Macrophage Scavenger Receptor 1 (Msr1, SR-A) Influences B Cell Autoimmunity by Regulating Soluble Autoantigen Concentration

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Macrophage Scavenger Receptor 1 (Msr1, SR-A) Influences B Cell Autoimmunity by Regulating Soluble Autoantigen Concentration

Stefanie Haasken,*,1 Jennifer L. Auger,*,† Justin J. Taylor,† Patricia M. Hobday,* Brian D. Goudy,* Philip J. Titcombe,‡ Daniel L. Mueller,‡ and Bryce A. Binstadt*

The class A macrophage scavenger receptor Msr1 (SR-A, CD204) has been reported to participate in the maintenance of immunological tolerance. We investigated the role of Msr1 in a mouse model of autoantibody-dependent arthritis. Genetic deficiency of Msr1 in K/BxN TCR transgenic mice decreased the incidence and severity of arthritis because of decreased autoantibody production. Despite normal initial activation of autoreactive CD4⁺ T cells, potentially autoreactive B cells in Msr1⁻/⁻ K/BxN mice retained a naive phenotype and did not expand. This was not due to an intrinsic B cell defect. Rather, we found that macrophages lacking Msr1 were inefficient at taking up the key autoantigen glucose-6-phosphate isomerase and that mice retained a naive phenotype and did not expand. This was not due to an intrinsic B cell defect. Rather, we found that macrophages lacking Msr1 were inefficient at taking up the key autoantigen glucose-6-phosphate isomerase and that Msr1-deficient mice had elevated serum concentrations of glucose-6-phosphate isomerase. Arthritis developed normally when bone marrow from Msr1⁻/⁻ K/BxN mice was transplanted into hosts whose macrophages did express Msr1. Thus, Msr1 can regulate the concentration of a soluble autoantigen. In this model, the absence of Msr1 led to higher levels of soluble autoantigen and protected mice from developing pathogenic autoantibodies, likely because of altered cognate interactions of autoreactive T and B cells with impaired differentiation of follicular Th cells. The Journal of Immunology, 2013, 191: 000–000.

The generation of autoreactive lymphocytes is a consequence of having a diverse cellular immune repertoire capable of responding to threats of a wide range of specificities. Both cell-intrinsic and -extrinsic modes of immunological tolerance exist to constrain lymphocyte clones that recognize self-Ags (1, 2). For B cells, central tolerance occurs in the bone marrow, where most self-reactive clones are either deleted or undergo receptor editing (3–5). In the periphery, autoactive B cells can be rendered tolerant (anergic) or induced to undergo apoptosis (6). Weakly autoreactive B cells can be maintained in a seemingly naive state of clonal ignorance if protected from cognate interactions with activated T cells (7). Autoimmunity can arise when these B cell tolerance mechanisms fail.

The class A scavenger receptor, macrophage scavenger receptor 1 (Msr1, SR-A, CD204, encoded by the murine gene, Msr1) is a multifunctional receptor that is expressed primarily on cells of the myeloid lineage and that binds modified self- and pathogen-associated Ags (8). Several lines of evidence support a role for Msr1 in peripheral tolerance. For example, one group reported a role for Msr1 in the maintenance of peripheral tolerance via tonic cross-presentation of self-Ags “nibbled” from the membranes of adjacent cells to CD8⁺ T cells (9). In addition, Msr1 may sequester sources of danger signals present among autoantigens by taking up apoptotic cellular debris. Of note, impaired clearance of apoptotic Ags has been implicated in the breakdown of tolerance in systemic lupus erythematosus (SLE) (10). Msr1-deficient mice do not develop spontaneous autoimmune diseases, however, suggesting that these reported “housekeeping” functions of Msr1 are not essential to maintain immunological tolerance and highlighting the fact that multiple layers of peripheral tolerance exist (11). The K/BxN TCR transgenic mouse model of spontaneous autoimmune arthritis is well suited to address questions regarding mechanisms of immunological tolerance. In this model, CD4⁺ T cells bearing the KRN transgene—encoded TCR recognize peptides derived from the ubiquitously expressed glycolytic enzyme, glucose 6-phosphate isomerase (GPI), presented on the MHC class II (MHCII) molecule I-A^B7. These activated T cells provide help to GPI-specific B cells, leading to the sustained production of high-titer arthritogenic autoantibodies (12, 13). Joint pathology arises via Ab-mediated activation of the innate immune system, and transfer of K/BxN serum to naive recipient mice is sufficient to provoke transient arthritis (termed K/BxN serum–transferred arthritis) (14). Arthritis in K/BxN TCR transgenic mice develops reliably between 3 and 4 wk of age when autoreactive KRN CD4⁺ T cells begin to emerge from the thymus and when anti-GPI autoantibody production can be detected (12, 13), demonstrating that both T cell and B cell tolerance are breached in K/BxN mice.

