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Functional aspects of the adaptive immune system in arthritis

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CHAPTER 3

ABATACEPT DECREASES DISEASE ACTIVITY IN THE ABSENCE OF CD4+ T CELLS IN THE COLLAGEN INDUCED ARTHRITIS MODEL

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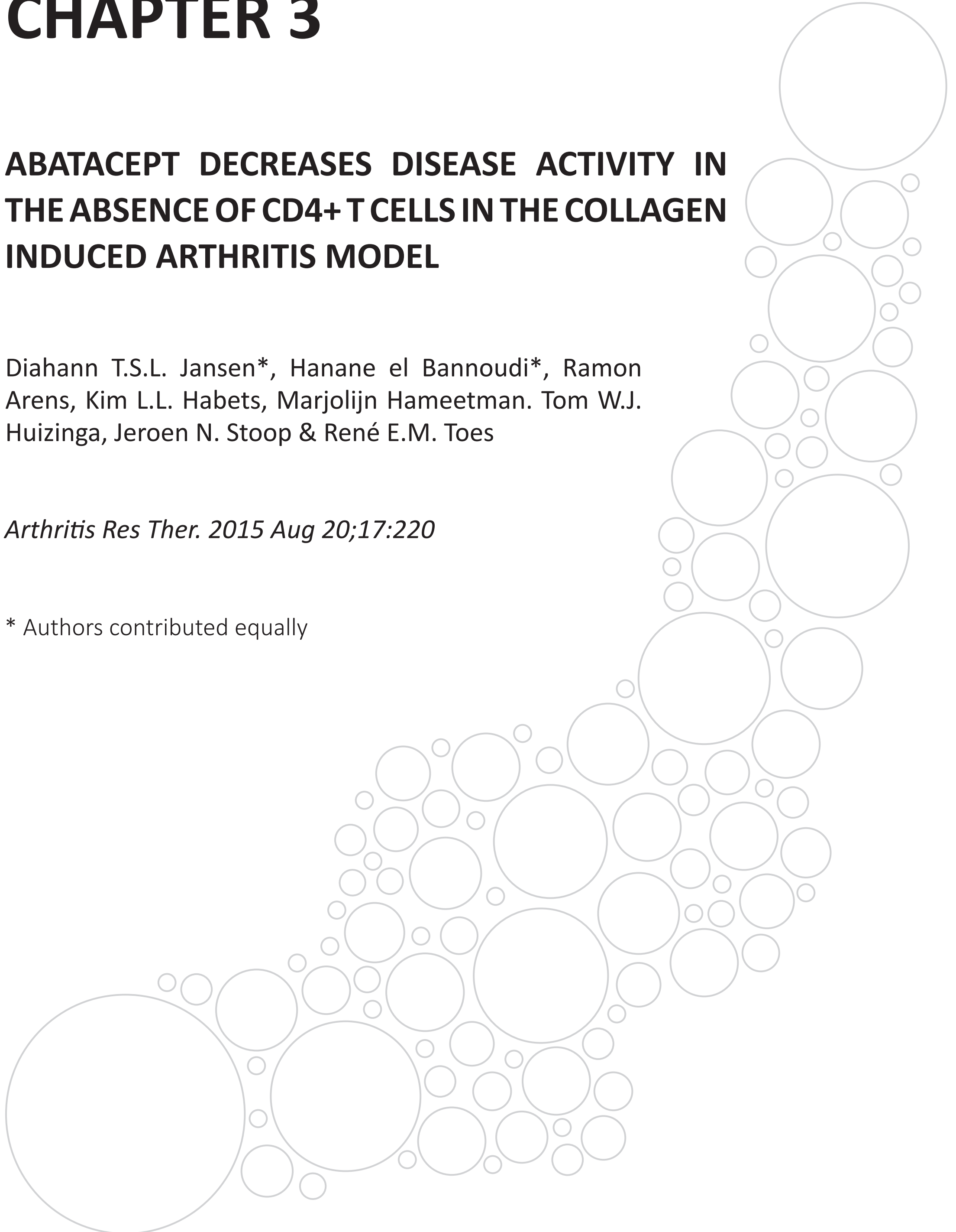

ABSTRACT

Introduction Abatacept is a fusion protein of human CTLA-4 and the Fc portion of human IgG1. It is believed to be effective in the treatment of rheumatoid arthritis by inhibiting co-stimulation of T cells via blocking CD28-B7 interactions as CTLA-4 binds to both B7.1 (CD80) and B7.2 (CD86). However, the interaction of CD28 with B7 molecules is crucial for activation of naive cells, whereas it is unclear whether the action of already activated CD4+ T cells, which are readily present in established disease, also depends on this interaction.

The aim of this study was to determine whether the mode of action of Abatacept depends solely on its ability to halt T cell activation in established disease.

Methods Arthritis was induced in thymectomized male DBA/1 mice by immunisation with bovine collagen type II. The mice were subsequently depleted for CD4+ T cells. Abatacept or control treatment was started when 80% of the mice showed signs of arthritis. Arthritis severity was monitored by clinical scoring of the paws and anti-collagen antibody levels over time were determined by ELISA.

Results Treatment with Abatacept in the absence of CD4+ T cells resulted in lower disease activity. This was associated with decreasing levels of collagen specific IgG1 and IgG2a antibodies while the antibody levels in control or CD4+ T cell-depleted mice increased over time.

