A three-dimensional model of the Fas/APO-1 molecule: cross-reactivity of anti-Fas antibodies explained by structural mimicry of antigenic sites

Bengt Fadeel1, Jakob Lindberg, Adnane Achour and Francesca Chiodi
Microbiology and Tumorbiology Center, Karolinska Institutet, 171 77 Stockholm, Sweden
1Present address: Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, 171 77 Stockholm, Sweden

Keywords: epitope mapping, Fas/APO-1, homology-based modeling, structural mimicry, tumor necrosis factor/nerve growth factor receptor family

Abstract

Fas/APO-1 is a member of the tumor necrosis factor (TNF)/nerve growth factor receptor family. This cell surface protein, when associated with the Fas/APO-1 ligand or specific mAb, elicits an apoptotic response in susceptible cells via an oligomerization of its intracellular domains, termed the ‘death domains’. We have previously mapped the epitopes of a panel of Fas/APO-1-reactive mAb to a series of linear portions of the Fas/APO-1 molecule. In order to gain a greater understanding of the mode of interaction of these antibodies with the Fas/APO-1 antigen, we constructed a homology-based model of the extracellular portion of the molecule, based on the crystallographic coordinates of the TNF type I receptor. The model clearly demonstrates that the antibodies do not identically mimic the endogenous ligand to achieve their effect, but probably act in an analogous manner by recruiting Fas/APO-1 molecules into clusters which may lead to oligomerization of ‘death domains’. Moreover, the apparent cross-reactivity observed for the monoclonal anti-Fas antibodies between different linear regions of the Fas/APO-1 molecule was found to be due, most likely, to the structural mimicry of these epitopes. Reduction of the Fas/APO-1-derived cross-reactive peptides by dithiothreitol completely abrogated their antigenic reactivity.

Introduction

Apoptosis, or programmed cell death, is a process of selective cell deletion that is fundamental to the normal development and homeostatic maintenance of multicellular organisms (1). Cells undergoing apoptosis display several distinctive morphological and biochemical features, most notably cell shrinkage, chromatin condensation, formation of apoptotic bodies and cleavage of genomic DNA into nucleosomal length fragments of ~200 bp (2). Fas/APO-1 and the Fas/APO-1 ligand, a pair of interacting molecules expressed primarily on cells of immune system origin, were recently characterized and found to be members of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor family and the TNF family respectively (3–5). Mutational analysis of the cytoplasmic portion of Fas/APO-1 suggested the presence of an inhibitory as well as a signal transduction domain, the latter being significantly conserved in Fas/APO-1 and in the TNF type I receptor (6). The apoptotic signal is thought to be initiated upon the clustering of these intracellular domains, termed the ‘death domains’. Subsequent interaction with other cytoplasmic proteins known as TNF receptor 1-associated death domain protein (TRADD), Fas-associated death domain protein (FADD) [or mediator of receptor-induced toxicity (MORT1)] and receptor interacting protein (RIP), which also contain the ‘death domain’ motif, couples Fas/APO-1 and the TNF type I receptor to downstream signaling cascades (reviewed recently in 7). The importance of Fas/APO-1 and its ligand in...
<table>
<thead>
<tr>
<th>TNFR</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVCQGKYIH PQNSSICCTK CHKGYTYND CGPGQDQTDC RECESG-SFT</td>
<td>46</td>
</tr>
<tr>
<td>TQNLLEGH- -HDQGFCKPD CPFGKERKARD CTVNDEPDC VPCQEGKEYT</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TNFR</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASENHLRCL HSCSKRCRMEG QVEISSCTVD RDTVCGRKMN QYRHYWSENGL</td>
<td>93</td>
</tr>
<tr>
<td>DKAHFSSKR RCRCLDEHGQ LEVEINCRTTN QNTKCRCKPM FFPCN--STVC</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TNFR</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCQFNCSSLCL NGTVHLSCQE KQNTVCT</td>
<td>141</td>
</tr>
<tr>
<td>EHCQPCTKCE NQIIK-ECTIL TSNTKCK</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. (A) Alignment of the primary sequences of the extracellular domains of TNF type I receptor and Fas/APO-1. Gaps (–) were introduced to yield maximal homology. Sequence numbering refers to Fas/APO-1 (3). Conserved amino acids, including the structurally important cysteine residues, are shown in bold face. The level of sequence identity is 24.4%. Amino acids included in the linear antibody binding epitope of Fas/APO-1 are arrowed. Putative N-linked glycosylation sites are indicated with an asterisk. Residues identified by Starling et al. (31) as being important for ligand binding to Fas/APO-1 are indicated with ‘$’. (B) Amino acid sequence alignment of TNF-β and the extracellular domain of the Fas/APO-1 ligand. Sequence numbering refers to the Fas/APO-1 ligand (5). Conserved residues and putative N-linked glycosylation sites are indicated as above. The level of sequence identity is 28.9%. Residues implicated in receptor-ligand interactions as deduced from recent mutagenesis experiments (32) are indicated with ‘#’. 

