were effective for mononeuritis multiplex in eight (73 %) of 11 patients. However, even combined steroids and cyclophosphamide do not always yield satisfactory therapeutic results, as seen in patient 1.

The use of IVIG may be beneficial for patients with motor or sensory neuropathy that does not respond to steroids or other immunosuppressive therapies. The possible value of IVIG has been suggested in several small uncontrolled studies. The following data are illustrative: five patients with SS and ataxic sensory neuropathy were treated with IVIG; four improved, with two improving following the first infusion [31]. However, there are some patients who do not respond to IVIG, such as patient 5. Because the clinical course of multiple mononeuropathy is acute or subacute, IVIG therapy is likely to be applied in the early stage. With respect to ataxic sensory neuropathy, there are some patients in the chronic stage who respond to IVIG treatment, such as patient 4. Thus, we consider it worthwhile to attempt IVIG therapy at least once, even in patients with chronic advanced disease. According to Morozumi et al. [32], IVIG therapy improved the visual analogue scale (VAS) scores in five patients with painful sensory neuropathy, but needed to be repeated because of recurrences. In the present study, IVIG was administered to patient 6 with painful sensory neuropathy, but did not prove effective. However, because the pathogenic mechanisms of painful sensory neuropathy and ataxic sensory neuropathy are expected to be similar, IVIG may be effective. Potential mechanisms underlying the efficacy of IVIG include the action of anti-idiotype antibody blocking or the inhibition of autoantibody production, the down-regulation of immunoglobulin production, the saturation of Fc receptors on macrophages or the inhibition of various immunological reactions, such as lymphocyte proliferation, and the inhibition of T-cell function [33, 34].

Thus, both steroids and IVIG can be expected to be effective, at least to some extent, for SS-associated peripheral neuropathy. Whether these therapeutic effects are sustained has not yet been sufficiently studied. If steroid or IVIG therapy is ineffective, other immunosuppressive therapy can be attempted. At present, only a few limited cases of immunosuppressive therapy have been reported. While the therapeutic strategy should be tailored to individual cases, studies on large numbers of patients according to the type of neuropathy are necessary.

Conclusions

A number of types of peripheral neuropathy are associated with PSS. Because the therapeutic strategies and prognosis vary depending on the type of neuropathy, a precise diagnosis is important. In clinical practice, when patients with peripheral neuropathy are encountered, it is important to consider the possibility of SS, even in patients without sicca symptoms. However, the pathological conditions of SS and its associations still remain largely unknown. Further studies are necessary.

Acknowledgments The present work was supported by Grants-in-Aid for Research on Intractable Diseases from the Ministry of Health, Labor, and Welfare of Japan.

Conflict of interest None.

References

1. Alexander EL, Malinow K, Lejewski JE. Primary Sjögren's syndrome with central nervous system disease mimicking multiple sclerosis. Ann Intern Med. 1986;104:323–30.
2. Rapoport P, Merle H, Smadja D, Gérard M, Alliot E. Bilateral optic neuropathy disclosing primary Gougerot–Sjögren syndrome. J Fr Ophtalmol. 1997;20:767–70.
3. Youinou Y. Sjögren's syndrome: a quintessential B cell-induced autoimmune disease. Joint Bone Spine. 2008;75:1–2.
4. Hietaharju A, Yli-Kerttula U, Häkkinen V, Frey H. Nervous system manifestations in Sjogren's syndrome. Acta Neurol Scand. 1990;81:144–52.
5. Visser LH, Koudstaal PJ, Van De Merwe JP. Hemiparkinsonism in a patient with primary Sjögren's syndrome. A case report and a review of the literature. Clin Neurol Neurosurg. 1993;95:141–5.
6. Gemignani F, Marbini A, Pavesi G, Di Vittorio S, Manganelli P, Cenacchi G, et al. Peripheral neuropathy associated with primary Sjogren's syndrome. J Neurol Neurosurg Psychiatry. 1994;57:983–6.
7. Delalande S, de Seze J, Fauchais AL, Hachulla E, Stojkovic T, Ferriby D, et al. Neurologic manifestations in primary Sjogren syndrome: a study of 82 patients. Medicine. 2004;83:280–91.
8. Fujibayashi T, Sugai S, Miyasaka N, Toujou T, Miyawaki S, Ichikawa Y, et al. Revised Japanese diagnostic criteria for Sjogren's syndrome. Annual Report of Research Committee for Immune Disease. Tokyo: Japanese Ministry of Health and Welfare; 1999. p. 135–8.
9. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61:554–8.
10. Harboe E, Tjensvoll AB, Maroni S, Gøransson LG, Greve OJ, Beyer MK, et al. Neuropsychiatric syndromes in patients with systemic lupus erythematosus and primary Sjögren syndrome: a comparative population-based study. Ann Rheum Dis. 2009;68:1541–6.
11. Skopouli FN, Dafni U, Ioannidis JP, Moutsopoulos HM. Clinical evolution, and morbidity and mortality of primary Sjögren's syndrome. Semin Arthritis Rheum. 2000;29:296–304.
12. Pavlakis PP, Alexopoulos H, Kosmidis ML, Mamali I, Moutsopoulos HM, Tzioufas AG, et al. Peripheral neuropathies in Sjögren's syndrome: a critical update on clinical features and pathogenetic mechanisms. J Autoimmun. 2012;39:27–33.
13. Amoura Z, Lafitte C, Piette JC. Gougerot–Sjogren syndrome. Neurologic complications. Presse Med. 1999;28:1209e13.
14. Mrabet D, Meddeb N, Ajlani H, et al. Cerebral vasculitis in a patient with rheumatoid arthritis. Joint Bone Spine. 2007;74:201–4.

15. Mellgren SI, Conn DL, Stevens JC, Dyck PJ. Peripheral neuropathy in primary Sjögren's syndrome. Neurology. 1989;39: 390–4.
16. Mori K, Iijima M, Koike H, Hattori N, Tanaka F, Watanabe H, et al. The wide spectrum of clinical manifestations in Sjögren's syndrome-associated neuropathy. Brain. 2005;128:2518.
17. Mori K, Koike H, Misu K, Hattori N, Ichimura M, Sobue G. Spinal cord magnetic resonance imaging demonstrates sensory neuronal involvement and clinical severity in neuronopathy associated with Sjögren's syndrome. J Neurol Neurosurg Psychiatry. 2001;71:488–92.
18. Chai J, Herrmann DN, Stanton M, Barbano RL, Logigian EL. Painful small-fiber neuropathy in Sjögren syndrome. Neurology. 2005;65:925.
19. Font J, Ramos-Casals M, de la Red G, Pou A, Casanova A, García-Carrasco M, et al. Pure sensory neuropathy in primary Sjögren's syndrome. Long-term prospective follow-up and review of the literature. J Rheumatol. 2003;30:1552.
20. Waterschoot MP, Guerit JM, Lambert M, de Barsy T. Bilateral tonic pupils and polyneuropathy in Sjogren's syndrome: a common pathophysiological mechanism? Eur Neurol. 1991;31: 114–6.
21. Moutsopoulos HM, Sarmas JH, Talal N. Is central nervous system involvement a systemic manifestation of primary Sjögren's syndrome? Rheum Dis Clin North Am. 1993;19:909.
22. Alexander EL. Neurologic disease in Sjögren's syndrome: mononuclear inflammatory vasculopathy affecting central/peripheral nervous system and muscle. A clinical review and update of immunopathogenesis. Rheum Dis Clin North Am. 1993;19:869.
23. Alexander EL, Provost TT, Stevens MB, Alexander GE. Neurologic complications of primary Sjogren's syndrome. Medicine. 1982;61:247–57.
24. Konttinen YT, Kinnunen E, Bonsdorff MV. Acute transverse myelopathy successfully treated with plasmapheresis and prednisone in a patient with primary Sjögren's syndrome. Arthritis Rheum. 1987;30:339–43.
25. Williams CS, Butler E, Roman GC. Treatment of myelopathy in Sjögren syndrome with a combination of prednisone and cyclophosphamide. Arch Neurol. 2001;58:815e9.
26. Sobue G, Yasuda T, Kachi T, Sakakibara T, Mitsuma T. Chronic progressive sensory ataxic neuropathy: clinicopathological features of idiopathic and Sjogren's syndrome-associated cases. J Neurol. 1993;240:1–7.
27. Grant IA, Hunder GG, Homburger HA, Dyck PJ. Peripheral neuropathy associated with sicca complex. Neurology. 1997;48: 855–62.
28. Mori K, Iijima M, Sugiura M, Koike H, Hattori N, Ito H, et al. Sjögren's syndrome associated painful sensory neuropathy without sensory ataxia. J Neurol Neurosurg Psychiatry. 2003;74: 1320–2.
29. Amoura Z, Lafitte C, Piette JC. Gougerot–Sjogren syndrome. Neurologic complications. Presse Med. 1999;28:1209–1.
30. Alexander EL. Neurologic complications of Sjogren's syndrome. Rheum Dis Clin North Am. 1992;18:637–72.
31. Takahashi Y, Takata T, Hoshino M, Sakurai M, Kanazawa I. Benefit of IVIG for long-standing ataxic sensory neuronopathy with Sjögren's syndrome, IV immunoglobulin. Neurology. 2003; 60:503.
32. Morozumi S, Kawagashira Y, Iijima M, Koike H, Hattori N, Katsuno M, et al. Intravenous immunoglobulin treatment for painful sensory neuropathy associated with Sjögren's syndrome. J Neurol Sci. 2009;15(279):57–61.
33. Thornton CA, Griggs RC. Plasma exchange and intravenous immunoglobulin treatment of neuromuscular disease. Ann Neurol. 1994;35:260–8.
34. Hartung HP, Pollard JD, Harvey GK, Toyka KV. Immunopathogenesis and treatment of the Guillain–Barré syndrome—part II. Muscle Nerve. 1995;18:154–64.

