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8. Immunoaffinity purification of 13S condensin was done as described (*7*); for more details, see *Science* Online (www.sciencemag.org). 13S condensin preparations from two different metaphase extracts [extracts from metaphase II–arrested eggs and extracts activated by cyclin BΔ90 (a nondegradable form of cyclin B)] had identical subunit compositions and supercoiling activities. We do not distinguish between the two preparations and refer to them as the mitotic form throughout this report. Similarly, 13S condensin purified from two different interphase extracts (extracts from eggs activated with the calcium ionophore A23187 and extracts activated in vitro by addition of $CaCl_2$) were indistinguishable. The cell cycle–specific extracts were prepared as described [A. W. Murray, *Methods Cell Biol.* **36**, 581 (1991); T. Hirano and T. J. Mitchison, *J. Cell Biol.* **115**, 1479 (1991)].
9. Supercoiling assay was done as described (*7*); for more details, see *Science* Online (www.sciencemag.org). Linking number is a parameter that describes the topological state of a closed circular DNA and corresponds to the number of times that the two chains of DNA twist around one another.
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15. A mixture of 8S and 13S condensins was immunoprecipitated with anti–XCAP-E, and the XCAP-D2 subunit was gel-purified and processed for microsequencing as described (*4*). On the basis of two peptide sequences obtained (MEDDFQTPKPPASRK and ENPDIYMAK) (*16*), we cloned XCAP-D2 cDNA by the reverse transcription–polymerase chain reaction (RT-PCR), two rounds of library screening, and nested PCRs. The full-length cDNA was constructed from multiple overlapping cDNAs and sequenced. The cDNA predicted a 1364–amino acid polypeptide with a calculated molecular weight of 154 kD and a pI of 5.46 (GenBank accession number AF067969). A database search identified homologs of unknown functions from yeast (YLR272C), *C. elegans* (AL021482), and human (D63880).
16. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
17. 13S condensin (~90 ng) purified from an interphase extract was incubated at 22°C for 30 min in 5 μl of buffer [10 mM Hepes-KCl (pH 7.7), 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM MgATP, 5 mM EGTA, 1 mM dithiothreitol, and ovalbumin (1 mg/ml)] containing purified Cdc2–cyclin B (~0.1 ng) (*13*). A 3-μl aliquot was used in a 5-μl supercoiling reaction (*9*). Purified Cdc2–cyclin B alone displayed no supercoiling activity. Two other kinases were used as controls. Casein kinase II phosphorylated XCAP-D2 and XCAP-H, but none of the three Cdc2 consensus sites of XCAP-D2 were phosphorylated, as judged by cross-reactivity to the phosphopeptide antibodies (*19*). MAP kinase (Erk2) barely phosphorylated the condensin subunits. Neither kinase activated the supercoiling activity of 13S condensin.
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19. A synthetic peptide corresponding to the COOH-terminal sequences of XCAP-D2 (CNPTPIRRTARSRAK) was used to prepare an antibody that recognizes both the mitotic and interphase forms of XCAP-D2. To prepare phospho-specific antibodies, we synthesized three phosphopeptides and the corresponding unphosphorylated peptides. The sequences were as follows: DP1, CEDDFQphosphoTPKPPA; DU1, CEDDFQTPKPPA; DP2, CLSEAEphosphoTPKNPT; DU2, CLSEAETPKNPT; DP3, CTPKNPphosphoTPIRRT; DU3, CTPKNPTPIRRT (*16*). A crude serum raised against the DPx (x = 1, 2, or 3) peptide was passed through an Affi-Gel 10 (Bio-Rad) column conjugated with DUx, and then its flowthrough fraction was loaded onto a second column conjugated with DPx. After extensive washing, phospho-specific antibody was eluted by low pH and used as an affinity-purified anti-DPx. Conjugation of the peptides to a carrier protein and affinity purification of specific antibodies were done as described [K. E. Sawin, T. J. Mitchison, L. G. Wordeman, *J. Cell Sci.* **102**, 303 (1992)].
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Inhibition of Toxic Epidermal Necrolysis by Blockade of CD95 with Human Intravenous Immunoglobulin