the homeostatic regulation of the immune system is underscored by the abnormalities observed in mice homozygous for the lpr and gld loci. As a result of mutations in Fas/APO-1 and the Fas/APO-1 ligand respectively, these mice develop profound autoimmune disease which resembles systemic lupus erythematosus (8,9). Fas/APO-1 is also important for the generation of T cell-mediated cytotoxicity (10) and has been implicated in several human diseases, such as HIV infection and various forms of liver damage (11–13).

In a previous report (14) we presented the sequence of the linear epitope corresponding to the major determinant of the original anti-Fas IgM mAb CH-11, which induces apoptosis upon its interaction with the Fas/APO-1 molecule. We also demonstrated that additional peptides derived from the amino acid sequence of the extracellular portion of Fas/APO-1 were able to bind to the CH-11 antibody, yet unlike the dominant peptide, were not able to block apoptosis induced by this antibody. In a subsequent study, designed to map the epitopes targeted by a panel of anti-Fas mAb of the IgG1 subclass, we obtained similar results (15). In order to resolve questions pertaining to these results, i.e. the apparent cross-reactivity of the anti-Fas mAb, we produced a molecular model of Fas/APO-1, based on the recently determined three-dimensional structure of the TNF type I receptor. Similarly, we generated a model of Fas/APO-1 in complex with its ligand. Our modeling suggests that internal molecular mimicry may account for the cross-reactivity we have observed. Formation of intramolecular disulphide bonds appears essential for antigenic reactivity, as evaluated in peptide ELISA performed under reducing and non-reducing conditions. Furthermore, our results indicate that anti-Fas antibodies only partially mimic the mode of action of the endogenous ligand in order to induce an apoptotic response.

Methods

Computer modeling of the Fas/APO-1 antigen

An automated alignment of the human TNF receptor type I and human Fas/APO-1 sequences was generated using the SIM algorithm (16) as implemented in the BCM search launcher (Human Genome Center, Baylor College of Medicine,
Houston, TX). Some manual adjustments were made to optimize the positions of the structurally important cysteine residues. Based on this alignment, a molecular model of the extracellular domain of the Fas/APO-1 antigen was constructed using the crystallographic coordinates of the soluble TNF receptor type I (17). All steps of the modeling procedure were performed with the COMPOSER homology modeling program (18,19) operating on a Silicon Graphics Indigo© workstation. The model was subsequently geometry optimized within the SYBYL 6.0 package using the following optimization regime. All disulphide linkages were joined, including those linkages which were intrinsically novel to the Fas/APO-1 molecule. The backbone and the Cα atoms of the molecule and the side chains which were conserved between the homologue and the template which were not involved in loop regions were fixed. All other side chains were allowed to move and a molecular mechanics optimization using the SYBYL implementation of the Amber 3.0a forcefield was performed on the model structure with charges invoked from the internal Amber dictionary (20). The following dielectric model was used: a distance-dependent dielectric function with a dielectric constant $\varepsilon = 4$ and a cut-off of 9 Å. The model was optimized until a convergence criterion of a deviation $<$0.5 kcal/mol in the total energy was achieved. At this point all constraints were removed and the entire molecule was refined for 500 cycles.

