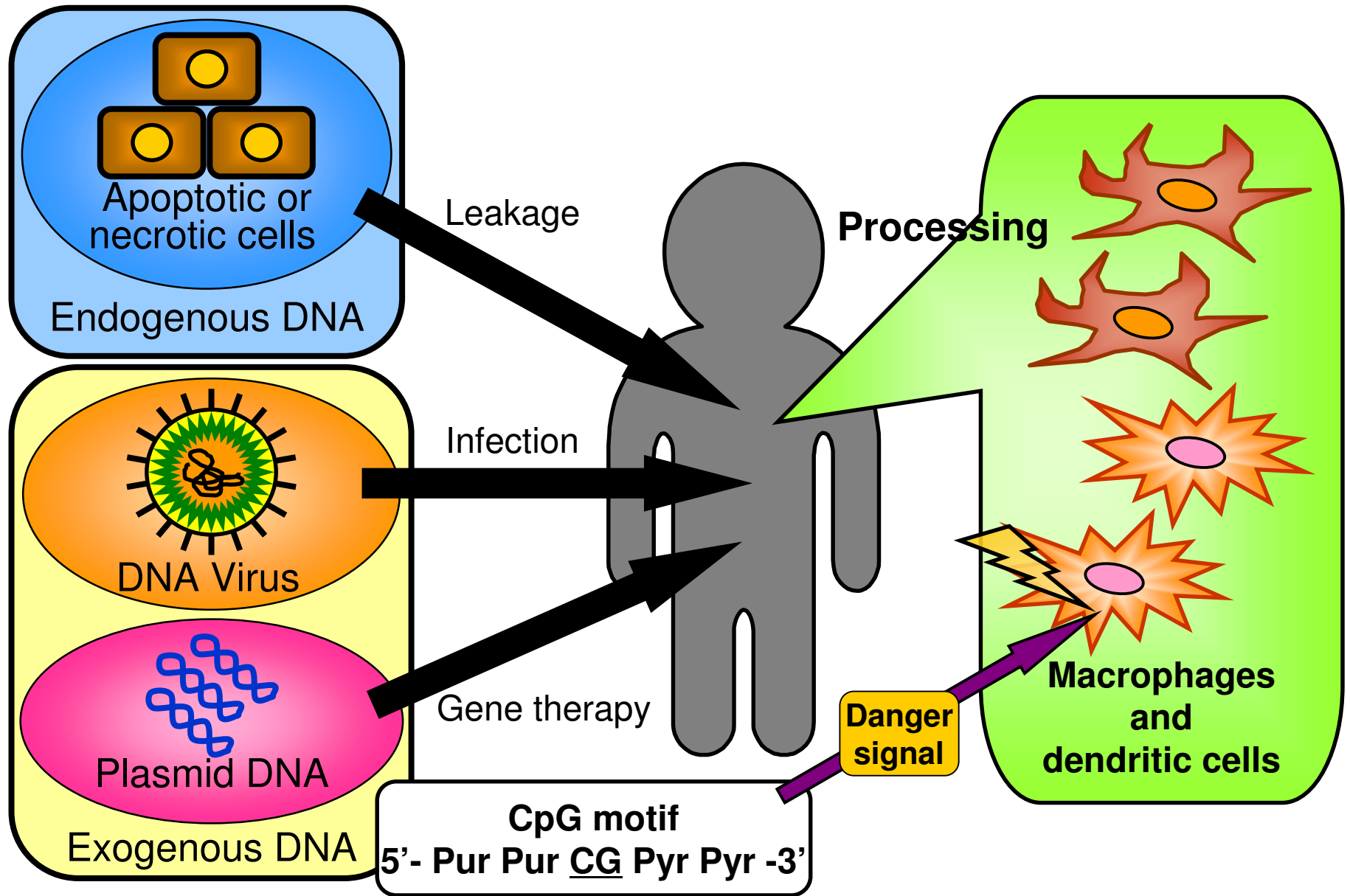


# **Cytokine Secretion Profiles in Primary Cultured Cells and in Mice Stimulated with Plasmid DNA**

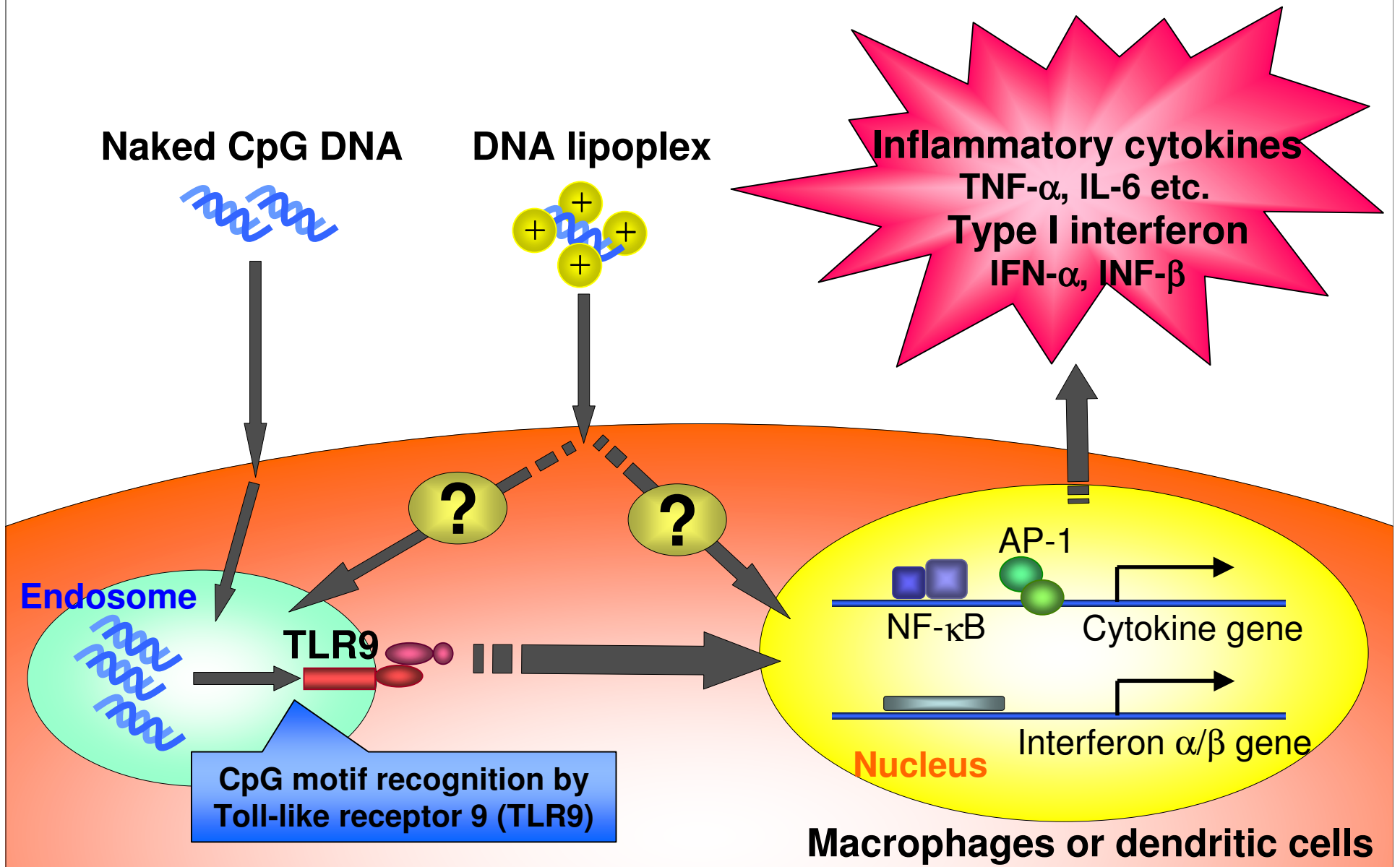
**Hiroyuki Yoshida, Makiya Nishikawa, Sachiyo Yasuda,  
Keiko Kako and Yoshinobu Takakura**

