Protection Factors are Ratios

To the Editor:

In their abstract, Davenport *et al* (1997) state that "Results consistently demonstrated that all the test sunscreens protected (against immunosuppression) beyond their designated protections factors." Protection factors are ratios by definition, and if the above statement by Davenport *et al* (1997) means anything at all, it is that the sunscreens' immune protection factors were greater than their corresponding sun protection factors (SPF). Chu *et al* (1998) restate their claim in their recent letter to the *Journal of Investigative Dermatology* in which they also state that they did not calculate immune protection factors in their original paper but would have to concede, from their own data, that had they done so, as suggested in letters by Gasparro (1998) and Wolf and Kripke (1998), these would have been lower than the corresponding SPF. If the immune protection factor is lower than the SPF, it is just not possible for sunscreens "to protect beyond their designated protection factors." The authors tested products with SPF ranging from 3.6 to 5.7, and defend their original conclusions by arguing that although *ex vivo* skin exposed to 5 minimal erythema doses (MED) results in 50% immunosuppression, a sunscreen of SPF = 5 completely protects against immunosuppression when skin is exposed to 5 MED. This is hardly surprising as 5 MED with an SPF of 5 is equivalent to 1 MED without a sunscreen, and 1 MED without sunscreen almost certainly does not cause immunosuppression in their model. All the authors can conclude from this analysis is that 1 MED delivered to the skin, with or without sunscreen, is not immunosuppressive. Furthermore, the UV dose-threshold for immunosuppression in their *in vitro* assay is at least 3 MED. Therefore any sunscreen that protects against erythema will protect against immunosuppression if tested using their model. In order to evaluate an endpoint, one has to give a UVR dose (with and without sunscreen) that induces the endpoint (e.g., 50% immunosuppression). For example, it would be impossible to assess SPF without giving an erythemal dose. In their recent letter, Chu *et al* express concern about the possibility of sunscreens giving better protection against erythema than immunosuppression, but the only way to evaluate this legitimate concern is to compare immune protection factor with SPF. Any conclusions on photoprotection (of any endpoint) without the use of ratios will depend on the sensitivity of the assay and are therefore invalid. In this context, it is worth stating that the SPF of the products tested were determined *in vitro* and it is well known that such determinations do not necessarily accord with *in vivo* assessments.

In summary, we totally agree with the letters by Gasparro (1998) and Wolf and Kripke (1998), that the data of Davenport *et al* (1997) do not show that sunscreens protect against immunosuppression beyond their designated protection factors; in fact their data show exactly the opposite.

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PUVA Therapy and Human Papillomavirus Type 5 Detection in Psoriasis

To the Editor:

Michel Favre *et al* reported in this Journal a high prevalence of human papillomavirus 5 (HPV5) infection in psoriatic patients and proposed that psoriasis is a reservoir for this virus (Favre *et al*, 1998). These conclusions were based on (i) the presence of antibodies against HPV5 VLP in 24.5% of 155 psoriatic patients, contrasting with the 2%–5% observed in the two other groups (atopic dermatitis and renal transplant recipients); (ii) the detection of HPV5 DNA sequences by nested polymerase chain reaction in lesional and uninvolved skin of 91.9% psoriatic patients and in no patient in the atopic dermatitis group, respectively.

These results are very exciting but we question whether the high prevalence of HPV5 infection is related to the psoriasis itself or to a possible irradiation with ultraviolet (UV) via phototherapy. Indeed, Bayle-Lebey *et al* have already reported the detection of HPV5 DNA sequences in a keratosic skin lesion in a psoriatic patient, although this patient had been treated for a long time with UVA phototherapy (Bayle-Lebey *et al*, 1994). Unfortunately, data on the previous treatments of Favre psoriatic patients are unknown. Moreover, the reported detection of HPV DNA sequences in both involved and nonlesional skin in psoriatic patients suggests the role of an extrinsic factor such as UV.

