行政院國家科學委員會補助專題研究計畫成果報告

計劃名稱：第一型及第二型T輔助細胞分化及活化之調控--發炎反應的細胞激素對嗜細胞內病菌所引起之疾病的保護效性

Regulation of the Differentiation and Activation of T helper 1/ T helper 2 cells--The effect of Proinflammatory Cytokines on Protective Immune Responses to an intracellular Pathogen of the Macrophages

計劃類別: ☑個別型計畫 □整合型計畫

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執行期間: 89 年 8 月 1 日 至 90 年 7 月 31 日

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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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1. 中文摘要

影響 Th1/Th2 分化的許多因素中，在微環境中的細胞激素佔有極為重要的角色。本研究題目是研究 Th2 型的細胞激素對於抵抗 *H. capsulatum* 之 Th1 型細胞發展的影響。由於 *H. capsulatum* 為胞內病菌，需有效地產生 Th1 細胞激素如: IFN-γ 干擾素，才能有效的幫助宿主清除此病菌。由此計劃的研究結果顯示，當以 goat anti-mouse IgD 抗血清注射於正常小鼠，在脾臟中的 Th2 型細胞激素如: IL-4, IL-10 介白素 mRNA 均會大量增加，且使得小鼠對於清除 *H. capsulatum* 的能力大為降低，呈現出延緩現象。在接受 anti-IgD 抗血清注射並感染的小鼠體內，其脾臟中所含產生 IFN-γ干擾素的 CD4 幫助型 T 細胞及 CD8 毒殺型 T 細胞數目減低，同時在胞內之 IFN-γ干擾素的量亦減少。有趣的是，當 IL-4 介白素基因缺陷小鼠同樣接受 anti-IgD 抗血清注射後並以 *H. capsulatum* 感染，小鼠對於清除 *H. capsulatum* 的能力恢復正常，但是其脾臟中所含產生 IFN-γ干擾素的 CD4 幫助型 T 細胞及 CD8 毒殺型 T 細胞數目雖仍降低，然而在 CD8 毒殺型 T 細胞胞內之 IFN-γ干擾素的量卻較不受影響。由體外細胞培養重覆以胞內菌抗原刺激實驗亦顯示，IL-4 介白素基因缺陷小鼠其脾臟細胞對胞內菌抗原產生 IFN-γ干擾素的總量，並不會因接受 anti-IgD 抗血清注射而被抑制。因此，本研究結果假設 anti-IgD 抗血清可能藉由 IL-4 介白素來抑制 CD8 毒殺型 T 細胞產生 IFN-γ干擾素的能力，進而影響宿主對胞內病菌的保護效性。

2. English Abstract

It has been established that goat anti-mouse IgD antiserum induces IL-4 and IL-10 mRNA expression and IgE production. In this study, we employed anti-mouse IgD antiserum to investigate the influence of T2 immune response on the susceptibility to histoplasmosis.

Histoplasma capsulatum, a dimorphic fungus, is an intracellular pathogen of the macrophage. Macrophage activated by IFN-γ is armed to control the growth of the intracellular fungus. Mice given two intraperitoneal injections of anti-IgD antiserum at days -6 and -1 were infected with *H. capsulatum*. At day 14 after infection, pretreated mice had about 50~70-fold greater fungal burden in their spleens and lungs as compared their untreated counterparts. The numbers of IFN-γ- producing $CD4^+$ and $CD8^+$ T cells were reduced in pretreated mice to about half of what was in the untreated control. Total amounts of IFN-γ in culture supernatants were also reduced in the pretreated mice as compared to their untreated counterparts. To examine whether IL-4 or IL-10 was responsible for anti-IgD antiserum-induced effect, we repeated the experiments in mice with either IL-4 or IL-10 gene defect. Similar to their normal

counterparts, mice with IL-10 gene deficiency given anti-IgD treatment exhibited enhanced susceptibility to histoplasmosis. Their IFN-γ response to *H. capsulatum* infection was also reduced after the treatment. In contrast, treating IL-4 deficient mice with anti-IgD antiserum did not enhance their susceptibility to histoplasmosis. The treatment also reduces the number of $CD4^+$ or $CD8^+$ IFN-γ-producing T cells in $IL\text{-}4^{-/-}$ mice similar with wild-type mice. However, the mean fluorescence intensity of a single $CD8^+$ rather than $CD4^+$ T cells did not significantly reduced. Nor did it significantly reduce the levels of IFN-γ produced by spleen cells in culture. In conclusion, our data suggested that IL-4 induced by anti-IgD treatment enhanced the susceptibility to histoplasmosis by reducing the intensity of IFN-γ producing $CD8^+$ T cells and the amounts of IFN-γ produced.

3. Introduction

Histoplasma capsulatum is a dimorphic fungus. Yeast cell of *H. capsulatum* is facultative intracellular pathogen of the macrophage. Murine macrophages from the peritoneal cavity or those from the spleen are activated by IFN-γ or IFN-γ plus LPS respectively to inhibit the replication of the fungus (1, 2).We and others have shown that the ability of animals to mount a T1 type cytokine response is vital clearance of the fungus (3, 4). IL-4 or IL-10 gene defect does not affect the ability of the host to clear histoplasmosis (unpublished data).

