MUCOSAL IMMUNOLOGY

Diet modulates colonic T cell responses by regulating the expression of a *Bacteroides thetaiotaomicron* antigen

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T cell responses to symbionts in the intestine drive tolerance or inflammation depending on the genetic background of the host. These symbionts in the gut sense the available nutrients and adapt their metabolic programs to use these nutrients efficiently. Here, we ask whether diet can alter the expression of a bacterial antigen to modulate adaptive immune responses. We generated a CD4$^+$ T cell hybridoma, BθOM, specific for *Bacteroides thetaiotaomicron* (*B. theta*). Adoptively transferred transgenic T cells expressing the BθOM TCR proliferated in the colon, colon-draining lymph node, and spleen in *B. theta*–colonized healthy mice and differentiated into regulatory T cells (T_{regs}) and effector T cells (T_{effs}). Depletion of *B. theta*–specific T_{regs} resulted in colitis, showing that a single protein expressed by *B. theta* can drive differentiation of T_{regs} that self-regulate T_{effs} to prevent disease. We found that BθOM T cells recognized a peptide derived from a single *B. theta* protein, BT4295, whose expression is regulated by nutrients, with glucose being a strong catabolite repressor. Mice fed a high-glucose diet had a greatly reduced activation of BθOM T cells in the colon. These studies establish that the immune response to specific bacterial antigens can be modified by changes in the diet by altering antigen expression in the microbe.

INTRODUCTION

Dietary components and metabolites produced by host and microbial enzymes modulate the function of a variety of host immune cells including T cells (*1*–*3*). These products can have local effects on the intestinal immune system and in more distant organs (*4*). For instance, host enzymes break down starch and various disaccharides in the diet to produce glucose, which is required systemically for maximal effector T cell (T_{eff}) stimulation (*5*, *6*). Microbial metabolites derived from dietary fiber, flavonoids, and amino acids such as tryptophan have immunomodulatory activities (*3*, *7*–*10*). As examples, short-chain fatty acids from fiber fermentation promote the development of intestinal regulatory T cells (T_{regs}) (*3*), modulate macrophage polarization (*11*), and suppress innate lymphoid cell development (*12*). Further, tryptophan catabolites act via the aryl hydrocarbon receptor to induce T cell cytokine production (*13*); taurine-conjugated bile acids formed from milk-derived dietary fat induce a proinflammatory T helper type 1 (T_H1) immune response (*14*), and, last, the microbial metabolite desaminotyrosine derived from flavonoids stimulates type I interferons (IFNs) and modulates macrophage activation and cytokine production (*15*). Recently, ascorbate, a microbial metabolite altered in Crohn's disease, has been shown to modulate T cell activity (*16*). Other dietary components such as excess salt can change the composition of the microbiome and favor pathogenic T helper 17 (T_H17) responses (*17*). Conversely, an iron-deficient diet can dampen intestinal inflammation (*18*). Collectively, these studies reveal the dominant effects of dietary components and their immediate or downstream metabolites on the immune system.

CD4$^+$ T cells play a critical role in the response to specific microbial antigens in the intestine (*19*–*23*). Symbiotic bacteria that do not damage the host produce tolerogenic T_{reg} responses, whereas pathogens that cause damage elicit T_{eff} responses. In both cases, microbe-specific antigens drive these responses, and these intestinal bacteria are well known to be modulated by diet. However, the effect of diet on T cells that recognize these different groups of symbionts has not been tested. This latter question is of importance due to the effects of diet on the composition and physiology of the microbiome, which has a multitude of effects on the host. It is unclear whether specific dietary components have effects at the level of specific bacterial antigens and the T cells that recognize them.

We hypothesized that the CD4$^+$ immune response to specific bacterial antigens can be modified by changes in the diet through effects on antigen expression of the microbe. Progress in this area has been hampered by the lack of a model system in which a CD4$^+$ T cell response against a specific gut symbiont can be examined. To this end, we developed a CD4$^+$ T cell model, termed BθOM, specific for an outer membrane (OM) antigen from *Bacteroides thetaiotaomicron* (*B. theta*, Bθ). *B. theta* is a prototypic gut symbiont that degrades a wide variety of dietary, host, and microbial glycans and is a representative of a prominent genus found in most human microbiomes (*24*). In healthy mice gavaged with *B. theta*, we found that TCR (T cell receptor) transgenic BθOM T cells responded in vivo by differentiating into T_{regs} and T_{effs}. Deletion of the BθOM T_{regs} induced colitis by activated BθOM T cells, revealing that the symbiont-specific CD4$^+$ T cells were no longer able to self-regulate to prevent T cell–mediated disease. The *B. theta* antigen recognized by BθOM T cells was identified to be BT4295, an OM protein contained in one of *B. theta*'s many polysaccharide utilization loci (PULs). We found that we can modify

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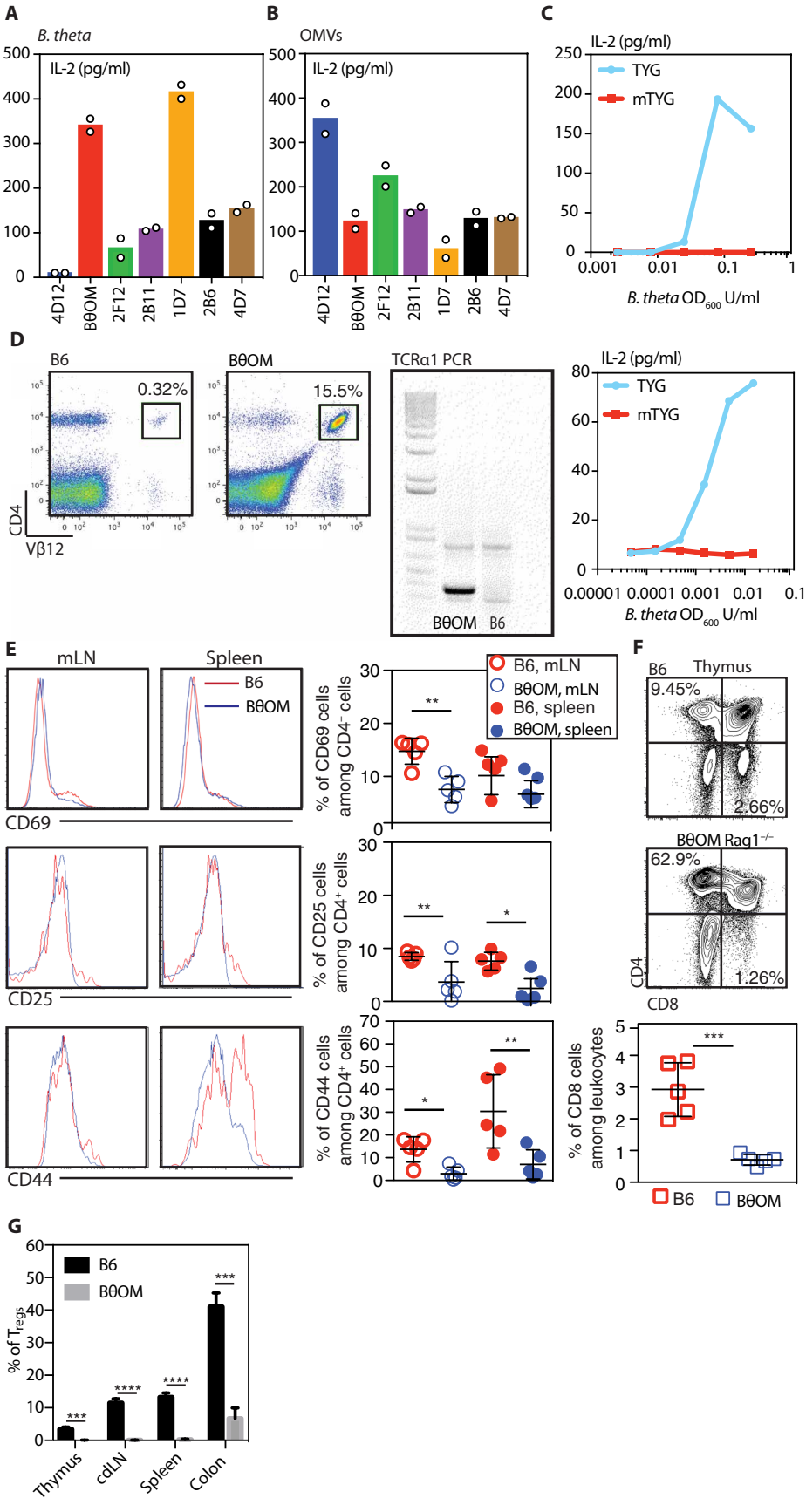

Fig. 1. Generation and characterization of the BθOM TCR transgenic mouse. (**A** and **B**) IL-2 levels in picogram per milliliter after generated T cell hybrid clones were cultured with BMDMs loaded with (A) *B. theta* ($n = 2$, one experiment) or (B) OMVs ($n = 2$, one experiment). (**C**) IL-2 levels in picogram per milliliter after the BθOM T cell hybrid was cultured with BMDMs loaded with *B. theta* grown in TYG or mTYG ($n = 2$; both replicates are shown). (**D**) Representative flow cytometry plot with Vβ12 staining on blood leukocytes of C57BL/6J mice (left) or BθOM transgenic mice (middle) ($n = 3$, three experiments). Representative TCRα1 PCR on DNA isolated from tails of C57BL/6J mice and BθOM transgenic mice (right) ($x = 3$, three experiments). (**E**) Representative histograms of CD69, CD25, and CD44 expression (left) and quantification of the percentage of CD69, CD25, and CD44 cells among all CD4 cells (right) isolated from the mLNs and spleen of C57BL/6J mice (red) or BθOM transgenic mice (blue) ($x = 5$, three experiments). (**F**) Representative flow cytometry plots of CD4 and CD8 staining of thymic cells isolated from C57BL/6J mice or BθOM transgenic mice ($x = 5$, three experiments) and quantification of the percentage of CD8 T cells among the thymic leukocyte population. (**G**) Percentage of T_{regs} in the thymus ($n \geq 6$, three experiments), cdLN ($n \geq 10$, six experiments), spleen ($n \geq 10$, six experiments), and colon ($n = 4$, four experiments) of C57BL/6J mice (black) or BθOM transgenic mice (gray). Student's *t* test: (E) $^{*}P < 0.1$ and $^{**}P < 0.01$; (F) $^{***}P = 0.0004$; (G) $^{****}P < 0.0001$ and $^{***}P = 0.0001$.

the response of BθOM T cells to their cognate antigen by altering the salts and glycans available to *B. theta*. Glucose was identified as a catabolite repressor of BT4295 expression. Mice fed a high-glucose diet had greatly reduced activation of BθOM T cells, establishing a direct link between dietary regulation of a microbial antigen and $CD4^+$ T cell activation. These results show that specific dietary components can alter the T cell–driven immune response to dominant symbiotic antigens.

