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CpG Oligodeoxynucleotides Down-Regulate Macrophage Class II MHC Antigen Processing

Rose S. Chu,* David Askew,* Erika H. Noss,*† Aaron Tobian,* Arthur M. Krieg,‡ and Clifford V. Harding2*†

Unmethylated CpG motifs in bacterial DNA or short oligodeoxynucleotides (ODN) stimulate cells of the immune system and provide adjuvant activity. CpG DNA directly activates macrophages to secrete IL-12 and TNF-α and increases transcription of various genes, but its effects on macrophage Ag processing remain uncertain. The effects of CpG ODN on class II MHC (MHC-II) Ag processing and presentation were examined using peritoneal macrophages that were cultured for 18 h with CpG ODN and then pulsed with protein Ags. T cell hybridomas were used to detect presentation of specific peptide:MHC-II complexes. Both CpG ODN and LPS inhibited processing of bovine RNase and hen egg lysozyme. Presentation of exogenous peptides was inhibited to a lesser degree. Treatment of macrophages for 18 h with CpG ODN decreased surface MHC-II expression, as measured by flow cytometry. Furthermore, Northern blot analysis revealed that treatment with CpG ODN decreased I-Ak mRNA. Endocytosis by macrophages, as measured by uptake of fluorescent dextran, was not altered by treatment with CpG ODN. The inhibitory effect of CpG ODN on Ag processing was seen after prolonged (18 h) treatment of macrophages, but not after short treatment (e.g., 2 h) with CpG ODN and protein Ag. Enhancement of macrophage Ag processing was not seen at any time point of CpG ODN exposure, in contrast to data from other studies with dendritic cells. In summary, exposure of macrophages to CpG ODN results in a decrease in macrophage Ag processing and presentation, which is largely mediated by a decrease in synthesis of MHC-II molecules. The Journal of Immunology, 1999, 163: 1188–1194.

Induction of CD4 helper T cell responses is mediated by T cell recognition of antigenic peptides bound to class II MHC (MHC-II) molecules. Macrophages are among the APCs that are able to process exogenous protein Ag and present peptide: MHC-II complexes to CD4+ T cells. Macrophages endocytose Ag, which is then proteolytically degraded into peptides within the endocytic pathway (1). Peptide binding to MHC-II molecules occurs in various endocytic compartments, particularly in the MHC class II compartment (MIIC), a late endosomal compartment containing MHC-II and the nonclassical MHC molecule, HLA-DM (H-2M in mouse). HLA-DM and H-2M facilitate the removal of a fragment of invariant chain, class II-associated invariant chain peptide (CLIP), from the peptide binding groove of nascent MHC-II molecules, allowing for the binding of antigenic peptides (2). Peptide:MHC-II complexes are then transported to the cell surface, where they can be recognized by CD4+ T cells.

For the purposes of this analysis, we define Ag processing as the set of mechanisms that convert native protein Ags to peptide: MHC-II complexes that are expressed on the cell surface. Synthesis of MHC-II molecules is required for processing of most antigenic epitopes, although some epitopes are presented by recycling MHC-II molecules in the absence of newly synthesized MHC-II molecules (3–9). Thus, for most epitopes, the level of MHC-II synthesis and expression is a major determinant of Ag processing.

Expression of MHC-II molecules (and therefore Ag presentation) by macrophages is closely regulated and varies depending upon the activation state, with resting macrophages having low levels of surface MHC-II, compared with activated macrophages (e.g., those activated with IFN-γ). In addition to Ag processing and presentation, other functions of macrophages, such as secretion of cytokines, microbicidal activity, and tumoricidal activity, are also regulated by activation state (10). Moreover, macrophages can be activated in different ways, resulting in different activation states with expression of different functions. Thus, a stimulus that activates one macrophage function can inhibit another function, and it is important to carefully define what is meant by macrophage activation in the context of different functions. Macrophages can be activated by cytokines and other substances, including microbial products, such as bacterial DNA.