Conclusion These results show that Abatacept decreased disease activity in the absence of CD4+ T cells indicating that the mode of action of Abatacept in established arthritis does not entirely depend on its effects on CD4+ T cell activation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting the joints in approximately 1% of the world population[1,2]. Patients with RA can be treated with non-steroidal anti-inflammatory drugs (NSAIDs) or with disease-modifying anti-rheumatic drugs (DMARDs). NSAIDs can alleviate disease symptoms, but do not impede the underlying inflammatory events or inhibit joint destruction, however, DMARDs do affect the disease process in all these respects[3]. Abatacept, a fusion protein of human CTLA-4 and the Fc portion of human IgG1, is a (biological) DMARD and is an effective therapy for established RA[4,5]. It is believed to be effective by blocking the co-stimulation of T cells through disruption of CD28-B7 interactions as CTLA-4 binds to B7.1 (CD80) and B7.2 (CD86) on antigen presenting cells (APC)[6].

CTLA-4-Ig has been tested in the collagen induced arthritis (CIA) model in mice and rats as a preventative treatment and on the first day of clinical onset resulting in lower clinical scores and reduced joint damage[7-9]. However, Abatacept is used to treat RA patients that failed anti-TNF treatment. It is likely that, in this phase of disease, the underlying autoimmune response is fully matured. Likewise, it is conceivable that the action of Abatacept does not fully depend on its ability to inhibit T cell responses as fully developed T cell responses are less dependent on CD28 co-stimulation. Indeed, CD28-B7 interactions are important for the activation of naive T cells, but this is less well-established for the activation of memory CD4+ T cells[10]. CD28-B7 costimulation of memory CD4+ T cells has been described to disturb IL-2 production and proliferation, however, production of other cytokines and expression of activation markers CD25 and CD69 are not affected indicating an incomplete dependence on this pathway[11]. Therefore, it is of interest to study the effect of Abatacept in the established phase of arthritis models as it is more alike the human situation with respect to the developmental phase of the underlying auto-immune response. In addition, much can be learned about the pathogenesis of human disease by understanding the mode of action of therapeutic interventions. The latter is exemplified through the use of e.g. anti-TNF or IL-6R blocking agents showing the pivotal role of these cytokines in inflammation. Nonetheless, the exact mode of action of several DMARDs used in RA treatment is still largely unclear, such as methotrexate or sulfasalazine.

A recent study described the comparison of anti-TNF treatment (Adalimumab) and Abatacept in a head-to-head study revealing similar efficacy in time based on clinical, functional and radiographic outcomes[12]. Intriguingly, anti-TNF therapy is thought to have a quick mode of action as it directly inhibits inflammation by blocking TNF, while Abatacept is thought to be effective after a longer time period as the effect of costimulation blockade will not become apparent directly. Consequently, similar efficacy of Adalimumab and Abatacept indicates a different mode of action of Abatacept in addition to costimulation blockade. Therefore, we now investigated whether the mode of action of Abatacept solely depends on its ability to halt T cell activation. We report a decrease in disease progression and activity after Abatacept

treatment in the absence of CD4+ T cells indicating that the mode of action of Abatacept in established arthritis does not entirely depend on its effects on CD4+ T cell activation.

METHODS

Mice

Male DBA/1 mice were obtained from our own breeding colony (originally obtained from Charles River). Thymectomized DBA/1 mice were purchased from Harlan. All mice were housed under specific pathogen-free conditions in individually ventilated cages at the animal facility of LUMC. All experiments were performed in accordance with national legislation and approved by the Ethical Committee for Animal Experimentation of Leiden University (approval number 11085 and 12217).

Induction of CIA and evaluation of arthritis

CIA was induced in 8-10 week old male DBA/1 mice as described before[13]. A clinical score was assigned based on a scoring protocol were each swollen or red phalanx was given 0.5 point and 1 point per toe. A red or swollen knuckle was given 1 point as well as a red or swollen footpad and a swollen ankle/wrist was given 5 points. The maximum score for each paw is 15 points resulting in a maximum score of 60 points per mouse. Disease progression was monitored till a maximum of 90 days after induction of CIA. Change in clinical score was calculated by subtracting the clinical score at start of treatment for every scoring time point after start of treatment till the end of follow up to correct for the difference in clinical score at start of treatment as the mice did not develop arthritis at the same time.

Treatment

Treatment was started when 80% of the mice showed signs of arthritis. The mice were randomized over the different treatment groups according to their score to assure that the mean clinical score of all groups was comparable at start of treatment. On day 0 of treatment, 100 µg GK1.5 (rat anti-mouse CD4 mAb), was administered intraperitoneally to the mice that received CD4+ T cell depletion to acquire CD4 depletion at start of treatment. This was weekly continued till the end of the experiment. For the different treatment regimens, mice were injected intraperitoneally with 500 µl PBS, 100 µg GK1.5, 1 mg Abatacept (Bristol-Myers Squibb), 100 µg GK1.5 in combination with 1 mg Abatacept, 1 mg isotype for Abatacept (Roche) or 1 mg isotype in combination with 100 µg GK1.5 on day 1, 3, 5, 8, 12 and 19.

Evaluation of CD4+ T cell counts

To confirm that CD4+ T cell depletion after GK1.5 treatment was complete, blood was collected on day 0, 12, 22, 35 and at the end of the experiment. The blood was lysed and subsequently stained with CD3 PerCP Cy5.5 (145-2C11), CD4 FITC (RM4-4), CD8 APC (Ly 2 53-6.7) from BD Pharmingen and CD45 efluor 450 (30-F11) from eBioscience. All samples were evaluated by a BD LSRFortessa cell analyser (BD Biosciences) and analysed using BD FACSDIVA

software (BD Biosciences) and FlowJo version 7.6.5 (Tree Star Inc).

