

Evaluation of TNF as antiviral, antibacterial and antiparasitic agent

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Introduction

The toxic effects associated with release of TNF during acute infections have been well publicised. They are very striking, but make little “biological sense” in evolutionary terms. However it is now becoming clear that TNF plays an important role in *resistance* to infection, and the toxic effects may represent protective mechanisms which have got out of control. In this paper we first review the evidence for this protective role and the mechanisms which are likely to be involved, and then consider whether it may be possible to exploit the beneficial effects without concomitant toxicity.

Evidence for the protective role of TNF *in vivo*

Protective effects in vivo deduced from effects of neutralizing antibody to TNF

A lethal infection with *Listeria monocytogenes* causes detectable levels of TNF to appear in the serum of mice, whereas a sublethal infection does not [1]. Nevertheless TNF plays a role in these sublethally infected animals, because neutralising antibody raised against murine TNF will exacerbate the disease [1–3] and 1 mg of rabbit antibody to murine TNF given 2 hours before infection

with 0.1 LD50 of *L. monocytogenes* resulted in 100% mortality by 7 days. On the other hand such antibody had little effect if given on day 3 or later, suggesting that the protective role of TNF is mostly during the early phase of the infection. Similarly neutralising anti-TNF caused enhanced proliferation of *M. bovis* (BCG) in mice if given early, before the classical T cell-dependent granulomata have formed [4].

This effect of antibody to TNF is not confined to experiments with facultative intracellular bacteria, since infections with *Plasmodium vinckei* [5] and *Leishmania major* [6] were also exacerbated.

Protective effects in vivo demonstrated by the administration of TNF

Direct evidence for the protective role of TNF *in vivo* has been obtained by the injection of recombinant TNF. This has been found to limit infection with *Leishmania major* [6], *Plasmodium spp.* [7, 8], *Trypanosoma cruzi* [9], *Toxoplasma gondii* [9] and *Mycobacterium avium* [10], and to accelerate clearance of *Legionella pneumophila* [11]. It will also protect mice from *Streptococcus pneumoniae* and from *Klebsiella* [12]. Moreover, it was observed that C3H/HeJ mice, which produce little cytokine in response to the LPS of Gram negative bacteria, were 1000-fold more susceptible to a

lethal infection with *Escherichia coli* than the congenic, LPS-sensitive C3H/HeN mice. The C3H/HeJ mice could be protected from > 20 LD50's by pretreatment with a combination of IL-1 and TNF [13]. Therefore, in this model of Gram negative infection the TNF itself seems to be protective. Conversely, TNF can be an essential component of a lethal pathway which accompanies Gram negative septicaemia, and *neutralisation* of TNF, rather than administration of yet more of the cytokine, was protective in a baboon model [14]. Such apparent discrepancies indicate that the *timing* and dose of TNF may be critical. There is evidence for this in other models. Thus TNF can protect mice from Lymphocytic Choriomeningitis Virus (LCM) if it is given before severe inflammation has developed in the brain, but causes accelerated death if given after this has occurred [15].

The timing of the administration of TNF to mice infected with *Trypanosoma musculi* is also critical, though the reverse of the situations described above. In the case early treatment seems to result in increased growth of the parasite, while if given late, after the parasitaemia has stabilised, the TNF can enhance clearance [16].

In order to make sense of these apparently conflicting observations, it is necessary to consider the source of TNF during infection, and the mechanisms by which TNF may exert its protective effects.

