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Ocular Immune Privilege Promoted by the Presentation of Peptide on Tolerogenic B Cells in the Spleen. II. Evidence for Presentation by Qa-1

Thomas J. D’Orazio,* Elizabeth Mayhew, † and Jerry Y. Niederkorn²†

Ocular immune privilege is the result of several unique features of the eye, including the systemic down-regulation of Th1 immune responses to Ags encountered in the anterior chamber of the eye—a phenomenon termed anterior chamber-associated immune deviation (ACAID). The induction of ACAID requires the participation of three cell populations: the ocular ACAID APC, the splenic B cell, and the splenic T cell. Because B cells have been implicated in tolerogenic Ag presentation in other systems, we hypothesized that B cells were responsible for the induction of regulatory T cells in ACAID. The central hypothesis for this study is that APC from the eye migrate to the spleen where they release antigenic peptides (OVA) that are captured and presented to T cells by splenic B cells. A combination of in vitro and in vivo studies demonstrated that splenic B cells, incubated with ACAID APC in vitro, were capable of inducing ACAID when transferred to naive mice. The induction of ACAID required the normal expression of β2-microglobulin on both the B cell and ACAID APC, but not on the T suppressor cells. Moreover, the induction of ACAID regulatory cells required histocompatibility between the B cells and regulatory T cells at the TL/Qa region. The results indicate that: 1) B cells are necessary for the induction of ACAID; 2) ACAID B cells do not directly suppress the expression of delayed-type hypersensitivity; and 3) the induction of Ag-specific regulatory T cells by ACAID B cells requires histocompatibility at the TL/Qa region. The Journal of Immunology, 2001, 166: 26–32.

Many of the tissues within the eye are incapable of regeneration and are vulnerable to immune-mediated injury. However, the anterior chamber of the eye is protected from immune-mediated damage by a number of overlapping protective mechanisms. Among these are the ubiquitous expression of Fas ligand on intraocular tissues (1), the presence of multiple immunosuppressive agents in the aqueous humor (2), the absence of lymph vessels draining the interior of the eye (3), and the systemic down-regulation of cellular and humoral immune responses to Ag encountered in the anterior chamber of the eye, a phenomenon termed anterior chamber-associated immune deviation (ACAID)³ (4). One hypothesis of the initiation of ACAID predicts that Ag encountered in the eye is processed by resident APCs. These APCs then migrate from the eye to the spleen, where they induce the formation of T cells that down-regulate multiple immune processes, including Th1-mediated immunity (5, 6).

The mechanisms involved in the generation of ACAID suppressor T cells are only beginning to be elucidated. Initial findings suggest that ACAID APC present Ag in a tolerogenic manner to T cells in the spleen (7). Subsequent studies have shown that B cells are also necessary for the development of ACAID (8). Recently, it has been shown that the splenic B cell is necessary for the in vitro generation of at least one population of ACAID regulatory T cells (9). In other models of tolerance, B cells induce CD8⁺ suppressor T cells when peptides are presented in the context of the nonclassical class I molecule Qa-1 (10). Coupled with other studies showing that B cells can serve as tolerogenic APC (11–16), these results suggested that the B cell might present Ag in the ACAID spleen. The hypothesis that B cells serve as APC in the ACAID spleen requires the transportation of Ag from the eye to the spleen. Studies have shown that Ag encountered in the anterior chamber is processed and transported to the spleen by resident APC expressing the mature macrophage marker, F4/80 (17, 18). Macrophages in other sites, such as the lung and peritoneum, have the capacity to process Ag and reaggregate antigenic peptides that can be captured by a variety of cells and presented to T cells (19, 20). We postulated that this mechanism might be responsible for the delivery of peptides to B cells in the ACAID spleen. In this study, we further examined the role of the B cell in the induction of ACAID and considered the role of classical and nonclassical class I molecules on three of the key cell populations involved in the induction of ACAID—the ocular APC, the splenic B cell, and the splenic T cell.

