行政院國家科學委員會補助專題研究計畫成果報告

CD8 T 細胞在胞內菌保護性免疫反應的角色

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC89－2320－B002－195

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：伍安怡　台大醫學院免疫所

共同主持人：

計畫參與人員：林志萱　台大醫學院免疫所

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□國際合作研究計畫國外研究報告書一份

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中　華　民　國　90　年　10　月　31　日

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1. 中文摘要

許多新的資料顯示“ 外源性的“ 抗原可以在抗原呈現細胞經由 MHC class I 的路徑呈現給 CD8 T 細胞。因此 CD8 T 細胞在對非病毒胞內寄生菌保護性免疫反應的角色有必要再深入探討。在許多病毒感染中，以 HIV 為例，寄主體內之 CD4 T 細胞數目會逐漸減少，因此了解 CD8 T 細胞的功能乃是一個重要課題。本實驗室初步的研究結果顯示，在 MHC class II 缺陷的老鼠被 *Histoplasma capsulatum* 感染後，可以將病菌負荷量維持在一定量而不使其升高，但卻無法清除。本篇研究發現，在 wild type 老鼠受到感染後 CD4 T 細胞和 CD8 T 細胞都能被活化而產生 IFNγ；二種細胞的 kinetics 亦相似。另外也研究產生 IFNγ 細胞 TCR 的使用。

2. English Abstract

As new evidence showed that exogenous antigens can be presented via MHC class I pathway in professional antigen-presenting cells, the role of CD8 T cells in protective immunity against endosomal pathogen of the macrophage needs to be re-examined. In many viral diseases, such as HIV infection, the major complication of the disease is the progressive loss of CD4 T cells. In the face of functionally active CD8 T cells the individual develops AIDS and succumbed to opportunistic infections. It is thus important to understand how CD8 T cells function. Our preliminary results showed that MHC class II-deficient mice were able to contain but not to clear an infection by *Histoplasma capsulatum*, an endosomal pathogen of the macrophage. In this study, we demonstrated that even in the wild type B6 mice, the CD8 T cells were activated to produce IFNγ and the kinetics was similar with the CD4 T cells. The Vβ usage of the IFNγ-producing T cells is also examined.

3. Introduction

It is widely accepted that cellular immune response via the activation of CD4 T cells plays an important role in the protection against intracellular non-viral pathogens of the macrophages. Therefore, much attention has been focused on CD4 T cells and the contribution of CD8 T cells has been neglected. During the past few years, more and more evidence shows that professional antigen-presenting cells can present "exogenous" antigens to activate CD8 T cells. It became clear that not only MHC

class-II- restricted CD4 T cells, but also MHC class I- restricted CD8 T cells are activated during an infection of intracellular non-viral pathogens. Both CD4 and CD8 T cells can contribute to the protective immunity (1).

Histoplasma capsulatum is an intracellular dimorphic fungus. When infecting a host, the fungus resides in the phagolysosome of the macrophage. Upon activation by cytokines released by activated T cells, macrophages acquire the ability to inhibit the growth of the organism. IFN-γ produced by activated T cells is the most important cytokine contributing to protective immunity (2, 3). TNF-α, released by activated macrophages is also important in defense against the pathogen (4). The roles of CD4 and CD8 T cells in immune response against histoplasmosis were examined by use of depleting antibodies, adoptive transfer experiments and β2m knockout mice (5, 6, 7). These studies established that CD4 T cells, the major producers of IFN-γ, are of vital importance to host defense against *H. capsulatum* and the importance of CD8 T cells is almost negligible. However, preliminary data of our work using MHC II-deficient mice indicate that CD8 T cell alone, not sufficient to clear but are competent to contain the infection. These results strongly suggest that the function of CD8 T cells in histoplasmosis needs re-examination.

