# Characterization of an Autoantigen Associated With Chronic Ulcerative Stomatitis: The CUSP Autoantigen is a Member of the p53 Family[1]

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**A unique clinical syndrome has been described in which patients have chronic oral ulceration and autoantibodies to nuclei of stratified squamous epithelium. We have characterized the autoantibodies from patient sera and found that the major autoantigen is a 70 kDa epithelial nuclear protein. Sequencing of the cDNA for this protein, chronic ulcerative stomatitis protein, revealed it to be homologous to the *p53* tumor suppressor and to the *p73* putative tumor suppressor, and to be a splicing variant of the *KET* gene. The *p53*-like genes, *p73* and the several *KET* splicing variants, are recently described genes of uncertain biologic and pathologic significance. This study provides the first clear association of a p53-like protein with a disease process.** ***Key words: KET/p73/tumor suppressor. J Invest Dermatol 113:146–151, 1999***

In 1990, Jaremko and coworkers reported a unique clinical syndrome in which patients had chronic ulcerative stomatitis (hereafter referred to as CUS) and IgG antibodies to keratinocyte nuclei (Jaremko *et al*, 1990). The antibodies were present in the circulation, the skin, and the oral mucosa of affected individuals. As the circulating autoantibodies bound a nuclear antigen that was present in epidermis and in esophageal epithelium but was undetectable in kidney or in HEp-2 cells, it was speculated that the expression of the autoantigen was limited to stratified squamous epithelium. Parodi and Cardo (1990), reporting two cases similar to those of Jaremko and coworkers, identified an epithelial autoantigen migrating in the range of 70–75 kDa. Antigenicity was adversely affected by DNAses, indicating that the antigen may bind DNA. In this report, using sera from patients with autoantibody-associated CUS, we characterize CUSP (chronic ulcerative stomatitis protein), a 70 kDa protein bound by the autoantibodies. Sequencing of the cDNA for *CUSP* reveals it to be homologous to the *p53* tumor suppressor and to the *p73* putative tumor suppressor, and to be a splicing variant of the newly described p53-like gene, *KET*.

## MATERIALS AND METHODS

**Sera** Serum samples were obtained from nine patients seen at the Warsaw School of Medicine who had CUS and a particulate pattern of IgG deposition in keratinocyte nuclei in direct and indirect immunofluorescence studies. Control samples were from 10 healthy volunteers and from patients with one of the following diseases: recurrent aphthous stomatitis (24 subjects), oral lichen planus (six subjects), autoantibody-positive dermatomyositis (two subjects), and autoantibody-positive lupus erythematosus. Of the autoantibody-positive subjects with lupus, 15 had discoid skin lesions, 15 had subacute cutaneous skin lesions, and two had systemic disease with no skin lesions. Of the patients with discoid lesions, five had systemic disease. Of the patients with subacute cutaneous lesions, six had systemic disease.

**Cell culture** Normal human neonatal foreskin keratinocytes were cultured in keratinocyte serum-free medium purchased from GibcoBRL (Gaithersburg, MD). Contaminating melanocytes were removed by a 5 min incubation with 0.025% trypsin + 0.02% ethylenediamine tetraacetic acid and recultured. HeLa cells (cervical carcinoma), A431 cells (epidermoid carcinoma), and COS-1 cells (monkey kidney cells) were obtained from American Type Culture Collection (Manassas, VA), HaCaT cells (transformed keratinocytes) were obtained originally from Professor Norbert Fusenig (German Cancer Research Center, Heidelberg, Germany), and human WM1617 melanoma cells were obtained from the Wistar Institute (Philadelphia, PA). Unless stated otherwise, cultures were used for experiments at ≈80% confluence.

**Immunofluorescence** Cultured keratinocytes, melanocytes, A431, HaCaT, HeLa, COS, and WM1617 melanoma cells were plated on to eight-well LabTek slides (Nalgene, Naperville, IL) for microscopy studies. Cells were permeabilized with cold acetone for 1 min prior to incubation with sera. HEp-2 cells on microscope slides were purchased ready for use from INOVA (San Diego, CA). Sera were diluted 1:100, 1:1000, and 1:10,000 in phosphate-buffered saline and overlaid on the slides for 1 h at room temperature. Antibody binding was identified using a 1:250 dilution of fluorescein-conjugated anti-human IgG purchased from Dako (Carpenteria, CA).

**Immunoblotting and immunoprecipitation** Protein extracts were prepared from cultured normal human neonatal keratinocytes and from

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Abbreviations: CUS, chronic ulcerative stomatitis; CUSP, chronic ulcerative stomatitis protein.

