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(54)名稱

包含細菌菌株之組合物

COMPOSITIONS COMPRISING BACTERIAL STRAINS

(57)摘要

本發明提供包含細菌菌株之組合物，其用於治療及預防發炎及自體免疫疾病。

The invention provides compositions comprising bacterial strains for treating and preventing inflammatory and autoimmune diseases.

指定代表圖：

總中性粒細胞。

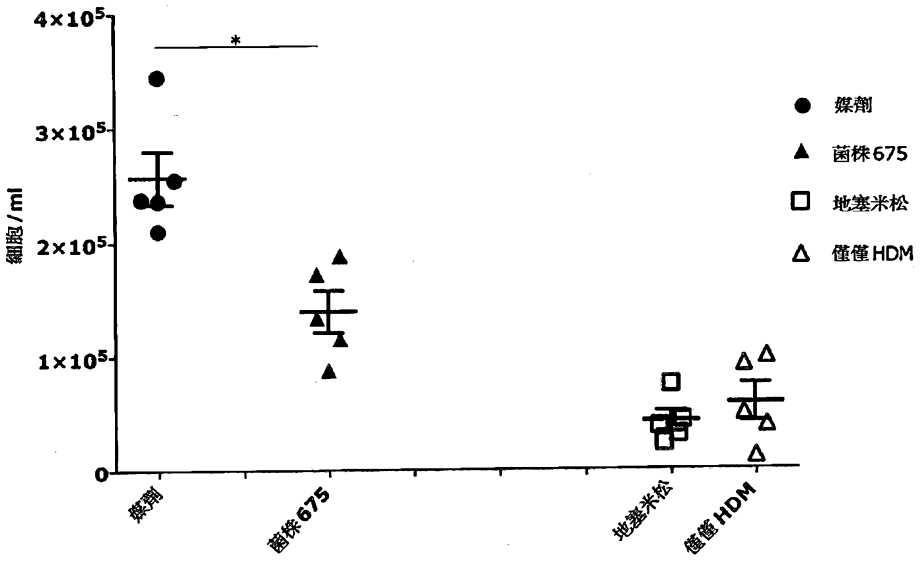

4×10^5
3×10^5
2×10^5
1×10^5
0
細胞/ml
*
媒劑
菌株675
地塞米松
僅僅HDM
媒劑
菌株675
地塞米松
僅僅HDM

圖 6

發明專利說明書

（本說明書格式、順序，請勿任意更動）

【發明名稱】（中文/英文）

包含細菌菌株之組合物

COMPOSITIONS COMPRISING BACTERIAL STRAINS

【技術領域】

【0001】 本發明屬於包含自哺乳動物消化道分離之細菌菌株之組合物及此類組合物用於治療疾病之用途的領域。

【先前技術】

【0002】 雖然認為人類腸道在子宮內無菌，但在出生後其立刻暴露於大量母體及環境微生物。此後，出現微生物定殖及演替的動態期，其受諸如分娩模式、環境、飲食及宿主基因型之因素影響，所有因素均影響腸微生物叢之組成，特別在童年期間。隨後，微生物叢穩定化且變成成人樣[1]。人類腸微生物叢含有超過 500-1000 個基本上屬於兩大細菌分類脆弱擬桿菌(Bacteroidete)及厚壁門菌(Firmicute)之不同種系型[2]。由人類腸之細菌定殖產生的成功共生關係產生多種代謝、結構、保護及其他有益功能。定殖於腸之增強代謝活性確保降解以其他方式無法攝取之飲食組分，同時釋放副產物，為宿主提供重要的營養物來源。類似地，充分認識到腸微生物叢之免疫重要性，且在免疫系統減弱之無菌動物中例示，其免疫系統在引入共生細菌後在功能上復原[3-5]。

【0003】 微生物叢組成之顯著變化已在諸如發炎性腸病(IBD)之腸胃病症中證明。舉例而言，在 IBD 患者中梭菌屬(*Clostridium*)XIVa 簇細菌之含量降低，而大腸桿菌數目增加，表明腸內共生體與病原性共生體之平衡的變化[6-9]。有趣地，此微生物生態失調亦與效應 T 細胞群體之不平衡相關。