4. Results and Discussion

(A) CD8 T cells as well as CD4 T cells are active IFN-γ producers. By intracellular cytokine staining methods, we analyzed the number and percentage of IFN-γ producing cells in C57BL/6 mice after *H. capsulatum* infection. Splenocytes harvested from mice at different time points of infection were stimulated with heat-killed *H. capsulatum* for 24 hours and stained with anti- IFN-γ and anti-CD4 or anti-CD8 antibodies. Data in Figure 1 show that CD8 T cells as well as CD4 T cells can produce IFN-g. At the peak of response (14 days after infection), there were 8.4 % and 3.9 % of CD4 T and CD8 T cells, respectively, actively producing IFN-γ (**Figure 1A**). The numbers of both CD4 and CD8 T IFN-γ-producing cells reached the peak at 14 days of infection (**Figure 1B**). The numbers fell to the basal level after 63 days of infection. These data indicate that not only CD4 T cells but also CD8 T cells are involved in the production of IFN-γ, suggesting that CD8 T cells may play an important role in defense against histoplasmosis.

(B) Most IFN-γ producing cells express $CD44^{hi}$ phenotype. To evaluate the phenotypic marker of IFN-g-producing cells, we triple-stained cells with anti-IFN-γ, anti-CD4 or-CD8, and anti-CD25,-CD44, or –1B11 antibodies. It has been demonstrated that 1B11 is upregulated in LCMV-specific $CD8^{+}$ effector T

cells and it has a strong correlation with CTL activity (8). It can also be used to distinguish effector from memory T cells (8). Interestingly, our data in **Figure 2** show that in response to *Histoplasma* infection almost all IFN-γ -producing CD4 and CD8 T cells express $CD44^{hi}$ phenotype. Only a fraction of them express CD25 or 1B11.

(C) Vβ usage by IFN-γ producing cells. To determine Vβ usage by IFN-γ producing cells, we tripled stained splenocytes with anti-CD4 or –CD8, anti-various Vβ, and anti-IFN-γ antibodies. Data in **Figure 3** show that $V\beta6^+$ and $V\beta8.1$ & 8.2^+ CD4 T cells constituted the major IFN-γ- producing cell population. Since the populations of $V\beta8.1^+$ and $V\beta8.2^+$ cells can not be separated by currently available antibody, the proportion of each them contributing to IFN-γ production could not be delineated.

(D) Chronic histoplasmosis is developed in MHC class II-deficient mice. To investigate the role of CD8 T cells in defense against histoplasmosis, we infected mice with MHC class II defects. Interestingly, Data in **Figure 4** show that mice with MHC class II-deficiency sustained a chronic state of histoplasmosis. The animals were not able to clear the infection, yet kept the fungus in check. These data indicate that CD8 T cells contributed to host defense, yet insufficient to clear the fungus.

