damage-associated molecules, such as HMGB1, heat shock protein and ATP, leads to the release of inflammatory cytokines. IL-1, TNF-α and, to a lesser extent, IL-6 stimulate the HPA axis at the level of vagus afferent nerves, the hypothalamus, the pituitary and the adrenal glands to release GC as well as the SNS to release NA, providing a negative feedback loop to stop inflammation. Cytokines produced in the periphery activate primary afferent nerves, such as the vagus nerves, enter the brain through the areas with a poorly developed blood–brain barrier, such as the circumventricular region or are actively transported. In addition, neurons, glial cells and endothelial cells produce these cytokines in the CNS.[153] In contrast to HPA axis, inflammatory cytokines have negative effects on the HPG axis, leading to the reduction of gonadal functions.[154]

The effects of cytokines on the CNS are not limited to the HPA axis and SNS, but are also involved in behavior induced by sickness including changes in behaviour that occur in ill patients, and even in depression. Systemic or intrathecal administration of IL-1β or TNF-α induces signs of behavior resulting from sickness, such as decreased motor activity, social withdrawal, altered cognition and fatigue. Although administration of IL-6 does not induce these behavioral changes, LPS-induced sickness behaviors are reduced in IL-6$^{-/-}$ mice, suggesting its involvement in these behavioral changes, although the degree is less compared with IL-1β or TNF-α. In contrast, anti-inflammatory cytokines, such as IL-10, attenuate LPS-induced sickness behaviors.[155–158]

Type I interferon, IFN-α and IFN-β, are used for the treatment of hepatitis C and MS, respectively. These cytokines show neuropsychiatric complications, including sleep disorders and depression, which serves as evidence of the cytokine-mediated modulation of neural activities. The potential link between inflammatory cytokines and depression is tryptophan metabolism. Tryptophan is an essential amino acid and is a source of serotonin, as it is metabolized to serotonin and kynurenine. In the latter pathway, tryptophan is metabolized by tryptophan 2,3 dioxygenase (TDO) and indoleamine 2,3 dioxygenase (IDO) to kynurenine, and then metabolized either to 3-hydroxykynurenine or kynurenic acid, an antagonist of NMDA receptors. 3-Hydroxykynurenine is further metabolized to 3-hydroxyanthranilic acid and quinolinic acid, an agonist of NMDA receptors. TDO is primarily located in the liver and is activated by GC, whereas IDO is widely expressed and is activated by inflammatory cytokines and downregulated by IL-4.[153,159] In patients treated with type I IFN, plasma levels of kyurenic acid as well as serotonin are decreased, suggesting that the behavior and depression caused by inflammatory cytokines including IFN might be a result of an alteration in glutamatergic neurotransmission.

Although much of the focus on T cells in a variety of pathogenic conditions, has been on classical immune-mediated inflammation, including infection and autoimmune disorders, in diseases newly related to inflammation, such as ischemia, neurogenerative and psychiatric disorders, a neuroprotective role has emerged as an important task for T cells. The production of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) from T cells,[160] reduced learning capacity in T cell-deficient mice and its restoration by passive T cell transfer[161] and enhanced hippocampal neurogenesis by T cells,[162,163] suggest a fundamental function of T cells in the maintenance of cognitive functions. Anti-inflammatory cytokines, such as IL-4 and TGF-β, are detectable in the CNS, and IL-4 is downregulated in a mouse model of Alzheimer's disease.[164] These cytokines, in addition to BDNF, might contribute to the maintenance of homeostasis of the CNS. Although the precise mechanisms remain elusive, T cells might serve as important players in the maintenance of neuronal integrity.

Future directions

Acute stress responses in the autonomic and peripheral nervous systems amplify local immune responses to eliminate pathogens and other dangerous occurrences. Subsequent to these initial responses, the neuroendocrine and autonomic systems act to inhibit immune responses and terminate inflammation. In contrast, chronically sustained stress induces unusual conditions, such as inadequate secretion of GC, as well as resistance to GC, increased sympathetic tone propelling the RAAS, functional loss of sympathetic nerve fibers at the inflammation site and a local β to α adrenergic shift.[165,166] Further studies to clarify the consequences of stress on chronic inflammatory conditions will provide novel strategies for the control of complex pathogenic conditions including autoimmune diseases, and neurogenerative and psychiatric disorders.

References

1. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol.* 2006; **6**: 318–28.

2. Calogero AE, Gallucci WT, Gole PW, Chrousos GP. Multiple feedback regulatory loops upon rat hypothalamic corticotrophin-releasing hormone secretion. Potential clinical implications. *J Clin Invest.* 1998; **82**: 767–74.
3. Schule R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, et al. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell.* 1990; **62**: 1217–26.
4. Ghosh S, Ma MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol.* 1998; **16**: 225–60.
5. Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. *Annu Rev Immunol.* 2002; **20**: 125–63.
6. Baker C, Richards LJ, Dayan CM, Jessop DS. Corticotropin-releasing hormone immunoreactivity in human T and B cells and macrophages: co-localization with arginine vasopressin. *J Neuroendocrinol.* 2003; **15**: 1070–4.
7. Crofford LJ, Sano H, Karalis K, Webster EL, Goldmuntz EA, Chrousos GP, et al. Local secretion of corticotropin-releasing hormone in the joints of Lewis rats with inflammatory arthritis. *J Clin Invest.* 1992; **90**: 2555–64.
8. Kempuraj D, Papadopoulou NG, Lytinas M, Huang M, Kandere-Grzybowska K, Madhappan B, et al. Corticotropin-releasing hormone and its structurally related urocortin are synthesized and secreted by human mast cells. *Endocrinology.* 2004; **145**: 43–8.
9. Zheng PY, Feng BS, Oluwole C, Struiksma S, Chen X, Li P, et al. Psychological stress induces eosinophils to produce corticotropin releasing hormone in the intestine. *Gut.* 2009; **58**: 1473–9.
10. Agelaki S, Tsatsanis C, Gravanis A, Margioris AN. Corticotropin-releasing hormone augments proinflammatory cytokine production from macrophages in vitro and in lipopolysaccharide-induced endotoxin shock in mice. *Infect Immun.* 2002; **70**: 6068–74.
11. Tanriverdi F, Silveira LF, MacColl GS, Bouloux PM. The hypothalamic-pituitary-gonadal axis: immune function and autoimmunity. *J Endocrinol.* 2003; **176**: 293–304.
12. Chen A, Ganor Y, Rahimipour S, Ben-Aroya N, Koch Y, Levite M. The neuropeptides GnRH-II and GnRH-I are produced by human T cells and trigger laminin receptor gene expression, adhesion, chemotaxis and homing to specific organs. *Nat Med.* 2002; **8**: 1421–6.
13. Jacobson JD, Ansari MA, Kinealy M, Muthukrishnan V. Gender-specific exacerbation of murine lupus by gonadotropin-releasing hormone: potential role of G alpha q/11. *Endocrinology.* 1999; **140**: 3429–37.
14. Cheskis BJ, Greger JG, Ngpal S, Freedman LP. Signaling by estrogens. *J Cell Physiol.* 2007; **213**: 610–7.
15. Wilder RL, Elenkov IJ. Hormonal regulation of tumor necrosis factor-α, interleukin-12 and interleukin-10 production by activated macrophages. *Ann NY Acad Sci.* 1999; **876**: 14–31.
16. Chrousos PG, Torpy DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. *Ann Intern Med.* 1998; **129**: 229–40.
17. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naïve B cells. *Proc Natl Acad Sci USA.* 2000; **97**: 2703–8.
18. Grimaldi CM, Michael DJ, Diamond B. Expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. *J Immunol.* 2001; **167**: 1886–90.
19. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen alters thresholds for B cell apoptosis and activation. *J Clin Invest.* 2002; **109**: 1625–33.
20. Jansson L, Olsoon T, Holmdahl R. Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. *J Neuroimmunol.* 1994; **53**: 203–7.
21. Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. *Neurology.* 1999; **52**: 1230–8.
22. Bebo BF Jr, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. *J Immunol.* 2001; **166**: 2080–9.
23. Lin HB, Loo KK, Palasynski K, Ashouri J, Lubahn DB, Voskuhl RR. Estrongen receptor alpha mediates estrogen's immune protection in autoimmune disease. *J Immunol.* 2003; **171**: 6936–40.
24. Palaszynski KM, Liu H, Loo KK, Voskuhl RR. Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *J Neuroimmunol.* 2004; **149**: 84–9.
25. Wang C, Dehghani B, Li Y, Laurie J, Kaler J, Arthur A, et al. Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death-1. *Immunology.* 2009; **126**: 329–35.
26. Polanczyk M, Zamora A, Subramanian S, Matejuk A, Hess DL, Blankenhorn EP, et al. The protective effect of 17β-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-α. *Am J Pathol.* 2003; **163**: 1599–605.
27. Elloso MM, Phiel K, Henderson RA, Harris HA, Adelman SJ. Suppression of experimental autoimmune encephalomyelitis using estrogen receptor-selective ligands. *J Endocrinol.* 2005; **185**: 243–52.
28. Morales LB, Loo KK, Liu H-b, Peterson C, Tiwari-Woodruff S, Voskuhl RR. Treatment with an estrogen receptor α ligand is neuroprotective in experimental

autoimmune encephalomyelitis. *J Neurosci.* 2006; **26**: 6823–33.
29. Tiwari-Woodruff S, Morales LB, Lee R, Voskuhl RR. Differential neuroprotective and anti-inflmmatory effects of estrogen receptor (ER)alpha and ERbeta lingad treatment. *Proc Natl Acad Sci USA.* 2007; **104**: 14813–8.
30. Crawford DK, Mangiardi M, Song B, Patel R, Du S, Sofroniew MV, et al. Oestrogen receptor β ligand: a novel treatment to enhance endogenous functional remyelination. *Brain.* 2010; **133**: 2999–3016.
31. Wang C, Dehghani B, Li Y, Kaler LJ, Proctor T, Vandenbark AA. Membrane estrogen receptor regulates EAE through upregulation of programmed death 1. *J Immunol.* 2009; **182**: 3294–303.
32. Blasko E, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, et al. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. *J Neuroimmunol.* 2009; **214**: 67–77.
33. Yates MA, Li Y, Chlebeck PJ, Offner H. GPR30, but not estrogen receptor-α, is crucial in the treatment of experimental autoimmune encephalomyelitis by oral ethinyl estradiol. *BMC Immunol.* 2010; **11**: 20.
34. Gold SM, Voskuhl RR. Estrogen treatment in multiple sclerosis. *J Neurol Sci.* 2009; **86**: 99–103.
35. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, et al. Treatment of multiple sclerosis with the pregnancy hormone estriol. *Ann Neurol.* 2002; **52**: 421–8.
36. Vukusic S, Ionescu I, El-Etr M, Schumacher M, Baulieu EE, Cornu C, et al. The Prevention of Post-Partum Relapses with Progestin and Estradiol in Multiple Sclerosis (POPART'MUS) trial: rationale, objectives and state of advancement. *J Neurol Sci.* 2009; **286**: 114–8.
37. Kalkhoven E, Wissink S, van der Saag PT, van der Burg B. Negative interaction between the RelA(p65) subunit of the NF-kappaB and the progesterone receptor. *J Biol Chem.* 1996; **271**: 6217–24.
38. Araneo BA, Dowell T, Diegel M, Daynes RA. Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and gamma-interferon, but not IL-2 by activated murine T cells. *Blood.* 1991; **78**: 688–99.
39. Gerlo S, Verdood P, Hooghe-Peters EL, Kooijman R. Modulation of prolactin expression in human T lymphocytes by cytokines. *J Neuroimmunol.* 2005; **162**: 190–3.
40. Matalka KZ. Prolactin enhances production of interferon-γ, interleukin-12, and interleukin-10, but not of tumor necrosis factor-alpha, in a stimulus-specific manner. *Cytokine.* 2003; **21**: 187–94.
41. Peeva E, Michael D, Cleary J, Rice J, Chen X, Diamond B. Prolactin modulates the naïve B cell repertoire. *J Clin Invest.* 2003; **111**: 275–83.
42. Peeva E, Grimaldi C, Spatz L, Diamond B. Bromocriptine restores tolerance in estrogen-treated mice. *J Clin Invest.* 2000; **106**: 1373–9.
43. Saha S, Gonzalez J, Rosenfeld G, Keiser H, Peeva E. Prolactin alters the mechanisms of B cell tolerance induction. *Arthritis Rheum.* 2009; **60**: 1743–52.
44. Arpin C, Pihlgren M, Fraichard A, Aubert D, Samarut J, Chassande O, et al. Effects of T3Ra1 and T3Ra2 gene deletion on T and B lymphocyte development. *J Immunol.* 2000; **164**: 152–60.
45. Schoenfeld PS, Myers JW, Myers L, LaRocque JC. Suppression of cell-mediated immunity in hypothyroidism. *South Med J.* 1995; **88**: 347–9.
46. Klecha AJ, Genaro AM, Lysionek AE, Caro LA, Coluccia AG, Cremaschi GA. Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis. *Int J Immunopharmacol.* 2000; **22**: 491–500.
47. Klecha AJ, Genaro AM, Gorelik G, Arcos MLB, Silberman DM, Schuman M, et al. Integrative study of hypothalamus–pituitary–thyroid–immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J Endocrinol.* 2006; **189**: 45–55.
48. Frick LR, Rapanelli M, Bussmann UA, Klecha AJ, Arcos ML, Genaro AM, et al. Involvement of thyroid hormones in the alterations of T-cell immunity and tumor progression induced by chronic stress. *Biol Psychiatry.* 2009; **65**: 935–42.
49. Kitazono T, Padgett RC, Armstrong M, Tompkins PK, Heistad DD. Evidence that angiotensin II is present in human monocytes. *Circulation.* 1995; **91**: 1129–34.
50. Okamura A, Rakugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, et al. Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages. *J Hypertens.* 1999; **17**: 537–45.
51. Nahmod KA, Vermeulen ME, Raiden S, Salamone G, Gamberale R, Fernández-Calotti P, et al. Control of dendritic cell differentiation by angiotensin II. *FASEB J.* 2003; **17**: 491–3.
52. Ruiz-Ortega M, Bustos C, Hernandez-Presa MA, Lorenzo O, Plaza JJ, Egido J. Angiotensin II participates in mononuclear cell recruitment in experimental immune complex nephritis through nuclear factor-kappa B activation and monocyte chemoattractant protein-1 synthesis. *J Immunol.* 1998; **161**: 430–9.
53. Kranzhofer R, Browatzki M, Schmidt J, Kubler W. Angiotensin II activates the proinflammatory transcription factor nuclear factor-kappa B in human monocytes. *Biochem Biophys Res Commun.* 1999; **257**: 826–8.
54. Potter DD, Sobey CG, Tompkins PK, Rossen JD, Heistad DD. Evidence that macrophages in atherosclerotic lesions contain angiotensin II. *Circulation.* 1998; **98**: 800–7.
55. Nabah YNA, Mateo T, Estellés R, Mata M, Zagorski J, Sarau H, et al. Angitotensin II induces neutrophil accumulation in vivo through generation and release of CXC chemokines. *Circulation.* 2004; **110**: 3581–6.