**Department of Biopharmaceutics and Drug Metabolism  
Graduate School of Pharmaceutical Sciences,  
Kyoto University**

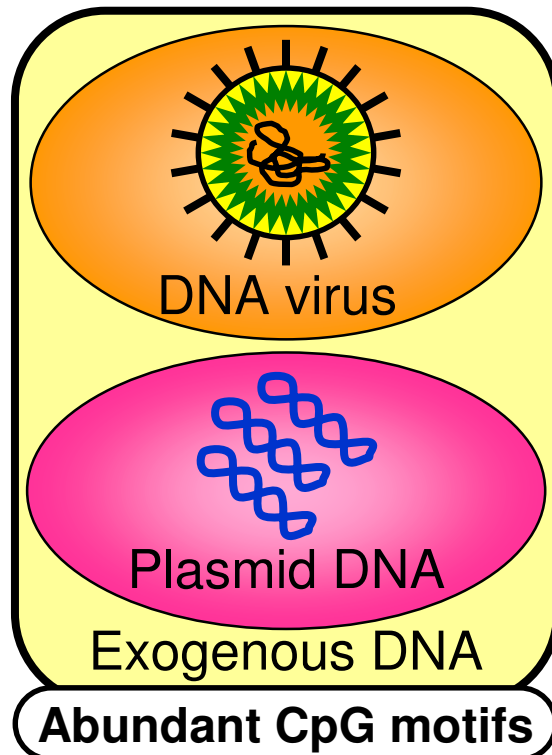
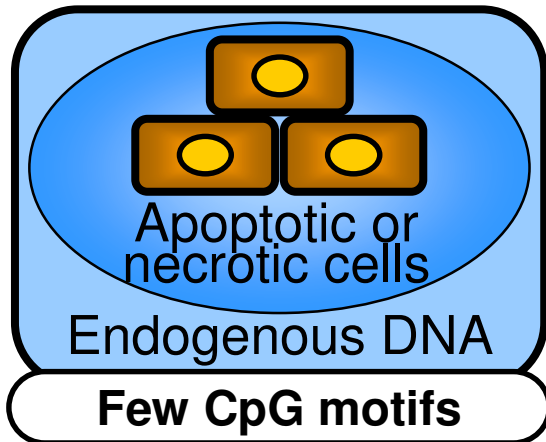
# Elimination of DNA by antigen presenting cells



# Mechanisms of immune response induced by naked CpG DNA or DNA/cationic liposome complex (lipoplex)



# Significance of the immune response to DNA



## Systemic autoimmune disease

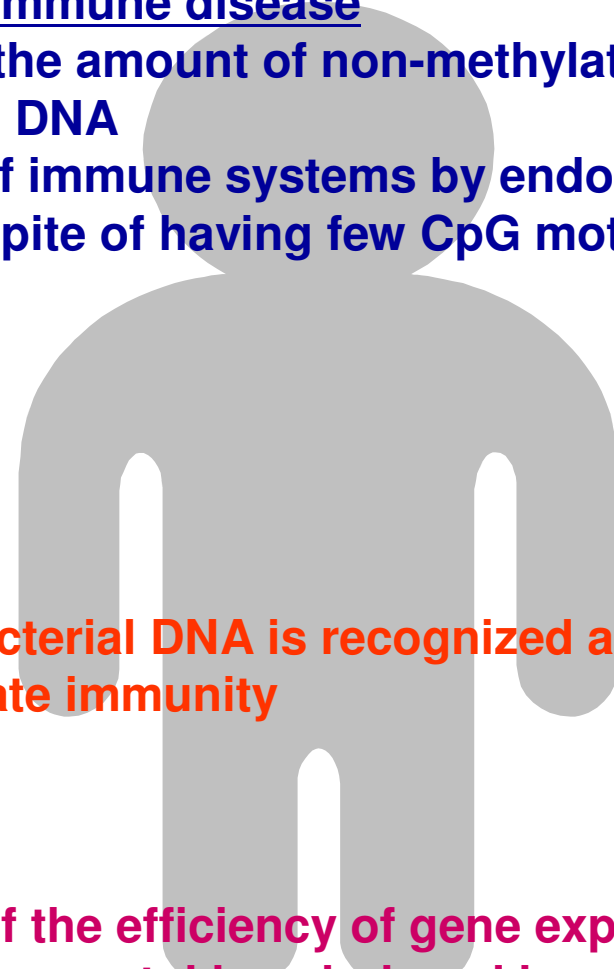
- Increase of the amount of non-methylated CpG motifs in circulating DNA
- Activation of immune systems by endogenous self-DNA in spite of having few CpG motifs

## Infection

- Viral and bacterial DNA is recognized as a danger signal in innate immunity

## Gene therapy

- Reduction of the efficiency of gene expression by inflammatory cytokines induced by plasmid DNA



# **Cytokine secretion profiles induced by...**

**Chapter. 1 Intravenous injection of DNA into mice in vivo.**

**Chapter. 2 DNA stimulation in primary cultured cells in vitro.**

# **Cytokine secretion profiles induced by...**

**Chapter. 1 Intravenous injection of DNA into mice in vivo.**

**Chapter. 2 DNA stimulation in primary cultured cells in vitro.**

# Materials and methods in vivo study

## DNA

### CpG DNA

Plasmid DNA (pDNA): 119 CpGs/ kbp

CpG-reduced pDNA: 63 CpGs/ kbp

### Non-CpG DNA

Calf thymus (CT) DNA: vertebrate DNA

Methylated pDNA: Inactivated CpG DNA

## DNA lipoplex formation

Cationic liposome: DOTMA/chol liposome  
(molar ratio=1/1)

DNA: cationic liposome=1/7.18 (w/w)  
(charge ratio=+2.24)

## Macrophage depletion

Clodronate liposome pre-administration

### Tissue distribution



← **Intravenous injection**

( Naked [<sup>32</sup>P]pDNA  
[<sup>32</sup>P]pDNA lipoplex )

10 min

30 min

60 min

**Collection of blood and organs**

**Radioactivity**

### Cytokine assay



← **Intravenous injection**

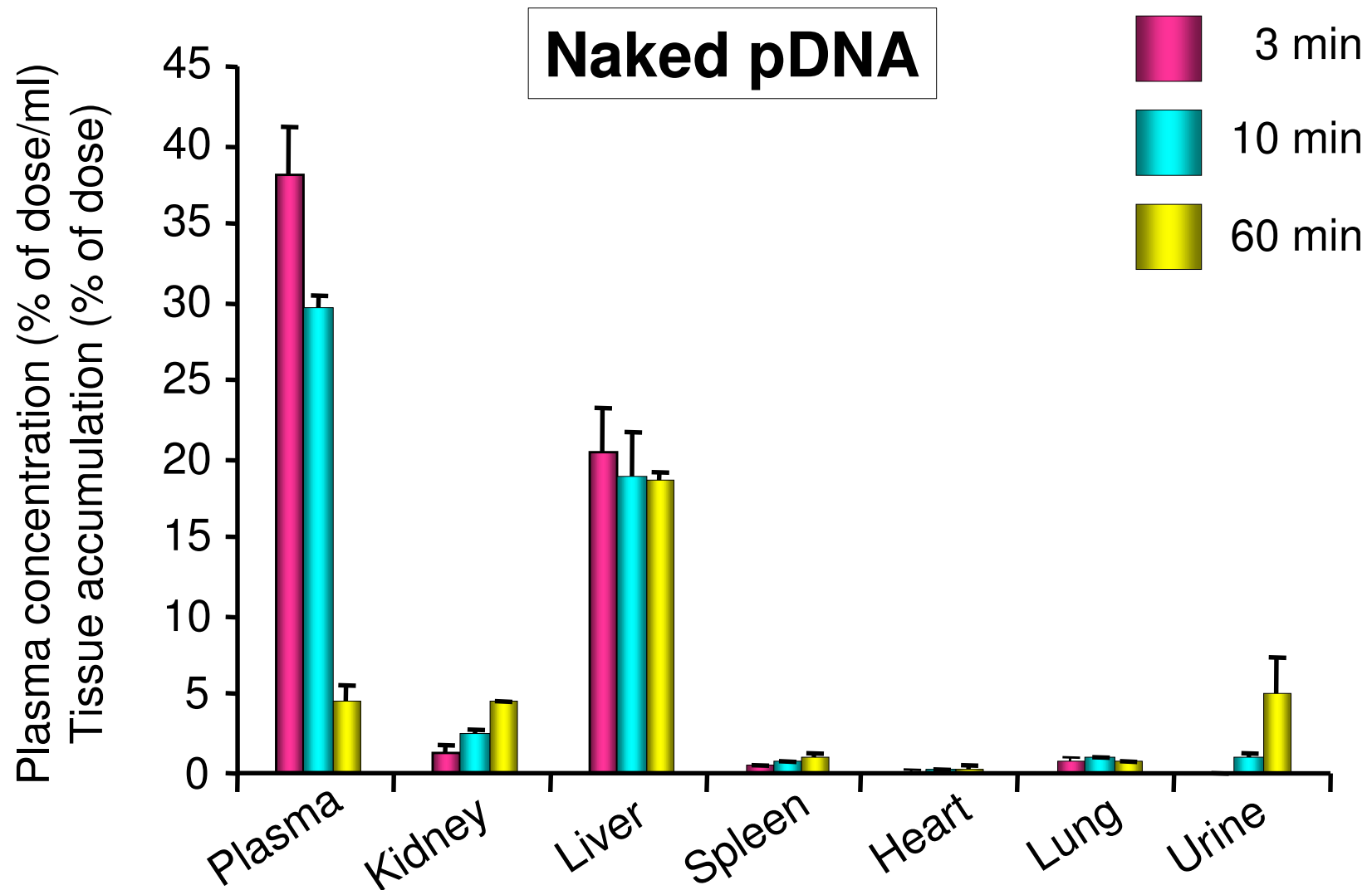
( Naked DNA  
DNA lipoplex )

