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Biomarkers for diagnosing and monitoring autoimmune diseases

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Abstract

The goal of studies of autoimmune disease biomarkers is to identity markers that fluctuate with disease development and severity, but then normalize following successful therapy. The perfect marker could thus serve as a diagnostic tool, as well as a monitoring device for therapeutic drug efficacy. Current biomarker discovery efforts are focused on three groups of proteins reflective of the autoimmune disease process: (1) degradation products arising from destruction of affected tissues, (2) enzymes that play a role in tissue degradation and (3) cytokines and other proteins associated with immune activation. Potential biomarkers for two autoimmune diseases, rheumatoid arthritis and multiple sclerosis, have been described in recent publications. For rheumatoid arthritis, these markers (by group) include (1) aggrecan fragments, C-propeptide of type II collagen and cartilage oligomeric matrix protein, (2) matrix metalloprotease (MMP)-1, MMP-3 and MMP-1/inhibitor complexes and (3) thioredoxin, IL-16 and tumour necrosis factor (TNF)-α. For multiple sclerosis, they include (1) neurofilament light protein and glial fibrillary acidic protein, (2) MMP-2 and MMP-9 and (3) TNF-α and soluble vascular adhesion molecule-1. The utility of most of these markers is limited by their restriction to relatively inaccessible anatomic sites (synovial or cerebrospinal fluid). Thus, from a practical standpoint, the most useful autoimmune biomarkers will be those measurable in serum or plasma.

Keywords: *Biomarkers, rheumatoid arthritis, multiple sclerosis*

Introduction

Autoimmune disease results when the immune system goes awry and recognizes self tissues as foreign. Both autoantibodies and autoreactive cellular responses contribute to the ongoing autoimmune disease process and the damage may be organ-specific or systemic. Inflammatory cells are recruited to the affected organ(s), where they either release tissue-degrading proteolytic enzymes or upregulate the release of proteolytic enzymes from other cells.

The field of biomarker discovery in autoimmune diseases is still in its infancy compared to other areas such as cardiovascular disease and cancer (see related articles in this issue). The most useful biomarker for autoimmune disease would be a marker that reaches abnormal levels (either increased or decreased compared to controls) in conjunction with disease development, fluctuates in relation to disease severity and normalizes following successful treatment. Such a marker would not only be a valuable diagnostic tool but also a device for monitoring the response to therapy.

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Autoantibodies are typically not good biomarkers, mainly because they tend to remain detectable, even after successful treatment.

Current efforts to identify autoimmune disease biomarkers are focused on three groups of proteins reflective of the autoimmune disease process. These groups include: (1) degradation products arising from destruction of the affected tissue(s), (2) enzymes that play a role in tissue degradation and (3) cytokines and other proteins associated with immune system activation and the inflammatory response. This article will briefly discuss possible biomarkers within these three groups of proteins for two representative autoimmune diseases targeted for biomarker discovery, namely rheumatoid arthritis and multiple sclerosis.

Rheumatoid arthritis (RA)

RA is characterized by persistent inflammation of joint synovium, leading to erosion of cartilage and bone. Inflammatory cells, recruited to the synovium by an unknown stimulus, release cytokines that initiate a cascade of synovial fibroblast proliferation and joint destruction (Poole and Dieppe 1994, Jikimoto et al. 2002).

Degradation products of affected tissues

Aggrecan fragments. Along with collagen, aggrecan is a major structural component of cartilage. It is cleaved by metalloproteases to produce fragments that express neoepitopes due to the exposure of new terminal amino acid sequences (Poole and Dieppe 1994). Researchers have created these fragments *in vitro* and raised antibodies to the neoepitopes in mice; the mouse antibodies are then used to identify specific aggregan fragments in synovial fluid (SF). Aggregan fragments are increased in SF of RA patients and the levels are related to disease severity (Fosang et al. 2003). However, no data are yet available on what happens to aggregan fragment levels in SF following treatment.