A recent report documented the results of an extensive epidemiological survey conducted in Japanese school children (5). The results showed a strong inverse association between positive tuberculin response and atopic characteristics in school children. Positive tuberculin-responders had significantly lower levels of type 2 cytokines (IL-4, IL-10, and IL-13) and higher level of the type 1 cytokine IFN-γ in the sera. IFN-γ mediated suppression of T2 responses in the lung has also been documented (6, 7, 8). With the use of experimental animals (9) showed that intranasal infection with *Mycobacterium bovis* BCG which induced IFN-γ production in the lungs suppressed the development of type 2 cytokine responses in mice after airway challenge with OVA. The inhibition did not extend to the systemic Ig response against OVA but was confined to the lungs. These reports strongly evidenced that a strong T1 response inhibits the development of T2 response in the microenvironment of the lungs. However, there has been no report on using disease model to study the effect of T2 cytokines on the development of T1 type cytokine response.

In this study, we employed goat anti-mouse IgD antiserum to induce T2 cytokines. By using goat anti-mouse antiserum, in an animal model of histoplasmosis, we examined how T2 cytokines regulate a host defense mechanism that is dependent on a T1 response.

4. Results and Discussion

a. T2-type cytokines are preferentially induced by anti-IgD antiserum treatment. We tested goat anti-mouse IgD antiserum obtained from Dr. Fred Finkleman (University of Cincinnati) for its ability to induce T2-type cytokines. C57BL/6 mice were given one intraperitoneal injection of anti-IgD antiserum six days before their spleen cells were harvested. Data in **Figure 1A** show that anti-IgD antiserum treatment upregulated the expression of IL-4 and IL-10 mRNA by 20- and 12.8-fold, respectively, as compared to untreated control mice. With two

injections of anti-IgD antiserum, 6 days apart, the expressions of IL-4 and IL-10 were sustained for up to 12 days after the first injection. In addition, IL-4-producing cells increased by 1-3% in the spleen as determined by intracellular cytokine staining and flow cytometric analysis (**Figure 1B**). These data indicate that T2 type cytokines are indeed induced by anti-IgD antiserum treatment.

b. Anti-IgD treatment retards fungal clearance. To investigate the influence of T2 cytokine microenvironment on host defense, mice were infection with *Histoplasma capsulatum* intravenously after two doses of anti-IgD antiersum treatment. Results in Figure 2 show that mice treated with anti-IgD antiserum had 52-fold and 68-fold higher fungal burden in their spleens (**Figure 2A**) and lungs (**Figure 2B**), respectively, than their untreated counterparts at 14 days after infection. It is thus apparent that anti-fungal immune response is suppressed by anti-IgD antiserum.

c. IFN-γ is reduced in mice treated with anti-IgD antiserum. In previous studies, we have shown that murine macrophages from the peritoneal cavity or those from the spleen are activated by IFN-γ or IFN-γ plus LPS, respectively, to inhibit the replication of the fungus (1, 2). Besides, it is of vital importance to mount a T1 type cytokine response to clear the fungus (3, 4). We examined whether anti-IgD antiserum induced retardation in fungal clearance was related to reduction in IFN-γ production. Data in **Figure 3** show that splenocytes from anti-IgD antiserum treated mice produced significantly lower levels of IFN-γ than that without anti-IgD treatment at days 7 and 14 after infection. Moreover, the number of IFN-γ -producing cells in the spleen of anti-IgD treated mice ($2.94\pm1.92 \times 10^5$) was much lower than that in mice without treatment ($1.03\pm0.49 \times 10^6$) (**Figure 4A**). Interestingly, as assessed by the fluorescence intensity, IFN-γ production by each cell in the anti-IgD antiserum treated mice was also lower than that in mice without treatment (**Figure 4B**). These data together indicate that anti-IgD treatment affected IFN-γ production both by inhibiting the generation of IFN-γ producing cells and by reducing the IFN-γ produced by each cell.

d. The effect of anti-IgD treatment on fungal clearance in wild-type mice is null in IL-$4^{-/-}$ mice but not in IL-$10^{-/-}$ mice. We used IL-$4^{-/-}$ and IL-$10^{-/-}$ mice to investigate whether the effect of anti-IgD antiserum on fungal clearance was mediated by either IL-4 or IL-10. Data in **Figure 5A** and **5B** show that anti-IgD treatment did not increase fungal burden in the spleen or lungs of IL-$4^{-/-}$ mice. However, the effect of anti-IgD antiserum treatment on fungal burden of IL-$10^{-/-}$ was similar to that in wild-type mice. These data indicate that the effect of anti-IgD treatment on fungal clearance is mediated by IL-4 but not by IL-10.

