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B Cells Capturing Antigen Conjugated with CpG Oligodeoxynucleotides Induce Th1 Cells by Elaborating IL-12

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APCs initiate T cell-mediated immune responses against foreign Ags. Dendritic cells are professional APCs that play unique roles, including Ag-nonspecific capture, priming of naive T cells, and Th1 induction, whereas B cells generally lack these functions. In this study we uncovered novel aspects of murine B cells as APCs using CpG oligodeoxynucleotides (CpG) conjugated with an Ag. B cells served as efficient APCs independently of surface Igs. This characteristic was underlaid by the CpG-mediated Ag uptake and presentation, which were functional only when CpG were covalently conjugated to Ag. The B cells cultured with CpG-conjugated Ag not only enhanced IFN-γ formation by Th1 cells, but also induced Th1 differentiation from unprimed T cells. These effects paralleled with the increase in the expression of CD40, CD86, and class II molecules on B cells and the coordinated production of IL-12 by the cells. To our knowledge this is the first report revealing that B cells share with dendritic cells common intrinsic characteristics, such as the Ag-nonspecific capture and presentation, and the induction of Th1 differentiation from unprimed T cells. The Journal of Immunology, 2002, 169: 787–794.

Antigen-presenting cells play a critical role in initiating T cell-mediated immune responses. Dendritic cells (DCs) are characterized by their ability to capture a large diversity of Ags in an Ag-nonspecific manner and to present them in a highly immunogenic form (1–4). These features highlight DCs as professional APCs capable of priming naive T cells (5, 6). B cells also serve as APCs (7–9). Resting B cells are poor at presenting Ag (10–12) or are tolerogenic to T cells (13, 14), and turn into effective APCs with the increased expression of costimulatory molecules after the activation (15–17). However, Ags specifically bound to surface Ig (sIg) on B cells are presented to T cells 103- to 104-fold more efficiently than those entering the cells in an sIg-independent manner (10, 18, 19). B cells are efficient APCs for Ag-primed T cells (13, 20) and are likely to induce Th2-dominant responses (21–27). It has long been debated whether B cells can prime naive T cells (28–32). Recent in vitro experiments using Ig transgenic (tg) mice demonstrated that B cells have the capacity of activating naive T cells for proliferation (33, 34) and the development of Th2 cells (35) or unpolarized effector T cells (36). However, little is known about the ability of B cells to prime naive T cells for differentiation toward Th1 cells.

The nature of DNAs as immune stimulators has recently been attracting much attention. Initially, CpG oligodeoxynucleotides (CpG) were found to trigger B cells to proliferate and differentiate into Ig-secreting cells (37). CpG were also found to activate monocytes, macrophages, and DCs to produce IL-12, which facilitates the development of Th1 cells (38–45). We reported that the ability of Ag to induce the differentiation of Ag-specific Th1 cells was greatly enhanced when CpG were covalently conjugated to the Ag (46, 47). The underlying mechanisms included the augmented capture of the CpG-tagged Ag by DCs in a CpG-guided manner and the expression of costimulatory molecules and IL-12 by the Ag-pulsed DCs (47). While the binding of CpG to B cells has recently been extensively studied (48), the functional relevance of CpG binding has not been addressed.

In this report we examined the role of CpG in the CpG-Ag conjugate in Ag capture and T cell stimulation by B cells. We observed that the CpG-Ag conjugates were efficiently captured by B cells regardless of the Ag specificity of sIg. The CpG-activated B cells could, in turn, present the Ag and induce the differentiation of Th1 cells from unprimed tg T cells by elaborating IL-12.

Materials and Methods

Animals

BALB/c mice were bred in our animal facility and were used at 7–12 wk of age. BALB/c mice tg for TCR specific for OVA323-339 and I-Aβ were supplied by Dr. S. Habu (Tokai University, Kanagawa, Japan) (49).