1.5 hr

**Collection of blood**

**TNF- $\alpha$**

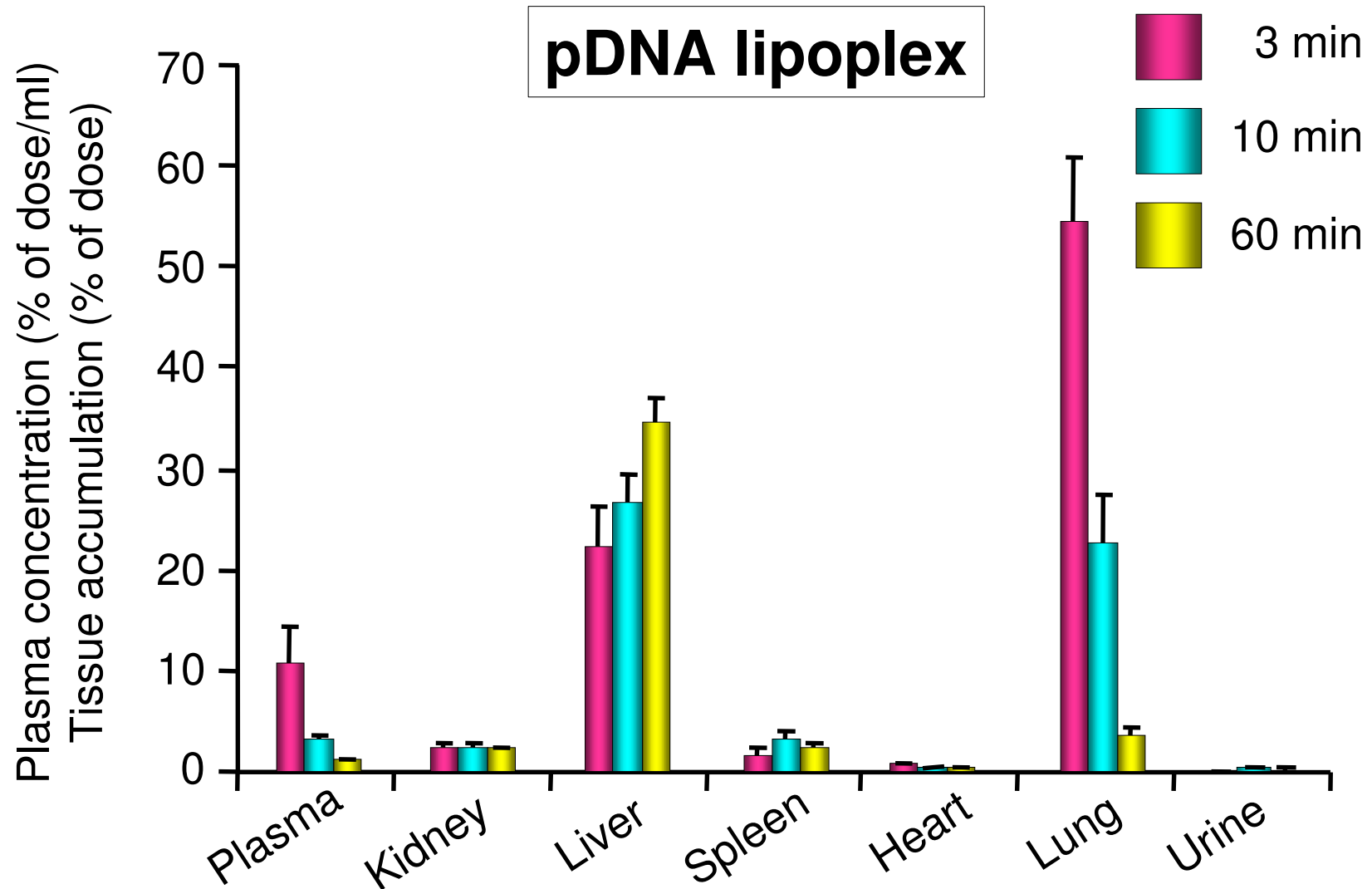
# Tissue distribution of naked $[^{32}\text{P}]$ pDNA after intravenous injection into mice



Kako et al., unpublished data.

