



Rapid Communication

OT-II TCR transgenic mice fail to produce anti-ovalbumin antibodies upon vaccination

Shelly Leung^a, Douglas Smith^b, Andrzej Myc^{b,c}, Jingga Morry^{b,1}, James R. Baker Jr.^{b,*}^a Graduate Program in Immunology, University of Michigan Medical School, Ann Arbor, MI 48109, United States^b Michigan Nanotechnology Institute for Medicine and Biological Sciences, Department of Internal Medicine, Division of Allergy, University of Michigan, Ann Arbor, MI 48109, United States^c Ludwik Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

ARTICLE INFO

Article history:

Received 20 July 2012

Accepted 27 December 2012

Available online 26 January 2013

Keywords:

T-B-cell interaction

Helper T-lymphocyte

B-cell

APC

Th1

Th2

Th17

Intranasal vaccine

ABSTRACT

OT-II mice were evaluated as a transgenic strain-specific model to assess T-cell help for B-cell responses. OT-II CD4⁺ T-cells express transgenic OVA-specific $\alpha\beta$ -TCRs. This high frequency of antigen-specific helper T-lymphocytes may augment induction of B-cell responses. Unexpectedly, OT-II mice did not produce OVA-specific antibodies after intranasal immunization. However, B-cells expressed normal antigen-presenting function *in vitro* for activation of OVA-specific T-cell responses. These OT-II T-cell responses produced a Th1-type cytokine profile with significantly reduced Th2 or Th17 responses. These data suggest that OT-II B-cells are not defective as APCs, however, downstream antibody responses are abrogated in this transgenic strain.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

T-cell receptor (TCR) transgenic (tg) mouse models have vastly improved our understanding of T-cell biology and immunity. For the past decade, T-cell receptor (TCR) transgenic (tg) mice have been used to study T-cell development, immunological tolerance, and various autoimmune disorders. The population of antigen-specific T-cells is often low in non-immunized individuals (<0.001% of the total T-cell population for many antigens) and after vaccination (~0.01% T-cells) depending on the immunodominance of the antigenic epitope under evaluation [1–3]. Thus, the availability of TCR transgenic mice has facilitated the phenotypic and functional characterization of antigen-specific T-cell populations. Furthermore, the elucidation of T-B cellular interactions required for induction of antibody responses is important for vaccine development.

The classical paradigm for T-cell-dependent B-cell activation in the immune response to most protein antigens involves early cell-to-cell contact-dependent signaling events initiated by

antigen-primed B-cells that present peptide epitope(s) in the context of class II MHC molecules to responding antigen-specific CD4⁺ T-cells. Antigen presentation by the B-cell to the helper T-lymphocyte (HTL) leads to CD4-receptor co-localization with the T-cell receptor (TCR), antigen-specific TCR triggering, and T-cell activation. Additional T-B cell contact-mediated costimulatory signaling interactions are provided by CD40/CD40L [4,5], B7/CD28 [6], TNF-family members [7,8] and other receptor-ligand pairs [9–11]. Specific binding of antigen to the B-cell antigen-receptor (BCR) also has been shown to activate antigen-presenting function. These early T-B-cell contact-mediated interactions activate cytokine secretion by the HTL which provides activating signals collectively termed “help” to the B-cell [12]. Thus, T-cell help both in the form of cell–cell contact-dependent interactions and secreted cytokines is required for B-cell proliferation, affinity maturation, Ig-class-switching and immunoglobulin secretion. Several cytokines are involved in this process including IL-2, IL-4, IL-5, IL-6, IL-10, TGF- β and IFN- γ , which act at various stages in the sequence of B-cell activation, proliferation, and differentiation leading to T-cell-dependent antibody production [12–14].

The current studies were designed to assess the OT-II TCR-transgenic mouse model for helper T-lymphocyte-dependent ovalbumin-specific B-cell activation. OT-II CD4⁺ T-cells express transgenic $\alpha\beta$ -TCRs that are specific for chicken ovalbumin (OVA) 323–339 in the context of H-2-I-A^b [15]. A high frequency

* Corresponding author. Address: 1150 W. Medical Center Dr., 9220 MSRB III, Ann Arbor, MI 48109, United States. Fax: +1 734 936 2990.

E-mail addresses: sheleung@umich.edu (S. Leung), smitdoug@umich.edu (D. Smith), myca@umich.edu (A. Myc), jmorry@gmail.com (J. Morry), jbakerjr@umich.edu, jbakerjr@med.umich.edu (J.R. Baker Jr.).

¹ Present address: Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR 97239, United States.

