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CTLA4-Ig suppresses development of experimental autoimmune uveitis in the induction and effector phases: Comparison with blockade of interleukin-6

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ABSTRACT

Recently, a number of biologics have been used in the treatment of autoimmune diseases. However, in the treatment of severe autoimmune uveitis, only TNF-alpha inhibitors are preferably used and the effect of other biologics such as interleukin-6 (IL-6) signaling blockade or cytotoxic T-lymphocyte antigen-4 −immunoglobulin fusion protein (CTLA4-Ig) has not been well studied. Previously, we reported that IL-6 blockade effectively suppresses the development of experimental autoimmune uveitis (EAU), a mouse model for uveitis, by inhibiting Th17 cell development. In this study, we investigated the effect of CTLA4-Ig on EAU development and compared it with the effect of anti-IL-6 receptor monoclonal antibody (MR16-1). C57BL/6J mice were immunized with interphotoreceptor retinoid-binding protein (IRBP) and treated once with CTLA4-Ig or MR16-1. Both CTLA4-Ig and MR16-1 administered in the induction phase (the same day as immunization) significantly reduced the clinical and histopathological scores of EAU. Fluorescence-activated cell sorting studies using draining lymph node (LN) cells from EAU mice 10 days after immunization showed that CTLA4-Ig can suppress early T-helper cell activation. CTLA4-Ig administered in the effector phase of the disease (one week after immunization), when IRBP-reactive T cells have been primed, also significantly reduced the clinical and histopathological scores of EAU. In contrast, MR16-1 administered in the effector phase did not ameliorate EAU. To investigate the differences between these biologics in the effector phase, in vitro restimulation analysis of LN cells obtained from EAU mice one week after immunization was performed and revealed that CTLA4-Ig, but not MR16-1, added to culture media could inhibit the proliferation of IRBP-specific $CD4^+$ T cells which possessed capacities of producing IFN-gamma and/or IL-17. Collectively, CTLA4-Ig ameliorated EAU through preventing initial T-cell activation in the induction phase and suppressing proliferation of IRBP-specific T cells in the effector phase. Blockade of IL-6 signaling did not have such inhibitory effects after T-cell priming. CTLA4-Ig may have therapeutic effects on human chronic uveitis.

1. Introduction

Autoimmune uveitis is a significant cause of morbidity and vision loss. Conventional treatments of uveitis such as corticosteroids and immunosuppressive agents are highly efficacious; however, such treatments can cause serious systemic side effects. Recently, as alternatives to conventional therapeutic agents, biologics targeting specific mediators of the immune-inflammatory system have been widely used for treating autoimmune disease. Biological agents, including tumor necrosis factor-alpha (TNF-α) inhibitors, anti-interleukin-6 receptor monoclonal antibody (anti-IL-6R mAb), and cytotoxic T-lymphocyte antigen-4−immunoglobulin fusion protein (CTLA4-Ig), have significantly improved the course of rheumatoid arthritis (RA) in the last decade

Abbreviations: CTLA4-Ig, cytotoxic T-lymphocyte antigen-4-immunoglobulin fusion protein; EAE, experimental autoimmune encephalomyelitis; EAU, experimental autoimmune uveitis; IRBP, interphotoreceptor retoid-binding protein.

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(Kievit et al., 2007; Maini et al., 2004; Nishimoto et al., 2009; Schiff et al., 2011). In autoimmune uveitis, TNF-α inhibitors have been effective in suppression of refractory uveoretinitis caused by Behcet's disease (Okada et al., 2012). However, in some cases, treatment with TNF-α inhibitors alone has been insufficient to suppress ocular inflammation owing to an unsatisfactory response or drug intolerance (Deighton, 2009). Therefore, alternative treatments are required for such cases. However, few studies have assessed therapeutic effects of other biologic treatments (Adan et al., 2013; Angeles-Han et al., 2008; Hirano et al., 2012; Kenawy et al., 2011; Muselier et al., 2011; Zulian et al., 2010).

Experimental autoimmune uveitis (EAU) is an animal model that shares many clinical and histological features with human uveitis (Caspi et al., 1988; Chan et al., 1990). EAU is induced by immunization with interphotoreceptor-binding protein (IRBP) and both Th1 and Th17 are pathogenic effectors that develop autoreactivity in EAU. Previously, using EAU mice, we and others demonstrated the efficacy of anti-IL-6R mAb (Haruta et al., 2011; Hohki et al., 2010; Yoshimura et al., 2009). We found that blockade of IL-6 signaling resulted in the downregulation of Th17 cells and antigen-specific Th1 cells. However, treatment of anti-IL-6R mAb after the T-cell priming was not very effective. These results suggest that the major mechanism of IL-6 blockade in EAU is to prevent Th17/Th1 cell induction by modulating T-helper cell differentiation.

