REPORT

What does tumour necrosis factor excess do to the immune system long term?

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Members of the tumour necrosis factor (TNF)/TNF-receptor (TNF-R) superfamily coordinate the immune response at multiple levels. For example, TNF, LTα, LTβ and RANKL provide signals required for lymphoid neogenesis, CD27, OX-40, 4-1BB and CD30 deliver costimulatory signals to augment immune responses, while pro-apoptotic members such as TNF, CD95L and TRAIL may contribute to the termination of the response. Biological identity of individual family members has been revealed through studies of gain of function or gene deficient mutants. Most notable are the development of spontaneous inflammatory polyarthritis in human TNF-globin transgenic mice, the auto-inflammatory syndromes resulting from mutations in the 55-kDa TNF-R, and, in particular, the obligatory role for the RANKL/RANK axis in osteoclastogenesis and bone remodelling. A growing appreciation of the molecular basis of signalling pathways transduced by TNF-R has provided a framework for better understanding the biology of this expanding family. For while the rapid and robust activation of NF-κB and MAPK pathways is typical of acute TNF-R engagement, the molecular basis of sustained receptor signalling remains a mystery, in spite of its relevance to chronic inflammatory and immune responses. Focusing on T cells, this report describes some of the molecular footprints of sustained TNF-R engagement and illustrates how these may influence immune function. A common theme arising is that prolonged TNF stimulation alters signalling thresholds over time. The authors propose that one major outcome of long term exposure to TNF is a state of localised IL-2 deficiency at sites of inflammation. The implications of this deficiency are discussed.

Chronic inflammation is the hallmark of a wide variety of diseases that, in addition to autoimmune inflammatory diseases, includes degenerative diseases, vascular disease, and a number of cancers. In spite of our growing knowledge of the cellular and molecular components of the innate and adaptive immune system that contribute to the initiation, evolution, and progression of chronic inflammatory diseases, our understanding of the reciprocal relation, namely how the inflammatory process influences immune responses, remains poorly characterised. This is especially important in an era where the use of biological agents to block key inflammatory mediators such as interleukin (IL)-1, tumour necrosis factor α (TNFα), and IL-6 is becoming routine. To treat patients safely requires an indepth understanding of not only how the immune system behaves when exposed to inflammatory mediators over periods of months and years, and how this influences the disease process, but also how it impinges upon host defence against foreign pathogens. This has become a topic of particular interest with respect to TNF blockade where concerns regarding immunity to intracellular pathogens and tumour immunity abound.

For some years it has been our belief that long term, as distinct from short term, effects of TNF in vivo may be relevant to the chronic inflammatory process (reviewed in reference 1). Elucidating these effects should help us identify how TNF blockade works, why it lasts, and why it is effective for treating a range of diverse immune mediated diseases. If we can couple this knowledge with judicious monitoring with biomarkers specific for the disease process we will be in a much stronger position to treat patients with targeted biological agents safely. This review focuses on new developments that provide some insight into how TNF imposes itself on the immune system over prolonged periods of time, and, in particular, how this may explain the profound and longlasting effects of TNF in a subset of patients with rheumatoid arthritis (RA).

STRUCTURAL AND FUNCTIONAL FOOTPRINTS OF THE TNF/TNF-R SUPERFAMILY

Our understanding of the biology of TNF and its close relatives has evolved from a detailed analysis of the structure and function of the core family, which includes TNFα, lymphotoxin α (LTα), LTβ, LIGHT, and Fas ligands, together with their cognate receptors.[2] With the exception of LTβ, these ligands are type II integral membrane proteins which share the "TNF homology domain", a stretch of ~150 amino acids with a conserved structural framework and virtually identical tertiary fold, that facilitates the association of monomeric subunits into the homotrimers or heterotrimers required to deliver biological signals. The receptors are mostly type I transmembrane proteins comprising a variable number of cysteine-rich domains in their extracellular domains each in turn comprising structural modules based on disulphide bridges. More recently, a pre-ligand assembly domain (PLAD) has been described for a subset of receptors, including Fas, TNF-receptor I (TNF-RI), and CD40.[3] This N-terminal structure is thought to facilitate homotypic association between monomeric receptor subunits, thereby providing receptor complexes capable of responding rapidly to environmental cues. Like their ligands, the receptors can be cleaved to form decoy or inhibitory receptors, providing another dimension to immune regulation.