The role of UV in epidermodysplasia verruciformis (EV) is well known (Orth, 1987), and excessive sun exposure is avoided in EV patients. Pityriasis versicolor-like skin lesions observed in EV and associated with HPV infection are particularly located in sun-exposed areas. The role of UV is clearly demonstrated in the induction of HPV5-associated skin carcinomas in EV and in renal transplant recipients.

Few data are available concerning the search of EV-associated HPV infection in patients under phototherapy. Spradbrow *et al* reported the presence of a possible papillomavirus in sun-exposed skin (Spradbrow *et al*, 1983); but most reported data are related to HPV16.

UV may act either via a local immunosuppression that could facilitate HPV DNA replication, or transcription of HPV. It can be hypothesized that EV-related HPV are widespread and that some factors such as local (UV) or general immunosuppression (EV, immunosuppressive agents, AIDS) may facilitate their replication and/or persistence.

On the other hand, it is difficult to understand the general good efficiency of phototherapy in psoriatic patients and its detrimental effect

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in EV patients if EV-related HPV play a pathogenic role in these two disorders. If the high prevalence of EV-related HPV in psoriasis was confirmed, the use of phototherapy or immunosuppressive agents might be reconsidered.

It would be of interest to look for an HPV5 infection in other skin disorders such as mycosis fongoid or prurigo, which are currently treated with phototherapy.

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Reply:

In response to the letter by Mahé and Descamps, we wish to discuss further certain important problems raised by our study on psoriasis (Favre *et al*, 1998).

The first question is whether the presence of HPV5 and other epidermodysplasia verruciformis (EV)-associated HPV genotypes in psoriatic lesions is related to the disease itself or to phototherapy acting via a local immunosuppression. As shown in **Table I**, only 17 of the 37 patients previously studied both serologically and virologically were currently under PUVA therapy (doses of UVA irradiation between 1 and 212 J per cm^2), and six had received a treatment 1–11 y before (doses between 40 and 150 J per cm^2). The prevalence of anti-HPV5 antibodies and the detection rate of HPV5 DNA were similar among the 23 patients receiving or having received PUVA therapy (26.1% and 91.3%, respectively) and among the 14 untreated patients (28.6% and 92.8%, respectively). Furthermore, HPV5-positive specimens of uninvolved skin (Favre *et al*, 1998) were collected from unexposed areas. The data strongly argue against phototherapy playing a role in the persistence and expression of HPV5 in psoriasis. Our data on the lack of detection of HPV5 in patients suffering from atopic dermatitis (prurigo), a disease frequently treated by photochemotherapy, further strengthen this conclusion.

Table I. HPV5 status and PUVA therapy in psoriatic patients[a]

	Proportion of patients	
PUVA treatment	HPV5 seropositive	HPV5 DNA positive
Present	4/17	16/17
Previous	2/6	5/6
None	4/14	13/14

[a]Thirty-seven patients virologically studied in Favre *et al* (1998).

A second important problem is the actual role played by HPV5 and EV HPV in the increased and dose-dependent risk of nonmelanoma skin cancer associated with PUVA therapy. Such a role could be suggested by the case described by Bayle-Lebey *et al* (1994) and by the data recently reported by Harwood *et al* (1998). In the latter study, 40% of the specimens of dysplastic keratoses and 50% of the specimens of invasive carcinomas taken from patients treated by PUVA therapy with high doses of UVA (essentially psoriasis patients) were found to harbor EV HPV DNA, using highly sensitive nested polymerase chain reaction methods. Similar or even higher rates of EV HPV detection have been reported for precancerous skin lesions and nonmelanoma skin cancers, for plucked hairs, and for specimens of normal skin from immunocompetent individuals or immunosuppressed allograft recipients (Boxman *et al*, 1997; Pfister and ter Schegget, 1997; Astori *et al*, 1998). It is interesting to stress that, in contrast with its rare detection in these studies, HPV5 was the EV HPV genotype most frequently detected in dysplastic keratoses and skin carcinomas associated with PUVA therapy (Harwood *et al*, 1998). Evidence available thus demonstrates that EV HPV are widespread but, in the absence of any data on the expression of the viral genome, their role in skin carcinogenesis in non-EV patients remains unclear.

