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TNF Pathophysiology in Murine Models of Chronic Inflammation and Autoimmunity

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Experimental work in animal models is providing important clues on the specific function of tumor necrosis factor (TNF) and its receptors in disease, especially on the molecular and cellular pathways through which TNF mediates beneficial and deleterious responses. Emerging data on the posttranscriptional regulatory processes, secretion, and postreceptor actions of TNF indicate a variety of mechanisms that may be causative of disease. More recent evidence in murine disease models has indicated heterogeneity of TNF receptor usage in autoimmune disease suppression versus inflammatory tissue damage, suggesting that selective TNF receptor inhibition may be advantageous to anti-TNF treatments in combating chronic inflammatory disease.

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Tumor necrosis factor α (TNF-α) is a major mediator of inflammation and, when overexpressed, can give rise to chronic inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), Crohn's inflammatory bowel disease (IBD), and multiple sclerosis (MS)(1-3). While pharmacological inhibition of TNF in experimental animal models for these diseases, and in a large number of RA and IBD patients, has yielded encouraging results, in MS patients the systemic blockade of TNF has led to immune activation and increased disease activity (4,5).

The inherent complexity of TNF-mediated immune pathophysiology is implicit in the differential bioactivities of its transmembrane and soluble forms (6), and the differential functioning of its p55 or p75 receptors (7). Secondarily, the timing, quantity, and quality of TNF released; the timing, location, and duration of expression in cells; and the genetic background of the organism may modulate to a great extent the biological function of TNF. Based on the context in which TNF-α is produced and expressed, its specific functions may cover a large spectrum of immunological phenomena ranging from innate immune functions to direct cell effects and immune activation or even suppression (2).

Genetically manipulated animal models that show deregulated expression of TNF present a powerful investigative tool and indicate that the window of TNF-α level where the risk of disease is minimized may be quite narrow. Within this working model, the overexpression of TNF-α is associated with inflammatory states, such as RA and IBD (1,3), whereas underexpression of TNF is the hallmark of autoimmune diseases, such as type 1 diabetes (8,9) and systemic lupus erythematosus (10).

TNF Overexpression: Proinflammatory Functions

Mutant mice engineered to lack the AU-rich element (ARE), which consists of 69 base-pairs of AT-rich nucleotides in the 3′-untranslated region of the TNF mRNA ($TNF^{\Delta ARE}$), develop chronic inflammatory polyarthritis and IBD (11). In this animal model, the absence of TNF ARE leads to (a) increased TNF mRNA stability; (b) constitutively active translation of the TNF mRNA; (c) chronic overproduction of TNF also at ectopic sites (eg, in synovial fibroblasts); (d) loss of anti-inflammatory translational controls; and (e) chronic inflammatory arthritis and Crohn's-like IBD. Expression of different pathologies in mice with functional ARE mutations implicates the role of differential regulation of stability and translation of TNF mRNA in the manifestation of disease states. Most interestingly, lack of the ARE regulatory sequences confers a state of anti-inflammatory unresponsiveness to TNF mRNA translation, allowing for sustained overexpression of TNF protein, which is apparently causative of disease.

For example, in mouse macrophages, interleukin-10 (IL-

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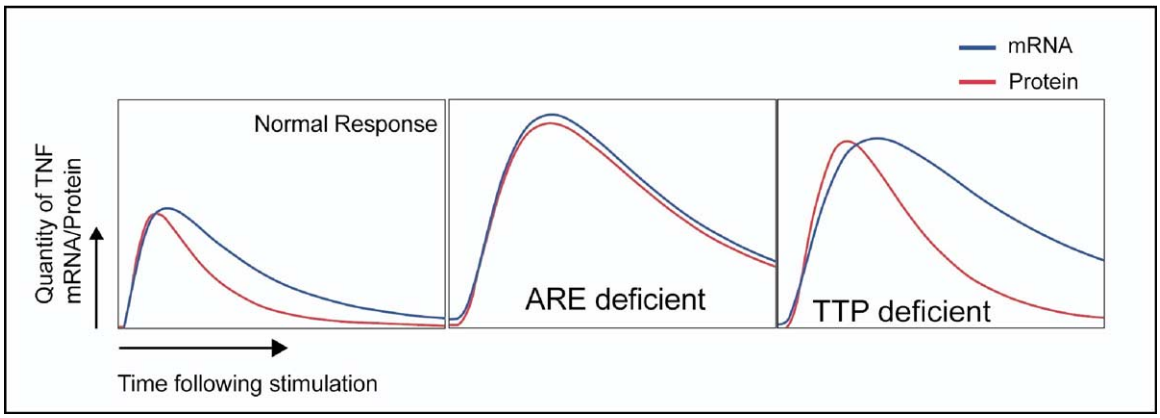

Figure 1 Differential regulation of TNF mRNA stability and translation.