Ags, CpG, and direct conjugation to Ags

OVA (Sigma, St. Louis, MO), BSA (Sigma-Aldrich), and keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) were conjugated with 2,4,6-trinitrobenzenesulfonate (Wako Pure Chemical, Osaka, Japan). The degree of substitution was 10 trinitrophenyl (TNP) residues/100-kDa Ag. The CpG (1826) used throughout this study consisted of 20 bases containing two CpG motifs (TCCATGACGTTCCTGACGTT) (50) and were fully phosphorothioated (underlining indicates a CpG motif). The control non-CpG oligodeoxynucleotides (ODNs; 1745) were identical, except that the CpG motifs were rearranged (TCCATGAGCTTCCTGAGTCT) (50) and were fully phosphorothioated (underlining indicates a CpG motif). The control non-CpG oligodeoxynucleotides (ODNs; 1745) were identical, except that the CpG motifs were rearranged (TCCATGACGTTCCTGACGTT) (50) and were fully phosphorothioated (underlining indicates a CpG motif). The control non-CpG oligodeoxynucleotides (ODNs; 1745) were identical, except that the CpG motifs were rearranged (TCCATGACGTTCCTGACGTT) (50) and were fully phosphorothioated (underlining indicates a CpG motif). The control non-CpG oligodeoxynucleotides (ODNs; 1745) were identical, except that the CpG motifs were rearranged (TCCATGACGTTCCTGACGTT) (50) and were fully phosphorothioated (underlining indicates a CpG motif).
OR) by mixing SH-conjugated ODNs at the 5' end and maleimid-activated Ag using sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carbodiyi (Pierce, Rockford, IL). Uncoujugated ODNs were removed by extensive dialysis. Aliquots of Cpg-OVA conjugate were purified by gel filtration chromatography to minimize the effects of the contaminating aggregates, as described previously (47). The molar and weight ratios of the ODN to Ag are listed in Table I.

**Purification of B cells and the B cell line**

Spleen or LN cells depleted of RBC by hypotonic treatment were layered onto 50% Percoll (Pharmacia Biotech, Uppsala, Sweden) and centrifuged for 10 min at 2000 rpm. The cells under the 50% Percoll layer were incubated with anti-B220 magnetic microbeads and enriched for B220+ B cells using a MACS magnetic separation system (Miltenyi Biotec, Auburn, CA). B cells used as APCs for the stimulation of Th cells were further purified by depleting them of CD11c+ DCs with anti-CD11c microbeads before enrichment for B220+ cells. Reanalysis of the recovered B220+CD11c− cells revealed that B220− B cells and CD11c− DCs comprised >98% and <0.5%, respectively (Fig. 1B). Low and high buoyant density B cells were prepared by centrifugation over a discontinuous Percoll gradient containing 55–60 and 60/70% interface were collected separately and used as large and small B cells, respectively. Where indicated, the BCL1 B cell leukemia line (51) (provided by Cell Resource for Biomedical Research, Tohoku University) was used as alternative APCs for the Th1 induction or activation.

**Stimulation of naive OVA-specific T cells with Cpg and OVA for the Th1 differentiation**

CD4+ T cells were prepared from spleens of unimmunized OVA-specific TCR tg mice by depletion of CD8+ and Ia+ cells using a panning method (47). B220−CD11c− B cells (2.5 × 10⁶) purified from unimmunized BALB/c mouse spleens or the BCL1 B cell line were pulsed with OVA and/or Cpg for 3 h. The BCL1 cell line was then fixed with 0.5% paraformaldehyde at 37°C for 15 min. After extensive washing they were cocultured with 2.5 × 10⁶ OVA-specific CD4+ T cells in 2 ml medium in 12-well plates. After 6 days of culture, viable lymphocytes (1 × 10⁶) recovered by Ficoll-Paque (Pharmacia Biotech) density-gradient centrifugation were restimulated with 2 × 10⁶ APCs in the presence or the absence of OVA (100 μg/ml) in quadruplicate in 96-well plates. APCs were prepared by treating spleen cells from unimmunized BALB/c mice with mitomycin C (MMC, 50 μg/ml; Wako Pure Chemical) for 30 min at 37°C. After 2 days of culture, the culture supernatants were assayed for IFN-γ and IL-4. To neutralize IL-12 activity, 10 μg/ml anti-IL-12 mAb (BioSource International, Camarillo, CA) was used as alternative APCs for the Th1 induction or activation.