(~80%) of OVA-specific CD4⁺ HTL precursors [15] in OT-II transgenic compared to wild-type mice was expected to provide augmented cellular interactions and signal transduction to induce strong B-cell responses leading to high-titer antibody production. To test this hypothesis, OT-II transgenic or wild-type C57BL/6 mice were immunized with ovalbumin. Remarkably, OT-II mice did not produce detectable anti-OVA-specific antibody responses after immunization.

2. Materials and methods

2.1. Mice

C57BL/6J wild-type and OT-II transgenic mice designated B6.Cg-Tg(TcraTcrb)425Cbn/J were purchased from The Jackson Laboratory, Bar Harbor ME. All studies were performed on female mice at ages 8–10 weeks old. All procedures were approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

2.2. Vaccines and immunization schedule

The vaccine was prepared by mixing 1 µg cholera toxin (CT) (List Biological Laboratories, Inc., Campbell, CA) with 20 µg endotoxin-free OVA (Hyglos, Germany) in a total volume of 12 µl PBS per mouse (6 µl per nare). The control group received PBS (12 µl) alone. Mice were primed at week 0 and boosted at weeks 2, 4, 6, and 8 for a total of 5 intranasal vaccinations. Sera were obtained 2 days prior to each vaccination.

2.3. Serum IgG endpoint titer by ELISA

Mouse sera collected from experimental animals were evaluated for OVA-specific IgG antibodies using ELISA. Nunc MaxiSorp flat-bottom 96-well plates were coated with 5 µg/ml OVA in carbonate-bicarbonate coating buffer and incubated overnight at 4 °C. Plates were washed 3 times with wash buffer (0.05% Tween 20 in PBS) and blocked with 1% dry milk in PBS for 30 min at 37 °C. Serum dilutions were prepared in 0.1% BSA in PBS, ranging from 500-fold to 10⁷-fold dilutions in a series of 2- or 5-fold dilutions, and incubated on OVA-coated plates overnight (18–24 h) at 4 °C. The ELISA plates were washed 3 times with wash buffer and incubated with alkaline phosphatase-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 h at 37 °C. Para-nitrophenylphosphate (PNPP) substrate (Sigma–Aldrich) was added to the wells and incubated at room temperature for 1 h to detect OVA-specific IgG. Plates were read using the Spectra MAX 340 plate reader (Molecular Devices) and data was analyzed with SoftMax Pro 5 software. The endpoint titer cutoff is calculated as three standard deviations above background (conjugate plus substrate only, without sera).

2.4. T-B cell co-cultures and cytokine profiling

CD4⁺ T-cells and CD19⁺ B-cells were isolated from spleens of mice ($n = 3$ per group) by magnetic cell sorting (Miltenyi Biotec). 10⁵ T-cells and 10⁵ B-cells were co-cultured in complete RPMI medium (10% FBS, 2 mM L-glutamine, 50 µM β-mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin, 1% nonessential amino acids, 1% HEPES, 1% sodium pyruvate) with OVA (Hyglos, Germany) at 0.1, 1, 10, and 100 µg/ml for 4 days at 37 °C. Cell-free supernatants were harvested, and cytokine secretion was assayed by Luminex[®] multiplex analysis (Millipore Corporation, Billerica, MA) and ELISA (R&D Systems) according to the manufacturers' instructions.

2.5. Intracellular cytokine staining

Splenocytes were seeded at 10⁶ cells/ml into 24-well plates coated with anti-CD3 (10 µg/ml, BD Pharmingen) and anti-CD28 (2 µg/ml, BD Pharmingen). OT-II cells were polarized toward the Th17 phenotype for 4 days using conditions described below, and then stimulated with cognate OVA protein (20 µg/ml) or with Pam3Csk4 (10 µg/ml) for 24 h. WT cells were polarized with Th17 polarizing cytokines as described below for 4 days, and then stimulated with PMA/Ionomycin (5 ng/ml and 500 ng/ml, respectively) for 5 h. The following antibodies and cytokines were used to polarize toward Th17 cells: anti-IFNγ (20 µg/ml) and anti-IL-4 (20 µg/ml) were purchased from BD Pharmingen; TGF-β (10 ng/ml), IL-6 (40 ng/ml), and IL-23 (40 ng/ml) were purchased from PeproTech. During the last 5 h of stimulation, OT-II and WT cells were treated with GolgiPlug protein transport inhibitor containing brefeldin A (BD Biosciences) to inhibit secretion of cytokines. Cells were stained with surface marker anti-mouse CD4 conjugated to FITC (BD Pharmingen) for 30 min at 4 °C. The cells were fixed and permeabilized using the BD Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's manual and stained with anti-mouse IL-17 conjugated to Alexa Fluor 647 (BioLegend). Cells were analyzed using the Accuri c6 flow cytometer and CFlow Plus software.

