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Differential MHC Class II Presentation of a Pathogenic Autoantigen during Health and Disease

Fei F. Shih,*† Jennifer Racz,* and Paul M. Allen2*

Glucose-6-phosphate isomerase (GPI) is the target autoantigen recognized by KRN T cells in the K/BxN model of rheumatoid arthritis. T cell reactivity to this ubiquitous Ag results in the recruitment of anti-GPI B cells and subsequent immune complex-mediated arthritis. Because all APCs have the capacity to process and present this autoantigen, it is unclear why systemic autoimmunity with polyclonal B cell activation does not ensue. To this end, we examined how GPI is presented by B cells relative to other immunologically relevant APCs such as dendritic cells (DCs) and macrophages in the steady state, during different phases of arthritis development, and after TLR stimulation. Although all APCs can process and present the GPI:I-A^b complex, they do so with different efficiencies. DCs are the most potent at baseline and become progressively more potent with disease development correlating with immune complex uptake. Interestingly, in vivo and in vitro maturation of DCs did not enhance GPI presentation, suggesting that DCs use mechanisms to regulate the presentation of self-peptides. Non-GPI-specific B cells are the weakest APCs (100-fold less potent than DCs) and fail to productively engage KRN T cells at steady state and during arthritis. However, the ability to stimulate KRN T cells is strongly enhanced in B cells after TLR ligation and provides a mechanism whereby polyclonal B cells may be activated in the wake of an acute infection. The Journal of Immunology, 2006, 176: 3438–3448.

A spontaneous murine model of rheumatoid arthritis, K/BxN, mimics many of the clinical and histologic features of human disease with arthritis, predominantly in the distal small joints and systemic features of hypergammaglobulinemia and splenomegaly (1). K/BxN mice were generated by crossing KRN TCR transgenic (Tg)3 mice to the NOD background. Although the initial specificity of the KRN TCR was directed to RNase(42–56)/I-Ak, the KRN TCR was shown serendipitously to recognize peptide 281–293 of the glycolytic enzyme glucose-6-phosphate isomerase (GPI) bound to I-A^b2 (2). Failure in T cell tolerance allowed KRN T cells to become activated by endogenously presented GPI and to provide help to anti-GPI B cells, giving rise to arthritogenic autoantibodies.

Because GPI is a critical glycolytic enzyme, it is expressed in the cytoplasm of all cells and is found circulating at ~400 ng/ml in the blood (3). Every APC can potentially process and present GPI to KRN T cells; therefore, KRN T cells should be available to provide cognate help to any B cell. Yet, the pathology seen in K/BxN mice is limited to arthritis mediated by anti-GPI Abs. In K/BxN mice, anti-GPI B cells exhibit selective growth advantage such that, by 11 wk of age, up to 50% of the B cells are anti-GPI Ab-secreting cells (4). Why are other autoreactive B cells, such as rheumatoid factor or antinuclear Ab B cells whose Ags are abundantly available, not similarly stimulated by the overabundance of KRN T cell help? Because MHC class II (MHC II) complexes are specialized for the presentation of Ags derived from exogenous sources taken up through endocytosis and processed in the endosomes, the presentation of cytotoxic Ags such as GPI may be inefficient in B cells that lack GPI-specific BCRs (5). However, many studies showed that transformed B cell lines can present peptides derived from cytoplasmic Ags for presentation to CD4+ T cells (6–12). Moreover, biochemically up to 30% of self-peptides eluted from I-A^d and I-A^b complexes in B cells were found to be derived from cytoplasmic Ags (11, 12). Indeed, both studies found peptides derived from GPI; thus, GPI is capable of being processed and presented by B cells. GPI(281–293) was, however, not among the peptides eluted from I-A^b7, indicating that it was not an abundantly processed self-peptide (12). Therefore, we sought to directly evaluate how GPI is presented by B cells as compared with other immunologically relevant APCs such as dendritic cells (DCs) and macrophages (Mφs). Because GPI expression is linked to the metabolic state of the cell and the disease state (3, 13), we examined its presentation under physiologic conditions and as the disease evolved to account for the disease phenotype exhibited by K/BxN mice. It is unclear whether and how presentation of GPI is modulated to accommodate conditions of increased metabolic demand such as during arthritis or acute inflammation. Because polyclonal autoantibody production, e.g., antinuclear Ab and rheumatoid factor, has been reported following an antecedent infection (14–19), we examined the ability of B cells and DCs to present GPI following TLR ligation to assess the role of bystander activation in modulating self-Ag presentation.

Materials and Methods

Mice

KRN TCR Tg mice on a C57BL/6 background have been previously described (1). NOD Lt/J and B6.AK-H-2k/FlaEg (stock no. 001148; hereafter referred to as B6.K) mice were purchased from The Jackson Laboratory. C57BL/6 mice (B6) were bred in our facility. The original K/BxN mice were generated on a heterozygous H-2^b7 background (1). In our laboratory we maintained these mice on the heterozygous H-2^b7 background; hence, we referred to them as KRN(B6.K)xNOD(F1), mice. The KRN(B6.K)xNOD(F1) mice developed arthritis at 4–5 wk of age and were used at 6–7 wk for acute arthritis or at >12 wk for chronic arthritis (20).
All other mice were used at 8–12 wk of age unless specified otherwise. All mice were bred and housed under specific pathogen-free conditions in the animal facility at the Washington University Medical Center (St. Louis, MO) in accordance with the rules of the Washington University Animal Studies Committee.