Bacterial DNA differs from vertebrate DNA in that it contains an increased number of unmethylated CpG dinucleotides. The unmethylated CpG is able to directly stimulate macrophages (11, 12), NK cells (13, 14), dendritic cells (15, 16), and B cells (17, 18), especially when present in a specific 6-bp motif where the central CpG is flanked preferentially by two 5’ purines and two 3’ pyrimidines (17). Short oligodeoxynucleotides (ODN) that contain CpG motifs (CpG ODN) have similar activity to, and are more potent than, genomic bacterial DNA. Because of their immune stimulatory properties, CpG ODN have been successfully used as adjuvants in several experimental systems (19–23).

CpG DNA affects a variety of macrophage functions. Macrophages exposed to CpG DNA rapidly secrete the inflammatory cytokines TNF-α and IL-12 (11, 12, 24, 25). The IL-12 acts...
synergistically with CpG DNA to induce IFN-γ production by NK cells, causing further activation of macrophages (24, 25). The induction of IL-12 and IFN-γ by CpG DNA is a possible mechanism for its Th1 adjuvant activity (19–23). In addition, CpG DNA may up-regulate antimicrobial activity of macrophages, as it induces the transcription of inducible NO synthase after pretreatment of macrophages with IFN-γ (11).

CpG DNA could affect Ag processing and presentation by both indirect mechanisms (e.g., the induction of IFN-γ secretion by other cells) and direct effects on macrophages. The direct effects of CpG DNA on Ag processing and presentation functions of macrophages have not been previously examined. The studies presented here examine the direct effects of CpG ODN on isolated macrophages in vitro. Theoretically, an increase in Ag presentation could partially explain the adjuvant effects of CpG DNA. In contrast, in this study, we demonstrate that CpG ODN do not directly enhance macrophage Ag processing. In fact, our results show that treatment of macrophages with CpG ODN for 18 h inhibits Ag processing, and the primary mechanism for this inhibition appears to be a CpG ODN-induced decrease in synthesis of MHC-II molecules. These results show that the macrophage response to CpG ODN differs significantly from that of dendritic cells, which are stimulated by CpG ODN to express higher levels of MHC-II and costimulator molecules (15, 16, 26) and to manifest a transient increase in Ag processing activity (26).

Materials and Methods

Reagents

Synthetic ODN were purchased from Operon Technologies (Alameda, CA) or Oligos Etc. (Wilsonville, OR). ODN were phosphorothioate-modified to increase their resistance to nuclease degradation. ODN with the following sequences were used: non-CpG motifs or alternate non-CpG motifs (underlined): CpG ODN 1826; TCAAGCTTCTCTCGGT; non-CpG ODN 1982; TCCAGGCTTTTCGGTAGT; CpG ODN 1760; AATAACGACGTCAGCAAG; non-CpG ODN 1908; TCCATGGCTTTCCAGC; non-CpG ODN 1960; TCCATGGCTTTCCAGCT; non-CpG ODN 1980; TCCATGGCTTTCCAGCT. ODN were dissolved in TE (10 mM Tris, 1 mM EDTA). ODN with the following sequences were used: 24-mer oligonucleotide, BamHi digest of the human cardiac actin gene, which cross-hybridizes with human cardiac actin gene sequences. Peptide: MHC-II complexes were detected with the following T hybridomas obtained from Drs. Paul Allen and Emil Unanue (Washington University, St. Louis, MO): TS12, specific for RNase (42–56):I-Aκ; WA.23, specific for RNase (90–105):I-Aκ; A6.A2, specific for HEL (34–45):I-Aκ; 3A9, specific for HEL (48–61):I-Aκ. All cells were cultured in DMEM supplemented with 10% FCS, glutamine, 2-ME, and antibiotics.