56. Fukuzawa M, Satoh J, Sagara M, Muto G, Muto Y, Nishimura S, et al. Angiotensin converting enzyme inhibitors suppress production of tumor necrosis factor-alpha in vitro and in vivo. *Immunopharmacology.* 1997; **36**: 49–55.
57. Lapteva N, Ide K, Nieda M, Ando Y, Hatta-Ohashi Y, Minami M, et al. Activation and suppression of rennin-angiotensisn system in human dendritic cells. *Biochem Biophys Res Commun.* 2002; **296**: 194–200.
58. Sánchez-Lemus E, Benicky J, Pavel J, Saavedra JM. In vivo angiotensisn II AT1 receptor blockade selectively inhibits LPS-induced innate immune response and ACTH release in rat pituitary gland. *Brain Behav Immun.* 2009; **23**: 945–57.
59. Dalbeth N, Edwards J, Fairchild S, Callan M, Hall FC. The non-thiol-angiotensin-converting enzyme inhibitor quinapril suppresses inflammatory arthritis. *Rheumatology.* 2005; **44**: 24–31.
60. Sagawa K, Nagatani K, Komagata Y, Yamamoto K. Angiotensin receptor blockers suppress antigen-specific T cell responses and ameliorate collagen-induced arthritis in mice. *Arthritis Rheum.* 2005; **52**: 1920–8.
61. Okunuki Y, Usui Y, Nagai N, Kezuka T, Ishida S, Takeuchi M, et al. Suppression of experimental autoimmune uveitis by angiotensin II type 1 receptor blocker telmisartan. *Invest Ophthalmol Vis Sci.* 2009; **50**: 2255–61.
62. Platten M, Youssef S, Hur EM, Ho PP, Han MH, Lanz TV, et al. Blocking antigotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. *Proc Natl Acad Sci USA.* 2009; **106**: 14948–53.
63. Lanz TV, Ding Z, Ho PP, Luo J, Agrawal AN, Srinagesh H, et al. Angiotensin II sustains brain inflammation in mice via TGF-β. *J Clin Invest.* 2010; **120**: 2782–94.
64. Guzik TJ, Mangalat D, Korbut R. Adipocytokines – novel link between inflammation and vascular function? *J Physiol Pharmacol.* 2006; **57**: 505–28.
65. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol.* 2006; **6**: 772–83.
66. Wong CK, Cheung PF, Lam CW. Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. *Eur J Immunol.* 2007; **37**: 2337–48.
67. Caldefie-Chezet F, Poulin A, Vasson MP. Leptin regulates functional capacities of polymorphonuclear neutrophils. *Free Radic Res.* 2003; **37**: 809–14.
68. Montecucco F, Bianchi G, Gnerre P, Bertolotto M, Dallegri F, Ottonello L. Induction of neutrophil chemotaxis by leptin: crucial role for p38 and Src kinases. *Ann NY Acad Sci.* 2006; **1069**: 463–71.
69. Lord GM, Matarese G, Howard JK, Baker RJ, Bloon SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature.* 1998; **394**: 897–901.
70. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. *J Clin Invest.* 2003; **111**: 241–50.
71. Okamoto Y, Folco EJ, Minami M, Wara AK, Feinberg MW, Sukhova GK, et al. Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. *Circ Res.* 2008; **102**: 218–25.
72. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood.* 2000; **96**: 1723–32.
73. Shwarz H, Villiger PM, von Kempis J, Lotz M. Neuropeptide Y is an inducible gene in the human immune system. *J Neuroimmunol.* 1994; **51**: 53–61.
74. Straub RH, Schaller T, Miller LE, von Hörsten S, Jessop DS, Falk W, et al. Neuropeptide Y cotransmission with norepinephrine in the sympathetic nerve-macrophage interplay. *J Neurochem.* 2000; **7**: 2464–71.
75. Bedoui S, Miyake S, Lin Y, Miyamoto K, Oki S, Kawamura N, et al. Neuropeptide Y (NPY) suppresses experimental autoimmune encephalomyelitis: NPY1 receptor-specific inhibition of autoreactive Th1 responses in vivo. *J Immunol.* 2003; **171**: 3451–8.
76. Wheway J, Mackay CR, Newton RA, Sainsbury A, Boey D, Herzog H, et al. A fundamental bimodal role for neuropeptide Y1 receptor in the immune system. *J Exp Med.* 2005; **202**: 1527–38.
77. Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grouve KL, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron.* 2003; **37**: 649–61.
78. Kalra SP, Ueno N, Kalra PS. Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action. *J Nutr.* 2005; **135**: 1331–5.
79. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature.* 2001; **409**: 194–8.
80. Wu JTR, Dral J. Ghrelin integrative neuroendocrine peptide in health and disease. *Ann Surg.* 2004; **239**: 464–74.
81. Koo GC, Huang C, Camacho R, Trainor C, Blake JT, Sirotina-Meisher A, et al. Immune enhancing effect of a growth hormone secretagogue. *J Immunol.* 2001; **16**: 4195–201.
82. Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kB activation in human endothelial cells. *Circulation.* 2004; **109**: 2221–6.
83. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and

activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest.* 2004; **114**: 57–66.
84. Gonzalez-Rey E, Chorny A, Delgado M. Therapeutic action of ghrelin in a mouse model of colitis. *Gastroenterology.* 2006; **130**: 1707–20.
85. Granado M, Priego T, Martin AI, Villanua A, Lopez-Caldron A. Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. *Am J Physiol Endocrinol Metab.* 2005; **288**: E486–92.
86. Chorny A, Anderson P, Gonzalez-Rey E, Delgado M. Ghrelin protects against experimental sepsis by inhibiting high-mobility group box 1 release and by killing bacteria. *J Immunol.* 2008; **180**: 8369–77.
87. Wu R, Dong W, Cui X, Zhou M, Simms HH, Ravikumar TS, et al. Ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. *Ann Surg.* 2007; **245**: 480–6.
88. Theil M-M, Miyake S, Mizuno M, Tomi C, Croxford JL, Hosoda H, et al. Suppression of experimental autoimmune encephalomyelitis by ghrelin. *J Immunol.* 2009; **183**: 2859–66.
89. Miyake S, Yamamura T. Ghrelin: friend or foe for neuroinflammation. *Discov Med.* 2009; **8**: 64–7.
90. Sloan EK, Capitanio JP, Tarara RP, Mendoza SP, Mason WA, Cole SW. Social stress enhances sympathetic innervation of primate lymph nodes: mechanisms and implications for viral pathogenesis. *J Neurosci.* 2007; **27**: 8857–65.
91. Sloan EK, Capitanio JP, Cole SW. Stress-induced remodeling of lymphoid innervation. *Brain Behav Immun.* 2008; **22**: 15–21.
92. Straub RH, Rauch L, Fassold A, Lowin T, Pongratz G. Neuronally released sympathetic neurotransmitters stimulate splenic interferon-gamma secretion from T cells in early type II collagen-induced arthritis. *Arthritis Rheum.* 2008; **58**: 3450–60.
93. Sloan EK, Nguyen CT, Cox BF, Tarara RP, Capitanio JP, Cole SW. SIV infection decreases sympathetic innervation of primate lymph nodes: the role of neurotrophins. *Brain Behav Immun.* 2008; **22**: 185–94.
94. Elenkov IJ, Papanicolaou DA, Wilder RL, Chrousos GP. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proc Assoc Am Physicians.* 1996; **108**: 374–81.
95. Le Tulzo Y, Shenkar R, Kaneko D, Moine P, Fantuzzi G, Dinarello CA, et al. Hemorrhage increases cytokine expression in lung mononuclear cells in mice: involvement of catecholamines in nuclear factor-kappaB regulation and cytokine expression. *J Clin Invest.* 1997; **99**: 1516–24.
96. Panina-Bordignon P, Mazzeo D, Lucia PD, D'Ambrosio D, Lang R, Fabbri L, et al. Beta2-agonists prevent Th1 developmenet by selective inhibition of interleukin 12. *J Clin Invest.* 1997; **100**: 1513–9.
97. Kin NW, Sanders VM. It takes nerve to tell T and B cells what to do. *J Leukoc Biol.* 2006; **79**: 1093–104.
98. Strell C, Sievers A, Bastian P, Lang K, Niggemann B, Zänker KS, et al. Divergent effects of norepinephrine, dopamine and substance P on the activation, differentiation and effector functions of human cytotoxic T lymphocytes. *BMC Immunol.* 2009; **8**: 10.
99. Bhowmick S, Singh A, Flavell RA, Clark RB, O'Rourke J, Cone RE. The sympathetic nervous system modulates CD4(+)FoxP3(+) regulatory T cells via a TGF-beta-dependent mechanism. *J Leukoc Biol.* 2009; **86**: 1275–83.
100. Grebe KM, Hickman HD, Irvine KR, Takeda K, Bennink JR, Yewdell JW. Sympathetic nervous system control of anti-influenza CD8+ T cell responses. *Proc Natl Acad Sci USA.* 2009; **106**: 5300–5.
101. Kohm AP, Sanders VM. Norepinephrine and beta 2-adrenergic receptor stimulation regulate CD4+ T and B lymphocyte function in vitro and in vivo. *Pharm Rev.* 2001; **53**: 487–525.
102. Lucin KM, Sanders VM, Jones TB, Malarkey WB, Popovich PG. Impaired antibody synthesis after spinal cord injury is level dependent and is due to sympathetic nervous system dysregulation. *Exp Neurol.* 2007; **207**: 75–84.
103. Harvath L, Robbins JD, Russell AA, Seamon KB. cAMP and human neutrophil chemotaxis. Elevation of cAMP differentially affects chemotactic responsiveness. *J Immunol.* 1991; **146**: 224–32.
104. Barnett CC, Moore EE, Partrick DA, Silliman CC. Beta-adrenergic stimulation down-regulates neutrophil priming for superoxide generation, but not elastase release. *J Surg Res.* 1997; **70**: 166–70.
105. Layé S, Bluthé RM, Kent S, Combe C, Médina C, Parnet P, et al. Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. *Am J Physiol.* 1995; **268**: R1327–31.
106. van Maanen MA, Lebre MC, van der Poll T, LaRosa GJ, Elbaum D, Vervoordeldonk MJ, et al. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. *Arthritis Rheum.* 2009; **60**: 114–22.
107. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature.* 2000; **405**: 458–62.
108. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susara S, et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature.* 2003; **421**: 384–8.
109. Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med.* 2004; **10**: 1216–21.

