A CD8$^{+}$/CD103$^{\text{high}}$ T Cell Subset Regulates TNF-Mediated Chronic Murine Ileitis

Johnson Ho, Courtney C. Kurtz, Makoto Naganuma, Peter B. Ernst, Fabio Cominelli and Jesús Rivera-Nieves

*J Immunol* 2008; 180:2573-2580; doi: 10.4049/jimmunol.180.4.2573

http://www.jimmunol.org/content/180/4/2573

Why *The JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

### References

This article cites 49 articles, 26 of which you can access for free at:
http://www.jimmunol.org/content/180/4/2573.full#ref-list-1

### Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

### Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

### Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
A CD8+/CD103\textsuperscript{high} T Cell Subset Regulates TNF-Mediated Chronic Murine Ileitis\textsuperscript{1}

Johnson Ho,* Courtney C. Kurtz,* Makoto Naganuma,* Peter B. Ernst,* Fabio Cominelli,* and Jesús Rivera-Nieves\textsuperscript{2,}\textsuperscript{*}\textsuperscript{†}

Recruitment of lymphocytes to sites of inflammation requires the sequential engagement of adhesion molecules and chemokine receptors. Of these, the lectin-like molecule CD44 has been particularly implicated in inflammatory trafficking. Using a TNF-driven model of chronic ileitis (i.e., B6.129P-Tnf\textsuperscript{ARE} mice) that recapitulates many features of Crohn’s disease, we demonstrate dynamic changes in the expression and functional state of CD44 on CD8\textsuperscript{+} T cells. These cells coexpress CD44 and L-selectin, giving them a surface phenotype similar to that of central memory T cells. Yet functionally they exhibit the phenotype of effector T cells, because they produce IFN-\(\gamma\). Unexpectedly, depletion of the CD8\textsuperscript{+} population had no effect on the severity of ileitis. Further analyses showed a second CD8\textsuperscript{+} population that lacked CD44, but expressed CD103, produced TGF-\(\beta\), inhibited the proliferation of CD4\textsuperscript{+} in vitro, and attenuated adoptively transferred ileitis in vivo, most likely counteracting the proinflammatory role of the CD44\textsuperscript{high} subset. Collectively, these data suggest that the presence or absence of CD44 and CD103 on the CD8\textsuperscript{+} lymphocyte surface defines functionally distinct subsets of CD8\textsuperscript{+} T cells in vivo. These inflammation-driven populations exert distinct roles during the development of chronic ileitis, and influence the balance of effector and regulatory functions in the chronically inflamed small intestine. The Journal of Immunology, 2008, 180: 2573–2580.

*Digestive Health Center of Excellence, University of Virginia Health Sciences Center, Charlottesville, VA 22908; and *Mucosal Inflammation Program, Division of Gastroenterology, University of Colorado Health Sciences Center, Denver, CO 80206

Received for publication March 7, 2007. Accepted for publication November 30, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by U.S. Public Health Service/National Institutes of Health Grants K08DK067254 and R01DK073280 (to J.R.-N.), RO1DK069880 (to P.B.E.), and R37DK2191-16 (to F.C.), and by the Morphology and Immunology Cores of the University of Virginia Silvio Conte Digestive Health Research Center (DK56703).

2 Address correspondence and reprint requests to Dr. Jesús Rivera-Nieves, Mucosal Inflammation Program, Division of Gastroenterology, University of Colorado Health Sciences Center, BRB, Room 742A, 4200 East 9th Avenue, Denver, CO 80206, E-mail address: jesus.rivera-nieves@uchsc.edu

3 Abbreviations used in this paper: IBID, inflammatory bowel disease; ARE, AU-rich region; CD, Crohn’s disease; FSC, forward scatter; MFI, mean fluorescence intensity; MLN, mesenteric lymph node; SSC, side scatter; \(T_{\text{reg}}\), regulatory T cell; WT, wild type.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
this latter population counteracts the proinflammatory role played by the CD8⁺/CD44\(\text{high}\) subset. Thus, the CD8⁺ population is not homogeneous, but rather composed of two discrete populations that have opposing effector and regulatory activities.

