Direct suppression of autoaggressive CD8+ T cells with CD80/86 blockade in CD8+ T cell-mediated polymyositis models of mice

H. Hasegawa, K. Kawahata, F. Mizoguchi, N. Okiyama, N. Miyasaka, H. Kohsaka

Department of Rheumatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan.

Abstract

Objective

CD80/86 blockade to inhibit CD28 costimulation suppressed alloreactive human and murine CD4+ T cells but not alloreactive CD8+ T cells. In contrast, CD28 costimulation augments CD8+ T cell-mediated cell lysis in antigen-nonspecific stimulation. The present study was conducted to discern whether the CD80/86 blockade exerts therapeutic effects on CD8+ T cell-mediated polymyositis (PM) models of mice and whether the effects could be attributable to direct suppression of autoantigen-specific CD8+ T cells.

Methods

C protein-induced myositis (CIM) was induced in mice with intradermal injection of C protein fragments. C protein peptide-induced myositis (CPIM), in which autoaggressive CD8+ T cells are activated without CD4+ T cell help, was induced in mice with intravenous injection of dendritic cells (DCs) loaded with CD8+ T cell-epitope peptides derived from the C protein fragment. The immunised mice were treated with CTLA4-Ig or anti-CD80 and anti-CD86 antibodies (anti-CD80/86 Abs). The muscles were evaluated histologically 21 days after the C protein immunisation or 7 days after the DC injection.

Results

CIM was suppressed in the mice treated with CTLA4-Ig or anti-CD80/86 Abs administered prophylactically from the day of immunisation and therapeutically after the disease onset. CPIM was suppressed when CTLA4-Ig was administered concurrently with the DC injection.

Conclusion

The CD80/86 blockade was effective in PM models of mice. Amelioration of CPIM indicates direct suppression of CD8+ T cells by the CD80/86 blockade. CTLA4-Ig should be a potential therapeutic agent of PM and other CD8+ T cell-mediated diseases by suppressing both autoantigen-specific CD4+ and CD8+ T cells.

Key words

polymyositis, mouse models, autoantigen-specific CD8+ T cells, CD28-CD80/86 costimulation, CTLA4-Ig

Hisanori Hasegawa, MD
Kimito Kawahata, MD, PhD
Fumitaka Mizoguchi, MD, PhD
Naoko Okiyama, MD, PhD
Nobuyuki Miyasaka, MD, PhD
Hitoshi Kohsaka, MD, PhD

Please address correspondence to:
Dr Hitoshi Kohsaka, ,
Department of Rheumatology,
Graduate School of Medical
and Dental Sciences,
Tokyo Medical and Dental
University (TMDU),
1-5-45 Yushima, Bunkyo-ku,
Tokyo 113-8519, Japan.
E-mail: kohsaka.rheu@tmd.ac.jp

Address reprint requests to:
Dr Hisanori Hasegawa,
E-mail: hasegawa.rheu@tmd.ac.jp

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Introduction

Muscle injury in polymyositis (PM) is suggested to be mediated by autoaggressive cytotoxic CD8+ T cells (1). Nonetheless, conventional treatment for PM depends on high-dose glucocorticoids with or without other non-specific immunosuppressants such as azathioprine and methotrexate. In addition, anecdotal use of rituximab, which is a B cell depleting agent, is increasing for PM recently (2). However, some patients are refractory to these treatments while others suffer from adverse effects. T cell activity can be suppressed with calcineurin inhibitors but often insufficiently because of their renal toxicity at high dose ranges. Agents that can suppress autoaggressive CD8+ T cells directly without significant adverse effects should offer specific and safe treatment for PM.

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) Ig binds to CD80/CD86 on antigen presenting cells and suppresses T cell functions by blocking CD28-CD80/86 costimulation (3). Abatacept, which is a human CTLA4-Ig with mutations in the hinge region of its Fc portion (4), is used in the treatment of rheumatoid arthritis (RA) with low rate of discontinuation for safety reasons.

Abatacept was used with success in 2 PM patients and 1 dermatomyositis (DM) patient who had not responded to the conventional immunosuppressive treatments (5-7). This fact indicates that CTLA4-Ig is effective in the tissue injury driven by autoaggressive cytotoxic CD8+ T cells as the final effector cells. Muscle injury in C protein-induced myositis (CIM), a murine model of PM, is mediated by autoaggressive CD8+ T cells (1). By treating CIM with CTLA4-Ig, we should be able to discern whether CD80/86 blockade suppresses tissue injury driven by autoaggressive CD8+ T cells.

