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CD8 T 細胞在對胞內菌保護性免疫反應的角色(2/3)

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中文摘要

許多新的資料顯示”外源性的”抗原可以在抗原呈獻細胞經由 MHC class I 的路徑呈獻給 CD8 T 細胞。因此 CD8 T 細胞在對非病毒胞內寄生菌保護免疫反應的角色有必要再探討。本實驗室的實驗結果顯示，除了 CD4 T 細胞外，CD8 T 細胞也參與對胞內菌 *Histoplasma* 感染產生干擾素γ的反應中。雖然 CD4 T 細胞的反應強度較 CD8 T 細胞強，二種細胞反應之擴張與收縮的動力學則是相同的模式。更進一步的研究結果則顯示，CD8 T 細胞在缺乏 CD4 T 細胞存在的情況下，對於抵抗 *Histoplasma* 感染的保護性免疫反應非常重要。在 MHC class II 基因缺陷小鼠體內去除 CD8 T 細胞，不但會破壞小鼠脾臟內黴菌量的維持使之增加，還會造成小鼠在感染後五週 100% 死亡。另外，除了會分泌干擾素γ，CD8 T 細胞也具有細胞毒殺的功能，能夠毒殺 *Histoplasma* 細胞均質液處理過的骨髓衍生巨噬細胞。本實驗室的結果顯示，CD8 T 細胞利用產生干擾素γ及毒殺功能參與抵抗擴散性 *Histoplasma* 感染的保護性免疫反應。雖然其反應較 CD4 T 細胞反應弱，但當缺乏 CD4 T 細胞時，CD8 T 細胞則是非常重要的。

Abstract

As new evidence showed that exogenous antigens can be presented via MHC class I pathway in professional antigen-presentation cells, the role of CD8 T cells in protective immunity against endosomal pathogen of the macrophages needs to be re-examined. Our data have shown that not only CD4 but also CD8 T cells were actively engaged in IFN γ production in response to intracellular *Histoplasma* infection. Although the magnitude of CD4 T cell response was greater than that of CD8 T cells, the kinetics of the expansion and contraction of both cell types followed the same pattern. Furthermore, CD8 T cells were of critical importance for the protective response against *Histoplasma* infection in the absence of CD4 T cells. Depletion of CD8 T cells in IIKO mice not only disrupted the maintenance of fungal load in spleens, but also caused 100% mortality after 5 weeks of infection. Moreover, as well as IFN γ production, CD8 T cells had cytotoxic activity that could lyse *Histoplasma* lysate-pulsed bone marrow-derived macrophages. Our results demonstrated that CD8 T cells participate in the protective immune response to disseminated infection with *Histoplasma* by IFN γ secretion and cytotoxicity. Although the response is minor as compared with CD4 T cells, CD8 T cells are important in the absence of CD4 T cells.

Results & Discussion

The magnitude and kinetics of specific CD4 and CD8 T cell response.

We have previously demonstrated that CD8 as well as CD4 T cells are actively producing IFN γ in response to *Histoplasma* infection. At the peak of response, 8.4% and 3.9% of total CD4 and CD8 T cells, respectively, are actively engaged in IFN γ production. These IFN γ -producing cell populations can represent the populations of antigen-specific T cells (1-3). Furthermore, we shown here that the percentage and the number of IFN γ -producing cells in both the CD4 and CD8 T cell populations changed during the course of infection **(Fig.1)**. Interestingly, although the magnitude of CD4 T cell response was greater than that of CD8 T cells, the kinetics of the expansion and contraction of both cell types followed the same pattern **(Fig. 1)**. It is worth noting that spleen cells from *Histopalsma*-infected mice made no IFN γ in the absence of antigenic stimulation and that very few T cells from naïve mice stimulated with heat-killed *Histoplasma* produced IFN γ (data not shown). These results of these control experiments indicate that intracytoplasmic IFN γ staining method detects antigenic specific T cell response.

By use of tetramers of MHC class I molecules containing antigenic peptides and functional assays measuring IFN γ production at a single cell level, many studies quantify the antigen-specific T cell response (1-3) Murali-Krishna et al. enumerated virus-specific CD8 T cells in mice infected with LCMV (3). Although the magnitude of response to *Histoplasma* infection was not as impressive as that in the LCMV model, a substantial response was staged by the murine host to fight against histoplasmosis.