Processing and presentation assays

Peritoneal cells were added to 96-well plates (2 × 10^5/well), incubated at 37°C for 2–4 h, and washed to remove nonadherent cells. ODN or LPS was added to the adherent cells in a total volume of 200 μl and incubated overnight (18–24 h) at 37°C. In Ag processing assays, cells were washed and pulsed with HEL or RNase. For detection of HEL (34–45):I-Aκ, HEL (48–61):I-Aκ, or RNase (90–105):I-Eκ, cells were pulsed with Ag for 4 h. For detection of RNase (90–105):I-Eκ, cells were pulsed with Ag for 2 h. After the Ag pulse, cells were washed and fixed in 0.5% paraformaldehyde. The fixed cells were washed extensively before the addition of T hybridoma cells (10^5/well). In assays using exogenous peptides, macrophages were incubated in medium alone for the duration of the Ag pulse, fixed, and incubated with synthetic peptides (Princeton Biomolecules, Columbus, OH) and T hybridoma cells. In other assays, ODN and protein Ag were added simultaneously to macrophages for 2 h, the macrophages were washed and fixed, and T hybridoma cells were added. Supernatants were collected after a 20- to 24-h incubation of macrophages with T hybridoma cells, and IL-2 content was assessed by a CTLL-2 bioassay. Proliferation of CTL-2 cells was measured by a colorimetric assay (27, 28) using Alamar Blue (Alamar Biosciences, Sacramento, CA) and a Bio-Rad 550 microplate reader (Bio-Rad Laboratories, Hercules, CA). All T cell assays were performed in triplicate.

Detection of surface I-Aκ by flow cytometry

Macrophages were plated in 100-mm tissue culture dishes with or without ODN or LPS for 18–24 h, and cells were removed by incubation with Versene (0.53 mM EDTA in PBS; Life Technologies, Gaithersburg, MD) and washed in PBS. Cells were plated in 96-well round-bottom plates (2.5 × 10^5/well) and subsequent steps were performed at 4°C in a volume of 50 μl. Cells were blocked in Fc Block (rat anti-mouse CD16/CD32; PharMingen, San Diego, CA) plus 20% normal mouse serum (NMS; Jackson ImmunoResearch Laboratories, West Grove, PA) and then stained with biotinylated anti-I-Aκ Ab (10-3.6.2) or a biotinylated IgG2a isotype control (AF6-88.5.3; anti-H-2Kk) at 10 μg/ml in PBS/10% NMS. The cells were washed, incubated with streptavidin-conjugated PE (1:100; PharMingen) in PBS with 10% NMS, washed again, and resuspended in 1% parafomaldehyde. The cells were analyzed with a FACScan flow cytometer (Becton Dickinson Immucytometry Systems, San Jose, CA).

Northern blot for detection of I-Aκ mRNA

Macrophages were treated with ODN or LPS for 18–24 h, and total cellular RNA was isolated using an RNaseasy kit (Qiagen, Valencia, CA) according to the manufacturer’s directions. A total of 6 μg of total RNA was run on a formaldehyde-agarose gel, blotted, and probed with an internal 32P-labeled probe derived from a 1-kb Aκ insert of plasmid pCExv-3 (29). Blots were then stripped and reprobed with a 32P-labeled probe generated by BamHI digestion of the human cardiac actin gene, which cross-hybridizes with mouse β-actin mRNA (30). Densitometry was performed on digital images of the probed blots, using the Kodak Digital Science 1D program to calculate net intensity (N.I.) values for each band. Relative expression was determined by the following equation: 

\[
\frac{[\text{I-Aκ N.I. sample} - \text{I-Aκ N.I. control}]}{[\text{I-Aκ N.I. control} - \text{β-actin N.I. control}]}\]

with control being untreated cells.

Endocytosis assay

Adherent macrophages in 24-well plates were treated with ODN or LPS overnight (18–24 h), washed, and incubated with FITC-conjugated dextran (m.w. of 20,000; Sigma) for 2 h at 4°C (as a control for background binding of FITC-dextran to cells) or 37°C. The cells were removed from the wells using 3 mM EDTA in PBS, and uptake of FITC-dextran was assessed by flow cytometry.

Results

CpG ODN inhibit production of peptide:MHC-II complexes from protein Ags

To examine the effects of CpG ODN on Ag processing and presentation functions of macrophages, activated macrophages were isolated by adherence from Con A-elicited peritoneal exudate cells. The macrophages were incubated overnight (18–24 h) with CpG ODN 1826 (1 μg/ml), non-CpG ODN 1982 (1 μg/ml), LPS (100 ng/ml), or control medium. After washing to remove ODN or LPS, the cells were pulsed with protein Ag, bovine RNase, or HEL for 1 or 2 h (see Materials and Methods). After the Ag pulse, the cells were washed and fixed to prevent further processing of Ag. T hybridoma cells were then added for the detection of specific peptide:MHC-II complexes (Table I). These epitopes also vary in their dependence upon DM/H-2M for peptide binding to MHC-II (Table I).

