TEXT S1

TNF Biology

TNF forms and its receptors

All soluble TNF is derived from tmTNF by proteolytic cleavage, but not all tmTNF goes on to be cleaved to generate sTNF[58]. Macrophages produce more sTNF than lymphocytes and the expression and effect of tmTNF is transient considering that it is degraded very fast[41]. Whether this translates to more tmTNF expressed on T cells is not known. Both forms of TNF function by binding to one of two receptors, TNFR1 (TNFRp55) and TNFR2 (TNFRp75)[13]. TNFR1 is constitutively expressed in most tissues, whereas expression of TNFR2 is highly regulated and is typically found in cells of the immune system[4]. The receptor TNFR2 has a lower affinity for sTNF than does TNFR1. Soluble TNF therefore stimulates TNFR1 more efficiently and effectively than TNFR2 (by some estimates as much as 20 fold differential[58]). Trans-membrane TNF stimulates TNFR1 and TNFR2 equally well. Anti-TNF Ab treated wild type mice and TNFR1 KO mice do not survive and are essentially identical in acute infection[4]. There is no evidence that in the absence of TNFR1, TNFR2 would signal through NFκβ and elicit an acceptable inflammatory response. In fact, only TNFR1 KO mice are susceptible to *M. tuberculosis*. Known effects of soluble TNF and tmTNF on macrophages and T cells are summarized in Table 1 (see [62] for a review).

Reverse signaling

Transmembrane TNF also contains a casein kinase I motif and itself can act as a receptor[SN2]. Transmembrane TNF engagement can be triggered both by TNFR positive cells and by TNF specific antibodies[SN3]. Reverse signaling (RS) of tmTNF confers resistance to LPS in monocytes and macrophages[18], causing an anergic state of

monocytic cells. Under pathological conditions tmTNF might additionally transmit apoptotic signals, as has been observed for monocytes and T cells from patients with Crohn's disease[19,SN4]. Trans-membrane TNF stimulation on CD4+ T cells can reversely induce the production of higher amounts of IL-2 and adhesion molecules, such as E-selectin[SN5,SN6,16].

Clearly more experimental evidence is needed to validate whether reverse signaling of TNF ligand family members is important *in vivo*. Bi-directional signaling requires direct cell-to-cell contact and could be relevant in situations where cells cluster, such as granulomas. The effect of reverse signaling so far described in B and T cells is of a stimulatory nature[16,17], whereas monocytes are mainly inhibited in their effector functions[18,19,20,SN7]. Based on the literature, the overall effects of reverse signaling by tmTNF seem to be minimal in the absence of anti-TNF treatment. Under pathological conditions (chronic inflammatory states), the presence of anti-TNF antibodies (and not TNF-receptor fusion molecules) and the consequent binding to tmTNF can induce:

i) Activation of the complement cascade (due to high concentration of Abs)[SN8]

ii) Apoptosis induced by RS through tmTNF binding[19,SN4]

Activation of complement cascade is supported by data on Crohn's disease and it might not be a mechanism shared among all the TNF-related pathologies (such as Rheumatoid Arthritis and Ankylosing Spondylitis). The likely consequence of triggering the complement cascade is the release of intracellular bacteria, while apoptosis kills most of the intracellular bacteria[SN9,53].

The role of lymphotoxin in M. tuberculosis infection

Lymphotoxin (LT) comprises two members of the TNF superfamily, LTα and LTß. LT is active as a secreted homotrimeric molecule (LT_3, also known as TNF-ß)[SN10], which binds

to both of the TNF receptors (TNFR1 and TNFR2)[SN11,SN12]. LTα_3 is produced mainly by $CD4^+$ T cells, B cells, and NK cells[SN13]. As LTα_3 and TNF bind to TNFR1 with similar affinity[SN14] and have 30% homology in amino acid sequence[SN15], the functional activity for LTα_3 independent of TNF is incompletely understood. Inhibition of LTα_3 and LTβ has been shown to result in delayed granuloma formation, structural lymph node defects[SN16], delayed macrophage activation and increased bacterial load[SN17], indicating that the TNF superfamily plays a pivotal and non-redundant role in containing infection.

The main effect described in the literature regarding LTα KO mice is abnormal granuloma formation[SN16], with similar numbers of T cells recruited in the lungs but with different localization than in wild type mice[SN16]. In fact, LTα_3 KO chimeras recruited normal numbers of T cells into their lungs, but the lymphocytes were restricted to perivascular and peribronchial areas and were not colocalized with macrophages in granulomas**.** That makes those mice more susceptible. LTα alone cannot rescue TNF KO mice[2]. A critical role for LTα lies not in the activation of T cells and macrophages per se but in the local organization of the granulomatous response.

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