計畫名稱 ： 噬細胞在發炎反應中的角色
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主持人 ： 伍安怡　台大醫學院免疫所
參與人員 ： 王大維 台大醫學院免疫所

1. 中文摘要

巨噬細胞(macrophage)**在** *H.capsulatum* **感染的過程中扮演了一個非常重要的角色。在感染的個體當中，病菌會轉變成以酵母菌**(yeast form)**的型式在巨噬細胞內生長及繁殖。免疫系統正常的個體會產生細胞性免疫反應以及活化巨噬細胞以清除** *H.capsulatum*。**活化後的巨噬細胞會藉著** iNOS (inducible nitric oxide synthase)**產生** NO **而直接毒殺** *H.capsulatum*。**感染早期，未活化的巨噬細胞並無法控制細胞內** *H. capsulatum* **的生長。巨噬細胞可能會因為** *H. capsulatum* **大量的繁殖而死亡。近來發現** DC **可以很有效地將少量的抗原呈現給少量專一性** T **細胞，進而使專一性** T **細胞活化，分化並增加數量。在** *H. capsulatum* **感染過程中，免疫反應的發生是否可以經** DC **直接吞噬** *H. capsulatum* **而引發，或者由吞噬** *H.capsulatum* **而死亡的巨噬細胞能經由**"crossing priming"**的機制，經由** DC **將這些死亡的巨噬細胞中** *H. capsulatum* **的抗原呈現而產生接續的免疫反應。**

2. English Abstract

Dendritic cells (DC) are potent antigen presenting cells activating antigen-specific T cells. Recent reports showed that DC can take up antigens from apoptotic macrophage and DC in turn activate CD8+ T cells for cytotoxic function. Based on our understanding of the interaction between *Histoplasma capsulatum* and macrophage, we studied whether or not DC acquire antigen by ingestion of apoptotic macrophages would also activate CD4+ T cells.

3. Introduction

Histoplasma capsulatum resides primarily in the macrophage of an infected host. The organism replicates uninhibitedly in a normal macrophage. Only after the macrophage is activated to express inducible nitric oxide synthase that it is armed to kill the intracellular pathogen (1, 2). Dendric cell is recognized as a potent antigen-presenting cell (3). DC can effectively ingest antigen and present it to T cells to induce the activation of T cells. How does macrophage, the primary residence of *H. capsulatum*, affect DC antigen presentation functions is an interesting question to be addressed.

A recent report showed that influenza A virus-infected macrophage become apoptotic thus lose the ability to present antigen to T cells (4). However, apoptotic

macrophage primes DC with its antigen in a coculture experiment in which induces CD8 T cell cytotoxic activity, a phenomenon coined as 'cross-priming'. Ingestion of *Salmonella* also found to induce macrophage apoptosis (5). Apoptotic macrophage can induce CTL activity via the activation of DC through cross priming. From these studies it appears that DC taking up antigen from apoptotic macrophages activate CD8+ T cells. It is our interest to study whether DC thus primed can also activate CD4+ T cells. CD4+ T cell activation and production of IFN-γ is essential to the clearance of *H. capsulatum*. The aim of this study is to determine whether or not cross-priming of DC by apoptotic macrophage can induce activation of CD4+ T cells. The results presented in this report describes the progress we made toward this aim.

4. Results and Discussion

a. The source and characterization of dendric cells. The source of DC was mouse bone marrow. Bone marrow cells were cultured in RPMI 1640 conatining 10% supernatants from X-63 cell line which was transfected with genes encoding for GM-CSF. Three days after incubation, non adherent cells were removed and adherent cells were further cultured in the medium containing 10% X-63 supernatant until day 6-9. The nonadherent cells obtained from this culture had short dendrite extensions on the surface resembling the morphological description of dendritic cells. Phenotypic characterization of the cells is currently underway.

b. DC pulsed with heat-killed yeasts of *H. capsulatum* stimulates immune T cells. Immune T cells were obtained from mice at 14 days after infection and were purified by nylon wool column. Nylon wool-purified T cells from normal or immune mice were cultured with heat-killed yeasts of *H. capsulatum* (hk-Hc) or with DC pulsed with hk-Hc. Shown in **Figure 1a**, upon stimulation with DC pulsed with hk-Hc, immune T cells proliferation with stimulation index (SI) of 12.3. Since T cell purification by nylon wool had its limitations, controls such as immune T cells stimulated with hk-Hc alone, normal T cells stimulated with hk-Hc or cocultured with DC and hk-Hc all proliferated with SI>1. Data in **Figure 1b** show that immune T cells stimulated by hk-Hc pulsed DC secreted 21.9 ng/ml of IFN-γ, while cultures with immune T cells and hk-Hc alone also had 11.5 ng/ml of IFN-γ produced. Panning methods to isolate T cells with >95% purity have been worked out. Lowered background SI values and IFN-γ secretion are expected. With lower background stimulation, the antigen-presenting function of DC can be shown more clearly.

c. Immune T cell response to hk-Hc pulsed DC is affected by macrophages with hk-

Hc internalized. To study whether macrophages affect DC antigen presentation functions, we gave hk-Hc to peritoneal macrophages for ingestion at a macrophage:hk-Hc ratio that 80% of macrophages had Hc internalized. These macrophages were added to the DC and T cell co-culture system. Results in **Figure 2** show that addition of macrophages decreased T cell response to DC stimulation. Macrophages not only decreased DC-stimulated T cell proliferation from SI of 5.2 to 1.6 (**Figure 2a**) but also decreased IFN-g production from 15.2 ng/ml to 10.9 ng/ml (**Figure 2b**). It appears that macrophage ingesting hk Hc supressed DC-induced immune T cell responses. We will study whether or not apoptotic macrophage would cross-prime DC to induce T cell activation. Preliminary results showed that replication of viable *H. capsulatum* induced macrophage apoptosis (data not shown). Experiments are currently undertaken to study the effect of apoptotic macrophage on DC antigen-presentation functions.

