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Conserved residues of tumour necrosis factor and lymphotoxin constitute the framework of the trimeric structure

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Four distinct areas of primary sequence conservation between known tumour necrosis factor and lymphotoxin polypeptides from various species can be recognized. When these amino acid sequences are highlighted in the three-dimensional structure, all are found in the same region, constituting the framework of the trimeric structure.

Tumor necrosis factor; Lymphotoxin; Computer modelling; Structural homology

1. INTRODUCTION

Tumour necrosis factor (TNF) and lymphotoxin (LT) are related cytokines, the former produced by activated macrophages or T-lymphocytes, and the latter by activated T-cells exclusively. Both cytokines bind to the same cell surface receptor and exert a wide variety of different biological functions. Both are believed to play a key role in diverse host defense mechanisms, but it is also recognized that they may be involved in certain disease states.

The genes coding for TNF and LT of different species have been cloned (see [1,2] for review), allowing a detailed comparison of the primary sequences. As can be deduced from fig.1, 4 areas of higher structural homology can be indicated: amino acids 11–18, 48–64, 119–133 and the 6 C-terminal residues. These regions encompass almost two-thirds of the conserved residues between TNF and LT (these 2 polypeptides are about 30% related). On the contrary, the interspecies conservation of either TNF or LT is very high: about 80%.

Crystals have been obtained using recombinant TNF from *E. coli* [3,4] or *S. cerevisiae* [5] and recently, the three-dimensional structure has been elucidated at a 2.9 Å resolution [4]. TNF is a compact trimer, with subunits having an all-β conformation. Each monomer consists of two antiparallel β-pleated sheets with the viral 'jelly-roll' motif. Here we report on the localization of the conserved residues between TNF and LT within this three-dimensional structure.

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2. RESULTS AND DISCUSSION

We have modelled the TNF structure using the published data. First, since the atomic coordinates are not available, by reconstructing the Cα-backbone, and followed by refinement of the model using an energy-minimization program [6]. When we highlight the 4 aforementioned conserved areas on this model it is clear that the residues involved are mainly grouped in the inner core of the structure, suggesting a structural role (fig.2). Interestingly, all these residues are situated in the lower half of the molecule. In addition, almost all of the conserved residues located outside of the 4 homology boxes are also found in this lower part.

It is tempting to speculate on the consequences of this finding. The fact that almost all conserved residues reside in the lower half of the structure suggest a likewise location of the receptor binding site. Several data are in agreement with this hypothesis.

(i) We have been able, by epitope mapping, to pinpoint the residue Arg-131 as being involved in the binding of a neutralizing monoclonal antibody directed against human TNF [7]. Another study showed that antibodies against a synthetic peptide spanning the N-terminal 15 amino acids could also neutralize the biological activity [8]. Both results point towards the lower half of the trimeric structure as being involved in receptor binding. In the two cases, however, the effects on the biological activity are most likely due to topological shielding of the receptor binding site by the antibodies.

(ii) A single disulfide bridge between residues 69 and 101 is located at the top of the molecule as shown in fig.2A. TNF variants, in which this disulfide bridge has

