1990).

It has been reported that, in humans, both the size of the chain and the number of unsaturated bonds influence the immunogenicity of these molecules. Aliphatic chains with 15 to 17 carbons are more immunogenic than shorter ones (Keil *et al*, 1944; Johnson *et al*, 1972). Moreover, the monoolefin and diolefin are more allergenic than the saturated chains. The findings indicate that both the lack of saturation and the length of the aliphatic chain play an important role in the immunogenicity of urushiols.

It is generally accepted that the aliphatic chain of urushiols allows the hapten to bind and to be inserted on the cytoplasmic membrane of epidermal cells, which allows the allergen to react with membrane proteins and/or to be internalized by skin cells for activation (Benezra, 1990; Kalish, 1991). Little attention has been paid, however, to the effect of the bulky aliphatic chain on antigen processing and presentation of urushiol-modified self-proteins. We propose that the activation of urushiols by antigen-presenting cells involves the partial or complete shortening of the hydrocarbon chain by means of the fatty acid oxidative metabolism. This hypothesis associates the catabolism of the aliphatic chain with the activation of the catecholic ring to the reactive quinone or the radical intermediate, either in peroxisomes or mitochondria. To test this model for the activation and processing of urushiols, we investigated the effect of drugs that influence fatty acid metabolism on the onset of the contact sensitivity induced by those allergens in mice. In accord with our hypothesis, clofibrate, a peroxisomal and P-450 inducer, enhances the response to urushiol. Conversely, tetradecyl-glycidic acid (TDGA), an inhibitor of fatty acid uptake by mitochondria, decreases the response, and 2-oxo-2-thiazolidine carboxylate (OTC), a stimulator of the glutathione synthesis, also decreases the intensity of the inflammatory reaction.

MATERIALS AND METHODS

Experimental Animals Balb/c mice (Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas and BIOSONDA S.A.) 1 to 2 mo of age and maintained under approved institutional guidelines were used.

Allergens 3-Pentadecyl (10-enyl) catechol (PDC) was extracted with petroleum ether from air-dried stem, bark, and leaves of *Lithraea caustica* from trees grown in the Olmué area (V region of Chile) in 1995. The extract containing urushiols was purified by silica gel column chromatography as described elsewhere (Gambaro *et al*, 1986). The product was shown to be homogeneous by thin layer chromatography. Dinitrochlorobenzene was of pharmacologic grade.

Contact Sensitization Unless otherwise indicated, mice were painted with 50 μ l of a solution containing the allergen in chloroform, distributed on 0.75 cm² of shaved abdomen. We used stock solutions of 1% PDC and 5% dinitrochlorobenzene (DNCB).

Testing for Contact Sensitivity After measurement of the baseline thickness of both ears, mice were challenged with 10 μ l of either 0.5% PDC

or 0.6% DNCB dissolved in chloroform applied to both sides of one ear; the other ear was used as control, being treated only with the solvent. This basic protocol was used with minor modifications depending upon specific experiments. The response to PDC and DNCB was determined by measuring the ear thickness using an engineering micrometer (model 7326, Mitutoyo, Dolla Eastern Co. 36-08 48th Avenue, Long Island City, NY) equipped with a gauge dial model 2804. The ear-swelling response was calculated by subtracting the ear thickness prior to testing from the thickness observed during the experiment. The control ear was challenged with chloroform alone. The results are expressed as mean increases in ear thickness \pm SEM for each group of mice over baseline values in microns. All experimental groups comprised five animals.

Drugs Clofibrate, Azionyl (ethyl p-chloro phenoxy butirate), was from Imperial Chemical Industries, Pharmaceuticals (Macclesfield, Cheshire, England). TDGA from McNeil Pharmaceutical (Spring House, PA) was a generous gift from Dr. Federico Leighton (Facultad de Ciencias Biológicas, Pontificia Universidad Católica). OTC was purchased from Sigma Chemical Co. (St. Louis, MO).

Enzyme Assays The activity of the peroxisomal catalase was determined as described by Leighton *et al* (1968). Fatty acid oxidase was measured as described by Inestrosa *et al* (1979).

