Clonorchis sinensis tames immune responses via anti-inflammatory cytokine production in murine antigen presenting cells

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Clonorchiasis

- **Caused by *Clonorchis sinensis,* Looss, 1907.**
- **Definitive host: human, dogs, cats, pigs and minks.**
- **Geographic distribution:**

  -Most common in eastern Asia, including China, Japan, Korea, Taiwan, Vietnam and far-eastern Russia

- **Prevalence of human clonorchiasis**

  - At least 20 million people were infected with *C. sinensis* in endemic areas all over the world. (Hong & Fang 2012)

National survey of intestinal parasitic infections in Korea, 8th Report 2013

(Centers for Disease Control and Prevention & National Institute of Health, Korea)

- **- 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 2.7% in 1986, 2.2% in 1992,**

  1.4% in 1997, 2.4% in 2004, 1.9% in 2013 (n=23,956).

Factors contributing to the slow decrease of *C. sinensis* in Korea:

- **Non-specific symptoms, no symptoms in light infection (Hong & Fang 2012)**
- **The habit of eating raw fish is obstinate (Oh et al. 2014)**
- **Since the life span of *C. sinensis* is around 26 years, infection accumulates through life of humans (Attwood et al. 1978)**
- **Humans are susceptible to infection and re-infection by *C. sinensis* (Sohn et al. 2006)**

Questions and Hypothesis

- How does the *Clonorchis sinensis* escape from the host immune system and survive long time?
- Which cell is the key modulator?

- Hypothesis:

  "Antigen presenting cells might have a critical role in immune regulation of *Clonorchis sinensis* infection"

Materials ● Methods

- **Preparation of crude antigen(CA) of *Clonorchis sinensis***
- **Cell culture and antigen stimulation**

  -DC2.4, BMDC, RAW264.7, BMDM
- **Flow cytometric analysis(Becton-Dickinson)**

  - CD11c, CD80, CD86, CD40, MHCII, TLR2, TLR4, Mac-3, CD14.
- **Cytokine enzyme-linked immunosorbent assay(e-Bioscience)**

  -IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN- γ, TNF-α, TGF-β
- **Protein extraction and western blot analysis**

  -phospho-ERK1/2, phospho-JNK, phospho-P38, total-ERK1/2, P38, JNK, actin

Agenda

PART I

Immune responses to *C. sinensis* crude antigen in Dendritic cells

PART II

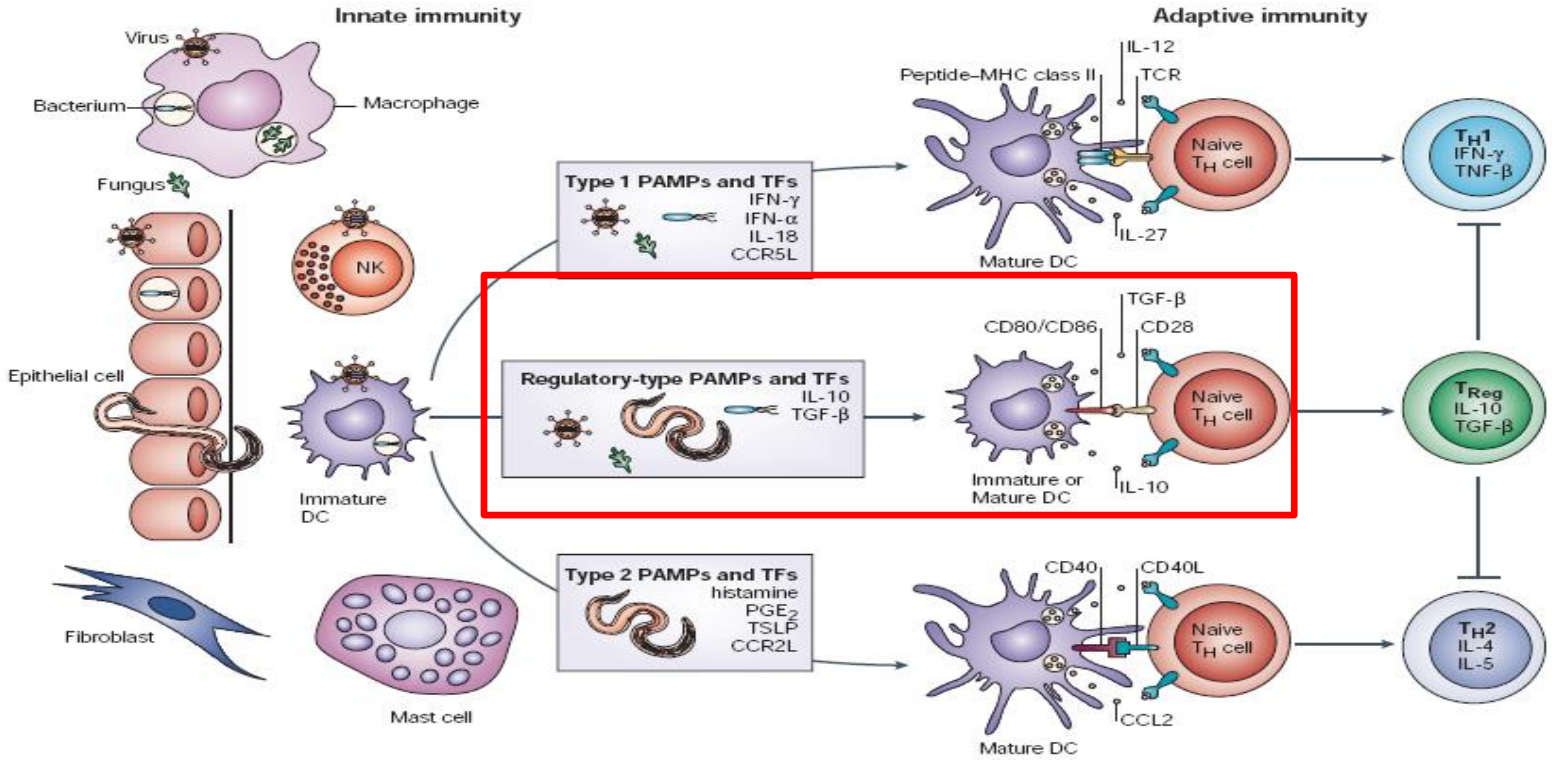
Immune responses to *C. sinensis* crude antigen in Macrophages