Materials and Methods

Mice

Six- to 10-wk-old C57BL/6 and BALB/c mice were obtained from the mouse colony at the University of Texas Southwestern Medical Center at Dallas. B6.A-H2-T18/BoyEg congenic mice (B6.A), C57BL/6-Igh-6tm1Cgn B cell knockout mice, B6.129P2-β2m+/- (B6-β2-microglobulin (β2m)-deficient C57BL/6 mice, and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). BALB/c mice transgenic for the D011.10 TCR (specific for the OVA peptide fragment, 323–339, in the context of I-Ad (21, 22) were kindly provided by Dr. J. Wayne Streilein (Scheepens Eye Research Institute, Harvard Medical School, Boston, MA).
All animal studies were approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas.

**Preparation of peritoneal exudate cells (PECs)**

PECs were collected by peritoneal lavage of mice that were injected i.p. with 2 ml of 3% thioglycolate (Sigma, St. Louis, MO) medium 3–4 days earlier.

**In vitro model of the anterior chamber (“eye-in-a-dish”)**

In a previously described model of the anterior chamber of the eye, TGF-β-treated PECs were found to mimic the function of ACAID APC (23). PEC from normal mice were collected and suspended in complete RPMI 1640 medium (cRPMI; JRH Biosciences, Lenexa, KS) containing 10% FBS (HyClone Laboratories, Logan, UT), 2 mM l-glutamine (JRH Biosciences), 10 mM HEPES buffer solution (JRH Biosciences), 1 mM sodium pyruvate solution (JRH Biosciences), 1% nonessential amino acids solution (BioWhittaker, Walkersville, MD), and 1% penicillin-streptomycin-fungizone solution (BioWhittaker). PEC were incubated on plastic tissue culture dishes (Falcon 3803; Becton Dickinson Labware, Lincoln Park, NJ) at 37°C in 5% CO2 for 2.5 h. Plastic nonadherent cells were washed off with HBSS (BioWhittaker). Plastic adherent cells, the vast majority of which are macrophages (90%–95%), were collected by gently dislodging the cells with a Nitek filter swab (Tekno, Briarcliff Manor, NY). Our results have shown that macrophages, and not contaminating dendritic cells, are the effector cells in this model (data not shown). The macrophages were resuspended in cRPMI medium and aliquoted at 1.5 × 106 cells/well in a 24-well plastic tissue culture plate (Falcon 3047; Becton Dickinson Labware). OVA (Sigma, St. Louis, MO) was added at 5 mg/ml to each well (24 h prior to harvesting), and the cultures were incubated at 37°C for another 24 h. OVA was added to some wells (at 2 ng/ml). This treatment caused these cells to subsequently function as ocular ACAID APC (25, 26). Control wells received an identical aliquot (100 μl) of cRPMI alone (normal APC). Cell cultures were incubated at 37°C in 5% CO2 overnight. The next day, all cells were collected, washed with HBSS, resuspended in cRPMI, and used in subsequent experiments.

**In vitro coculture of APC with B cells**

ACAID and normal APCs were prepared as described, and 3–4 × 106 cells placed into medium petri dishes (Falcon 3002; Becton Dickinson Labware) in cRPMI. Splenocytes were harvested and erythrocytes lysed. BALB/c B cells were isolated by panning on plastic dishes coated with goat anti-mouse γ globulin (ICN Pharmaceuticals, Aurora, OH) as described (8). B cells (80–100 × 106 cells) were cocultured with APC and incubated at 37°C for 7 days. The nonadherent population of cells was collected and resuspended in cRPMI. B cells (4–5 × 106 cells) were injected i.v. into naive, syngeneic mice. In some experiments, the nonadherent population of cells was plated on plastic tissue culture dishes (Falcon 3803; Becton Dickinson Labware) as described above to remove any adherent APC contaminant. Nonadherent B cells (3–5 × 106 cells) were injected i.v. into naive, syngeneic mice. In other experiments, the B cells were harvested from a complement lysis procedure using 10 μg/ml rat anti-mouse B220 mAb (IgG2a; Pharmingen, San Diego, CA) and 1:20 rabbit complement (Accurate Chemicals, Westbury, NY). Control populations were treated with complement alone.