【0004】 認識到某些細菌菌株對動物腸可具有潛在積極作用，已提議各種菌株用於治療各種疾病(參見例如[10-13])。亦已提議某些菌株，包括大部分乳桿菌屬(*Lactobacillus*)及雙歧桿菌屬(*Bifidobacterium*)菌株，用於治療不直接與腸相關之各種發炎及自體免疫疾病(綜述參見[14]及[15])。然而，

不同疾病及不同細菌菌株之間的關係及在全身層面上特定細菌菌株對腸及對任何特定類型疾病之準確作用尚未很好地表徵。

【0005】 此項技術中需要治療發炎及自體免疫疾病之新方法。亦需要待表徵之腸細菌之潛在作用，以可研發使用腸細菌之新療法。

【發明內容】

【0006】 本發明者已研發用於治療及預防發炎及自體免疫疾病之新療法。詳言之，本發明者已研發用於治療及預防由 IL-17 或 Th17 路徑介導之疾病及病狀的新療法。詳言之，本發明者已鑑別來自擬桿菌屬(*Bacteroide*)之細菌菌株可有效減少 Th17 發炎反應。如實例中所描述，經口投與包含人糞擬桿菌(*Bacteroides coprocola*)之組合物可降低氣喘、類風濕性關節炎及多發性硬化之小鼠模型中包括 Th17 發炎反應之發炎反應之嚴重程度。

【0007】 因此，在第一實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀的方法中。本發明者已鑑別用來自該屬之細菌菌株治療可降低作為 Th17 路徑(包括 IL-17)之一部分之細胞介素的含量，可減輕 Th17 發炎反應且在由 IL-17 及 Th17 路徑介導之發炎及自體免疫疾病之小鼠模型中可提供臨床益處。

【0008】 在特定實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防選自由以下組成之群之疾病或病狀的方法中：多發性硬化；關節炎，諸如類風濕性關節炎、骨關節炎、牛皮癬性關節炎或幼年特發性關節炎；視神經脊髓炎(德維克氏病(Devic's disease))；強直性脊椎炎；脊椎關節炎；牛皮癬；全身性紅斑狼瘡；發炎性腸病，諸如克羅恩氏病(Crohn's disease)或潰瘍性結腸炎；乳糜瀉；氣喘，諸如過敏性氣喘或中性粒細胞性氣喘；慢性阻塞性肺病(COPD)；癌症，諸如乳癌、結腸癌、肺癌或卵巢癌；葡萄膜炎；鞏膜炎；血管炎；白塞氏病(Behcet's disease)；動脈粥樣硬化；異位性皮炎；肺氣腫；牙周炎；過敏性鼻炎；及同種異體移植排斥。來自擬桿菌屬之細菌菌株展示之對 Th17 發炎反應的作用可為由 IL-17 及 Th17 路徑介導之疾病及病狀，諸如上列疾病及病狀提供治療益處。

【0009】 在較佳實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防氣喘，諸如中性粒細胞性氣喘或過敏性氣喘的方法中。本發明者已鑑別用擬桿菌屬菌株治療可減少中性粒細胞及嗜酸細胞募集至肺中，此可幫助治療或預防氣喘。此外，本發明者已測試及證明擬桿菌屬菌株在氣喘之小鼠模型中的功效。在某些實施例中，組合物用於治療或預防中性粒細胞性氣喘或嗜酸性粒細胞性氣喘之方法中。本發明之組合物展示的對中性粒細胞及嗜酸性粒細胞之作用意謂其可特別有效治療或預防中性粒細胞性氣喘及嗜酸性粒細胞性氣喘。實際上，在某些實施例中，組合物用於在治療或預防氣喘中降低中性粒細胞性發炎反應之方法中，或組合物用於在治療或預防氣喘中降低嗜酸性粒細胞性發炎反應之方法中。在較佳實施例中，本發明提供一種包含物種人糞擬桿菌之細菌菌株的組合物，其用於治療氣喘，且尤其中性粒細胞性氣喘。人糞擬桿菌在氣喘模型中展示對中性粒細胞具有特別深遠之作用，且用人糞擬桿菌治療可特別有效治療中性粒細胞性氣喘。在某些實施例中，本發明提供一種包含物種多形擬桿菌(*Bacteroides thetaiotaomicron*)之細菌菌株的組合物，其用於治療氣喘，且尤其嗜酸性粒細胞性氣喘或過敏性氣喘。在某些實施例中，本發明提供一種包含物種脆弱擬桿菌(*Bacteroides fragilis*)之細菌菌株的組合物，其用於治療氣喘，且尤其嗜酸性粒細胞性氣喘或過敏性氣喘。