Pre-treatment with Drugs

Clofibrate Animals were sensitized as described above. The drug treatment was started 8 d after PDC sensitization, consisting of six daily intraperitoneal injections of 20 mg of clofibrate in 200 μ l of olive oil. Control animals received 200 μ l of olive oil. In parallel, another group of mice was sensitized with DNCB, as described above, and submitted to the same pharmacologic treatment. Fourteen days after sensitization the mice were challenged with PDC or DNCB, and the contact dermatitis response was measured as described above.

TDGA Animals were sensitized as described above. Drug treatment was started 8 d after PDC sensitization, consisting of four doses of 400 μ g of TDGA dissolved in 200 μ l of a solution of bovine serum albumin-10 mg 10% n-butanol per ml in water. The injections were administered at 8-h intervals (24-h treatment). Control animals received 200 μ l of a solution of bovine serum albumin in 10% n-butanol in water. In parallel, another group of mice was sensitized with DNCB, as described above, and submitted to the same pharmacologic treatment. Six hours after the first dose of TDGA, mice were challenged with PDC or DNCB, and the contact dermatitis response was measured.

OTC Animals were sensitized as described above and treated with OTC by following published methods (Williamson and Meister, 1981; Schmidt *et al*, 1990). Drug treatment started 7 d after sensitization with PDC. Mice were injected twice a day (12-h intervals) with 10 mg of neutralized OTC dissolved in 100 μ l of distilled water. The treatment was extended for 8 d, and controls received 100 μ l of distilled water. In parallel, another group of mice was sensitized with DNCB and submitted to the same pharmacologic treatment. At day 14, when the last administration of OTC was given to mice, the animals were challenged respectively with PDC or DNCB, and the contact dermatitis response was measured.

RESULTS

Time Course of the Contact Sensitivity to Urushiols Protocols that lead to the induction of contact dermatitis to haptens such as dinitrofluorobenzene (Gocinski and Tigelaar, 1990; Waldorf *et al*, 1991) are well established, but there are few reports describing the conditions for effective sensitization of mice to urushiols (Dunn *et al*, 1982). The time course of the contact dermatitis in mice previously sensitized to PDC is shown in **Fig 1**. The response is characterized by ear redness and swelling, and the thickness of the ear increases up to 72 h post-challenge. Primary epicutaneous exposure of mice to urushiols generates an inflammatory process of about 20% of the secondary response. Naïve mice sensitized to DNCB by epicutaneous sensitization on the abdomen show a primary inflammatory response to PDC after a challenge. In contrast to guinea pigs and human beings, mice show a strong innate response to PDC and other contact sensitizers (data not shown).

The presence of an inflammatory response to contact sensitizers in naive mice may be explained by the release of tumor necrosis factor- α and interleukin-1 by keratinocytes (Hass *et al*, 1992); these inflammatory cytoquines may play an important role in the early

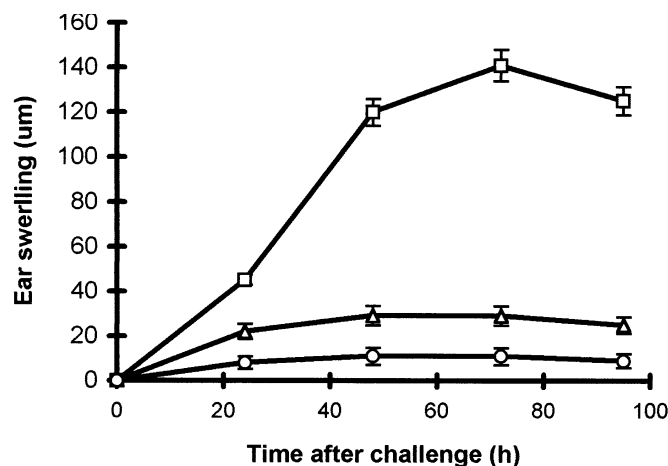


Figure 1. Kinetics of the contact dermatitis to PDC in mice. Each experimental group consisted of five mice. Mice were sensitized with PDC on the abdominal skin and challenged 5 d later (\square - \square -) and naive mice (∇ - ∇ -) were challenged with PDC as described in *Materials and Methods*. Mice challenged with pure solvent (\circ - \circ -). The results are expressed as mean increments in ear thickness (micrometers \pm SEM) at various times after exposure to PDC.

response to immunogenic or inflammatory signals *in vivo*. In addition, it has been shown that keratinocytes exposed to urushiols increase the expression of adhesion molecules, enhancing the inflammatory response (Barker, 1992).