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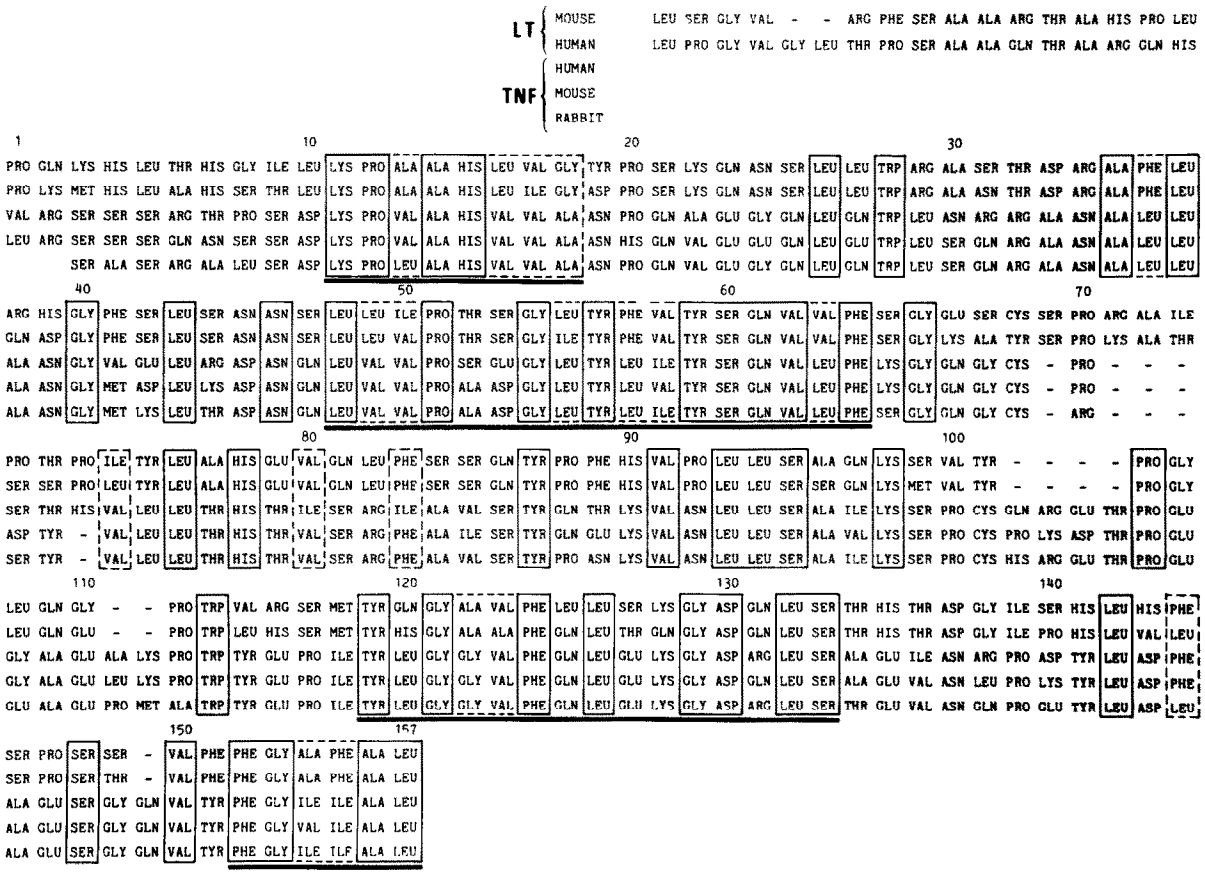

Fig.1. Primary sequence conservation amongst TNF and LT. The complete amino acid sequences are shown of mature lymphotoxin (mouse [10] and human [11]) and tumour necrosis factor (human [12], mouse [13] and rabbit [14]). Numbering refers to the human TNF molecule. Gaps have been introduced to optimize alignment. Full boxes indicate conserved residues between all 5 polypeptides; dashed boxes add conservative changes.

been removed by site-specific mutagenesis (substitution of the Cys residues by Ser) have an only slightly reduced specific activity when compared to wild-type TNF ([9], our own unpublished data). Also, crystal analysis data have indicated that this upper region of the structure has a high main-chain mobility [4].

(iii) Using a random mutagenesis approach, we located inactivating, single amino acid mutations in close vicinity of the 131 Arg residue (Van Ostade, manuscript in preparation) suggesting that the receptor binding site is a constellation of residues located in the lower half of the TNF trimer.

Interestingly, none of these inactivating mutations correspond to conserved residues between TNF and LT, suggesting that many of the conserved residues might have a mainly structural role. One explanation for the remarkable conservation of the core might be that this structure is essential in the folding process to yield the correct conformation, or to maintain the correct positioning of the residues involved in receptor binding. Alternatively, it cannot be excluded that the conserved areas constitute a sofar unknown functional domain within the TNF molecule which perhaps is only activated after the internalization process. It remains intriguing to know how the less conserved areas between TNF and LT are still responsible for binding to the same surface receptor. The elucidation of the structure of LT certainly will help to understand this.

