The Macrophage as a Validated Pharmaceutical Target

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Abstract "Therapeutic validation" is a utilitarian classification of the application of the basic science base of the macrophage. Reduction to therapeutic practice represents the cutting edge of therapy, but rests upon a decades-old basic science foundation. Macrophage-targeted therapeutics have now added significant value to the lives and quality of life of patients, without undue adverse effects in multiple disease settings. These are exemplified by the impact of macrophage enzyme replacement for a lysosomal storage disease (Gaucher's), the modulation of osteoclast-dependent bone destruction by bisphosphonates, and revolutionary impact of TNF sequestrants on both rheumatoid arthritis, as well as the delineation of new mechanisms in the understanding of Crohn's diseases. The macrophage, as a cell, is now beginning to reach a full measure of therapeutic maturity in the application of the understanding of the particular rate-limiting roles that it plays in the maintenance of health or the induction of diseases.

Keywords Bisphosphonates, Crohn's disease, Enzyme replacement, Gaucher's disease, Macrophage, Osteoporosis, Rheumatoid arthritis, TNF sequestration

The concept of therapeutic target validation bears disproportionate weight in the conduct of applied science in the pharmaceutical and biotechnology sectors, where research decisions are significantly influenced by perceptions of market size. These organizations measure the success of their scientific output by products launched and the expansion of revenues earned and not by the successful testing of meaningful hypotheses and the resulting high-impact publications. To do this, however, the products must add significant value to the lives and quality of life of patients, without undue adverse effects. The ability to regard the macrophage as a "validated" therapeutic target suggests the macrophage, as a cell, is now beginning to reach a full measure of maturity in the application of the understanding of the particular rate-limiting roles that it plays in the maintenance of health or the induction of diseases.

The notion of "therapeutic validation" is simply a utilitarian classification of the application of the basic science base of the macrophage. In many respects, the reduction to therapeutic practice may represent the cutting edge of therapy, but rests upon a decades-old basic science foundation. It provides an objective, although retrospective, validation of the importance of the study of macrophages to advancing human therapeutics. The view is, however, skewed towards the major medical needs that drive economic activity, and does not necessarily reflect the full contribution of the study of macrophages, where the advancing science base has had major impacts upon the understanding of immunity, vaccination, tissue remodeling, and embryology, although in areas that have not yet been brought to therapeutic fruition. In this essay, I have used a broad definition of macrophage study that includes cells of the monocytic lineage such as monocytes, macrophages, osteoclasts, and have also included discussion of inherited metabolic defects in which macrophages are central to the expression of tissue pathology and dysfunction. I will also show how the quality of the basic science base was prospectively predictive of both efficacy and the adverse experience profiles associated with therapeutic approaches to molecules affecting macrophage function.

1
Modification of Macrophage Function Can Now Be Said to Modify Disease Outcome, Rather than Provide Symptomatic Relief

The validation of the macrophage as a proven therapeutic target has crossed multiple treatment modalities. In the macrophage-specialized function of lysosomal degradation, the lysosomal storage defects of Gaucher's disease have led to the relatively successful replacement of a heritable defective enzyme, glucocerebrosidase, using recombinant enzyme, as an orphan disease. In contrast, the treatment of rheumatoid arthritis and Crohn's disease has been revolutionized by the advent of tumor necrosis factor (TNF) sequestrants. These have not only improved patients' lives but have provided unique insights into human disease mechanisms not readily modeled in animal models (Podolsky 2002). The successful treatment of osteoporosis and Paget's disease of bone by the inhibition

of osteoclast-dependent bone resorption represents the successful use of small molecules (bisphosphonates) in the alteration of outcome of an event entirely dependent upon cells of the monocytic lineage. These all represent highly specific macrophage-related events in pathology usefully modulated therapeutically, and are much more compelling evidence for the specific role of macrophages in pathology than is, for example, the expression and upregulation of cyclooxygenase 2 in macrophages, where therapeutic efficacy is only partly attributable to macrophage effects, and where such efficacy is largely symptomatic, rather than disease modifying in terms of long-term impact upon patient outcomes.

