

Heat Shock Protein 65 Immunoreactivity in Experimentally Induced Polymorphic Light Eruption

J. P. McFADDEN, P. G. NORRIS, R. CERIO, G. ORCHARD and J. L. M. HAWK

St. John's Institute of Dermatology, St. Thomas's Hospital, London, U.K.

The expression of 65 kiloDalton heat shock protein (HSP65) immunoreactivity of skin biopsies from experimentally induced polymorphic light eruption (PLE) lesions was studied, to investigate its possible role as a photo-induced antigen responsible for precipitating lesions. In each subject the 24-h minimal erythema dose of solar simulated radiation was determined and an area of skin previously affected by PLE subjected to 70% of the minimal erythema dose in order to induce PLE lesions. The irradiated areas were sequentially biopsied between 0 and 6 days. ML-30, a monoclonal antibody which recognises heat shock protein 65, was used to label the sections by means of an indirect immunoperoxidase technique. In PLE patients clinical inflammation was noted by 5 h post-irradiation, with subsequent evolution of PLE-like lesions; these were still present at 6 days. Increased ML-30 antibody labelling in epidermal keratinocyte and endothelial cell cytoplasm was recognisable from 1 h post-irradiation, and in dermal dendritic cells from 5 h sustained through to 6 days. In normal subjects neither clinical nor histological features of inflammation were noted after irradiation, nor any increase in HSP65 labelling. *Key word: immunohistochemistry.*

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J. P. McFadden, St. John's Institute of Dermatology, St Thomas' Hospital, SE1 7EH London, U.K.

Polymorphic light eruption (PLE) is an acquired, intermittent, transient, non-scarring, papular reaction of the skin to sunlight of delayed onset, and varied morphology, usually beginning during the second to third decade of life. It affects 10–20% of the population in temperate climates (1, 2). A delayed type hypersensitivity reaction to a UV-induced cutaneous antigen has been postulated as being important in the pathogenesis (3). Heat shock proteins (HSPs), produced when a cell is confronted with a sudden increase in temperature or a variety of noxious stimuli (4), are candidates for such an antigen. They are classified according to their molecular weight and may be induced by UV radiation by mechanisms not yet understood but possibly through the production of oxygen free radicals (5). These induced proteins may be protective against noxious insults but, importantly, may also play a pivotal role in the immune response to injury (6). Since the 60–65 kiloDalton family of proteins (HSP65) have immunogenic properties and are important allergens in the tuberculin reaction, which has similar characteristics to the DTH reaction of PLE, we studied the expression of HSP65 in PLE, by using the monoclonal antibody ML-30.

METHODS

Subjects

7 subjects with PLE of 1 to 43 years' duration were studied. The condition was defined as a fully resolving, non-scarring papular photo-eruption occurring on exposed skin 1 to 48 h after sun exposure, in which the following investigations were normal or negative: full blood count and erythrocyte sedimentation rate, circulating antinuclear factor concentration and SSA and SSB antibody titres, and blood, urine and stool porphyrin concentrations. No subject had received any topical or oral therapy during the 2 weeks preceding the study. Seven normal controls were also studied; 2 of these subjects went on to have an area of their skin irradiated. All subjects gave informed consent and the approval of the hospital Ethical Committee was obtained.

Induction of PLE lesions

PLE was successfully induced in all 7 disease subjects, as previously described (7). Three 4 × 4 cm adjacent sites, previously affected by the eruption, were exposed to up to 70% of a normal MED solar simulated radiation (Kratos 2500 W, xenon arc filtered, Model No. LH 152/3SS) at a constant distance of 25 cm from the output port. The UV intensity at the test site was 2.8–3.0 MED/h, measured with a digital erythema UV intensity meter Model 2D (Solar Light Co. Inc, PA, USA), with a spectral response closely resembling the human erythema action spectrum.

