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# Overexpression of *Helicobacter pylori* VacA N-terminal fragment induces proinflammatory cytokine expression and apoptosis in human monocytic cell line through activation of NF- $\kappa$ B

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**Keywords:** *H. pylori*; VacA; transfection; proinflammatory cytokines; apoptosis.

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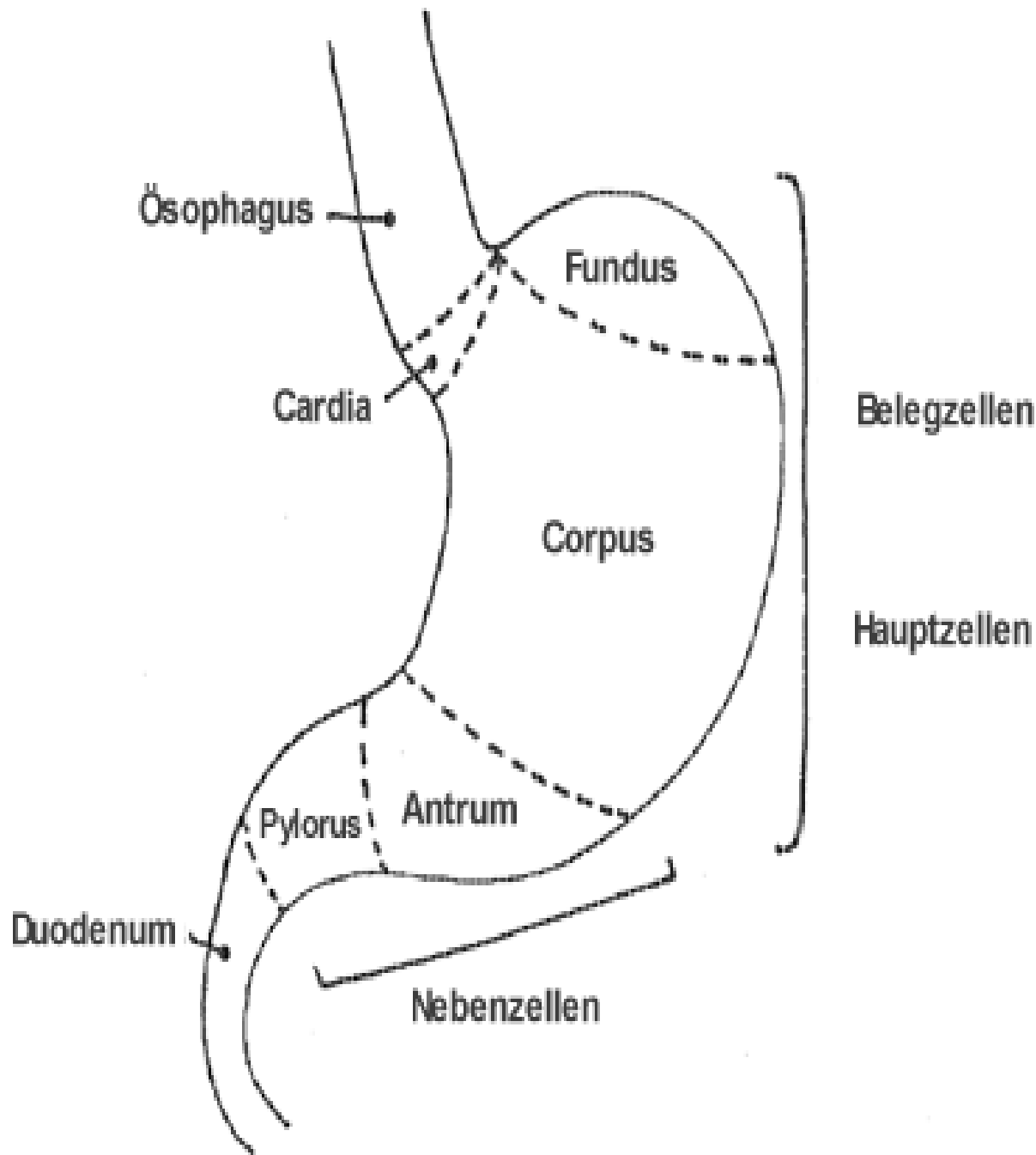






# Pathogenesis of *H. pylori*

- ❖ Infection is acquired in childhood & persists throughout life.
- ❖ In  $\leq 10\%$  of infected population, *H. pylori* infection causes peptic ulcer disease, gastric cancer, or lymphoma originated in MALT.
- ❖ Infection is usually located in the antrum and corpus of stomach.
- ❖ Identified virulence factors for *H. pylori* include:
  - Urease
  - VacA
  - CagA
- ❖ Adherence of bacteria to the gastric epithelium induces activation of the innate immune response, tyrosine phosphorylation, and cytokines production (IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-8) that lead to adaptive IR.



## Gross Anatomy of the human stomach



# Vacuolating cytotoxin

- ❖ VacA is an 88 kDa vacuolating cytotoxin.
- ❖ Induces the formation of large cytoplasmic vacuoles (*Leunk et al., 1988*).
- ❖ Induces apoptosis (*Manente et al., 2008*).
- ❖ Interferes with Ag presentation (*Molinari et al., 1998*).
- ❖ Activation of MAPK (*Nakyama et al., 2004*).
- ❖ Inhibition of activation-induced proliferation of T cells (*Torres et al., 2007*).