**Computer modeling of the Fas/APO-1 ligand**

The model for the Fas/APO-1 ligand was produced using an identical procedure as the one described for the receptor. An automated alignment of the human TNF-β and human Fas/APO-1 ligand sequences was generated and modeling was based on the previously determined crystallographic structure of the TNF-β molecule (17). The models of Fas/APO-1 and its receptor were combined by superposition onto the structure of the trimeric TNF receptor–TNF-β complex in order to form a pastiche model upon which the assessment of location of
antibody binding epitopes, in relation to the location of cognate ligand binding, could be made.

**Peptide synthesis**

Fifteen peptides, corresponding to the extracellular region of human Fas/APO-1 (residues 16–175) (3), were synthesized using the solid-phase multiple peptide synthesis method (21) and cleaved by liquid hydrogen fluoride in a multivessel apparatus (22). Peptides were 20 amino acids in length, each overlapping the neighboring peptide by 10 amino acids. We also synthesized additional sets of peptides of constant length in which the amino acid residues present in Fas/APO-1 peptides 5, 11 and 12 were sequentially substituted with a glycine.

**Peptide ELISA**

Peptide ELISA was performed as previously described (14). Briefly, microtiter plates (Titertek; Flow, MacLean, VA) were coated with 2 µg/well of peptide dissolved in 0.01 mol/l carbonate buffer and then blocked with 5% BSA. The mAb were added at a concentration of 0.5 µg/well. Peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts; Dako, Glostrup, Denmark) were diluted 1:1000. Absorbance values were determined at 490 nm.

Some ELISA experiments were performed under reducing conditions. Microtiter plates were coated with 1 µg/well of peptide and incubated overnight at room temperature. The plates were then incubated with the reducing agent dithiothreitol (DTT; Sigma) dissolved in 0.01 mol/l carbonate buffer at 1 nmol/well. After 1 h at 37°C, the DTT solution was discarded and the plates were alkylated with iodoacetic acid dissolved in 0.01 mol/l carbonate buffer at 1 nmol/well for 1 h at 37°C. Blocking solution, antibodies and conjugate were all left on the plates for 1 h at 37°C. Primary antibodies were added at 1 µg/well and guinea pig sera were diluted 1:100 in PBS containing 0.5% BSA and 0.05% Tween 20. Peroxidase-conjugated rabbit anti-guinea pig Ig (Dakopatts) were diluted 1:2000 and anti-mouse Ig were employed as above. Peptides were assayed under reducing and non-reducing conditions on the same plate.

**Serological reagents**

The prototypic anti-Fas mAb (clone CH-11) was purchased from Medical & Biological Laboratories (Nagoya, Japan).
Fig. 4. (A) Epitope specificities of anti-Fas mAb CH-11 against Fas/APO-1 peptides 5 (Gly56–Val75), 11 (Glu116–Cys135) and 12 (Lys126–Pro145) determined by substitution set analyses. The anti-Fas mAb was tested against sets of peptides in which individual amino acids had been substituted for by a glycine. The particular amino acid substituted from a peptide is indicated in the bottom part of each figure. Criteria for the demonstration of amino acid dependence are given in the text. (B) Epitope specificities of hyperimmune guinea pig sera generated against Fas/APO-1 peptides 5 (Gly56–Val75) and 12 (Lys126–Pro145) respectively. Substitution set analyses were performed as above.

