

Secreted or not secreted: an important question in TNF function and therapy

The effects of the secreted cytokine TNF were described long before it was first identified in 1975. Perhaps best known for its roles in anti-tumor immunity, wasting, and septic shock, TNF is a key component of inflammation, and as such, autoimmunity. However, not all of the inflammatory effects of TNF are due to the secreted molecule. The membrane-bound form of TNF has important and distinct roles, which require further investigation. Current anti-TNF therapies highlight the need to better understand this distinction, as their differing clinical outcomes may result in part from different interactions with transmembrane TNF.

In the late 1800's it was observed that killed bacteria could be administered to induce tumor regression, albeit with intolerable side effects, but it would take about a century to begin to understand why. [1] Tumor Necrosis Factor (TNF), also called TNF- α , TNFSF2, DIF, and cachectin, is a pro-inflammatory cytokine secreted by M1 macrophages, Th1 cells, and many other cell types in response to LPS and other inducers of inflammation. [2] TNF signals through two TNF family receptors, TNFR1 (TNFRSF1A) and TNFR2 or TNFR1B (TNFRSF1B), which have many similar functions, but one critical difference is the presence of a death domain in TNFR1. [3] The importance of TNF mediated apoptosis is emphasized by its conservation across species. In the invertebrate drosophila the TNF-like gene Eiger has been shown to mediate apoptosis in a JNK-dependent manner. [4] Further, TNF orthologs are well-conserved among mammals, with greater than 80% sequence homology demonstrated between bat, horse, dog, cat, cattle, swine and human TNF genes. [5]

In addition to its role in inducing apoptosis of tumor cells, infected cells, and endothelial cells, TNF is a critical component of a pro-inflammatory cascade known as the acute phase reaction, which also includes IL-6 and IL-1. Together, these cytokines mediate a host of immune functions, including cell proliferation, differentiation, and trafficking, antibody production, coagulation and lipid metabolism. [2, 3] Although these diverse functions have been

TNF- α Products

Antibodies

- Bovine TNF- α PABs
- Swine TNF- α PABs
- Canine TNF- α PABs
- Feline TNF- α PABs
- Dolphin TNF- α PABs
- Guinea Pig TNF- α PABs
- Mouse TNF- α Pabs

ELISAs

- Bovine TNF- α VetSet
- Canine TNF- α VetSet
- Bovine TNF- α Do-It-Yourself ELISA
- Canine TNF- α Do-It-Yourself ELISA
- Dolphin TNF- α Do-It-Yourself ELISA
- Feline TNF- α Do-It-Yourself ELISA
- Guinea Pig TNF- α Do-It-Yourself ELISA
- Mouse TNF- α Do-It-Yourself ELISA
- Swine TNF- α Do-It-Yourself ELISA

Proteins

- Bovine TNF- α
- Canine TNF- α
- Dolphin TNF- α
- Equine TNF- α
- Feline TNF- α
- Guinea Pig TNF- α
- Human TNF- α
- Mouse TNF- α
- Ovine TNF- α
- Rabbit TNF- α
- Swine TNF- α

identified, there is still limited understanding of how such diversity is achieved. While differences between the TNF receptors account for some of these varied functions, differences in TNF itself also contribute. Unlike most cytokines, TNF is initially expressed as a transmembrane protein, which can then be cleaved by the metalloproteinase TACE to release the soluble cytokine. However, transmembrane TNF can also act as a ligand for the TNF receptors for cell-to-cell interactions, and may preferentially bind TNFR2, unlike soluble TNF. Further, some evidence suggests that transmembrane TNF may be able to signal internally upon binding the TNFRs, thus itself acting like a receptor. [3] These additional combinations of signaling interactions may help to explain the diversity of functions TNF is capable of eliciting.