110. Shi FD, Piao WH, Kuo YP, Campagnolo DI, Vollmer TL, Lukas RJ. Nicotinic attenuation of central nervous system inflammation and autoimmunity. *J Immunol.* 2009; **182**: 1730–9.
111. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. *J Immunol.* 2009; **183**: 6681–8.
112. Fujii YX, Fujigaya H, Moriwaki Y, Misawa H, Kasahara T, Grando SA, et al. Enhanced serum antigen-specific IgG_1 and proinflammatory cytokine production in nicotinic acetylcholine receptor α7 subunit gene knockout mice. *J Neuroimmunol.* 2007; **189**: 69–74.
113. van der Zanden EP, Snoek SA, Heinsbroek SE, Stanisor OI, Verseijden C, Boeckxstaens GE, et al. Vagus nerve activity augments intestinal macrophage phagocytosis via nicotinic acetylcholine receptor α4β2. *Gastroenterology.* 2009; **189**: 1029–39.
114. Shaked I, Meerson A, Wolf Y, Avni R, Greenberg D, Gilboa-Geffen A, et al. MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. *Immunity.* 2009; **131**: 965–73.
115. Levite M. Neurotransmitters activate T-cells and elicit crucial functions via neurotransmitter receptors. *Curr Opin Pharmacol.* 2008; **8**: 460–71.
116. Nakano K, Higashi T, Takagi R, Hashimoto K, Tanaka Y, Matsushita S. Dopamine released by dendritic cells polarizes Th2 differentiation. *Int Immunol.* 2009; **21**: 645–54.
117. Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. *Proc Natl Acad Sci USA.* 1994; **91**: 12912–6.
118. Flierl MA, Rittirsch D, Nadeau BA, Chen AJ, Sarma V, Zetoune F, et al. Phagocyte-derived catecholamines enhance acute inflammatory injury. *Nature.* 2007; **449**: 721–6.
119. Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, et al. Human $CD4^+CD25^+$ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. *Blood.* 2007; **109**: 632–42.
120. Agulia MC, Dees WL, Haensly WE, McCann M. Evidence that somatostatin is localized and synthesized in lymphoid organs. *Proc Natl Acad Sci USA.* 1991; **88**: 11485–9.
121. De Giorgio R, Tazzari PL, Barbara G, Stanhellini V, Corinaldesi R. Detection of substance P immunoreactivity in human peripheral leukocytes. *J Neuroimmunol.* 1998; **82**: 175–81.
122. Li Y, Tian S, Douglas SD, Ho WZ. Morphine up-regulates expression of substance P and its receptor in human blood mononuclear phagocytes and lymphocytes. *Cell Immunol.* 2000; **205**: 120–7.
123. Qian BF, Zhou GQ, Hammarstrom ML, Danielsson A. Both substance P and its receptor are expressed in mouse intestinal T lymphocytes. *Neuroendocrinology.* 2001; **73**: 358–68.
124. Arsenescu R, Blum AM, Metwali A, Elliott DE, Weinstock JV. IL-12 induction of mRNA encoding substance P in murine macrophages from the spleen and sites of inflammation. *J Immunol.* 2005; **174**: 3906–11.
125. Mousa SAStraub R, Schafer M, Stein C. Beta-endorphin, Met-enkephalin and corresponding opioid receptors within synovium of patients with joint trauma, osteoarthritis and rheumatoid arthritis. *Ann Rheum Dis.* 2007; **66**: 871–9.
126. Bracci-Laudiero L, Aloe L, Buanne P, Finn A, Stenfors C, Vigneti E, et al. NGF modulates CGRP synthesis in human B-lympho cytes: a possible anti-inflammatroy action on NGF? *J Neuroimmunol.* 2002; **123**: 58–65.
127. Bracci-Laudiero L, Aloe L, Caroleo MC, Buanne P, Costa NStarace G, et al. Endogenous NGF regulates CGRP expression in human monocytes, and affects HLA-DR and CD86 expression and IL-10 production. *Blood.* 2005; **106**: 3507–14.
128. Xing L, Guo J, Wang X. Induction and expression of β-calcitonin gene-related peptide in rat T lymphocytes and its significance. *J Immunol.* 2000; **165**: 4359–66.
129. Gomariz RP, Leceta J, Garrido E, Garrido T, Delgado M. Vasoactive intestinal peptide (VIP) mRNA expression in rat T and B lymphocytes. *Regul Pept.* 1994; **50**: 177–84.
130. Martinez C, Delgado M, Abad C, Gomariz RP, Ganea D, Leceta J. Regulation of VIP production and secretion by murine lymphocytes. *J Neuroimmunol.* 1999; **93**: 126–38.
131. Ganor Y, Besser M, Ben-Zakay N, Unger T, Levite M. Human T cells express a functional ionotropic glutamate receptor GluR3, and glutamate by itself triggers integrin-mediated adhesion to laminin and fibronectin and chemotactic migration. *J Immunol.* 2003; **170**: 4362–72.
132. Pitt D, Werner P, Paine CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat Med.* 2000; **6**: 67–70.
133. Pacheco R, Oliva H, Martinez-Navio JM, Climent N, Ciruela F, Gatell JM, et al. Glutamate released by dendritic cells as a novel modulator of T cell activation. *J Immunol.* 2006; **177**: 6695–704.
134. Pacheco R, Ciruela F, Casado V, Mallol J, Gallart T, Lluis C, et al. Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. *J Biol Chem.* 2004; **279**: 33352–8.
135. Fallarino F, Volpi C, Fazio F, Notartomaso S, Vacca C, Busceti C, et al. Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. *Nat Med.* 2010; **16**: 897–902.
136. Bergquist J, Josefsson E, Tarkowski A, Ekman R, Ewing A. Measurements of catecholamine-mediated apoptosis of immunocompetent cells by capillary electrophoresis. *Electrophoresis.* 1997; **18**: 1760–6.

137. Ghosh MC, Mondal AC, Basu S, Banerjee S, Majumdar J, Bhattacharya D, et al. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. *Int Immunopharmacol.* 2003; **3**: 1019–26.
138. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. *Physiol Rev.* 1998; **78**: 189–225.
139. Saha B, Mondal AC, Majumder J, Basu S, Dasgupta PS. Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4+ and CD8+ T cells in vitro: a receptor-mediated mechanism. *Neuroimmunomodulation.* 2001; **9**: 23–33.
140. Kipnis J, Cardon M, Avidan H, Lewitus GM, Mordechay S, Rolls A, et al. Dopamine, through the extracellular signal regulated kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity: implications for neurodegeneration. *J Neurosci.* 2004; **4**: 6133–43.
141. Levite M, Chowers Y, Ganor Y, Besser M, Hershkovits R, Cahalon L. Dopamine interacts directly with its D3 and D2 receptors on normal human T cells, and activates beta1 integrin function. *Eur J Immunol.* 2001; **31**: 3504–12.
142. Besser MJ, Ganor Y, Levite M. Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNFalpha or both. *J Neuroimmunol.* 2005; **169**: 161–71.
143. Watanabe Y, Nakayama T, Nagakubo D, Hieshima K, Jin Z, Katou F, et al. Dopamine selectively induces migration and homing of naive CD8+ T cells via dopamine receptor D3. *J Immunol.* 2006; **176**: 848–56.
144. Nakano K, Higashi T, Hashimoto K, Takagi Ri, Tanaka Y, Matsushita S. Antagonizing dopamine D1-like receptor inhibits Th17 cell differentiation: preventive and therapeutic effects on experimental autoimmune encephalomyelitis. *Biochem Biophys Res Commun.* 2008; **373**: 286–91.
145. Nakano K, Yamaoka K, Hanami K, Saito K, Sasaguri Y, Yanagihara N, et al. Dopamine induces IL-6-dependent IL-17 production via D1-like receptor on CD4 Naïve T cells and D1-like receptor antagonist SCH-23390 inhibits cartilage destruction in a human rheumatoid arthritis/SCID mouse chimera model. *J Immunol.* 2011; **186**: 3745–52.
146. Lotz M, Vaughan JH, Carson DA. Effects of neuropeptides on production of inflammatory cytokines by human monocytes. *Science.* 1988; **241**: 1218–21.
147. Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T cells. *J Immunol.* 1992; **148**: 3498–504.
148. Mathers AR, Tckacheva OA, Janelsins BM, Shufesky WJ, Morelli AE, Larregina AT. In vivo signaling through the neurokinin 1 receptor favors transgene expression by Langerhans cells and promotes the generation of Th1- and Tc1-biased immune responses. *J Immunol.* 2007; **178**: 7006–17.
149. Cunin P, Caillon A, Corvaisier M, Garo E, Scotet M, Blanchard S, et al. The tachykinins substance P and hemokinin-1 favor the generation of human memory Th17 cells by inducing IL-1β, Il-23, and TNF-like 1A expression by monocytes. *J Immunol.* 2011; **186**: 4175–82.
150. Hegde A, Zhang H, Moochhala SM, Bhatia M. Neurokinin-1 receptor antagonist treatment protects mice against lung injury in polymicrobial sepsis. *J Leukoc Biol.* 2007; **82**: 678–85.
151. Gad M, Pedersen AE, Kristensen NN, Fernandez Cde F, Claesson MH. Blockage of the neurokinin 1 receptor and capsaicin-induced ablation of the enteric afferent nerves protect SCID mice against T-cell-induced chronic colitis. *Inflamm Bowel Dis.* 2009; **15**: 1174–82.
152. Hegde A, Koh YH, Moochhala SM, Bhatia M. Neurokinin-1 receptor antagonist treatment in polymicrobial sepsis: molecular insights. *Int J Inflam.* 2010; **2010**: 601098.
153. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci.* 2008; **9**: 46–57.
154. Rettori V, Gimeno MF, Karara A, Gonzalez MC, McCann SM. Interleukin 1 alpha inhibits prostaglandin E2 release to suppress pulsatile release of luteinizing hormone but not follicle-stimulating hormone. *Proc Natl Acad Sci USA.* 1991; **88**: 2763–7.
155. Bluthé RM, Kelley KW, Dantzer R. Effects of insulin-like growth factor-I on cytokine-induced sickness behavior in mice. *Brain Behav Immun.* 2006; **20**: 57–63.
156. Dantzer R, Gheusi G, Johnson RW, Kelley KW. Central administration of insulin-like growth factor-1 inhibits lipopolysaccharide-induced sickness behavior in mice. *Neuroreport.* 1999; **10**: 289–92.
157. Dantzer R, Gheusi G, Johnson RW, Kelley KW. Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. *Psychoneuroendocrinology.* 1999; **24**: 301–11.
158. Leon LR, Kozak W, Rudolph K, Kluger MJ. An antipyretic role for interleukin-10 in LPS fever in mice. *Am J Physiol.* 1999; **276**: R81–9.
159. Perkins MN, Stone TW. An iontophoretic investigation of actions of convulsant kynurenines and their interaction with endogenous excitant quinolinic acid. *Brain Res.* 1982; **247**: 184–7.
160. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med.* 1999; **189**: 865–70.
161. Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M. T cell deficieny leads to cognitive dysfunction: implications

for therapeutic vaccination for schizophrenia and other psychiatric conditions. *Proc Natl Acad Sci USA*. 2004; **101**: 8180–5.
162. Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, et al. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci*. 2006; **9**: 268–75.
163. Wolf SA, Steiner B, Akpinarli A, Kammertoens T, Nassenstein C, Braun A, et al. CD4-positive T lymphocytes provide a neuroimmunological link in the control of adult hippocampal neurogenesis. *J Immunol*. 2009; **182**: 3979–84.
164. Abbas N, Bednar I, Mix E, Marie S, Paterson DL, Jungberg A, et al. Up-regulation of the inflammatory cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP(SWE) transgenic mice. *J Neuroimmunol*. 2002; **126**: 50–7.
165. Straub RH, Dhabhar FS, Bijlsma WJ, Cutolo M. How psychological stress via hormones and nerve fibers may exacerbate rheumatoid arthritis. *Arthritis Rheum*. 2005; **52**: 16–26.
166. Jara LJ, Navarro C, Medina G, Vera-Lastra O, Blanco F. Immune-neuroendocrine interactions and autoimmune diseases. *Clin Dev Immunol*. 2006; **13**: 109–23.

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Mucosal-Associated Invariant T Cells Promote Inflammation and Exacerbate Disease in Murine Models of Arthritis

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Objective. The function of mucosal-associated invariant T (MAIT) cells remains largely unknown. We previously reported an immunoregulatory role of MAIT cells in an animal model of multiple sclerosis. The aim of this study was to use animal models to determine whether MAIT cells are involved in the pathogenesis of arthritis.

Methods. $MR1^{-/-}$ and $MR1^{+/+}$ DBA/1J mice were immunized with bovine type II collagen (CII) in complete Freund's adjuvant to trigger collagen-induced arthritis (CIA). To assess CII-specific T cell recall responses, lymph node cells from mice with CIA were challenged with CII ex vivo, and cytokine production and proliferation were evaluated. Serum levels of CII-specific antibodies were measured by enzyme-linked immunosorbent assay. Collagen antibody–induced arthritis (CAIA) was induced in $MR1^{-/-}$ and $MR1^{+/+}$ C57BL/6 mice by injection of anti-CII antibodies followed by injection of lipopolysaccharide. To demonstrate the involvement of MAIT cells in arthritis, we induced CAIA in $MR1^{-/-}$ C57BL/6 mice that had been reconstituted with adoptively transferred MAIT cells. MAIT cell activation in response to cytokine stimulation was investigated.

Results. The severity of CIA was reduced in $MR1^{-/-}$ DBA/1J mice. However, T and B cell responses to CII were comparable in $MR1^{-/-}$ and $MR1^{+/+}$ DBA/1J mice. $MR1^{-/-}$ C57BL/6 mice were less susceptible to CAIA, and reconstitution with MAIT cells induced severe arthritis in $MR1^{-/-}$ C57BL/6 mice, demonstrating an effector role of MAIT cells in arthritis. MAIT cells became activated upon stimulation with interleukin-23 (IL-23) or IL-1β in the absence of T cell receptor stimuli.

Conclusion. These results indicate that MAIT cells exacerbate arthritis by enhancing the inflammation.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation in the joints. It has been suggested that environmental factors influence autoimmunity, and in particular, increasing evidence highlights the important role of gut flora in the development of autoimmune diseases (1), including arthritis. For example, differences in the intestinal microbiota of patients with early RA have been described, and tetracycline treatment was shown to reduce disease activity in RA (2,3). In addition, oral vancomycin treatment significantly decreased the severity of adjuvant-induced arthritis (4). More recently, it was demonstrated that germ-free conditions strongly inhibit arthritis in the K/BxN arthritis model and that the introduction of segmented filamentous bacteria induced severe arthritis in germ-free K/BxN mice (5). Thus, mucosal immunity plays an important role in the development and progression of arthritis.

