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# Genomic DNA Released by Dying Cells Induces the Maturation of APCs<sup>1,2</sup>

Ken J. Ishii,\* Koichi Suzuki,<sup>‡</sup> Cevayir Coban,\* Fumihiko Takeshita,\* Yasushi Itoh,<sup>§</sup> Hana Matoba,<sup>‡</sup> Leonard D. Kohn,<sup>‡</sup> and Dennis M. Klinman<sup>3\*</sup>

Mature APCs play a key role in the induction of Ag-specific immunity. This work examines whether genomic DNA released by dying cells provides a stimulus for APC maturation. Double-stranded but not single-stranded genomic DNA triggered APC to up-regulate expression of MHC class I/II and various costimulatory molecules. Functionally, dsDNA enhanced APC function in vitro and improved primary cellular and humoral immune responses in vivo. These effects were dependent on the length and concentration of the dsDNA but were independent of nucleotide sequence. The maturation of APC induced by dsDNA may promote host survival by improving immune surveillance at sites of tissue injury/infection. *The Journal of Immunology*, 2001, 167: 2602–2607.

Macrophages and dendritic cells play a critical role in the generation of adaptive immune responses (1–3). Mature APCs excel at presenting Ag to naive T lymphocytes (1–4). Although the transition from immature to mature APC is triggered by contact with infectious microorganisms (5–8), recent evidence suggests that components of dead/dying host cells may serve the same function (9–13).

This work examines whether genomic DNA released by injured cells contributes to APC maturation. Although DNA was historically believed to be immunologically inert, it is now appreciated that DNA can be recognized by the immune system. For example, unmethylated “CpG motifs” expressed at high frequency in bacterial DNA activate lymphocytes, NK cells, and macrophages to proliferate and secrete Ig and/or cytokines (14, 15), whereas dsDNA triggers stromal cells to up-regulate surface expression of MHC molecules (16). DNA is normally sequestered in the nucleus, but can be released into the systemic circulation when cells undergo necrosis/apoptosis (10–12). Exposure to DNA has been implicated in the development of autoimmune disease, as in DNase-I-deficient mice (17). This set of findings led us to hypothesize that double-stranded genomic DNA released by injured host cells may act as a “danger signal,” promoting APC maturation or other forms of immune stimulation (13, 18).

Results indicate that introducing dsDNA into the cytoplasm of macrophages and bone marrow-derived dendritic cells (BMDC)<sup>4</sup>

induces them to mature phenotypically and functionally. These APCs support the development of primary immune responses against coadministered Ag in vivo. These findings are consistent with the hypothesis that genomic DNA may promote host survival by improving immune recognition of pathogens at sites of tissue damage/infection.

## Materials and Methods

### Mice and cell cultures

Female BALB/c and C57BL/6 mice (6- to 10-wk old) were obtained from The Jackson Laboratory (Bar Harbor, ME). All studies were approved by the Center for Biologics Evaluation and Research Animal Care and Use Committee. BMDC were obtained by collecting bone marrow and depleting T, B, and NK cells by MACS (Miltenyi Biotec, Indianapolis, IN) using mAbs against Thy1.2, B220, and DX-5 conjugated to magnet microbeads as previously described (19, 20). The remaining cells were incubated for 5 days in complete medium (DMEM supplemented with 5% FBS, 2 mM L-glutamine, 100 nM nonessential amino acids, 1 mM sodium pyruvate, and 20 mM HEPES) supplemented with 10 ng/ml recombinant mouse GM-CSF (BD PharMingen, San Diego, CA). At day 5, cells were treated with 3 mM EDTA for 5 min and harvested. More than 96% of the cells isolated by this technique were immature dendritic cells (CD11c<sup>+</sup>, I-Ad<sup>dim/high</sup>, CD40<sup>low</sup>, and CD80<sup>low</sup>), whereas the others were macrophages (CD11b<sup>+</sup>/CD11c<sup>-</sup>).

OVA<sub>323–339</sub>-specific T cells were generated from BALB/c DO11.10 TCR transgenic mice (National Institute of Allergy and Infectious Diseases-Taconic, Bethesda, MD) as previously described (21). Briefly, CD4<sup>+</sup> T cells were enriched by negative selection using magnetic beads (R&D Systems, Minneapolis, MN). Cells were maintained in vitro by repeated stimulation with irradiated BALB/c spleen cells pulsed with OVA<sub>323–339</sub> peptide (ISQAVHAAHAEINEAGR) in medium supplemented with 10 ng/ml IL-12, 10 μg/ml anti-IL-4, and 10 U/ml rIL-2 (all obtained from R&D Systems). Viable cells were isolated from resting cultures by density gradient centrifugation over Lympholyte M (Cedarlane Laboratories, Bethesda, MD) immediately before assay. The RAW 264.7 (H-2<sup>d</sup>) mouse macrophage cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in complete DMEM supplemented with 10% FBS.