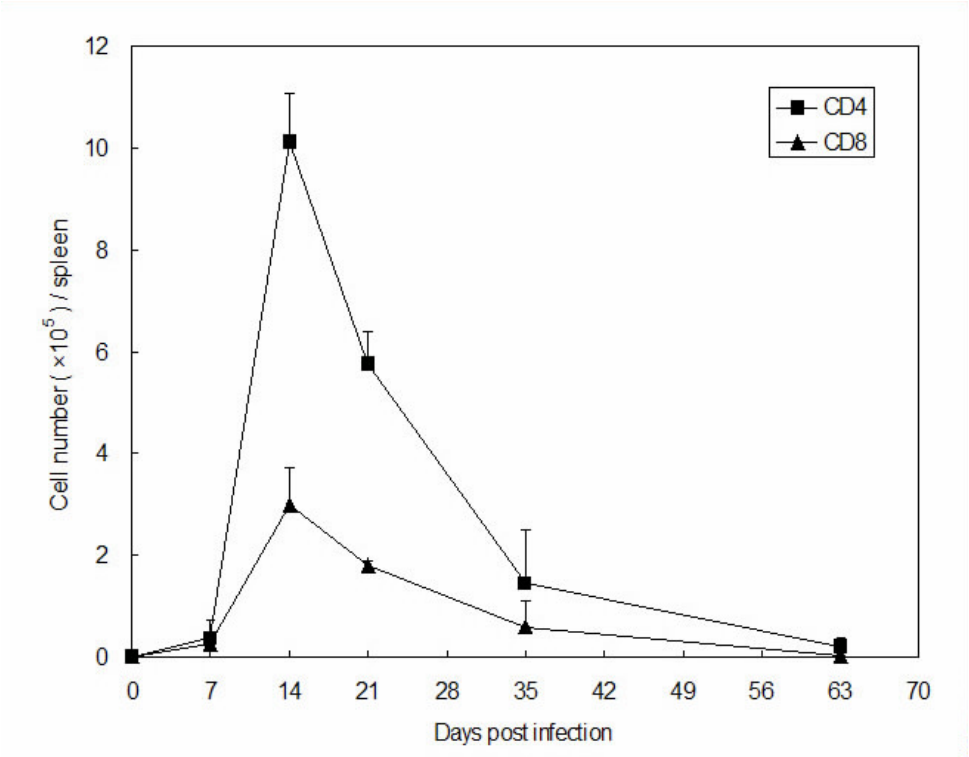

Figure 1. The magnitude and kinetics of antigen-specific functional CD4 and CD8 T cells in histoplasmosis. Spleen cells were harvested from mice at different time points after infection. Cells were stimulated with heat-killed *Histoplasma* yeast cells for 24 h and stained for intracellular IFN γ . The number of IFN γ -producing cells were calculated from the percentage and the total cell number. Four mice were included in each group.

CD8 T cells are critical for not only keeping fungal load in check, but also viability of IIKO mice in the absence of CD4 T cells.

To further investigate the role of CD8 T cells in the protective immune response against histoplasmosis, we used MHC class II-deficient mice (IIKO) to study the response of CD8 T cells in the absence of CD4 T cells. Our previously data shown that, as IFN γ is of critical importance to host defense against *Histoplasma*, mice deficient in IFN γ gene (GKO) were not able to clear the infection and succumbed to

this sublethal dose after 3 weeks. In contrast, although not as efficient as WT B6 mice in getting rid of the fungus, IIKO mice were able to keep the fungal burden at a level comparable to that in the first week after infection up to 16 weeks, developing a chronic histoplasmosis. Since IIKO mice have intact CD8 T cells and are depleted of almost all functionally active CD4 T cells, these data implicate that CD8 T cells contribute to host defense against an intracellular non-viral pathogen of macrophages, yet insufficient to clear the infection.

In order to role out the possibility that the sustain of fungal load in IIKO mice is not directly dependent on CD8 T cells, we depleted mouse CD8 T cells by injecting anti-CD8 mAb clone 2.43 twice a week during the infection. More than 99% CD8 T cells were depleted as determined by flow cytometry (data not shown). **Fig. 2** revealed that the loss of CD8 T cells was associated with an increase number of *Histoplasma* CFU in spleens. Treatment with 2.43 mAb disrupts the maintenance of fungal load in spleens of IIKO mice ($P < 0.05$). The fungus in IIKO mice increased one log when CD8 T cells were absent and kept increasing at day21 after infection ($P < 0.05$). This demonstrates the maintenance of fungal burden in IIKO mice is dependent on CD8 T cells.

