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Crystal structure of TNF α complexed with a poxvirus MHC-related TNF binding protein

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Abstract

The poxvirus 2L protein binds tumor necrosis factor- α (TNF α) to inhibit host antiviral and immune responses. The 2.8-Å 2L–TNF α structure reveals three symmetrically arranged 2L molecules per TNF α trimer. 2L resembles class I major histocompatibility complex (MHC) molecules but lacks a peptide-binding groove and β 2-microglobulin light chain. Overlap between the 2L and host TNF receptor-binding sites on TNF α rationalizes 2L inhibition of TNF α –TNF receptor interactions and prevention of TNF α -induced immune responses.

Poxviruses express a wide range of secreted proteins that interfere with the vertebrate immune response¹. The pro-inflammatory cytokine TNF α is a common target for inhibition by secreted poxviral proteins, including virally encoded homologs of TNF receptors (TNFRs)^{2,3}. A class of secreted TNF α inhibitor that is not homologous to viral or host TNFRs was recently identified from Tanapox virus (TPV)⁴ and related viruses (Supplementary Fig. 1). The TPV 2L protein binds human TNF α with very high affinity ($K_d = 43$ pM), thereby effectively sequestering TNF α to prevent activation of TNF receptors and downstream antiviral effects such as TNF-induced apoptosis⁴.

Although 2L proteins are distantly related to extracellular regions of MHC class I molecules ($\sim 13\%$ sequence identity)⁴ (Supplementary Fig. 2), 2L from the Yaba-like disease virus (98% identical to TPV 2L; Supplementary Fig. 1) did not co-purify with β 2-microglobulin (β 2m, the class I MHC light chain) when expressed in insect cells (data not shown). We determined the structure of the 2L–TNF α complex to 2.8-Å resolution by molecular replacement (Supplementary Table 1 and Supplementary Methods; $R_{\text{cryst}} = 23.6\%$, $R_{\text{free}} = 26.6\%$). The structure revealed a central TNF α trimer bound by three symmetrically arranged 2L molecules, analogous to a three-fold symmetric host TNFR–TNF complex (TNFR1 bound to TNF β (lymphotoxin α)⁵) (Fig. 1).

In common with classical class I MHC molecules and MHC homologs, a diverse family that includes host and viral proteins with immune and non-immune functions⁶, the 2L structure

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Accession codes. Protein Data Bank: Coordinates and X-ray crystallographic data for the 2L–TNF α complex have been deposited with accession code **3IT8**.

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also includes an $\alpha 1$ - $\alpha 2$ superdomain (an antiparallel β -sheet that is topped by two α -helices) and an $\alpha 3$ domain (an immunoglobulin constant region fold) (Supplementary Fig. 3a,b). Disulfide bonds are found in the expected positions for class I MHC molecules and homologs: one linking the $\alpha 2$ domain helix to the β -sheet floor of the $\alpha 1$ - $\alpha 2$ platform and one joining the two β -sheets in the $\alpha 3$ domain. An additional disulfide bond near the C terminus joins residues separated by 3 residues (Supplementary Fig. 2).

Similar to many class I MHC homologs, 2L does not contain a noticeable groove between its $\alpha 1$ and $\alpha 2$ helices (groove surface area of $60 \text{ \AA}^2$) (Supplementary Fig. 3c and Supplementary Table 2), and there was no unexplained electron density in this region. In this regard, 2L is more similar to HFE and FcRn (groove surface areas of $415 \text{ \AA}^2$ and $235 \text{ \AA}^2$, respectively)⁷, which do not present endogenous peptides, than it is to classical peptide-binding class I MHC molecules, the peptide-binding viral homolog UL18 or the lipid-presenting CD1d1 protein (groove surface areas of $\sim 760 \text{ \AA}^2$, $900 \text{ \AA}^2$ and $1440 \text{ \AA}^2$, respectively)⁷⁻⁹. Thus 2L is not expected to bind/present endogenous peptides or other antigens.

Groove closure in 2L is accomplished by displacement of the N-terminal half of each helix toward the opposite helix and insertion of large side chains, including Trp69 and Met140, which would prevent binding of a peptide antigen (Supplementary Fig. 3b). The 2L $\alpha 1$ helix is shorter than its MHC counterpart (Supplementary Fig. 2) because it includes irregular structure extending toward the $\alpha 2$ domain. The beginning of the 2L $\alpha 1$ domain helix is anchored by the insertion of Tyr64 into a hydrophobic pocket within the $\alpha 1$ domain. The irregular structure preceding the long helix positions His58 of the $\alpha 1$ domain almost over the $\alpha 2$ helix, where it interacts with Tyr87 of TNF α .

