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Inhibition of very late antigen-4 and leukocyte function-associated antigen-1 in experimental autoimmune uveoretinitis

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Abstract B10.RIII mice were immunized with interphotoreceptor retinoid binding protein peptide to induce uveitis. Mice were injected intraperitoneally with anti-very late antigen-4 (VLA-4), anti-leukocyte function-associated antigen-1 (LFA-1), or a control Ab every other day from Day 5 to Day 13 post-immunization. The eyes and spleens were harvested on Day 14 or 28. The eyes were used for histologic/cytokine mRNA expression analyses. The spleens were used for Ag-recall cytokine production assays and intracellular cytokine assays. Treatment with both Abs led to a profoundly significant reduction in severity of uveitis and cytokine mRNA expression in the eye. However, cytokine production by splenocytes was significantly upregulated. Discontinuation of Ab treatment led to an increase in uveitis severity and cytokine mRNA expression in the eye, but led to a decrease in cytokine production and intracellular IFN-γ^+ and IL-17A$^+$cytokine profile by splenocytes. Thus, blockade of these molecules using specific Abs may be a therapeutic option for patients with uveitis; however, such treatment must be continued.

1. Introduction

Uveitis is a form of intraocular inflammation that is classified as either exogenous or endogenous [1]. Exogenous uveitis is generally caused by infections such as toxoplasma, and therapeutic approaches are focused on eliminating the infectious agent(s). By contrast, the etiology of endogenous uveitis is not fully understood [2]. A recent report suggests that endogenous intermediate or posterior uveitis leads not only to severe loss of vision but also to meaningful reductions in mental health outcomes and health-related quality of life [3]. Thus, effective therapy for endogenous uveitis is urgently required.

To understand the mechanism(s) underlying the development of endogenous uveitis, animal models of the disease have been established [4]. The most common which is also

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relevant to human uveitic patients, is experimental autoimmune uveoretinitis (EAU) [5]. Analyses of EAU indicate that endogenous uveitis is generally induced by T cell-mediated autoimmune responses [6]. Recent studies show that helper T (Th) cells, particularly Th1 and Th17 cells, are central players in the development of EAU [7,8]. During the development of EAU, Ag-specific Th cells are recruited to target tissues in the retina, a process that is regulated by the expression of cell surface adhesion molecules. Therefore, targeting adhesion molecules is a logical strategy for treating uveitis. In experimental settings, the development of EAU can be prevented by blocking LFA-1, ICAM-1 or VLA-4 [9–12]. Antibodies (Abs) that target these adhesion molecules have been approved by the US Federal Drug Administration for use in patients with Crohn's disease [13], multiple sclerosis (MS) [14] (anti-VLA-4; i.e. natalizumab) and psoriasis [15] (anti-LFA-1; i.e. efalizumab); however, these Abs are not approved for the treatment of uveitis.

A recent study examined the effect of efalizumab in a patient suffering from endogenous uveitis with macular edema. The authors reported that uveitis with macular edema was well controlled after 37 weekly injections of efalizumab and that no recurrence was noted at 6 months after the end of the treatment [16]. A more recent study examined the effects of efalizumab in an open-label, prospective, non-comparative phase I/II trial. Patients received 16 weekly injections of efalizumab, which improved visual function and macular edema with no evidence of severe side effects [17]. These reports indicate that targeting adhesion molecules is an attractive strategy for patients with endogenous uveitis. Although the former report did not find any evidence of disease recurrence, it should be noted that these therapeutic approaches do not induce Ag-specific tolerance, and that the disease-suppressive effects are thought to be transient. To address this issue, we examined whether anti-LFA-1 and anti-VLA-4 Abs continued to suppress EAU after treatment was discontinued.