**Subcutaneous inoculations**

Mice were immunized by s.c. injection of OVA (125–150 μg) emulsified 1:1 in CFA (0.5 mg Mycobacterium/ ml; Behring Diagnostics, Deerfield, IL) in a total volume of 100 μl.

**Delayed-type hypersensitivity (DTH) assay**

Seven days after s.c. immunization, both ear pinnae of experimental and control animals were measured with a Mitutoyo engineer’s micrometer immediately before challenge. OVA (400 μg) in 20 μl PBS was injected s.c. into the left ear pinnae. The right ear pinnae received 20 μl sterile PBS alone (negative control). Both ear pinnae were measured 24 h later, and the difference in ear pinnae size was used as a measure of DTH. Results are expressed as: specific ear pinnae swelling = (24 h measurement − 0 h measurement) for experimental ear − (24 h measurement − 0 h measurement) for control ear.

**In vitro model of the ACAID spleen (“spleen-in-a-dish”)**

ACAID APC (3 × 106 cells) in cRPMI were placed into large petri dishes (Falcon 3003; Becton Dickinson Labware). Splenocytes were harvested and erythrocytes lysed. Whole splenocytes (100 × 106 cells) were added to the cultures. OVA (20 μg) was added to simulate Ag escape from the eye in vivo. cRPMI was added to a total volume of 15 ml. The dishes were incubated at 37°C in 5% CO2 for 7 days with frequent agitation. B cells were isolated from the cultures by panning on plastic dishes coated with goat anti-mouse γ globulin as described above. T cells were collected in the nonadherent population. The generation of cells capable of suppressing the expression of DTH was assessed with a local adoptive transfer assay. Briefly, positive immune effector T cells were generated by immunizing BALB/c mice s.c. with OVA (250 μg) emulsified 1:1 in CFA. After 7–14 days, the spleen and peripheral lymph nodes were harvested and erythrocytes lysed. T cells were collected by incubating the cell suspension on scrubbed nylon wool (Fenwal Laboratories, Deerfield, IL) at 37°C in 5% CO2 for 1 h. T cells were eluted with 30 ml HBSS. The positive immune effector T cells were resuspended at 106 cells/ml in 10 mg/ml OVA in PBS. B or T cells from the spleen-in-a-dish cultures were resuspended at the same concentration (106 cells/ml) in 10 mg/ml OVA in PBS. Equivalent volumes of positive and test cells were mixed. Positive controls included positive immune effector T cells mixed with naive splenocytes (106 cells/ml in 10 mg/ml OVA in PBS). Negative controls included an equivalent number of naive splenocytes alone. Both ear pinnae of naive, syngeneic mice were measured with a Mitutoyo engineer’s micrometer. In all cases, 20 μl of the cell suspension (2 × 106 cells) was injected s.c. into the left ear pinnae. The right ear pinnae received 20 μl of 10 mg/ml OVA in PBS (negative control). Both ear pinnae were measured 24 h later, and the difference in ear pinnae size was used as a measure of DTH due to the reactivity of the positive immune effector T cells. A significant reduction in ear swelling was indicative of direct suppression of this response.

**Reconstitution of B cell-deficient mice**

C57BL/6 wild-type and B6.A spleen cells were harvested and erythrocytes lysed. B cells were isolated by panning on plastic dishes coated with goat anti-mouse γ globulin (ICN Pharmaceuticals) as described (8). The purified B cells were resuspended at 5 × 106 cells/ml in cRPMI, filtered through sterile Nitex (Tekto), and 200 μl injected (107 B cells) i.v. into B cell-deficient mice. This procedure was shown to effectively reconstitute B cell knockout mice (9).

