Measurement of Sunscreen Immune Protection Factors in Humans: A Consensus Paper

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It is increasingly accepted that sunscreens should protect against ultraviolet radiation (UVR)-induced immunosuppression, with an index of protection that can be compared with the sun protection factor (SPF). Five groups of immunoprotection researchers met to discuss the status of immune protection factor (IPF) evaluation in human skin *in vivo*. Current methods rely on a suncreen's inhibition of UVR-induced local suppression of the contact hypersensitivity (CHS) response or the delayed-type hypersensitivity (DTH) response, using either the induction or the elicitation arms of these responses. The induction arm of the CHS response has the advantage of being sensitive to a single sub-erythemal exposure of solar-simulating radiation (SSR) that allows a direct comparison with the SPF. This approach, which necessitates sensitization, requires a large number of volunteers and is too labor intensive and time consuming to become a routine method. The elicitation arm of the CHS or DTH responses exploits prior sensitization to contact or recall antigens and has the advantage of being possible to apply on small groups of volunteers. Some current protocols, however, require repeat SSR exposures, which invalidates a direct comparison with SPF that is based on a single exposure. There is a need for a new simpler method of IPF that will have to be validated against existing models.

Key words: consensus paper/immune protection/sunscreens/ultraviolet radiation
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Human ultraviolet radiation (UVR)-induced immunosuppression (Cooper *et al*, 1992; Kelly *et al*, 1998, 2000) probably plays a role in skin cancer (Nishigori *et al*, 1996; de Gruijl, 2002; Ullrich, 2002). The standard method of assessing sunscreen protection is based on erythema and is expressed as the sun protection factor (SPF). It is recognized that labelled SPF is often not achieved, because users typically apply sunscreens at lower application densities than the 2 mg per cm^2 required by regulatory bodies (Diffey, 1996). Quite apart from the behavioral issues that determine the actual SPF achieved from a product, erythema is a poor indicator of immunosuppression (Kelly *et al*, 2000). This raises the question: "Is the immune protection factor (IPF) of a sunscreen equivalent to its SPF?" Several studies (e.g., Ullrich *et al*, 1999; Cooper et al, 2002) indicate that sunscreens afford some protection against UVR-induced immunosuppression, but wide variations in experimental design and data management make it difficult to standardize the assessment and definition of IPF. An expert panel (Table I), convened by L'Oréal Recherche in Paris on 5th of July 2002, discussed these issues, which form the basis of this paper.

Abbreviations: CHS, contact hypersensitivity; DNCB, dinitrochlorobenzene; DTH, delayed-type hypersensitivity; EI, erythema index; ID_{50}, UVR dose that results in 50% immunosuppression; IPF, immune protection factor; MED, minimal erythemal dose; MISD, minimal immunosuppressive dose; PAR, primary allergic response; SFT, skin fold thickness; SPF, sun protection factor; SSR, solar-simulated radiation; ts, total score; UVR, ultraviolet radiation

Immunological Background to IPF Methodology

UVR suppresses the induction and elicitation arms of the contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses. CHS is a response to topically applied antigens, whereas DTH is the reaction to intracutaneously delivered antigens. Exposing naïve volunteers to UVR before antigen sensitization assesses suppression of the induction arm. This assessment is made by challenge with the same antigen 2–3 wk later. The assessment of the suppression of the elicitation arm is made on volunteers with prior sensitization via vaccination or environmental exposure to common contact allergens such as nickel. In this case, the volunteers are exposed to UVR and re-challenged with the relevant antigens or contact allergen. Failure to induce or elicit sensitization by applying or delivering the antigen to the UVR-exposed site is called local immunosuppression and failure to induce or elicit sensitization by applying or delivering the antigen to a non-UVR exposed distant site is called systemic or distal immunosuppression.

Table I. Investigative groups and techniques used to assess immunoprotection

Group	Techniques used for IPF assessment	Relevant references
Australian	Suppression of elicitation response to nickel CHS	Poon *et al* (2003)
Austrian	Suppression of sensitization to DNCB	Wolf *et al* (2003)
French	Suppression of elicitation response to recall DTH	Moyal and Fourtanier (2003)
UK	Suppression of sensitization to DNCB	Kelly *et al* (2003)
USA	Suppression of sensitization to DNCB	Baron *et al* (2003)

DNCB, dinitrochlorobenzene; IPF, immune protection factor; CHS, contact hypersensitivity; DTH, delayed type hypersensitivity.

The IPF of a sunscreen has been evaluated using the induction or elicitation arm of the local CHS or DTH response (see Table I), and the systemic DTH response (Moyal and Fourtanier, 2001).