Contact Sensitivity to PDC Is Augmented in Mice Pre-treated with Clofibrate This drug is a potent peroxisomal proliferator in mice (Lazarow, 1978; Foxworthy *et al*, 1990). The effectiveness of the drug treatment in inducing peroxisome proliferation was assessed by determining the activity of catalase and fatty acid oxidase, two peroxisomal enzymes. These enzymes increased in activity 50% and 570% over the control animals, confirming the efficacy of the treatment (data not shown). The inflammatory response to PDC of pre-treated animals (Fig 2) was 50% higher

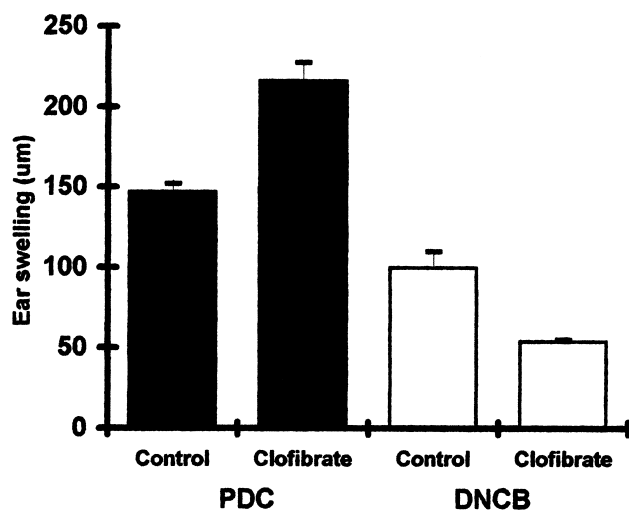


Figure 2. Clofibrate increases contact dermatitis to PDC (solid bars) and DNCB (open bars). Mice sensitized with either PDC or DNCB were treated with clofibrate and challenged with the respective allergen as described in *Materials and Methods*. Control animals were submitted to the same immunization and challenge protocol but treated only with the drug vehicle. The results are expressed as mean increments in ear thickness (micrometers \pm SEM) at 24 h after challenge.

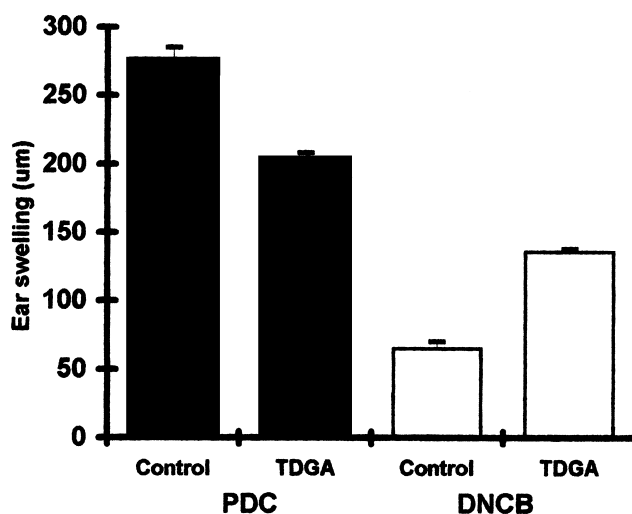


Figure 3. TDGA decreases contact dermatitis to PDC (solid bars) and DNCB (open bars). Mice sensitized with either PDC or DNCB were treated with TDGA and challenged with the respective allergen as described in *Materials and Methods*. Control animals were submitted to the same immunization and challenge protocol but treated only with the drug vehicle. The results are expressed as mean increments in ear thickness (micrometers \pm SEM) at 24 h after challenge.

than that of the control mice group treated with the vehicle ($p < 0.01$). The maximum difference was observed between 48 and 72 h after challenge. This pharmacologic effect was specific for the PDC response, because clofibrate-pre-treated mice, sensitized and challenged with DNCB, showed a significantly decreased response (Fig 2).