CTLA4-Ig is a fusion protein of the extracellular domain of CTLA4 and IgG1. CTLA4-Ig binds to both CD80 and CD86 (also referred to as B7-1 and B7-2 or collectively as B7 proteins) (Xu et al., 2012) and prevents interaction of B7 proteins with their counterreceptors, CD28, expressed on T cells. Previous studies have shown that the CD28/B7 pathway plays an important role in experimental autoimmune diseases such as arthritis, lupus, experimental autoimmune encephalomyelitis (EAE), and EAU (Fukai et al., 1999; Kuchroo et al., 1995; Nakajima et al., 1995; Silver et al., 2000; Webb et al., 1996). A local administration of retinal Muller glial cells infected *ex vivo* with adenovirus expressing CTLA4-Ig have shown to be effective against EAU (Verwaerde et al., 2003). However, it remains unclear that systemic administration of CTLA4-Ig fusion protein itself can also suppress ocular inflammation in murine uveitis. Moreover, whether CTLA4-Ig administration in the effector phase can suppress EAU development is not known.

Here, we analyzed the efficacy of CTLA4-Ig in the induction phase (covering the antigen-priming phase) and the effector phase (after the priming phase) and compared with that of anti-IL-6R mAb. To compare the mechanism of both treatments, we analyzed characteristics of $CD4^+$ T cells in draining lymph nodes (LNs) in vivo. We also restimulated LN cells obtained from untreated EAU mice with IRBP in the presence of CTLA4-Ig or MR16-1 and assessed the expansion of IRBP-specific T-helper subsets in vitro.

2. Materials and methods

2.1. Mice

Wild-type female C57BL/6J mice were purchased from Charles River Laboratories (Yokohama, Japan) and used at 7–9 weeks of age. All mice were maintained under specific pathogen-free conditions at The National Institute of Biomedical Innovation (NIBIO). Animal experiments were approved by the Animal Care and Use Committee of NIBIO (DS25-34). All animals were treated humanely, and all experimental procedures conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. EAU induction

All mice were immunized with 100 μg of a human IRBP epitope (the $IRBP_{1-20}$ peptide GPTHLFQPSLVLDMAKVLLD) in 0.2 mL emulsion of complete Freund's adjuvant (Difco Laboratories, Detroit, MI) supplemented with a suspension of *Mycobacterium tuberculosis* H37Ra (Difco) to a final volume of 2.5 mg/mL. In addition, 500 ng of pertussis toxin (Sigma–Aldrich, St. Louis, MO) was injected intraperitoneally (Avichezer et al., 2000).

2.3. Clinical evaluation

Ophthalmic examinations were performed after immunization. Tropicamide (0.5%) was applied to the eyes to induce mydriasis, and the fundus of the eyes was examined using a slit lamp microscope. Every 48 h from 9 to 27 days after immunization, the animals were clinically evaluated in a masked fashion by experienced ophthalmologists who examined the animals for the presence of dilation, white focal lesions, white linear lesions affecting blood vessels, retinal hemorrhage, and retinal detachment. Clinical scores between 0 and 4 were assigned according to the severity, as described previously (Thurau et al., 1999).

2.4. Histopathological evaluation

The eyes were enucleated from each mouse at the peak of EAU (15–17 days after immunization) and fixed with formalin. Approximately 8–10-μm-thick tissue sections were stained with standard hematoxylin and eosin. Sections were histopathologically evaluated in a masked fashion by experienced pathologists. Histopathological scores between 0 and 4 were assigned according to the severity, as described previously (Caspi, 2003).

2.5. Treatment with CTLA4-Ig

CTLA4-Ig (Orencia®, abatacept) is a fusion protein of human cytotoxic T-lymphocyte-associated antigen 4 and the modified Fc portion of human IgG1, which targets T-cell costimulation. CTLA4-Ig was obtained from Bristol-Myers Squibb (New Hampshire, USA) and 1 mg per mouse was intraperitoneally injected on day 0 (induction phase of the disease) or day 7 (effector phase of the disease) after immunization. A preliminary experiment to determine the optimal dose of CTLA4-Ig revealed that an effective amount of CTLA4-Ig was 1 mg or more (data are not shown); therefore, in this study, we decided to inject 1 mg of CTLA4-Ig.

2.6. Treatment with anti-IL-6R monoclonal antibody

A neutralizing anti-IL-6R antibody (clone MR16-1), which is a chimeric (rat–mouse) monoclonal IgG1 antibody against murine IL-6R, was obtained from Chugai Pharmaceutical Co. (Gotemba, Japan). MR16-1 (1 mg per mouse) was intraperitoneally injected on day 0 or day 7 after immunization. The suitable dose of MR16-1 was determined according to a previous report (Terabe et al., 2011).

2.7. Assays for IRBP-specific antibody production

Serum levels of anti-IRBP antibodies of the IgG2c subclass from EAU mice at 26–28 days after immunization were determined using an enzyme-linked immunosorbent assay (ELISA), as described previously (Commodaro et al., 2010), with some modifications. In brief, 96-well plates (Nunc®, Roskilde, Denmark) were coated with IRBP (1 μg/well). After blocking plates with a bovine serum albumin solution (Sigma–Aldrich) for 2 h and incubating them with serum samples for 1 h, we developed signals in the plates using