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Toxic epidermal necrolysis (TEN, Lyell's syndrome) is a severe adverse drug reaction in which keratinocytes die and large sections of epidermis separate from the dermis. Keratinocytes normally express the death receptor Fas (CD95); those from TEN patients were found to express lytically active Fas ligand (FasL). Antibodies present in pooled human intravenous immunoglobulins (IVIG) blocked Fas-mediated keratinocyte death in vitro. In a pilot study, 10 consecutive individuals with clinically and histologically confirmed TEN were treated with IVIG; disease progression was rapidly reversed and the outcome was favorable in all cases. Thus, Fas-FasL interactions are directly involved in the epidermal necrolysis of TEN, and IVIG may be an effective treatment.

Alterations in the control of apoptosis, a type of cell death, are involved in the pathogenesis of several human diseases (*1, 2*). Apoptosis can be triggered by interaction between a cell-surface death receptor such as Fas and its respective ligand (Fas ligand: FasL or CD95L).

TEN (or Lyell's syndrome) is a severe drug-induced skin disease in which apoptotic epidermal cell death results in the separation of large areas of skin at the dermo-epidermal junction (Fig. 1A), producing the appearance of scalded skin (*3, 4*). TEN occurs at an estimated incidence of 0.4 to 1.2 cases per million, most frequently as a result of sulfonamide, anticonvulsant, or nonsteroidal anti-inflammatory drug use, and is associated with a mortality rate of about 30% (*3, 5*). There is no known effective treatment for TEN. Keratinocyte apoptosis is rare in the normal epidermis, but is abnormally increased during TEN (*3, 4*). The mechanisms responsible for enhanced keratinocyte apoptosis in TEN remain unclear (*4*).

We screened serum samples from patients with clinically and histologically confirmed TEN (*6*), extensive drug-induced maculopapular rash (MPR), and healthy controls for soluble FasL (sFasL) content (*7, 8*). Using a specific enzyme-linked immunosorbent assay (ELISA), we detected high concentrations of sFasL in the sera of patients with TEN, whereas sFasL was virtually undetected in patients with MPR or in healthy controls (Fig. 1B).

Keratinocyte apoptosis, which precedes epidermal detachment, is an early morphologic feature of TEN (*4*). Therefore, we investigated if the sFasL detected in the sera of patients with TEN resulted from cleavage of membrane-bound FasL produced in their epidermis (*7, 9, 10*). Fas signaling is known to be functional in human keratinocytes in vitro

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(*11*). Under basal conditions, keratinocytes express little FasL, and it is nonlytic in vitro (*12–14*). Following appropriate stimulation however, keratinocyte FasL can be induced to become lytic (*12*). To assess the possible involvement of Fas and its ligand in the process of keratinocyte cell death that leads to TEN, we analyzed Fas and FasL expression in skin samples from individuals with TEN (n = 7), MPR (n = 4), and in healthy controls (n = 5). Skin sections from individuals with TEN showed keratinocyte apoptosis (Fig. 2A), and immunohistochemical analysis of adjacent frozen skin sections with monoclonal antibody (mAb) A11 to FasL (Fig. 2B) revealed that keratinocytes in all the TEN samples, but not control or MPR samples, expressed large amounts of FasL, whereas Fas expression remained unchanged compared to controls (Fig. 2C).

To determine if keratinocyte FasL in patients with TEN could induce Fas-mediated cell death, we examined the lytic capacity of cutaneous FasL in vitro. Frozen skin sections from healthy controls (n = 5) and patients with MPR (n = 3) or TEN (n = 3) were overlaid with Fas-sensitive Jurkat cells for 6 hours, and Jurkat cell apoptosis was subsequently assessed using an annexin–fluorescein isothiocyanate (FITC) (Fig. 2D) or cytochrome c assay (*15*). With both assays, TEN skin sections were reproducibly found to induce three- to fourfold more target cell death than did skin sections from healthy controls or patients with MPR (Fig. 2D). The cytotoxicity induced by TEN skin sections