Contact Sensitivity to PDC Is Decreased in Mice Pre-treated with TDGA The effect of TDGA in contact hypersensitivity to PDC is shown in Fig 3. Mice sensitized with PDC and treated with TDGA, an inhibitor of the palmitoyl carnitine transferase (Skorin *et al*, 1992), show a significant decrease in the inflammatory response after challenge with the allergen, compared to animals treated with vehicle only. At 24 h after challenge, treated mice reached only 74% of the response of the control group (Fig 3). This difference is statistically significant ($p < 0.001$). In contrast to the inhibitory effect of TDGA on the response to PDC, this drug potentiates the inflammatory response to DNCB up to 100% over the control group (Fig 3) ($p < 0.001$), indicating that the inhibitory effect of TDGA is specific for the contact dermatitis response to PDC.

Contact Sensitivity to PDC Is Decreased in Mice Pre-treated with 2-Oxo-2-thiazolidine Carboxylate Figure 4 shows the effect of OTC, a drug that increases the level of intracellular glutathione, on the contact dermatitis response in mice (Williamson and Meister, 1981; Schmidt *et al*, 1990). The drug treatment decreased the response to PDC in 65% ($p < 0.05$) with respect to the control group. This effect seemed to be specific for the response to PDC, because the response to DNCB in mice was not significantly affected by the administration of OTC.

DISCUSSION

Contact dermatitis due to pro-electrophilic haptens belonging to the family of urushiols is the major cause of occupational dermatitis in widespread rural areas of the world (Benezra and Dupuis, 1983; Benezra, 1990). Since the early work of Baer *et al* (1966), little has been learned about the immunochimistry and the *in vivo* activation of these molecules during the cognitive and effector phases of the Rhus dermatitis. Pioneering work from the group of Kalish and associates (Kalish and Morimoto, 1988; Kalish and Morimoto,

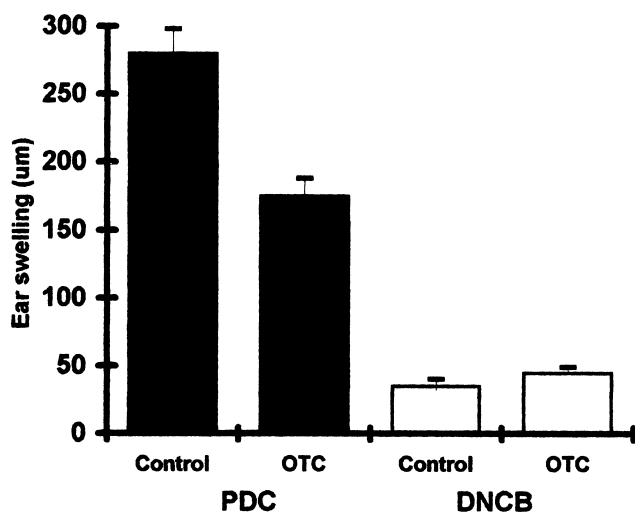


Figure 4. OTC decreases contact dermatitis to PDC (solid bars) and DNCB (open bars). Mice sensitized either with PDC or DNCB were treated with OTC and challenged with the respective allergen as described in *Materials and Methods*. Control animals were submitted to the same immunization and challenge protocol but treated only with the drug vehicle. The results are expressed as mean increments in ear thickness (micrometers \pm SEM) at 24 h after challenge.

1989) indicated that most of the T-cell lines derived from sensitized individuals belonged to the CD8⁺ phenotype, suggesting that the activation of urushiols occurs mainly via the endogenous pathway of antigen presentation. Thus, CD8⁺ effector T cells should play a critical role at the effector phase of the response in humans. Furthermore, the same authors demonstrate that *in vitro*, urushiol-self-modified peptides can be presented via the endogenous pathway to CD8⁺ positive T cells as well as via the endocytic pathway to CD4⁺ T cell clones (Kalish *et al*, 1994).

Because it is difficult to experimentally manipulate the human immune system *in vivo* and to maintain human T cell clones *in vitro* (Meinl *et al*, 1995), it has been difficult to address key questions regarding contact dermatitis. Those questions include the role of (i) unsaturation of the aliphatic chain in the allergenicity of urushiols; (ii) the mechanisms and metabolic pathways involved in urushiol activation; (iii) the processing of PDC-self-modified peptides by antigen-presenting cells.

