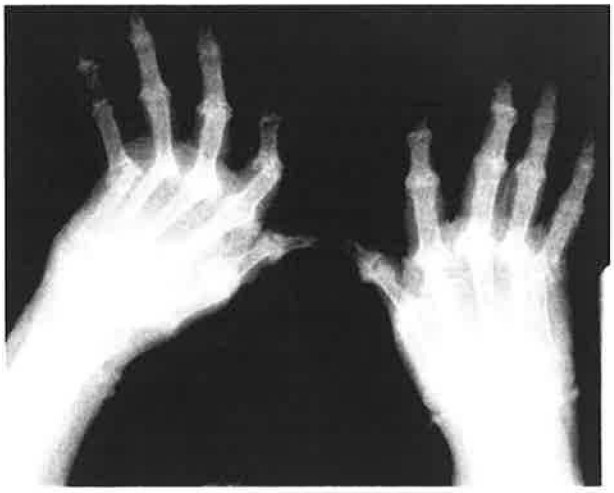
Can Patients With Rheumatoid Arthritis Be Cured?



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Internal Medicine Grand Rounds
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This is to acknowledge that Dr. Karp has disclosed financial interests of other relationships with commercial concerns related directly to this program. Dr. Karp will be discussing off-label uses in his presentation

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Dr. Karp's research interests include:

- The effect of oxidative stress in the development and function of the immune system
- The role of complement in adaptive and autoimmunity
- Development of novel therapies for autoimmune diseases

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Why is Early Diagnosis of Rheumatoid Arthritis Important?

Rheumatoid arthritis (RA) affects nearly 1% of the population. It causes significant pain, dysfunction, and disability. As both an autoimmune and inflammatory disorder, RA has attracted the study of clinicians and immunologists for the last half century. The result has been the development of new pharmacological and biological therapies that have transformed the lives of RA patients in the last five years. Clinical trials to study the effectiveness of medications such as leflunomide, etanercept, infliximab, and adalimumab demonstrated the ability of prompt and aggressive treatment to not only alleviate the signs and symptoms of RA, but more importantly, to retard the progression of radiological damage.

The success of these agents and their relative safety has prompted rheumatologists to speculate about "curing" what is currently a lifelong condition. Is it possible to intervene in the immune pathogenesis of this disease with the appropriate drug or combination of drugs in such a manner so that joint inflammation does not return? If that hypothetical treatment were applied before joint damage has occurred, then would a lifetime of dysfunction be prevented?

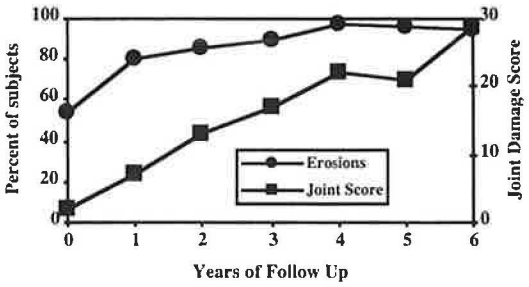
Most rheumatologists agree that if such a therapeutic strategy to be possible, it needs to be implemented early on in the course of RA. This "window of opportunity" may represent a time when the patient's condition is not clearly defined as RA by current criteria. The classic appearance of symmetric, inflammatory polyarthritis may not be present. Rheumatoid factor, the most commonly used serological marker for RA, may be absent or present only in equivocal quantities. The clinical and laboratory features of these patients will overlap with those who eventually will turn out to have other conditions: other autoimmune diseases, viral infections, endocrinopathies, crystal-induced arthropathies, paraneoplastic syndromes, etc. Subjecting these patients to intense anti-rheumatic therapy will increase their risk and delay proper treatment.

The costs of failing to diagnose and treat RA early are very high. In economic terms, the annual direct costs of RA have recently been estimated at ~$6,000 per person (1). Annual indirect costs such as work disability have been estimated at ~$3,200 per person in a recent Spanish study (2), while employer costs were nearly $18,000 per year in a study of US patients (3). Together, these data suggest that the overall economic cost of RA in the US alone is nearly $20 billion. Moreover, there is a direct relation between loss of function and costs, such that direct medical costs to patients in the fourth quartile of functional ability were more than 2.5 times greater than those in the first quartile. The distribution of costs is highly skewed, with those individuals in the 95th and 100th percentiles incurring costs of ~$31,000 and ~$85,000 per year.

Of course, it is human costs that drive the economic costs. Even early in the course of RA, up to 42% of patients are work disabled in the first 36 months of disease (4). Over 25% have had income reductions due to their RA. Patients with RA have more co-morbid disease than do age matched controls. The incidence of infection and lymphoma are increased in RA, irrespective of therapy. One of the most striking recent observations is the risk of cardiovascular disease in RA (5-8). Data in this regard was recently published from the Nurses Health Study (9). In that analysis, 114,342 women between the ages of 30 to 55 were studied for the acute myocardial infarction rates. In this study, women with RA had a two-fold increase in the risk of MI compared to controls after adjustment for other cardiovascular disease risk factors. Thus, RA is an independent risk for atherosclerosis, with evidence pointing to the role of inflammatory

mediators and endothelial dysfunction (5, 10-14). The morbidity of RA combined with increased co-morbidities leads to a shortened life span of up to 10 years in RA patients compared to controls (15, 16). While therapy with methotrexate has appeared to improve the mortality associated with RA, the effect of more specific biological agents is not yet known (17).

Joint Damage Occurs Early

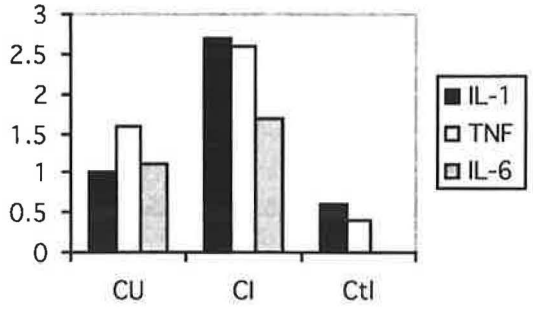


Underlying much of the economic costs, disability, and morbidity of RA is joint destruction. Approximately 70% of patients develop radiographic joint damage in the first two years of their disease (18, 19). 40% of patients will have evidence of at least one bone erosion in the first six months of symptomatic RA (20). Joint damage occurs in a linear, progressive manner during the earliest years of disease. 502 patients who had RA for less than one year were followed prospectively (21). 54% had one or more eroded or one or more narrowed joint on radiographs at baseline. After six years of follow up, over 95% had an eroded or narrowed joint. While the percent of patients with radiographic damage reached a plateau, the number of new abnormalities continued to accumulate over time. This study was ended in 1996, and therefore reflects the natural history of joint damage before the use of more aggressive or targeted therapies.