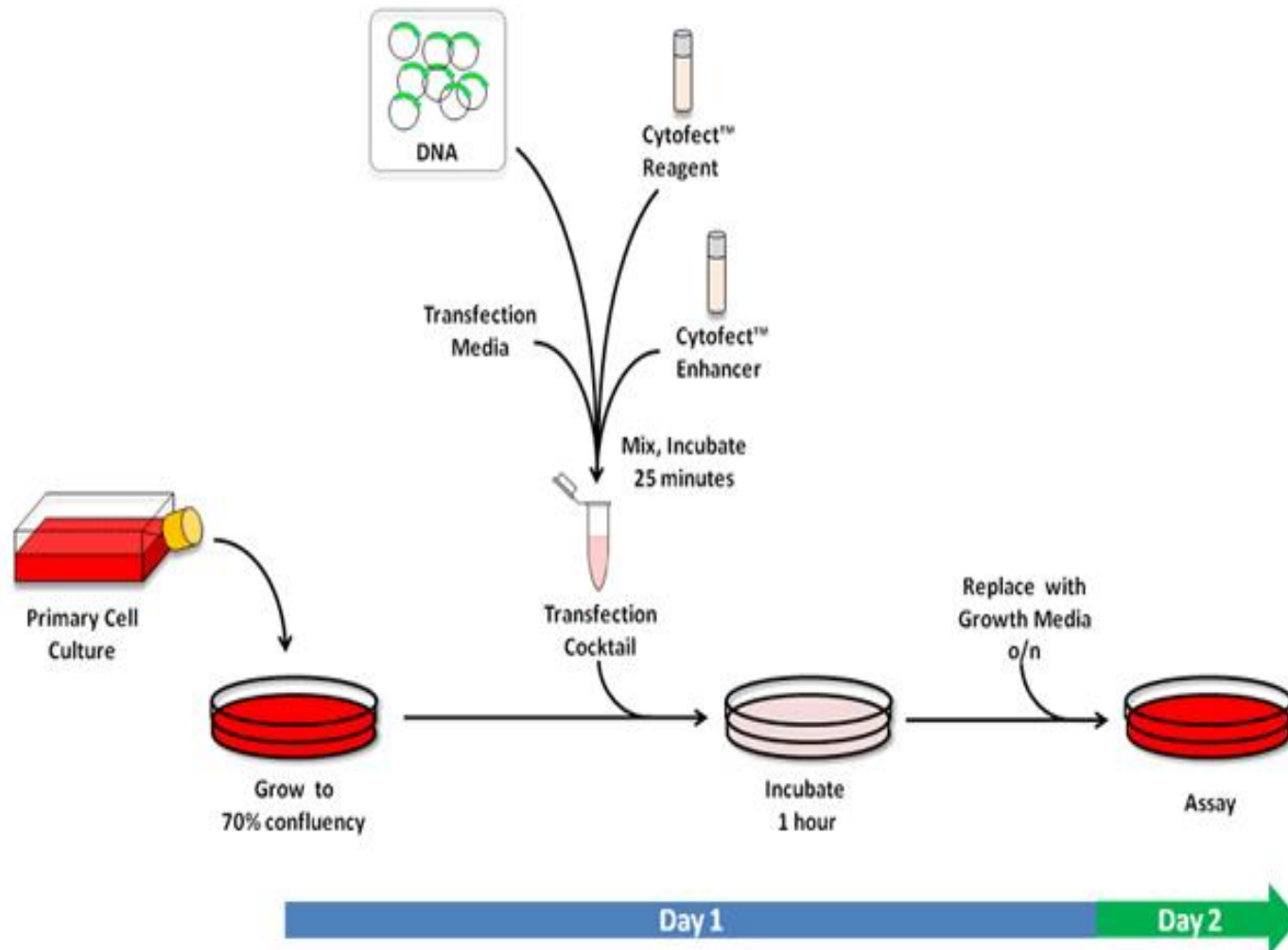
# OBJECTIVES

- ❖ To investigate the effects of *H. pylori* VacA NH<sub>3</sub>-terminal p52 fragment on cytokine secretion, ROS production, & apoptosis of PMA-differentiated THP-1 cells.
- ❖ To elucidate the molecular mechanisms by which *H. pylori* evades the immune system to establish chronic infection in humans.



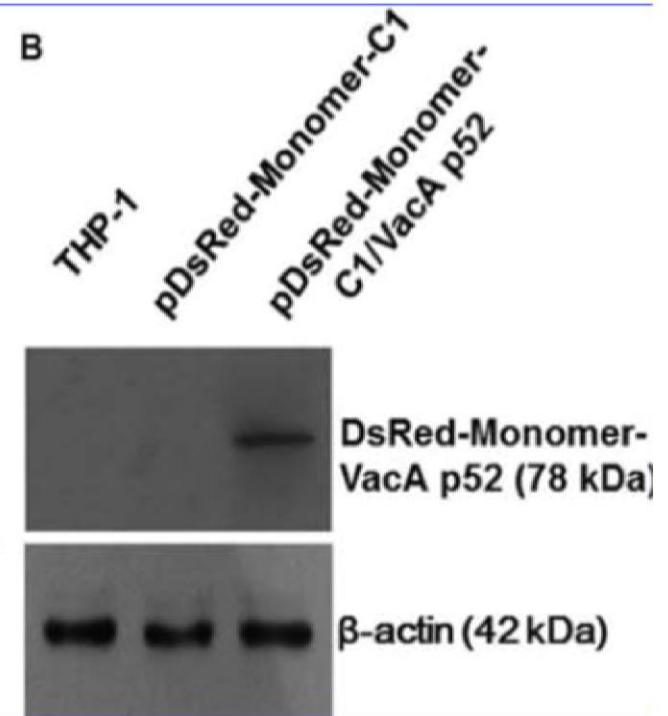
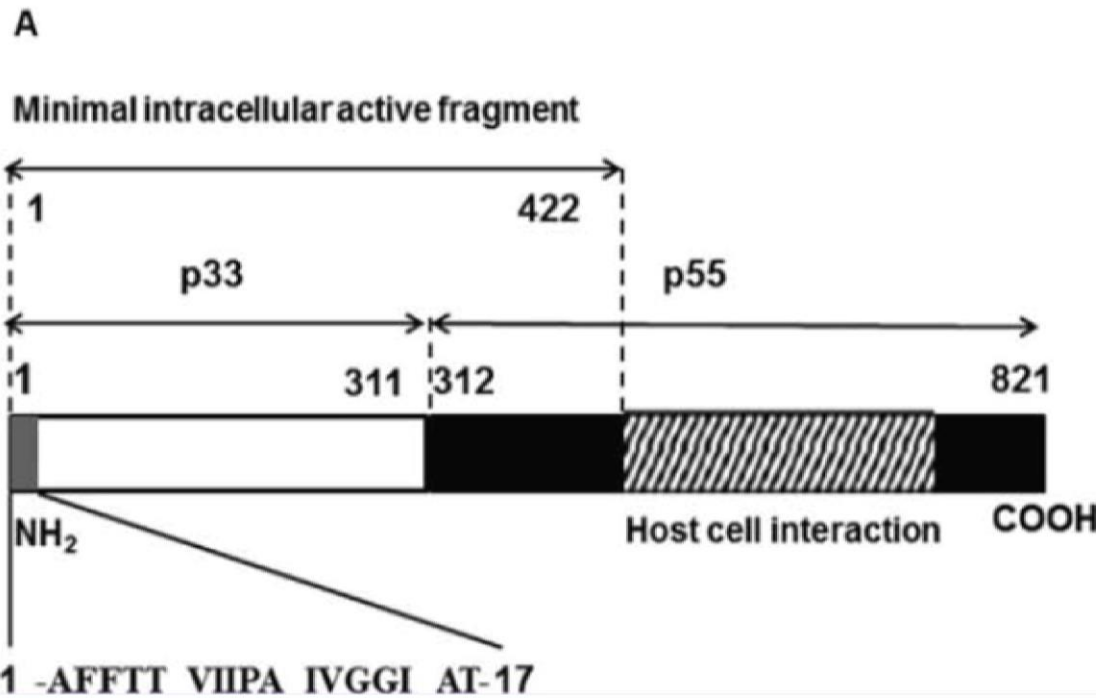
# Materials & Methods