【0010】 在其他較佳實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防類風濕性關節炎之方法中。本發明者已確定用擬桿菌屬菌株治療可在類風濕性關節炎之小鼠模型中提供臨床益處且可減少關節腫脹。在較佳實施例中，本發明提供一種包含物種人糞擬桿菌之細菌菌株的組合物，其用於治療類風濕性關節炎。使用人糞擬桿菌之組合物可特別有效治療類風濕性關節炎。在某些實施例中，本發明提供一種包含物種多形擬桿菌之細菌菌株的組合物，其用於治療類風濕性關節炎。在某些實施例中，本發明提供一種包含物種脆弱擬桿菌之細菌菌株的組合物，其用於治療類風濕性關節炎。

【0011】 在其他較佳實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防多發性硬化之方法中。本發明者已確定

用擬桿菌屬菌株治療可在多發性硬化之小鼠模型中降低發病率及疾病嚴重程度。在較佳實施例中，本發明提供一種包含物種人糞擬桿菌之細菌菌株的組合物，其用於治療多發性硬化。使用人糞擬桿菌的組合物可特別有效治療多發性硬化。在某些實施例中，本發明提供一種包含物種多形擬桿菌之細菌菌株的組合物，其用於治療多發性硬化。在某些實施例中，本發明提供一種包含物種脆弱擬桿菌之細菌菌株的組合物，其用於治療多發性硬化。

【0012】 在其他較佳實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防癌症、諸如乳癌、肺癌或肝癌之方法中。包含擬桿菌屬之細菌菌株的組合物可在乳癌、肺癌或肝癌之小鼠模型中降低腫瘤生長。在某些實施例中，組合物用於癌症治療中減小腫瘤尺寸或預防腫瘤生長之方法中。在某些實施例中，本發明提供一種包含物種人糞擬桿菌之細菌菌株的組合物，其用於治療癌症。在某些實施例中，本發明提供一種包含物種多形擬桿菌之細菌菌株的組合物，其用於治療癌症。在某些實施例中，本發明提供一種包含物種脆弱擬桿菌之細菌菌株的組合物，其用於治療癌症。

【0013】 在其他較佳實施例中，本發明提供一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防葡萄膜炎、諸如後葡萄膜炎之方法中。在某些實施例中，本發明提供一種包含物種人糞擬桿菌之細菌菌株的組合物，其用於治療葡萄膜炎。在某些實施例中，本發明提供一種包含物種多形擬桿菌之細菌菌株的組合物，其用於治療葡萄膜炎。在某些實施例中，本發明提供一種包含物種脆弱擬桿菌之細菌菌株的組合物，其用於治療葡萄膜炎。

【0014】 在某些實施例中，本發明之組合物用於由 IL-17 或 Th17 路徑介導之疾病或病狀的治療或預防中減少 IL-17 產生或減少 Th17 細胞分化之方法中。詳言之，本發明之組合物可用於氣喘、類風濕性關節炎或多發性硬化之治療或預防中減少 IL-17 產生或減少 Th17 細胞分化。較佳地，本發明提供包含物種人糞擬桿菌之細菌菌株的組合物，其用於氣喘、類風濕性關節炎或多發性硬化或氣喘、類風濕性關節炎、多發性硬化、葡萄膜炎

或癌症之治療或預防中減少 IL-17 產生或減少 Th17 細胞分化。在某些實施例中，本發明提供包含物種多形擬桿菌之細菌菌株的組合物，其用於氣喘、類風濕性關節炎、多發性硬化、葡萄膜炎或癌症之治療或預防中減少 IL-17 產生或減少 Th17 細胞分化。在某些實施例中，本發明提供包含物種脆弱擬桿菌之細菌菌株的組合物，其用於氣喘、類風濕性關節炎、多發性硬化、葡萄膜炎或癌症之治療或預防中減少 IL-17 產生或減少 Th17 細胞分化。

【0015】 在某些實施例中，組合物用於具有升高之 IL-17 含量或 Th17 細胞的患者中。擬桿菌屬菌株展示之對 Th17 發炎反應的作用可特別有益於此類患者。

