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*Oriental Pharmacy and Experimental Medicine* 2006 **6(2)**, 134-143

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# Effects of bacterial LPS and DNA on the induction of IL-1β, IL-10 and IL-12 by mouse peritoneal macrophages *in vitro*

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## SUMMARY

The capacities of bacterial DNA, extracted from *Salmonella typhimurium*, and lipopolysaccharide (LPS), extracted from *Salmonella minnesota*, to activate mouse peritoneal macrophages *in vitro* were compared. Activation was assessed by estimating the levels of 3 cytokines, IL-10, IL-12, and IL-1β, at time intervals of 3, 6, 9, and 24 h after addition of LPS and/or DNA to macrophage cultures. Cytokine levels in culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) and cytokine mRNA levels were estimated based on band intensity in cultured cells by reverse transcriptase-polymerase chain reaction (RT-PCR). Results obtained demonstrated the ability of DNA and LPS to elicit increased production of all 3 cytokines as compared to controls. In the amount tested, LPS appeared to be a more potent inducer of IL-12, and IL-1β, whereas DNA induced higher levels of IL-10. DNA and LPS, used in combination, exhibited neither an additive nor a synergistic effect. Rather, an antagonist effect appeared to occur. RT-PCR results correlated well with ELISA.

**Key words:** Lipopolysaccharide; Bacterial DNA; CpG motifs; TLR; Cytokines; ELISA; RT-PCR

## INTRODUCTION

The innate immune system is an ancient defense system found in all multi-cellular organisms. It is comprised of cells and proteins which are able to recognize molecular patterns common to large groups of microorganisms and known as pathogen-associated molecular patterns (PAMPs) (Aderem and Ulevitch, 2000; Medzhitov and Janeway, 2000; Diks *et al.*, 2001). Cells of the innate immune system, such as macrophages and dendritic cells (DCs) recognize such 'danger signals' through a set of receptors known as pattern recognition receptors (PRRs) (Krieg, 1999; Aderem and Ulevitch, 2000). Defined examples of PAMPs include lipopolysaccharide (LPS), bacterial lipoproteins, and bacterial DNA. Recently, it has been shown that DNA and LPS are able to activate cells of the innate immune system through a family of PRRs known as the Toll-like receptors (TLRs) (Bauer *et al.*, 2001; Diks *et al.*, 2001). So far, ten members of the mammalian TLR family have been discovered (TLR 1-10) (Diks *et al.*, 2001; Krug *et al.*, 2001). Signaling through TLRs leads, after several steps, to the nuclear translocation of nuclear factor (NF)-κB, where it acts as a crucial transcription factor for the expression of several pro-inflammatory cytokines. In addition to NF-κB, activation of activator protein-1 (AP-1) leads to the transcription of several effector genes and, consequently, cytokine production (Anderson, 2000; Hemmi *et al.*, 2000;

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Medzhitov and Janeway, 2000; Bauer *et al.*, 2001).

Bacterial DNA has stimulatory effects on mammalian immune cells leading to a coordinated set of responses that includes innate and acquired immunity (Kreig, 2002). Recognition of DNA depends on the presence of unmethylated CpG dinucleotides, or immunostimulatory sequences (ISS), in certain base contexts (Kreig *et al.*, 1995; Ballas *et al.*, 1996; Klinman *et al.*, 1997), and its cellular effects are mediated by a TLR, namely TLR9 (Hemmi *et al.*, 2000; Bauer *et al.*, 2001; Kreig, 2002). CpG DNA can activate macrophages (Chu *et al.*, 1999), DCs, natural killer (NK) cells, and B-cells (Hemmi *et al.*, 2000; Kreig, 2002) leading to the production of IgM (Kreig *et al.*, 1995; Ballas *et al.*, 1996; Kreig, 2002), interferon gamma (IFN-γ), interleukin-6 (IL-6), IL-12, IL-18, and TNF-α (Chu *et al.*, 1999; Klinnman *et al.*, 1999; Krieg, 1999; Hemmi *et al.*, 2000; Krieg, 2000). Cytokines such as IFN-γ, IL-12, and IL-18 are known to favor the progression of Th1 immune responses (Krieg, 2000; Bauer *et al.*; 2001) which are usually protective. Thus, CpG DNA might be useful for immunotherapy of allergy, infectious diseases, and cancer (Hemmi *et al.*, 2000; Krieg, 2000).