PART I

Regulation of anti-inflammatory cytokines IL-10 and TGF-β in mouse dendritic cells through treatment with *Clonorchis sinensis* antigen

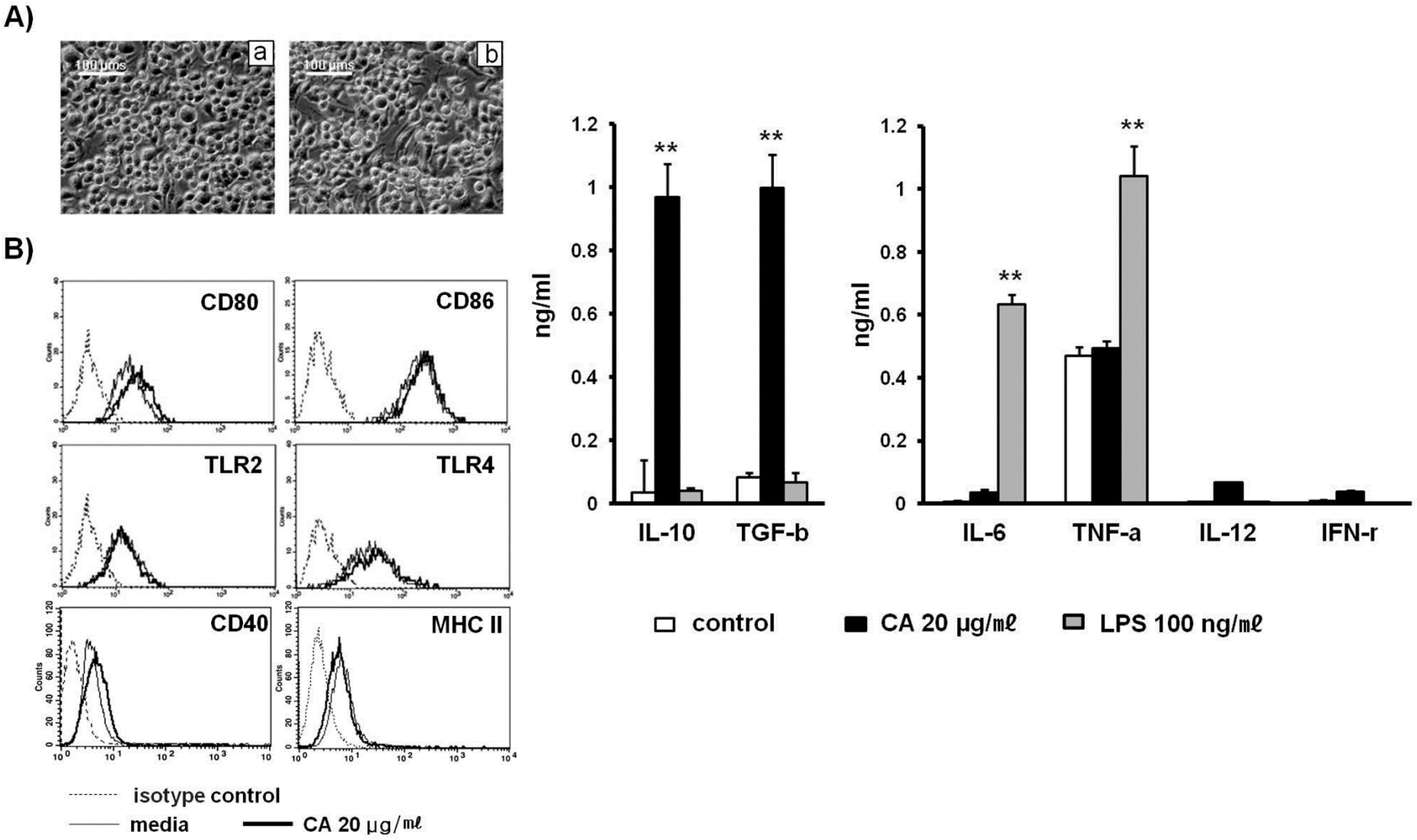
Dendritic cells

- **Dendritic cells(DCs) are the primary professional antigen presenting cells, important in balancing immune response between tolerance induction and immune activation**