European Journal of Immunology

Phenotype conversion from rheumatoid arthritis to systemic lupus erythematosus by introduction of *Yaa* mutation into FcγRIIB-deficient C57BL/6 mice

Shinya Kawano[*1], *Qingshun Lin*[*2], *Hirofumi Amano*[1], *Toshiyuki Kaneko*[1], *Keiko Nishikawa*[2], *Hiromichi Tsurui*[2], *Norihiro Tada*[3], *Hiroyuki Nishimura*[4], *Toshiyuki Takai*[5], *Toshikazu Shirai*[2], *Yoshinari Takasaki*[1] *and Sachiko Hirose*[2]

[1] Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan
[2] Department of Pathology, Juntendo University School of Medicine, Tokyo, Japan
[3] Atopy Research Center, Juntendo University School of Medicine, Tokyo, Japan
[4] Toin Human Science and Technology Center, Department of Biomedical Engineering, Toin University of Yokohama, Yokohama, Japan
[5] Department of Experimental Immunology and CREST of JST, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

We previously established an IgG Fc receptor IIB (FcγRIIB)-deficient C57BL/6 (B6)-congenic mouse strain (KO1), which spontaneously develops rheumatoid arthritis (RA), but not systemic lupus erythematosus (SLE). Here, we show that when Y chromosome-linked autoimmune acceleration (*Yaa*) mutation was introduced in KO1 strain (KO1.*Yaa*), the majority of KO1.*Yaa* mice did not develop RA, but instead did develop SLE. This phenotype conversion did not depend on autoantibody specificity, since KO1.*Yaa* mice, compared with KO1, showed a marked increase in serum levels of both lupus-related and RA-related autoantibodies. The increase in frequencies of CD69$^+$ activated B cells and T cells, and the spontaneous splenic GC formation with T follicular helper cell generation were manifest early in life of KO1.*Yaa*, but not KO1 and B6.*Yaa*, mice. Activated CD4$^+$ T cells from KO1.*Yaa* mice showed upregulated production of IL-21 and IL-10, compared with the finding in KO1 mice, indicating the possibility that this aberrant cytokine milieu relates to the disease phenotype conversion. Thus, our model is useful to clarify the shared and the disease-specific mechanisms underlying the clinically distinct systemic autoimmune diseases RA and SLE.

Keywords: Cytokines · FcγRIIB receptor · Rheumatoid arthritis · Systemic lupus erythematosus · *Yaa* mutation

Introduction

IgG Fc receptor IIB (FcγRIIB) is a major negative regulator of BCR-mediated activation signals in B cells [1]. We previously found that the *Fcgr2b* gene encoding FcγRIIB is polymorphic, and that autoimmune disease-prone mouse strains, such as NZB, BXSB, MRL, and NOD, all share deletion polymorphism in the AP-4-binding site in the *Fcgr2b* promoter region [2]. Because of the

Correspondence: Prof. Sachiko Hirose
e-mail: sacchi@juntendo.ac.jp

*These authors contributed equally to this work.

defective AP-4 binding, mice with this autoimmune-type allele polymorphism show downregulation of FcγRIIB expression levels in activated GC B cells, resulting in upregulation of IgG autoantibody production [3, 4]. These observations suggested that the autoimmune-type *Fcgr2b* confers the basis of susceptibility to autoimmune diseases. Consistent was our earlier finding that systemic lupus erythematosus (SLE) phenotypes in BXSB male mice carrying Y chromosome-linked autoimmune acceleration (*Yaa*) mutation were almost completely inhibited by the substitution of the autoimmune-type *Fcgr2b* for the wild C57BL/6 (B6)-type *Fcgr2b* [5]. However, because BXSB female mice carrying the autoimmune-type *Fcgr2b* but lacking *Yaa* did not develop SLE, it is likely that the autoimmune-type *Fcgr2b* contributes to SLE susceptibility through a strong epistatic interaction with *Yaa* mutation.

To examine further the role of the downregulated expression of FcγRIIB in autoimmune diseases, we recently established an FcγRIIB-deficient B6 mouse strain, KO1, by gene targeting in 129-derived embrionic stem cells and selective backcrossing to a B6 background. Intriguingly, KO1 did not develop SLE, but instead developed severe rheumatoid arthritis (RA), as reported previously [6]. This KO1 strain carried a 129-derived approximately 6.3 Mb interval distal from the null-mutated *Fcgr2b* gene within the *Sle16* locus, which is shown to induce loss of self-tolerance in the B6 background [7]. Boross et al. [8] reported that FcγRIIB-deficient B6 mice generated by gene targeting in B6-derived embryonic stem cells, thus lacking the 129-derived flanking *Sle16* locus, fail to develop any sign of autoimmune diseases. Thus, the development of RA in KO1 mice may be due to the epistatic interaction of FcγRIIB deficiency and *Sle16* locus.

Boross et al. [8] also reported that their FcγRIIB-deficient B6 mice develop lethal lupus nephritis in the presence of *Yaa* mutation, indicating the epistatsis between FcγRIIB-deficiency and *Yaa* in the development of full-blown autoimmune diseases. In addition, Subramanian et al. [9] reported that the strong epistatic interaction between *Yaa* and *Sle1*, which contains the autoimmune-predisposing *Slam/Cd2* haplotype, contributes to severe lupus nephritis. The *Sle16* locus also contains this autoimmune-predisposing *Slam/Cd2* haplotype [10].

In contrast to the accelerated effect of *Yaa* on lupus nephritis, Jansson and Holmdahl [11] reported the suppressive effect of *Yaa* on collagen-induced arthritis. In the present study, we have introduced *Yaa* mutation into FcγRIIB-deficient RA-prone KO1 mice to examine how *Yaa* affects the disease phenotypes in these mice. We found that the majority of KO1.*Yaa* mice did not develop RA, but instead did develop severe SLE early in life, and that this phenotype conversion did not depend on the shift of autoantibody specificity from RA-related to lupus-related one.

Characteristic clinical features differ between RA and SLE; however, both diseases share aberrant activation of immune processes associated with the production of a variety of autoantibodies and subsequent immune complex-mediated tissue inflammation. Our model is useful to investigate the shared and the disease-specific factors contributing to the clinically distinct systemic autoimmune diseases RA and SLE.

Results

Disease phenotype in Yaa-carrying FcγRIIB-deficient KO1 mice

KO1 mice developed arthritis after 4 months of age and the disease incidence and severity were increased with age. At 8 months of age, 67% of KO1 mice showed arthritis with marked swelling and stiffness of forepaws and hindpaws. In contrast, the incidence and severity of arthritis were markedly suppressed in KO1.*Yaa* mice and 88% of KO1.*Yaa* mice were free from arthritis (Fig. 1A). Representative macroscopic findings of forepaws and hindpaws of KO1 and KO1.*Yaa* mice at 8 months of age are shown in Figure 1B. Intriguingly, KO1 strain did not develop proteinuria; however, KO1.*Yaa* began to be positive for proteinuria at 2 months of age and the incidence of positive proteinuria reached 63% (Fig. 1C) with 46% mortality rate at 8 months of age (Fig. 1D).

Figure 1E shows a comparison of representative histopathological and immunofluorescent findings of renal glomeruli among B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice at 4 months of age. In KO1.*Yaa* mice, glomeruli were significantly enlarged even at 4 months of age (Fig. 1F), because of a marked cellular proliferation in glomeruli and a large amount of IgG deposition in mesangial area and along glomerular capillary walls. These glomerular lesions were seldom observed in B6, B6.*Yaa*, and KO1 mice even at 8 months of age.

Serum levels of autoantibodies

To examine the relationship between the disease phenotype conversion from RA to SLE and the specificity of autoantibodies, we compared serum levels of lupus-related autoantibodies against dsDNA, chromatin, and RNP, and RA-related rheumatoid factor (RF), anti-type II collagen (CII), and -cyclic citrullinated peptide (CCP) antibodies at 2 and 6 months of age among B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice (Fig. 2). KO1.*Yaa* mice showed higher serum levels of both lupus-related and RA-related autoantibodies than the other three strains of mice even at 2 months of age. The levels of all these antibodies were increased with age in KO1.*Yaa* mice. Age-associated increase was also observed in KO1 mice; however, the levels were remarkably higher in KO1.*Yaa* mice than those in KO1 mice at 6 months of age. Thus, the conversion of disease phenotype from RA to SLE was not explained by the shift of antibody specificity from RA-type to lupus-type.

Splenomegaly, subpopulation, and maturation/activation status of splenic lymphocytes

The spleen weight in B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice was compared at 4 months of age. Splenomegaly was only observed in KO1.*Yaa* mice (Fig. 3A). Consistently, spontaneous GC formation was observed only in KO1.*Yaa* mice at 4 months of age (Fig. 3B).