e. The effect of anti-IgD antiserum treatment on IFN-γ production is null in IL-$4^{-/-}$ mice. In line with the finding with fungal clearance, at days 7 and 14 after infection, spleen cells from anti-IgD antiserum treated IL-$4^{-/-}$ mice produced same levels of IFN-γ as those without treatment (**Figure 6**). These results indicate the anti-IgD antiserum - induced IL-4 negatively regulates IFN-γ production. To investigate how IL-4 negatively regulates IFN-γ production, we sought to determine whether IL-4 reduces the number of IFN-γ producing cells.

f. The number of IFN-γ producing T cells in IL-$4^{-/-}$ mice is no different from that in wild-type mice after anti-IgD antiserum treatment. **Figure 7A** and **7B** show that treatment with anti-IgD antiserum reduced the number of IFN-γ producing CD4 and CD8 T cells in wild-typed mice as well as in IL-4-deficient mice, indicating that IL-4-mediated reduction of IFN-γ production after anti-IgD treatment was not caused by reduction of the number of IFN-γ producing cells.

g. IFN-γ produced by CD8 T but not CD4 T cell is affected by IL-4. We took a step further to study the level of IFN-γ production by CD4 as well as CD8 T cells in IL-$4^{-/-}$ mice. In **Figure 8** we showed the mean fluorescence intensity of each cell population after intracytoplamic staining with anti-IFN-γ antibody. The level of IFN-γ produced by each CD4 T cell in anti-IgD treated mice was significantly lower than that by CD4 T cells in mice with infection alone without treatment. However, the level of IFN-γ produced by CD8 T cells in IL-$4^{-/-}$ mice was not significantly different from mice with infection alone without treatment. Thus, it appears that IL-4 production induced by anti-IgD treatment reduces the level of IFN-γ produced by each CD8 T cell and results in the reduction of the level of total IFN-γ produced.

5. References

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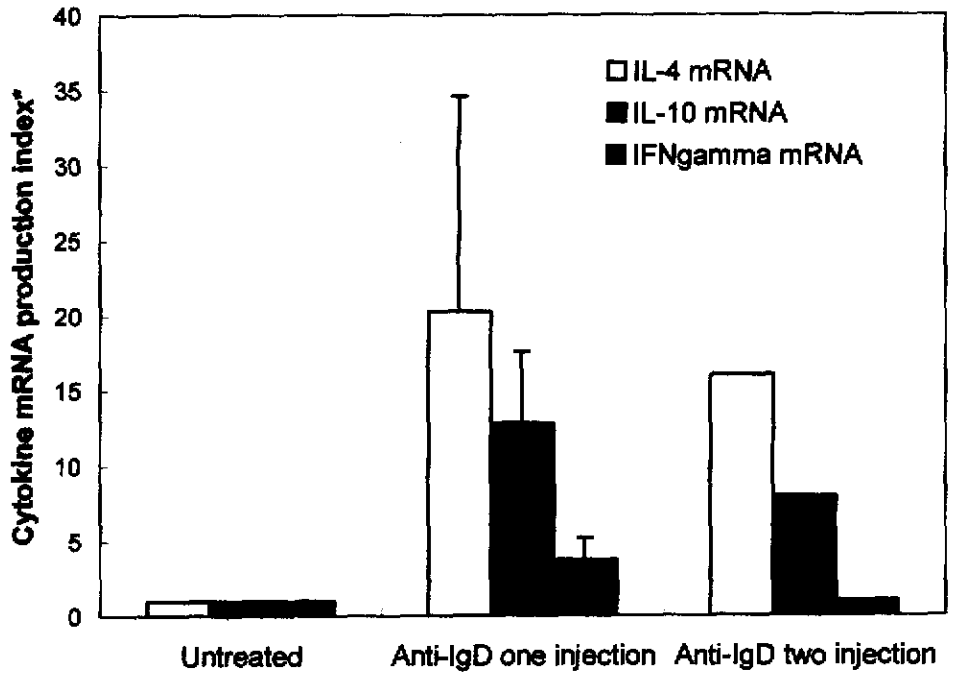
Figure 1. T2-type cytokines are preferentially induced by anti-IgD antiserum treatment.

(a)



(b)