2
Enzyme Replacement in Gaucher's Disease

Gaucher's disease is an autosomal recessive disease with its highest prevalence (type I) in Ashkenazi Jews (Balicki and Beutler 2002). It is a typical lysosomal storage disease caused by a deficiency in glucocerebrosidase, an essential step in the cleavage of glucose from ceramide (Inoue and Lupski 2002). Glucocerebroside substrate, therefore, accumulates within cells of the mononuclear phagocyte system and within the central nervous system. (Dwek et al. 2002) Three clinical subtypes of Gaucher's disease are described. The adult or type I Gaucher's is a non-cerebral form of storage disease, with some residual enzymatic activity, that accounts for its emphasis on splenic, hepatic, and skeletal involvement. Infantile Gaucher's (type II) is an acute disease dominated by cerebral accumulation of substrate, as well as hepatosplenic involvement. No enzyme activity is usually detected. Type III Gaucher's is a juvenile disease intermediate in signs and symptoms from types I (adult) and II (infantile). The involvement of the CNS and pre-existing skeletal muscle lesions has significant impact on the efficacy of enzyme replacement therapy.

The disease pathology results directly from either CNS dysfunction, or the physical effects of the accumulation of distended macrophage "Gaucher's cells" in spleen, liver, marrow, lymph nodes, thymus, and Peyer's patches. These long-term effects include anemia and thrombocytopenia. Longitudinal studies with 2–5 years of clinical follow-up have been published in type I Gaucher's disease, using recombinant glucocerebrosidase targeted to macrophage lysosomes through the endocytic route (Weinreb et al. 2002). Enzyme replacement is highly effective in ameliorating extra-CNS disease manifestations. Patients show long-term improvements and maintenance of improvement in anemia and thrombocytopenia. There is also a measurable benefit in bone erosions and bone pain, with more than 50% of patients showing measurable improvement even after the presence of radiologically documented bone lesions and bone pain (Bembi et al. 2002; Poll et al. 2002). This is a pleasing and rational approach to the correction of a primary heritable peripheral defect of macrophages, and illustrates the therapeutic accessibility of macrophages that have access to the circulation. Large molecules equilibrate inefficiently across the blood–brain barrier in the absence of specific transcytotic mechanisms or leak-

age, and therefore the primary CNS defect is not amenable to approach via the circulation, and will likely require the development of effective gene therapies or alternate approaches to the modification of glycosylation pathways (Dwek et al. 2002). The disease, because of its low prevalence, will always be considered an orphan. This ultimately limits the resources that could be brought to therapeutic approaches, and reflects that market forces will always favor the highly prevalent, and patients and physicians will need to rely upon small companies seeking market niches, or academic institutions with governmental or charitable funding to approach these diseases.

3
TNF Sequestrant Therapy Improves the Outcome of RA and Sheds Critical Mechanistic Insights into Crohn's Disease

The central role of the macrophage in the mechanism of human disease has emerged from the clinical evaluation of TNF sequestrants in rheumatoid arthritis and Crohn's disease. The impact of the elucidation of the pathophysiology of TNF on therapeutics, sheds light into the long lag phase between the leading edge of macrophage biology and its therapeutic application. TNF-α was discovered in the 1970s by Old and colleagues (Feldmann and Maini 2001) and cloned in the early 1980s, when it was shown that cells of the monocytic lineage were the major source of TNF production (Tracey and Cerami 1994). TNF is a rapidly produced proinflammatory cytokine, with serum levels detectable within 30 min of lipopolysaccharide (LPS) stimulus, and likely reflects the cleavage of preformed membrane-bound TNF-α by TNF-α-converting enzyme (TACE). Blockade of TNF release blunts the release of other proinflammatory cytokines such as interleukin (IL)-1 and IL-6, suggesting that TNF plays a role as a molecular trip wire for the activation of stress responses to noxious stimuli, that engage a cascade of events culminating in spatially and temporally regulated recruitment of inflammatory and immune leukocytes at sites of injury. In keeping with these data was the demonstration of the pivotal role of TNF-α in the early control of intracellular bacterial pathogens. The work of Havell in listeriosis (Havell 1989) and Vassalli (Kindler et al. 1989) in early granuloma formation and the control of Bacillus Calmette-Guerin (BCG) infection in mice, both accurately showed the role of TNF-α as an essential organizer of the granulomatous response, and foreshadowed the efficacy of TNF sequestration in Crohn's disease, (Sandborn and Targan 2002) as well as the deleterious potentiation of bacterial diseases including tuberculosis (Tb) by TNF sequestration. This remains one of the few areas of basic biology where early studies have so accurately prospectively predicted the adverse effects expected upon clinical usage. The lag phase between characterization of the role of TNF and the central role of the macrophage as organizer and participant in the granuloma, and the clinical exploitation of these data was almost 15 years. The features that contributed to the gap between the basic discovery and the clinical exploitation included developing adequate methods of production and manufacture, as well as the lengthy re-