C-propeptide of type II collagen. This protein is released from procollagen as collagen is incorporated into forming fibrils outside the cell; thus, C-propeptide levels in SF are directly proportional to the rate of collagen synthesis (Poole and Dieppe 1994). SF levels of C-propeptide are increased in RA, perhaps indicating increased collagen synthesis in response to increased collagen degradation (Mansson et al. 1995). Post-treatment levels of C-propeptide in SF have not yet been extensively characterized.

Cartilage oligomeric matrix protein (COMP). COMP is a non-collagenous protein component of cartilage that is released as cartilage is degraded. COMP is increased in both serum and SF from RA patients and is a marker of early destruction of cartilage; increased levels of COMP levels are usually detectable before there is radiologic evidence of cartilage damage. In newly diagnosed RA patients, increased COMP is a strong predictor of oncoming aggressive disease; these patients are usually targeted for more aggressive therapy (Poole and Dieppe 1994, Skoumal et al. 2003, Christgau and Cloos 2004). Long-term treatment with glucocorticoids or monoclonal anti-tumour necrosis factor (TNF)-α leads to decreased levels of COMP in serum and SF (den Broeder et al. 2002). COMP, thus, represents one of the most promising biomarker candidates for RA.

Enzymes involved in tissue degradation

Matrix metalloproteases (MMPs). MMPs are produced by macrophages found in the inflamed rheumatoid joint, as well as by synovial fibroblasts activated by inflammatory cytokines. MMP-1, also known as collagenase, is increased in serum from RA patients. However, this elevation is not specific to RA; increased serum levels are also observed in osteoarthritis (OA) and systemic lupus erythematosus (SLE). MMP-3, also known as stromelysin, is increased in serum from RA and SLE patients, but not OA patients. Levels of MMP-3 are strongly correlated with inflammatory markers such as C-reactive protein and reflect disease severity in RA (Keyszer et al. 1999). Serum levels of MMP-3 decrease following treatment of RA patients with anti-TNF-α (Christgau and Cloos 2004).

MMP-1/inhibitor complexes. Recent studies have demonstrated that complexes of MMP-1 and its inhibitor are increased only in RA patients (Keyszer et al. 1999). However, data on the relationship of complex levels to disease severity and changes in response to treatment are not yet available.

Immune activation markers

Thioredoxin. Thioredoxin is a ubiquitous protein that regulates cellular functions via thiol redox control. It is induced by reactive oxygen species produced by inflammatory leukocytes. Extra-cellular levels of thioredoxin are increased in SF and plasma from RA patients and the levels correlate with disease activity (Jikimoto et al. 2002). However, no studies have characterized changes in thioredoxin levels following therapy in RA.

Interleukin (IL)-16. This cytokine, produced by activated synovial fibroblasts, induces chemotaxis of T-helper cells and monocytes. Plasma levels of IL-16 significantly correlate with joint damage in RA, but not disease activity (Blaschke et al. 2001, Lard et al. 2004). Further studies are needed to systematically assess changes in SF IL-16 levels following therapy.

TNF-alpha. The pro-inflammatory cytokine TNF-α is a major mediator of leukocyte recruitment to the rheumatoid joint and synovial inflammation. It stimulates synthesis of collagenases by chondrocytes and activates fibroblasts to secrete metalloproteases. SF levels of TNF-α are increased in RA and the levels correlate significantly with markers of cartilage degradation (Mansson et al. 1995, Maricourt et al. 2000). As reviewed by Song et al. (2002), monoclonal antibodies to TNF-α and recombinant TNF receptors are highly effective therapies for RA.