Measurement of serum antibodies by ELISA

Anti-collagen type II and total IgG antibody levels were determined as described before[14]. In short, Nunc Maxisorp plates (Thermo Scientific) were coated with 2 µg/ml bovine type II collagen (Chondrex) or 3 µg/ml murine type II collagen (Chondrex) for antigen specific antibodies or with 0.5 µg/ml goat anti-mouse IgG (Southern Biotechnology) for total antibodies. IgG, IgG1 and IgG2a were detected using goat anti-mouse IgG HRP, goat anti-mouse IgG1 HRP and goat anti-mouse IgG2a HRP respectively (all Southern Biotech). Enzyme activity was visualized using ABTS. Serial dilutions from pooled sera of arthritic mice were used as standard to calculate arbitrary units (AU).

Measurement of supernatant antibody titers

At sacrifice bone marrow and spleen cells were isolated and 200.000 cells per well were cultured in IMDM (Lonza) containing 10% fetal calf serum (Gibco), glutamax, penicillin, streptomycin (Invitrogen) and 2-mercaptoethanol. After 7 or 14 days of culture supernatant was harvested and total IgG levels were determined by ELISA.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc.). The Abatacept and CD4 depletion combination treated group and control treated group were compared using the Student's T-test or the Mann-Whitney U test as appropriate according to data distribution. *p*-values <0.05 were considered to be significant.

RESULTS

Abatacept decreased disease activity in mice depleted of CD4+ T cells by GK1.5

CTLA-4-Ig treatment has been used in the CIA model as a preventative intervention[7-9]. However, CTLA-4-Ig has not been tested in established disease where most of the disease-associated T cells are thought to be already activated or to have differentiated into memory T cells. Hence, it is not known whether its mode of action is also mediated through T cell inhibition in this disease phase. To investigate this, CIA was induced and treatment was started when 80% of the mice showed signs of arthritis. One day before start of treatment, CD4+ T cells were depleted by intraperitoneal administration of the CD4+ T cell depleting mAb GK1.5 and GK1.5 treatment was continued weekly until the end of follow up. Intriguingly, mice treated with the combination of CD4 depletion and Abatacept showed a significant decrease in disease activity compared to the mice treated with GK1.5 only or PBS control (Figure 1B and 1C). In contrast, CD4 depletion only did not significantly alter arthritis development compared to the control group (Figure 1B and 1C). Abatacept treatment in combination with CD4 depletion did not result in a lower number of affected paws, however, combination treatment did reduce the number of severely affected paws (clinical score >=5) (Figure 1D and 1E). In addition, novel paws that developed inflammation after start of therapy, displayed a lower disease score.