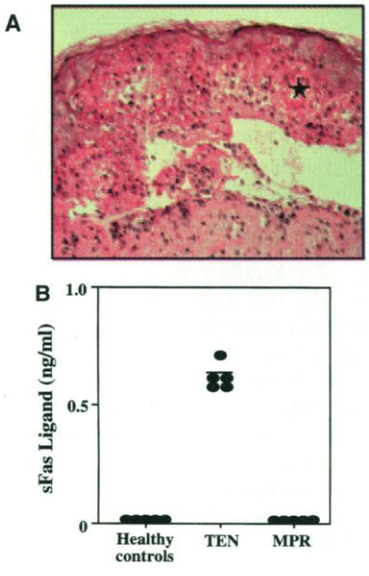

Fig. 1. (**A**) Detachment of the epidermis (star) from the dermis as a result of extensive keratinocyte death in a skin sample from an individual with TEN. (**B**) Detection of sFasL in serum samples from patients with TEN (n = 5), MPR (n = 5), and healthy controls (n = 5), using an sFasL ELISA (*25*).

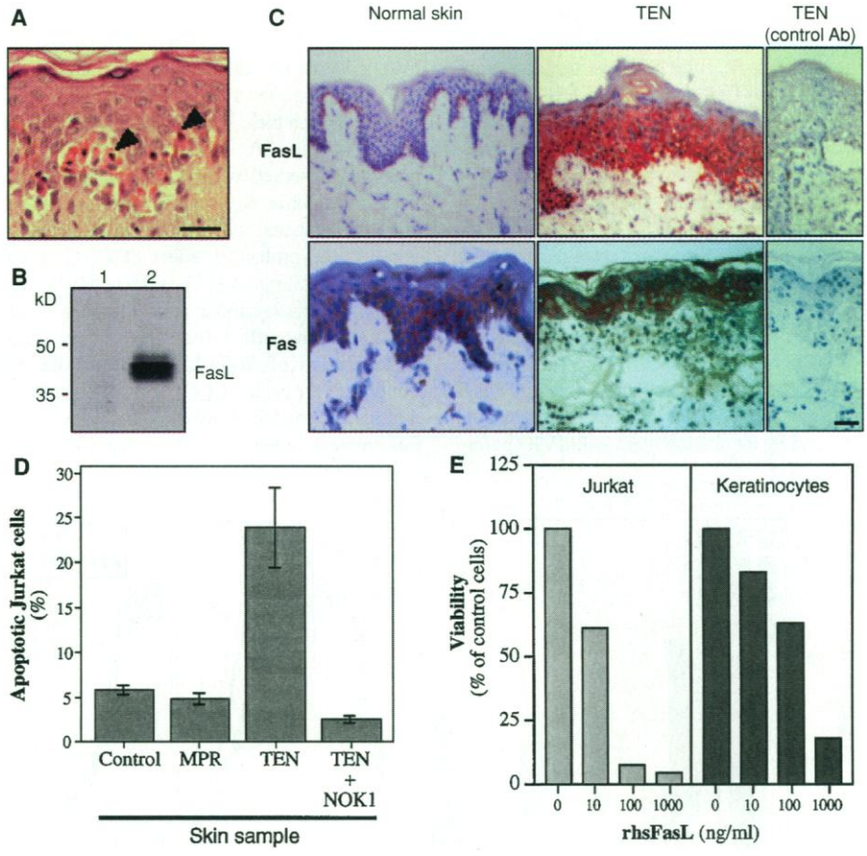

Fig. 2. Up-regulated keratinocyte FasL expression in patients with TEN induces Fas-mediated target cell death. (**A**) Histological analysis of a skin section from a patient with TEN [patient BG (Table 1)]. Arrows, apoptotic keratinocytes. Bar, 35 μm. (**B**) Immunoblot of mAb A11 to FasL. Lane 1, 293T-mock–transfected cell lysate; lane 2, 293T-FasL–transfected cell lysate. (**C**) Immunohistochemical analysis of skin sections from a representative healthy control and a TEN patient exposed to anti-Fas, anti-FasL, or isotype control antibodies (*26*). Bar, 35 μm. (**D**) Flow cytometric analysis of apoptosis (annexin-FITC) in Fas-sensitive Jurkat cells overlaid for 6 hours on skin cryosections from healthy controls, patients with MPR, or TEN in the absence or presence of FasL-blocking antibody (NOK1) (*27*). Results of a representative experiment performed in duplicate (error bars) in three to five separate individuals (five controls, three MPR, three TEN). (**E**) Susceptibility of Jurkat cells and primary human epidermal keratinocytes (HEK) to rhsFasL. Cells were incubated with FLAG-tagged sFasL (rhsFasL, Alexis Corp.) plus anti-FLAG, [enhancer (1 μg/ml), Alexis Corp.] for 6 and 16 hours, respectively. Viability was determined using a cell proliferation assay (WST-1, Boehringer GmbH, Mannheim, Germany).

Table 1. Clinical characteristics and evolution of 10 consecutive patients with TEN treated by IVIG (*6*).

Patient/ age (years)/ sex	Erythema/ detachment (%)*	Causal drug	Dose of IVIG (g/kg/day)/ duration (days)	Time from onset to treatment (days)†	Time to response/ skin healing (days)‡
1. ME/23/M	50/50	Ibuprofen	0.75/4	5	2/7
2. BG/22/F	50/30	Carbamazepin	0.75/4	4	1/7
3. ER/57/F	40/20	Ciprofloxacin	0.375/4	3	2/5
4. MP/11/M	70/20	Paracetamol	0.75/4	4	1/6
5. BK/26/M	20/60	Ceftriaxon	0.75/4	2	1/10