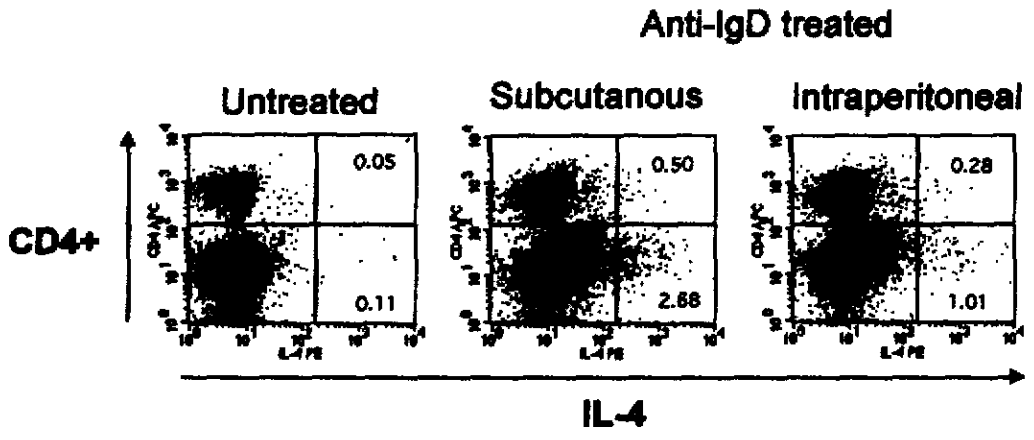
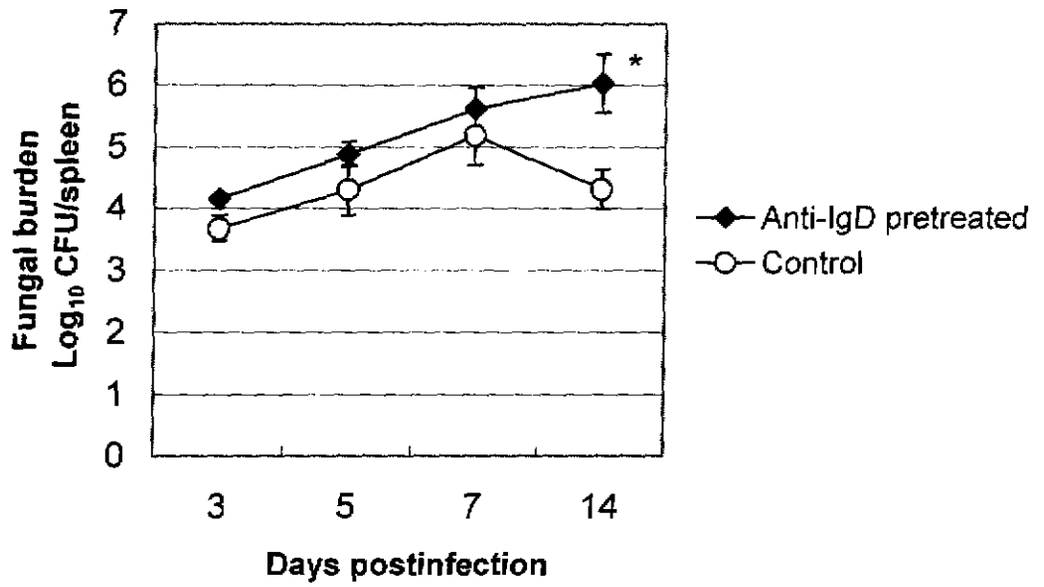


Figure 2. Fungal clearance is retarded in mice treated with anti-IgD antiserum

(a)



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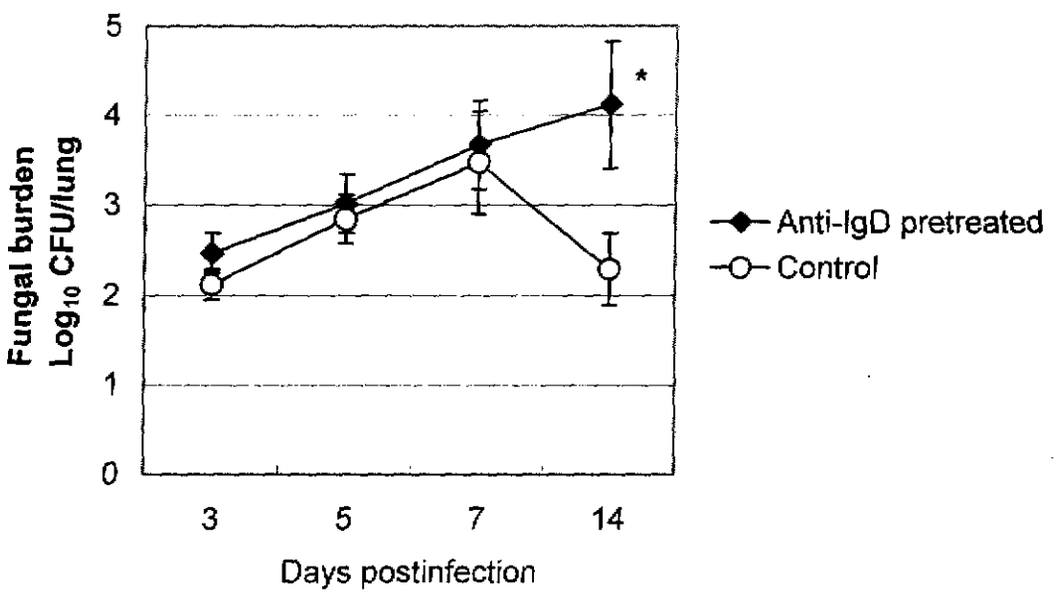


Figure 3. Anti-IgD antiserum treatment reduces IFN-γ-production by splenocytes after infection.