**Intracamer al inoculation**

Mice were anesthetized with 0.66 mg of ketamine hydrochloride (Vetalar; Park-Davis and Co., Detroit, MI) given i.p. A glass micropipette (~80 μm diameter) was fitted onto a sterile infant-feeding tube (no. 5 French; Professional Medical Products, Greenwood, SC) and mounted onto a 0.1-ml Hamilton syringe (Hamilton, Whittier, CA). A Hamilton automatic dispensing apparatus was used to inject 5 μl of 20 mg/ml OVA in PBS (~100 μg OVA) into the anterior chamber via the glass micropipette.

**Statistics**

All experimental and control groups contained five to eight animals per group with the majority of groups having an n = 4 or 5. Differences between groups were analyzed by Student’s t test. Values of p < 0.05 were considered significant.

**Results**

**B cell transfer of ACAID**

If the hypothesis that tolerogenic B cells capture peptides released by ACAID APC and present Ag to T cells is true, then one would expect B cells to transfer ACAID. This hypothesis was tested by coculturing ACAID APC with syngeneic B cells and transferring the B cells into naive hosts. Briefly, ACAID and normal APCs were generated and placed into medium petri dishes. Splenic B cells were collected as described above and cocultured with the APC overnight. The next day, nonadherent B cells were collected and injected (5 × 106 cells) into naive, syngeneic mice. Seven days later, the mice were immunized s.c. with 125 μg OVA in CFA. Seven days later, DTH was assessed. B cells cocultured with ACAID APC (ACAID B cells) were able to transfer ACAID to naive hosts (Fig. 1). By contrast, B cells cocultured with normal APC (normal B cells) did not induce ACAID (Fig. 1). These data show that ACAID APC are able to transfer an ACAID signal to splenic B cells and support the hypothesis that B cells serve as tolerogenic APC in ACAID.

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It was previously reported that as few as 20 ACAID APC could transfer ACAID when injected i.v. into naive hosts (28). The next experiments were designed to confirm that B cells, and not contaminating ACAID APCs, were responsible for the transfer of ACAID. The next experiment tested the hypothesis that the transfer of ACAID could be blocked by lysing the B cells. Accordingly, ACAID APCs were generated and incubated with splenic B cells for 2 days. Nonadherent cells were collected and treated with complement or Ab and complement to lyse B220+ B cells before i.v. injection. As a control, ACAID APCs were injected i.v. into naive mice. Seven days later, all mice were immunized s.c. with 125 μg OVA in CFA. Seven days later, DTH was assessed. As shown in Fig. 2, ACAID B cells treated with complement alone were able to induce ACAID in naive mice (Fig. 2).

An additional experiment was performed to confirm that the ACAID-inducing B cells generated in vitro were Ag specific. ACAID APC were pulsed with OVA and incubated with splenic B cells as described above. Plastic nonadherent B cells were collected, and 5 × 10⁶ B cells were injected i.v. into naive, syngeneic mice. Seven days later, mice were immunized s.c. with 125 μg OVA in CFA or 200 μg of hen egg lysozyme (HEL) in CFA. Seven days later, DTH to either OVA or HEL was assessed. The results indicate that the B cells pulsed with ACAID APC and OVA suppressed the DTH responses to OVA, but not to HEL (Fig. 3). In summary, these results show that the B cell is responsible for the transfer of ACAID. Moreover, they demonstrate that ACAID APCs are able to transfer an Ag-specific ACAID-inducing signal to B cells. This is consistent with the notion that the signal is a peptide that can be presented by tolerogenic B cells in the spleen.