【0016】 在本發明之較佳實施例中，組合物中之細菌菌株為人糞擬桿菌。亦可使用緊密相關之菌株，諸如具有與人糞擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列的細菌菌株。較佳地，細菌菌株具有與 SEQ ID NO:1、2、3 或 4 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，序列一致性係針對 SEQ ID NO:4。較佳地，用於本發明之細菌菌株具有由 SEQ ID NO:4 表示之 16s rRNA 序列。

【0017】 在本發明之進一步較佳實施例中，組合物中之細菌菌株為多形擬桿菌。亦可使用緊密相關之菌株，諸如具有與多形擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列的細菌菌株。較佳地，細菌菌株具有與 SEQ ID NO:5 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，用於本發明之細菌菌株具有由 SEQ ID NO:5 表示之 16s rRNA 序列。

【0018】 在本發明之進一步較佳實施例中，組合物中之細菌菌株為脆弱擬桿菌。亦可使用緊密相關之菌株，諸如具有與 CR626927.1 具有序列一致性之基因組的細菌菌株。

【0019】 在某些實施例中，本發明之組合物用於經口投與。經口投與本發明之菌株可有效治療 IL-17 或 Th17 路徑介導之疾病及病狀。此外，經口投與便於患者及開業醫師且允許傳遞至腸及/或部分或全部定殖於腸。

【0020】 在某些實施例中，本發明之組合物包含一或多種醫藥學上可

接受之賦形劑或載劑。

【0021】 在某些實施例中，本發明之組合物包含已凍乾之細菌菌株。凍乾為一項製備允許傳遞細菌之穩定組合物的有效及便利技術。

【0022】 在某些實施例中，本發明提供一種包含如上所述之組合物的食品。

【0023】 在某些實施例中，本發明提供一種包含如上所述之組合物的疫苗組合物。

【0024】 另外，本發明提供一種治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀的方法，其包括投與包含擬桿菌屬之細菌菌株的組合物。

【0025】 在研發以上發明時，本發明者已鑑別及表徵特別適用於療法之細菌菌株。本發明之人糞擬桿菌菌株展示有效治療本文中描述之疾病，諸如關節炎、氣喘及多發性硬化。因此，在另一個態樣中，本發明提供一種以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株或其衍生物的細胞。本發明亦提供包含此類細胞之組合物或此類細胞之生物純培養物。本發明亦提供一種以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株或其衍生物的細胞，其用於治療中，尤其用於本文中描述之疾病。

【圖式簡單說明】

【0026】 **圖 1**：房塵蟎誘發之氣喘的小鼠模型-總 BAL 流體細胞計數。

【0027】 **圖 2**：房塵蟎誘發之氣喘的小鼠模型-BALF 中總嗜酸性粒細胞計數。

【0028】 **圖 3**：房塵蟎誘發之氣喘的小鼠模型-BALF 中嗜酸性粒細胞比例。

【0029】 **圖 4**：房塵蟎誘發之氣喘的小鼠模型-BALF 中總巨噬細胞計數。

【0030】 **圖 5**：房塵蟎誘發之氣喘的小鼠模型-BALF 中巨噬細胞比例。

【0031】 **圖 6**：房塵蟎誘發之氣喘的小鼠模型-BALF 中總中性粒細胞計數。

【0032】 **圖 7**：房塵蟎誘發之氣喘的小鼠模型-BALF 中中性粒細胞比

例。

【0033】 **圖 8**：房塵蟎誘發之氣喘的小鼠模型-BALF 中總淋巴細胞計數。

【0034】 **圖 9**：房塵蟎誘發之氣喘的小鼠模型-BALF 中淋巴細胞比例。

【0035】 **圖 10**：嚴重中性粒細胞性氣喘之小鼠模型-總 BAL 流體細胞計數。

【0036】 **圖 11**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中總嗜酸性粒細胞計數。

【0037】 **圖 12**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中嗜酸性粒細胞比例。

【0038】 **圖 13**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中總巨噬細胞計數。

【0039】 **圖 14**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中巨噬細胞比例。

【0040】 **圖 15**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中總中性粒細胞計數。

【0041】 **圖 16**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中中性粒細胞比例。

【0042】 **圖 17**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中總淋巴細胞計數。

【0043】 **圖 18**：嚴重中性粒細胞性氣喘之小鼠模型-BALF 中淋巴細胞比例。

【0044】 **圖 19**：類風濕性關節炎之小鼠模型-體重，第-14 天至第 0 天。資料呈現為初始(第-14 天)體重之平均值±SEM 百分比。統計顯著性：▲ $p < 0.05$ 及▲▲▲▲ $p < 0.0001$，當與媒劑處理組比較時。