CTLA4-Ig suppressed allogeneic reaction of CD4+ T cells in human and mice, but not that of CD8+ T cells (3, 8-10). In contrast, CD28 costimulation augments CD8+ T cell-mediated cell lysis in antigen-nonspecific stimulation with anti-CD3 antibodies (11). CD28-CD80/86 costimulation can be important for activation of autoantigen-specific CD8+ T cells. If it is dispensable, therapeutic effects of CTLA4-Ig on PM and DM (PM/DM) should be mediated solely by suppression of CD4+ T cells that promote CD8+ T cell differentiation.

C protein peptide-induced myositis (CPIM) is another autoimmune myositis model we established (12). CPIM was induced with intravenous transfer of activated bone marrow-derived dendritic cells (BMDCs) loaded with CD8+ T cell-epitope peptides that derive from the murine C protein fragment. It was inhibited in CD8+ T cell-depleted mice, but not in CD4+ T cell-depleted mice. These facts indicated that autoaggressive CD8+ T cells should be activated without CD4+ T cell help in CPIM (12), which should be useful in developing therapeutic approaches to direct suppression of autoaggressive CD8+ T cells. If CD80/86 blockade suppresses autoaggressive CD8+ T cells directly, CTLA4-Ig should be a potential therapeutic agent of PM and other CD8+ T cell-mediated diseases by suppressing both autoaggressive CD4+ and CD8+ T cells.

Methods

Mice

Female C57BL/6 (B6) mice were purchased from Charles River Japan (Yokohama, Japan). All animal experiments were carried out under specific pathogen-free conditions and approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (TMDU).

Reagents

CTLA4-Ig (abatacept) was purchased from Bristol-Myers Squibb (New York, NY). Anti-CD80 antibodies (RM80, rat IgG2a) and anti-CD86 antibodies (PO3, rat IgG2b) were kindly provided by Dr M Azuma (TMDU, Tokyo, Japan). Bovine serum albumin (BSA), rat polyclonal IgG and HILIYSDV, a CD8 T cell-epitope peptide, were purchased from Sigma-Aldrich (St. Louis, MO) (12).

Induction of myositis

CIM was induced in 8-week-old B6 mice with intradermal injection of re-

combinant human C protein fragments emulsified in complete Freund's adjuvant (CFA) at the back and hind footpads (1). To induce CPIM, first, leukocytes from femurs and tibiae of 6-week-old B6 mice were differentiated into DCs with culture supernatant of Chinese Hamster Ovary cells transfected with a murine granulocyte-macrophage colony-stimulating factor gene (13). The BMDCs were activated by 24-hour of culture with lipopolysaccharides (Sigma-Aldrich) and incubated with HILIYSDV in the last 1 hour of the culture (12). Then, two million of the activated BMDCs loaded with HILIYSDV were transferred intravenously twice with a three-day interval to 8-week-old B6 mice together with intradermal injection of CFA at the hind footpads (12).

Histological evaluation of myositis

Haematoxylin and eosin (HE)-stained 10 μm sections of the quadriceps and hamstring were examined histologically in a blinded manner for the presence of mononuclear cell infiltration and degeneration of the muscle fibres. Histological severities of myositis in the sections were graded as described previously (14). Histological score of each muscle block was calculated by averaging grades of 2 different sections of the muscle for CIM or 4 different sections for CPIM, and was added to represent the final histological score of each mouse.

Statistical analysis

Histological scores of CIM and CPIM were analysed statistically using Mann-Whitney *U*-test.

Results

Prophylactic and therapeutic effects of CTLA4-Ig on mice with CIM

To discern whether blockade of CD28-CD80/86 costimulation ameliorates CIM, which is mediated by $CD8^+$ effector T cells, CTLA4-Ig was administered to mice immunised with C protein fragments. BSA acted as a control. As treatment in a prophylactic protocol, CTLA4-Ig or BSA was administered from the day of the C protein immunisation (day 0). Histological evaluation of the quadriceps and hamstring revealed multiple lesions of mononuclear cell infiltration with degenerated muscle fibres in the muscle section from the control mice (Fig. 1A). These lesions were suppressed in the mice treated with CTLA4-Ig (Fig. 1B-C).

