International Immunology, Vol. 7, No. 9, pp. 1381–1386

Characterization of the non-functional Fas ligand of *gld* mice

Michael Hahne, Manuel C. Peitsch[2], Martin Irmler, Michael Schröter, Bente Lowin, Marga Rousseau, Claude Bron, Toufic Renno[1], Lars French[3] and **Jürg Tschopp**

Institute of Biochemistry, University of Lausanne and [1]Ludwig Institute for Cancer Research, BIL Research Center, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland
[2]Glaxo Institute for Molecular Biology, Chemin des Aulx 14, CH-1228 Plan-les-Ouates, Switzerland
[3]Division of Dermatology, Geneva University Hospital, CH-1211 Geneva 14, Switzerland

Keywords: activity, Fas, *gld*, structure

Abstract

Mice homozygous for either the *gld* or *lpr* mutation develop autoimmune diseases and progressive lymphadenopathy. The *lpr* mutation is characterized by the absence of functional Fas, whereas *gld* mice exhibit an inactive FasL due to a point mutation proximal to the extracellular C-terminus. The structural repercussions of this amino acid substitution remain unknown. Here we report that FasL is expressed at similar levels on the surface of activated T lymphocytes from *gld* and wild-type mice. Using a polyclonal anti-FasL antibody, indistinguishable amounts of a 40 kDa protein are detected in both *gld* and wild-type splenocytes. The molecular model of FasL, based on the known structure of TNF-α, predicts that the Phe→Leu *gld* mutation is located at the protomer interface which is close to the FasR interaction site. We conclude that the *gld* mutation allows normal FasL biosynthesis, surface expression and oligomerization, but induces structural alterations to the Fas binding region leading to the phenotypic changes observed.

Introduction

FasL is a member of the type II membrane protein superfamily consisting of TNF-α and -β (lymphotoxin-α), lymphotoxin-β, CD27L, CD30L, CD40L and 4-1BB (1). Despite the diversity of the biological activities elicited by these ligands, most of them are known to induce cellular differentiation or proliferation with two exceptions. FasL and, under some circumstances, TNF are responsible for rapid induction of apoptosis of receptor-bearing cells (2,3).

The importance of a functional Fas–Fas ligand (FasL) system is illustrated by the phenotype of *lpr* (lymphoproliferation)and *gld* (generalized disease) mutant mice. MLR mice homozygous for *lpr* or *gld* develop a progressive autoimmune disorder, resembling systemic lupus erythematosis in human (4). These mice also suffer from a large accumulation of non-malignant $CD4^-CD8^-$ T cells in the spleen and in lymph nodes. Genetic analysis indicated that the *gld* phenotype results from a mutation in FasL (5,6). The *lpr* mutation has been correlated with a defect in Fas (7).

The Fas system unquestionably plays a crucial role in the deletion of autoimmune cells. Fas-mediated elimination occurs in the periphery, since both negative and positive selection in the thymus proceed normally in *gld* (or *lpr*) animals (8,9).

Fas also plays an important role in T cell cytotoxicity. Cytolytic T cells use two major cytolytic pathways, one based on perforin-granzymes and one dependent on FasL (10–13). Cytotoxic T cells derived from perforin knock-out mice have considerable killing activity left (14) which is attributable to Fas (10–12). In turn, perforin-containing *gld* cytotoxic T cells still kill tumor cells, but with reduced efficiency (10).

FasL in *gld/gld* mice displays no killing activity upon stimulation with phorbol ester (PMA) and calcium ionophore (ionomycin) (5,6), in spite of normal mRNA levels present (5,6), Moreover, activated *gld* T cells fail to interact with a FasR.Fc hybrid protein (6,15). This functional loss is a result of a single point mutation changing Phe273 to Leu proximal to the extracellular C-terminus of this type II membrane protein (5,6). In the present study we report on the structural implications of this fatal single amino acid replacement.

Methods

Expression of bacterial recombinant FasL

cDNA coding for the extracellular domain of FasL was amplified from 1 ng mouse FasL cDNA (GenBank accession no. MMU10984) using the following primers: primer 1, 5′-TTC

Correspondence to: J. Tschopp

Transmitting editor: L. Moratta

Received 23 January, *accepted* 24 April

GCT CGA GAA CTG GCA GAA CTC CGT GAG-3′; primer 2, 5′-AAG GAT CCT AGC TGA CCT GTT GGA CCT TGC-3′. The amplified product was cloned into the pCRII vector using the AT cloning kit (Invitrogen, Heidelberg, Germany) and subsequently subcloned into the page-KG expression vector (kindly provided by Dr A. Quest at our institute) at the *Eco*RI site. The glutathione-S-transferase (GST)–FasL fusion protein was expressed in bacteria upon induction with IPTG.