【0045】 **圖 20**：類風濕性關節炎之小鼠模型-體重，第 0 天至第 42 天。資料呈現為初始(第 0 天)體重之平均值±SEM 百分比。▲ $p < 0.05$，♦ $p < 0.05$，▲▲▲ $p < 0.001$，•••• $p < 0.0001$，當與媒劑處理組比較時。

【0046】 **圖 21**：類風濕性關節炎之小鼠模型-臨床評分。資料呈現為平均值±SEM。****$p < 0.0001$，當與媒劑處理組中第 21 天比較時。♦,O $p < 0.05$，當既定日與媒劑處理組比較時。

【0047】 **圖 22**：類風濕性關節炎之小鼠模型-對 II 型膠原蛋白之脾細胞增殖反應。基於 3H-TdR 併入之每分鐘減去培養基背景[CII 刺激-培養基背景]計數。所有資料呈現為平均值±SEM。

【0048】 **圖 23**：類風濕性關節炎之小鼠模型-來自媒劑處理組之組織培養上清液中 IFNγ 之含量。線表示組中位值。

【0049】 **圖 24**：類風濕性關節炎之小鼠模型-來自媒劑處理組之組織培養上清液中 IL-17A 之含量。線表示組中位值。

【0050】 **圖 25**：類風濕性關節炎之小鼠模型-來自媒劑處理組之組織培養上清液中 IL-10 之含量。線表示組中位值。

【0051】 **圖 26**：類風濕性關節炎之小鼠模型-來自媒劑處理組之組織培養上清液中 IL-6 之含量。線表示組中位值。

【0052】 **圖 27**：類風濕性關節炎之小鼠模型-來自生物治療劑#675 處理組(組 4)之組織培養上清液中細胞介素之含量。線表示組中位值。

【0053】 **圖 28**：房塵蟎誘發之氣喘的小鼠模型-血清中總 IgE。

【0054】 **圖 29**：房塵蟎誘發之氣喘的小鼠模型-血清中 HDM 特異性 IgG1。

【0055】 **圖 30**：房塵蟎誘發之氣喘的小鼠模型-BALF 中總 IgE。

【0056】 **圖 31**：房塵蟎誘發之氣喘的小鼠模型-BALF 中 HDM 特異性 IgG1。

【0057】 **圖 32**：房塵蟎誘發之氣喘的小鼠模型-組織學分析-平均細枝氣管周浸潤評分。

【0058】 **圖 33**：房塵蟎誘發之氣喘的小鼠模型-組織學分析-平均血管周浸潤評分。

【0059】 **圖 34**：房塵蟎誘發之氣喘的小鼠模型-組織學分析-平均發炎評分(細枝氣管周與血管周浸潤評分之平均值)。

【0060】 **圖 35**：房塵蟎誘發之氣喘的小鼠模型-組織學分析-黏液評

分。

【0061】 圖 **36**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-9 含量。

【0062】 圖 **37**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-1a 含量。

【0063】 圖 **38**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IFNγ 含量。

【0064】 圖 **39**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-17A 含量。

【0065】 圖 **40**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-4 含量。

【0066】 圖 **41**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-5 含量。

【0067】 圖 **42**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 IL-1b 含量。

【0068】 圖 **43**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 RANTES 含量。

【0069】 圖 **44**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 MIP-1a 含量。

【0070】 圖 **45**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 KC 含量。

【0071】 圖 **46**：房塵蟎誘發之氣喘的小鼠模型-肺組織中 MIP-2 含量。

【0072】 圖 **47**：嚴重中性粒細胞性氣喘之小鼠模型-血清中 HDM 特異性 IgG1。

【0073】 圖 **48**：嚴重中性粒細胞性氣喘-血清中 HDM 特異性 IgG2a。

【0074】 圖 **49**：嚴重中性粒細胞性氣喘-BALF 中 HDM 特異性 IgG1。

【0075】 圖 **50**：嚴重中性粒細胞性氣喘-BALF 中 HDM 特異性 IgG2a。

【0076】 圖 **51**：嚴重中性粒細胞性氣喘之小鼠模型-組織學分析-平均細枝氣管周浸潤評分

【0077】 圖 **52**：嚴重中性粒細胞性氣喘之小鼠模型-組織學分析-平均血管周浸潤評分