2.6. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical significance between groups was calculated using a two-tailed Student's *t*-test. The analyses were based on 95% confidence limits, where a *p* value < 0.05 is considered statistically significant.

3. Results and discussion

3.1. Intranasal administration of ovalbumin with CT does not elicit an IgG antibody response in OT-II mice

Adjuvants augment immunity and therefore greatly enhance the efficacy of vaccines. Mucosal administration of vaccine antigens together with an adjuvant, such as CT, has been shown to elicit strong humoral immunity as indicated by high (>10⁵–10⁶) serum antibody titers. Therefore, a CT-OVA vaccine, formulated by mixing 20 µg OVA with 1 µg CT in a total volume of 12 µl in PBS (6 µl per nare) was administered intranasally to wild-type (WT) C57BL/6 and OT-II mice. Mock immunized OT-II mice were given 6 µl per nare (12 µl total volume) of PBS. These mice were primed at week 0 and boosted at weeks 2, 4, 6, and 8 for a total of 5 vaccinations with CT-OVA or PBS as a control. Antibody titers in the sera of these mice were determined by ELISA. A high frequency of OT-II antigen-specific T helper cells was expected to enhance the humoral immune response. However, as shown in Fig. 1, OVA-specific IgG titers for CT-OVA immunized OT-II mice were not significantly different from mock (PBS) immunized mice. In contrast, OVA-specific serum IgG antibody titers were approximately 1000-fold higher for WT compared to OT-II transgenic mice ($p < 0.0001$) (Fig. 1A).

The endpoint titers of IgG1 and IgG2c immunoglobulin heavy chain subclasses of OVA-specific antibodies were evaluated as a means to assess the Th1 and Th2 polarity of the immune response in WT mice (Fig. 1B and C). The IgG2a isotype (Igh-1^a) is not expressed in the C57BL/6 mouse strain (Igh-1^b) due to deletion of the Igh-1^a gene [16]. Instead, C57BL/6 mice express a separate gene for IgG2c (Igh-1^b) [16–18] and expression of this isotype is closely associated with Th1-type responses [19,20], including production of IFNγ by antigen-specific T-cells. B-cell class switching to the

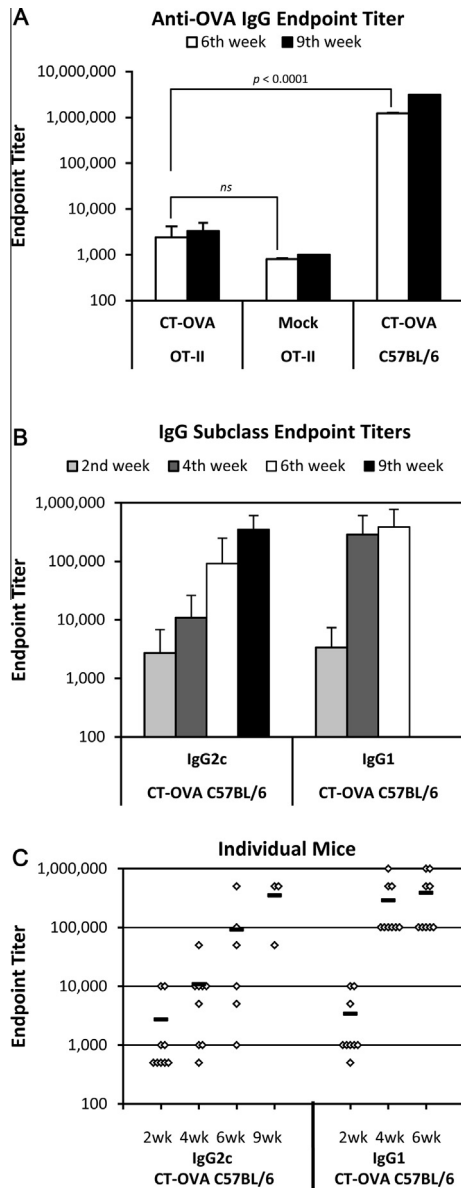


Fig. 1. Cholera toxin fails to elicit humoral immunity against ovalbumin in OT-II transgenic mice. OT-II and WT (C57BL/6) mice ($n = 3-8$ per group) were primed intranasally at week 0 and boosted at weeks 2, 4, 6, and 8 for a total of 5 vaccinations against OVA. (A) Serum antibody titers were determined by ELISA at 2 days prior to the 6th-week boost (6th week bleed) and 7 days after the 8th-week boost (9th-week bleed). CT-OVA immunized OT-II mice did not produce significantly different antibody titers compared to mock (PBS immunized) OT-II mice (ns, not significant). WT mice produced significantly higher antibody titers compared to OT-II mice ($p < 0.0001$) in response to CT-OVA. (B) Evaluation of IgG2c and IgG1 heavy chain isotype profiles for the IgG anti-OVA response by wild type C57BL/6 B6 mice to OVA. Data are shown as mean endpoint titer with standard deviation; (C) Evaluation of IgG2c and IgG1 heavy chain isotype profiles for wild type C57BL/6 B6 mice to OVA. Data are shown as a dot plot to demonstrate the distribution of responses by individual mice within each group.

