

Expansion of B Cells Is Necessary for the Induction of T-Cell Tolerance Elicited through the Anterior Chamber of the Eye

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Key Words

B cells • Immune privilege • Peripheral tolerance • T-regulatory cells

Abstract

Antigens injected into the anterior chamber (AC) of the eye induce a form of peripheral immune tolerance termed anterior chamber-associated immune deviation (ACAID). ACAID is initiated by F4/80+ ocular antigen-presenting cells (APC) which capture ocular antigens and migrate to the spleen where they transfer antigenic peptides to B cells, which act as ancillary APC for the induction of T-regulatory cells (T_{reg}) that inhibit delayed-type hypersensitivity (DTH) responses. Here we show that ocular-like APC induce the expansion of tolerogenic splenic B cells. Furthermore, we show that inhibiting B-cell proliferation with either mitomycin-c or γ -irradiation abolishes the ability of B cells to induce T_{reg} . To our knowledge, this is the first study to report that B-cell proliferation is needed for B-cell-induced T-cell tolerance.

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Introduction

Immune privilege in the anterior chamber (AC) of the eye is due in large part to the induction of T-regulatory cells (T_{reg}) that suppress antigen-specific delayed-type hypersensitivity (DTH) responses to antigens introduced into the AC [1]. This anterior chamber-associated immune deviation (ACAID) protects the eye from immune-mediated inflammation and injury to ocular tissues that have little capacity to regenerate [1]. ACAID also mitigates ocular autoimmune diseases, Th2-mediated inflammation, and promotes corneal allograft survival. ACAID is induced when antigens are injected into the AC and are processed by F4/80+ ocular antigen-presenting cells (APC). Ocular APC migrate to the spleen and release modified antigenic peptides that are captured by splenic B cells expressing the B-cell receptor (BCR) specific for the antigen that was introduced into the AC [2]. ACAID B cells present antigen to CD8+ T cells that develop into T_{reg} that suppress DTH in an antigen-specific manner [2, 3]. Ocular-like APC and ACAID B cells can be generated in vitro, and, when adoptively transferred to naïve mice, induce ACAID [2–8]. Moreover, adoptively transferred ocular-like APC promote corneal allograft survival [6, 9].

Induction of ACAID requires a specific subset of B cells that express the relevant BCR that recognizes the antigen injected into the AC [2, 3]. Thus, the population

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of B cells expressing the relevant BCR is limited and would seem to be inadequate for presenting antigen to T cells. Accordingly, we tested the hypothesis that expansion of the splenic B-cell population is required for the induction of ACAID.

Materials and Methods

C57BL/6 mice were immunized by subcutaneous injection of 250 μ g of ovalbumin (OVA) emulsified in complete Freund's adjuvant (CFA) [4]. Seven days later, DTH responses to OVA were measured using a conventional ear swelling assay [4].

Ocular-like APC were generated in vitro by culturing peritoneal macrophages (1×10^6 /ml) from C57BL/6 mice in medium containing 10 mg/ml OVA and 2 ng/ml TGF- β_2 [4, 10, 11]. Normal APC (macrophages) were pulsed with OVA in the absence of TGF- β_2 . The in vitro generated ocular-like APC induce peripheral tolerance that is indistinguishable from ACAID and identical to the tolerance induced by F4/80+ APC isolated from the AC of the eye. By contrast, normal APC induce positive DTH [2, 4, 12].

An in vitro culture system was used to generate B cells capable of inducing T_{reg} that express the same phenotype as those induced by AC injection of the antigen [2, 4]. OVA-pulsed ocular-like APC (2×10^6 /ml) were generated as described above, washed in media, and cocultured for 48 h in the absence of TGF- β_2 with B cells (4×10^7) isolated from spleens of normal C57BL/6 mice. B-cell suspensions (4×10^7 /ml) were treated with anti-F4/80+ (10 μ g/ml) and complement (1:10) to kill any residual macrophages [3]. This protocol kills >99% of macrophages as determined by trypan blue exclusion (not shown). The in vitro generated ACAID B cells were then used for in vitro and in vivo experiments. In one experiment, B cells were treated with either 50 μ g/ml mitomycin-C or γ -irradiation (2,000 rad) prior to incubation with OVA-pulsed ocular-like APC. B-cell viability was determined by trypan blue exclusion immediately prior to adoptive transfer and was >95%. For in vitro experiments, B-cell proliferation was measured by ^{3} H-thymidine incorporation.