【0078】 圖 **53**：嚴重中性粒細胞性氣喘之小鼠模型-組織學分析-平均發炎評分(細枝氣管周與血管周浸潤評分之平均值)。

【0079】 圖 **54**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 TNFα 含量。

【0080】 圖 **55**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IL-1a

含量。

【0081】 圖 **56**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IFNγ 含量。

【0082】 圖 **57**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IL-17F 含量。

【0083】 圖 **58**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IL-1b 含量。

【0084】 圖 **59**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 RANTES 含量。

【0085】 圖 **60**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 MIP-2 含量。

【0086】 圖 **61**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 KC 含量。

【0087】 圖 **62**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IL-17A 含量。

【0088】 圖 **63**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 MIP-1a 含量。

【0089】 圖 **64**：嚴重中性粒細胞性氣喘之小鼠模型-肺組織中 IL-33 含量。

【0090】 圖 **65**：類風濕性關節炎之小鼠模型-組織病理學評分之目測模板。代表性影像展示膠原蛋白誘發之關節炎研究中來自小鼠跗關節之綜合評分。

【0091】 圖 **66**：類風濕性關節炎之小鼠模型-組織病理學：發炎評分。資料呈現為平均值±SEM。**$p < 0.01$，當與媒劑處理組比較時。

【0092】 圖 **67**：類風濕性關節炎之小鼠模型-組織病理學：媒劑處理組軟骨評分。資料呈現為平均值±SEM。

【0093】 圖 **68**：類風濕性關節炎之小鼠模型-組織病理學：骨骼評分。資料呈現為平均值±SEM。

【0094】 圖 **69**：類風濕性關節炎之小鼠模型-組織病理學：總評分。

資料呈現為平均值±SEM。

【0095】 **圖 70**：類風濕性關節炎之小鼠模型-組織病理學：菌株#675。資料呈現為平均值±SEM。

【0096】 **圖 71**：類風濕性關節炎之小鼠模型-組織病理學：代表性圖。在括弧之間指示動物 ID (#n.n)及四肢(R 為右，L 為左)。左上影像(媒劑)：發炎及纖維化延伸至關節周圍之軟組織之大範圍關節及骨骼破壞。下影像(菌株#675)：滑膜炎及滑囊炎病灶性延伸至關節周組織、輕度關節軟骨損傷及關節內碎片，骨結構未受影響。

【0097】 **圖 72**：多發性硬化之小鼠模型-臨床評分。

【0098】 **圖 73**：多發性硬化之小鼠模型-發病率。

【實施方式】

細菌菌株

【0099】 本發明之組合物包含擬桿菌屬之細菌菌株。該等實例證明該屬之細菌可用於治療或預防由 IL-17 或 Th17 路徑介導之疾病及病狀。較佳細菌菌株為物種人糞擬桿菌。其他較佳細菌菌株為物種多形擬桿菌或脆弱擬桿菌。

【0100】 用於本發明之擬桿菌屬物種之實例包括馬賽擬桿菌(*Bacteroides massiliensis*)、人糞擬桿菌、多形擬桿菌及糞擬桿菌(*Bacteroides caccae*)。用於本發明之擬桿菌屬物種之另一實例為脆弱擬桿菌。擬桿菌屬為一種革蘭氏陰性、專性厭氧之細菌屬。擬桿菌屬物種為不形成內生孢子之桿菌，且可為運動或不運動的，視物種而定。擬桿菌屬物種構成哺乳動物腸胃細菌叢之一大部分，且為加工複雜分子所不可或缺的。

【0101】 在 EG 血瓊脂盤上培養之人糞擬桿菌細胞非常厭氧，不形成孢子，不運動且為革蘭氏陰性。短桿或桿狀細胞寬度為約 0·8 μm 且長度可變，一般在 1-4 μm 範圍內。物種人糞擬桿菌之示例菌株描述於[16]中。模式菌株 M16^{T} (=JCM 12979^{T}=DSM 17136^{T})自健康人類之糞便分離。此物種中包括另兩種菌株[M11 (=JCM 12980)及 M156 (=JCM 12981)]。此等人糞擬桿菌菌株之 16S rRNA 基因序列的 GenBank/EMBL/DDBJ 登記號為 AB200223、AB200224 及 AB200225 (本文中揭示為 SEQ ID NO:1、SEQ ID