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HeLa cells, and from epidermal sheets obtained immediately after trypsin separation of epidermis from dermis. Extracts were subjected to standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis, then proteins were transferred on to nitrocellulose paper and immunoblotted with 1:100 dilutions of CUS sera and control sera (Targoff *et al*, 1993). Immunoprecipitation of $^{35}$S-labeled keratinocytes and HeLa cells and RNA immunoprecipitation were performed according to previously published techniques (Targoff *et al*, 1993).

**Keratinocyte cDNA library preparation** A keratinocyte cDNA library was prepared from a culture of normal human neonatal keratinocytes using a ZAP Express cDNA Synthesis Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. The library was packaged using the Gigapack II Gold packaging extract from Stratagene and plated on the *Escherichia coli* cell line XL1-Blue MRF′.

**Cloning and sequencing** CUS sera were used at a 1:1000 dilution to screen the human keratinocyte cDNA library. Candidate clones were subcloned and tested for reactivity with other CUS sera and with control sera. Sequencing was performed by the University of Colorado Cancer Center DNA Sequencing and Analysis Core Facility.

Sequencing of the cDNA 5′ of the cloned cDNA was accomplished using a 5′ Rapid Amplication of cDNA Ends (RACE) technique (Kozak, 1987). Epidermal sheets were separated from the dermis by overnight incubation in 0.5 M ammonium thiocyanate in 0.1 M sodium phosphate buffer, pH 6.8. The epidermal sheets were suspended in TRIzol reagent (a monophasic solution of phenol and guanidine isothiocyanate, GibcoBRL), subjected to several freeze/thaw cycles, and processed according to the manufacturer's specifications. First-strand cDNA synthesis used the Super-Script II RNase H$^-$ Reverse Transcriptase (GibcoBRL) with gene-specific primers. After cDNA synthesis, the RNA template was degraded using a mixture of RNase H and RNase T1 and the RNA-free cDNA was then used as a template for the RACE technique. A homopolymeric tail was added to the 3′ end of the cDNA (corresponding to the 5′ end of the mRNA) using TdT and dCTP. Polymerase chain reaction amplification was accomplished using Taq DNA polymerase, a nested, gene-specific primer that annealed to a site located within the cDNA molecule, and a deoxyinosine-containing anchor primer.

The deduced amino acid sequences and the sequence comparisons were performed using the GenBank database and the BLAST 2.0.4 program (Altschul *et al*, 1997). Sequence alignment was performed using the ClustalW Multiple Sequence Alignment program obtained from Kim Worley of the Human Genome Center, Baylor College of Medicine, Houston, TX.

**Transfection of COS cells** Full-length CUSP was expressed in COS cells, which do not normally express CUSP, using cationic liposome-mediated transfection. The transfection procedure utilized the FuGENE 6 Transfection Reagent (Roche Molecular Biochemicals; Basel, Switzerland) according to the manufacturer's directions. Expression of CUSP was examined using CUS patient sera and affinity-purified anti-CUSP in an immunofluorescence technique.

**Affinity purification of anti-CUSP** Antibodies to the protein expressed by the clone (a partial sequence consisting of the 3′ end of CUSP) were affinity purified by the following technique. A nitrocellulose filter of ≈50 cm$^2$ was overlaid on *E. coli* colonies containing the clone. Patient serum diluted 1:100 was incubated with the nitrocellulose for 2 h. Following washing, antibodies bound to the nitrocellulose were eluted for 10 min with 1 ml of 100 mM glycine, pH 2.5, then 30 μl of 1 M Tris, pH 9, was added. These affinity-purified antibodies were then used in immunoblotting. Control antibodies were obtained by affinity purification against *E. coli* containing vector without insert.

Antibodies to the full-length CUSP protein were prepared using a Sepharose 4B column (Pharmacia, Piscataway, NJ) containing the protein product of full-length CUSP cDNA. Antibodies were eluted with glycine as above, and then dialyzed against phosphate-buffered saline prior to use in immunoblotting or immunofluorescence.

**Immunization of rabbits** Two adult New Zealand white rabbits were immunized with a peptide containing amino acids 4–17 from the N′-terminus of CUSP, using a standard immunization protocol with the KLH-conjugated CUSP peptide diluted in the adjuvant TiterMax Classic (Sigma, St. Louis, MO). Boosting was performed every 3 wk. Antibody responses were assessed preimmunization, at 3 wk, and at 6 wk, using immunofluorescence and immunoblotting.

