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Differential contributions of B7-1 and B7-2 to the development of murine experimental allergic conjunctivitis

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Abstract

B7-1 and B7-2 are the co-stimulatory molecules that are involved in activation of T cells. We investigated whether B7-1 and B7-2 play a role in the development of T cell-mediated experimental allergic conjunctivitis (EC). EC was induced in Balb/c mice by active immunization with ragweed (RW) followed by RW challenge in eye drops. These mice were treated with neutralizing anti-B7-1 Ab, anti-B7-2 Ab, both Abs, anti-cytotoxic T lymphocyte-associated Ag-4 (CTLA-4) Ab or normal IgGs as controls either during the induction phase or the effector phase. With regard to the induction phase treatment, EC was significantly attenuated when both anti-B7-1 and anti-B7-2 Abs were injected. In contrast, anti-CTLA-4 Ab treatment significantly exacerbated EC. With regard to the effector phase treatment, anti-B7-2 Ab alone significantly attenuated EC, while anti-CTLA-4 Ab tended to exacerbate EC. Collectively, B7-1 and B7-2 differently contribute to the development of EC during the induction and effector phases.

Keywords: Allergic conjunctivitis; B7-1 (CD80); B7-2 (CD86); Cytotoxic T lymphocyte-associated Ag-4 (CD152); T cell

1. Introduction

Allergic conjunctivitis (AC) is mediated by mast cell activation in the conjunctiva that is induced by cross-linking of the Fcε receptor by IgE and the antigen (Ag) [1]. The mast cells, subsequently, release various inflammatory mediators such as histamine [1]. Eosinophils also play a role in the development of AC [2]. The infiltration of eosinophils is mediated by mast cell activation and it is therefore called a late phase reaction [3]. Previously, we used a murine model of AC (experimental conjunctivitis, EC) to investigate whether the activation of mast cells by IgE alone can induce conjunctival eosinophil infiltration. This study revealed that IgE-induced mast cell activation only provokes mild conjunctival eosinophil infiltration [4]. In contrast, the transfer of Ag-primed T cells followed by Ag challenge in the conjunctiva induced severe conjunctival eosinophil infiltration [4,5]. Since the severity of AC is dependent on the numbers of infiltrating eosinophils [6], it appears that T cells play more important roles than IgE in the severe forms of AC.

Naïve T cells can be activated when they receive two signals, namely, the signal from T cell receptor and the signal from one or more co-stimulatory molecules [7]. We previously analyzed in a series of experiments the roles different co-stimulatory molecules play in the development of EC. These studies have shown that agonistic stimulation of the co-stimulatory molecule 4-1BB [8] and OX40 [9] suppressed and augmented EC, respectively. Thus, these two TNF receptor family molecules appear to participate in the development of EC. In contrast, blockade of the interaction between inducible co-stimulator (ICOS), a molecule belonging to the CD28 family, and B7-related protein-1 (B7RP-1) did not affect EC [10]. In experimental airway inflammation, however, ICOS/B7RP-1 interactions are important for the development of this disease [11]. Thus, it is likely that co-stimulatory molecules act differently in the development of EC and airway inflammation.

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Of all the co-stimulatory molecules that are known to be involved in activating T cells, the interaction between CD28/cytotoxic T lymphocyte-associated Ag-4 (CTLA-4, CD152) on T cells and B7-1 (CD80)/B7-2 (CD86) on Ag-presenting cells has been the most extensively investigated [12]. CD28 is constitutively expressed on naïve T cells and transmit a positive costimulatory signal upregulating proliferation and cytokine production upon engagement by B7-1 or B7-2 [13]. In contrast, CTLA-4 is expressed on activated T cells and transmits a negative signal downregulating ongoing T cell responses upon engagement by B7-1 or B7-2 [14]. With regard to experimental airway inflammation, several reports have confirmed that B7-1 and/or B7-2 play a role in its development [15–21]. In addition, it was reported that blockade of CTLA-4 enhanced eosinophilic airway inflammation in Balb/c mice [22]. However, with regard to AC, little information has been available concerning the interaction between these molecules. This study aimed to investigate whether B7-1 and B7-2 play a role in the development of EC. In addition, we investigated a role of CTLA-4 in the development of EC, since CTLA-4 has both stimulatory and inhibitory roles depending on the timing of Ag presentation [23].