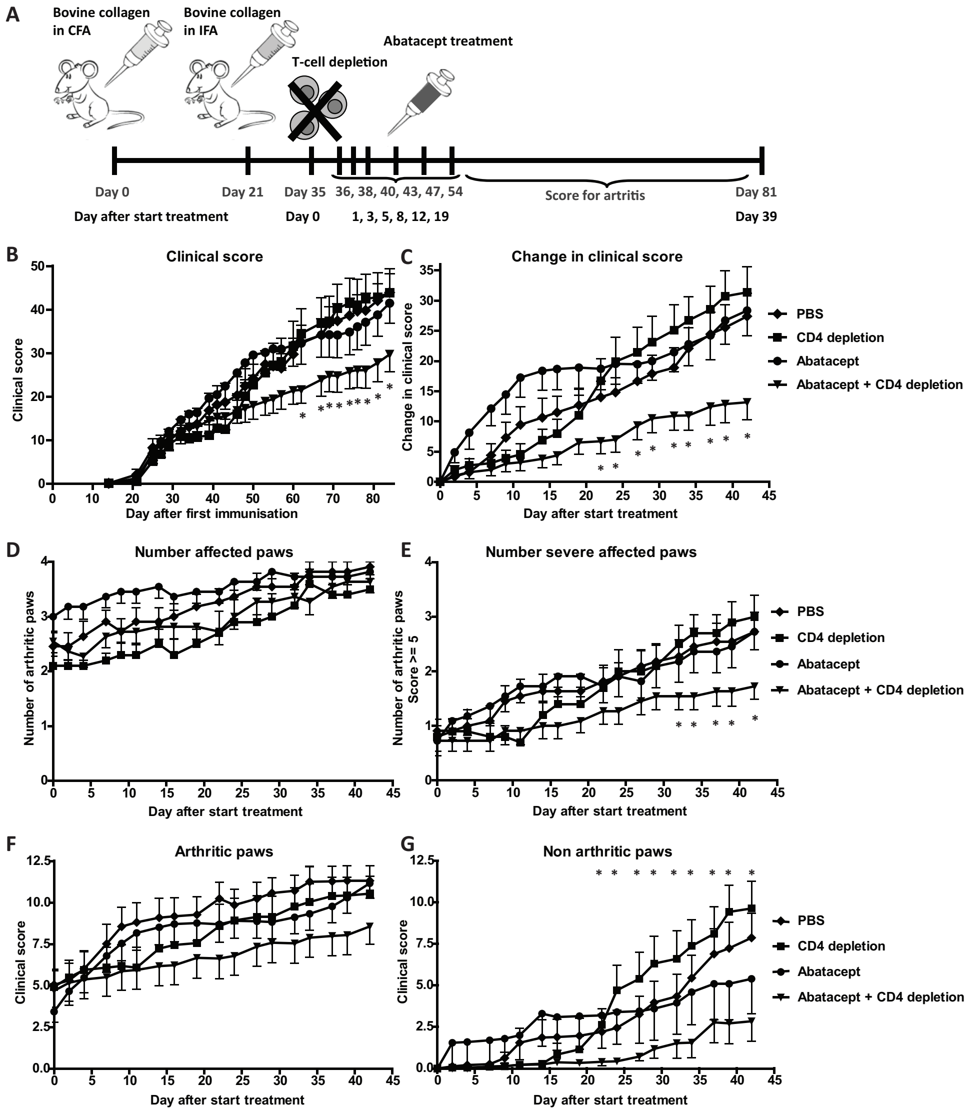

Figure 1. Abatacept decreased disease activity in mice depleted of CD4+ T cells by GK1.5. (A) Collagen induced arthritis was induced in male DBA/1 mice and when 80% of the mice showed signs of arthritis, treatment was started. One day before start of treatment CD4+ T cells were depleted by intraperitoneal administration of GK1.5 and the depletion was continued until the end of follow up. Treatment was administered by intraperitoneal injection of either PBS (diamonds), GK1.5 (CD4 depletion) (squares), Abatacept (circles) or the combination of GK1.5 and Abatacept (triangle). The mice were scored 3 times per week for inflammation in the paws to monitor disease progression. (B) Clinical score over time of the different treatment groups. (C) Change in clinical score from day of start of treatment over time of the different treatment groups. (D) Number of affected paws per treatment from the day of start of treatment over time. (E) Number of affected paws with a clinical score of 5 or higher (considered as severe affected paws) per treatment from the day of start of treatment. (F) Clinical score of paws that showed signs of arthritis at start of treatment over time per treatment. (G) Clinical score of paws that did not show signs of arthritis at start of treatment per treatment over time. Values are mean ± SEM, n=11 per treatment group. Statistical analysis was performed using the Student's T-test. * p<0.05 Abatacept + CD4 depletion vs CD4 depletion

Thus Abatacept treatment did not prevent arthritis development in non-affected joints, but it did reduce clinical score of affected joints (Figure 1F and 1G). Similar results were also obtained in an independent replication experiment (data not shown).

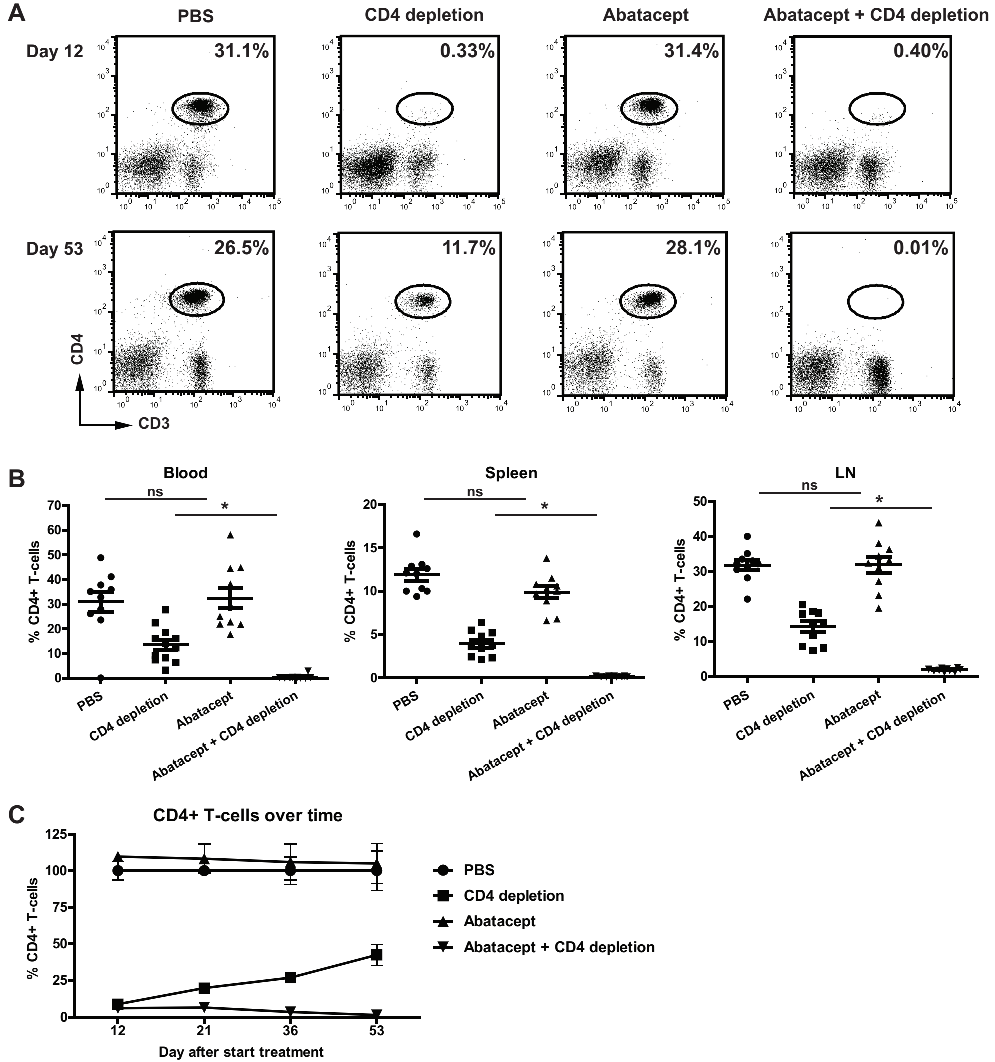

Figure 2. Incomplete CD4+ T cell depletion by GK1.5 over time. The presence of CD4+ T cells in the blood was monitored over time using flow cytometry. Blood was collected by tail incision during the experiment or cardiac puncture at sacrifice. After red blood cell lysis, blood mononuclear cells were cell-surface stained for CD45, CD3, CD4 and CD8. Cells were gated on CD45 and subsequently on CD3 and CD4. (A) Dotplots of representative mice of each treatment group on day 12 and day 53 after start of treatment (end of experiment). (B) Summary of the percentage of CD4+ T cells per treatment at the end of follow up is depicted for the blood, spleen and inguinal lymph node (LN). Each symbol represents 1 mouse. (C) Summary of CD4+ T cells in the blood over time as a percentage of the PBS treated group. Values are mean ± SEM, n=11 per treatment group. Statistical analysis was performed using the Student's T-test. * p<0.05 Abatacept + CD4 depletion vs CD4 depletion, ns=not significant