RESULTS

The *B. theta*–specific $CD4^+$ T cell response is sensitive to changes in *B. theta* growth media

To determine how dietary components and metabolites can affect the interactions between a symbiont and the host immune system, we developed a bacteria-specific $CD4^+$ T cell model. We chose to focus our study on *B. theta*, a model gut symbiont that is known to adapt to changes in the available nutrients, especially by changing expression of carbohydrate utilization gene loci. We immunized C57BL/6J mice with the human *B. theta* strain VPI-5482 (herein referred to as *B. theta*) and produced T cell hybridoma cell lines that responded to *B. theta*. We screened the T cell hybridomas for reactivity against *B. theta* outer membrane vesicles (OMVs), which have been shown to be a source of antigen to the immune system (*25*). To identify a T cell sensitive to changes in available nutrients, we took advantage of a fortuitous observation that *B. theta* grown in two different formulations of tryptone-yeast-glucose (TYG) media—classic TYG (TYG) and modified TYG (mTYG) (table S1)—stimulated T cells differently. We chose one T cell hybridoma clone (herein denoted as *B. theta* outer membrane or "BθOM") that showed a robust response to both *B. theta* and OMVs in T cell stimulation assays (Fig. 1, A and B). When we cultured BθOM T cell hybridomas with bone marrow–derived macrophages (BMDMs) along with *B. theta* grown in the different media, BθOM T cell activation was highest with *B. theta* grown in TYG media (Fig. 1C); no stimulation of these T cells was observed when *B. theta* was grown in mTYG media (Fig. 1C). Thus, BθOM T cells were sensitive to changes in the nutrients in the media used to grow *B. theta*.

We next created a transgenic mouse line expressing the BθOM TCR genes on a C57BL/6J-*Rag1*$^{-/-}$-CD45.1 genetic background (BθOM *Rag1*$^{-/-}$ mouse strain). The TCR transgenic T cells from this line were I-A^b restricted, expressed Vα1 and Vβ12 (Fig. 1D), and were specific for *B. theta* (human or mouse isolates) (Fig. 2A). The peripheral T cells from BθOM *Rag1*$^{-/-}$ mice were essentially all naive, expressing low levels of CD69, CD25, and CD44 proteins (Fig. 1E); the thymus was also devoid of $CD8^+$ T cells (Fig. 1F). We found that BθOM transgenic mice develop few, if any, thymic or peripheral T_{regs} compared with nontransgenic C57BL/6J mice (Fig. 1G). Isolated naive T cells from BθOM *Rag1*$^{-/-}$ mice could be activated when stimulated in vitro with BMDM incubated with either *B. theta* or OMVs (Fig. 2, A and B). Stimulation of the BθOM TCRtg T cells by *B. theta* was confirmed to be sensitive to nutrients in TYG media (Fig. 2C), enabling the use of BθOM T cells to study the effect of diet on symbiont-host interactions.

We then evaluated the function of BθOM T cells in vivo by transferring them into antibiotic pretreated *Rag1*$^{-/-}$ mice. Mice were pretreated with antibiotics for 3 weeks to allow colonization with the subsequently gavaged human isolate of *B. theta*, which we previously showed colonize mice under these conditions (*26*). Sorted naive ($CD44^{lo}CD62L^{hi}$) $CD25^-CD4^+CD45.1^+$ BθOM T cells (fig. S1A) were transferred into *Rag1*$^{-/-}$ mice that had been previously colonized by *B. theta* for 4 days (fig. S1B). We identified $CD4^+CD45.1^+$ T cells in the lamina propria, colon-draining lymph node (cdLN), which refers to the lymph node within the mesenteric lymph node (mLN) that drains the colon, and spleen 7 days after T cell transfer (Fig. 3, A and B). In these mice, BθOM T cell localization in the colon lamina propria and cdLNs was dependent on *B. theta* colonization (Fig. 3C). We also found BθOM T cells in the spleen of *B. theta*–colonized *Rag1*$^{-/-}$ mice (Fig. 3C). The BθOM T cells proliferated in the lamina propria, cdLN, and spleen, revealing that they were exposed to their cognate antigen (Fig. 3, D and E). *B. theta*–gavaged BθOM *Rag1*$^{-/-}$ mice did not have obvious signs of disease such as weight loss (fig. S2A).

BθOM T cells differentiate into T_{effs} and T_{regs} that self-regulate to prevent colitis

Because *Bacteroides* have been previously shown to be strong drivers of T_{reg} induction (*27*), we reasoned that the BθOM T_{regs} would mediate

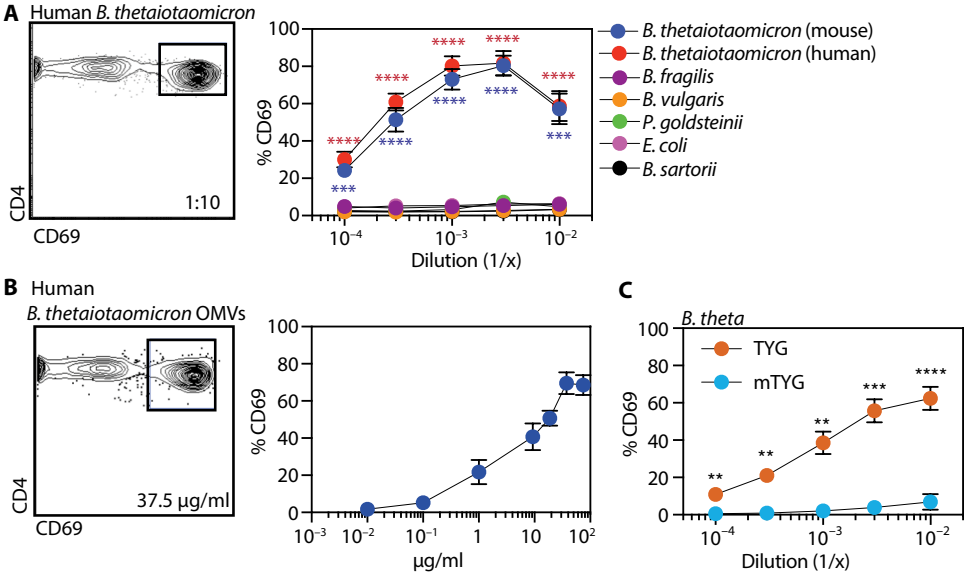

Fig. 2. *B. theta* activates BθOM T cells in a nutrient-dependent manner. (**A** and **B**) Percentage of CD69 expressing BθOM T cells after a 24-hour culture with BMDM loaded with (A) Bacteroidaceae family [human: *B. thetaiotaomicron* ($n = 4$, four experiments); mouse: *B. fragilis*, *B. vulgaris*, *Parabacteroides goldsteinii*, *E. coli*, *B. sartorii* ($n = 3$, three experiments)] or (B) human *B. theta* OMVs (75 µg/ml: $n = 7$, six experiments; 37.5 µg/ml: $n = 6$, six experiments; 18.75 µg/ml: $n = 5$, four experiments; 10 µg/ml: $n = 8$, six experiments; 1 µg/ml: $n = 3$, three experiments; 0.1 µg/ml: $n = 4$, four experiments; 0.01 µg/ml: $n = 3$, three experiments). Flow cytometry plots are gated on $CD4^+$ $CD45.1^+$ leukocytes. (**C**) Percentage of CD69 expressing BθOM hybridoma T cells after a 24-hour culture with BMDM loaded with human *B. theta* grown in TYG ($n = 13$, five experiments) or mTYG medium ($n = 5$, five experiments). One-way ANOVA analysis: (A) ***$P < 0.001$ and ****$P < 0.0001$. Means with asterisks are significantly different by Tukey's multiple comparisons test. Student's *t* test: (C) ****$P < 0.0001$, ***$P = 0.0001$, and **$P < 0.01$.