To address those questions we developed a murine system to test *in vivo* the effect of drugs that interfered with fatty acid metabolism and redox potential on the immune response to PDC *in vivo*. Consistent with our hypothesis, clofibrate, a potent P-450 and peroxisomal inducer in mice (Lazarow, 1978; Foxworthy, 1990), specifically potentiated the response to PDC, suggesting that the activity of the P-450 system and/or peroxisomal activity are involved in PDC activation. Most of the studies describing the role of peroxisomes in fatty acid metabolism, as well as the effect of peroxisomal proliferators, have been performed in murine hepatocytes. In addition, the presence of peroxisomes in the cell line J-774.3, derived from antigen-presenting cells (Ralph *et al*, 1975; Sein *et al*, 1993), has been recently described (Couve *et al*, 1992). The authors demonstrated that the addition of *cis*-clofibrate to the culture medium increased the number of peroxisomes and the activity of catalase and fatty acid oxidase on the J-774.3 line, supporting a possible role for peroxisomes in antigen-presenting cells. The small DNCB-associated decrease in inflammation observed in clofibrate-treated mice may be due to the increased degradation of prostaglandins by peroxisomes, as has been described recently for hepatocytes (Brass and Ruff, 1991).

In addition to immunogenic factors, members of the P-450 family have been involved in allergic reactions to several drugs

(Mauri-Hellweg *et al*, 1995). For sulfonamides and aromatic anti-convulsants such as phenytoin and carbamazepin, it has been suggested that the metabolism of the drugs into a reactive intermediate by P-450 is a crucial step in the generation of immunogenic adducts. The responder phenotype has been associated with a high level of expression of components of the P-450 system because of a polymorphism in the promoter controlling the expression of this cytochrome. This factor, in conjunction with a slow acetylator phenotype, generates high levels of reactive drug intermediaries that can modify self-proteins (Mauri-Hellweg *et al*, 1995).

The inhibitory effect of OTC observed by us is in keeping with the work of Schmidt *et al* (1990) on the response to urushiols and support a scavenger role for glutathione over toxic oxidative compounds generated inside the cell (Reed, 1990); also, a high level of glutathione may prevent an efficient conversion of the catechol ring to the reactive quinone by lowering the redox potential.

The inhibition of the response to PDC after treatment of mice with TDGA suggests that the metabolism of urushiols by mitochondria is important in the generation of a reactive intermediate necessary to modify self-peptides. TDGA is a potent inhibitor of carnitine palmitoyl transferase, a key enzyme involved in the entry of fatty acids into the mitochondria, and for carnitine acyltransferase, a translocase involved in the transportation of short fatty acids to the mitochondria (Skorin *et al*, 1992). A further peculiar finding is that TDGA potentiated the response to the electrophilic hapten DNCB. Because TDGA is an inhibitor of the entry of fatty acid to the mitochondria, it may lower the catabolism of inflammatory mediators such as prostaglandins and leukotrienes after peroxisomal β -oxidation (Schepers *et al*, 1988), thus augmenting the inflammatory response in a nonspecific fashion.

Our results, taken together, strongly suggest that fatty acid metabolism is involved in the *in vivo* activation of urushiols. The specific role of members of the microsomal P-450 system, however, the participation of the peroxisomal β -oxidation in the catabolism of the aliphatic chain, and the involvement of those organelles in the intracellular traffic and activation of urushiols by antigen-presenting cells and target cells must be analyzed further in cell-free systems to determine the metabolic intermediaries responsible for the modification of self-proteins. Preliminary results show that PDC is metabolized to H₂O₂ by isolated rat hepatocytes, suggesting that the compound may be a substrate for peroxisomal catabolism (data not shown).

Antigen processing and presentation of endogenous peptides require the participation of proteolytic enzymes and transporter-associated proteins that finally deliver the immunogenic peptides to nascent MHC class I molecules at the lumen of the endoplasmic reticulum (Germain, 1994). It is well known that class I antigens are able to present peptides of about eight to 10 amino acids in length (Ploegh and Benaroch, 1993) and that transporter-associated proteins also contribute to the specificity by transporting peptides of a similar size and specific physicochemical properties (Germain, 1994). Therefore, it is unlikely that a self-modified peptide bearing the bulky aliphatic chain from urushiols may be efficiently processed, transported, and loaded onto the MHC class I antigen-presenting cavity via transporter-associated protein-dependent processing. In this context, the shortening of the long aliphatic chain of urushiols by intracellular metabolic machinery may increase the efficiency of processing and presentation of urushiol-modified self-peptides by reducing the steric hindrance of the self-modified peptides at different levels of the process.