As shown in Fig. 1, both CpG ODN 1826 and LPS down-regulated formation of peptide:MHC-II complexes from protein Ag.
Thus, despite the known stimulatory effects of CpG ODN on immune responses, we found that CpG ODN had inhibitory effects on Ag processing after overnight incubation. Another inflammatory bacterial product and macrophage activator, LPS, had a similar inhibitory effect. Formation of RNase (42–56):I-Ak and RNase (90–105):I-Ek complexes (Fig. 1, A and B) was inhibited to a greater degree than formation of HEL (34–45):I-Ak and HEL (48–61):I-Ak complexes (Fig. 1, C and D). The inhibition by CpG ODN 1826 was specific to the CpG motif, since non-CpG ODN 1982 did not affect Ag processing (Fig. 1), and inhibition was also seen with CpG ODN 1760 but not non-CpG ODN 1908 (data not shown; see Materials and Methods for ODN sequences). Although LPS inhibited Ag processing, LPS contamination was not the cause of the Ag processing inhibition associated with CpG ODN; ODN preparations contained undetectable levels of LPS, and among multiple lots of similarly prepared ODN, only CpG ODN were inhibitory. CpG ODN similarly inhibited processing by macrophages that were elicited by i.p. injection of mice with L. monocytogenes (data not shown). Thus, CpG ODN inhibited processing of four epitopes in two different exogenous protein Ags by two different types of activated macrophages.

Additional studies tested for potential differences in the responses of macrophages with different baseline activation states to exposure to CpG ODN. These experiments showed that results with less-activated macrophages (e.g., resident peritoneal macrophages from naive mice, Fig. 2, or thioglycollate-elicited peritoneal macrophages) were similar in principle to those described above with more activated macrophages. The baseline efficiency of MHC-II Ag processing with these systems was less than that seen with Con A- or Listeria-elicited macrophages, but the overall effect of CpG ODN was basically similar: inhibition of protein Ag processing from the baseline level (Fig. 2). These results were confirmed with all macrophage preparations for processing to produce both HEL (48–61):I-Ak and RNase (42–56):I-Ak complexes. In summary, experiments with each of four different macrophage systems with widely varying states of activation (resting resident, thioglycollate-elicited, Con A-elicited, and

Table I. Characteristics of specific peptide:MHC-II complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>T Cell Hybridoma</th>
<th>HLA-DM Dependencea,b</th>
<th>Compartment of Complex Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase(42–56):I-Ak</td>
<td>TS12</td>
<td>No</td>
<td>Early endosome</td>
</tr>
<tr>
<td>RNase(90–105):I-Ek</td>
<td>WA.23</td>
<td>?</td>
<td>Late endosome/MIC</td>
</tr>
<tr>
<td>HEL (34–45):I-Ak</td>
<td>A6.A2</td>
<td>No</td>
<td>Late endosome/MIC</td>
</tr>
<tr>
<td>HEL (48–61):I-Ak</td>
<td>3A9</td>
<td>Yes</td>
<td>Late endosome/MIC</td>
</tr>
</tbody>
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a See Ref. 9.

b Tested in human hybridoma cell lines expressing I-Ak (9).

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Overnight culture with CpG ODN decreases levels of specific peptide:MHC-II complexes formed from protein Ag. Con A-elicited macrophages were cultured overnight in medium alone or with CpG ODN 1826 (1 μg/ml), non-CpG ODN 1982 (1 μg/ml), or LPS (100 ng/ml). Cells were washed and pulsed with RNase (A and B) or HEL (C and D) at varying doses for 1 or 2 h before fixation and addition of T hybridoma cells. A, Detection of RNase (42–56):I-Ak by TS12 T hybridoma cells. B, Detection of RNase (90–105):I-Ek by WA.23 T hybridoma cells. C, Detection of HEL (34–45):I-Ak by A6.A2 T hybridoma cells. D, Detection of HEL (48–61):I-Ak by 3A9 T hybridoma cells. Data points represent the means of triplicate wells with SDs shown (error bars are less than symbol width when they are not apparent).