**Restimulation of OVA-specific Th1 cells with Cpg and OVA**

OVA-specific TCR tg Th1 (hereafter referred to as Th1) cells were induced in vitro and enriched for CD4+ cells as described previously (47). In brief, spleen cells from unimmunized OVA-specific TCR tg mice were cultured with OVA (100 μg/ml) and IL-12 (1 ng/ml). After 6 days of culture, viable lymphocytes were enriched for CD4+ T cells by a panning method. CD4+ Th1 cells (1 × 10⁵) were cultured in 96-well plates with 2 × 10⁵ untreated spleen cells or B220−CD11c− B cells from BALB/c mice or the BCL1 B cell line as APCs in the presence of OVA and/or Cpg in quadruplicate. After 2 days of culture, the culture supernatants were assayed for IFN-γ and IL-4. The enriched T cells failed to produce IFN-γ or IL-4 in response to OVA or Cpg-OVA in the absence of additional APCs.

**Restimulation of anti-OVA Th1 cells by TNP-primed B cells**

BALB/c mice were immunized in the hind footpads with TNP-KLH or KLH emulsified in CFA (Difco, Detroit, MI). After 1 wk, ppoliteal LN cells were pooled from three mice and purified for B220−CD11c− B cells as described above. CD4+ Th1 cells (1 × 10⁵) were cultured with 2 × 10⁵ purified B cells in the presence of OVA conjugated with Cpg, TNP, or both in quadruplicate in 96-well plates for 2 days, and the culture supernatants were assayed for IFN-γ.

**Stimulation of the purified B cells with Cpg**

The purified B220−CD11c− B cells (2 × 10⁵/well) from unimmunized BALB/c mice were cultured with LPS (Sigma-Aldrich), Cpg, or control non-Cpg ODNs in quadruplicate in 96-well plates. After 2 days of culture, the culture supernatants were assayed for IL-12 by ELISA as described below.

**Cytokine assay**

The concentrations of IFN-γ and IL-4 in the culture supernatants were determined using ELISA as described previously (52). The concentrations of IL-12 were determined using paired anti-IL-12 mAbs (BioSource International, Camarillo, CA) according to the manufacturer’s instructions. Tetramethylbenzidine reagent (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used for color development, and ODs determined at 450 nm were converted to concentrations (nanograms per milliliter) according to a standard curve. Standard recombiant mouse IL-12 was purchased from PeproTech (Rocky Hill, NJ).

**Reagents used for flow cytometry**

FITC-conjugated anti-B220 mAb, PE-conjugated anti-IL-12 mAb, and allophycocyanin-conjugated streptavidin (SA) were purchased from Immunotech (Westbrook, ME), BD PharMingen, and Biomedica (Foster City, CA), respectively. Biotinylated anti-CD40 and anti-CD86 mAbs were purchased from Caltag Laboratories (Burlingame, CA). Anti-IFN-γ mAb (MAB-788) was partially purified from ascites by ammonium sulfate precipitation and conjugated to biotin (Sigma-Aldrich) in our laboratory.

**Analyses of B cells by flow cytometry**

The B220− B cells from unimmunized spleens were incubated with PE alone, a mixture of PE and Cpg, or graded doses of PE-Cpg conjugates overnight. The cells were stained with FITC-conjugated B220 mAb together with biotinylated anti-CD40, anti-CD86, or anti-IFN-γ mAb. The binding of biotinylated mAbs was detected with allophycocyanin-SA. The correlations between PE staining and the CD40, CD86, or IFN-γ expression on the viable B220− B cells were analyzed using FACS Calibur (BD Bioscience, Mountain View, CA). Propidium iodide (Sigma-Aldrich)-stained dead cells were excluded from analyses. For staining of intracytoplasmic IL-12, the purified B220− B cells were cultured with Cpg or LPS overnight, with 10 μg/ml brefeldin A (Wako Pure Chemical) added for the final 4 h. After staining with FITC-labeled anti-B220 mAb, the cells were treated with cell permeabilization solution (Immunotech, Minneapolis, MN) and then stained with PE-labeled anti-IL-12 mAb (0.3 μg/ml). Where indicated, a 20-fold excess of unlabeled anti-IL-12 mAb (BD PharMingen) was also added. They were analyzed by flow cytometry.

**Statistics**

Data from in vitro culture experiments are expressed as the mean ± SEM. Each experiment was repeated at least twice. Student’s t test was used in the analysis of the results.