Purification of APCs
DCs, B cells, and MØs were isolated from thymi, lymph nodes, and spleens of 5–10 mice. The organs were dissociated and digested in RPMI 1640 with Liberase Blendzyme 3 (Roche) and DNase I (Sigma-Aldrich) for 1 h at 37°C with intermittent agitation. APCs were isolated by sequential selection using Miltenyi Biotec cell separation MicroBeads. First, DCs were isolated using positive selection on CD11c MicroBeads (Miltenyi Biotec) according to manufacturer’s directions, except that the positively selected cells were purified using two sequential LS columns to obtain >90% purity. MØs were subsequently isolated from the DC flow through via positive selection using CD11b MicroBeads (Miltenyi Biotec). Finally, B cells were obtained by negative selection using CD43 MicroBeads (Miltenyi Biotec). Purity of preparation was assayed for DCs, MØs, and B cells using CD11c, CD11b, and B220, respectively.

Flow cytometry
Single-cell suspensions of splenic APCs (2–10 × 10⁶) were FcR blocked with 1 μg of 2.4G2 Ab (anti-CD16) from BD Pharmingen before surface staining with specific Abs according to standard protocols. The Abs/Reagents were used: 53-6.7-FITC (anti-CD8α), 14.4.4-FITC (anti-CD4), HL3-PE and HL3-biotin (anti-CD11c), MI70-PE (anti-CD11b), RA3-6B2-biotin, RA3-6B2-APC (anti-CD11c), 1G10-FITC (anti-CD80), GL1-PE (anti-CD86), and 3/23-FITC (anti-CD40) from BD Pharmingen or BioLegend. AG2.42.7 (anti-I-Aα) was a gift from E. Unanue (Washington University School of Medicine, St. Louis, MO). Streptavidin-allophycocyanin was obtained from Caltag Laboratories. All samples were analyzed on a FACSCaliber flow cytometer (BD Biosciences) with CellQuest software. Gating on live lymphocytes was based on forward and side scatter and propidium iodide exclusion. Fifty thousand to 500,000 gated live events were collected per sample.

Primary T cell proliferation
CD4+ T cells were purified from the spleens of 10–20 KRNk mouse by positive selection using Miltenyi Biotec MicroBeads. Proliferation assays were performed in triplicate with 1–2 × 10⁴ CD4+ T cells/well in round-bottom plates with Iscove’s medium containing 10% heat-inactivated bovine serum and 2 mM GlutaMAX (Invitrogen Life Technologies), 2 × 10⁻⁵ M 2-ME, and 50 μg/ml gentamicin (referred to henceforth as ISC-10). APCs received 2000 rad of gamma irradiation before use.

Generation of bone marrow-derived DCs (BMDCs)
BMDCs were prepared from the femurs of mice aged 8–12 wk of age as described (22). Single-cell suspensions of bone marrow cells were plated at 1 × 10⁶ cells/ml in R10 with 3% supernatant from GM-CSF-producing J774 cells and 100 U/ml IL-4 in 2 ml. Half of the culture medium was replaced at days 2, 4, and 6 with the addition of 2 × GM-CSF and IL-4. On day 6, 1 μg/ml LPS (Sigma-Aldrich) or 10 μg/ml CpG 1826 (Operon) was added to mature the BMDCs. On day 7, nonadherent cells were harvested using a Pasteur pipette. Previous studies reported that BMDCs from NOD mice developed into macrophage-like DCs that were poor APCs (23). We purposely enriched for the more DC-like population through positive selection on CD11c MicroBeads. Approximately 20% of the day 7 BMDCs were CD11c⁺/I-Aα⁺, and these cells were purified to >90% purity for the studies described here.

In vitro stimulation of DCs and B cells
Purified DCs and B cells were cultured at 10⁶ cells/ml in 2 ml of ISC-10 in 24-well plates. DCs were stimulated with either 1 μg/ml LPS or 10 μg/ml CpG. B cells were stimulated with either 50 μg/ml LPS or 3 μg/ml CpG. After 16 h, DCs and B cells were harvested, washed three times, and irradiated before use in T cell stimulation assays.