Flow cytometric analysis of spleen cells from 4-month-old mice revealed that, while there were no differences in

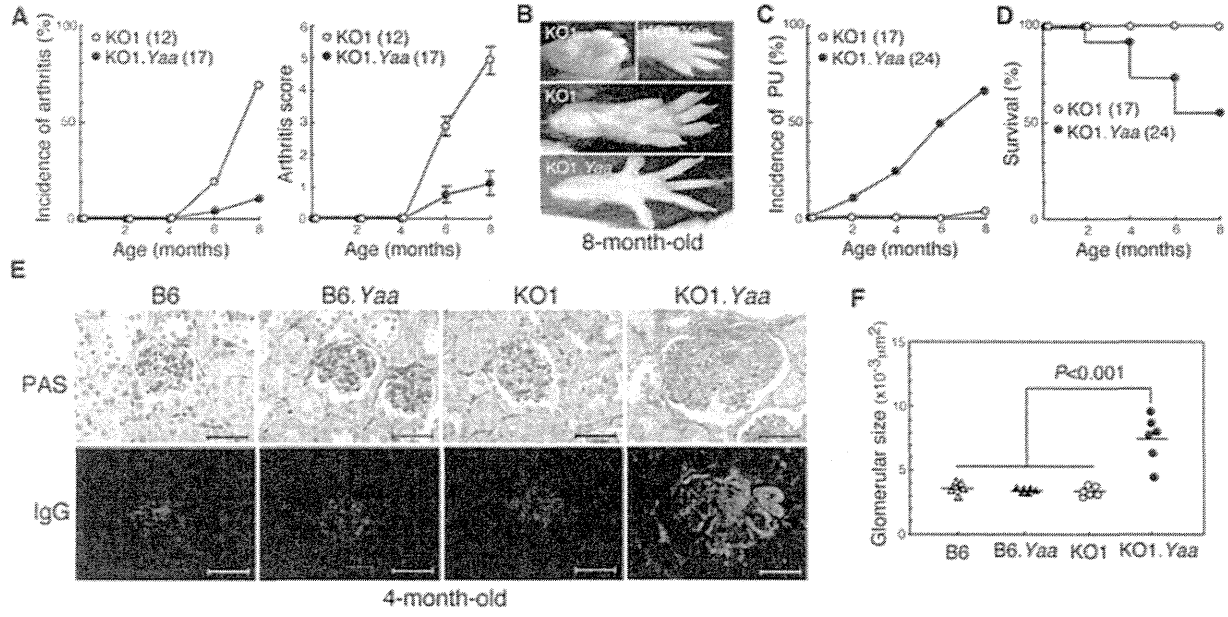

Figure 1. Disease phenotype shift from RA to SLE in KO1.*Yaa* mice. (A) Comparison of the cumulative incidence and score of arthritis between KO1 and KO1.*Yaa* mice. Score is shown as mean ± SE. (B) Representative macroscopic findings of forepaws and hindpaws in KO1 and KO1.*Yaa* mice at 8 months of age. The former mice show marked swelling and stiffness of the wrist and ankle joints. (C) Comparison of the cumulative incidence of proteinuria (PU) between KO1 and KO1.*Yaa* mice. (D) Comparison of survival rate between KO1 and KO1.*Yaa* mice. (E) Histopathological findings of glomeruli in B6, B6.*Yaa*, KO1, and KO1.*Yaa* at 4 months of age. Formalin-fixed sections were stained with periodic acid-Schiff/hematoxylin (PAS) (top). Frozen sections were stained with anti-mouse IgG (bottom) to evaluate the deposition of IgG in renal glomeruli. Scale bars = 50 μm. Representative results obtained from six mice in each strain. (F) Comparison of glomerular size as an indicator of the severity of glomerular lesion. The horizontal bar represents the mean. (A–F) All data are shown as the mean of the indicate numbers of mice in each panel and are representative of three experiments performed. Statistical significance was determined by Mann–Whitney's *U* test.

frequencies of B220$^+$ B cells per total spleen cells among four strains of mice (Table 1), there was a significant decrease in frequencies of CD21$^+$CD23$^-$ marginal zone B cells in *Yaa*-bearing B6.*Yaa* and KO1.*Yaa* mice (Fig. 4A and Table 1). This decrease is thought to be due to the effect of *Yaa* mutation, as reported previously [12], and not directly related to SLE phenotype. As for the activation/maturation status of B cells, frequencies of CD69$^+$ activated B cells, peanut agglutinin (PNA)$^+$ GC B cells, and CD138$^+$ plasma cells were significantly higher in KO1.*Yaa* mice than those found in other strains of mice (Fig. 4B and Table 1). As for T cells, while total CD3$^+$ T cells per total cells was significantly decreased in KO1.*Yaa* mice, the frequency of CD69$^+$ activated

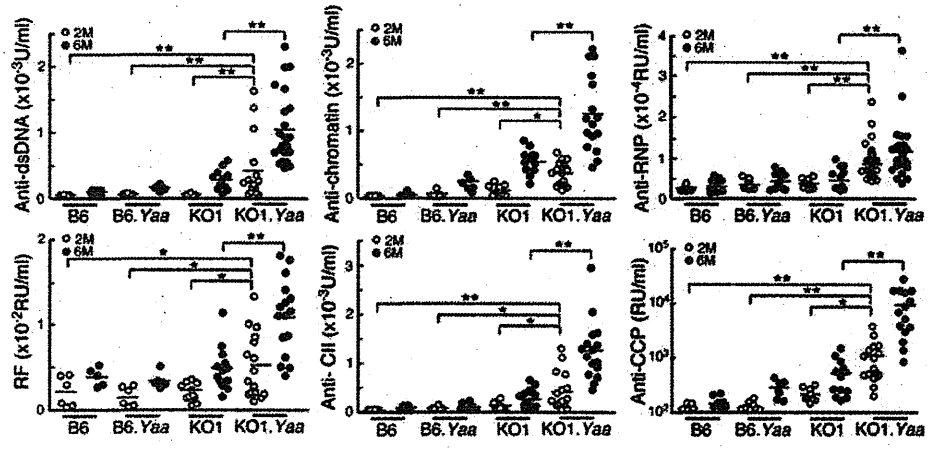

Figure 2. Comparisons of serum levels of lupus-related IgG autoantibodies against dsDNA, chromatin, and RNP, and RA-related IgG RF, anti-CII and -CCP antibodies among B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice at 2 and 6 months of age. Each symbol represents an individual mouse and the bar represents the mean. Data shown are representative of three experiments performed. Statistical significance was determined by Mann–Whitney's *U* test (**$p < 0.001$, *$p < 0.05$).

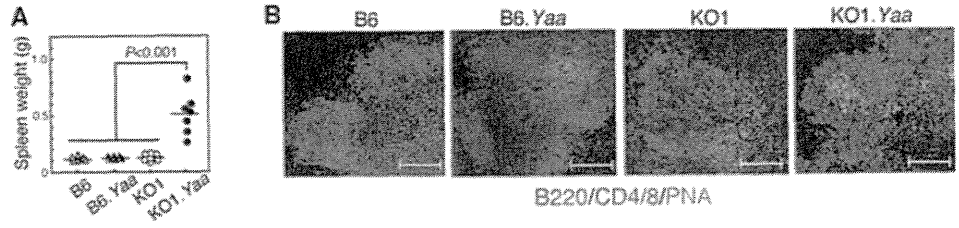

Figure 3. Splenomegaly with spontaneous GC formation in KO1.*Yaa* mice. (A) Comparison of spleen weight among B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice at 4 months of age. Each symbol represents a single mouse and the bar represents the mean. Data shown are representative of three experiments performed. Statistical significance was determined by Mann–Whitney's *U* test. (B) Frozen spleen sections of 4-month-old mice were triple stained with a mixture of anti-CD4 and anti-CD8 mAbs (green), anti-B220 mAb (blue), and PNA (red) to examine the extent of GC formation. Representative results obtained from six mice in each strain are shown. Scale bar = 100 μm.

T cells was markedly increased in KO1.*Yaa* mice (Fig. 4C and Table 1). This activation of T cells may reflect the increases in the $CD4^+/CD8^+$ T-cell ratio and in the frequency of T_{FH} cells with $PD1^+ICOS^+CXCR5^+CD4^+$ phenotype (Fig. 4C and Table 1). Because the frequencies of $PD1^+ICOS^+CXCR5^+CD4^+$ T_{FH} cells in B6, B6.*Yaa*, and KO1 mice were within normal range (Table 1), the observed abnormal increase in $PD1^+ICOS^+CXCR5^+CD4^+$ T_{FH} cells in KO1.*Yaa* mice with overt SLE was thought to be due to the combined effect of the FcγRIIB-deficiency, *Sle16* locus, and *Yaa* mutation. Table 1 also shows that the frequency of $CD11b^+$ monocyte/macrophage population was significantly increased in KO1.*Yaa* mice with a comparable level observed in BXSB male mice [5].

Cytokine profile in spleen from KO1 and KO1.*Yaa* mice

To examine the difference in in vivo cytokine expression levels associated with phenotype conversion from RA to SLE, quantitative real-time PCR (qRT-PCR) analysis was performed to compare mRNA expression levels of notable cytokines in spleen between KO1 and KO1.*Yaa* mice at 4 months of age (Fig. 5A). The result showed that the expression of IL-6, IL-10, and IL-21 was significantly upregulated in KO1.*Yaa* mice compared with that in KO1 mice. Among these, the increase in IL-10 expression was prominent, with more than tenfold increase in KO1.*Yaa* mice. There was no significant difference in expression levels of other cytokines such as IL-2, IL-4, IL-17, IFN-γ, TNF-α, and IFN-α between two strains of mice.