Antibody production

A peptide spanning amino acids 196–220 of the mouse Fas ligand (RGQSCNNQPLNHKVYMRNSKYPEDL), synthesized using the multiple antigen technology (16), was used as antigen. Spleen cells of an immunized rat were fused to NF1 mouse myeloma cells and hybridoma cells secreting peptide specific IgG were selected. Two clones, H11 and A11, were further analyzed. The same peptide construct was also injected into rabbits for polyclonal antibody production. The antiserum (PE62) was affinity purified on FasL peptide coupled to CNBr–Sepharose (Pharmacia, Zürich, Switzerland).

Splenocytes and their activation

B6.gld mice were purchased from Jackson Laboratories (Bar Harbor, ME). Splenocytes were isolated from 6-week-old mice and freed from red blood cells by 10 min incubation in an ice cold buffer containing 13 mM sodium bicarbonate, 156 mM ammonium chloride and 127 μM EDTA, and then resuspended in complete medium. Cells were cultured for 4 h at ~1×10^7 cells/ml in either the presence or the absence of PMA (5 ng/ml) and ionomycin (500 ng/ml) (Sigma, Buchs, Switzerland).

Flow cytometry

Analysis was performed on a FACScan cytometer (Becton Dickinson, Mountain View, CA) using the Lysys II software Cells were stained with the anti-FasL antibody PE62 followed by donkey anti-rabbit coupled to fluorescein (Dianova, Hamburg, Germany) and the PE-labeled mAb H 129.19 for L3T3/CD4 and 53-6.7 for Ly-2/CD8 staining (Boehringer, Mannheim, Germany).

SDS–PAGE, Western blot and native electrophoresis

Splenocytes were washed in ice cold PBS and then boiled in sample buffer for 5 min. Cellular proteins of ~2×10^6 splenocytes per lane were electrophoretically separated on a 10% polyacrylamide gel in the presence of SDS under non-reducing conditions (17) and subsequently transferred to nitrocellulose. Immunoblot analysis was conducted using plain supernatant of the mAb H11 and A11, and 50 μg/ml of affinity-purified PE62 antibody. First antibodies were detected using anti-peroxidase-conjugated donkey anti-rat and anti-rabbit antibodies (Dianova, Hamburg, Germany) followed by a chemiluminescence reaction using the ECL system (Amersham, Bucks, UK).

For native electrophoresis the Phast separation system of Pharmacia (Zürich, Switzerland) was employed. Approximately 10^7 splenocytes were lysed in 50 μl lysis buffer (100 mM NaCl, 1% NP-40 in 50 mM Tris, pH 8.0) containing 1 mM PMSF. Cell lysate (1 μl) was applied per lane and the proteins were electrophoretically separated on 4–15% gradient gels and analyzed by immunoblot analysis as described above. Complement C8 and C9 components were used as molecular weight standards.

Results

Production of FasL specific antibodies

Polyclonal and monoclonal antibodies were generated against a peptide corresponding to residues 196–220 of the mouse FasL, predicted to lay on the loop forming the outer tip of the ligand (see below). Affinity-purified polyclonal antiserum (PE62) and two independent mAb (H11 and A11) were used throughout this study. To confirm that these anti-peptide antibodies specifically recognize FasL, the reactivity of the antibodies was tested against the recombinant extracellular domain of FasL. As shown in Fig. 1, both the monoclonal and polyclonal antibodies recognized bacterial lysates containing the GST–FasL fusion protein in Western blots, whereas no band was detectable in lysates transfected with the expression vector containing FasL insert in the inverse orientation. Flow cytometric analysis showed reactivity of the polyclonal antibody PE62 with the native ligand on the cell surface (Fig. 2), while the use of the mAb was restricted to the analysis of denatured protein in Western blots .

Expression of FasL in T lymphocytes of wild-type and gld mutant mice

FasL mRNA has been detected in activated splenocytes (3,6). Flow cytometric analysis of activated T lymphocytes derived from spleens indeed showed high expression of FasL (Fig. 2). The $CD4^+$ and the $CD8^+$ subsets showed equally intense FasL staining (data not shown). FasL expression was critically dependent on lymphocyte activation. Only cells that had been previously treated with PMA/ionomycin were positive, while marginal staining of T lymphocytes was observed with non-activated T cells. Interestingly, only a subpopulation of activated T lymphocytes was FasL positive. Approximately 70%

