Combination therapy with DMARDs and biological agents in collagen-induced arthritis

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ABSTRACT

There is increasing interest in the use of combination therapy for rheumatoid arthritis and in the possibility of combining the conventional drug approach with newer biological therapies. Animal models of arthritis provide important tools for evaluating novel forms of therapy and for eludicating mechanisms of drug action. In this paper, we review the results of our own research into combination therapy in collagen-induced arthritis using biological therapies such as anti-tumor necrosis factor α, anti-CD4, and anti-interleukin 12 monoclonal antibodies, and small molecular weight compounds such as cyclosporin and the phosphodiesterase IV (PDE IV) inhibitor rolipram.

Introduction

The drive towards the use of combination therapy in rheumatoid arthritis (RA) has come about for a variety of different reasons. First, clinical experience has shown that a significant proportion of patients treated with disease-modifying antirheumatic drugs (DMARDs) either show a poor initial response or respond well but subsequently suffer flares in disease activity, despite continued therapy. Furthermore, it has become increasingly apparent that even when DMARDs are effective in reducing the level of disease activity, in many cases they are unable to halt the progressive development of joint erosions (1). Combination therapy may offer the possibility of more effective control of disease symptoms, as well as a greater degree of protection from joint damage. Another consideration is that DMARDs may have unwanted side effects, particularly at high doses, and by using DMARD combinations at lower doses than are conventionally used in monotherapy, it may be possible to expand the margin between efficacy and toxicity. The arrival of effective biological agents for the treatment of RA greatly expands the opportunities for combination therapy by allowing, for example, combinations of DMARDs with monoclonal antibodies (mAbs) directed at pro-inflammatory cytokines.

Animal models of arthritis represent important tools for the evaluation and optimisation of novel forms of combination therapy for RA. In this review, we will summarise some of the experiments carried out at the Kennedy Institute on combination therapy in murine collagen-induced arthritis (CIA).

CIA: Synopsis of the model

CIA is known to occur in rats (2), mice (3), and primates (4) following immunisation with type II collagen. The CIA model has been widely studied as a model of arthritis, largely on the basis of the pathological similarities between CIA and RA (5). Thus, both RA and CIA exhibit similar patterns of synovitis, pannus formation, erosion of cartilage and bone, fibrosis, and loss of joint mobility (6). Another key similarity between RA and CIA is that susceptibility to both diseases is strongly associated with genes encoding major histocompatibility complex (MHC) class II molecules, suggesting the involvement of CD4+ T cells in the pathogenesis of both forms of arthritis. However, it is also recognised that, as in human RA, the humoral arm of the immune response plays a significant role in the pathogenesis of CIA (5).

Although there are similarities between CIA and RA, it is clear that there are also differences. For example, the inflammatory cell infiltrate in CIA tends to be dominated by polymorphonuclear cells, whereas the infiltrate in RA contains a higher proportion of mononuclear cells. Furthermore, periosteal inflammation is commonly observed in CIA but not in RA. Most importantly, CIA (induced with heterologous type II collagen) re-

