How the Discovery of TNF- α Has Advanced Gastrointestinal Diseases and Treatment Regimes

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Introduction

Tumor necrosis factor (TNF)- α , identified over 30 years ago as a potent antitumor agent, is also a key component of the inflammatory cascade in addition to its namesake ability to regress certain malignant tumors. Indeed, the main clinical utility of TNF has been in the inhibition of its effects by neutralizing antibodies in the therapy of chronic inflammatory conditions, including the inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC). Nearly 15 years after the first clinical trial to treat CD with the TNF- α monoclonal antibody infliximab, many other biological agents which block its activity have appeared in treatment protocols. The mechanisms involved in anti-TNF- α therapy for the treatment of CD and UC are not well established, although its action may lie beyond simple blockage of TNF- α interaction with its receptors. Further research on the effects of TNF- α in gastrointestinal (GI) diseases, as well as on other potential inhibitors of the inflammatory pathway, is essential for the diversification of our therapeutic arsenal against this cytokine's effects. This review underlines the major impact the discovery of TNF- α has had on the treatment of GI diseases such as IBD.

Discovery

Like most scientific discoveries, a series of isolated, seminal contributions from many researchers and clinicians in diverse fields paved the way for the identification and isolation of TNF- α over 30 years ago (Fig. 1). The effects of bacterial infections or bacterial extracts on tumors were commented on in past centuries with observations published as early as the 19th century, notably by the German physicians Busch [1], Fehleisen [2] and Bruns [3], who reported spontaneous tumor regression in patients following streptococcal bacterial infections. Coley [4], an American bone sarcoma surgeon and researcher known as one of the pioneers of cancer immunotherapy, reported antitumor activity in bacterially infected patients, either by incidental infection, by direct inoculation of patients with inoperable tumors with *Streptococcus pyogenes* or with inactivated bacterial cultures derived from *S. pyogenes* and *S. marcescens* known as “Coley's toxins.” Shear et al. [5], via thorough laborious and painstaking purification of bacterial products, later identified the factor responsible for inducing this antitumor response by gram-negative bacteria, now known as endotoxin or lipopolysaccharide (LPS). O'Malley et al. [6] subsequently established that LPS induced an antitumor host factor in serum by injecting tumor-bearing mice with serum of LPS-treated normal mice. Lloyd Old and colleagues were the first to identify TNF- α as the host factor responsible for tumor necrosis upon LPS treatment. Indeed, they showed that TNF- α , isolated from mice, rats and rabbits treated with LPS, was toxic to subcutaneous tumors in mice and neoplastic cell lines [7]. They also identified macrophages as one of the sources for TNF- α . Nearly 10 years after its discovery by the Old group, a major step in TNF research was achieved when TNF- α was purified, sequenced and cloned by the

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Fig. 1 Timeline of discoveries leading up to and following the first report of the isolation of tumor necrosis factor (TNF). Dates signify the publication year of the first published report. *LPS* lipopolysaccharide, *Ab* antibody, *Mab* monoclonal Ab, *IFX* infliximab, *RA* rheumatoid arthritis, *FDA* US Food and Drug Administration

Aggarwal group [8, 9], which was key in determining that cachectin, a factor responsible for cachexia in mice, was in fact murine TNF- α [10, 11]. Sequencing of TNF- α was also a critical step in the identification of homologous proteins, now part of the TNF superfamily. The identity of the TNF receptors on the surface of a human cervical carcinoma cell line and mouse fibroblasts was also reported at that time [12, 13].

TNF- α and the TNF Superfamily

Currently, the human TNF superfamily is comprised of 19 ligands and 29 receptors (reviewed in [14]). Most of the superfamily ligands, including TNF- α , are type II transmembrane proteins with an extracellular TNF homology domain, a single transmembrane domain and an amino terminal intracellular domain. The TNF homology domain, responsible for receptor binding, shares 20–30 % amino acid identity among superfamily members. The extracellular domain of the 26-kDa transmembrane form is released from the cell surface by proteolytic cleavage by the metalloproteinase TNF- α converting enzyme (TACE) into the 17 kDa-secreted form [15, 16]. The biologically active transmembrane and the soluble forms of TNF- α are self-assembling non-covalent homotrimers [17]. Nearly all TNF ligands are expressed by immune cells, including

macrophages, monocytes, dendritic cells, T-cells, B-cells and natural killer (NK) cells, with the exception of vascular endothelial cell-growth inhibitor (VEGI), expressed chiefly by endothelial cells [14]. The greatest producers of TNF- α are activated macrophages and monocytes, particularly when stimulated with LPS.

TNF superfamily ligands trigger cell responses through ligation of receptors belonging to the TNF receptor superfamily (TNFR; reviewed in [14]). Presently, 29 receptors have been identified in humans, of which two are receptors to TNF- α : TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). Expression of TNF receptors varies considerably; for example, TNFR1 (CD120a) is ubiquitously expressed, whereas TNFR2 (CD120b) expression is restricted to immune and endothelial cells. TNF superfamily receptors are type I transmembrane proteins with most containing tandem cysteine-rich domains in their extracellular domain. The highly variable intracellular domain can contain death domains (DD), as is the case for TNFR1, and/or binding motifs for TNFR-associated factors (TRAFs). Signaling triggered by ligation of TNF receptors induces apoptosis, differentiation, proliferation, and pro-inflammatory responses due to the activation of nuclear factor (NF)- κ B.