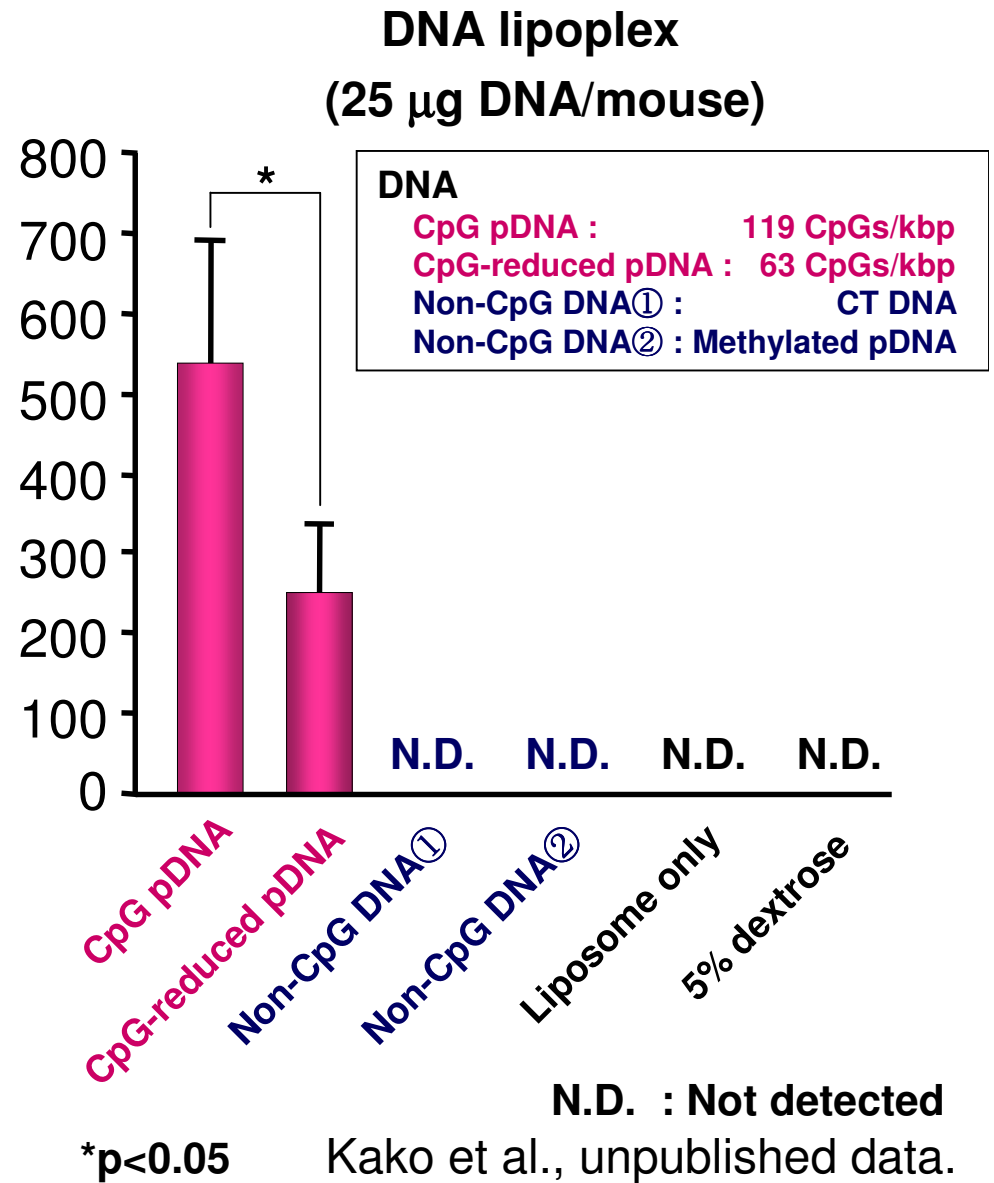
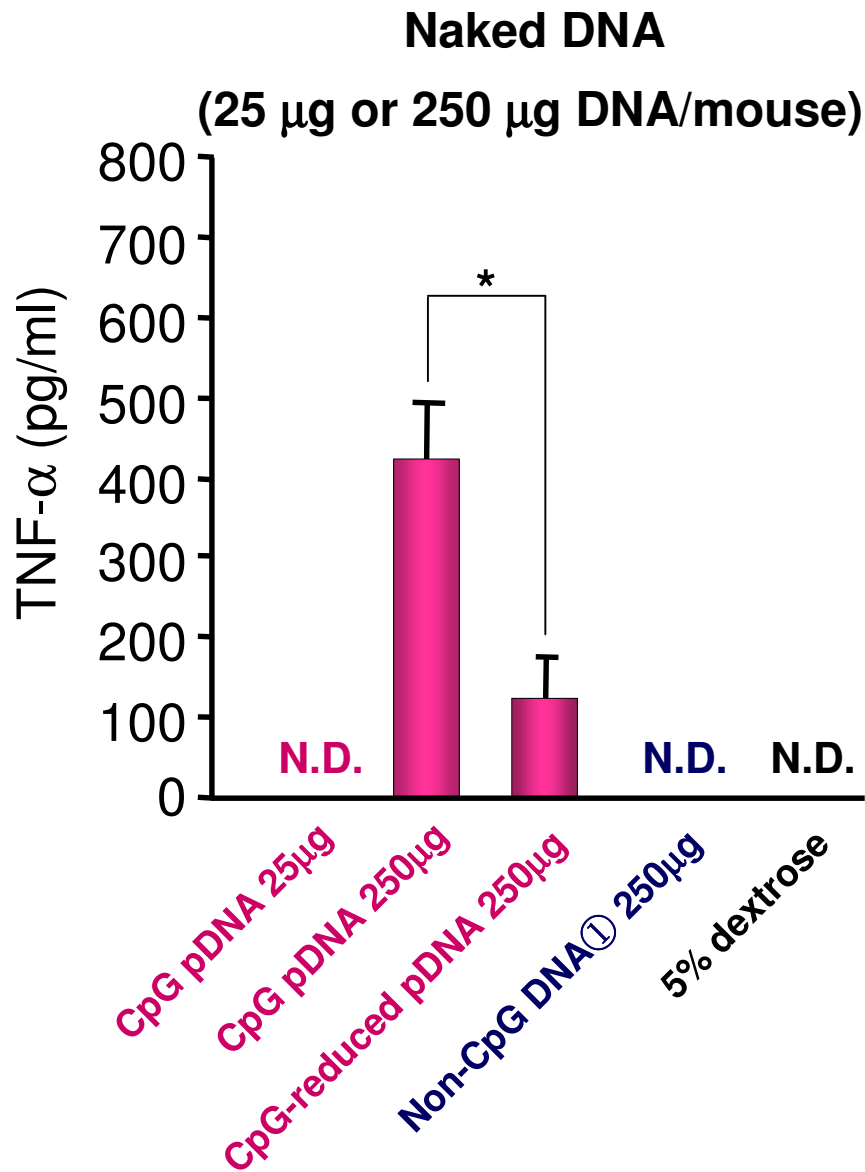


# Tissue distribution of [<sup>32</sup>P]pDNA lipoplex after intravenous injection into mice

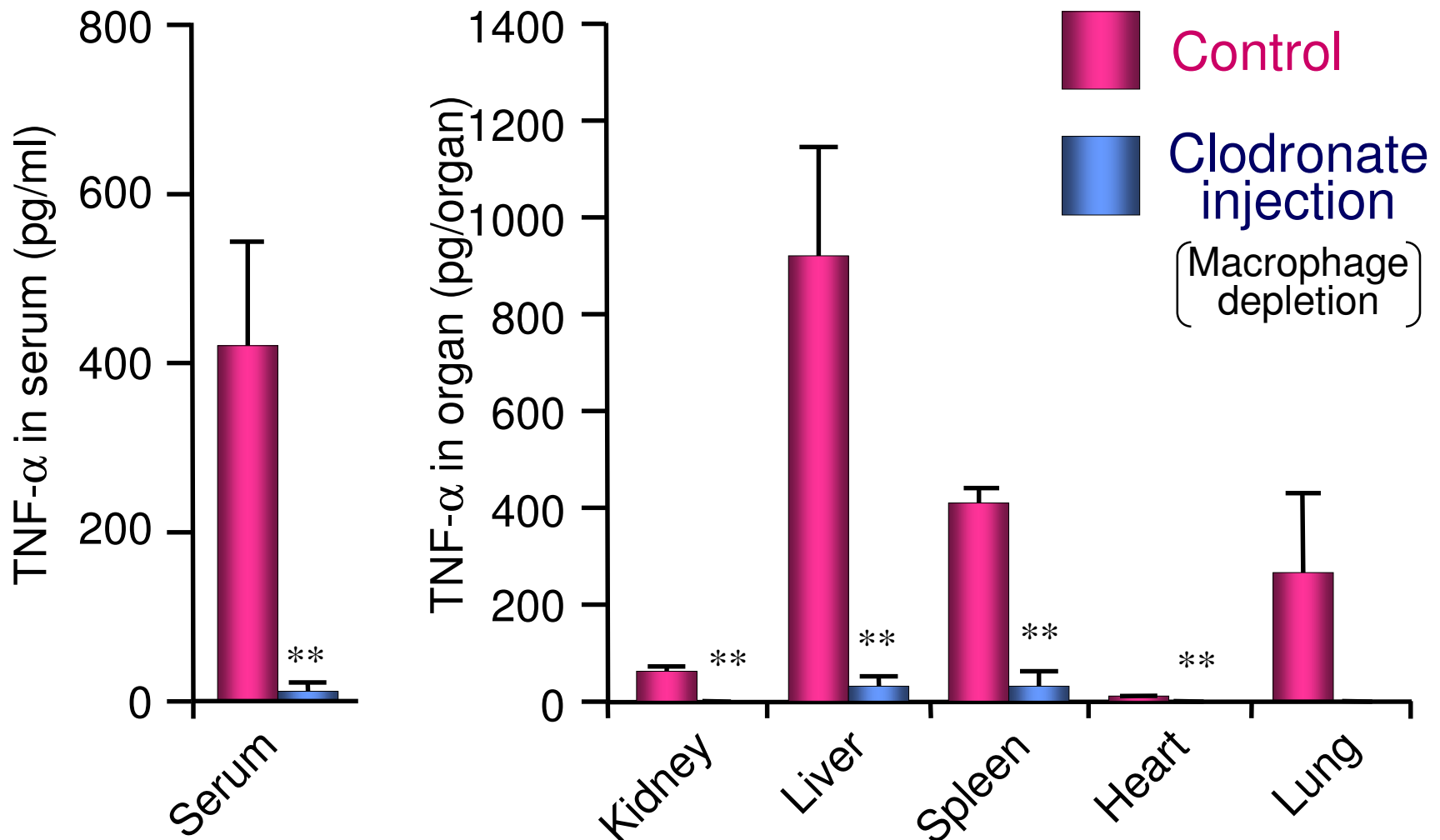


Kako et al., unpublished data.