The 2L $\alpha 3$ domain includes an unusual large insertion between $\alpha 3$ strands C and D (residues 220–252) (Fig. 1 and Supplementary Figs. 2 and 3a,d). This insertion makes extensive contacts with the $\alpha 1$ - $\alpha 2$ platform underside, resulting in an $\alpha 3$ domain and platform quaternary arrangement that differs markedly from $\beta 2m$ -binding MHC molecules and homologs. Whereas the distal end of the $\alpha 3$ domain in $\beta 2m$ -binding MHC molecules is positioned almost directly under the $\alpha 1$ - $\alpha 2$ platform center, the 2L $\alpha 3$ domain is tilted $\sim 42^\circ$ toward the $\alpha 2$ portion of the platform and also rotated $\sim 40^\circ$ about its long axis as compared with its counterpart in HLA-A2 (Supplementary Fig. 3e).

The solvent-accessible surface area buried at the 2L platform- $\alpha 3$ interface ($1,970 \text{ \AA}^2$) is comparable to the area buried at the HLA-A2 platform interface with both its $\alpha 3$ and $\beta 2m$ domains ($2,080 \text{ \AA}^2$), but most of the HLA-A2 interface area results from platform interactions with $\beta 2m$ ($1,520 \text{ \AA}^2$). The large interdomain area in 2L suggests that the $\alpha 3$ domain C-D loop, particularly residues 242–246, has a role analogous to $\beta 2m$ in other MHC molecules; that is, it establishes the relative orientation of $\alpha 3$ with respect to the $\alpha 1$ - $\alpha 2$ platform (Supplementary Fig. 3d). In 2L, extensive contacts between the platform and the $\alpha 3$ insertion, interactions between $\alpha 3$ and the $\alpha 2$ domain $\beta 5$ - $\beta 6$ loop and ionic interactions between the N-terminal amino group and acidic residues (Asp211 and Asp286) in proximal loops of the $\alpha 3$ domain presumably lock the position of $\alpha 3$ with respect to the $\alpha 1$ - $\alpha 2$ platform. This effectively pre-organizes the TNF α -binding surface, which involves residues from all three 2L domains (Supplementary Fig. 3e).

Although the position of the 2L $\alpha 3$ domain does not overlap with $\beta 2m$'s location in MHC class I-like molecules, N-linked glycosylation at Asn6 under the 2L $\alpha 1$ - $\alpha 2$ platform (Supplementary Fig. 3a) would prevent the canonical $\beta 2m$ interaction. The corresponding residue in the non- $\beta 2m$ -binding protein MIC-A (suggested to be the source of 2L proteins by horizontal gene transfer¹⁰) is also glycosylated¹¹. In addition, the altered position of the 2L $\alpha 3$ domain is not compatible with simultaneous binding of $\beta 2m$ to the $\alpha 1$ - $\alpha 2$ platform and to $\alpha 3$ (Supplementary

Fig. 3f). Thus, it seems that 2L lacks $\beta 2m$ to allow the $\alpha 3$ domain to be brought closer to the $\alpha 2$ helix to form a larger TNF α -binding site (Fig. 2).

2L binds to TNF α using a large and highly complementary interface (Fig. 2a,b), rationalizing the picomolar affinity between 2L and TNF α ⁴. The binding site spans nearly 40 Å, and the total buried surface area at the 2L–TNF α interface (2,060 Å², of which 2L contributes 1,030 Å² and adjacent TNF α subunits contribute 710 Å² and 320 Å²) is larger than typical protein-protein interfaces (1,560–1,700 Å²)¹². The interface is also highly complementary, as assessed by the shape complementarity index (S_C)¹³ ($S_C = 0.72$ for 2L–TNF α , where a value of 0.0 indicates no complementarity and 1.0 indicates a perfect fit), compared with antibody-antigen interfaces ($S_C = 0.64$ – 0.68)¹³. The TNFR1–TNF β interface is less complementary ($S_C = 0.64$), but buries a similar surface area (2,120 Å², with 1,090 Å² from TNFR1 and 530 Å² and 510 Å² from adjacent TNF β subunits).