Materials and Methods

Mice

The B6.129P-\(\text{Tg}^{\text{ARE}}\) strain was generated by over 20 generations of continuous backcrosses between \(\text{Tg}^{\text{ARE}}\) mice on mixed genetic background (i.e., C57BL6 and 129S6, generated as previously described (9)) to C57BL6/6 mice. Genetic analysis of this B6.129P-\(\text{Tg}^{\text{ARE}}\) congenic strain showed no detectable 129S6 loci, except for those flanking the TNF locus, which screened for 40 informative microsatellite loci. The maximal interval of 129S6-derived DNA flanking the TNF gene was <10.7 Mb (~5 cm), which was less than that expected for most nongenotypically selected congenic strains. Mice were kept under specific pathogen-free conditions. All progeny generated through this breeding strategy and used for these experiments were either heterozygous (\(\text{Tg}^{\text{ARE}}\)) or carried no mutated alleles (wild type (WT)). The latter were used as the noninflamed controls. CD44+, integrin \(\beta_7\), CD103-, and L-selectin-deficient mice on the C57BL6/J background were obtained from The Jackson Laboratory. Fetal samples from all mouse strains were negative for \textit{Helicobacter hepaticus}, \textit{Helicobacter bilis}, and other murine \textit{Helicobacter} species, and for protozoa and helminthes. All animals were handled according to procedures approved by the institutional committee for animal use of the Universities of Virginia and Colorado.

Tissue collection and histological analyses

Mice were anesthetized and euthanized at the times required by the experimental design. The distal ilea (10 cm) were resected, rinsed of debris, and oriented from distal to proximal over a glass slide using HistoGel (Richard-Allan Scientific) to prevent recoiling of intestinal tissue. Tissues were fixed in 10% buffered formalin or Bouin’s, embedded in paraffin, and sectioned to 5-μm sections, and stained with H&E. Histological assessment of ileal inflammation was performed by a single pathologist in a blinded fashion, using a standardized semiqualitative scoring system, as described previously (10).

Lymphocyte isolation

MLN and spleens were aseptically removed at the time of necropsy. Single-cell suspensions were obtained by gently pressing the MLN or spleen against a 100-μm cell strainer. Splenic RBC were lysed by incubating for 15 min in ammonium chloride lysing reagent (BD Pharm Lyse; BD Biosciences).

Flow cytometry

Cells from the indicated compartments were incubated with fluorescent rat anti-mouse Abs, including against the following: mouse CD8\(\alpha\) (53-6.7) for gating of lymphocyte populations, and CD44 (IM7), L-selectin (MEL-14), CD103 (M293; BD Biosciences), or their respective isotype controls for further subset evaluation. Additional controls included cells isolated from mice deficient for CD44, L-selectin, CD103, and integrin \(\beta_7\). Cells were washed and fixed with 2% paraformaldehyde, and four- to five-color analyses were performed using the FACSCompuler system (BD Immunocytometry Systems; modified by Cytek Development). Further analyses were performed using FLOWJo software (Tree Star).

Intracellular staining for IFN-γ

Staining was performed using the BD Cytofix/Cytoperm kit, as per the manufacturer’s instructions (BD Biosciences). Cells were fixed with 2% paraformaldehyde, and four-color analyses were performed using the FACSCompuler system (BD Immunocytometry Systems). Further analysis was performed using FLOWJo software (Tree Star).

T cell culture and cytokine assay

Lymphocytes were cultured in anti-CD3e-coated (clone 145-2C11, 5 μg/ml; BD Biosciences), 96-well round-bottom plates at a density of 5\(^{10^4}\) cells/ml in complete medium (RPMI 1640 with 10% FBS, 2 mM l-glutamine, and 1% penicillin/streptomycin). Supernatants were collected after 48 h and stored at ~70°C. A bead-based multiplex immunoassay (Upstate Biotechnology) was used to determine cytokine concentrations from cell culture supernatants. Bound cytokines were detected using a LumineX 100 array reader (Bio-Rad), and results were analyzed using the BioPlex Manager bead array software (Bio-Rad).