LPS, on the other hand, the prototypic activator of innate immunity (Aderem and Ulevitch, 2000), is recognized by TLR4 (Kaisho *et al.*, 2001), which is primarily expressed on monocytes/macrophages and neutrophils (Diks *et al.*, 2001; Rigato *et al.*, 2001). It is active at concentrations below 1 nM (Aderem and Ulevitch, 2000), whereby it induces the production of cytokines such as TNF-α, IL-1, IL-6, IL-10, and IL-12 (Kirschning *et al.*, 1998; Hemmi *et al.*, 2000; Rigato *et al.*, 2001).

In the current study, LPS extracted from *Salmonella minnesota* and DNA extracted from *Salmonella typhimurium* were used to activate mouse peritoneal macrophages *in vitro*. The two preparations were tested each alone, and in combination, for their ability to induce cytokine production. Relative differences in the transcriptional levels of the different cytokines were also investigated using reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA extracted from murine macrophages. This was done in an attempt to compare the effects of DNA and LPS, and to gain some insight in relation to the resulting synergy or antagonism when both are used in combination.

## MATERIALS AND METHODS

### Bacterial LPS and DNA

LPS (obtained in pure form from Sigma Chemical Co., St. Louis, MO, USA), lyophilized, extracted from *Salmonella minnesota* was suspended in phosphate buffered saline (PBS) (Sigma Chemical Co., St. Louis, MO, USA). DNA was extracted from *Salmonella typhimurium* using the PUREGENE Kit (Gentra Systems, Inc., MN, USA). The extraction procedure was performed exactly as described by the manufacturer. About 600 μg of DNA were obtained from 1 ml of an overnight culture of *S. typhimurium.* The A260/A280 ratio ranged from 1.7 to 1.87 indicating no protein or RNA contamination. Moreover, the electrophoretic pattern of the extracted DNA indicated a homogenous preparation with no ladder formation. The Limulus Amebocyte Lysate (LAL) (Cape Code Inc., LAL) assay was used for detection of Gram- negative bacterial endotoxin (ET) in the DNA preparations and a negative result was obtained (i.e. no gel was formed at the bottom of the tube). Sensitivity of the assay is 0.25 EU/ml.

### Harvesting and culture of macrophages

Ten female BALB/c mice (4 to 8 weeks old), obtained from the Animal House, Faculty of Medicine, American University of Beirut were injected intraperitoneally with 1 ml of 3% thioglycollate medium.

Forty-eight h after intraperitoneal injection, mice were sacrificed and peritoneal exudate cells were aseptically harvested, by lavage with RPMI 1640 medium (GIBCO Laboratories™, Grand Island, NY). Cells were washed three times with RPMI 1640 medium, and staining was then performed using Leishman stain to confirm that the cells obtained were mainly macrophages. Viability was also determined using trypan blue and it ranged between 50 and 80%.

Total cell counts were performed using a hemocytometer.

Cells were suspended at a concentration of $3 \times 10^6$ cells/ml into 16 microcentrifuge tubes, each containing 1 ml of RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (Hyclone Laboratories, Inc., Utah, USA), 100 unit/ml of penicillin, and 100 μg/ml of streptomycin and cultured overnight at 37°C in a humidified, 5% $CO_2$ incubator (Sheldon manufacturing, Inc., model IR 2424). It is worth mentioning that the culture medium was filtered before addition of the cells (Takasuka *et al.*, 1991; Gao *et al.*, 1999; Liu, 2000; Gao *et al.*, 2001).