5. Reference

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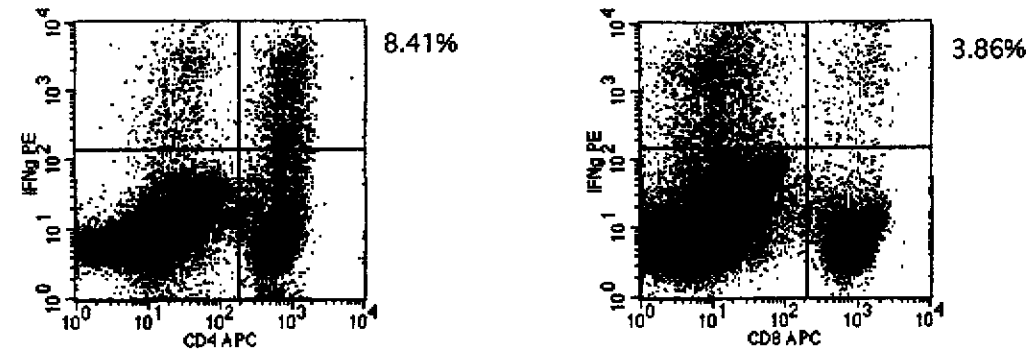
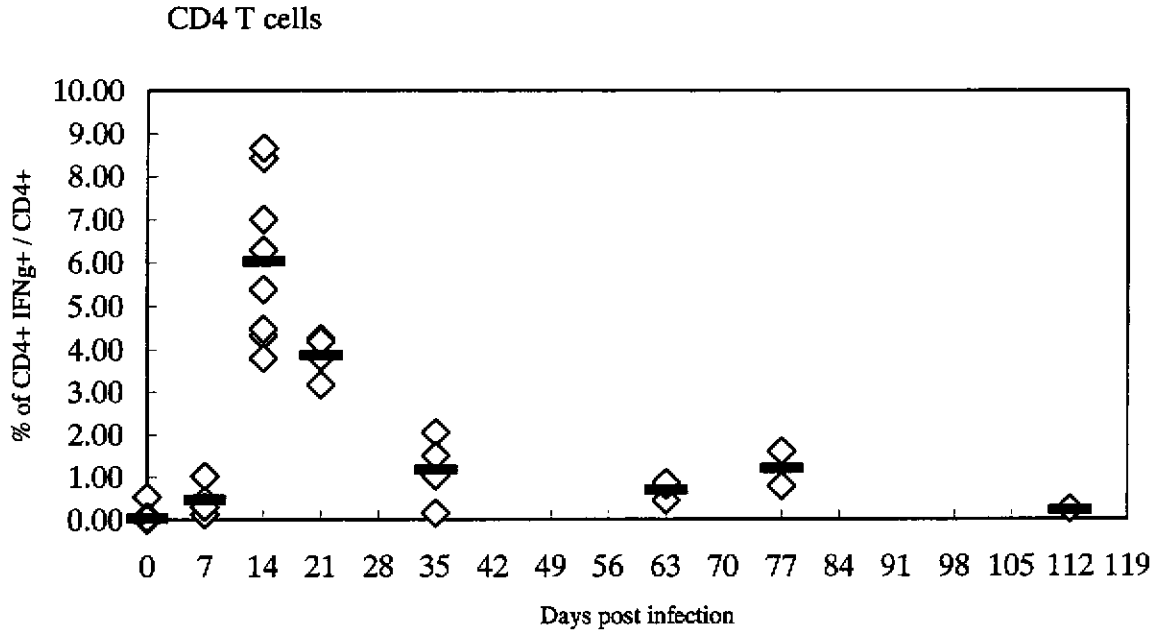
(A)


CD4
CD8
8.41%
3.86%
IFNg PE
CD4 APC
CD8 APC

(B)


CD4 T cells
% of CD4+ IFNg+ / CD4+
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119
Days post infection

