Present and future of combination therapy of autoimmune diseases

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'A dual therapeutic strategy targeting the dominating proinflammatory milieu as well as the more specific immune response is, in our view, especially promising.'

Treatment regimens for rheumatological diseases have changed considerably over the past 20 years. To date, treatment of rheumatoid arthritis (RA) mainly focused on the inflammatory pathogenic component of the disease. Significant progress in the induction of disease remission has been achieved by the use of powerful immunosuppressants and cytostatic drugs, such as corticosteroids, methotrexate (MTX) and leflunomide, in the early stage of the disease [1,2]. Biologics have recently been added for treatment-resistant disease, more specifically targeting components that play a role in the disease pathogenesis [3,4]. Unfortunately, persistent disease remission can only be achieved as long as the drugs are administered, and only then can progression of joint damage be prevented. In addition, since most of these agents induce a generalized and nonspecific inhibition of immune response and inflammation, they can cause considerable immunodepression, leading to complications that prompted US FDA to formulate black-box warnings for some of these products. A more specific modulation of the immune response could theoretically overcome these pitfalls. Some of these novel strategies are in the clinical development stage.

The combination of treatment strategies with different and complementary mechanisms of action is likely to be more successful and is thus being introduced into the standard of practice; by acting complementarily or synergistically, they can enhance efficacy. Cost and toxicity can be decreased, owing to the opportunity to administer lower amounts of drugs than when they are given separately. Combination of nonbiologics, currently approved biologics and novel biologics will be discussed in this editorial. A dual therapeutic strategy targeting the dominating proinflammatory milieu as well as the more specific immune response is, in our view, especially promising. Induction of specific immune tolerance may provide a long-lasting disease remission, enabling changes in dosing and scheduling of those drugs with the least convenient safety profile, leading certainly to a more individualized approach to medicine and opening the possibility of identifying a strategy leading to long-lasting remission.

Combination of nonbiologics

It has become common practice, that patients are started on disease-modifying antirheumatic drugs (DMARDs) therapy early in the course of their disease. In order to achieve synergy without subsequent increase in toxicity, long-term remission and slowing of radiological damage, the combination of several DMARDs with different mechanisms of action was tested and found to be effective in some combinations [5,6]. For instance, in two open-label randomized trials in patients with early RA, the combination of MTX, sulfasalazine, hydroxychloroquine (HCQ) and prednisolone demonstrated greater clinical improvement and significantly less radiographic progression compared with a single DMARD with or without prednisone [2,7]. Many other combinations are used in common practice, with a general direct correlation between increased clinical efficacy and overlapping undesired effects, which limit the use of such combinations.

The underlying reason for the synergistic effect of these DMARDs is not fully known at present. This effect may may be partly due to the influence on each other's pharmacokinetics; for example, HCQ leads to slower clearance and uptake with a greater area under the curve for MTX in patients taking the combination of HCQ and MTX [8]. The synergy may also be mechanistic and affect both the adaptive and innate arms of the immune response. For instance, MTX, as a folate inhibitor, acts on rapidly proliferating cells, which are purportedly composing the pool of effector cells that may fuel autoimmune inflammation. Conversely, HCQ is an inhibitor of intracellular processes involved in antigen presentation, thus potentially affecting the repertoire of autoantigens available to effector cells [9].

Unfortunately, a proportion of patients still do not respond sufficiently to DMARD therapy, and complementary approaches are needed.

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Major disadvantages of the use of DMARDs are that they are not specific (and therefore lead to side effects) and that they must be administered continuously to retain efficacy.

Combination of currently approved biologics

Greater success with fewer side effects can be expected by more specifically targeting pro-inflammatory cytokines that are known to play a role in the autoimmune process, such as tumor necoris factor (TNF)α, interleukin (IL)-1 or -6. Several biological agents have been identified that can block these cytokines:

- Enbrel (Etanercept®), a soluble TNF receptor fusion protein, targets TNFα;
- Remicade (Infliximab®), a chimeric anti-TNFα antibody, targets TNFα;
- Humira (Adalimumab®), a recombinant human anti-TNFα antibody, targets TNFα;
- Kineret (Anakinra®), an IL-1 receptor antagonist (IL1-RA), neutralizes IL-1;
- MRA® (a humanized IL-6 receptor antibody) targets IL-6.