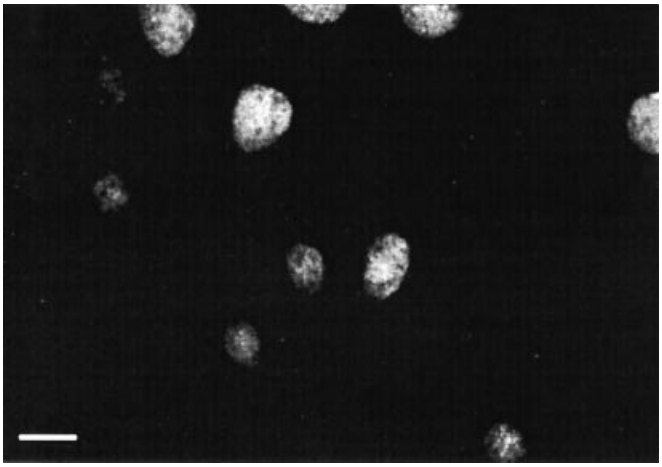

**Figure 1. Cellular location of autoantibodies in chronic ulcerative stomatitis.** Cultured normal human keratinocytes were incubated with serum from a patient with chronic ulcerative stomatitis. Antibody binding is identified using a fluoresceinated probe. There is particulate staining of keratinocyte nuclei. *Scale bar*: 10 μm.

**Northern blot analysis** The expression of CUSP mRNA was examined using a standard northern blotting technique. Total RNA was extracted from cultured human keratinocytes, human melanoma cells, COS cells, A431 cells, and HaCaT keratinocytes. Following electrophoresis, the RNA was probed with a digoxygenin-labeled probe derived from the 5′ sequence of *CUSP*. The probe consisted of 630 bp extending from 60 bp into the 5′ UTR through bp 570 in the coding region.

## RESULTS

**Antibodies in CUS sera bind keratinocyte nuclei in immunofluorescence** IgG antibodies in CUS sera bound nuclei of normal human keratinocytes, A431 cells, and HaCaT cells with a particulate pattern (**Fig 1**). Nuclear staining was absent when HEp-2, HeLa, COS cells, normal human melanocytes, and WM1617 melanoma cells were used. Normal human keratinocyte cultures were examined at ≈50%, 80%, and 100% confluence, and at each of these conditions strong nuclear staining was observed.

**Antibodies in CUS sera identify a 70 kDa keratinocyte protein** Each of the CUS sera contained antibodies to a protein migrating at ≈70 kDa in immunoblotting (**Fig 2*A***). The protein was present in keratinocytes and epidermal sheets but absent in HeLa cells. Although other antibody specificities were present in the sera, the specificity common to all the sera was anti-70 kDa. Six of nine sera also contained antibodies to a protein of ≈52 kDa present in keratinocytes but not detected in HeLa cells.

In the evaluation of the control sera, all samples from normal individuals and all samples from patients with autoimmune diseases (dermatomyositis and lupus erythematosus) were tested in both immunofluorescence and immunoblotting. The samples from patients with recurrent aphthous stomatitis and oral lichen planus were first screened with immunofluorescence, and those showing nuclear staining (two samples) were then tested in immunoblotting to determine whether the autoantibodies were specific for CUSP. Antibodies to CUSP were not detected in control sera from these 74 controls consisting of 10 healthy subjects, 24 patients with recurrent aphthous stomatitis, six patients with oral lichen planus, two patients with dermatomyositis, 15 patients with subacute cutaneous lupus, 15 patients with discoid lupus, and two patients with systemic lupus erythematosus without cutaneous lesions.

Similar results were obtained with protein immunoprecipitation performed with five of the CUS sera and five control sera (**Fig 2*B***). A 70 kDa protein was precipitated from keratinocyte but not HeLa cell extract. The 70 kDa protein was the only protein precipitated by all CUS sera and no control sera, and detected in keratinocytes but not HeLa cells. The precipitates were also examined for RNA complexed with the autoantigen. No RNAs were present in the