In this study, we investigated how Msr1 impacts immunological tolerance in the K/BxN mouse model of spontaneous autoimmune arthritis.

Materials and Methods

Mice

KRN TCR transgenic mice on the C57BL/6 (B6) background (12) were a gift from Drs. D. Mathis and C. Benoist (Harvard Medical School,...
Intracellular cytokine staining
Lymph node cells were isolated, stimulated with PMA and ionomycin, and stained intracellularly for IL-17 and IFN-γ as described previously (24).

Ag uptake by macrophages
Peritoneal macrophages were elicited by i.p. injection of 1 ml Brewer thioglycollate medium (Fluka Analytical). Macrophages were collected 5 d later via peritoneal lavage, incubated with 5 µg GPI or BSA labeled with AF647 (Life Technologies) in complete media for 20 min at 37°C/5% CO2, washed, and analyzed by flow cytometry.

Western blotting
Serum samples were separated by SDS-PAGE and transferred to Immobilon-FL membranes (Millipore). GPI was detected using serum from K/BxN mice, followed by peroxidase-conjugated goat anti-mouse IgG1 (Jackson Immunoresearch Laboratories), developed with ECL Prime Western blotting Detection Reagent (GE Healthcare), and imaged quantitatively on an ImageQuant LAS4000 workstation (GE Healthcare). Because Ab H chain comigrates with GPI, Ab-deficient µMT−/− mice were used to allow detection of GPI.

Determination of serum albumin concentration
Serum albumin concentrations were determined in the same mice by ELISA, according to the manufacturer’s instructions (Bethyl Laboratories) The plates were read on a Bio-Rad Model 680 Microplate reader at 415 nm.

Cell purification and adoptive transfer
Cells were purified from spleens and lymph nodes. CD4+ T cells were purified with T cell isolation kit II (Miltenyi Biotec). B cells were purified by negative enrichment using a mixture of biotinylated Abs (CD4, CD8, CD11b, CD11c, F4/80, and GR-1) in combination with anti-biotin beads (Miltenyi Biotec). Lymphocytes were labeled with CFSE (where indicated) and injected intravenously into recipient mice. At the experimental end points, the lymphocytes were analyzed by flow cytometry.

Bone marrow chimeric mouse
Rag1-deficient recipient mice were sublethally irradiated with 300 rad. The following day, 10 × 10^6 bone marrow cells from Msr1-sufficient or -deficient K/BxN mice (combined with 5 × 10^6 bone marrow cells from unirradiated Rag1-deficient mice where indicated) and injected i.v. into recipient mice. The recipient mice were maintained on sulfamethoxazole and trimethoprim (Sigma Chemical Co.) and aseptically housed. Aspirated BM was analyzed by flow cytometry, and serum and lymphoid organs were harvested for analysis.