B cells as suppressors in ACAID

The results from the previous experiments strongly suggested that the B cells induced ACAID by presenting peptides in a manner that culminated in the generation of regulatory T cells. However, an additional experiment was performed to rule out the possibility that the adoptively transferred ACAID B cells were acting as direct suppressors of DTH. We tested this possibility using a previously described in vitro model of the ACAID spleen (9). In this model, Ag-pulsed ACAID APCs are incubated for 5–7 days with spleen cell cultures containing both T cells and B cells. The ensuing nonadherent T cell population contains cells capable of suppressing the expression of DTH (9). For these experiments, ACAID APCs were generated and placed into culture with normal, unfractionated spleen cells. After 7 days, the nonadherent population of cells was collected and plated on plastic dishes coated with Ab to mouse Ig. This procedure allowed for the separation of adherent B cells from the nonadherent T cells. Each population was tested for the ability to suppress the expression of DTH in a local adoptive transfer assay. Briefly, the test population of cells was mixed with Ag (OVA) and an equal number of OVA-primed, effector T cells. The cells were then injected s.c. into the ear pinnae of naive mice, and ear swelling was used as a measure of DTH. As shown in Fig. 4,
the B cell population was not able to suppress the expression of DTH. Instead, this activity was restricted to the T cell population (Fig. 4). These data show that ACAID APCs do not induce B cells to become direct effector suppressors of DTH.

**Induction of ACAID B cells requires MHC compatibility and is MHC class I restricted**

Previous studies have shown that ACAID APC do not induce ACAID when injected into allogeneic hosts (7, 23, 26). Accordingly, an experiment was performed to test whether MHC restriction was important at the level of the ACAID APC interaction with B cells. C57BL/6 and BALB/c ACAID APC were generated and placed into culture with BALB/c splenic B cells. Two days later, plastic nonadherent B cells were collected and injected i.v. into naive BALB/c mice. Seven days later, all mice were immunized s.c. with 125 μg OVA in CFA or 200 μg HEL in CFA. All results are expressed as mean ear swelling ± SEM. +, p = 0.02, compared with positive control; **, p = 0.0001 for B cells vs negative control; p > 0.05 vs positive control.

**The role of nonclassical class I molecules in ACAID**

The results summarized above, along with previous data, indicate that ACAID is abrogated in mice deficient in the β2m molecule (7). Specifically, β2-m knockout mice were unable to generate at least one population of ACAID suppressor T cells. In addition to having little to no functional class I, β2-m knockout mice do not express nonclassical class I molecules, such as Qa-1 (27, 28). Ag presentation by Qa-1 has long been known to induce suppression of various immune responses (29, 30). It was recently reported that B cells presenting peptide in the context of Qa-1 induce CD8+ suppressor T cells (10). Based on these findings, we hypothesized that B cells generate ACAID by presenting antigenic peptides to T cells in the context of Qa-1. Previous studies had shown that ACAID is abrogated in B cell knockout mice and that reconstitution with normal, syngeneic B cells restores ACAID (9). Our hypothesis was tested by reconstituting B cell knockout mice with B cells purified from syngeneic, B6.A congenic mice differing at the TL/Qa region (Qa-1a). If ACAID B cells present peptide in the context of Qa-1, then TL/Qa congenic B cells would be unable to restore ACAID in B cell knockout mice. Splenic B cells from C57BL/6 and B6.A mice were harvested by panning on petri dishes coated with Ab to mouse γ globulin. Adherent B cells were collected and injected (10^7 cells) i.v. into naive, B cell knockout mice. Five days later, all mice were primed in the anterior chamber.
FIGURE 5. ACAID APC do not transfer an ACAID signal to allogeneic B cells. BALB/c and C57BL/6 ACAID APC were generated and cocultured with B cells from BALB/c mice. Two days later, all nonadherent cells were collected, washed, and 4 x 10⁶ C57BL/6 B cells (=allo-pulsed ACAID B cells) were injected i.v. into naive BALB/c mice. ACAID B cells from BALB/c mice were generated as described in Fig. 1 (=syn-pulsed ACAID B cells) and injected i.v. into naive BALB/c mice. As controls, BALB/c ACAID APCs (=syn ACAID APC) or C57BL/6 ACAID APCs (=allo ACAID APC) were injected i.v. into naive BALB/c mice. Seven days later, mice were immunized s.c. with OVA in CFA. DTH was assessed 7 days after the s.c. immunization. All results are expressed as mean ear swelling ± SEM. *, p > 0.05 for ACAID B cells vs negative control. **, p ≤ 0.0003 for MHC mismatch and allogeneic ACAID APC vs negative control.