Prior to the development of joint damage, it is the pain and dysfunction from synovial inflammation that brings RA patients to seek medical care. It has been clear for over twenty-five years that there is a relationship between inflammation (as judged by CRP or ESR) and the rate of radiographic progression (22-26). More importantly, therapies that control inflammation also retard radiographic progression. For example, the use of what are now considered 'first-line' treatments for RA (methotrexate and/or sulfasalazine) was shown decrease cumulative CRP values over the first 24 months of disease by 35% when compared to what was once the traditional stepwise treatment with non-steroidal anti-inflammatory drugs, parenteral gold, and late use of more efficacious drugs (23). This was accompanied by 26% decrease in radiographic progression over this period of time. While some of these retrospective and prospective follow-up studies showed only modest gains, they were sufficiently persuasive such that radiographic stabilization, and not just improvement in signs and symptoms, is a required outcome for large clinical trials of new therapies (see below).

Unfortunately, clinical symptoms may not be present event in the face of sub-clinical synovitis. While the physician writes, "Doing well," in the chart, the patient may be developing radiographic damage and losing function.

It is also clear that synovial inflammation occurs before there is clinical evidence of RA. Arthroscopic biopsies of clinically un-inflamed joints revealed early proliferation of the synovial lining layer and infiltration with mononuclear cells (27). In study of paired synovial biopsies from ten RA patients with one clinically involved knee and one uninvolved knee, there was a accumulation of macrophages and T cells in the sublining layer of the uninvolved knee that was



Expression of cytokines in clinically uninvolved (CU), clinically involved (CI). and control (CtI) knees (28).

not seen in control subjects (28). Immunohistochemical staining for cytokines revealed that IL-1β, TNF-α, and IL-6 were all present in increased quantity in the clinically normal knees. Finally, newer imaging techniques such as musculoskeletal ultrasound and magnetic resonance imaging have been able to demonstrate extensive synovitis and bony changes early in the course of rheumatoid arthritis (29).

Once established, true rheumatoid arthritis rarely (never?) undergoes sustained remission. In a 1996 analysis of a community based early (< 1 yr) arthritis inception cohort, only 5% of 258 RA patients had a sustained remission at both one and two years after disease onset. Approximately 23% had remission at some time during the study (30). Patients with inflammatory arthritis who did not meet formal criteria for RA did somewhat better, with 19% in long-term remission and 50% achieving remission at some time. In this study, remission was defined as the absence of objective synovitis while off medication for at least three months. A Swedish study looked at 183 patients with definite RA who started treatment within the first two years of symptoms (31). Remission was defined as the two-month absence of signs and symptoms of synovitis, as well as normalization of the ESR. Patients were treated with disease modifying anti-rheumatic drugs (DMARDS) as needed. During a five-year follow up, 20% of patients had at least one episode of remission lasting a mean of 20.5 months. However, remission represented only 7% of the entire follow up time. Thus, for most patients, sustained disease activity is the rule not the exception. Current research using more detailed analysis (synovial biopsy, MRI, etc) may show that even fewer patients truly undergo complete remission of their synovitis.

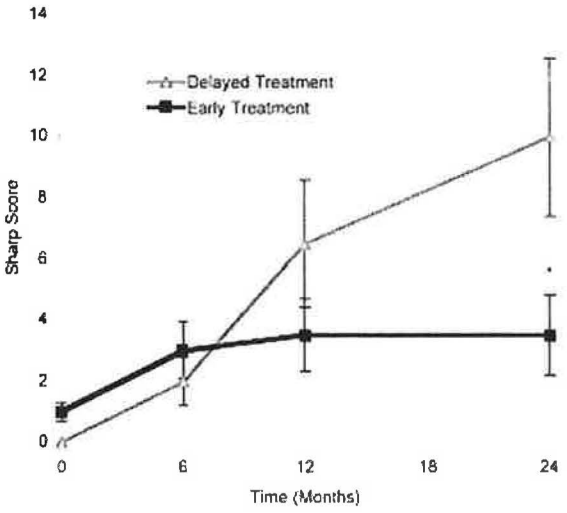
In summary, early diagnosis and treatment of RA is essential to prevent long-term joint destruction and disability. Synovitis is present even in clinically normal joints, and uncontrolled synovitis leads to joint damage. Such progression is typical as spontaneous remission is rare. Synovitis can be suppressed and joint destruction halted. It has taken rheumatologists over fifty years to realize that RA has features of a malignancy, and that the principles of oncologic management – early detection, prompt treatment, and aggressive, multi-faceted therapy – are needed to achieve a remission.

KEY POINTS:

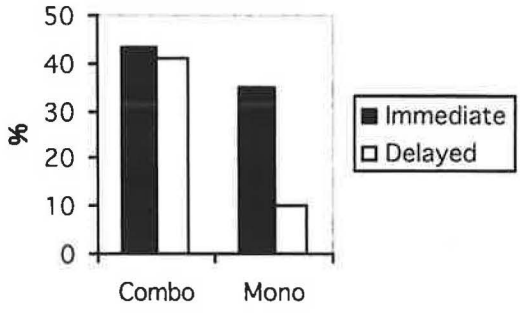
- **Joint damage occurs early**
- **RA is typically a progressive disease**
- **Subclinical synovitis is widespread**
- **Remission is rare**
- **Inflammation x Time = Damage**
- **RA is a "medical emergency" worthy of prompt and aggressive care**