Natural killer (NK) cells, invariant NK T (iNKT) cells, γ/δ T cells, mucosal-associated invariant T (MAIT) cells, B-1 B cells, and marginal-zone B cells are categorized as innate-like lymphocytes. Such lymphocytes reside in unique locations, including the marginal zone of the spleen and epithelial and mucosal tissues and rapidly exert effector functions in the absence of clonal expansion (6–15). Therefore, these innate-like lymphocytes are thought to play important roles in "first-line" im-

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mune responses against exogenous stimuli. As MAIT cells are preferentially located in the gut lamina propria, there is a growing interest in the function of MAIT cells in various types of immune responses, including autoimmunity (16–20).

MAIT cells are restricted by a nonpolymorphic class IB major histocompatibility complex (MHC) molecule, the class I MHC–related molecule (MR1), and express an invariant T cell receptor (TCR) α-chain: V_α7.2–J_α33 in humans and V_α19–J_α33 in mice. The invariant TCRα chain associates with a limited set of V_β chains (14,21,22). MAIT cells are selected in the thymus in an MR1-dependent manner, but, interestingly, MAIT cells require B cells as well as commensal flora for their peripheral expansion (14,23). Our group previously demonstrated a protective role of MAIT cells against autoimmune encephalomyelitis (EAE), an animal model of human multiple sclerosis. The suppression of EAE was accompanied by increased production of interleukin-10 (IL-10) by B cells, which was induced in part by ICOS costimulation (17). Because the invariant V_α7.2–J_α33 TCR is highly expressed in central nervous system lesions of multiple sclerosis patients, human MAIT cells may also be involved in the pathogenesis of multiple sclerosis (16).

In addition to their regulatory function, MAIT cells also possess proinflammatory functions like other innate-like lymphocytes. Le Bourhis et al (20) demonstrated that MAIT cells display antimicrobial capacity. Both human and mouse MAIT cells are activated by *Escherichia coli*–infected antigen-presenting cells in an MR1-dependent manner. MAIT cells show a protective role against *Mycobacterium abscessus* or *E coli* infections in mice. Human MAIT cells are capable of producing interferon-γ (IFNγ) and IL-17 and are found in *Mycobacterium tuberculosis*–infected lung tissues. Thus, MAIT cells play an antimicrobial function under these infectious conditions. Although accumulating evidence suggests that certain subsets of innate-like lymphocytes, such as NK cells, iNKT cells, and γ/δ T cells, are involved in the pathogenesis of arthritis in animal models of the disease, the role of MAIT cells in arthritis remains unknown (24–31).

We report herein that MAIT cells play a pathogenic role in murine models of arthritis. The disease severity of collagen-induced arthritis (CIA) in MAIT cell–deficient $MR1^{-/-}$ DBA/1J mice was ameliorated compared with that of $MR1^{+/+}$ DBA/1J mice. However, T cell responses to type II collagen (CII) and CII-specific serum antibody levels were comparable between CIA-induced $MR1^{-/-}$ and $MR1^{+/+}$ DBA/1J mice. We found that $MR1^{-/-}$ C57BL/6J mice are much less susceptible to collagen antibody–induced arthritis (CAIA) as compared to $MR1^{+/+}$ C57BL/6J mice. $MR1^{-/-}$ C57BL/6J mice reconstituted with adoptively transferred MAIT cells developed severe arthritis, suggesting that MAIT cells may be one of the effectors contributing to inflammation in arthritis. Finally, we investigated the cytokine-producing capacity of MAIT cells. No differences in IFNγ production by liver mononuclear cells (LMNCs) from $MR1^{-/-}$ C57BL/6J and $MR1^{+/+}$ C57BL/6J mice were observed upon TCR stimulation, but the level of IL-17 produced by LMNCs from $MR1^{+/+}$ C57BL/6J mice was much higher than that produced by cells from $MR1^{-/-}$ C57BL/6J mice. We further demonstrated that sorted murine MAIT cells produce IL-17 upon TCR engagement. Surprisingly, IL-17 production by MAIT cells was observed after exposure to IL-23 without TCR stimulation, and IL-1β alone induced proliferation of MAIT cells, indicating that MAIT cells may be activated by cytokines and may enhance the inflammation in arthritis.

MATERIALS AND METHODS

Mice. DBA/1J mice were purchased from the Oriental Yeast Company. C57BL/6J mice were obtained from CLEA Laboratory Animal Corporation. $MR1^{-/-}$ mice (14) were provided by S. Gilfillan (Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO), and V_α19i-transgenic mice (32) on a C57BL/6J background were provided by M. Shimamura (University of Tsukuba, Ibaraki, Japan). $MR1^{-/-}$ mice were backcrossed to DBA/1J mice for 10 generations to obtain $MR1^{-/-}$ DBA/1J mice. V_α19i-transgenic $CD1d1^{-/-}$ C57BL/6J mice were generated by backcrossing V_α19i-transgenic mice with $CD1d1^{-/-}$ C57BL/6J mice for 7 generations. Mice were maintained under specific pathogen–free conditions in accordance with institutional guidelines and used in the experiments at 7–12 weeks of age.

Induction of CIA. Both $MR1^{-/-}$ DBA/1J mice and their littermate controls ($MR1^{+/+}$ DBA/1J mice) (n = 5–6 per group; ages 7–8 weeks old) were immunized intradermally at the base of the tail with 150 μg of CII (Collagen Research Center) emulsified with an equal volume of complete Freund's adjuvant containing 250 μg of heat-killed *Mycobacterium tuberculosis* H37Ra (Difco). Three weeks after the primary immunization, mice were given an intradermal booster injection of 150 μg of CII emulsified in incomplete Freund's adjuvant (Difco).

Induction of CAIA. $MR1^{-/-}$ C57BL/6J mice and their littermate controls ($MR1^{+/+}$ C57BL/6J mice) were injected intravenously with a mixture of anti-CII monoclonal antibodies (mAb) (Arthrogen-CIA mAb, 2 mg; Chondrex) followed 2 days later by an intraperitoneal injection of 50 μg of lipopolysaccharide.

Clinical assessment of arthritis. Mice were examined for signs of joint inflammation, which was scored on a scale of 0–4, where 0 = no change, 1 = significant swelling and redness

of 1 digit, 2 = mild swelling and erythema of the limb or swelling of ≥2 digits, 3 = marked swelling and erythema of the limb, and 4 = maximal swelling and redness of the limb and later, ankylosis. The average macroscopic score was expressed as a cumulative value for all paws, with a maximum possible score of 16.

Histopathologic assessment. Arthritic mice were killed, and all 4 paws were fixed in buffered formalin, decalcified, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin. Histologic assessment of joint inflammation was scored on a scale of 0–3 as follows: 0 = normal joint, 1 = mild arthritis (minimal synovitis without cartilage/bone erosion), 2 = moderate arthritis (synovitis and erosion but joint architecture maintained), and 3 = severe arthritis (synovitis, erosion, and loss of joint integrity). The average of the macroscopic scores was expressed as a cumulative value for all paws, with a maximum possible score of 12.

CII-specific T cell response. Lymph node cells were collected on days 35–42 after immunization and suspended in complete RPMI 1640 medium (Life Technologies) containing 1% syngeneic mouse serum. The cells were cultured for 72 hours in 96-well flat-bottomed plates at a density of 1×10^6/well in the presence of CII. Proliferative responses were measured using a β-1205 counter (Pharmacia) to detect the incorporation of ^{3}H-thymidine (1 μCi/well) during the final 16 hours of culture.

Measurement of CII-specific total IgG, IgG1, and IgG2a. Bovine CII (1 mg/ml) was coated onto enzyme-linked immunosorbent assay (ELISA) plates (Sumitomo Bakelite) overnight at 4°C. After blocking with 1% bovine serum albumin in PBS, serially diluted serum samples were added to CII-coated wells. For detection of anti-CII antibodies, the plates were incubated with biotin-labeled anti-IgG1 and anti-IgG2a (SouthernBiotech) or anti-IgG antibody (CN/Cappel) for 1 hour and were then incubated with streptavidin–peroxidase. After adding substrate, the reaction was evaluated as the optical density values at 450 nm (OD_{450}).

Adoptive transfer and in vitro stimulation of V_α19i T cells. LMNCs were purified from V_α19i-transgenic CD1d1$^{-/-}$ C57BL/6J mice by use of Percoll density-gradient centrifugation, and erythrocytes and B cells were depleted with phycoerythrin (PE)–conjugated anti–Ter-119 and PE-conjugated anti-CD19 (BD) followed by separation with anti–PE-conjugated magnetic-activated cell sorter beads (Miltenyi Biotec). Cells were stained with fluorescein isothiocyanate–conjugated anti-TCRβ and PerCP–Cy5.5 anti-NK1.1 (BD), and TCRβ+ NK1.1+ cells were sorted using a FACSAria cell sorter (BD). The purity of isolated NK1.1+ T cells (MAIT cells) was >95%, as assessed by flow cytometry.

In adoptive transfer experiments, 5×10^5 MAIT cells or NK1.1– T cells (T cells) were injected intravenously into naive MR1$^{-/-}$ C57BL/6 recipient mice 1 day before administration of CII mAb. LMNCs or sorted MAIT cells were resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 50 units/ml of penicillin/streptomycin, and 55 μM β-mercaptoethanol (Life Technologies) and stimulated with immobilized anti-CD3 mAb (2C11, 1 μg/ml) and/or the following cytokines: IL-1β, tumor necrosis factor α (TNFα), IL-6, and transforming growth factor β (TGFβ) (all from PeproTech) and IL-23 (R&D Systems).

Detection of cytokines. Cytokine levels in the culture supernatant were determined using a sandwich ELISA. The

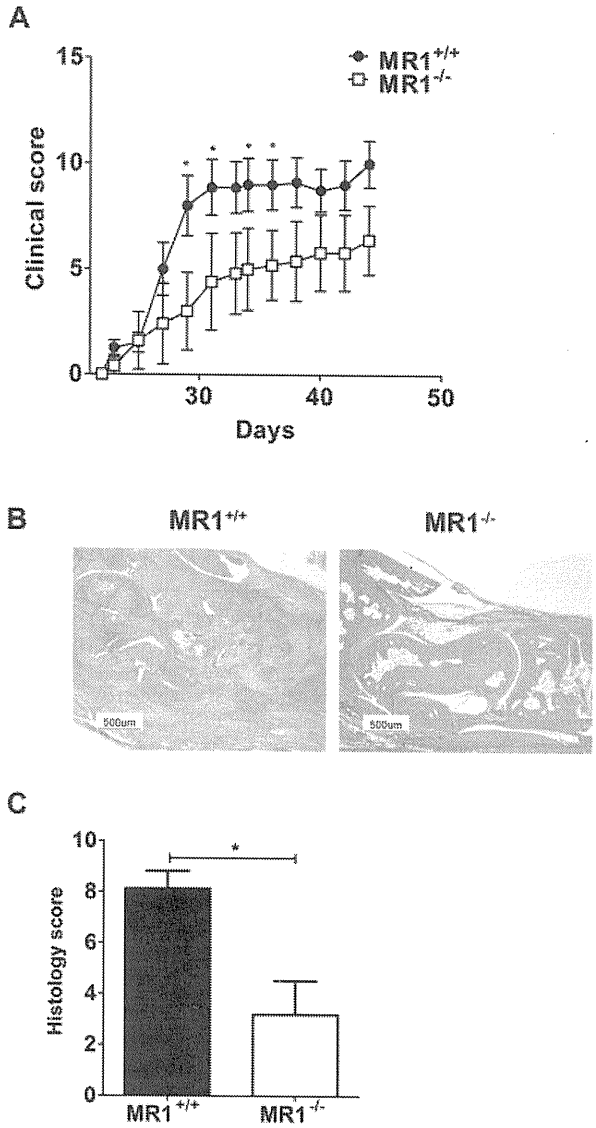

Figure 1. Amelioration of collagen-induced arthritis (CIA) in MR1$^{-/-}$ mice. **A,** Clinical scores for CIA in MR1$^{-/-}$ DBA/1J mice and in MR1$^{+/+}$ DBA/1J mice. Values are the mean ± SEM of 5–8 mice per group. * = $P < 0.05$ versus MR1$^{-/-}$ DBA/1J mice. **B,** Representative histologic sections of the joints of MR1$^{+/+}$ DBA/1J mice and MR1$^{-/-}$ DBA/1J mice. Hematoxylin and eosin stained; original magnification × 40. **C,** Histology scores in MR1$^{-/-}$ DBA/1J mice and in MR1$^{+/+}$ DBA/1J mice, expressed as the sum of the scores in the 4 paws. Results from a single representative experiment of 2 similar experiments performed are shown. Values are the mean ± SEM. * = $P < 0.05$.

ELISA antibodies for IFNγ were purchased from BD. Levels of IL-17 were determined using an IL-17 ELISA kit (R&D Systems).