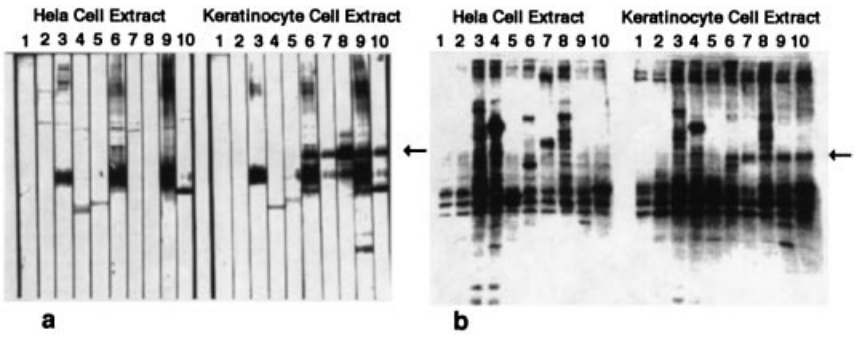


**Figure 2. CUS antibodies bind a 70 kD keratinocyte protein.** (*a*) Immunoblot with HeLa cell extract (*left side*) and cultured keratinocyte extract (*right side*). Reactivity of cell extracts with control normal sera is shown in *lanes 1* and *2*. Reactivity with control dermatomyositis and lupus sera is shown in *lanes 3–5*. Reactivity with CUS sera is shown in *lanes 6–10*. A different serum is used for each lane. The CUS sera bind a protein migrating at 70 kDa (*arrow*) present in the keratinocyte extract but not the HeLa cell extract. (*b*) Immunoprecipitation of proteins from HeLa cells (*left side*) and cultured keratinocytes (*right side*). Proteins precipitated by normal sera are shown in *lanes 1* and *2*, and proteins precipitated by dermatomyositis and lupus sera are shown in *lanes 3–5*. Proteins precipitated by CUS sera are shown in *lanes 6–10*. The notable finding is the presence of a protein at 70 kDa precipitated from keratinocytes but not HeLa cells by CUS antibodies.

precipitates using the CUS sera, indicating that the CUS-associated autoantigen is not a ribonucleoprotein (data not shown).

**Sequencing of the 70 kDa antigen, CUSP** A keratinocyte cDNA expression library was screened using CUS sera. A candidate clone was identified which bound six of six CUS and none of five normal sera. Antibodies affinity purified to the clone reacted with a single band of 70 kDa in immunoblotting. The cDNA consisted of ≈3500 nucleotides. Approximately 700 nucleotides were in the coding region, and the remaining 2800 nucleotides were in a large 3′ UTR. In order to obtain the 1100 nucleotides that were in the coding region for CUSP but were 5′ of the clone, 5′ RACE was used.

Once the full coding sequence of CUSP was obtained, a full-length CUSP insert was used to affinity purify anti-CUSP antibodies. The affinity-purified antibodies produced nuclear staining of cultured keratinocytes in immunofluorescence. Affinity purification to the full-length CUSP protein produced a 70 kDa band in immunoblotting and nuclear staining of keratinocytes in immunofluorescence.

As further evidence confirming that the sequence represents CUSP, the cDNA sequence was used for polycationic transfection of COS cells. COS cells do not normally express CUSP, as was verified by immunofluorescence, immunoblotting, and northern blotting. Following transfection of COS cells with the cDNA, there was nuclear expression of the protein (**Fig 3**).

Finally, rabbits immunized to a peptide of CUSP developed antibodies to a 70 kDa keratinocyte protein and their sera produced staining of keratinocyte nuclei in immunofluorescence.

**Homology of CUSP to *p53* and *p53*-like genes** The predicted amino acid sequence of CUSP is homologous to rat KET, p73 and p53 (**Fig 4**). In humans, several genes have been reported recently which are human homologs of rat *KET*, including *p51*, *p40*, and *p73L*. Their predicted amino acid sequences are shown in **Fig 4**, with the exception of p73L, which differs from CUSP only at three amino acid positions, and rat KET, which is nearly identical to human p51B. CUSP and p40 are virtually identical at the 5′ end, but CUSP contains an additional 230 amino acids at the C-terminus. Two variants of p51, p51A (predicted to be 51 kDa) and p51B (predicted to be 72 kDa) have been described (Osada *et al*, 1998). CUSP is identical to the p51B variant except for the 5′-most amino acids. An overview of the KET variants is presented in **Fig 5**. There are at least three splicing variants of KET: CUSP/p73L, p51B, and p51A. It is not clear whether p40 is a distinct splice variant or an incompletely sequenced gene. Two variants of p73, α and β, have been reported, with p73β being a splicing variant lacking exon 13 (Kaghad *et al*, 1997). CUSP more closely resembles the p73α variant, having an ≈60% identity to p73α in its predicted amino acid sequence.

A comparison of the KET variants to p73 and human p53 in the four conserved regions (II–V) of the DNA-binding domain can be seen from examination of **Fig 4**. In these regions, the sequences of CUSP and all the other KET variants are identical. The amino acid identities between KET and p73 and human p53, respectively, are as follows: 92% and 65% for region II; 90% and 90% for region III; 96% and 81% for region IV; and 94% and 71% for region V. Thus, the KET gene is more closely related to p73 than to p53.