We next examined the cellular source of IL-10 and IL-21, using flow cytometric analysis of PMA/ionomycin-stimulated spleen cells from 4-month-old KO1 and KO1.*Yaa* mice. Both IL-10 and IL-21 were secreted from $CD4^+$ T cells and the frequencies of IL-10 and IL-21-secreting cells per total $CD4^+$ T cells were significantly higher in KO1.*Yaa* than those in KO1 mice (mean ± SE of KO1 versus KO1.*Yaa*; IL-10: 7.56 ± 1.25 versus 14.74 ± 0.43, $p < 0.01$, IL-21: 5.09 ± 0.22 versus 9.91 ± 0.60, $p < 0.01$) (Fig. 5B), consistent with the results of qRT-PCR analysis. PD1 and ICOS expression levels were upregulated in in vitro stimulated $CD4^+$ T cells. Most IL-10 and IL-21-secreting cells showed high PD1 expression levels; however, the ICOS expression level was broadly distributed in these cytokine-secreting cells (Fig. 5B). As shown in Figure 5C, in addition to IL-10 and IL-21 single producers, the significant frequency of $CD4^+$ T cells secreted both cytokines.

Table 1. Subpopulations of splenocytes in KO1, KO1.*Yaa*, B6, and B6.*Yaa* mice at 4 months of age[a)]

	B6	B6.*Yaa*	KO1	KO1.*Yaa*
$B220^+$ B/total cells	50.5 ± 3.3	54.6 ± 3.4	53.5 ± 3.2	41.2 ± 5.7
$CD21^+CD23^-$ MZ B/total B	9.8 ± 0.8	2.7 ± 0.3[b)]	11.3 ± 1.2	2.3 ± 0.4[b)]
$CD69^+B220^+$ B/total B	2.7 ± 0.7	6.1 ± 1.8	2.3 ± 0.7	15.4 ± 2.8[c)]
PNA^+B220^+ B/total B	2.4 ± 0.2	2.4 ± 0.6	1.2 ± 0.1	8.1 ± 0.8[c)]
$CD138^+$ plasma/total cells	0.5 ± 0.0	1.0 ± 0.5	0.4 ± 0.1	2.6 ± 0.7[c)]
$CD3^+$ T/total cells	32.2 ± 1.6	27.9 ± 1.5	28.3 ± 2.1	18.8 ± 1.6[c)]
$CD69^+CD4^+$ T/total T	14.3 ± 2.1	19.3 ± 2.2	14.1 ± 1.3	44.6 ± 3.9[c)]
$CD4^+/CD8^+$ ratio	1.4 ± 0.1	1.6 ± 0.2	1.1 ± 0.1	6.1 ± 2.4[c)]
$CD25^+FoxP3^+CD4^+$ T/total T	18.0 ± 1.3	17.0 ± 1.3	16.0 ± 1.1	18.2 ± 0.2
$PD1^+ICOS^+CD4^+$ T/total T	2.6 ± 0.7	3.7 ± 1.2	2.1 ± 0.7	32.3 ± 3.4[c)]
$CXCR5^+PD1^+CD4^+$ T/total T	3.4 ± 0.8	3.7 ± 1.3	2.1 ± 0.7	13.2 ± 3.2[c)]
$CD11b^+$ cells/total cells	4.4 ± 0.5	4.7 ± 0.1	5.1 ± 0.2	13.3 ± 0.1[c)]

[a)] Results were obtained from six mice in each strain, and are shown as mean and SE.
[b)] The value is significantly different from B6 mice or KO1 mice ($p < 0.005$, Student's *t*-test).
[c)] The value is significantly different from other strains of mice ($p < 0.05$, Student's *t*-test).

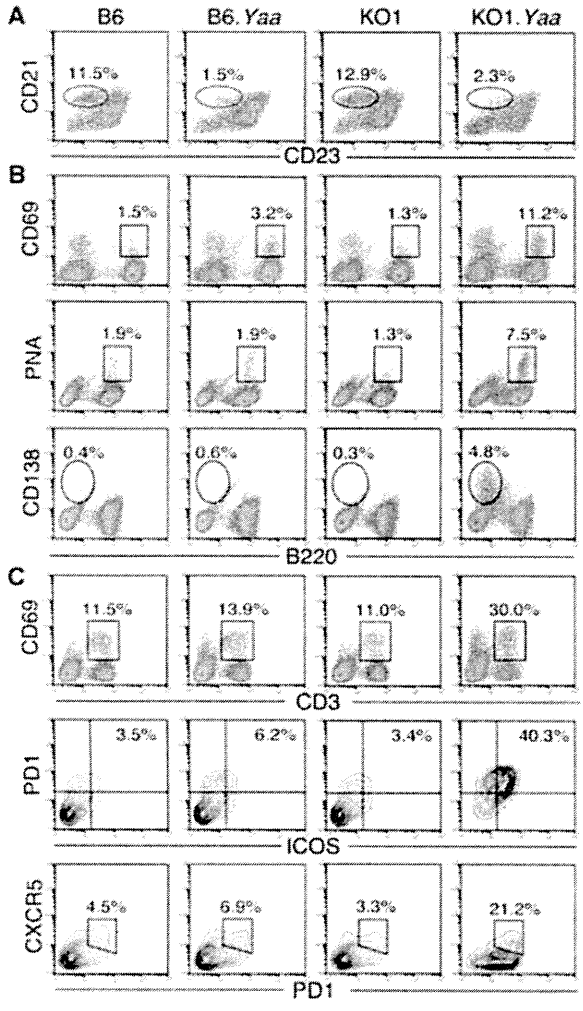

Figure 4. Comparisons of cell surface phenotypes of splenic lymphocytes among B6, B6.*Yaa*, KO1, and KO1.*Yaa* mice at 4 months of age, using flow cytometry. (A) Spleen cells were triple-stained with anti-B220, -CD21, and -CD23 mAbs, and CD21 and CD23 expression levels on $B220^+$ B cells were examined. The frequency of $CD21^+CD23^-$ marginal zone (MZ) B cells is shown. (B) Activation/maturation status of B cells. Spleen cells were stained with anti-CD69, -CD138, -B220 mAbs, and PNA. Frequencies of $CD69^+$ activated B cells per total B cells, PNA^+ GC B cells per total B cells, and $CD138^+$ plasma cells per total cells are shown. (C) Activation/maturation status of T cells. Spleen cells were stained with anti-CD69 and -CD3 mAbs, and the frequency of $CD69^+$ activated T cells per total $CD3^+$ T cells is shown. Cells were further stained with anti-CD4, -PD1, -ICOS, and -CXCR5 mAbs, and the frequencies of $PD1^+ICOS^+CD4^+$ and $PD1^+CXCR5^+CD4^+$ T_{FH} cells per total $CD4^+$ T cells are shown. Representative results obtained from six mice in each strain are shown.

Discussion

The current study showed that introduction of *Yaa* mutation into RA-prone KO1 mice leads to conversion of disease phenotypes from RA to SLE. RA and SLE are both classified as systemic autoimmune diseases. Since features of RA are occasionally associated with the clinical pictures of SLE [13], it has long been suggested that certain shared genetic pathways, as well as disease-specific ones, underlie the pathogenesis of both RA and SLE [14]. Our current model provided a clue to investigate this issue and suggested that, while the FcγRIIB deficiency and *Sle16* locus in KO1 genetic background confers predisposition to RA [6], an additional epistatic effect of *Yaa* mutation induces conversion of the disease phenotype from RA to SLE.

It has been shown that an etiology of *Yaa*-mediated B-cell activation is the duplication of the *Tlr7* gene [9, 15, 16]. The ligand for TLR7 is single-stranded RNA, thus suggesting that overexpression of TLR7 activates B cells by RNA-containing autoantigens, resulting in RNA-associated lupus autoantibody production. However, in the present study, *Yaa*-mediated disease phenotype conversion from RA to SLE was not explained by the shift of autoantibody specificity, and rather *Yaa*-mediated B-cell activation seems to be polyclonal in KO1.*Yaa* mice. This polyclonal B-cell activation may relate to the marked spontaneous GC formation and the T_{FH}-cell generation that developed in the spleen early in life of KO1.*Yaa* mice. The formation of GC depends on intrafollicular localization of antigen, activated B cells and T cells [17, 18]. Among subsets of $CD4^+$ T cells, T_{FH} cells are the specialized subset to help B cells to generate affinity-matured antibodies [17]. In addition to the B-cell help by T_{FH} cells in GC reaction, it has been shown that the relationship between B cells and T_{FH} cells is a reciprocal dependency, and that the cognate interaction with activated B cells is required for the maintenance of $PD\text{-}1^+ICOS^+CXCR5^+$ T_{FH} cells [19]. This is consistent with the present study, in which the combined effect of FcγRIIB-deficiency, *Sle16* locus, and *Yaa* mutation accelerated not only spontaneous PNA^+ B-cell generation and GC formation but also T_{FH}-cell generation in KO1.*Yaa* mice. As this vicious cycle of activated B cells and T_{FH} cells promotes polyclonal B-cell activation, KO1.*Yaa* mice showed the marked increase in serum levels of both lupus-related and RA-related autoantibodies.

Anti-CCP antibodies are currently considered to be the most specific autoantibodies for RA patients, although some patients with SLE and Sjögren's syndrome were found to have these autoantibodies [20]. Anti-CCP antibodies react with citrullinated proteins, which are the product of posttranslational modification. Citrullination of protein is a physiological process and is catalyzed by peptidyl arginine deiminase enzymes. Anti-CCP antibodies may thus gain the arthritogenicity when citrullinated proteins are increased, particularly in the arthritic region [20]. In mouse models, an increased serum level of anti-CCP antibodies was observed in SLE-prone and arthritis-free *bcl-2*-transgenic (NZW × B6)F1 mice [21], as in the case of KO1.*Yaa* mice in the current study. Thus, it appears that this autoantibody specificity is not exclusively associated with inflammatory joint diseases.