IgG1 isotype is associated with production of Th2-type cytokines, including IL-4, IL-5 and IL-10 during the HTL response to antigen [21]. As shown in Fig. 1B and C, WT mice produced high endpoint titers ($>10^5$) of OVA-specific antibodies expressing either IgG1 or IgG2c heavy chain subclasses after successive boosting with CT-OVA, indicative of a mixed Th1 and Th2-type immune response. Maximum titers of IgG1 antibodies were achieved by 4 weeks compared to 9 weeks for the IgG2c isotype. This result is consistent with a modest bias toward Th2 responses as previously reported

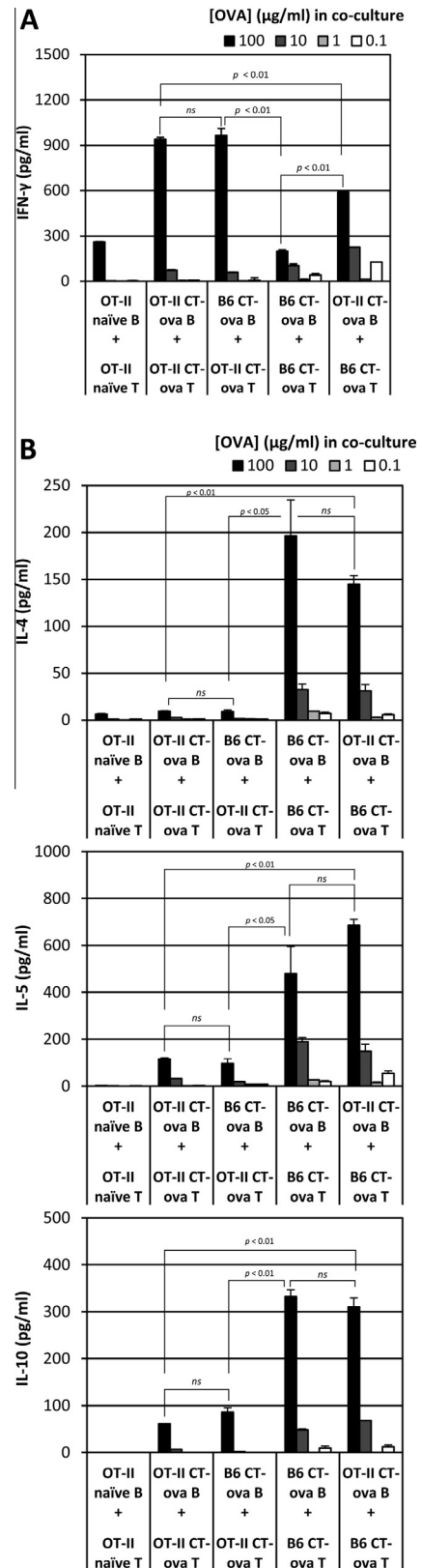


Fig. 2. Re-stimulation of OT-II transgenic T-cells with OVA *in vitro* demonstrates polarization toward Th1 responses. CD4⁺ T-cells and CD19⁺ B-cells were isolated from spleens of mice ($n = 3$ per group) as described in the Materials and Methods section. T-cells (10^5) and B-cells (10^5) were co-cultured with 0.1–100 μg/ml OVA for 4 days. Cytokine secretion was quantified by Luminex[®] analysis in cell-free culture supernatants on day 4. (A) Concentration of IFN-γ, a hallmark of Th1 cell differentiation; (B) Concentrations of IL-4, IL-5, and IL-10 cytokines associated with Th2 cell differentiation. B6 is the designation for wild type (WT) C57BL/6 mice.

by other investigators for the CT adjuvant [22]. In contrast, repeated intranasal administration of CT-OVA to OT-II mice failed to induce a significant IgG humoral immune response against OVA (Fig. 1A). Therefore, skewing or biasing of the HTL response for OT-II mice was assessed next at the T-cell level by comparison of OT-II and WT T-cells from immunized mice for OVA-specific cytokine secretion profiles in subsequent studies.