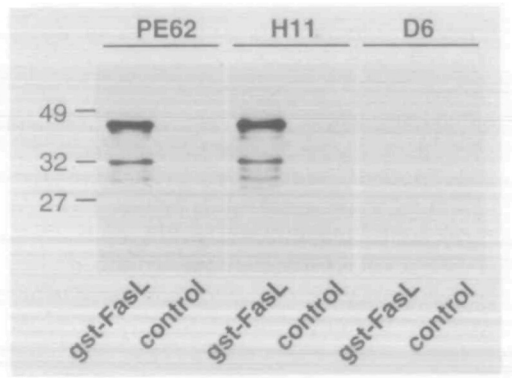


Fig. 1. Characterization of anti-mouse FasL antibodies. Western blot analysis of a bacterial lysate containing recombinant mouse FasL–GST fusion protein (46 kDa) using the affinity-purified PE62 rabbit polyclonal antibody to FasL or rat monoclonal anti-mFasL H11. The low molecular weight bands most likely correspond to degradation products. Bacterial lysate expressing GST alone served as a negative control. The D6 mAb antibody detects the peptide 62 in ELISA, but does not react with the recombinant FasL (control lanes).

remained FasL low. We are currently investigating the phenotype of the FasL positive lymphocyte subpopulation in greater detail.

The relative surface expression of FasL on splenic lymphocytes isolated from wild-type B6 mice with that from *gld* B6 mice was compared Previously, Ramsdell (15) showed that the FasR.Fc hybrid protein detected the ligand only on wild-type lymphocytes, whereas the *gld* T lymphocytes were negative. Using FasL specific antibodies, however, staining on PMA/ionomycin treated *gld* T lymphocytes was comparable to that of wild-type T lymphocytes (Fig. 2). Only 30% of activated *gld* T lymphocytes expressed high levels of FasL.

This was confirmed by Western blot analysis An equal quantity of protein and an identical size was found in extracts from activated T lymphocytes of wild-type and of *gld* origin (Fig. 3). FasL exhibited a molecular weight of ~40 kDa, in agreement with the molecular weight of post-translationally modified FasL in transfected COS cells (18).

Structure of the gld-FasL

The inability of the FasR.Fc hybrid protein to detect *gld*-FasL points to structural dissimilarities in the Fas binding region of the ligand. Other members of the TNF family have been shown to be active only as trimers and not as monomers (19,20). Similarly, FasL, although present on the surface, could show impaired dimer or trimer formation on the surface of *gld* T cells. This would be compatible with the observed difference of FasL surface expression, which differs depending on whether a FasL antibody or the FasR.Fc hybrid protein is used as tool for detection. Whereas the antibody most likely detects multimers and monomers, the receptor.Fc hybrids are known to interact only with multimeric ligands with high affinity.

This hypothesis was supported by the spatial localization of FasL Phe273 to Leu mutation. Although the level of sequence identity between the individual members of the TNF superfamily does not exceed 35%, their three-dimensional structures are expected to be very similar as observed for TNF-α and -β for which X-ray structures are available. Both factors crystallize in the trimeric form and are detected as such in solution (20–22). Knowledge-based protein modeling of FasL was therefore performed (23). Figure 4(A and B) shows the predicted three-dimensional structure of the presumptive FasL trimer. The trimer interface is largely dominated by hydrophobic residues which are well conserved throughout the TNF family (23). In the *gld* mutation the bulky aromatic side chain of Phe273, which is part of the subunit interface, is replaced by the shorter leucine (Fig. 5A). Phe273 is positioned at the center of a 12 Å sphere where several of the best conserved residues of the TNF family are clustered (V144, A145, H146, L158, W160, Y190, V193, G244, A245, F247, F273, G274), although they are quite distant in the sequence. This suggests an important functional role for this particular region. Based on our model of murine FasL, Phe273 (located on strand H) interacts mainly with Ala245 (on strand F) of the adjacent subunit (Figs 4 and 5). It is responsible for ~50 $Å^2$ of the hydrophobic contact surface between individual protomers. The *gld*-FasL model predicts that the contribution of amino acid 273 is reduced by >20 $Å^2$ when replaced by a leucine. This would in turn result in a decrease of hydrophobic interactions between the subunits and suggest that either trimer formation of FasL itself is impaired or that local distortions of the polypeptide backbone occur in the *gld* mutation. As in the *gld* mutation, this position is occupied by shorter hydrophobic residues, i.e. isoleucine and valine, in human and mouse CD30L respectively (Fig. 5A and B). The molecular model of the CD30L (23) shows that the consequent difference