A third problem raised by Mahé and Descamps is the opposite effects of UV radiations in EV and psoriasis, although both diseases are associated with a high prevalence of HPV5. EV results from a genetic predisposition to infection by the oncogenic HPV5 and related genotypes. Numerous copies of HPV5 genome and abundant transcripts of the E6 and E7 viral oncogenes are detected in EV benign lesions and squamous cell carcinomas (Orth, 1987). Ultraviolet radiations of sunlight may favor the persistence of EV lesions by their immunosuppressive effect and, by inducing mutations within oncogenes or tumor suppressor genes (like the p53 gene), may lead to the early development of skin cancers, in synergy with the E6 and E7 viral oncogenes. In contrast, HPV5 sequences are only detected in low amounts in psoriatic lesions and no data are yet available on the expression of the E6 and E7 viral genes. The detection of antibodies against L1 and L2 capsid proteins in a significant proportion of patients, however, can only be explained by the occurrence of a complete life cycle of the virus in some keratinocytes (Favre *et al*, 1998; Majewski *et al*, 1988). UV radiations and photochemotherapy have a beneficial effect in patients with psoriasis, due to their immunosuppressive and anti-proliferative actions. Because HPV5 infection is likely to be favored by epidermal cell hyperproliferation (Majewski *et al*, 1998), it can be assumed that phototherapy has a negative effect on HPV5 persistence and expression. It should be stressed that cases of psoriasis selected for PUVA therapy are those active and extensive, in whom epidermal proliferation may have already allowed a significant level of viral expression.

Immunosuppressive therapies are methods of choice in the treatment of psoriasis (Stern, 1997), and we strongly believe that the use of phototherapy and immunosuppressive agents should not be reconsidered because of HPV5 detection. They are the best treatment modalities for decreasing T cell activation as well as epidermal hyperproliferation, and, thus, expression and replication of EV HPV.

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A Novel Homozygous Mutation Affecting Integrin α6 in a Case of Junctional Epidermolysis Bullosa with Pyloric Atresia Detected *In Utero* by Ultrasound Examination

To the Editor:

We report a new case of junctional epidermolysis bullosa with pyloric atresia (PA-JEB) in the product of a consanguineous union of healthy parents that had previously generated a clinically normal child. At 24 wk of gestation, an amniocentesis was requested because of polyhydramnios. The karyotype revealed a normal 46XX haplotype, and analysis of the amniotic fluid detected high levels of acetylcholinesterase. A week later, ultrasound examination of the fetus using an Aloka 2000–5 MHz transducer apparatus confirmed the presence of polyhydramnios with dilated stomach. Careful examination detected abnormal limbs with fisted hands, overlapping fingers, and malposition of the first toe. Ears were hypoplastic, with the helix merged with the temporal muscle, absent lobule and the concha opening obstruded by collapsed epidermis. Facial deformities and extensive desquamation of the integument were observed.

Five skin biopsies were obtained by fetoscopy at 28 wk gestation under local anesthesia and ultrasound control. Electromicroscopic analysis of the skin samples showed detachment of the epithelium, displaying a cleavage plane within the lamina lucida of the basement membrane zone. No hemidesmosomal structure could be detected, which correlated with the extreme fragility of the fetal skin. The pregnancy was terminated. At the delivery the fetus presented with aplasia cutis congenita and almost total detachment of the integument, involving the complete separation of the pilosebaceous units, nails, and nailbeds from the dermis. The oral and nasal mucosa were detached from the mesenchyme, and the vaginal mucosa had prolapsed externally. Pathologic examination confirmed the presence of the pyloric obstruction.

