Sudden and Gradual Molluscan Extinctions in the Latest Cretaceous of Western European Tethys

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Incompleteness of the fossil record has confounded attempts to establish the role of the end-Cretaceous bolide impact in the Late Cretaceous mass extinctions. Statistical analysis of latest Cretaceous outer-shelf macrofossils from western European Tethys reveals (i) a major extinction at or near the Cretaceous-Tertiary (K-T) boundary, probably caused by the impact, (ii) either a faunal abundance change or an extinction of up to nine ammonite species associated with a regression event shortly before the boundary, (iii) gradual extinction of most inoceramid bivalves well before the K-T boundary, and (iv) background extinction of approximately six ammonites throughout the latest Cretaceous.

The end Cretaceous is marked by the extinction of 70% of species worldwide (*1*), which apparently coincided with the large bolide impact that created the 180- to 300-km-wide Chicxulub impact structure (*2*). Although much attention has centered on the possibility that the impact was the primary cause of the extinctions (*3*), there remains considerable skepticism about the importance of the impact event. Other proposed explanations for the extinctions include sea-level change, global cooling, volcanic eruption, and evolutionary transitions (*4*).

The fossil record yields information about the times and rates of extinction and thus has the potential to distinguish between different extinction scenarios (*5*). However, because observed patterns of extinction can be markedly compromised by incomplete preservation [making abrupt extinctions look gradual, the Signor-Lipps effect (*6*)], the paleontological record has been difficult to interpret. In an attempt to minimize this difficulty, most detailed paleontological studies of the end-Cretaceous extinction have focused on the particularly rich fossil record of plankton (*7*). Here we use confidence intervals on stratigraphic ranges (*8–10*) to address the Signor-Lipps effect (*11, 12*).

We analyzed the fossil record of latest Cretaceous macroinvertebrates from the outer shelf environment of western European Tethys. Our data has been accumulated over 12 years from seven measured sections in the Bay of Biscay, France and Spain. The sections are stratigraphically complete [no missing microfossil or macrofossil zones (*13*)], have only a few minor faults, and reflect high sedimentation rates. The sampled rock types are offshore limestone and marl-dominated rhythmites (*14*). They are divided into five distinct lithological members of Maastrichtian age (*15*) that can be readily identified over 200 km within the basin, as well as in regions as distant as Gubbio, Italy; Caravaca and Agost, Spain; and the Republic of Georgia (*16*). Water depths were probably between 100 and 500 m (*17*). For this study we sampled from within the Early Maastrichtian *Globotruncana gansseri* zone to the end of the Maastrichtian (top of the *Abathomphalus mayaroensis* zone), encompassing a time interval of about 3 to 4 million years (Fig. 1).

The statistical methods used depended on high-precision stratigraphic data; the occurrences of each taxon were tabulated to the nearest meter (Fig. 1), except for the last 1.5 m of Maastrichtian strata, where they were recorded at centimeter scale. The boundary itself is well marked in each section by a reduced layer 1 to 3 mm thick containing abundant microspherules and enhanced concentrations of iridium (*18*); it is overlain by a boundary clay 10 to 20 cm thick. We projected all occurrences onto a single standard composite section (*19*).

Of the 40 molluscan species recovered, only the bivalve *Ostrea* sp. and the nautiloid *Eutrephoceras* sp. may have survived into the Tertiary (current taxonomies are too poorly developed to be certain) (*20*). Three species of ammonites [*Kossmaticeras* (*Natalites*) sp., *Nostoceras* sp., and *Pseudokossmaticeras tercense*] are each known from a single fossil and were excluded from the statistical analysis. We analyzed the fossil records of the remaining 28 species of ammonites (known from 271 fossil horizons) and seven species of inoceramid bivalve [known from more than 70 fossil horizons from the Zumayan section alone (*21*)] (Fig. 1).