Methodology Used by the Five Groups

Sunscreens It is important to characterize the sunscreens studied. Apart from their names and concentrations, actives such as antioxidants should be noted as these may influence UVR-induced immunosuppression. Sunscreen absorption spectra should be determined spectrophotometrically *in vitro*, using either a Transpore tape (3M, Reuil, Malmaison, France) (Diffey and Robson, 1989) or a roughened quartz plate (Moyal and Fourtanier, 2001) as a substrate. Such spectral data enable the calculation of different *in vitro* factors such as SPF and critical wavelength (λ_c) (Diffey, 1994).

It is very important to verify the *in vivo* assigned SPF because this depends on the solar-simulating radiation (SSR) source used for its assessment (LeVee *et al*, 1980), as well as the method of sunscreen application and the clinical evaluation of erythema. In all cases, the same SSR source and similar volunteers (phototype, age range, sex ratio, and body site) as those included for IPF assessment should be used. Furthermore, the same investigator should apply the sunscreen for SPF and IPF assessments. The European Cosmetic Toiletry and Perfumery Association (Colipa, 1994) or the Food and Drug Administration (FDA, 1999) recommendations should be followed for SPF determination. Erythema intensity can be assessed clinically or quantified using colorimetric measurements (Chromameter Minolta, Osaker, Japan; Diastron Dia-stron, Andover, Oklahoma) (Colipa, 1994).

UVR sources and dosimetry SSR spectrum must comply with a standard for SPF determination (e.g., Colipa, 1994). It is important to measure SSR spectral irradiance at skin level with a calibrated spectroradiometer. Routine spectroradiometry is time consuming so calibrated broadband radiometers are usually used for day-to-day measurements.

Volunteers and test sites Inclusion criteria, such as sex, skin type, and test site, may influence the results. Immunity decreases with age but the effect of age on photoimmunosuppression is unknown. The volunteers in the studies of Table I ranged from 18 to 71 y old (UK: 18–35, Austria: 18–60, France 18–40, Australia 18–71, USA: 18–60). Menstruating women undergo marked monthly fluctuations in their immune responsiveness but at mid-cycle, their immune response is similar to men (Oberhelman *et al*, 1992). The UK and US groups sensitized females at mid-cycle to control for this. The Austrian and French groups dropped females from the sunscreen IPF testing because of variability in response observed in the first part of their study. It is easier to find females for nickel elicitation studies because more women (15%) than men (5%) develop allergic dermatitis to nickel. The UK group has shown that susceptibility to immunosuppression (induction of CHS) is skin type dependent (Kelly *et al*, 2000), with skin types I/II being more readily suppressed than skin types III/IV. But the Australian group found no relationship between susceptibility to sunburn, which is roughly skin type dependent, and susceptibility to suppression of the elicitation of CHS to nickel (Damian *et al*, 2001).

All groups, except the Australians, assessed individual sensitivity to SSR by determination of the minimal erythemal dose (MED) 48 h to 2 wk before the immune function assays. The MED is the SSR dose (J per m^2) required to induce a just visibly perceptible erythema or an erythema with well-defined borders 24 h after exposure. The buttock was the sensitization site for induction of CHS studies because this area is relatively flat with an even color and, in general, UVR naïve. The buttock is not suitable, however, for DTH because of its softness makes it difficult to give a homogeneous intracutaneous delivery of allergens. In this case, the back is preferred, which was also used for the elicitation of CHS to nickel because it offers a larger flat area than buttock skin. For induction studies, the challenge (or elicitation) was always performed on the UVR-protected upper inner arm, opposite to the UVR-exposed site (left arm when right buttock).

It is important that both SPF and IPF determinations are based on a set of homogeneous volunteers with the same inclusion criteria. Moreover, for IPF determination in elicitation studies, the initial immune response of the volunteers has to be considered for randomization of different groups, and for selection of nickel concentrations used in the challenge patches. It is important to note that comparisons between IPF and SPF must be made in the same anatomical sites.

SSR doses and group design In general, the group size was 6–15 volunteers. All groups except one (Australian) based SSR doses on individual MED, giving fractions or multiples of the individual MED that were determined prior to the immunological protocols. Single SSR exposure protocols used doses between 0.25 and 3 individualized MED on unprotected sites. In repeated exposure protocols, which assessed the suppression of nickel-induced CHS, all volunteers received the same SSR doses that were not greater than 1 mean MED per exposure. This limits the SSR-induced erythema, which could otherwise interfere with the assessment of CHS. The mean MED was determined on a different but comparable group of volunteers.

The sunscreen-treated sites received MED increments that were comparable with unprotected skin after the test