The use of these agents has shown clinical efficacy in several clinical trials, but the fact that the effects are only temporary, their cost is high and severe side effects may occur, such as an increase in serious infections and possibly lymphomas due to anti-TNFα treatment, is hampering their success [3,10].

The potential for additive or synergistic effects of two biologics has been tested in the treatment of RA by adding kineret to treatment with enbrel. However, no additional clinical effect was demonstrated, whereas an increased safety risk did become apparent, due to higher incidence of serious infections, injection site reactions and neutropenia [11]. Therefore, the combination of two anticytokine biologics is not recommended.

Conversely, a field with great potential is the combination of biologics that target different immunopathogenic pathways; therefore exploiting a potential complementarity in mechanism of action. However, these approaches are at the initial stage of clinical testing to ascertain both efficacy and tolerability.

Combination of nonbiologics with currently approved biologics

Promising results were obtained when combining MTX with a biological agent. The combination of MTX with anti-TNFα or IL-1RA treatment is found to improve the clinical outcome significantly, compared with MTX or anti-TNFα treatment alone [12–20]. The combination treatments led to a decrease in disease activity, reduction of disability, an increase in remission rates and even a decrease in progression of joint damage. Unfortunately, the exact mechanism underlying this synergistic effect is not currently known, but may be explained in part by a decreased amount of neutralizing antibodies against TNF antagonists [21]. Furthermore, knowledge on the influence of MTX on T cells and the inflammatory process has been expanding in recent years, and may also provide some explanation for the combinatory effect of MTX with anticytokine treatment. MTX promotes the release of the endogenous anti-inflammatory mediator adenosine, presumably through its capacity to increase intracellular 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) concentrations [22]. Adenosine has a central role in regulation of inflammatory responses, one of which is the contribution to the resolution of inflammation, both by down-regulating macrophage activation and by advancing T-helper (Th)2 versus Th1 cell development [23]. Direct evidence for the promotion of a more anti-inflammatory environment by MTX was gained in early RA patients, where a decrease in T-cell-derived TNFα was observed, together with an increase in IL-10-producing T cells [24]. In *in vitro* experiments, MTX was demonstrated to increase *IL-10* and *-4* gene transcription as well as decrease *IFNγ* gene transcription [25]. Therefore the combination of blocking proinflammatory cytokines by anticytokine biologics with the promotion of a more anti-inflammatory environment by MTX may work synergistically.

Hence, an important step towards obtaining disease remission and slowing of radiological damage has been obtained by combining several DMARDs or combining MTX and anticytokine biologics. However, the effects only last while the drugs are administered.

Combination of currently approved biologics with novel biologics

The induction of specific immune tolerance would ideally spare the patient generalized immune suppression and could be expected to provide a long-lasting effect (and maybe even a cure) that is devoid of side effects. The induction of immune tolerance requires the identification of the appropriate target. Efforts at inducing tolerance independent of a specific antigenic target

are mainly based on molecules interfering with either T (CD3 or CD80/CTLA-4) or B cells (CD20/CD22). These attempts have led to very encouraging results, and some of these drugs are already available on the market. In recent onset Type 1 diabetes, two humanized anti-CD3 monoclonal antibodies were able to maintain residual β cell function better than placebo or a control group, as demonstrated by increased C peptide responses and decreased insulin needs. Short-term treatment even produced lasting effects for up to 2 years [26,27]. Orencia (Abatacept®), a recombinant cytotoxic T-lymphocyte antigen (CTLA)4-immunoglobulin (Ig) fusion protein, blocks the costimulatory signal required for T-cell activation by competing with CD28 for CD80 and CD86 binding. It demonstrated effectiveness in active RA patients, in improvement of signs and symptoms of disease, physical function and the quality of life over a period of 12 months in two Phase II studies [28,29]. In a Phase III randomized trial, it also demonstrated clinical and functional benefits in nonresponders to TNFα therapy [30]. Although the role of B cells in RA is not fully understood, selective depletion of B cells by a monoclonal antibody against CD20, rituxan (Rituximab®), led to sustained clinical improvement in an open label study [31]. In a subsequent randomized, double-blind, controlled study in 161 RA patients, treatment with rituxan, alone or in combination with cyclophosphamide or MTX, led to significant improvement of disease symptoms [32]. This category of molecules represents a significant step forward with their mechanism of action, because in most cases they aim to modulate certain aspects of adaptive immunity rather than suppress an individual pathway (i.e., a cytokine).