HSP65 labelling

Murine monoclonal antibody ML-30 (gift from M. Ivanyi, Royal Postgraduate Medical School, Hammersmith Hospital, London), raised originally to *M. leprae* and reacting with the human homologue of HSP65, thus labelling cells expressing HSP65 in paraffin sections (8), was used to label paraffin-embedded 5 µ thick tissue sections of skin biopsies. After having been dewaxed with xylene and absolute ethanol and washed with phosphate buffered saline, the sections were labelled at 1/250 dilution of the mouse antibody. The second layer consisted of peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako) and the reaction product revealed with diaminobenzidine and 0.03% hydrogen peroxide (9). Control incubations omitting the primary antibody in all patient biopsies and in normal skin were performed. The distribution of the labelling was assessed by light microscopy.

Quantification of labelling

This was performed by blinded assessment of the slides and grading of the labelling of various skin structures. A semi-quantitative scale (0 = negative labelling; + = minimal labelling; ++ = moderate labelling; +++ = marked labelling) was used to give a mean overall labelling for each time point.

Skin specimens

4-mm diameter punch biopsies were obtained during local anaesthesia from the exposed sites at 0 h (n=3), 1 h (n=2), 5 h (n=3), 1 day (n=3), 3 days (n=2) and 6 days (n=1) on a limited number of occasions for each subject to avoid multiple scars. In the 7 normal subjects biopsies were taken of uninvolved skin and in 2 of these subjects 1 day post-irradiation.