FIGURE 6. ACAID requires MHC class I on the ACAID APC and B cells. ACAID APC were generated using PEC from either normal C57BL/6 or β₂m knockout donors. PECs (3 x 10⁶) were placed into culture with 5 x 10⁷ splenic B cells and 5 x 10⁷ splenic T cells from either normal C57BL/6 or β₂m knockout mice. Cultures were incubated for 7 days with frequent agitation. After 7 days, each group of cells was washed and resuspended to 1 x 10⁶ cells/ml in 10 mg/ml OVA and then mixed with an equal number of immune T cells that were generated in vivo by immunizing normal mice with 500 μg OVA. Each cell mixture was used in a local adoptive transfer assay. The results are expressed as mean ear swelling ± SEM. *, p ≤ 0.01, compared with first two groups.

FIGURE 7. Induction of ACAID requires histocompatibility at the TL/Qa region. B cell knockout mice were reconstituted with 10⁷ splenic B cells collected from syngeneic B6 or B6.A mice. Five days later, mice were primed in the anterior chamber with 100 μg OVA. Seven days later, all mice were immunized s.c. with 150 μg OVA in CFA. Seven days later, DTH was assessed. Positive control animals (Pos) were immunized s.c. with 150 μg OVA in CFA. Negative control animals (Neg) were not immunized. All results are expressed as mean ear swelling ± SEM. *, p > 0.05 for B6 vs negative control. **, p = 0.0001 for B6.A vs negative control.

Discussion

The eye is comprised of tissues that are incapable of regeneration and is, therefore, potentially susceptible to immune-mediated injury, such as DTH, which acts in an Ag-nonspecific manner. However, the eye is endowed with multiple mechanisms that minimize immune-mediated damage. Among these is the Ag-specific down-regulation of Th1 responses by ACAID (4). A current model of the initiation of ACAID suggests that resident F4/80⁺ APC process Ag in the eye and migrate to the spleen where they induce the generation of multiple regulatory T cell subsets (5, 6). The cells and mechanisms responsible for this tolerogenic presentation of Ag remain unknown. Previous data have suggested a necessary role for a triad of cells: the ACAID APC, the splenic T cell, and the splenic B cell. The results presented here have attempted to define the potential role of splenic B cells in the generation of ACAID suppressors of DTH.

Using the in vitro model of the ACAID spleen, we have shown that ACAID APCs can induce the generation of efferent suppressor T cells, but only if splenic cells are also present (9). We have hypothesized that ACAID APCs release antigenic peptides to B cells, which present the peptides to T cells in a manner that generates T regulatory cells. Therefore, if B cells function as tolerogenic APC in the ACAID spleen, then one would predict that B cells could transfer ACAID to naive hosts. This hypothesis was tested by coculturing ACAID APC with splenic B cells and transferring the B cells into naive hosts. The results showed that B cells could generate efferent suppressors of DTH when injected i.v. into naive, syngeneic mice. However, B cells cocultured with normal Ag-pulsed APC did not transfer ACAID. Furthermore, an in vitro model of the ACAID spleen showed that T cells, and not B cells, were directly responsible for the suppression of DTH. It was previously reported that B cells are necessary for the generation of ACAID regulatory T cells (8, 9). These results suggest that the...
required role of the B cell is to accept an ACAID-inducing signal from immigrant ACAID APC. We predict that this signal is a regurgitated peptide that the tolerogenic B cells presents to T cells in the ACAID spleen.