Statistical analysis
Statistical differences between mean values for groups were calculated using Student’s two-tailed t test. Arthritis severity scores were compared with a repeated-measures ANOVA. Relative risk was used to calculate differences in the incidence of arthritis between the genetic model and mixed bone marrow chimeric mice. A p value < 0.05 was considered significant. Analysis was performed with SPSS 19.0.

Results
K/BxN mice lacking Msr1 are protected from arthritis and endocarditis
To address how Msr1 might affect the development of autoantibody-associated arthritis, we generated K/BxN TCR transgenic mice lacking Msr1 (Msr1−/− K/BxN). Surprisingly, half of the Msr1−/− K/BxN mice developed subtle to no arthritis by 8 wk of age (Fig. 1A, 1B, Supplemental Fig. 1A). Some of the Msr1−/− K/BxN mice developed subtle to no arthritis by 8 wk of age (Fig. 1A, 1B, Supplemental Fig. 1A). Some of the Msr1−/− K/BxN mice developed subtle to no arthritis by 8 wk of age (Fig. 1A, 1B, Supplemental Fig. 1A). Some of the Msr1−/− K/BxN mice developed subtle to no arthritis by 8 wk of age (Fig. 1A, 1B, Supplemental Fig. 1A).
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The reduced incidence and severity of both arthritis and endocarditis suggested that Msr1 deficiency influenced the T and B cell–dependent initiation phase of autoimmunity in K/BxN mice. To exclude formally the possibility that Msr1 deficiency interfered with the innate immune system–mediated effector phase of arthritogenesis, we asked whether Msr1 deficiency affected T and B cell autoreactivity.

Decreased autoantibody production in Msr1-deficient K/BxN mice

K/BxN mice have elevated levels of total serum IgG as well as high-titer, arthritogenic anti-GPI autoantibodies (13, 14). We found that K/BxN mice lacking Msr1 had lower total IgG levels and lower anti-GPI IgG titers compared with controls. Furthermore, among the Msr1−/− K/BxN mice, those with arthritis tended to have higher total IgG levels and anti-GPI titers than those without arthritis (Fig. 2), consistent with the tight link between autoantibody production and arthritis development in this model. We next sought to determine how Msr1 deficiency interfered with autoantibody production.

The T cell compartment is unaltered in Msr1-deficient K/BxN mice

Production of anti-GPI autoantibodies in K/BxN mice depends on the interaction of CD4+ T cells expressing the transgene-encoded KRN TCR with GPI-specific B cells. We therefore asked whether the impaired autoantibody production in Msr1−− K/BxN mice was due to altered T cell development or activation, particularly in view of the restricted expression of Msr1 on macrophages, a cell type capable of presenting peptide:MHCII Ags to CD4+ T cells (8, 25). We first evaluated T cell subsets in the thymus and periphery from Msr1−/− and control K/BxN mice, but found no difference in their numbers or relative frequencies (Fig. 3A, 3B). Peripheral CD4+ T cells from both groups of mice expressed comparable levels of the T cell activation marker, CD44, (Fig. 3D), demonstrating that the activation of pathogenic Th cells was not impaired (24, 26).

Msr1 has been reported to confer Ag presentation capacity to anergic Msr1−/− CD4+ T cells (25). To address directly whether the absence of Msr1 led to reduced Ag presentation in vivo, we adoptively transferred CFSE-labeled naive CD4+ T cells from KRN/B6 (H-2b) mice into Msr1−/− K/BxN recipients (Fig. 3F). Furthermore, there was no apparent difference in the expression of the CD4+ T cell anergy markers CD73 and folate receptor 4 (FR4) in the two groups of mice (Fig. 3G) (27). Collectively, these findings suggest that the reduction in anti-GPI autoantibody production in Msr1−/− K/BxN mice was not significantly different.

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Decreased autoantibody production in Msr1-deficient K/BxN mice

K/BxN mice have elevated levels of total serum IgG as well as high-titer, arthritogenic anti-GPI autoantibodies (13, 14). We found
mice was not due to impaired T cell development, activation, Ag-induced expansion, or anergy. We next focused on B cells.

