

Summary of Major Scientific Achievements

Overview

George Kollias has pioneered genetic approaches to study the function of cytokines in animal models of human diseases, with specific focus on Tumor Necrosis Factor (TNF). He is renowned for providing the scientific basis for TNF function in arthritis and for establishing the human TNF transgenic mouse model, which mimics the human disease. His discoveries led to the successful introduction of biological anti-TNF therapies for the treatment of Rheumatoid arthritis and other chronic inflammatory diseases.

Scientific Achievements

George Kollias is internationally credited not only for providing fundamental *in vivo* tools and knowledge of the molecular mechanisms underlying the development of chronic inflammatory diseases, but more notably the imminent and successful translation of these findings into innovative treatments that are considered pioneering approaches to the clinical pharmacological regimes for a broad spectrum of diseases. Through the development and characterization of novel genetically-modified animal models mimicking human disease, studies performed in the Kollias lab established TNF as a key molecule in the development of rheumatoid arthritis (RA), spondyloarthropathies, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus and septic shock. These models have been disseminated to hundreds of academic and industrial labs around the world, and have supported numerous research and drug development projects.

In 1991, the Kollias lab was first to provide *in vivo*, proof-of-principle, studies confirming that deregulated TNF production is causal to the development of chronic polyarthritis in a transgenic animal model, and the original finding that anti-TNF antibody treatment is efficacious for treating the modeled disease. These studies were instrumental in mobilizing the interest of the anti-TNF industry and drove the development of the first successful clinical trials performed initially in RA (1994), followed by other diseases such as Crohn's disease, psoriasis, psoriatic arthritis, juvenile idiopathic arthritis, spondylarthritis and Behçet's disease, which collectively affect 2-3% of the population. This original publication (Keffer *et al.*, EMBO 1991, Transgenic mice expressing human tumor necrosis factor: a predictive genetic model of arthritis) has been cited more than 1.000 times. **Brennan and McInnes** wrote: "*It was soon shown, using immunohistochemistry, that TNF- α and receptors for TNF- α (TNFRs) were expressed in human rheumatoid joint tissue and, using the collagen-induced arthritis (CIA) model of RA, that administration of a mAb specific for mouse TNF- α after disease onset ameliorated both inflammation and joint damage* (Williams *et al.*, PNAS 1992). Separately, Kollias and colleagues found that transgenic mice expressing the modified human TNFA gene (with replacement of the 5'-UTR regulatory sequences) spontaneously developed peripheral

arthritis. This arthritis was characterized by increased human TNF- α protein, joint inflammation, bone erosion, and cartilage destruction (all hallmarks of RA), and disease could be ameliorated with antibodies specific for human, but not mouse, TNF- α (EMBO J 1991). Together these data provided the rationale for developing therapeutics that block TNF- α , *The Journal of Clinical Investigation*, 2008, 118:3537. Similarly, **Li and Schwarz** report: "Twelve years ago George Kollias' laboratory generated a transgenic (Tg) mouse that over-expresses human TNF-alpha, and develops an erosive polyarthritis with many characteristics observed in rheumatoid arthritis patients. The phenotype of this mouse model validated the theory that TNF-alpha is at the apex of the pro-inflammatory cascade in rheumatoid arthritis, and foreshadowed the remarkable success of anti-TNF-alpha therapy that has transformed the effective management of this disease", Springer Seminars Immunopathology. 2003, 25(1):19-33. In a recent review in *Nature Reviews in Immunology* (2015 Jun;15(6):362-74), **Brenner et al.** stated that "with respect to rheumatoid arthritis, strong preclinical data from mouse models of arthritis supported the testing of TNF-specific antibodies in clinical trials and confirmed the effectiveness of blocking TNF-mediated signaling", referring to the Tg197 animal model.

Further work performed in the Kollias lab provided the first description of physiological functions of TNF in host defense and the structure and function of secondary lymphoid organs (Pasparakis *et al.*, JEM 1996), work that was recently expanded to confirm that establishment of TNFR1 and NF- κ B signals specifically in follicular dendritic cells (FDCs) is of pivotal significance in the regulation of humoral B cell responses and autoimmunity (Victoratos *et al.*, Immunity 2006; Immunity 2009). Importantly, pharmacological interference in the maintenance of FDCs ameliorated disease development, suggesting the FDCs as a potential target for dampening autoimmunity. **These studies offered a deeper understanding of the associated side-effects of anti-TNF therapy, such as patient susceptibility to infection, and introduced new concepts for treatment of autoimmune disorders, for example by suppressing autoantibody production mechanisms.**

