

Structure and Heterogeneity of the Human Gene for Epiplakin (EPPK1)

To the Editor:

Almost all known members of the plakin family, for example BPAG1, desmoplakin, envoplakin, periplakin, and plectin, can be targets of a patient's antibodies in several autoimmune blistering diseases and these plakins are, in fact, used as markers for specific diseases (Sawamura *et al*, 1991; Tanaka *et al*, 1991; Oursler *et al*, 1992; Kim *et al*, 1997; Mahony *et al*, 1998; Proby *et al*, 1999). Some members of this family have a common structure, with predicted globular amino-terminal and carboxy-terminal domains that are separated by a central rod domain. Some homologous domain structures have been identified in both globular domains of many plakins, whereas the central domain is rich in heptad repeats and is believed to form a parallel α -helical coiled-coil structure with a dimerization partner (Green *et al*, 1992; Leung *et al*, 2002).

Epiplakin, which was recently identified as an autoantigen in serum from a patient with subepidermal blistering diseases, is also a member of the plakin family and the message size of this protein was more than 12 to 13 kb (Fujiwara *et al*, 1992, 1996). Molecular cloning of epiplakin demonstrated that it is a protein of 5065 amino acids with a molecular mass of 552 kDa. Moreover, it has some very unusual features; for example, it has 13 domains that are homologous to the B domain in the carboxy-terminal region of desmoplakin. The last five of these 13 B domains, together with their associated linker regions, are particularly strongly conserved. Moreover, epiplakin lacks a coiled-coil rod domain and an amino-terminal domain, both of which are found in all other known members of the plakin family and, in addition, no dimerization motif is evident in the sequence. Thus, it is likely that epiplakin exists *in vivo* as a single-chain structure (Fujiwara *et al*, 2001).

We report here the isolation of the human gene for epiplakin from a genomic library and an analysis of its structure. We screened a human genomic library, constructed from leukocytes, with the insert of the cDNA clone Ep21, which were obtained from a HeLa cell library, as described in a previous report (Fujiwara *et al*, 2001). We isolated one genomic clone, NT2, and this clone hybridized with another cDNA clone pE450-D, confirming that we had isolated a gene for epiplakin. We used the 3' end of Ep12 cDNA for isolation of another genomic clone, NT4. This clone overlapped with NT2 (Fig 1). In order to confirm the extent of the strongly homologous repeats in the 3' region, we performed genomic polymerase chain reaction to amplify the repetitive region, using DNA from HeLa cells (HeLa5) or leukocytes from a single individual (NTnt) as described previously (Fujiwara *et al*, 2001). The lengths of DNA fragments obtained from both amplifications was identical and was slightly more than 9 kb. The contig sequence that included the sequences of NT2 and NT4 was 21.5 kb long and it included the entire open reading frame for epiplakin (15,195 kb). No interruptions were found from ATG to TGA and, thus, no introns were present. There were 30 nucleotide differences between the cDNA from HeLa cells and the gene from the leukocyte library, 19 of which would be expected to lead to amino acid substitutions.

There were nine or fewer GCC repeats that encoded polyalanine in the linker regions of the five homologous repeats at the 3' end of epiplakin cDNA. When we focused on the diversity of this region, we found only two sites of differences (repeats 4 and 5) between the cDNA and the HeLa DNA; however, we found at least four sites of differences between genes from two different sources namely, NT2 and NT4 versus HeLa5 or NT2 and NT4 versus NTnt (Table 1a). These data suggested diversity among individuals in these microsatellite regions, which we were able to demonstrate as follows. We isolated DNA from 15 genetically nonrelated healthy Japanese and amplified the first of five homologous repeats in the gene for epiplakin by nested polymerase chain reaction. Eight GCC repeats were the most frequent and were found in 15 chromosomes and, in five of these chromosomes, there was a G to A transition in the fifth GCC repeat. Five GCC repeats were found in nine chromosomes, and nine GCC repeats were found in six chromosomes, in one of which there was a G to A transition in the seventh GCC repeat (Table 1b).