B cells remain relatively naïve in Msr1<sup>+/−</sup> K/BxN mice

Genetic absence of Msr1 did not affect the total number of splenic B cells in K/BxN or B6 mice (Fig. 4A). We therefore used a recently developed tetramer enrichment strategy to enumerate Ag-specific (i.e., GPI-specific) B cells (20). We found that mice lacking the KRN transgene contained very low numbers of GPI-specific B cells. In contrast, Msr1<sup>+/+</sup> K/BxN mice contained significantly more GPI-specific B cells relative to Msr1<sup>+/−</sup> K/BxN mice (300,000 versus 80,000 cells/mouse; Fig. 4B). Notably, there was no difference in the number of GPI-specific B cells in arthritic versus nonarthritic Msr1<sup>/−</sup> K/BxN mice (Fig. 4B). In addition, we were unable to detect anti-GPI IgG-secreting cells by ELISPOT in either arthritic or nonarthritic Msr1<sup>/−</sup> K/BxN mice (27). In this setting, GPI-specific B cells were readily detected among both the Msr1<sup>+/+</sup> and Msr1<sup>/−</sup> K/BxN mice (Fig. 4C). We therefore investigated the expression of B cell surface markers. We found that the expression of MHCII was essentially equivalent among GPI-specific B cells from Msr1<sup>+/+</sup> and Msr1<sup>/−</sup> mice. In contrast, GPI-specific B cell surface expression of IgM and IgD was substantially lower in Msr1<sup>+/+</sup> K/BxN mice relative to Msr1<sup>/−</sup> K/BxN mice, consistent with an activated B cell phenotype in the Msr1<sup>+/+</sup> group and a more naive phenotype in the Msr1<sup>/−</sup> group (Fig. 4D). Further analysis confirmed this impression. Specifically, more GPI-specific B cells in Msr1<sup>+/+</sup> K/BxN mice demonstrated isotype switching as demonstrated by increased intracellular IgG1 expression and also contained a significantly larger population of GPI-specific IgM<sup>+</sup>/CD38<sup>−</sup> GL7<sup>+</sup> germinal center cells relative to Msr1<sup>/−</sup> K/BxN mice (Fig. 4E, 4F) (28).

No intrinsic defect in Msr1-deficient B cells

We next asked why the GPI-specific B cells in Msr1<sup>/−</sup> K/BxN mice remained relatively naive in the presence of apparently normally activated autoreactive CD4<sup>+</sup> T cells. Two scenarios seemed likely—defects that were either B cell–intrinsic or B cell–extrinsic. First, it was possible that Msr1<sup>/−</sup> B cells have an intrinsic activation defect. Arguing against this possibility is the fact that Msr1 is not expressed by B cells, in addition to a prior study demonstrating normal T cell–dependent humoral immune responses in the presence of pharmacologic blockade of Msr1 (29). Nonetheless, we formally considered the hypothesis that the B cells from Msr1<sup>/−</sup> mice were less efficient responders than wildtype B cells. To test this, we isolated congenically marked B cells from Msr1<sup>+/+</sup> and Msr1<sup>/−</sup> mice (H-2<sup>b</sup>) and transferred them together with naive KRN<sup>+</sup>CD4<sup>+</sup> T cells (H-2<sup>b</sup>) into T cell–deficient H-2<sup>b</sup>/g7 hosts and harvested 3 d later for flow cytometric analysis. Data represent two experiments with a total of seven recipient mice. No CFSE dilution was seen in H-2<sup>b</sup> hosts (data not shown). (G) Expression of the T cell anergy markers CD73 and FR4 was evaluated among CD4<sup>+</sup> splenocytes and lymph node cells in the indicated mice. Data represent two experiments with a total of three mice per group.