TNF- α in Gastrointestinal Diseases

Uncontrolled TNF- α release, which has many harmful effects such as chronic inflammation, cachexia, and septic shock, underlies many inflammatory diseases, including IBD (reviewed in [18]). IBD is characterized by unregulated inflammation of the intestinal tract. Although triggers of this inflammatory response are not known, genetic predisposition, disruptions of gut homeostasis, and an uncontrolled immune response are considered to be contributory factors [19, 20]. Although cytokine profiles differ for CD and UC with CD associated with a Th1-type response whereas UC is associated with a Th2 type response, TNF- α is implicated in both disorders (reviewed in [21]). Indeed, serum and stool TNF- α concentrations and the frequency of TNF- α -secreting mononuclear cells are higher in IBD patients than in healthy controls [22–24]. Kontoyiannis et al. [25] reported that dysregulation of TNF- α expression in mice induces Crohn's-like IBD and chronic inflammatory arthritis. Furthermore, the beneficial effects of neutralizing TNF- α in animal models of intestinal inflammation and in clinical trials of anti-TNF- α treatments further support that TNF- α is a key component of CD and UC pathogenesis [26, 27].

Although TNF- α was first identified as an anti-cancer agent, it has also been implicated in the promotion of gastric cancer induced by chronic infection by

Helicobacter pylori (reviewed in [28]). *H. pylori* induces secretion of TNF- α and other pro-inflammatory cytokines in the gastric microenvironment by the expression of Tip α , a bacterial protein unique to *H. pylori* required for colonization that triggers macrophage TNF- α secretion and activates Ras, a well-known oncogene [28, 29]. The mechanisms by which TNF- α promote gastric cancer, however, are still unclear and are likely to be multifactorial.

Inhibition of TNF- α in the Treatment of IBD

Many therapies inhibit inflammatory cytokine function in IBD patients. In the past few decades, the mainstays of treatment have been systemic and topical corticosteroids and 5-aminosalicylates (5-ASA), and immunomodulators such as 6-mercaptopurine (6-MP), azathioprene, cyclosporine, and methotrexate. Although inexpensive and generally well tolerated, steroids are unsuitable for long-term use, 5-ASA are mostly useful only for mild-moderate disease, and 6-MP and azathioprene are incapable of inducing clinical remission, whereas cyclosporine, though useful in acute severe disease, is not suitable for chronic treatment [30]. Infliximab was the first biological inhibitor of TNF- α to be approved for treatment of IBD (reviewed in [31]). Infliximab is a chimeric human–mouse monoclonal antibody designed to maximize efficacy: its variable region, derived from a mouse monoclonal antibody, binds TNF- α with high affinity whereas the constant region is based on the human sequence in order to reduce its immunogenicity [32]. The first clinical trial to report its efficacy in the treatment of fistulizing CD was published in 1995 [33]. Subsequent clinical trials have reported efficacy in the treatment of active CD and UC using a variety of endpoints such as induction of clinical and endoscopic remission, corticosteroid use, risk of colectomy, and need for hospitalization (reviewed in [26] and [27]). A major advantage of infliximab and other anti-TNF agents is that they can be used as “top-down” therapy for moderate–severe disease, in that they can be used as monotherapy to induce and maintain remission, with resultant advantages in terms of obviating the need to “step up” therapies and perhaps avoid complications such as toxic megacolon with resultant colectomy associated with uncontrolled inflammation [34].

One of the main problems with infliximab is diminishing efficacy due to the development of idiotypic antibodies. To reduce the risk of mounting a host response to anti-TNF- α , similar human monoclonal antibodies have been approved for clinical use including adalimumab and certolizumab pegol. Adalimumab was the first human IgG1 monoclonal antibody to TNF- α which has similar efficacy to that of

infliximab [26, 27], effective in CD and UC patients who have become intolerant or have lost responsiveness to Infliximab. Certolizumab pegol is a pegylated (polyethylene glycol adduct) human monoclonal antibody to TNF- α , designed to further reduce the potential risk of host intolerance. This antibody, however, has not performed as well as the first two in clinical trials. This could be due to its inability of the antibody to induce antibody-dependent cell-mediated cytotoxicity and apoptosis [35]. Finally, etanercept is a recombinant fusion protein or TNFR2 with IgG1 that acts as a TNF- α blocking agent. Although it is currently used for the treatment of inflammatory diseases other than UC, it did not show any significant effects in the treatment of Crohn’s disease [36]. Therefore, the only TNF- α neutralizing agents in the treatment of CD and UC remain limited to the monoclonal antibodies listed above. The success of these anti-TNF- α agents has nonetheless drastically changed treatment protocols for patients with UC and CD, with clinical remission more probable than with agents used previously. A future challenge is to use biomarkers to help select patient’s response to powerful, targeted therapies such as anti-TNF agents [37, 38]. Yet, since their use is associated with undesirable adverse effects such as development of intolerance and increased infection risk, further research into how TNF- α is involved in IBD pathogenesis is still warranted to uncover mechanisms that could provide novel therapeutic targets.

Conclusions

Clearly, the impact of the discovery of TNF- α by the Old group and others on the field of gastroenterology is immeasurable. Today, the use of anti-TNF- α biological agents has been validated in the treatment of CD and UC and constitutes a suitable option in the treatment of IBD and many other inflammatory diseases such as rheumatoid arthritis and psoriasis. Moreover, further investigation in the implication of TNF- α in *H. pylori*-induced gastric cancer may lead to interesting treatment options with regards to the modulation of this cytokine. Thus, the role of TNF- α in many diseases make it a prime target for therapeutics and the current treatment options may represent the tip of the iceberg in regards to TNF- α -targeting biological agents.

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