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** CpG ODN inhibit protein Ag processing by resident peritoneal macrophages from naive mice as well as Con A-activated macrophages. Ag processing was examined as in Fig. 1 using resident peritoneal macrophages from naive mice and Con A-elicited peritoneal macrophages. A, Detection of RNase (42–56):I-Ak by TS12 T hybridoma cells. B, Detection of HEL (48–61):I-Ak by 3A9 T hybridoma cells.
Listeria-elicited) showed that CpG ODN inhibit processing of multiple antigenic epitopes in all four macrophage systems.

These effects were studied using CpG ODN at various concentrations, and macrophages were exposed to CpG ODN for various periods. Maximal inhibitory effect occurred when macrophages were treated with CpG ODN at 1 µg/ml, but partial inhibition was observed with doses as low as 0.1 µg/ml (data not shown). The inhibitory effect was seen after treatment of macrophages with CpG ODN by 18 h, but not after short-term (e.g., 2 h) treatment with CpG ODN and protein Ag (data not shown) or when CpG ODN was added to macrophages after a 2-h Ag pulse (data not shown). CpG-specific enhancement of Ag processing was not observed with any period of treatment of either activated or resident peritoneal macrophages.

The down-regulation of Ag processing seen in Fig. 1 could be a result of CpG ODN-mediated changes in internal components of the Ag processing pathway or changes in the synthesis, expression, or availability of MHC-II molecules. To begin to address this, we examined the effects of CpG ODN on the ability of macrophages to present exogenous peptides, using the same protocol as was used for examination of protein Ag processing. Macrophages were treated with ODN or LPS, as above, and then incubated in medium alone for the length of the corresponding protein Ag pulse (1 or 2 h). The cells were then fixed, and synthetic peptides were added together with T hybridoma cells. In this approach, the number of specific peptide:MHC-II complexes formed should reflect the level of peptide-receptive MHC-II molecules on the cell surface.

Fig. 3 shows that treatment with CpG ODN 1826 caused a slight but consistent reduction in presentation of exogenous peptides by Con A-elicited macrophages. Inhibition of exogenous peptide presentation was seen using doses of CpG ODN as low as 0.1 µg/ml (data not shown). A similar inhibition of peptide presentation was produced by LPS but not by non-CpG ODN 1982 (Fig. 3). These results indicate that CpG ODN may negatively regulate Ag processing and presentation by decreasing the expression of peptide-receptive MHC-II molecules. However, CpG ODN only slightly inhibited the presentation of exogenous peptide, in contrast to the strong inhibition of protein Ag processing. This suggests that CpG ODN inhibit intracellular mechanisms of Ag processing and/or intracellular supplies of MHC-II molecules.

CpG ODN down-regulate synthesis of MHC-II molecules

To further examine the effect of CpG ODN on expression of MHC-II molecules, levels of surface I-A^b were measured by flow cytometry after overnight treatment of Con A-elicited macrophages with CpG ODN 1826, non-CpG ODN 1982, or LPS (Fig. 4). Within 18 h, both CpG ODN 1826 and LPS down-regulated surface expression of I-A^b molecules to less than half of the level on control untreated cells (Fig. 4). Non-CpG ODN 1982, however, had no effect on surface expression of I-A^b (Fig. 4C).

In addition, Northern blot analysis showed that CpG ODN 1826 caused a marked decrease in I-A^b mRNA to only 24% of the level seen in untreated control cells, as quantitated by densitometry (I-A^b levels were standardized by the β-actin mRNA signal; Fig. 5). Cells treated with non-CpG ODN 1982 showed only minor variation from control cells in the expression of I-A^b mRNA (78% of control cells). Thus, in accordance with the previous results, CpG ODN caused a decrease in macrophage expression of MHC-II mRNA, as well as MHC-II protein. These results indicate that treatment with CpG ODN decreased transcription and/or stability of MHC-II mRNA.