It has been suggested from studies of *Drosophila* orthologues that this core family of ligand/receptors has evolved into

Abbreviations: AP-1, activator protein-1; CCR, chemokine receptor; JNK, Jun N-terminal kinase; LAT, linker for activation of T cells; LT, lymphotoxin; MAPK, mitogen activated protein kinase; MKP, mitogen activated protein kinase phosphatase; NF-κB, nuclear factor kappa B; PLC, phospholipase C; RA, rheumatoid arthritis; RANK, receptor activator of nuclear factor kappa B; ROS, reactive oxygen species; TCR, T cell receptor; TNF, tumour necrosis factor; TNF-R, tumour necrosis factor receptor; TNF-RSF, tumour necrosis factor receptor superfamily; TNFSF, tumour necrosis factor superfamily

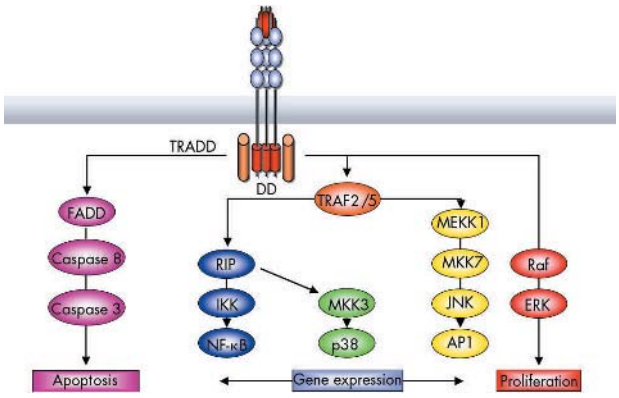

Figure 1 Signalling footprints of the TNF/TNF-R superfamily. Prototype signalling modules recruited to death domain expressing TNF-R family members, including TNF-R1. Each pathway is associated with specific functions, but does not signal in isolation. AP, activator protein; ERK, extracellular regulating kinase; FADD, Fas associated death domain protein; IKK, IκB kinase; JNK, Jun N-terminal kinase; MEKK, MAP/ERK kinase kinase; MKK, MAP kinase kinase; NF-κB, nuclear factor kappa B; RIP, receptor-interacting protein; TRADD, TNF receptor-associated death domain protein; TRAF, TNF receptor-associated factor.

an extended family with the emergence of the adaptive immune system partly through ''en bloc'' gene duplication events from single ancestral genes, perhaps Eiger (the TNF superfamily (TNFSF) homologue) and Wengen (the TNF-RSF homologue).[4] What has emerged is that members of this family are widely expressed in the immune system and, as such, play important roles in immune homoeostasis, including lymphocyte activation, survival, and differentiation.[5] They also play a crucial role in lymphoid neogenesis which, while pivotal to host defence, may predispose to chronic autoimmune inflammatory syndromes when dysregulated.[6] This idea is supported by the informative phenotypes of spontaneous germline mutations of TNFSF/TNF-RSF family members in mice and man, as well as the engineered gain or loss of function mutant animals. For the extended TNFSF/TNF-RSF family other good examples have been the contribution of receptor activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL) to bone homoeostasis,[7] and the key role of B lymphocyte activating factor (BAFF) and its receptors in B cell survival and differentiation.[8]