2. Materials and methods

2.1. Mice

Inbred female Balb/c mice were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). The mice were kept in specific pathogen-free conditions at the animal facility of Kochi Medical School and were used when they were 6–12-week-old. All research adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Reagents

Short ragweed pollen (RW) was purchased from Polysciences Inc. (Warrington, PA). RW extract was obtained from LSL Co. Ltd. (Tokyo, Japan). Aluminum hydroxide (alum) was purchased from Sigma (St. Louis, MO). Anti-B7-1 (RM80, rat IgG2a) [24], anti-B7-2 (PO.3, rat IgG2b) [24] and anti-CTLA-4 (UC10-4F10, hamster IgG; ATCC, Manassas, VA) [25] Abs were generated as described previously. Normal rat IgG (nrIgG) and normal hamster IgG (nhIgG) were purchased from MP Biomedicals Inc. (Aurora, OH).

2.3. EC induction by active immunization and treatment with Abs

RW adsorbed on alum was injected into the left hind-footpad and at the base of the tail. Fifty microliters of the emulsion (50 μg of RW and 2 mg of alum) was injected into each site. The mice were also injected intraperitoneally on days 0, 2, 4, 6 and 8 after RW immunization (induction phase treatment) or on day 10 only (2 h before RW challenge; effector phase treatment) with 200 μg of anti-B7-1 Ab, anti-B7-2 Ab, anti-CTLA-4 Ab, both anti-B7-1 and anti-B7-2 Abs (200 μg each), or control Abs (nrIgG or nhIgG). Each group contained 13 animals except for the groups receiving both B7 Abs, which consisted of nine mice. On day 10, the eyes of the immunized mice were challenged with RW in PBS (2 mg in 10 μl per eye). Twenty-four hours after the RW challenge, the eyes and spleens were harvested for histological analysis and cytokine production, respectively.

2.4. Histological analysis

The eyes including the conjunctivas were harvested and fixed in 10% buffered formalin. Vertical 2 μm-thick sections were cut and stained with Giemsa. Since the severity of AC is dependent on the numbers of infiltrating eosinophils [6], infiltrating eosinophils in the lamina propria mucosae of the tarsal and bulbar conjunctivas in the entire section were counted by two observers given blind samples. Three sections of each eye (26 eyes per group except the group receiving both B7 Abs (18 eyes)) were prepared for counting. The sections counted were those from the central portion of the eye, which included the pupil and optic nerve head, as described previously [26]. The data are presented as averages ± S.D. of all the mice examined.

2.5. Measurement of cytokines in the culture supernatants

RBC-depleted splenocytes (10^7 cells/ml) were cultured for 48 h with RW extract (25 μg/ml) in 96-well flat-bottom plates in a final volume of 0.2 ml RPMI 1640 medium supplemented with 10% FCS and 50 μM 2-ME. Then the culture supernatants were collected and kept frozen until use. The levels of IL-4, IL-5, IL-10, IL-13 and IFN-γ in the culture supernatants produced were measured by the Bioplex system (Bio-Rad, Hercules, CA) according to the manufacturer's recommendations.

2.6. Statistical analysis

Significant differences between the four groups (nrIgG, anti-B7-1 Ab, anti-B7-2 Ab and anti-B7-1/anti-B7-2 Abs) with regard to the infiltrating eosinophil numbers and splenocyte cytokine production were evaluated by ANOVA and then by Fisher's protected least significant difference method, while Student's *t*-test was used to compare nhIgG- and anti-CTLA-4 Ab-treated groups. *P* values less than 0.05 were considered significant.

3. Results

3.1. During the induction phase, treatment with both anti-B7-1 and anti-B7-2 Abs attenuates conjunctival eosinophil infiltration, while treatment with anti-CTLA-4 Ab augments conjunctival eosinophil infiltration

Treatment with either anti-B7-1 Ab or anti-B7-2 Ab alone did not significantly affect conjunctival eosinophil infiltration as compared to the nrIgG-treated control group (Fig. 1). Anti-B7-1 Ab alone tended to enhance eosinophil infiltration,