3.2. Re-stimulation of OT-II transgenic T-cells with OVA *in vitro* drives Th1 responses

B-lymphocytes function as antigen-presenting cells that express class I and class II MHC molecules, co-stimulatory molecules, and a clonally distributed antigen receptor (BCR) which enhances antigen capture leading to efficient uptake, processing and

presentation to responding antigen-specific helper-T-cells [12]. The failure of OT-II mice to produce antigen-specific IgG antibody responses after immunization with CT-OVA may indicate a defect in antigen presentation by B-cells, and/or biasing at the T-cell level leading to polarization away from the Th2-type of response associated with generation of help for B-cells. To evaluate these hypotheses, CD4⁺ T-cells and CD19⁺ B-cells were isolated from CT-OVA immunized WT and OT-II transgenic mice for co-culture together with 0.1–100 µg/ml of OVA as described in Section 2. As shown in Figs. 2 and 3, OT-II T-cells co-cultured with either OT-II B-cells or WT B-cells secreted similar concentrations of specific cytokines when comparing IFN γ (Fig. 2A); IL-4, -5 or IL-10 (Fig. 2B); and, TGF β , IL-6 or IL-17 (Fig. 3A) in response to OVA (all differences were not statistically significant when comparing the response of OT-II T-cells against OT-II vs. WT B-cells at 100 µg/ml OVA for each