2L inhibition of TNF α –TNFR interactions is rationalized by overlap between the 2L- and TNFR-binding sites on the TNF trimer (Fig. 1). Residues from all three 2L domains contribute to binding to TNF α at the shallow groove between adjacent TNF α subunits, burying loops D-E and A-A' (also crucial for TNF α binding to TNFR1 and TNFR2 (ref. 14) and TNF β binding to TNFR1 (ref. 5)) (Figs. 1 and 2 and Supplementary Table 3). Given the high (picomolar) affinity of the 2L–TNF α interaction, we assume that 3:3 2L–TNF α complexes are formed *in vivo*. However, formation of 3:3 2L–TNF α complexes may not be necessary for inhibition of signaling through TNFR because this kind of signaling requires multiple intact TNFR-binding sites on TNF, as demonstrated by inhibition of signaling when TNF exchanges subunits with dominant-negative TNF variants¹⁵.

The structure of TNF α shows few changes upon binding to 2L (Supplementary Fig. 4). The 2L–TNF α interface primarily involves TNF α D-E loop residues 86–87 and A-A' loop residues 30–34 from the neighboring subunit interacting with 2L $\alpha 2$ helix residues 157–175; for example, the side chain of TNF α Tyr87 in the D-E loop, a crucial residue for binding both TNFRs¹⁴, contacts the kink in the 2L $\alpha 2$ domain helix and makes a hydrogen bond with His58 from the $\alpha 1$ helix (Fig. 2c). Some features of the TNF-binding sites for 2L and host TNFRs differ: G-H loop residues (Asp143 and Glu146) and residues in the E-F loop (part of the TNF α -binding site for TNFR1 (ref. 16)) do not contact 2L, and TNF α strand B', which interacts with the insertion in the 2L $\alpha 3$ domain, is not part of the site for host TNFR binding.

Unlike host TNFRs, 2L does not bind to TNF β or other TNF family members⁴. Superimposing TNF β onto the 2L–TNF α structure shows no main chain steric clashes (data not shown), suggesting the specificity of 2L for TNF α is related to complementarity between acidic (2L Glu99 and Asp289) and basic (TNF α A'-A'' loop Arg31 and Arg32) residues, which are not present in TNF β (Supplementary Fig. 5). Binding of TNF β may be also prevented by different positions of the TNF D-E loops (residues 85–89 in TNF α and residues 106–110 in TNF β), which differ by ~2.5 Å (measured between the C α atoms of analogous residues: TNF α Tyr87–TNF β Tyr108).

Anti-TNF α antibodies and TNF receptors are used clinically to inhibit autoimmune inflammatory processes¹⁷. The 2L–TNF α structure reveals the molecular mechanism used by poxviruses to attenuate host immune responses and thus provides further insight into how TNF α may be modulated. The structure could facilitate efforts to develop 2L-based anti-TNF α pharmaceuticals; specifically, efforts to minimize the immunogenicity of this viral protein by removing T and B cell epitopes¹⁸ could be guided by identification of 2L residues involved in TNF α binding and maintaining its tertiary structure.

Supplementary Material

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Acknowledgments

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Figure 1.

Structure of 2L–TNF α and comparison with TNFR1–TNF β . Disulfide bonds and ordered carbohydrates on 2L are shown as yellow sticks. Left, the 2L–TNF α complex shown as a ribbon diagram in two orientations: looking down the three-fold symmetry axis (above) and with the three-fold axis vertical (middle). Below left, a surface representation of TNF α from the complex structure, with the 2L-binding site highlighted in red at one of three interfaces on the TNF α trimer (TNF α subunits are different shades of blue). Right, The TNFR1–TNF β structure⁵ shown as a ribbon diagram in analogous orientations (top and middle). Below right, a surface representation of TNF β from the complex structure with one of the TNFR1-binding sites highlighted in red.

Figure 2.

The 2L-TNF α interface. **(a)** Surface representations of 2L (left) and the 2L-TNF α complex (right). 2L is magenta and TNF α subunits are different shades of blue-green. Contact surfaces (≤ 4.0 Å) are highlighted in blue on 2L and red on TNF α . **(b)** Ribbon diagrams corresponding to the surfaces shown above. The C terminus of 2L is distant from the TNF α -binding site, rationalizing why truncations in this region did not affect binding to TNF α ¹⁹. **(c)** Stereo close-up view of 2L-TNF α interface, with the $\alpha 3$ domain insertion highlighted in blue.