FIGURE 3. Msr1 deficiency does not affect KRN T cell activation. T cell subsets from the thymus (A) and spleen (B) were enumerated among K/BxN mice of the indicated Msr1 genotypes. The values plotted are means ± SEM, n = 5 mice/genotype. (C) Surface expression of CD44 measured by mean fluorescence intensity (MFI) on splenic CD4<sup>+</sup> effector T cells from Msr1<sup>+/+</sup> (squares) and Msr1<sup>/−</sup> (circles) K/BxN mice was determined by flow cytometry. (D) The number of CD4<sup>+</sup> lymph node T cells expressing intracellular IL-17 was determined by flow cytometry. In both (C) and (D), filled shapes indicate individual arthritic animals, open shapes indicate nonarthritic animals; bars represent mean values. (E) CFSE-labeled naive CD4<sup>+</sup> T cells from KRN/B6 donor mice were adoptively transferred into Msr1<sup>+/+</sup> (thick line) or Msr1<sup>/−</sup> (thin line) H-2<sup>b</sup>/g7–expressing recipient mice or control B6 (H-2<sup>b</sup>) mice (shaded histogram) and harvested 48 h later for flow cytometric analysis. The data shown are representative of three separate experiments (n = 8 total mice/group). (F) CFSE-labeled, congenically marked CD4<sup>+</sup> T cells (10<sup>5</sup>) from Msr1<sup>+/+</sup> K/BxN mice (thick line) or Msr1<sup>/−</sup> K/BxN mice (thin line) were adoptively transferred together into H-2<sup>b</sup>/g7–expressing hosts and harvested 3 d later for flow cytometric analysis. Data represent two experiments with a total of seven recipient mice. No CFSE dilution was seen in H-2<sup>b</sup> hosts (data not shown). (G) Expression of the T cell anergy markers CD73 and FR4 was evaluated among CD4<sup>+</sup> splenocytes and lymph node cells in the indicated mice. Data represent two experiments with a total of three mice per group.

Msr1 regulates serum GPI concentration

Although normally found in the cytoplasm, low levels of soluble GPI can also be detected in the serum of mice and humans (30–32). Because Msr1 functions as a scavenger receptor, we hypothesized that Msr1 might bind and clear excess Ag (GPI) from the circulation. To address this, we incubated peritoneal macrophages from Msr1<sup>+/+</sup> and Msr1<sup>/−</sup> with fluorescently labeled GPI or an irrelevant protein (bovine albumin), using fluorescence as a measure of uptake. Macrophages lacking Msr1 took up both GPI and albumin less efficiently than did wild-type macrophages, and this defect
appeared more pronounced for GPI (Fig. 6A). Correspondingly, we found elevated levels of GPI in the serum of Msr1-deficient mice, which ranged between 2 and 20 μg/ml by Western blot analysis (Fig. 6B). Although the GPI standards detected as little as 23 ng/ml, the serum concentration of GPI in Msr1+/+ mice was below the level of detection for the assay, indicating that the serum concentration of GPI in Msr1−/− mice was a log or more higher than in Msr1+/+ mice. Deficiency of Msr1 did not impact the serum concentration of mouse albumin (42.9 ± 16 mg/ml in Msr1+/+ versus 45.9 ± 14 mg/ml in Msr1−/− [mean ± S.D., p = 0.76, n = 5 mice/group]). We speculate that although Msr1 may be able to mediate uptake of albumin (as in Fig. 6A), this effect does not measurably affect the concentration of a protein in high abundance in the serum. In contrast, the absence of Msr1-mediated uptake of a low abundance protein such as GPI can cause a detectable and immunologically significant increase in its serum concentration. These results demonstrate that Msr1 normally acts to maintain low levels of soluble GPI in the circulation.