# TNF- $\alpha$ level in mouse serum after intravenous injection of naked DNA and DNA lipoplex



# Effect of macrophage depletion on TNF- $\alpha$ production after intravenous injection of pDNA lipoplex



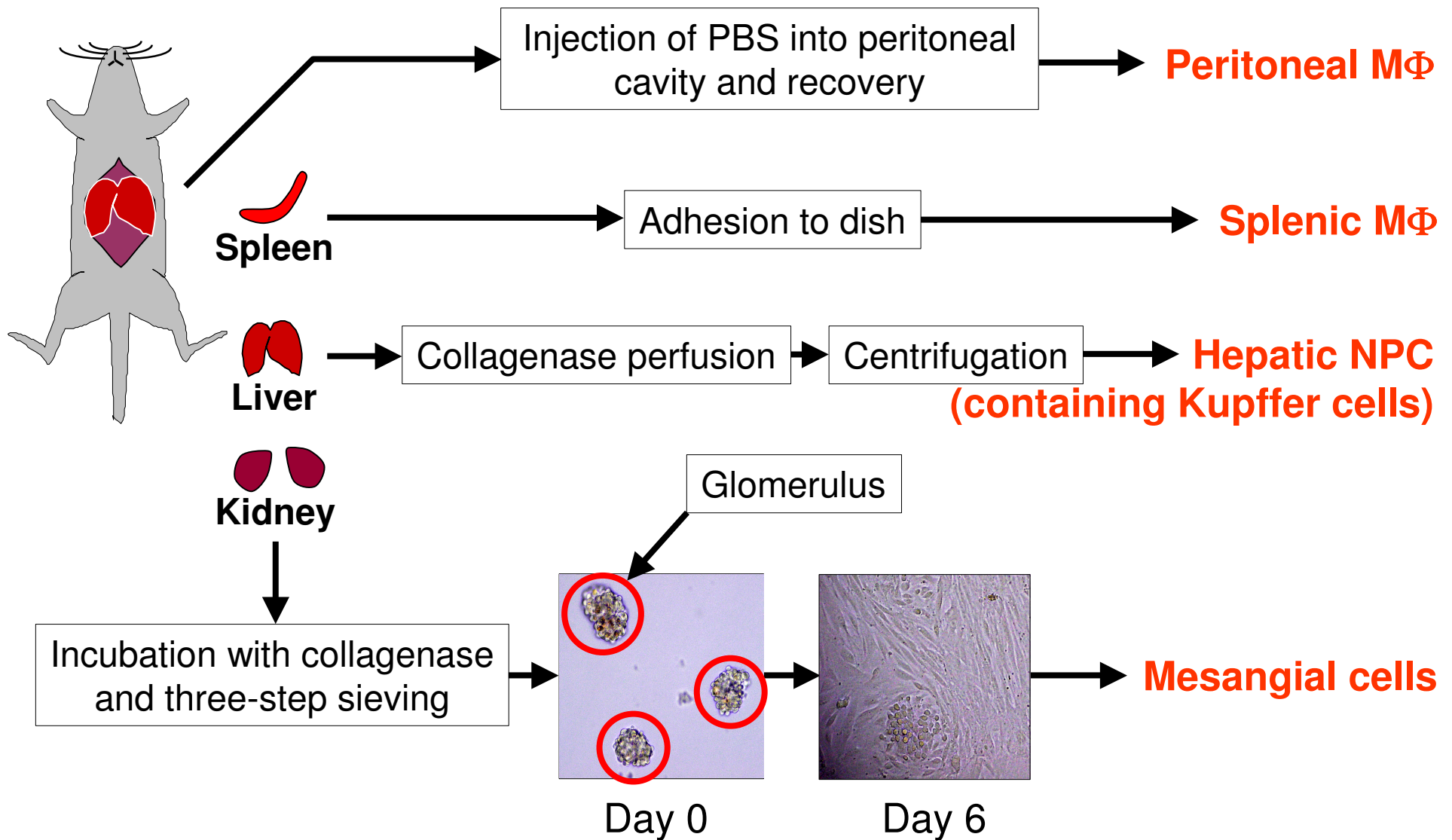
\*\* P < 0.01 vs. control    Kako et al., unpublished data.

# **Cytokine secretion profiles induced by...**

**Chapter. 1 Intravenous injection of DNA into mice in vivo.**

**Chapter. 2 DNA stimulation in primary cultured cells in vitro.**

# Isolation procedures of primary cultured cells



# Materials and methods in vitro study

## DNA

CpG DNA

pDNA: 119 CpGs/ kbp

Non-CpG DNA

Calf thymus (CT) DNA

## Cationic liposome

DOTMA/cholesterol liposome

## Cells

Primary cultured cells

Resident peritoneal macrophages ( M $\Phi$  )

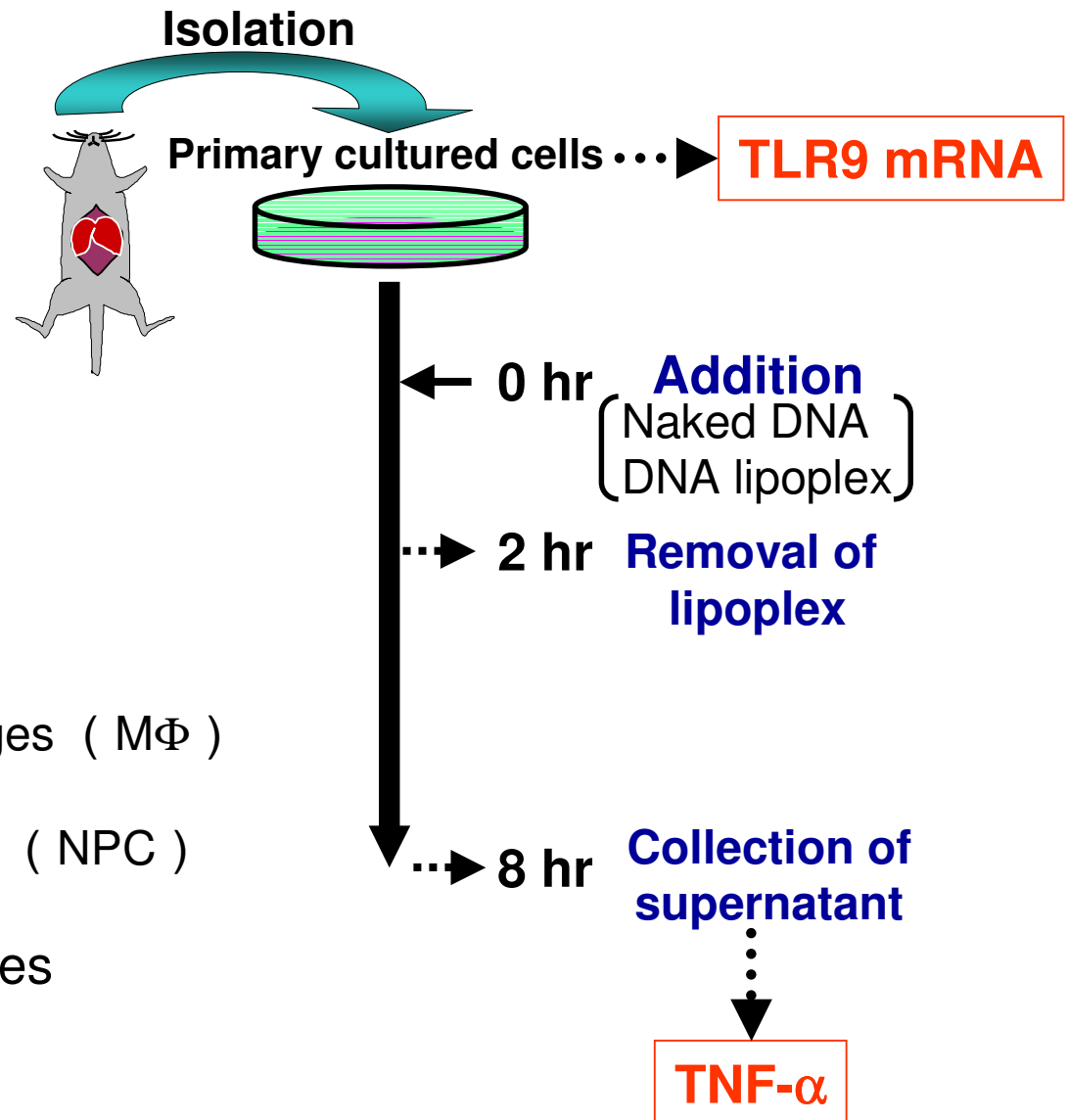
Splenic macrophages

Hepatic non-parenchymal cells ( NPC )