**Results**

**Activation of Th1 cells by unprimed B cells and Cpg-OVA**

We first determined whether unprimed B cells could present Ag to OVA-specific Th1 cells. Spleen cells from unimmunized BALB/c mice (Fig. 1A) were enriched for B cells using magnetic beads. The purity of B220− B cells was >98%, and the contamination by CD11c+ DCs was <0.5% (Fig. 1B). When spleen cells were employed for APCs, the Th1 cells predominantly produced IFN-γ in response to OVA. With the purified B cells, however, Th1 cells failed to produce IFN-γ in response to 10–100 μg/ml OVA, verifying the depletion of DCs and the high purity of the B cell fraction (Fig. 1C). The lack of cytokine production from the Th1 cells cultured with Cpg-OVA in the absence of APCs substantiated the depletion of DCs in the Th1 population (data not shown). The purified B cells failed to present OVA in the presence of 10 μg/ml Cpg, whereas the same B cells cultured with Cpg-OVA

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Table I. Molar and weight ratios of ODN to Ag

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<th>ODN</th>
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<th>Weight Ratio</th>
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<td>Non-Cpg-OVA</td>
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<td>Non-Cpg-PE</td>
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conjugate potently activated Th1 cells for the production of IFN-γ, but not IL-4, in a dose-dependent manner. We also employed purified CpG-OVA that contained minimal amounts of aggregates (47) and found that the monomeric CpG-OVA and CpG-OVA before purification were comparable in their ability to activate Th1 cells (Fig. 1D), indicating that the Th1 activation reflects the feature of the monomeric CpG-conjugated OVA, but not the aggregates. Although B cells could also present non-CpG ODN-conjugated OVA to Th1 cells, the IFN-γ levels induced by non-CpG ODN-conjugated OVA were significantly lower than those induced by CpG-OVA (Fig. 1E). Neither large nor small B cells presented OVA to Th1 cells, whereas both B cell populations presented CpG-OVA to stimulate Th1 cells to comparable levels at the doses tested (Fig. 1F).

**Differentiation of Th1 cells from naive T cells by stimulation with CpG-OVA and unpurified B cells**

We next examined whether purified B cells could induce the differentiation of Ag-specific Th1 cells from naive T cells. CD4 T cells precultured with MMC-treated spleen cells and OVA developed into effector Th cells that produced comparable levels of IFN-γ and IL-4 upon antigenic stimulation. However, CD4 T cells precultured with purified B cells in the presence of 100 μg/ml OVA did not produce cytokines upon restimulation with Ag in the presence of APCs (Fig. 2A). The B cells failed to present OVA to naive T cells for the development of effector Th cells even after stimulation with a mixture of OVA and CpG. In contrast, the conjugate of the corresponding doses of CpG and OVA induced naive T cells for the development of Th1 cells. Purified monomeric CpG-OVA devoid of aggregates had a Th1-inducing activity comparable to that of CpG-OVA before fractionation, indicating that the Th1 development can be ascribed to the monomeric CpG-OVA conjugates. The non-CpG-OVA conjugate failed to induce the differentiation of naive T cells. It was also found in an additional experiment that IFN-γ production of Th1 cells induced by CpG-OVA increased in a dose-dependent manner (Fig. 2B). The results clearly showed that unpurified B cells could present Ag to induce the Th1 differentiation from unpurified T cells if the Ag was in the form of a conjugate with CpG.

**Dose-dependent and coordinated increases in Ag uptake and the expression of costimulatory molecules on B cells by CpG-Ag conjugate**

We then determined the effects of CpG in the conjugate on Ag uptake by and the expression of costimulatory molecules on B cells. To track the fate of Ag, CpG-conjugated PE was employed. Previous experiments showed that CpG-PE contained no discernible amounts of aggregates, as determined by SDS-PAGE, and that free PE or CpG in the CpG-PE preparation did not affect the function of DC (47). Splenic B cells were incubated overnight with either the conjugate or a mixture of PE and CpG, and PE in B220-CD11c- B cells (C–E), or fractionated B cells using Percoll density centrifugation (F). After 2 days of culture, the culture supernatants were assayed for IFN-γ and IL-4 by ELISA. Flow cytometric results are representative of multiple independent experiments. The in vitro culture experiments were repeated independently two or three times with similar results. * p < 0.005; ** p < 10^{-4} (compared with the CpG + OVA group).