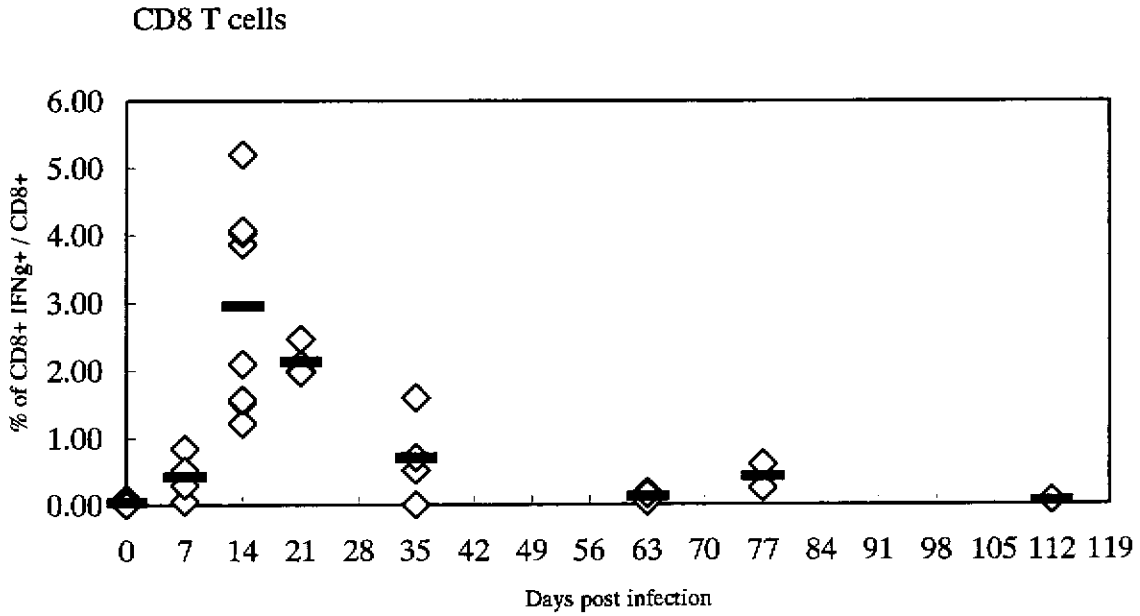

CD8 T cells
% of CD8+ IFNg+ / CD8+
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119
Days post infection

Figure 1. Both CD8 and CD4 T cells produce IFN γ in response to *H. capsulatum* infection. (A) Dotplots show the percentage of $CD4^+$ and $CD8^+$ T cells producing IFN γ at 14 days of infection. (B) The percentage of IFN γ -producing $CD4^+$ and $CD8^+$ T cells in the course of infection.