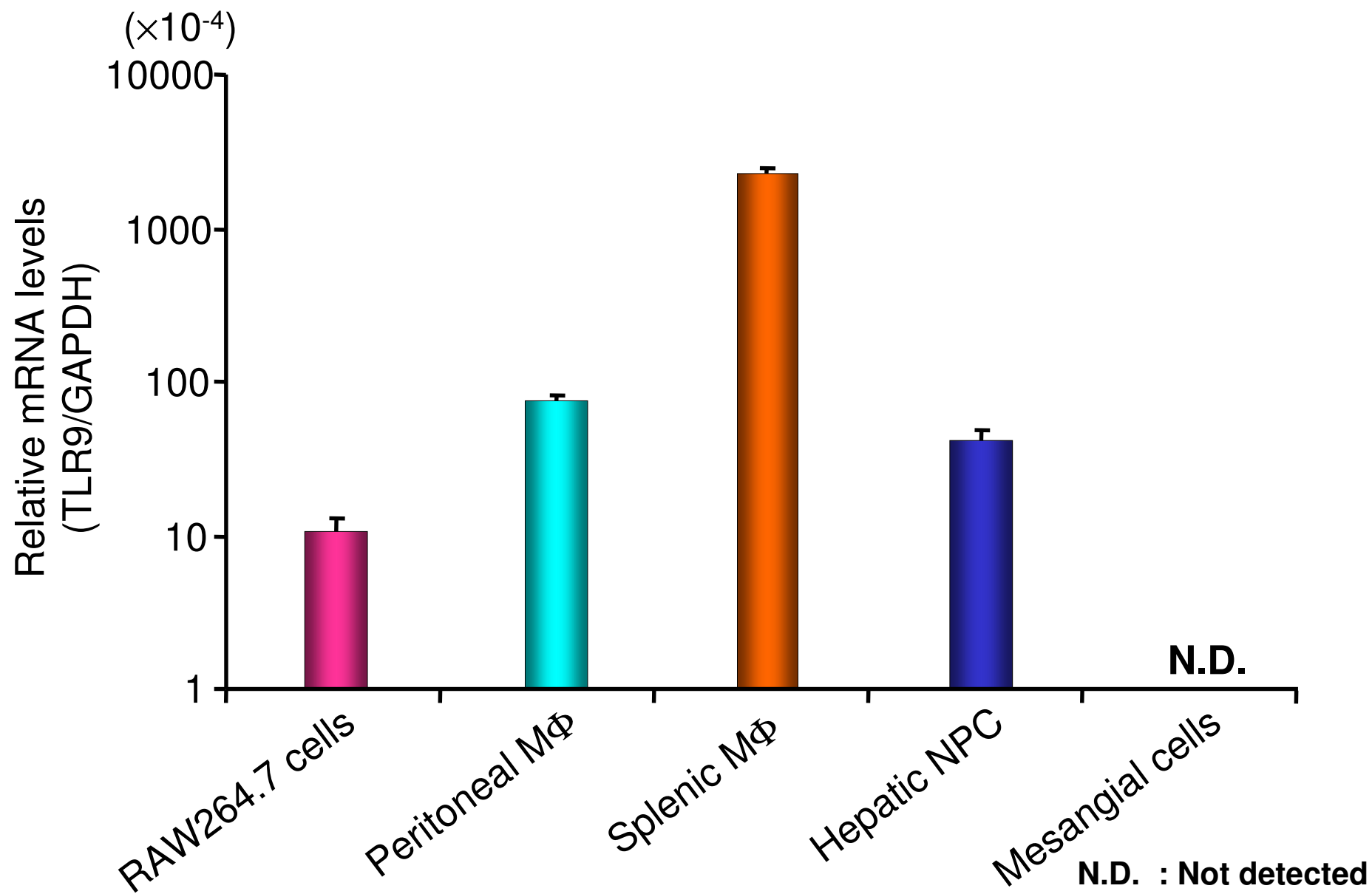
Mesangial cells

Mouse macrophage-like cell lines

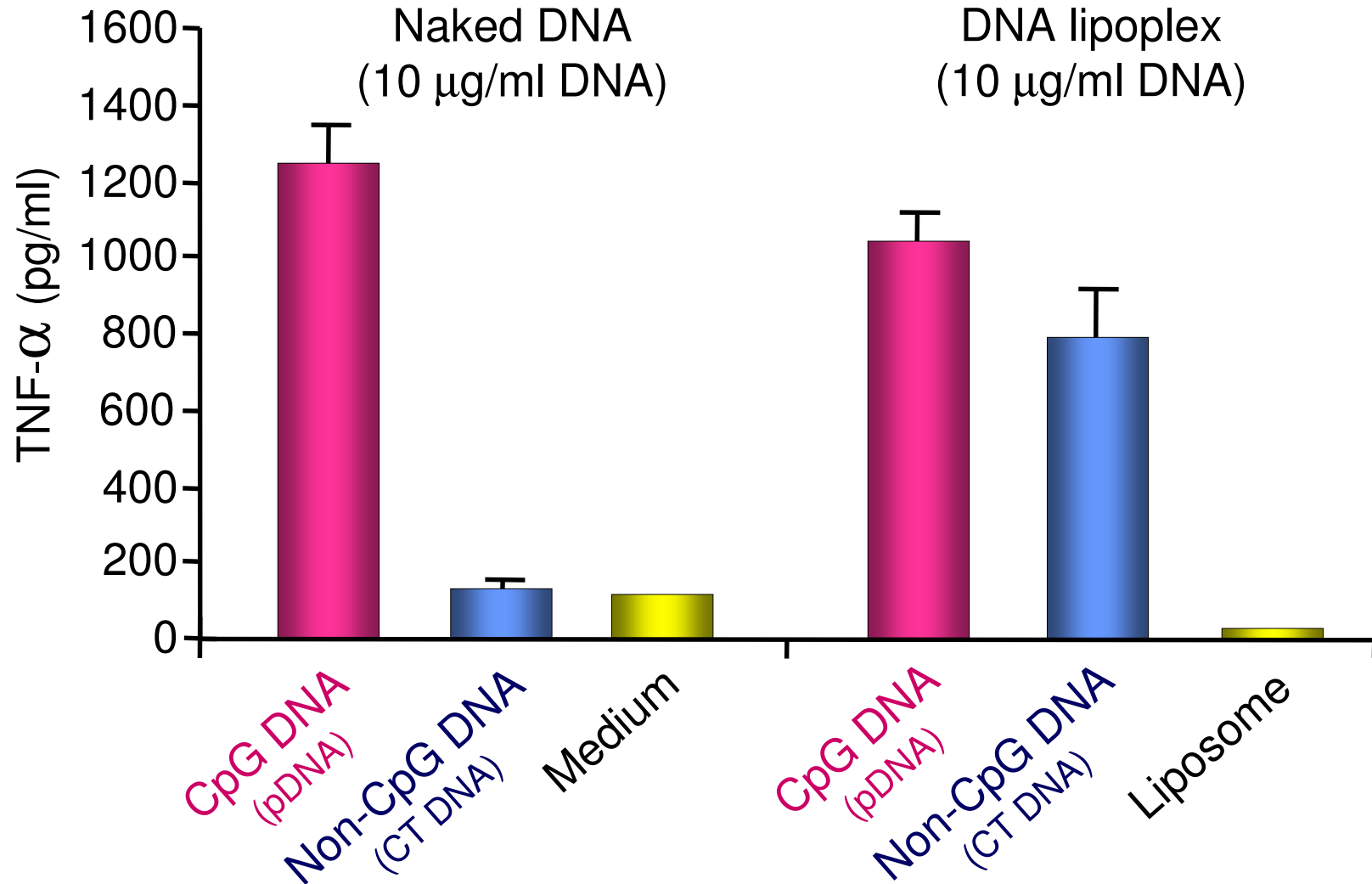
RAW264.7 cells



# TLR9 mRNA expression in various cells

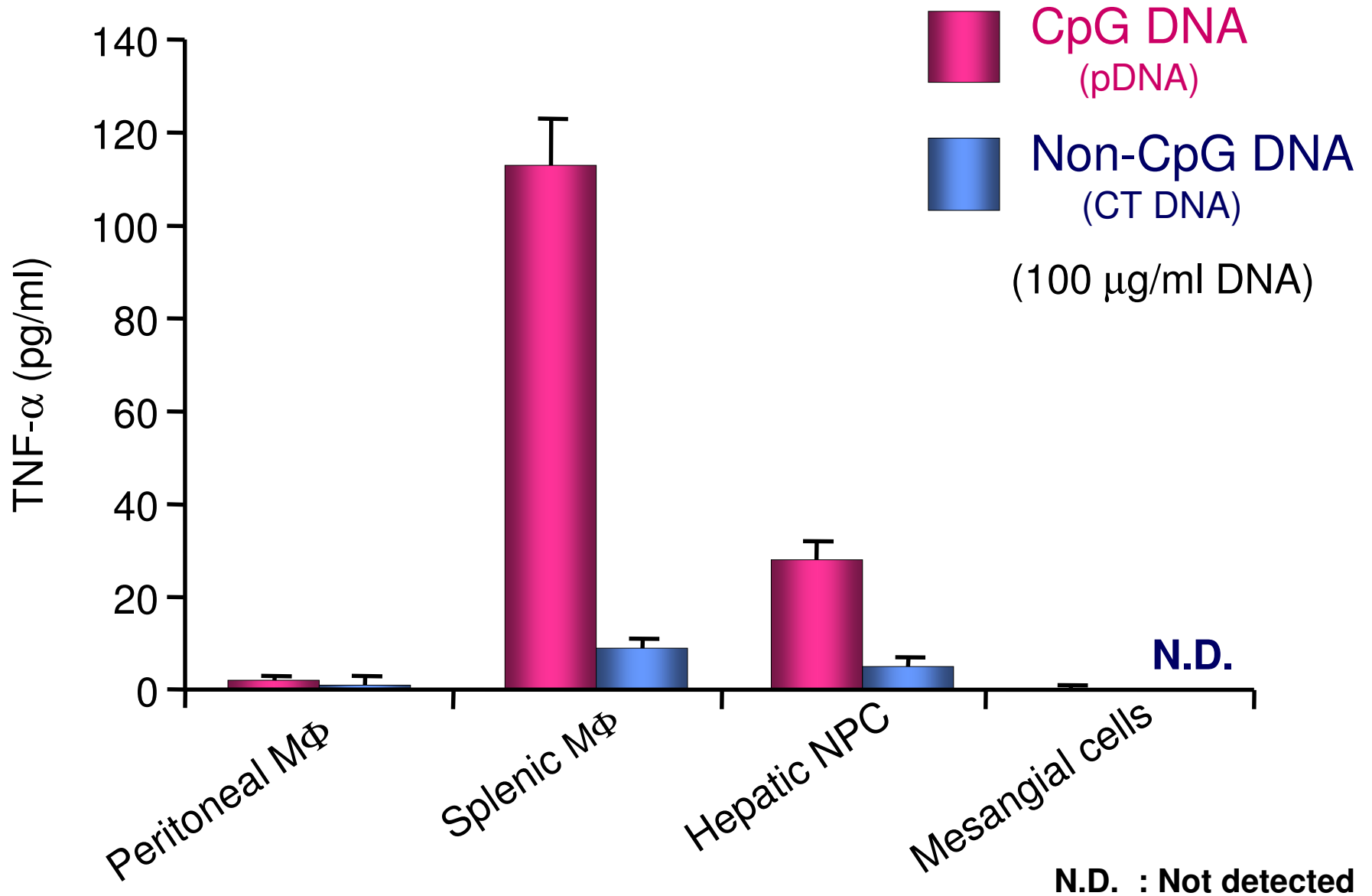


# TNF- $\alpha$ secretion from RAW264.7 cells induced by naked DNA or DNA lipoplex

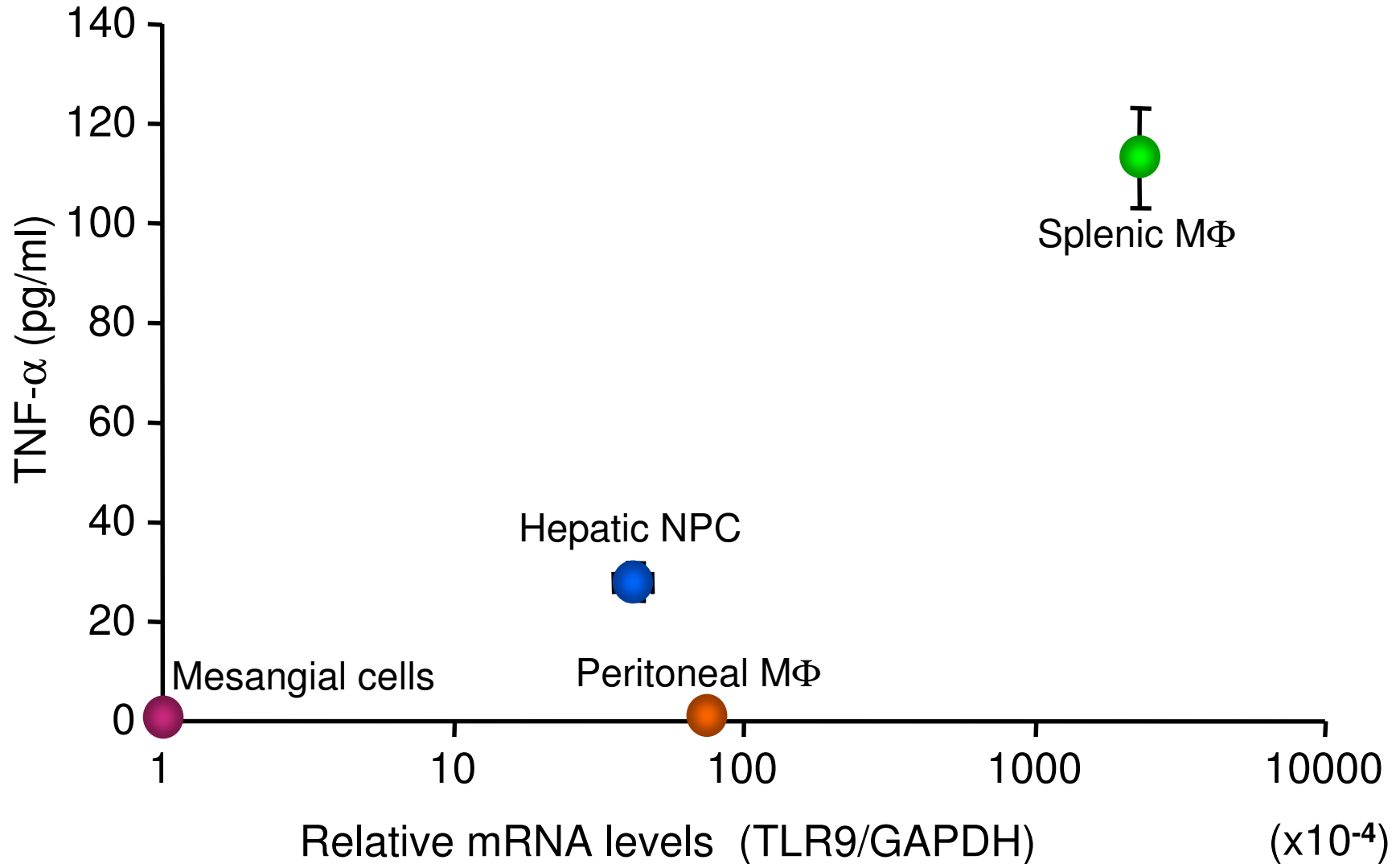




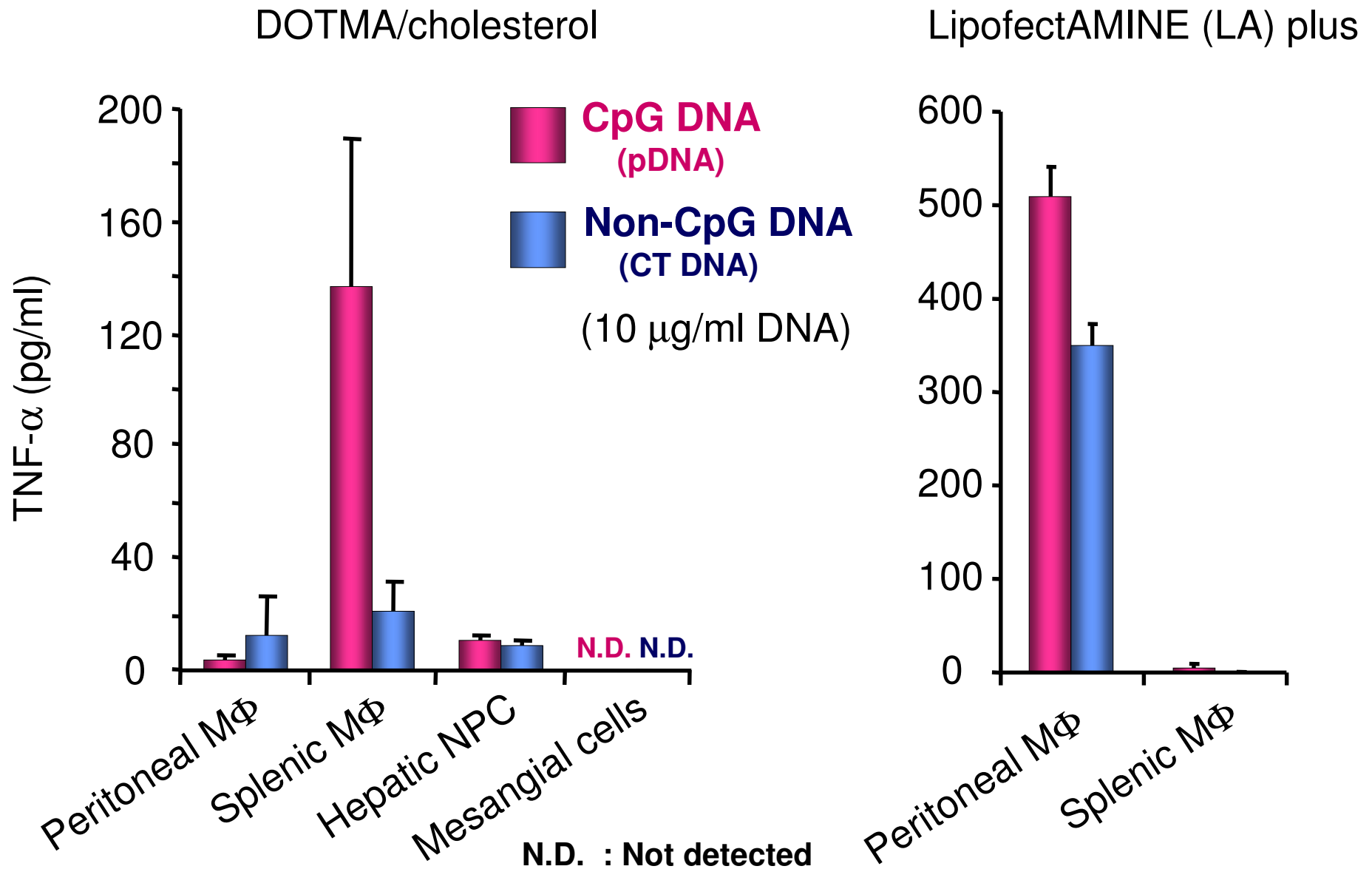