Results

ACAID can be induced by adoptively transferring either in vitro generated or in vivo generated ACAID B cells to naïve recipients [2, 3]. Accordingly, experiments were performed to determine the minimum number of ACAID B cells that are required for the generation of ACAID. ACAID-inducing B cells were generated in vitro and were adoptively transferred into naïve C57BL/6 mice. One week later, recipient mice were immunized subcutaneously with OVA and CFA. On day 14, mice were challenged in the ears with OVA. On day 15, an ear swelling assay was performed. Results show that $>1 \times 10^6$ ACAID B cells were needed for the induction of ACAID (fig. 1).

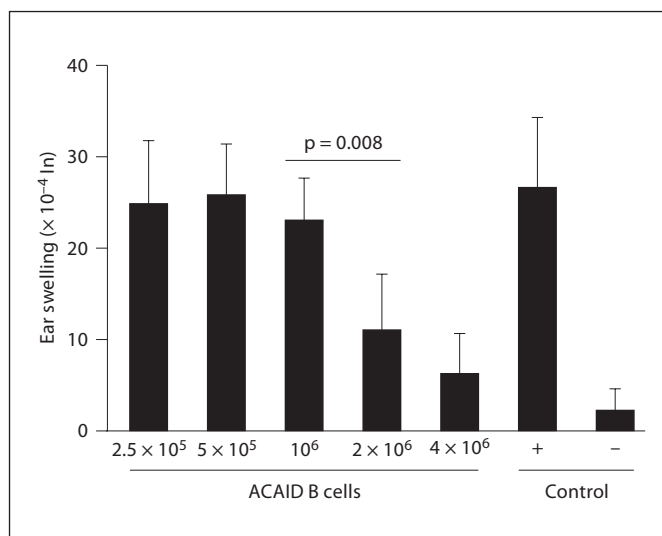


Fig. 1. Number of B cells required for the induction of ACAID. ACAID B cells were generated in vitro and were adoptively transferred to different groups of normal C57BL/6 mice. Seven days later, recipient mice were subcutaneously injected with OVA (250 μ g) emulsified in CFA, and a DTH assay was performed on day 15. This experiment was performed twice with similar results both times. $n = 5$ mice/group. In a separate experiment, 4×10^6 CFSE-labeled B cells were intravenously injected into naïve mice and were detected in the spleens 7 days later by FACS indicating that a significant number B cells were alive following adoptive transfer.

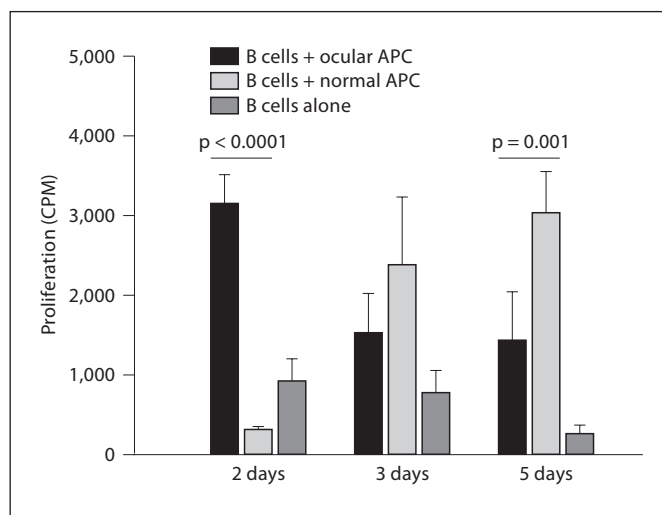


Fig. 2. ACAID APC stimulate B-cell proliferation in vitro. Splenic B cells were cocultured with either OVA-pulsed ocular-like APC or with OVA-pulsed normal APC. B-cell proliferation was evaluated by ^{3} H-thymidine incorporation 2, 3, and 5 days later. This experiment was performed twice with similar results both times. $n = 5$ cultures/group.