quirements for clinical trials in chronic diseases with complex clinical endpoints such as rheumatoid arthritis (Feldmann and Maini 2001; Bresnihan 2002; Jenkins and Hardy 2002; Kalden 2002; Scott 2002; Weisman 2002) and Crohn's disease. In addition, the essential role of TNF in host defense coupled with the long half-lives of TNF sequestrants required significant empirical approaches to dose ranging. The choice of dose in attempting to validate a pharmacological mechanism is always difficult, in this case especially so because of the need to walk a tightrope between suppression of the host response sufficient for efficacy, and suppression of macrophage function to the point where host defense impairment results in catastrophic potentiation of rapid bacterial infections. The advantage of the rheumatoid arthritis field is that standard therapies including methotrexate and corticosteroids also have well-documented risks of potentiation of infection. With appropriate patient exclusion criteria, the clinical safety of TNF sequestration has been acceptable, with a somewhat higher prevalence of upper respiratory infection in First World usage. Recent reports have included the reactivation of miliary Tb in patients by TNF sequestrants, and more than 70 reports of Tb reactivation can now be found within the Adverse Experience database maintained by the Food and Drug Administration (FDA) (Keane et al. 2001; Mayordomo et al. 2002).

An unexpected clinical finding has been the development of demyelinating disease in a small number of patients receiving TNF sequestrant therapy for adult or juvenile rheumatoid arthritis (Sicotte and Voskuhl 2001). Trials of lenercept in multiple sclerosis (Lenercept 1999) showed that there were no significant differences between groups on any magnetic resonance imaging (MRI) study measure, but the number of lenercept-treated patients experiencing exacerbations was significantly increased compared with patients receiving placebo (p=0.007) and their exacerbations occurred earlier (p=0.006). These findings suggest that one of the roles of TNF may be in the control of demyelination, and that the macrophage, by extension, can play pleiotropic roles in human pathology, exacerbating rheumatoid arthritis and Crohn's disease, while playing a suppressive role in multiple sclerosis. Another hint towards a suppressive role for TNF in disease is the small incidence of drug-induced lupus on TNF sequestrant therapy. This again is in keeping with the predictive value of basic studies, where TNF-α deletant mice also developed elevated levels of anti-double stranded (DS) DNA antibodies (Ettinger and Daniel 2000).

The findings that TNF sequestration can have beneficial effects upon the expression of Crohn's disease but not ulcerative colitis, has again sharpened the focus on the role of the macrophage in Crohn's disease, and strongly differentiated the pathogenesis of Crohn's disease from ulcerative colitis (Podolsky 2002; Sandborn and Targan 2002). One of the strengths of the pharmacological approach is this ability, through controlled clinical trials, to provide insights into human as opposed to model diseases. It is, however, salutary that in this field at least, disease models in lower species including rodents have in large part been prospectively predictive of both efficacy and adverse experience.

4
Bisphosphonates Enhance Bone Mass Retention and Protect Against Fractures by Inhibition of Osteoclast Formation

Whereas the two previous examples deal with macrophage enzyme replacement using recombinant enzyme, or sequestration of a macrophage-secretory product TNF with antibodies or receptor fusion proteins, the efficacy of small synthetic chemical moieties, specifically nitrogen-containing bisphosphonates, impact upon the resorption of bone mass, by inducing osteoclast and macrophage apoptosis.