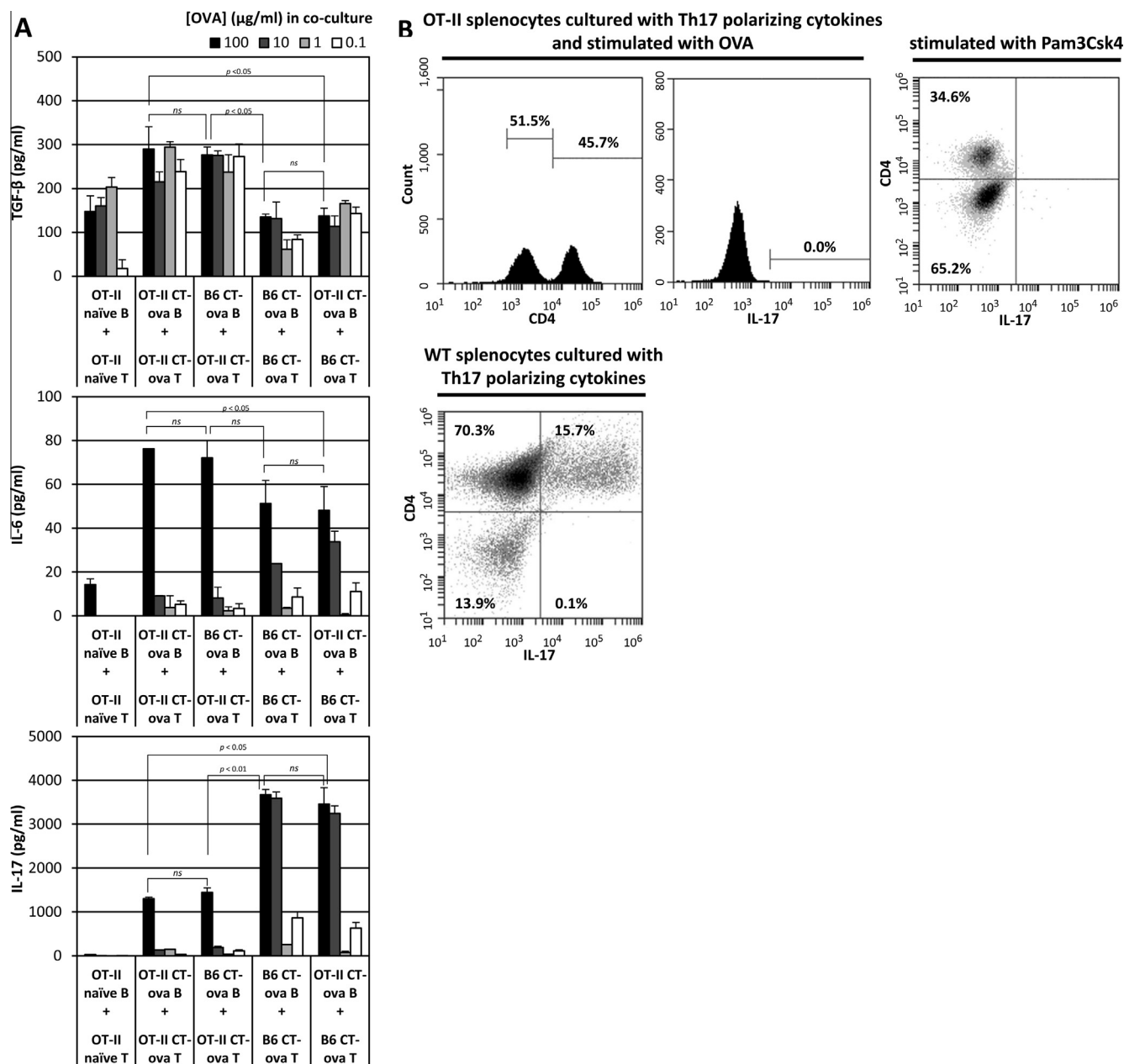


Fig. 3. Re-stimulation of OT-II transgenic T-cells with OVA *in vitro* does not elicit a robust Th17 response. (A) TGF- β , IL-6 and IL-17 secretion in co-cultures of CD4⁺ T-cells and CD19⁺ B-cells. (B) OT-II CD4⁺ T-cells do not differentiate *in vitro* into Th17 cells. Naïve OT-II splenocytes were cultured with Th17-polarizing cytokines for 4 days and stimulated with their cognate protein OVA for 24 h. The IL-17⁺ population were analyzed within a CD4⁺ gate. OT-II CD4⁺ T-cells did not produce IL-17 (middle panel) in contrast to WT CD4⁺ T-cells (bottom, left) as determined by intracellular cytokine analysis. OT-II splenocytes stimulated with the TLR2 agonist Pam3Csk4 also did not express IL-17 as expected for this negative control (right panel) (ns, not significant).

individual cytokine). Furthermore, WT T-cells also produced similar levels of each cytokine, with the possible exception of IFN- γ , in response to OT-II or WT B-cells plus OVA (Figs. 2 and 3). Collectively, these results indicate that both OT-II and WT B-cells are capable of presenting antigen *in vitro* to OT-II transgenic or WT T-cells from mice immunized against CT-OVA.

In contrast to antigen presentation by B-cells, the overall profile and biasing of cytokines produced *in vitro* by T-cells from CT-OVA immunized OT-II transgenic compared to WT mice in response to OVA was profoundly different. As shown in Fig. 2A, OT-II T-cells secreted significantly higher levels of IFN- γ , the prototypical Th1 effector cytokine, compared to WT (C57BL/6 or B6) mice regardless of the source of B-cells used to present antigen. Conversely, T-cells from CT-OVA immunized WT mice secreted significantly higher levels of Th2-type cytokines including IL-4, IL-5 and IL-10, compared to OT-II transgenic mice (Fig. 2B). These observations indicate strong T-cell polarization toward Th1 differentiation and concomitantly away from Th2 type responses (Fig. 2A) by OT-II T-cells in response to OVA.

3.3. Re-stimulation of OT-II transgenic T-cells with OVA *in vitro* does not elicit a robust Th17 response

Th17 cells have been identified as a subset of CD4⁺ T-cells that are induced by IL-6 and TGF- β , and secrete IL-17 in response to TCR activation [23]. This T-cell subset has been implicated to cause autoimmunity and also mediates the control of certain extracellular pathogens, particularly at mucosal sites of infection [24]. Recent reports have identified the ability of CT to induce Th17 cells [25]. Therefore, the induction of Th17 cells by CT-OVA was evaluated in OT-II transgenic compared to WT mice as shown in Fig. 3. OT-II T-cells secreted marginally higher (<2-fold) quantities of TGF- β and IL-6 compared to WT T-cells *in vitro* in response to OVA. However, production of IL-17 by these OT-II T-cells was reduced by 3-fold relative to WT T-cells regardless of the source of B-cells used as APCs for OVA (Fig. 3A).

Innate production of Th17-polarizing cytokines such as TGF- β and IL-6 by APCs and other accessory cells at the time of T-cell priming rather than post-T-cell activation likely is critical for Th17 differentiation. Therefore, the effect of Th17-polarizing cytokines on differentiation of naïve OT-II T-cells was assessed by addition of TGF- β , IL-6 and IL-23 cytokines to spleen cell cultures at the time of activation using anti-CD3 and anti-CD28 mAbs. Anti-IFN γ and anti-IL-4 mAbs were added to the cultures to minimize Th1 and Th2 cell differentiation. The cultures were harvested after 4 days, re-stimulated as required for intracellular cytokine analysis, and evaluated by flow cytometry. As shown in Fig. 3B, OT-II CD4⁺ T-cells derived from naïve OT-II mice under Th17-polarizing culture conditions did not express IL-17. As a negative control, OT-II splenocytes stimulated with the TLR2 agonist Pam3Csk4, also did not express IL-17, confirming that TLR2-stimulation does not maintain a Th17 phenotype [26]. In contrast, WT CD4⁺ T-cells produced IL-17 (15.7%) upon re-stimulation at the time of harvest (Fig. 3B). These findings suggest that OT-II T-cells may have reduced plasticity for differentiation in response to stimuli for Th17 polarization. Collectively, these results are consistent with strong biasing of the T-cell immune response toward Th1 responses and away from Th2 or Th17 cell responses in OT-II mice after immunization with CT-OVA.