Th1 and Th17 cells participate in the development of EAU. We [18] and others [19] reported that Th17 cells infiltrate the retina at an earlier time point than Th1 cells. However, it is not known how the timing of Th1 and Th17 cells infiltration is regulated. A recent study used a mouse model of experimental autoimmune encephalomyelitis (EAE) (which is a model for MS) to examine whether Th1 and Th17 cell infiltration into the central nervous system (CNS) is selectively regulated by adhesion molecules [20]. The report demonstrated that treatment with an anti-VLA-4 Ab inhibited Th1-mediated EAE, without affecting CNS infiltration by Th17 cells. Th17 cell infiltration was completely dependent on the expression of LFA-1. This led us to examine whether LFA-1 and VLA-4 regulate the infiltration of Th17 and Th1 cells, respectively, into the retina during the development of EAU.

2. Methods

2.1. Mice and materials

B10.RIII mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed under standard (specific pathogen-free) conditions at the Kochi Medical School animal facility. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Interphotoreceptor retinoid binding protein (IRBP) peptide 161–180 (SGIPYIISYLHPGNTILHV) was purchased from Scrum Inc. (Tokyo, Japan) by synthesis on consignment. The purity of the peptide was greater than 95% by high performance liquid chromatography. Complete Freund's adjuvant (CFA) containing 2.5 mg/ml *Mycobacterium tuberculosis* was obtained from Difco (Baltimore, MD), and *Bordetella pertussis* toxin (PTX) [21] was obtained from Sigma-Aldrich (St, Louis, MO). The anti-mouse VLA-4 (PS/2) and anti-mouse LFA-1(KBA) rat monoclonal Abs (mAbs) [22,23] were purified from ascites by using protein G columns as previously described. The mAb preparations contained <100 pg/ml endotoxin. Normal rat IgG (nrIgG) was obtained from MP Biomedicals Inc. (Aurora, OH).

2.2. Immunization

To induce EAU, mice (6–12 weeks old) were immunized subcutaneously on both sides of the neck with an emulsion (200 μl) containing 100 μg IRBP peptide 161–180 in CFA (1:1 v/v). The mice also received an intraperitoneal injection of *B. pertussis* toxin (1.0 μg) as an additional adjuvant. The day of immunization was designated Day 0.

2.3. Immunohistochemistry

Eyes were enucleated on Day 14. Negative control eyeballs were collected from naive mice which had not been received adjuvants. The eyes were embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetek Japan, Tokyo, Japan) and frozen in liquid nitrogen. Vertical (8-μm) serial sections were prepared and fixed in cold acetone (for anti-VLA-4 and anti-LFA-1) or 4% buffered paraformaldehyde (for anti-IFN-γ and anti-IL-17A). Endogenous peroxidase activity was inhibited by incubation with 3% H_2O_2 in methanol for 15 min at room temperature. The samples were then incubated with anti-VLA-4 (2 μg/ml, rat mAb), anti-LFA-1 (5 μg/ml, rat mAb), anti-VCAM-1 (10 μg/ml, rat mAb), anti-ICAM-1 (2.5 μg/ml, rat mAb) (BioLegend, San Diego, CA), FITC-conjugated anti-IFN-γ or FITC-conjugated anti-IL-17A (5 μg/ml, rabbit polyclonal; Bioss, Inc., Woburn, MA) for 1 h at room temperature. Rat mAbs were incubated with biotinylated anti-rat IgG Ab (5 μg/ml) (BD Pharmingen, San Diego, CA) for a further 1 h. Control samples were exposed to the rat or rabbit isotype control Ab. Ab binding was revealed by an Avidin-Biotin-Complex kit (Vector Laboratories, Burlingame, CA) and Mayer's hematoxylin solution (Wako Pure Chemical Industries, Osaka, Japan) or VECTASHIELD Mounting Medium contains 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA) was used as a nuclear counterstain.

2.4. Treatment with Abs

EAU was induced as described above. EAU-developing mice were injected intraperitoneally with 200 μg of anti-VLA-4, anti-LFA-1, or anti-VLA-4/LFA-1 (combined treatment), or with nrIgG alone (control) every other day from Day 5 to Day 13 post-immunization.