Statistical analysis. Clinical or pathologic scores for CIA and CAIA in the various groups of mice are presented as

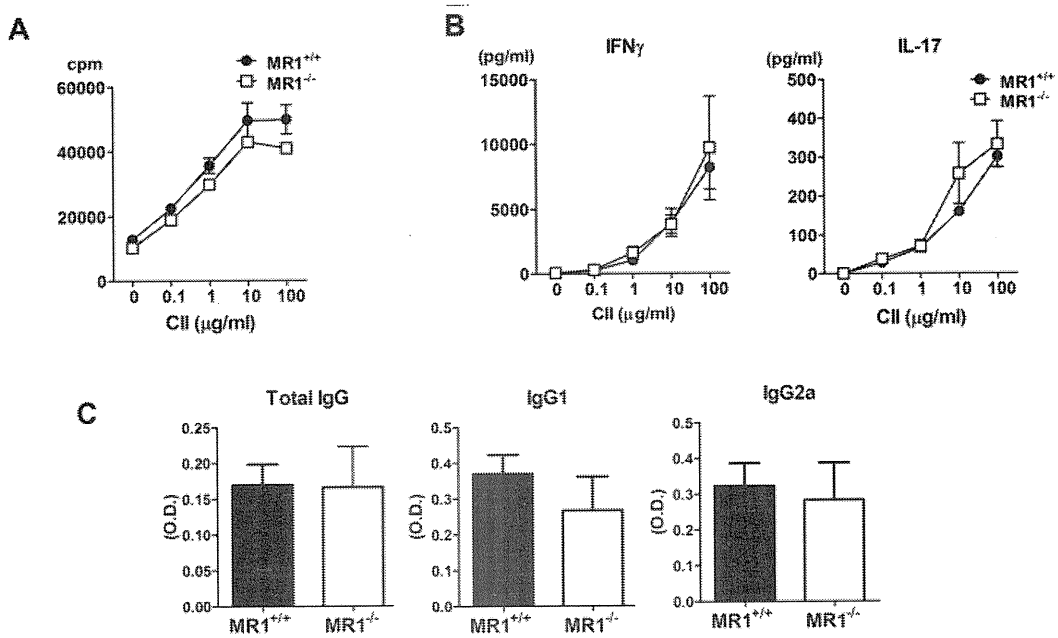

Figure 2. Type II collagen (CII) responses in MR1$^{-/-}$ DBA/1J mice. A and B, Inguinal lymph node cells from MR1$^{-/-}$ DBA/1J mice and MR1$^{+/+}$ DBA/1J mice with collagen-induced arthritis were incubated for 48 hours in the presence of CII. Proliferative responses were determined by the uptake of ^{3}H-thymidine (A), and the levels of interferon-γ (IFNγ) and interleukin-17 (IL-17) in culture supernatants were measured by enzyme-linked immunosorbent assay (B). C, CII-specific antibody levels in individual serum samples obtained at the end of the experiment were analyzed as described in Materials and Methods. Results from a single representative experiment of 2 similar experiments performed are shown. Values are the mean ± SEM of 5–8 mice per group. OD = optical density

the mean ± SEM clinical score for the group, and statistical differences were analyzed with a nonparametric Mann-Whitney U test. Data for cytokines and proliferation were analyzed with an unpaired *t*-test.

RESULTS

Amelioration of CIA in MR1$^{-/-}$ mice. To investigate whether MAIT cells play a role in the pathogenesis of arthritis, we first evaluated the involvement of MAIT cells in CIA using MR1$^{-/-}$ mice lacking MAIT cells. Because DBA/1J mice bearing the H-2q haplotype are the most susceptible strain for CIA, MR1$^{-/-}$ C57BL/6J mice were backcrossed to DBA/1J mice for 10 generations to obtain MR1$^{-/-}$ DBA/1J mice. Both MR1$^{-/-}$ DBA/1J mice and littermate MR1$^{+/+}$ DBA/1J mice were immunized with CII to induce CIA, and the clinical severity of arthritis was evaluated by visual scoring of each paw. As shown in Figure 1A, the clinical scores in MR1$^{-/-}$ DBA/1J mice were reduced in comparison to those in MR1$^{+/+}$ DBA/1J mice. Histologic examination of the joints of the 4 paws 44 days after CIA induction showed less cell infiltration, cartilage erosion, and bone destruction in MR1$^{-/-}$ DBA/1J mice than in the MR1$^{+/+}$ DBA/1J mice (Figure 1B). Quantification of the histologic severity of arthritis revealed that MR1$^{-/-}$ DBA/1J mice developed milder joint inflammation than MR1$^{+/+}$ DBA/1J mice (Figure 1C). These results suggest that MAIT cells contribute to the exacerbation of the disease course of CIA.

CII responses in MR1$^{-/-}$ DBA/1J mice. As the presence of MAIT cells augmented the severity of CIA, we next asked whether MAIT cells influence the CII-specific responses of T and B cells. Lymph node cells from CIA-induced animals were rechallenged with CII ex vivo. As shown in Figure 2A, the proliferative responses of lymph node cells upon stimulation with CII were similar in the two groups. Lymph node cells from both MR1$^{-/-}$ DBA/1J mice and MR1$^{+/+}$ DBA/1J mice produced comparable amounts of IL-17 and IFNγ in response to CII in a dose-dependent manner (Figure 2B). We also evaluated CII-specific immunoglobulin levels in serum obtained 35-42 days after arthritis induction. We observed a trend of reduced levels of CII-specific IgG1 in MR1$^{-/-}$ DBA/1J mice compared to the

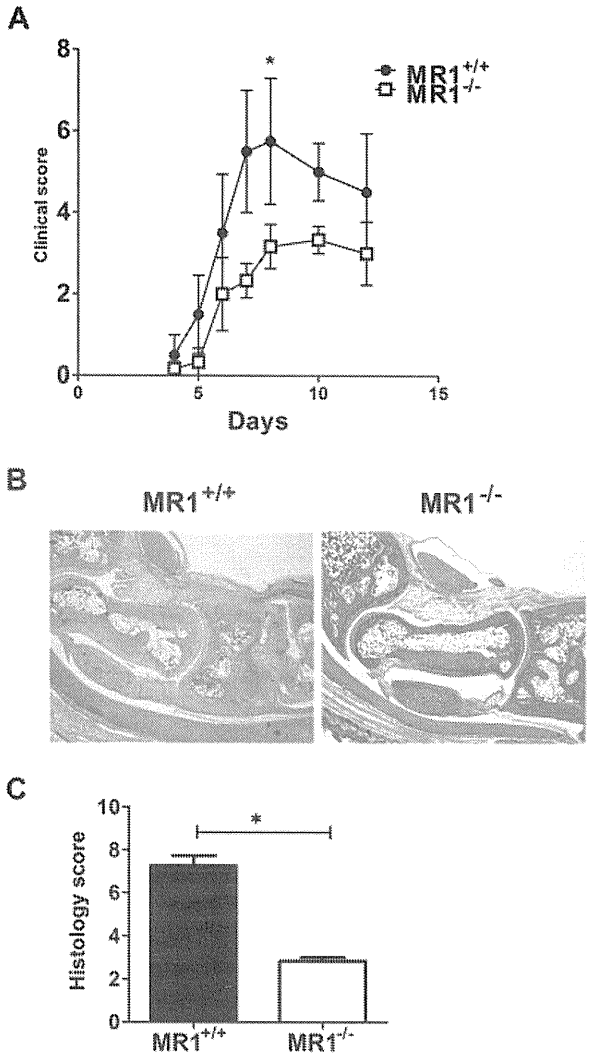

Figure 3. Amelioration of collagen antibody–induced arthritis (CAIA) in $MR1^{-/-}$ mice. A, Clinical scores for CAIA in $MR1^{-/-}$ C57BL/6J mice and $MR1^{+/+}$ C57BL/6J mice. Values are the mean ± SEM of 4–6 mice per group. * = $P < 0.05$ versus $MR1^{-/-}$ C57BL/6J mice. B, Representative histologic sections of the joints of $MR1^{+/+}$ C57BL/6J mice and $MR1^{-/-}$ C57BL/6J mice. Hematoxylin and eosin stained; original magnification × 40. C, Histology scores in $MR1^{-/-}$ C57BL/6J mice and in $MR1^{+/+}$ C57BL/6J mice, expressed as the sum of the scores in the 4 paws. Results from a single representative experiment of 2 similar experiments performed are shown. Values are the mean ± SEM. * = $P < 0.05$.

levels in $MR1^{+/+}$ DBA/1J mice, but the difference did not reach statistical significance (Figure 2C). These results indicate that the presence of MAIT cells has little effect on CII-specific responses.

Amelioration of CAIA in $MR1^{-/-}$ mice. The CIA model requires both adaptive and innate immune responses for disease development, and T cells and B cells responding to CII are the major players in the initiation of the disease. Although we observed significant differences in both the clinical and pathologic severity of arthritis when comparing $MR1^{-/-}$ DBA/1J mice to $MR1^{+/+}$ DBA/1J mice (Figure 1), the CII-specific responses of T and B cells appeared not to depend on the presence of MAIT cells (Figure 2). Thus, we hypothesized that MAIT cells may influence the effector phase of arthritis. To test this hypothesis, we induced CAIA in $MR1^{-/-}$ and $MR1^{+/+}$ C57BL/6J mice. By 7 days after injection of anti-CII mAb, $MR1^{+/+}$ C57BL/6J mice had developed severe arthritis, as assessed by clinical scores (Figure 3A). In contrast, the clinical scores in the $MR1^{-/-}$ C57BL/6J mice were lower compared to those in the $MR1^{+/+}$ C57BL/6J mice. Histologic assessment 10 days after arthritis induction revealed severe arthritis with leukocyte infiltration, synovial hyperplasia, pannus formation, cartilage erosion, and bone destruction in $MR1^{+/+}$ C57BL/6J mice, whereas these features were milder in $MR1^{-/-}$ C57BL/6J mice (Figures 3B and C).

Augmentation of arthritis in $MR1^{-/-}$ mice by adoptive transfer of MAIT cells. To demonstrate that MAIT cells actually enhance disease severity in the

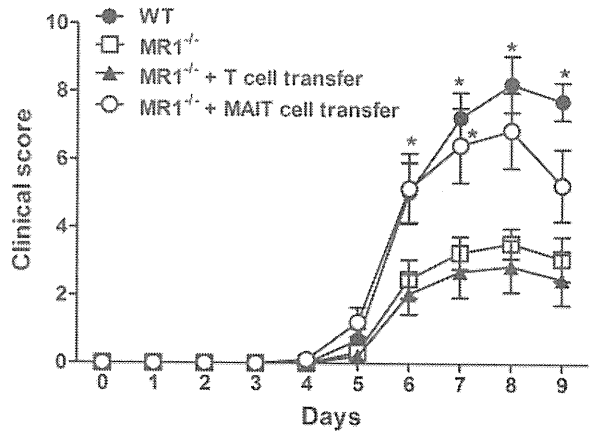

Figure 4. Augmentation of arthritis by adoptive transfer of mucosal-associated invariant T (MAIT) cells in $MR1^{-/-}$ mice. $MR1^{-/-}$ C57BL/6J mice received 5 × 10^5 NK1.1+TCRβ+ T cells (MAIT cells) or an equal number of NK1.1–TCRβ+ cells (T cells) from V_α19-transgenic $CD1d1^{-/-}$ mice. One day later, collagen antibody–induced arthritis was induced in wild-type (WT) C57BL/6J mice, $MR1^{-/-}$ C57BL/6J mice, and $MR1^{-/-}$ C57BL/6J mice reconstituted with T cells or MAIT cells. Results pooled from 2 similar experiments performed are shown. Values are the mean ± SEM of 8–10 mice per group. * = $P < 0.05$ versus $MR1^{-/-}$ C57BL/6J mice.

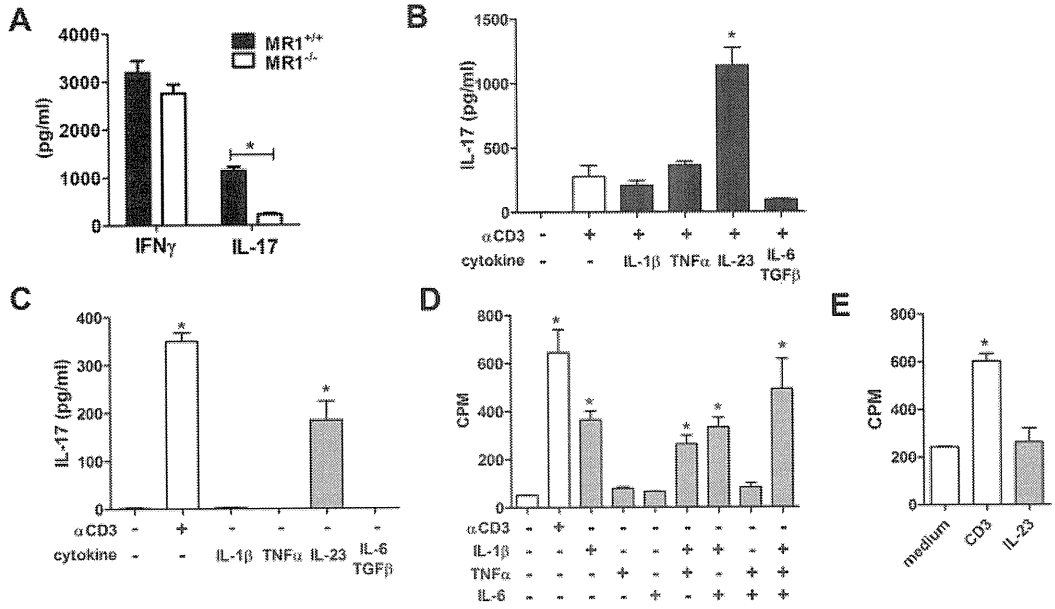

Figure 5. Cytokine-mediated mucosal-associated invariant T (MAIT) cell activation. **A**, Liver mononuclear cells from MR1$^{+/+}$ C57BL/6J mice and MR1$^{-/-}$ C57BL/6J mice were stimulated for 48 hours with immobilized anti-CD3 (αCD3) monoclonal antibody (mAb). The levels of interferon-γ (IFNγ) and interleukin-17 (IL-17) in culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA). * = $P < 0.05$. **B**, MAIT cells were stimulated for 48 hours with immobilized anti-CD3 mAb, with or without IL-1β, tumor necrosis factor α (TNFα), IL-23, or IL-6 plus transforming growth factor β (TGFβ), and the levels of IL-17 were measured by ELISA. * = $P < 0.05$ versus anti-CD3 mAb stimulation alone. **C**, MAIT cells were stimulated with immobilized anti-CD3 mAb or the indicated cytokines, and IL-17 levels were measured. * = $P < 0.05$ versus unstimulated control. **D** and **E**, Proliferative responses after 48 hours of exposure to the indicated cytokines were determined as the uptake of ^{3}H-thymidine. Results from a single representative experiment of 2 similar experiments performed are shown. * = $P < 0.05$ versus unstimulated control. Values in **A–E** are the mean ± SEM.