Although we have only just begun to understand the complexity of TNF family interactions, the importance of these distinctions has been highlighted by the advent of anti-TNF therapies. There are now many different kinds of anti-TNF agents available for use in humans, including monoclonal antibodies such as infliximab and adalimumab, and a soluble TNFR2 linked to the Fc portion of human IgG1, called etanercept. [1, 3] While both types of therapy have proven effective in Rheumatoid Arthritis treatment, granulomatous diseases such as Crohn's, Wegener's and sarcoidosis respond to monoclonal antibodies but not the soluble TNF receptor. Infliximab is associated with a greater risk of granulomatous diseases such as tuberculosis, listeriosis and histoplasmosis, while etanercept is not. This suggests that the soluble receptor is not able to impair granuloma formation, while the monoclonal antibodies do. While the Fc portions of the monoclonal antibody therapies are able to attract complement, the Fc of etanercept can not, limiting its ability to induce complement-dependent cytotoxicity when bound to a cell. [3] Etanercept also induced less apoptosis and less production of IL-10 in vitro upon binding to transmembrane TNF [6]. Finally, expression of transmembrane TNF on T cells has been shown to play a role in defense against mycobacterial infection. [7] Taken together, these data present the possibility that transmembrane TNF signaling is an integral component of granuloma formation, and that differences in these therapies are in part due to differences in the way they interact with transmembrane TNF. [3]

TNF is a critical pro-inflammatory cytokine that has been extensively characterized. However, as its complicated network of interactions begin to be elaborated it becomes clear that a great deal remains to be discovered about how TNF functions in disease, immunopathology, and cancer pathways. Understanding the significance of transmembrane TNF will be important to future clinical applications targeting TNF signaling.

References

1. M. A. Palladino *et al*, *Nat. Rev. Drug Disc.* 2: 736 (2003).
2. NCBI. Gene ID:7124, TNF [*Homo sapiens*]. [Last update: 3 Dec, 2013].
3. T. Horiuchi *et al*, *Rheumatology*. 49: 1215 (2010).
4. E. Moreno *et al*, *Curr. Biol.* 12: 1263 (2002).
5. K. Iha *et al*, *J. Vet. Med. Sci.* 71: 1691 (2009).
6. H. Mitoma *et al*, *Gastroenterology*. 128: 376 (2005).
7. H. Bruns *et al*, *J. Clin. Invest.* 119: 1167 (2009).

REAGENTS FOR ANIMAL MODEL AND VETERINARY RESEARCH



TNF- α Products

Antibodies

Product Description	Catalog #	Quantity	Host	Purification	Applications
Bovine TNF- α PAb	PB0275B-100	100 μ g	Rabbit	Antigen-affinity	Western Blot, ELISA
Biotinylated Bovine TNF- α PAb	PBB0278B-050	50 μ g	Rabbit	Antigen-affinity	ELISA
Canine TNF- α PAb	PB0389D-100	100 μ g	Goat	Antigen-affinity	Western Blot, ELISA
Biotinylated Canine TNF- α PAb	PBB0397D-050	50 μ g	Goat	Antigen-affinity	ELISA
Dolphin TNF- α PAb	PB0443P-100	100 μ g	Goat	Antigen-affinity	Western Blot, ELISA
Biotinylated Dolphin TNF- α PAb	PBB0449P-050	50 μ g	Goat	Antigen-affinity	ELISA
Feline TNF- α PAb	PB0419F-100	100 μ g	Rabbit	Antigen-affinity	Western Blot, ELISA
Guinea Pig TNF- α PAb	PB0446GP-100	100 μ g	Goat	Antigen-affinity	Western Blot, ELISA
Biotinylated Guinea Pig TNF- α PAb	PBB0452GP-050	50 μ g	Goat	Antigen-affinity	ELISA
Mouse TNF- α Pab	KP0740M-100	100 μ g	Rabbit	Antigen-affinity	Western Blot, ELISA
Biotinylated Mouse TNF- α Pab	KPB0741M-050	50 μ g	Rabbit	Antigen-affinity	ELISA
Swine TNF- α PAb	PA0144S-100	100 μ g	Rabbit	Protein A	Western Blot
Swine TNF- α PAb	PB0317S-100	100 μ g	Rabbit	Antigen-affinity	Western Blot, ELISA
Biotinylated Swine TNF- α PAb	PBB0318S-050	50 μ g	Rabbit	Antigen-affinity	ELISA

ELISAs

Each VetSet contains 2 coated plates, standard, detection antibody and streptavidin HRP.