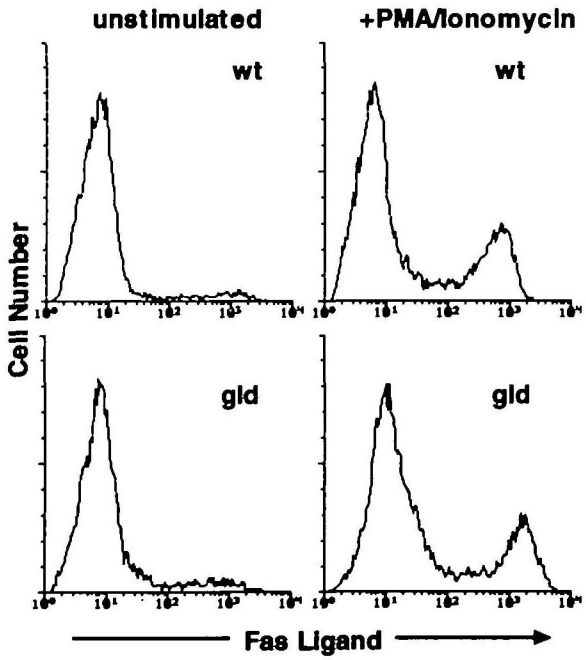


Fig. 2. Expression of FasL on wild-type and *gld* lymphocytes. FACS analysis of cell surface FasL expression on unstimulated (left panels) and PMA/Ionomycin (4 h) stimulated (right panels) $CD4^+$ or $CD8^+$ splenocytes of C57BL/6J (B6) (A) or B6-*gld* origin (B). The flow cytometric profiles show the combined staining of $CD4^+$ and $CD8^+$ splenocytes stained with the anti-FasL polyclonal antibody PE62. Unrelated antibody control is identical with the profiles of unstimulated splenocytes (upper left panel).

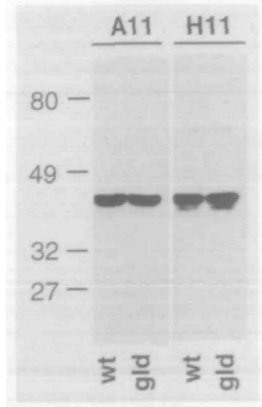


Fig. 3. FasL in splenocytes. Western blot analysis of PMA/ionomycin (4 h) stimulated wild-type and *gld* splenocytes. FasL-detecting antibodies were H11 and A11. No staining is seen with an irrelevant mAb (data not shown).

in subunit contact surface is, however, compensated by CD30L:Leu199 which corresponds to FasL:Ala245 (Fig. 5B).

Oligomerization of FasL

To determine if there is a failure of trimerization of the *gld*-FasL, Western blot analysis was performed with the native FasL-recognizing polyclonal PE62 antibody. This antibody normally detected at least three bands under non-reducing conditions, the lowest of 40 kDa co-migrating with that detected by the mAb (Fig. 6A). The two larger bands had apparent molecular weights of ~75 and 105 kDa respectively. Their molecular masses correlate with the predicted mass of FasL dimers and trimers, and most likely reflect non-dissociated oligomeric forms of the ligand.

To obtain additional information with respect to the aggregation state of FasL in the presence of non-denaturing detergents, activated splenocytes were disrupted with the mild detergent NP-40. The detergent extract was separated by native gradient gel electrophoresis. Subsequent immunoblot analysis revealed multiple FasL–antibody reactive bands. The lowest one had an apparent molecular size of ~200 kDa, but two additional FasL antibody-reactive species with apparent molecular masses of ~400 and 600 kDa were detected. These absolute values have to be interpreted with caution, since NP-40 forms large micellar structures. However, they correspond to dimeric and trimeric forms of the ligand. Importantly, the pattern of these bands was similar in PMA/ionomycin activated splenocytes of wild-type and *gld* origin.