Regardless of the specific ligand/receptor families, the functions of these receptors derive from distinct motifs present in the intracytoplasmic domains of the receptors which, though lacking intrinsic kinase activity act as docking sites for groups of adaptor proteins, most notably TNF receptor-associated death domain protein (TRADD), Fas associated death domain protein (FADD) and TNF receptor-associated factors (TRAFs) (fig 1).[9] By associating with specific adaptors, these motifs facilitate the association of trimerised receptor complexes. We now know that the signals transduced through these adaptor proteins have become associated with distinct functions, notable examples being FADD dependent assembly of the death receptor complex, activation of the caspase cascade and induction of apoptosis, and the association of TRAF and receptor-interacting protein (RIP) leading to activation of nuclear factor kappa B (NF-κB) and Jun N-terminal kinase (JNK), both of which are key regulators of gene expression. Like their receptors, these pathways do not function in isolation. There is evidence of signal pathway cross-talk that not only provides signal amplification and facilitates a rapid, sensitive response to environmental signals but also provides a means of attenuating potentially harmful chronic responses. It follows from this that changes or aberrations in these signalling pathways could potentially lead to profound effects on immunity and immune tolerance. To illustrate this, we now describe some of the less predictable biological effects of chronic TNF stimulation derived from a detailed biochemical and molecular analysis of sustained TNF-R engagement in CD4+ T lymphocytes. Some of the earliest observations regarding TNF and its effects on cells of the immune system have been reviewed extensively elsewhere, so will not be discussed further.[10–12] The effects of chronic TNF administration in animal models of autoimmune disease have also been reviewed previously.[13]

SUSTAINED TNF STIMULATION ALTERS TNF-R SIGNALLING THRESHOLDS

One of the major factors that determines the extent to which the TNF-R superfamily transduces death signals relates to the capacity to activate NF-κB.[14] TNF is a robust activator of NF-κB but, at least for primary cells, is a poor inducer of cell death pathways. However, if NF-κB is inhibited, or cells are stimulated in the presence of mRNA or protein synthesis inhibitors with agonists that induce NF–κB-dependent transcription of several antiapoptotic genes, cells will undergo rapid apoptosis. Fas signals, on the other hand, are relatively weak activators of NF-κB but are robust inducers of apoptotic cell death. Thus, one predicted outcome of long term TNF signalling would be persistent activation of NF-κB and the expression of genes that promote cell survival and effector responses.[15] In T cells, as in many other cell types studied to date, this appears to be the case. Thus, chronic TNF signals contribute to the survival of effector T cells at sites of inflammation.

More recently, there has been great interest in understanding another aspect of TNF-R1 signalling cross-talk, namely the relation between the activation of NF-κB and Jun N-terminal kinase (JNK).[16] For example, it has been documented for many years that activation of JNK in response to inflammatory stimuli, including IL-1, TNF, and toll receptor ligands is transient. Until recently the mechanisms and functional significance of this were poorly understood. Nevertheless, previous studies of TNF-R signals in RelA or IκB kinase (IKKβ) deficient mouse embryonic fibroblasts (MEFs) demonstrated prolonged activation of JNK when compared to that observed in wild-type MEFs.[17 18] Several explanations were proposed, including the induction by NF-κB of XIAP and GADD45β which in turn negatively regulated kinase pathways upstream of JNK; according to this model, expression of these negative regulators would be absent in RelA or IKKβ deficient cells. However, further analysis of cells deficient in XIAP and GADD45β failed to demonstrate anomalies of JNK activation,[19 20] and so alternative mechanisms were sought. Attempts to establish a relation between NF-κB activation and the activity of specific phosphatases which regulate mitogen activated protein kinase (MAPK) activation, including JNK, proved to be more fruitful. Recent published data have demonstrated unambiguously that the missing link is the induction by TNF of reactive oxygen species (ROS).[21] In competent cells accumulation of ROS is controlled by mitochondrial superoxide dismutase and oxidation of cellular components is suppressed. Under these circumstances, sustained JNK activation, which can contribute to cell death, is prevented by JNK phosphatases, specifically mitogen activated protein kinase phosphatase (MKP) 1, 3, 5, and 7. In NF-κB deficient cells there is massive accumulation of hydrogen peroxide, increased oxidative stress, and inactivation of phosphatases through oxidation of a critical cysteine residue in their catalytic domain. This leads to sustained activation of JNK and other MAPK such as p38 MAPK. When taken in conjunction with the interactions between NF-κB and the