In KO1.*Yaa* mice, there was a significant increase in the IL-21 expression level early in life compared with that in KO1 mice. IL-21 is a potent immunoregulatory cytokine produced by NKT cells and $CD4^+$ T cells, and it has recently been shown that IL-21 is an autocrine growth factor for T_{FH} cells [17, 22]. Many cell types express the receptors for IL-21, but the level of expression on B cells is the highest and drives terminal differentiation of B cells and plasma cells [19, 22]. Intriguingly, Bubier et al. [23]

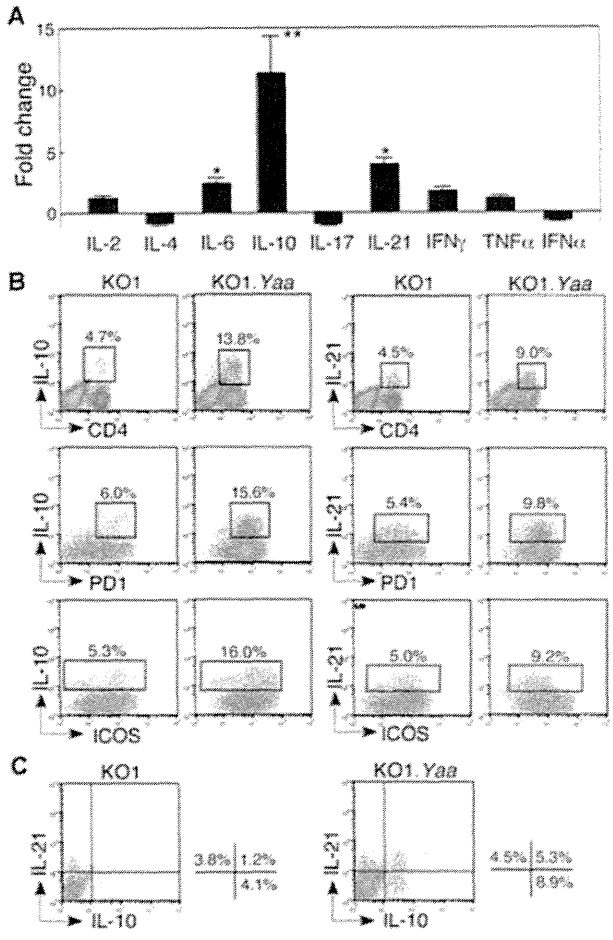

Figure 5. Comparisons of cytokine synthesis between KO1 and KO1.*Yaa* mice at 4 months of age. (A) Quantitative real-time PCR analysis of cytokine mRNA expression levels in spleen from KO1 and KO1.*Yaa* mice. Value of KO1 mice was designated as 1, and values of KO1.*Yaa* mice were evaluated as fold change compared with the values in KO1 mice. Data are shown as mean + SE of four mice for each strain and representative of three experiments performed. Statistical significance was determined by Mann–Whitney's *U* (*$p < 0.05$, **$p < 0.01$). (B) Flow cytometric analysis of IL-10 and IL-21-secreting cells in PMA/ionomycin-stimulated spleen cells from KO1 and KO1.*Yaa* mice. Frequencies of each cytokine secreting cells per total $CD4^+$ T cells, $CD4^+PD1^+$ T cells and $CD4^+ICOS^+$ T cells are shown. (C) Flow cytometric analysis of IL-10 and L-21-secreting cells, using gated $CD4^+$ T cells. Representative results obtained from six mice in each strain are shown.

reported that SLE phenotypes including autoantibody production in BXSB male mice were almost completely inhibited in the mice with the deficient IL-21 receptor. Furthermore, Rankin et al. [24] recently reported that IL-21 receptor-deficient MRL/*lpr* mice were devoid of abnormal systemic accumulation of activated B cells and T cells. These findings suggest that IL-21-mediated signals play an essential role for the pathogeneses of SLE.

It has been shown that IL-21 is a potent regulator of IL-10, since IL-10 production decreases in IL-21 receptor knockout mice, while it increases in IL-21-transgenic mice [25]. IL-10 was first described as a factor produced by Th2 cells, which inhibited the production of cytokines by Th1 cells [26]. Accumulating evidence, however, have shown that IL-10 is actually produced by many types of cells, and that, although IL-10 shows antiinflammatory properties against T cells and macrophages through inhibiting the production of inflammatory cytokines, it promotes B-cell function to induce antibody production [27, 28]. Considering these dual effects with immunosuppressive and immunostimulatory properties, IL-10 may confer different effects on the disease progression processes of RA and SLE. Indeed, the hallmark of RA is the excess production of inflammatory cytokines by T cells and macrophages at inflammatory foci, while SLE is characterized by increased production of high-affinity autoantibodies and deposition of their immune complexes in a wide variety of tissues, particularly in renal glomeruli. Consistently, there are several reports indicating the suppressive effect of IL-10 on RA [27, 29] and the promoting effect of IL-10 on lupus pathogenesis [30]. Further studies are needed to define the role of IL-10 in the conversion of disease phenotypes observed in the present study. These studies are underway in our laboratory.

Peripheral blood mononuclear cells from patients with active SLE show up-regulated expression of a group of type I IFN-induced genes [31–33]. Thus, IFN-α seems to be an important cytokine in SLE pathogenesis. In pristane-induced lupus model, the disease was shown to be associated with excess IFN-α production [34], as in the case of human SLE. However, overexpression of IFN-α is not likely to be involved in SLE pathogenesis in KO1.*Yaa* mice, since there were no differences in IFN-α expression levels between RA-prone KO1 mice and SLE-prone KO1.*Yaa* mice. Accumulating evidence shows that IL-6, IL-17, and TNF-α are important contributing cytokines to the pathogenesis of RA [35–37]. In the present studies, however, IL-6 expression levels were increased in SLE-prone KO1.*Yaa* mice compared with those in RA-prone KO1 mice, and there was no significant difference in expression levels of IL-17 and TNF-α between KO1 and KO1.*Yaa* mice. Thus, these cytokines are suggested to be unrelated to the observed phenotype conversion from RA to SLE in our model.

In conclusion, we introduced *Yaa* mutation into RA-prone KO1 strain and found that the disease phenotype converted from RA to SLE in KO1.*Yaa* mice. This phenotype conversion was likely to be due to the changes in cytokine milieus rather than the shift of autoantibody specificity from RA-related to lupus-related one. Further studies for the clarification and identification of the mechanism underlying this phenotype conversion are of paramount importance for shedding light on the mechanisms that control the development of clinically distinct systemic autoimmune diseases RA and SLE.

Materials and methods

Mice

FcγRIIB-deficient KO1 mice were generated by gene targeting in 129-derived embryonic stem cells and by backcrossing to B6 for over 12 generations [6]. The *Yaa* mutation was introduced into

KO1 mice by crossing with B6.*Yaa* mice. B6.*Yaa* mice were purchased from the Jackson Laboratory. All mice were housed under identical conditions. Experiments were performed in accordance with our institutional guidelines. Male mice were analyzed in the current study.

Incidence of arthritis

Ankle joint swelling was examined by inspection and arbitrarily scored as follows: 0, no swelling; 1, mild swelling; 2, moderated swelling; 3, severe swelling. Scores of both ankle joints are put together, and mice with scores over 2 were considered positive for arthritis.

Measurement of proteinuria

The proteinuria was monitored by biweekly testing and scored as previously descrobed [38]. Briefly, urine samples (10 μL) spotted on filter paper were air dried, fixed in 70% ethanol and stained with bromophenol blue solution. A series of standard three-fold dilution of BSA were processed as the same way, and the degree of proteinuria was assessed by visually comparing the color intensity of urine spot with that of the spot of BSA standards. Scores are as follows; 0:<37 mg/100 mL 1:≧37 mg/100 mL, 2:≧74 mg/mL, 3:≧111 mg/100 mL, 4:≧333 mg/100 mL, 5:≧1000 mg/100 mL, and 6:≧3000 mg/100 mL. Mice with urinary protein levels of four or more in repeated tests were considered as positive for proteinuria.

Histopathology and tissue immunofluorescence

Tissues fixed in 4% paraformaldehyde and embedded in paraffin were sectioned at 2 μm thickness, and tissue sections were stained with periodic acid-Schiff and hematoxylin (PAS). For immunofluorescence, tissues were embedded in Tissue-Tek OCT compound, frozen in liquid nitrogen, and sectined at 4 μm thickness. Frozen kidney sections were stained with FITC-labeled polyclonal goat anti-mouse IgG for 60 min at room temperature. For analysis of splenic tissues, frozen sections were three-color stained with Alexa 488-labeled anti-CD4 and -CD8 mAbs, Alexa 647-labeled anti-B220 mAb, and Alexa 546-labeled PNA. Antibodies and PNA were purchased from BD Pharmingen (San Diego, CA) and Vector Laboratories Inc. (Burlingame, CA), respectively. The labeling of these reagents was performed in our laboratory. Color images were obtained using laser scanning microscopy (Zeiss LSM510, Carl Zeiss Co., Ltd., Germany).