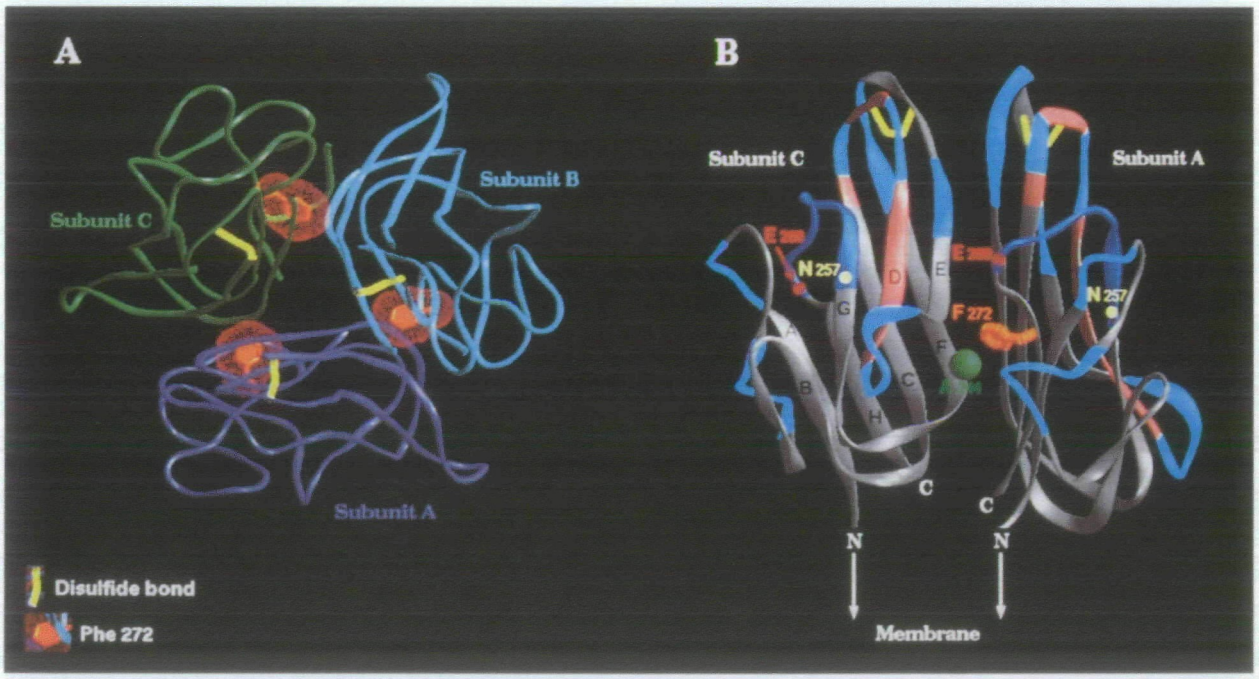
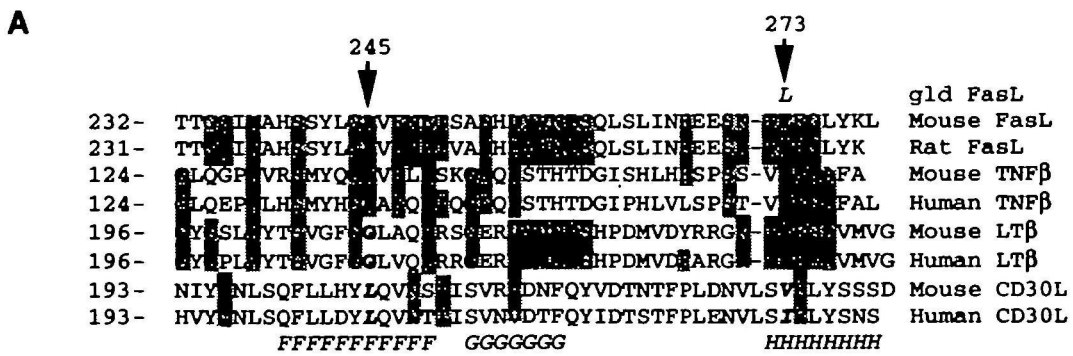


Fig. 4. Molecular model of the murine FasL. Ribbons representation of the murine FasL. In the top view (A), all three subunits, their disulfide bonds and Phe273 mutated in *gld*-FasL are represented, while in the side view (B) only the subunits A and C are shown for clarity. The receptor-binding loops are depicted in blue (darker blue for the loop linking the G and H strands) while the sequence corresponding to peptide 62 is colored in pink. The green sphere represents the side chain of Ala245 (see Fig. 5A), which is in contact with Phe273 (orange side chain). The residues at the beginning and end of the receptor-binding GH loop are indicated with light yellow and red circles. The N- and C-terminal ends of the extracellular FasL sequence which displays homology to the TNF molecule are indicated with white letters, and the β-strands are identified by black letters on the C subunit.