# TNF- $\alpha$ secretion from primary cultured cells induced by naked DNA



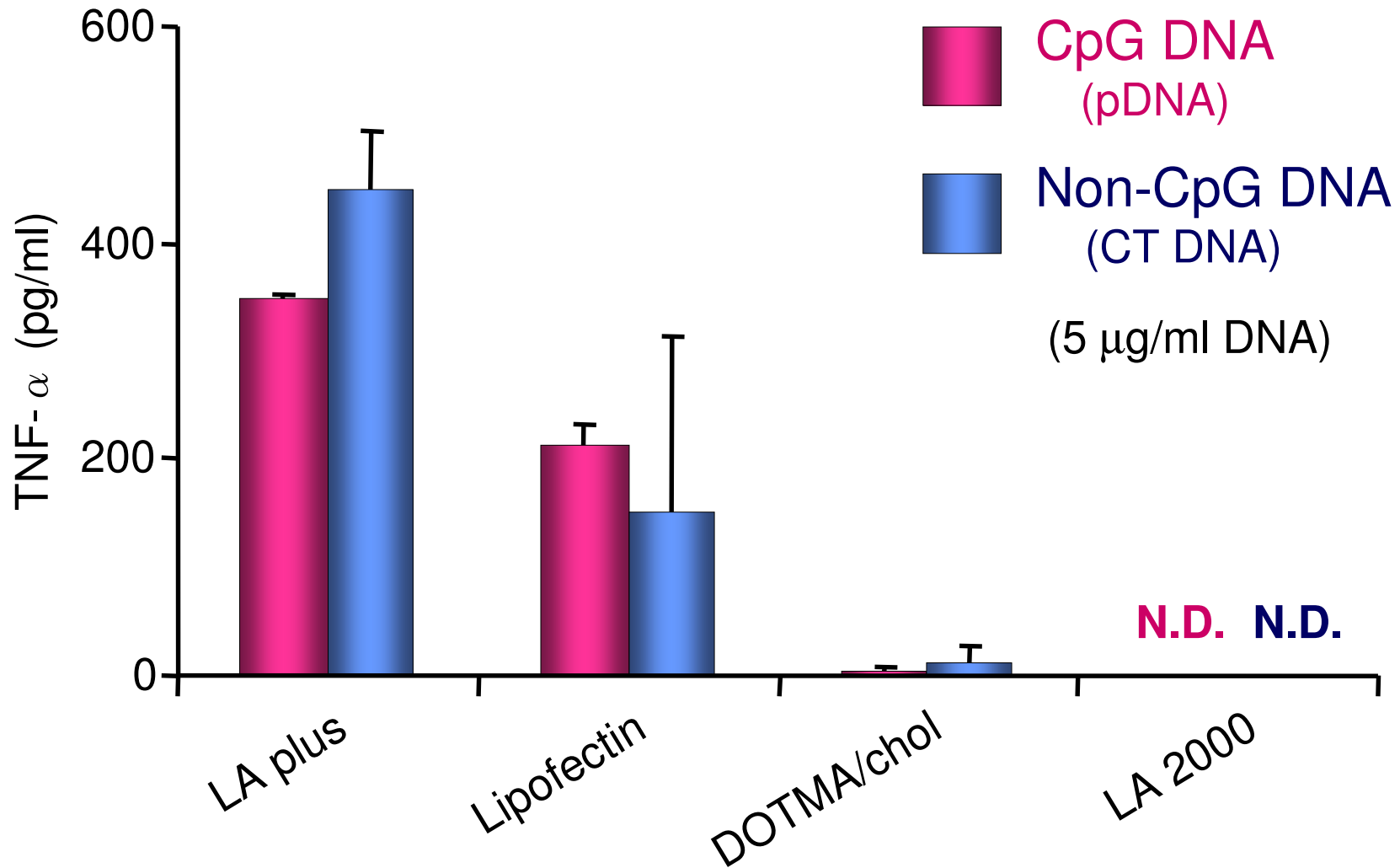
# Correlation between TLR9 mRNA expression and TNF- $\alpha$ secretion induced by naked CpG DNA in primary cultured cells



# TNF- $\alpha$ secretion from primary cultured cells induced by DNA lipoplex



# Cytokine release induced by DNA complexed with various cationic liposomes from peritoneal macrophages



N.D. : Not detected

# Summary

- Intravenous injection of either naked DNA or DNA lipoplex into mice induced TNF- $\alpha$  production dependently on the amount of CpG motifs.
- Splenic macrophages and hepatic non-parenchymal cells released the cytokine stimulated with naked CpG DNA.
- Peritoneal resident macrophages showed abnormal response to naked DNA and DNA lipoplex.