The Evidence For Early Treatment

A large number of clinical trials have been done to support the hypothesis that early and aggressive treatment of RA prevents joint damage and disability (reviewed in (32, 33)). It is not surprising now that the original placebo-controlled studies showed reductions in both inflammation and radiographic changes in patients treated with hydroxychloroquine or sulfasalazine. More impressive are the studies showing that even a short lag in the institution of treatment has profound effects (34, 35). Lard, *et al.*, described the treatment of two groups of early RA patients seen in the same clinic (35). The first group was seen from 1993 to 1995 and had symptoms for 162 days at the time of the first visit. They were treated with analgesics alone for until it was determined that they definitely had persistent disease. At a median of 123 days of therapy, they were switched to chloroquine or sulfasalazine. The second group of patients was seen from 1996 through 1998. At that time it was decided to treat all patients with the same disease-modifying drugs immediately. This group had symptoms for a median of 128 days at first visit, and began chloroquine or sulfasalazine 15 days after that (in addition to analgesics). Patients were followed for two years. There was a similar decline in disease activity for both groups, although the CRP level decreased faster in the patients treated immediately. For the first six months, the score of radiographic damage was the same in both groups of patients. At that time, it leveled off in the immediate treatment group, and continued to progress in a linear manner for the delayed treatment group. Even though these patients received what is now considered relatively mild therapy, the short (4 month) delay in treatment caused them to miss a critical window to prevent permanent joint damage.



Fin-RACo



Percent of patients in remission after 2 yr according to combination or mono-therapy and immediate or 4 mo delayed treatment (36).

The effect of delayed treatment has also been seen in randomized trials of therapy. The Fin-RACo study looked at 195 consecutive Finnish RA patients with onset of disease less than six months (36). They were randomly assigned to either monotherapy with sulfasalazine (± prednisolone) alone, or combination therapy with sulfasalazine, hydroxychloroquine, methotrexate, and prednisolone. The primary outcome was remission of disease on treatment. The initial analysis of the data clearly showed that combination therapy was more efficacious (37% vs. 18% in remission at two years), and was

associated with less radiographic progression. The data were then analyzed for factors that could predict remission (37). A multivariate analysis was done using age, gender, RF positivity, diagnostic criteria fulfilled, HLA haplotype, and delay in institution of treatment as variables. Of these, only a delay in treatment was statistically significant in predicting which patients would achieve remission in the monotherapy group. Those RA patients treated in the first four months of their symptoms were three times more likely to achieve remission than were those whose treatment started later. There was no effect of a delay of therapy in the patients receiving combination therapy, suggesting that more aggressive treatment is required to make up for even a short delay in treatment.

COBRA

Another example of how aggressive therapy has long-term benefits is the COBRA (Combinatietherapie Bij Rheumatoide Artritis) study, a multicenter, randomized trial in the Netherlands and Belgium (38). The goal of this trial was to determine the effect of very aggressive (at the time) therapy early in RA and then to taper medications to a potentially less toxic regimen. Between 1993 and 1995, 155 patients with early (median duration, 4 months) RA were randomly assigned to either sulfasalazine alone, or a combination of drugs. Prednisolone, was started at 60 mg/d, and then tapered to 7.5 mg/d for a total of 28 weeks. Methotrexate (7.5 mg/wk) was given for 40 weeks, and sulfasalazine was administered throughout the 56-week trial. As expected, the clinical outcome (a composite of physical exam, ESR, and patient questionnaires) improved more rapidly and to a greater extent in the combination therapy group than in the single therapy group. However, by the end of the trial, the two groups had converged, indicating a loss of efficacy after withdrawal of the methotrexate and corticosteroids (39). At the end of the randomized trial, the patients were returned to their own rheumatologist for routine care. This therapy was not specified, but patients were followed for up to five years after that. During this time, disease activity and disability measures remained constant for both groups of patients. However, there was significantly greater progression in radiographic damage in the patients originally treated with sulfasalazine alone. Thus, it appears that early, aggressive therapy can have long-lasting effects on joint destruction.

A meta-analysis performed by Anderson, *et al.*, underscores the need to start treatment early (40). They analyzed the primary data from 14 randomized and controlled trials of non-biological agents in RA. The strongest predictor for clinical response was disease duration. In 1,435 patients studied, 53% of patients treated within the first year of symptoms had a measurable response, while only 35% of patients enrolled in trials after 10 years of disease did so. The overall response rate was mirrored by individual outcome measures such as tender and swollen joint counts and ESR. These data have importance not only in the design and interpretation of clinical trials, but also the expectations of individual patients and physicians starting or changing therapy.

ATTRACT

These studies set the stage for the inclusion of radiographic progression as an outcome in the Phase III clinical trials of biologic agents. The ATTRACT trial was a randomized, double-blind trial of methotrexate plus placebo compared to methotrexate plus the monoclonal anti-TNF antibody, infliximab (41). 428 patients with long-standing RA that was active despite

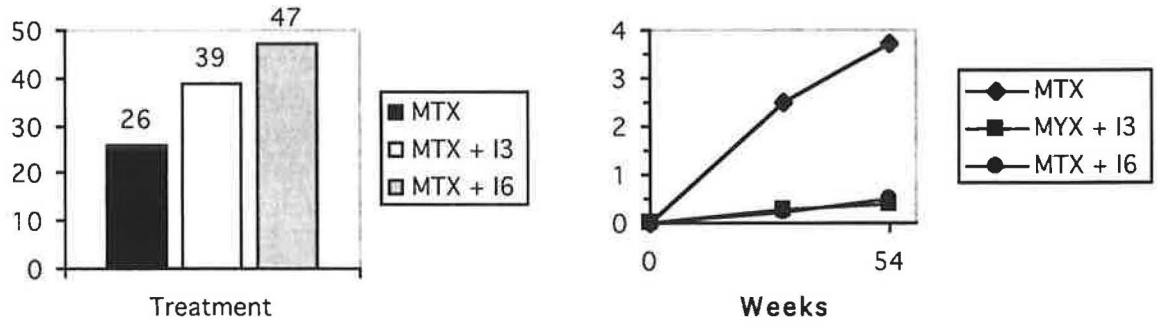
methotrexate therapy were randomized to either placebo or four different dose schedules of infliximab. The clinical responses to methotrexate plus the anti-TNF agent were far superior to methotrexate alone. One of the most striking features of the trial was the effect on radiographic progression. Radiographs of the hands were obtained at baseline and intervals throughout the 54-week trial. While patients treated with methotrexate had continued erosion and joint space narrowing, these changes were practically halted in all patients receiving infliximab. The magnitude of these differences was unlike any other study in RA, and set a new standard for disease management.