Depletion of CD8⁺ T cells

Eight-week-old TNF\(\Delta\)ARE mice were administered i.p. doses every other day of mAbs (100 μg each) against CD8\(\alpha\) (clone 2.43, rat IgG2b; American Type Culture Collection) or the corresponding isotype control mAb. Lymphocytes isolated from peripheral blood at treatment day 6 were enriched by positive or negative selection with anti-mouse CD4, CD8, or CD25, or mixture microbeads (Miltenyi Biotec). All selections were confirmed by flow cytometry from peripheral blood at treatment day 6 and from MLN and spleen at day 12. Intestinal tissues were collected at day 12, and the severity of ileitis was assessed, as previously described (10).

In vitro T cell proliferation assay

Splenic APCs (10\(^{5}\)/well) were irradiated (3000 rad) and cocultured for 96 h with soluble anti-CD3 (1 μg/ml) and CD4⁺ T cells from TNF\(\Delta\)ARE mice alone (10\(^{5}\)/well) or with TNF\(\Delta\)ARE mice following the strategy delineated for the in vivo transfer (see next paragraph) and as illustrated in the top two panels of Fig. 8. Each condition was assayed in triplicate. Incorporation of [\(\text{\text{[H]}\text{thymidine}}\)1 μCi/well; MP Biomedicals) during the last 24 h of culture was measured with a Harvester 96 (Tomtec) and a 1450 Microbeta scintillation counter (PerkinElmer).

Induction of ileitis by transfer of T cell subsets to RAG⁻/− mice

CD4⁺ and CD8⁺ T cells from the MLN and spleen of TNF\(\Delta\)ARE or WT mice were enriched by positive or negative selection with anti-mouse CD4, CD8, or CD25, or mixture microbeads (Miltenyi Biotec). All selections were performed, as per the manufacturer’s instructions. T cell fractions were determined to be >97% pure by flow cytometry. The CD8⁺ population was stained with PE-labeled rat anti-mouse CD103 (M290) and allophycocyanin-labeled rat anti-mouse CD44 (IM7), and then separated into CD103\(\text{high}\)/CD44\(\text{low}\) and CD103\(\text{low}\)/CD44\(\text{high}\) subsets using a FACS-Vantage SE Diva system (BD Biosciences). Identical subsets used for in vitro proliferation assays were also adoptively transferred into RAG⁻/− mice, as illustrated in the top panels of Fig. 8. Cells were counted, washed, and suspended in 500 μl of PBS for injection into 6-wk-old female RAG⁻/− recipients at doses of 5 × 10\(^5\) for CD4⁺ cells and 2 × 10\(^5\) for CD8⁺. The ilea and colon of RAG⁻/− recipients were harvested 6 wk after transfer, and the severity of inflammation was assessed, as previously described (10).

Statistics

Statistical analyses were performed using two-tailed Student’s t test or two-way ANOVA. Data were expressed as mean ± SEM. Statistical significance was set at \(p < 0.001\) for in vitro proliferation assays and \(p < 0.05\) for all other studies.
controls, in both the spleen (75 vs 35%) and MLN (46 vs 24%).

We examined the surface expression of CD44 on CD8+ T cells compared with WT littermates from four mice per strain at representative density plots were obtained from three experiments using cells from four mice per strain at ≥20 wk of age run in triplicate. Mean hemagglutinin binding ± SEM pooled from three experiments, p < 0.01.