- **Growth of *H. pylori* (Wt. 26695 strain) & DNA extraction.**
- **Cloning of the sequence encoding p-52 N-terminal domain of VacA.**
- **Construction of recombinant plasmid: **pDsRed-Monomer-C1/VacA p52.****
- **Preparation of THP-1 for transfection:**
  - (i) growth to confluency in complete RPMI;
  - (ii) stimulation of THP-1 with PMA for 48 hrs
  - (iii) growth of PMA-differentiated THP-1 cells in 1 % FBS O/N.
  - (iv) transfection of rec plasmid or empty vector into THP-1 cells by using transfection reagent.
- **Subsequent use of transfected cells for:**
  - **Western blot;**
  - **TEM;**
  - **cytokine assay;**
  - **NO measurement;**
  - **detection of apoptosis; and**
  - **EMSA.**



# Transfection protocol

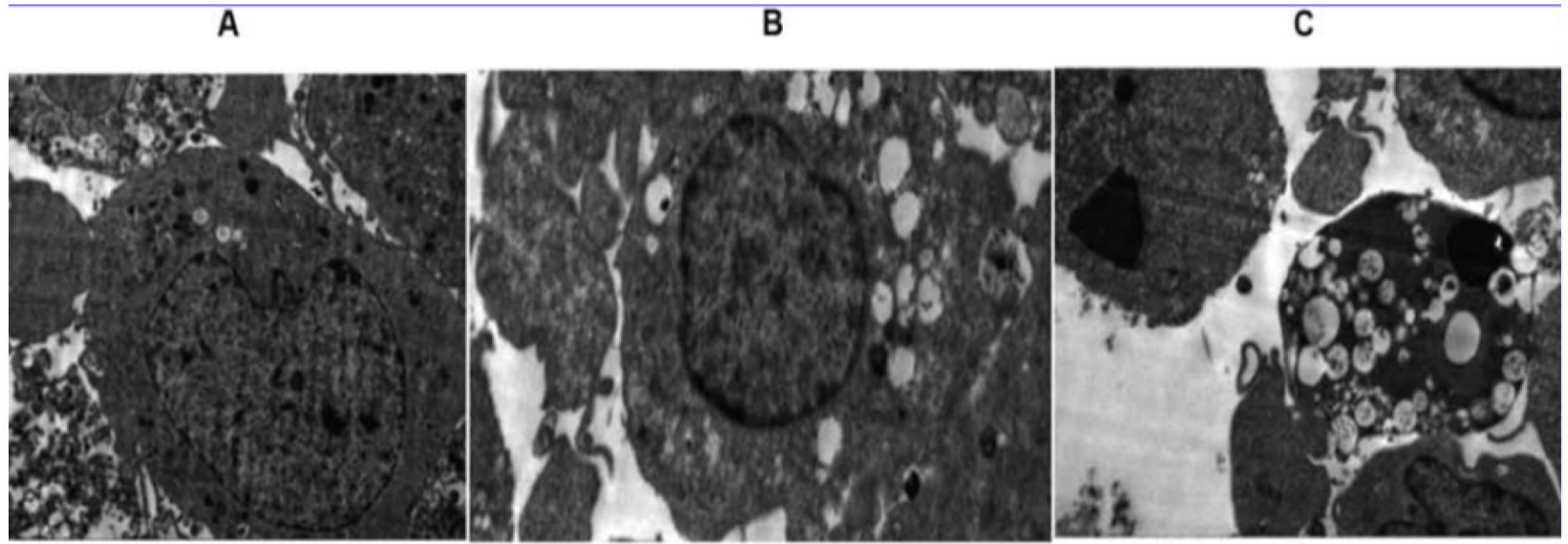




## Expression of DsRed-Monomer-VacA p52 in THP-1 cells.

(A) Structure of mature VacA.

(B) Western-blot analysis of p52 VacA in THP-1 cells.