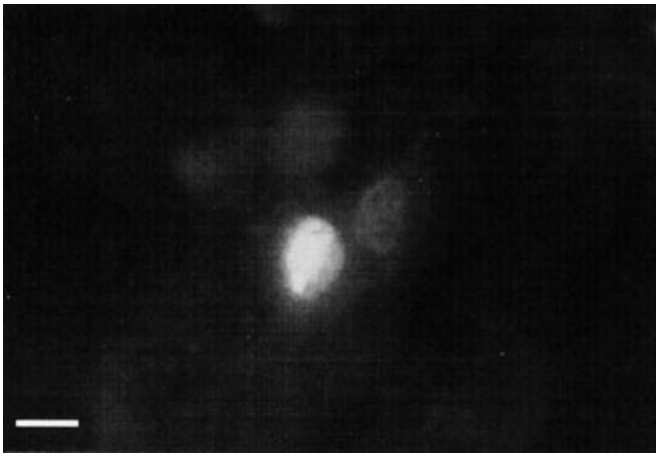

**Figure 3. Expression of CUSP protein in COS cells.** Following polycationic transfection of full-length CUSP, CUSP expression was identified using CUS serum and an immunofluorescence technique. The transfected COS cells show nuclear fluorescence. *Scale bar*: 10 μm.

**Expression of CUSP mRNA** Northern blotting of cultured keratinocytes revealed a strong band of ≈4.7 kb, and a weak band of ≈5.8 kb (**Fig 6**). No bands were detected in COS cells or human melanoma cells. The CUSP 4.7 kb transcript was readily detected in A431 cells and HaCaT cells, but the 5.8 kb transcript was not detected in either of those cell lines.

## DISCUSSION

The syndrome of chronic ulcerative stomatitis with antibodies to a nuclear antigen of stratified epithelium is defined both by its clinical phenotype and its autoantibody specificity. CUS is characterized clinically by erosive and exfoliative oral lesions, a chronic course with exacerbations and remissions, predominance of females and older individuals, and a therapeutic response to chloroquine (Chorzelski *et al*, 1998). Circulating and tissue-bound IgG antibodies to a keratinocyte nuclear antigen are required for diagnosis (Jaremko *et al*, 1990; Beutner *et al*, 1991). We have characterized the CUSP autoantigen and shown it to be homologous to p53 and p73, and to be a variant of the p53-like *KET* gene.

The p53 tumor suppressor plays a crucial part in the prevention of tumorigenesis, allowing cells that are damaged to repair that damage before cell division takes place and directing cells that are overwhelmingly damaged to commit suicide through apoptosis

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CUSP                                                                MLYLE
p51        MSQS-TQTNEFLSPEVFQHIWDFLEQPICSVQPIDLNFVDEPSEDGATNKIEISMDCIRMQ
p73β       MAQS--TATSPDGGTTFEHLWSSLE-------PDSTYFDLPQSSRGNN--EVVGGTDSSMD
p73α                        I                                       MD
p53      MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMD----------------------

CUSP      NNAQTQFSEPQYTNLGLLNSMDQQIQNG--SSSTSPYNTDHAQNSVTAPSPYAQPSSTFDAL
p51       DSDLSDPMWPQYTNLGLLNSMDQQIQNG--SSSTSPYNTDHAQNSVTAPSPYAQPSSTFDAL
p73       VFHLEGMTTSVMAQFNLLSSTMDQMSSR--AASASPYTPEHAA-SVPTHSPYAQPSSTFDTM
p53       -------PSQAMDDLMLSPDDIEQWFTEDPGPDEAPRMPEAAP--RVAPAPAA-PTPAAPAP

CUSP/p51  SP----SPAIPSNTDYPGPHSFDVSFQQSSTAKSATWTYSTELKKLYCQIAKTCPIQIKVMTPP
p73       SP----APVIPSNTDYPGPHHFEVTFQQSSTAKSATWTYSPLLKKLYCQIAKTCPIQIKVSTPP
p53       APSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCEVQLWVDSTP
                                          II

CUSP/p51   PQGAVIRAMPVYKKAEHVTEVVKRCPNHELSREFNEGQIAPPSHLIRVEGNSHAQYVEDP
p73        PPGTAIRAMPVYKKAEHVTDVVKRCPNHELGRDFNEGQSAPASHLIRVEGNNLSQYVDDP
p53        PPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSD-G-LAPPQRLIRVEGNLRVEYLDDR
                              III

CUSP/p51   ITGRQSVLVPYEPPQVGTEFTTVLYNFMCNSSCVGGMNRRPILIIVTLETRDGQVLGRRC
p73        VTGRQSVVVPYEPPQVGTEFTTILYNFMCNSSCVGGMNRRPILIIITLEMRDGQVLGRRS
p53        NTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNS
                                    IV

