Editorial

Mechanisms of Action of Tumor Necrosis Factor Antagonists and Granulomatous Infections

The discovery that high levels of tumor necrosis factor (TNF) may mediate chronic inflammation and joint destruction in rheumatoid arthritis (RA) has heralded a new era of targeted and highly effective biologics for RA and other chronic inflammatory diseases[1-7]. These therapeutics currently fall into 2 classes: (i) monoclonal antibodies (mAb), including infliximab (Remicade®) and adalimumab (Humira®), and (ii) the soluble TNF receptor (sTNFR) etanercept (Enbrel®). Although these agents have significant advantages over older therapies in both efficacy and safety, an increase in granulomatous infections such as tuberculosis and histoplasmosis has been associated with the use of TNF antagonists[8-16]. Observational studies and voluntary reporting systems document a higher incidence of granulomatous infections associated with the use of infliximab than with etanercept, although only one such study found a statistically significant difference between rates seen with these agents[8,11,17]. This difference may be due in part to the reporting methods used, but may also reflect the different mechanisms of action of the mAb and the soluble receptor, and how these mechanisms may affect granuloma formation and maintenance.

The primary sources of TNF in immunity and inflammatory diseases are cells of the monocyte/macrophage lineage, which secrete TNF in response to exogenous molecules such as lipopolysaccharide (LPS) and endogenous mediators such as interleukin (IL) 1ß and interferon. TNF is expressed as a homotrimer on the cell surface and is released as a soluble cytokine following cleavage of membrane-anchoring domains[18]. TNF plays a significant role in resistance to infections through activation of neutrophils and enhancement of macrophage and natural killer (NK) cell killing. It is a potent proinflammatory cytokine that stimulates release of inflammatory cytokines (IL-1ß, IL-6, IL-8, and granulocyte monocyte colony stimulating factor, GM-CSF), endothelial adhesion molecules (ICAM-1, VCAM-1, E-selectin) and chemokines (monocyte chemotractant proteins, MCP-1, MIP-2, RANTES and macrophage inflammatory protein-1α)[19]. In healthy humans, circulating TNF is not detectable, but in patients with inflammatory diseases such as RA[20], Crohn's disease (CD)[21], and bacterial meningitis[22], TNF is easily detectable. Increased expression of TNF has been noted in inflamed joints of animals with experimentally induced arthritis[23] and in the synovia of RA patients[24]. In CD, elevated TNF levels are found in both serum and stool, and other proinflammatory cytokines have been observed in the colonic mucosa[21,25]. However, the most convincing evidence for the central pathogenic role of TNF in these and other inflammatory diseases has been the efficacy of TNF antagonists observed in clinical trials.

TNF also plays an essential role in granuloma formation. Granulomas are comprised of macrophages (epithelioid and multinucleated giant cells) encircled by lymphocytes, primarily Th1-biased CD4+ T cells[26]. Granuloma formation typically occurs when acute inflammatory processes cannot destroy invading agents[27]. Animals deficient in TNF are highly susceptible to granulomatous infections[28-31]. Neutralization of TNF has been shown to decrease both the recruitment of inflammatory cells and the formation of granulomas[19,32]. TNF orchestrates the early induction of chemokines and subsequent leukocyte recruitment to infected organs[33]. In addition to its role in initial cellular recruitment, TNF plays an essential role in regulation of the inflammatory response, and in particular the juxtaposition of macrophages and lymphocytes to form granulomas[33]. TNF is also required for establishing and maintaining granuloma architecture: it regulates the tight association between macrophages and lymphocytes within granulomas, either as secreted or membrane-bound forms[33]. Human mycobacterial immunity reflects growth inhibitory mechanisms requiring direct cell contact and activation[34-36]. As a result, the continuous recruitment of antigen-specific T cells is required for maintenance of granulomas and prevention of progression of latent infection to active disease.