### Addition of LPS and/or DNA to macrophage cultures

After the overnight culture, the 16 tubes were divided into 4 groups, each containing 4 tubes, and macrophages were stimulated using either LPS extracted from *Salmonella minnesota*, or DNA extracted from *Salmonella typhimurium*, or both. The first group received 0.1 ml of 1 μg/ml LPS. The second group received 0.1 ml of 3 μg/ml DNA. The third group received 0.05 ml of 1 μg/ml LPS and 0.05 ml of 3 μg/ml DNA. The last group received nothing, and consequently was used as a negative control.

Macrophages were incubated again at 37°C in a humidified, 5% $CO_2$ environment and a batch of 4 tubes, one from each group was removed sequentially, at time intervals of 3, 6, 9, and 24 h. Viability was determined again each time a group of 4 tubes was removed from the incubator (Viability remained between 50 and 80%).

The tubes were centrifuged at 4°C for 5 min at a speed of 8,000 rpm. Culture supernatants were removed and frozen at -20°C for use in cytokine assays (enzyme-linked immunosorbent assay (ELISA)), and the cell pellet was used to perform RNA extraction using Trizol reagent (Life Technologies, Grand Island, NY).

### ELISA

Supernatant concentrations of IL-10, IL-1β, and IL-12p70 (the bioactive form of IL-12 which is composed of 2 subunits p35 and p40) were measured by ELISA (BioSource International, Inc., CA, USA) and the intensity of the color produced was read at 450 nm. The minimum detectable doses of mouse IL-1β, IL-10, and IL-12p70 were less than 7 pg/ml, less than 13 pg/ml, and less than 40 pg/ml, respectively.

### RT-PCR

RT-PCR was performed on the RNA extracts using the Ready-To-Go™ You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer's specifications. RT-PCR was done to detect relative differences in the transcriptional levels of IL-10, IL-1β, IL-12p35, and IL-12p40.

Total RNA concentration was determined by spectrophotometry and the amount of RNA to be used was adjusted to 900 ng for all samples. RNA was then reverse transcribed into cDNA. Reverse transcription was followed by PCR amplification of IL-10, IL-1β, IL-12p35, and IL-12p40 cDNA. Previously published primers (Todt *et al.*, 2000) provided by Amersham Pharmacia Biotech were utilized in this protocol. The sizes of the amplified sequences from the IL-10 gene (Anonymous, 2005), IL-1β gene (Krieg *et al.*, 1995), IL-12p35 gene (Schoenhaut *et al.*, 1992; Anonymous, 2005), and IL-12p40 gene (Schoenhaut *et al.*, 1992) are 213 bp, 377 bp, 315 bp, and 431 bp, respectively. PCR amplification was performed in a thermal cycler (PTC-100™, MJ Research Inc., Watertown, MA) as follows: 5 min at 95°C, followed by up to 35 cycles of 15 s each at 95°C (denaturation step), 20 s at 58°C (primer annealing), and 30 s at 72°C (extension step). After cycling, there was a final DNA extension period of 6 min at 72°C (Todt *et al.*, 2000). PCR products were subjected to agarose gel electrophoresis and photographed using a 667 polaroid camera.

## RESULTS

### Cytokine levels in supernatants determined by ELISA

1- Macrophages cultured in the presence of LPS (Table 1a). There was an elevation of all three

**Table 1a.** Cell culture supernatant concentrations of IL-10, IL-12 and IL-1β at different time intervals following exposure of macrophages to LPS

| Time (h) following exposure to LPS | Cell culture supernatant concentration(pg/ml) of | | |
|---|---|---|---|
| | IL-10 | IL-12 | IL-1β |
| 3 | 30.0 ± 3.74 | 5.0 ± 0.64 | 208.50 ± 10.92 |
| 6 | 20.0 ± 3.94 | 3.75 ± 0.37 | 82.50 ± 5.60 |
| 9 | 19.0 ± 3.63 | 4.0 ± 0.40 | 77.50 ± 5.65 |
| 24 | 20.50 ± 2.56 | 5.25 ± 0.71 | 360.0 ± 26.10 |