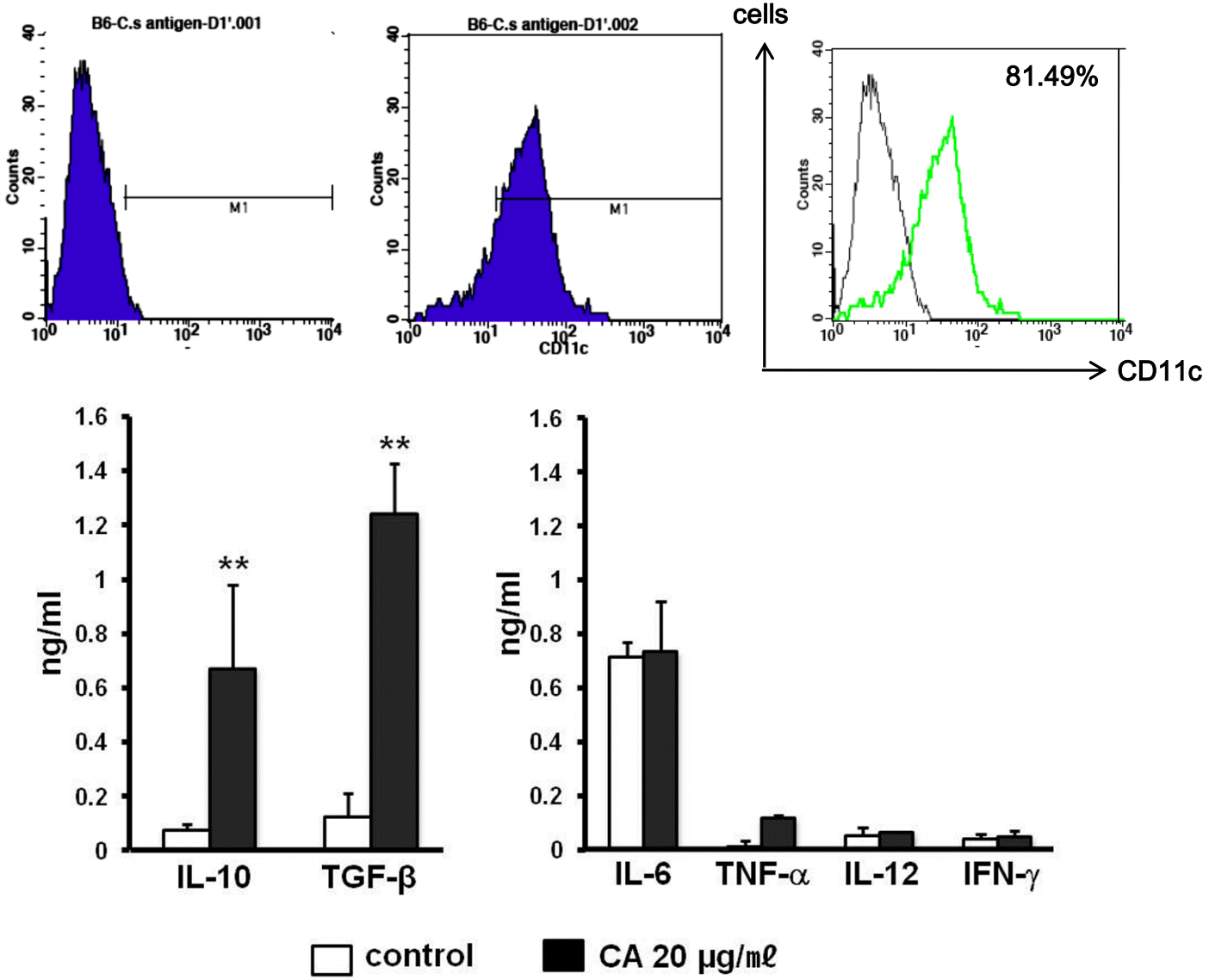


Nat Rev Immunol. 2003 Dec;3(12):984-993

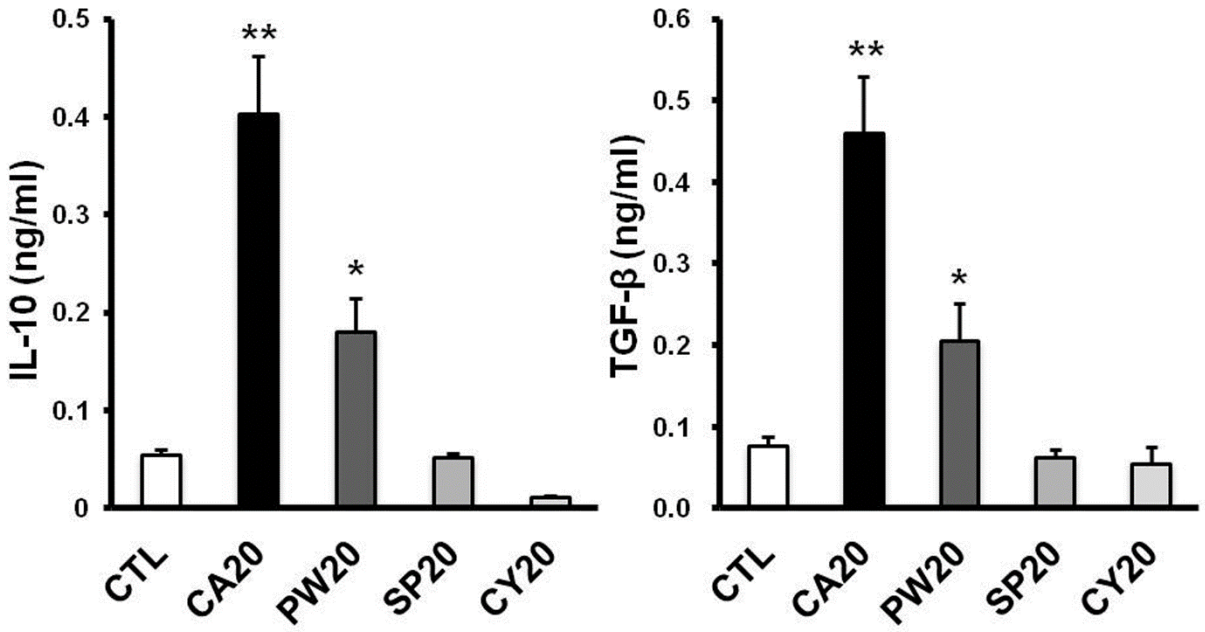
C. sinensis CA regulates cytokine production in DC2.4