Induction of TNF release by microbial products

TNF plays a protective role during infection with such a wide variety of organisms, that it is not surprising to find a similarly wide distribution of microbial components able to induce its production. The lipopolysaccharides (LPS) of the Gram negative organisms are the most studied, but the

somewhat analogous Lipoteichoic acids (LTA) of Gram positive cocci [17] and phosphatidyl inositol mannosides (Lipoarabinomannan or LAM) of the mycobacteria [18] are equally potent. Other bacterial components which appear to have this property include the Toxic Shock Syndrome Toxin of Staphylococci (TSST-1) [19], a streptococcal cell wall preparation [20], and muramyl dipeptide (MDP), a synthetic analogue of part of the ubiquitous bacterial cell wall peptidoglycan appears to prime for enhanced release of TNF by other bacterial components [21]. Perhaps all micro-organisms trigger release of TNF, and in addition to the organisms already discussed above, *Legionella* [11], *Listeria* [1], malaria parasites [22], and *Candida* [23] all have this property though the active components have not been identified. In the case of malaria, both the blood-stage parasite and released antigens are active, and there is some evidence that the latter may be predominantly glycolipid in nature and act as T-independent antigens [24]. It remains possible that some organisms do not have the ability to trigger release of TNF. However there have been sporadic reports of lymphokines able to cause release of TNF by pathways which do not appear to involve triggering of appropriately activated cells by a microbial component. If this is so, TNF could be involved in protection against organisms which lack a TNF "trigger". Similarly it is possible that membrane-associated TNF is involved in local events which do not require release of free TNF.

Induction of TNF by the human immunodeficiency virus

Macrophages and monocytes infected with HIV spontaneously release TNF [25, 26]. Moreover, the free virus is able to trigger TNF release from uninfected monocytes by cross-linking membrane CD4 [27]. The importance of these observations is discussed later.

Mechanisms of the protective effects of TNF *in vivo*

1. Activation of the cells of the immune system

Many workers have investigated the possibility that TNF is directly toxic for microorganisms. Most of the results have been negative, and remain unpublished. However TNF has been reported to be toxic for *Trypanosoma musculi in vitro* [16].

TNF can indirectly cause killing of organisms by activating phagocytes.

Activation of neutrophils

There is strong evidence that TNF can prime neutrophils, so that they subsequently give an exaggerated burst of superoxide or H_2O_2 production when exposed to stimuli such as zymosan [28], phorbol myristate acetate (PMA) or f-met-leu-phe [29]. The increased oxidative response to zymosan [28], and to opsonised amoebae [30] was attributed to increased expression of CR3 [28] which may also be responsible for the increase in adhesion of TNF-exposed neutrophils to endothelial cells [31]. These priming effects are rapid. Increased adhesion to endothelial cells [31] is apparent within 5 minutes of exposure to TNF, and priming for enhanced superoxide production is apparent within 20 mins. Longer exposure to TNF (20 mins to several hours) increased phagocytosis of latex, and ADCC [32], and in some reports, leads directly to superoxide or H_2O_2 release without any further stimulus [28, 33]. However some release of lactate dehydrogenase (LDH) is seen at this time [28], implying cell death, so the interpretation is not clear. Perhaps the neutrophils are triggered to release TNF while phagocytosing their dying colleagues. Nevertheless this may not be an *in vitro* artefact, and such events could presumably occur *in vivo*. Neutrophils exposed to TNF for several hours in this way are able to disrupt endothelial cell monolayers *in vitro* [33].

Whatever the details of the activation pro-

cess, it is clearly rapid, and can result in enhanced clearance of *Candida* [34], and *Legionella* [11].

Activation of platelets by TNF

There is a report that platelets can also be activated by TNF. If platelets are incubated overnight with Schistosomulae in the presence of TNF, the percentage killing is increased, though TNF is not itself toxic to the larvae [35].

Activation of macrophages by TNF

Exposure to TNF will cause inhibition of multiplication of *Trypanosoma cruzi* in murine peritoneal macrophages. The TNF can be added after infection has taken place [36]. Other workers found that the effect was not seen using LPS-insensitive C3H/HeJ macrophages, and that it could be blocked by adding catalase [37]. They suggested that the inhibition was dependent on release of H_2O_2 from the TNF-activated cells by contaminating LPS. TNF did not inhibit growth of *T. cruzi* in human fibroblasts [36], perhaps because these cells do not make H_2O_2 .