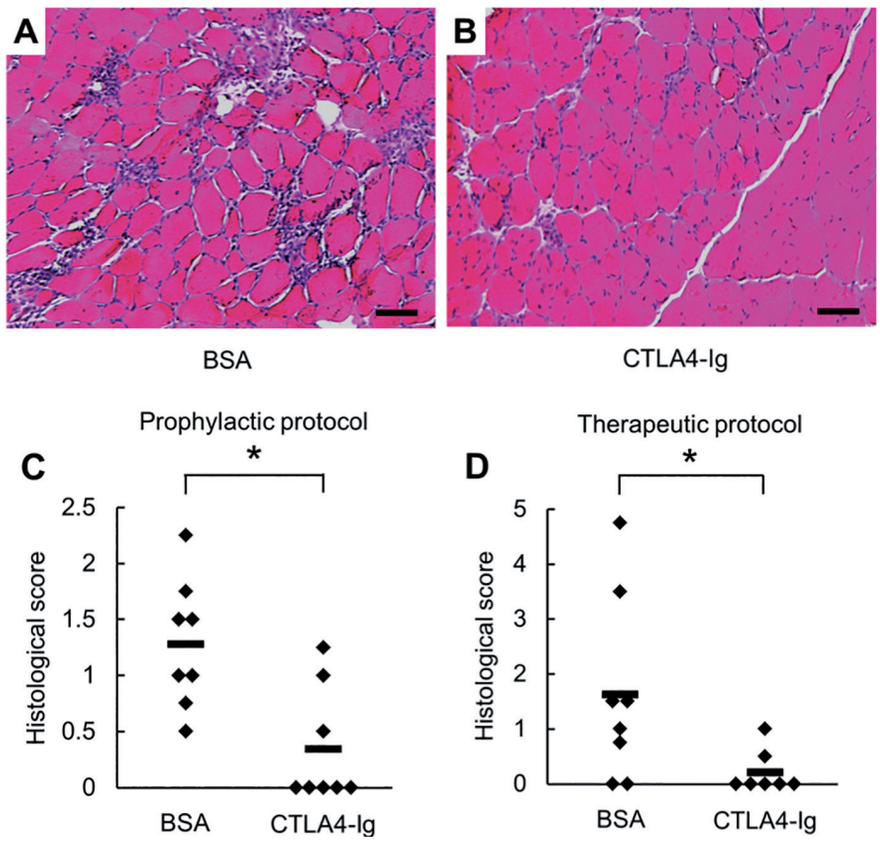

Fig. 1. Prophylactic and therapeutic effects of CTLA4-Ig on CIM. In a prophylactic protocol, 1000 μg of CTLA4-Ig or BSA was administered intraperitoneally to mice with CIM every 3 days for 4 times from the day of C protein immunisation (day 0). Eight mice were engaged in each experimental group. Histological evaluation of the quadriceps and hamstring from the CIM mice was performed on day 21. Sections of the muscles from the CIM mice treated with BSA (A) or with CTLA4-Ig (B) were stained with haematoxylin and eosin (HE). Bars = 100 μm. Histological severities of myositis were graded for the CIM mice treated with either agent in the prophylactic protocol (C). In a therapeutic protocol, 1000 μg of CTLA4-Ig or BSA was administered to CIM mice every 3 days for 4 times from day 7. Seven or eight mice were engaged in each experimental group. Histological severities of myositis were graded for the CIM mice treated with either agent in the therapeutic protocol (D). Horizontal bars represent the mean scores of the individual groups. *$p<0.05$.

We next evaluated the effect of CTLA4-Ig when administered after the onset of CIM. Since histological inflammation is evident in CIM mice 7 days after the immunisation (1), treatment was initiated on day 7 as a therapeutic protocol. CTLA4-Ig suppressed the severity of the ongoing myositis (Fig. 1D).

The therapeutic effect of anti-CD80 antibodies and anti-CD86 antibodies on mice with CIM

To investigate the effects of CD80/86 blockade on CIM in a different method, CIM mice were treated with anti-CD80 and anti-CD86 antibodies (anti-CD80/86 Abs) in the therapeutic protocol. Rat polyclonal IgG acted as a control. Histological evaluation revealed that anti-CD80/86 Abs suppressed the severity of CIM (Fig. 2A-C).

The effect of CTLA4-Ig on mice with CPIM

As stated earlier, $CD4^+$ T cells promote $CD8^+$ T cells to differentiate into cytotoxic T lymphocytes. This should be the case with CIM since it was inhibited in $CD4^+$ T cell-depleted mice (1). The therapeutic effects on CIM with CTLA4-Ig and anti-CD80/86 Abs may thus depend on suppression of $CD4^+$ T cell responses.