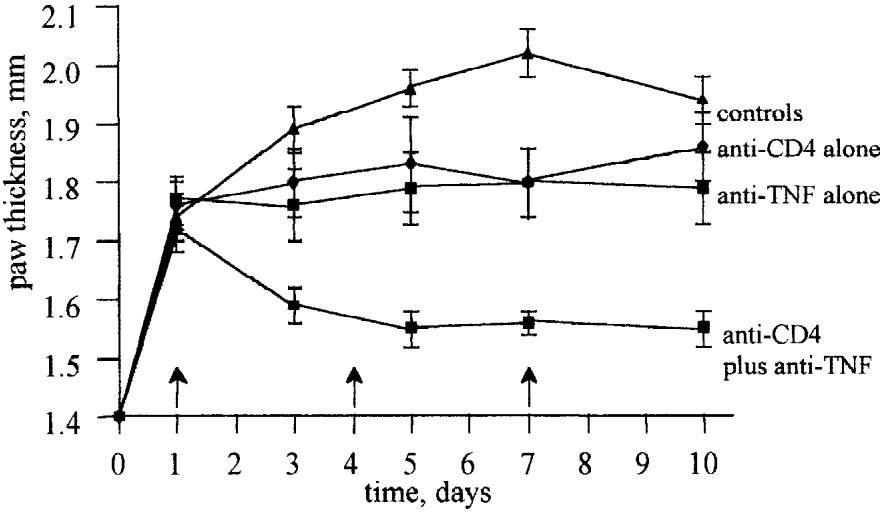

Fig. 1. Combination therapy in CIA. Mice with established arthritis were treated with a sub-optimal dose of anti-TNFα mAb (TN3-19.12; 50 µg/mouse) alone, anti-CD4 mAb cocktail (YTS 191.1.2/YTA 3.1.2; 200 µg/mouse) alone, or anti-TNFα plus anti-CD4. Arrows indicate the times of injection. Modified from (24).

sults in a relatively acute and self-limiting form of arthritis, whereas RA is a truly chronic disease. However, arthritis induced with autologous type II collagen results in a more protracted disease course with a fluctuating level of disease activity that more accurately resembles human RA (7, 8).

Most studies of immunotherapy in CIA involve treatment either before or after the onset of clinical arthritis, and these different experimental approaches may not necessarily provide the same results. For example, when given prior to disease onset, a number of T-cell targeted therapies (e.g., anti-CD4, anti-interleukin 12 [anti-IL-12], CTLA-4-Ig) have been shown to be effective in blocking the development of arthritis by inhibiting or altering the immune response that precedes the development of the disease. However, such treatments are usually found to be less effective in halting the progression of disease once the inflammatory response is underway (9-12).

Combination therapy using mAb

Anti-TNFα plus anti-CD4

The first form of combination therapy tested by our group was anti-tumour necrosis factor α (TNFα) mAb plus anti-CD4 mAb. Previously, we and others had shown that anti-TNFα therapy was effective in reducing the severity of established CIA (13-15), a finding that was subsequently confirmed in human RA (16-19). Anti-CD4 therapy, as discussed above, had been shown to be relatively ineffective in established CIA (10), although it was effective in preventing the induction of arthritis if given around the time of collagen immunisation (9). From these findings, it was concluded that CD4+ T cells played a more prominent role in the induction phase of arthritis, whereas the role of TNFα was more prominent in the effector phase of the disease. To test the effect of a combined therapeutic strategy that targets both induction and effector mechanisms we used the anti-TNFα mAb, TN3-19.12 (20), in combination with a cocktail of two lytic anti-CD4 mAb, YTS 191.1.2 and YTA 3.1.2 (21-23).

DBA/1 mice with established CIA were treated with either an optimal or a sub-optimal dose of anti-TNFα alone, anti-CD4 alone, or anti-TNFα plus anti-CD4. Controls received mAb of irrelevant specificities. Anti-CD4 mAb alone had a relatively minor impact on the disease compared to the controls, whereas anti-TNFα alone was effective at the optimal dose as shown in previous studies (14). However, combined anti-CD4/anti-TNFα treatment caused a much more significant decrease in the severity of arthritis than either anti-TNFα alone or anti-CD4 alone (24). The synergistic effects of anti-TNFα and anti-CD4 were particularly apparent at the sub-optimal dose of anti-TNFα, which on its own was relatively ineffective (Fig. 1). For example, it was shown by histology that sub-optimal anti-TNFα treatment alone reduced the number of erosions in the proximal interphalangeal joints by 20%, and anti-CD4 alone reduced joint erosions by 22%. In contrast, combined anti-TNFα/anti-CD4 treatment reduced the number of joint erosions by 72% (24). Another finding was that in the mice treated with anti-TNFα plus anti-CD4 there was a reduction in the IgM antibody response to the anti-TNFα antibody (a hamster IgG1 mAb), a potentially significant finding, as the development of antiglobulin responses represents an obstacle to the repeated, long-term use of murine mAbs in humans (25).