Results
Characterization of APCs isolated from B6.KxNOD F1 mice
Because the arthritic KRNkxg⁷ mice were maintained on the heterozygous H-2kxg⁷ background in our colony, we made use of B6.KxNODF1 mice to examine the presentation of endogenous GPI during steady state, i.e., in a nonarthritic state. Moreover, this allowed us to assess the ability of these APCs to present endogenously derived GPI as well as the exogenously supplied cognate peptides GPI and RNase presented by I-Aα and I-Aβ, respectively. Because previous studies have reported quantitative and qualitative differences in DCs and B cells isolated from NOD mice (24–26), we sought to evaluate the APC populations that are present in our B6.KxNODF1 mice relative to the parental B6.K and NOD mice. As shown in Table I, the percentages of DC subsets, MØs, and B cells were similar among these three strains of mice. Just as others have reported a predominance of CD8α⁺ DCs in NOD mice, we found a similar enrichment of myeloid DCs in our NOD and (B6.KxNOD)F1 mice. Interestingly, our B6.K parental mice also showed a similar lack of CD8α⁺ DCs. Overall, we did not see gross differences in the percentage of CD8α⁺, CD8α⁻, CD11b⁺, or B220⁺ subsets in the DCs from (B6.KxNOD)F1 vs parental NOD or B6.K mice. We also did not see significant differences in the percentages of MØ and B cells among these three strains. The presence of the KRN TCR transgene did not alter the APC subsets compared with (B6.KxNOD)F1 mice (Table I).

As phenotypic differences had been reported in DCs and B cells from NOD mice compared with nonautoimmune strains (24, 25, 26), we cloned and sequenced the relevant transgene in our KRN and NOD backgrounds. As shown in Figure 1A, we found a single major rearrangement event, which we have designated KRNKxG7. As shown in Table I, the percentages of CD8α⁺, CD8α⁻, CD11b⁺, or B220⁺ subsets in the DCs from (B6.KxNOD)F1 vs parental NOD or B6.K mice. We also did not see significant differences in the percentages of MØ and B cells among these three strains. The presence of the KRN TCR transgene did not alter the APC subsets compared with (B6.KxNOD)F1 mice (Table I).

Table I. APC subsets of splenocytes from (B6.KxNOD)F1, KRN(B6.KxNOD)F1, and parental B6.K and NOD strains

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<tr>
<td>CD4⁺ T cells</td>
<td>1.65 ± 0.60</td>
<td>1.26 ± 0.21</td>
<td>1.83 ± 0.33</td>
<td>1.51 ± 0.46</td>
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<tr>
<td>CD8α⁺</td>
<td>0.08 ± 0.03 (5.1)</td>
<td>0.06 ± 0.01 (5.2)</td>
<td>0.08 ± 0.01 (4.4)</td>
<td>0.08 ± 0.03 (5.3)</td>
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<tr>
<td>CD8α⁻</td>
<td>1.56 ± 0.57</td>
<td>1.19 ± 0.22</td>
<td>1.75 ± 0.34</td>
<td>1.43 ± 0.43</td>
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<tr>
<td>CD11b⁺</td>
<td>0.59 ± 0.12 (37.6)</td>
<td>0.46 ± 0.09 (37.9)</td>
<td>0.53 ± 0.03 (29.6)</td>
<td>0.55 ± 0.04 (37.9)</td>
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<tr>
<td>CD220⁻</td>
<td>1.00 ± 0.04 (44.2)</td>
<td>0.44 ± 0.01 (31.7)</td>
<td>0.65 ± 0.00 (35.2)</td>
<td>0.63 ± 0.01 (40.0)</td>
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<td>Macrophages</td>
<td>1.62 ± 0.08</td>
<td>1.10 ± 0.12</td>
<td>1.37 ± 0.04</td>
<td>1.86 ± 0.23</td>
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<tr>
<td>B cells</td>
<td>68.13 ± 4.80</td>
<td>61.47 ± 1.69</td>
<td>55.80 ± 1.31</td>
<td>66.45 ± 6.01</td>
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a. Spleens from individual mice were analyzed by flow cytometry. Percentages are representative of total DCs in that subset. Numbers represent percentage of total live splenocytes. Numbers in parentheses represent percentage of total DCs in that subset. n = 3 mice for B6.K and NOD and 5 mice for (B6.KxNOD)F1 and KRN(B6.KxNOD)F1.
27), we compared the expression of MHC II, CD40, CD80, and CD86 in each of the APCs from (B6.KxNOD)F1, B6.K, and NOD mice (Fig. 1). Because the parental strains were homozygous for the MHC II haplotypes whereas the (B6.KxNOD)F1 cells were heterozygotes, comparison of overall MHC II expression between strains is difficult to interpret. However, we noted more heterogeneous MHC II expression in both DC and Mφs compared with the more uniform MHC II expression in B cells. As shown in Fig. 1, NOD DCs expressed slightly higher CD40 levels compared with the B6.K and (B6.KxNOD)F1 DCs. The difference was less prominent among the Mφs and B cells. Similarly, slightly higher CD80 expression was found among NOD DCs and Mφs compared with their B6.K and (B6.KxNOD)F1 counterparts. As was previously reported, CD86 expression was lower in NOD APCs compared with B6.K APCs (27). We found CD86 expression to be even lower among (B6.KxNOD)F1 APCs compared with B6.K DCs, Mφs, and B cells. As DCs and Mφs comprised a minute proportion of splenocytes (Table I), DCs, Mφs, and B cells were enriched using one round of Miltenyi Biotec MicroBead selection before flow cytometric analysis to facilitate direct comparison between the rare DC and Mφ cells and more abundant B cells. These enriched cells also more closely reflected the cells used in the subsequent Ag presentation studies. Experiments using unfractionated splenocytes yielded similar results (Fig. 1). DCs, as defined by CD11c<sup>high</sup>, comprised a heterogeneous population; approximately half of the cells expressed high levels of I-A<sup>q2</sup>, and the other half expressed low to intermediate levels of I-A<sup>q7</sup> (Fig. 2A). Whereas the I-A<sup>q7</sup>hi DCs were CD40<sup>+</sup> and therefore mature DCs, only half of the I-A<sup>q7</sup>lo cells were CD40<sup>+</sup> (Fig. 2A). Thus, a substantial proportion of splenic DCs displayed markers indicative of an immature phenotype. Splenic Mφs (as defined as CD11b<sup>−</sup>CD11c<sup>+</sup>) predominately expressed low levels of MHC II and CD40. A small population (10%) of these Mφs exhibited a more mature phenotype with higher levels of MHC II and CD40 expression. This phenotype varied from that seen in the bulk splenocytic population, where 16–30% expressed high levels of MHC II, and may reflect differences due to mouse-to-mouse variation and/or MACS enrichment. B cells were a uniform population expressing high levels of both MHC II and CD40 compared with both DCs and Mφs (Fig. 2A). In contrast, expressions of the costimulatory molecules CD80 and CD86 were slightly lower in B cells compared with DCs and Mφs.