CpG ODN do not specifically affect endocytosis by macrophages

In addition to changes in MHC-II expression, another possible mechanism for the CpG ODN-mediated inhibition of Ag processing is a decrease in Ag uptake by endocytosis. We investigated endocytosis by macrophages by measuring uptake of fluoresceinated dextran using a flow cytometry assay (see Materials and Methods). Macrophages treated overnight with CpG ODN 1826, non-CpG ODN 1982, or LPS all showed slight decreases in endocytosis of fluoresceinated dextran relative to untreated control cells, but there was no CpG-specific component of this effect (e.g., the decrease seen with non-CpG ODN 1982 was greater than that seen with CpG ODN 1826 in the experiment shown in Fig. 6). These results indicate that treatment with CpG ODN did not specifically reduce the endocytic function of macrophages.
Discussion

The immune stimulatory properties of CpG DNA have resulted in its successful use as an adjuvant in a number of systems using protein Ag. CpG DNA is capable of augmenting Ag-specific Ab responses (22, 23), CTL responses (21, 22), and IFN-\(\gamma\)-dominated Th1 responses (19, 20, 23). The mechanism for the various adjuvant effects of CpG DNA have not been elucidated but are likely to involve the direct activation of B cells and cells of the innate immune system, such as macrophages, NK cells, and dendritic cells. These cells are stimulated to secrete a variety of inflammatory cytokines (12, 15, 16, 31) and, as has been shown with B cells and dendritic cells, to up-regulate costimulatory molecules (15, 16, 22). Thus, activation of innate immune cells by CpG DNA has the capacity to profoundly affect development of Ag-specific responses by influencing activation and differentiation of CD4\(^+\) T cells.

The direct effect of CpG DNA on macrophage Ag processing and presentation has not previously been examined, and modulation of Ag presentation is another potential mechanism to explain the adjuvant activity of CpG DNA. An increase in Ag processing and presentation in response to bacterial DNA would seem appropriate in the context of bacterial infection, since this would allow more bacterial epitopes to be presented to T cells. CpG DNA is capable of directly activating macrophages, causing up-regulation of certain functions, such as cytokine secretion, and possibly microbicidal activity. However, in this study we demonstrate that CpG ODN actually down-regulate Ag processing and presentation functions of macrophages. In addition, another potent inflammatory bacterial product, LPS, has inhibitory effects on Ag processing and presentation that are similar to the effects of CpG ODN. The CpG ODN themselves contained undetectable amounts of LPS, as measured by Limulus amebocyte assay (<1 ng LPS/mg of ODN, resulting in <1 pg/ml LPS in the cultures). Moreover, multiple preparations of CpG ODN caused inhibition, while similarly prepared non-CpG ODN did not produce inhibition, demonstrating that the inhibitory effect was specifically associated with CpG DNA sequences.

The effects of CpG ODN on Ag processing were examined in four different macrophage preparations with widely varying states of activation, from less-activated resident peritoneal macrophages (from naive mice) and thioglycollate-elicited macrophages to more activated macrophages elicited by Con A or \textit{Listeria monocytogenes}. CpG ODN inhibited Ag processing from the baseline control level with all macrophages tested. The consistency of the results with multiple different macrophage populations supports the significance and physiological relevance of the observations to varying macrophage populations involved in Ag presentation.

The negative effect of CpG ODN on Ag processing is due largely to a decrease in synthesis of MHC-II molecules. Interestingly, previous studies have shown that LPS mediates a decrease in MHC-II synthesis and expression when macrophages are simultaneously treated with IFN-\(\gamma\) (32, 33). Although we did not treat cells with IFN-\(\gamma\) in our studies, our results also demonstrated an inhibitory effect of LPS, similar to CpG ODN, on MHC-II expression (Fig. 4). The effects of LPS on MHC-II synthesis have been proposed to be a result of increased levels of prostaglandin (32) or NO secretion (34), and this may be the case for CpG ODN as well.

\textbf{FIGURE 4.} CpG ODN down-regulate surface expression of MHC-II molecules. Con A-elicited macrophages were cultured without (A) or with (B–D) bacterial products, as in Fig. 1. Cells were then removed from culture dishes and analyzed for I-A\(^k\) expression by flow cytometry. m, mean fluorescence.