CAIA model, we performed adoptive transfer experiments. Most NK1.1+ TCRβ T cells within liver lymphocytes from CD1d1$^{+/+}$ mice are iNKT cells, and we and other investigators previously demonstrated that the NK1.1+ TCRβ T cell population in V_α19i-transgenic CD1d1$^{-/-}$ mice is highly enriched in V_α19i TCR+ cells (15,17). Thus, to obtain MAIT cells, we isolated NK1.1+ TCRβ T cells from V_α19i-transgenic CD1d1$^{-/-}$ mice. We adoptively transferred these MAIT cells into MR1$^{-/-}$ C57BL/6J mice, and 1 day later, we injected these mice with anti-CII mAb to induce CAIA. MR1$^{-/-}$ C57BL/6J mice reconstituted with MAIT cells developed severe arthritis at a level similar to that of wild-type (WT) C57BL/6J mice (Figure 4). However, the transfer of an equal number of T cells into MR1$^{-/-}$ C57BL/6J mice had little effect on the clinical arthritis scores. Taken together, these results suggest that the presence of MAIT cells augmented arthritis mainly by enhancing the inflammation in arthritis.

Cytokine-mediated MAIT cell activation. To understand the mechanism by which MAIT cells exacerbate the disease course of arthritis, we first compared the cytokine-producing capacity of T cells from MR1$^{-/-}$ and WT C57BL/6J mice. Upon anti-CD3 mAb stimulation, LMNCs from MR1$^{-/-}$ and WT C57BL/6J mice produced comparable amounts of IFNγ. However, the level of IL-17 was lower in MR1$^{-/-}$ C57BL/6J mice than in WT C57BL/6J mice (Figure 5A).

It was recently demonstrated that human MAIT cells express the Th17-associated transcription factor retinoic acid receptor–related orphan nuclear receptor (ROR) and produce high levels of IL-17 (33). We therefore sought to determine whether mouse MAIT cells produce IL-17, which is known to play a pathogenic role in arthritis. MAIT cells were sorted from LMNCs obtained from V_α19i-transgenic CD1d1$^{-/-}$ mice and were stimulated ex vivo with anti-CD3 mAb. As previously shown (34), MAIT cells produced large amounts of IL-17. In addition, IL-17 production by anti-CD3 mAb–stimulated MAIT cells was augmented in the presence of IL-23 (Figure 5B).

Innate-like lymphocytes such as iNKT cells and

γ/δ T cells are known to be activated by cytokines directly, without TCR stimulation. A combination of IL-12 and IL-18 activates iNKT cells to produce IFNγ, and IL-1 together with IL-23 induces IL-17 production by γ/δ T cells (31,35,36). We therefore next asked whether MAIT cells are activated directly by cytokines. MAIT cells were incubated with various cytokines without TCR stimulation, and cytokine concentrations in the culture supernatants were evaluated. Surprisingly, MAIT cells produced high levels of IL-17 after exposure to IL-23 in the absence of TCR stimulation (Figure 5C).

Inflammatory cytokines such as IL-1β, TNFα, and IL-6 play critical roles in arthritis models and in human RA. Therefore, we next tested whether MAIT cells could be activated by these cytokines. As shown in Figure 5D, IL-1β induced robust proliferation of MAIT cells, although cytokine production was not observed after exposure to these cytokines, including IL-1β (data not shown). In addition, IL-23 did not induce proliferation of MAIT cells (Figure 5E). Thus, in the absence of TCR stimuli, IL-1β induced the proliferation of MAIT cells and IL-23 promoted the production of IL-17 by MAIT cells.

DISCUSSION

Previous studies by our group as well as others revealed that iNKT cells play pathogenic roles in CIA and CAIA by inducing a Th1 or Th17 shift of autoimmune T cells and by augmenting the inflammation in arthritis (25–27). In the present study, we demonstrated that MAIT cells contribute to the severity of CIA and CAIA mostly by augmenting joint inflammation during the effector phase of arthritis. $MR1^{-/-}$ mice were originally generated on the 129P2 background. Although $MR1^{-/-}$ mice were backcrossed onto C57BL/6 or DBA/1J, we are not able to exclude the possibility that some residual sequence from the 129P2 mice affects the arthritis susceptibility of $MR1^{-/-}$ C57BL/6 and $MR1^{-/-}$ DBA/1J mice. However, since the reconstitution of MAIT cells induced severe CAIA in $MR1^{-/-}$ C57BL/6 mice, the phenotype observed in $MR1^{-/-}$ mice seems to be dependent on the lack of MAIT cells.

It has been revealed that there are CD1d-restricted T cells that are different from iNKT cells and do not express an invariant TCRα chain ($V_\alpha14$–$J_\alpha18$ in mice and $V_\alpha24$–$J_\alpha18$ in humans). Such CD1d-restricted T cells are called type II NKT cells and possess different functions from iNKT cells. Recently, CD1d-restricted NKT cells, which recognize murine type II collagen peptide 707–721, were reported to suppress CIA (37). It is not known whether there are distinct subsets with different functions among MAIT cells or whether there are other T cells that are restricted by the MR1 molecule. As adoptively transferred $V_\alpha19i$ T cells augmented CAIA in $MR1^{-/-}$ mice, MAIT cells include the population that enhances the inflammation in arthritis.

It was recently shown that IL-17–producing γ/δ T cells were observed in the joints of mice with CIA and that blocking a certain subset of IL-17–producing γ/δ T cells suppressed CIA (29). However, γ/δ T cells have been shown to be dispensable for the development of CIA (38). In addition, anti-CII–specific antibody levels were comparable between γ/δ T cell–deficient and wild-type mice. These findings suggest that MAIT cells and γ/δ T cells share similar roles in arthritis and that both are involved mainly in the effector phase of arthritis. It is known that γ/δ T cells as well as iNKT cells are increased during CIA. Because MAIT cells share similar features with γ/δ T cells and iNKT cells, MAIT cells may also be increased during CIA.

We observed a significant decrease in IL-17 production by LMNCs upon stimulation with anti-CD3 mAb in $MR1^{-/-}$ mice compared to WT control mice. As sorted MAIT cells produced high amounts of IL-17 after anti-CD3 mAb stimulation, the major source of IL-17 responsible for the difference between $MR1^{-/-}$ and WT mice seems to be MAIT cells. Th17 cells and iNKT cells have been shown to produce IL-21, which enhanced IL-17 production or induced proliferation of IL-17–producing cells (39). It is not known whether MAIT cells produce IL-21, but MAIT cells might augment IL-17 production by other LMNCs, including γ/δ T cells, through such mechanisms. Further studies to determine whether MAIT cells regulate γ/δ T cells under both physiologic and pathologic conditions, including in the presence of arthritis, will be of interest.

The frequency of murine γ/δ T cells is 1–5% in blood lymphocytes and 25–60% in gut lymphocytes. Human γ/δ T cells also comprise up to 2–3% of peripheral T cells (9,10). Although the precise frequency of murine MAIT cells is not known, it has been speculated that MAIT cells may comprise up to 10% of double-negative T cells in the gut lamina propria and <2% of double-negative T cells in the mesenteric lymph nodes, indicating that the frequency of murine MAIT cells is much lower than that of mouse γ/δ T cells (15). It has been suggested that γ/δ T cells are the predominant source of IL-17 in the joints of CIA mice, but IL-17–producing γ/δ T cells could not be detected in RA synovial tissue (31). Recently, Martin et al (23) revealed that human MAIT cells can be identified as $V_\alpha7.2+$ $CD161^{high}$ T cells, which are abundant in blood. In addition, human MAIT cells produce IL-17 and express

tissue-homing chemokine receptors (23). An IL-17–producing CD161^{high} T cell population has been described in human arthritic joints (40). Thus, it is possible that MAIT cells rather than γ/δ T cells play a major role in the pathogenesis of human RA.

CD4+ Th17 cells require IL-6/STAT-3 activation for the expression of RORγt, which is a crucial transcription factor for IL-17 production (41). However, some innate-like lymphocyte subsets, such as iNKT cells, γ/δ T cells, and lymphoid tissue–inducer (LTi)–like cells, are known to constitutively express RORγt, IL-1 receptor type I, and IL-23R (42). In addition, these IL-17–producing innate-like lymphocytes, including LTi cells, γ/δ T cells, and iNKT cells, secrete IL-17 when stimulated by IL-23 with or without IL-1β. In this study, we demonstrated cytokine-mediated activation of MAIT cells. MAIT cells produced IL-17 in response to IL-23. Moreover, IL-1β induced proliferation of MAIT cells. Thus, it is possible that MAIT cells may contribute to the disease progression of arthritis through another mechanism in addition to IL-17 production. In adoptive transfer experiments, MAIT cells augmented the disease severity of CAIA in MR1-deficient mice. Thus, this result also indicates that MAIT cell–mediated exacerbation of arthritis may be induced by cytokines, without a requirement for TCR stimulation.

In EAE, disease suppression by MAIT cells was accompanied by a reduction in the production of cytokines, including IFNγ and IL-17, by T cells and increased IL-10 production by B cells. Encephalitogenic T cells play a major role in EAE (43,44). EAE can be induced in naive mice by transferring myelin-reactive T cells. T cell–targeted therapies, including anti–very late activation antigen 4 treatment, have been shown to suppress EAE. Although CIA was reduced in MR1$^{-/-}$ DBA/1J mice, we observed a significant decrease in CII-specific IgG1 antibody levels in these mice as compared with their WT controls in some experiments (data not shown), suggesting the inhibition of Th1 responses by MAIT cells. Therefore, it is still possible that MAIT cells suppress Th1 response during the early induction phase of CIA. MAIT cells may be functionally plastic, and thus exert different functions depending on the pathologic condition. Arthritis involves massive cytokine production due to various types of immune cell activation. Since MAIT cells can be activated by inflammatory cytokines, MAIT cells may contribute to augment the immune response once overt inflammation occurs.

In summary, we have shown that MAIT cells contribute to the progression of arthritis by enhancing the inflammation in CIA and CAIA models. In addition, we demonstrated that MAIT cells could be activated by cytokine stimulation even without TCR stimulation. We and others previously reported that, although iNKT cells play pathogenic roles in arthritis models, modulation of iNKT cell function by ligands successfully suppressed arthritis (45–47). The proportion of human MAIT cells appears to be much higher than that of mouse MAIT cells. Therefore, MAIT cells may play an important pathogenic role in human arthritis and MAIT cell–targeted therapy may hold promise as a new therapeutic intervention for arthritis, including RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Miyake had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Chiba, Tajima, Miyazaki, Miyake.
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Analysis and interpretation of data. Chiba, Tajima, Tomi, Miyazaki, Yamamura, Miyake.

REFERENCES

1. Chervonsky AV. Influence of microbial environment on autoimmunity [review]. Nat Immunol 2010;11:28–35.
2. Vaahtovuo J, Munukka E, Korkeamaki M, Luukkainen R, Toivanen P. Fecal microbiota in early rheumatoid arthritis. J Rheumatol 2008;35:1500–5.
3. Stone M, Fortin PR, Pacheco-Tena C, Inman RD. Should tetracycline treatment be used more extensively for rheumatoid arthritis? Metaanalysis demonstrates clinical benefit with reduction in disease activity. J Rheumatol 2003;30:2112–22.
4. Nieuwenhuis EE, Visser MR, Kavelaars A, Cobelens PM, Fleer A, Harmsen W, et al. Oral antibiotics as a novel therapy for arthritis: evidence for a beneficial effect of intestinal Escherichia coli. Arthritis Rheum 2000;43:2583–9.
5. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 2010;32:815–27.
6. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells [review]. Nat Immunol 2008;9:503–10.
7. Kronenberg M, Kinjo Y. Innate-like recognition of microbes by invariant natural killer T cells [review]. Curr Opin Immunol 2009;21:391–6.
8. Brigl M, Brenner MB. How invariant natural killer T cells respond to infection by recognizing microbial or endogenous lipid antigens [review]. Semin Immunol 2010;22:79–86.
9. Carding SR, Egan PJ. γδ T cells: functional plasticity and heterogeneity [review]. Nat Rev Immunol 2002;2:336–45.
10. Bonneville M, O'Brien RL, Born WK. γδ T cell effector functions: a blend of innate programming and acquired plasticity [review]. Nat Rev Immunol 2010;10:467–78.
11. Allman D, Pillai S. Peripheral B cell subsets [review]. Curr Opin Immunol 2008;20:149–57.