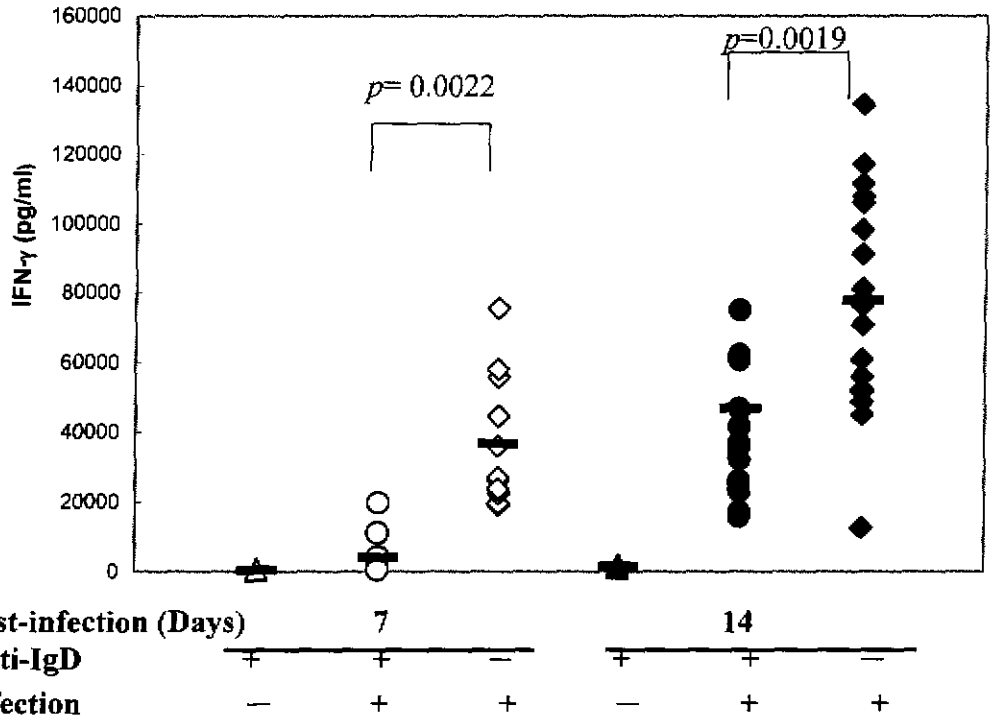
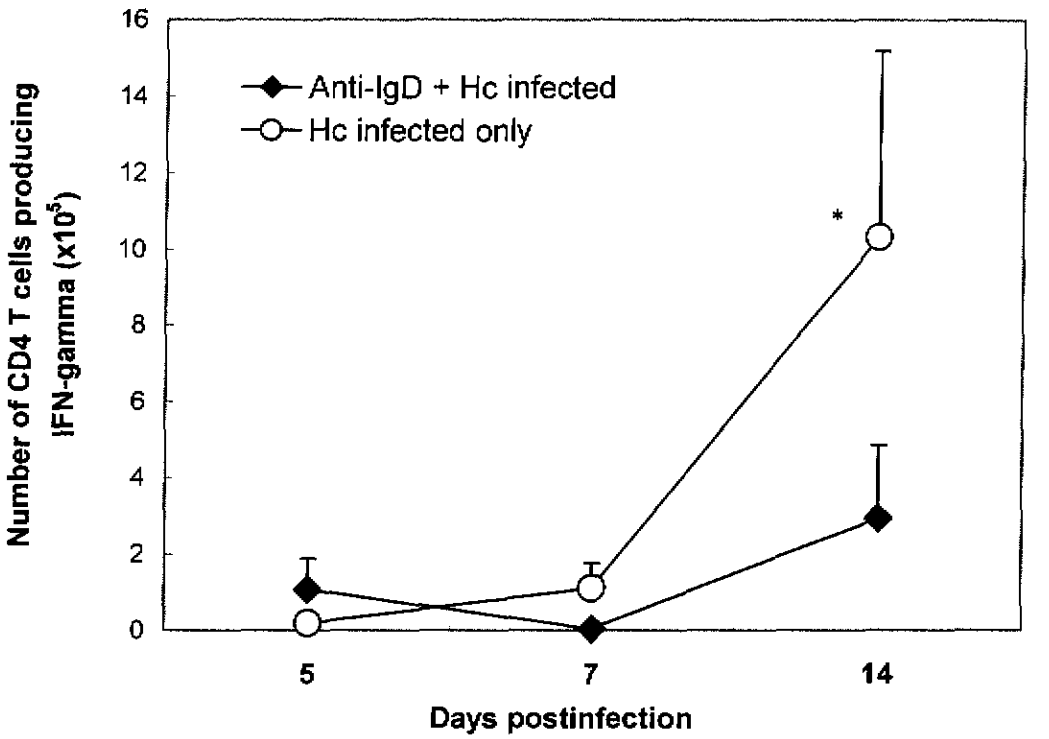


Figure 4. Anti-IgD antiserum treatment reduces the number of IFN-γ-producing CD4 T cells after *Histoplasma* infection.

