

Delayed-type hypersensitivity skin testing: variance among Trichophyton species extracts

Dear Sir,
Assessment of cellular immune response by delayed-type hypersensitivity skin testing requires quality materials. Fungal antigens are often used for this testing. Recently, *Candida albicans* extracts have been shown not to meet minimal standards (1). We wondered whether a similar problem existed for Trichophyton extracts.

Delayed-type hypersensitivity skin testing provides important information about cellular aspects of the immune system and the ability to process and present antigen to cellular effector cells. Certain delayed-type skin testing materials are regulated by the Center of Biologics Evaluation and Research of the Food and Drug Administration to ensure consistent potency. However, among Trichophyton extracts, only one product, the Merieux Multi-test CMI is currently licensed for this use. It delivers Trichophyton extract by percutaneous puncture.

However, many physicians have preferred standard intradermal technique because more predictable amounts of antigen are administered intracutaneously. Moreover, since fungal species are antigenically diverse, this variance

may also affect comparability. Thus, equivalence is difficult to ensure.

With antigens not licensed for delayed-type hypersensitivity skin testing, practitioners generally selected materials licensed for immediate skin testing or for allergy immunotherapy. We therefore investigated the potency of nine commercially available Trichophyton products. Potency and reactivity were assessed *in vitro* and with delayed-type hypersensitivity skin testing. Isoelectric focusing and laser densitometry were used to assess composition (2, 3, 4). Four healthy adult subjects were skin tested after obtaining informed consent under a protocol approved by the Walter Reed Army Medical Center Department of Clinical Investigation/Institutional Review Board.

Skin induration, banding with isoelectric focusing, and absorbance with laser densitometry showed these products to be variable. The data are displayed in the accompanying table. At one extreme, Product 1 failed to produce a positive skin test in any of the four patients. At the other extreme, Products 6, 7 and 8, gave positive skin tests in all four patients. Interme-

Table

Commercial extracts of Trichophyton species show varied activity as delayed hypersensitivity skin test agents

Products	CMI ¹	1	2	3	4	5	6	7	8
Induration diameter at 48 h in mm									
Patient	1	0	0	0	0	0	0	8	6
	2	8	0	13	15	15	17	16	17
	3	9	0	11	14	12	13	12	11
	4	ND ²	0	12	14	14	17	11	12
IEF band ³	+	-	-	-	+	+	+	-	+
LD absorbance ⁴	6	< 0.01	< 0.01	< 0.01	6	1.2	4	< 0.01	2

¹Multitest CMI, Merieux, Inc.; ² ND: not done; ³isoelectric focusing (visible band present: +, absent: -); ⁴laser densitometry absorbance units.

diate between these extremes, were Products 2, 3, 4, 5 and the Multi-test CMI which produced positive skin tests in two or three patients but not all four patients.

Products 4, 5, 6, 8 and the Multi-test CMI all exhibited definite banding and absorbance. Products 1, 2, 3 and 7 failed to exhibit visible protein bands or a corresponding absorbance peak. When present, peaks appeared 80–100 mm from the point of application. In contrast to an earlier observation (1), absence of banding on isoelectric focusing and absorbance on laser densitometry did not always indicate sub-potent extracts. Three Products, 2, 3 and 7 failed to exhibit visible protein bands or absorbance but did produce positive delayed-type hypersensitivity skin tests.

This discrepancy between skin test and isoelectric focusing suggests that the focusing material may not be antigenically relevant. Relevant material may have a carbohydrate component or be so small that it does not focus.

Nevertheless, the variation in skin test reactions observed in this study would have been clinically relevant. If extract potency were not known, the negative skin test obtained with Products 1, 2, 3, 5 and Multi-test CMI could not have been interpreted correctly. Misreading could be attributed to differences between extracts, differences in patient responsiveness, or both. More information about these delayed-

type hypersensitivity skin test materials needs to be routinely available. Screening with isoelectric focusing and laser densitometry was not adequate to predict acceptable potency. A method for standardization of all *Trichophyton* species extracts to bio-equivalency is sorely needed.

Edward N. Squire, Jr.

Susan E. Kosisky

Aparna S. Shah

REFERENCES

1. Kuthiala, C., Kosisky, S., Kirk, K. & Squire, E. N.: Delayed-type hypersensitivity skin testing: Variance among *Candida albicans* extracts. *JAMA* 1989, 261, 1883–1884.
2. Modified isoelectric focusing procedure using LKB multiphor apparatus and LKB ampholine PAG plates. In: *Methods of the allergenic products branch*, Washington D.C.: Food and Drug Administration, 1984.
3. Righetti, P. G. & Drysdale, J. W.: *Isoelectric focusing*. American Elsevier Science Publishing, New York 1976.
4. Stelander, M.: Quantification of protein in tracheobronchial alveolar lavage fluid by isoelectric focusing and scanning densitometry. *Eur. J. Respir. Dis.* 65 Suppl. 138, 1984.

Address:

Allergy/Immunology Service
Walter Reed Army Medical Center
Washington D.C. 20307-5001
U.S.A.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.