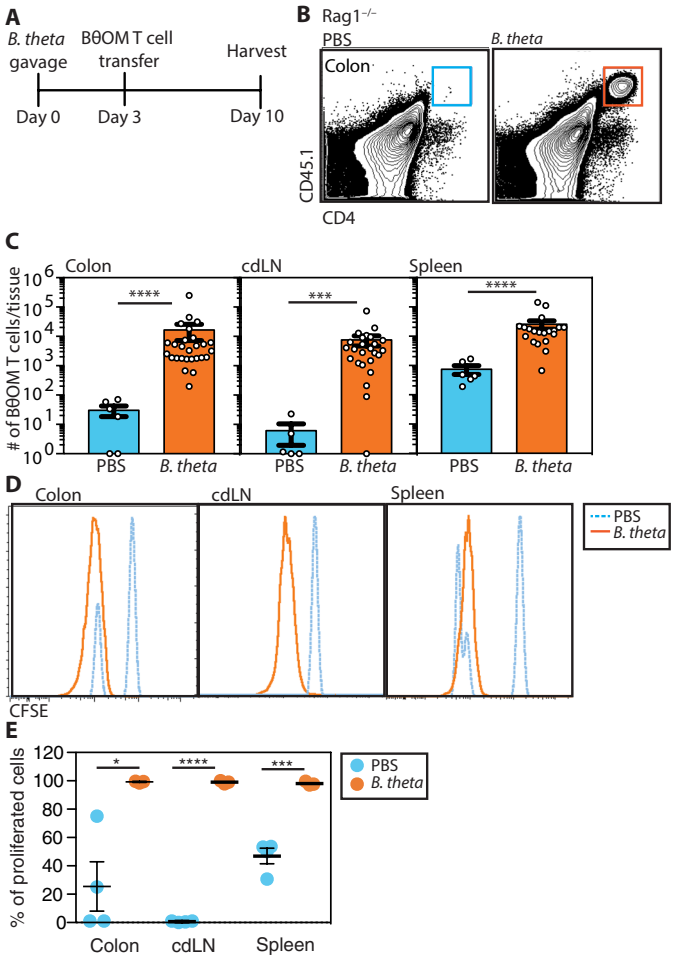

Fig. 3. BθOM T cells proliferate in the colon in *B. theta*–colonized mice. (**A**) Schematic of adoptive transfer of BθOM T cells into *Rag1*$^{-/-}$ mice gavaged with PBS or *B. theta*. (**B**) Representative flow cytometry plots of CD45.1^{+}CD4^{+} BθOM T cells in the colon of *B. theta*–gavaged mice compared with PBS-gavaged mice. (**C**) Number of BθOM T cells among live leukocytes that are CD45.2^{-}CD45.1^{+}CD4^{+} in PBS or *B. theta*–gavaged mice in the colon ($n \geq 6$, ≥ five experiments), cdLN ($n \geq 5$, ≥ three experiments), and spleen ($n \geq 6$, ≥ four experiments). (**D**) Representative histograms of adoptively transferred carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled BθOM T cells in the colon ($n \geq 3$, ≥ three experiments), cdLN ($n \geq 3$, three experiments), and spleen ($n \geq 3$, ≥ three experiments) of *B. theta*–gavaged mice compared with PBS-gavaged mice. (**E**) Quantification of the percentage of proliferated CFSE low CD45.2^{-}CD45.1^{+}CD4^{+} T cells in the colon ($n \geq 3$, ≥ three experiments), cdLN ($n \geq 3$, three experiments), and spleen ($n \geq 3$, ≥ three experiments). Mann-Whitney test for non-normally distributed data: (C) ****$P < 0.0001$ and ***$P = 0.0006$. Student's *t* test: (E) ****$P < 0.0001$, ***$P = 0.0005$, and *$P = 0.0160$.

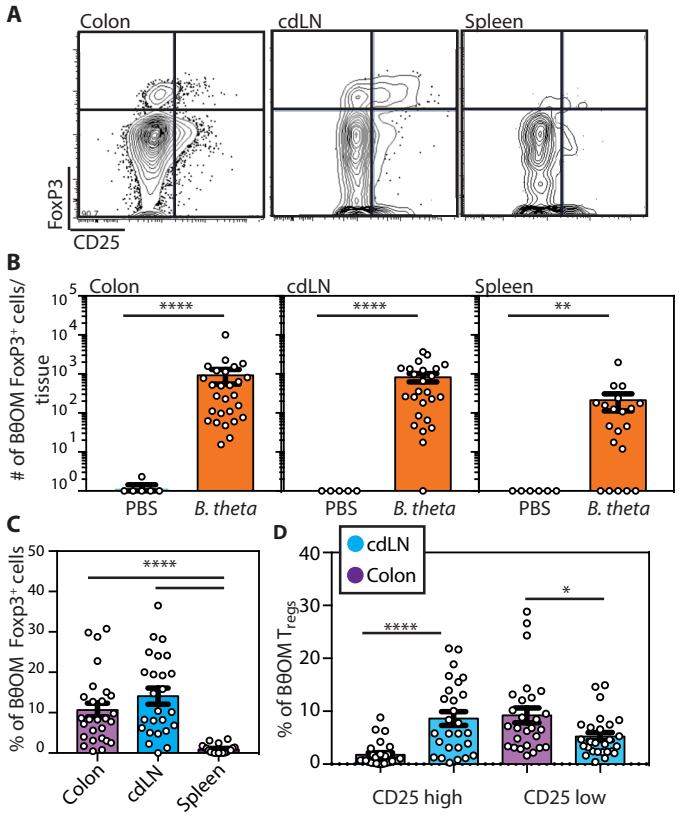

Fig. 4. BθOM T cells in the colon differentiate into T_{regs}. (**A**) Flow cytometry plots of CD45.1^{+}CD4^{+} BθOM T cells in the colon, cdLN, and spleen of PBS or *B. theta*–gavaged *Rag1*$^{-/-}$ mice transferred with naive CD25^{-} BθOM T cells. (**B**) The number of CD4^{+} CD45.1^{+}FoxP3^{+} BθOM T_{regs} cells in the colon ($n \geq 6$, ≥ five experiments), cdLN ($n \geq 5$, ≥ three experiments), and spleen ($n \geq 6$, ≥ four experiments) of PBS or *B. theta*–gavaged *Rag1*$^{-/-}$ mice after CD25^{-} BθOM T cell transfer. (**C**) Percentage of FoxP3^{+} T_{regs} in the colon ($n = 27$, nine experiments), cdLNs ($n = 25$, seven experiments), and spleen ($n = 20$, seven experiments) of *Rag1*$^{-/-}$ mice that received naive CD25^{-} BθOM T cells and were gavaged with *B. theta*. (**D**) Percentage of CD25^{high} versus CD25^{low} CD4^{+}FoxP3^{+} T_{regs} in the colon ($n = 27$, nine experiments) and cdLNs ($n = 25$, seven experiments) of *Rag1*$^{-/-}$ mice gavaged with *B. theta* and injected with naive BθOM T cells. Mann-Whitney test for non-normally distributed data: (B) ****$P < 0.0001$ and **$P = 0.004$. Kruskal-Wallis with Dunn's posttest for non-normally distributed data: (C) ****$P < 0.0001$. Two-way ANOVA analysis: (D) ****$P < 0.0001$ and *$P = 0.0161$.

tolerance to *B. theta*. We transferred BθOM T cells into *Rag1*$^{-/-}$ mice; the transferred cells were presorted for CD4^{+}CD44^{lo}CD62L^{hi}CD25^{-} to ensure that there was no transfer of preexisting T_{regs} into recipients (fig. S1A). Characterization of the BθOM T cells in multiple locations showed a mixture of T_{eff} and FoxP3^{+} T_{regs} in the lamina propria and cdLN with a lower percentage of T_{regs} found in the spleen (Fig. 4, A to C). T_{reg} development in the peripheral lymphatics and the colonic tissue was dependent on *B. theta* colonization because few to no T_{regs} were found in phosphate-buffered saline (PBS)–gavaged mice (Fig. 4B and fig. S1B). Despite the presence of T_{regs} in both the cdLN and colonic lamina propria, the cdLNs had many more T_{regs} expressing CD25 than the colon, where most of the T_{regs} expressing FoxP3 lacked CD25 expression (Fig. 4D). Consistent with previous reports with polyclonal T_{regs} exposed to *Bacteroides* in the lamina propria (*28*, *29*), 50% of BθOM FoxP3^{+} T_{regs} express RORγt (fig. S1C). This finding is also consistent with a report showing that, in healthy wild-type mice, pathobiont-specific T cells differentiate into RORγt-expressing specific induced T_{regs} (iT_{regs}) in the large intestine (*30*). Together, these data reveal that the same TCR can differentiate into both T_{effs} and T_{regs} (*19*).

We hypothesized that *B. theta*–specific T_{regs} produced sufficient regulation in the colonic mucosa to prevent *B. theta*–specific CD4^{+} T cells from inducing colitis upon exposure to *B. theta*. To test this hypothesis, we crossed the BθOM transgenic mouse to FoxP3-DTR-GFP mice, which permits the in vivo depletion of T_{regs} upon diphtheria

toxin (DT) (*31*) treatment and includes a green fluorescent protein (GFP) marker for T_{reg} identification (*30*). We transferred naive, GFP^{lo} BθOM T cells into *Rag1*$^{-/-}$ mice colonized with *B. theta* that were treated with DT on days 9, 11, and 13 (Fig. 5A). We confirmed depletion of T_{regs} in the cdLNs and spleen (Fig. 5B). We found that *Rag1*$^{-/-}$ mice that received BθOM-FoxP3-DTR cells and DT developed colitis, with an increase in hyperproliferative crypts, epithelial proliferation, lymphocyte infiltrate, mitotic figures, and crypt height compared with control mice that received BθOM T cells and DT (Fig. 5, C to E). Cells isolated from the mLN of *Rag1*$^{-/-}$ mice transferred with BθOM-FoxP3-DTR T cells and treated with DT to deplete T_{regs} showed an increase in proinflammatory cytokines [interleukin-17A (IL-17A), IFN-γ, and IL-6] compared with cells isolated from *Rag1*$^{-/-}$ mice receiving wild-type BθOM T cells and treated with DT (Fig. 5F and fig. S3, A and B). Both BθOM-FoxP3-DTR T cells and wild-type BθOM T cells isolated from the colon lamina propria and mLN differentiated into T_H1 cells (fig. S4A). BθOM-FoxP3-DTR T cells can also differentiate into T_H17 cells; however, variable levels of T_H17 induction were observed between experiments (fig. S4, B and C). These findings are a direct demonstration that symbiont-specific $CD4^+$ T cells can develop into both T_{effs} and T_{regs} and that these T_{regs} can self-regulate.