We hypothesized that adding Msr1-expressing APCs to capture and “normalize” the concentration of GPI should reverse the defect in B cell activation seen in Msr1−/− K/BxN mice. To test this, we created bone marrow chimeric mice in which bone marrow from nonarthritic Msr1−/− K/BxN or arthritic Msr1+/+ K/BxN mice was mixed with bone marrow from Rag1-deficient mice (as an additional source of Msr1-expressing APCs) and injected into sublethally irradiated Rag1-deficient (Msr1-expressing) recipient mice. Indeed, mice transplanted with both Msr1−/− K/BxN marrow and Msr1-sufficient APCs developed arthritis equivalently to control mice (Fig. 6C). Furthermore, the incidence of arthritis among the mice transplanted with Msr1−/− K/BxN bone marrow was significantly higher than the incidence among the Msr1−/− K/BxN TCR transgenic animals (90 versus 52.5%; p = 0.0033). In subsequent experiments in which the additional donor Rag1-deficient bone marrow was omitted, all Rag1-deficient recipients of either Msr1−/− K/BxN or Msr1+/+ K/BxN bone marrow developed arthritis, al-
concentrations were determined by Western blot in 8-wk-old Rag1−/− Msr1−/− mice to restore B cell autoreactivity to GPI. These bone marrow recipients were entirely donor-derived (Supplemental Fig. 2B). We found that 38–63% of the macrophages in the reconstituted mice were derived from the donor genotypes (Supplemental Fig. 2B). We found that Msr1−/− mice lacking the KRN TCR transgene (Fig. 7C). However, our finding that GPI-reactive B cells from Msr1−/− mice lacking the KRN TCR transgene (Fig. 7C). Below we discuss potential mechanisms by which a relatively autoantigen-rich environment might paradoxically lead to less efficient T-B cell collaboration and decreased differentiation to helper Th cells.

Discussion

In this paper, we report that Msr1 deficiency ameliorates systemic autoimmune disease in the K/BxN TCR transgenic mouse model of autoantibody-dependent arthritis. Specifically, in the absence of Msr1, circulating levels of the autoantigen GPI were increased. Despite normal activation of autoreactive CD4+ T cells, GPI-specific B cells remained relatively naive, resulting in decreased autoantibody production and protection against arthritis development.

The increased circulating concentration of GPI in Msr1-deficient mice could theoretically impact the fate of GPI-specific B cells in a number of ways. For example, high concentrations of GPI could lead to more efficient clonal deletion of high-affinity, GPI-specific B cells or induce them to become anergic. Notably, naive B cells may be rendered anergic, or hyporesponsive to antigenic stimuli, if they encounter their cognate Ag (signal 1) in the absence of TLR ligation or appropriate costimulation (signal 2) (6, 35). However, in Msr1−/− mice lacking autoreactive T cells, the number of GPI-specific B cells was not reduced (see Fig. 4B), suggesting that clonal deletion was not more efficient. It is possible that the GPI-specific B cells in the Msr1−/− mice bind GPI with lower affinity or are relatively anergic compared with their counterparts in Msr1+/+ mice. However, our finding that GPI-reactive B cells from Msr1−/− proliferated equivalently to those derived from Msr1+/+ mice following adoptive transfer suggests that they were neither anergic nor of substantially lower affinity.

We favor a model in which the higher concentration of circulating GPI in Msr1-deficient mice impairs B cell activation indirectly by altering the cognate interactions between autoreactive CD4+ T cells and B cells. In the Msr1−/− environment of relative GPI excess, T cells are more likely to encounter professional APCs presenting GPI peptide/MHCII complexes, resulting in T cell “stop” signals (36). Widespread, sustained arrest of the autoreactive T cells would in turn reduce the likelihood that an activated autoreactive T cell would encounter a rare GPI-reactive B cell, leading to the reduced number of Th cells that we observed. The net effect of this disruption of cognate T-B cell collaborations would be that GPI-reactive B cells would paradoxically appear to be in a clonally ignorant, naive state despite the high concentration of soluble self Ag. As a corollary, because the precursor frequency of autoreactive
A