Since activation of autoantigen-specif-

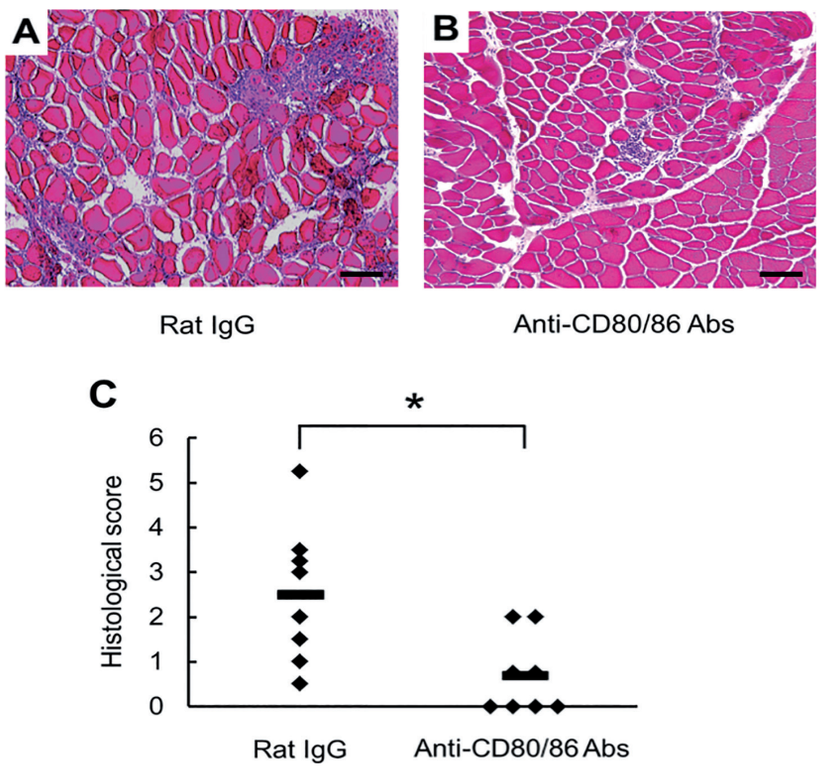

Fig. 2. The therapeutic effect of anti-CD80 antibodies and anti-CD86 antibodies on CIM. Mice with CIM were treated in the therapeutic protocol with 250 μg of anti-CD80 antibodies and of anti-CD86 antibodies (anti-CD80/86 Abs) or with 500 μg of rat polyclonal IgG. Eight mice were engaged in each experimental group. Histological evaluation of the muscles was performed on day 21. Sections of the muscles from the CIM mice treated with rat IgG (**A**) or with anti-CD80/86 Abs (**B**) were stained with HE. Bars = 100 μm. Histological severities of myositis were graded for the CIM mice treated with either agent (**C**). Horizontal bars represent the mean scores of the individual groups. *p<0.05.