12. Lopes-Carvalho T, Foote J, Kearney JF. Marginal zone B cells in lymphocyte activation and regulation [review]. Curr Opin Immunol 2005;17:244–50.
13. Le Bourhis L, Guerri L, Dusseaux M, Martin E, Soudais C, Lantz O. Mucosal-associated invariant T cells: unconventional development and function [review]. Trends Immunol 2011;32:212–8.
14. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 2003;422:164–9.
15. Kawachi I, Maldonado J, Strader C, Gilfillan S. MR1-restricted Vα19i mucosal-associated invariant T cells are innate T cells in the gut lamina propria that provide a rapid and diverse cytokine response. J Immunol 2006;176:1618–27.
16. Illes Z, Shimamura M, Newcombe J, Oka N, Yamamura T. Accumulation of Vα7.2-Jα33 invariant T cells in human autoimmune inflammatory lesions in the nervous system. Int Immunol 2004;16:223–30.
17. Croxford JL, Miyake S, Huang YY, Shimamura M, Yamamura T. Invariant Vα19i T cells regulate autoimmune inflammation. Nat Immunol 2006;7:987–94.
18. Peterfalvi A, Gomori E, Magyarlaki T, Pal J, Banati M, Javorhazy A, et al. Invariant Vα7.2-Jα33 TCR is expressed in human kidney and brain tumors indicating infiltration by mucosal-associated invariant T (MAIT) cells. Int Immunol 2008;20:1517–25.
19. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol 2010;8:e1000407.
20. Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010;11:701–8.
21. Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, et al. An invariant T cell receptor α chain defines a novel TAP-independent major histocompatibility complex class IB-restricted α/β T cell subpopulation in mammals. J Exp Med 1999; 189:1907–21.
22. Lantz O, Bendelac A. An invariant T cell receptor α chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4−CD8− T cells in mice and humans. J Exp Med 1994;180:1097–106.
23. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, et al. Stepwise development of MAIT cells in mouse and human. PLoS Biol 2009;7:e54.
24. Lo CK, Lam QL, Sun L, Wang S, Ko KH, Xu H, et al. Natural killer cell degeneration exacerbates experimental arthritis in mice via enhanced interleukin-17 production. Arthritis Rheum 2008;58: 2700–11.
25. Chiba A, Kaieda S, Oki S, Yamamura T, Miyake S. The involvement of V_α14 natural killer T cells in the pathogenesis of arthritis in murine models. Arthritis Rheum 2005;52:1941–8.
26. Kim HY, Kim HJ, Min HS, Kim S, Park WS, Park SH, et al. NKT cells promote antibody-induced joint inflammation by suppressing transforming growth factor β1 production. J Exp Med 2005;201: 41–7.
27. Ohnishi Y, Tsutsumi A, Goto D, Itoh S, Matsumoto I, Taniguchi M, et al. TCR Vα14 natural killer T cells function as effector T cells in mice with collagen-induced arthritis. Clin Exp Immunol 2005;141:47–53.
28. Teige A, Bockermann R, Hasan M, Olofsson KE, Liu Y, Issazadeh-Navikas S. CD1d-dependent NKT cells play a protective role in acute and chronic arthritis models by ameliorating antigen-specific Th1 responses. J Immunol 2010;185:345–56.
29. Roark CL, French JD, Taylor MA, Bendele AM, Born WK, O'Brien RL. Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing γδ T cells. J Immunol 2007;179:5576–83.
30. Peterman GM, Spencer C, Sperling AI, Bluestone JA. Role of γδ T cells in murine collagen-induced arthritis. J Immunol 1993;151: 6546–58.
31. Ito Y, Usui T, Kobayashi S, Iguchi-Hashimoto M, Ito H, Yoshitomi H, et al. Gamma/delta T cells are the predominant source of interleukin-17 in affected joints in collagen-induced arthritis, but not in rheumatoid arthritis. Arthritis Rheum 2009;60:2294–303.
32. Okamoto N, Kanie O, Huang YY, Fujii R, Watanabe H, Shimamura M. Synthetic α-mannosyl ceramide as a potent stimulant for an NKT cell repertoire bearing the invariant Vα19-Jα26 TCR α chain. Chem Biol 2005;12:677–83.
33. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, $CD161^{hi}$ IL-17-secreting T cells. Blood 2011;117:1250–9.
34. Shimamura M, Huang YY, Kobayashi M, Goji H. Altered production of immunoregulatory cytokines by invariant Vα19 TCR-bearing cells dependent on the duration and intensity of TCR engagement. Int Immunol 2009;21:179–85.
35. Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. J Immunol 2007; 178:2706-13.
36. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from γδ T cells, amplifying Th17 responses and autoimmunity. Immunity 2009;31:331–41.
37. Liu Y, Teige A, Mondoc E, Ibrahim S, Holmdahl R, Issazadeh-Navikas S. Endogenous collagen peptide activation of CD1d-restricted NKT cells ameliorates tissue-specific inflammation in mice. J Clin Invest 2011;121:249–64.
38. Corthay A, Johansson A, Vestberg M, Holmdahl R. Collagen-induced arthritis development requires αβT cells but not γδ T cells: studies with T cell-deficient (TCR mutant) mice. Int Immunol 1999;11:1065–73.
39. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity [review]. Annu Rev Immunol 2008;26:57–79.
40. Billerbeck E, Kang YH, Walker L, Lockstone H, Grafmueller S, Fleming V, et al. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. Proc Natl Acad Sci U S A 2010;107:3006–11.
41. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations [review]. Annu Rev Immunol 2010:445–89.
42. Cua DJ, Tato CM. Innate IL-17–producing cells: the sentinels of the immune system [review]. Nat Rev Immunol 2010;10:479–89.
43. Wekerle H. Lessons from multiple sclerosis: models, concepts, observations [review]. Ann Rheum Dis 2008;67 Suppl III:iii56–60.
44. Fletcher JM, Lalor SJ, Sewwney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis [review]. Clin Exp Immunol 2010;162:1–11.
45. Chiba A, Oki S, Miyamoto K, Hashimoto H, Yamamura T, Miyake S. Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosine-truncated analog of α-galactosylceramide. Arthritis Rheum 2004;50:305–13.
46. Coppieters K, Van Beneden K, Jacques P, Dewint P, Vervloet A, Vander Cruyssen B, et al. A single early activation of invariant NK T cells confers long-term protection against collagen-induced arthritis in a ligand-specific manner. J Immunol 2007;179:2300–9.
47. Kaieda S, Tomi C, Oki S, Yamamura T, Miyake S. Activation of invariant natural killer T cells by synthetic glycolipid ligands suppresses autoantibody-induced arthritis. Arthritis Rheum 2007; 56:1836–45.

Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica

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Neuromyelitis optica (NMO) is an inflammatory disease affecting the optic nerve and spinal cord, in which autoantibodies against aquaporin 4 (AQP4) water channel protein probably play a pathogenic role. Here we show that a B-cell subpopulation, exhibiting the $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ phenotype, is selectively increased in the peripheral blood of NMO patients and that anti-AQP4 antibodies (AQP4-Abs) are mainly produced by these cells in the blood of these patients. These B cells showed the morphological as well as the phenotypical characteristics of plasmablasts (PB) and were further expanded during NMO relapse. We also demonstrate that interleukin 6 (IL-6), shown to be increased in NMO, enhanced the survival of PB as well as their AQP4-Ab secretion, whereas the blockade of IL-6 receptor (IL-6R) signaling by anti–IL-6R antibody reduced the survival of PB in vitro. These results indicate that the IL-6–dependent B-cell subpopulation is involved in the pathogenesis of NMO, thereby providing a therapeutic strategy for targeting IL-6R signaling.

neuroinflamatory disease | autoimmunity | multiple sclerosis | central nervous system | IL-6 receptor blockade

Neuromyelitis optica (NMO) is an inflammatory demyelinating disorder characterized by recurrent attacks of severe optic neuritis and myelitis. Unlike the conventional form of multiple sclerosis (CMS), the lesions of NMO tend to spare the cerebral white matter, especially during the early stage (1), and even a single episode of attack can cause serious neurological deficits such as total blindness and paraplegia. Accordingly, accumulation of irreversible damage to the central nervous system (CNS) along with rapid progression of disability is more frequently found in NMO compared with CMS (2).

NMO can be distinguished from CMS by clinical, neuroimaging, and serological criteria (3). It is now known that serum anti-aquaporin 4 (AQP4) autoantibodies can be used as a disease marker of NMO (1, 2). AQP4 is the most abundantly expressed water channel protein in the CNS and is highly expressed in the perimicrovessel astrocyte foot processes, glia limitans, and ependyma (4). Emerging clinical and pathological observations suggest that anti-AQP4 antibodies (AQP4-Abs) play a key role in the pathogenesis of NMO. Prior studies have documented a significant correlation of serum AQP4-Ab levels with the therapeutic efficacy of plasma exchange during clinical exacerbations of NMO (2, 5). In the CNS lesions of NMO, reduced expression of AQP4 on astrocytes is evident even during the early stage (6), which is followed by the occurrence of vasculocentric destruction of astrocytes associated with perivascular deposition of complement and IgG (7).

On the other hand, recent studies have suggested that AQP4-Abs alone are incapable of causing the clinical and pathological features of NMO. In fact, Hinson et al. emphasized the role of cellular immunity in combination with AQP4-Abs by showing that the attack severity of NMO was not correlated with serum AQP4-Ab levels (8). It was also demonstrated that direct injection of IgGs derived from NMO patients into the brains of naïve mice did not cause NMO-like lesions, although brain tissue destruction associated with leukocyte infiltration was elicited by coinjecting human complement (9). Other groups have shown that the passive transfer of IgGs from NMO patients to rats challenged with induction of experimental autoimmune encephalomyelitis (EAE) may cause a decrease in the expression of AQP4 in astrocytes along with worsening of clinical EAE (10–12). In contrast, the transfer of IgGs to unimmunized rats did not cause any pathology. These results suggest that induction of AQP4-Ab–mediated pathology in NMO depends on the presence of complement, leukocytes, and T cells.

Although AQP4-Ab–secreting cells are a potential target for therapy, detailed characteristics of AQP4-Ab–producing cells have not been clarified yet. Because some NMO patients have elevated serum anti-nuclear and anti–SS-A/SS-B Abs (1), as found in patients with systemic lupus erythematosus (SLE) or Sjögren syndrome, NMO might share common pathological mechanisms with these autoimmune diseases. Kikuchi et al. previously reported that $CD180^{-}$ B cells are activated B cells capable of producing autoantibodies in SLE (13). CD180 is a member of the leucine-rich repeat family of molecules with homology to Toll-like receptor 4 (14), which is highly expressed by naïve and memory B cells but not by plasma cells (15). Odendahl et al. demonstrated that $CD27^{high}CD38^{+}$ B cells, capable of producing high-affinity IgG (16), are increased in the peripheral blood of SLE patients with some correlation to disease activity (17). Considering the phenotypes of autoantibody-producing cells reported in SLE, we analyzed the expression of CD27, CD38, and CD180 on $CD19^{+}$ B cells in the peripheral blood of NMO patients. We found that $CD27^{high}CD38^{high}CD180^{-}$ B cells were significantly increased in AQP4-Ab seropositive patients diagnosed with NMO or NMO spectrum disorder (1) compared with healthy subjects (HS) or CMS patients. Notably, this B-cell subpopulation was found to be a major source of AQP4-Abs in the peripheral blood of AQP4-Ab seropositive patients and depended on interleukin-6 receptor (IL-6R) signaling for survival.

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Results

$CD27^{high}CD38^{high}CD180^{-}$ B Cells Were Increased in the Peripheral Blood of NMO Patients. Although AQP4-Abs are identified as IgGs (18), no prior study has focused on proportional changes of B-cell subsets in NMO. We therefore performed multicolor flow cytometric analysis of peripheral blood mononuclear cells (PBMC) derived from patients and controls. After starting the study, we soon noticed a remarkable expansion of a distinct B-cell subset in some patients with NMO. The expanded B cells were identified as a population of $CD27^{high}$, $CD38^{high}$, and $CD180^{-}$, and showed lower expression of CD19 than other B cells (Fig. 1*A*). Notably, this population did not express the B-cell marker CD20 (Fig. S1). First, we collected samples from patients in remission and analyzed the pooled data. We found that the proportion of this subpopulation among $CD19^{+}$ B cells was significantly increased in AQP4-Ab seropositive patients with NMO or NMO spectrum disorder (Fig. 1*B*) compared with HS or CMS patients. There was no significant difference in the proportion of this B-cell subpopulation between those with typical NMO and those with NMO spectrum disorder. Furthermore, the frequency of this B-cell subpopulation was correlated with the serum AQP4-Ab titer (Fig. S2). Comparison of paired samples obtained from the same patients during relapse and in remission showed that the $CD27^{high}CD38^{high}CD180^{-}$ B cells further increased during relapse (Fig. 1*C*). In contrast, the frequencies of $CD27^{-}$ naïve B cells (nB) and $CD27^{+}CD38^{-low}$ memory B cells (mB) were not altered in AQP4-Ab seropositive patients compared with controls (Fig. S3). The large majority of seropositive patients were treated with corticosteroids. However, the frequency of $CD27^{high}CD38^{high}CD180^{-}$ cells among $CD19^{+}$ B cells was not correlated with the daily corticosteroid dose given to patients (Fig. S4). Moreover, the increase in cells in NMO patients was still evident compared with that in CMS patients similarly treated with corticosteroids (Fig. S5). Taken together, the selective increase in $CD27^{high}CD38^{high}CD180^{-}$ B cells in seropositive patients was thought to reflect their role in the pathogenesis of NMO but not to be an effect of the corticosteroid treatment.