For statistical analysis, it is desirable that the sections were collected with uniform sampling intensity (*8–10*), a condition that holds here, given that the fossils were recovered as part of reconnaissance biostratigraphic studies (*18, 21*). There are three exceptions. (i) No ammonites have been recovered from the "pre–K-T gap," a packet of difficult-to-collect rocks 8 to 1.5 m below the K-T boundary. This layer corresponds to the peak of a basin-wide regression that lies within the marls

Table 1. Confidence that Bay of Biscay ammonite species became extinct before the stratigraphic horizon 8 m below the K-T boundary. The null hypothesis of a random distribution of fossil horizons (assumed by Eq. 1) was only rejected for two species (Kolmogorov-Smirnov goodness-of-fit test, $P = 0.05$); their confidence values are in parentheses. Taxa in bold are those that most likely ($P = 0.05$) became extinct well before the K-T boundary (with this many taxa, one expects by chance alone to have approximately two taxa with confidence values near 0.99; hence, the two taxa labeled with a # have been included as candidates for extinction at or near the K-T boundary). The species with confidence levels of 0.000 are those for which fossils have been found in the last 1.5 m of the Maastrichtian (and therefore were extant 8 m below the boundary).

Ammonite species	Confidence	Ammonite species	Confidence
Pachydiscus epiplectus	**0.999**	*Pachydiscus* cf. *neubergicus dissitus*	0.357
Baculites anceps	**0.999**	*Phyllopachyceras forbesianum*	(0.454)
Hauericeras rembda	**0.994**	*Gaudryceras kayei*	0.000
Pachydiscus neubergicus	**0.997**	*Desmophyllites larteti*	0.000
Phylloptychoceras sipho #	0.992	*Zelandites* sp.	0.000
Baculites* cf. *B. vertebralis	**$>$0.999**	*Anagaudryceras politissimum*	(0.000)
Anapachydiscus fresvillensis	**0.998**	*Phylloceras* (*N.*) *ramosum*	0.000
Glyptoxoceras rugatum #	0.986	*Pseudophyllites indra*	0.000
Hoploscaphites constrictus	0.824	*Diplomoceras cylindraceum*	0.000
Eubaculites carinatus	0.293	*Brahmaites brahma*	0.000
Saghalinites wrighti	0.269	*Pachydiscus armenicus*	0.000
Anagaudryceras sp.	0.282	*Pachydiscus jacquoti*	0.000
Pachydiscus gollevillensis	0.415	*Pseudokossmaticeras dureri*	0.000
Phylloceras (*N.*) *surya*	0.237	*Anapachydiscus terminus*	0.000

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of Member V, which constitutes the last 15 m of the Cretaceous. Echinoids and the inoceramid *Tenuipteria argentea*, however, are common in this gap. (ii) The last 1 to 1.5 m of the Cretaceous was collected much more intensively than the rest of the Maastrichtian (*22*) in an effort to determine which ammonite species survived until the end of the Cretaceous. The strata that predate the pre–K-T gap were analyzed separately from this last 1.5 m of the Cretaceous. (iii) Collection intensity was much lower in the overlying Danian strata, which contain few fossils and are difficult to sample. Thus, some of the taxa thought to have become extinct at or near the K-T boundary may have survived into the Tertiary in the Bay of Biscay, although none of these taxa have been found above the K-T boundary elsewhere. All analyses were based on stratigraphic ranges measured in rock thicknesses, rather than time. About 300 rhythmites of relatively uniform thickness dominate the sections, suggesting that rock thickness closely correlates with time for these sections.

We identified species that most likely became extinct well before the K-T boundary using the equation

$$C = 1 - \left(\frac{G}{R} + 1\right)^{-(H-1)} \qquad (1)$$

where C is the confidence that the species became extinct somewhere within the interval G above its last appearance, *R* is the observed stratigraphic range, and *H* the number of fossiliferous strata that make up that range (*8*). The quantity (1 − C) reflects the confidence that the species was extant near the time of the K-T boundary.

Application of Eq. 1 to our data identified about six ammonite species that appear to have become extinct before the pre–K-T gap (Table 1). These species were most likely victims of background extinction processes. Raup (*23*) also recognized the possibility that the disappearance of some ammonites from these sections was the result of background extinction. Application of Springer's statistical method (*11*) shows that the fossil record is inconsistent with a sudden extinction of these six ammonites species anywhere within the section.

Application of Eq. 1 to the inoceramid bivalve fossil record shows that all but the small and unusual bivalve *T. argentea* became extinct well before the pre–K-T gap (Table 2). The almost complete elimination of the inoceramids occurred over a narrow stratigraphic range both in western Tethys and globally (*24–26*) and thus was a major evolutionary event. Statistical analysis, as well as the stratigraphic distribution of inoceramid prisms (*24*), shows that the fossil record of these six inoceramids is inconsistent with their sudden extinction; their observed gradual disappearance, at least in this basin, is real (Fig. 2). This period of elevated inoceramid extinction does not correspond to the time of extinction of any other taxa analyzed in these sections and is thought to have stemmed from global changes in deep-sea circulation (*26*).