Because our objective is to compare the presentation of GPI by various APCs in (B6.KxNOD)F1 mice, we next compared the levels of MHCII, CD40, CD80, and CD86 between the different APCs. As DCs and Mφs comprised a minute proportion of splenocytes (Table I), DCs, Mφs, and B cells were enriched using one round of Miltenyi Biotec MicroBead selection before flow cytometric analysis to facilitate direct comparison between the rare DC and Mφ cells and more abundant B cells. These enriched cells also more closely reflected the cells used in the subsequent Ag presentation studies. Experiments using unfractionated splenocytes yielded similar results (Fig. 1). DCs, as defined by CD11c<sup>high</sup>, comprised a heterogeneous population; approximately half of the cells expressed high levels of I-A<sup>q2</sup>, and the other half expressed low to intermediate levels of I-A<sup>q7</sup> (Fig. 2A). Whereas the I-A<sup>q7</sup>hi DCs were CD40<sup>+</sup> and therefore mature DCs, only half of the I-A<sup>q7</sup>lo cells were CD40<sup>+</sup> (Fig. 2A). Thus, a substantial proportion of splenic DCs displayed markers indicative of an immature phenotype. Splenic Mφs (as defined as CD11b<sup>−</sup>CD11c<sup>+</sup>) predominately expressed low levels of MHC II and CD40. A small population (10%) of these Mφs exhibited a more mature phenotype with higher levels of MHC II and CD40 expression. This phenotype varied from that seen in the bulk splenocytic population, where 16–30% expressed high levels of MHC II, and may reflect differences due to mouse-to-mouse variation and/or MACS enrichment. B cells were a uniform population expressing high levels of both MHC II and CD40 compared with both DCs and Mφs (Fig. 2A). In contrast, expressions of the costimulatory molecules CD80 and CD86 were slightly lower in B cells compared with DCs and Mφs.

**Differential stimulation of KRN T cells by APCs from (B6.KxNOD)F1 mice**

To assess how various APC subsets from (B6.KxNOD)F1 present endogenous GPI under steady-state conditions, purified KRN CD4<sup>+</sup> T cells were cultured with graded numbers of DCs, Mφs, and B cells. We made use of a unique feature of KRN T cells in that they can be positively selected on the H-2<sup>b</sup> background (1).

**FIGURE 1.** Characterization of splenic APCs from B6.K, NOD and (B6.KxNOD)F1 mice. Splenocytes from B6.K, NOD, and (B6.KxNOD)F1 mice were analyzed by flow cytometry. DCs defined by CD11c<sup>high</sup>, Mφs defined by CD11c<sup>low</sup> and CD11b<sup>high</sup>, and B cells defined by B220 were analyzed by flow cytometry for I-E<sup>k</sup> (in B6.K and (B6.KxNOD)F1 splenocytes), I-A<sup>q7</sup> (in NOD and (B6.KxNOD)F1 splenocytes), CD40, CD80, and CD86. Filled areas denote B6.K, thin lines denote (B6.KxNOD)F1, and thick lines denote NOD. The experiment shown is representative of three.