Estimation of the severity of glomerular lesion

The extent of cellular proliferation in glomerular lesion was estimated by the measurement of glomerular size. Kidney section stained by PAS was photographed under a microscope (Biozero, KEYENCE, Osaka, Japan) with ×50 magnification. Ten glomeruli in each field were randomly selected in order of size, and the size of each glomerulus was calculated using BZ-II analyzer software (KEYENCE). Mean size of 10 glomeruli was used as an indicator of histological severity of lupus nephritis in each individual mouse.

Serum levels of autoantibodies

Serum levels of IgG anti-dsDNA and -chromatin antibodies were measured using ELISA, as previously described [39]. Serum antibody levels are expressed in units, referring to a standard curve obtained by the serial dilution of a pooled serum of (NZB × NZW) F1 mice over 8 months, containing 1000 units/mL. Serum levels of IgG anti-RNP antibodies were measured by employing a commercially available kit (Alpha Diagnostic Intl. Inc., San Antonio, TX), and are expressed as relative units according to the manufacturer's instructions.

Serum levels of IgG RF and IgG anti-CCP antibodies were measured employing commercially available kits (Shibayagi Co. Ltd., Gunma, Japan and Cosmic Corporation, Tokyo, Japan, respectively), and are expressed as relative units according to the manufacturer's instructions. Serum levels of IgG anti-CII antibodies were measured using an ELISA plate precoated with bovine CII (Sigma-Aldrich, St. Louis, MO). CII-binding activities are expressed in units, referring to a standard curve obtained by serial dilution of a standard serum pool from KO1 mice hyper-immunized with CII, containing 1000 unit activities/mL.

Flow cytometric analysis

For the analysis of splenic lymphocytes, spleen cells were stained with the following reagents: FITC-conjugated anti-CD3, -CD21, -ICOS, -Foxp3, and -CD11b mAbs, Pacific blue-conjugated anti-B220, -CD4 mAbs, and PNA, PE-conjugated anti-B220, -CD138, -PD1, and -CD25 mAbs, and biotin-conjugated anti-CD69, -CD23, -CD8, and -CXCR5 mAbs, followed by streptavidin allophycocyanin. mAbs for CD25 and those for Foxp3, PD1, and ICOS were obtained from BioLegend (San Diego, CA) and eBioscience (San Diego, CA), respectively. Others were from BD PharMingen. Stained cells were four-color analyzed using a FACSAria cytometer and FlowJo software (Tree Star, Inc., Ashland, OR) with whole cell-gate excluding dead cells in forward and side scatter cytogram.

For intracellular cytokine staining of spleen cells, cells were stimulated with PMA (0.2 μg/mL)/ionomycin (2 μg/mL) in the presence of Golgi-Stop (BD Bioscience, San Jose, CA) for 5 h and stained with Pacific Blue-labeled anti-CD4 and biotin-labeled anti-PD1 or anti-ICOS mAbs followed by streptavidine allophycocyanin. Stained cells were then fixed and permeabilized using BD Cytofix/Cytoperm (BD Bioscience), followed by staining with FITC-labeled anti-IL-10, and PE-labeled anti-IL-21 mAbs. Stained cells were analyzed as above.

qRT-PCR analysis

Total RNA was isolated from spleen and first-stranded cDNA was synthesized using an oligo(dT)-primer with Superscript II First-Strand Synthesis kit (Invitrogen, Carlsbad, CA). The cDNA product was used for each qRT-PCR sample. The data were normalized to β-actin reference. Primer pairs used were as follows: IL-2 (forward) 5′-AACCTGAAACTCCCCAGGAT-3′, (reverse) 5′-AGGGCTTGTTGAGATGATGC-3′; IL-4 (forward) 5′-CCTCACAGCAACGAAGAACA-3′, (reverse) 5′-AAGTTAAAGCATGGTGGCTCA-3′; IL-6 (forward) 5′-GACAAAGCCAGAGTCCTTCAGAGAG-3′, (reverse) 5′-CTAGGTTTGCCGAGTAGATCTC-3′; IL-10 (forward) 5′-CCAAGCCTTATCGGAAATGA-3′, (reverse) 5′-TGGCCTTGTAGACACCTTGG-3′; IL-17 (forward) 5′-TCTCTGATGCTGTTGCTGCT-3′, (reverse) 5′-GACCAGGATCTCTTGCTGGA-3′; IL-21 (forward) 5′-ATCCTGAACTTCTATCAGCTCCAC-3′, (reverse) 5′-GCATTTAGCTATGTGCTTCTGTTTC-3′; IFNγ (forward) 5′-AAGACAATCAGGCCATCAGC-3′, (reverse) 5′-ATCAGCAGCGACTCCTTTTC-3′; TNF-α (forward) 5′-GGCAGGTCTACTTTGGAGTCATTGC-3′, (reverse) 5′-ACATTCGAGGCTGCTCCAGTGAATTCGG-3′; consensus IFNα (forward) 5′-ATGGCTAGRCTCTGTGCTTTCCT-3′, (reverse) 5′-AGGGCTCTCCAGAYTTCTGCTCTG-3′; β-actin (forward) 5′-AGCCATGTACGTAGCCATCC-3′, and (reverse) 5′-CTCTCAGCTGTGGTGGTGAA-3′. The quantity was normalized using the formula of the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Statistical analysis was performed using Mann–Whitney's *U* test for disease phenotypes and Student's *t*-test for flow cytometric analysis. A value of $p < 0.05$ was considered as statistically significant.

Acknowledgments: The authors thank Dr. A. Sato-Hayashizaki, Mr. N. Ishihara, Ms. K. Kojyo, Ms. N. Ohtsuji, and Ms. T. Ikegami for excellent technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Technology, Sports and Culture of Japan, and Grant for Research on Intractable Diseases from the Ministry of Health, Labour, and Welfare of Japan.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

1 **Ravetch, J. V. and Kinet, J.-P.**, Fc receptors. *Annu. Rev. Immunol.* 1991. 9: 457–492.

2 **Jiang, Y., Hirose, S., Abe, M., Sanokawa-Akakura, R., Ohtsuji, M., Mi, X., Li, N. et al.**, Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. *Immunogenetics* 2000. 51: 429–435.

3 **Jiang, Y., Hirose, S., Sanokawa-Akakura, R., Abe, M., Mi, X., Li, N., Miura, Y. et al.**, Genetically determined aberrant down-regulation of FcgRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. *Int. Immunol.* 1999. 11: 1685–1691.

4 **Xiu, Y., Nakamura, K., Abe, M., Li, N., Wen, X-S., Jiang, Y., Zhang, D. et al.**, Transcriptional regulation of *Fcgr2b* gene by polymorphic promoter region and its contribution to humoral immune responses. *J. Immunol.* 2002. 169: 4340–4346.

5 **Lin, Q., Xiu, Y., Jiang, Y., Tsurui, H., Nakamura, K., Kodera, S., Ohtsuhi, M. et al.**, Genetic dissection of the effects of stimulatory and inhibitory IgG Fc receptors on murine lupus. *J. Immunol.* 2006. 177: 1646–1654.

6 **Sato-Hayashizaki, A., Ohtsuji, M., Lin, Q., Hou, R., Ohtsuji, N., Nishikawa, K., Tsurui, H. et al.**, Presumptive role of 129 strain-derived *Sle16* locus for rheumatoid arthritis in a new mouse model with FcgRIIB-deficient C57BL/6 genetic background. *Arthritis Rheum.* 2011. 63: 2930–2938.

7 **Carlucci, F., Cortes-Hernandez, J., Fossati-Jimack, L., Bygrave, A. E., walport, M. J., Vyse, T. J., Cook, H. T. et al.**, Genetic dissection of spontaneous autoimmunity driven by 129-derived chromosome 1 loci when expressed on C57BL/6 mice. *J. Immunol.* 2007. 178: 2352–2360.

8 **Boross, P., Arandhara, V. L., Martín-Ramirez, J., Santiago-Rober, M-L., Carlucci, F., Flierman, R., van der Kass, J. et al.**, The inhibiting Fc receptor for IgG, FcgRIIB, is a modifier of autoimmune susceptibility. *J. Immunol.* 2011. 187: 1304–1313.

9 **Subramanian, S., Tus, K., Li, Q. Z., Wang, A., Tian, X. H., Zhou, J., Liang, C. et al.**, A *Tlr7* translocation accelerates systemic autoimmunity in murine lupus. *Proc. Natl. Acad. Sci. USA* 2006. 103: 9970–9975.

10 **Wandstrat, A. E., Nguyen, C., Limaye, N., Chan, A. Y., Subramanian, S., Tian, X.-H., Yim, Y.-S. et al.**, Association of extensive polymorphisms in the SLAM/CD20 gene cluster with murine lupus. *Immunity* 2004. 21: 769–780.

11 **Jansson, L. and Holmdahl, R.**, The Y chromosome-linked "autoimmune accelerating" *Yaa* gene suppresses collagen-induced arthritis. *Eur. J. Immunol.* 1994. 24: 1213–1217.

12 **Amano, H., Amano, E., Moll, T., Marinkovic, D., Ibnou-Zekri, N., Martinez-Soria, E., Semac, I. et al.**, The *Yaa* mutation promoting murine lupus causes defective development of marginal zone B cells. *J. Immunol.* 2003. 170: 2293–2301.

13 **Panush, R. S., Edwards, N. L., Longley, S. and Webster, E.**, 'Rhupus' syndrome. *Arch. Intern. Med.* 1988, 148: 1633–1636.

14 **Jawaheer, D., Seldin, M. F., Amos, C. I., Chen, W. V., Shigeta, R., Monteio, J., Kern, M. et al.**, A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with autoimmune diseases. *Am. J. Hum. Genet.* 2001. 68: 927–936.