Etanercept in Early RA (ERA)

The findings of the ATTRACT trial were replicated in two other studies involving only early RA patients. The first looked at the soluble TNF receptor, etanercept, in 632 patients with RA for less than 3 years (42). They were randomized to either 25 mg of etanercept twice a week or up to 20 mg per week of methotrexate. In the first year of the trial, both groups had substantial clinical responses to either treatment arm, suggesting that aggressive use of methotrexate may be just as efficacious as the biological response modifier. With regard to radiographic progression, however, the patients treated for two years with etanercept had significantly fewer erosions and joint space narrowing that the methotrexate patients, although both groups had less progression than predicted.

ASPIRE

Finally, the ASPIRE trial was a 54 week study of infliximab plus methotrexate vs. methotrexate alone in over a thousand clinically active early RA patients (mean duration 7 months). Infliximab was given at either 3 mg/kg or 6 mg/kg, and the methotrexate dose was increased to 20 mg/week as tolerated. At the end of 54 weeks, all treatment groups showed a significant clinical improvement that ranged from 26% in the methotrexate group to 47% in the high dose infliximab plus methotrexate group. Once again, the most striking outcome from this study was the fact that while both methotrexate alone and in combination with infliximab slowed the radiographic damage compared to historical controls, those patients receiving the anti-TNF antibody had virtually no change in the structure of their joints during the trial.



Data from ASPIRE: *Left panel* – Percent improvement from baseline in signs and symptoms of RA. *Right panel* – Change in radiographic score. MTX: methotrexate; I3: infliximab, 3 mg/kg; I6, infliximab, 6 mg/kg.

RA PATHOPHYSIOLOGY – A BRIEF REVIEW

A major obstacle in the early diagnosis and treatment of RA is the fact that, after half a century of study, there is still no clear cause of the disease identified. All that we have are increasingly detailed models based on accumulation of evidence from different types of study. In effect, the models of RA pathophysiology over time reflected the increasing use of more detailed experimental techniques. The discovery of rheumatoid factor (RF) in the 1930's and 40's led to the discovery that RA was, in fact, a systemic autoimmune disease. RF binds to the Fc portion of immunoglobulin, forming immune complexes that can activate complement. This discovery focused attention on autoreactive B cells as the underlying cause of RA. However, RF is not unique to RA, and immune complexes are often found in other conditions that do not produce chronic arthritis. Thus B cells and autoantibodies do not adequately explain the features of the rheumatoid synovium.

In 1976, Stastny, *et al.*, demonstrated the association between HLA-DR genes and RA (43). The function of HLA-DR molecules is to bind antigenic peptides and present them to T lymphocytes. Subsequent studies have shown that the association of HLA-DR with RA actually resides in a specific set of amino acid residues found in the DR β chain (44, 45). This sequence – Q(K/R)RAA – is termed the 'shared epitope' and is present in multiple HLA-DR alleles that are associated with RA: DRB1*0101, *0401, *0404, and *1402. Its presence not only raises the risk of developing RA, but also is associated with severity and extra-articular manifestations. The link between HLA-DR and rheumatoid arthritis implies the involvement of 'arthritogenic' T cells. Such autoreactive T cells are the *sine qua non* of animal models of RA in which rodents are hyperimmunized with collagen to produce both cellular and humoral autoimmunity. CD4 T cells make up a large proportion of the synovial infiltrate in established RA. Although the T cells in the synovium are polyclonal, T cells from patients have also been identified that can be stimulated *in vitro* by collagen, proteoglycans, heat shock proteins and other candidate antigens. Unfortunately, no unique T cell has been isolated from RA patients.

In the 1980's molecular techniques for the quantification of cytokines became available. It was then realized that despite large numbers of T cells in RA synovium, the levels of T cell derived cytokines (IL-2, IFN-γ) were low. By contrast, inflammatory products of macrophages and fibroblasts (TNF-α, IL-1β, IL-6, IL-8, IL-15, IL-18, and GM-CSF) were high (see (46, 47)). In addition, levels of anti-inflammatory proteins (IL-1Ra, IL-10, TGF-β) were low, leading to an imbalanced cytokine network. This hypothesis was refined in the 1990's with the development of anti-cytokine therapies: anti-TNF monoclonal antibodies, soluble TNF receptors, and recombinant IL-1 receptor antagonist. These agents work by targeting 'master cytokines' whose effects are not only as inflammatory mediators, but also as inducers of other cytokines. Their ability to control synovitis is substantial, but not universal and disease recurs if the anti-cytokine therapy is stopped. Thus, the cytokine network is not the only answer to RA pathophysiology.