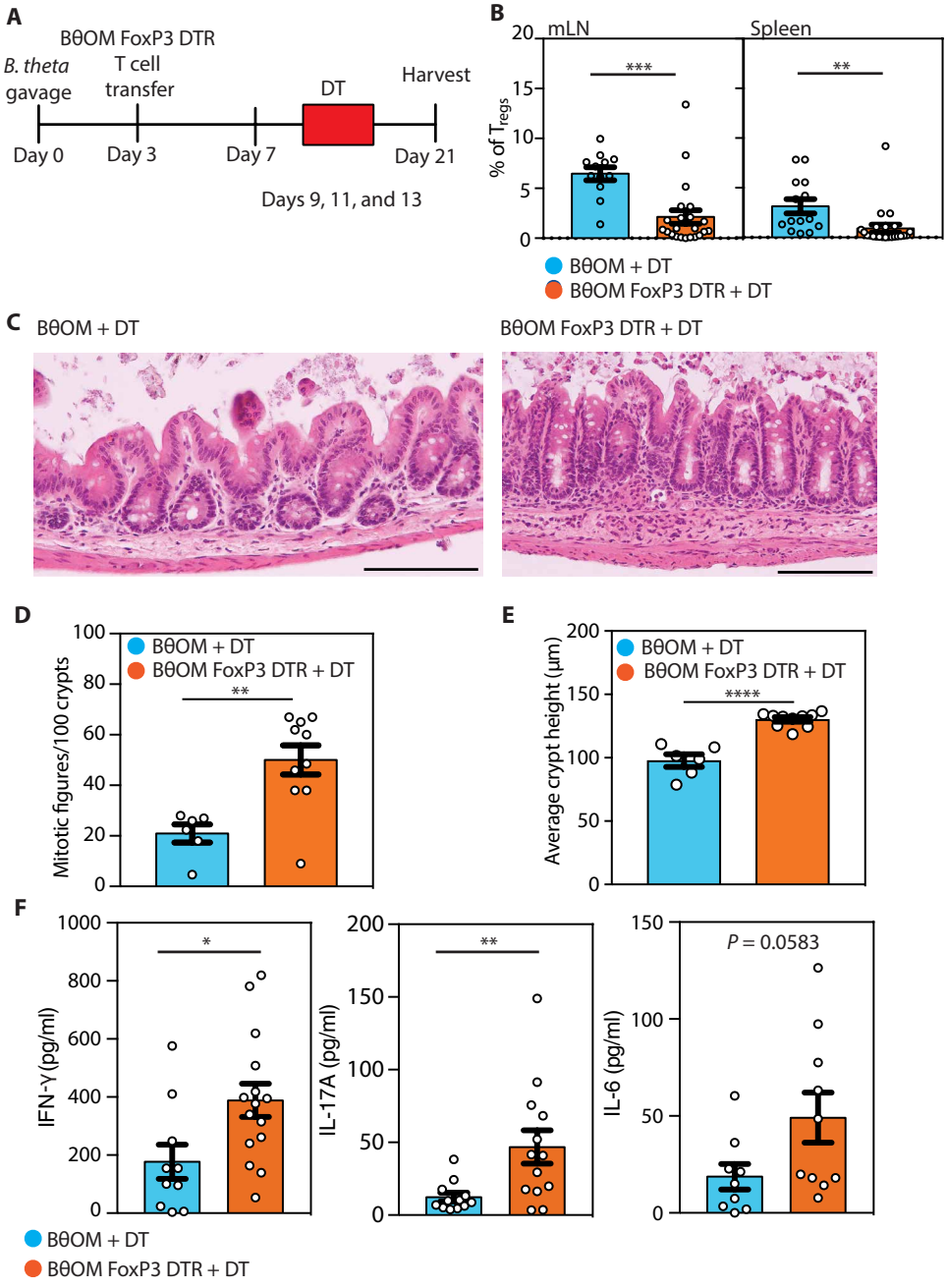

Fig. 5. Depletion of BθOM T_{regs} drives BθOM $CD4^+$ T_{eff} to cause colitis. (**A**) Schematic of adoptive transfer of BθOM or BθOM-FoxP3-DTR T cells into *Rag1*$^{-/-}$ mice gavaged with PBS or *B. theta* and treated with DT (*31*) to deplete BθOM T_{regs}. (**B**) Percentage of BθOM T_{regs} after depletion in the mLN ($n \geq 12$, five experiments) or spleen ($n \geq 14$, five experiments). (**C** to **E**) Histology (C), quantification of the number of mitotic figures/10 crypts (D), and average crypt height (E) in cecal sections from *Rag1*$^{-/-}$ mice given BθOM T cells and DT ($n = 6$, three experiments) compared with those given BθOM-FoxP3-DTR T cells and DT ($n = 10$, three experiments). Scale bars, 120 μm. (**F**) Cytometric bead array used to quantify IFN-γ ($n \geq 10$, three experiments), IL-17A ($n \geq 10$, three experiments), and IL-6 ($n \geq 10$, three experiments) after cells isolated from the mLN were stimulated with PMA for 5 hours. Student's *t* test: (B) ***$P = 0.0002$ and **$P = 0.0055$; (D) **$P = 0.0029$; (E) ****$P < 0.0001$; (F) *$P = 0.0205$ and **$P = 0.098$.

The antigen recognized by BθOM T cells, BT4295, is expressed in a PUL

To elucidate how diet could affect a bacterial antigen expression, we needed to identify the antigen recognized by BθOM T cells. To identify this *B. theta* antigen, we used positive functional fractionation, mass spectrometry, and a loss-of-function screen. Using *B. theta* OMVs as the starting material, we performed a T cell activation assay from 20 fractions of isolated proteins separated on the basis of molecular weight (Fig. 6A and fig. S5A). We found a single fraction of *B. theta* OMV proteins that stimulated BθOM T cells (Fig. 6A). Mass spectrometry analysis of this fraction identified 322 distinct proteins (Fig. 6A). To refine the list of potential antigens, we generated a *B. theta* transposon insertion library (*32*) and screened individual clones using the in vitro T cell activation assay for BθOM T cells (Fig. 6A). In a screen of 2300 clones, we identified five genes that, when knocked out, no longer stimulated BθOM T cells (Fig. 6A and fig. S5B). One of the five *B. theta* gene candidates (BT4298) was identified in the mass spectrometry analysis (Fig. 6A). The other four hits were all in one additional unlinked locus (BT1220-23) containing genes encoding enzymes in the pentose phosphate pathway.