6. IF/88/F	50/10	Allopurinol	0.2/4	4	2/5
7. FD/13/M	60/40	Cefuroxim	0.45/4	2	2/9
8. HW/65/M	60/30	Doxycyclin	0.75/4	4	2/12
9. CM/28/F	80/5	Undetermined	0.75/4	4	1/4
10. PE/61/M	40/20	Phenytoin	0.75/4	4	1/4

*Percent of total body surface was evaluated using the rule of 9 on the first day of IVIG treatment; detached surface was not counted as erythematous. †Time from onset to treatment = time between appearance of first skin lesions and beginning of IVIG treatment. ‡Time to response = time between onset of IVIG treatment and interruption of further epidermal detachment; time to skin healing = time between onset of IVIG treatment and complete skin healing (reepithelialization).

was completely abrogated by incubation with FasL blocking mAb (Fig. 2D) or by the addition of Fas-Fc, which shows that keratinocyte FasL in TEN can induce Fas-mediated apoptosis. Human keratinocytes were also sensitive to recombinant human sFasL (rhsFasL) (Fig. 2E). These results suggest a role for keratinocyte FasL in the keratinocyte apoptosis observed in TEN.

IVIG is a blood product prepared from the pooled plasma of healthy donors. IVIG can modulate cytokine release, induce functional blockade of certain receptors, and is increasingly used to treat inflammatory and autoimmune diseases (*16*). We investigated whether IVIG could interfere with Fas-mediated keratinocyte cell death by incubating them with IVIG before rhsFasL exposure. Using concentrations of rhsFasL that induce 75% keratinocyte apoptosis, IVIG (30 mg/ml; calculated equivalent of the daily dose used for treating a 60-kg individual) completely inhibited Fas-mediated keratinocyte apoptosis (Fig. 3A). Protection from rhsFasL was also observed when human keratinocyte, hepatocarcinoma or lymphoblastoid cell lines were preincubated with IVIG (Fig. 3A), showing that the protective effect of IVIG is not limited to keratinocytes. The effect of IVIG was specific, because similar concentrations of albumin had no effect (Fig. 3A). In contrast, preincubation of rhsFasL with IVIG did not inhibit its lytic capacity (*17*), showing that IVIG inhibits Fas-mediated cell death by blocking the Fas receptor rather than interacting with FasL.