### Preparation of cell lysates

Primary fibroblasts were obtained from newborn BALB/c mice as previously described (10). These cells were passaged several times and used in log growth phase. Necrosis was induced by aspirating washed cells through a 30-gauge needle or by suspending cells in distilled water for 10 min at 4°C. Lysis was confirmed by microscopic examination. Some lysates were treated with 400 μg/ml proteinase K (Sigma, St. Louis, MO) for 18 h and/or 100 U/ml DNase-I (Boehringer Mannheim, Indianapolis, IN) for

\*Section of Retroviral Immunology, Center for Biologics and Evaluation Research, Food and Drug Administration; <sup>‡</sup>Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, and <sup>§</sup>Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

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<sup>2</sup> The assertions herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Food and Drug Administration at large.

<sup>3</sup> Address correspondence and reprint requests to Dr. Dennis M. Klinman, Building 29A Room 3 D 10, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892. E-mail address: Klinman@CBER.FDA.GOV

<sup>4</sup> Abbreviations used in this paper: BMDC, bone marrow-derived dendritic cells; PN, polynucleotide; HSP, heat shock protein; ssPN, single-stranded PN; dsPN, double-stranded PN.

1 h. The enzymes were inactivated by incubating samples at 65°C for 15 min. BMDC and fibroblasts were mixed at a 1:1 ratio.

### DNA preparation

Genomic DNA was purified from BALB/c liver using the Wizard Genomic DNA purification kit as recommended by the manufacturer (Promega, Madison, WI) and then repurified by repeated phenol-chloroform extraction. Synthetic polynucleotides (PN) were purchased from Amersham Pharmacia (Piscataway, NJ). Double-stranded *Escherichia coli* DNA was obtained from Sigma (St. Louis, MO). ssDNA was prepared by boiling dsDNA for 5 min followed by immediate cooling on ice. All DNA was repurified by treatment with Triton X-114 to remove endotoxin as previously described (22). Less than 0.01 U/ $\mu$ g endotoxin or protein was present in any of the DNA samples based on a *Limulus* amoebocyte lysate assay (BioWhittaker, Walkersville, MD).

dsDNA fragments (75, 150, and 300 bp) were obtained by PCR amplifying the pBR322 plasmid (Boehringer Mannheim) using the following primers: 75 bp; sense 5'-ACTCGCTCGGTCG-3', antisense 5'-CTGTGGATAACCGTATTA-3', 150 bp; sense same as 75 bp, antisense ACGCGCCTTTTACGGT-3', 300 bp; sense CGGATACCTGTCCGC CTT-3', antisense ACTGTCCTTCTAGTGTAG-3'. The DNA fragments were isolated using Sephaglass (Amersham Pharmacia) after electrophoresis in 2% GTG agarose gel (Bioproducts, Rockland, ME).

In some experiments, genomic DNA was methylated using SssI CpG methylase (New England Biolabs, Beverly, MA) in NEB buffer supplemented with 160  $\mu$ M S-adenosylmethionine. Methylation of genomic DNA was confirmed by the loss of *HpaII* cleavage susceptibility (data not shown).

### Transfection

DNA (1  $\mu$ g) was mixed with 3  $\mu$ l of Fugene 6 transfection reagent (non-liposomal; Boehringer Mannheim) in 100  $\mu$ l of OptiMEM (Life Technologies, Gaithersburg, MD) for 15 min at room temperature. This solution was resuspended in 1 ml of complete DMEM containing  $10^6$  cells. The viability of cells was not affected by this treatment.

### Flow cytometric analysis of cell surface molecule expression

Cells were washed with PBS, fixed with 4% paraformaldehyde for 5 min at 37°C, and stained with FITC or PE-labeled Abs for 30 min at room temperature (23, 24). Stained cells ( $10^6$ ) were washed, resuspended in PBS/0.1% BSA plus azide, and analyzed by FACSORT (BD Biosciences, San Jose, CA). Abs specific for the following molecules were obtained from BD PharMingen: I-A<sup>d</sup>, I-A<sup>d</sup>/I-E<sup>d</sup>, CD40, CD54 (ICAM-1), TCR- $\beta$ , IL-2, IFN- $\gamma$ , control rat IgG2a for I-A<sup>d</sup>/E<sup>d</sup>, CD40, and hamster IgG for CD54.