All other species (22 ammonites and the inoceramid *T. argentea*) are possible victims of the K-T mass extinction, but the inhomogeneity in preservation and collecting intensity prevented us from de-

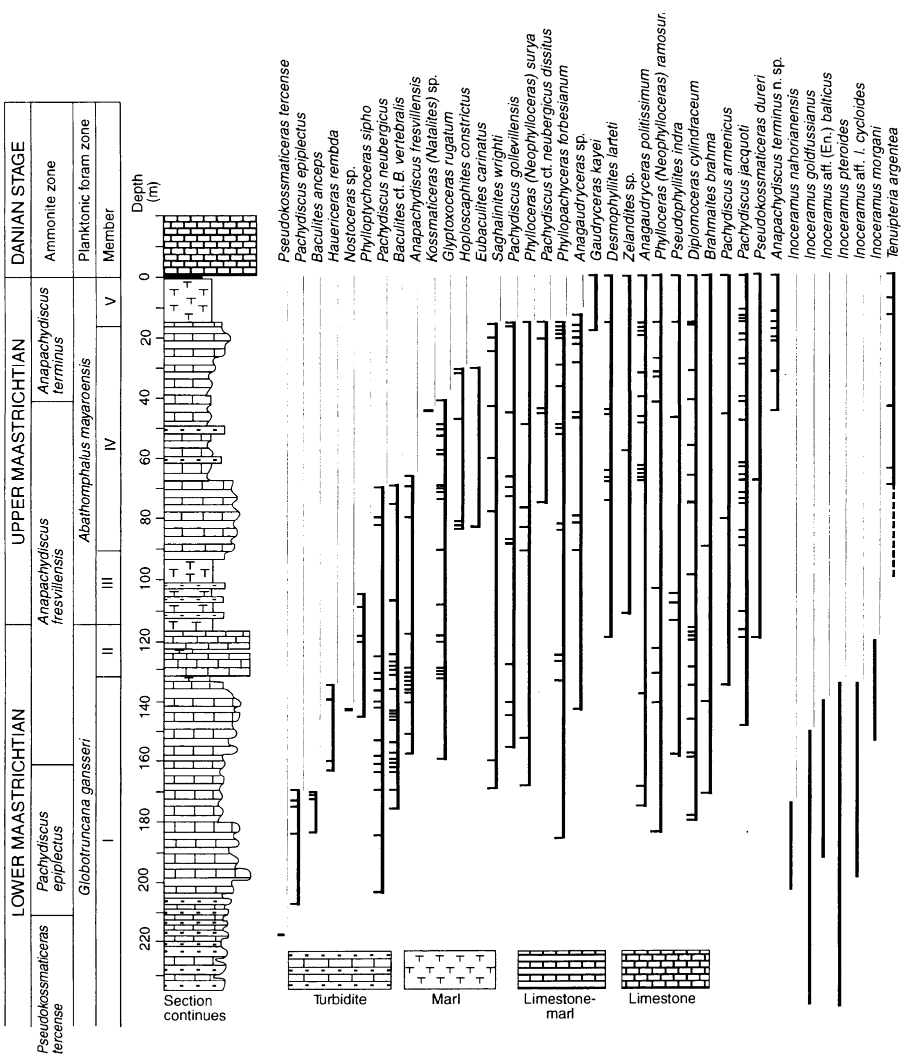


Fig. 1. Composite ranges of molluscan macrofossils from all Bay of Biscay sections, projected onto the Zumaya measured section. The positions of the fossiliferous beds used in this analysis are indicated by the bars, except for *Inoceramus* species (see Fig. 2). The first 31 species are ammonites. The dashed line for *T. argentea* indicates that stratigraphic range extends beyond the range analyzed here. All fossil specimens collected are now deposited in the Thomas Burke Museum, University of Washington. Taxonomic treatments of all taxa were completed before this analysis (*18, 21, 25*).