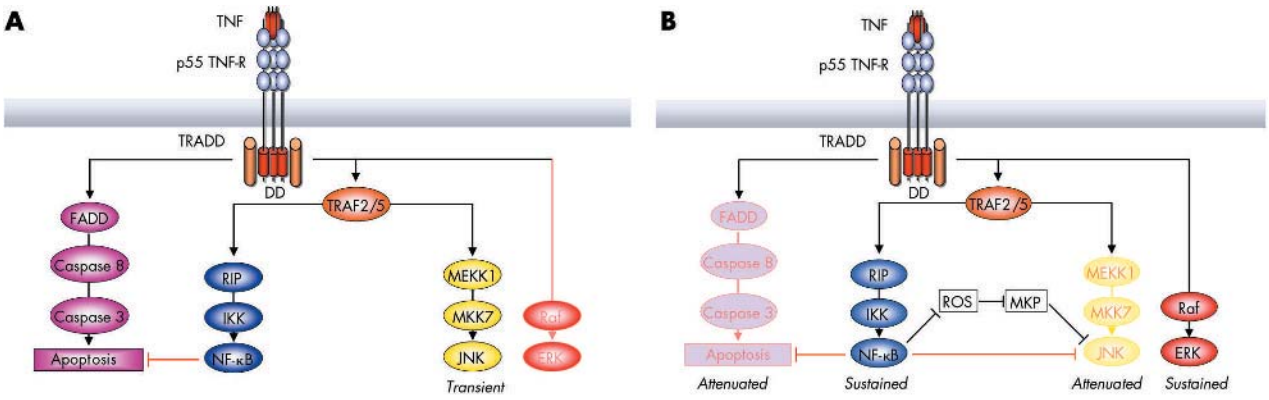

Figure 2 (A) A signalling footprint for acute tumour necrosis factor. For TNF-R1 activation of NF-κB leads to a programme of gene transcription which includes antiapoptotic genes. In primary cells, the default response to TNF favours NF-κB over caspase activation. (B) A signalling footprint for chronic TNF. Here, attenuation of apoptotic pathways is maintained, while NF-κB dependent attenuation of reactive oxidative species (ROS) production enhances mitogen activated protein kinase phosphatase (MKP) activity thereby suppressing activation of JNK. For abbreviations see Figure I legend.

caspase pathway, we can now begin to think of acute and chronic TNF responses as being rather distinct at a biochemical level (fig 2).

What are the implications of these distinct signalling footprints for immunity and inflammation? We can make several predictions based on what we know about the function of NF-κB, JNK, and caspase pathways. Inhibition of caspase activation by sustained activation of NF-κB will generate a survival signal. For rapid, innate immune responses to foreign pathogens this is crucial, providing populations of effector cells to act as a first line of defence. This may also allow for sustained effector responses of the adaptive immune system. On the other hand, inappropriate survival and expansion of self-reactive lymphocyte populations at sites of inflammation would clearly be detrimental. Inhibition of JNK activation by NF-κB could have similar antiapoptotic effects, while at the same time attenuating the expression of genes dependent on AP-1 family members, the downstream targets of the JNK signalling cascade. Although the genes that follow this pattern of expression are not well defined, it follows that any gene whose expression is repressed by AP-1 should be upregulated. According to the available data, there appears to be a switch in signalling thresholds which, after an initial wave of NF-κB and JNK activation, favours sustained NF-κB activity (fig 2B). The extent to which the inhibitory IκB family of proteins modulates activation of NF-κB will determine how long an NF-κB dependent programme of gene transcription may last. Indeed, one study in fibroblasts suggests that continued stimulation of TNF impairs IκBα re-synthesis and, thereby, complete resolution of the NF-κB response.[22] Temporal shifts in NF-κB dimer composition and DNA binding imposes another layer of complexity.[23] These findings, if they can be extrapolated to other cell types, have important implications for chronic inflammatory responses, especially in the light of data from mouse models showing that IKKβ plays a crucial role in T cell effector function.[15] Gene expression profiling in cells stimulated with TNF for different periods of time would provide valuable insight into the distinct footprint of sustained TNF-R signals in different cell types.