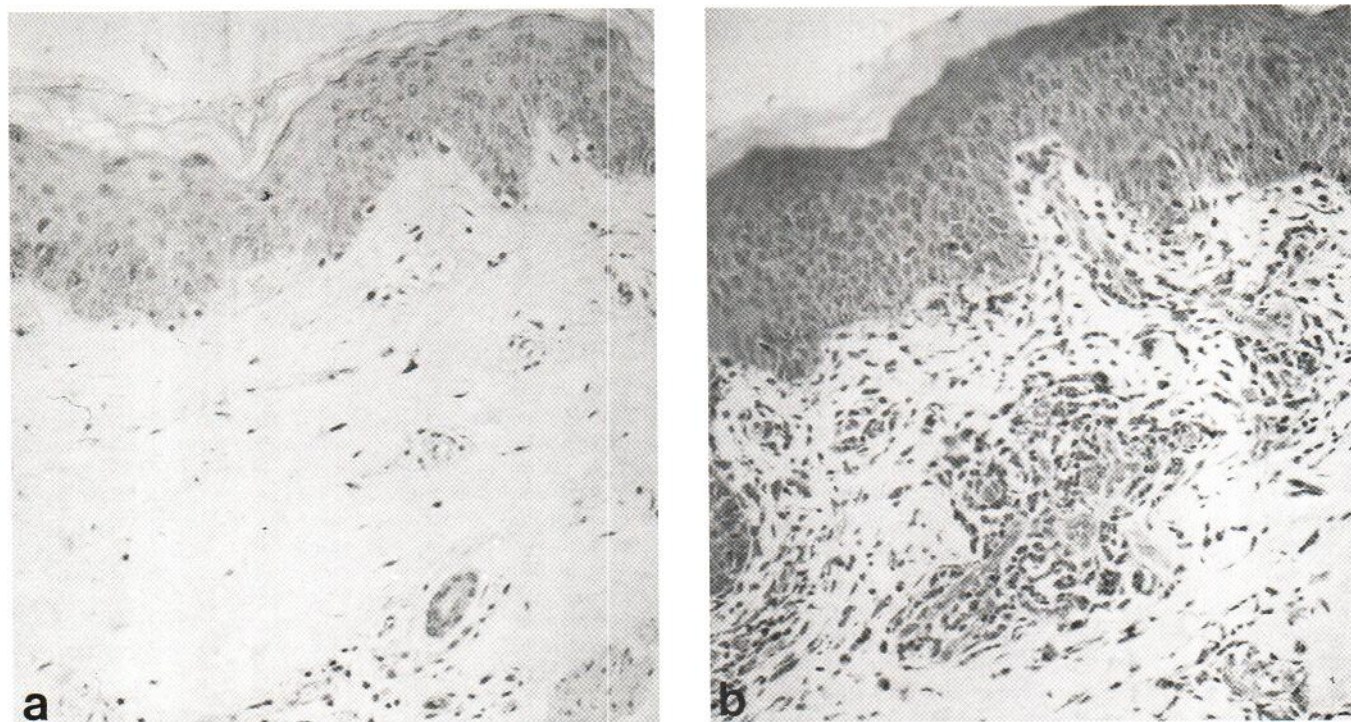


Fig. 1. HSP65 expression in PLE at a) 0 h and b) 72 h. There is increased epidermal and dermal immunoreactivity at 72 h.

RESULTS

Biopsies of pre-irradiated skin (Fig. 1a) and normal skin of 5 more normal controls showed minimal ML-30 labelling of keratinocytes and endothelial cells, moderate labelling of dermal dendritic cells (recognised as resident antigen-presenting cells in the dermis) and moderate to marked labelling of apocrine and eccrine glands and ducts. Increased labelling of both basal and suprabasal keratinocyte and endothelial cell cytoplasm was noted from 1 h post-irradiation, and of dermal dendritic cells from 5 h (Table I). This increase in labelling was sustained at 3 and 6 days (Fig. 1b and Table I). Inflammatory cell infiltration was noted from 5 h through to 6 days, with labelling of the mononuclear cellular infiltration. Labelling of biopsies of PLE lesions from patients clinically presenting with the rash showed

labelling similar to that found in experimentally induced lesions. No increase in ML-30 labelling was seen in the post-irradiation skin samples from the 2 normal volunteers (Table I). A control mouse monoclonal antibody, UCHL-1, which labels only certain T lymphocytes, was used concurrently to label PLE sections, and this failed to show any background labelling.

DISCUSSION

This study has demonstrated increased HSP65 immunoreactivity, determined by ML-30 labelling of keratinocytes, endothelial cells and dermal dendritic cells in evolving lesions of experimentally induced PLE. This has also been found in a variety of inflammatory dermatoses (J. McFadden & R. Cerio, in preparation), and in the inflamed pannus cartilage of rheumatoid arthri-

Table I. HSP65 expression in experimentally induced PLE lesions: mean scores for each time given
0 = negative labelling; + = minimal labelling; ++ = moderate labelling; +++ = marked labelling.

H post irradiation	Basal epidermis	Suprabasal epidermis	Mononuclear cell infiltrate	Endothelium	Dermal dendrocytes
PLE					
0 h	+	+	+	+	++
1 h	++	++	+	+/+++	++
5 h	++	++	+++	+++	+++
24 h	++	+++	+	++	+++
72 h	++	++	+/+++	++	++
144 h	++	++	+++	+++	+++
Control					
0 h	+/+++	+/+++	+	+/+++	+/+++
24 h	+	+	+	+	+

tis joints (10) but not, to our knowledge, in a photosensitive disorder. One has to interpret the data with caution, as ML-30 is raised against lepromatous rather than human HSP65, but our findings of increased labelling in clinically inflamed tissue agree with previous studies (10). In addition, immunolabelling with ML-30 correlates with that found with antisera raised against the human homologue (11).

Delayed type hypersensitivity to a light-induced antigen was first proposed by Epstein as important in the pathogenesis of PLE (12). Early evidence for this was conflicting, but further work has now shown that lymphocyte immunophenotype and adhesion molecule expression in a time-course study of PLE strongly suggest a delayed type hypersensitivity response (7).

Immune priming may need to occur during the first episode of PLE, which is often triggered by particularly intense sun exposure (2), and locally retained specific T cells may be very possibly activated following subsequent experimental UV exposure, a process analogous to elicitation of the contact hypersensitivity response (13).