Immunofluorescence analysis of samples of the proband's skin obtained at the delivery showed a complete absence of reactivity to antibodies directed against integrins α6 and β4, and also against the bullous pemphigoid antigen PB230 (**Fig 1*B***, ***D***, ***H***). Labeling of bullous pemphigoid antigen BP180 was strongly reduced and displayed a faint pericellular staining of basal keratinocytes (**Fig 1*F***). Staining of plectin was decreased and irregular (**Fig 1*J***). In view of these results, and considering that PA-JEB has been associated with mutations in the genes for integrin α6 and β4, expression of these integrin subunits was assessed by northern analysis of total RNA purified from secondary cultures of epidermal keratinocytes obtained from the proband. Hybridization with an integrin β4 cDNA probe resulted in a strong signal, whereas with an integrin α6 cDNA probe no hybridization band was detectable (**Fig 2*A***). Expression of the major hemidesmosome components was then examined by western analysis of protein extracts obtained from the proband's cultured keratinocytes. Absence of integrin α6 was confirmed, whereas integrin β4, BP230, plectin, and BP180 were synthesized *in vitro* by the PA-JEB keratinocytes (**Fig 2*B***).

To identify the causative mutation hampering the expression of integrin α6, a search for mutations was performed on the corresponding cDNA obtained by reverse transcriptase-polymerase chain reaction amplifications of total RNA purified from cultured keratinocytes isolated from the proband. Overlapping polymerase chain reaction amplimers spanning the entire open reading frame of integrin α6 cDNA were obtained using eight pairs of primers (Ruzzi *et al*, 1997). Direct sequence analysis of the cDNA segment spanning nucleotides 1064–1815 detected a homozygous C-to-T