A number of granulomatous infections caused by intracellular organisms, including *Aspergillus*[15], *Cryptococcus neoformans*[14], *Histoplasma capsulatum*[11,13], *Listeria monocytogenes*[9,10,37,38], and *Mycobacterium tuberculosis*[8,12,16] have been reported in association with the use of some or all of the TNF antagonists adalimumab, etanercept, and infliximab. Early studies done with adalimumab suggested a dose-response relationship with the occurrence of tuberculosis[39]. Patients who contracted tuberculosis were receiving higher doses than the licensed dose of 40 mg every other week. Reducing the treatment dose and screening for the

presence of latent tuberculosis reduced the frequency of activated tuberculosis to 1 or 2 cases in the next approximately 2500 patients[39].

Passive surveillance studies have suggested a numerically higher incidence of certain granulomatous infections with the use of infliximab than with etanercept[8,11,40]. The US Food and Drug Administration monitors the safety of all drugs through its Adverse Event Reporting System (AERS), a surveillance system to which drug manufacturers are required to submit reports of adverse events, and to which healthcare professionals and consumers voluntarily report adverse events. One study analyzed all infliximab-related tuberculosis reports received through the AERS from the licensure of infliximab in 1998 to May 29, 2001; the authors concluded that the rate of reported tuberculosis cases was numerically, but not statistically, higher than the available background rates[8]. Recently, results from an analysis of reports from a European surveillance database supported these findings[41]. Another study analyzed all reports received through the AERS of histoplasmosis following infliximab or etanercept therapy from licensure of the 2 drugs in 1998 to July 2001[11]. Histoplasmosis was reported in about 6 out of 100,000 infliximab-treated patients and about 1 out of 100,000 etanercept-treated patients, suggesting that patients treated with infliximab may have a higher risk for developing histoplasmosis compared to patients treated with etanercept, although no statistically significant differences exist to date.

A more comprehensive review of the AERS database searched for all reports of granulomatous infections associated with the use of infliximab and etanercept from their licensure through the second quarter of 2002 (adalimumab was not approved until December 2002)[17]. Fifteen types of granulomatous infections were reported in association with one or both drugs. Overall, more cases of granulomatous infections were reported in infliximab-treated patients than in etanercept-treated patients. During the period covered by this analysis in the US, approximately 197,000 patients had been treated with infliximab and 113,000 with etanercept, with an incidence of 13 granulomatous infections per 10,000 infliximab patients and 6 per 10,000 for etanercept. In both infliximab- and etanercept-treated patients, tuberculosis was the most frequently reported granulomatous infection, accounting for 106/255 (42%) of all infections in infliximab-treated patients and 32/68 (47%) in etanercept-treated patients. Additionally, 13 cases of tuberculosis and 6 cases of invasive infections caused by *Histoplasma*, *Aspergillus*, and *Nocardia* species were reported in adalimumab clinical trials[42].

Although treatment with any of the 3 TNF antagonists results in modulation or neutralization of TNF-induced and regulated biological responses, there are differences in the mechanisms of action of the soluble TNFR and of the mAb to TNF. One or more of these differences may explain the relative differences in granulomatous infections seen with the different TNF antagonists.

Etanercept is a dimeric fusion protein that consists of 2 molecules of the extracellular ligand-binding portion of human TNFR2 attached to the Fc portion of human IgG1[43]. Infliximab is a chimeric IgG1 mAb composed of human constant and murine variable regions[44], whereas adalimumab is a recombinant human IgG1 mAb[45]. All 3 molecules prevent TNF signaling by blocking interaction with TNFR. Each mAb molecule can bind up to 2 TNF molecules; a single TNF homotrimer can bind up to 3 molecules of infliximab or adalimumab[46,47]. In contrast, etanercept binds to the interface of 2 TNF subunits in a 1:1 ratio[46]. While affinity measurements for these molecules are particularly complex given the multiple binding sites on each, the mAb appear to have significantly higher affinity for TNF. However, these *in vitro* data with varying conditions often describe conflicting results[46-49]. TNF knockout mice are highly susceptible to granulomatous infection, and complete TNF inhibition interferes with normal immunity, yet partial TNF inhibition can improve inflammatory disease processes such as RA. Since etanercept dissociates from TNF much faster than the mAb do, one interpretation is that it may be easier to titrate TNF suppression with etanercept to inhibit inflammation while retaining greater resistance to infection. Another interpretation is that infliximab and adalimumab are more potent inhibitors of TNF actions than is etanercept.