An isotype-matched mAb directed against Aspergillus niger glucose oxidase was obtained from Dako. For the production of guinea pig hyperimmune sera, peptides 5 and 12 derived from the extracellular portion of Fas/APO-1 were dissolved in PBS and mixed with keyhole limpet hemocyanin (KLH; Sigma) at a final concentration of 1 mg/ml of both peptide and carrier. The coupling reaction was performed in the presence of 0.5% glutaraldehyde in PBS and the peptide–KLH conjugate was rotated end-over-end overnight at room temperature. To terminate the coupling reaction, Tris–HCl (pH 7.5) was added at a final concentration of 50 mM. Pairs of guinea pigs were immunized with 250 µg of the peptide–KLH conjugate in complete Freund's adjuvant. Booster immunizations with the KLH-coupled peptides in incomplete Freund's adjuvant were performed on day 14 and 21 after the initial intramuscular injection, and on day 31 the animals were sacrificed and
**Results**

**Evaluation of the molecular model of Fas/APO-1 and the Fas/APO-1 ligand**

Similarity between members of the TNF/NGF receptor family is found within the extracellular cysteine-rich domains, whereas their cytoplasmic domains are almost entirely unrelated (17,23). The TNF type I receptor which we used as template for our Fas/APO-1 model contains four such cysteine-rich domains, while Fas/APO-1 has only three; nevertheless, it was possible to align the Fas/APO-1 sequence with the sequence corresponding to the first three domains of the TNF type I receptor without major insertions or deletions (Fig. 1A). Subsequent superposition of Cα backbones of the original TNF type I receptor structure and the Fas/APO-1 model reveals only minor deviations of the model from the template structure (Fig. 2A). Hence, the loop comprised of amino acids Phe134 to His142 differs slightly from the original structure, whereas the loop corresponding to the dominant antibody binding epitope of Fas/APO-1 (discussed below) is highly conserved, indicating that the modeling of this part of the molecule is reliable and likely to reflect the true structure of the molecule.

The level of sequence identity between the TNF family members does not exceed 35%, yet their three-dimensional folds are expected to be very similar (24). Therefore, it appeared feasible to generate a model of the Fas/APO-1 ligand based on the known structure of TNF-β. Figure 1(B) shows the alignment of the human TNF-β and human Fas/APO-1 ligand sequences, and in Fig. 2(B) the Cα backbones of the TNF-β structure and the Fas/APO-1 ligand model are superimposed. The all-β conformation of the TNF-β monomer is accurately maintained in the Fas/APO-1 ligand model, with deviations in backbone conformation occuring only in the intervening loops between the β-strands. Banner et al. (17) have described in detail the receptor-ligand interface of the TNF type I receptor and TNF-β and found that two regions, the d–e loop of the C subunit and the a–a0 region of the A subunit of the trimeric TNF-β ligand are those regions which are primarily involved in receptor contact. Interestingly, the corresponding regions of the Fas/APO-1 ligand model are highly conserved (Fig. 2B).

Additional support for the homology based model was provided by the location of the five putative N-linked glycosylation sites in Fas/APO-1 and the Fas/APO-1 ligand respectively (3,25). The side chains of the Asn residues from all of these sites were exposed to solvent in our model structure.

**Analysis of the location of peptide epitopes within the three-dimensional structure of the Fas/APO-1 antigen**

In a previous report we defined the primary epitope of the prototypic apoptosis inducing anti-Fas mAb CH-11 (14). We noted that in our peptide-based epitope mapping the antibody bound in a cross-reactive manner to three different peptides, yet only one peptide was capable of blocking the apoptotic effect of the antibody in Fas/APO-1-expressing Jurkat cells. There was no apparent sequence identity observed for the different peptides. Initially, we considered two different explanations for these anomalous findings. The first explanation was that the peptides, when in the context of the entire Fas/APO-1 protein, formed a contiguous surface and that the blocking peptide was the dominant determinant of this surface. The second explanation which we considered was that the peptides formed a discontinuous series of epitopes which were able to adopt similar conformations in solution as free peptides, or by induction in the antibody binding site.
Fig. 6. (A) Homology-based model of Fas/APO-1 and the Fas/APO-1 ligand. The trimeric Fas/APO-1 receptor complex is depicted in grey and the Fas/APO-1 ligand trimer is colored bright red. The N- and C-termini of the Fas/APO-1 molecule are indicated. (B) The same Fas/APO-1–Fas/APO-1 ligand complex as in (A) viewed down the 3-fold axis from the top. The dominant antibody binding epitope is indicated with an arrow. The amino acid corresponding to the point mutation in the Fas/APO-1 ligand of gld mice is also indicated (Phe275), together with the side chain of the amino acid proposed to interact with this phenylalanine residue (Ala247; 38). These pictures were produced using the program SETOR (37).