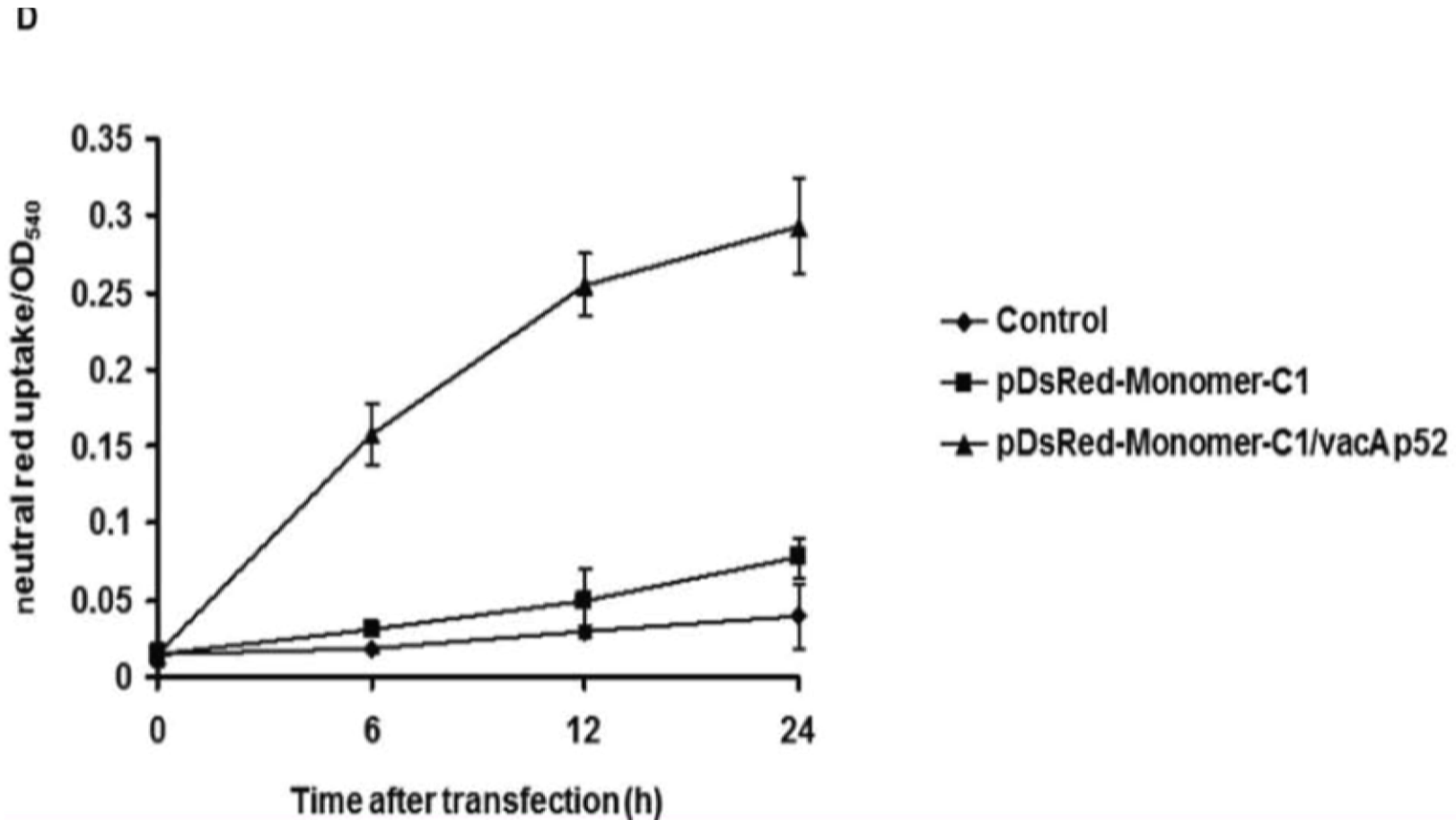
**Transmission electron micrographs showing vacuolating cells.**