**Results**

The percentage of CD8+ T cells that express high levels of CD44 increased from 4 to ≥20 wk of age in TNFΔARE mice compared with WT littermates

We examined the surface expression of CD44 on CD8+ T cells isolated from the spleen and MLN of TNFΔARE mice (Fig. 1, solid line) during early (4 wk) and late disease (≥20 wk), and compared it with that of age-matched noninflamed C57BL/6J (WT) littermates (gray histograms). Allophycocyanin-labeled isotype-matched Ab (mean fluorescence intensity (MFI) < 104; data not shown) and lymphocytes isolated from CD44-deficient mice (Fig. 1, dotted histograms) were used as controls. CD44-expressing cells were identified in the spleen and MLN of both the control WT and TNFΔARE mice. At 4 wk of age, few CD8 expressed high levels of CD44 in both WT and TNFΔARE mice (±4%). An overall shift to the right from 4 to ≥20 wk of age was observed in WT mice, whereas in TNFΔARE mice at ≥20 wk of age the CD44high subset (MFI ~ 105) doubled in frequency compared with controls, in both the spleen (75 vs 35%) and MLN (46 vs 24%). In addition, the intermediate population virtually disappeared in TNFΔARE mice, with almost complete polarization as CD44high or CD44low/negative. By contrast, in age-matched WT mice, the mean fluorescence intensities shifted to the right, consistent with an age-related overall increase in CD44 expression. Interestingly, when the absolute numbers of CD44high cells were calculated by correcting the percentages of expression against the cellularity of both organs, the largest increase in frequency of CD44-expressing cells in TNFΔARE mice occurs within the MLN, at ≥20 wk of age (WT = 4.1 ± 0.9 × 106 vs TNFΔARE = 12.1 ± 0.6 × 106, p < 0.01), whereas within the spleen the difference did not reach statistical significance, because the cellularity of the spleen decreases during advanced disease (WT = 24.6 ± 4.9 × 106 vs TNFΔARE = 30.5 ± 7.8 × 106).

**Binding of hyaluronate is increased in CD8+ T cells from TNFΔARE mice compared with WT mice**

To determine whether chronic inflammation influenced the activation state of CD44 in vivo, we assayed the ability of freshly isolated CD8+ T cells from mice with or without inflammation to

**FIGURE 2.** Enhanced hyaluronate binding by CD8+ T cells from TNFΔARE mice compared with noninflamed WT littermates. Freshly isolated CD8+ T cells from the indicated organs were incubated with FITC-labeled hyaluronate (HA FITC) at 37°C for 30 min and analyzed by flow cytometry. Preincubation with nonfluorescent hyaluronate was used to test the specificity of binding (data not shown). Cells were gated on FSC, SSC, and CD8+. Representative density plots were obtained from three experiments using cells from four mice per strain at ≥20 wk of age run in triplicate. Mean hemagglutinin binding ± SEM pooled from three experiments, p < 0.01.

**FIGURE 3.** The CD8+/CD44high T cell population coexpresses L-selectin and CD45RB. A and B. Lymphocytes isolated from the indicated lymphoid compartments were incubated with anti-CD8, anti-CD44, anti-CD45RB, and anti-L-selectin mAbs and analyzed by flow cytometry. Representative density plots and mean ± SEM are provided for the indicated subsets of cells gated on FSC, SSC, and CD8, obtained from at least four mice per strain at ≥20 wk of age.

**FIGURE 4.** The proportion of CD8+/CD44high T cells that produce IFN-γ was increased in TNFΔARE mice. Lymphocytes isolated from indicated populations of TNFΔARE mice or WT littermates (WT) were cultured under PMA/ionomycin stimulation and incubated with Abs against CD8, CD44, and IFN-γ, as per manufacturer’s instructions, and then were analyzed by flow cytometry. Cells were gated on FSC, SSC, and CD8 using CD44-deficient lymphocytes for surface staining and isotype Ab for intracellular staining (MFI < 104; data not shown). Representative density plots were obtained from three experiments using three to four mice per strain at ≥20 wk of age and run in duplicate.
The majority of the CD8⁺/CD44<sup>high</sup> T cells coexpress L-selectin

We compared the phenotype of CD8⁺ T cells from the spleen and MLN of TNFARE and WT mice. The majority of the CD8⁺/CD44<sup>high</sup> T cells also expressed high levels of L-selectin in both the spleen (25 ± 7 vs 61 ± 9, p < 0.01) and MLN (22 ± 7 vs 49 ± 8, p < 0.01) of TNFARE mice compared with the levels found in WT littermates (Fig. 3A). Comparable differences were observed in the CD44<sup>high</sup>/CD45RB<sup>high</sup> subset in the spleen (29 ± 9 vs 74 ± 8, p < 0.01) and MLN (18 ± 7 vs 52 ± 10, p < 0.01) of TNFARE mice compared with the levels found in WT littermates (Fig. 3B). Thus, the CD8⁺/CD44<sup>high</sup> population in TNFARE mice exhibited predominantly a central memory-like (i.e., CD44<sup>high</sup>/L-selectin<sup>high</sup>) surface phenotype.