### In vitro analysis of APC function

BMDC were incubated with 50  $\mu$ g/ml DNA plus 2 ng/ml OVA<sub>323-339</sub> peptide for 24 h. The cells were then collected, washed, and mixed with an equal number of D11.10 T cells (specific for OVA<sub>323-339</sub> peptide presented in the context of MHC class II). After 5 h of stimulation at 37°C in the presence of 2  $\mu$ M monensin (Sigma), activated T cells were identified by surface staining for TCR- $\beta$  coupled with intracytoplasmic staining for IL-2 and IFN- $\gamma$  (24).

### Cytokine-specific ELISA

Immulon 2 plates (96-well) were coated with anti-IL-6 or anti-IL-12 (BD PharMingen) in PBS (pH 7.2) for 4 h as described (15). After the plates were blocked and washed, supernatants from DNA-stimulated RAW 264.7 cells were added for 2 h at room temperature. The plates were then washed and treated with biotinylated anti-cytokine Ab followed by phosphatase-streptavidin (BD PharMingen), as described (15). The concentration of cytokine was determined by comparison to purified control cytokine included in the same experiment.

### Immunization and Ag-specific Ab responses

C57BL/6 mice (10/group) were immunized i.p. with 2  $\mu$ g of soluble OVA (Sigma). In some cases, 100  $\mu$ g of free DNA was included in the injection mixture. Two weeks after immunization, serum was collected from these mice and stored at -20°C (25). Anti-OVA Abs were detected by Ag-specific ELISA, as described (25, 26).

### Ag-specific CTL assays

Spleen cells were collected from C57BL/6 mice 3 wk after immunization as described above. Spleen cells ( $5 \times 10^6$ ) were cultured in 2 ml of complete medium with 5  $\mu$ g/ml SIINFEKL peptide as described (23). Recombinant human IL-2 (10 U/ml; R&D Systems) was added after 2 days of culture, and cells were harvested on day 5. EL4 thymoma (H-2<sup>b</sup>) and P815 mastocytoma (H-2<sup>d</sup>) target cells were cultured overnight with <sup>51</sup>Cr (DuPont-NEN, Boston, MA)  $\pm$  1  $\mu$ M SIINFEKL peptide. Target cells ( $5 \times 10^3$ ) were cultured with increasing numbers of effectors. After 6 h, released <sup>51</sup>Cr was measured by gamma counting (Wallac, Gaithersburg, MD).

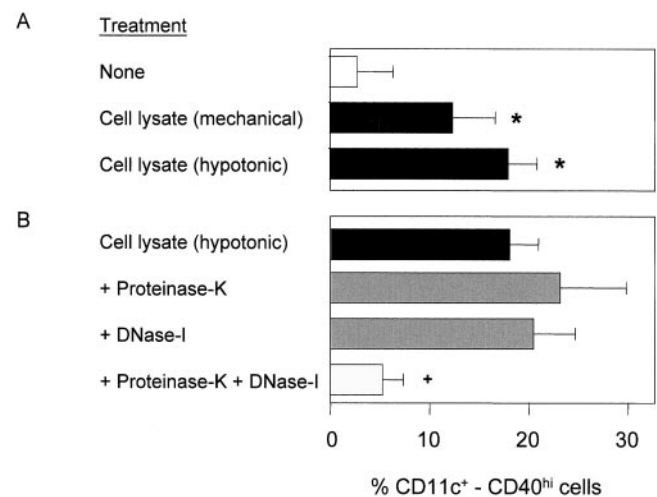
binant human IL-2 (10 U/ml; R&D Systems) was added after 2 days of culture, and cells were harvested on day 5. EL4 thymoma (H-2<sup>b</sup>) and P815 mastocytoma (H-2<sup>d</sup>) target cells were cultured overnight with <sup>51</sup>Cr (DuPont-NEN, Boston, MA)  $\pm$  1  $\mu$ M SIINFEKL peptide. Target cells ( $5 \times 10^3$ ) were cultured with increasing numbers of effectors. After 6 h, released <sup>51</sup>Cr was measured by gamma counting (Wallac, Gaithersburg, MD).