The Do-It-Yourself ELISA kits are the stand-alone unconjugated antibody (capture antibody), recombinant protein (standard), and biotinylated antibody (detection antibody). Each of these components can be bought individually, if desired (or as components run out). These antibodies have been shown to function together with the standard provided in an ELISA.

Product Description	Catalog #	Quantity
Bovine TNF- α VetSet	VS0285B-002	2 plates
Canine TNF- α VetSet	VS0408D-002	2 plates
Bovine TNF- α Do-It-Yourself ELISA	DIY0675B-003	1 Set
Canine TNF- α Do-It-Yourself ELISA	DIY0682D-003	1 Set
Dolphin TNF- α Do-It-Yourself ELISA	DIY0693P-003	1 Set
Feline TNF- α Do-It-Yourself ELISA	DIY0708F-003	1 Set
Guinea Pig TNF- α Do-It-Yourself ELISA	DIY0709GP-003	1 Set
Mouse TNF- α Do-It-Yourself ELISA	DIY0745M-003	1 Set
Swine TNF- α Do-It-Yourself ELISA	DIY0732S-003	1 Set

REAGENTS FOR ANIMAL MODEL AND VETERINARY RESEARCH



Proteins

Product Description	Catalog #	Quantity	MW	Gene ID	AA Sequence
Bovine TNF-α	RP0055B-005 RP0055B-025 RP0055B-100	5 μ g 25 μ g 100 μ g	17.4 kDa	280943	LRSSSQASSN KPVAVHVADI NSPGQLRWWD SYANALMANG VKLEDNQLVW PADGLYLIYS QVLFKGGQCP STPLFLTHTI SRIAVSYQTK VNLLSAIKSP CHRETPAEAE AKPWYEPIYQ GGVFQLEKGD RLSAEINLDP YLDYAESGQV YFGIIAL
Canine TNF-α	RP0261D-005 RP0261D-025 RP0261D-100	5 μ g 25 μ g 100 μ g	17.3 kDa	403922	VKSSSRTPSD KPVAVHVANP EABGQLQWLS RRANALLANG VELTDNQLIV PSDGLYLIYS QVLFKGGQCP STHVLLTHTI SRFAVSYQTK VNLLSAIKSP CQRETPEGTE AKPWYEPIYL GGVFQLEKGD RLSAEINLPA YLDFAESGQV YFGIIAL
Dolphin TNF-α	RP0307P-005 RP0307P-025	5 μ g 25 μ g	17.4 kDa	101318178	LRSSSKTSSN KPVAVHVANL STQGQLRWLN TYANTLLANS VKLEDNQLVW PTDGLYLIYS QVLFKGGQCP STHLFLTHTI SRIAVSYPTK VNLLSAIKSP CQRETPEGAE AKPWYEPIYQ GGVFQLEKGD RLSAEINLDP YLDFAESGQV YFGIIAL
Equine TNF-α	RP0137E-005 RP0137E-025	5 μ g 25 μ g	17.1 kDa	100033834	LRSSSRTPSD KPVAVHVANP QABGQLQWLS GRANALLANG VKLTDNQLVW PLDGLYLIYS QVLFKGGQCP STHVLLTHTI SRLAVSYPSK VNLLSAIKSP CHTESPEQAE AKPWYEPIYL GGVFQLEKGD QLSAEINQPN YLDFAESGQV YLGIIAL