TNFR-IgG plus anti-CD4

In a follow-up study, a similar synergistic therapeutic effect was demonstrated between anti-CD4 and a recombinant human p55 TNF receptor-IgG (TNFR-IgG) fusion protein, which neutralises mouse TNFα (26). An additional finding to emerge from this study was that human TNFR-IgG alone was found to induce a strong neutralising antibody response in mice, and this response was profoundly suppressed by concurrent anti-CD4 treat-

Table I. Anti-TNFR-IgG responses and levels of TNFR-IgG in the sera of mice treated with TNFR-IgG alone or in combination with anti-CD4. The mice were treated on days 1, 4, and 7 of arthritis, and the sera were collected on day 10 of arthritis. Modified from (26).

Treatment	Anti-TNFR-IgG response (titres)		TNFR-IgG level
	IgM	IgG	
PBS	1:20	1:35	-
TNFR-IgG alone (2 µg)	1:50	1:590	< 0.2 µg/ml
TNFR-IgG alone (20 µg)	1:232	1:3,924	< 0.2 µg/ml
TNFR-IgG alone (100 µg)	1:336	1:5,100	< 0.2 µg/ml
TNFR-IgG (100 µg) plus anti-CD4	1:15	1:200	12.3 ± 1.1 µg/ml

ment. Secondly, it was found that by abrogating the anti-globulin response, higher levels of free TNFR-IgG could be detected in the circulation (Table I). From this finding, it was concluded that at least one of the mechanisms involved in the synergism between anti-CD4 and TNFR-IgG was the inhibition of anti-globulin responses leading to an increased half-life of the TNFR-IgG. A similar effect was observed in a study of lupus in NZW/NZB F1 mice in which a short-term pulse of anti-CD4 treatment was found to inhibit anti-globulin responses to concurrently administered anti-IL-6 mAb, leading to a dramatic increase in the therapeutic effects (27). However, it is very likely that other mechanisms of synergy operate, because in our first study synergy was observed between anti-CD4 and hamster anti-TNFα mAb (which is relatively non-immunogenic) without any significant augmentation of serum levels of anti-TNFα mAb (24).

Anti-TNFα plus anti-IL-12

Having established the therapeutic potential of a combined strategy using anti-TNFα mAb and lytic (depleting) anti-CD4 mAb, we then set out to identify a more selective approach to targeting the pathogenic T cell response in CIA that would avoid causing any significant depletion of the peripheral T cell pool. A number of studies have established that CIA, like RA, is a predominantly T-helper cell type 1 (Th1)-mediated disease. For example, it was shown that the period of induction of CIA is associated with a highly polarised Th1-like T cell cytokine profile, with high levels of interferon γ (IFNγ) and minimal IL-4 and IL-10 (28, 29). Subsequently, it was shown that by suppressing the Th1 response during the induction phase of CIA (prior to disease onset) by blockade of IL-12, it was possible to modulate, though not abrogate, subsequent disease development (Table II).

Next, a study was carried out to determine the effect of anti-IL-12 treatment combined with anti-TNFα treatment after the onset of clinical arthritis. Mice with established CIA were therefore treated with anti-IL-12 alone, anti-TNFα alone, or anti-IL-12 plus anti-TNFα. As in the case of anti-CD4 treatment, anti-IL-12 alone did not significantly affect the course of the disease. On the other hand, anti-TNFα treatment, as in previous studies, caused a significant reduction in the severity of arthritis, relative to controls (30). However, the combination of anti-IL-12 plus anti-TNFα led to a significantly greater reduction in both the clinical and histologic severity of arthritis than either anti-IL-12 alone or anti-TNFα alone (Table III). These results are clearly consistent with earlier findings concerning anti-CD4 plus anti-TNFα and further support the concept of combination therapy based on TNFα blockade coupled with the suppression of pathogenic T helper cell activity. In addition, these findings have clearly identified a form of combination therapy that is potentially applicable to human RA by virtue of the fact that it is effective but does not involve the depletion of CD4+ T cells.