EFFECTS OF TNF ON THE EVOLUTION OF ADAPTIVE IMMUNE RESPONSES

There is a growing appreciation that those members of the TNF-RSF with costimulatory function, including CD27, OX-40, 4-1BB, and CD30 play an important and non-redundant role in promoting longlasting adaptive immunity (reviewed in reference 24). They may augment signalling from TCR by promoting the accumulation of common pathway intermediates, or by recruiting distinct signalling modules that amplify proliferative and effector responses of T lymphocytes. Does TNF fit this paradigm? Previous studies, documenting the costimulatory effects of acute stimulation of TNF on T cells after T cell receptor (TCR) ligation, would suggest that it does.[25] However, more recent studies from our own laboratory, and those of others, have demonstrated that TNF has important immunomodulatory functions in vivo and in vitro.[1 12 13] Probably the most convincing data are from those studies in TNF transgenic and TNF deficient mice, which, supported by analysis of cellular immune responses of patients with RA during anti-TNF treatment,[26–28] revealed that sustained exposure to TNF attenuated T cell responses. Thus, using a TCR transgenic mouse model we discovered that repeated injections of TNF at doses sufficient to suppress type 1 diabetes in non-obese diabetic mice or lupus nephritis in susceptible mouse strains suppressed subsequent recall responses to specific antigen.[29] Peptide responses of mice double transgenic for TCR and the hTNF-globin transgene, which leads to sustained p55 TNF-R signals in vivo, were also attenuated when compared with those of mice single transgenic for TCR. In contrast, anti-TNF injections for periods of up to three weeks enhanced the reactivity of T cells. This effect was especially striking with respect to interleukin (IL)-2 production, but the suppressive effects were also noted for T cells differentiating in vivo or in vitro along either T helper (Th) 1 or Th 2 pathway.

WHAT HAPPENS TO THE IMMUNE RESPONSE IN THE CONTEXT OF TNF BLOCKADE?

An analysis of the function and responsiveness of T cells from TNF deficient mice has confirmed that although TNF may be required for the initial priming phase of antigen specific immunity and effector responses, it is also required for the resolution phase. This has been most elegantly shown in murine models of multiple sclerosis.[30] On a C57BL/6×129 genetic background, which is resistant to experimental autoimmune encephalomyelitis (EAE), Kollias and colleagues demonstrated that priming to myelin antigens in TNF deficient mice is depressed. However, antigen reactivity is maintained for periods of up to 77 days when compared with the very poor memory responses of wild-type mice, where myelin reactivity is no longer detectable at these later time points. Using a disease inducing regimen effective in susceptible strains, non-susceptible wild-type mice failed to develop disease, whereas their $TNF^{-/-}$ littermates developed EAE. The chronic phase of EAE in C57/BL6 mice after

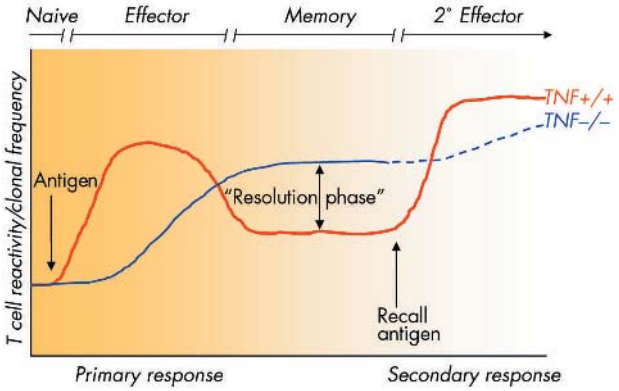

Figure 3 TNF – a potent immunomodulator of adaptive immunity. Like other costimulatory TNF-R superfamily members, acute TNF exposure can enhance antigen specific responses. As the immune response evolves, sustained expression of TNF plays a non-redundant role in resolution of adaptive immunity. However, this may lead to sustained reactivity of T cells to self-antigens and an increased predisposition to autoimmunity.

immunisation with myelin oligodendrocyte glycoprotein (MOG) was also exacerbated in $TNF^{-/-}$ mice. These results are intriguing in the light of clinical observations demonstrating disease exacerbations, documented in patients with MS treated with TNF blockade,[31] and the rare demyelinating syndromes reported in patients after prolonged TNF blockade.[12] A similar mechanism, implicating the immunosuppressive effects of TNF, might be ascribed to the development of antinuclear antibodies, as well as lupus syndromes, in a significant proportion of patients with RA receiving anti-TNF.[32] Whether TNF has similar direct immunomodulatory effects on B cells awaits further investigation. Based on the available data, we can conclude that TNF has early costimulatory and late immunomodulatory effects on the evolution of adaptive immunity (fig 3).