Expression in *Escherichia coli* of the BT4298 protein identified in both the

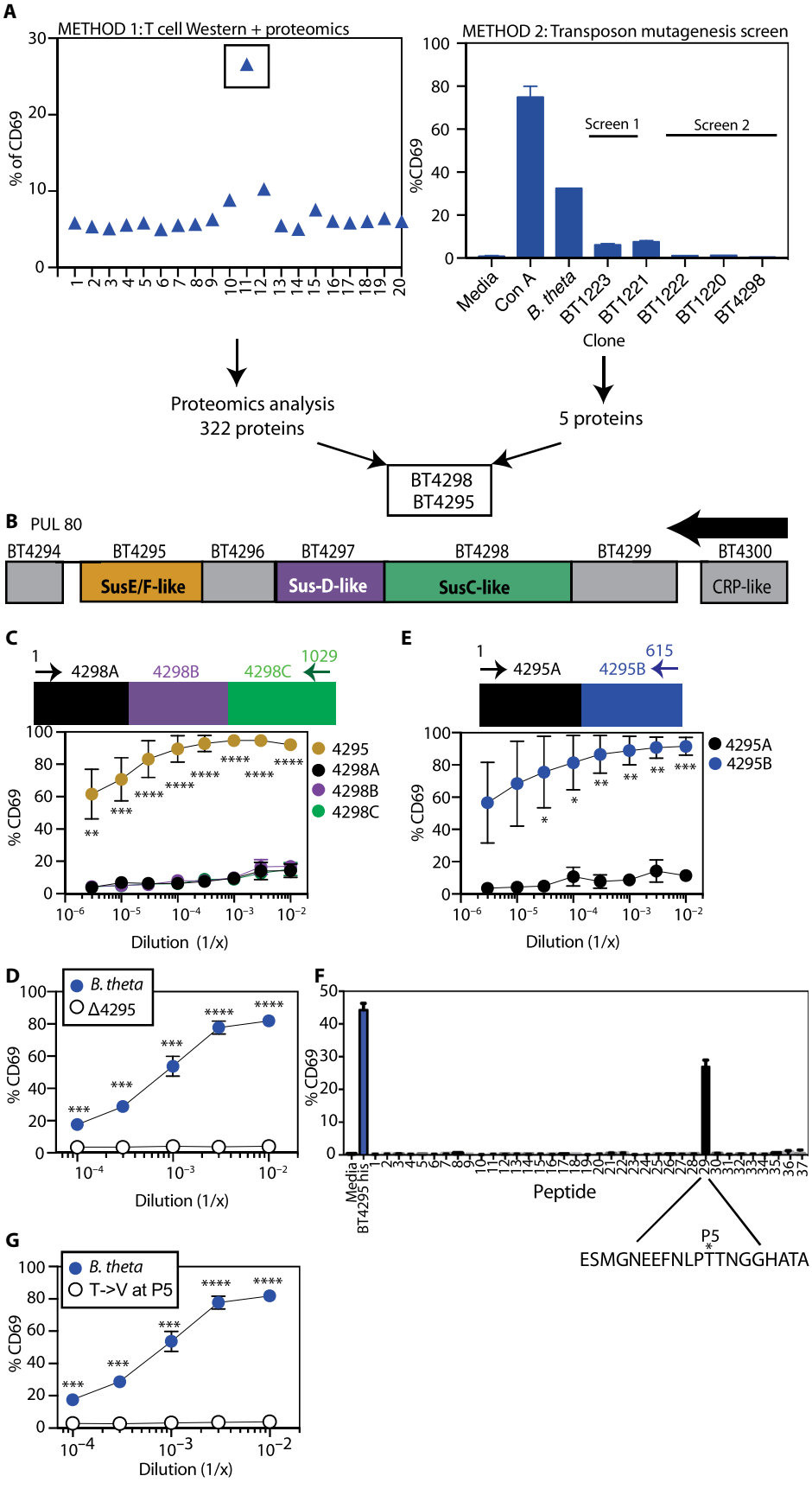

Fig. 6. BθOM T cells specifically recognize the BT4295$_{(541–554)}$ epitope. (**A**) Two parallel methods, T cell Western with proteomics (left) and transposon mutagenesis (TM) screen (*20*) (right), used to identify the antigen that stimulates BθOM T cells. (**B**) Schematic of the PUL80 affected by BT4298 disruption by TM. The arrow represents the direction of transcription. (**C** to **G**) Percentage of CD69 expressing BθOM T cells after culture with BMDM loaded with (C) *E. coli* expressing the full-length BT4295 ($n = 3$, three experiments for each dilution) or three consecutive segments of BT4298 (BT4298A, BT4298B, and BT4298C) ($n = 3$, three experiments for each dilution), (D) *B. theta* ($n = 4$, four experiments) or Δ4295 ($n = 4$, four experiments), or (E) *E. coli* expressing two consecutive segments of BT4295 (BT4295A and BT4295B) ($n = 3$, three experiments for each dilution). (F) Synthetic 20-amino acid peptides overlapping by 12 amino acids. The asterisks represent the P5 position. (G) *B. theta* ($n = 4$, four experiments, same data as Fig. 2E) or Δ4295 ($n = 3$, three experiments). One-way ANOVA analysis: (C) $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. Means with asterisks are significantly different by Tukey's multiple comparisons test. Student's *t* test: (D) $^{***}P < 0.001$ and $^{****}P < 0.0001$; (E) $^{*}P < 0.1$, $^{**}P < 0.01$, and $^{***}P < 0.001$; (G) $^{***}P < 0.001$ and $^{****}P < 0.0001$.

mass spectrometry and transposon library, unexpectedly, did not stimulate BθOM T cells (Fig. 6C). However, many bacterial genes are organized into cotranscribed operons, and this is likely to be true for *B. theta*. For example, the BT4294-4300 PUL was previously shown to be coordinately activated in response to mucus O-linked glycans (*26*, *33*). We therefore reasoned that the transposon insertion in the BT4298 gene exerts loss-of-function effects on downstream genes due to polarity (Fig. 6B), including BT4295, which was also identified in our mass spectroscopy analysis (Fig. 6A). Expression of BT4295 in *E. coli* resulted in strong stimulation of BθOM T cells (Fig. 6C), demonstrating that the BT4295 was the antigen recognized by BθOM T cells. BT4295 is predicted to be a SusE/SusF lipoprotein that is ultimately trafficked to the OM, including OMVs (fig. S6A) (*26*). We confirmed that BT4295 was the only antigen recognized by BθOM T cells by generating an in-frame deletion mutant of BT4295 that disrupted its expression (BTΔ4295) and abolished its ability to stimulate BθOM T cells (Fig. 6D).

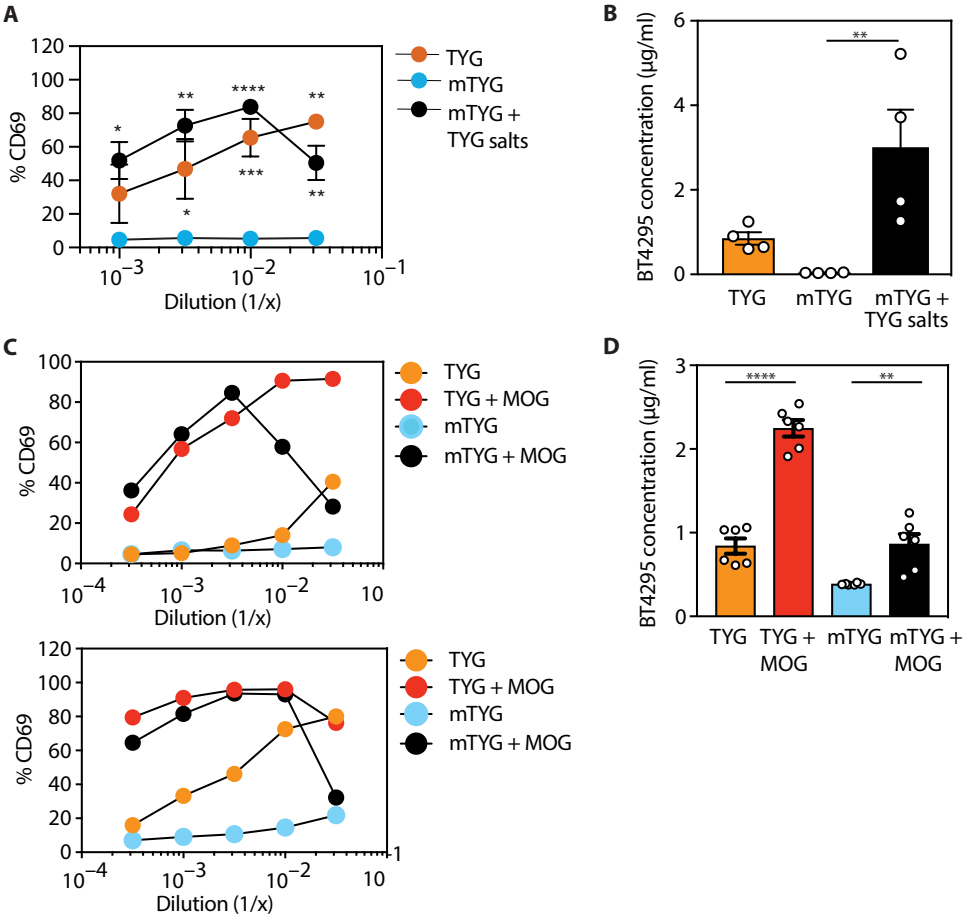

Fig. 7. Salt and glycan regulate BT4295 expression and alter BθOM T cell activation. (**A**) Percentage of CD69 expressing BθOM T cells after a 24-hour culture with BMDM loaded with *B. theta* grown in mTYG ($n = 4$, four experiments), TYG ($n = 2$, two experiments), and mTYG supplemented with TYG salts ($n = 4$, four experiments). (**B**) The concentration in microgram per milliliter of BT4295 protein expressed in *B. theta* grown in TYG, mTYG, and mTYG supplemented with TYG salts ($n = 4$, four experiments) as determined by a quantitative ELISA. (**C**) Percentage of CD69 expressing BθOM T cells after a 24-hour culture with BMDM loaded with *B. theta* grown in mTYG, TYG, mTYG supplemented with MOG and TYG supplemented with MOG ($n = 2$, two experiments). (**D**) The concentration in microgram per milliliter of BT4295 protein expressed in *B. theta* grown in mTYG, TYG, mTYG supplemented with MOG and TYG supplemented with MOG ($n = 3$, three experiments) as determined by a quantitative ELISA. One-way ANOVA analysis: (A) $^{*}P < 0.1$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$; (B) $^{**}P = 0.0093$; (D) $^{****}P < 0.0001$ and $^{**}P = 0.0065$. Means with asterisks are significantly different by Tukey's multiple comparisons test.

To identify the epitope in BT4295 recognized by BθOM T cells, we expressed amino and carboxyl halves of the protein in *E. coli* (Fig. 6E). We found that the carboxyl half of the protein activated BθOM T cells (Fig. 6E). We then generated overlapping 20-mer peptides for the entire carboxyl half of BT4295 and tested them for their ability to activate BθOM T cells. A single peptide (536 to 555) stimulated BθOM T cells (Fig. 6F). The antigenic epitope was further defined to be the highly stimulatory 14-mer (541 to 554) (EEFNLPTTNGGHAT), which contains a strong predicted I-A^b binding motif (P1 = F543) (fig. S6B). We identified the threonine at the P5 position (T547) to be critical for TCR recognition and generated a point mutation at the P5 position (a threonine to a valine substitution, T547V) that resulted in the complete loss of BθOM T cell activation (Fig. 6G). Together, these findings demonstrate that BθOM T cells strongly and specifically recognize a single peptide epitope ($BT4295_{541–554}$) in the BT4295 protein, which is expressed in the *B. theta* OM in response to mucin-type O-glycan (MOG) cues.

Expression of BT4295 is regulated by available nutrients

Having identified BT4295 as the antigen recognized by BθOM T cells, we determined how specific nutrients altered its expression. On the basis of the differential ability of *B. theta* grown in TYG versus in mTYG media to stimulate BθOM T cells (Fig. 2C), we asked whether removing specific components (table S1) from the TYG media or adding them to the mTYG media would alter the stimulatory ability of *B. theta* grown in these modified media. Individually removing vitamin B_{12}, vitamin K_3, histidine, cysteine, $FeSO_4$, or $MgCl_2$ from TYG media had no effect on the ability of *B. theta* to stimulate BθOM T cells (fig. S7, A and B). However, when we removed salts [KH_2PO_4, $(NH_2)_4SO_4$, and NaCl] from TYG, *B. theta* grown in this altered media no longer stimulated BθOM T cells (fig. S7, A and B). Because removing salts from the TYG media did reduce *B. theta* growth to some extent, we also tested the addition of these salts to mTYG media that contained notably lower concentration of salts (KH_2PO_4, K_2HPO_4, and NaCl) (Fig. 7A). Adding TYG salts to mTYG media resulted in a significant increase in BθOM T cell activation (Fig. 7A). The ability of *B. theta* grown in TYG, mTYG, and mTYG with TYG salts to stimulate BθOM T cells directly correlated with the level of BT4295 protein expression as determined by a quantitative enzyme-linked immunosorbent assay (ELISA; Fig. 7B and fig. S7C).

Previous transcriptional analysis showed that, in the absence of dietary glycans, *B. theta* in vivo increases the expression of the BT4294-4300 PUL likely to break down endogenous mucin glycans, which is supported by in vitro expression of this PUL in response to purified mucin glycans (*34*, *35*). Therefore, we tested whether growing *B. theta* in mTYG with porcine MOG would increase the expression of BT4295 and drive BθOM T cell activation. We found that *B. theta* grown in mTYG supplemented with MOG now strongly activated BθOM T cells (Fig. 7C) and led to increased BT4295 protein expression (Fig. 7D). Thus, BT4295 expression can be up-regulated by MOG in mTYG media, which alone did not induce expression. Together, these findings demonstrate that, by changing available nutrients (salts or glycans), the expression of a specific symbiont-derived antigen can be markedly affected.