To confirm complete CD4+ T cell depletion CD4+ T cell frequencies were evaluated in blood of GK1.5-treated mice. On day 12 after start of treatment CD4+ T cells were correctly depleted as expected. However, on day 53 CD4+ T cell depletion was not complete anymore. In contrast, mice receiving the combination of Abatacept and CD4 depletion were still depleted of CD4+ T cells (Figure 2A). This was also the case in the spleen and inguinal lymph node (Figure 2B). Examining the frequency of CD4+ T cells over time indicated that from day 12 onwards the CD4+ T cell frequencies gradually increased until the end of the experiment. Nonetheless, at this time point CD4+ T cell frequencies were still significantly lower compared to the groups that did not receive GK1.5 (Figure 2C). In contrast, CD4+ T cells in the mice receiving the combination of GK1.5 and Abatacept treatment remained depleted (Figure 2C), conceivably as a result from the prevention of the development of an anti-rat antibody response against GK1.5 by Abatacept. Together, these results show that Abatacept is also effective in a situation where CD4+ T cell number is greatly reduced, suggesting a T cell-independent effect of Abatacept that inhibits the progression of arthritis.

Abatacept decreased disease activity in thymectomized mice depleted of CD4+ T cells

Although the data presented above point to a CD4+ T cell independent effect of Abatacept in the treatment of arthritis, they do not show such effects in a conclusive manner as CD4+ T cells returned after initial depletion. To ascertain that the CD4+ T cells were completely depleted during the treatment, a more stringent method of CD4+ T cell depletion was implemented. CIA was induced in mice that were thymectomized at 6 weeks after birth. After immunisation with collagen type II and arthritis development, CD4+ T cell depletion was performed using GK1.5. Because the mice were thymectomized, no new T cells could reappear in CD4+ T cell-depleted mice. Again, we observed that treatment with Abatacept resulted in reduced disease activity in CD4+ T cell-depleted mice (Figure 3B). Likewise, a reduced clinical score of paws that were not arthritic at the start of treatment was observed (Figure 3C) as well as a reduced number of severely affected paws, but Abatacept treatment did not prevent arthritis development in joints not affected at start of therapy (data not shown). Abatacept only treatment did not modulate the clinical score compared to PBS treatment (data not shown). To confirm that CD4+ T cells were completely depleted, CD4+ T cell frequencies were monitored over time by flow cytometry. Contrary to treatment with GK1.5 only (Figure 2), thymectomy in combination with GK1.5 treatment resulted in complete depletion of CD4+ T cells in mice receiving CD4 depletion (alone or in combination with Abatacept) (Figure 3D). These results indicate that Abatacept treatment results in decreased disease activity in the absence of CD4+ T cells.

Reduced antibody levels after treatment with Abatacept in the absence of CD4+ T cells

Collagen type II specific antibodies play a crucial role in the development of CIA[15]. Therefore, collagen type II specific and total IgG levels were determined over time in serum of the thymectomized mice by ELISA. Treatment with Abatacept in the absence of CD4+ T cells