TNF had no effect on the intracellular growth of *Toxoplasma gondii* [36]. Nevertheless the same group detected a protective effect of TNF during infection with *T. gondii in vivo* [9], suggesting that direct activation of macrophages is likely to be only one of several protective pathways enhanced by TNF.

Bermudez and Young report that TNF will cause kill of AIDS-derived strains of *Mycobacterium avium* by human monocyte-derived macrophages, and by murine peritoneal macrophages *in vitro* [38], while gamma interferon caused increased growth of the organisms in both human and murine cells. These findings are in conflict with much previously published data. Gamma interferon induced total stasis of *M. tuberculosis* [39] and of *M. avium* strains not derived from patients with AIDS (unpublished observations) in murine peritoneal cells. On the other

hand TNF had no effect at all on growth of *M. tuberculosis* in these cell types (Rook et al., unpublished) though a small effect is seen using bone-marrow-derived murine macrophages (S. Kaufmann, personal communication). Therefore, either the AIDS-derived strains are quite different, or technical problems are significant.

As outlined earlier, TNF does exert some protective effect against *M. avium* *in vivo* [10], but for reasons to be explained below, this does *not* constitute evidence that its mode of action is by direct activation of the macrophages. As in the case of *Toxoplasma* [9], there is more than one mechanism at work.

Activation of NK cells by TNF, and protective effects secondary to release of IFN-gamma from TNF-activated NK cells

TNF is able to activate NK cells [40], and it has been suggested that in synergy with unidentified microbial components, TNF causes release of gamma interferon from these cells [3]. This could be an important pathway providing the source of gamma interferon in SCID mice [3], and a simple rationale for the role of TNF in the early T cell-independent phase of response to *Listeria* [1, 2], and BCG [4] *in vivo*. Moreover it provides a possible explanation for the protective effect of TNF against *M. avium* [10] and *Toxoplasma* [9] in mice. This pathway "makes sense" from an evolutionary point of view, because it means that IFN-gamma is available early after infection, before the T cell response has developed. It will clearly be essential to check whether it is involved in the experiments in mice where TNF or anti-TNF administered early after infection, are found to modify the disease [1-16]. It should be noted that if this is how TNF protects mice against *M. avium*, it is irrelevant to human infections with mycobacteria, since all authors agree that IFN-gamma has no effect on the growth of mycobacteria in human macrophages [39].

Activation of eosinophils by TNF

TNF increases the killing of schistosomulae by eosinophils [41] but a study of direct influences of TNF on degranulation, enzyme release, and oxidative metabolism of eosinophils revealed minimal effects so the mechanism of the TNF-enhanced cytotoxicity is not known [42].

Basophils and mast cells

TNF does not appear to activate these cell types [43].

2. Protective effects of TNF not involving activation of the cells of the immune system

Other sections of this issue deal in detail with the immunomodulatory and pro-inflammatory effects of TNF, and the relationships between TNF and the production of other cytokines and mediators. Clearly these topics are all relevant to its role in protection against infection, but they are not reviewed again here. Only certain important experiments directly involving infection are outlined.

The effect of TNF on growth of organisms in tumour cells and fibroblasts

Exposure of HEp-2 cells (derived from a human carcinoma of the larynx) to TNF renders them resistant to invasion by *Salmonella typhimurium* [44] and inhibits intracellular growth of *Chlamydia trachomatis* [45]. The latter effect is partly reversed by neutralising antibodies to IFN-beta, and also by addition of tryptophan. The authors concluded that the TNF induced secretion of IFN-beta, which in synergy with the TNF augmented tryptophan-depleting enzyme activity. TNF did not inhibit growth of *T. cruzi* in human fibroblasts [36].

Anti-viral effects of TNF

The ability of TNF to influence cells not belonging to the immune system is particularly relevant in the context of viral infections.

