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THERAPEUTIC STRATEGIES FOR RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis is one of the most common systemic autoimmune diseases, and one of the least understood. It is an autoimmune disease where the body's immune system which is designed to fight infections and help healing wounds goes haywire and attacks its own tissues, especially joints. It is caused by an interaction between a polygenic susceptibility and unknown environmental factors. Usually young adults develop this type of joint involvement and the sufferer feels ill with the small joints, usually fingers, swelling and becoming painful. Later, it often involves all other major joints of the body. With passage of time the joints get destroyed and produce deformities.

Drugs often control the pain, but as of today there is no definite cure. Different therapeutic strategies for the treatment of this disease apart from drugs are immunotherapy, gene therapy and siRNA therapy. RNA interference has rapidly become the method of choice for gene silencing, and from this standpoint siRNA can be an important tool to study gene function in Rheumatoid arthritis. In addition, it may have potential as a novel therapeutic strategy.

Keywords: Rheumatoid Arthritis, immunotherapy, gene therapy, siRNA.

INTRODUCTION

Rheumatoid Arthritis is a crippling autoimmune disease commonly associated with chronic inflammation of the joints. Although considered to be a systematic disorder, the symptoms of rheumatoid arthritis are most prominent in the wrist, knees, proximal interphalangeal and metacarpophalangeal joints. The debilitating effects of the disease occur progressively with time. The synovium normally a thin layer of tissue that lines the internal surfaces of the joint capsule, becomes dramatically thickened and hypercellular from infiltrating leucocytes and proliferating synovial cells. Rheumatoid Arthritis synovial fluid is enriched predominantly with neutrophils, but macrophages, T lymphocytes and dendritic cells are also present. Chronic secretion of inflammatory cytokines by monocytes and macrophages causes the cells in the synovium to become activated, giving the hypertrophied tissue an

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aggressive phenotype. The synovium enlarges to a pannus that attaches to, invades and erodes the articular cartilage and subcondral bone. With time, the cumulative degradation of the joint structures often results in severe disfigurement and loss of function (Ghivizzani *et al*., 2000).

Progression of this disease leads to irreversible joint destruction (Pincus *et al*., 1984; Wolfe, 1996; Wolfe *et al*., 1994). Intra-articular expression of pro-inflammatory cytokines in particular tumor necrosis factor - a (TNF-a) and interleukin-1 (IL-1) b plays a critical role in the pathogenesis of rheumatoid arthritis (Alvaro-Gracia *et al*., 1990). Cytokines are local protein mediators, now known to be involved in almost all important biological processes, including cell activation, growth, immunity and differentiation. Thus, it is not surprising that they have a role in an autoimmune disease such as rheumatoid arthritis, in which there is chronic inflammation, with fibrosis and the eventual destruction of cartilage and bone. The pathology of rheumatoid arthritis extends throughout the synovial joint and in severe cases involves many other organs.

The major site of irreversible tissue damage originates at the junction of the synovium lining the joint capsule with the cartilage and bone, a region often called as the pannus, an area rich in macrophages. The cells of the pannus migrate over the underlying cartilage and into the subcondrial bone causing the subsequent erosion of the tissues (Allard *et al.,* 1987).

The destruction of the cartilage seen in rheumatic disease is now considered to be mostly due to the activity of matrix metalloproteinases (MMPs), the enzymes produced by activated macrophages and fibroblasts in response to proinflammatory cytokines such as IL-1 and TNF-a. These enzymes are synthesized and secreted as latent molecules, which are activated by proteolytic cleavage of the polypeptide domain. In arthritis, the MMP enzyme collegenase (MMP-1) and stromelysin 1 (MMP-3), whose production is increased, play an important role in the destruction process (Vincenti *et al.,* 1994).

The activity of MMPs is regulated to some extent by Tissue Inhibitors of metalloproteinase (TIMP), three forms of which have been cloned in humans. These irreversibly bind the active MMP to form a 1:1 complex with the enzyme. The fact that the TIMPs are produced by same cells that produce the MMPs suggests an intimate role for these inhibitors in regulating matrix turnover, and also that much of the connective tissue destruction associated with arthritic disease is due to an imbalance between the production of the MMPs and that of the specific TIMPs.

Of interest in this regard is the observation that transforming growth factor b (TGFb) and IL-10, two immune - regulatory and anti - inflammatory cytokines produced in rheumatoid arthritis joint, not only inhibit the production of pro - inflammatory cytokines that induce MMPs, but also induce the production of their native inhibitor TIMPs (Wright *et al.,* 1991). IL-1 and TNF-*a* protein are readily detected in synovial fluid (Fontana *et al.,* 1982; Di Giovine *et al.,* 1988; Saxne *et al.,* 1988; Hopkins and Meager, 1988; Hopkins *et al.,* 1988).