Another approach would be induction of tolerance using an antigen. However, the search for the disease-triggering antigen has not been successful to date, and attempts to induce tolerance to candidates in this respect, such as chicken and bovine Type II collagen and human cartilage glycoprotein (HCgp)39, major constituents of articular cartilage, were also not encouraging [33–36]. In fact, we feel that the focus of antigen-specific therapy should move away from the one disease-triggering antigen and should focus mainly on key players at the site of inflammation, which play a role in disease perpetuation. Heat-shock proteins (HSPs) are present in all cells and are upregulated during stress. As ubiquitous and bacterial-derived products, HSP-derived peptides are perceived as a danger signal and elicit a default proinflammatory physiological response, which involves both the adaptive and the innate arms of the immune system [37–39]. Such a response contributes to the clearing of a possible pathogen invasion, but also induces increased availability of self-HSP-derived peptides through cellular stress. These peptides then form a new target for the immune system and induce self-perpetuating cycles of inflammation, fueled by the self-antigens and self-reactive T cells. We have previously demonstrated, that HSP peptides are recognized by T cells with regulatory function, which are then capable of preventing further tissue damage. If such regulatory function is impaired, loops of inflammation continue and autoimmune arthritis prevails [40,41].

'Promising results of antigen-specific immunomodulation by HSP in experimental disease models of arthritis and Type 1 diabetes warranted subsequent clinical trials in human disease.'

Promising results of antigen-specific immunomodulation by HSP in experimental disease models of arthritis and Type 1 diabetes warranted subsequent clinical trials in human disease [42,43]. In RA, we have recently reported the results of a Phase I/IIa clinical trial with a dnaJ-derived peptide, dnaJP1, administered orally to 15 patients with early, active disease [44]. Interestingly, with this treatment, we were able to induce immune deviation from proinflammatory to modulatory T-cell responses, leading to significant reduction in TNFα and IFNγ production, and an increase in IL-10 and -4. These effects were mediated via restoration of function of CD4CD25 bright regulatory T cells (Treg), producing IL-10 and expressing FOXP3. Recently, we completed the Phase II clinical trial with dnaJP1 [Submitted]. This study focused on safety and clinical efficacy of the drug. It involved 160 patients who received dnaJP1 or placebo orally once per day for 6 months. The dnaJP1 peptide treatment demonstrated encouraging clinical and immunological effects, suggesting that induction of immune tolerance to an inflammatory ubiquitous antigen may translate into clinical improvement of the disease.

Due to safety and specificity in mechanism of action, epitope-specific immunotherapy has the profile of an ideal 'work with' approach. As such, it could exploit synergy and complementarity in mechanisms of action with both biologics and more traditional DMARDs.

We have obtained the first results in the combination of anti-TNFα therapy and antigen-specific immunomodulation to an HSP60 peptide in an experimental form of arthritis: adjuvant arthritis (AA). AA is a T-cell-dependent disease that can be passively transferred by a T-cell clone that is specific for the 180–188 amino acid sequence of mycobacterial HSP60 [45,46]. In previous studies, we demonstrated that nasal administration of peptide 180–188 after the induction of AA is mildly effective [43]. Interestingly, by giving a single low dose of enbrel before mucosal tolerance induction to HSP60 peptide 180–188, significant suppression of arthritis was observed to the same extent as a full course of enbrel therapy [47]. This implies that lower doses of anti-TNFα can be given, resulting in lower cost and less long-term side effects. Interestingly, two distinctly different immunological mechanisms were at the basis of equivalent clinical suppression of arthritis when comparing full dose enbrel therapy with the combination treatment of anti-TNFα and 180–188 peptide. Where anti-TNFα treatment induced mainly immune suppression, combination treatment was able to induce active modulation through induction of IL-10 production by effector T cells, as well as the induction of $CD4^+CD25^+$ regulatory T cells, again producing IL-10 and expressing FOXP3.