CUSP/p51   FEARICACPGRDRKADEDSIRKQQVSD--STKNGDGTKRPFRQNTHGIQMT--SIKKRRS
p73        FEGRICACPGRDRKADEDHYREQQALNESSAKNGAASKRAFKQSPPAVPALGAGVKKRRH
p53        FEVRVCACPGRDRRTEEENLRKKGEPH--HELPPGSTKRALPNNTSSSPQP---KKKPLD
           V                                                    OLIG

CUSP/p51B  PDDELLYLPVRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQTSIQS
p51A       PDDELLYLPVRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQK------
p73        GDEDTYYLQVRGRENFEILMKLKESLELMELVPQPLVDSYRQQQQ-----LLQRPSHLQP
p53        G--EYFTLQIRGRERFEMFRELNEALELKDAQAGKEPGGSRAHSS----------HLKS
                          OLIG

CUSP/p51B  PSSYGNSSPPLNKMN-SMNKLPSVSQLIN--PQQRNALTPTTIPDGMGANIPMMGTHMPM
p51A       --------------H--LLSACFRNELV-EPRRE----TPKQSDV----FFR---HSKPP
p73        PS-YGPVLSPMNKVHGGMNKLPSVNQLVGQPPPHSSAATPNLGPVGPG-MLNNHGHAVPA
p53        KK--GQSTSRHKKLM-FKTEGPDSD

CUSP/p51B  AGDMNGLSPTQALPPPLSMPSTSHCTPPPPYPTDCSIVSFLARLGCSSCLDYFTTQGLTT
p51A       N-------------------------RSVYP
p73α       NGEMSSSHSAQ------SMVSGSHCTPPPPYHADPSLVSFLTGLGCPNCIEYFTSQGLQS
p73β       NGEMSSSHSAQ------SMVSGSHCTPPPPYHADPSLVRTWGP

CUSP/p51B  IYQIEHYSMDDLASLKIPEQFRHAIWKGILDHRQLHEFSSPSHLLRTPSSASTVSVGSSE
p73α       IYHLQNLTIEDLGALKIPEQYRMTIWRGLQDLKQGHDYSTAQQLLRSSNAATISIGGSGE

CUSP/p51B  TRGERVIDAVRFTLRQTISFPPR-------DEWNDFNFDMDARRNKQQRIKEEGE
p73α       LQRQRVMEAVHFRVRHTITIPNRGGPGGGPDEWADFGFDLPDCKARKQPIKEEFTEAEIH
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**Figure 4. Predicted amino acid sequence of CUSP and its comparison with p51A, p51B, p73α, p73β, and human p53.** The cDNA sequence of CUSP is available through GenBank, accession number AF091627. The predicted amino acid sequences of p51A, p51B, p73α, p73β, and p53 were transcribed directly from GenBank. The sequence alignment was performed by the ClustalW Multiple Sequence Alignment program. The boxed areas represent the transactivation (I) domain, the conserved regions (II–V) of the sequence-specific DNA binding domain, and the oligomerization (OLIG) domain.

(Chang *et al*, 1995; Nataraj *et al*, 1995; Brash and Bale, 1997). Recently, *p53*-like genes have been discovered, either fortuitously or through a directed search using degenerate primers from highly conserved regions of *p53*. We have found a *p53*-like gene through a different pathway, as a result of characterizing the autoantigen of a unique clinical syndrome in which patients have CUS and IgG antibodies to keratinocyte nuclei (Jaremko *et al*, 1990). The autoantigen, which we termed "CUSP", for chronic ulcerative stomatitis protein, is homologous to rat KET. The p53-like rat *KET* gene was identified fortuitously in rat tongue papillae and shown to be restricted in its expression to oral epithelium, skin, and thymus (Schmale and Bamberger, 1997). During the course of this work, several human homologs of *KET* have been reported as a result of a search for *p53*-like genes. These include: *p40*, which contains the 5′ sequence of *CUSP* (Trink *et al*, 1998); *p73L*, which is almost identical to *CUSP* (Senoo *et al*, 1998); and *p51B*, which contains the 3′ sequence of *CUSP* (Osada *et al*, 1998). In general, unlike p53 itself, the p53-like proteins are not expressed in all tissues, but rather are restricted in their tissue expression (Kaelin, 1998). Although these genes are clearly homologous to *p53*, their role as tumor suppressors is uncertain (Kaelin, 1998).

The 5′ identity of *CUSP* to *p40* and the 3′ identity of *CUSP* to *p51B* indicate that *CUSP/p73L*, *p40*, and *p51* are likely to be splicing variants of the human homolog of rat *KET* (see **Fig 5**).