**b.** Cell culture supernatant concentrations of IL-10, IL-12 and IL-1β at different time intervals following exposure of macrophages to DNA

| Time (h) following exposure to DNA | Cell culture supernatant concentration (pg/ml) of | | |
|---|---|---|---|
| | IL-10 | IL-12 | IL-1β |
| 3 | 50.0 ± 9.60 | 3.25 ± 0.45 | 42.50 ± 3.36 |
| 6 | 33.0 ± 3.27 | 4.0 ± 0.94 | 70.0 ± 5.02 |
| 9 | 60.0 ± 7.46 | 3.20 ± 0.49 | 45.0 ± 6.60 |
| 24 | 53.0 ± 3.77 | 3.50 ± 0.35 | 55.0 ± 5.77 |

**c.** Cell culture supernatants concentrations of IL-10, IL-12 and IL-1β at different time intervals following exposure of macrophages to LPS and DNA

| Time (h) following exposure to LPS + DNA | Cell culture supernatant concentration (pg/ml) of | | |
|---|---|---|---|
| | IL-10 | IL-12 | IL-1β |
| 3 | 33.0 ± 3.20 | 4.0 ± 0.11 | 66.50 ± 2.56 |
| 6 | 25.0 ± 2.90 | 4.25 ± 0.17 | 74.0 ± 3.84 |
| 9 | 23.0 ± 3.04 | 4.0 ± 0.17 | 90.0 ± 1.78 |
| 24 | 27.0 ± 4.81 | 4.0 ± 0.06 | 111.0 ± 7.45 |

Controls: at each time interval cytokine levels were determined in culture supernatants obtained from cell cultures that were not exposed to neither LPS nor DNA. The values obtained were: IL-10 = 9.60 ± 2.77; IL-12 = 2.20 ± 0.21; IL-1β = 20.90 ± 3.32.

cytokine levels in the supernatants 3 h post-culture of macrophages with LPS as compared to controls. IL-10 level declined at 6 h culture and levels were maintained at 9 and 24 h culture with LPS. With the exception of a slight increase in IL-12 and a marked increase in IL-1β levels at 24 h culture with LPS, patterns similar to that of IL-10 levels were obtained. At all time intervals tested cytokine levels remained higher than that in controls.

2- Macrophages cultured in the presence of DNA (Table 1b). There was an elevation of all three cytokine levels in the supernatants at 3 h culture of macrophages with DNA as compared to controls. IL-10 level declined at 6 h culture but increased at 9 h, and the level was maintained at 24 h culture. IL-12 levels remained elevated and unchanged at 6, 9 and 24 h culture. IL-1β level increased at 6 h culture, declined at 9 h and remained stable at 24 h culture. At all time intervals tested cytokine levels remained higher than that in controls.

3- Macrophages cultured in the presence of LPS and DNA (Table 1c). DNA and LPS used in combination induced neither a synergetic nor an additive effect as compared to DNA or LPS used alone. On the contrary, it appeared that in certain instances an inhibitory effect was observed. Transcriptional levels determined by RT-PCR (Fig. 1a, 1b, 2a, 2b, 3a, and 3b). Interpretation of RT-PCR patterns was based on relative differences in band intensities observed visually. Results of RT-PCR correlated well and appeared to be consistent with the findings obtained by ELISA. Cytokine mRNA