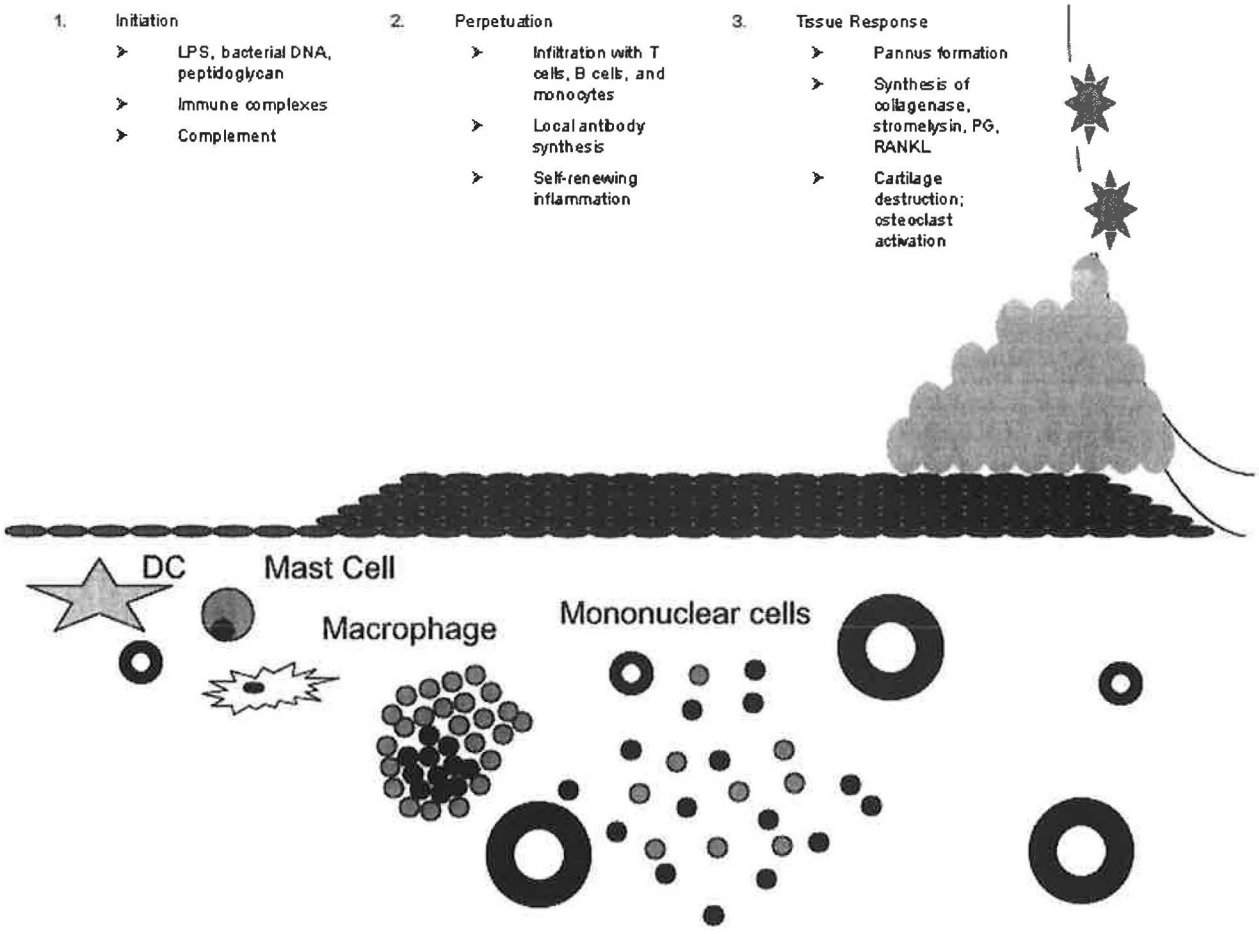
The current view of rheumatoid synovitis takes into account data from both early and advanced RA histopathology, as well as animal models and clinical trials (48). The normal synovium consists of lining layer one or two cells thick, composed of fibroblasts and resident macrophages. The sub-lining is a layer of loose connective tissue containing a few blood vessels, adipocytes,

and nerves. The few mononuclear cells that are present in the sublining are part of the innate immune system – macrophages, dendritic cells, and mast cells.

The first step in the production of synovitis is probably a non-specific stimulus in the synovial sub-lining. This could be trauma, viral infection, the deposition of immune complexes, or activation of macrophages through Toll-like receptors that recognize bacterial products. This non-specific joint inflammation may typically be a self-limited phenomenon. If however, activation of the innate immune system is such that sufficient GM-CSF and TNF-α are produced, there will be maturation of dendritic cells that will travel to draining lymph nodes where they will activate autoreactive T cells. This might only occur in the presence of certain HLA-DR alleles, or Fc receptor or cytokine polymorphisms. Meanwhile, cytokines, chemokines and angiogenic factors are produced that lead to new blood vessel growth and differentiation, tissue edema, macrophage accumulation, and influx of autoreactive T and B cells.

The second phase of synovial inflammation involves a unique interplay between the lining and the sub-lining. The lining layer becomes hypertrophic, with fibroblasts secreting cytokines such as IL-6 and IL-8. There is a massive influx of memory phenotype T cells and B cells that form aggregates or even germinal centers that resemble lymph node or spleen. These T cells can secrete cytokines that promote local immunoglobulin synthesis and further stimulate macrophages to produce large quantities of TNF-α and IL-1. Continued stimulation of antigen presenting cells leads to activation of other autoreactive T cells and development of self-perpetuating inflammation. Unfortunately, this is likely to be the point when RA becomes clinically evident. At this point, substantial changes have occurred in the synovium and it is unlikely that they are reversible.

The last stage of rheumatoid synovitis is the tissue response. Fibroblasts in the lining layer proliferate in a tumor-like condition to form the 'pannus', a poorly differentiated tissue that invades articular cartilage. These fibroblasts synthesize matrix metalloproteinases such as collagenase and stromelysin, as well as prostaglandins, leukotrienes, and the cytokines IL-6 and TNF-α. They also secrete RANK (receptor activator of NF-κB) ligand. RANK ligand, which is also expressed by activated T cells, regulates the differentiation of osteoclast precursors into mature osteoclasts. Under constant stimulation from the sub-lining, the synovial lining is thus transformed into an invasive tissue that degrades cartilage and promotes bone erosion.



Schematic model of rheumatoid synovitis

Serum Transfer Mouse Model – A Clue to Human Disease?

While current therapies have been targeted to the cell types and pathology that is present in established RA, a recently described animal model has re-shaped current hypotheses. The group headed by Mathis and Benoist, now at Harvard, had created a transgenic mouse in which all the T cells expressed a T cell receptor for bovine ribonuclease (49). When this T cell was present in mice of the correct MHC haplotype, all the mice developed spontaneous, destructive, inflammatory arthritis of the peripheral joints. The histopathology of this arthritis mirrors that of spontaneous human RA. Subsequent experiments revealed that this T cell was recognizing a self-antigen that turned out to be glucose-6-phosphate isomerase (GPI) (50). This is a ubiquitous intracellular enzyme that is part of the Krebs cycle. The auto-reactive T cells provide help for anti-GPI B cells that secrete immunoglobulin. GPI is released from dying cells and binds non-specifically to articular cartilage (51). There, it forms immune complexes with anti-GPI antibodies that initiate a self-perpetuating joint inflammation. It should be noted that some, but not all, RA patients have antibodies to human GPI (52). Nor is it specific for rheumatoid arthritis. Thus, it is probably not the cause of human disease.