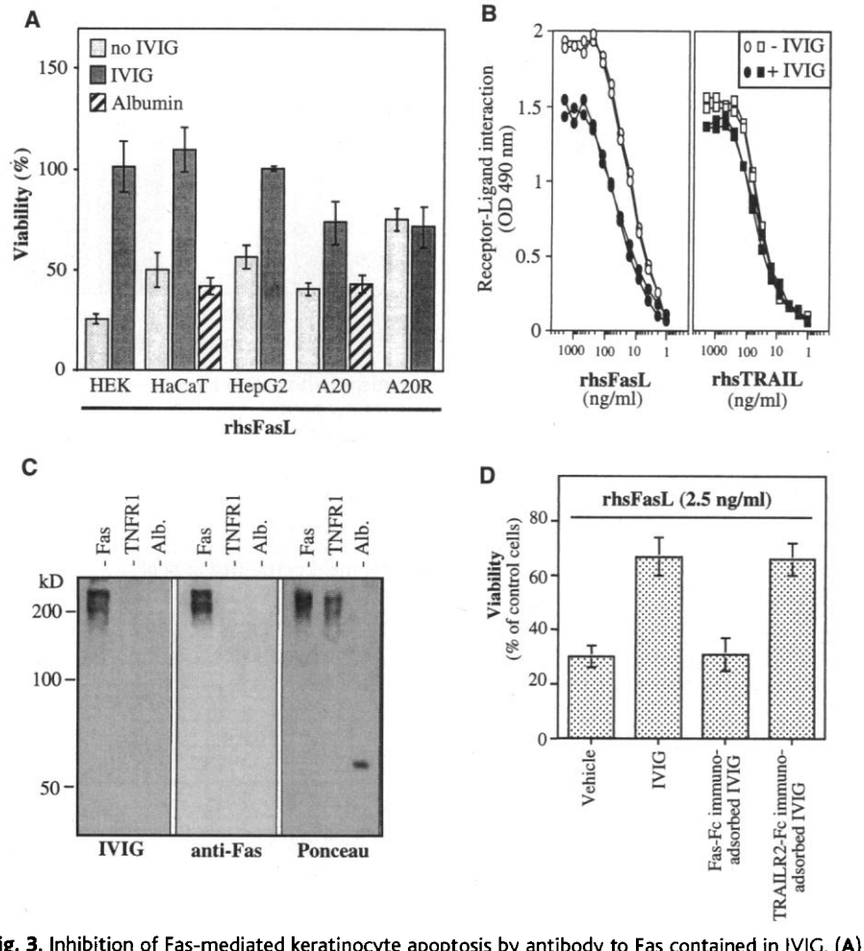

Fig. 3. Inhibition of Fas-mediated keratinocyte apoptosis by antibody to Fas contained in IVIG. (**A**) Effect of preincubation of primary human keratinocytes (HEK), or the human keratinocyte (HaCaT), hepatocarcinoma (HepG2), Fas-sensitive (A20), and resistant (A20R) lymphoblastoid cell lines with IVIG, vehicle (no IVIG), or albumin on the susceptibility to rhsFasL (*28*). Viability is the percent of the viability observed in the absence of rhsFasL. Shown are results of a representative experiment performed twice separately in triplicate (error bars). (**B**) Effect of IVIG on Fas-FasL (left) and TRAIL-TRAILR2 (right) interaction. Microtiter plates coated with Fas-Fc or TRAILR2-Fc fusion proteins were incubated with rhsFasL or rhsTRAIL in the presence or absence of IVIG (30 mg/ml), and ligand-receptor binding was measured as described (*10, 24*). OD, optical density measured at 490 nm wavelength. (**C**) Immunodetection of Fas-comp (Fas), but not related TNFR1-comp (TNFR1) or albumin (Alb.), with IVIG. Left, IVIG staining; middle, anti-Fas staining; right, ponceau staining (*29*). (**D**) Susceptibility of A20 cells to rhsFasL after preincubation with vehicle, IVIG, or Fas-Fc or TRAIL-R2-Fc (as control) immunoabsorbed IVIG. Viability was determined as previously described. Results of a representative experiment performed twice in triplicate (error bars).

The inhibition of Fas-FasL interaction by IVIG was further confirmed by using an ELISA in which receptor-ligand interaction was measured in the presence or absence of IVIG. Binding of rhsFasL to Fas-Fc, but not binding of rhsTRAIL [recombinant human soluble TRAIL (TNF-related apoptosis-inducing ligand)] to TRAILR2-Fc (control), was significantly inhibited by IVIG (Fig. 3B).