Finally, better understanding of the pharmacogenetic and immunologic factors involved in Rhus dermatitis may help to develop desensitizing strategies and to control the onset of the allergy in a more specific way. Moreover, because the basic mechanisms involved in contact hypersensitivities are similar to those of several autoimmune and viral diseases, the elucidation of these mechanisms might contribute to a better knowledge of the pathologic mechanisms of some human diseases.

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REFERENCES

- Baer H, Watkins RC, Bowser RT: Delayed contact sensitivity to catechols and resorcinols. The relationship of structure and immunization procedure to sensitizing capacity. *Immunochemistry* 3:479-485, 1966
- Barker JN: Role of keratinocytes in allergic contact dermatitis. *Contact Dermatitis* 26:145-148, 1992
- Benezra C: Molecular recognition in allergic contact dermatitis to natural products. *Pure Appl Chem* 62:1251-1258, 1990
- Benezra C, Dupuis G: L'allergie de contact. *La Recherche* 14:1062-1072, 1983
- Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D, Revillard JP, Nicolas JF: Major histocompatibility complex class I-restricted CD8⁺ T cells and class II-restricted CD4⁺ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *Eur J Immunol* 25:3006-3010, 1995
- Brass EP, Ruff LJ: Effect of clofibrate treatment on hepatic prostaglandin catabolism and action. *J Pharmacol Exp Ther* 257:1034-1038, 1991
- Cavani A, Hackett CJ, Wilson K, Rothbard JB, Katz SI: Characterization of epitopes recognized by hapten-specific CD4⁺ T cells. *J Immunol* 154:1232-1238, 1995
- Couve AO, König C, Santos MJ: Induction of peroxisomal enzymes and a 54 Kd. peptide in cultured mouse macrophages treated with clofibrate. *Exp Cell Res* 202:541-544, 1992
- Dawson CR: Final report on the chemistry of poison ivy (Contract PH-43-64-76), US Department of Commerce. National Technical Information Service, Springfield, VA, 1973
- Dunn IS, Liberato DJ, Dennik R, Castagnoli N, Byers VS: A murine model for contact sensitization to poison oak or ivy urushiol components. *Cell Immunol* 68:377-388, 1982
- Foxworthy PS, Withe SL, Hoover DM, Eacho PI: Effect of ciprofibrate, bezafibrate and LY171883 on peroxisomal β -oxidation in cultured rat, dog, and rhesus monkey hepatocytes. *Toxicol Applied Pharmacol* 104:386-394, 1990
- Gambaro V, Chamy MC, von Brand E, Garbarino JA: 3-(Pentadec-10-enyl)-catechol, a new allergenic compound from *Lithraea caustica* (Anacardiaceae). *Planta Med* 15:20-22, 1986
- Germain RN: MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76:287-299, 1994
- Gillis W: The systematics and ecology of poison ivy and poison oak. *Rhodora* 74:465-470, 1971
- Gocinski BL, Tigelaar RE: Roles of CD4⁺ and CD8⁺ T cells in murine contact sensitivity revealed by *in vivo* monoclonal antibody depletion. *J Immunol* 144:4121-4128, 1990
- Haas J, Lipkow T, Mohamadzadeh M, Kolde G, Knop J: Induction of inflammatory cytokines in murine keratinocytes upon *in vivo* stimulation with contact sensitizers and tolerizing analogues. *Exp Dermatol* 1:76-83, 1992
- Inestrosa NC, Bronffan M, Leighton F: Detection of peroxisomal fatty acyl-coenzyme A oxidase activity. *Biochem J* 182:779-788, 1979
- Johnson RA, Baer H, Kirkpatrick CH, Dawson CR, Khurana RG: Comparison of the contact allergenicity of the four pentadecylcatechols derived from poison ivy urushiol in human subjects. *J Allergy Clin Immunol* 49:27-35, 1972
- Kalish RF, Wood JA, LaPorte A: Processing of urushiol (poison ivy) hapten by both endogenous pathways for presentation to cells *in vitro*. *J Clin Invest* 93:2039-2047, 1994
- Kalish RS: Recent developments in the pathogenesis of allergic contact dermatitis. *Arch Dermatol* 127:1558-1563, 1991