Table II. Effect of anti-IL-12 treatment during the induction phase of CIA. Anti-IL-12 mAb (10F6) was administered twice weekly (500 µg/mouse) from the time of immunisation until the onset of arthritis. Modified from (12).

Treatment	Incidence of arthritis	Day of onset	Clinical score (day 10 of arthritis)
Controls	24/27 (89%)	23.5 ± 3.5	6.1 ± 0.9
Anti-IL-12	39/42 (93%)	26.2 ± 4.8	2.0 ± 0.5 (P < 0.001)

Table III. Synergistic effect of anti-TNFα and anti-IL-12 in CIA. Mice with established arthritis were treated every 2 days with anti-TNFα (cV1q; 300 µg) alone, anti-IL-12 (17.8; 500 µg) alone, or anti-TNFα plus anti-IL-12. After 10 days, the paws were processed for histology and analysed in a blinded fashion. Modified from reference 30.

Treatment	Histological severity of arthritis	
	Normal/Mild	Moderate/Severe
Controls (n = 18)	16%	84%
Anti-TNFα alone (n = 18)	38%	62%
Anti-IL-12 alone (n = 18)	30%	70%
Anti-TNFα plus anti-IL-12 (n = 18)	73%	27%

Drug/mAb combination therapy

Anti-cytokine mAb have a number of potential advantages over conventional DMARD therapy. One such advantage is that mAb therapy is likely to have a much more rapid therapeutic effect than most DMARDs. For example, in clinical trials of anti-TNFα treatment in RA, reductions in disease activity took place very rapidly, with significant reductions in pain, joint stiffness, and serum levels of C-reactive protein being detected as soon as 24 hours after treatment (17). Another advantage of mAb therapy is that it does not have any hemaetological, liver, or renal toxicity, one or more of which are associated with the use of DMARDs, nor does it have the toxicity of high-dose long-term corticosteroids. These considerations are particularly important in a chronic disease such as RA, which often requires treatment over a period of years.

However, a significant disadvantage of long-term treatment with mAb, even humanised mAb, is the potential for the development of anti-globulin responses, which leads to reduced efficacy of the mAb and the risk of anaphylaxis. This problem does not occur with DMARDs because of their small molecular size. It may be possible, therefore, to combine the mAb approach with the conventional DMARD approach so as to minimise the problems of the respective therapeutic agents whilst maximising the therapeutic effect. For example, concurrent mAb therapy may allow for a reduction in the doses of DMARDs used. Alternatively, mAbs may be used for "induction therapy," i.e. to bring patients with active

disease into a state of remission by means of a short pulse of mAb therapy, so that DMARDs can then be administered at lower doses in order to maintain the state of remission in the long term.

We have utilised the CIA model to study the effects of therapy using drug/antibody combinations, partly to identify forms of combination therapy that may be applicable to human RA and partly to analyse the mechanisms of drug action. For example, if a particular drug acts solely by suppressing TNFα activity, then little additional benefit would be obtained from administering the drug in combination with anti-TNFα mAb compared to the drug alone. On the other hand, an additive or synergistic effect between anti-TNFα mAb and the drug in question suggests that the drug is acting through a different, but complementary, pathway.

Anti-TNFα plus cyclosporin

Cyclosporin has been found in placebo-controlled clinical trials to be an effective therapeutic agent for the treatment of RA (31). It is thought that cyclosporin acts principally by blocking the activity of calcineurin, leading to a reduced level of activity of the nuclear transcription factor of activated T cells. This, in turn, causes a reduction in the level of activity of T cells (32). However, there is also a limited amount of evidence to suggest that cyclosporin suppresses macrophage function, including the production of TNFα (33). We examined the therapeutic effects of cyclosporin in combination with anti-TNFα, with the aim of finding out whether the two reagents act synergistically, as has been shown previously for anti-CD4 plus anti-TNFα (24). A comparison was also made in this study between the effects of cyclosporin and anti-TNFα in terms of their effects on Th1 cell activity, as judged by the production of IFNγ by type II collagen-stimulated T cells from treated mice.