CD8⁺/CD44<sup>high</sup> T cells produce IFN-γ

To aid our understanding of the role played by the CD8⁺/CD44<sup>high</sup> subset in the disease process, we assessed whether they produce the Th1 cytokine IFN-γ. We found that the majority of the IFN-γ-producing cells were CD44<sup>high</sup> in both WT and TNFARE mice. The percentage of IFN-γ-producing cells increased in the MLN (25 vs 58%) and in the spleen (12 vs 28%) of TNFARE mice compared with WT littermates (Fig. 4).

Depletion of the CD8⁺ T cell population did not alter the severity of ileitis in TNFARE mice

To assess whether the CD8⁺ T cell population exerted a predominant pro- or anti-inflammatory role during the maintenance of ileitis in TNFARE mice, we depleted the CD8⁺ T cells using a standard CD8-depleting Ab (clone 2.43) and assessed its effect on the severity of the ileitis. Unexpectedly, this treatment did not affect any of the indices of disease severity in TNFARE mice (active index, isotype NS; chronic index, isotype 3.5 ± 0.7 vs treatment 3.6 ± 0.7, NS; transmural index, isotype 3.3 ± 1.2 vs treatment 3.6 ± 0.7, NS; total index, isotype 15.7 ± 3.8 vs treatment 16.5 ± 1.5, NS; Fig. 5A), despite adequate depletion of CD8⁺ T cells, as confirmed by flow cytometry on peripheral blood at treatment day 6 and at both the MLN and spleen at day 12 (Fig. 5B).

The CD8⁺ population polarized into CD44<sup>high</sup>/CD103<sup>low</sup> and CD44<sup>low</sup>/CD103<sup>high</sup> subsets in TNFARE mice

We subsequently investigated the surface phenotype of the CD44<sup>low/negative</sup> subset in TNFARE mice with advanced disease.
bated with Abs against CD8, CD103, and IFN-γ indicated populations of TNF-ARE mice or WT littermates were incubated with [3H]thymidine and analyzed by flow cytometry. Cells were gated on FSC, SSC, and CD8 using CD103-deficient lymphocytes for surface staining and isotype Ab for intracellular staining. Cells were sorted by FACS as per the strategy illustrated in Fig. 8 (top density plots). This indicates that inflammatory mediators may potentiate the regulatory function of the CD8+/CD103high subset.

Adoptive transfer of CD8+/CD103high T cells from either TNFARE or WT mice attenuates the ileitis that results from adoptive transfer of CD4+ T cells

To confirm that the CD8+/CD103high T cell population plays a regulatory role in vivo, we adoptively transferred CD4+ T cells from TNFARE mice into immunodeficient RAG−/− mice alone or combined with CD8+ T cell subsets (CD8+/CD103high or

FIGURE 8. CD8+/CD103high, but not CD8+/CD44high T cells isolated from TNFARE mice decreased proliferation of CD4+ T cells more efficiently than CD8+/CD103high T cells isolated from WT littermates. A and B, CD4+ T cells from TNFARE mice were isolated magnetically, sorted by flow cytometry as delineated in the top plots, and cocultured with APCs in the presence or absence of CD8+/CD103high or CD8+/CD44high T cells from TNFARE or WT mice. Incorporation of [3H]thymidine was determined, as described in Materials and Methods. Data are presented as mean ± SEM from two independent experiments run in triplicate; *, p < 0.001.