TNF UNCOUPLES TCR SIGNAL TRANSDUCTION PATHWAYS

What have we learnt about the mechanisms of immune modulation by TNF? Initial clues came from studies of TCR transgenic mice injected with TNF, or TCR transgenic T cells stimulated over periods of up to 10 days in the presence of TNF, which suggested that TNF attenuated peptide specific calcium flux.[29] Using a T cell hybridoma model that recapitulated our findings in primary T cells we undertook a systematic analysis of TCR proximal signalling in control and TNF treated T cells. We reported several unexpected observations.[33] Chronic but not acute TNF lowered expression of the TCR/CD3 complex at the cell surface. Biochemical analysis then revealed that TNF selectively targeted the expression of the TCRζ chain, an invariant subunit of the TCR/CD3 complex involved in the assembly of the TCR, while expression of the CD3ε, γ, and δ subunits was spared. The TCRζ chain, which contains three immunoreceptor tyrosine based activation motifs, functions thereby as an amplification module for signals from the TCR complex to downstream pathways. Chronic but not acute TNF attenuated receptor-proximal tyrosine phosphorylation of the protein tyrosine kinase ZAP-70, the transmembrane adaptor protein p36 LAT and PLCγ, and Ca^{2+} mobilisation induced by TCR was suppressed by 80%. Interestingly, *N*-acetylcysteine, which replenishes intracellular glutathione, completely restored TCR responses of cells treated with TNF. To see if reconstitution of TNF treated T cells with receptors using TCRζ chains to signal restored both TCR expression and receptor specific IL-2 production, we expressed a series of chimeric receptors as well as wild-type TCRζ cDNA in T cells by retroviral-mediated gene transduction. The results demonstrated that although overexpression of TCRζ restored TCR/CD3 expression in TNF treated cells, IL-2 gene transcription was still significantly suppressed, suggesting that TNF targets the expression and/or function of additional downstream signalling pathways.[34] The specific molecular targets are currently under investigation.

TNF INDUCES A STATE OF RELATIVE IL-2 DEFICIENCY AT SITES OF INFLAMMATION

The findings described above are entirely consistent with observations made many years ago that synovial T cells are hyporesponsive to TCR engagement.[1] They are also consistent with a model of T cell differentiation characterised by a switch from ''antigen mode'', where antigen driven IL-2 expression is abundant, to ''inflammation mode'', where responses to antigen signals are attenuated, IL-2 gene transcription is deficient, and responses to cytokine signals may predominate (fig 4). Analysis of synovial T cells has also demonstrated loss of TCRζ expression,[35] impaired phosphorylation of the linker for activation of T cells (LAT),[36] and dysregulation of the activation of small GTP binding proteins

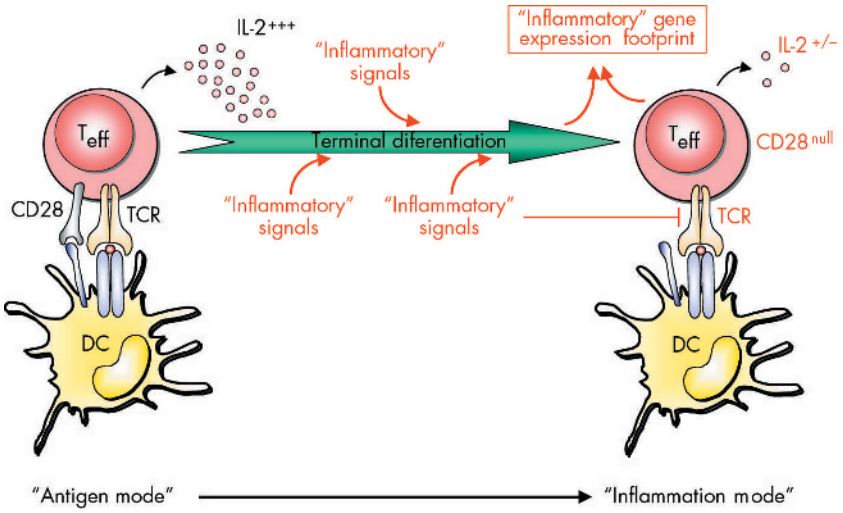

Figure 4 Tumour necrosis factor (TNF) regulates the switch from ''antigen mode'' to ''inflammation mode'' during terminal T cell differentiation. This model proposes that during the evolution of immune responses, CD4+ T cells become progressively refractory to T cell receptor (TCR) engagement. At the same time, cytokine signals may promote a unique gene expression signature favouring cell survival, migration, and effector responses. This change in thresholds of activation of signalling pathways will also lead to a state of relative interleukin (IL)-2 deficiency at sites of inflammation, with implications for maintaining immune homoeostasis. DC, dendritic cell; T_{eff}, T effector cell.