The study confirmed that cyclosporin at a dose of 20 mg/kg body weight was effective in reducing the severity of established CIA. Treatment with cyclosporin also caused marked suppression of IFNγ production by type II collagen-stimulated CD4+ T cells, a finding that is consistent with the reported inhibitory effects of cyclosporin on T cells. However, cyclosporin was also found to reduce the expression of TNFα by macrophage-like cells in the joints of mice with CIA (34). This immediately raised the question as to whether cyclosporin was exerting a direct effect on macrophage function. However, subsequent studies showed that cyclosporin did not have a direct inhibitory effect on TNFα production by macrophages *in vitro*, and it was concluded that the suppression of TNFα expression observed *in vivo* was probably a consequence of the reduced level of T cell activity following treatment with cyclosporin.

A further finding of interest was that anti-TNFα, like cyclosporin, caused a significant reduction in the production of IFNγ by collagen-stimulated CD4+ T cells, indicating that TNFα is involved in the T cell response to type II collagen in CIA, in addition to its pro-inflammatory role. Finally, it was shown that the therapeutic effects of combined treatment with cyclosporin and anti-TNFα mAb were additive, as judged by the reductions in the clinical score (Fig. 2). A similar additive effect was observed in the protection against joint erosion. For example, cyclosporin alone and anti-TNFα alone reduced the proportions of proximal interphalangeal joints with erosions by 60% and 54%, respectively, whereas combined treatment with cyclosporin plus anti-TNFα reduced the proportion of joints with erosions by 86% (34).

Anti-TNFα plus rolipram

Rolipram is a selective type IV (PDE IV) inhibitor that affects the level of activity of a variety of different cell types through a cyclic AMP-dependent mechanism. Apart from its activity as an antidepressant, rolipram has been shown to reduce inflammation and demyelination in two animal models of autoimmune encephalomyelitis (35, 36), and we have demonstrated in mice that the drug is effective in reducing the severity of established CIA (37).

The PDE IV family of enzymes are expressed by a variety of different cells of the immune system, and the consequences of inhibiting PDE IV activity include the suppression of TNFα production by macrophages and the down-regulation of T cell proliferation. In view of this dual ability of rolipram to affect the functioning of both macrophages and T cells, we studied the effect of rolipram in combination with either anti-TNFα mAb or anti-CD4 mAb, in order to identify the principal mechanism of action of rolipram in CIA. Treatment with rolipram plus anti-TNFα was found not to provide a significantly greater therapeutic effect than either rolipram alone or anti-