Moreover, depletion of CD8 T cells in IIKO mice not only disrupted the maintenance of fungal load in spleens, but also caused 100% mortality after 5 weeks of infection while untreated IIKO mice were 100% survived **(Fig. 3)**. This data further demonstrated that in the absence of CD4 T cells, CD8 T cells are of critical importance for protection and survival in response to *Histoplasma* infection.

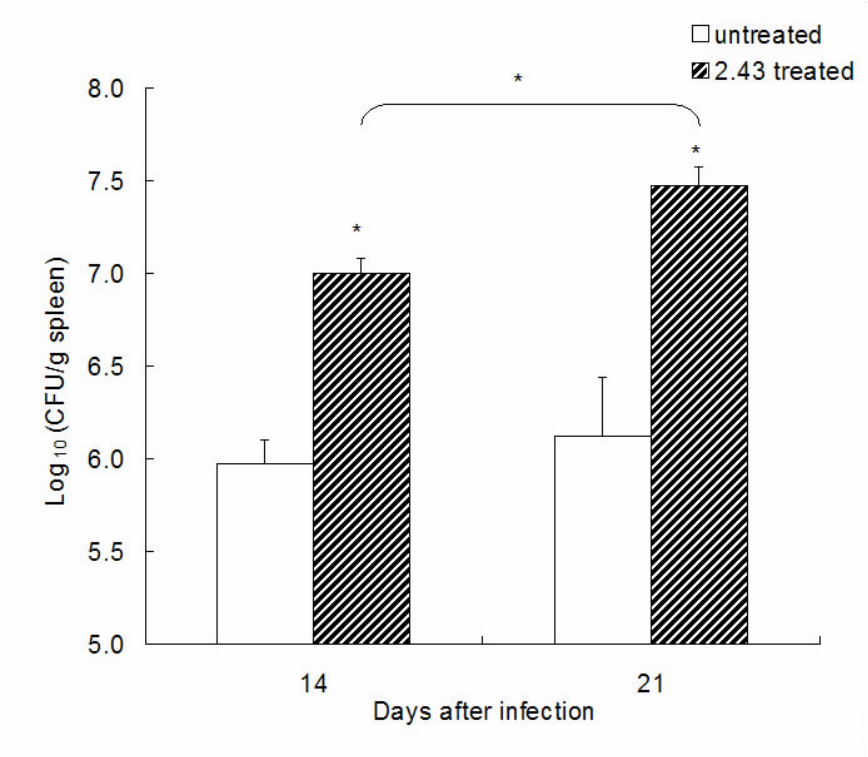

Figure 2. Depletion of CD8 T cells in IIKO mice disrupts the maintenance of fungal load in spleens. IIKO mice injected with 0.4ml concentrated 2.43 hybridoma cell line culture supernatant twice a week from the day of infection. At day14 and day21, spleens were harvest and fungal load was determined by CFU assay. (* $P < 0.05$, n = 3)