(namely constitutive Ras activation and inhibition of Rap1 activation)[37]—all these studies implicating a role for chronic oxidative stress in perpetuating this apparent state of anergy. Loss of CD28 expression both in vitro in response to chronic TNF stimulation, as well as in vivo, might also contribute to this distinct T cell phenotype.[38] How can we reconcile the loss of TCR responsiveness at sites of inflammations with the paradigm of an antigen driven effector response in autoimmune chronic inflammatory disease? Several scenarios might account for the data, albeit speculative. On the one hand, attenuation of T cell reactivity might reflect an adaptive response to attenuate T cell reactivity at sites of tissue damage. According to this hypothesis, hyporesponsive T cells might exist as inert senescent cells at sites of inflammation contributing little to the chronic phase of the disease. An alternative and much more intriguing possibility is that hyporesponsiveness to TCR ligation results in a deficit in immune regulation under circumstances where such regulation depends on intact pathways of TCR signal transduction.

Is there evidence to support this hypothesis? One unambiguous fact accepted by many investigators is that chronically activated synovial T cells paradoxically fail to express many cytokines at the mRNA and protein level. This includes IL-2, and accounts for the poor proliferative responses of synovial T cell in situ. Is this observation relevant? An overriding theme beginning to emerge from the field of regulatory T cell biology is that the crucial non-redundant role for IL-2 in vivo is the generation and maintenance of CD4+CD25+ regulatory T cells (reviewed in reference 39). This idea first became apparent when it was found unexpectedly that IL-2 deficient mice show evidence of lymphoproliferation which precedes lethal autoimmunity. A similar predisposition to autoimmunity was subsequently documented in mice deficient for IL-2Rα, IL-2Rβ or STAT5A/B. Although there was initial speculation that this susceptibility was due to failure of IL-2 to sensitise autoreactive T cells to apoptotic signals, it subsequently became apparent that these deficient strains all lacked sufficient numbers of CD4+CD25+ T cells, with reductions of 50% and up to 90% in the thymus and peripheral lymphoid compartments, respectively.[40] More recently, neutralisation of IL-2 has been shown to be sufficient to inhibit physiological proliferation of CD4+CD25+ T cells,[41] whereas administration of IL-2 or thymic expression of IL-2Rβ in IL-2Rβ$^{-/-}$ mice restores lymphoid homoeostasis.[42] Consistent with these findings, high doses of IL-2 are required to promote growth and suppressor function of CD4+CD25+ regulatory T cells in vitro.

A specific characteristic of CD4+CD25+ T cells is that they themselves do not express IL-2 mRNA or protein. This may be due, in part, to the failure of chromatin remodelling at the IL-2 promoter after TCR stimulation. It turns out that the principal source of IL-2 for maintaining CD4+CD25+ T cells in vivo may reside within the CD4+CD25− effector T cell population.[41] This makes some teleological sense, given the requirement for resolution of immune responses, after an initial wave of antigen specific effector function, to maintain immune homoeostasis. If this model can be substantiated, then it follows that the IL-2 deficient rheumatoid synovial joint could provide a haven for unbridled effector function where both the numbers and/or function of CD4+CD25+ T regulatory cells should be deficient. Do the data support such a model?