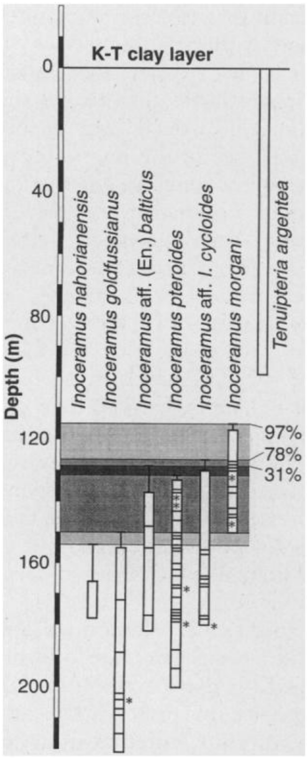
Table 2. Confidence inoceramid species became extinct before the stratigraphic horizon 8 m below the K-T boundary [using Eq. 1 and the Zumayan fossil record (*21*) only]. The null hypothesis of a random distribution of fossil horizons was not rejected for any species (Kolmogorov-Smirnov goodness-of-fit test, $P = 0.05$). Taxa in bold are those that most likely became extinct well before the K-T boundary. *Inoceramus morgani* is included in this group given that with the addition of the numerous fossils collected from other sections (*21*), its confidence value closely approaches 1.0. *Tenuipteria argentea* has a confidence level of 0.000 because it has been found in the last 1.5 m of the Maastrichtian.

Inoceramid species	Confidence
I. nahorianensis	>0.99999
I. goldfussianus	>0.99999
I. balticus	>0.99999
I. pteroides	>0.996
I. cycloides	>0.99999
I. morgani	>0.93
T. argentea	0.000

termining directly if their fossil records are consistent with their sudden extinction at the K-T boundary. We addressed this problem by first analyzing their fossil records below the K-T gap, which we found to be consistent with all 23 species being extant at the time of onset of the pre–K-T gap (Fig. 3) and thus consistent with the hypothesis that all 23 species became extinct at the K-T boundary or within the last 1.5 m of the Cretaceous. However, only 13 of these species are known from the last 1.5 m of the Cretaceous: 10 ammonites species are "missing."

Estimating the approximate time of extinction of the 10 missing species of ammonite is difficult. Rarefaction analysis (*27*) was used to test the null hypothesis that they were actually extant during the last 1.5 m of the Cretaceous and that their absence is simply the result of collection failure. The 22 ammonites thought to be extant at the onset of the pre–K-T gap are known from 205 fossils from pre–K-T gap strata, and 42 ammonite fossils are known from the last 1.5 m of the Cretaceous. Rarefaction predicts with 95% confidence that these 42 fossil should have yielded 16.5 ± 3 species if all 22 species were extant in the last 1.5 m of the Cretaceous. Because only 12 are known, we reject the null hypothesis that collection failure is the sole cause of the absence of the missing taxa.

There are two ways to account for the observed drop in ammonite diversity across the pre–K-T gap. Either (i) all 22 ammonite species were actually present in the last 1.5 m of the Cretaceous but some have evaded detection because of a significant drop in abundance (*28*) or (ii) there was an ammonite extinction event just before, or associated with, the pre–K-T gap. Assuming that there was an extinction and were no changes in the abundances of the taxa that survived the pre–



