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The *in vivo* and *in vitro* induction of anterior chamber associated immune deviation to myelin antigens in C57BL/6 mice

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ABSTRACT

Introduction of antigens into the anterior chamber (AC) of the eye generates a specific systemic form of tolerance that is termed AC-associated immune deviation (ACAID). Experimental autoimmune encephalomyelitis (EAE) is an animal model of the human CNS demyelinating diseases, including multiple sclerosis (MS) and acute disseminated encephalomyelitis. We investigated whether the encephalitogenic antigens myelin oligodendrocyte glycoprotein (MOG_{35-55}) or myelin basic protein (MBP) induce ACAID in the EAE-prone C57BL/6 mice. We hypothesized that injection of MOG_{35-55}/MBP induces antigen-specific tolerance whether via the AC route, the adoptive transfer of *in vitro*-generated MOG_{35-55}-specific/MBP-specific ACAID antigen presenting cells (APCs), or the adoptive transfer of MOG_{35-55}-specific/MBP-specific ACAID T regulatory cells (Tregs). ACAID is characterized by the specific impairment of delayed-type hypersensitivity (DTH) responses. Thus, DTH assays were used to test for ACAID following the AC injection of MOG_{35-55}/MBP, or the intravenous injection of MOG_{35-55}-specific/MBP-specific ACAID APCs. The functional local adoptive transfer (LAT) assays were used to examine the putative regulatory functions of *in vitro* generated MOG_{35-55}-specific/MBP-specific Tregs. This report is the first to demonstrate the *in vivo* and *in vitro* induction of MOG_{35-55}-specific/MBP-specific ACAID-mediated tolerance in C57BL/6 mice. These findings highlight the need for novel immunotherapeutic strategies for MS and optic neuritis.

1. Introduction

Immunologically privileged sites regulate deleterious inflammatory immune mechanisms by upregulating the expression of immune inhibitory molecules especially in ocular tissues and fluids (Dahal et al., 2013; Taylor, 2009). In case of breach of regulatory mechanisms, substantial injury or damage to sensitive ocular tissues could ensue. Apart from conferring protection to the ocular compartment, antigen inoculation into the anterior chamber (AC) of the eye promotes a form of immune tolerance known as AC-associated immune deviation (ACAID) that induces systemic regulation of antigen-specific cell-mediated responses (Ashour and Niederkorn, 2006a,b; Streilein, 2003). Furthermore, ocular infection could also possibly trigger ACAID induction. As a result of ACAID-mediated tolerance, individuals with virus-induced acute retinal necrosis seldom generate cell-mediated immune responses although antigen-independent circulating antiviral antibodies could still be detectable (Kezuka et al., 2007). Antigen-specific regulation of delayed-type hypersensitivity (DTH) responses and complement-fixing antibody isotypes appear to be the main hallmarks for the induction of ACAID (Niederkorn, 2007a,b). ACAID is functionally controlled by both antigen-specific $CD4^+$ $CD25^+$ T regulatory cells (Tregs) and antigen-specific $CD8^+$ Tregs (Niederkorn, 2007a,b).

Multiple Sclerosis (MS) is one of the most commonly reported inflammatory disorders afflicting the central nervous system (CNS). The major distinctive feature for MS is neuronal demyelination. Experimental autoimmune encephalomyelitis (EAE) is an experimentally induced autoimmune disease in rodents resulting

Abbreviations: AC, anterior chamber; ACAID, anterior chamber associated immune deviation; APCs, antigen presenting cells; BSA, bovine serum albumin; CMI, cell mediated immune response; DTH, delayed-type hypersensitivity; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; PBS, phosphate buffered saline; LAT, local adoptive transfer; Tregs, regulatory T cells.

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in substantial inflammatory demyelinating damage to CNS by activation of autoantigen-specific $CD4^+$ T cells. Autoantigens include Myelin oligodendrocyte glycoprotein (MOG) and Myelin basic protein (MBP). We have recently shown that the encephalitogenic antigens MOG_{35-55} or MBP induced peripheral tolerance in Balb/c mice following inoculation of the antigens via the AC (Farooq and Ashour, 2013) or after the intravenous injections of MOG_{35-55}-specific/MBP-specific ACAID antigen presenting cells (APCs), MOG_{35-55}-specific/MBP-specific ACAID B cells, and MOG_{35-55}-specific/MBP-specific ACAID Tregs (Farooq and Ashour, 2014). It is noteworthy that EAE induction is possible in Balb/c mice after targeted disruption of ST2 molecule but is entirely possible in C57BL/6 mice (Milovanovic et al., 2012). Therefore, we aimed to investigate whether MOG_{35-55}-specific/MBP-specific ACAID could be induced in C57BL/6 mice. We hypothesized that injection of MOG_{35-55} or MBP induces antigen-specific immune tolerance in C57BL/6 mice whether via the AC route, the adoptive transfer of *in vitro*-generated MOG_{35-55}-specific/MBP-specific ACAID APCs, or the adoptive transfer of MOG_{35-55}-specific/MBP-specific ACAID Tregs.

2. Materials and methods

2.1. Mice

C57BL/6. mice (6–8 weeks of age) were procured from Jackson Laboratories (Bar Harbor, ME). Animals were maintained at Animal care facility of the Eugene Applebaum College of Pharmacy and Health Sciences. The guidelines of the Institutional Animal Care and Use Committee (IACUC), Wayne State University were followed.