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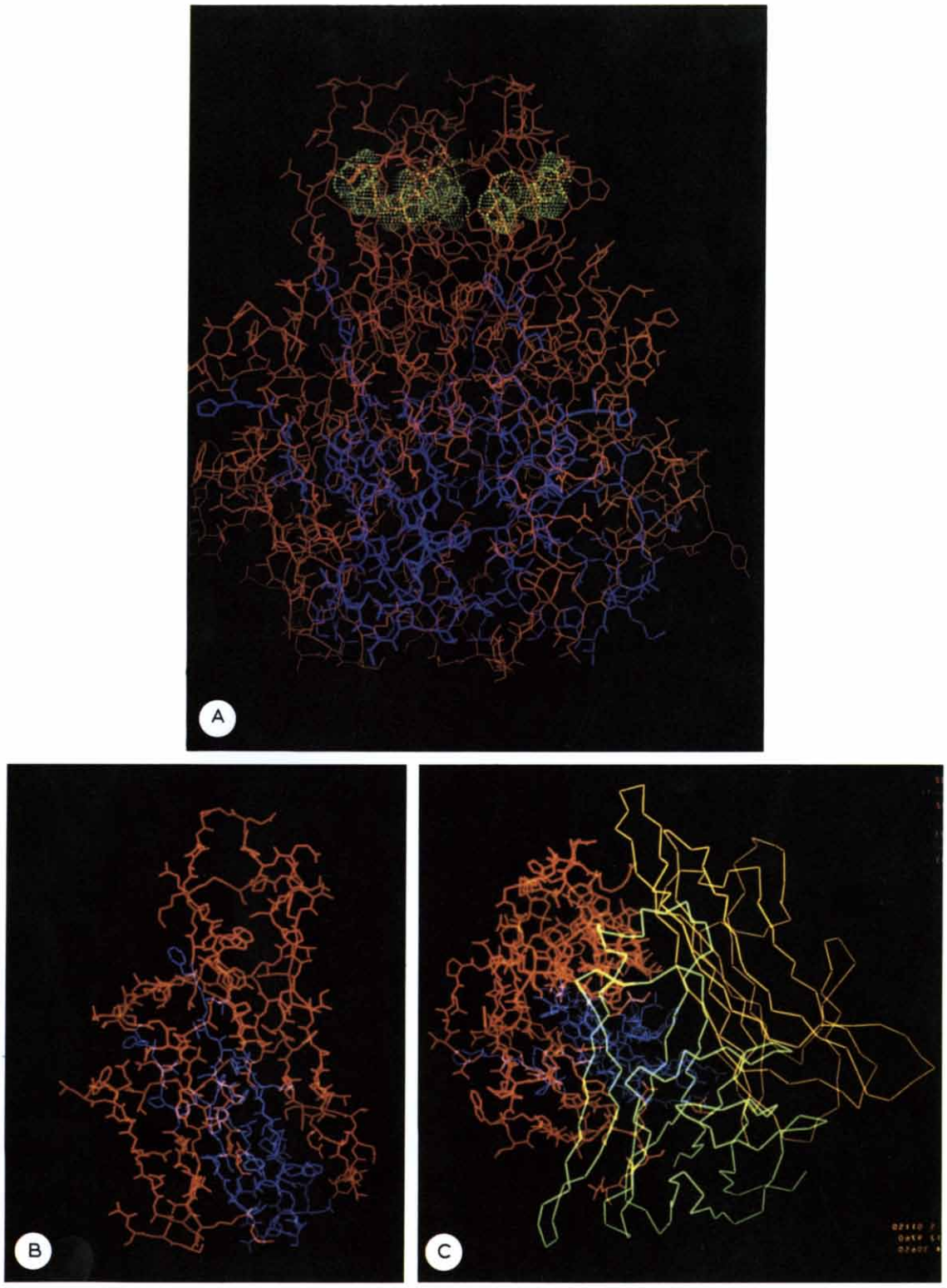

Fig.2. Localization of conserved residues within the TNF structure. (A) The TNF trimer is presented with the Cα backbone and side chains, and is shown with the 3-fold axis vertical. Highlighted in blue are the residues conserved between TNFs and LTs. The green spheres near the top show the positions of the disulphide bridges. (B) Same as A but the monomeric structure is shown. (C) Same as A but the view is from above. For reasons of clarity, two monomers are only presented with their Cα backbone (yellow).

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