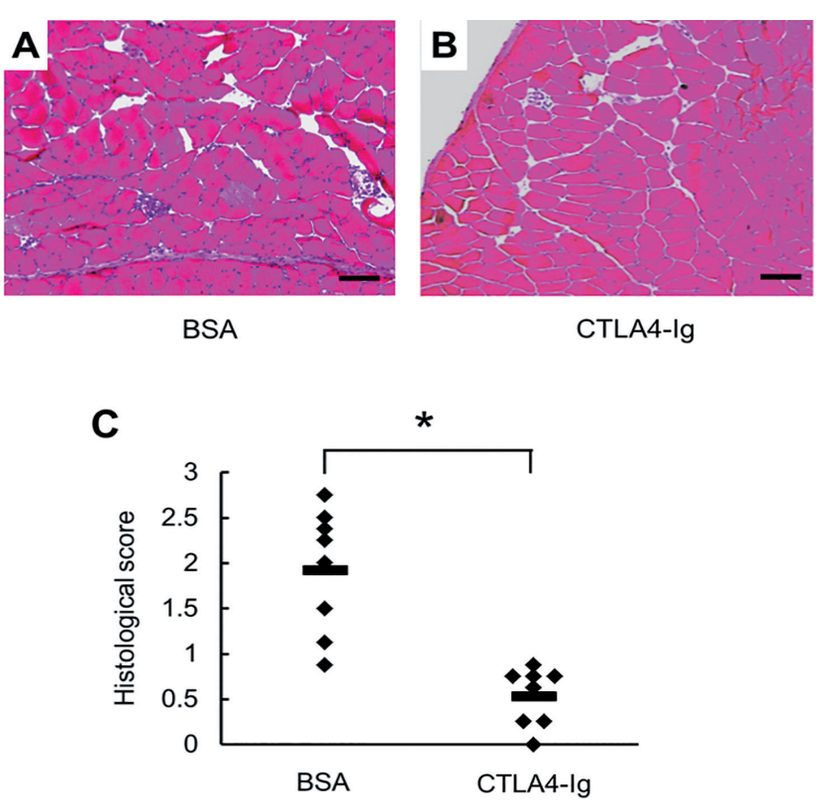

Fig. 3. The effect of CTLA4-Ig on CPIM. One thousand microgram of CTLA4-Ig or BSA was administered to mice with CPIM intraperitoneally every 3 days for 3 times from the day of the first BMDC transfer. BMDCs, which were activated with 1 μg/ml of lipopolysaccharides, were loaded with 50μg/ml of $CD8^+$ T cell-epitope peptides (HILIYSDV) prior to the intravenous transfer. Eight mice were engaged in each experimental group. Histological evaluation of the muscles from the CPIM mice was performed 7 days after the first BMDC transfer. Sections of the muscles from the CPIM mice treated with BSA (**A**) or with CTLA4-Ig (**B**) were stained with HE. Bars = 100 μm. Histological severities of myositis were graded for the CPIM mice treated with either agent (**C**). Horizontal bars represent the mean scores of the individual groups. *p<0.05.

ic $CD8^+$ T cells in CPIM are mediated with BMDCs presenting $CD8^+$ T cell-epitope peptides and are independent of $CD4^+$ T cell help (12), CPIM were employed to asses the direct effect of the CD80/86 blockade on the $CD8^+$ effector cells. When CTLA4-Ig or BSA was administered from the day of the first BMDC transfer, histological evaluation of their muscles revealed that CTLA4-Ig suppressed the severity of CPIM (Fig. 3A-C).

Discussion

Blockade of CD28-CD80/86 costimulation ameliorated CIM as well as CPIM, a myositis model that is mediated by autoaggressive $CD8^+$ T cells. CTLA4-Ig, abatacept, should exert its

therapeutic effect by suppressing both autoantigen-specific CD4+ and CD8+ T cells. It can be a potential therapeutic agent of PM and other CD8+ T cell-mediated diseases.

Successful treatment of CIM mice in the therapeutic protocol and of 3 refractory PM/DM cases with CTLA4-Ig indicate that CD80/86 blockade should have ameliorated myositis by suppressing continuous immune responses of autoaggressive CD28+ CD8+ T cells and subsequent muscle injury (5-7). Concerning CD28, CD28- CD8+ T cells predominated in the muscles of PM/DM (15). These cells from PM patients were cytotoxic to autologous muscle cells (16). CD28- CD8+ T cells differentiate from CD28+ CD8+ T cells with repeated CD80/86 stimulation (17). Hence, favorable therapeutic effects of CTLA4-Ig on PM/DM should be mediated via suppressing activation of CD28+ CD8+ T cells and preventing generation of CD28- CD8+ T cells. Actually, circulating CD28- CD8+ T cells decreased in RA patients after the CTLA4-Ig treatment (17).

CTLA4-Ig was effective in PM/DM that were resistant to calcineurin inhibitors (5-7). While TCR and CD28 signaling induce cytotoxic activity of CD4+ and CD8+ T cells, the induction could be blocked only partially with cyclosporine A (CsA) (11). Additionally, the cytotoxicity of CD8+ T cells was more resistant to inhibition by CsA than that of CD4+ T cells (11). In the reported cases, CTLA4-Ig should have suppressed the cytotoxic T cell activity resistant to calcineurin inhibitors and suppressed muscle injury.

Abatacept has mutations in its Fc portion and does not bind to complements or binds minimally to Fc receptors (4). Human immunoglobulin preparations that are effective in treating CIM as well as PM/DM do not have mutations in their Fc portions (1). Fc portion of the IgG plays an important role in the proposed mode of action of human IgG in PM/DM (1). Hence, we chose BSA, which does not bind to complements and Fc receptors or to CD80/86, as a control. Furthermore, rat anti-CD80/86 Abs, but not rat polyclonal IgG, ameliorated CIM. This fact supports that the effects of abatacept on CIM and CPIM were not mediated by the Fc portion of abatacept.

We evaluated the therapeutic effects on CIM and CPIM by histological scoring. Reproducible techniques to measure muscle power of CIM or CPIM mice are yet to be developed. Although creatine kinase (CK) is elevated in CIM mice, CK elevation is also observed in some healthy control mice (1). It was probably due to uncontrollable muscle exercise of the mice. The histological scoring is still the most practical method.

CTLA4-Ig exerted therapeutic effects on PM models of mice. An ongoing phase II randomised clinical trial in PM/DM (ARTEMIS study) will give us further evidence of the efficacy of CTLA4-Ig on PM.

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