and as a result of this conformation were able to present a surface capable of structural mimicry of the cognate epitope. In order to resolve which of these tentative explanations was the most likely and, furthermore, to localize these epitopes onto

Fig. 7. The cross-reactive Fas/APO-1 peptide epitopes and the Fas/APO-1 ligand interface are situated on opposite sides of the Fas/APO-1 receptor. The location of Fas/APO-1 peptides 5 and 14 and the overlapping amino acids common to peptides 11 and 12 is indicated with asterisks. Three different projections of the same ligand–receptor complex are shown. For the purpose of clarity, only one receptor monomer and one ligand subunit are shown; in the trimeric receptor–ligand complex, the peptide epitopes are situated on the exterior surface and the receptor–ligand interface on the interior surface of the receptor (cf. Fig. 6). These images were produced using SYBYL, a Tripos, Inc. product.
the three-dimensional fold of the molecule, we constructed a homology model of the Fas/APO-1 molecule based on the crystal structure of the closely related TNF type I receptor.

An analysis of the Fas/APO-1 model in relation to the location of the antibody epitopes demonstrates that all the antigenic regions which we previously identified are located within spatially separate loops of the protein (Fig. 3). Thus the model largely disproves the hypothesis that the cross-reactive peptides form part of a singular non-linear antigenic surface. Rather, the locations of the peptides suggest that some form of internal molecular mimicry is involved.

**The role of cysteine residues within the Fas/APO-1 peptide epitopes**

We previously noted that the cross-reactive Fas/APO-1 peptides were cysteine rich (15). In order to elucidate the role of these cysteine residues, we synthesized sets of peptides in which the individual amino acid residues present in the native sequences were sequentially substituted by a glycine. These substitution sets of peptides were then analyzed for their capacity to bind the anti-Fas mAb CH-11 in a peptide ELISA. The absorbance value of the mAb against the intact peptide was chosen to represent 100% and the reactivity against the substituted peptides was expressed in relation to this number. A reduction of 60% or more indicated that the deleted amino acid was important for binding of the anti-Fas mAb. This analysis indicated that the cysteine residues, together with the asparagine and the two phenylalanine residues, were critical for antibody binding against the overlapping peptides 11 and 12 (Fig. 4A). Furthermore, in the cross-reactive peptide 5 the cysteine residues were clearly the most important determinants for antibody reactivity. We have previously analyzed these peptide sequences to resolve the cross-reactive behavior of a panel of anti-Fas antibodies of the IgG1 subclass and found that a basic surface may be involved in the peptide–antibody interactions (15). The peptides all contain at least two basic residues and if a basic surface was involved, the substitution of only one of the basic residues may not suffice to yield a decrease in antibody binding. Interestingly, the fact that we observed a similar reduction in peptide antigenicity following substitution of either one of the different cysteine residues in peptide 5 (Fig. 4A) suggests that all three possible intramolecular disulphide bonds are equally important for antibody binding. However, based on our previous molecular graphics analysis of peptide 5 in its 'native' conformation, we would predict that the outlying cysteine residues Cys59 and Cys73 are those which are principally involved in the formation of the intrapeptide disulphide bonds (15).