We mapped the gene for epiplakin on human chromosomes by the published procedure (Feng *et al*, 1994) using the genomic clone NT2. Under our conditions, the efficiency of detection of signals due to fluorescence *in situ* hybridization was approximately 87% with our chosen probe (among 100 mitotic figures examined, 87 yielded hybridization signals on one pair of chromosomes). The gene was mapped to the q24.3 region of human chromosome 8 (data not shown).

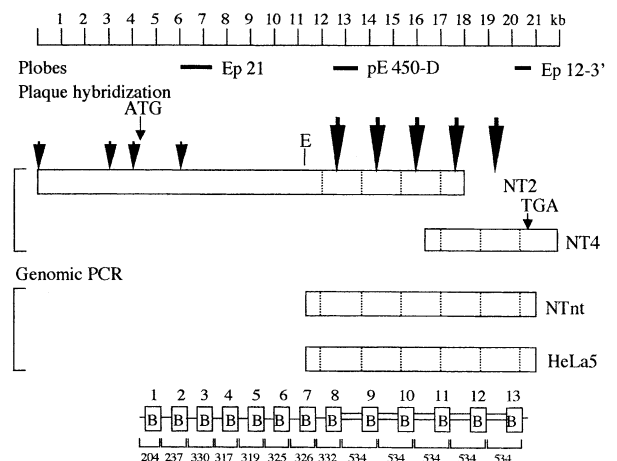


Figure 1. Cloning strategy for the isolation and sequencing of the human gene for epiplakin and schematic representation of the deduced protein. The genomic clones isolated in this study allowed identification of one exon, which corresponded to contiguous cDNA sequences. Three cDNA probes, namely Ep21, pE450-D, and the 3' end of Ep12 (Ep12-3'), are also shown. The five homologous repeats at the 3' end of the gene for epiplakin were amplified by polymerase chain reaction using DNA from leukocytes (NTnt) and from HeLa cells (HeLa5). On the lowest diagram, the numbers at the top are domain numbers; the numbers under the line below the boxes show the distance between domains (in terms of amino acid residues). Boxes indicate B domains that are 70-46% homologous to the B domain in desmoplakin (Fujiwara *et al*, 2001). Double-lined linker regions and the five B domains (9-13) to their right are almost identical. *Small arrowheads* indicate *Xho* sites and *E* indicates an *EcoRI* site. *Large arrowheads* indicate the positions of GCC repeats. Dashed lines indicate borders of five strongly conserved repeats at the 3' end.

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The sequence of gene for Epiplakin (EPPK1) has been submitted to the DNA Data Bank of Japan, accession number, AB107036.

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Table Ia. Variations between microsatellite sequences in genes and the cDNA for epiplakin.

There are GCC repeats in the five homologous repeats that encode linker regions between B domains (Fig. 1). There was a G to A transition in some fifth and fourth repeat. Numbers after parentheses indicate the numbers of GCC or ACC repeats. Only two sites of differences (repeats 4 and 5) were found when we compared cDNA and HeLa DNA. At least four sites of differences were found between genes from two different sources, NT2 plus NT4 versus HeLa5 or NTnt

Sequence/Repeat	cDNA	NT2 & NT4	NTnt	HeLa5
1st	(GCC) 8	(GCC) 5	(GCC) 9	(GCC) 8
2nd	(GCC) 8	(GCC) 4 ACC (GCC) 3	(GCC) 9	(GCC) 8
3rd	(GCC) 8	(GCC) 4 ACC (GCC) 3	(GCC) 8	(GCC) 8
4th	(GCC) 4 ACC (GCC) 3	(GCC) 5 or (GCC) 9	(GCC) 8	(GCC) 8
5th	(GCC) 8	(GCC) 8	(GCC) 8	(GCC) 3 ACC 2 (GCC) 3

Table Ib. Variations in the microsatellite sequence in the first repeat of the gene for epiplakin among 15 individuals.