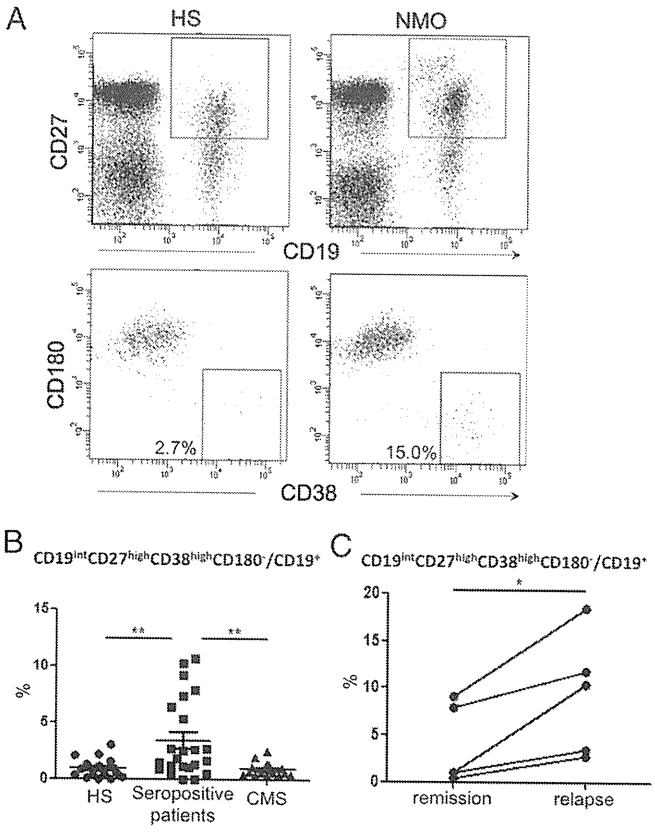

Fig. 1. $CD27^{high}CD38^{high}CD180^{-}$ B cells increased in NMO patients. (*A*) A flow cytometric scheme for the analysis of B-cell subpopulations. PBMC from HS and NMO in remission were stained with fluorescence-conjugated anti-CD19, -CD27, -CD38, and -CD180 mAbs. $CD19^{+}CD27^{+}$ cells were partitioned (*Upper*) and analyzed for expression of CD38 and CD180 (*Lower*). Values represent the percentage of $CD38^{high}CD180^{-}$ cells within $CD19^{+}CD27^{+}$ cells. (*B*) Analysis of the pooled data derived from patients in clinical remission. This shows the percentages of $CD27^{high}CD38^{high}CD180^{-}$ cells within $CD19^{+}$ cells from HS, seropositive patients, and CMS patients (**$P < 0.01$; Tukey's post hoc test). (*C*) Comparison of remission and relapse of NMO. Data obtained from the same patients are connected with lines (*$P < 0.05$; Wilcoxon signed rank test).

Expanded Cells Resemble Early Plasma Cells in Gene Expression and Morphology. To gain insights into the developmental stage of the $CD27^{high}CD38^{high}CD180^{-}$ B cells, we quantified the mRNA expression of B-cell–associated transcription factors in sorted cell populations. Compared with nB and mB, this population showed much higher expression of B-lymphocyte-induced maturation protein 1 (Blimp-1) and IFN regulatory factor 4 (IRF4), which are essential for the regulation of plasma cell differentiation (19, 20) (Fig. 2*A*). In contrast, the expression of paired box gene 5 (PAX5), known to be down-regulated in early plasma cell differentiation (21), was reciprocally reduced in the B-cell subset. This gene expression pattern is very similar to that of plasma cells. However, it was notable that the cells of interest expressed CD19, which is not detected in mature plasma cells. Moreover, only 40% of this population expressed the most reliable plasma cell marker CD138 (22). Morphological analysis also confirmed the similarity of this population to plasma cells: they exhibit eccentric nucleus, perinuclear hof region, and abundant cytoplasm. However, they possess a larger nucleus with a lower extent of chromatin clumping compared with $CD138^{+}$ plasma cells derived from HS (Fig. 2*B*). Notably, the $CD138^{+}$ population among $CD27^{high}CD38^{high}CD180^{-}$ cells in NMO patients was

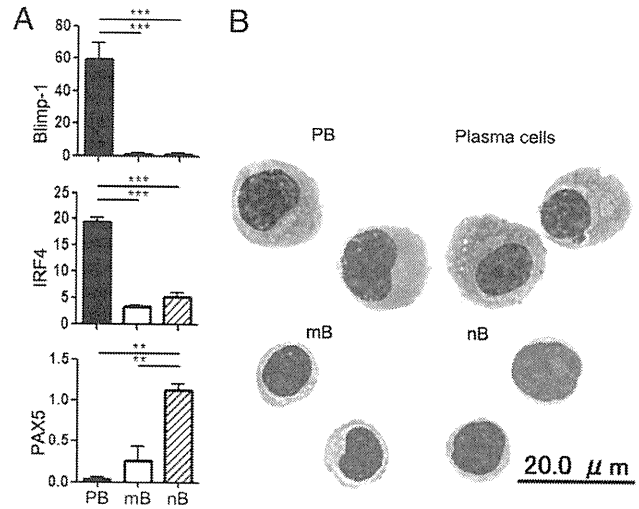

Fig. 2. Resemblance of $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ cells to plasma cells. (*A*) mRNA expression of Blimp-1, IRF4, and PAX5. B-cell subpopulations [$CD27^{high}CD38^{high}CD180^{-}$ (PB), $CD27^{-}$ naïve (nB), $CD27^{+}CD38^{-low}$ memory (mB)] were sorted by FACS and total RNA was extracted for qRT-PCR analysis. RNA levels were normalized to ACTB for each sample (**$P < 0.01$; ***$P < 0.001$; Tukey's post hoc test). (*B*) May–Grünwald–Giemsa staining of B-cell subpopulations. PB (*Upper Left*), mB (*Lower Left*), and nB (*Lower Right*) from NMO are presented along with morphologically identified plasma cells ($CD19^{int}CD27^{high}CD38^{high}CD138^{+}$) from HS (*Upper Right*).

morphologically indistinguishable from the CD138$^-$ population in NMO patients or HS, indicating the immature characteristic of CD27^{high}CD38^{high}CD180$^-$ cells (Fig. S6). These phenotypical and morphological characteristics as well as the results of the quantitative real-time PCR (qRT-PCR) analysis indicate that this B-cell population is equivalent to plasmablasts (PB) (22–26). Hereafter, we use the term "PB" to distinguish this population from other B cells.

Expression of B-Cell Cytokine Receptors on PB. Prior studies have identified cytokines that are critically involved in the differentiation and/or survival of plasma cells, including IL-6 and B-cell–activating factor (BAFF). IL-6 induces B-cell differentiation into plasma cells, maintains early plasma cell survival, and enhances plasma cell IgG secretion (24). Besides, IL-6 is elevated in the cerebrospinal fluid (CSF) or peripheral blood of NMO patients compared with that of CMS patients and HS (27, 28). In a rodent autoimmunity model, IL-6 deficiency caused impaired autoantibody secretion by B cells (29). Given the potential role of IL-6 in NMO, we performed flow cytometry analysis for the expression of IL-6R. Results showed remarkable expression of IL-6R on PB, although it was only marginal or absent on mB and nB (Fig. S7). Because BAFF and A proliferation-inducing ligand (APRIL) can also promote the survival of PB (25, 26), we next evaluated the expression of the receptors for BAFF and APRIL, BAFF receptor (BAFF-R), B-cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). Expression of BCMA and TACI was selectively up-regulated in PB in parallel with IL-6R. In contrast, BAFF-R was up-regulated in mB and nB, but not in PB (Fig. S7).

PB Is a Selective Source of AQP4-Abs in Peripheral Blood. We were interested to know whether PB were capable of producing AQP4-Abs upon stimulation with cytokines and, therefore, examined the ability of IL-6, BAFF, and APRIL to enhance AQP4-Ab secretion by PB. We cultured the isolated PB for 6 d in the presence or absence of each cytokine, and evaluated the presence of AQP4-Abs in the supernatants by measuring IgG binding to Chinese hamster ovary (CHO) cells transfected with the human AQP4 vector (CHO^{AQP4}) or the vector control (CHO^{VC}). We found that IL-6, but not BAFF or APRIL, could significantly enhance AQP4-Ab secretion from PB (Fig. S8), as assessed by specific IgG binding to CHO^{AQP4}. Further study focusing on IL-6 showed that exogenous IL-6 promoted the production of AQP4-specific IgGs from PB (Fig. 3*A*), but not from the other B-cell subpopulations. Similar results were obtained from six independent experiments (Fig. S9), indicating that PB could be major AQP4-Ab producers in PBMC. In the absence of addition of IL-6, supernatants from PB did not show any significant reactivity to CHO^{AQP4}. To further analyze the AQP4-Ab–secreting potential of each B-cell subpopulation, we next stimulated the cells with a combination of IL-6, IL-21, and anti-CD40 that efficiently induces B-cell differentiation and IgG production (30). This polyclonal stimulation induced the secretion of similar amounts of IgGs from mB and PB. However, only the supernatant of PB specifically reacted to CHO^{AQP4} cell transfectants, indicating that AQP4-Ab–producing B cells were highly enriched in PB (Fig. 3*B*).

Survival and Functions of PB Depend on IL-6 Signaling. We evaluated the influence of IL-6, BAFF, and APRIL on the survival of PB after 2 d of in vitro culture (Fig. 4*A*). Among the added cytokines, only IL-6 was found to significantly promote the survival of PB (Fig. 4*B*). We also assessed the expression levels of X-box-binding protein 1 (XBP-1) in PB by qRT-PCR after 24 h of culture with or without IL-6. XBP-1 is a transcription factor critical for IgG secretion (31), and the splicing process of XBP-1 mRNA yields a more active and stable protein. We found that the expression of both unspliced [XBP-1(u)] and spliced [XBP-1(s)] forms of XBP-1 mRNA was augmented in PB by the ad-

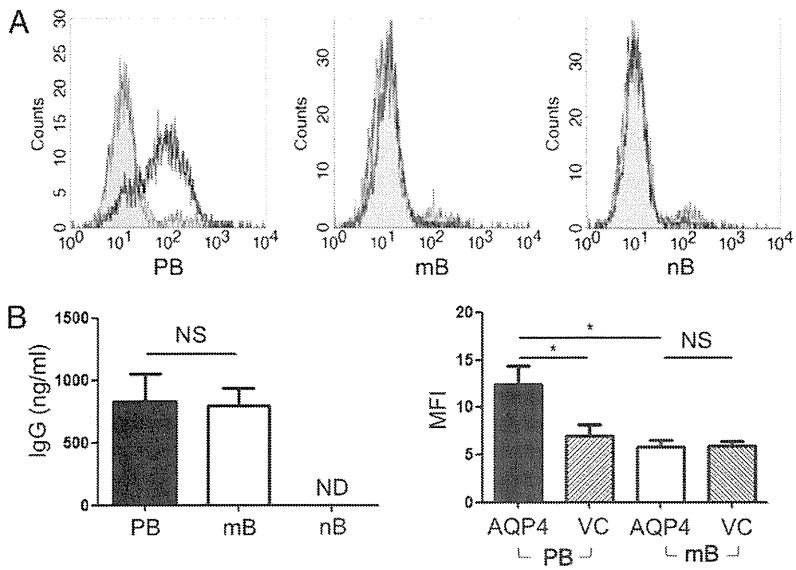

Fig. 3. Production of AQP4-Abs by PB. (*A*) Using flow cytometry, we examined whether AQP4-Abs could be produced by PB, mB, or nB cells. FACS-sorted cells were cultured with IL-6 (1 ng/mL) for 6 d and supernatants were collected. Supernatant IgGs reactive to CHO^{AQP4} (open histogram) and CHO^{VC} cells (closed histogram) were detected by anti-human IgG secondary antibody. The supernatant from PB (*Left*), but not from mB or nB, contains IgGs reactive to CHO^{AQP4}, indicating that only PB secrete AQP4-Abs after stimulation with IL-6. (*B*) Memory B cells (mB) produce IgGs but not AQP4-Abs. B-cell subpopulations were cultured in the presence of IL-6 (1 ng/mL), IL-21 (50 ng/mL), and anti-CD40 mAb (1 μg/mL) for 6 d. IgGs in the culture supernatants were measured by sandwich ELISA (*Left*) (each assay was performed in quadruplicate). Data from three patients are expressed as mean ± SD. The activity of AQP4-Abs in the culture supernatants from PB and mB was also measured by flow cytometry (*Right*). Aliquots of CHO^{AQP4} cells (AQP4) and CHO^{VC} cells (VC) (n = 4 for each) were stained with the supernatant of PB or mB from every patient. Data are expressed as median fluorescence intensity values from the results of three patients (*P < 0.05; Tukey's post hoc test). ND, not detected; NS, not significant.