Generation of BMDCs and production of cytokines following treatment of BMDCs with *C. sinensis* CA



Regulation of IL-10 and TGF-β expression in response to parasite antigens



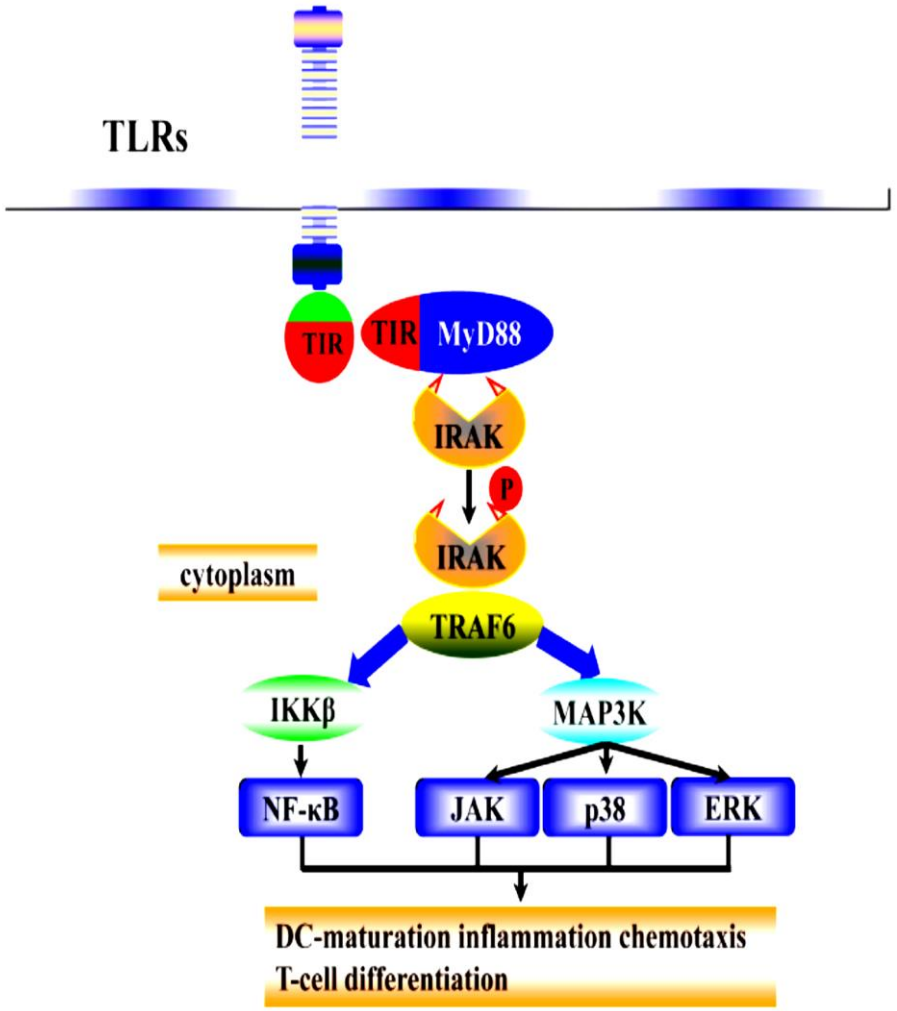
CA- curde antigen of *Clonorchis sinensis*

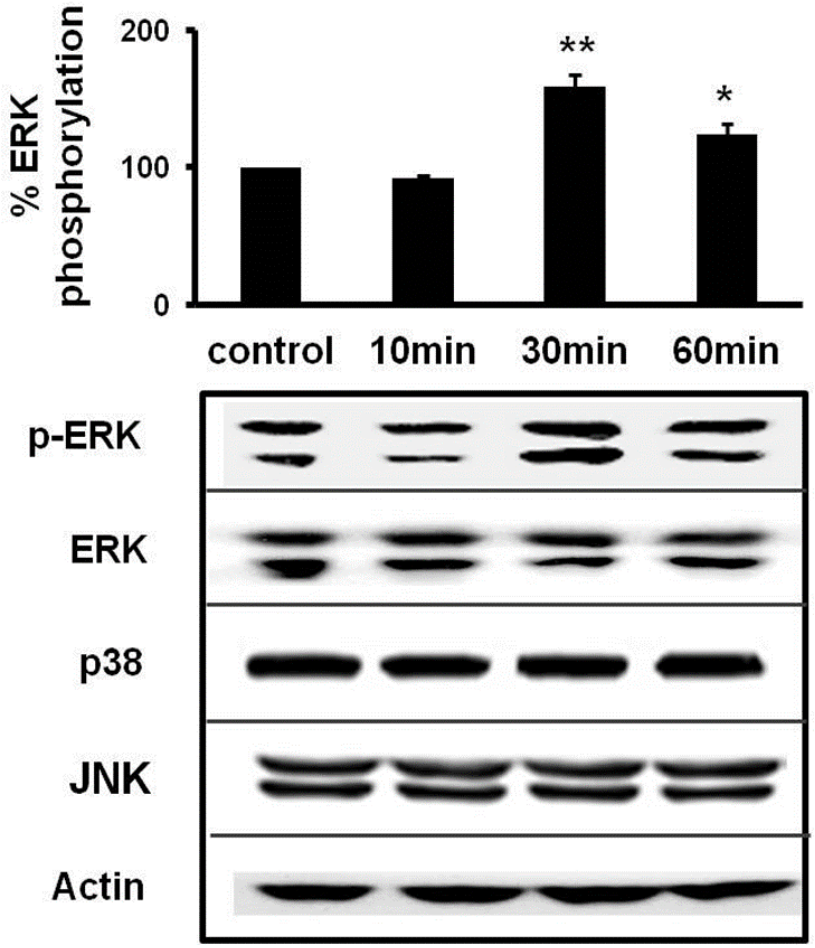
PW- crude antigen from *Paragonimus westermani*