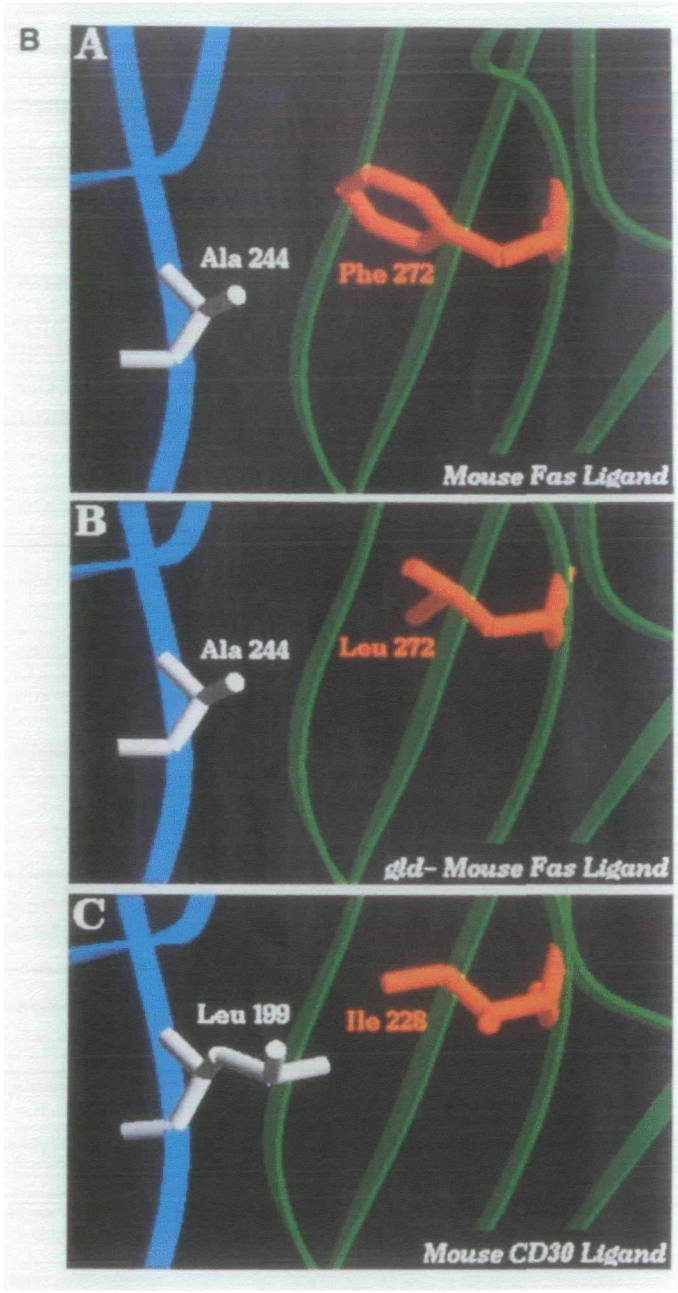


Fig. 5. The interface of the *gld*-FasL trimer. (A) Multiple amino acid sequence alignment of TNF superfamily members that are most similar to FasL (>22% overall identity), i.e. TNF-β, lymphotoxin-β (LTβ) and CD30L. Only the C-terminal region of FasL is shown. Residues identical in 50% of the sequences are indicated with a shaded background, while those involved in interface formation in the *gld* mutation are labeled in bold face and by arrows. The β-strands are indicated in italics. (B) Representation of a section of the interface between the A (green) and C (blue) subunits of (A) mouse FasL, (B) mouse *gld*-FasL and (C) human CD30L. The side chains of FasL:Phe273 and FasL.Ala245 are shown in orange and white respectively. The corresponding residues of murine CD30L are shown in the lower panel. The space left empty by the replacement of Phe by Ile228 in CD30L is filled by the compensatory replacement of Ala by Leu199. In *gld*-FasL, the mutation of Phe273 to Leu is not compensated by the replacement of Ala244 by a larger hydrophobic residue, leaving a large empty space in the structure. To fill this cavity in the trimer structure, *gld*-FasL may undergo a distortion with dramatic effect on its FasR-binding activity.

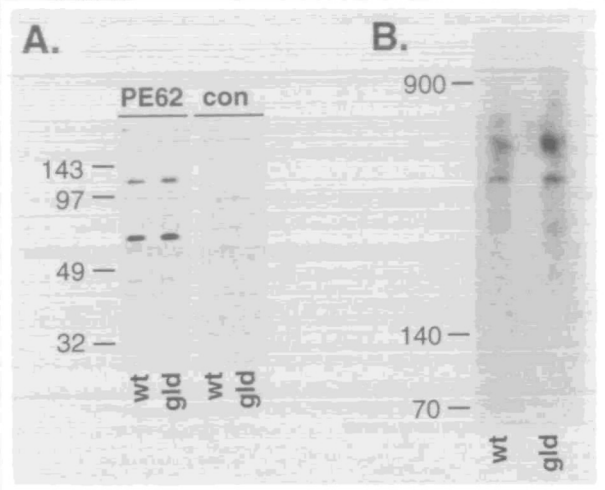


Fig. 6. Oligomer formation by FasL. (A) Western blot analysis of PMA/ionomycin activated splenocytes using the polyclonal affinity-purified PE62 antibody. In the control lanes (con), the first antibody (PE62) was left out during the incubations. Negative results were also obtained with irrelevant rabbit IgG. (B) Separation of splenocyte proteins by native gel electrophoresis. FasL antigen was detected by Western blot analysis using the PE62 antibody.

