Immunomodulatory Effects by a Heat Shock Protein dnaJ-Derived Peptide in Rheumatoid Arthritis

Abstract

Peptides derived from the E. coli heat shock protein (hsp) dnaJ share the ‘shared epitope’ sequence with HLA DR alleles associated with rheumatoid arthritis. These peptides are antigenic in human autoimmune arthritis. T cell recognition of these peptides is associated with TH-1 type and pro-inflammatory responses, including production of TNFα, suggesting an involvement of these abnormal responses in the pathogenesis of autoimmune inflammation. In a pilot clinical trial, we attempted to modulate these pro-inflammatory responses by oral administration of various doses (.25, 2.5, 25 mg po qd for 6 months) of the target antigen in 15 patients with rheumatoid arthritis. We measured the percentage of CD3+ cells producing the pro-inflammatory cytokines IL2, IFNγ, TNFα, and the tolerogenic cytokines IL4 and IL10, by FACS analysis of the intracellular products. In addition, we measured the cytokine concentrations, including TGFβ, by ELISA in culture supernatant. The observed decline in pro-inflammatory cytokines production during treatment was accompanied by IL4, IL10 and TGFβ production, suggesting an effective immunomodulation of these disease-specific responses.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease with an estimated prevalence of 0.8-1.0% (1). Despite the collection of a large amount of data, the role of T cells in the pathogenesis of RA is still poorly understood. Lack of identification of inciting antigens, and difficulties in evaluating antigen-specific T cell responses are the major problems encountered. These restrictions have also limited approaches for manipulations of pro-inflammatory T cells through conventional drug therapy or by immunotherapy.

A large body of evidence points to a role for heat shock proteins (hsp) in the pathogenesis of arthritis (2-8) (Table I). Other groups and we have showed that heat shock proteins are highly immunogenic molecules and are capable of modulating autoimmune inflammation (9-12). The work performed on hsp60 and dnaJ in human arthritis is particularly intriguing, in this respect. These abnormal responses contribute...
Table I. Associations of Heat Shock Proteins with Autoimmune Disease

<table>
<thead>
<tr>
<th>Heat Shock Protein</th>
<th>Autoimmune Disease</th>
<th>Disease Induction</th>
<th>Target Organ</th>
</tr>
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<tbody>
<tr>
<td>Hsp65</td>
<td>AA</td>
<td>M. Tuberculosis</td>
<td>Joint</td>
</tr>
<tr>
<td>Hsp65</td>
<td>Avridine Arthritis</td>
<td>Avridine (CP20961)</td>
<td>Joint</td>
</tr>
<tr>
<td>Hsp65</td>
<td>CIA</td>
<td>Collagen type II</td>
<td>Joint</td>
</tr>
<tr>
<td>Hsp60</td>
<td>Diabetes</td>
<td>NOD mouse</td>
<td>Pancreas</td>
</tr>
<tr>
<td>αB-crystallin</td>
<td>MS</td>
<td>?</td>
<td>CNS</td>
</tr>
<tr>
<td>dnaJ</td>
<td>JRA, early RA</td>
<td>?</td>
<td>Joint</td>
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to the generation of pro-inflammatory stimuli, which may be responsible for the induction, or perpetuation of RA.

We have previously demonstrated that a peptide (dnaJp1) derived from the E. coli heat shock protein dnaJ shares sequence homology with HLA DR alleles associated with RA. These alleles have in common a 5 amino acid stretch called ‘shared epitope’ (11,13). dnaJp1 is a target of pro-inflammatory T cell responses in RA patients (11,14,15).

We also observed that immune responses to peptides containing the ‘shared epitope’ from dnaJ generate pro-inflammatory stimuli such as increased production of IFNγ and TNFα. Hence, modulating these stimuli through oral tolerization with dnaJ peptides seems an attractive approach in affecting pathogenic T cell populations involved in the autoimmune disorder while keeping the physiological immune function intact as had been demonstrated in earlier studies with oral tolerance (16-21).

Based upon these observations, we conducted a pilot in the attempt to immunomodulate the pathogenic reactivity of T cells to the ‘shared epitope’, as expressed on the dnaJ molecule. The objectives of the phase I trial were to determine the optimal dose of the peptide to be administered, to show lack of adverse effects to the peptide, and to collect immunological data towards defining outcome measurements of immune reactivity.

Preliminary results of the phase I study suggest immunomodulatory effects by dnaJp1 peptide. In vitro responses to dnaJp1 peptide of peripheral blood lymphocytes (PBLs) from untreated RA patients are accompanied by production of pro-inflammatory cytokines IL2, IFNγ, and TNFα. FACS analysis of CD3+ gated cells from PBLs of the same RA patients receiving the dnaJp1 peptide throughout the treatment period of six months showed a declining trend in these pro-inflammatory cytokine productions. This observation is also supported by ELISA analysis from in vitro cultures.