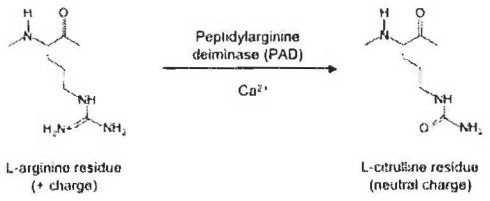
The most important part of this model is the fact that since GPI is normally bound to cartilage, arthritis can be induced in mice of any genetic makeup by the transfer of anti-GPI containing

serum or purified antibodies. Using this technique, the Mathis group and others have shown the primary role of the innate immune system in the generation of inflammatory arthritis. Inflammation does not occur in mice that lack neutrophils (53), certain Fc receptors (54), and complement components, particularly C5 or the C5a receptor (55). Interestingly, arthritis does not occur in mice that lack mast cells, suggesting that these cells, which are resident in the synovial sub-lining and express both Fc and C5a receptors, are crucial for initiation of innate inflammation (56). Mast cells not only secrete vasoactive compounds that lead to tissue edema, but also chymases and tryptases that promote tissue destruction and inflammatory cytokines such as TNF-α. Lastly, mice that lack cadherin 11, the protein responsible for the intracellular adhesion between synovial lining cells, fail to develop synovial inflammation in this model. This underscores the interplay between the lining and the inflammatory infiltrate in the synovial sub-lining that must take place for chronic inflammation and tissue destruction to occur.

Antibodies to Citrullinated Proteins – Role in RA Pathology and Diagnosis

As with other rheumatic diseases, there is no diagnostic test that is specific for RA. Criteria exist that segregate RA patients from those with other forms of inflammatory arthritis for the purposes of research studies. These 'classification criteria' are not designed for the diagnosis of the individual patient, but are often used as such. Currently, the only laboratory criterion is the presence of rheumatoid factor. The test for this serological marker is problematic, especially in early disease. In established RA, rheumatoid factor (RF) is seen in 75-80% of patients. The remaining patients are classified as having RA on the basis of clinical findings and erosive changes on radiographs. However, RF is not specific for RA, as it is found in other chronic infectious or inflammatory disorders such as bacterial endocarditis, syphilis, tuberculosis, hepatitis, and pulmonary fibrosis. It can also be found in up to 30% of healthy elderly subjects. In early RA, it may only be present in 50% of cases, making it a useless test.

Enzymatic Conversion of
Peptidylarginine to Peptidylcitrulline



A number of other autoantibodies have been found in patients with RA. These include antibodies to ribonucleoproteins, calpastatin (a calpain inhibitor), type II collagen, ANCA (anti-neutrophil cytoplasmic antibody) and the heavy chain binding protein, BiP (57). In 1964, antisera from RA patients were found to stain the keratohyaline granules in the cytoplasm of differentiating buccal mucosa (58). These antibodies were termed 'anti-perinuclear factor' (APF) and were found to be the same antibodies that bound to keratin-like structures in esophageal epithelium (anti-keratin antibodies, AKA) (59). The antigen recognized by each of these antisera turned out to be filaggrin, a widely expressed epithelial protein (60). During terminal differentiation of epithelial cells approximately 20% of the arginine residues in filaggrin are converted to citrulline (61). Using short peptides derived from filaggrin, it was determined that APF and AKA both recognize citrulline in the context of specific amino acid residues (62).

While the APF, AKA and anti-filaggrin assays could only be done in research laboratories, a commercially available ELISA using a cyclic citrullinated peptide (CCP) has been developed (63). With established RA, the sensitivity of the anti-CCP was 68% and the specificity was 96%. In patients referred to an Early Arthritis Clinic the sensitivity and specificity were 48% and 96%, respectively. In this group of 486 patients, 31% had a diagnosis of RA at the end of one year, giving a positive predictive value of 84% and negative predictive value of 81%. In a total of 815 other individuals, anti-CCP was detected in 2% of patients with other autoimmune diseases, in 2% of patients with infectious diseases, and 0.8% of healthy controls (63).

A recently published study looked at stored serum samples from 83 Swedish RA patients who had previously given blood for various blood banking or population cohort studies (64). Anti-CCP antibodies were found in 34% of patients a median of 2.5 years **before** the onset of symptoms. Titers of the autoantibody increased in samples obtained closer to onset of clinical RA. Each patient was compared to 4 health controls, allowing test statistics to be calculated. The sensitivity was 25% for samples taken > 1.5 yr before symptoms, 52% for ≤ 1.5 yr, and 70% at the onset of symptoms. The specificity was 98% at all time points. Only IgA-RF, an isotype of rheumatoid factor not typically measured, had similar diagnostic utility.

Diagnostic Properties of Anti-CCP testing in Undifferentiated Polyarthritis

Sensitivity	**50%**	**CI (41-59%)**
Specificity	**97%**	**CI (95-99%)**
PPV	**93%**	**CI (87-99%)**
NPV	**75%**	**CI (69-80%)**
+LR	**16.7**	

Data from (65)

The diagnostic utility of anti-CCP testing to predict the development of RA in undifferentiated polyarthritis patients was recently reported. A prospective study analyzed 936 patients referred to an Early Arthritis Clinic within 2 years of the onset of symptoms (65). An initial diagnosis was made two weeks after examination. 21.9% had RA based on clinical criteria and/or RF positivity. A total of 40.6% of patients were diagnosed with other conditions such as psoriatic arthritis, crystal-induced arthritis, reactive arthritis, spondyloarthropathy, and others. 37% of patients had undifferentiated arthritis. At three years of follow up, 93% of anti-CCP positive had developed RA, while only 25% of anti-CCP negative patients had RA. As with the previous studies, the sensitivity and specificity were 50% and 97%, respectively, and the positive likelihood ratio was 16.7. In a multivariate analysis, anti-CCP positivity was the strongest risk factor for progression from undifferentiated arthritis to RA with an odds ratio of 38.6. Lesser risk factors included the presence of polyarthritis, symmetric arthritis, or erosions on radiographs. When anti-CCP is included in the analysis, IgM-RF is no longer a risk factor for progression to RA. The authors concluded that anti-CCP was helpful in predicting the progression to RA when one, two, or three criteria for the diagnosis were present initially. When four or more clinical criteria were met at baseline, anti-CCP did not add to the diagnostic model.