**A. Untreated THP-1 macrophage.**

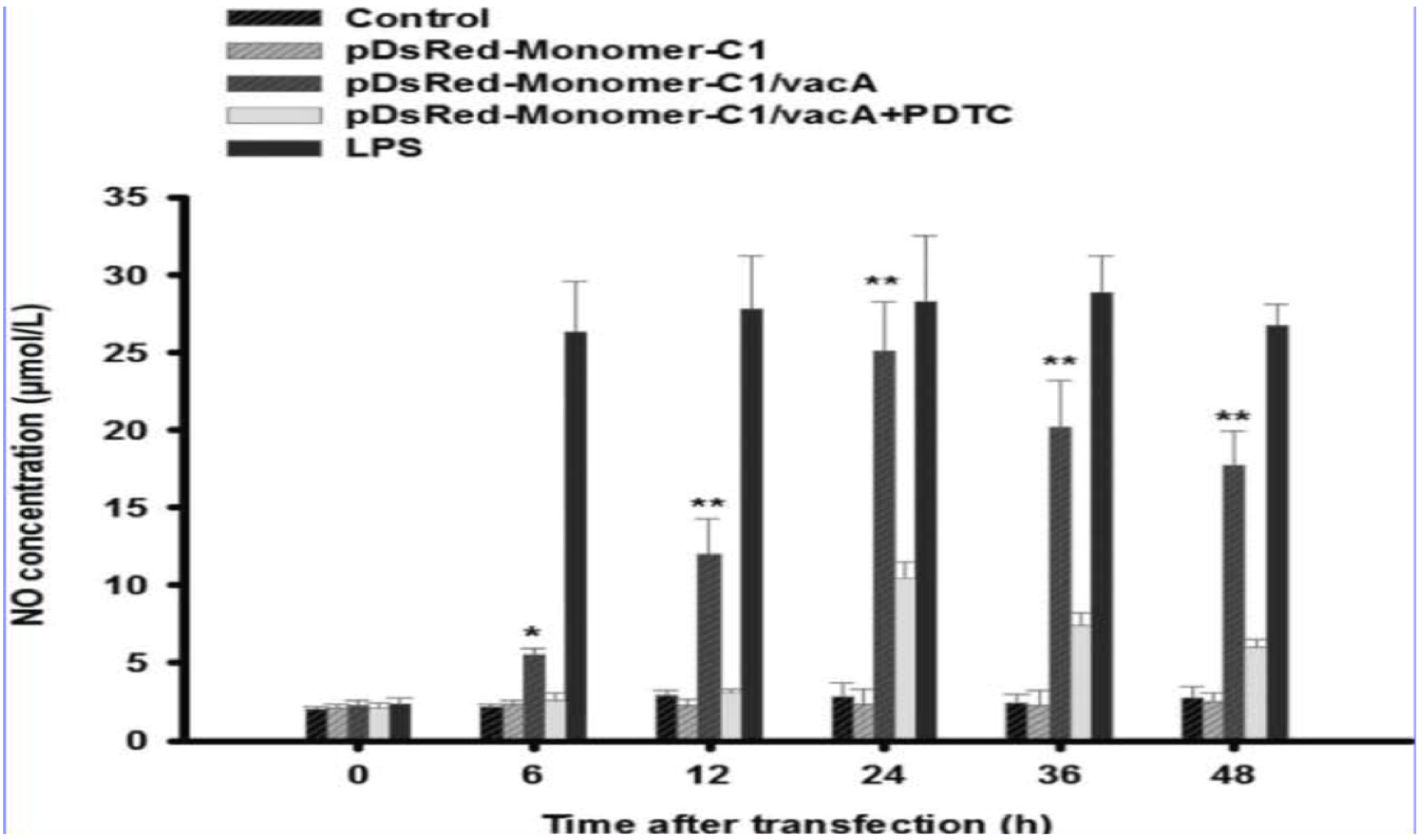
**B-C. THP-1 cells transfected with VacA p52 plasmid for 24 hrs.**

**Noticeable  $\uparrow$  vacuoles in the cytoplasm of THP-1 macrophage (B&C) with a nucleus pushed aside in C.**





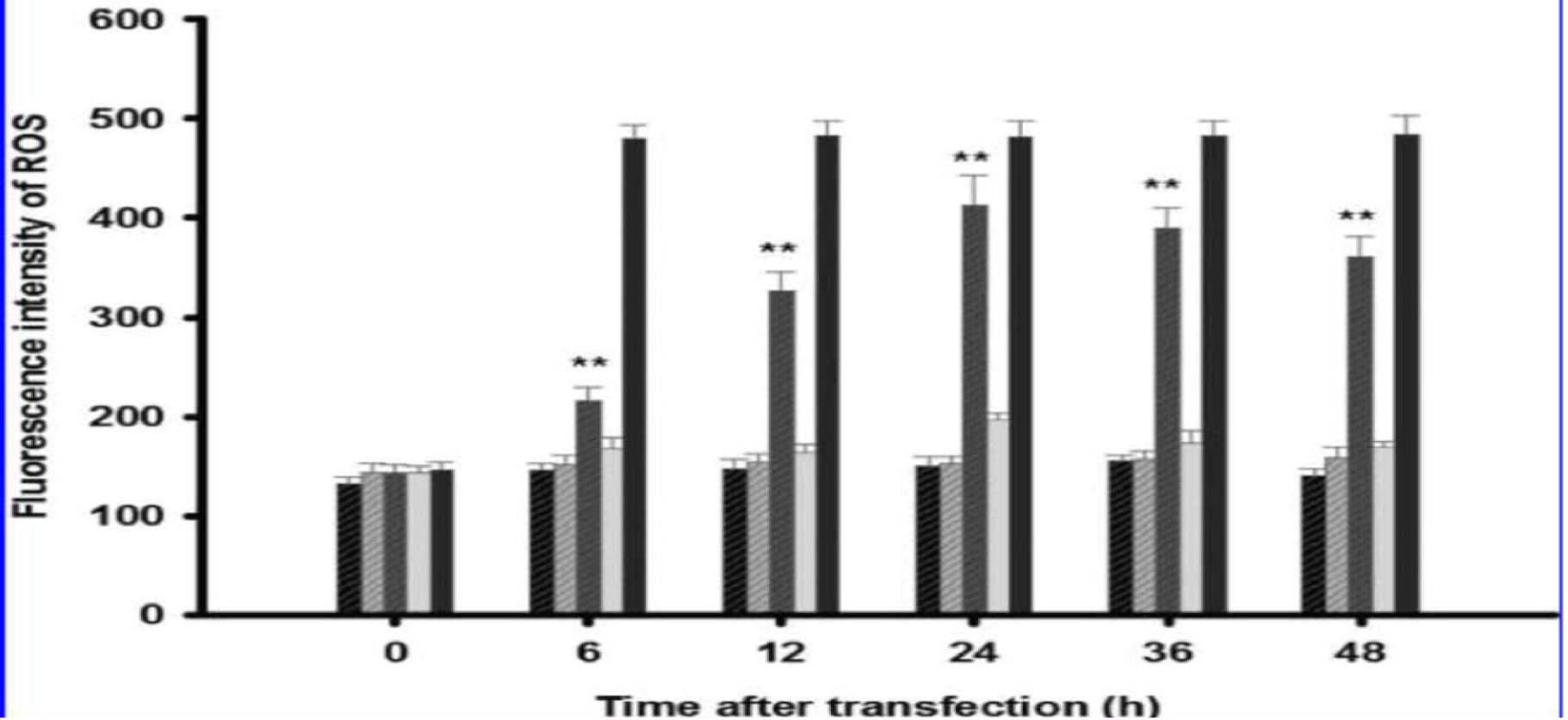
**Vacuolating cytotoxic activity of p52 VacA in THP-1 cells. Results showed the mean  $\pm$  SD of 3 expts for the uptake of neutral red by the vacuoles.**