SP- crude antigen of *Spirometra erinacei* plerocercoid

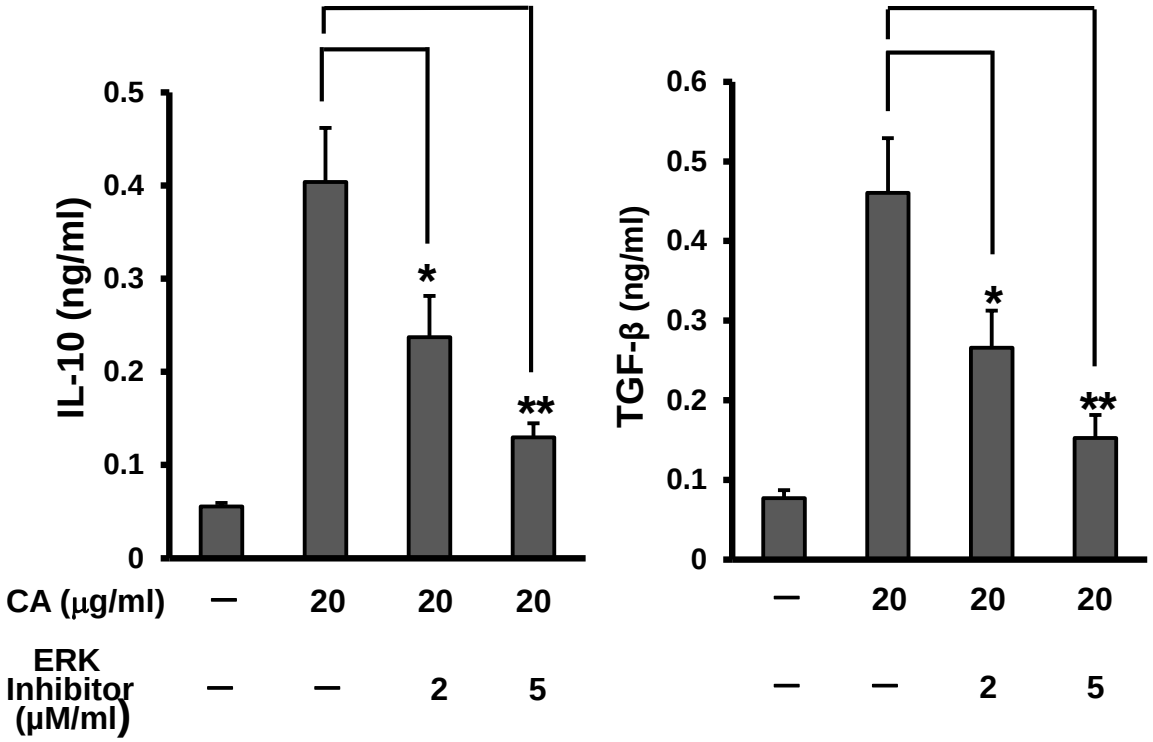
CY- *Echinococcus granulosus* cystic fluid antigen

Activation of ERK in DC2.4 cells upon treatment with *C. sinensis* CA





ERK is essential for upregulation of IL-10 and TGF- β in DC2.4 cells



Summary 1.

1. ***C. sinensis*** **may evade the host's defenses by promoting an anti-inflammatory response in the dendritic cells.**