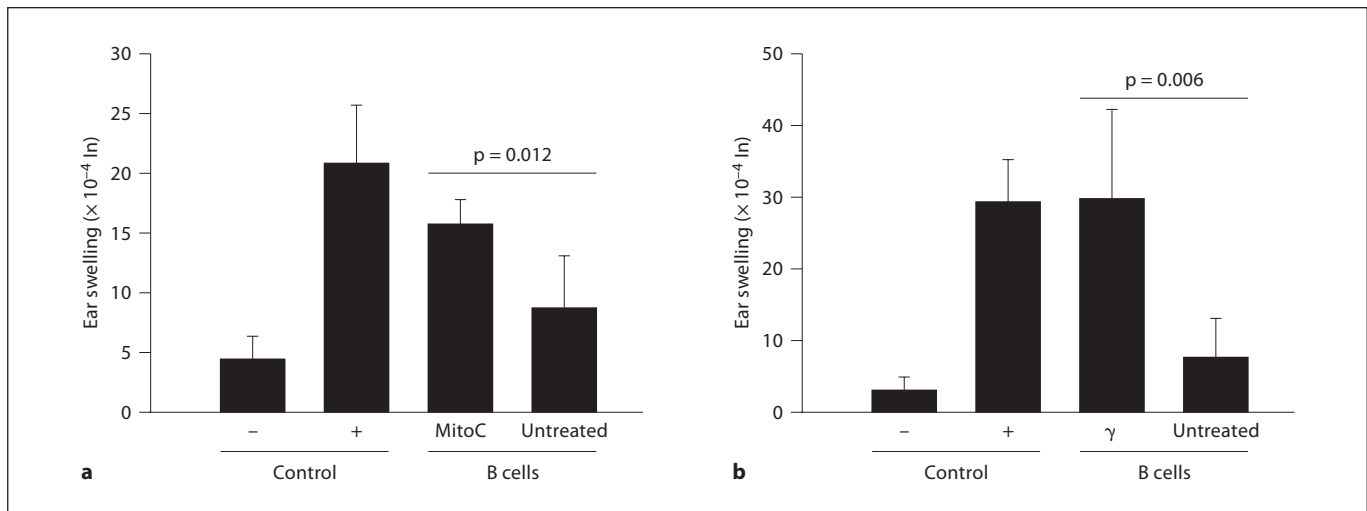


Fig. 3. Inhibition of B-cell proliferation prevents the induction of ACAID. OVA-pulsed ocular-like APC were cocultured for 5 days with untreated B cells or B cells treated with: mitomycin-C (MitoC; **a**) or γ -irradiation (γ ; **b**). B-cell suspensions were treated with anti-F4/80 antibody plus complement before 4×10^6 B cells

were intravenously injected into normal C57BL/6 mice that were immunized subcutaneously with OVA (250 μ g) and CFA on day 7. DTH responses to OVA were measured on day 14. These experiments were performed three times with similar results in all three experiments. $n = 5$ mice/group.

This suggests that unlike the extraordinarily small number of ocular APC (i.e. 20) that are needed to induce the initial step of ACAID [7], a significantly larger (i.e. 5 logs more) number of B cells is needed for the second step of antigen presentation that culminates in the generation of T_{reg} . Considering the limited number of B cells that express the BCR specific for OVA, we suspected that B-cell proliferation was needed to generate $>10^6$ OVA BCR+ B cells that are needed for the induction of ACAID.

Accordingly, splenic B cells were cocultured with either OVA-pulsed ocular-like APC or OVA-pulsed normal APC, and B-cell proliferation was assessed by the uptake of ^3H -thymidine. OVA-pulsed ACAID APC stimulated a spike in B-cell proliferation within 48 h (fig. 2). The proliferation rate was significantly higher than that with non-ACAID APC after 2 days. Thus, tolerance induction by ACAID B cells coincides with the B cell proliferation that is elicited by ACAID APC.

The in vitro model for the generation of ACAID B cells was used to determine if B-cell proliferation was necessary for ACAID. Accordingly, ACAID B-cell suspensions generated in vitro were treated with either mitomycin-C or γ -irradiation prior to adoptively transferring them to naïve mice. Inhibition of B-cell proliferation by treatment with either mitomycin-C or γ -irradiation prevented the induction of ACAID by B cells (fig. 3).

Discussion

Although only 20 ACAID APC are needed to induce ACAID [13], we found that over two million ACAID B cells are needed to produce the same effect. ACAID B cells must express the relevant BCR that corresponds to the antigen introduced into the AC or processed by ACAID APC in the in vitro model of ACAID [2]. Thus, in order for the spleen to harbor ≥ 2 million OVA-specific B cells, B-cell proliferation or recruitment must occur. The in vitro ACAID B-cell proliferation results suggest that antigen-pulsed ocular-like APC induce the clonal expansion of splenic B cells. Moreover, inhibition of B-cell proliferation with either mitomycin-c or γ -irradiation confirmed that B-cell proliferation was needed for the induction of ACAID.

The role of B cells as APC in the induction of other forms of T-cell tolerance has been reported [2, 14, 15]. In these models, T-cell tolerance could be induced by delivering antigen intravenously, orally, or via the AC of the eye [2, 14, 15]. However, in each case, B cells acted as APC for the induction of T-cell tolerance suggesting that this may be a broadly utilized mechanism for inducing T-cell tolerance. To our knowledge, the present study is the first to report that B-cell expansion is required for antigen-presenting B cells to induce the generation of T_{reg} and thus the induction of T-cell tolerance.

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