15 **Pisitkunm, P., Deane, J. A., Difilippantonio, M. J., Tarasenko, T., Satterthwaite, A. B. and Bolland, S.**, Autoreactive B cell responses to RNA-related antigens due to *TLR7* gene duplication. *Science* 2006. 312: 1669–1672.

16 **Deane, J. A., Pisitkun, P., Barrett, R. S., Feigenbaum, L., Town, T., Ward, J. M., Flavell, R. A. et al.**, Control of Toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* 2007. 27: 801–810.

17 **King, C., Tangye, S. G. and Mackay, C. R.**, T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu. Rev. Immunol.* 2008. 26: 741–766.

18 Vinuesa, C. G., Sanz, I. and Cook, M. C., Dysregulation of germinal centers in autoimmune disease. *Nat. Rev. Immunol.* 2009. 9: 845–857.

19 King, C., A fine romance: T follicular helper cells and B cells. *Immunity* 2011. 34: 827–829.

20 Uysal, H., Nandakumar, K. S., Kessel, C., Carlsen, S., Burkhardt, H. and Holmdahl, R., Antibodies to citrullinated proteins: molecular interactions and arthritogenicity. *Immunol. Rev.* 2010. 133: 9–33.

21 López-Hoyos, M., Marquina, R., Tamayo, E., González-Rojas, J., Izui, S., Merino, R. and Merino, J., Defects in the regulation of B cell apoptosis are required for the production of citrullinated peptide autoantibodies in mice. *Arthritis Rheum.* 2003. 48: 2353–2361.

22 Spolski, R. and Leonard, W. J., Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu. Rev. Immunol.* 2008. 26: 57–79.

23 Bubier, J. A., Sproule, T. J., Foreman, O., Spolski, R., Shaffer, D. J., Morse, H. C. III, Leonard, W. J. et al., A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-*Yaa* mice. *Proc. Natl. Acad. Sci. USA* 2009. 106: 1518–1523.

24 Rankin, A. L., Guay, H., Herber, D., Bertino, S. A., Duzanski, T. A., Carrier, Y., Keegan, S. et al., IL-21 receptor is required for the systemic accumulation of activated B and T lymphocytes in MRL/MpJ-Fas$^{lpr/lpr}$/J mice. *J. Immunol.* 2012. 188: 1656–1667.

25 Spolski, R., Kim, H.-R., Zhu, W., Levy, D. E. and Leonard, W. J., IL-21 mediates suppressive effects via its induction of IL-10. *J. Immunol.* 2009. 182: 2859–2867.

26 Fiorentino, D. F., Bond, M. W. and Moamann, T. R., Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 1989. 170: 2081–2095.

27 Moore, K. W., de Waal Malefyt, R., Coffman, R. L. and O'Garra, A., Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 2001. 19: 683–765.

28 O'Garra, A., Barret, F. J., Castro, A. G., Vicari, A. and Hawrylowicz, C., Strategies for use of IL-10 or its antagonists in human disease. *Immunol. Rev.* 2008. 223: 114–131.

29 Hata, H., Sakaguchi, N., Yoshitomi, H., Iwakura, Y., Sekikawa, K., Azuma, Y., Kanai, C. et al., Distinct contribution of IL-6, TNF-a, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. *J. Clin. Invest.* 2004. 114: 582–588.

30 Ishida, H., Muchamuel, T., Sakaguchi, S., Andrade, S., Menon, S. and Howard, M., Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* 1994. 179: 305–210.

31 Baechler, E. C., Batliwalla, F. M., Karypis, G., Gaffney, P. M., Ortmann, W. A., Espe, K. J., Shark, K. B. et al., Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. USA* 2003. 100: 2610–2615.

32 Bennett, L., Palucka, A. K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J. and Pascual, V., Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 2003. 197: 711–723.

33 Kirou, K. A., Lee, C., George, S., Louca, K., Papagiannis, I. G., Peterson, M. G., Ly, N. et al., Coordinate overexpression of interferon-a-induced genes in systemic lupus erythematosus. *Arthritis Rheum.* 2004. 50: 3958–3967.

34 Reeve, W. H., Lee, P. Y., Weinstein, J. S., Aatoh, M. and Lu, L., Induction of autoimmunity by pristine and other naturally occurring hydrocarbons. *Trends in Immunol.* 2009. 30: 455–464.

35 Ishihara, K. and Hirano, T., IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev.* 2002. 13: 357–368.

36 Iwakura, Y., Nakae, S., Saijo, S. and Ishigame, H., The role of IL-17A in inflammatory immune responses and host defence against pathogens. *Immunol. Rev.* 2008. 226: 57–79.

37 Banchereau, J., Pascual, V. and Palucka, A. K., Autoimmunity through cytokine-induced dendritic cell activation. *Immunity* 2004. 20: 539–550.

38 Knight, J. G., Adams, D. D. and Purves, H. D., The genetic contribution of the NZB mouse to the renal disease of the NZB x NZW hybrid. *Clin. Exp. Immunol.* 1977. 28: 352–358.

39 Zhang, D., Fujio, K., Jiang, Y., Zhao, J., Tada, N., Sudo, K., Tsurui, H. et al., Dissection of the role of MHC class II A and E genes in autoimmune susceptibility in murine lupus models with intragenic recombination. *Proc. Natl. Acad. Sci. USA* 2004. 101: 13838–13843.

Abbreviations: **B6**: C57BL/6 mice · **CII**: type II collagen · **CCP**: cyclic citrullinated peptide · **FcγRIIB**: IgG Fc receptor IIB · **PNA**: peanut agglutinin · **RA**: rheumatoid arthritis · **RF**: rheumatic factor · **SLE**: systemic lupus erythematosus · **qRT-PCR**: quantitative real-time PCR · ***Yaa***: Y chromosome-linked autoimmune acceleration mutation

Full correspondence: Prof. Sachiko Hirose, Department of Pathology, Juntendo University School of Medicine, 2–1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Fax: +81-3-3813-3164
e-mail: sacchi@juntendo.ac.jp

Received: 10/10/2012
Revised: 28/11/2012
Accepted: 17/12/2012
Accepted article online: 26/12/2012

ARTHRITIS & RHEUMATISM
Vol. 64, No. 1, January 2012, pp 153–161
DOI 10.1002/art.33314

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

Asako Chiba, Ryohsuke Tajima, Chiharu Tomi, Yusei Miyazaki, Takashi Yamamura, and Sachiko Miyake

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-

Supported by the Japan Foundation for Neuroscience and Mental Health (to Dr. Chiba), the Japan Rheumatism Foundation (to Dr. Chiba), the Japan Society for the Promotion of Science (Grants-in-Aid for Scientific Research B: 20390284 and 23390261 to Dr. Miyake), and the Ministry of Health, Labor, and Welfare of Japan (Health and Labor Sciences Research Grants on Intractable Diseases).

Asako Chiba, MD, PhD, Ryohsuke Tajima, MS, Chiharu Tomi, Yusei Miyazaki MD, PhD, Takashi Yamamura MD, PhD, Sachiko Miyake MD, PhD: National Institute of Neuroscience, National Centre of Neurology and Psychiatry, Tokyo, Japan.

Address correspondence to Sachiko Miyake MD, PhD, Department of Immunology, National Institute of Neuroscience, National Centre of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan. E-mail: miyake@ncnp.go.jp.

Submitted for publication May 15, 2011; accepted in revised form August 25, 2011.

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 μ*M* 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 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

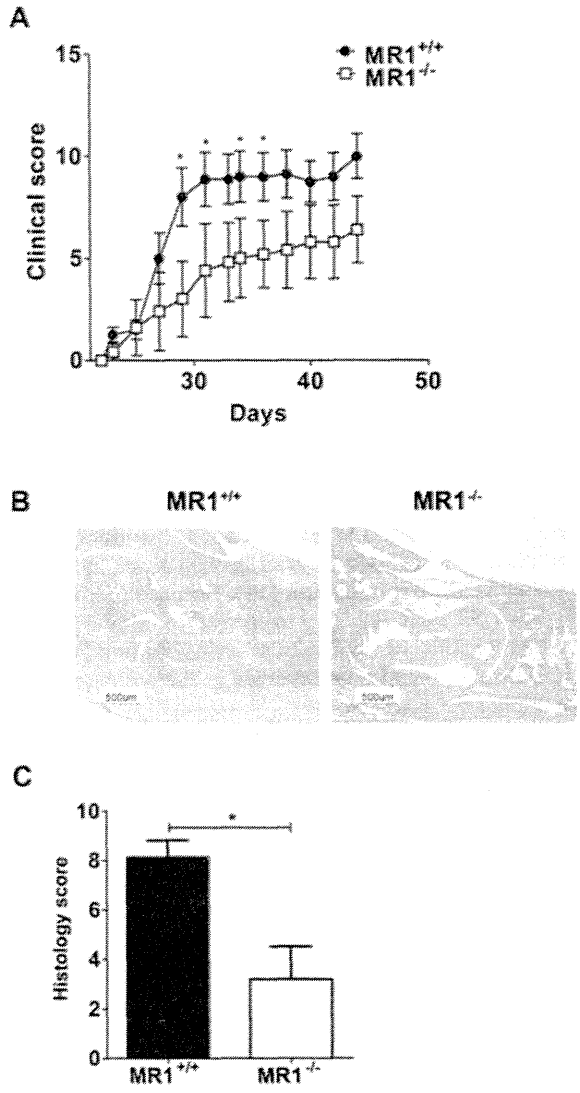

Figure 1. Amelioration of collagen-induced arthritis (CIA) in MR1$^{-/-}$ mice. **A,** Clinical scores for CIA in MR1$^{-/-}$ DBA/1J mice and in MR1$^{+/+}$ DBA/1J mice. Values are the mean ± SEM of 5–8 mice per group. * = $P < 0.05$ versus MR1$^{-/-}$ DBA/1J mice. **B,** Representative histologic sections 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. * = $P < 0.05$.