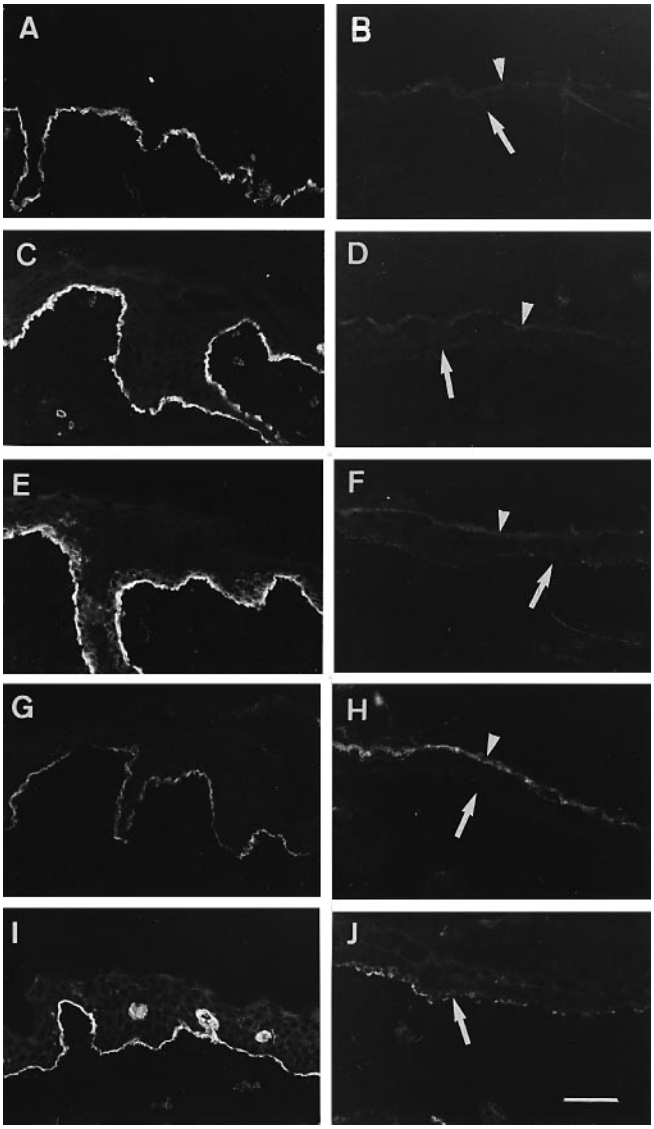

Figure 1. Immunofluorescence analysis of frozen skin biopsies. Cryostat sections of control (*A*, *C*, *E*, *G*, *I*) and proband (*B*, *D*, *F*, *H*, *J*) skin were stained with monoclonal antibodies GoH3 (Sonnenberg *et al*, 1987) (*A*, *B*) and 3E1 (Life Technologies, Cergy Pontoise, France) (*C*, *D*) specific to integrin α6 and β4, respectively, and with monoclonal antibodies 1A8C (Nishizawa *et al*, 1993) (*E*, *F*), FP1 (Tanaka *et al*, 1990) (*G*, *H*) and HD121 (Nishizawa *et al*, 1993) (*I*, *J*) directed against BP180, BP230, and plectin, respectively, as reported (Gagnoux-Palacios *et al*, 1997). The dermal–epidermal junction (*arrows*) and the stratum corneum (*arrowheads*) are indicated. *Scale bar*: 200 μm.

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transversion (CGA to TGA) at position 1764 (Genbank no X53586), resulting in a nonsense mutation (R540X) within the extracellular domain of the polypeptide. Direct nucleotide sequencing of genomic DNA obtained from the nuclear family, confirmed the presence of a homozygous C-to-T substitution in the patient, and disclosed the heterozygous state of the parents (**Fig 2*C***).

A prenatal testing of the fetus at risk for PA-JEB was requested for the third pregnancy of the mother. A search for mutation R540X was performed by polymerase chain reaction amplification of genomic DNA extracted from chorionic villous samples, as described in the legend to **Fig 2**. The mutation was not detected in the fetus, and the prenatal diagnosis was confirmed by the delivery of an unaffected child.

Integrin α6β4 plays a pivotal role in the nucleation of hemidesmosomes, therefore genetic mutations hampering the expression of this adhesion receptor interfere with the proper assembly of these adhesion structures and the stabilization of the dermal–epidermal junction. Two cases of PA-JEB caused by genomic mutations leading to premature termination codons in the gene for integrin α6 have thus far been reported (Pulkkinen *et al*, 1997; Ruzzi *et al*, 1997). In the two patients, the genetic defects predict an α6 polypeptide truncated in the extracellular domain. Immunohistologic examination of the skin of one of these patients detected a normal labeling pattern for BP180 and BP230, but ultrastructurally, the hemidesmosomes were rudimentary with a reduced or absent inner plaque and subbasal dense plate (Ruzzi *et al*, 1997). In contrast, in the PA-JEB patient presented in this study, the staining pattern of all hemidesmosomal components was altered. This may indicate an abnormal structuration of hemidesmosomes that would well correlate with the extreme fragility of the integument detected *in utero* by ultrasound examination of the fetus. Because ultrastructural studies of fetal skin have revealed that morphogenesis of the hemidesmosomes proceeds rapidly between 9 and 15 wk of gestation (McMillan and Eady, 1996), it remains unclear why, at the molecular level, the α6-defective fetus is distinct from the two α6-defective PA-JEB newborns described so far. Comparative analysis of fetal and newborn keratinocytes obtained from cases of lethal JEB is in progress and it may help to elucidate possible subtle mechanisms involved in the structuration of the hemidesmosomes during the different stages of development.

This study increases the repertory of mutations affecting integrin α6 in PA-JEB, and confirms that sonographic observations may provide clues to prenatal diagnosis of the disease.