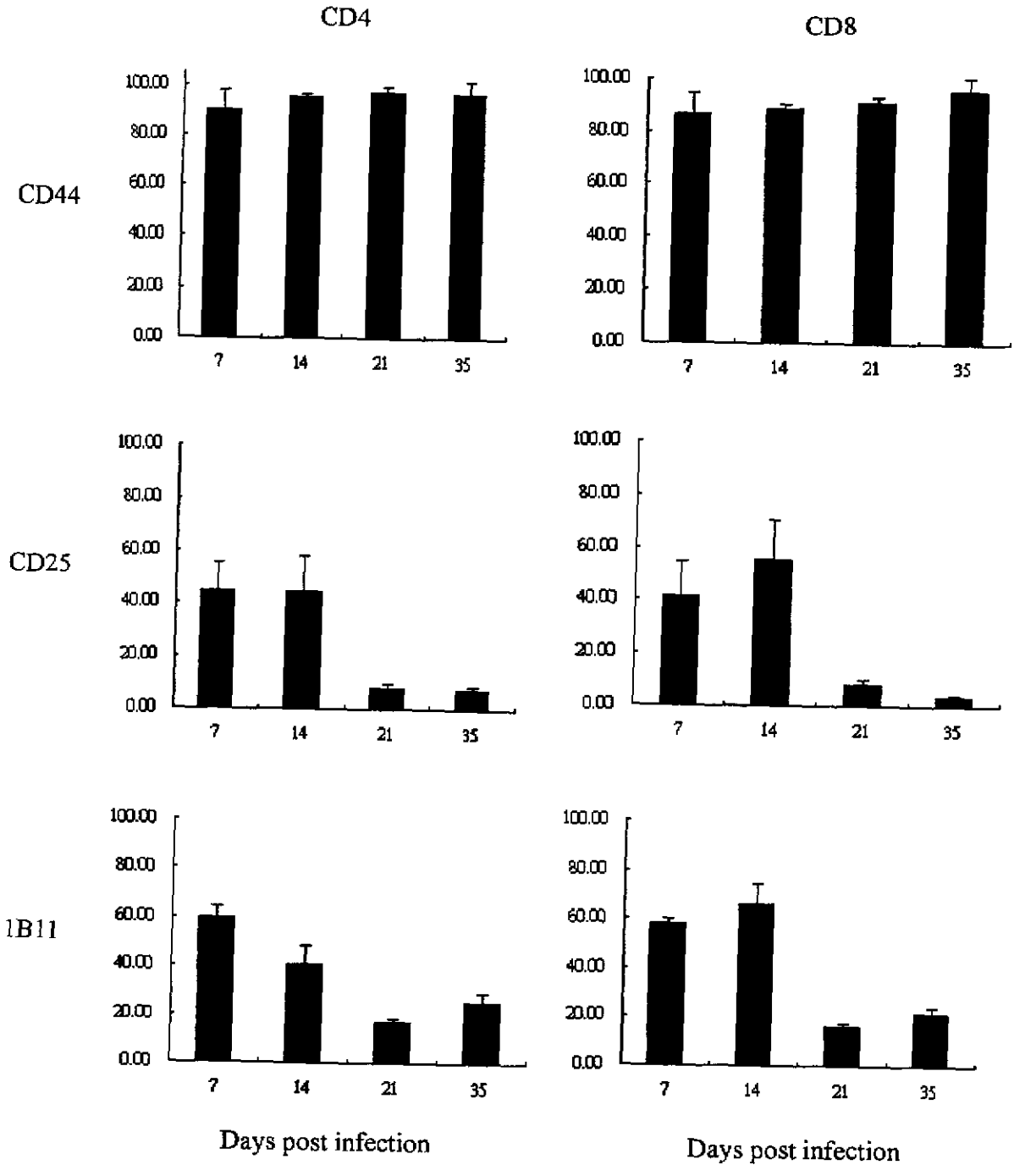


Figure 2. Almost all IFN γ -producing cells express CD44^{hi} phenotype. Splenocytes were stained with anti-CD4/8, anti- IFN γ and anti-CD25/CD44 or clone 1B11 antibodies. The y-axis represents the percentage of triple positive cells in the population of CD4/8- IFN γ double positive cells.