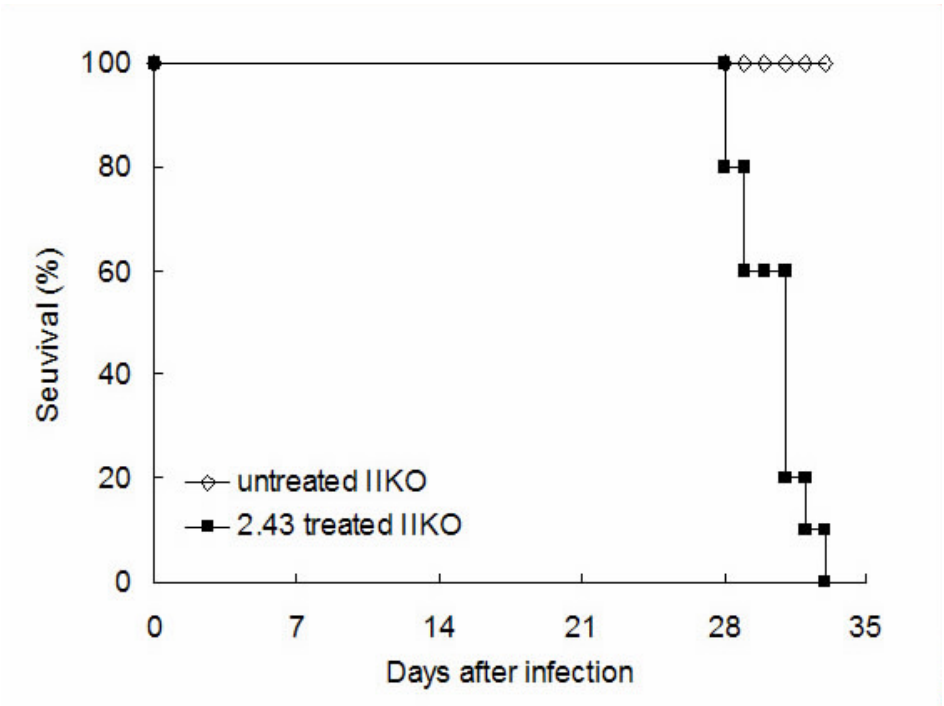

Figure 3. Depletion of CD8 T cells in IIKO mice causes 100% mortality. IIKO mice injected with 0.4ml concentrated 2.43 hybridoma cell line culture supernatant

twice a week from the day of infection. The viability of mice was checked every day (n = 10).

Perforin-deficient mice have higher fungus in spleens compare to WT mice, indicating cytotoxic function may involve in the resistance to Histoplasma infection.

Besides cytokine secretion, CD8 T cells contribute to host defense against infection by lysis of infected target cells. Perforin-deficient mice (PKO) have been used to study the role of cytotoxicity in infections. *Histoplasma*-infected PKO mice showed increase CFU in spleens comparing to WT mice (**Fig. 4**, $P < 0.05$), indicating cytotoxic function may play a role in defense against histoplasmosis. At day14, CFU in PKO mice slight increased to the similar level compared to that of β_2m-knockout mice ($P = 0.43$ when compared with β_2m-knockout mice; $P < 0.05$ when compared with WT mice) , while at day21 fungal clearance in PKO mice was still impaired but better than in β_2m-knockout mice ($P < 0.05$). PKO mice had higher fungal load in spleens suggested that perforin-dependent mechanism is involved in protective immune response to *Hisoplasma*. Comparing β_2m-knockout mice and PKO mice suggested both perforin-dependent and –independent mechanisms are used by CD8 T cells to clear this intracellular pathogen.

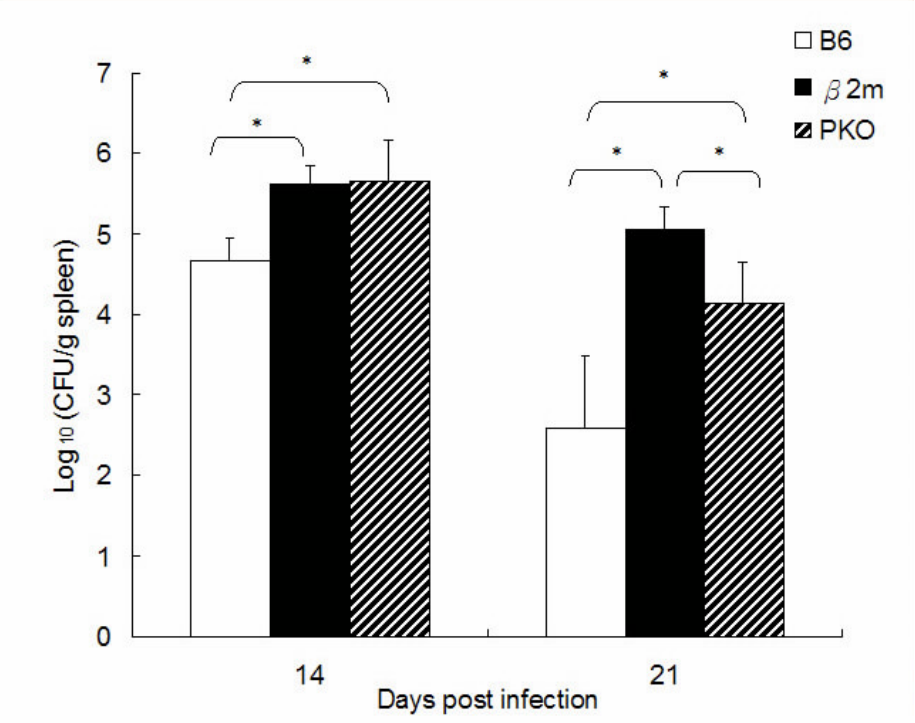

Figure 4. Perforin-deficient mice have higher fungus in spleens compare to WT mice, indicating cytotoxic function may involve in the resistance to *Histoplasma* infection. Perforin-deficient mice (PKO), β_2m-knockout mice (β_2m), and wild-type B6 mice were infected with 2.5×10^4 yeast cells of *Histoplasma*. Spleens were harvested at day14 and day21. Fungal load were determined by CFU assay *(** $P < 0.05$, $n = 3\sim5$).