To determine if the Fas inhibitory activity of IVIG is due to the presence of naturally occuring anti-Fas immunoglobulin (Ig), we determined whether IVIG bound to human Fas, and whether depletion of anti-Fas Ig from IVIG abrogated their capacity to inhibit Fas-mediated apoptosis. Immunodetection with IVIG of albumin or purified recombinant protein constructs containing the extracellular domains of human Fas (Fas-comp) or tumor necrosis factor receptor (TNFR1-comp) fused to a 55–amino acid linker [comp: cartilage oligomeric matrix protein pentamerization domain (*18*)] showed that IVIG bound to Fas-comp (Fig. 3C), not to albumin, and weakly to TNFR1-comp, suggesting that there may also be some anti-TNFR1 activity in IVIG. Depletion of anti-Fas Ig from IVIG by several passages on a Fas-Fc affinity column specifically abrogated the ability of IVIG to bind Fas-comp and block Fas-mediated cell death (Fig. 3D).

In an open, noncontrolled pilot study, 10 consecutive patients with TEN were treated in three clinical centers (Geneva, Lausanne, and Bern University Hospitals) with IVIG at doses ranging from 0.2 to 0.75 g/kg of body weight per day for four consecutive days. In all 10 patients, the progression of skin disease was rapidly interrupted after IVIG infusion (within 24 to 48 hours), which was accompanied by rapid skin healing and a favorable vital outcome without significant adverse effects (Table 1). Confirmation of the efficacy of IVIG in the treatment of TEN will require a controlled multicentric clinical trial.

Our results suggest that up-regulation of keratinocyte FasL expression is the critical trigger for keratinocyte destruction during TEN. The observed therapeutic effect of IVIG in patients with TEN is likely to involve inhibition of Fas-mediated keratinocyte death by naturally occurring Fas-blocking antibodies contained within IVIG preparations. IVIG may also prove useful in the treatment of other diseases that are due to Fas-mediated tissue destruction, including graft-versus-host disease, Hashimoto's thyroiditis, and fulminant hepatitis (*2, 19*).

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25. Serum aliquots from patients with full-blown TEN or MPR and from healthy controls were assessed using a sFasL ELISA kit (Medical & Biological Laboratories Ltd., Nagoya, Japan; uses anti-FasL mAb 4H9 and 4A5). TEN was defined as previously described (*6*). MPR was defined as a drug-related symmetrical confluent cutaneous maculo-papular eruption without clinical signs of epidermal detachment, which spontaneously resolved after drug withdrawal. All included cases of MPR had a rash affecting at least 50% of the body surface. Healthy controls were patients less than 40 years of age who were free of cutaneous or systemic disease.
26. Skin biopsies were taken at the time of referral from patients with TEN (at the interface between detached and nondetached skin), MPR (lesional skin), and from healthy controls (non–sun-exposed skin) following informed consent, with one part snap-frozen in liquid nitrogen and the other fixed in 4% paraformaldehyde and routinely processed. Immunohistochemistry was performed on cryosections as described (*20*), using mAb to FasL [A11, Alexis Corp., San Diego, CA (*8*)], mAb to Fas (UB2, Immunotech), and isotype controls.
27. Frozen tissue sections were overlaid with Fas-sensitive Jurkat (human T cell leukemia) cells as described (*21, 22*), with or without preincubation for 30 min with mAb to FasL (NOK1, 2.5 μg/ml, Pharmingen). Jurkat cell apoptosis was determined by flow cytometry using annexin-FITC (Pharmingen) (*23*).
28. Cells were preincubated for 24 hours with IVIG (30 mg/ml; Sando globulin, Novartis, Bern, Switzerland), vehicle [0.9% NaCl and saccharose (51 mg/ml)], or albumin (30 mg/ml in 0.9% NaCl), and thereafter susceptibility to rhsFasL was assessed (*24*).
29. Equimolar amounts of purified fusion proteins Fas-comp (*18*), TNFR1-comp, or albumin were immunoblotted with either IVIG or monoclonal mouse anti-human Fas antibody (ZB4, Immunotech), and then were revealed by using ECL (Amersham). IVIG did not bind to purified comp alone.
30. We thank G. Radlgruber-Steiger for technical assistance; A. Limat and D. Masson for advice; M. Pechère, I. Masouyé, J. Pugin, D. Guggisberg, and P. de Viragh for data and samples from patients; D. Wohlwend for help with fluorescence-activated cell sorting analysis; N. Fusenig for HaCaT cells; and P. Vassalli for discussions. Supported by grants from the Swiss National Science Foundation (L.E.F. and J.T.), the Ernst Schering Research Foundation, the Sir Jules Thorn Charitable Trust, the Ernst and Lucie Schmidheiny Foundation, the Ligue Genevoise contre le Cancer, and the Fondation Medic.