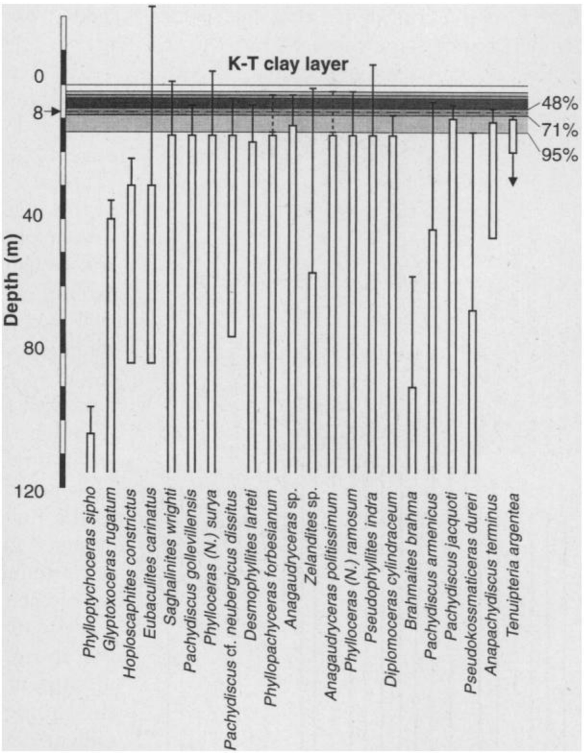


Fig. 2 (**left**). The 50% confidence-interval method (*12*) rejects the hypothesis that there was a sudden extinction of the six species of *Inoceramus*. Contours indicate predicted position of the true extinction horizon, assuming there was one. The contoured interval should lie above all taxa if all species became extinct simultaneously, but *I. morgani* has an observed stratigraphic range that extends through most of the contoured region: The null hypothesis that there was sudden extinction of all taxa is rejected [actually the 50% confidence intervals admit the possibility of a sudden extinction between 115 and 117 m below the K-T boundary; however, with the use of Springer's method (*11*), the hypothesis of a sudden extinction in this interval is rejected ($P < 0.01$)]. Stippling from darkest to lightest shows stratigraphic intervals bracketed by three, two, and one 50% confidence-interval endpoint. The confidence that an extinction horizon lies within each interval, assuming there was one, is given adjacent to the upper bound of the stippled intervals (calculated using the binomial distribution). Stratigraphic ranges were based on the fossil record at Zumaya only (*21*). Horizontal bars indicate the position of fossil horizons. An asterisk indicates the presence of two horizons too close to resolve. **Fig. 3** (**right**). Fossil records of the candidates for extinction at the K-T boundary, here artificially truncated 8 m below the K-T boundary, are consistent with the hypothesis that all were extant 8 m below the K-T boundary, the time of onset of the pre–K-T gap. Contours indicate predicted position of the 8-m truncation horizon. Stippling from darkest to lightest shows stratigraphic intervals bracketed by 10, nine, and seven 50% confidence-interval end points. The confidence that the truncation horizon lies within each interval is given adjacent to the lower bound of the stippled intervals. The 8-m truncation horizon lies within the 71% band. For the two taxa with nonrandom distributions of fossil horizons (Table 1), distribution-free confidence intervals were used (*9*); the dashed line indicates the uncertainty in the length of the 50% confidence interval. *Gaudryceras kayei* is known from only one fossil in this interval and was not included in the analysis; however, we infer it was extant at the onset of the pre–K-T gap because it is known from the last 1.5 m of the Maastrichtian. The exact stratigraphic positions of *T. argentea* were only recorded from the uppermost 20 to 30 m at Zumaya; the arrow indicates that it ranges below the first occurrence shown here.

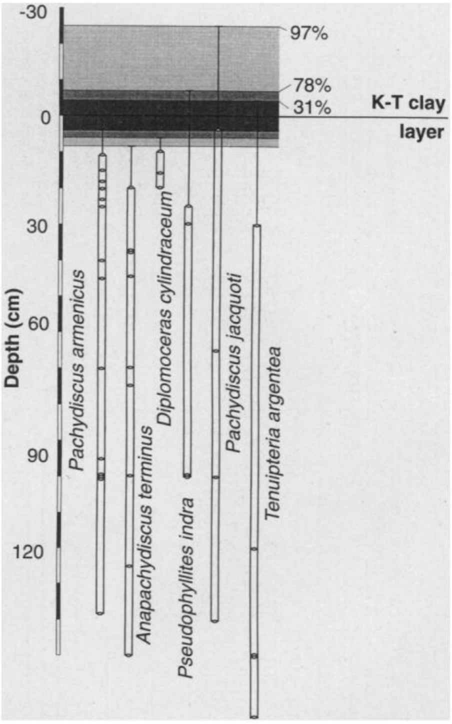


Fig. 4. Fifty percent confidence interval method (*12*) indicates that the fossil records of the six molluscan species known from more than one fossil in the last 1.5 m of the Cretaceous are consistent with their sudden extinction at the K-T boundary. Contours indicating predicted position of extinction horizon, assuming there was one, are shown. Stippling from darkest to lightest shows stratigraphic intervals bracketed by three, two, and one 50% confidence interval end point, respectively. The confidence that the truncation horizon lies within each interval is given adjacent to the upper bound of the stippled intervals. The K-T boundary clay lies in the middle of the contour interval: the apparent gradual decline in diversity over the last 25 cm appears to be due to the Signor-Lipps effect. Positions of the fossil horizons indicated within ranges.