**FIGURE 2.** Characterization of splenic APCs from (B6.KxNOD)F1 mice. DCs defined by CD11c<sup>high</sup>, Mφs defined by CD11c<sup>low</sup> and CD11b<sup>high</sup>, and B cells defined by B220 were analyzed by flow cytometry for I-A<sup>q7</sup>, CD40, CD80, and CD86. A. Coexpression levels of CD40 and I-A<sup>q7</sup> on DCs, Mφs, and B cells. B. Comparison of I-A<sup>q7</sup>, CD40, CD80, and CD86 levels among the three APC populations. Thick lines denote DC, thin lines denote Mφs, and dashed lines denote B cells. The experiment shown is representative of four.
This allowed us to obtain a population of T cells in which any contaminating APCs cannot present Ag to the responder KRN T cells because they lack both I-Ak and I-Ag7. The Ag-presenting function is therefore exclusively derived from the added APCs. For the presentation of endogenous GPI, DCs were the most potent APCs on a per cell basis. They stimulated KRN T cells with at least 100-fold greater efficiency compared with B cells (Fig. 3A). Mφs were of intermediate potency, 3- to 5-fold more stimulatory than B cells. We also isolated resident peritoneal Mφs as an additional source of Mφs and found them to be of equivalent potency to their splenic counterparts. Because we used naive KRN T cells as responder T cells, it was possible that the different potencies reflected differences in costimulatory molecules rather than differences in GPI:1-Ag7 complexes. To address this issue, we made use of KRN T cell hybridomas, which are insensitive to levels of costimuli (11, 28, 29). We elicited the same hierarchy of T cell stimulation using T cell hybridomas as with KRN T cells (Fig. 3B).

Because GPI is ubiquitous and its loss is lethal, we cannot definitively identify the source of GPI in this model. As GPI can be secreted from live cells or released from dying cells, we cannot remove exogenous GPI as a source for Ag presentation. We also cannot exclude the uptake of apoptotic cells as a source of exogenous GPI. To address this issue of Ag uptake, we examined GPI presentation in the presence of excess exogenous GPI. Because B cells were poor APCs on a per cell basis relative to DCs and Mφs, we attempted to normalize their presentation capability by assessing their ability to present exogenous peptides. We found that 10-fold more B cells than either DCs or Mφs were necessary to stimulate equivalent KRN T cell proliferation when supplied with the exogenous KRN peptide ligands GPI and RNase (Fig. 3C). In the absence of exogenous Ag, KRN T cell response to this higher number of B cells (2x10^5) was consistently half of that seen with DCs (2x10^4) and similar in magnitude to that seen with Mφs (2x10^5) (Fig. 3C). Using these APC numbers, we showed that the extent of free GPI uptake was limited, as the addition of 50 μg/ml recombinant GPI (>100-fold excess of serum level) to all three APC populations (DCs, Mφs, and B cells) increased KRN T cell proliferation by <2-fold (Fig. 3C) over that achieved with the APCs alone. This was in agreement with previously published data showing poor uptake of free GPI by non-GPI B cells (30). Therefore exogenous GPI likely contributed minimally to the number of GPI:1-Ag7 complexes. Moreover, because 10-fold more B cells were capable of stimulating KRN T cells to equivalent levels with exogenous peptides, the 100-fold difference between DCs and B cells therefore reflect differences in the processing and presentation of endogenous GPI. Our results are in agreement with previous reports comparing the stimulatory function of DCs vs B cells with cytosolic Ags and extend the studies to include Mφs (10, 11, 29).

Thus, despite expressing higher levels of CD40 and I-Ag7, B cells were inefficient at processing and presenting GPI, such that they were incapable of presenting GPI to KRN T cells. The differential GPI presentation by various APCs is illustrated in Fig. 3. A and B, Differential stimulation of KRN naive T cells (A) and hybridomas (B) by graded numbers of DCs (●), Mφs (△), peritoneal resident macrophages (■), and B cells (★). C, Stimulation of naive KRN T cells by exogenously added Ags. Recombinant GPI protein and hen egg lysozyme (HEL) (Sigma-Aldrich) were added at 50 μg/ml. KRN agonists GPI(281–293), RNase(42–56), and irrelevant peptide BDC (I-Ag7 restricted) were added at 1μM. Filled squares denote DCs, open squares denote peritoneal Mφs, striped squares denote Mφs, and stippled squares denote B cells. Each data point represents mean of triplicate wells with error bars denoting SD. The experiment shown is representative of at least four experiments.
the weakest APCs for KRN T cells. Although B cells were the pathogenic effectors in this disease model, DCs are most likely the initial activator of the KRN T cell in vivo with subsequent recruitment of anti-GPI B cells and Møs for amplification of the anti-GPI response.