The production of anti-CCP antibodies may not only be a marker for RA, but may also play a role in the pathogenesis of disease. Citrullinated proteins are readily demonstrated by immunohistochemistry (66, 67). Macrophages and fibroblasts within the lining and sub-lining areas of the synovium stain intensely with anti-citrulline antibodies, as well as amorphous interstitial deposits in the deep synovium. The major synovial targets of these antibodies are deiminated alpha and beta chains of fibrinogen (67). These variable regions of synovial anti-

CCP antibodies are highly mutated, indicating a specific antigen driven reaction rather than cross reactivity with another antigen (68).

The generation of citrullinated peptides may also explain some of the genetic risk of RA. Citrulline residues are generated from arginine by the enzyme pepdidylarginine deiminase (PAD). Of the five mammalian PAD isotypes, two (PAD2 and PAD4) are expressed in hemopoietic cells. In particular, PAD4 is expressed in the nucleus of neutrophils and monocytes. Suzuki, *et al.*, recently reported an analysis of the region on human chromosome 1 that encodes PAD4 (*PADI4*). Single nucleotide polymorphisms (SNPs) within the *PADI4* gene are associated with RA (case control ratio of 1.28 for the susceptible haplotype vs. 0.87 for the non-susceptible haplotype) (69). The susceptible *PADI4* haplotype was associated with greater PAD4 mRNA stability and 30% increase in the prevalence of anti-CCP antibodies, indicating the functional significance of this genetic variation. The authors estimate that up to 17% of attributable genetic risk for developing RA can be explained by the *PADI4* association.

A recent report has demonstrated that the presence of citrulline in certain peptides derived from synovial peptides increases their binding to HLA-DR alleles bearing the RA-associated 'shared epitope' by 10- to 100-fold (70). No increase in binding is seen with HLA-DR alleles not associated with RA. In transgenic mice expressing human HLA-DR4, T cells proliferate to a citrulline-containing peptide from vimentin and secrete gamma interferon, while there is no response to unmodified vimentin.

Together, these data suggest a pathogenic mechanism that incorporates many known features of RA (71). Since citrulline-modified peptides are only expressed as post-translational modifications on a minority of proteins, the T and B cells that can recognize them escape deletion in the thymus and bone marrow, respectively. In the correct genetic background (e.g., HLA-DR4, high PAD4, 'high' cytokine production), synovial proteins are deiminated and the citrulline-containing peptides presented to DR4-restricted, autoreactive T cells. These cells then provide specific help for the production of anti-citrulline antibodies by synovial plasma cells. In a manner analogous to the serum transfer model of murine arthritis, these locally produced immune complexes trigger the innate immune system, causing further protein degradation and deimination, driving a chronic multicellular autoimmune and inflammatory reaction.

EARLY DIAGNOSIS AND TREATMENT GOALS

If early treatment is a goal, then early diagnosis is a requirement. The barriers to early diagnosis are several. Patients must first seek medical attention. It has been estimated that one third of people in the US with chronic joint pain never see a physician or other provider. What percentage of those 'non-patients' have RA is not known, but every rheumatologist has seen people with an insidious onset of arthritis who treat themselves with NSAIDs or acetaminophen for years until they present with advanced, destructive disease. Second, initial providers must make an accurate diagnosis and initiate appropriately aggressive therapy. In most instances, this hinges on the referral to a rheumatologist or other specialist for confirmation of RA and institution of DMARD treatment (72). In one US center analyzed from 1997 to 2000, the average time from symptom onset to rheumatologist appointment was 28.6 weeks vs. 38.5 weeks for patients in Birmingham, UK during the same time period. Education of primary care providers is effective, as a study of rheumatology referrals in Glasgow showed. The median

delay from symptom onset to specialist referral declined from 23 months in 1989 to 4 months in 1997 (73). During the same time period, there was a decline in the delay to DMARD use from over six years to almost 6 months. Nevertheless, 35% of patients seen within three months of the onset of their symptoms already had erosive radiographic changes.

1987 ACR Criteria for the Classification of Rheumatoid Arthritis

1. **Morning stiffness ≥ 1 hr**
2. **Arthritis in ≥ joint area**
3. **Arthritis of the hands**
4. **Symmetric arthritis**
5. **Rheumatoid nodules**
6. **Elevated RF**
7. **Radiographic changes**

4 of 7 required for classification, with 1-4 present for ≥ 6 wk

The two diagnostic challenges faced by both primary care providers and rheumatologists are to identify patients with self-limited disease from those who will have persistent synovitis, and to accurately predict who will have erosive disease. As noted above, the 1987 American College of Rheumatology (ACR) criteria were designed to separate populations of patients with prevalent RA from those with other diseases. Since the development of the criteria required patients to have been diagnosed already, their application to the diagnosis of individual patients invokes circular reasoning. When applied as a diagnostic tool at the onset of early RA, the ACR criteria are fairly specific (82%), but rather insensitive (66%).

Other scoring systems have been developed specifically for early arthritis. For example, a model based on the clinical, laboratory, and radiographic characteristics of 524 Dutch patients with early RA generates a model that predicts for a given patient the probability of self-limited disease, persistent non-erosive disease, and erosive synovitis in the ensuing 24 month period (74). Statistical analyses demonstrated that this model was superior to the traditional ACR criteria. Patients in this study were evaluated within 3 months of the onset of symptoms and were not stratified as to treatment. Whether this methodology can be validated in other populations or can be correlated with other outcome measures must be done before it can be used to base individual treatment decisions.

A group of patients apt to be lost in any attempt to diagnose early RA are those with undifferentiated polyarthritis (UPA). This group typically has only a few joints affected, lacks RF, and may have episodic rather than continuous disease. In series of early arthritis, this group may make up 30-40% of patients. Because they do not meet criteria for RA, these patients may be under treated. In fact, only a small number of patients with UPA enter remission (absence of symptoms without treatment). Persistent synovitis is seen in approximately 40% of patients and ~80% of these patients require DMARD therapy (75). The strongest predictor of persistent synovitis at one year was the duration of symptoms > 12 weeks. Roughly 30% of patients with UPA at baseline can be classified as having RA at the end of one year. Therefore, even mild polyarthritis should be taken seriously, so that progressive disease can be treated early.