(a)



(b)

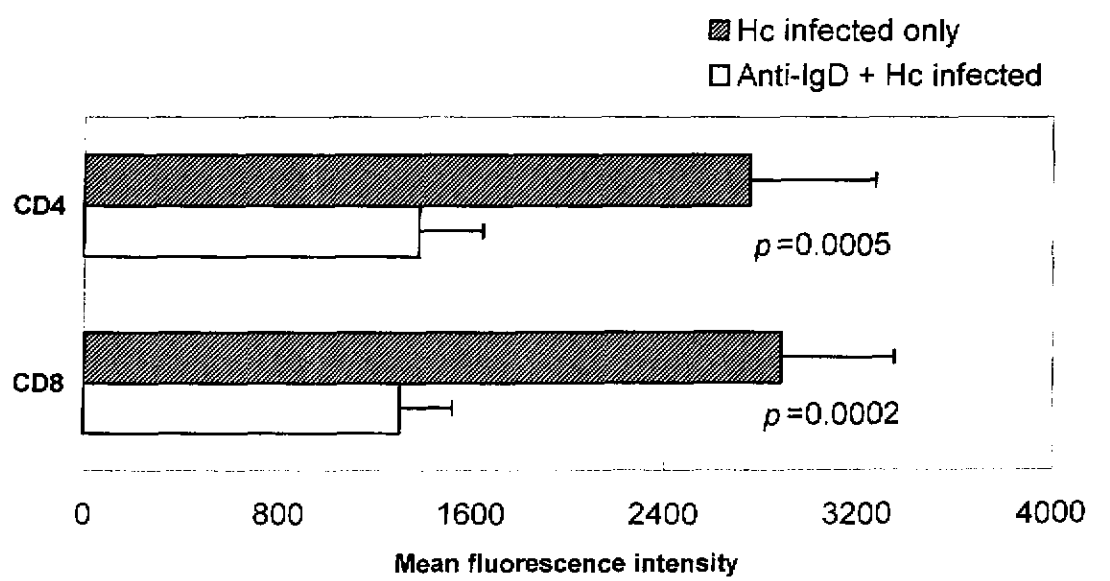
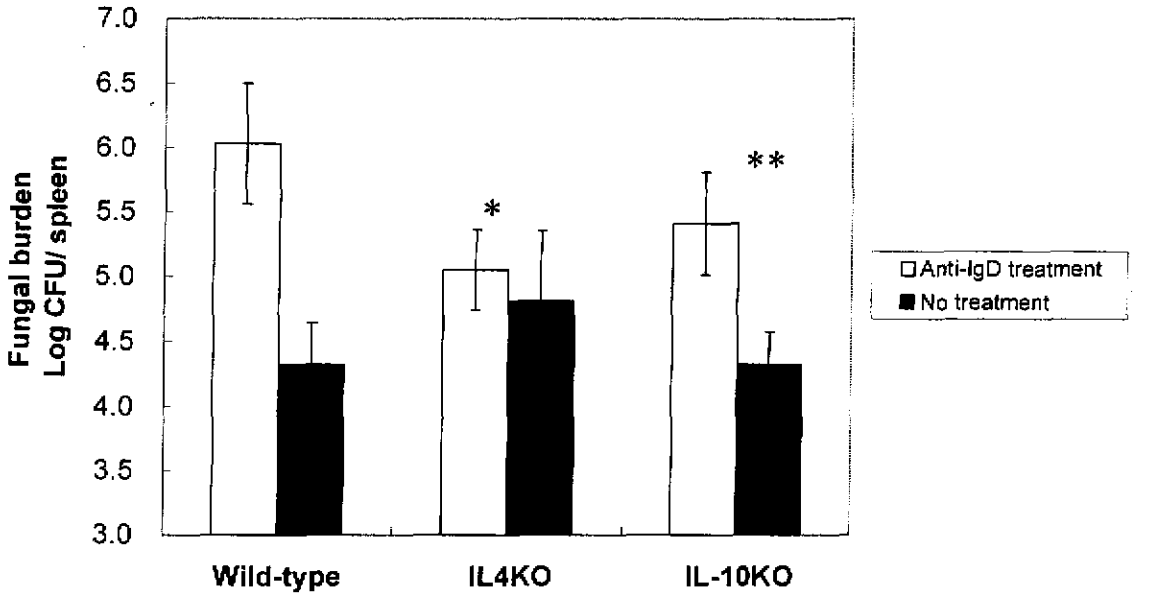


Figure 5. The effect of anti-IgD on fungal burden in wild type B6 mice is reversed in IL-4$^{-/-}$ mice but not in IL-10$^{-/-}$ mice.