- Kalish RS, Morimoto C: Urushiol (poison ivy)-triggered suppressor T cell clone generated from peripheral blood. *J Clin Invest* 82:825-832, 1988
- Kalish RS, Morimoto C: Quantitation and cloning of human urushiol specific peripheral blood T- cells: isolation of urushiol triggered suppressor T- cells. *J Invest Dermatol* 92:46-52, 1989
- Keil H, Wasserman D, Dawson C: The relation of chemical structure in catechol compounds and derivatives of poison ivy hypersensitivity in man as shown by the patch test. *J Exp Med* 80:275-287, 1944
- Lazarow PB: Rat liver peroxisomal system of fatty acid β -oxidation: elevation by clofibrate and other hypolipidemic drugs. In: Carlson LA, Padetti R, Sirtori CR, Weber G (eds.) International Conference on Atherosclerosis. Raven Press, New York, pp 15-17, 1978
- Leighton F, Pooley B, Beaufay H, Baudhuin P, Coffey J, Fowler S, de Duve C: Automated method for the large scale isolation and analysis of peroxisomes and lysosomes from liver of rats injected with Triton WR 1339. *J Cell Biol* 37:482-514, 1968
- Martin S, von Bonin A, Fessler C, Pflugfelder U, Weltzien HU: Structural complexity of antigenic determinants for class I MHC-restricted, hapten-specific T cells: two qualitatively different types of H-2K^b restricted TNP epitopes. *J Immunol* 151:678-687, 1993
- Martin S, Weltzien HU: T cell recognition of haptens, a molecular view. *Int Arch Allergy Immunol* 104:10-16, 1994
- Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ: Activation of drug-specific CD4⁺ and CD8⁺ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepin. *J Immunol* 155:462-472, 1995
- Meinl E, Hohlfeld R, Wekerle H, Fleckenstein B: Immortalization of human T cells by *Herpesvirus saimiri*. *Immunol Today* 16:55-58, 1995
- Meltzer MS, Nacy CA: Delayed-type hypersensitivity and the induction of activated, cytotoxic macrophages. In: Paul WE (ed) *Fundamental Immunology*. 2nd ed. Raven Press, New York, pp 765-777, 1989
- Ploegh H, Benaroch P: Immunology of MHC class II dimer or dimers. *Nature* 364:16-17, 1993
- Ptak W, Herzog WR, Askenase PW: Delayed-type hypersensitivity initiation by early-acting cells that are antigen mismatched or MHC incompatible with late-acting, delayed-type hypersensitivity effector T cells. *J Immunol* 146:469-475, 1991
- Ralph P, Prichard J, Cohn M: Reticulum cell sarcoma: an effector cell in antibody dependent immunity. *J Immunol* 114:898-905, 1975
- Reed DJ: Glutathione: toxicological Implications. *Annu Rev Pharmacol Toxicol* 30:603-631, 1990
- Schepers L, Casteels M, Vamecq J, Parmentier G, van Vedhoven PP, Mannaerts GP: β -oxidation of the carboxyl side chain of prostaglandins E₂ in rat liver peroxisomes and mitochondria. *J Biol Chem* 263:2724-2731, 1988
- Schmidt RJ, Khan L, Chung LY: Are free radicals and not quinones the haptenic species derived from urushiols and other contact allergenic mono and dihydric alkylbenzenes? The significance of NADH, glutathione, and redox cycling in the skin. *Arch Dermatol Res* 282:56-64, 1990
- Sein J, Cachicas V, Becker MI, De Ioannes AE: Mucin allows survival of *Salmonella typhi* within mouse peritoneal macrophages. *Biol Res* 26:372-380, 1993
- Skorin C, Necochea C, Johow V, Soto U, Grau AM, Bremer J, Leighton F: Peroxisomal fatty acid oxidation and inhibitors of the mitochondrial carnitine palmitoyltransferase I in isolated rat hepatocytes. *Biochem J* 281:561-567, 1992
- Waldorf HA, Walsh LJ, Schechter NM, Murphy GF: Early cellular events in evolving cutaneous delayed hypersensitivity in humans. *Am J Pathol* 138:477-486, 1991
- Williamson JM, Meister A: Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-proline substrate. *Proc Natl Acad Sci USA* 78:936-939, 1981