Bisphosphonates are the most effective inhibitors of bone resorption and are extensively used for the treatment of systemic or local bone loss including postmenopausal osteoporosis and tumor bone disease. Bisphosphonates are pyrophosphate analogs, which bind to bone. Bisphosphonates are then removed from the mineral matrix in the acidic compartment formed between the osteoclast and the bone surface, analogous to the sealed compartments described in macrophages upon immune complexes or complement ligands (Wright and Silverstein 1984). Labeled bisphosphonates accumulate in osteoclasts and inhibit further bone resorption (Reszka et al. 1999). Evidence has accumulated that all bisphosphonates that inhibit the resorption of bone induce the caspase-dependent formation of pyknotic nuclei and the cleavage of Mst1 kinase. This cleavage of Mst1 kinase and caspase activation is dependent upon the bisphosphonate inhibition of the mevalonate pathway, and is specifically blocked by the addition of geranylgeraniol, a key precursor for geranylgeranyl diphosphate (Benford et al. 1999; Reszka et al. 1999). It emerges from these studies that the flux through the mevalonate pathway to geranylgeraniol is essential for the formation of osteoclasts from macrophages, and for the long-term maintenance of the osteoclast population (Fisher et al. 1999; Fisher et al. 2000). In the absence of protein geranylgeranylation, osteoclasts fail to differentiate from macrophages in murine, rabbit, and chicken systems, and that this failure can be attributed to the effects of bisphosphonates on geranylgeranylation and not farnesylation of proteins (Coxon et al. 2000). The effects of bisphosphonates are therefore complex. Effective pharmacodynamic inhibition of bone resorption that can be measured both as the retention of bone mass, as well as by the inhibition of fractures in long-term clinical use (Karpf et al. 1997) can be achieved by the modulation of osteoclast differentiation from macrophages, as well as by the inhibition of osteoclast function through the activation of caspase-mediated apoptotic events.

5
Future Directions

These areas have shown the therapeutic feasibility of macrophage enzyme replacement, secretory product sequestration, and inhibition of osteoclast differentiation and function. Efficacious therapies with direct effects upon macro-

phages now target rheumatoid arthritis, Crohn's disease, osteoporosis, tumor disease of bone, and inherited metabolic defects of the mononuclear phagocyte system. The important role of the macrophage as a regulator and remodeler of tissue, and regulator of the migratory and differentiative events of tissue cells has not yet been exploited. That the macrophage plays a central role in atherosclerosis is clear. The therapies effectively directed towards the role of the macrophage in that disease still requires reduction to practice. It may be that many of the therapeutically advantageous effects of peroxisome proliferator-activated receptors (PPAR) agonists in vascular disease will prove to be useful probes of macrophage function in human pathology (Berger and Moller 2002).

6 References

Balicki D, Beutler E (2002) Gene therapy of human disease. Medicine 81:69

Bembi B, Ciana G, Mengel E, Terk M, Martini C, Wenstrup R (2002) Bone complications in children with Gaucher disease. Br J Radiol 75:A37

Benford HL, Frith JC, Auriola S, Monkkonen J, Rogers MJ (1999) Farnesol and Geranylgeraniol Prevent Activation of Caspases by Aminobisphosphonates: Biochemical Evidence for Two Distinct Pharmicological Classes of Bisphosphonate Drugs. Molecular Pharmacology 56:131

Berger J, Moller DE (2002) The Mechanisms of Action of PPARS. Annu Rev Medicine 53, no. 1:409

Bresnihan B (2002) Preventing joint damage as the best measure of biologic drug therapy. J Rheumatol 65, no. 29 Sept. Suppl.:39

Coxon FP, Helfrich MH, Van'T Hof R, Sebti S, Ralston HSH A, Rogers MA (2000) Protein Geranylgeranylation is Required for Osteoclast Formation, Function, and Survival: Inhibition by Bisphosphonates and GGTI-298. Journal of Bone and Mineral Research 15, no. 8:1467

Dwek R, Butters T, Platt F, Zitzmann N (2002) Targeting glycosylation as a therapeutic approach. Nature Reviews Drug Discovery 1:65