Macrophages subjected to various stress stimuli, including γ -interferon activation and viral infection, are immunologically recognised by T cells raised against HSP65 (14). γ -interferon treatment of human monocytes increases their HSP65 messenger RNA and also their ML-30 monoclonal antibody labelling, indicative of increased HSP65 protein production (15). Dermal dendrocytes are known to belong to the monocyte-macrophage line, and capable of antigen presentation, differing from infiltrating mononuclear cells in being part of the resident macrophage system in the dermis (16). It is therefore possible that the increased HSP65 expression found in relation to dermal dendrocytes may be associated with a similar autoantigen presentation and immune response. Induction of autoreactive lymphocyte clones to HSP65 is a possible occurrence under conditions of cellular stress, and this is a theoretically possible forerunner of the cell-mediated immune response of PLE in predisposed individuals. Mechanisms to modify or reduce such a reaction could be sought to prevent this extremely common, annoying and sometimes debilitating condition.

REFERENCES

1. Morison WL, Stern RS. Polymorphous light eruption: a common reaction uncommonly recognised. *Acta Derm Venereol (Stockh)* 1982; 62: 237–240.
2. Ros A, Wennersten G. Current aspects of polymorphous light eruptions in Sweden. *Photodermatol* 1987; 3: 298–302.
3. Epstein JH. Polymorphous light eruption. *J Am Acad Dermatol* 1980; 3: 298–302.
4. Lindquist S, Craig EA. The heat shock proteins. *Ann Rev Genet* 1988; 22: 631–677.
5. Polla BS. Heat shock and the skin. *Dermatologica* 1990; 180: 113–117.
6. Welch WJ. The mammalian heat shock (or stress) response: a cellular defence mechanism. *Adv Exp Biol Med* 1987; 225: 287–304.
7. Norris PG, Morris J, McGibbon DM, Chu AC, Hawk JLM. Polymorphic light eruption: an immunopathological study of evolving lesions. *Br J Dermatol* 1989; 120: 173–183.
8. Evans DJ, Norton P, Ivanyi J. Distribution in tissue sections of the human groEL stress-protein homologue. *Acta Path Microbiol Immunol Scand* 1990; 98: 437–441.
9. Heyderman E. Immunoperoxidase technique in histopathology: applications, methods and controls. *J Clin Pathol* 1979; 32: 971–978.
10. Karlsson-Parra A, Söderström K, Ferm M, Ivanyi J, Kiessling R, Klareskog LK. Presence of human 65 kDa heat shock protein in inflamed joints and subcutaneous nodules of rheumatoid arthritis patients. *Scand J Immunol* 1990; 31: 283–288, 1990.
11. Ferm MT, Söderström K, Jindal S, Grönberg A, Ivanyi J, Young R, et al. Induction of human hsp60 expression in monocytic cell lines. *Int Immunol* 1992; 4: 305–311.
12. Epstein S. Studies in abnormal human sensitivity to light. IV. Photoallergic concept in prurigo aestivalis. *J Invest Dermatol* 1942; 5: 289–295.
13. Scheper RJ, Von Blumberg M, Boerrigter GH, Bruynzeel D, van Dither A, Vos A. Induction of immunological memory in the skin. Role of local T cell retention. *Clin Exp Immunol* 1983; 51: 141–148.
14. Koga T, Wand-Wurttenberger A, DeBruyn J, Munk ME, Schoel B, Kaufman SHE. T cells against a bacterial heat shock protein recognise stressed macrophages. *Science* 1989; 245: 1112–1115.
15. Kiesling R, Grönberg A, Ivanyi J, Söderström K, Ferm M, Kleinau S, et al. Role of hsp60 during autoimmune and bacterial inflammation. *Immunol Rev* 1991; 121: 91–111.
16. Weber-Matthiesen, W, Sterry K. Organisation of the monocyte-macrophage system of normal human skin. *J Invest Dermatol* 1990; 95: 83–89.