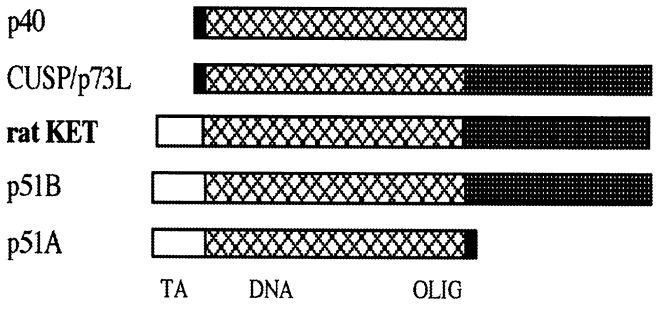


**Figure 5. Diagram of the relationship of *CUSP* to *p40*, *p51A*, *p51B*, and rat *KET*.** Regions where the sequences are virtually identical are depicted as identically filled boxes. The approximate locations of the transactivation, DNA binding, and oligomerization domains are noted as TA, DNA, and OLIG, respectively. Rat *KET*, the originally described gene, is shown in the middle, with the human homologs of *KET* above and below. Rat *KET* and *p51B* are virtually identical. *CUSP/p73L* and *p40* have a different 5′ sequence than rat *KET*, with *p40* having a truncated 3′ region. *CUSP/p73L* have the same 3′ sequence as rat *KET* and *p51B*. *p51A* differs from *p51B* and rat *KET* at the 3′ end.

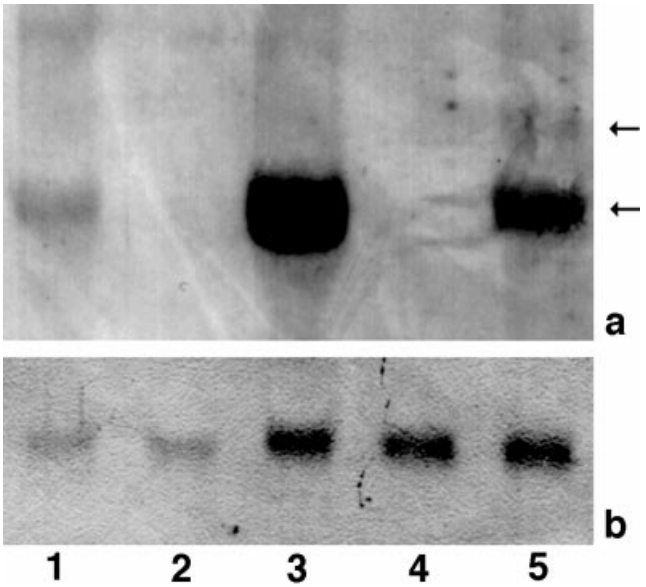


**Figure 6. Expression of CUSP mRNA in cultured cells.** Shown is a northern blot hybridized to a CUSP probe in the upper panel (*a*) and to an actin probe in the lower panel (*b*). The lanes contain RNA from the following cultured cells: *lane 1*, A431 epithelial carcinoma cells; *lane 2*, COS monkey kidney cells; *lane 3*, HaCaT transformed keratinocytes; *lane 4*, melanoma cells; and *lane 5*, normal human keratinocytes. The 4.7 kb CUSP transcript (*lower arrow*) is readily identified in the keratinocyte-derived cells. In *lane 5*, there is also a weak band at ≈5.9 kb (*upper arrow*).

(CUSP and p73L are so nearly identical in sequence that they must represent the same splicing variant; the minimal difference in sequence can probably be attributed to sequencing artifact or to sequence polymorphisms.) The likelihood that the human *KET* homologs are splicing variants is rendered almost certain by the localization of *p40*, *p51*, and *p73L* to the same area of chromosome 3 (Osada *et al*, 1998; Senoo *et al*, 1998; Trink *et al*, 1998). Keratinocytes express at least one splicing variant of human KET, but whether they express other splicing variants is not clear. When we used the RACE technique to obtain the 5′ sequence of CUSP, three different reactions revealed the same sequence and did not indicate 5′ splicing variants. We have also failed to detect other splice variants using the reverse transcriptase–polymerase chain reaction with freshly obtained human epidermal sheets or cultured human keratinocytes. Northern blotting of cultured keratinocytes showed a strong band at 4.7 kb, which is consistent with the size of CUSP, and a weak band of 5.8 kb. Transcripts of sizes previously reported for other human KET variants were not detected. The weak band of 5.8 kb may represent a polyadenylation variant of CUSP, an unidentified splicing variant of human KET, or a homologous gene hybridized to the probe. In any case, CUSP appears to be the major transcript detected in keratinocytes.