B

C

FIGURE 7. Msr1-deficient K/BxN mice have few Tfh cells and fail to form robust germinal centers. (A) Representative flow cytometric plots of lymph node cells from Msr1+/+ K/BxN and Msr1−/− K/BxN mice analyzed for the presence of CXCR5hi,PD-1hi Tfh cells. Upstream gating included dump (B220, CD11b, CD11c, and F4/80) negative, CD3+, CD44hi, and Foxp3+. The number indicates the percentage of cells within the gate. (B) Numbers and percentages of CXCR5hi,PD-1hi Tfh cells in lymph nodes from the indicated mice, identified as in (A). Each point represents one mouse, line represents mean. Data compiled from two independent experiments. (C) Sections of inguinal lymph nodes from the indicated mice were analyzed for the distribution of T cells (detected by TCRβ and CD3, green) and B cells (B220, orange). Nucleated cells were detected with DAPI (blue). In Msr1+/+ K/BxN mice, T cells are in close proximity to B cells in germinal centers (lower left panel, white arrows), in contrast to Msr1−/− K/BxN mice in which the T cells remain in the paracortical area (lower right panel) as in naïve, nonarthritic animals (top two panels). Original magnification ×10. Images are representative of three independent experiments with lymph nodes from two mice per genotype.

(KRN+) CD4+ T cells is high in this system, our findings suggest that GPI peptide:MHCII-expressing APCs are scarce in Msr1+/+ K/BxN mice, permitting activated CD4+ T cells to move unhindered by APCs to encounter and activate GPI-specific B cells. Importantly, the proposed reduction in autoreactive T-B cell collaborations in Msr1−/− versus Msr1+/+ K/BxN mice is relative rather than absolute, meaning that autoreactive T-B cell collaborations can still occur, but less frequently and may appear to occur stochastically. Our data, including our finding that autoimmune arthritis develops in some but not all Msr1−/− K/BxN mice, are consistent with this scenario.

A similar dichotomy in arthritis susceptibility has been reported in K/BxN mice lacking IL-4 and in K/BxN mice lacking the neonatal FcR (21, 37). In these studies and the present one, the development of arthritis correlated closely with the production of anti-GPI IgG autoantibodies, and even very low titers of anti-GPI IgG were sufficient to provoke arthritis (see Fig. 2B). These findings are consistent with the notion that the threshold for developing arthritis can be crossed relatively easily, and that stochastic events, such as those discussed above, likely impact if and when that crossing occurs.

Consistent with our findings, Manula and colleagues (38) recently noted decreased pathology and autoantibody titers in autoimmune-prone MRL mice genetically lacking Msr1, perhaps attributable to defects in Msr1-mediated trafficking of Ag from B cells to macrophages, resulting in decreased T cell activation (39). In our model system, however, the loss of Msr1 did not appreciably impair T cell activation.

A main reported function of Msr1 is the clearance of apoptotic debris. Impaired clearance of apoptotic debris is implicated in the breakdown of tolerance in SLE, the prototypic autoantibody-dependent disease (10, 40). The recent description of anti-Msr1 (anti–SR-A) Abs among patients with SLE is notable. It has been suggested that anti-Msr1 Abs might interfere with the uptake of apoptotic debris, thereby promoting autoimmunity (41, 42). On the basis of these studies, one might reasonably expect autoimmunity to arise more easily in the absence of Msr1. Yet we found just the opposite—Msr1 deficiency protected against autoimmunity in K/BxN mice. This discrepancy might be related more to timing, rather than to peculiarities of the model system. That is, Msr1 deficiency early in lymphocyte development may result in impaired initial activation of autoreactive B cells, even in the presence of activated, autoreactive T cells. In contrast, in an organism with pre-existing activated, self-reactive B cells, inhibiting Msr1 and increasing the load of Ag might fuel sustained autoreactivity and inflammation (41, 42). These scenarios are consistent with Mitchison’s framework of “high zone tolerance” (43). Understanding how anti-Msr1 Abs might influence SLE disease pathogenesis will likely require detailed knowledge of the timing of when the anti-Msr1 Abs appear and whether or how they affect the interaction of Msr1 with specific autoantigens.