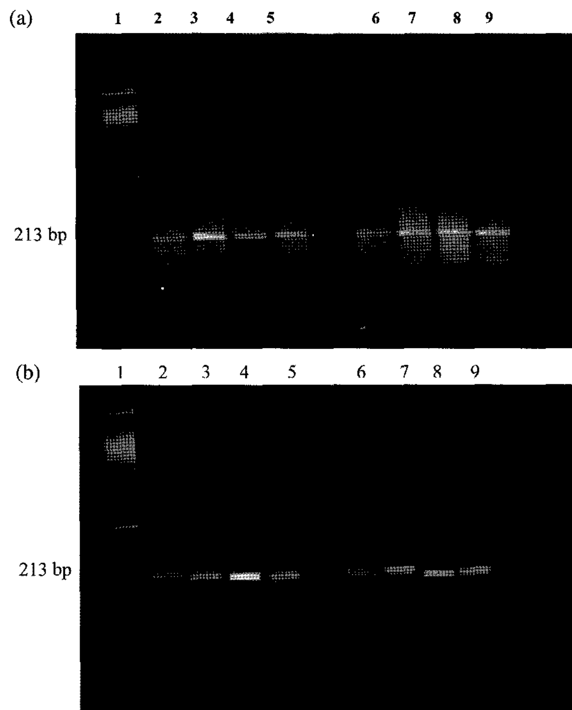


**Fig. 1(a).** RT-PCR of IL-10 after 3 and 6 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 3 h (culture medium), Lane 3: culture medium + LPS (3 h), Lane 4: culture medium + DNA (3 h), Lane 5: culture medium + DNA + LPS (3 h), Lane 6: control after 6 h, Lane 7: culture medium + LPS (6 h), Lane 8: culture medium + DNA (6 h), Lane 9: culture medium + DNA + LPS (6 h). **(b)** RT-PCR of IL-10 after 9 and 24 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 9 h (culture medium), Lane 3: culture medium + LPS (9 h), Lane 4: culture medium + DNA (9 h), Lane 5: culture medium + DNA + LPS (9 h), Lane 6: control after 24 h, Lane 7: culture medium + LPS (24 h), Lane 8: culture medium + DNA (24 h), Lane 9: culture medium + DNA + LPS (24 h).

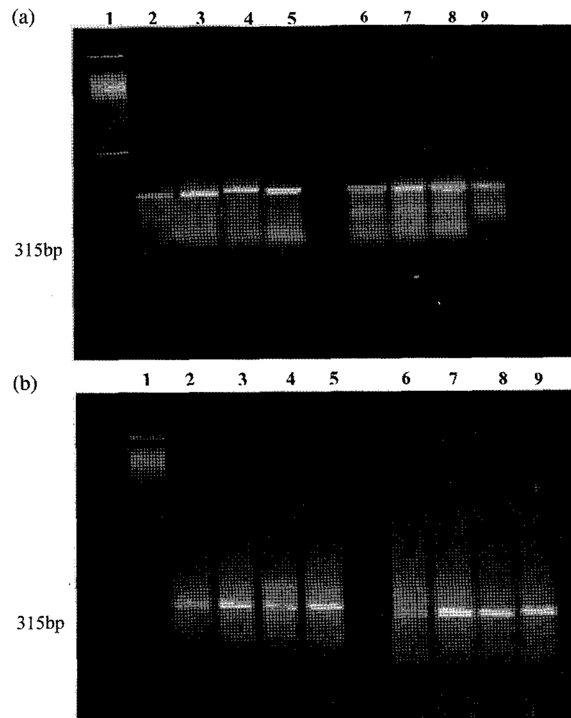


**Fig. 2(a).** RT-PCR of IL-12p35 after 3 and 6 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 3 h (culture medium), Lane 3: culture medium + LPS (3 h), Lane 4: culture medium + DNA (3 h), Lane 5: culture medium + DNA + LPS (3 h), Lane 6: control after 6 h, Lane 7: culture medium + LPS (6 h), Lane 8: culture medium + DNA (6 h), Lane 9: culture medium + DNA + LPS (6 h). **(b)** RT-PCR of IL-12p35 after 9 and 24 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 9 h (culture medium), Lane 3: culture medium + LPS (9 h), Lane 4: culture medium + DNA (9 h), Lane 5: culture medium + DNA + LPS (9 h), Lane 6: control after 24 h, Lane 7: culture medium + LPS (24 h), Lane 8: culture medium + DNA (24 h), Lane 9: culture medium + DNA + LPS (24 h).

levels in macrophages cultured in the presence of DNA, LPS, or both were elevated, as detected by a high-intensity band, compared to mRNA from control macrophages cultured in their absence. The only exception was IL-12p40 where RNA transcription was detected, neither in the control nor in the stimulated samples.