FIGURE 7. CD8+/CD103high T cells from TNFARE mice produce TGF-β, but not IL-10 or IFN-γ. A, CD4+ T cells were isolated from the MLN and spleen of TNFARE mice using magnetic beads and used as positive controls for cytokine production. The CD4+ fraction was then sorted by FACS as per the strategy illustrated in Fig. 8 (top density plots). The CD8+/CD103high and CD4+ fractions were cultured as described, and cytokine concentrations in the supernatants of cultured cells (mean ± SEM from two experiments) pooled from six mice per experiment, run in triplicate, as per Materials and Methods. B, Lymphocytes isolated from the indicated populations of TNFARE mice or WT littermates were incubated with Abs against CD8, CD103, and IFN-γ and analyzed by flow cytometry. Cells were gated on FSC, SSC, and CD8 using CD103-deficient lymphocytes for surface staining and isotype Ab for intracellular staining (MFI < 10; data not shown). Representative density plots were obtained from three experiments using three to four mice per strain at ≥20 wk of age and run in duplicate.

and in age-matched WT littermates. We found that in inflamed mice the CD8+ T cells polarized into two major populations that expressed either CD44 or CD103 with a marked diminution of the intermediates for both markers (Fig. 6, left-hand panels). In addition, we found that the majority of CD44low/CD103low T cells from TNFARE mice are known to produce regulatory cytokines. We assayed the supernatants of sorted CD8+/CD103high that had been cultured for 48 h. These were compared with the supernatants of unfraccionated CD4+ T cells, which contain known regulatory subsets. We found that the CD8+/CD103high produced TGF-β, yet little IL-10 (Fig. 7A). In addition, unlike the CD44high subset, few CD8+/CD103high T cells from the MLN or spleen produced IFN-γ (Fig. 7B). Thus, the overall cytokine profile of the CD8+/CD103high T cell is similar to that of a Th3 regulatory cell.

Fewer CD8+/CD103high isolated from TNFARE than from WT mice were required to decrease proliferation of CD4+ T cells

To assess the role of the inflammation-driven CD8+ T cell subsets in ileitis, we investigated whether CD8+/CD103high cells isolated from TNFARE or WT mice (as per the strategy shown in Fig. 8, top density plots) could affect the proliferation of CD4+ T cells from TNFARE, as has been shown for CD4+/CD103high T cells (11). There was strong proliferation of CD4+ T cells when they were cocultured with APCs alone (bare). Addition of the CD8+/CD103high subset ( ), but not of the CD8+/CD44high subset ( ), significantly reduced proliferation of the CD4+ T cells (p < 0.001). Yet, whereas a CD8+/CD44high/CD103high ratio of 5:1 was sufficient to decrease proliferation when the CD8+/CD103high T cells originated from TNFARE mice (Fig. 8A, bottom left), a ratio of 1:2 was required to achieve significant suppression when the CD8+/CD103high T cells originated from WT mice (Fig. 8A, bottom right). This indicates that inflammatory mediators may potentiate the regulatory function of the CD8+/CD103high subset.

CD8+/CD103high T cells produce TGF-β, but not IL-10 or IFN-γ

We then evaluated the functional phenotype of the CD8+/CD103high subset in TNFARE mice. CD4/CD103high Treg cells are known to produce regulatory cytokines. We assayed the supernatants of sorted CD8+/CD103high that had been cultured for 48 h. These were compared with the supernatants of unfraccionated CD4+ T cells, which contain known regulatory subsets. We found that the CD8+/CD103high produced TGF-β, yet little IL-10 (Fig. 7A). In addition, unlike the CD44high subset, few CD8+/CD103high T cells from the MLN or spleen produced IFN-γ (Fig. 7B). Thus, the overall cytokine profile of the CD8+/CD103high T cell is similar to that of a Th3 regulatory cell.