## Results

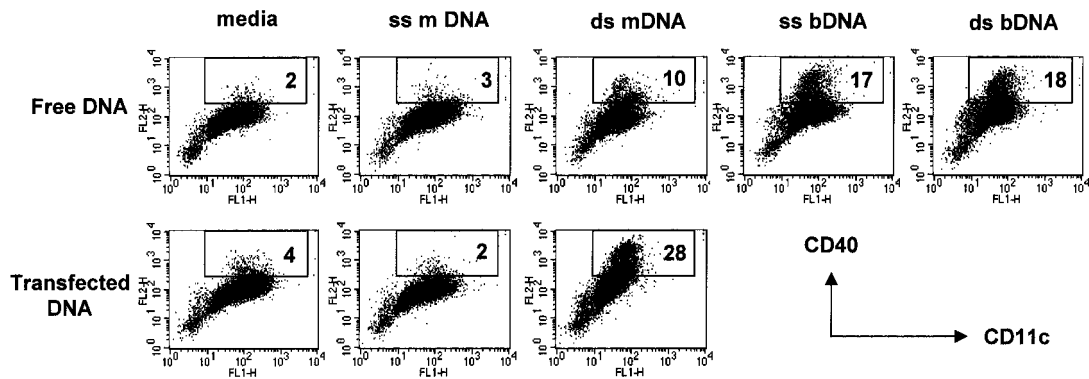
### Genomic DNA released by dying cells stimulates DC maturation

Dying cells leak factors capable of activating the immune system of the host (13). To identify the elements responsible for this activity, fibroblasts were mechanically or osmotically lysed and then added to BMDC cultures. These cellular lysates stimulated BMDC to mature, as manifest by a significant increase in the number of CD11c<sup>+</sup> cells expressing the CD40 maturation marker ( $p < 0.02$ , Fig. 1A). Treating cell lysates with both proteinase K and DNase-I abrogated this effect, suggesting that both self-proteins and DNA contributed to DC maturation ( $p < 0.01$ , Fig. 1B). Unfortunately, nuclear proteins present in these lysates protected genomic DNA from digestion, complicating efforts to clarify the independent contribution of DNA to this process.

To pursue this issue, dsDNA was purified from contaminating host proteins. All DNA preparations used in this study were endotoxin free. Pure genomic dsDNA stimulated a significant increase in the number of BMDC expressing high levels of CD40 ( $p < 0.001$ , Fig. 2). This genomic DNA lost its ability to induce APC maturation when reduced to single-stranded form by boiling. In contrast, APC maturation was induced by CpG-containing bacterial DNA in both single- and double-stranded form (Fig. 2). To determine whether cell surface binding was required for DC maturation, Fugene 6 was used to transfect dsDNA directly into the cytoplasm of target cells. Dose-response experiments showed that 100-fold less DNA was required to optimally up-regulate CD40 expression following transfection than after adding free DNA, suggesting that intracytoplasmic dsDNA was responsible for the observed maturation of APC (Fig. 2 and data not shown).



**FIGURE 1.** Effect of cell lysates on DC maturation. Immature BMDC were cultured with fibroblast lysates (A) or lysates digested with proteinase K and/or DNase-I (B) for 24 h. Numbers represent the percentage of CD40<sup>+</sup>/CD11c<sup>+</sup> double-positive cells after each treatment. Data are representative of two independent experiments. \*, Significantly different from untreated cells,  $p < 0.02$ ; +, significantly different from hypotonic fibroblast lysate,  $p < 0.01$ .



**FIGURE 2.** Murine dsDNA induces BMDC maturation. Immature BMDC were incubated for 24 h with 100  $\mu\text{g/ml}$  free murine or bacterial DNA (*upper panel*) or transfected with 1  $\mu\text{g/ml}$  DNA in Fugene 6 (*lower panel*). Numbers represent the percentage of  $\text{CD40}^+/\text{CD11c}^+$  double-positive cells after each treatment. Data are representative of four independent experiments.

#### Effect of dsDNA on RAW 264.7 macrophages

Consistent with results involving BMDC, murine dsDNA (but not ssDNA) stimulated RAW 264.7 murine macrophages to significantly up-regulate expression of CD40 (Fig. 3). Genomic dsDNA also increased APC expression of MHC class II (I-A/Ed) and CD54, with the maximal effect being observed at 24 h and persisting through 96 h (Fig. 3 and data not shown).