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**FIGURE 1.** Activation of Th1 cells by unpurified B cells and CpG-OVA. A and B, Spleen cells from unprimed BALB/c mice were stained with mAbs against B220 and CD11c before (A) and after (B) the purification for B220-CD11c- B cells. Contamination of the purified B cell preparation with CD11c- DC was reproducibly <0.5% (B). C–F, Th1 cells were induced by culturing spleen cells of OVA-specific TCR tg mice in the presence of OVA (100 μg/ml) and IL-12 (1 ng/ml) for 6 days, and 1 × 10^5 CD4+ Th1 cells were restimulated with OVA, CpG, a mixture of CpG (10 μg/ml) and OVA (10 μg/ml)), graded doses of the CpG-conjugated OVA, purified CpG-OVA, or control ODN-conjugated OVA in the presence of 2 × 10^5 APCs. The APCs used were spleen cells (C), purified B220-CD11c- B cells (C–E), or fractionated B cells using Percoll density centrifugation (F). After 2 days of culture, the culture supernatants were assayed for IFN-γ and IL-4 by ELISA. Flow cytometric results are representative of multiple independent experiments. The in vitro culture experiments were repeated independently two or three times with similar results. * p < 0.005; ** p < 10^{-4} (compared with the CpG + OVA group).
the increase in the expression of costimulatory molecules. Most notable was CD86 expression. The results show that the CpG-PE conjugate induced concomitant increases in CD86 expression and PE uptake in a dose-dependent fashion. CpG in the mixture with PE induced CD86 expression without an accompanying increase in PE uptake. After activation with CpG-PE conjugate, B cells with higher levels of PE expression exhibited concomitant increases in the expression of CD40 and class II molecules. In additional experiments, the effects of non-CpG control ODN were examined. The control ODN-conjugated PE did not increase the expression of CD86 (Fig. 3B).

**IL-12 production by CpG-activated B cells**

We determined whether IL-12 secreted from B cells facilitated Th1 differentiation by the CpG-OVA conjugate. First, we assessed IL-12 production by CpG-activated B cells. CpG stimulated purified B cells to form IL-12 in a dose-dependent manner (Fig. 4A). The CpG-OVA conjugate also induced IL-12 formation to comparable levels as CpG alone at two different doses. Non-CpG ODNs failed to induce IL-12 formation. The unstimulated or LPS-stimulated B cells failed to produce IL-12, as reported previously (54). The results confirmed the exclusion of DCs, which produce IL-12 in response to LPS stimulation (55), in the B cell preparation.

Additional support came from the experiment with neutralizing anti-IL-12 mAb. The purified B cell population, which failed to present OVA to Th cells, presented the CpG-OVA conjugate to induce OVA-specific Th1 cells (Fig. 4B). The induction of Th1 differentiation by purified B cells was neutralized by anti-IL-12 mAb, but not by the isotype-matched control. Control non-CpG ODNs failed to induce Th1 differentiation.

More concrete evidence for the formation of IL-12 by B cells was obtained by the staining of intracytoplasmic IL-12 in gated B220+ B cells (Fig. 5). Neither unstimulated B220+ B cells nor LPS-activated B cells were stained with PE-labeled anti-IL-12 mAb, substantiating the lack of nonspecific staining with PE-labeled anti-IL-12 mAb. In contrast, the staining of CpG-activated B cells with PE-labeled anti-IL-12 mAb shifted the staining of the whole population by nearly 4 times, as judged by the increase in the mean fluorescence intensity (from 11.42 to 44.67). The proportion of B cells scored as positive for IL-12 staining increased to 16.5%. IL-12 staining was specific, because pretreatment of CpG-activated B cells with unconjugated anti-IL-12 mAb inhibited binding of PE-labeled anti-IL-12 mAb. Thus, we concluded that CpG-conjugated Ag induced Ag-specific Th1 cell differentiation through the elaboration of IL-12 in B cells.
FIGURE 4. CpG-activated B cells elaborate IL-12 and induce the differentiation of Th1 cells. A, Purified B220<sup>−</sup>CD11c<sup>−</sup> B cells (2 × 10<sup>5</sup>) were stimulated with LPS, CpG, CpG-OVA, or non-CpG ODN (ODN) in 96-well plates for 2 days, and culture supernatants were assayed for IL-12 by ELISA. CpG and CpG-OVA induced B cells to produce IL-12 to a comparable extent. B, Unprimed OVA-specific CD4<sup>+</sup> T cells (2.5 × 10<sup>5</sup>) were cocultured with 2.5 × 10<sup>6</sup> purified B220<sup>−</sup>CD11c<sup>−</sup> B cells pulsed with OVA (100 μg/ml), CpG-OVA (10 μg/ml), or non-CpG ODN-OVA (ODN-OVA; 10 μg/ml) in the presence or the absence of anti-IL-12 (αIL-12) or isotype-matched control (CTRL) mAb (10 μg/ml). After 6 days of culture, 1 × 10<sup>6</sup> viable lymphocytes were restimulated with OVA (100 μg/ml) and 2 × 10<sup>5</sup> MMC-treated spleen cells for 2 days, and culture supernatants were assayed for IFN-γ and IL-4. *, p < 0.01; **, p < 10<sup>−</sup>3 (compared with the unstimulated group).