2.2. ACAID induction via AC injection of MOG_{35-55} and MBP

ACAID was induced in C57BL/6 mice using Hamilton automatic dispensing apparatus (Hamilton, Whittier, CA) as described in our previous publications (Ashour and Niederkorn, 2006a,b). C57BL/6 mice were anesthetized using 2–3% isoflurane with an oxygen supply. About 50–100 μg of MOG_{35-55} or MBP in 5 μl PBS (Sigma, St. Louis, MO) was injected into the AC of the eye. Mice injected with PBS alone via AC injection were used as controls. On day 7, mice were immunized with a subcutaneous injection of 250 μg of MOG_{35-55} or MBP (Sigma–Aldrich). MOG_{35-55} or MBP was emulsified 1:1 in complete Freund's adjuvant (CFA; Sigma–Aldrich). 200 μl of an emulsion of MOG_{35-55}/CFA or MBP/CFA was injected into each animal. On day 14 post AC injection of MOG_{35-55} or MBP, either a DTH assay or a LAT assay was performed as explained below.

2.3. Generation of ACAID APCs

ACAID APCs were generated *in vitro* as described previously (Ashour and Niederkorn, 2006a,b). C57BL/6 mice were used to generate ACAID APCs as described before (Ashour and Seif, 2007). APCs

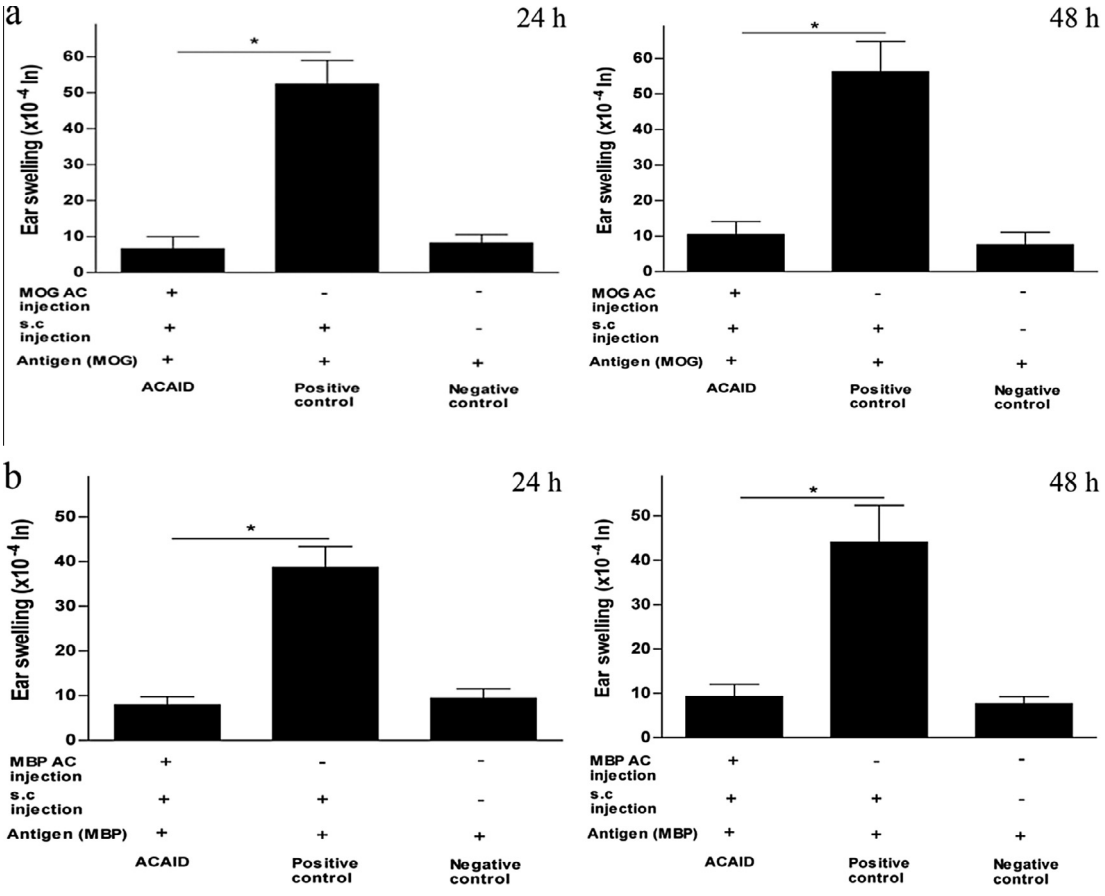

Fig. 1. Introduction of MOG_{35-55} or MBP into the AC triggers ACAID in C57BL/6 mice (a) The AC injection of MOG_{35-55} induced ACAID in C57BL/6 mice. (b) The AC injection of MBP induced ACAID in C57BL/6 mice. DTH assays were performed as described in the materials and methods section. The AC injection of MOG_{35-55} or MBP was followed by subcutaneous immunization on day 7 with MOG_{35-55}/CFA or MBP/CFA. Mice were challenged with MOG_{35-55} or MBP (500 μg in 20 μl) intradermally in the left ear pinna, and 20 μl PBS was injected into the right ear pinna as an internal control on day 14. Inhibition of ear-swelling responses after 24 h and 48 h was used to assess the induction of ACAID. Positive control mice were subcutaneously immunized with MOG_{35-55}/CFA or MBP/CFA on day 7 and with MOG_{35-55} or MBP on day 14 whereas negative control mice only received the day 14 intradermal injection of MOG_{35-55} or MBP. P values < 0.05 were considered significant (*).