The effect of dsDNA was dose- and length-dependent. As little as 50 ng/ml synthetic double-stranded PN (dsPN) triggered APC activation, whereas a 100-fold higher concentration of ssDNA was ineffective (Fig. 4A). Longer strands of dsDNA more efficiently induced APC maturation. Only background levels of MHC class II expression were observed when RAW 264.7 cells were transfected with dsDNA  $\leq 75$  bp in length. By comparison, class II expression increased significantly when cells were transfected with dsDNA 150 bp long ( $p < 0.05$ , Fig. 4B).

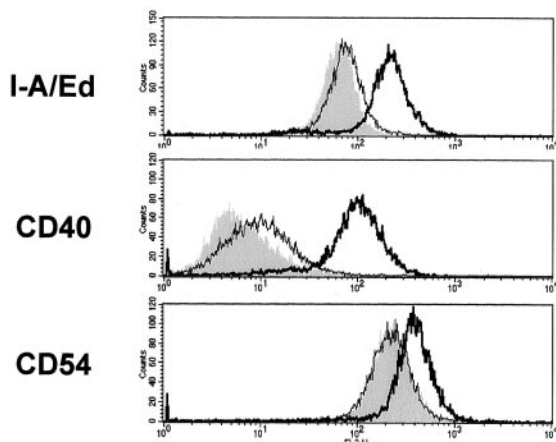
#### Differences in the stimulatory properties of bacterial vs mammalian DNA

Bacterial DNA contains immunostimulatory CpG motifs that activate B cells to secrete Ig, NK cells to produce  $\text{IFN-}\gamma$ , and macrophages/monocytes to up-regulate expression of MHC and costimulatory molecules (Table I and Ref. 27). Methylation of the

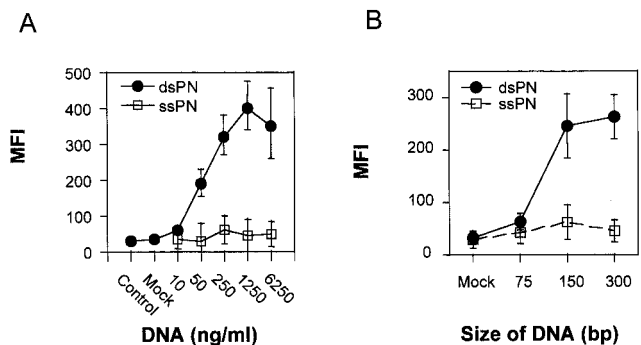
CpG motifs in single-stranded bacterial DNA abrogates this immune stimulation (Table I and data not shown).

CpG motifs are present in mammalian DNA, but at a 20-fold lower frequency than in bacterial DNA (28). Studies were performed to determine whether these rare CpG motifs were responsible for the activity of double-stranded murine DNA. Consistent with earlier experiments, both mammalian and bacterial dsDNA triggered RAW 264.7 cells to up-regulate expression of MHC class II, MHC class II, and costimulatory molecules (Table I). Similar APC activation was observed when freshly isolated spleen cells were used in these experiments (data not shown). However, unlike bacterial DNA, treating murine DNA with CpG methylase did not reduce its effect on APC maturation (Table I). An additional difference between CpG DNA and double-stranded murine DNA was the inability of the latter to induce cytokine production. These findings suggest that 1) unmethylated CpG motifs are not responsible for the immune activation triggered by genomic dsDNA and 2) the nature of the immune response elicited by genomic DNA differs from that induced by CpG motifs.

To confirm these conclusions, PN of known sequence were synthesized and tested. Mock transfected cells, and cells transfected with single-stranded PN (ssPN) (poly(dG) + poly(dC); poly(dA) + poly(dT)) did not induce RAW 264.7 cells to up-regulate expression of MHC class II, CD40, or CD54 (Fig. 5). However, when the same ssPN were mixed together to form double-stranded structures, stimulation was observed. Of note, dsPN



**FIGURE 3.** Effect of dsDNA on RAW 264.7 cells. RAW 264.7 cells were transfected with murine ssDNA (thin line) or dsDNA (thick line). Mock transfected cells are shown in gray. Data represent surface expression of MHC and costimulatory molecules 24 h after transfection. Experiments were repeated three times with similar results.