Synergism between Ag-nonspecific CpG-mediated and Ag-specific sIg-mediated Ag capture by primed B cells

We next examined the effects of sIg-mediated capture of CpG-conjugated Ag on Th1 cell activation. TNP-primed and control B cells were prepared from TNP-KLH- and KLH-primed LN cells, respectively, and cultured with OVA-specific Th1 cells and OVA conjugated with CpG, TNP, or both. Th1 cells produced low levels of IFN-γ in response to TNP- or CpG-conjugated OVA presented by TNP-primed B cells, whereas the same concentration of OVA conjugated with both TNP and CpG stimulated Th1 cells when TNP-primed, but not control, B cells were used as APCs (Fig. 6). In addition, TNP-KLH-primed B cells induced IFN-γ production to a level comparable to that induced by KLH-primed B cells when cultured with Th1 cells and the combination of TNP-BSA-CpG plus OVA-CpG (Fig. 6A). These results indicate that the Ag-specific sIg-mediated Ag capture enhanced Ag presentation and Th1 activation by primed B cells in a synergistic manner with Ag-nonspecific CpG-mediated Ag capture.

Activation and induction of Th1 cells by the BCL1 B cell line as APCs

We examined the Ag-presenting ability of the B cell leukemia line to exclude the possible contribution of DCs that might have contaminated the purified B cell population. The BCL1 B cell line activated Th1 cells by presenting CpG-OVA in a dose-dependent manner (Fig. 7A), as did the purified B cells shown in Fig. 2C. Similarly, the BCL1 B cell line also induced Th1 cells from naive T cells in the presence of CpG-OVA (Fig. 7B), as did the purified

FIGURE 5. Expression of intracytoplasmic IL-12 in CpG-activated B cells. Purified B220<sup>−</sup> B cells were cultured with LPS or CpG overnight, with 10 μg/ml brefeldin A added for the final 4 h. After staining with FITC-labeled anti-B220 mAb, the cells were treated with cell permeabilization solution and then stained with PE-labeled anti-IL-12 mAb (0.3 μg). Where indicated, a 20-fold excess of free anti-IL-12 mAb was added at the beginning of the incubation. They were analyzed for intracytoplasmic IL-12 by flow cytometry. The IL-12 expressions in the gated B220<sup>−</sup> B cells are shown. Data are representative of four independently performed experiments with similar results.

FIGURE 6. Synergism between Ag-nonspecific CpG-mediated and Ag-specific sIg-mediated Ag capture by primed B cells. B220<sup>−</sup>CD11c<sup>−</sup> B cells were purified from the popliteal LN cells primed with TNP-KLH (III) or KLH (I) in CFA. B cells (2 × 10<sup>5</sup>) were cultured with 1 × 10<sup>5</sup> CD4<sup>+</sup> Th1 cells in the presence of 0.1 μg/ml (A) or 1.0 μg/ml (B) of the indicated stimulants for 2 days, and culture supernatants were assayed for IFN-γ by ELISA. IL-4 levels were not detected in any culture. Experiments were repeated independently three times with similar results. *, p < 0.05; **, p < 10<sup>−</sup>4 (compared with the CpG-OVA-TNP group).
B cells shown in Fig. 2A. These results reinforce our contention that purified unprimed B cells, but not contaminating DCs, induce or activate Th1 cells.

Discussion

APCs are initiators of the T cell-mediated immune surveillance system. DCs are well equipped with devices to capture diverse Ags and present antigenic peptides to T cells (1–4). DCs produce IL-12 upon encountering microbes, thereby inducing protective Th1 responses (56–58). B cells also serve as APCs (7–9). Characteristics of B cells include clonally expressed slgs that promote the capture of Ag (10, 18, 19). B cells tend to induce Th2 responses (21–27) and are unable to induce Th1 cells. Thus, DCs and B cells appear to play distinctive roles in directing immune responses.