As is the case with all the human *KET* variants, *CUSP* has a greater similarity to *p73* than to *p53*. CUSP has a 60% identity with p73 throughout the coding region and a 94% identity with p73 in the predicted amino acid sequence in four conserved regions of the DNA-binding domain. Because *p73* has greater similarity to squid and to trout *p53* than to human *p53*, it is speculated that *p73* and *p53* diverged early in evolution, perhaps from an ancestral *p73*-like gene (Kaghad *et al*, 1997) or *KET*-like gene.

Currently, the p53-like proteins have neither defined functions nor defined roles in disease processes. This study provides a link, albeit not completely elucidated, between a p53-like protein and a disease process. Although it has not been established that autoantibodies to CUSP cause the disease, the association of the CUSP autoantigen with the CUS syndrome is clear. It is intriguing that the CUS antigen is expressed in oral epithelium and skin, and patients with CUS have lesions in oral epithelium and, occasionally, skin. A direct pathogenic effect of anti-CUSP, however, has not been addressed by these studies, and a possible mechanism of pathogenesis is clearly open to speculation. It is notable that the CUSP gene does not have an identifiable transactivation (I) domain (**Fig 4**). The lack of a transactivation domain, together with highly conserved sequence-specific DNA domains and oligomerization domains, may mean that CUSP binds DNA but does not activate genes. Theoretically, CUSP could compete with p53 or other p53-like proteins for DNA binding sites, and could therefore downregulate the effects of p53 or other p53-like proteins. If so, as expression of p53, p73, and p51 has been shown to produce apoptosis, CUSP may be in effect an anti-apoptotic protein. Should autoantibody binding to CUSP alter its function, apoptotic epithelial cell injury could conceivably result. The function of CUSP has yet to be defined, however, and the link, if any, between anti-CUSP and alteration of CUSP function has yet to be demonstrated.

In support of a relationship between autoantibodies to CUSP and CUS is the apparent disease specificity of the autoantibodies. Clearly, patients are classified as having CUS only if they have antibodies to a nuclear epithelial antigen together with chronic oral lesions, so a relationship between autoantibodies and disease phenotype is implicit in the definition of CUS. Anti-CUSP autoantibodies, however, are not commonly seen in other settings, as confirmed by the lack of detection of anti-CUSP in 74 control sera. Anti-CUSP autoantibodies are not linked simply to autoimmunity or to chronic oral lesions, as we did not find anti-CUSP in sera from patients with other autoantibody specificities (dermatomyositis and lupus patients) and in sera from patients with other oral diseases (aphthous stomatitis and lichen planus). Our studies do not, however, define exactly the specificity of anti-CUSP for CUS, and we would not conclude that all individuals with anti-CUSP must have or develop CUS.

It has not been established that the CUSP autoantigen is the only autoantigen in CUS. Clearly, some patients' autoantibodies bind other proteins present in keratinocytes. The sequence similarity between CUSP, p*40*, p51A, p51B, and even p73 and p53 could result in cross-reactivities between CUSP and those proteins, and such cross-reactivities could be of clinical significance. At the present time, to our knowledge, only the p73 and p53 proteins have been characterized in gel electrophoresis, and the proteins encoded by the KET splicing variants, p40 and p51, have not been characterized. The predicted amino acid sequences indicate though that CUSP should have a somewhat different molecular weight than the other p53-like proteins. The observation that the CUSP band at 70 kDa in immunoblotting is the only band consistently identified by all our patient sera indicates that it is likely to be the major autoantigen. If other autoantigens are associated with CUS, the frequent presence of a band at ≈ 52 kDa in immunoblotting indicates that the ≈52 kDa protein is the strongest candidate for an additional autoantigen associated with CUS. We have affinity-purified antibodies to 52 kDa protein from nitrocellulose and reacted these antibodies in immunoblotting and immunofluorescence (data not shown). The antibodies bind only the 52 kDa protein and not 70 kDa CUSP, and produce no staining of keratinocytes in immunofluorescence. Also, antibodies affinity purified to 70 kDa CUSP do not bind the 52 kDa protein in immunoblotting. The identity of the 52 kDa protein has not been determined. Based on the preceding results, however, it appears unlikely that the 52 kDa protein is simply a degradation product of 70 kDa CUSP.

In summary, autoantibodies to the KET splicing variant, CUSP, are associated with a distinctive clinical syndrome of chronic oral ulceration. The *p53*-like genes, *p73* and the several *KET* splicing variants, are recently described genes of uncertain biologic and pathologic significance (Kaelin, 1998). This study provides the clear association of a p53-like protein with a disease process.

**Note in proof.** Recently, a gene called p63 has been reported by Yaug *et al.* This gene is also the human homolog of KET.

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