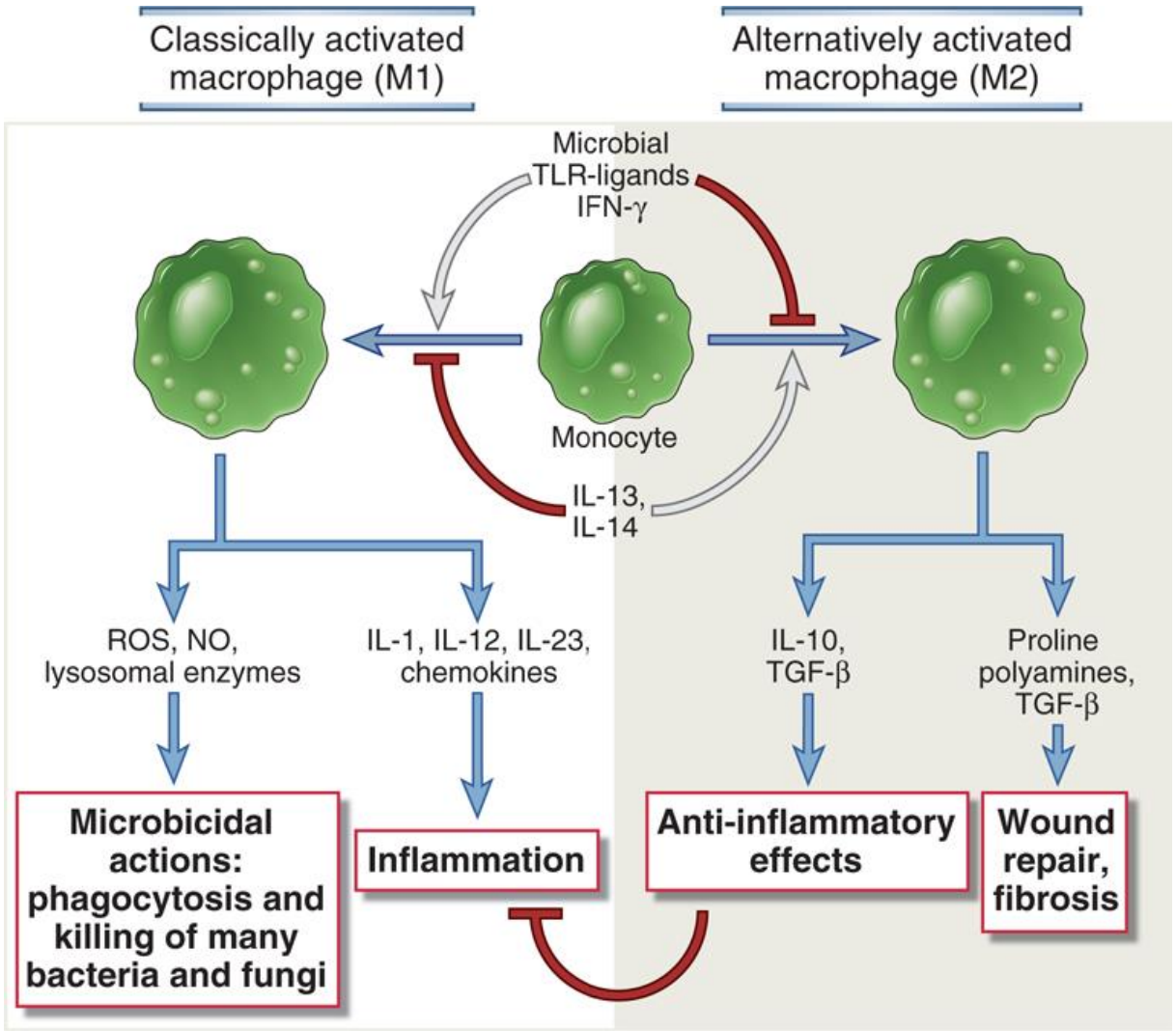
2. **Proteins from *C. sinensis* and *Paragonimus westermani* significantly increased levels of IL-10 and TGF-β in both DC2.4 cells and BMDC while *Spirometra erinacei* and *Echinococcus granulosus* had little effect.**

3. **Proteins derived from *C. sinensis* can activate ERK 1/2 helped to promote the secretion of the anti-inflammatory cytokines from the dendritic cells.**

PART II

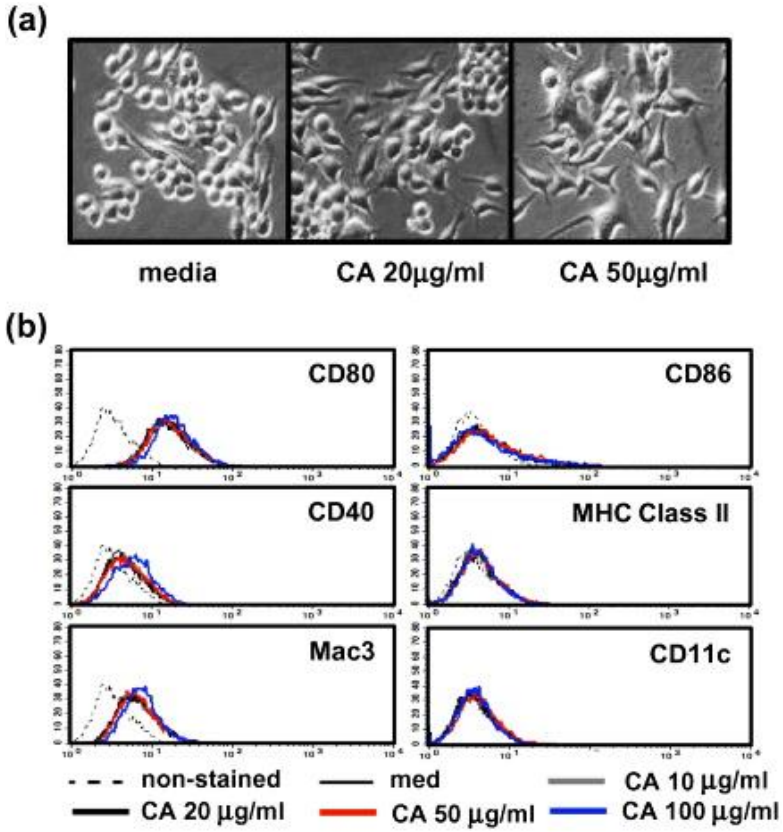
Predominance of IL-10 and TGF-β production from the mouse macrophage cell line, RAW264.7, in response to crude antigens from *Clonorchis sinensis*