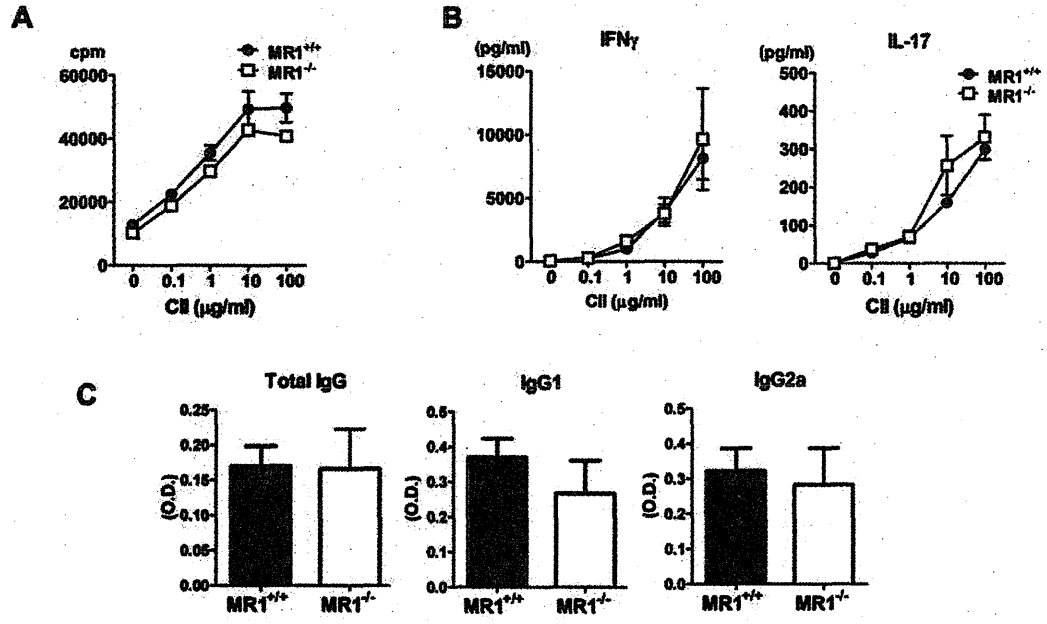

Figure 2. Type II collagen (CII) responses in MR1$^{-/-}$ DBA/1J mice. A and B, Inguinal lymph node cells from MR1$^{-/-}$ DBA/1J mice and MR1$^{+/+}$ DBA/1J mice with collagen-induced arthritis were incubated for 48 hours in the presence of CII. Proliferative responses were determined by the uptake 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

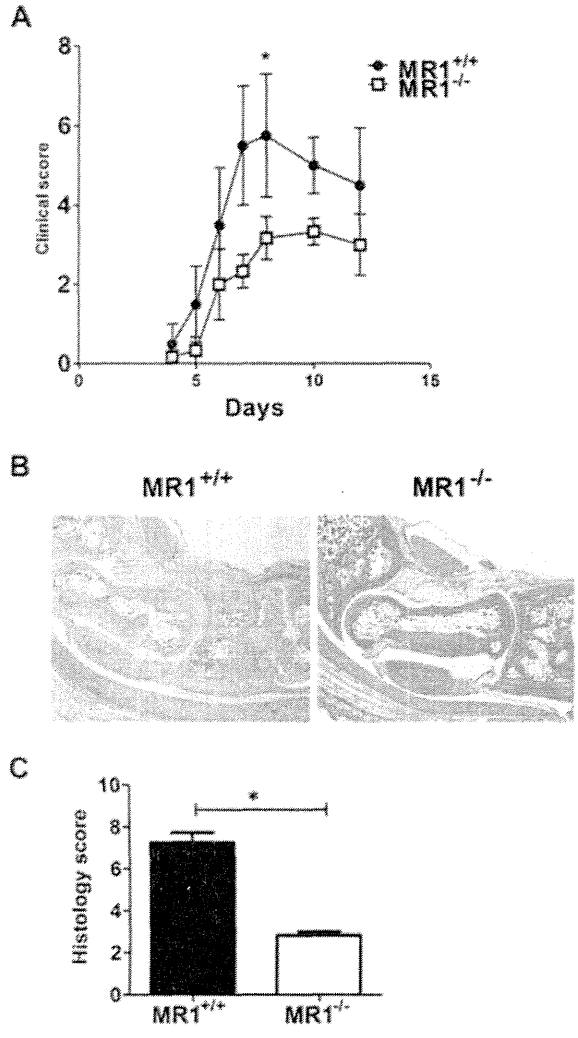

Figure 3. Amelioration of collagen antibody–induced arthritis (CAIA) in MR1$^{-/-}$ mice. **A,** Clinical scores for CAIA in MR1$^{-/-}$ C57BL/6J mice and MR1$^{+/+}$ C57BL/6J mice. Values are the mean ± SEM of 4–6 mice per group. * = $P < 0.05$ versus MR1$^{-/-}$ C57BL/6J mice. **B,** Representative 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

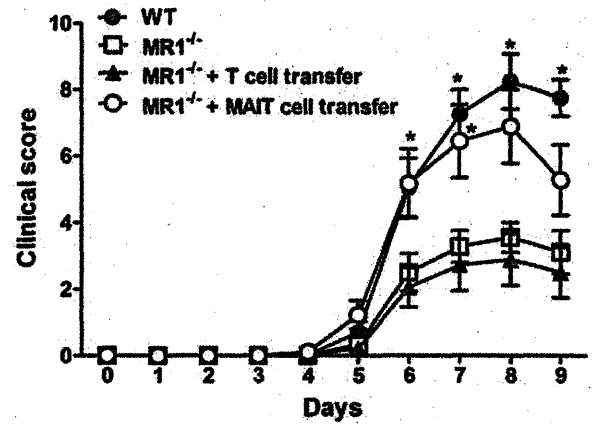

Figure 4. Augmentation of arthritis by adoptive transfer of mucosal-associated invariant T (MAIT) cells in MR1$^{-/-}$ mice. MR1$^{-/-}$ C57BL/6J mice received 5 × 10^5 NK1.1+TCRβ+ T cells (MAIT cells) or an equal number of NK1.1–TCRβ+ cells (T cells) from V_α19-transgenic CD1d1$^{-/-}$ mice. One day 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.

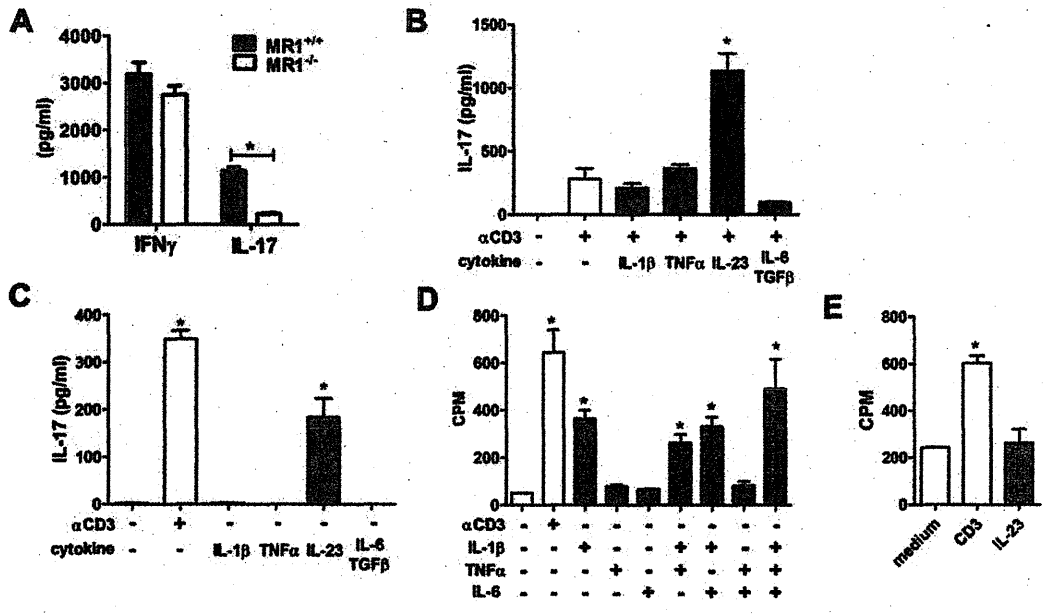

Figure 5. Cytokine-mediated mucosal-associated invariant T (MAIT) cell activation. **A**, Liver mononuclear cells from $MR1^{+/+}$ C57BL/6J mice and $MR1^{-/-}$ C57BL/6J mice were stimulated for 48 hours with immobilized anti-CD3 (αCD3) monoclonal antibody (mAb). The levels of interferon-γ (IFNγ) and 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.

ACKNOWLEDGMENTS

The authors thank S. Gilfillan (Washington University School of Medicine, St. Louis, MO) for MR1$^{-/-}$ mice and M. Shimamura (University of Tsukuba, Ibaraki, Japan) for $V_{\alpha}19$-transgenic mice.

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.
Acquisition of data. Chiba, Tajima, Tomi, Miyazaki.
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.