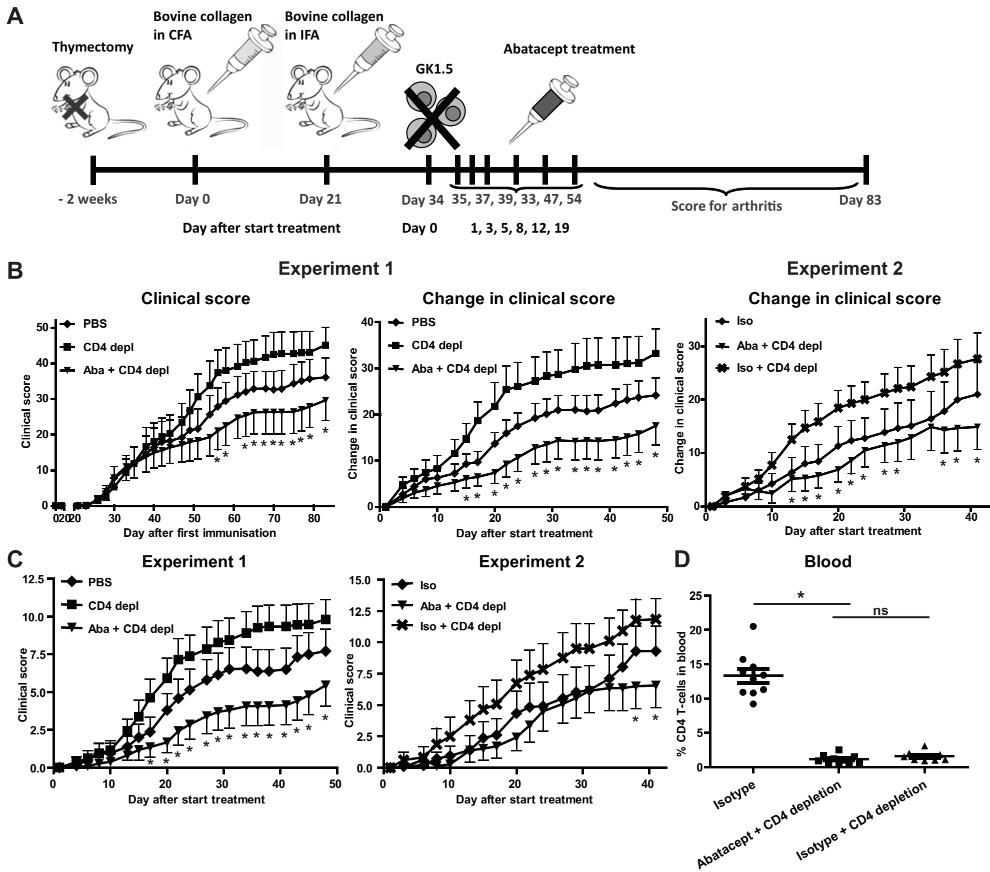

Figure 3. Abatacept decreased disease activity in thymectomized mice depleted of CD4+ T cells. (A) Collagen induced arthritis was induced in male DBA/1 mice 2 weeks after they were thymectomized. When 80% of the mice showed signs of arthritis, treatment was started. One day before start of treatment CD4+ T cells were depleted by intraperitoneal administration of GK1.5 and the depletion was continued till the end of follow up. Treatment was administered by intraperitoneal injection of PBS (diamonds), GK1.5 (squares; CD4 depl) or the combination of GK1.5 and Abatacept (triangle; Aba + CD4 depl). The mice were scored 3 times per week for inflammation in the paws to monitor disease progression. (B) Clinical score and change in clinical score from day of start of treatment over time of the different treatment groups (Experiment 1). *n*=10 per treatment group. The same experiment was independently repeated in another 10 mice per treatment group and an isotype for Abatacept was used as control treatment (diamond; iso) and in combination with CD4 depletion (cross; iso + CD4 depl) (Experiment 2). Change in clinical score from day of start of treatment is depicted. In C the clinical score of the paws that did not show signs of arthritis at the start of treatment is depicted for Experiment 1 and 2. The frequency of CD4+ T cells in blood at the end of follow up was determined by flow cytometry depicted in D. Abatacept only treatment is not depicted for readability of the graphs. Values are mean ± SEM. Statistical analysis was performed using the Student's T-test. * $p<0.05$ Abatacept + CD4 depletion vs control group, ns=not significant

resulted in decreased total IgG2a levels over time compared to control (Figure 4). More importantly, also a decrease in bovine collagen type II (immunisation antigen) and murine collagen type II (autoantigen) levels was detected after treatment with Abatacept and GK1.5 (Figure 4). This result was not specific for the IgG2a isotype as decreased IgG1 levels were

observed as well (Figure 4). Together, these results indicate that Abatacept treatment leads to a reduction in disease activity as well as collagen specific antibody levels in the absence of CD4+ T cells.

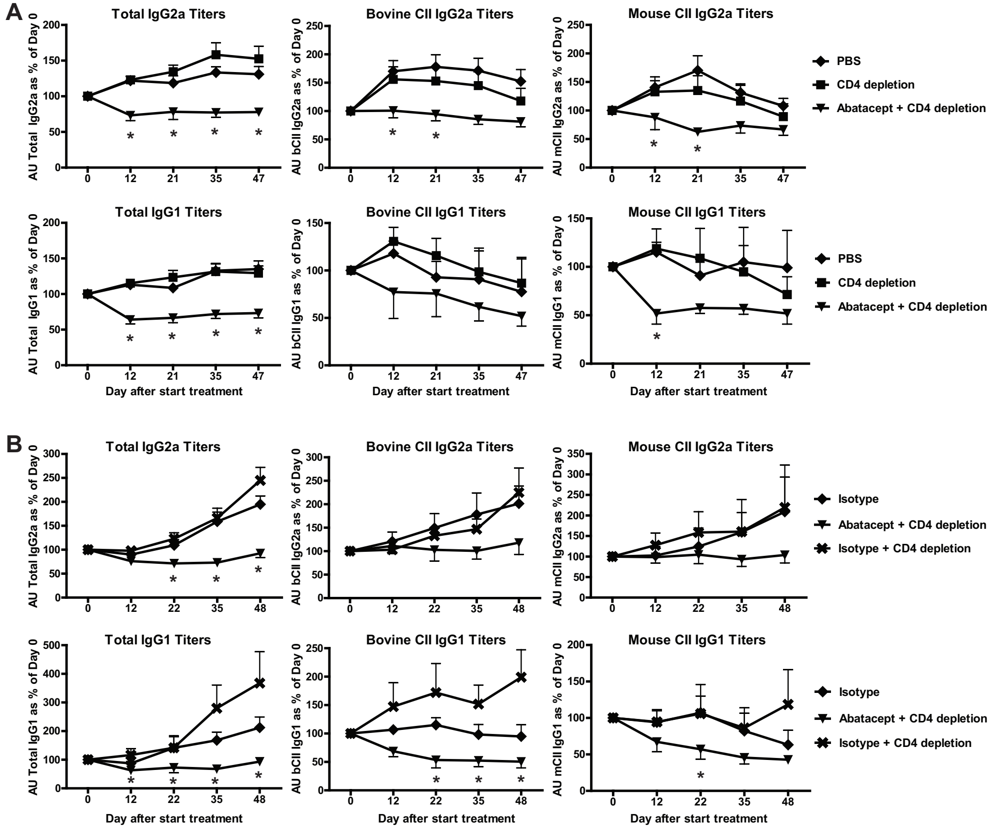

Figure 4. Reduced antibody levels after treatment with Abatacept in the absence of CD4+ T cells. Antibody levels were determined by ELISA over time. Serum samples were collected on day 0, 12, 21-22, 35 and 47-48 after start of treatment and total IgG2a and IgG1 levels were determined in the thymectomized mice. In addition, bovine and mouse collagen type II specific IgG2a and IgG1 levels were determined. Levels are depicted as percentage of day 0 in A. Figure B depicts an independent experiment including the isotype for Abatacept. Abatacept only treatment is not depicted for readability of the graphs, but showed comparable results to Abatacept + CD4 depletion. Levels are depicted as percentage of day 0. Values are mean ± SEM, *n*=10 per treatment group. Statistical analysis was performed using the Mann-Whitney U test. * $p<0.05$ Abatacept + CD4 depletion vs control group