4. Conclusions

These studies were designed to evaluate the OT-II TCR-transgenic mouse model for helper T-lymphocyte-dependent ovalbumin-specific B-cell activation. Remarkably, OT-II mice failed to

produce OVA-specific IgG antibodies in response to intranasal vaccination against CT-OVA (Fig. 1). B-cells from both wild-type C57BL/6 and OT-II mice were fully competent to present antigen *in vitro* to OT-II T-cells from immunized mice (Fig. 2). However, the cytokine profile produced by these OT-II T-cells in response to OVA was highly polarized toward production of Th1-type cytokines (IFN γ) with greatly reduced production of Th2 (IL-4, -5, -10) or Th17-type (IL-17a) cytokines, regardless of the source of B-cells (wild-type or OT-II) used to present antigen (Fig. 2). These data indicate that B-cells from OT-II mice are not generally defective for antigen-presenting activity, however, subsequent pathways leading to antibody production are abrogated. The studies conducted using relatively high concentrations of OVA *in vitro* (1–100 μ g/ml) may not eliminate the possibility that antigen capture or presentation by B-cells is defective at very low concentrations of antigen *in vivo* during initiation of cellular and humoral immune responses. Strong polarization of the OT-II CD4⁺ T-cell response toward Th1-type responses and away from the Th2 cytokine profile, which is generally associated with providing help for B-cells and antibody-mediated immunity, is consistent with the failure to produce OVA-specific antibody responses in this transgenic mouse strain.

TCR-transgenic mice have not previously been reported to express abnormalities in B-cell development or T-B cell interactions. Positive selection of CD4⁺ T-lymphocytes is significantly greater in the thymus and lymph nodes of OT-II compared to WT mice [15]. T-cells from peripheral lymphoid tissues exhibit a 4-fold increase in the CD4–CD8 ratio in OT-II compared to WT mice. This altered T-cell phenotype in OT-II transgenic mice may contribute to differences in B-cell responses.

Helper T-cell interactions with B-cells are facilitated in the germinal centers of lymphoid tissues which provide a specialized milieu for interaction between B-cells and helper T-lymphocytes expressing CD40/CD40L and other receptor/ligand pairs and cytokines for induction of B-cell responses [9,10]. Additional studies will be required to determine the mechanism for the failure of OT-II transgenic mice to produce IgG responses against OVA, its relationship to potential defects at the level of the HTL, B-cell or other accessory cell interactions, and the potential for broader generalization of these results to provide insight into T-B collaboration in non-transgenic mice. Future analysis of the OT-II model may focus on specific receptor–ligand mediated T-B interactions, the cellular and cytokine milieu required for T-cell polarization in the OT-II tissue microenvironment, and the germinal center response involving interaction of OT-II B-cells with follicular helper T-cells as specialized HTL for T-cell-dependent antibody responses. Interestingly, immunodeficiencies have been identified for B-cell responses both in mice and humans as a result of defects in many of these pathways [27,28].

Acknowledgments

This research was supported in part by the Bill and Melinda Gates Foundation, under award 37868; by the Barbara Padnos Breast and Karlene Kulp Breast Cancer Research Funds; and by Grant Number 2T32AI007413 from the National Institute of Allergy and Infectious Diseases.

References

- [1] V. Kouskoff, K. Signorelli, C. Benoist, D. Mathis, Cassette vectors directing expression of T cell receptor genes in transgenic mice, *J. Immunol. Methods* 180 (1995) 273–280.
- [2] P. Kisielow, H. Bluthmann, U.D. Staerz, M. Steinmetz, H. von Boehmer, Tolerance in T-cell receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes, *Nature* 333 (1988) 742–746.