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Two Modes of Survival of Fission Yeast Without Telomerase

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Deletion of the telomerase catalytic subunit gene *trt1*$^+$ in *Schizosaccharomyces pombe* results in death for the majority of cells, but a subpopulation survives. Here it is shown that most survivors have circularized all of their chromosomes, whereas a smaller number maintain their telomeres presumably through recombination. When the telomeric DNA-binding gene *taz1*$^+$ is also deleted, *trt1*$^-$ *taz1*$^-$ survivors use the recombinational mode more frequently. Moreover, the massive elongation of telomeres in *taz1*$^-$ cells is absent in the double mutant. Thus, Taz1p appears to regulate telomeric recombination as well as telomerase activity in fission yeast.

In most organisms, the DNA at chromosome ends (telomeres) consists of short GT-rich repeats that are synthesized by a ribonucleoprotein reverse transcriptase called telomerase. In the absence of telomerase, cells continuously lose their telomeric DNA because of incomplete DNA replication and eventually lose the ability to divide. This state is known as senescence.

The catalytic protein subunit of telomerase, TERT (telomerase reverse transcriptase), is phylogenetically conserved (*1*). In the fission yeast *Schizosaccharomyces pombe*, TERT is encoded by the *trt1*$^+$ gene (*2*). As expected for a telomerase mutant, *trt1*$^-$ cells progressively lose their telomeric DNA. They also lose viability, as evidenced by the appearance of irregularly shaped microcolonies, consisting mainly of elongated nondividing cells. Viability of *trt1*$^-$ cells drops to the lowest level around 120 divisions after germination. However, a subpopulation of cells survives: Larger, round colonies containing mostly normal-sized cells eventually reappear upon further restreaking of senescing colonies. Once formed, these survivor strains can continue forming round colonies and divide indefinitely (*2*).

We hypothesized that these *trt1*$^-$ cells might survive by a recombinational mode of telomere maintenance, which is mediated by the Rad52 recombination protein in telomerase-negative budding yeast *Saccharomyces cerevisiae* and *Kluveromyces lactis* (*3*). A characteristic feature of this mode of survival is the generation of rearranged and amplified telomeric or subtelomeric regions or both. We therefore isolated *trt1*$^-$ survivors by successively streaking for single colonies on plates and looked for telomere amplification by Southern (DNA) blot hybridization. A telomere-repeat probe and three additional probes that recognize distinct subregions of the telomere-associated sequence (TAS) were used (Fig. 1A) (*4*). Unexpectedly, DNA from *trt1*$^-$ survivors showed no hybridization signals with probes to telomeric repeats, to TAS1, or to TAS2 (Fig. 1B, left), indicating that at least 4 kilobases (kb) of telomeric and subtelomeric DNA was lost. A hybridization signal was observed with a TAS3 probe, which recognizes subtelomeric DNA located at least 5 kb from the chromosome end (Fig. 1B, right). Each independent isolate of survivors showed a uniquely rearranged pattern of subtelomeric DNA restriction fragments that was stably maintained. However, neither telomeric nor subtelomeric regions were amplified in survivors, and thus this mode of survival of *trt1*$^-$ cells differs from the recombination-dependent telomerase-negative survival described

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