Kollias and colleagues also provided further mechanistic insights into the differential functions of transmembrane and soluble TNF in pathophysiology. By blocking the shedding of endogenous murine TNF through deletion of its cleavage site, they demonstrated for the first time that transmembrane TNF protects mutant mice against intracellular bacterial infections, chronic inflammation and autoimmunity (Alexopoulou *et al.*, Eur. J. Immunol. 2006). These data supported the hypothesis that **selective targeting of soluble TNF may offer several advantages over complete blockade of TNF in the treatment of chronic inflammation and autoimmunity, thus rationalizing potential complications or optimizations of anti-TNF therapies in TNF-driven diseases.**

The Kollias lab provided the first mechanistic evidence of the multi-layered roles of TNF and its receptors, and were the first to hypothesize that anti-TNFR1 therapies will be advantageous and safer than current anti-TNF treatments, particularly for organ-specific autoimmune diseases such as multiple sclerosis (Kassiotis *et al.*, JEM 2001). Using knock-in mice expressing a mutated non-sheddable TNF receptor I, they subsequently showed that TNF receptor shedding controls thresholds of innate immune activation, which balance opposing TNF

functions in infectious and inflammatory diseases (Xanthoulea *et al.*, JEM 2004). Recently, the Kollias lab introduced a novel pathogenic principle to explain the cellular basis of TNF function in gut/joint axis diseases by showing that the **mesenchymal cell compartment, namely synovial fibroblasts and intestinal subepithelial myofibroblasts, are common pathogenic targets of TNF sufficient to drive the chronic inflammatory pathologies**. These studies offered a novel mechanistic perspective for TNF function in gut and joint pathologies, established the sufficiency of synovial and intestinal subepithelial fibroblasts in mediating TNF signals and driving the complete arthritic and intestinal pathologies, and indicated early common cellular pathways that explain the often observed synovial–gut axis diseases in humans (Armaka *et al.*, JEM 2008). These findings not only provided a more complete understanding of the cellular function of TNF, but **also introduced new therapeutic innovations, such as mesenchymal stem cell transplantation in combination with impaired TNF receptor function in these cells, which could also lead to a new generation of more effective disease treatments for chronic inflammatory bowel disease**.

Moreover, the Kollias group provided the first genetic evidence of the physiology and post-transcriptional role of AU-rich elements (AREs) in the regulation of TNF expression, and introduced new animal models that develop combined joint and gut pathologies (Kontoyiannis *et al.*, Immunity 1999; EMBO 2001; JEM 2002).

More recently, the Kollias lab has explored the role of intestinal epithelial cells (IEC) as a potential source of TNF in the TNF Δ ARE model of IBD and showed that TNF overexpression specifically by IEC leads to early activation of the underlying mesenchymal cells and is sufficient for complete induction of Crohn's-like pathology in the mouse. These findings provided insight into the mechanisms of Crohn's disease pathogenesis by showing that IEC are potential TNF producers and that **IEC and mesenchymal cells can form a cellular axis of TNF function in the gut sufficient to cause the full spectrum of pathology seen in relevant human diseases** (Roulis *et al.*, PNAS 2011). Furthermore, the lab has recently demonstrated that intestinal myofibroblast (IMF)-specific signals play an important physiological role in colitis-associated cancer, in line with a novel hypothesis that **dysregulations in the mesenchymal compartment (MC) may be causal to chronic inflammatory and tumorigenic pathologies** (Koliaraki *et al.*, JCI 2012; Roulis *et al.*, PNAS 2014; Koliaraki *et al.*, JEM 2015).

Recent studies in the Kollias lab focused on the cellular mechanisms that are responsible for TNF-induced systemic toxicity and addressed the question of whether the anticancer and proinflammatory effects of TNF can be uncoupled, leading to safer anticancer effects. The lab showed that reducing the expression or availability of TNFR1 in intestinal epithelial cells strongly dampen the proinflammatory signal without affecting the induction of apoptosis and antitumor effects. Furthermore, the lab succeeded in treating melanoma tumors in mice by administering TNF and simultaneously decreasing TNF receptor levels. These findings uncovered **an important cellular basis of systemic TNF toxicity and laid the groundwork for safe and effective TNF-based antitumor treatment strategies** (Van Hauwermeiren, Armaka *et al.*, JCI 2013).