IS IMMUNE HOMOEOSTASIS PERTURBED IN RA

There remains little agreement as to whether the frequency of CD4+CD25+ T cells are altered in RA peripheral blood when compared with healthy donors.[43 44] This might be explained by the different phenotypes used to define CD4+CD25+ T cell numbers in vitro. With one major exception (see below), there is also little evidence that the numbers of circulating CD4+CD25+ T cells change with disease activity or following treatment. In contrast, a detailed analysis of CD4+CD25^{bright} T cells in progressive and self-limiting subsets of juvenile idiopathic arthritis demonstrated reductions of CD4+CD25+ T cells and FoxP3 expressing cells in those children with the more progressive form of the disease, suggesting that, at least in vivo, adequate numbers of functional CD4+CD25+ T cells are sufficient for downregulating the inflammatory process.[45] With regard to the synovial compartment, there is a stronger consensus that the frequency of synovial fluid CD4+CD25+ T cells is significantly increased as compared with that in peripheral blood.[43 46] These CD4+CD25+ T cells express a distinct phenotype with high levels of CTLA4, GITR, CD69, and major histocompatibility complex (MHC) class II. Several explanations have been offered, including preferential trafficking or retention of CXCR4+CD4+CD25+ T cells also expressing CCR4 and CCR8 to, or in, the synovial compartment,[47] and increased survival of this subset due to the expression of interferon β (IFNβ) derived from stromal cells.[48] On the other hand, the numbers appear to be relatively stable, in spite of disease flares or treatment. However, there is less agreement regarding their functional capacity. For example, several studies have documented potent suppressor function of synovial fluid CD4+CD25+ T cells in terms of inhibition of proliferation of CD4+CD25− effector T cells.[43 44 46] Others have suggested that there may be some level of resistance to suppression by these CD4+CD25− effector T cells, perhaps in response to an IL-6-rich environment.[49] An alternative possibility is that the TCR signals required for regulatory T cell function may be deficient. Together these data support the idea that while there is evidence for an attempt at homoeostasis through increased numbers of CD4+CD25+ T cells at sites of inflammation, their levels are insufficient to suppress fully the inflammatory process, a situation somewhat analogous to the increased, albeit inadequate, levels of soluble TNF-R detected in inflamed joints.

Is there evidence that TNF may alter the numbers or function of CD4+CD25+ T cells? Several studies have examined the effects of TNF in vitro.[50] The results have shown modest or no effects. Similarly, analysis of CD4+CD25+ T cell numbers in patients treated with anti-TNF have failed to show consistent effects. Recently, Ehrenstein and coworkers undertook a more comprehensive analysis of CD4+CD25+ T cell function in patients with RA before and after therapy with infliximab.[51] Although their studies were confined to the analysis of CD4+CD25+ T cells in peripheral blood, they showed that although these T cells derived from RA patients could suppress the proliferative capacity of effector T cells, they failed to regulate production of effector cytokines such as IFNγ and TNFα. After anti-TNF, peripheral blood CD4+CD25+ T cells from treatment responders, but not non-responders or patients remaining on methotrexate, reacquired the capacity to suppress cytokine production by effector T cells; CD4+CD25+ T cell numbers also increased in responders. Perhaps the crucial experiments were those showing that anti-TNF conferred on CD4+CD25− T cells the capacity to generate new CD4+CD25+ T cells. If the mouse data, which show an absolute requirement for IL-2 for regulatory T cell homoeostasis,[41] turn out to be true for human CD4+CD25+ T cells, then it is tempting to speculate that anti-TNF may contribute to immune homoeostasis by restoring the capacity of CD4+CD25− T cells to produce IL-2. There is ample evidence that, at least over the short term, anti-TNF does lead to enhanced IL-2 expression,[26–28] but this has yet to be demonstrated in the context of the generation or function of CD4+CD25+ T cells. Nevertheless, the studies by Ehrenstein and Mauri highlight the need for comparing both

numbers and different aspects of function of CD4+CD25+ T cells both in peripheral blood and at sites of inflammation, and this is underscored by the distinct phenotypes of this T cell subset in each compartment. The discrepancy between this study and others also indicates that a more rigorous analysis of clinical or laboratory parameters may be required to establish whether a true relation exists between inflammation and defective function of CD4+CD25+ T cells, and to what extent this is reversible with biological therapy.

CONCLUDING REMARKS

Precisely what TNF does long term to the immune system will depend on the signal input and the type of responder cell. We know that thresholds of signalling pathway activation change over time as an initial strong wave of activation is commonly modulated by the regulatory feedback pathways which serve to attenuate potentially harmful responses. Only by understanding the relation between these changing thresholds and cell function will we be able to identify ways of manipulating the system to the benefit of our patients.

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