Reduced antibody levels in supernatant of *ex vivo* cultured spleen and bone marrow cells after treatment with Abatacept in the absence of CD4+ T cells

The data presented above suggests that Abatacept treatment could directly affect the number and/or activity of antibody-secreting B cells. To study whether Abatacept in addition to the inhibition of costimulation of T cells also affected the antibody producing capacity of spleen and bone marrow derived B cells, we next isolated spleen- and bone marrow cells from

treated animals. Total IgG levels in the supernatant of *ex vivo* cultured, but not stimulated, spleen and bone marrow cells were subsequently analysed by ELISA (Figure 5). Spleen cells of mice treated with Abatacept and CD4 depletion produced lower IgG levels after 7 and 14 days of culture compared to spleen cells of mice receiving only CD4 depletion, although the percentage of B cells and plasma cells, as analysed by flow cytometry, was comparable between the different treatment groups (data not shown). This reduction in IgG production was also observed in the supernatant of cultured bone marrow cells indicating a loss of antibody producing capacity after Abatacept treatment in the absence of CD4+ T cells.

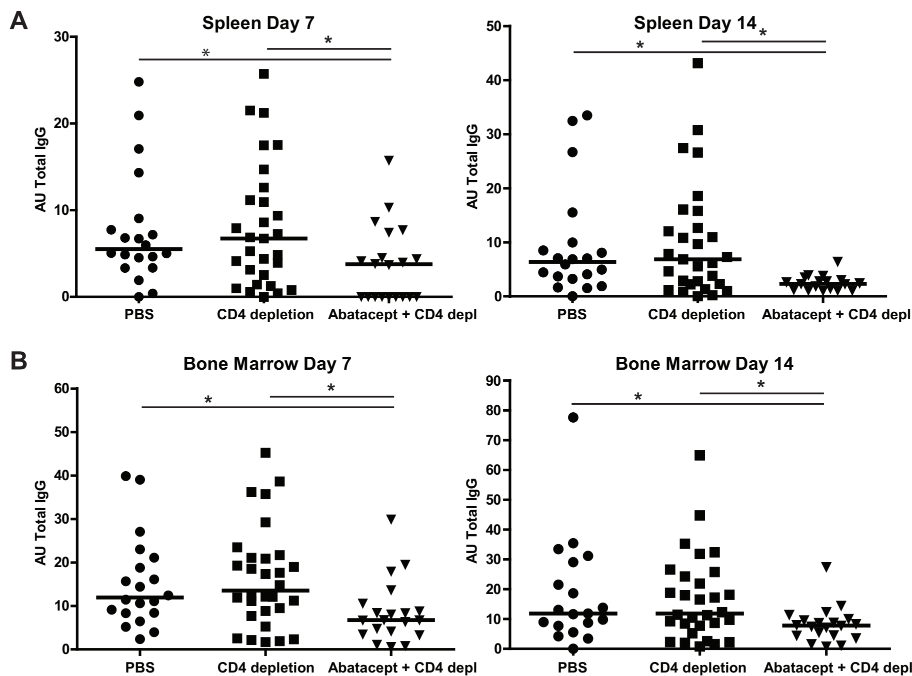

Figure 5. Abatacept decreased antibody levels detected in supernatant of *ex vivo* spleen and bone marrow cells in the absence of CD4+ T cells. At sacrifice spleen and bone marrow cells were collected and cultured without stimulation for 7 or 14 days and IgG production was measured in the supernatant by ELISA. (A) Summary of the IgG levels detected in supernatant of spleen cells of Experiment 1 and 2. The IgG levels of the PBS and isotype treated mice are combined and depicted as 'PBS' and the CD4 depletion and combination of isotype and CD4 depletion treated groups are combined and depicted as 'CD4 depletion'. (B) Summary of the IgG levels detected in supernatant of bone marrow cells cultured for 7 or 14 days of Experiment 1 and 2. Statistical analysis was performed using the Mann-Whitney U test. * $p<0.05$ Abatacept + CD4 depletion vs control group

DISCUSSION

Abatacept is an effective treatment in RA and is thought to block costimulation of T cells by inhibiting CD28-B7 interactions as Abatacept binds to both B7.1 and B7.2[6]. The interaction of CD28 with both B7 molecules is crucially important for the activation of naive T cells whereas its role in the activation of already activated/memory CD4+ T cells is less clear. As such CD4+ T cells are readily present in established disease, we investigated whether Abatacept is still

effective in the absence of CD4+ T cells in the established disease phase of the CIA model. Our study revealed that Abatacept treatment is able to decrease disease activity in the absence of CD4+ T cells, indicating that the mode of action mediated by Abatacept in CIA does not solely depend on its ability to block costimulation of T cells. In addition, Abatacept treatment is also capable of reducing collagen-specific and total antibody levels in a T cell independent setting. To evaluate the mode of action of Abatacept in established disease, CD4+ T cells were depleted using the rat anti-mouse CD4 antibody, GK1.5. Remarkably, after 12 days of depletion the CD4+ T cells gradually reappeared in mice treated with GK1.5 only. Interestingly, the CD4+ T cells remained properly depleted in mice treated with the combination of Abatacept and GK1.5. This observation is most likely explained by the notion that mice treated with GK1.5 develop an anti-rat antibody response ultimately neutralizing the CD4 depleting antibodies. This illustrates that Abatacept is also capable of blocking costimulation and thereby the activation of naive T cells, preventing the development of the anti-rat response. Therefore, the depletion of CD4+ T cells by GK1.5 in mice treated with Abatacept resulting in the complete and sustained depletion of CD4+ T cells until the end of follow up. For this reason, we also depleted CD4+ T cells in thymectomized mice to prevent the reappearance of new T cells when the GK1.5 treatment became less effective and we obtained similar results.