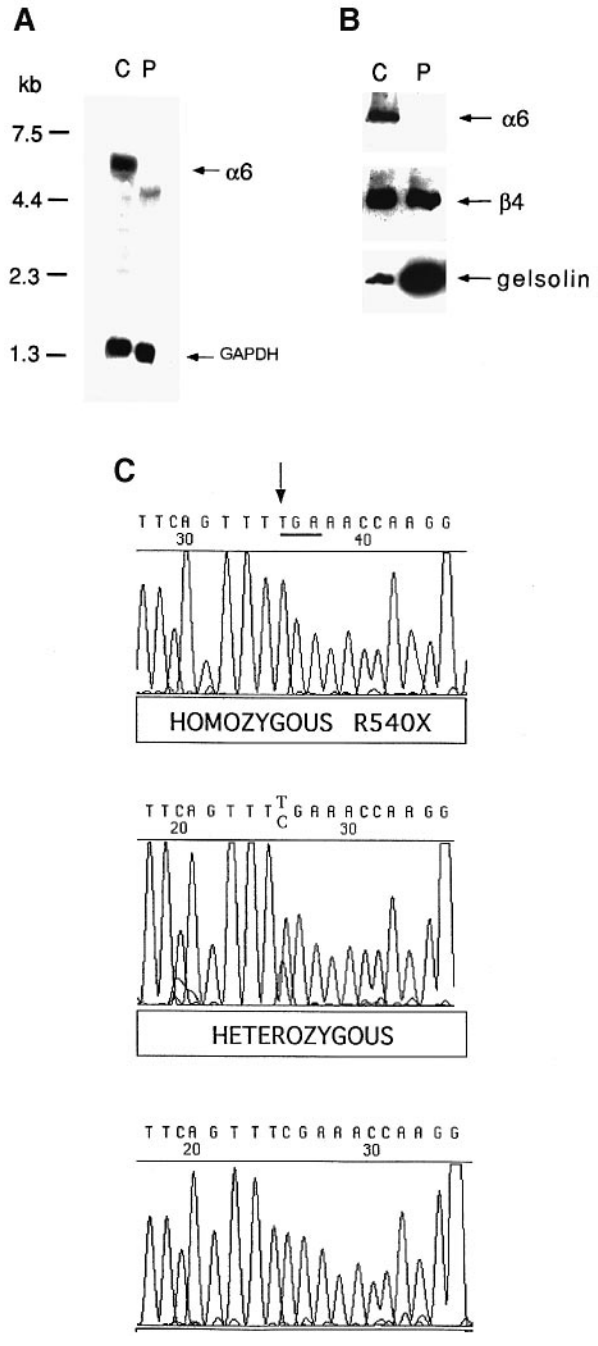

Figure 2. Identification of the genetic defect associated with this case of PA-JEB. (*A*) Northern analysis of total RNA. 30 μg of RNA was electrophoresed in a 1% agarose-formaldehyde denaturing gel, transferred onto a nitrocellulose membrane and hybridized with integrin α6 and GAPDH specific P^{32}-labeled cDNA probes. The α6 integrin mRNA signal is absent in PA-JEB keratinocytes. The faint 4.4 kb band visible in *lane P* is not specific and results from the overexposition of the northern blot. (*B*) Western analysis of cell extracts. Thirty micrograms of total proteins was fractionated on a 10% sodium dodecyl sulfate-polyacrylamide gel in nonreducing conditions, transferred onto a nitrocellulose filter, and reacted with monoclonal antibody 1A10, specific to integrin α6, or with a polyclonal antibody specific to integrin β4. An anti-gelsolin antibody was used as an internal control. Note the absent synthesis of the α6 polypeptide in proband cells. (*C*) Identification of tmutation R540X in the PA-JEB kindred: polymerase chain reaction amplification and direct sequencing of the proband's genomic DNA identified a homozygous C to T substitution in the patient (upper), and demonstrated heterozygosity of the parents for the mutation (middle) when compared with the normal sequence (lower). To detect the mutation at the genomic level, a DNA fragment was polymerase chain reaction-amplified using primers (L) 5′-CACTTGAAGC-TGAAAAAGAAAG-3′ and (R) 5′-GCCTCTTCAGAGTTAGTTC-3′ and, as a template, genomic DNA (100 ng) obtained from the proband and the proband's parents. The polymerase chain reaction conditions were: 94°C for 5 min, 94°C for 30 s; 54°C for 45 s; 72°C for 10 s (30 cycles). The amplified products were submitted to direct sequence analysis.

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