K-T gap, rarefaction analysis indicates that anywhere from 3 to all 10 of the missing ammonites may have become extinct before the last 1.5 m of the Cretaceous ($P = 0.05$). We are unable to distinguish between these two end-member possibilities. Thus, there was at least a minor biological change, and perhaps a fairly large extinction event, associated with the regression that peaked shortly before the K-T boundary. The fossil record from outside the Bay of Biscay basin does not help distinguish between these possibilities, as only one of the 10 missing species is known to have survived to the K-T boundary elsewhere [*Hoploscaphites constrictus* in Denmark (*29*)].

The ammonites in the Bay of Biscay are preserved as impressions only and hence can only be seen on bedding surfaces. Within the marly pre–K-T gap there are essentially no bedding surfaces exposed. Hence, it is difficult to confirm that there was a drop in abundance, if not diversity, during the interval, or whether their absence in the pre–K-T gap simply reflects collection failure (*30*). However, near Hendaye in southern France, it is relatively easy to excavate bedding surfaces in the top 2 m of the pre–K-T gap. Quantitative analysis of collecting intensity at this locality does indeed support the hypothesis that, minimally, the abundance of ammonites within the pre–K-T gap dropped in comparison with the last 1.5 m of the Cretaceous (*31*).

Of the 13 molluscan species known from the last 1.5 m of the Cretaceous, only six are known from two or more fossils: five ammonites and *T. argentea*. Statistical analysis of the fossil records of these six species failed to reject the null hypothesis that they became extinct at the K-T boundary (Fig. 4). Thus, using the results of the six species as a proxy for all 13 found in the last 1.5 m of the Cretaceous, as well as any of the 10 missing ammonite species that may have survived the pre–K-T gap, we cannot reject the hypothesis that all these species became extinct as a result of the impact. However, the data are also consistent with a range of gradual extinction scenarios (*12*) not enumerated here.

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30. The absence of ammonites in the marls of Member V is unlikely to be due to preservation failure, given that ammonites are found in the similar marls of Member III, which occurs lower in the section.
31. Ammonite fossils have been found, on average, once every 18 m^2 of outcrop in the top 1.3 m of the Cretaceous at Hendaye, France. Echinoids are recovered at a comparable rate of one specimen per 19 m^2 of outcrop. At least one echinoid per collecting season, but no ammonites over seven seasons, has been recovered in about 50 m^2 of outcrop excavated within the pre–K-T gap (1.3 to 3.0 m below the K-T boundary). At these recovery rates, the probability that ammonites were as abundant in the excavated interval as they were above the pre–K-T gap is only 0.06; however, the probability that the ammonites were a third as abundant in the excavated interval as they were above the pre–K-T gap is 0.39.

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Melanoma Cell Expression of Fas(Apo-1/CD95) Ligand: Implications for Tumor Immune Escape

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Malignant melanoma accounts for most of the increasing mortality from skin cancer. Melanoma cells were found to express Fas (also called Apo-1 or CD95) ligand (FasL). In metastatic lesions, Fas-expressing T cell infiltrates were proximal to FasL$^+$ tumor cells. In vitro, apoptosis of Fas-sensitive target cells occurred upon incubation with melanoma tumor cells; and in vivo, injection of FasL$^+$ mouse melanoma cells in mice led to rapid tumor formation. In contrast, tumorigenesis was delayed in Fas-deficient *lpr* mutant mice in which immune effector cells cannot be killed by FasL. Thus, FasL may contribute to the immune privilege of tumors.

Membrane-bound FasL (mFasL) induces rapid cell death of Fas-sensitive cells (*1*). FasL is not only one of three major cytolytic pathways used by cytolytic T cells to kill target cells (*2–4*), but is also a key element in the elimination of activated T cells during the downregulation of the immune response (*5, 6*). Similar to the structurally homologous tumor necrosis factor α (TNFα) (*7, 8*), processing of the membrane-bound form of FasL by metalloproteases results in shedding of the extracellular portion (sFasL) (*9–11*). Patients with diseases characterized by pathological cell death, such as alcoholic hepatitis, contain high concentrations of sFasL in their serum (*12*). In the process of screening serum samples from patients with other diseases, elevated concentrations of sFasL

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