Infliximab and adalimumab have longer serum half-lives (8-10 days[44] and 10-20 days[45], respectively) than etanercept (3-5 days). In clinical studies, infliximab could still be detected in the serum after 8 weeks following a single 3 mg/kg dose[44], and detectable concentrations of free infliximab have been observed for up to 28 weeks[50]. If absorption and distribution occur rapidly after intravenous administration, the maximum serum concentrations for the majority of the dosing period for infliximab would be significantly higher than the maximum serum concentration for etanercept. Therefore, TNF suppression could be greater and more prolonged during infliximab treatment than during etanercept treatment. The clinical implications of differences in pharmacokinetics are difficult to understand based on the available evidence. Ongoing studies may determine whether the etanercept's shorter half life interferes with monocyte/macrophage activity less than the longer half life of the mAb.

Cells coated with aggregates of antibody isotypes that fix complement and bind Fc receptors, such as human IgG1, can activate complement-dependent cytolysis (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Infliximab has been shown to induce CDC and ADCC in a murine myeloma cell line expressing mutant human TNF that remains membrane associated (ma-TNF)[51]. The observation that infliximab can cause the direct killing of cells expressing ma-TNF, such as macrophages and monocytes, via CDC and/or ADCC may explain the monocytopenia observed in patients following treatment with infliximab

that can persist for weeks following infusion[52]. More importantly, monocytes are an essential component of granulomas and monocyte destruction might lead to greater susceptibility to granulomatous infections. Given the identical effector portion of both molecules, it is likely that adalimumab would have activity similar to that of infliximab in this regard. While etanercept contains the Fc portion of IgG1, it does not fix complement[53]; perhaps because steric hindrance impedes binding of C1q, thereby preventing activation of the classical complement cascade. Furthermore, because etanercept binds only single molecules of TNF, it should not form immune complexes necessary to activate CDC and ADCC. In addition, etanercept can bind lymphotoxin alpha and infliximab and adalimumab cannot; the clinical significance of this difference is unclear.

An unexpectedly sustained clinical response in patients with CD treated with infliximab[54] prompted a search for additional mechanisms of action for infliximab, including induction of apoptosis. Twenty-four hours after infliximab infusion in CD patients, an increase in CD3+ apoptotic cells was observed with a TUNEL assay of intestinal biopsies from these patients[55]. These results suggest that infliximab may exert its sustained therapeutic effects by causing apoptosis of T lymphocytes, at least in inflammatory bowel disease (IBD), since uncontrolled T-cell activation plays a central role in IBD pathogenesis. Apoptosis has also been observed in circulating monocytes from CD patients following infliximab infusion. While preliminary in nature, these studies suggest a role for infliximab in inducing apoptosis in activated monocytes and T cells[56]. Removal of activated monocytes and CD4+ T cells might be desirable in chronic inflammation. However, since these 2 cell types play an essential role in granuloma formation and maintenance, infliximab-induced apoptosis may lead to more potent inhibition of the granulomatous response than can be expected from TNF neutralization alone.

Anti-TNF monoclonal antibodies and soluble TNFR are effective in treating RA and have dramatically improved the lives of people with this disease. However, there are significant differences between the 2 classes of TNF inhibitors. The mAb tend to suppress TNF levels more profoundly than do the soluble receptors, and the mAb may also eliminate activated T cells and macrophages, either directly by cell lysis or by inducing apoptosis. These differences may explain the greater efficacy of infliximab compared with etanercept in CD on one hand, and on the other hand, the apparent trend toward greater susceptibility to granulomatous infections such as tuberculosis with infliximab versus etanercept therapy. PPD testing before beginning therapy with any anti-TNF agent is suggested by experts[57]. Screening tests are not currently available for many other granulomatous infections, some of which (e.g., histoplasmosis and coccidioidomycosis) are endemic in parts of the US. Careful surveillance will be needed to ensure that an increase in the incidence of these infections does not outweigh the benefit of anti-TNF therapy. Further investigations into class differences may help clinicians choose among different biologic therapies.

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