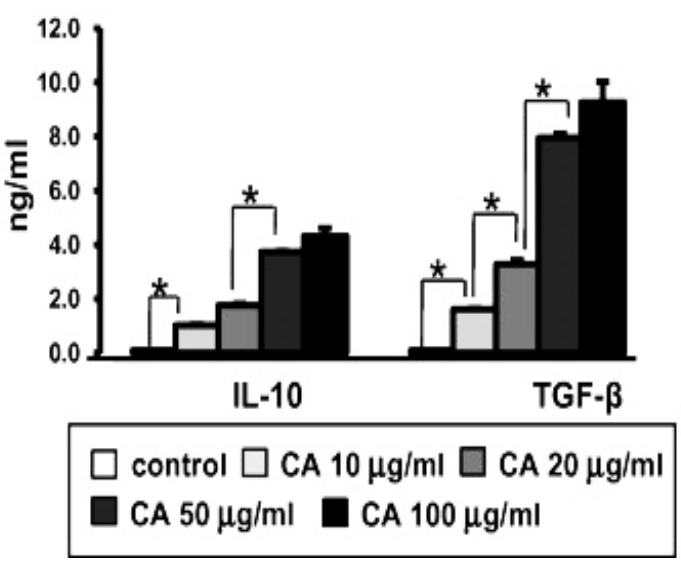
Macrophage



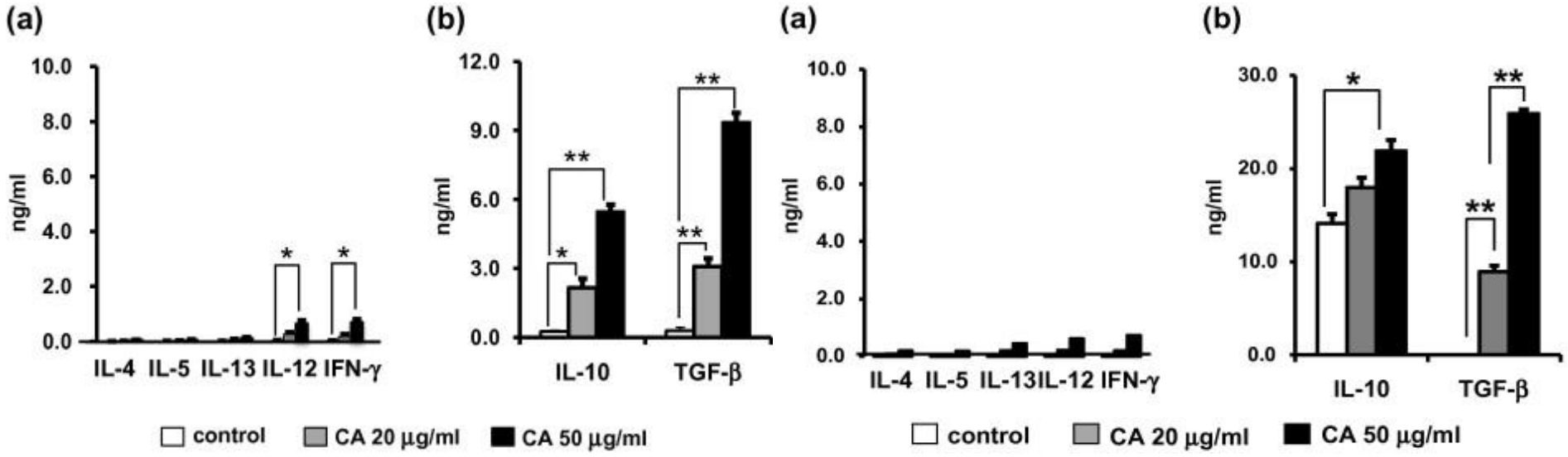
Abbas et al: Cellular and Molecular Immunology, 7e.
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CA induces morphological changes of RAW cells into dendritic -like cells





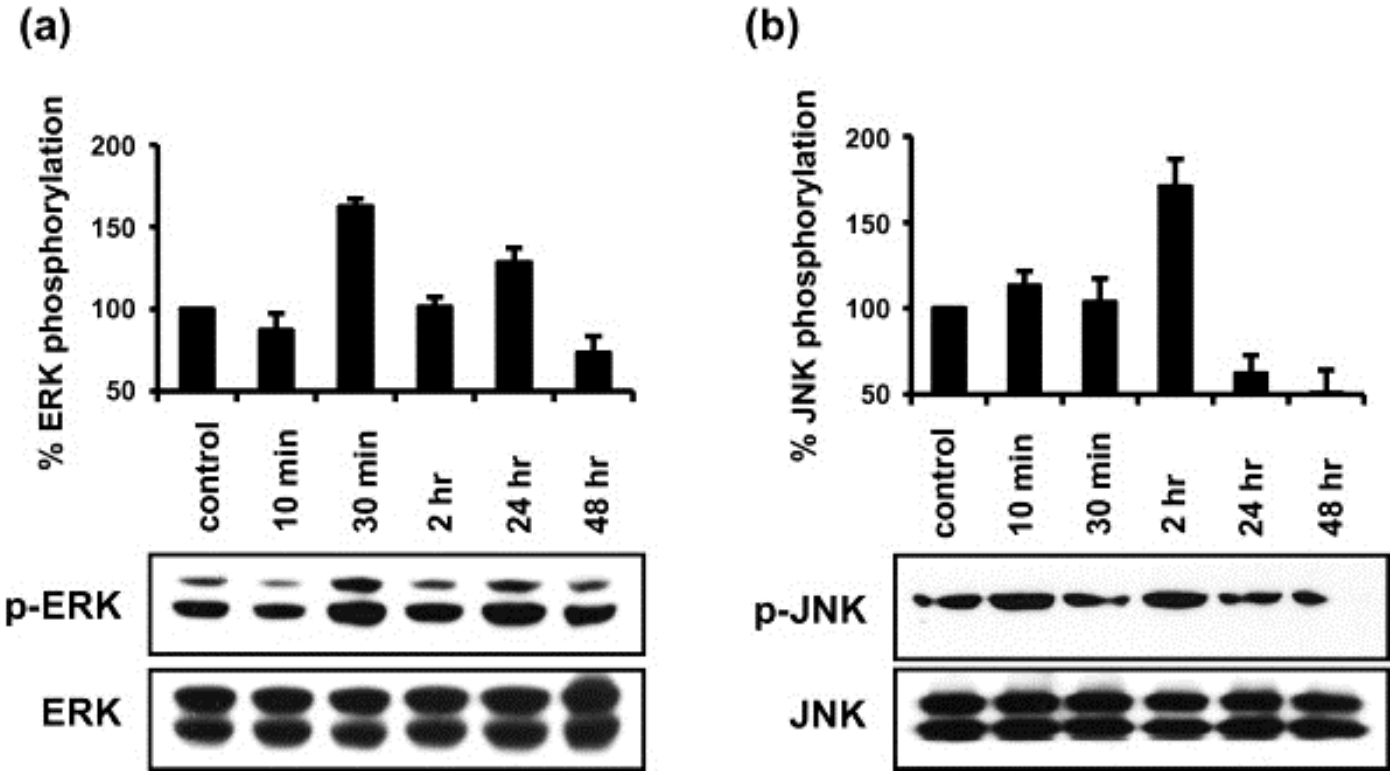
Increase of IL-10 and TGF- β secretion from Raw and BMDMs by CA addition