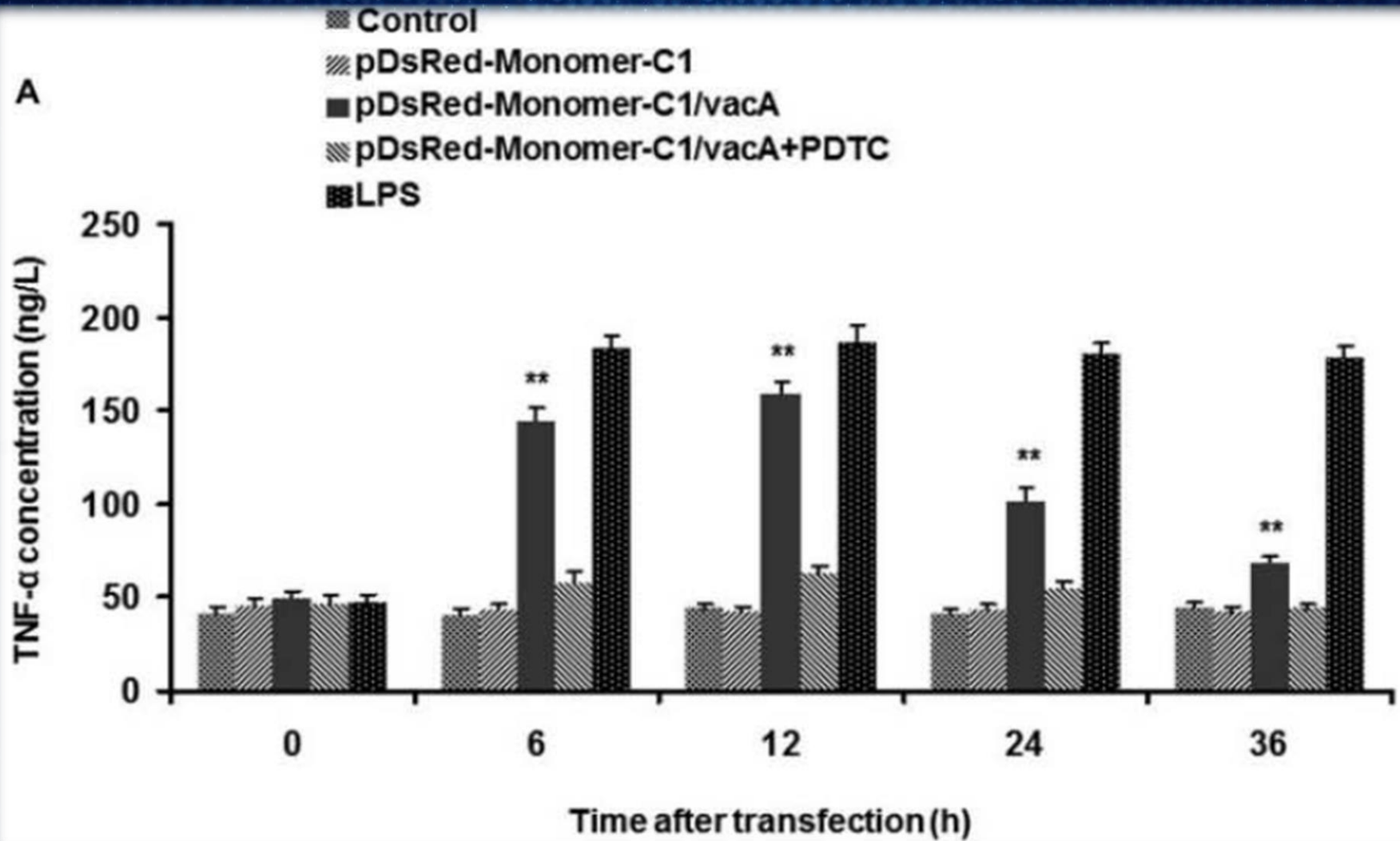
**Kinetics of NO expression in VacA p52 transfected THP-1 cells.**



- Control
- pDsRed-Monomer-C1
- pDsRed-Monomer-C1/vacA p52
- pDsRed-Monomer-C1/vacA p52+PDTC
- LPS