### Comparison of LPS and DNA activity

In general DNA induced higher levels of IL-10 than LPS at all time intervals. On the other hand, levels of IL-12 and IL-1β induced by LPS were higher than those induced by DNA at all time intervals.

## DISCUSSION

Optimal immunity to viruses and intracellular bacteria and parasites is typically mediated by the Th1 subset of CD4+ T lymphocytes. Th1 cells are characterized by the production of cytokines including IL-2 and IFN-γ, the ability to help cytotoxic T-lymphocyte

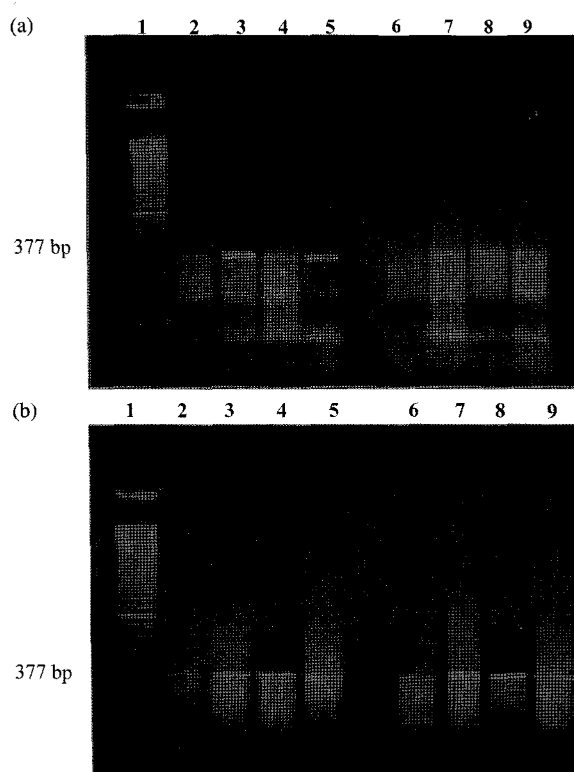


**Fig. 3(a).** RT-PCR of IL-1β after 3 and 6 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 3 h (culture medium), Lane 3: culture medium + LPS (3 h), Lane 4: culture medium + DNA (3 h), Lane 5: culture medium + DNA + LPS (3 h), Lane 6: control after 6 h, Lane 7: culture medium + LPS (6 h), Lane 8: culture medium+DNA (6 h), Lane 9: culture medium + DNA + LPS (6 h). **(b)** RT-PCR of IL-1β after 9 and 24 h of macrophage stimulation. Lane 1: 100 bp ladder, Lane 2: control after 9 h (culture medium), Lane 3: culture medium + LPS (9 h), Lane 4: culture medium + DNA (9 h), Lane 5: culture medium + DNA + LPS (9 h), Lane 6: control after 24 h, Lane 7: culture medium + LPS (24 h), Lane 8: culture medium + DNA (24 h), Lane 9: culture medium + DNA + LPS (24 h).

(CTL) responses, and the promotion of complement-fixing antibody isotypes such as IgG2a. IL-12, chiefly a product of Antigen Processing/Presenting cells (APC), is also critical for the development of Th1 responses. Intense interest is being directed toward the use of bacterial derivatives, such as DNA and LPS, for promotion of Th1-like responses and for their use as enhancers of CTL responses (Huang *et al.*, 1999). The purpose of this study was to compare the stimulatory effect of DNA and LPS by studying the cytokine response of macrophages to these different constituents used either alone or in combination. It is worth mentioning that *S. typhimurium* was used as a source of DNA because *S. minnesota* was not available. However, LPS should be generally conserved among most Gram negative bacteria.