**DCs and B cells from different lymphoid organs stimulate KRN T cells equivalently**

In K/BxN mice, KRN T cells were incompletely deleted in the thymus, such that they persisted in the periphery where they became activated. We demonstrated previously that expression of a KRN agonist in the thymus resulted in complete deletion of KRN T cells (20). Thus, thymic presentation of endogenous GPI is insufficient for complete negative selection. Therefore, it is plausible that the disparity in GPI presentation between the thymus and periphery allows KRN T cells to escape deletion and be activated by higher GPI presentation in the periphery. We have also shown previously that despite the ubiquitous expression of this autoantigen, the draining lymph nodes were the initial sites where the anti-GPI B cells were detected (4). Because different DC and B cell subsets are found in the various lymphoid organs (31–33), the pattern of anti-GPI reactivity may reflect differences in the level of GPI: I-A^d^ complexes presented by the resident APCs. To test this hypothesis, DCs were isolated from thymi, lymph nodes, and spleens of steady state nonarthritic (B6.KxNOD)F1 mice and (B6xB6.K)F1 mice as negative controls. Thymic DCs stimulated KRN T cells with equivalent potency as that of DCs isolated from the lymph nodes or spleens (Fig. 4A). Therefore, the peripheral activation of KRN T cells in K/BxN mice does not reflect a disparity in the presentation of GPI in the thymus relative to the secondary lymphoid organs.

Previous studies from our laboratory (4) have demonstrated that anti-GPI Ab-secreting cells were first detected in the lymph nodes draining arthritic joints. This raised the question of whether lymph node B cells were better than splenic B cells at presenting GPI to KRN T cells. Neither population strongly stimulated KRN T cells; however, the lymph node B cells were consistently less stimulatory than splenic B cells (Fig. 4B). A likely explanation for the difference is the presence of marginal zone B cells, members of a small B cell population in the spleen shown to be potent APCs for naive T cells (34, 35). These results showed that non-GPI B cells in both the spleen and lymph nodes weakly stimulate KRN T cells and that their engagement does not result in productive B cell activation and polyclonal Ab generation.

**Presentation of GPI during various phases of arthritis**

In K/BxN mice, the development of arthritis presents three significant perturbations to the processing and presentation of the autoantigen GPI. First, GPI is a glycolytic enzyme; thus, its expression is linked to the metabolic state of the cell. Therefore, the expression of GPI could vary throughout the different phases of arthritis. Second, elaboration of anti-GPI Abs results in the production of immune complexes that can serve both as sources of Ag and as modulators of APC functions. Third, the preferential growth of anti-GPI B cells during arthritis results in an increasing proportion of B cells bearing GPI-specific BCRs, which can augment Ag presentation through BCR-mediated endocytosis of GPI. It is unclear how GPI is presented during the evolution of arthritis. In this model, acute synovitis arises at 4–5 wk of age, reaches a peak at 6–7 wk of age, and becomes quiescent after 10–12 wk of age (20). To assess how this autoantigen is presented through the various phases of arthritis, we isolated splenic DCs and B cells from nonarthritic, acutely arthritic (6–7 wk), and chronically arthritic (>12 wk) mice. We found that, as the disease progressed, DCs from arthritic mice became progressively more stimulatory compared with DCs from nonarthritic mice (Fig. 5A). DCs from chronically inflamed mice were 10-fold more stimulatory than non-arthritic DCs. This was not due to elevated expression of MHCII, CD40, or costimulatory molecules CD80/86 (Fig. 5C). As reported...
FIGURE 5. Presentation of GPI by DCs and B cells during different phases of arthritis. A, Graded number of splenic DCs from nonarthritic (Non; □), acutely arthritic (Acute; □), and chronic arthritic (Chronic; ■) mice were used to stimulate KRN naive T cells. Each data point represents mean of triplicate wells with error bars denoting SD. The experiment shown is representative of three experiments. B, Graded number of splenic B cells from nonarthritic (Non; gray circles), acutely arthritic (Acute; ○), and chronically arthritic (Chronic; ●) mice were used to stimulate KRN naive T cells. Each data point represents mean of triplicate wells, with error bars denoting SD. The experiment shown is representative of three experiments. C, Expression of I-A^{	ext{d}}\text{, CD40, CD80, and CD86 on DCs and B cells from varying phases of arthritis. Gray-filled areas denote nonarthritic mice, thin lines denote acutely arthritic mice, and thick lines denote chronically arthritic mice. Experiment shown is representative of three experiments.} D, Surface binding of GPI or hen egg lysozyme (HEL) by DCs and B cells from varying phases of arthritis. Splenocytes were incubated with 1 μg of biotinylated recombinant GPI or biotinylated hen egg lysozyme (negative control) followed by streptavidin-allophycocyanin to detect the presence of anti-GPI Igs on the surface of DCs and B cells. DCs that have captured anti-GPI Igs through the FcR would therefore bind GPI. Gray-filled areas denote nonarthritic mice, thin lines denote acutely arthritic mice, and thick lines denote chronically arthritic mice. The experiment shown is representative of three experiments. E, Presentation of GPI by DCs and B cells from thymi (T; □), lymph nodes (LN; ■ and ●) and spleen (□ and ○) to KRN T cells. Squares denote DCs. Circles denote B cells. Each data point represents mean of triplicate wells, with error bars denoting SD. The experiment shown is representative of three experiments.
previously by Matsumoto et al. (3), in nonartritic (B6.KxNOD)F1 mice, the level of serum GPI was high (μg/ml range) at birth and dropped to ~0.4 μg/ml as the mice matured. In the arthritic mice, free GPI was low at week 1 but became undetectable by week 3, coinciding with the appearance of anti-GPI Abs and immune complexes. To assess whether DCs from arthritic mice have captured GPI-anti-GPI immune complexes, we used biotinylated recombinant GPI to detect anti-GPI Abs bound on FcRs on DC surfaces. As shown in Fig. 5D, we found significant numbers of DCs with surface anti-GPI Abs. The accumulation of surface GPI-anti-GPI immune complexes on DCs correlated with the rising titers of anti-GPI Abs and arthritis development. No staining was seen using similarly biotinylated hen egg lysozyme (Fig. 5D). Similar accumulation of surface GPI binding was found on purified DCs from nonarthritic (B6.KxNOD)F1 mice incubated with sera from arthritic mice for 16 h, showing that DCs actively acquire GPI:anti-GPI complexes from serum (data not shown). Consistent with increased GPI presentation through uptake of GPI immune complexes, we found that peripheral DCs were significantly better at stimulating KRN T cells than thymic DCs, correlating with surface GPI (Fig. 5E).