Our results indicate that CD4+ T cells are not required for disease progression once arthritis is established, since the mice treated with GK1.5 only showed comparable or a trend towards more severe disease progression as compared to the control groups. These observations are in line with the observation by Morgan *et al.* describing that lethal irradiation of mice with CIA, followed by syngeneic bone marrow transplantation, results in continuation of the disease even though the T cells were depleted[16]. Thus, CD4+ T cells are not required for arthritis progression once the disease has become clinically manifest in the CIA model. The presented results are obtained in a mouse model, however, it is not known whether Abatacept has a direct effect on other cell types in addition to T cells in the human situation. Nonetheless, a recent study in RA, comparing head-to-head anti-TNF and Abatacept treatment reported comparable efficacy based on clinical, functional and radiographic outcomes[12]. Intriguingly, no difference in the rate of response was noted as similar improvements were observed over time. As one could speculate that a T cell targeting drug would require more time to mediate its beneficial effects compared to a TNF inhibitor, this observation could be compatible with the notion that Abatacept has a different mode of action in addition to the blocking effect on T cell costimulation in humans as well.

The observation that Abatacept is capable of decreasing disease activity in the absence of CD4+ T cells does not contradict with the ‘dogma’ that the mode of action of Abatacept is mediated through blockade of costimulation and thereby activation of (naive) CD4+ T cells. However, our results do indicate that Abatacept also has another mode of action in addition to its effects on costimulation. A limitation of our study is that we did not elucidate the mechanism responsible for the inhibitory effect of Abatacept on arthritis. In this respect

Abatacept is no different from other DMARDs as methotrexate for which the exact mode of action is also not elucidated. Nonetheless, it would be interesting to delineate these additional modes of action[17] as it could allow for a more refined targeted therapy and additional insights into the etiological pathways of disease.

Recently, Rozanski *et al.* have described that CD28 serves as a survival factor for long lived plasma cells. Loss of CD28 or B7.1 (CD80) and B7.2 (CD86) caused significant loss of long lived plasma cells resulting in decreased antibody titers[18]. As Abatacept prevents the binding of CD28 to CD80/86, this survival signal could be abrogated leading to loss of plasma cells and consequently a decrease in antibody titers. Indeed, this would be in line with our observation of decreased antibody levels after Abatacept treatment, reduction of clinical scores as well as our observation of decreased antibody production by cultured spleen and bone marrow cells from CD4+ T cell depleted mice treated with Abatacept. Likewise, in the BXD2 mouse model of autoimmune disease it has been reported that elevated expression of activation induced cytidine deaminase (AID) in recirculating follicular CD86+ B cells and increased germinal centre activity are associated with the production of autoantibodies[19]. Treatment with CTLA-4-Ig resulted in normalization of AID expression in the B cells and suppression of IgG autoantibodies, which could explain the decrease in IgG titers we observed after Abatacept treatment in the absence of CD4+ T cells.

The CD4+ T cell independent effect of Abatacept could also be explained by the induction of nitric oxide synthase (NOS) or indoleamine 2,3-dioxygenase (IDO) by antigen presenting cells[20-22]. IDO is an enzyme that degrades the essential amino acid tryptophan resulting in local depletion of tryptophan[23], which leads to cell cycle arrest[24,25] and thereby inhibition of T cell proliferation and expansion of the immune response[26-28]. IDO has been implicated in disease aetiology as, for example, it has been reported that CTLA-4 on regulatory T cells can induce IDO in APCs[29], but that regulatory T cells from RA patients fail to induce such expression due to low CTLA-4 expression[30]. In addition to the suppressing effect of IDO on proliferating effector T cells, IDO expressing dendritic cells are able to promote the activation of regulatory T cells[31] and the differentiation of naïve T cells to regulatory T cells[23,32] which could explain the inflammation suppressing effects of Abatacept. However, Davis *et al.*[33] reported inhibition of naïve and memory T cell proliferation and effector function in the absence of IDO induction, indicating that Abatacept could also have other mechanisms of action.

Abatacept could also have an effect on osteoclast precursors explaining the anti-erosive effects of Abatacept[34,35] or on monocytes by modulating their migratory capacity)[36]. Direct effects on macrophages have also been described resulting in decreased cytokine production and reduction of the inflammatory reaction[37-40] which could account for the beneficial effects in the treatment of RA.

In conclusion, Abatacept is thought to block costimulation of T cells by blocking the interaction between CD28 and B7 resulting in inhibition of T cell activation. However, Abatacept reduced disease progression and activity in the absence of CD4+ T cells in the CIA mouse model

indicating that Abatacept can exert its action in established arthritis independent from its effects on CD4+ T cell activation. Since a head-to-head comparison of anti-TNF and Abatacept treatment resulted in comparable efficacy with a similar time course, it is tempting to speculate that Abatacept could have an effect on other cell types in addition to T cells in human RA as well.

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