Several cell lines have been shown to be protected from viral cytopathic effect by TNF, and virus yield and viral protein synthesis were reduced. Cell lines in which these effects were observed include HEp-2, Human Embryonic Lung Fibroblasts (HEL & WI-38), and mouse embryonic fibroblasts (MEF) [46], and human lung (A549) and renal (7860) carcinomas [47]. Susceptibility to the TNF-mediated effect was not related to the transformed phenotype, or to inherent sensitivity to the cytotoxic effect of TNF [46]. TNF will protect from both RNA (EMCV and VSV) and DNA viruses (adenovirus-2, HSV-2) and acts synergistically with gamma interferon [47]. In at least one cell line the protection is mediated indirectly via induction of IFN-beta [48] though this is probably not so in every case, and several pathways may exist. TNF inhibits activation of human B cells by Epstein-Barr virus in the presence of macrophages. This seems to be due to production by the macrophages of an unidentified soluble factor [49], and might represent a useful role for TNF in malaria, which can act as a cofactor with EBV in the genesis of B cell (Burkitt's) lymphoma.

Kill of virus-infected cells

TNF may protect against spread of virus infection *in vivo* by killing infected cells. Thus the A547 human lung carcinoma is rendered sensitive to the cytotoxic effect of TNF by infection with Adenovirus or VSV [47], and similar observations have been made with herpes virus [50] and HIV [51]. It seems likely that this mechanism can be either protective or severely disabling depending on how many cells in any one vital organ are infected, and so killed, at the time when TNF is released or administered. This could also partly explain the detrimental effect of late administration of TNF to mice infected with LCM virus [15].

Does TNF kill cells infected with organisms other than viruses?

It is possible that the ability of TNF to kill some transformed or virus-infected cells is

part of a broader property enabling it to destroy cells which are functionally disturbed. We have found recently that if L929 fibroblasts are infected with *M. tuberculosis* (which they take up in large numbers) they become sensitive to killing by very low levels of TNF in the absence of emetine or actinomycin D. The intracellular mycobacteria do not cause any obvious alterations in protein synthesis, (assessed by ³⁵S-methionine labelling, followed by analysis of the SDS-PAGE-separated proteins in a beta scanner), but when such cells are exposed to a normally non-toxic level of TNF, protein synthesis ceases promptly (Filley, Rook et al., in preparation). This phenomenon is also seen using a normally TNF-resistant subclone of the L929 line supplied by Dr. N. Matthews.

A possible role for TNF in granuloma formation

Since neutralising antibody to TNF exacerbates infection with *Listeria* or *M. bovis* (BCG) if given early, before granulomata have formed [1, 2, 4], it was suggested that induction of granuloma formation might be a function of TNF. In fact these experiments could equally be explained by the TNF/NK cell/IFN-gamma pathway described earlier. Nevertheless it has been shown that TNF conjugated to sepharose beads can cause granuloma formation in mouse lungs [52].

Induction of tolerance to TNF

Treatment of rats with a single low intravenous dose of TNF protected them from a potentially lethal dose given 24 hours later. Similarly it protected against the lethal effects of LPS, or caecal ligation and puncture [53]. This suggests that TNF could conceivably be used prophylactically in patients in whom septicaemic episodes were anticipated. The mode of action may be tachyphylaxis. On the other hand TNF is known to lead secondarily to production of IL-6 [54], and this cytokine induces a pattern of acute phase response involving protease inhibitors, caeruloplasmin

and haptoglobin, which may have an anti-inflammatory effect [55].

Detrimental effects of TNF

Endogeneously raised levels of TNF correlating with bad clinical outcome in vivo

In numerous clinical situations high serum levels of TNF correlate with a poor clinical outcome. These include septicaemia [56], and the adult respiratory distress syndrome (ARDS) [57]. Similarly in malaria TNF has been implicated in severe disease [58], and in such complications as anaemia [59], and abortion [60], and in cerebral malaria in a mouse model [61]. This has given rise to suggestions for the treatment of Gram-negative shock, severe malaria, etc., with regimes aimed at reducing TNF levels, either directly by antibodies or inhibitors, or indirectly by vaccine-induced immunity against the triggering molecules [24].