(a) Spleen



(b) Lung

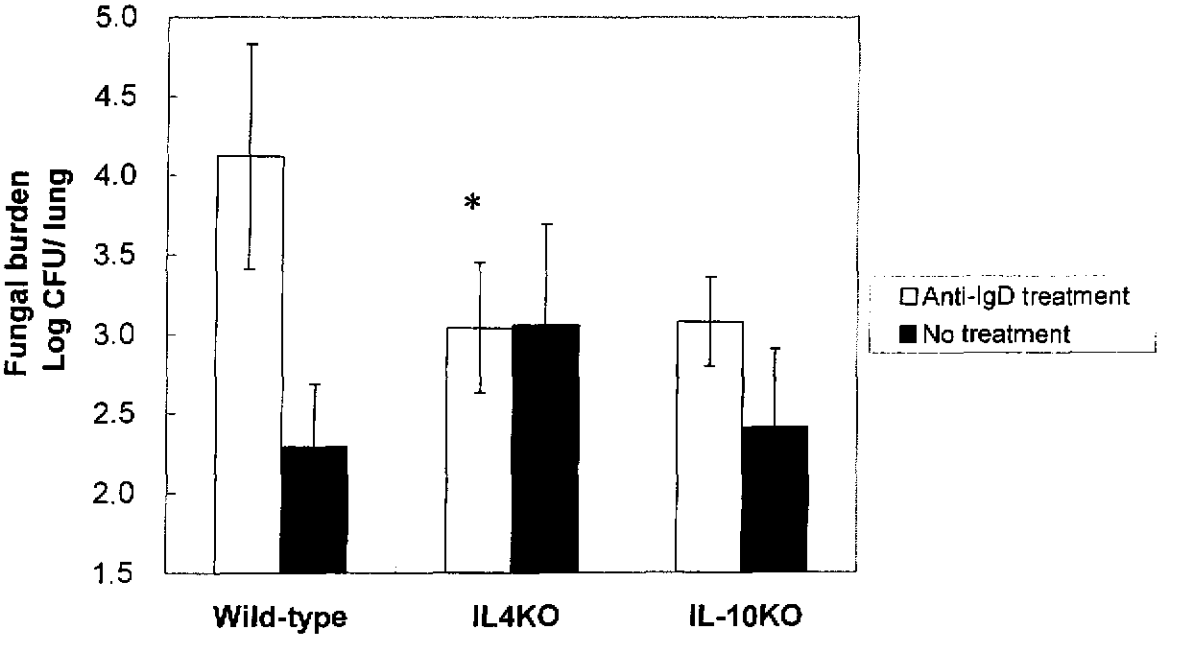


Figure 6. The effect of anti-IgD antiserum on IFN-γ production is reversed in IL-4$^{-/-}$ mice.

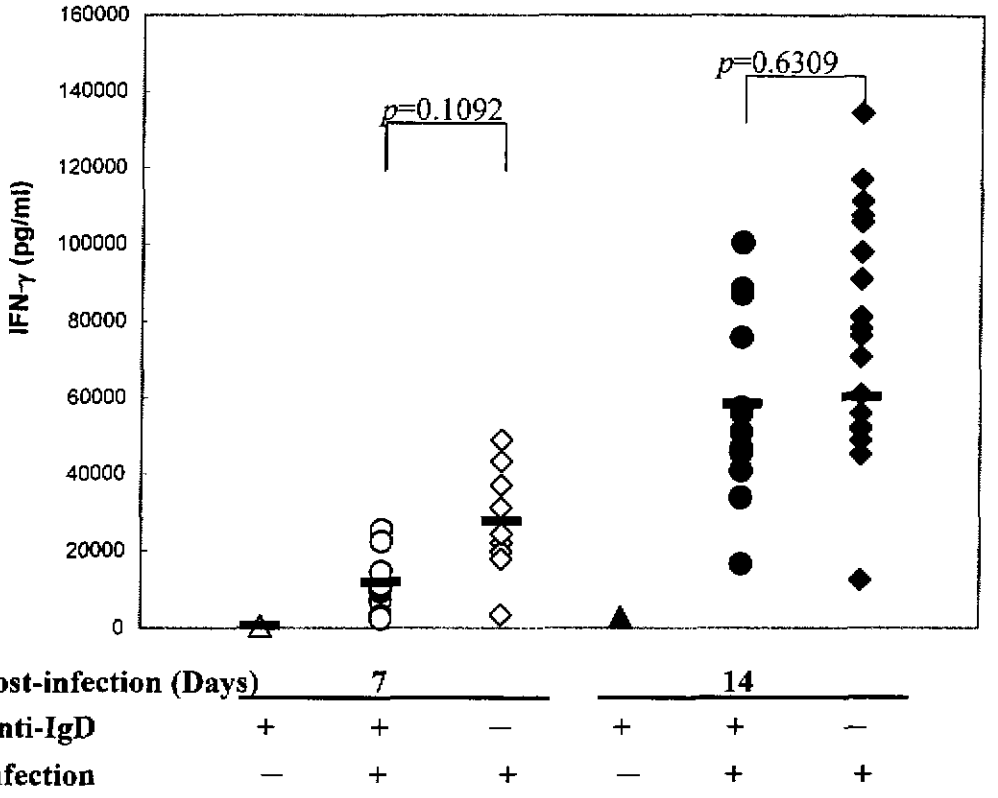
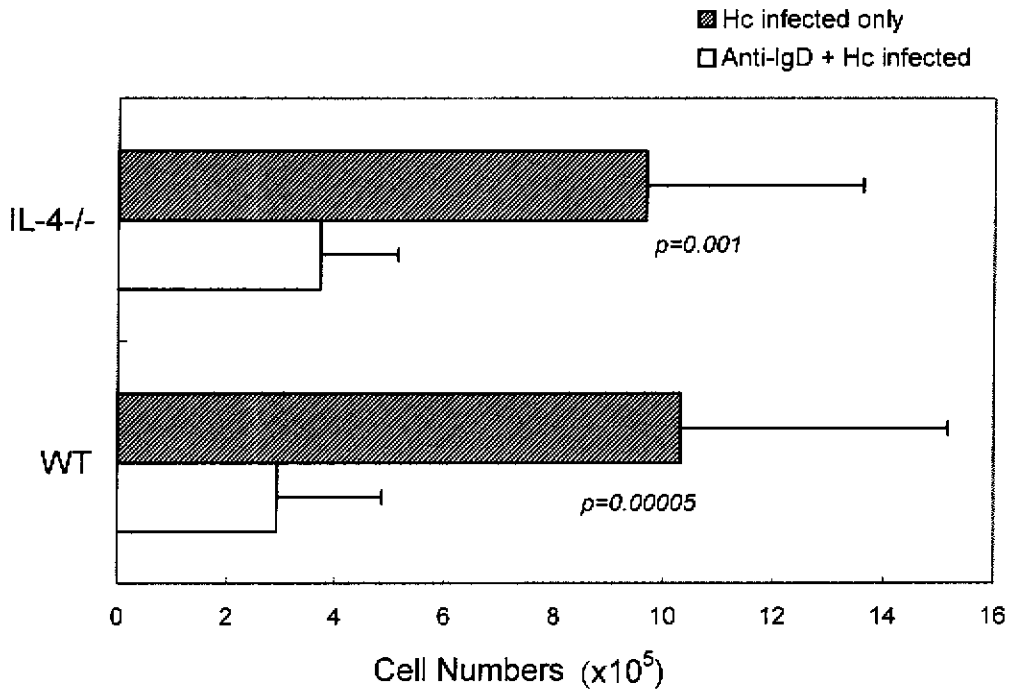