Previous data have shown that ACAID is abrogated in mice deficient in the b2m molecule (7). Specifically, b2m knockout mice are unable to generate the CD8\(^{+}\) effector suppressors of DTH. The present in vitro data indicate that the generation of regulatory T cells requires the expression of the b2m molecule on the ACAID APC and the B cell, but not the T cell. Because Ag presentation by the b2m-dependent, nonclassical class I molecule, Qa-1, has been implicated in immune regulation and the generation of CD8\(^{+}\) suppressor T cells (10, 29, 30), we hypothesized that ACAID B cells presented antigenic peptides in the context of Qa-1. The present data show that B cells disparate at the TL/Qa region are unable to restore ACAID in B cell-deficient mice. This suggests that B cells generate ACAID T suppressors in the spleen by presenting antigenic peptides in the context of the nonclassical class Ib molecule, Qa-1. This is reminiscent of previous studies demonstrating that Qa-1\(^{+}\) B cells are needed for the generation of CD8\(^{+}\) regulatory T cells that inhibit Ab responses to sheep erythrocytes (10). However, the suppression of IgG and IgM Ab responses in that study was due to IFN-\(\gamma\) produced by Qa-1-restricted CD8\(^{+}\) regulatory T cells (10). By contrast, ACAID involves the down-regulation of IFN-\(\gamma\) and a concomitant induction of IL-10 production (31–33). In other systems, Qa-1-restricted CD8\(^{+}\) regulatory T cells have been shown to down-regulate Th1 immune responses and mitigate Th1-mediated autoimmune diseases, such as experimental allergic encephalomyelitis (34, 35). Using T cell vaccination as a means of suppressing Th1 immune responses, Jiang and coworkers (34, 35) have analyzed CD8\(^{+}\) regulatory T cells that down-regulate CD4\(^{+}\) T responses via recognition of Qa-1/TCR peptide complexes on the surface of CD4\(^{+}\) T cells. The CD4\(^{+}\) T cells are believed to process and express relevant Qa-1/TCR complexes on their surface. The CD4\(^{+}\) T cells express the relevant TCR peptide coupled to Qa-1 molecules and serve as targets for cytotoxic CD8\(^{+}\) regulatory T cells. In this paradigm, Qa-1/peptide complex on the CD4\(^{+}\) T cells acts as a target and restricting element for CD8\(^{+}\) regulatory T cells. Thus, down-regulation of the immune response is produced by deletion of the relevant CD4\(^{+}\) Th cells. Experiments are underway to determine whether a similar Qa-1-restricted cytotoxic regulatory cell is an integral component of ACAID.

The present study has attempted to define the potential role of splenic B cells in the generation of ACAID suppressors of DTH. The data are consistent with the hypothesis that ACAID APC release antigenic peptides, which are preferentially or exclusively captured by splenic B cells. Moreover, B cells are able to transfer an ACAID-inducing signal to T cells, which either differentiate into effector suppressor cells or induce the generation of suppressor T cells. In this regard, it is noteworthy that Sonoda et al. (36) have demonstrated that CD1-reactive NK T cells are needed for the development of effector suppressor cells of ACAID. Interestingly, the APCs in the spleen that express the highest density of CD1 are the marginal zone B cells (37).

The present findings also demonstrate that the expression of class I, specifically Qa-1, on splenic B cells is necessary for the induction of ACAID. It will be important to show that the mechanism of peptide transfer is directly involved in the development of ACAID. Moreover, it is not known how the B cells capture peptide or whether the peptide is reprocessed in the B cell. We have previously demonstrated that B cells are necessary for the induction of ACAID to alloantigens. Accordingly, it will be critical to determine whether Qa-1 plays a role in the processing of alloantigens and other soluble Ags that are known to induce ACAID. Nevertheless, these data suggest a mechanism by which distinct ocular macrophages process Ag in the eye and migrate to the spleen, where they transfer the relevant peptide moiety to B cells in a class I-restricted, Qa-1-dependent manner.

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References