TREATMENT OF RHEUMATOID ARTHRITIS

Conventional anti-rheumatic drugs and recently developed biological agents aim primarily at the suppression of inflammatory mediators involved in rheumatoid arthritis. Most of the biological agents neutralize pro-inflammatory cytokines such as TNF-a & IL-1β, and the inhibition of these cytokines effectively ameliorates the synovitis of rheumatoid arthritis (Genovese, 2005). These therapies are effective in suppressing bone destruction, but not in all cases. Synovial hyperplasia has been characterized as a tumor-like proliferation and is thought to be a major cause of destruction of cartilage and bone (Karouzakis *et al*., 2006).

Increased proliferation and insufficient apoptosis of synovial cells might contribute to its expansion, and so elimination of proliferatory synoviocytes in the rheumatoid synovium seems to be a potentially effective treatment of rheumatoid arthritis (Wada *et al*., 2007).

Ideally, the treatment for rheumatoid arthritis would be directed at the etiologic mechanism. Since, there is no hard evidence of a discreet causative element, this type of approach is not feasible. Even without knowing the cause of rheumatoid arthritis, it will be possible to interrupt the inflammatory or proliferative cascade at common key points resulting in the down regulation of the entire disease process (Ghivizzani *et al*., 2000).

As rheumatoid arthritis is considered an autoimmune disorder, it may be possible to deliver genes whose products block stimulatory pathways of immune cells, or alternatively, selectively, remove auto reactive subpopulations of cells from the immune system (Ghivizzani *et al*., 2000).

A point of intervention would be at the level of inflammatory mediators. After activation, immune cells trigger the production and release of various cytokines and cellular signalling molecules that mediate the inflammatory response. Over expression of gene products that block the activity or modulate the production of these molecules has been shown to be an effective means of treating arthritis in animals. Several laboratories have found benefit in targeting *principal proinflammatory cytokines, IL-1 & TNF-a* (Bakker *et al*., 1997; Ghivizzani *et al*., 1998, Le CH *et al*., 1997; Mageed *et al*., 1998; Makarov *et al*., 1996; Otani *et al*., 1996; Quattrocchi *et al*., 1999).

Other options for treatment of rheumatoid arthritis would be to deliver genes whose products inhibit the activity of certain *proteases*, because after activation, synovial fibroblasts, chondrocytes and macrophages secrete large amounts of *stromelysin* and *collagenase* which are capable of degrading matrix proteins in bone and articular cartilage. Antagonists of these mediators include *Tissue Inhibitors of Metalloproteinases*, *TIMP-1, TIMP-2, TIMP-3 and TIMP-4* (Cawston, 1998; Vincenti *et al*., 1994).

Different biotherapeutic interventions apart from drugs are:

1. Immunotherapy for Rheumatoid Arthritis.

2. Gene Therapy.
3. siRNA Therapy.

1. **Immunotherapy for Rheumatoid Arthritis** : Over the past decade, advances in the understanding of the pathogenesis of rheumatoid arthritis, based on studies of human tissues and animal models of disease have led to the identification of a number of molecular targets for immunotherapeutic intervention. Of these TNF-a has been validated as a good target for treatment. Therapies inhibiting TNF-a in patients with active rheumatoid arthritis result in rapid and sustained improvement in symptoms and signs of disease, improvement in the quality of life and protection of joints from structural damage.

   Targeting another proinflammatory cytokine, IL-1 has the potential to retard structural damage to joints in inflammatory arthritis. Administration of IL-1R antagonist (IL-1Ra) results in less impressive control of symptoms than TNF-a blockade, but has demonstrated retardation of radiological progression of joint damage in the short term (Taylor *et al*., 2001). Studies in animal models of arthritis have also demonstrated the therapeutic potential of IL-1 blockade (van den Berg, 2000).

   Recently published studies confirm that the long term use of biologic targeting TNF-a in therapy for rheumatoid arthritis gives rise to rapid and sustained decrease in symptoms and signs of disease by 60-70% from baseline and improvements in the quality of life measurements (Maini *et al*., 1999; Weinblatt *et al*., 1999; Lipsky *et al*., 2000). Furthermore, it has been confirmed that anti-TNF-a therapy protects joints from structural damage (Lipsky *et al*., 2000; Bathon *et al*., 2000) as judged by a reduction in the rate of deterioration detected in the radiographs of the hands and feet, assessed by a scoring system that assesses cartilage and bone loss separately.