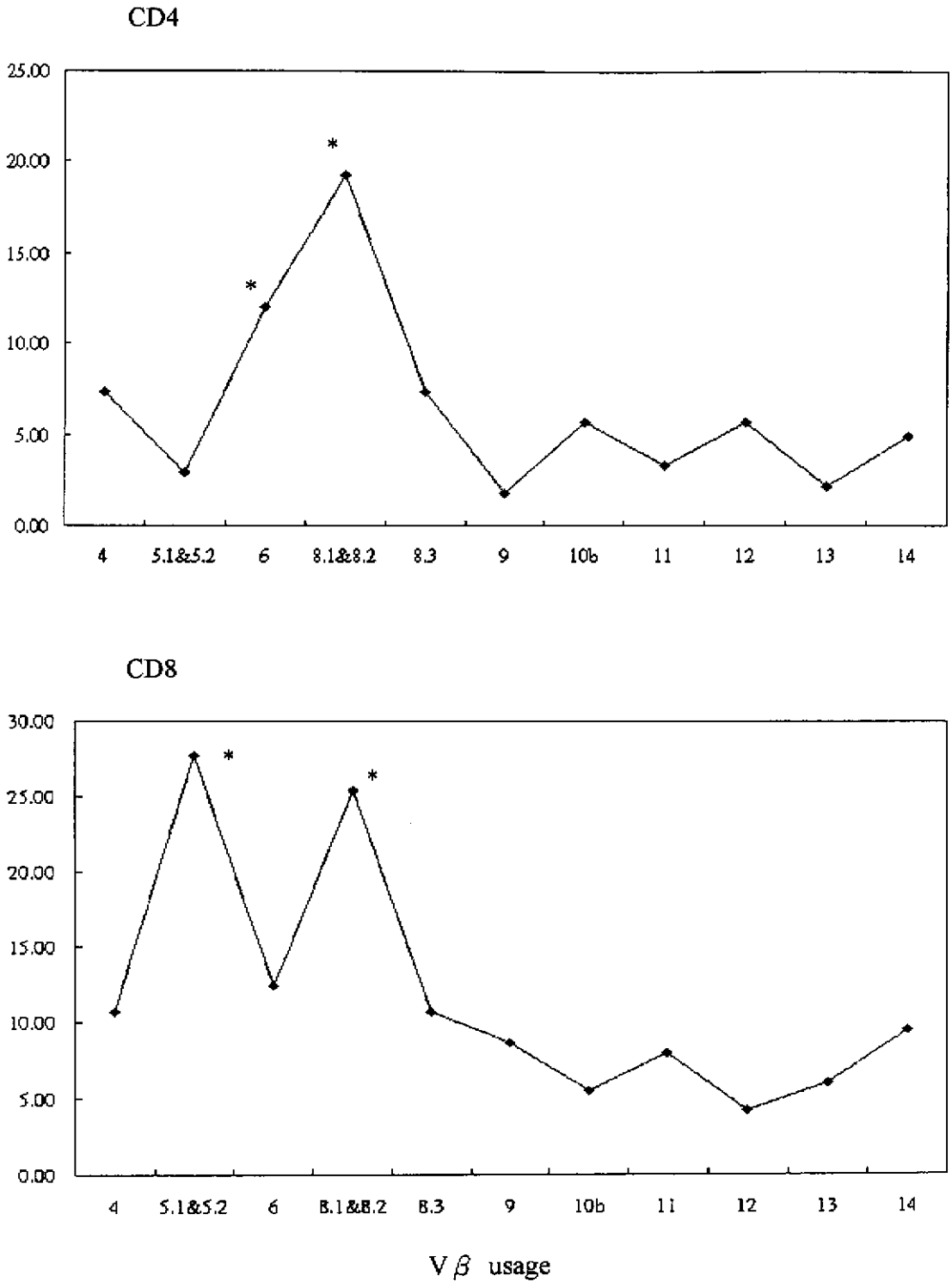


Figure 3. The V β usage of IFN γ -producing cells. The splenocytes were stained with anti-TCR V β, anti- IFN γ and anti-CD4/8 antibodies. The y-axis indicates the percentage of triple positive cells in the CD4+ IFN γ + cell population. Data show here are the means of that from six mice in three independent experiments.

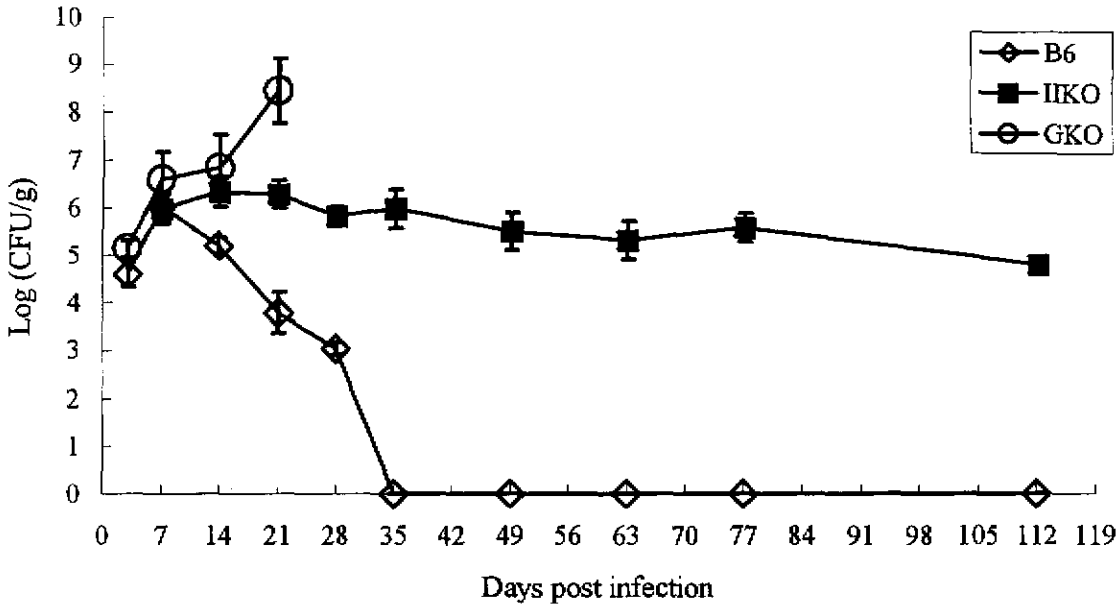


Figure 4. The fungal load in spleens of B6, IIKO and GKO mice.