Feline TNF-α	RP0294F-005 RP0294F-025	5 μ g 25 μ g	17.2 kDa	493755	LRSSSRTPSD KPVAVHVANP EABGQLQWLS RRANALLANG VELTDNQLKV PSDGLYLIYS QVLFKGGQCP STHVLLTHTI SRFAVSYQTK VNLLSAIKSP CQRETPEGAE AKPWYEPIYL GGVFQLEKGD RLSAEINLPA YLDFAESGQV YFGIIAL
Guinea Pig TNF-α	RP0345GP-005 RP0345GP-025 RP0345GP-100	5 μ g 25 μ g 100 μ g	17.0 kDa	100135630	SASQNDNDKP VAHVANQQA EEELQWLSKR ANALLANGMG LSDNQLVVP DGLYLIYSQV LFKGGQCP SY LLLTHTVSR AVSYPEKVN LSAIKSPCK ETPEGAERKP WYEPYLGGV FQLQKGDRLS AEVNLPQYLD FADSGQIYFG VIAL
Human TNF-α	RP0440H-005 RP0440H-025	5 μ g 25 μ g	17.2 kDa	7124	VRSSSRTPSD KPVAVHVANP QABGQLQWLN RRANALLANG VELRDNQLVW PSEGLYLIYS QVLFKGGQCP STHVLLTHTI SRIAVSYQTK VNLLSAIKSP CQRETPEGAE AKPWYEPIYL GGVFQLEKGD RLSAENRPDY LDFAESGQVY FGIIAL
Mouse TNF-α	RP0374M-005 RP0374M-025	5 μ g 25 μ g	17.3 kDa	21926	LRSSSQNSSD KPVAVHVANH QVEEQLEWLS QRANALLANG MDLKDNDQLVW PADGLYLVYS QVLFKGGQCP DYVLLTHTVS RFAISYQEKV NLLSAVKSPC PKDTPEGAE LKPWYEPYLG GVFQLEKGDQ LSAEVNLPKY LDFAESGQVY FGVIAL
Ovine TNF-α	RP0902V-005 RP0902V-025 RP0902V-100	5 μ g 25 μ g 100 μ g	17.2 kDa	443540	LRSSSQASNN KPVAVHVANI SAPGQLRWGD SYANALMANG VELKDNDQLVW PTDGLYLIYS QVLFKGGQCP STPLFLTHTI SRIAVSYQTK VNLLSAIKSP CHRETLGAE AKPWYEPIYQ GGVFQLEKGD RLSAEINLPE YLDYAESGQV YFGIIAL
Rabbit TNF-α	RP0429U-005 RP0429U-025	5 μ g 25 μ g	17.0 kDa	100009088	SASRALSDKP LAHVANPQV EGQLQWLSQR ANALLANGMK LTDNQLVPA DGLYLIYSQV LFSGGQCRSY VLLTHTVSRF AVSYPNKVN LSAIKSPCHR ETPEEAEPMA WYEPYLGGV FQLEKGDRLS TEVNQPEYLD LAESGQVYFG IIAL
Swine TNF-α	RP0080S-005 RP0080S-025	5 μ g 25 μ g	16.9 kDa	397086	SSSQTSQDPV AHVAVNKAE GQLQWQSGYA NALLANGVKL KDNQLVVPD GLYLIYSQVL FRGQGGPSTN VFLTHTISRI AVSYQTKVN LSAIKSPCQR ETPEGAEAKP WYEPYLGGV FQLEKDDRLS AEINLPDYLD FAESGQVYFG IIAL

Kingfisher Biotech, Inc. is committed to accelerating basic veterinary research and human disease research done in animal models by developing and commercializing research reagents specific for a variety of species. Currently, we offer over 1000 reagents and kits for veterinary and animal model research. If there is a product that you are looking for and can't find, please send us an email to info@kingfisherbiotech.com.

REAGENTS FOR ANIMAL MODEL AND VETERINARY RESEARCH