Discussion

Our results show that the single amino acid change in the *gld* mutant FasL allows correct post-translational modification and sorting of the ligand to the cellular surface. Furthermore, both the *gld*- and wild-type FasL seem to form SDS-resistant oligomers. As shown for soluble members of this ligand family (24), the membrane-associated form of FasL may require surface dimerization or trimerization to display functional activity. There are antibodies to Fas which have ligand-like activities and induce apoptosis in Fas-bearing cells. The two original antibodies which have led to the discovery of Fas (Apo-1) are aggregating antibodies, i.e. of either the IgM or IgG3 subtype (25,26). $F(ab')_2$ fragments alone are inactive (27). Thus, it is very likely that FasL homotrimer formation is required to induce Fas aggregation and signal transduction.

In the context that the apparent aggregation state of the *gld*- and wild-type FasL does not differ *in vitro*, we favor the conclusion that the *gld*-FasL's inability to interact with soluble Fas dimers (FasR.Fc) and to transmit the signal via Fas results from a local distortion in FasL–FasR interaction site. Closer examination of the structure shown in Fig. 4(A) shows that the critical Phe273 is part of a loop involved in FasR contact (23). This loop links the strands G and H (the GH loop) and ends only four residues before Phe273. The replacement of Phe273 by a leucine most likely alters the structure of strand H in the FasL trimer. It may then be that the modified structure of strand H changes the conformation of the GH loop giving rise to a FasL with impaired FasR-binding activity.

Several mutations in the TNF family leading to distinct phenotypes have been reported. CD40L mutants were discovered in connection with the X-linked hyper-IgM syndrome (28). The spatial mapping of human CD40L mutants discovered in connection with the X-linked hyper-IgM syndrome (summarized in 28) show that several of the reported point

mutations probably affect the folding of individual subunits or the interaction with the receptor (23). Two mutations, however, A123→E and G227→V, are also found at the subunit interface. Interestingly, these two amino acids are located in the same three dimensional region as FasL:Phe273 (12 Å sphere around Phe273) and correspond to two of the highly conserved residues throughout the TNF family Although more polar subunit interactions (CD40L:Ser256 instead of FasL:Phe273) replace the very hydrophobic ones in the other members of the TNF family, mutations in this region of CD40L lead to ligands with impaired receptor-binding function as evidenced by the appearance of the X-linked hyper-IgM syndrome in patients bearing such mutations (28). These mutations may lead to similar effects on the conformation of the GH loop (one of the receptor-binding loops) as in FasL and thus explain the lack of functionality of these mutants. In contrast to CD40L, no mutation affecting FasL activity has been described in humans to date. The pathological consequences of such a mutation, as seen in the *gld* mouse, may be too profound to allow its occurrence.

Acknowledgements

The authors gratefully acknowledge the technical assistance of H. Hasler, S. Hertig, T. Bornand and P Allegrini The helpful comments and suggestions of H. R. MacDonald and C. Kamel are also gratefully acknowledged. This work was supported by grants of the Swiss National Science Foundation