We also examined B cells for their ability to stimulate KRN T cells. In contrast to DCs, B cells from acutely inflamed mice were
the most stimulatory, activating KRN T cells with 10-fold fewer cells than either nonarthritic or chronically arthritic mice (Fig. 5B). However, the magnitude of the T cell response remained significantly lower than that induced by DCs. Again, we did not detect any changes in the expression of I-A<sup>d</sup>, CD40, or CD80/86 molecules among splenic B cells isolated at different phases of arthritis (Fig. 5C). There was no significant enhancement in GPI presentation in B cells in chronically arthritic mice despite the finding that the majority of splenic B cells displayed surface anti-GPI Abs (Fig. 5D). We were unable to differentiate between anti-GPI BCR or anti-GPI in the form of immune complexes bound to FcR, but from prior studies it was known the majority of these B cells represented anti-GPI-secreting B cells (4). In distinct contrast with DCs, the presence of GPI:anti-GPI immune complexes did not enhance GPI presentation. Indeed, we noted a slight decrease in the magnitude of T cell response elicited with B cells from chronically arthritic mice compared with steady-state B cells for which no GPI binding was seen (Fig. 5D). Lymph node and splenic B cells from chronically arthritic mice exhibited similar stimulatory potencies as those seen in steady-state B cells (Fig. 5E).

In summary, as arthritis progressed, peripheral DCs became more effective stimulators for KRN T cells through Ag uptake via immune complexes. B cells, despite the preferential growth of GPI-specific B cells, continued to be ineffective APCs for KRN T cells. Differences in FcR populations in DC and B cells likely account for this differential effect of immune complexes on APC functions, as B cells only express FcγRIIb, whose engagement serves to down-modulate B cell responses (36–39), whereas DCs also can use FcγRIII as a mechanism for Ag uptake to enhance presentation (40).

Presentation of GPI in acute inflammatory states

There is ample correlation between infection and the onset of autoimmune disease (41–44). A prominent mechanism posited bystander activation of resident APCs by inflammatory cytokines or microbial products, thereby altering the context in which self-Ags are processed and presented to autoreactive T cells (45). Infection can therefore provide an attractive mechanism where a previously innocuous TCR:peptide-MHC interaction can become pathogenic. TLRs play a critical role in adaptive immunity as well as in host innate immunity (reviewed in Ref. 46). TLR ligation can promote adaptive immunity by inducing DC maturation by up-regulating

![Figure 7](http://www.jimmunol.org)
MHC II and costimulatory molecules CD80/86 and altering Ag-processing capacity.

To analyze the effect of acute inflammation on endogenous GPI presentation, mice were injected i.p. with either LPS or PBS control. In vivo-matured DCs were harvested after 18 h and assayed for their ability to stimulate KRN T cells. Surprisingly, we found no enhancement in the ability of LPS-treated DCs to stimulate KRN T cells relative to PBS-treated controls (Fig. 6A). This is due to decreased endogenous GPI presentation, as LPS-treated DCs were more stimulatory than PBS-treated DCs when exogenous GPI and RNase peptides were added (Fig. 6B). Moreover, by flow cytometry, up-regulation of I-A, IL-4, CD40, CD80, and CD86 was seen in LPS-treated DCs (Fig. 6C). Given this unexpected result, we examined in vitro maturation of DCs by TLR9 as well as TLR4 engagement. Purified splenic DCs were cultured for 18 h with medium, LPS, or CpG and used to stimulate KRN T cells. As was seen with in vivo activation, we did not see a significant increase in the ability of either LPS- or CpG-treated DCs to stimulate KRN T cells (Fig. 7A). Because splenic DCs were a heterogeneous population, we used the BMDC as a more uniform source of immature DCs. Day 7 BMDCs were generated from bone marrow with GMCSF and IL-4 and matured in vitro with LPS or CpG 18 h before harvest. As was seen for in vitro-stimulated splenic DCs, BMDCs also did not increase GPI presentation upon maturation (Fig. 7B). In contrast, purified B cells stimulated in vitro with either LPS or CpG were 3–10-fold more stimulatory on a per cell basis than unstimulated B cells (Fig. 7C). In addition, the magnitude of the T cell response approached that induced by DCs. Thus, DCs and B cells differentially regulate MHC II presentation of an autoantigen in response to TLR signaling. Whereas DCs are more potent APCs at baseline, the processing of GPI is regulated such that there is no enhanced display of self-peptides during bystander activation. B cells, in contrast, showed significant increases in their ability to stimulate KRN T cells. This change in relative potencies between DCs and B cells may therefore provide a scenario in which polyclonal B cells may more effectively engage KRN T cells to receive help.