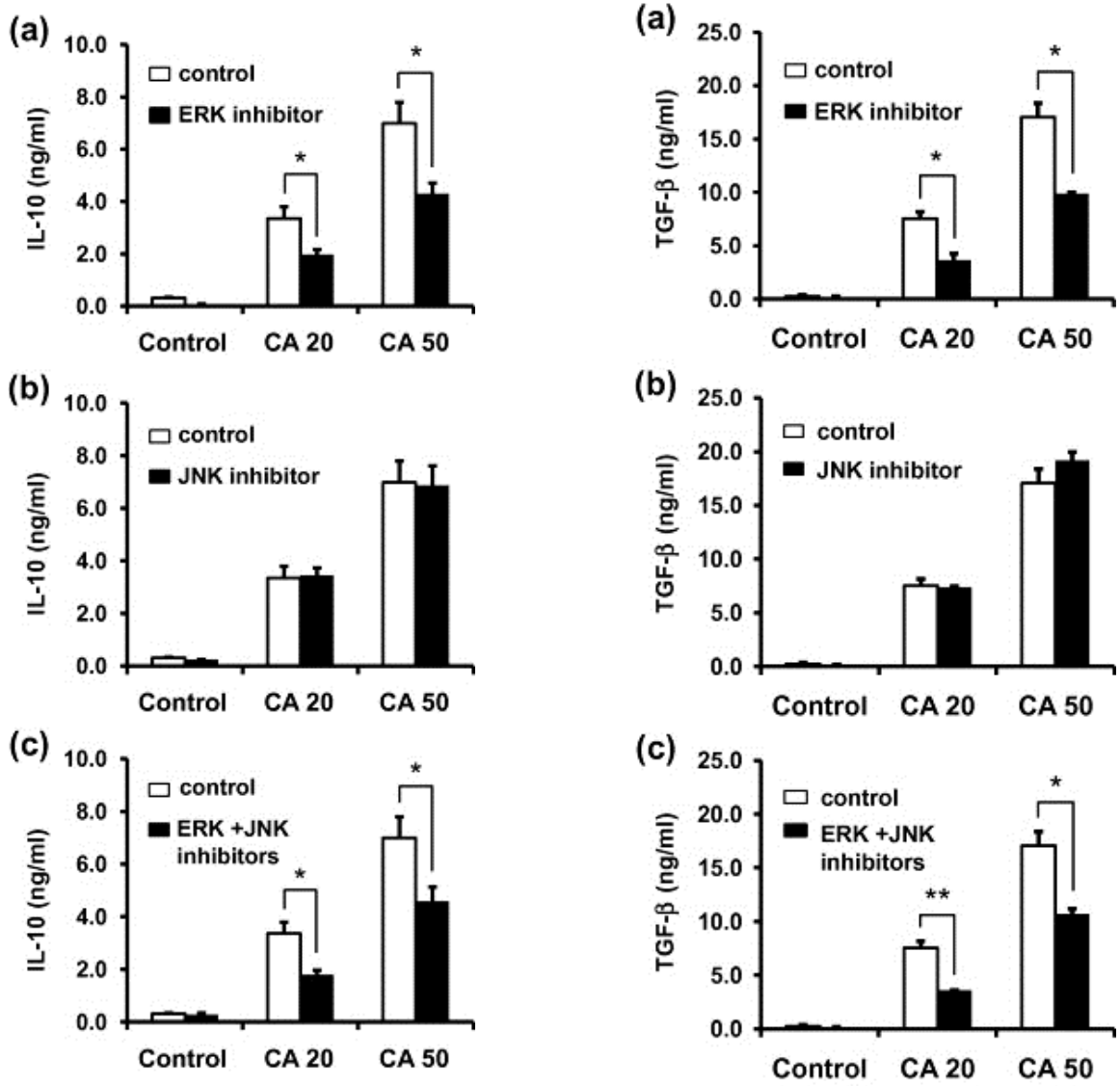
RAW cells

BMDMs

CA exerts its activational effects via ERK and JNK



The ERK signaling cascade is essential for the upregulation of IL-10 and TGF-β



Summary 2.

- ***C. sinensis*** **crude antigen (CA) stimulated differentiation of macrophage RAW cells into dendritic-like cells**
- **CA induced prominent secretion of anti-inflammatory cytokines such as IL-10 and TGF-b**
- ***C. sinensis*** **CA treatment induced ERK and JNK phosphorylation, and unexpectedly, elevated levels of IL-10 and TGF-b were inhibited by the addition of an ERK-specific inhibitor**

Conclusion

C. sinensis **can evade the host defenses by promoting an anti-inflammatory response in the antigen presenting cells via the regulation of ERK.**

Thanks for your attention!