**FIGURE 4.** dsPN stimulate RAW 264.7 cells. *A*, RAW 264.7 cells were transfected with increasing concentrations of dsPN (poly(dA)-poly(dT)) or ssPN (poly(dT)). Data represent the mean fluorescence intensity  $\pm$  SD of MHC class II expression. *B*, Raw 264.7 cells were transfected with 1  $\mu\text{g/ml}$  dsPN or ssPN of defined length. All data shown in this figure are representative of at least three independent experiments.

Table I. Comparative effects of bacterial vs murine DNA<sup>a</sup>

Stimulus	H-2D <sup>d</sup>	I-A/E <sup>d</sup>	CD40	IL-6	IL-12
Controls					
Medium	59 ± 21	71 ± 11	13 ± 4	2.1 ± 1.3	< 2
Mock transfection	77 ± 13	69 ± 7	9 ± 7	0.8 ± 0.1	< 2
Murine DNA					
ssDNA	69 ± 16	76 ± 9	11 ± 2	0.8 ± 0.4	< 2
Methylated ssDNA	72 ± 34	54 ± 21	10 ± 3	1.4 ± 1.0	< 2
dsDNA	212 ± 64*	244 ± 74*	125 ± 64*	2.2 ± 0.2	< 2
Methylated dsDNA	219 ± 51*	262 ± 19*	132 ± 29*	2.3 ± 1.3	< 2
<i>E. coli</i> DNA					
ssDNA	220 ± 22*	311 ± 59*	147 ± 33*	20.9 ± 0.6*	56.4 ± 9.7*
Methylated ssDNA	38 ± 51	72 ± 54	25 ± 12	3.6 ± 3.6	< 2
dsDNA	178 ± 31*	268 ± 34*	159 ± 55*	23.8 ± 2.4*	62.6 ± 8.5*
Methylated dsDNA	181 ± 74*	211 ± 92*	147 ± 39*	4.4 ± 2.3	< 2

<sup>a</sup> RAW 264.7 cells were transfected with 1 μg/ml the indicated DNA. Cytokine levels (picograms per milliliter) from 24-h culture supernatants were quantitated by ELISA. CD40, MHC class I, and MHC class II (H-2D<sup>d</sup>; I-A<sup>d</sup>) expression was monitored by FACS (mean fluorescence intensity is shown). Results represent the mean ± SD of triplicate cultures. \*, *p* < 0.01 when compared to mock transfected controls.

lacking CpG motifs (poly(dA)-poly(dT); poly(dI)-poly(dC); poly(dA-dT)-poly(dA-dT)) activated APC to the same extent as dsPN that were high in C/G content (poly(dG)-poly(dC); poly(dG-dC)-poly(dG-dC)) (Fig. 5 and data not shown). These findings confirm that the activity of dsDNA was not CpG dependent. They also provide evidence that the activity of dsDNA was not due to endotoxin contamination, because the same PN that were inactive in single-stranded form induced APC maturation when mixed to form dsPN.

#### Effect of dsDNA on APC function

The effect of dsDNA on the ability of APC to present Ag was investigated. BMDC were cultured in vitro with OVA<sub>323-339</sub> (an immunogenic peptide of OVA) and/or 50 μg/ml dsDNA. The cells were harvested 24 h later and mixed with DO11.10 T cells (these cells are specific for OVA<sub>323-339</sub> presented in the context of the

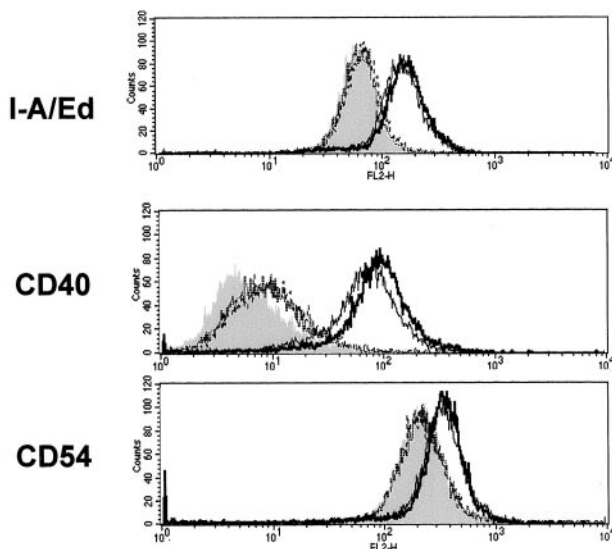
I-A<sup>d</sup> MHC class II molecule). BMDC pulsed with OVA<sub>323-339</sub> effectively presented Ag to DO11.10 cells (21, 29), resulting in a 2- to 3-fold increase in the number of T cells secreting IFN-γ and IL-2 (Fig. 6). When dsDNA was combined with OVA<sub>323-339</sub>, a further 3-fold increase in T cell activation was observed (Fig. 6). This improved ability to present exogenous Ag indicates that the APCs induced to mature by dsDNA were functionally active. This effect was Ag-specific and MHC-restricted; no increase in T cell activation was induced by BMDC treated with ssDNA, pulsed with irrelevant Ag, or by cells that present OVA<sub>323-339</sub> in the context of MHC class I (Fig. 6 and data not shown).