- [3] P. Kisielow, H.S. The, H. Bluthmann, H. von Boehmer, Positive selection of antigen-specific T cells in thymus by restricting MHC molecules, *Nature* 335 (1988) 730–733.
- [4] R. Elgueta, M.J. Benson, V.C. de Vries, A. Wasiuk, Y. Guo, R.J. Noelle, Molecular mechanism and function of CD40/CD40L engagement in the immune system, *Immunol. Rev.* 229 (2009) 152–172.
- [5] C. Van Kooten, J. Banchereau, CD40–CD40 ligand: a multifunctional receptor–ligand pair, *Adv. Immunol.* 61 (1996) 1–77.
- [6] N.K. Damle, P.S. Linsley, J.A. Ledbetter, Direct helper T cell-induced B cell differentiation involves interaction between T cell antigen CD28 and B cell activation antigen B7, *Eur. J. Immunol.* 21 (1991) 1277–1282.
- [7] T.H. Watts, TNF/TNFR family members in costimulation of T cell responses, *Annu. Rev. Immunol.* 23 (2005) 23–68.
- [8] G.A. Bishop, The multifaceted roles of TRAFs in the regulation of B-cell function, *Nat. Rev. Immunol.* 4 (2004) 775–786.
- [9] U. Klein, R. Dalla-Favera, Germinal centres: role in B-cell physiology and malignancy, *Nat. Rev. Immunol.* 8 (2008) 22–33.
- [10] C. King, S.G. Tangye, C.R. Mackay, T follicular helper (TFH) cells in normal and dysregulated immune responses, *Annu. Rev. Immunol.* 26 (2008) 741–766.
- [11] H. Qi, From SAP-less T cells to helpless B cells and back: dynamic T–B cell interactions underlie germinal center development and function, *Immunol. Rev.* 247 (2012) 24–35.
- [12] D.C. Parker, T cell-dependent B cell activation, *Annu. Rev. Immunol.* 11 (1993) 331–360.
- [13] R.E. Callard, M.W. Turner, Cytokines and Ig switching: evolutionary divergence between mice and humans, *Immunol. Today* 11 (1990) 200–203.
- [14] M. Howard, A. O'Garra, H. Ishida, Malefyt.R. de Waal, J. de Vries, Biological properties of interleukin 10, *J. Clin. Immunol.* 12 (1992) 239–247.
- [15] M.J. Barnden, J. Allison, W.R. Heath, F.R. Carbone, Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements, *Immunol. Cell Biol.* 76 (1998) 34–40.
- [16] E. Jouvin-Marche, M.G. Morgado, C. Leguern, D. Voegtli, F. Bonhomme, P.A. Cazenave, The mouse Igh-1^a and Igh-1^b H chain constant regions are derived from two distinct isotypic genes, *Immunogenetics* 29 (1989) 92–97.
- [17] R.M. Martin, A.M. Lew, Is IgG2a a good Th1 marker in mice?, *Immunol Today* 19 (1998) 49.
- [18] R.M. Martin, J.L. Brady, A.M. Lew, The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice, *J. Immunol. Methods* 212 (1998) 187–192.
- [19] T.A. Barr, S. Brown, P. Mastroeni, D. Gray, B cell intrinsic MyD88 signals drive IFN-gamma production from T cells and control switching to IgG2c, *J. Immunol.* 183 (2009) 1005–1012.
- [20] S.L. Lambert, C.Y. Okada, R. Levy, TCR vaccines against a murine T cell lymphoma: a primary role for antibodies of the IgG2c class in tumor protection, *J. Immunol.* 172 (2004) 929–936.
- [21] T.L. Stevens, A. Bossie, V.M. Sanders, R. Fernandez-Botran, R.L. Coffman, T.R. Mosmann, E.S. Vitetta, Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells, *Nature* 334 (1988) 255–258.
- [22] S. Yamamoto, H. Kiyono, M. Yamamoto, K. Imaoka, K. Fujihashi, F.W. Van Ginkel, M. Noda, Y. Takeda, J.R. McGhee, A nontoxic mutant of cholera toxin elicits Th2-type responses for enhanced mucosal immunity, *Proc. Natl. Acad. Sci. USA* 94 (1997) 5267–5272.
- [23] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, V.K. Kuchroo, Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells, *Nature* 441 (2006) 235–238.
- [24] S.K. Datta, M. Sabet, K.P.L. Nguyen, P.A. Valdez, J.M. Gonzalez-Navajas, S. Islam, I. Mihajlov, J. Fierer, P.A. Insel, N.J. Webster, D.G. Guiney, E. Raz, Mucosal adjuvant activity of cholera toxin requires Th17 cells and protects against inhalation anthrax, *Proc. Natl. Acad. Sci. USA* 107 (2010) 10638–10643.
- [25] J.B. Lee, J.E. Jang, M.K. Song, J. Chang, Intranasal delivery of cholera toxin induces th17-dominated T-cell response to bystander antigens, *PLoS One* 4 (2009) e5190.
- [26] R.J. Carmody, Q. Ruan, H.C. Liou, Y.H. Chen, Essential roles of c-Rel in TLR-induced IL-23 p19 gene expression in dendritic cells, *J. Immunol.* 178 (2007) 186–191.
- [27] H. Niu, E.S. Sobel, L. Morel, Defective B-cell response to T-dependent immunization in lupus-prone mice, *Eur. J. Immunol.* 38 (2008) 3028–3040.
- [28] S.G. Tangye, E.K. Deenick, U. Palendira, C.S. Ma, T cell–B cell interactions in primary immunodeficiencies, *Ann. NY Acad. Sci.* 1250 (2012) 1–13.