In patients with AIDS high levels of TNF correlate with encephalopathy [62].

Activation of HIV production in human cells by TNF

Although TNF is reported to kill HIV-infected cells [51] the consequences of the interaction of these cells with TNF may not be protective. Several authors agree that TNF increases virus yield from HIV-infected cells *in vitro* [51, 63–66], and the mechanism is partly understood [66]. Therefore administration of TNF to individuals infected with HIV may be contraindicated, and there is a report that TNF caused rising levels of circulating HIV antigen in patients undergoing a trial of TNF therapy for Kaposi's sarcoma [67]. Since HIV also induces TNF production [25–27], this positive feedback may constitute a significant part of the pathogenesis of the disease. It is also interesting that tuberculosis is an early complication of HIV, (unlike *M. avium* infection which occurs late when T cells are depleted), and seems to lead to fur-

ther aggravation of the HIV infection. Perhaps this is attributable to synergistic induction of TNF by HIV [25–27] and by mycobacterial Lipoarabinomannan [18] leading to further activation of the virus.

Detrimental consequences of administered TNF

Administration of TNF can induce cachexia, anaemia, inflammation and haemorrhagic necrosis [68]. However, the systemic toxicity of TNF is greatly increased in the presence of IL-1 or LPS [69]. This may be one of the obstacles which prevents its use in the treatment of infection, since these substances are likely to be present in relevant patients. Moreover microbial products, and certain types of inflammatory response "prepare" tissue sites so that they become exquisitely sensitive to TNF, and liable to undergo haemorrhagic necrosis in its presence [70–72]. This "preparation" of an inflamed site probably involves changes in the properties of endothelial cells (reviewed in [73]), and can be brought about both by T cell-independent (LPS, [70]) and T cell-dependent (low doses of soluble mycobacterial antigen [72]) responses to microbial products. This phenomenon probably explains the Shwartzman reaction (discussed in [72]) in which a skin site prepared by an injection of LPS undergoes necrosis if a further dose of LPS is given intravenously 24 hours later. This tendency for sites of microbial inflammation to undergo necrosis in the presence of TNF is largely a vascular phenomenon. However, it could be further aggravated if the infection involved resulted in the cells of the relevant organ becoming themselves sensitive to the cytotoxic effects of TNF as described earlier for virus-infected [47, 50, 51] and tuberculosis-infected cells. This has been postulated as the basis of fulminant hepatitis [74] and a combination of necrotising vasculitis and death of infected cells could help to explain the very rapidly fatal effect of TNF administered late to mice with LCM virus [15].

Conclusions

Should we conclude that the treatment of infectious disease with TNF will be impossible, in spite of the conclusive evidence that this cytokine is an essential part of the normal response to infection? The situation is not quite that bleak. Obviously it will be essential to avoid activating HIV, or triggering shock, Shwartzman reactions, or fulminant haemorrhagic necrosis of infected organs or foci. On the other hand several recent studies revealed that we know very little about the circumstances under which TNF is or is not toxic. Rodents can be desensitised to the toxic effects of TNF [13, 75] but we do not yet know whether such desensitisation also eliminates all the many protective effects of the cytokine. Still more remarkable is the observation that monophosphoryl lipid A (a non-toxic derivative of LPS) will induce serum levels of TNF in mice infected with *Listeria monocytogenes* which are comparable to the levels induced by LPS itself, and yet whereas the result is fatal in the LPS-treated animals, it is not in those receiving the monophosphoryl compound [76]. Again this implies that the toxicity of TNF involves other agonists, or can be blocked by regulatory substances, but we do not know which, or whether the protective effects would be similarly decreased. There is clearly much scope for further experiment.

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