Several other studies, done either *in vitro* or *in vivo*, and comparing as well bacterial DNA and LPS, showed that DNA had superior cytokine (especially IL-12) inducing capacities to LPS, and that LPS was able to induce high levels of cytokines only under the most favorable conditions, such as priming of cells or host with IFN-γ or neutralization of IL-10 which is known to inhibit IL-12 production (Flesch *et al.*, 1995; Huang *et al.*, 1999). However, in the amounts used in this study, LPS rather than DNA appeared to be a more potent inducer of IL-12 and IL-1β at all time intervals. This is probably due to the fact that unlike other studies mentioned, an APC-enriched environment using peritoneal macrophages which are known for their potent capacity to produce IL-12 and IL-1β were used. It should be mentioned here that IL-1β, though not classified as a typical Th1 cytokine, is able to play a role in the progression of a Th1-biased immune response.

Despite the fact that DNA and LPS induced higher levels of IL-10, IL-12, and IL-1β as compared to controls, the increase in cytokine concentration was generally more significant in the case of IL-10 and IL-1β. One explanation is that there could be less IL-12 molecules being secreted per cell as compared to IL-1β and IL-10 (Huang *et al.*, 1999; Crabtree *et al.*, 2001). In one study, however, Hessle *et al.* (2000) reported that much more IL-12 was induced by Gram-positive bacteria than Gram-negative bacteria, which preferentially stimulated secretion of IL-10. Since the LPS and DNA used were both derived from Salmonella, a Gram-negative bacterium, the relatively low levels of IL-12 observed could be attributed to this fact. LPS is a unique component of Gram-negative bacteria, but DNA of Gram-negative bacteria may not differentiate from that of Gram-positive bacteria. In addition, the optimal

CpG motifs which can activate different host cells possess different base sequences, high levels of a specific cytokine might be induced by CpG motifs of a certain base context but not by others (Zhang *et al.*, 2001).

Two key cytokines that bridge the gap between innate and acquired immunity are IL-10 and IL-12. Both are produced by monocytes, macrophages, and dendritic cells in response to microbes, but they have largely opposite cellular effects. IL-12 is a T-cell stimulatory cytokine which activates Th1 and NK cells. Whereas IL-10 down-regulates T-cell cytotoxicity, as well as IL-12 and IFN-γ production and decreases presentation of antigens to T cells, and stimulates B-cell maturation and antibody production (Hessle *et al.*, 2000). Consequently, the observed pattern of change in the concentrations of the pro-inflammatory cytokines, IL-12 and IL-1β, which paralleled that of the anti-inflammatory cytokine, IL-10 appears to be logical. As the level of IL-10 dropped from 30 pg/ml to 20.5 pg/ml between 3 and 24 h, using LPS, the level of IL-1β markedly increased from 208.5 pg/ml to 360 pg/ml. The increase, however, was not as significant in the case of IL-12. This pattern of change was better demonstrated when DNA was used, thus suggesting that IL-10 secretion is an essential component of a feedback mechanism resulting in the inhibition of IL-12 and IL-1β production and serving a counter-regulatory function to suppress macrophage activity and down-regulate Th1-like cytokine responses. A similar observation was made in several other studies, whereby the gradual accumulation of IL-10 over a certain time period resulted in down-modulation of Th1 cytokine production (Huang *et al.*, 1999; Hessle *et al.*, 2000; Crabtree *et al.*, 2001).