\textbf{FIGURE 5.} CpG ODN down-regulate transcription of MHC-II molecules. Con A-elicited macrophages were cultured with or without bacterial products as in Fig. 1. Total RNA was isolated and analyzed by Northern blotting for levels of I-A\(^k\) mRNA. Detection of \(\beta\)-actin mRNA was used as an internal control. For calculation of relative expression, see Materials and Methods.
A decrease in synthesis of MHC-II molecules may not be the only mechanism by which CpG ODN (and LPS) inhibit Ag processing, as not all the complexes examined are dependent upon newly synthesized MHC-II molecules. For example, formation of RNase (42–56):I-Ak is resistant to treatment of cells with brefeldin A and does not involve newly synthesized MHC-II molecules (3, 9), as summarized in Table I. This particular complex is formed in an early endosomal compartment and is likely to utilize MHC-II molecules recycled from the cell surface (3, 9). In fact, several different groups have demonstrated that certain complexes are formed using recycling MHC-II molecules (3–9). While overnight treatment with CpG ODN decreased surface levels of MHC-II by about one half (as measured by fluorescent staining; Fig. 4), it is not clear whether this decrease alone would affect levels of recycled MHC-II in early endosomes enough to explain the dramatic inhibition in the formation RNase (42–56):I-Ak (a two-log shift in the Ag dose-response curve) that was produced by CpG ODN (Fig. 1A). Thus, in addition to affecting synthesis and expression of MHC-II molecules, CpG ODN may also inhibit recycling of surface MHC-II or other aspects of the Ag processing pathway (e.g., Ag uptake, Ag degradation, Ag targeting inside the cell, traffic and function of compartments involved in formation of peptide/MHC-II complexes, and expression and/or function of DM/H-2M). In our studies, however, the uptake function of macrophages did not appear to be affected specifically by CpG ODN (Fig. 6). In summary, decreased synthesis of MHC-II molecules appears to be a fundamental mechanism for inhibition of Ag processing by CpG ODN, but the observation that CpG ODN also inhibit formation of complexes implicated by other studies to derive from recycling MHC-II molecules suggests the possibility that other Ag processing functions may also be decreased by exposure to CpG ODN.

It is unclear how the down-regulation of MHC-II expression and Ag processing produced by CpG ODN in vitro relates to either actual in vivo effects of bacterial DNA in a physiological context of infection or the in vivo effects of CpG ODN in the context of vaccine function. The inhibitory effect of CpG ODN on Ag processing was seen after prolonged (18 h) treatment of macrophages but not after short-term (2 h) treatment with CpG ODN and protein Ag (data not shown). This indicates the existence of a short period of time after exposure to CpG DNA where macrophages are still able to process Ag. This window of time may be sufficient to allow macrophages to process and present bacterial Ags to T cells in vivo. In addition, our in vitro system examines the direct effects of CpG ODN on processing and presentation functions of isolated macrophages. In vivo, CpG DNA may also have indirect effects on macrophages via stimulation of other cell types, e.g., the stimulation of NK cells to produce IFN-γ. For example, the up-regulation of inducible NO synthase transcription by treatment of macrophages with CpG DNA is detected only after pretreatment of macrophages with IFN-γ (11). However, when peritoneal exudate cells were treated with CpG ODN overnight before nonadherent cells were removed by washing, the macrophages still exhibited a down-regulation in HEL processing (data not shown). These results indicate that, within an 18-h time frame, exposure of other types of peritoneal exudate cells to CpG ODN does not result in indirect stimulation of macrophages to up-regulate Ag processing.

One explanation for these results is a model wherein CpG DNA does not up-regulate in vivo Ag presentation by macrophages, but Ag processing and presentation by other APCs may be enhanced in some manner. For instance, dendritic cells are APCs that are stimulated by CpG ODN and LPS to increase expression of MHC-II molecules (15, 16, 26) and therefore may be responsible for an up-regulation in Ag presentation following exposure to bacterial DNA. In fact, recent results have indicated that treatment with CpG ODN produces a transient up-regulation of Ag processing by dendritic cells (26). Together, these data show that there are important differences in the ways that macrophages and dendritic cells respond to CpG ODN, such that CpG ODN produce transient activation of Ag processing and presentation by dendritic cells but produce only inhibitory effects on macrophage Ag processing.

Even though CpG DNA may not increase the Ag processing activity of macrophages, it may activate them for other important functions involved in host resistance to infection. For example, macrophage recognition of CpG DNA from bacteria may increase microbialidal activity as well as cytokine secretion. Thus, down-regulation of macrophage Ag processing by CpG DNA may reflect induction of a macrophage activation state predominated by other beneficial antimicrobial effects, such as cytokine secretion and microbialidal activity.

References