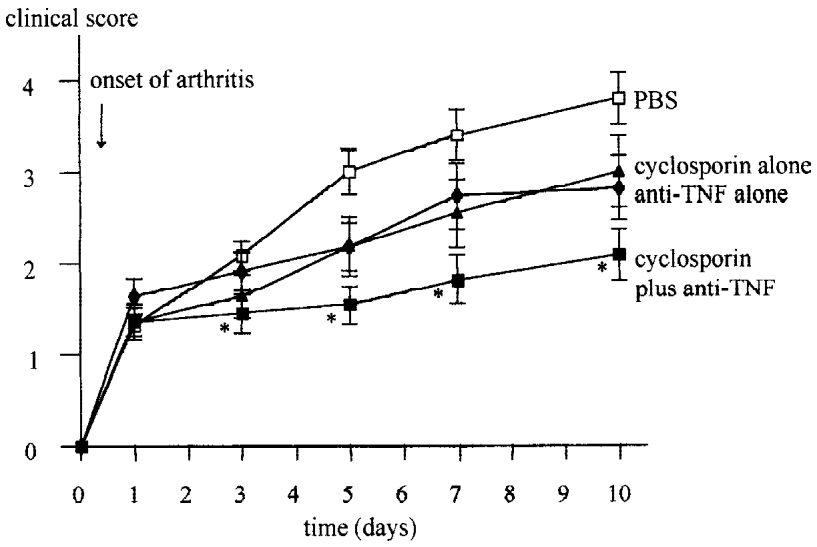

Fig. 2. Additive effect of anti-TNFα mAb (TN3-19.12) and cyclosporin in established CIA. Anti-TNFα was administered every third day at a dosage of 50 µg/mouse, and cyclosporin was administered daily at 250 µg/mouse (equivalent to approximately 10 mg/kg body weight). Treatment was initiated on day 1 of arthritis (the first day that clinical arthritis was observed) and continued until day 10. *P < 0.05 (versus the PBS-treated group). Modified from reference 34.

TNFα alone (37). In contrast, treatment with rolipram in combination with anti-CD4 resulted in a significantly reduced severity of arthritis compared to rolipram alone or anti-CD4 alone.

These findings suggest that the effects of rolipram overlap to a greater extent with those of anti-TNFα than anti-CD4, and we could infer therefore that one of the major mechanisms of action of rolipram in CIA is the suppression of TNFα activity. The fact that synergy was observed between rolipram and anti-CD4 mAb has potential implications for human therapy, since it might permit the use of lower doses of anti-CD4 mAb and non-emetic doses of a PDE IV inhibitor in RA.

Cytokines in combination therapy

It is now recognised that at the sites of inflammation, both pro- and anti-inflammatory cytokines are co-expressed. In fact, the chronic inflammation that is a characteristic feature of RA has been described as an imbalance between pro- and anti-inflammatory factors (38). IL-10 is an example of an anti-inflammatory cytokine that regulates the activity of macrophage and lymphocyte functioning, and that may, therefore, represent a potential therapeutic agent for RA. This concept is supported by a number of studies which have demonstrated amelioration of CIA by the administration of recombinant IL-10 and exacerbation of arthritis by anti-IL-10 treatment (39-43).

In one of these studies, the effect of combining IL-10 with anti-TNFα was studied in established CIA (39). The two treatments were found to produce an additive effect, particularly at the histological level. However, the apparent absence of synergy between the two treatments suggests that the therapeutic actions of IL-10 are due, at least in part, to the inhibition of TNFα activity. In a more recent study, a modest additive therapeutic effect was observed when IL-10 was administered in combination with IL-11, a cytokine belonging to the IL-6 family that possesses a number of anti-inflammatory properties (44).

Conclusions

It is clear from studies with animal models of arthritis as well as clinical trials in human RA that combination therapy offers the possibility of more effective disease control than conventional treatments (45). Increasingly, the goal in therapy will be to induce and extend the periods of remission, during which time drug therapy is not required. Most available evidence points to the involvement of T cells in the pathogenesis of RA, and it will probably be necessary to down-regulate or modulate the function of these cells if successful disease intervention is to be achieved. The challenge will be to identify and target pathogenic T cell subsets without debilitating the non-pathogenic peripheral T cell pool.

In view of the increasing emphasis on combination therapy for RA, two considerations are worth highlighting. The first is that evaluating a particular form of combination therapy in human clinical trials requires a relatively large number of patients, because the combination therapy should be tested against both forms of monotherapy as well as against placebo-treated controls. In addition, there would be logistical difficulties in performing clinical trials to test all of the potential combinations of DMARDs and biological therapies. For these reasons, the use of animal models to test combination therapies could be invaluable as a pre-screen, to pick out promising forms of combination therapy prior to testing in man.

The second consideration is that the design of effective forms of combination therapy requires some knowledge of the mechanisms of action of the available therapeutic agents. Thus, synergy between two therapeutic agents is most likely to occur if the agents affect different, yet complementary, pathways in the disease process, e.g., the immune and inflammatory pathways. Animal models of arthritis may provide valuable tools, not only for pre-clinical screening, but also to help determine the mechanisms of action of different drugs and to clarify how multiple therapeutic agents may interact *in vivo*.

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