In sum, we have shown that K/BxN mice lacking Msr1 are protected from autoantibody-mediated arthritis due to decreased autoreactive B cell activation in the presence of excess soluble autoantigen. The finding that factors that increase autoantigen load might alter T-B cell collaborations and reduce rather than enhance autoimmunity furthers our understanding of the complex temporal and mechanistic balance of immunological tolerance.

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Disclosures

The authors have no financial conflicts of interest.
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Supplemental Figure 1: Decreased incidence and severity of arthritis and endocarditis in \textit{Msr1}^{-/-} K/BxN mice. (A) Histological analysis of ankle (left panels) and mitral valve (right panels) pathology in \textit{Msr1}^{+/-}, +/-, and +/- K/BxN mice demonstrates protection from both joint and cardiac valve pathology in non-arthritic \textit{Msr1}^{-/-} K/BxN mice. For ankles, original magnification = 10x, bar (in fourth row) indicates 200 microns. For mitral valves, original magnification = 40x; bar (in fourth row) indicates 100 microns. (B) Five non-arthritic \textit{Msr1}^{-/-} K/BxN mice (filled circles) were aged up to 15 weeks, during which any mild arthritis resolved or remained equivalent to the 8-week time point, as demonstrated by changes in ankle thickness. The data from the \textit{Msr1}^{+/-} K/BxN mice (reproduced from Figure 1B) are shown for comparison. Plotted values are means \pm SEM. *$p \leq 0.05$, **$p \leq 0.01$.

Supplemental Figure 2: \textit{Msr1}-sufficient host environment permits \textit{Msr1}-deficient K/BxN hematopoietic compartment to drive arthritis and B cell activation

\textit{Rag1}^{-/-} recipient mice were sublethally irradiated (300 Rad) and transplanted with \textit{10x10^6} bone marrow cells from \textit{Msr1}^{+/-} K/BxN mice or \textit{Msr1}^{-/-} K/BxN mice as indicated. (A) The development of arthritis in both groups was determined by weekly arthritis scoring and ankle measurements. Values shown are means +/- SEM. n= 3 mice/group. (B) B cells in the reconstituted mice were analyzed for their ability to bind to GPI-tetramer, for having undergone isotype switching express IgG1 intracellularly, and transitioning to a CD38^{low} GL7^{+} activated
phenotype. Numbers indicate the percentage of cells in each quadrant or gate. (C) Representative flow cytometric plot of CD3− F4/80+ macrophages in the reconstituted mice analyzed for expression of the congenic markers differentiating the donor cells (CD45.1+ CD45.2+) from the host cells (CD45.1− CD45.2+). The numbers shown are percentages. In analysis of multiple mice, the percent of macrophages that were host-derived ranged from 38-63%. (D) Similar analysis for CD3+ T cells and B220+ B cells reveals that they are entirely donor-derived.
KRN negative

Msr1+/+ K/BxN

Msr1+/− K/BxN

Msr1−/− K/BxN (arthritic)

Msr1−/− K/BxN (non-arthritic)

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Supplemental Figure 1

A

ankle  mitral valve

B

Δ Ankle Width (mm)

Age of mice (weeks)
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Supplemental Figure 2

A

![Graph showing Arthritis Score over time after transplantation for different genotypes.]

B

![Flow cytometry plots showing gated B220+ and gated GPI+ cells with G1 and G2 gates.]

C

![Plots showing CD3, F4/80, CD45.1, and CD45.2 expressions on donor and host cells.]

D

![Plots showing CD3, CD45.1, and B220 expressions on donor and host cells.]