CD8 T cells of WT B6 mice can perform cytotoxic function against Hc lysate-pulsed bone marrow-derived macrophages.

However, complex factors are involved in the clearance of the fungus and the viability of mice. It has not been clearly demonstrated that CD8 T cells exhibit their specific cytotoxic function against *Histoplasma*-infected targets. Using non-radioactive cytotoxicity assay quantitatively measuring lactate dehydrogenase (LDH), a stable cytosolic enzyme that is realeased upon cell lysis, we demonstrated

CD8 T cells of WT mice specifically lysed *Histoplasma* lysate-pulsed bone marrow-derived macrophages **(Fig. 5)**. Nylon wool enriched T cells **(Fig. 5A)** and MACS purified $CD8^+$ T cells **(Fig. 5B)** could lyse Ag-pulsed macrophages. These data clearly elucidate the participation of CD8 T cell cytolytic activity in resistance to intracellular *Histoplasma* infection. It is also possible that NK cell has a similar direct role in mediating cell killing early in infection. But the cytolysis activity of NK cells would likely be early after infection, thus at day 14 of the highest cytokine response, the role of NK cells may not be considered. Whether the cytotoxic activity of CD8 T cells against *Histoplasma*-pulsed macrophages is perforin-dependent remains to be determined. Moreover, the relationship between perforin expression and IFN γ production of CD8 T cells at a single cell level has not been established. It is of interest to address whether cytotoxic activity mediates immunopathology.

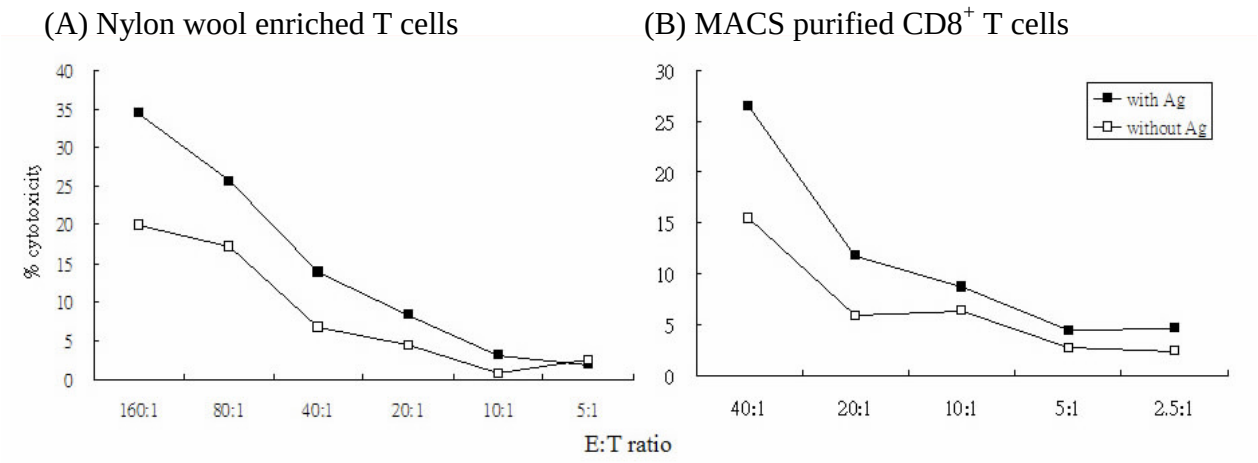

Figure 5. WT CD8 T cells can lyse *Histoplasma* lysate-pulsed bone marrow-derived macrophages. Bone marrow-derived macrophages were plused with 25 ì g/ml Hc lysate for 24 h. Nylon wool enriched T cells (A) and MACS purified $CD8^+$ T cells (B) were added to the culture. After 4h (A) or 5h (B) incubation, culture supernatant were harvested and LDH amount were measured by Non-Radioactive Cytotoxicity Assay. Percent of cytotoxicity was calculated as described in manule.

Reference

1. **Doherty, P. C.** 1998. The numbers game for virus-specific $CD8^+$ T cells. Science **280:** 227.
2. **Doherty, P. C. and J. P. Christensen.** 2000. Accessing complexity: the dynamics of virus-specific T cell responses. Annu. Rev. Immunol. **18:** 561-592.
3. **Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. D. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed.** 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity **8:** 177-187.