In this report we disclosed features of B cells that challenge the ideas described above if Ag is chemically conjugated with CpG. B cells could efficiently capture the conjugate and present antigenic peptides to Th cells regardless of the Ag specificity of slg (Figs. 1–3), and CpG-stimulated B cells could induce Th1 development by producing a sufficient amount of IL-12 (Figs. 2, 4, and 5). The efficient Ag uptake in an Ag-nonspecific manner and the ability to induce Th1 differentiation from naive T cells had been considered to be unique to DCs. Here, we demonstrate that B cells are also endowed with these characteristics and can work like DCs provided that Ag is linked to CpG.

During the course of our studies of regulatory CD4+ T cells that control Th2 responses, we found that the CpG-OVA conjugate induced Ag-specific Th1 cells and inhibited airway eosinophilia (46). One of underlying mechanisms was the augmented capture of the CpG-tagged Ag by DCs in a CpG-guided manner, because PE conjugated to CpG bound to DCs >100-fold more than PE mixed with CpG (47). In this study we found that the same mechanism for capturing Ag applies to B cells. B cells had been thought to be poor for nonspecific Ag uptake (10, 18, 19). Under physiological conditions, B cells neither efficiently processed Ag (Fig. 3) nor stimulated Th cells (Figs. 1 and 2). The activation of B cells by CpG failed to improve the uptake of Ag (Fig. 3) or the presentation of antigenic peptide to Th cells (Figs. 1 and 2). When CpG were conjugated to Ag, however, B cells could present the Ag and serve as efficient APCs in an Ig-independent manner (Figs. 1–3). The enhanced uptake of CpG-conjugated Ag by B cells is considered to reflect the efficient binding of CpG to surface receptors specific for ODNs (59), which, however, have not been defined yet.

IL-12 was initially identified as a product of human transformed B lines, whereas it had been controversial whether murine B cells produced IL-12 (54, 60). There has been accumulating evidence that B cells as APCs are likely to skew T cell immune responses toward the Th2-dominant phenotype (21–27). In sharp contrast to these earlier studies, we here demonstrate that B cells secreted IL-12 (Figs. 4 and 5) and can play a decisive role in Th1 differentiation from unprimed T cells (Fig. 2). Recently, B effector 1 (Be1) stimulated with Th1 cells was reported to produce IL-12, although IL-12 failed to polarize the naive T cells to differentiate into Th1 cells (61). IL-12 production from Be1 cells was detected upon restimulation following an initial 4-day culture with Th1 cells, whereas naive B cells could secrete IL-12 in response to overnight CpG stimulation. Thus, CpG-activated B cells and Be1 cells appear to represent the distinct activation status of B cells.

The nonspecific Ag uptake that is independent of slg specificity is mediated by other receptors on B cells. The most notable is CD21 (complement receptor type 2)-mediated endocytosis. The Ag coupled to C3 fragment is taken up in an Ag-nonspecific manner and presented to T cells as efficiently as those bound through slg (62–64). CD21 ligation failed to up-regulate costimulatory molecules (65, 66), whereas the activation of B cells by CpG enhanced the expression of costimulatory molecules (Fig. 3), which highlights the advantage of CpG as an immunostimulator.

What, then, could the physiological significance of the B cell responses to CpG be? Ag-primed B cells are known to be efficient APCs following Ag capture through slg (10, 18, 19) and activation (15–17). They are likely to induce Th2-dominant responses (21–27). However, we have found that CpG-activated B cells can initiate Th1 responses (Figs. 1 and 2). Thus, B cells as APCs can modulate the immune outcome by converting Th2-oriented responses to Th1-dominant responses in the presence of CpG. An additional surprising finding is that the Th1 inducibility of Ag-primed B cells was further amplified when the B cells bind to Ag in both CpG- and slg-mediated manners (Fig. 6). This mechanism might be very advantageous for the induction of definitive Th1 responses against microbial infections. Microbe-specific B cells could bind to bacteria through slg and CpG when bacteria are tagged with DNA expelled from degraded microbes. This scenario could be plausible, since bacteria express surface receptors specific for DNA (67).

In summary, we showed that B cells share common roles with APCs with DCs, including the Ag-nonspecific capture and the induction of Th1 differentiation from unprimed T cells.

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