Figure 7. The number of IFN-γ-producing T cells in IL-4$^{-/-}$ mice is no different from that in wild type mice after anti-IgD treatment.

(a) CD4



(b) CD8

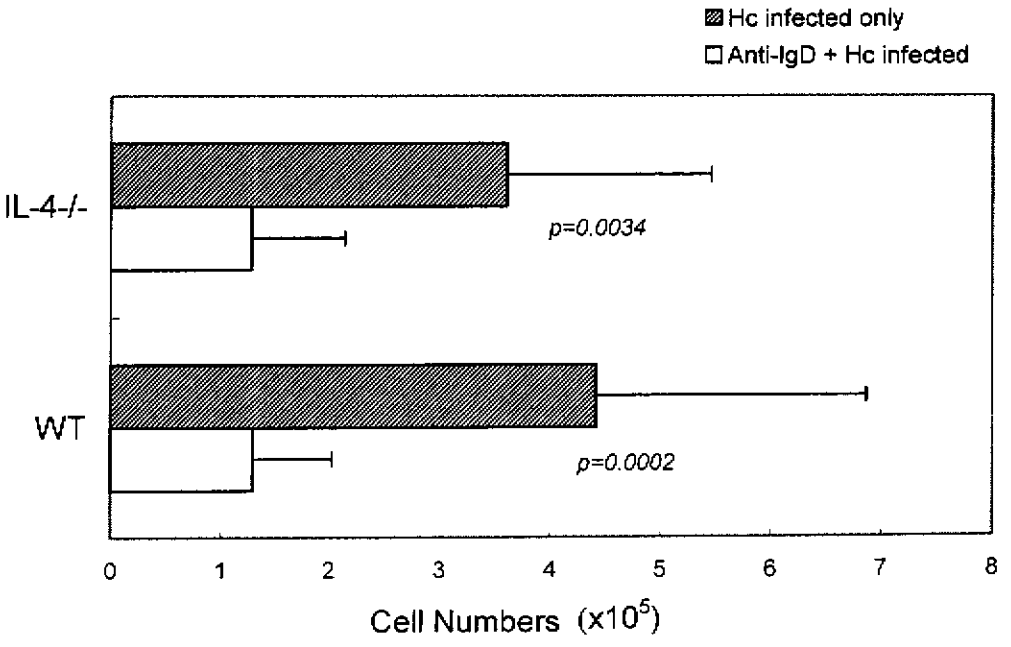


Figure 8. Analysis of the fluorescence intensity of cells stained by anti-IFNγ antibody.