Ettinger R, Daniel N (2000) TNF-deficient mice develop anti-nuclear autoantiboies. Scand J Immunol 51:S88

Feldmann M, Maini RN (2001) anti-TNF-α therapy of RHEUMATOID arthritis: what have we learned? Annu Rev Immunol 19, no. 1:163

Fisher JE, Rodan GA, Reszka AA (2000) In vivo Effects of Bisphosphonates on the Osteoclast Mevalonate Pathway. Endocrinology 141, no. 12:4793

Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ, Wesolowski G, Russell RGG, Rodan GA, Reszka AA (1999) Alendronate mechanism of action: geranylgerniol, and intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA 96:133

Havell E (1989) Evidence that tumor necrosis factor has an important role in antibacterial resistance. J Immunol 143:2894

Inoue K, Lupski JR (2002) Molecular mechanisms for genomic disorders. Annu Rev Genom Hum Genet 3, no. 1:199

Jenkins JK, Hardy KJ (2002) Biological modifier therapy for the treatment of rheumatoid arthritis. Am J Med Sci 323, no. 4:197

Kalden JR (2002) Emerging role of anti-tumor necrosis factor therapy in rheumatic diseases. Arthritis Res 2, no. 4 Suppl:S34

Karpf D, Shapiro D, Seeman E, Ernsrud K, Johnston CAS, Harris S, Santora A, Hirsch L, Oppenheimer L, Thompson D (1997) Prevention of nonvertebral fractures by alendronate: a meta-analysis. J Am Med Assoc 277:1159

Keane J, Gershon S, Wise R, Mirabilie-Levens E, Kasnica J, Schwieterman W, Siegel J, Braun M (2001) Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing ag4ent. N Engl J Med 345:1098

Kindler V, Sappino A, Grau G, Piguet P, Vassalli P (1989) The Inducing Role of Tumor Necrosis Factor In the Development of Bactericidal Granulomas during BCG Infection. Cell 56:731

Lenercept Msg (1999) TNF neutralization in MS: Results of a randomized, placebo-controlled multicenter study. Neurology 53, no. 3:457

Mayordomo L, Marenco J, Gomez-Mateos J, Rejon E (2002) Pulmonary miliary tuberculosis in a pateint with anti-TNF-alpha treatment. Scand J Rheumatol 31:44

Podolsky D (2002) Inflammatory bowel disease. N Engl J Med 347:417

Poll L, Maas M, Terk M, Roca-Espiau M, Bembi B, Ciana G, Weinreb R (2002) Response of Gaucher bone disease to enzyne replacent therapy. Br J Radiol 75:A25

Reszka AA, Halasy-Nagy J, Masarachia PJ, Rodan GA (1999) Bisphosphonates Act Directly on the Osteoclast to Induce Caspase Cleavage of Mst1 Kinase Apoptosis. The Journal of Biological Chemistry 274, no. 49:34967

Sandborn WJ, Targan SR (2002) Biologic therapy of inflammatory bowel disease. Gastroenerology 122, no. 6:1592

Scott DL (2002) Advances in the medical management of rheumatoid arthritis. Hosp Medicine 63, no. 5:1227

Sicotte NL, Voskuhl RR (2001) Onset of multiple sclerosis associated with anti-TNF therapy. Neurology 57, no. 10:1885

Tracey KJ, Cerami A (1994) Tumor necrosis factor: a pleiotropic cytokine and therapuetic target. Annu Rev Medicine 45, no. 1:491

Weinreb N, Charrow J, Andersson H, Kaplan P, Kolodny E, Mistry P, Pastores G, Rosenbloom B, Scott C, Wappner R, Zimran A. (2002) Effectiveness of enzyme replacement therapy in 1028 pateinest with type I Gaucher disease after 2 to 5 years of treatment. Am J Med 113:112

Weisman MH (2002) What are the risks of biologic therapy in rheumatoid arthritis? An update on safety. J Rheumatol 65, no. 29 Sept. Suppl:33

Wright S, Silverstein S (1984) Phagocytosing macrophages exclude proteins from the zones of contact with opsonized targets. Nature 309:359