Eight repeats were most frequently found in 15 chromosomes, in five of which there was a G to A transition in the fifth repeat. Five GCC repeats were found in nine chromosomes and nine repeats were found in six chromosomes, in one of which there was a G to A transition in the seventh repeat. Numbers after parentheses indicate the number of GCC repeats

Repeats	Variation	Number of chromosomes in which variation was found
5	(GCC) 5	9
8	(GCC) 8	10
	(GCC) 4ACC (GCC) 3	5
9	(GCC) 9	5
	(GCC) 6ACC (GCC) 2	1

Epiplakin contains 13 B domains, which are 70–46% homologous to the B domain at the carboxyl terminus of desmoplakin, and these domains are distributed along the sequence with relatively uniform spacing. In the genes of other known members of the plakin family, including desmoplakin, plectin, and BPAG1, the carboxy-terminal domain is also encoded by one exon (Green *et al.*, 1999) and this rule is not broken in the case of epiplakin. The polymorphism among individuals with respect to the five repeated structures in the gene for epiplakin might contribute to the functional diversity of this region. Nine or less than nine GCC repeats that encode polyalanine are localized in the linker regions of the five homologous repeats in the human gene for epiplakin. The B domain is strongly conserved between human and mouse, although the linker region differs considerably between these two species and the GCC repeats are unique to the human genome (unpublished data). Thus, if genetic cutaneous diseases with microsatellites of abnormal length, similar to those in a trinucleotide repeat expansion disease in the central nervous system (Ashley and Warren, 1995) were to exist, they would be restricted to humans.

The gene for epiplakin mapped to chromosomal band 8q24.3, which is close to the locus of the gene for plectin (Liu *et al.*, 1996; McLean *et al.*, 1996). According to the UCSC Human Genome Browser (<http://genome.ucsc.edu/>) in April 2003, the specific location of the gene for epiplakin is the telomeric end of the long arm of chromosome 8, consistent with our data, but the genomic size of the gene for epiplakin was given as 7524 bp, which is a major underestimate. Almost all the strongly conserved repeats at the 3' end were missing, perhaps due to the limited ability of the shot-gun sequencing method used in the international genome project. The distance between the gene for epiplakin and that for plectin was estimated to be about 60 kb and only one predicted gene is localized between them. Both genes are oriented in the same directions and are transcribed towards the centromeric end. The position of these two genes in relatively close proximity suggests that they might be regulated cooperatively.

Our mapping data exclude the possibility that the genomic clones that we isolated might represent processed pseudogene(s), even though they did not contain any introns. One mapped locus, the absence of a paralog, and the absence of a polyadenylated [poly(A)]tail suggest that retrotransposition of the gene for epiplakin, in which spliced mRNA would be reverse transcribed and reinserted into the genome, is unlikely to have occurred in the evolution of the gene for epiplakin.

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Propionibacterium acnes-Reactive T Helper-1 Cells in the Skin of Patients with Acne Vulgaris

To the Editor:

Acne vulgaris is the commonest skin disorder to affect humans, characterized by both noninflammatory (comedones) and inflammatory lesions (papules, pustules, and nodulocystic lesions). It is a disease of the pilosebaceous follicle with comedones resulting from the hypercornification of the keratinocytes of the duct wall and usually preceding inflammatory lesions (Cunliffe *et al*, 2000). Of particular interest in the pathophysiology of inflammatory acne is the role of the normal skin commensal bacterium *Propionibacterium acnes*. Although not a requirement for comedogenesis, a number of observations have suggested that *P. acnes* is implicated in the pathogenesis of inflammatory acne. The density of *P. acnes* increases markedly during puberty coinciding with the onset of the disease (Leyden *et al*, 1975). *P. acnes* is rarely found in animal skin and acne is not seen in animals (Webster *et al*, 1981; Kearney *et al*, 1982). Treatments that reduce *P. acnes* numbers lead to clinical improvement of acne (Thiboutot, 1997) and, finally, the emergence of antibiotic-resistant *P. acnes* strains are linked to the failure of antibiotic treatment (Eady *et al*, 1989). The immunostimulatory activity of *P. acnes* and resulting prototypic T helper 1 immune response has been widely studied in animal models (Matsui *et al*, 1997; Okazaki *et al*, 2001); however, the specificity and cytokine profiles of lesional T cells from skin of acne patients have not been investigated previously. Here we describe the generation of T cell lines (TCL) from inflamed acne lesions, and their proliferative and cytokine responses to *P. acnes* antigens. Approval was granted for this research project by the Hammett-Smith Queen Charlotte's & Chelsea and Actin Hospitals Research Ethics Committee. Full consent was granted by subjects.