Abbreviations

FasL	Fas ligand
GST	glutathione-S-transferase

References

1 Smith, C. A., Farrah, T. and Goodwin, R. G. 1994. The TNF-receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell* 76:959
2 Aggarwal, B. B. and Eessaul, T. E. 1987. Human tumor necrosis factor and lymphotoxin: their structural and functional similarities In *Tumor Necrosis Factor*, p. 297. Alan R. Liss, New York.
3 Suda, T , Takahashi, T , Golstein, P. and Nagata, S. 1993. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 75:1169.
4 Cohen, P. L. and Eisenberg, R. A. 1991. *Lpr* and *gld*: single gene models for of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9:243.
5 Takahashi, T., Tanaka, M., Brannan, C. I , Jenkins, N. A., Copeland, N. G., Suda, T. and Nagata, S. 1994 Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 76:969.
6 Lynch, D. H , Watson, L. M., Alderson, M. R., Baum, P. R., Miller, R. E., Tough, T. W., Gibson, M., Davis-Smith, T., Smith, C. A., Hunter, G., Bhat, D., Din, W., Goodwin, R. G. and Seldin, M. F. 1994. The mouse Fas-ligand gene is mutated in gld mice and is part of a TNF family gene cluster. *Immunity* 1:131.
7 Watanabe-Fukunaga, R., Brannan, C. I., Itoh, N., Yonehara, S., Copeland, N. G., Jenkins, N. A. and Nagata, S. 1992. The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J. Immunol.* 148:1274.
8 Singer, G. G. and Abbas, A. K. 1994. The Fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* 1:365.
9 Sidman, C. L., Marshall, J. D and von Boehmer, H. 1992. Transgenic T cell receptor interactions in the lymphoproliferative and autoimmune syndromes of *lpr* and *gld* mutant mice. *Eur. J. Immunol.* 146:3340
10 Lowin, B., Hahne, M., Mattmann, C. and Tschopp, J. 1994. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. *Nature* 370·650.
11 Kojima, H., Shinohara, N., Hanaoka, S., Someya-Shirota, Y., Takagaki, Y., Ohno, H., Saito, T., Katayama, T., Yagita, H , Okumura, K., Shinkai, Y., Alt, F. W., Masutzawa, A., Yonehara, S. and Takayama, H 1994. Two distinct pathways of specific killing revealed by perforin mutant cytotoxic T cells *Immunity* 1:357
12 Kagi, D , Vignaux, F., Ledermann, B., Burki, K., Depraetere, V., Nagata, S., Hengartner, H. and Golstein, P. 1994. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. *Science* 265:528.
13 Heusel, J. W., Wesselschmidt, R. L., Shresta, S , Russell, J. H. and Ley, T. J. 1994. Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells. *Cell* 76:977.
14 Lowin, B , Beermann, F., Schmidt, A. and Tschopp, J. 1994. A null mutation in the perforin gene impairs cytolytic T lymphocyte- and NK-mediated cytotoxicity. *Proc. Natl Acad. Sci. USA* 91 11151.
15 Ramsdell, F., Seaman, M. S., Miller, R. E., Tough, T. W., Alderson, M. R and Lynch, D H 1994. *gld/gld* mice are unable to express a functional ligand for Fas *Eur J Immunol* 24.928.
16 Francis, M. J., Hastings, G. Z , Brown, F., McDermed, J., Lu, Y. A. and Tam, J P 1991. Immunological evaluation of the multiple antigen peptide (MAP) system using the major immunogenic site of foot-and-mouth disease virus *Immunology* 73:249.
17 Laemmli, U. K 1970 Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* 227:680.
18 Suda, T. and Nagata, S. 1994 Purification and characterization of the Fas-ligand that induces apoptosis *J Exp. Med* 179·873.
19 Smith, R A and Baglioni, C. 1987. The active form of tumor necrosis factor is a trimer. *J. Biol. Chem.* 262·6951.
20 Eck, M. J., Ultsch, M., Rinderknecht, E., de, V A. M. and Sprang, S. R. 1992 The structure of human lymphotoxin (tumor necrosis factor-beta) at 1.9-Å resolution. *J. Biol. Chem.* 267:2119
21 Loetscher, H., Gentz, R., Zulauf, M., Lustig, A., Tabuchi, H., Schlaeger, E. J., Brockhaus, M , Gallati, H., Manneberg, M. and Lesslauer, W 1991 Recombinant 55-kDa tumor necrosis factor (TNF) receptor. Stoichiometry of binding to TNF alpha and TNF beta and inhibition of TNF activity. *J. Biol. Chem.* 266:18324.
22 Jones, E. Y., Stuart, D I. and Walker, N. P. 1989. Structure of tumour necrosis factor. *Nature* 338:225.
23 Peitsch, M. C. and Tschopp, J. 1995. Comperative molecular modelling of the Fas-ligand and other members of the TNF family *Mol. Immunol.* in press.
24 Smith, R. A. and Baglioni, C 1989. Multimeric structure of the tumor necrosis factor receptor of HeLa cells. *J. Biol. Chem.* 264.14646.
25 Yonehara, S., Ishii, A. and Yonehara, M. 1989. A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med.* 169:1747.
26 Trauth, B C., Klas, C., Peters, A. M., Matzku, S., Moller, P., Falk, W. and Krammer, P. H. 1989. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 245:301.
27 Dhein, J., Daniel, P. T., Trauth, B. C., Oehm, A., Moller, P. and Krammer, P. H. 1992. Induction of apoptosis by monoclonal antibody anti-APO-1 class switch variants is dependent on cross-linking of APO-1 cell surface antigens. *J. Immunol.* 149:3166.
28 Callard, R. E., Armitage, R. J., Fanslow, W. C. and Spriggs, M. K. 1993. CD40 ligand and its role in X-linked hyper-IgM syndrome *Immunol. Today* 14:559.