'Immune tolerance may still be the ultimate objective in the treatment of autoimmune diseases.'

Similarly promising results were recently published by Bresson and colleagues in experimental diabetes [48]. By combining anti-CD3 and intranasal proinsulin peptide treatment, recent onset diabetes could be reversed more potently than when anti-CD3 or the peptide was given alone. This combination treatment induced Tregs; the level of $CD4^+CD25^+FOXP3^+$ cells increased and insulin-specific production of IL-10, TGFβ and IL-4 by Tregs was enhanced. The Tregs were capable of suppressing autoaggressive $CD8^+$ responses *in vitro*. Furthermore, adoptive transfer of the peptide-specific Tregs suppressed disease in recent onset diabetic recipients to the same extent as in the donors.

The reason the induction of tolerance is facilitated by the combination with anti-TNFα and anti-CD3, may be partly because both have been demonstrated to create a tolerogenic environment. Several studies have demonstrated that anti-TNFα treatment can improve Treg function and numbers in RA patients [49–51]. Additionally, anti-TNFα treatment was shown to induce a shift to a more anti-inflammatory cytokine profile in peripheral blood mononuclear cells (PBMCs) and T cells of RA patients [52]. In non-obese diabetic mice, it was demonstrated that anti-CD3 treatment led to a decrease in the amount of autoaggressive T cells and an expansion of $CD4^+CD25^+$ cells in draining lymph nodes. Disease suppression was mediated through production of TGFβ [53].

As aforementioned, epitope-specific immunotherapy also has the potential to act in synergy with more traditional DMARDs. In our Phase II trial with dnaJP1 in human RA, we unexpectedly obtained the first results of combination treatment of a DMARD and mucosal tolerance induction. Interestingly, the clinical effect of dnaJP1 was clearly enhanced in patients using hydroxychloroquine (HCQ). HCQ is traditionally an antimalarial drug that, due to its immunomodulatory effects, is also used in the treatment of RA. The explanation of the enhanced effect of dnaJP1 tolerance induction due to HCQ, may be partly because it is known to decrease TNFα and IL-6 production. HCQ's main effect is exerted through blockade of the processing of proteins by antigen-presenting cells (APCs) [9,54,55]. This creates an environment of low self-presentation of proteins, whereby the dnaJP1 peptide might be more easily presented, as it does not need to be processed in order to be presented by APCs. In this way, such peptides may have a greater impact on the regulatory immune system.

Combination of MTX with antigen-specific tolerance induction may also be beneficial. MTX administration may create a better milieu for antigen-specific immunomodulation, by creating an anti-inflammatory environment and maybe even a more tolerogenic microenvironment via its action on rapidly proliferating effector T cells and the decrease in TNFα production [23,24].

Conclusion & future perspective

The combination of different treatments, especially of DMARDs or anticytokine biologics with novel biologics, appears to be effective through synergistic, as well as complementary, working mechanisms. These approaches may implicate important changes in the future management of autoimmunity.

Immune tolerance may still be the ultimate objective in the treatment of autoimmune diseases. The combination treatment approach may

exploit the strengths of complementary drugs and reduce the chances of developing side effects. This approach, which is associated with a more focused and effective approach toward modulation of adaptive immunity, may render true immune tolerization attainable.

Secondly, the increasing knowledge of the pathophysiology of disease may enable the choice of different associations of drugs, for example, based on genetic and pathological patterns. This may lead to the identification of subgroups of patients who may be more prone to responding to certain cocktails of drugs; thereby providing an important step towards individualized medicine.

Lastly, the complementarities in mechanisms of action and the diverse potency of various DMARDs and biologics may eventually lead to a progressive treatment design, where various drugs may be used at different times. In such a protocol, DMARDs and anticytokine biologics can be applied to induce disease remission, followed by epitope-specific therapy in order to maintain it.

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