Discussion
Systemic reactivity to the autoantigen GPI resulted in a joint specific, immune complex-mediated arthritis. GPI exhibits several features that distinguish it from other model Ags of autoimmunity. It is an actual autoantigen that is ubiquitously expressed in normal mice. We showed here that ex vivo-isolated DCs, MΦa, and B cells can all present this autoantigen through the MHC II pathway but with different efficiencies, reflecting differences in the processing of this autoantigen.

A myriad of studies have documented the superior APC function of DCs for naive T cell activation compared with the more abundant B cells (47). The majority of these experiments made use of exogenously added Ags. Our findings are in agreement with previous reports showing DCs to be 50–100-fold more potent than B cells at presenting peptides derived from a cytosolic source (10, 11). We extended the hierarchy to include MΦs, which are of an intermediate phenotype. Thus, at steady state, DCs are likely the initiators of the anti-GPI response in the K/BxN mice. Despite reported differences in DC subsets within the thymus, lymph nodes, and spleen (32, 33), we did not find any significant difference in the level of GPI presentation by DCs from these different sites. Therefore, the lack of T cell tolerance and the subsequent activation of KRN T cells cannot be attributed to differences in Ag presentation. A likely explanation for the lack of tolerance is the overabundance of KRN T cells in this TCR Tg system, which overwhelms its capacity to delete autoreactive T cells as has been demonstrated for other TCR Tg systems (48, 49).

Our finding that GPI presentation is not enhanced in DCs after TLR engagement is unexpected and conflicts with several reports (28, 29) showing enhanced presentation of autoantigens upon DC maturation attributed to increased expression of MHC II and costimulatory molecules. How do we account for this discrepancy? Previous studies made use of transmembrane Ags with mice on a B6 background (28, 29), whereas our studies assessed the presentation of a cytosolic Ag on a mixed (B6.KxNOD)F1 background. Because proteosomal processing is essential for processing of cytosolic Ags (10, 50, 51) and defects in proteosomes have been reported in NOD strains (52, 53), the use of APCs on a mixed (B6.KxNOD)F1 background may result in this lack of enhancement in GPI presentation. Other qualitative differences in NOD APCs have been described and may also be a determining factor (26, 27, 54–57).

B cells, despite their abundance, are the least efficient cells in processing and presenting GPI. Possible mechanisms to account for this deficiency include but are not limited to a differential capacity for Ag uptake, a selective expression of H-2O in B cells to favor peptide loading in the late endosomes (58–60), and a differential expression of proteases involved in Ag presentation (61). Because presentation of cytosolic Ag is inefficient in B cells, BCR-mediated endocytosis of GPI imparts a significant advantage to anti-GPI B cells, such that only they proliferate in the presence of KRN T cell help (62, 63). Other autospecific B cells receive signal 1 through the BCR but do not receive signal 2 because of intellectual engagement of KRN T cells and, hence, are not activated. The weak signal evoked by non-GPI B cells in steady state would not result in polyclonal autoantibody production.

As the disease progressed, the recruitment of anti-GPI B cells resulted in the production of anti-GPI Abs and amplification of GPI presentation by DCs through the uptake of immune complexes through FcyRIII. Thus, DCs became potent APCs during the progression of the disease and served to amplify the anti-GPI response. It is interesting that in aged KRNkxg7 mice inflammatory infiltrates appear in the liver and lungs (our unpublished observation), possibly reflecting a more T cell-mediated disease. B cells from chronically arthritic mice continued to be poor stimulators of KRN T cells despite the high proportion of GPI-specific B cells and the surface binding of anti-GPI immune complexes. In agreement with the role of FcyRII in limiting autoimmunity, the binding of immune complexes on B cell surfaces down-modulated GPI presentation in chronic arthritic B cells compared with B cells of acutely arthritic mice.

In the aftermath of an acute inflammatory stimulus, the détente between the stronger but fewer DCs and weaker but abundant B cells to stimulate KRN T cells is perturbed. Upon TLR4 or TLR9 ligation, activated B cells became more potent stimulators, approaching the potency of DCs. In our studies, DCs regulated the processing of the cytosolic Ag such that the overall presentation was kept constant despite the up-regulation of MHC II and costimulatory molecules. This shifting of powers now may allow autoreactive B cells to more effectively engage KRN T cells and allow for polyclonal B cell activation. This mechanism therefore provides an attractive mechanism for finding autoantibodies in the wake of an antecedent infection.

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Disclosures

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References