In order to further evaluate the importance of the formation of disulphide bridges in these Fas/APO-1-derived peptides, we performed ELISA experiments under reducing and non-reducing conditions. Following treatment of peptides 5, 11, 12 and 14 with the reducing agent DTT, the reactivity of the anti-Fas mAb CH-11 was markedly decreased (Fig. 5A). To rule out the possibility that this reduction in reactivity was due to loss of peptide from the plates, we also assayed polyclonal guinea pig serum against peptide 5 and 12 respectively. These hyperimmune sera were first subjected to substitution set analyses which indicated that the anti-peptide 5 serum had an almost identical amino acid dependency as the previously tested anti-Fas mAb, i.e. the cysteine residues appeared to be critical for binding of this guinea pig serum (Fig. 4B). On the other hand, the anti-peptide 12 serum showed no such pattern of amino acid preferences; instead, it appeared to recognize an epitope in the C-terminal part of the peptide (Fig. 4B). Consequently, we found that the reactivity of the hyperimmune serum recognizing peptide 12 was unaffected by DTT treatment of the corresponding peptide (Fig. 5B), demonstrating, in effect, that DTT treatment does not result in loss of peptide from the microtiter plates. On the other hand, the anti-peptide 5 serum showed a decrease in reactivity similar to that observed for the anti-Fas mAb.

**The location of antibody epitopes in relation to the cognate ligand binding surface**

The TNF-β molecule is a homotrimer (17) and it is likely that the trimeric nature of the ligand induces an oligimerization of the receptor. Evidence has been adduced for such a trimeric structure also for the Fas/APO-1 ligand. The evidence rests primarily on the homology of the Fas/APO-1 ligand with other members of the ligand family such as TNF-α, TNF-β and the CD40 ligand, all of which have been shown to form trimeric structures (17,26,27), and also on chemical cross-linking studies of the Fas/APO-1 ligand performed recently by Tanaka et al. (28). Furthermore, the fact that the multivalent anti-Fas IgM mAb is capable of inducing apoptosis while F(ab')2 fragments derived from the same antibody have no cytolytic activity (29) indicates that bivalency is insufficient for the induction of apoptosis, whereas the clustering of three (or more) receptor subunits efficiently mediates cell death. These findings are consistent with a trimeric structure of the Fas/APO-1 ligand.

In order to analyze the mode of interaction between the Fas/APO-1 molecule and the anti-Fas antibodies in relation to that of the cognate ligand, a pastiche model of the receptor–ligand complex based on the TNF type I receptor–TNF-β complex, was constructed (Fig. 6). This trimeric Fas/APO-1–Fas/APO-1 ligand model was analyzed in the light of the location of the linear epitopes mapped by synthetic peptides. Interestingly, all of the epitopes (i.e. regions corresponding to peptides 5 and 14 as well as the overlapping region of peptides 11 and 12) are on the external face of the trimeric complex (Fig. 7), with the internal face being that occupied by the Fas/APO-1 ligand. This poses some interesting questions regarding the mode of action of the anti-Fas mAb. The fact that an antibody can mimic the effect of the cognate ligand by binding to the exterior surface of the receptor molecule indicates that the sole function of the ligand is to promote receptor oligimerization. As such, the ligand would not be required to induce a conformational change in the receptor in order to exert its effect. Indeed, the clustering of receptors on the cell surface appears to be a common activation mechanism for several growth factors (30).

During the preparation of this manuscript, Starling and co-workers reported on the identification of several amino acid residues in the extracellular domain of Fas/APO-1 which were important for ligand binding (31). Interestingly, these amino acids are distinct from those which we have identified as critical for antibody binding (Fig. 1A) and they are all situated on the internal face of the Fas/APO-1 trimeric complex in our model (data not shown), thus corroborating our findings in the present study. Similarly, Schneider et al. recently reported on the identification of amino acids essential for interaction between Fas/
APO-1 and its ligand (32); the substitution of one of these amino acids (Phe275) was suggested to prevent the correct association of the trimeric ligand, whereas two additional amino acids (Pro206 and Tyr218) were shown to be important for receptor interaction. Again, these amino acids are predicted to interact with what is in our model the internal face of the trimeric receptor complex (data not shown).