10), an inhibitor of TNF production, exerts its anti-inflammatory and immunosuppressive action primarily by targeting TNF ARE to inhibit its translation. In the absence of ARE, the efficacy of IL-10 in suppressing TNF expression is severely compromised (12). In contrast, IL-10 has no effect on TNF mRNA decay, either with or without ARE, suggesting that TNF mRNA stability is not targeted by IL-10 in macrophages. IL-10 receptor-mediated signals most likely give rise to secondary mediators that block the MKK6/P38/MK2 pathway, which in turn affects the translation but not the stability of the TNF mRNA (12).

Distinct regulation of stability and translation of the TNF mRNA is also evident with tristetraprolin (TTP) and T-cell intracellular antigen (TIA), respectively. These TNF ARE binding proteins regulate TNF overexpression and may function as arthritis suppressors (13,14). TTP binds to TNF ARE and inhibits TNF production from macrophages by destabilizing its mRNA without imposing translational control (15), while TIA-1 represses the expression of TNF by inhibiting translational pathways (16). What has become evident using these knockout systems is that the pathways targeting stability or translational regulation on the TNF message are uncoupled (12), and therefore, levels and kinetics of TNF production are dependent on the specific regulatory stimuli that operate in each case. As a consequence, although TNF overexpression is present in all of these knockout mice, not all animals develop the same immunopathologies. ARE-deficient mice develop IBD and RA; TTP-knockout mice develop the arthritis phenotype, and TIA-1-deficient animals do not develop any pathology. Differential regulation and kinetics of TNF production may account for some of these differences. For example, while both ARE-deficient and TTP-deficient mice experience prolonged TNF production, anti-inflammatory controls are able to suppress TNF translation in the TTP-deficient mice and TNF levels quickly return to that characteristic of wild type. In contrast, loss of anti-inflammatory regulation on the ARE-deficient TNF message allows for more sustained overproduction of TNF, which may be causative of IBD (Fig. 1).

Molecular and Cellular Pathways Conferring TNF-Mediated Disease in Animal Models

Reciprocal bone marrow transplantation experiments provide preliminary evidence into the cell types that give rise to arthritis and Crohn's-like disease in the $TNF^{\Delta ARE}$ animal model. Bone marrow from a $TNF^{\Delta ARE}$ animal placed into an irradiated wild-type recipient carrying both receptors for TNF induces the development of arthritis. In contrast, arthritis does not develop when bone marrow is transferred into a TNF receptor-knockout animal, indicating that the targets for TNF function in arthritis are to be found in radiation-resistant cells. This is further confirmed by the development of arthritis in wild-type animals following bone marrow transplant from a $TNF^{\Delta ARE}$ and TNF receptor-knockout donor. Therefore, bone marrow cells are sufficient producers of TNF, and radiation-resistant target cells may be sufficient for the production of the arthritic phenotype (data to be presented elsewhere).

There are also redundancies in the cellular pathways leading to TNF-mediated Crohn's-like IBD. In the above experiments, all recipients of ARE-deficient bone marrow developed IBD independent of the restriction of TNF receptors in donors or recipients, suggesting that in IBD there are cellular targets, either in the radiation-resistant compartment or in the bone marrow, which are sufficient to produce TNF-mediated IBD (17). Therefore, multiple cell types and compartments can work independently to produce IBD and arthritis. Genetic inactivation experiments have revealed that at least 2 kinases, Tpl2 and JNK2, promote, whereas a third one, MK2, opposes the TNF-mediated induction of IBD (17).

In summary, 2 different levels of complexity seem to operate in TNF-mediated pathologies. First, different modes of aberrant TNF ARE function may lead to differential controls of TNF production, which in turn lead to different disease profiles. Second, redundant cellular and molecular pathways may function downstream of a single cytokine in disease.