Fewer CD8+/CD103high isolated from TNFARE than from WT mice were required to decrease proliferation of CD4+ T cells

To assess the role of the inflammation-driven CD8+ T cell subsets in ileitis, we investigated whether CD8+/CD103high cells isolated from TNFARE or WT mice (as per the strategy shown in Fig. 8, top density plots) could affect the proliferation of CD4+ T cells from TNFARE, as has been shown for CD4+/CD103high T cells (11). There was strong proliferation of CD4+ T cells when they were cocultured with APCs alone ( ). Addition of the CD8+/CD103high subset ( ), but not of the CD8+/CD44high subset ( ) significantly reduced proliferation of the CD4+ T cells (p < 0.001). Yet, whereas a CD8+/CD44high/CD103high ratio of 5:1 was sufficient to decrease proliferation when the CD8+/CD103high T cells originated from TNFARE mice (Fig. 8A, bottom left), a ratio of 1:2 was required to achieve significant suppression when the CD8+/CD103high T cells originated from WT mice (Fig. 8A, bottom right). This indicates that inflammatory mediators may potentiate the regulatory function of the CD8+/CD103high subset.

Adoptive transfer of CD8+/CD103high T cells from either TNFARE or WT mice attenuates the ileitis that results from adoptive transfer of CD4+ T cells

To confirm that the CD8+/CD103high T cell population plays a regulatory role in vivo, we adoptively transferred CD4+ T cells from TNFARE mice into immunodeficient RAG−/− mice alone or combined with CD8+ T cell subsets (CD8+/CD103high or

The Journal of Immunology

2577

Downloaded from http://www.jimmunol.org/ by Guest on January 14, 2018
CD8+/CD103high T cells isolated from WT or TNFΔARE mice. Transfer of the CD4+/CD103high (Fig. 9, D and E) but not of the CD8+ T cells (data not shown) induced ileitis in RAG−/− mice. In addition, ileitis was attenuated by cotransfer of CD8+/CD103high T cells isolated from TNFΔARE mice (inflammatory indices for CD4 transfer vs CD4+/ΔARECD8+/CD103high cotransfer, active = 1.5 ± 0.4 vs 0.3 ± 0.3, p < 0.05; chronic = 3.4 ± 0.8 vs 0.6 ± 0.3, p < 0.05; transmural = 0.6 ± 0.2 vs 0.3 ± 0.1, p < 0.05; total = 8.8 ± 1.4 vs 4.5 ± 1.1, p < 0.05). 

By contrast with the data that were obtained in vitro, CD8+/CD103high T cells from WT mice also attenuated ileitis (inflammatory indices for CD4 transfer vs CD4+/WTCD8+/CD103high cotransfer: active = 1.5 ± 0.4 vs 0.4 ± 0.2, p < 0.05; chronic = 3.4 ± 0.8 vs 0.4 ± 0.2, p < 0.05; transmural = 0.6 ± 0.2 vs 0.2 ± 0.1, p < 0.05; total = 8.8 ± 1.4 vs 2.6 ± 1.0, p < 0.05). However, cotransfer of the CD8+/CD44high subset did not attenuate ileitis (active = 3.0 ± 1.4, chronic = 4.0 ± 0.9, transmural = 1.3 ± 0.6, total = 12.3 ± 2.6, NS). These findings confirmed that the CD8+/CD103high T cell subset plays a regulatory role in vivo whether the cells originate from WT or from TNFΔARE mice in this adoptive transfer model of chronic ileitis.

Discussion

A prior study has demonstrated that the CD8+/CD44high T cell population increased in frequency in TNFΔARE mice with established inflammation (9). The current study extends those observations to show that not only the expression of CD44, but also its reactivity with its ligand is enhanced in diseased mice. In addition, we demonstrate that the increased surface expression of CD44 reflects expansion of a central memory-like T cell pool that coexpresses L-selectin, yet different from classic central memory T cells, they are major producers of IFN-γ (12). Most importantly, we describe a counterpart for the CD8+/CD44high population that has not been previously described. This second CD8+ subset lacked CD44, yet expressed CD103 and L-selectin, produced TGF-β, inhibited the proliferation of CD4+ in vitro, and attenuated adoptively transferred ileitis in vivo. Thus, in addition to effectors (9), the CD8+ population also comprises a regulatory subset.