**Kinetics of ROS induction in THP-1 cells after VacA p52 transfection.**

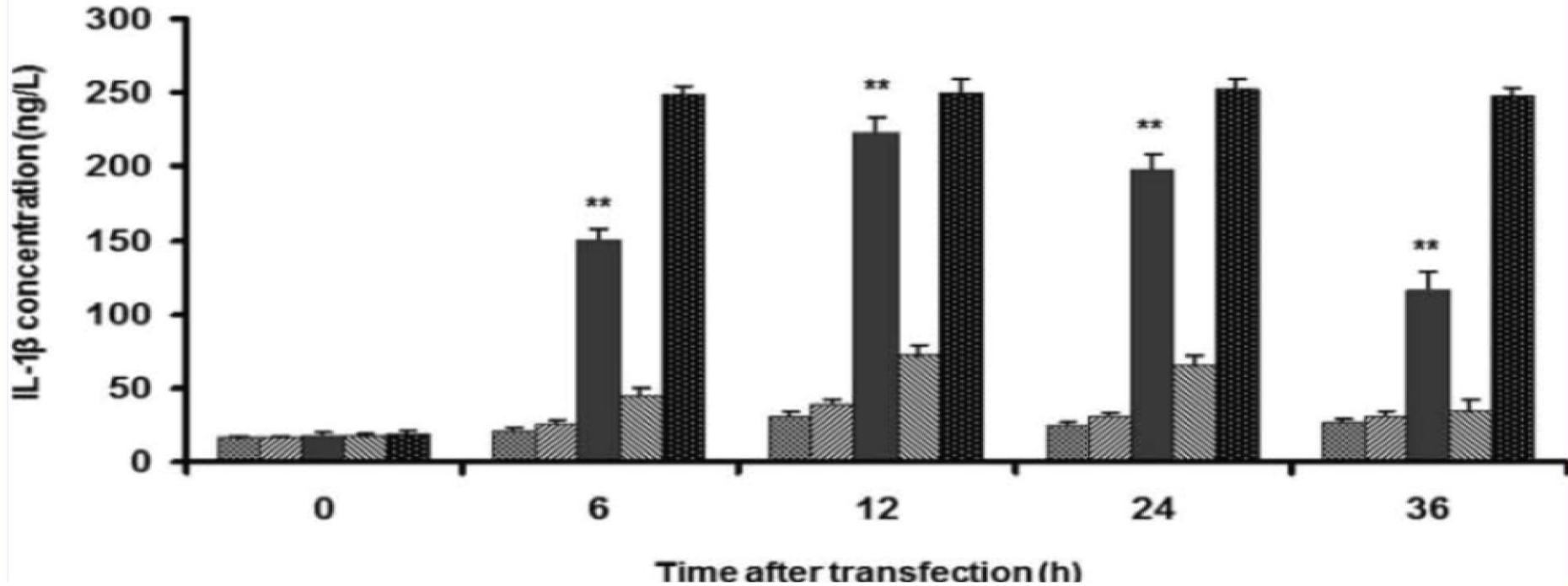


**Kinetics of TNF- $\alpha$  induction in THP-1 cells after VacA p52 transfection.**

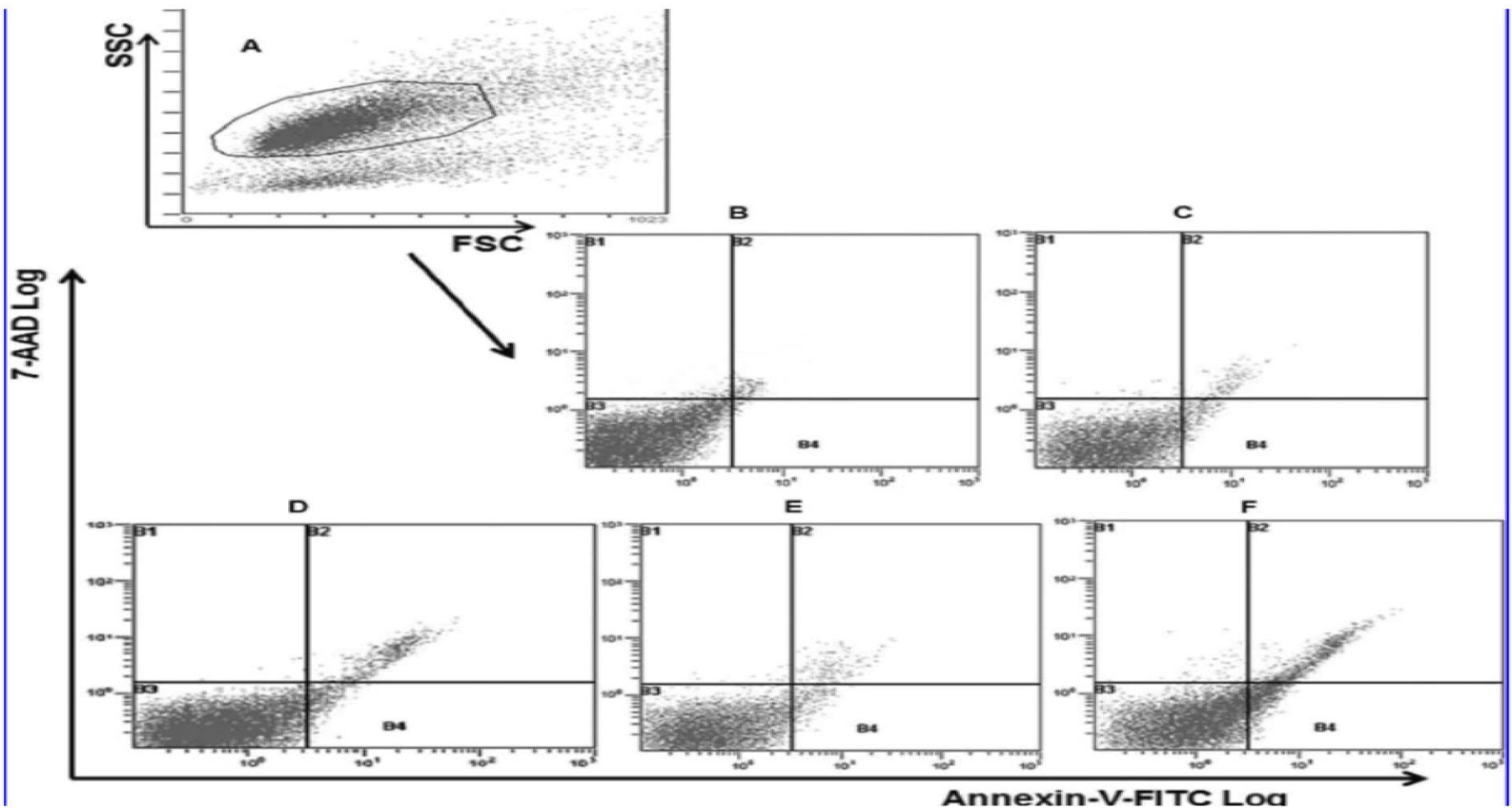


**B**

- Control
- pDsRed-Monomer-C1
- pDsRed-Monomer-C1/vacA
- pDsRed-Monomer-C1/vacA+PDTC
- LPS