Use of Ultrasound and Magnetic Resonance Imaging

Both musculoskeletal ultrasound (US) and magnetic resonance imaging (MRI) have been tested for their ability to aid in the diagnosis of early RA and to identify patients with erosive disease. Both modalities can detect soft tissue changes consistent with inflammation as well as bony

lesions that are not seen on plain radiographs. In one study that included both early and established RA, sonography was able to detect 39 early erosions while radiographs found only 6. In late disease, US found 88 erosions; radiographs only 26 (29). Overall, 37.5% of early patients had sonographic erosions. Radiographs detected erosions in only 5% of these patients. In late disease, two-thirds of patients had erosions by US while a quarter could be seen with radiographs. A prospective study of patients with established arthritis (mean duration of 42.5 months) showed that the prevalence of erosions increased from 6% to 8% by US while radiographs detected none of these changes (76). During this time, the clinical exam was unchanged and US determined synovitis actually decreased suggesting that structural damage does not always stop when inflammation is controlled. Power Doppler can be added to an ultrasound evaluation to assess the vascularity of the synovium as a quantifiable marker of inflammation (77-79). Despite good interobserver agreement, US suffers from operator dependence to get good images, and the requirement for specialized ultrasound transducers.

MRI is even more sensitive in documenting bone changes in inflammatory arthritis. In the study described above, bone edema or erosions were documented in 20% of patients at baseline and 50% patients at two years while radiographs found none of these changes (76). In a study of 31 patients with early RA (mean duration 6 months), bone edema was found in 68% of patients and gadolinium enhanced synovitis in 97% of patients (80). In a recent study, MRI was performed on early RA (median duration of 4 months) patients and then six years later (81). At inception, erosions were seen in 45% of patients while radiographs were positive in only 15%. The presence of bone edema and erosions on MRI at baseline predicted the radiographic presence of erosive disease at six years. Thus, MRI of the hand at baseline may be the most accurate predictor of long-term radiographic damage, even in cases of clinical improvement (82). The clinical use of MRI is being promoted by the marketing of dedicated hand and foot scanners that are portable and can fit in an exam room.

Proposed Guideline for Referral

Using an evidence-based review of the literature, Emery, *et al.*, published a clinical guide for the early recognition and referral of patients with RA (83). They note that much of the knowledge gained on the clinical outcome of patients with early disease comes from so-called 'Early Arthritis Clinics' (EAC) that have existed primarily in Europe for the past decade (84-92). The function of the EAC is to see patients whose symptoms have been present for a relatively short period of time – 12 to 24 months. Typically, EACs will evaluate patients within 1-2 weeks of a referral. In the Netherlands, institution of an EAC shortened the lag time for referral to a rheumatologist from 122 days to 31 days of symptoms (93). In ~70% of cases, the diagnosis of RA could be made within two weeks of the first visit to a rheumatologist, and did not change over the ensuing year. 25% of these Dutch patients seen in the EAC had bone erosions at presentation, causing the authors to declare early RA a 'medical emergency' that should motivate early referral and treatment.

Prognostic features of persistent RA have come from several EAC-based studies. While the underlying studies are somewhat heterogeneous, several common features for both radiographic joint damage and long-term disability emerge. They include disease duration greater than 12 weeks, involvement of two or more joints, especially hand joints, high disease activity at baseline, RF positivity, and elevated CRP (89). In an effort to provide a tool that can be used by

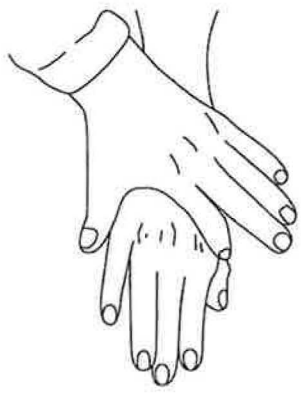
primary care providers to screen patients to be referred to an EAC, Emery, *et al.*, proposed the following criteria: 1) Three or more swollen joints, 2) Tenderness of the metacarpophangeal or metatarsophangeal joints assessed by compression across the hand or foot, and 3) Morning stiffness lasting at least 30 minutes. The presence of **any** of these findings suggests the need for more in-depth evaluation. RF, anti-CCP, erosions, and elevated CRP are poor prognostic factors,

Refer to a Rheumatologist if *ANY* of the following are present:

- **≥ 3 swollen joints**
- **≥ 30 minutes of morning stiffness**
- **Tenderness of MCP or MTP joints**

Lack of RF, or equivocal ESR/CRP, are not exclusionary

NSAIDs and steroids may delay diagnosis and have poor risk-benefit ratios



The MCP "Squeeze Test"

but their absence at baseline does not preclude a diagnosis of RA. The authors also point out that NSAIDs and corticosteroids will diminish inflammation and pain, delaying the institution of proven DMARDs with greater efficacy and lower toxicity.

THE FUTURE

Right now, there is no cure for established RA. What does the future hold? Better education can improve the recognition of early RA and Early Arthritis Clinics can improve the diagnosis of inflammatory arthritis, but key questions remain. Will genomic or proteomic analyses aid in the selection of those patients who will respond to monotherapy? Which patients will require combination or biological therapy? TNF inhibitors target the cytokine network only. Recent studies have shown highly effective therapy with agents that target the T and B cell activity in RA. CTLA4-Ig is a biological agent that disrupts the interaction between T cells and antigen presenting cells that has recently been shown to treat the signs and symptoms of RA (94). Anti-CD20 therapy causes depletion of peripheral B cells and has shown to treat a number of autoimmune diseases, including RA (95). It is possible that combination therapy with these agents can allow all parts of the immune system to be 'reset'.

What about treating very early disease? A large, investigator-initiated trial is in planning stages. This trial, termed DINORA (Definitive Intervention in New-Onset Rheumatoid Arthritis) is designed to enroll over 1,000 patients with less than 14 **weeks** of symptoms, within 2-6 weeks of diagnosis. Extensive patient characterization will be done to look for factors that predict progression to more aggressive disease. DINORA will have seven treatment arms, including placebo. Subjects who get active treatment will get methotrexate for three or twelve months, infliximab for three or twelve months, or a combination of methotrexate and infliximab for three or twelve months. At the end of one year there will be a four-year follow up with standard

treatments allowed. Patients in the placebo arm will be allowed to opt out to active therapy at any time. This study faces a number of challenges, not the least the establishment of Early Arthritis Clinics in the US to get early referral of subjects. Nonetheless, this type of study is required to define ways to **cure** rheumatoid arthritis, not just treat it.

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