The total surface expression of CD44 does not necessarily correlate with the molecule’s ability to bind hyaluronate (13, 14). Indeed, whereas many cell lines constitutively express active CD44, the functional state of CD44 in vivo is tightly regulated (15, 16). Because CD44-hyaluronate interactions are so ubiquitously relevant for many cellular processes, tight regulation in vivo is not surprising. Inducible conformational modifications control the ability of CD44 to interact with its ligands (16, 17). The increased proportion of T cells that carry CD44 in an activated state in inflamed mice demonstrates that inflammatory mediators affect the activation state of CD44 in vivo and allows CD44-expressing cells to readily interact with its ligands to perpetuate the dysregulated inflammatory recruitment.

β2-microglobulin-deficient TNFΔARE mice express low levels of MHC class I protein on the surface of cells (18) and develop attenuated ileitis compared with TNFΔARE mice (9). This finding supports a critical effector role for the CD8+ population in TNFΔARE mice. Yet, in the current studies, depletion of the CD8+ T cell population did not attenuate established ileitis. To reconcile this apparent discrepancy, we must recognize that the first set of data reflects prevention or possibly a delay of disease onset, whereas the current data show that CD8 depletion has no effect on established disease. A better understanding of the basic mechanisms involved in induction vs maintenance of ileitis would allow us to understand the different results. Alternatively, the relative contribution of Qa-1 and CD1d-mediated processes (also absent in β2-microglobulin-deficient mice) (19–22) must be elucidated, before we can fully understand the mechanisms underlying attenuation of ileitis in the β2-microglobulin-deficient TNFΔARE mice.

While characterizing the surface phenotype of the second subset of CD8+ T cells (i.e., CD44low/positive), we determined that these cells express CD103 and that the CD8+ T cells from mice with ileitis are polarized into two discrete populations (CD44high/CD103low or CD44low/CD103high), with very few cells expressing intermediate levels. The majority of the CD44low/CD103high T cells expressed L-selectin. Expression of L-selectin by these cells may suggest that they exert their suppressive role within secondary lymphoid organs, during the initial stages of T cell activation,
rather than at intestinal effector sites. Regulatory T cell dependency on L-selectin to home to secondary sites has been shown for other regulatory T cell subsets in several models of autoimmunity. For example, cardiac allograft survival was prevented by Ab blockade of L-selectin, which interfered with trafficking of regulatory subsets (23). In the NOD mouse model of diabetes, only Treg that expressed L-selectin were able to delay diabetes transfer (24, 25) and more recently to additionally ameliorate ongoing diabetes (26). In graft-vs-host disease, L-selectinhigh Treg interfere with activation and expansion of graft-vs-host disease effector T cells in secondary lymphoid organs after bone marrow transplantation (27, 28). Venturi et al. (29) formally demonstrated that Treg subsets require L-selectin expression for localization. In addition, an interesting subset of CD8/L-selectinhigh T cells potentially counteracts proinflammatory cytokine overproduction in the elderly (30). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.

Recently, retinoic acid, which mediates the induction of the integrin α6β1 and the small intestinal chemokine receptor CCR9 (35–38), was shown to inhibit IL-6-driven induction of proinflammatory Th17 cells, tilting the balance toward anti-inflammatory activity in the presence of TGF-β (39). Because TGF-β in turn promotes the induction of CD103 (40), these molecules may be functionally closely parallels that of Th3 cells (44) and CD8 T (40) and positively regulates its own production (48). The interplay that home to mucosal surfaces such as the intestine and lung (41). Thus, a common theme emerges in regulatory T cell biology in which L-selectin expression more accurately reflects their ability to home to lymphoid organs rather than their distinct state of activation. In addition, in the chronically inflamed small intestine, there is widespread proliferation of inducible lymphoid follicles and induction of the sulfotransferases (31) responsible for the activity of L-selectin ligands (our unpublished observations). Treg accumulate at these sites, where they may additionally exert regulatory functions (32, 33). Thus, L-selectin expression enables Treg subsets to migrate to both secondary and tertiary sites (34) to control dysregulated immune processes.