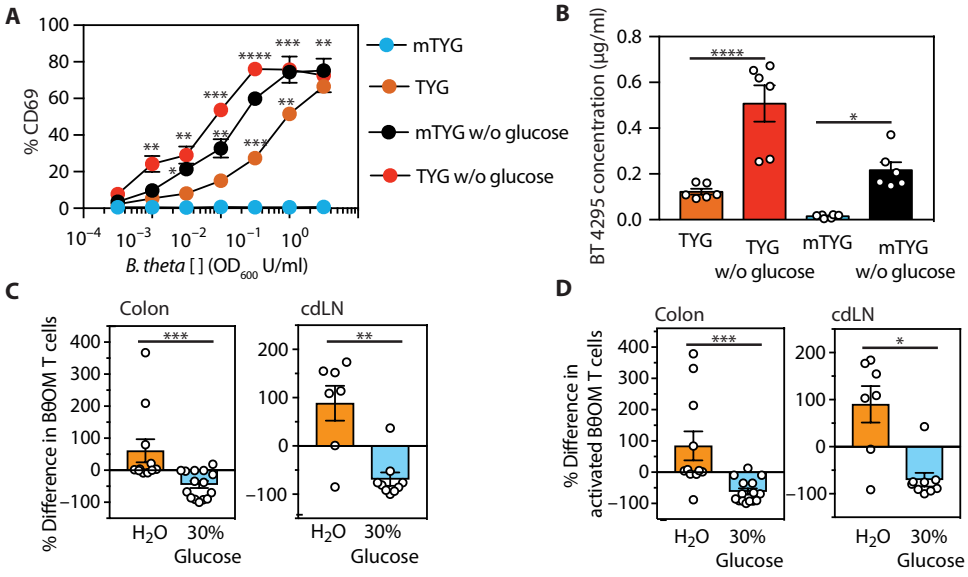

Fig. 8. Dietary glucose represses BT4295 expression, decreasing the activation of BθOM T cells in vivo. (**A**) Representative plot of the percentage of CD69 expressing BθOM T cells after culture with BMDM loaded with *B. theta* grown in TYG and mTYG media with or without glucose ($n = 6$, three experiments). (**B**) The concentration in microgram per milliliter of BT4295 protein expressed in *B. theta* grown in TYG and mTYG media with or without glucose ($n = 6$, three experiments). The percent difference in the number of (**C**) $CD4^+CD45.1^+$ BθOM T cells or (**D**) $CD4^+CD45.1^+CD44^+CD62L^-$ activated BθOM T cells in the colon ($n = 26$, $x = 3$ experiments) and cdLN ($n = 16$, two experiments) of *B. theta*–colonized mice given water or 30% glucose water and adoptively transferred with 200,000 CD4-enriched BθOM T cells. (C and D) The percent difference was calculated from the mean of each experiment. ANOVA multiple comparison analysis: (A) $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$; (B) $^{****}P < 0.0001$ and $^*P = 0.0190$. Means with asterisks are significantly different by Tukey's multiple comparisons test. Mann-Whitney test for non-normally distributed data: (C) $^{***}P = 0.0002$ and $^{**}P = 0.0052$; (D) $^{***}P = 0.0002$ and $^*P = 0.0115$.

Glucose catabolically represses BT4295

The four transposon mutant hits in the pentose phosphate pathway that significantly decreased expression of BT4295 (Fig. 6A) implicated glucose metabolism as another potential regulator of BT4295 expression. To test the involvement of glucose on the regulation of BT4295 expression, we eliminated glucose from the TYG and mTYG media (table S1). *B. theta* grew in both media in the absence of glucose, but at slightly reduced rates. We found that BθOM T cells were now stimulated by *B. theta* grown in mTYG in the absence of glucose (Fig. 8A). Similarly, *B. theta* grown in TYG without glucose also stimulated BθOM T cells, even stronger than in the presence of glucose (Fig. 8A). Thus, glucose appeared to be acting as a repressor of BT4295 expression. Catabolite repression is a well-established regulatory process in bacteria, including *B. theta*, in which other metabolic pathways are repressed in the presence of glucose or other high-priority nutrients (*36*, *37*). Using a quantitative ELISA for BT4295 protein, we tested whether the increase in stimulatory ability of *B. theta* grown in the absence of glucose was due to increased BT4295 protein expression. Removing glucose from the mTYG media resulted in a 14.5-fold increase in the expression of BT4295, and removing it from TYG media resulted in a 4-fold increase (Fig. 8B). This finding again shows a direct correlation between the level of BT4295 protein expression and the ability to stimulate BθOM T cells, providing proof that glucose is acting as a repressor of BT4295 expression. From these findings, we conclude that, in the presence of glucose, *B. theta* shuts down the expression of the BT4294-4300 PUL, thereby reducing production of the BT4295 antigen.

Dietary glucose decreases the stimulation of BθOM T cells in vivo

We next determined whether exogenous glucose affected the ability of BθOM T cells to be stimulated in vivo by decreasing BT4295 expression. We added 30% glucose to the drinking water of recipient mice and maintained them on the standard chow throughout the course of the experiment. The addition of 30% glucose to the drinking water had no effect on *B. theta* colonization levels (fig. S8A). The number of BθOM T cells in the colon and cdLN markedly decreased in the recipient mice fed 30% glucose drinking water (Fig. 8C). Although there was no difference in T_{regs} (fig. S8B), the number of activated BθOM T cells was also decreased (Fig. 8D). Thus, with a high-glucose diet, BT4295 antigen expression is decreased, resulting in weaker stimulation of the BθOM T cells. This finding establishes that diet can affect the expression of a specific symbiont antigen and modulate a $CD4^+$ T cell response in vivo.

DISCUSSION

We developed a symbiont-specific T cell model to study how diet could affect the interactions between a symbiont and the host immune system. We show that BθOM T cells respond to *B. theta* and OMVs but not to other *Bacteroides* family members. Next, we identified BT4295, a SusE/F homolog, as the BθOM T antigen. Transfer of BθOM T cells into *B. theta*–colonized *Rag1*$^{-/-}$ mice showed that antigen-specific T cells differentiate into T_{regs} and T_{effs}. Upon depletion of BθOM T_{regs}, the BθOM T_{effs} cause colitis. We show that the expression of BT4295 can be altered by glycans, salts, and glucose. A high-glucose diet reduced activation of the BθOM T cells, making BT4295 a nutrient-sensitive antigen able to alter T cell responses to microbes. This study definitively shows that diet can play a role in altering antigen expression thereby affecting immune responses.

TCR transgenic models have been previously developed to study antigen-specific responses to gut microbes. T cells specific for segmented filamentous bacteria (SFB) in the small intestine have revealed how symbiotic microbes contribute to driving organ-specific autoimmunity (*23*). The CBir1 TCR transgenic mice are widely used to study antigen-specific microbial interactions (*21*); however, CBir1 T cells do not recognize their antigen during homeostasis despite the abundance of microbial antigen in the lumen (*38*). More recently, *Helicobacter* species–specific transgenic T cells were shown to respond differently during homeostasis and mucosal injury/inflammation (*19*, *29*). In all of these cases, microbial antigens were not shown to cross the epithelial barrier except in the context of inflammation. Therefore, we developed a symbiont-specific T cell that responds to *B. theta* and OMVs, a relevant source of antigen that crosses the colonic epithelium and interacts with the host immune system during homeostasis (*25*, *39*).

Although our study focused on a single T cell and its cognate antigen, this approach is likely relevant because of the concept of immunodominance. Despite a theoretically large number of potential microbial epitopes, which can be recognized by $CD4^+$ T cells, the immune system generally focuses on a few immunodominant epitopes. As one example, the $CD4^+$ T cell response in mice to SFB focuses on two dominant antigens of this microbe (*23*). We propose that the TCR we identified in this study may be specific for a dominant *B. theta* antigen.

Our data directly show the conversion of a naive *B. theta*–specific T cell into T_{regs}. Using the diphtheria toxin receptor (DTR) system, we deplete *B. theta*–specific T_{regs} and show that, in the absence of these cells, symbiont-specific T cells cause colitis. To determine the mechanism of T_{reg} induction, we identified the antigen driving T cell activation. Previous reports on *B. fragilis* identified capsular polysaccharides on OMVs that induce T_{regs} (*40*), suggesting that bacterially derived polysaccharides have immunomodulatory effects on the host immune system. Our study extends the types of *Bacteroides* antigens that can participate in T cell development, including induction of T_{regs}.

One potential factor we have not controlled for is a direct effect of glucose on T cells. There is significant literature showing that glucose enhances T cell responses (*31*, *41*, *42*). To our knowledge, there are no reported studies showing that increased glucose in vivo would decrease T cell responses or homeostatic proliferation. Although we cannot definitively rule out that increased glucose in vivo was directly inhibiting BθOM T cells, the literature supports our conclusion that increased dietary glucose caused a decrease in T cell proliferation due to a direct effect on BT4295 protein expression.

Inflammatory bowel disease (IBD) involves a potentially definable number of chronically activated T cells and microbial antigen specificities. We now show that specific TCR/cognate antigen pairs can be modulated by altering dietary components to affect gene expression of such a key microbial antigen. Future work developing additional TCR/antigen systems from other symbionts, including those that are enriched in patients with IBD, will be valuable to test whether this paradigm established with *B. theta* can be extended to other key microbial antigens. If glucose repression or salt stimulation of dominant microbial antigens is widespread, then such dietary manipulations may become effective for therapy.

MATERIALS AND METHODS

Study design

The objective of this study was to generate a *B. theta*–specific T cell system (BθOM T cells) to identify the interactions between the immune system and an antigen expressed on a highly prevalent colonic symbiont and determine the role that diet plays in altering those interactions. We designed and performed experiments in cellular immunology, protein biochemistry, and mass spectrometry. The number of independent experiments is outlined in the figure legends.