To determine whether double-stranded genomic DNA had similar activity in vivo, C57BL/6 mice were immunized i.p. with 2 μg of OVA ± 100 μg of dsDNA. Mice treated with OVA alone (or OVA plus ssDNA) developed a low titrated serum IgG anti-OVA Ab response. Significantly higher IgG1 and IgG2a anti-OVA titers were induced by immunization with the combination of OVA plus dsDNA or dsPN (Fig. 7A, *p* < 0.01 vs ssDNA, *p* < 0.002 vs ssPN). Coadministering OVA plus dsDNA also led to a significant increase in Ag-specific CTL activity (*p* < 0.01 dsDNA vs ssDNA, Fig. 7B).

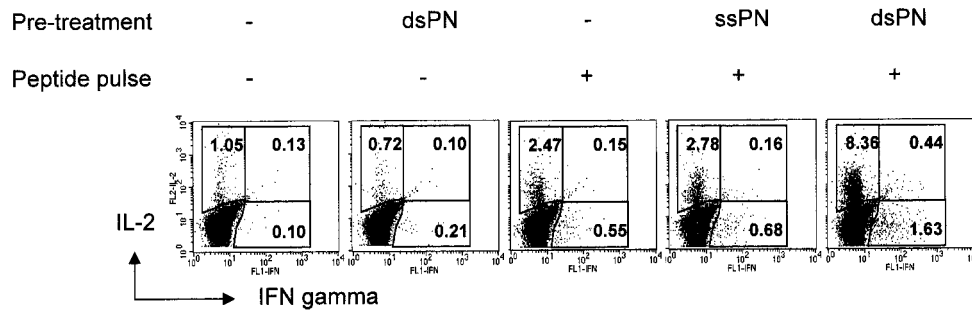
#### Discussion

This work suggests that double-stranded genomic DNA provides a danger signal to the immune system, triggering macrophages and dendritic cells to mature phenotypically and functionally. APCs stimulated by dsDNA increased their expression of MHC class II, CD40, and CD54. Moreover, the combination of dsDNA plus Ag triggered APC to support Ag-specific Ab and CTL responses in vivo.

Mature APCs play a critical role in the development of Ag-specific immunity (4, 13). Whereas molecules derived from infectious pathogens most commonly stimulate APC maturation (6), recent studies indicate that factors released by dying host cells may also serve this function. Heat shock proteins (HSPs) (including HSP70, 90, and gp96) have been implicated in this immunostimulatory process (30). For example, HSPs released by dying cells up-regulate expression of B7.1 and B7.2 by APC (30). Yet HSPs alone do not account for the range of immunostimulatory activity observed with whole cell lysates, indicating that other factors (alone or in combination with HSP) may contribute to these effects. Shi et al. (31) showed that coadministering OVA with cytoplasmic material from necrotic and/or apoptotic cells enhanced CTL activity. Those studies involved directly conjugating OVA to



**FIGURE 5.** Stimulation of RAW 264.7 cells by dsDNA is sequence independent. RAW 264.7 cells were transfected with 1 μg/ml PN and analyzed for expression of MHC class II (I-A/E<sup>d</sup>), CD40, and CD54 after 24 h. Results show the effect of the dsPN poly(dA)-poly(dT) (thick line) and poly(dG)-poly(dC) (thin line) or a mixture of ssPN composed of poly(dA) plus poly(dT) (dotted line) or poly(dG) plus poly(dC) (dashed line). ssPN were independently mixed with transfection reagent to avoid the formation of dsPN. Mock transfected cells are shown in gray.