Fourteen TCL were generated from early papular inflammatory acne lesions of 16 untreated patients, stained for CD4, CD8, and T cell receptor $\alpha\beta$ expression and tested in proliferation assays with *P. acnes* and control antigens (Fig 1). To determine their cytokine profile, TCL were stimulated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin or *P. acnes* extract and stained intracellularly for interferon (IFN)- γ , interleukin (IL)-4, IL-5, and IL-10 using flow cytometry. Results were compared with eight corresponding TCL generated in the same way from lesional skin of 11 psoriatic patients.

Acne TCL consisted of T cell receptor $\alpha\beta^+$ T cells ($96.4 \pm 4\%$) with a predominance of CD4 $^+$ ($92 \pm 7.5\%$) phenotype, whereas psoriatic TCL contained a higher percentage of CD8 $^+$ T cells

($24.8 + 21.4\%$; $p < 0.05$). This is in agreement with an earlier immunohistochemical study that demonstrated perivascular and periductal infiltrates of CD4 $^+$ T cells in early acne skin lesions (Layton *et al*, 1998); however, the stimulus for T cell infiltration into acne lesions remains to be determined. The initial T cell infiltrate may represent a specific cell-mediated immune response to *P. acnes* antigens within the ductal lumen. In support of this hypothesis, this study has shown that all acne TCL proliferated to *P. acnes* extract (optimal concentration 1:100) with a wide range of responses (mean 6420 ± 4545 cpm) (Fig 1). Anti-HLA-DR

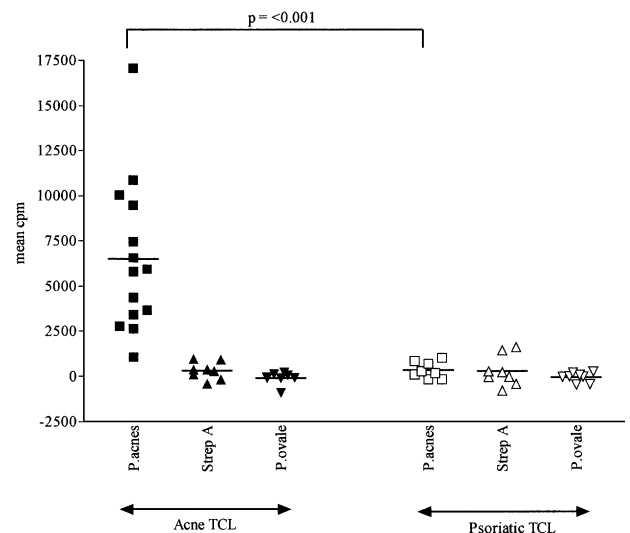


Figure 1. Increased proliferative response to *P. acnes* extract by acne versus psoriatic TCL. TCL were established by culturing skin fragments (4 mm acne skin biopsies or psoriasis shave biopsies) in RPMI 1640 medium (Gibco BRL, Invitrogen Ltd, Paisley, UK) supplemented with 2 mM L-glutamine, 100 U per mL penicillin and 100 μ g streptomycin per mL (Gibco BRL), and 10% human AB $^+$ serum (complete medium) containing *P. acnes* extract (1:100 dilution), adding 20 U per mL human recombinant IL-2 (Roche Molecular Biochemicals, Lewes, UK) every 3 d. After 14 d culture, TCL (2.5×10^4 per well) plus an equal number of irradiated, autologous peripheral blood mononuclear cells were cultured for 3 d in the presence or absence of optimal concentrations of each of the microbial preparations in 96-well plates. Tritiated thymidine (4 mCi per mL, specific activity 5 Ci per mmol; Amersham International, Amersham, UK) was added for the last 6 h of culture. The cells were harvested on to fiberglass paper, and the incorporated radioactivity measured in a β scintillation counter. Proliferation was expressed as the mean cpm of three replicates in the presence of microbial preparation minus the mean cpm of three replicates in the absence of microbial preparation. Bars indicate the mean proliferative response of each group.

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