Discussion

We recently mapped the epitope targeted by the prototypic apoptosis inducing anti-Fas mAb CH-11 (14). We demonstrated that this epitope is comprised of amino acids 126–135 of the extracellular portion of the Fas/APO-1 molecule. Furthermore, two additional peptides contained within the sequence of the protein, when tested, could also bind to the antibody, yet were unable to block its apoptotic effect in vitro. A similar pattern of cross-reactivity was observed with a panel of anti-Fas mAb of the IgG1 subclass (15). The cross-reactivity or promiscuity of these antibodies may be reconciled in one of three possible scenarios. Insufficient subcloning of the antibodies or the presence of contaminants may lead to the generation of bispecific sera. However, since all of the antibodies tested display similar patterns of cross-reactivity this would appear to be unlikely from a statistical standpoint. Moreover, the various antibody preparations were purely of the IgM and IgG1 subclasses respectively, a finding highly unlikely in the event of contamination. Alternatively, the cross-reactivity could be caused by the collection of cross-reactive peptides forming a nonlinear antigenic surface in the intact molecule. A precedent for this was recently reported in a study by Björling et al. (33), who demonstrated that even short peptides can adopt a secondary structure which may be important for antibody recognition. The importance of cysteine residues in particular in maintaining the secondary structure of peptides has been commented on by Åkerling-Stopner et al. (35), who concluded that the reactivity of mAb against the A and B subgroups of the respiratory syncytial virus was critically dependent upon intrapeptide disulphide bonds. In view of these findings, we conclude that the cross-reactivity observed for this panel of anti-Fas antibodies is due, at least in part, to intramolecular mimicry of spatially discrete antigenic regions.

Based on the crystallographic structure of the TNF type I receptor in complex with TNF-β, we proceeded to generate a molecular model of the putative trimeric Fas/APO-1 receptor in complex with its ligand, also depicted as a homotrimer. Interestingly, both the dominant and cross-reactive peptides are located on the opposite face of the Fas/APO-1 receptor complex when compared to the ligand binding site. This finding suggests that the antibody molecule does not directly mimic the action of the ligand. Furthermore, since the ligand and the antibodies bind to different surfaces of the receptor, it is unlikely that they should generate an identical conformational change in the receptor upon binding and thus unlikely that the generation of such an altered structure, either by the endogenous ligand or antibody, is the event which provides the apoptotic signal. In support of this view are the initial observations made by Banner et al. (17), who could find no indication in the structure of the TNF type I receptor for any hinge region and no direct evidence of structural change upon complex formation. Moreover, a recent comparison of the structure of the unliganded TNF receptor with that crystallized as a complex with TNF-β demonstrated that ligand binding does not induce global changes in the tertiary structure of the receptor (36).

To conclude, the homology-based model of Fas/APO-1, one of the molecular doyens within the field of apoptosis, has advanced our understanding of the interaction of anti-Fas antibodies with Fas/APO-1. The modeling strategy described herein has further elucidated the molecular forces which drive the induction of apoptosis by these agonistic mAb. Moreover, our model of Fas/APO-1 and its ligand may also serve to facilitate future studies of the structure–function relationship of this interacting pair of molecules, and to aid in the design of specific inhibitors of such interactions.

Acknowledgements

This work was supported by the Swedish Medical Research Council, the Swedish Cancer Society and the Magnus Bergvall Foundation.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>FADD</td>
<td>Fas-associated death domain protein</td>
</tr>
<tr>
<td>KLH</td>
<td>keyhole limpet hemocyanin</td>
</tr>
<tr>
<td>MORT1</td>
<td>mediator of receptor-induced toxicity</td>
</tr>
<tr>
<td>NGF</td>
<td>nerve growth factor</td>
</tr>
<tr>
<td>RIP</td>
<td>receptor interacting protein</td>
</tr>
<tr>
<td>TRADD</td>
<td>TNFR1-associated death domain protein</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
</tbody>
</table>

References

A structural model of Fas/APO-1

140

24 Peitsch, M. C. and Jongeneel, C. V. 1993. A 3-D model for the CD40 ligand predicts that it is a compact trimer similar to the tumor necrosis factors. Int. Immunol. 5:233.