Mice

All experimental procedures were performed under approval by Washington University's Animal Studies Committee. Mice were housed in an enhanced specific pathogen–free facility. BθOM transgenic mice on the $Rag1^{-/-}$ background were maintained by breeding to a non-transgenic $Rag1^{-/-}$ mouse. BθOM-FoxP3-DTR mice were generated by breeding BθOM transgenic mice with FoxP3-DTR mice (*30*).

Generation of the BθOM transgenic mouse

B. theta was grown to confluence and washed with PBS. C57BL/6J mice were immunized subcutaneously in the rear footpads with *B. theta* mixed with incomplete Freund's adjuvant (IFA; Difco) in a 1:1 ratio. One week later, draining popliteal lymph nodes were harvested and stimulated in vitro with *B. theta* for 3 days. Stimulated T cells were fused following a standard protocol. Hybridomas were selected for responsiveness to *B. theta* presented by IFN-γ–stimulated BMDMs. The BθOM clone was selected for further analysis, and its TCR genes were sequenced and cloned into TCR expression vectors (*43*). TCRα and TCRβ constructs were co-injected into C57BL/6J pronuclei in the Washington University Department of Pathology and Immunology's Transgenic Core Facility. Transgenic mice were identified by polymerase chain reaction (PCR) amplification of the Vα1 and Vβ12 transgenes from tail DNA (Vα1 forward primer GTTTCCAAGCAGGTGTGAGGAG and reverse primer CAAAACGTACCAGGGCTTACC; Vβ12 forward primer CTTCTCTTCTAGGTGATGCTG and reverse primer CCCAGCTCACCGAGAACAGTC).

Antibodies and reagents

The following reagents were purchased: CD62L (MEL-14) and CD45.1 (A20) (BD Biosciences); CD4 (GK1.5), CD69 (H1.2F3), CD45.1 (A20), CD44 (IM7), CD25 (PC61), CD45.2 (104), CD25 (PC61), Vβ12 (MRII-I), and Mouse $T_H1/T_H2/T_H17$ Cytometric Bead Array Kit (BioLegend); CD25 (eBio3C7), CD4 (RM4-5), FoxP3 (FJK-16 s), IFN-γ (XMG1.2), and IL-17A (TC11-18H10.1) (eBiosciences); CellTrace CFSE Cell Proliferation Kit and LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Life Technologies); deoxyribonuclease 1 from bovine pancreas grade II (Roche); and collagenase from *Clostridium histolyticum* (Sigma). Homemade cocktail antibodies for negative selection of $CD4^+$ T cells were purchased: anti-mouse Ter-119, CD11c (clone N418), CD11b (M1/70), CD8α (53-6.7), CD19 (1D3), and CD45R/B220 (RA3-6B2) (Tombo); CD49b (DX5) and CD24 (M1/69) (BioLegend); anti-biotin microbeads (Miltenyi Biotec).

Media recipes

TYG medium

The following components of TYG medium were purchased: tryptone (10 g/liter) and yeast extract (5 g/liter) (BD Bacto); D-glucose (4 g/liter), 100 mM KH_2PO_4, 8.5 mM $(NH_2)_4SO_4$, 15 mM NaCl, 10 μM vitamin K_3, 2.63 μM $FeSO_4 \cdot 7H_2O$, 0.1 mM $MgCl_2$, 1.9 μM hematin, 0.2 mM L-histidine, 3.69 nM vitamin B_{12}, and 413 μM L-cysteine (Sigma); 7.2 μM $CaCl_2 \cdot 2H_2O$ (Mallinckrodt).

mTYG medium

The following components of mTYG medium were purchased: tryptone (20 g/liter) and yeast extract (10 g/liter) (BD Bacto); D-glucose (5 g/liter), 8.25 mM L-cysteine, 78 μM $MgSO_4 \cdot 7H_2O$, 294 μM KH_2PO_4, 230 μM K_2HPO_4, 1.4 mM NaCl, 7.9 μM hemin (hematin), 4 μM resazurin, and 24 μM $NaHCO_3$ (Sigma); 68 μM $CaCl_2 \cdot 2H_2O$ (Mallinckrodt).

Preparation of OMVs

B. theta OMVs were purified with multiple rounds of centrifugation and filtering (*25*).

Functional in vitro macrophage T cell assay

BMDM was stimulated with IFN-γ at 2000 U/ml in I-10 medium [Iscove's Modified Dulbecco's Medium (IMDM) 10% fetal bovine serum, glutamine, and gentamicin] and plated on a 96-well plate at 1×10^5 cells per well. The cells were washed with PBS 24 hours later

and kept in 100 μl of fresh I-10 medium without IFN-γ for another 24 hours. A total of 5×10^5 splenocytes or 1×10^5 isolated BθOM $CD4^+$ T cells were added per well in 50 μl with 50 μl of half log dilutions of Bacteroidaceae strains and OMV. Bacteroidetes were grown in a 5-ml TYG or mTYG culture at 37°C overnight to mid-log phase. Cultures were washed twice with PBS and resuspended in medium before adding to the assay. Twenty-four hours later, the supernatant containing the T cells was transferred to a fresh 96-well plate and spun down at 1200 rpm. The cells were washed with fluorescence-activated cell sorting buffer and stained for CD69 expression.

In vivo experiments

Bacterial stocks

Bacteroidetes were grown anaerobically from single isolates in standing culture in TYG at 37°C for 24 hours (*33*). Each culture was concentrated by centrifugation, mixed with sterile, prereduced PBS and glycerol to a final concentration of 20% glycerol, and frozen at −80°C in single-use aliquots.

Gavage

Rag1$^{-/-}$ mice were placed on antibiotics at 3 to 4 weeks of age for 3 to 4 weeks. Antibiotic treatment consisted of ciprofloxacin (0.66 mg/ml), metronidazole (2.5 mg/ml; Sigma), and sugar-sweetened grape Kool-Aid Mix (20 mg/ml; Kraft Foods) in the drinking water (*44*). Mice were gavaged with 100 μl of antibiotic water on the first 2 days and the last 2 days of the 3- to 4-week duration. For the bulk of the experiments, mice were taken off antibiotic water and given Kool-Aid. For the in vivo glucose experiments, mice were taken off antibiotic water and given water or 30% glucose water. Two days later, mice were gavaged with 100 μl of *B. theta* strains at a concentration of 1×10^8 colony-forming units/ml. Fecal pellets were obtained on days 0, 4, and 7 to determine colonization.

BθOM T cell transfer

Three days after gavage, *Rag1*$^{-/-}$ mice were injected with BθOM T cells isolated from the peripheral lymph nodes (axillary, brachial, and inguinal), mLNs, and spleen. Cells were enriched by negative selection using a homemade cocktail of antibodies (see reagents) and sorted for $CD4^+CD44^{lo}CD62L^{hi}CD25^-$ T cells. Cells (1×10^5 to 2×10^5) were injected retrorbitally.

Lamina propria dissociation

Seven days after T cell transfer, mice were euthanized, and leukocytes were isolated from the lamina propria following the Lamina Propria Dissociation Kit protocol published by Miltenyi Biotec.

Peripheral tissue processing

The cdLN and spleen were removed and processed using frosted microscope slides (Thermo Fisher Scientific). Samples were filtered through a 70-μm filter.

DT depletion of BθOM FoxP3$^+$ T_{regs}

T_{reg} depletion

Antibiotic treated *Rag1*$^{-/-}$ mice were gavaged with *B. theta* and injected with enriched and sorted 1×10^5 BθOM-FoxP3-DTR or BθOM T cells. Intraperitoneal injections of DT (10 μg/kg) were performed on days 9, 11, and 13 after gavage. Depletion was confirmed by staining for T_{regs} on day 21 after gavage in mLNs and spleen.

Cytokines

On day 21 after gavage, 5×10^4 mLNs and 2×10^6 splenocytes were stimulated with phorbol 12-myristate 13-acetate (PMA; 50 ng/ml) and ionomycin (500 ng/ml) for 5 hours at 37°C. $T_H1/T_H2/T_H17$ cytokines were quantified in the supernatant using the BD Cytometric Bead Array following the manufacturer's instructions. Supernatants from splenocyte samples were diluted 1:2.

T cell differentiation

On day 24 after gavage, cells isolated from the colon lamina propria and mLN were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) for 1 hour at 37°C, Brefeldin A was added (5 μg/ml), and the cells were stimulated for four additional hours at 37°C. T_H1 and T_H17 cells were identified by intracellular staining with IFN-γ and IL-17A antibodies.

Tissue harvest, fixation, and preparation for histology

Ceca and colons were fixed in methacarn fixative for 12 to 16 hours at 24°C. Samples were washed two times with 100% methanol for 30 min, followed by 100% ethanol for 20 min (two times), and then stored in 70% ethanol. Five-micrometer sections were stained with hematoxylin and eosin (H&E). Representative images of cecal histology were taken with an Olympus BX51 microscope. Blinded microscopic analysis for mitotic figures using H&E-stained histologic sections was performed at 20× magnification on well-oriented crypts as previously described (*44*).

Fecal bacterial DNA extraction and quantitative PCR amplification

Fecal bacterial DNA extraction and quantitative PCR amplification were performed according to a previously published protocol (*25*, *45*).

T cell Western assay

B. theta OMV antigens were separated using a T cell Western blot assay as described (*46*). Briefly, 500 μg of OMVs was separated on a 10% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) gel on both the left and right sides of the gel with molecular weight standards on both sides. For the left side, the proteins were transferred to nitrocellulose (each lane cut into 20 strips), dissolved in dimethyl sulfoxide, and precipitated with sodium carbonate/sodium bicarbonate. The nitrocellulose particles from each strip were tested for their ability to stimulate BθOM T cells using BMDM as antigen presenting cells (APCs). The corresponding position of the active fraction on the right side of the SDS-PAGE gel was further analyzed by mass spectrometry.