   In preclinical studies, another highly effective approach is the combined use of biological agents targeting TNF- a & IL-1 (Bendele *et al*., 2000).

2. **Gene Therapy** : Gene therapy emerged as a novel antiarthritis strategy in the early 1990's as part of the wider movement towards biological therapy.In its most fundamental form, arthritis gene therapy involves the transfer to the body of complementary DNA (cDNA) encoding antiarthritis gene products that are otherwise difficult to administer in a sustained or targeted manner. In many instances, these will be proteins that act as cytokine antagonists, immunomodulators, growth factors, transcription regulators, enzyme inhibitors, antioxidants, antinociceptive agents and so forth. Gene transfer enables the body to synthesize these products endogenously in a continuous, biologically authentic and potentially regulated manner. Moreover, there is evidence that proteins synthesized endogenously as a result of gene transfer have greater biological activity than their recombinant counterparts (Gouze *et al*., 2003; Palmer *et al*.,

2005).There are two types of strategies for gene therapy for rheumatoid arthritis (Evans *et al*., 1999):

(i) The first involves a systematic approach in which exogenous genes are delivered to cells in a certain tissue, or tissues and the secreted gene products are released into the circulatory system where they could modulate disease processes throughout the body.In the systematic gene delivery approach to gene therapy for rheumatoid arthritis, cells in a certain tissue (s) would be modified genetically to secrete an antiarthritic protein that then is released into the circulatory system. The secreted agent then would be distributed throughout the body and serve to block disease at numerous sites simultaneously. The limitations of the systemic approach to gene therapy for rheumatoid arthritis are essentially the advantages of local delivery; exposure of non target tissues to the therapeutic agent may have toxic effects or may compromise the immune system of the patient. Certain proteins likely will require very high levels of synthesis to achieve therapeutic function. With respect to rheumatoid arthritis, this approach may only provide a technologically advanced method of achieving what already can be accomplished by existing routines of intravenous or subcutaneous infections.

(ii) The second approach involves local delivery of exogenous genes to affected joints, where only cells populating the articular tissues would be modified genetically to express the protein or biological agent of choice. With the use of local gene therapy for rheumatoid arthritis, the idea is to modify cells genetically within specific joints such that the activity of the afflicted cells would be redirected towards inhibiting disease pathogenesis. The target tissue for genetic modification primarily has been the synovium. With the exception of cartilage, synovial tissue covers all internal surfaces of the joint capsule. It has a large surface area; it readily captures particles infected intra articularly, and has no membrane separating synovial cells from the joint space. There are several potential advantages to intra articular gene delivery for rheumatoid arthritis. With the use of secreted or soluble gene products, the greatest concentration of the agent occurs at the site of the disease, and there is a reduced exposure on non-involved tissues. As the gene product accumulates within the joint capsule and is not diluted by a large blood volume, the amount of gene expression required to reach therapeutic levels is considerably less. Rheumatoid Arthritis is a systemic disease, usually involving numerous joints, thus, a distinct limitation of local gene delivery is that multiple joints likely will require individual treatment.

3. **siRNA Therapy** : RNA interference (RNAi) is a recently discovered process that utilizes either endogenous or exogenous double-stranded RNA species to inhibit expression of genes in a highly sequence specific manner and is rapidly supplanting antisense methods (Zamore, 2002; Tuschl & Borkhardt, 2002;

Schiffelers *et al*., 2004). In mammals, RNAi can be invoked by introduction of short (19-21 nucleotides) double stranded RNA oligonucleotides called small interfering RNA (siRNA). The siRNA is taken up by RNA- inducing silencing complex in the cytoplasm and silences expression of specific messenger RNA with a complementary sequence. Therefore, siRNAs offer promise as a novel therapeutic strategy and in addition, may be used as a tool for functional genomics to elucidate genes controlling disease pathways (Schiffelers *et al*., 2005).

Rheumatoid Arthritis may benefit from a therapeutic strategy based on the use of siRNA. The disease is characterized by involvement of multiple gene products, whose inhibition can strongly reduce joint inflammation (Arend, 2001). In addition, the exact roles of many of the genes that influence the disease are still unknown (Lubberts, 2003; D'Ambrosio *et al*., 2003) and siRNA could be a valuable tool to study the function of different genes in disease progression (Tuschl, 2002).Unfortunately, siRNA is a relatively large, highly charged molecule and does not readily enter cells to reach its intracellular site of action. Therefore, delivery strategies that enhance intracellular uptake and availability need to be devised. RNA interference had rapidly become the method of choice for gene silencing experiments, and from this standpoint siRNA can be an important tool to study gene function in rheumatoid arthritis. In addition, it may have potential as a novel therapeutic strategy.

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