Another critical point concerning the effects of DNA and LPS is that of synergy and antagonism. A body of conflicting data exists around this issue thus making it difficult to draw firm conclusions regarding the exact relationship between DNA and LPS. Several studies indicated that DNA and LPS induce the synergistic production of cytokines such as TNF-α and IL-1β. However, the observed synergy appeared to be dependent on the concentration of LPS, the temporal order of treatment by LPS and DNA, and the time of exposure to DNA and LPS. Regarding the concentration of LPS, DNA appeared to synergize with sub-threshold concentrations of LPS, in the order of 0.3 ng/ml. Pretreatment with either DNA or LPS, and then incubation of macrophages with the other stimulant or with both, enhanced synergy at times and suppressed it at others. In addition, prolonged exposure to DNA and LPS appeared to inhibit synergy, whereas brief periods of exposure appeared to enhance it (Gao *et al.*, 1999, 2001; Sester *et al.*, 1999; Crabtree *et al.*, 2001; Yi *et al.*, 2001). In a previous *in vivo* study done in our laboratory (Abdelnoor and Joukhadar, 2002), it was shown that DNA and LPS administered in combination exhibited neither an additive nor a synergistic effect. Instead, the degree of protection conferred by both was either similar to or lower than that detected when DNA or LPS were administered alone. Similar results were obtained in this *in vitro* study, whereby cytokine levels achieved when macrophages were incubated with DNA or LPS alone were in general higher than those attained upon co-stimulation with bDNA and LPS. Even band intensities, as observed by RT-PCR, were either the same as or slightly lower in cultures where both DNA and LPS were added to the macrophage-containing culture medium. RT-PCR, however, and as mentioned earlier in the section on 'results', cannot be really used in this case to compare DNA or LPS efficacy, for it is not totally quantitative, but rather based on the relative differences observed in band intensities. It has been reported that TLR1 inhibits the function of TLR2 (Hajjar *et al.*, 2001), so it could be conceivable that TLR9, the receptor for CpG, may inhibit TLR4, the receptor for LPS, thus diminishing its activity. In contrast, the findings of a study done by Hume *et al.* (2001) indicated that activation by any one TLR pathway does not preclude further activation by another, suggesting that common downstream regulatory components are not limiting. Such a diverse body of conflicting results indicates that the molecular mechanism lying behind the synergy and antagonism

between DNA and LPS is yet to be elucidated.

In contrast to IL-10, IL-1β, and IL-12p35, whose RT-PCR results correlated with those of ELISA, as a moderate or a high-intensity band was observed in parallel to a moderate or a significant increase in cytokine concentration as determined by ELISA, no cDNA amplicon was detected in case of IL-12p40 in all the samples that were analyzed. It is known that expression of a certain gene is regulated at multiple levels. It can be regulated at the transcriptional, post-transcriptional, or translational level. Since the levels of IL-12p70, the bioactive form of IL-12 which consists of 2 subunits IL-12p35 and IL-12p40, appeared to increase upon stimulation with DNA or LPS, as determined by ELISA, but with no parallel detection of an IL-12p40 amplicon by RT-PCR, a possible explanation would be that expression of IL-12p40 was induced by a post-transcriptional or a translational, rather than a transcriptional, mechanism.

LPS and DNA do not have the same effect on a dose basis (Huang *et al.*, 1999). No attempts were made to perform a dose-response analysis for LPS or DNA. However, repeated trials in our laboratory using 1 μg/ml LPS and 3 μg/ml DNA proved to be effective. As a final statement, it can be speculated from our results that both DNA and LPS can stimulate the production of IL-1β and IL-12. In the doses tested LPS was more efficacious at inducing these cytokines than DNA. On the other hand, DNA appeared to be a more potent inducer of IL-10, classically identified as an anti-inflammatory cytokine. However, recently published reports have indicated, contrary to all our previous knowledge that this classical categorization of cytokines as pro versus anti-inflammatory might not apply to the multiple effects of IL-10. These reports suggest that IL-10 may enhance the function of NK cells, thus leading, through pathogen lysis and destruction, to increased antigen availability. Moreover, through inhibition of APC maturation, IL-10 serves to retain their ability for antigen uptake while simultaneously hindering their migration to lymph nodes. This so-called 'antigen loading' phase may constitute a crucial step of the innate immune reaction to a pathogen. Further production of pro-inflammatory cytokines might then lead to maturation of APCs that would activate the adaptive immune response against the specific pathogen (Mocellin *et al.*, 2003). All of these studies show that DNA and LPS possess remarkable abilities for activation of the innate immune system. Further understanding of the molecular mechanisms mediating these abilities may lead to the design of more effective Th1-promoting adjuvants, which are needed for protective responses to viral, parasitic, and intracellular bacterial infections.

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