**Kinetics of IL-1 $\beta$  induction in THP-1 cells after VacA p52 transfection.**



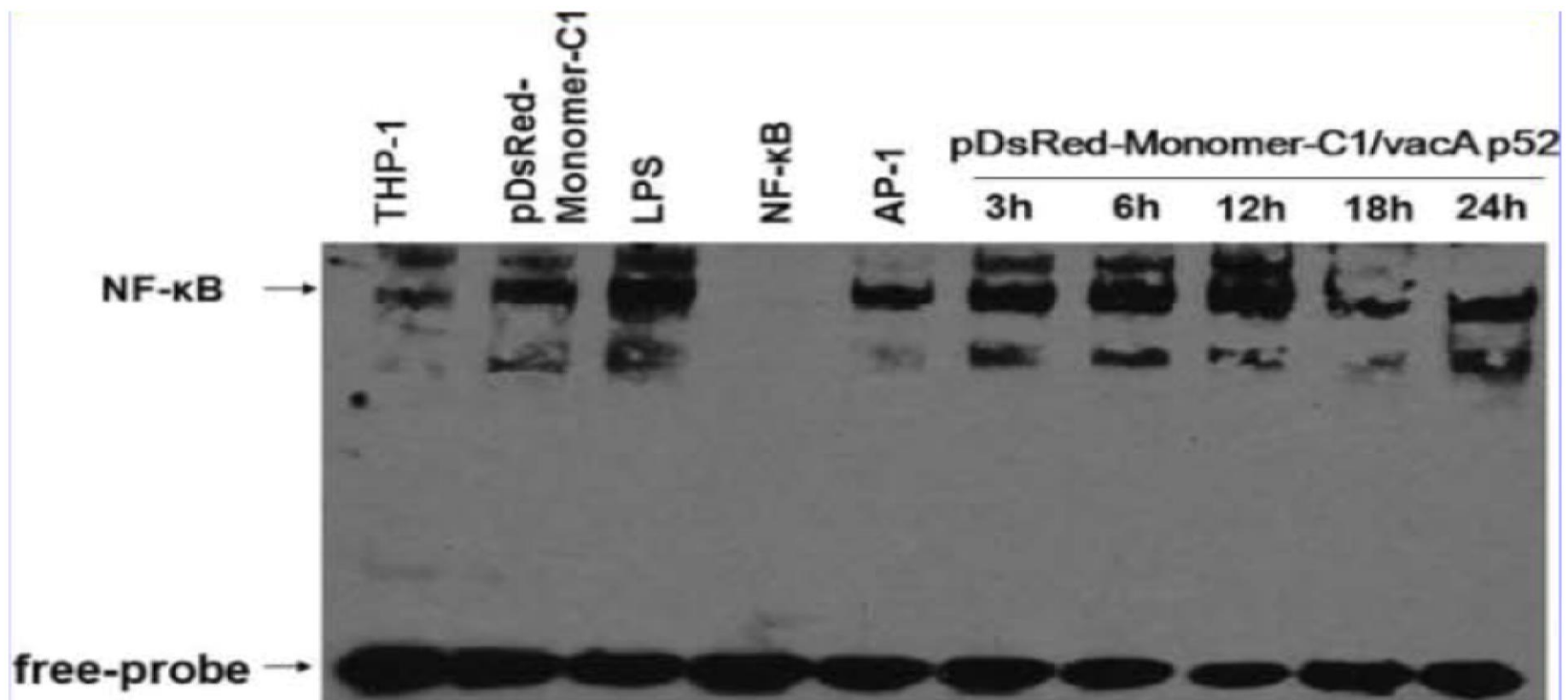
FACS analysis of apoptosis in THP-1 cells due to VacA p52 transfection. Apoptosis was analyzed 16 hrs. post transfection by using a combination of FITC-conjugated Annexin V & 7-aminoactinomycin D (7-AAD). **(A)** Dot plot depicting FSC versus SSC profile of THP-1 cells. **(B)** Untreated THP-1 cells. **(C-F)** THP-1 cells transfected with empty plasmid (C), plasmid plus VacA p52 (D), plasmid plus VacA p52 after prior treatment with PDC (E), or treated with hexadecadrol.



Table 1. Apoptosis rate of the THP-1 cells induced by VacA p52 with Annexin V-7-AAD staining method 16 h after transfection.

Group	Apoptosis rate (%) (mean $\pm$ SD, n = 3)
Control	2.78 $\pm$ 0.79
pDsRed-Monomer-C1	6.43 $\pm$ 1.36
pDsRed-Monomer-C1/VacA	16.42 $\pm$ 5.76*
pDsRed-Monomer-C1/VacA + PDTC	6.82 $\pm$ 0.79
Hexadecadrol	29.89 $\pm$ 4.08*

Note: 7-AAD, 7-aminoactinomycin D; PDTC, pyrrolidine dithiocarbamate. \*, significantly different from the control group at  $P < 0.05$ .



Electrophoretic mobility shift assay (EMSA) of NF- $\kappa$ B activity in different groups of THP-1 cells. NF- $\kappa$ B activation was measured by using biotin-labeled oligonucleotide encompassing the NF- $\kappa$ B consensus motif. The specificity of DNA binding was assessed by preincubating extracts with unlabeled specific NF- $\kappa$ B or unspecific (AP-1) competitor oligonucleotide.



# Conclusions

- ❖ *H. pylori* VacA p52 ↑ the production of proinflammatory cytokines & induces apoptosis in macrophages through a NF- $\kappa$ B-dependent pathway.
- ❖ Induction of macrophage apoptosis coupled with disruption of phagosome maturation & excessive release of inflammatory cytokines lead to chronic inflammation & impairment of the host immune response.



# DISCUSSION

- ❖ What are the goodies and woeful matters in the text of the presented paper.
- ❖ What is unique to our knowledge about the presented data.
- ❖ Where does the weakness exist on this paper.
- ❖ How do you rate the paper & the presenter.

**Think about it!**

**In science we develop & In honesty we flourish.**