Experiences with Immunomodulation: Anti-TNFα therapy, Oral tolerization to OM-89 and chicken collagen in patients with rheumatoid arthritis

Attempts at immunotherapy of RA are based either on i) modulation of an individual immune response pathway involved in the inflammation, such as suppression of pro-inflammatory cytokines (TNFα) or ii) tolerization to antigens such as E. coli lysates (OM 89) or chicken collagen.
There is a growing collection of evidence, which indicate that many immune-mediated diseases may involve an abnormal balance in regulations of pro-inflammatory cytokines such as TNFα or IFNγ. These cytokines regulate production of other pro-inflammatory mediators and are abundant in RA joints (22-25). Modulation of TNFα that is expressed by various cell types in rheumatoid synovium with use of monoclonal antibodies against TNFα, therefore, represents an attractive approach in ameliorating the inflammation at the joints. Recent report of TNFα blockade from 3 randomized, double-blind, placebo-controlled clinical trials of 101 patients with active RA have shown rapid and sustained improvement in disease activity when compared to methotrexate (26-29).

Engineered TNFα receptors (TNFR:Fc) of two p75 (type II TNF) receptors fused to Fc fragment of human IgG1 neutralized TNFα biologic effects in a randomized double-blind, placebo-controlled study (30,31) These studies substantiate the pivotal role played by TNFα in the pathogenesis of RA and illustrate the importance of modulating the effects of these pro-inflammatory cytokines. However, the clinical effects and safety of prolonged TNFα suppression are still unknown.

Studies with E.coli extract, OM-89, which contains heat shock proteins, including dnaJ and hsp60 has been employed in the oral treatment of rheumatoid arthritis. The preparation has passed phase III clinical trials and is currently licensed for treatment of rheumatoid arthritis in the European Union. Its efficacy is reportedly comparable to oral gold salts and inferior to methotrexate (32-34).

Three clinical trials using chicken type II collagen (CII) in an attempt to ameliorate rheumatoid arthritis have been published to date. In one study of 60 RA patients orally treated with different doses of the collagen, marginal beneficial effects were observed in the treatment group. The number of swollen and tender joints decreased after 3 months of oral treatment but not in any of the other efficacy variables (35). Similar findings were observed in another subsequent study of 90 patients with early RA that were orally treated with bovine CII at different dosage (36). There were no differences observed, however, in the various disease parameters in these studies among the different treatment groups. In a more recent phase II multicenter clinical trial of 274 patients with RA treated with 4 different doses of chicken CII orally, improvement in joint assessments (swelling and tenderness) were also observed. Improvement was detected when 1 of 3 outcome parameters was applied (37). The major limitations of the study were due to a small size of the 5 treatment cohorts of RA patients.

Problems with current antigen non-specific immunomodulation approaches and future considerations for immunotherapy

Although oral tolerance has been proven effective in experimental animal models, translation of this model to measurable outcome in human autoimmune diseases has been unsatisfactory. Several causes may explain for these discrepancies: i) Human autoimmune diseases are often multifactorial and genetic elements such as TCR usage, HLA type and restriction render a uniform and predictable outcome very difficult. ii) The studies performed to date in human rheumatoid arthritis are based on
administration of either bacterial lysates containing probably thousands of different proteins, most of them irrelevant, or on individual whole proteins, such as collagen, which may contain relevant epitopes in the context of a multitude of irrelevant ones. No epitope-specific approach based on synthetic peptides has yet to be attempted.

iii) Little is known in humans regarding the immune mechanisms related to induction of oral tolerance, as no sequential and detailed studies have been performed with the associated clinical picture in mind. A detailed pathogenic mechanism is further clouded by the polymorphic nature of any patient population, and by the consequent difficulty in identifying immune mechanisms specifically related to the treatment.

A possible flaw in the use of collagens for oral tolerization, besides the ill definition of the trigger-antigen, is the source of the antigens themselves. Since data from experimental animal models was most effective when homologous antigen was used for oral tolerization, perhaps human recombinant CII or CII derived peptides would be more appropriate than the use of either bovine or chicken collagen. A defined homology between the host and the trigger-antigen needs to be identified in order to induce tolerance as previous studies have demonstrated (38-40).

One of the key ‘bridging’ information necessary for successful immunotherapy through tolerization is the understanding of antigen specific and antigen independent events involving T cell responses which lead to chronic autoimmune inflammation. Thus, a model antigen needs to be identified which can trigger pro-inflammatory responses and which can be targeted for immunotherapy. Appropriate endpoints to evaluate efficacy of intervention are also lacking. Immunological changes induced by the treatment are usually not measured and compared to the clinical picture.