Proteomic analysis of OMVs

Proteomic analysis of the corresponding T cell stimulatory SDS-PAGE fraction of OMVs from TYG-grown *B. theta* was performed using standard procedures at MS Bioworks (Ann Arbor, MI). Briefly, the gel slices were digested with trypsin and analyzed by nano liquid chromatography–tandem mass spectrometry with a Waters NanoAcquity HPLC system interfaced to a Thermo Fisher Q Exactive. The data were searched using Mascot against the UniProt *B. theta* reference proteome. Mascot DAT files were parsed into Scaffold for validation, filtering, and creation of a nonredundant list per sample, requiring at least two unique peptides per protein.

B. thetaiotaomicron transposon mutagenesis library and screen

Transposon mutagenesis of *B. theta* was performed as described previously (*32*). Briefly, mutagenesis was carried out on an acapsular *B. theta* strain (ΔCPS) lacking all capsular polysaccharide loci, which was previously characterized (*37*). Here, we used the pSAM_Bt vector containing *mariner* transposon and an *ermG* cassette. S17 *E. coli*

was used to deliver the vector through conjugative transfer into *B. theta*. DNA isolation from selected mutants was performed using the Qiagen DNeasy Blood and Tissue Kit. Two-round PCR was performed to identify the transposon insertion site with the following conditions: round 1; 1 cycle at 95°C (3 min); 5 cycles at 95°C (30 s), 30°C (30 s), and 72°C (45 s); 32 cycles at 95°C (30 s), 55°C (30 s), and 72°C (45 s). The PCR reactions from step 1 were purified using the Qiagen PCR Purification Kit, and 100 to 200 ng of product were used as a template for round 2; 1 cycle at 95°C (3 min); 35 cycles at 95°C (30 s), 55°C (30 s), 72°C (45 s). Reactions from round 2 were run on a 2% agarose-Tris-Borate-EDTA gel, and bands were extracted using the Qiagen Gel Extraction Kit. These products were then sequenced using the primers previously described (*32*).

The library was frozen in 96-well plates. The plates were thawed and spun down, and the medium was removed, washed once in 200 μl of PBS, and then suspended in 100 μl of complete medium. Ten microliters of each was screened using the in vitro macrophage T cell assay during the primary screen, and hits were retested in duplicate for conformation before sequencing.

Generation of the BT4295 mutant

BT4295 gene deletion and amino acid substitutions within this gene were done using allelic exchange as described previously (*47*). Briefly, all manipulations were done in a Δ*tdk* strain background of *B. theta* using the pExchange-tdk vector (*48*), and primers are listed in table S2. All *Bacteroides* strains and mutants were grown in TYG medium or brain-heart infusion agar with 10% horse blood added. The following antibiotics were used as needed: gentamicin (200 μg/ml), erythromycin (25 μg/ml), and 5-fluoro-2′-deoxyuridine (200 μg/ml).

Generation of the BT4295 T->V mutant

Construction of the T547V mutation was done using site-directed mutagenesis via overlapping PCR. Forward and reverse primers were synthesized containing the desired mutation, and outside primers were constructed to contain the entire *BT4295* gene. Once a verified construct was sequenced as containing the mutation, we followed a similar strategy to construct the deletion mutants (e.g., 4295 or SPdeletion). *E. coli* containing the T547V construct was mated with the BT4295 deletion strain, therefore complementing the *BT4295* gene back, but with a T547V mutation so that it no longer stimulated T cells.

Expression of BT4295 and BT4298 in *E. coli*

To express BT4295 and BT4298 in *E. coli*, we used the Lucigen Expresso T7 Cloning and Expression System and followed the manufacturer's protocol. Briefly, we expressed BT4295 and BT4298 in the pETite N-His Kan vector and designed oligonucleotides for cloning full-length or partial proteins listed in table S2.

Sequence-confirmed clones of each were transformed into BL21(DE3) *E. coli* and grown overnight at 37°C with shaking. Fresh 2-ml cultures were inoculated and grown to an OD_{600} (optical density at 600 nm) of 0.5, induced with 1 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) and grown for 5 hours at 37°C with shaking, harvested by centrifugation, washed once with PBS, and suspended in 1 ml of PBS. Samples were heat-inactivated for 20 min at 95°C and then stored at 4°C until use.

Production of recombinant BT4295

BT4295 was expressed in Pet-ite expression vector by cloning the sequence distal to the SPII cleavage motif and including a 5′6 His tag using the oligos CATCATCACCACCATCACTCGCCCGATTACGAAACCGAGTT (forward) and GTGGCGGCCGCTCTATTATATACTGCAGTTAAATGCCTAG (reverse) (*49*). The construct was verified by sequencing and expressed in the *E. coli* strain BL21(DE3). Bacteria were grown at 37°C until mid-log phase growth was reached. The culture was induced with 1 mM IPTG and grown overnight at 19°C. Cells were collected by centrifugation, lysed [50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, lysozyme (1 mg/ml; HEL), and protease inhibitors at pH value of 8.0] for 30 min on ice, sonicated, and centrifuged to remove insoluble material. Supernatants were passed over a Qiagen NiNTA column, washed, and eluted in 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, and protease inhibitors at pH value of 8.0. Eluted material was buffer-exchanged into PBS with an Amicon Ultra 15 10-kDa concentrator to 1 to 2 ml of the final volume and quantified by absorbance at 280 nm (A_{280}).

Generation of monoclonal antibodies against BT4295

C57BL/6J mice were immunized subcutaneously with 100 μg of recombinant protein (rBT4295) emulsified in complete Freund's adjuvant and boosted twice with 100 μg of rBT4295 in IFA every 4 weeks, followed by an intravenous (IV) boost of 50 μg rBT4295 3 days before harvest. Splenic B cells were fused with P3Ag8.6.5.3 myeloma cells to create hybridomas. Hybridomas were screened by ELISA against rBT4295, and positives were screened against whole *B. theta* or OMV preparations to confirm specificity. Two clones (ERC-11 and 4E9) were selected for further characterization. They were subcloned by limit dilution, and both antibodies isotyped as IgG2b,κ. The antibodies were purified from culture supernatants on a Protein A–Sepharose column. Purified 4E9 was biotinylated using the Pierce Ez-Link Sulfo-NHS-SS-Biotin reagent following the manufacturer's protocol.

Quantitative ELISA for BT4295

BT4295 protein levels in *B. theta* samples were determined using a quantitative ELISA assay. Samples were obtained from equivalent numbers of *B. theta* from OD_{600}-measured cultures. Bacteria were lysed in 100 mM CHAPS detergent (Sigma) and incubated with agitation for 1 hour at room temperature (RT). Insoluble material was removed by centrifugation, and samples were stored at 4°C. Purified anti-BT4295 antibody, ERC11, was coated on an Immulon 2 ELISA plate overnight in carbonate coating buffer [5 μg/ml (pH value of 9.6)] at 4°C. Plates were washed and blocked with buffer (PBS with 0.5% bovine serum albumin and 0.1% Tween 20) for 1 hour at RT. Plates were washed and samples were added for 2 hours at RT, washed again, and then, the anti-BT4295 antibody biotin-4E9 (5 μg/ml) was added for 1.5 hours at RT. Plates were washed again, and 1:5000 dilution of streptavidin horseradish peroxidase (SouthernBiotech) was added for 1 hour at RT. Plates were washed and developed with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to completion, and A_{405} was determined. Unknown sample concentrations were quantitated by comparison to a standard curve of rBT4295 performed in the same ELISA using GraphPad Prism software.

Statistical analysis

Differences between two groups were evaluated using Student's *t* test (or Mann-Whitney test, for non-normally distributed data), and those among more than two groups were evaluated using analysis of variance (ANOVA) with Tukey's multiple comparisons test (or Kruskal-Wallis with Dunn's posttest for non-normally distributed

data) using GraphPad Prism software. *P* values of less than 0.05 were considered to be significant. Data are summarized as means ± SEM.

SUPPLEMENTARY MATERIALS

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Fig. S1. Sorting strategy and *B. theta* colonization for in vivo BθOM T cell transfer experiments.
Fig. S2. BθOM T cells do not cause weight loss in *B. theta*–colonized mice.
Fig. S3. Cytokines not altered by BθOM T_{reg} depletion.
Fig. S4. BθOM T cells primarily differentiate into T_H1 cells in vivo in the colon lamina propria and mLN.
Fig. S5. Identification of the epitope recognized by BθOM T cells.
Fig. S6. BθOM T cells recognize $BT4295_{(541–554)}$ and schematic of the BT4295 PUL.
Fig. S7. The effect of various nutrients on BθOM T cell activation.
Fig. S8. The addition of 30% glucose to the drinking water has no effect on *B. theta* colonization or T_{reg} differentiation.
Table S1. Composition of TYG medium versus mTYG medium.
Table S2. BT4295 and BT4298 primers.
Table S3. Raw data.

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Diet modulates colonic T cell responses by regulating the expression of a *Bacteroides thetaiotaomicron* antigen

Marta M. Wegorzewska, Robert W. P. Glowacki, Samantha A. Hsieh, David L. Donermeyer, Christina A. Hickey, Stephen C. Horvath, Eric C. Martens, Thaddeus S. Stappenbeck and Paul M. Allen

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Dietary modulation of T cell immunity

Commensal intestinal bacteria respond to dietary changes by modifying gene expression, leading to shifts in the levels of bacterial antigens encountered by the intestinal immune system. Wegorzewska *et al.* developed a mouse model system to investigate whether $CD4^+$ T cell recognition of protein antigens of the gut symbiont *Bacteroides thetaiotaomicron* is subject to dietary modulation. TCR transgenic T cells for an outer membrane vesicle protein differentiated into both regulatory and effector T cells, with colitis emerging after selective regulatory T cell depletion. Dietary glucose was observed to strongly repress the T cell–detected antigen. These findings suggest that dietary modifications that reduce expression of immunodominant antigens targeted by T cells could help ameliorate some forms of human inflammatory bowel disease.

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