Myeloid-related protein 8 (MRP8). Related to migration inhibition factor, MRP8 regulates leukocyte transmigration to sites of inflammation. Surface-enhanced laser desorption/ionization (SELDI) mass spectrometry has identified MRP8 as a protein expressed specifically in SF of RA patients, but not OA patients (Uchida et al. 2002). In the juvenile form of RA, serum levels of MRP8 are increased 120-fold compared to controls and levels decrease markedly following drug therapy or autologous stem cell transplantation (Frosch et al. 2003, Wulffraat et al. 2003). Thus, along with COMP, MRP8 is one of the most promising biomarker candidates for use in monitoring RA disease activity and treatment.

Multiple sclerosis (MS)

MS is associated with autoimmune-mediated inflammation of the central nervous system leading to demyelination and axonal damage. A clear role for T-cells and monocytes in myelin destruction has been identified (Kieseier et al. 1999).

Degradation products of affected tissues

Neurofilament light protein (NFL). NFL is the most abundant cytoskeletal component in large myelinated axons; its release into the cerebrospinal fluid (CSF) is thus a marker of axonal damage (Malmestrom et al. 2003). CSF levels of NFL are increased during all stages of MS; thus, NFL is not a particularly good marker of disease severity (Malmestrom et al. 2003, Uccelli et al. 2003, Haghighi et al. 2004). Changes in NFL levels following treatment have not been extensively characterized.

Glial fibrillary acidic protein (GFAP). This protein represents the intermediate filament of fibrillary astrocytes; it is the main protein constituent of the chronic MS lesion. The CSF level of GFAP is strongly correlated with the extent of neurologic deficits and is, thus, a good biomarker for clinical disability and perhaps disease progression (Malmestrom et al. 2003). However, changes in CSF GFAP levels following therapy have not been characterized.

Enzymes involved in tissue degradation

MMP-2. MMP-2 degrades extra-cellular proteins and disrupts the subendothelial basement membrane, thus enabling the transmigration of inflammatory cells. CSF levels of MMP-2 are increased in chronic progressive MS, but not relapsing and active forms of MS (Uccelli et al. 2003). No data are available on MMP-2 levels following treatment in MS.

MMP-9. CSF levels of MMP-9 are increased in relapsing and active forms of MS and levels correlate with blood–brain barrier breakdown and disease activity. CSF and serum levels of MMP-9 decrease in parallel with clinical improvement following treatment with interferon-β (Uccelli et al. 2003, Miller et al. 2004) or high-dose methylprednisolone (Kieseier et al. 1999). MMP-9 is, thus, a good candidate for a useful biomarker in MS.

Immune activation markers

TNF-alpha. Levels of this cytokine are increased in both serum and CSF of MS patients and the levels strongly correlate with clinical impairment and blood–brain barrier damage (Kieseier et al. 1999). Treatment with glucocorticoids reduces TNF-α levels for several months, whereas interferon-β treatment, known to be clinically effective (Sorensen et al. 2003), does not appear to alter TNF-α levels (Kieseier et al. 1999). Thus, the value of TNF-α as a biomarker is questionable with regard to its utility in monitoring therapeutic responses.

Soluble vascular adhesion molecule-1 (sVCAM-1). VCAM-1 is found on the surface of accessory cells and activated endothelial cells, where it plays a major role in movement of leukocytes from the circulation to the central nervous system. The soluble form results from either shedding or cleavage from the cell surface and probably plays a role in de-adhesion (Kieseier et al. 1999). This soluble form is increased in serum and

CSF of MS patients (Baraczka et al. 2003); however, longitudinal studies demonstrated no significant correlation between sVCAM-1 levels and clinical relapse (Kieseier et al. 1999). Methylprednisolone treatment had no effect on sVCAM-1 levels, whereas interferon-β treatment led to a further increase in sVCAM-1 levels (Kieseier et al. 1999).

Conclusion

Many proteins are increased at tissue sites affected by autoimmune diseases, but only a small number of them show promise as useful biomarkers. Although good progress has been made in relating the levels of these proteins to disease activity and severity, much more work is needed to characterize changes in response to treatment.

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