Epitope specific immunomodulatory approach: ‘shared epitope’ dnaJp1 peptide-driven immune modulation in patients with rheumatoid arthritis

We recently conducted a clinical trial to test the hypothesis that ‘shared epitope’ peptides derived from E. coli heat shock protein immunomodulates autoreactive lymphocytes in RA patients. Patients with rheumatoid arthritis who responded in vitro to dnaJ peptides were divided into three different dose groups for a phase I open label first year clinical trial. A synthetic peptide encompassing the ‘shared epitope’ in the context of dnaJ sequence was administered for six months at various doses of 0.25, 2.5, 25 mg po qd. Patients were evaluated on a monthly basis both clinically and immunologically. Peripheral blood lymphocytes (PBLs) isolated from these patients were stimulated with dnaJ peptide and proliferative responses were measured by standard thymidine (3H) incorporation. We measured the percentage CD3+ cells producing the pro-inflammatory IL2, IFNγ, TNFα, and tolerogenic cytokines IL4, IL10 and TGFβ by FACS analysis and ELISA.

Patients were enrolled into the study if they met the following criteria:

1. They fulfill 7 ACR criteria for diagnosis of RA.
2. Reactivity to dnaJp1 peptide, measured as stimulation index>2.0
3. CD3+ cells>2% (over non-stimulated cells) for IL2, IFNγ, and TNFα.
Patients must have active RA of less than 5 years, at least 18 years old, not pregnant, and are not in DMARDs.

Stimulation with dnaJp1 peptide induces production of pro-inflammatory cytokines in untreated RA patients, which is immunomodulated after oral administration of the peptide. Peripheral blood lymphocytes collected at monthly intervals from RA patients were coded and analyzed blindly. There was an observed reduction in proliferative response by the same cells to the peptide throughout the treatment. Loss of T cell proliferative responses to dnaJp1 cannot be restored by addition of IL-2 to PBMC from treated patients.

Productions of pro-inflammatory cytokines IL2, IFNγ, TNFα, decreased through the treatment period, while production of tolerogenic cytokines IL4, IL10 and TGFβ increased (See Figure 1). Control antigenic peptides and untreated RA patients were utilized as comparisons to the dnaJ and treated RA patients, respectively. The immunological response of dnaJ treated RA patients do not reflect regression to the mean in the sample studied (not shown).

Clinical data showed a subjective (RADAR and AIMS) and objective (joint count, joint score and swelling) improvement in the intermediate and high dose groups. However, given the open label nature of a Phase I, and the lack of a placebo group, any interpretation for these clinical data should be taken with caution.
As in accordance to the objectives of the Phase I clinical trial, treatment with dnaJp1 is safe. No side or toxic effects have been reported to date by the patients enrolled. This confirms the general consensus regarding the proven safety of oral tolerization approaches in humans.

Conclusion

The data generated by the clinical trial on oral administration of the ‘shared epitope’, dnaJ peptide, is too preliminary to draw any conclusive interpretation because the study is not blinded and placebo controlled. However, preliminary immunologic data from the study show an intriguing change in the functional characteristics of the ‘shared epitope’ specific T cells from pro-inflammatory TH-1 to tolerogenic TH-2 type of response. The declining trend in proliferative responses to the peptide and in pro-inflammatory cytokine productions of isolated PBMCs from treated patients suggests a treatment-specific response. Conversely, the increase in production of tolerogenic cytokines as measured by both FACS analysis and ELISA suggests an immunomodulatory response by CD3+ cells to the dnaJ peptide supporting the hypothesis of ‘shared epitope’ driven immunomodulation in RA patients.

This study identifies a model antigen, dnaJ derived peptide (dnaJp1) that can be used for better understanding the pathogenesis of RA and perhaps as a model for other autoimmune diseases. The results lend support to earlier findings with use of defined homologous antigen in producing tolerance through oral administration in both experimental and human models in RA as well as other autoimmune diseases.

Although these data suggests an antigen-specific immune response, the events leading to these responses such as antigen dependent and independent events still need to be defined. Pathogenic T cells involved in the recognition and presentation of the dnaJp1 peptide need to be isolated and the phenotypical and functional characteristics defined. To this end, we have developed a system based on artificial antigen presenting cells. This system, named ‘T cell capture’ has enabled us to identify dnaJp1-specific cells, and to discriminate between antigen specific and bystander effects of the immunomodulatory treatment. More work still needs to be performed on larger, placebo controlled and randomized trial.

Future work can also identify surrogate markers such as activation markers (CD25 and CD69) and chemokine receptors (CXCR3, CCR5, CCX3, and CCR4) to correlate T cell function with treatment efficacy. These membrane phenotypical markers will be useful in predicting disease status and clinical outcome of therapy in addition to the conventional method of joint assessment for disease activity.

We have simply scratched the surface in understanding the pathogenesis of the initial stages of rheumatoid arthritis. The model antigen (dnaJ peptide) has allowed us to view one of many mechanisms that contribute to the generation of chronic autoimmune inflammation in RA. Modulation of this debilitating disease through tolerization with one of the inciting antigens (shared epitope) holds some promise. Effective immunotherapy of rheumatoid arthritis will most likely comprise of a combination of several approaches, given the complexity of pathogenic mechanisms, the
likely multiplicity of the antigenic triggers, and the central role played by bystander, antigen non-specific mechanisms. Immunomodulation of the inflammatory process by one or more combined heat shock proteins through either oral or mucosal tolerization may have to be considered as future avenues for research.

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References


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