**FIGURE 6.** Murine dsDNA improves APC function in vitro. BMDC were incubated with 50  $\mu\text{g/ml}$  PN  $\pm$  2 ng of the I-A/E<sup>d</sup> restricted peptide OVA<sub>323-339</sub> for 24 h. BMDC were then mixed with an equal number ( $10^6$ ) of OVA-specific DO11.10 T cells for 5 h. T cells producing IL-2 and IFN- $\gamma$  were detected by intracytoplasmic staining. Data show the percentage of cells secreting each cytokine. Results are representative of two independent experiments.

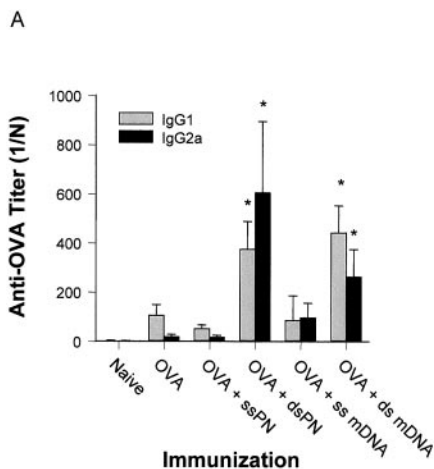
intracellular proteins, and thus did not examine the role of genomic DNA.

Our data suggest that introducing dsDNA into the cytoplasm of APCs stimulates their maturation. These effects were sequence independent, with dsPN of entirely different sequences showing equal activity. There was no evidence that CpG motifs contributed to this stimulation, because 1) dsPN lacking C or G nucleotides were stimulatory, 2) CpG methylation did not inhibit the activity of dsDNA, and 3) single-stranded genomic DNA was inactive. It is unlikely that bacterial contaminants were responsible for the activity ascribed to dsDNA or dsPN. All DNA preparations were endotoxin-free, and the same PN that were inactive in single-stranded form were stimulatory when combined to form dsDNA.

The mechanism by which dsDNA induces APC maturation is unknown. Because tissue damage results in the release of dsDNA and other immunostimulatory factors by dying host cells, we consider it likely that these agents may synergistically promote a local immune response. Studies are underway to determine whether Toll-like receptor 9 (the cell surface receptor responsible for the recognition of CpG DNA) or DNA-PK (an intracytoplasmic pro-

tein linked to CpG-mediated immune activation) are involved in the signaling cascade activated by dsDNA (32, 33). We are also investigating whether dsDNA-induced APC maturation proceeds through intermediates such as NF- $\kappa$ B, Stat-1, or other cellular factors (16).

Our results support the hypothesis that “factors” released by dying host cells facilitate the induction of immunity against foreign and/or self Ags (13). Several reports indicate that necrotic/apoptotic cellular debris promotes the development of autoimmune disease (17). For example, lupus autoantibodies preferentially react with nuclear Ags complexed to dsDNA (34), and DNase-I-deficient mice commonly develop autoimmune disease (17). These findings underscore the potential role of genomic DNA in the development or persistence of autoimmune disease. Yet, if immune recognition of dsDNA has been conserved, it must provide some benefit to the host. We postulate that dsDNA released following tissue injury/infection promotes APC maturation and thus facilitates the elimination of pathogens at the injury site. This mechanism might also improve host recognition of virus-infected APCs. During viral replication, pro-viral dsDNA commonly accumulates in the cytoplasm of infected cells (35, 36). By improving the presentation of viral Ags, APCs, the immune response elicited by dsDNA would facilitate the elimination of infected APCs and promote host survival.



**FIGURE 7.** Murine dsDNA improves APC function in vivo. A, C57BL/6 mice (10/group) were immunized IP with 2  $\mu\text{g}$  of OVA, or OVA plus 100  $\mu\text{g}$  of ssPN (poly(dT)), dsPN (poly(dA)-poly(dT)), or murine DNA. Serum IgG1 and IgG2a anti-OVA titers were measured 2 wk post immunization by ELISA. \*,  $p < 0.002$ ; \*\*,  $p < 0.01$ . B, C57BL/6 mice (10/group) were immunized i.p. with 2  $\mu\text{g}$  of OVA alone (○), OVA plus 100  $\mu\text{g}$  of poly(dT) (△), or OVA plus poly(dA)-poly(dT) (▲). Lysis of H-2-matched OVA peptide-pulsed EL-4 cells by CTL isolated 3 wk after immunization is shown. Lysis of EL4 cells without peptide, and of peptide-pulsed H-2 mismatched P815 cells, was uniformly  $p < 0.01$ ; dsPN vs ssPN.

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