5. References

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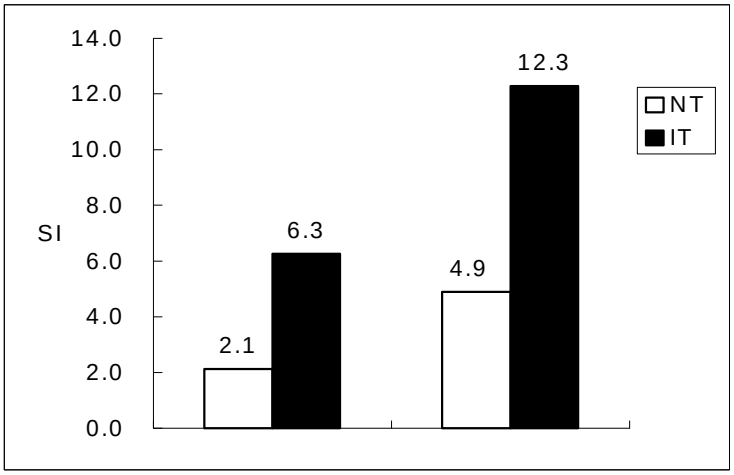
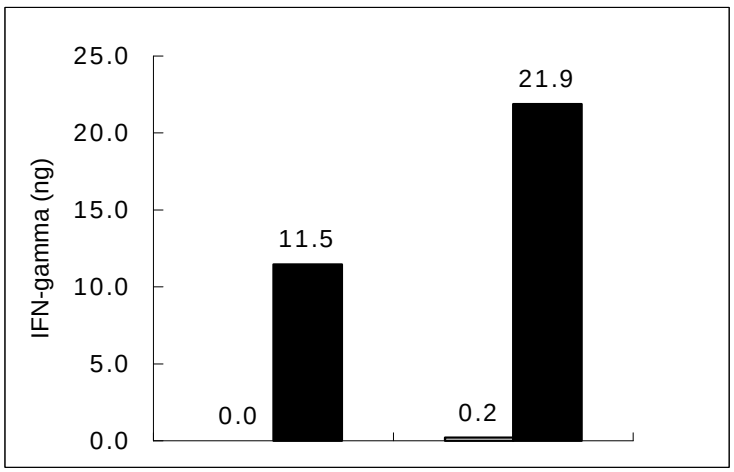
Figure 1 Immune T cells respond to the stimulation by dendritic cells with hk-HC ingested.

(a) Proliferative response



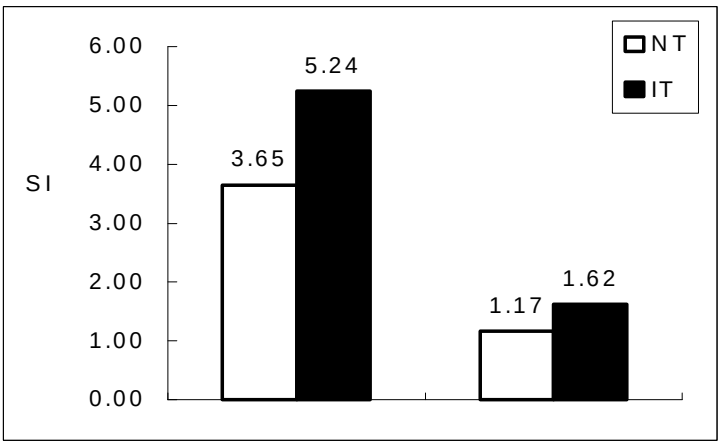
(b) IFN-γ production



T cell	+	+
DC	−	+
hkHC	+	+

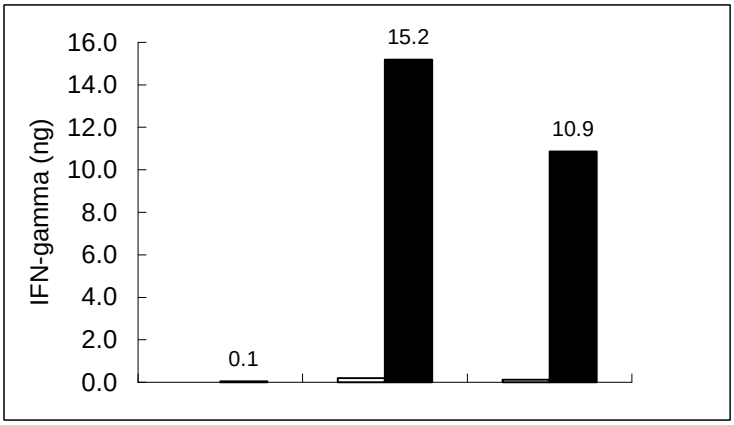
Figure 2 Macrophage with ingested hk-Hc inhibits dendritic cell antigen-presenting function.

(a)Proliferatiive response



T cell	+	+
DC	+	+
hkHC	+	−
PC+hkHC	−	+

(b)IFN-γ production



T cell	+	+	+
DC	+	+	+
hkHC	−	+	−
PC+hkHC	−	−	+