NO:2 及 SEQ ID NO:3)。

【0102】 以登記號 NCIMB 42408 寄存之人糞擬桿菌細菌在該等實例中進行測試且在本文中亦稱為菌株 675。測試之 675 菌株的 16S rRNA 序列在 SEQ ID NO:4 中提供。菌株 675 由 4D Pharma Research Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland)以「擬桿菌屬 675」在 2015 年 5 月 13 日寄存在國際寄存局 NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland)且分配登記號 NCIMB 42408。

【0103】 菌株 675 之基因組包含染色體組及質體。菌株 675 之染色體組序列提供於 SEQ ID NO:6 中。菌株 675 之質體序列提供於 SEQ ID NO:7 中。此序列使用 PacBio RS II 平臺產生。

【0104】 亦預期與實例中測試之菌株緊密相關的細菌菌株有效治療或預防由 IL-17 或 Th17 路徑介導之疾病及病狀。在某些實施例中，用於本發明之細菌菌株具有與人糞擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，用於本發明之細菌菌株具有與 SEQ ID NO:1、2、3 或 4 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，序列一致性係針對 SEQ ID NO:4。較佳地，用於本發明之細菌菌株具有由 SEQ ID NO:4 表示之 16s rRNA 序列。

【0105】 用於本發明之另一較佳細菌菌株為以登記號 NCIMB 42341 寄存之多形擬桿菌菌株。此菌株於 2014 年 12 月 3 日寄存在國際寄存局 NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland)。

【0106】 用於本發明之其他較佳多形擬桿菌菌株為模式菌株 ATCC 29148 = CCUG 10774 = CIP 104206 = DSM 2079 = JCM 5827 = NCTC 10582 = VPI 5482 及菌株 WAL 2926 = ATCC 29741。用於本發明之另一較佳多形擬桿菌菌株為描述於 EP1448995 中之菌株。多形擬桿菌菌株 WAL 2926 之 16S rRNA 基因序列之登記號為 M58763 (本文中揭示為 SEQ ID NO:5)。

【0107】 在某些實施例中，用於本發明之細菌菌株具有與多形擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，用於本發明之細菌菌株具有與 SEQ ID

NO:5 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。較佳地，用於本發明之細菌菌株具有由 SEQ ID NO:5 表示之 16s rRNA 序列。

【0108】 用於本發明之一較佳脆弱擬桿菌菌株為 ATCC 25285 = CCUG 4856 = CIP 77.16 = DSM 2151 = JCM 11019 = LMG 10263 = NCTC 9343。脆弱擬桿菌 NCTC 9343 菌株完整基因組之登記號為 CR626927.1 (型式：CR626927.1，GI：60491031)。

【0109】 在某些實施例中，用於本發明之細菌菌株具有與 CR626927.1 有序列一致性之基因組。在較佳實施例中，用於本發明之細菌菌株具有在 CR626927.1 之至少 60% (例如至少 65%、70%、75%、80%、85%、95%、96%、97%、98%、99%或 100%)上與 CR626927.1 具有至少 90%序列一致性(例如至少 92%、94%、95%、96%、97%、98%、99%或 100%序列一致性)的基因組。舉例而言，用於本發明之細菌菌株可具有在 CR626927.1 70%上與 CR626927.1 至少 90%序列一致，或在 CR626927.1 80%上與 CR626927.1 至少 90%序列一致，或在 CR626927.1 90%上與 CR626927.1 至少 90%序列一致，或在 CR626927.1 100%上與 CR626927.1 至少 90%序列一致，或在 CR626927.1 70%上與 CR626927.1 至少 95%序列一致，或在 CR626927.1 80%上與 CR626927.1 至少 95%序列一致，或在 CR626927.1 90%上與 CR626927.1 至少 95%序列一致，或在 CR626927.1 100%上與 CR626927.1 至少 95%序列一致，或在 CR626927.1 70%上與 CR626927.1 至少 98%序列一致，或在 CR626927.1 80%上與 CR626927.1 至少 98%序列一致，或在 CR626927.1 90%上與 CR626927.1 至少 98%序列一致，或在 CR626927.1 100%上與 CR626927.1 至少 98%序列一致的基因組。

【0110】 在某些實施例中，用於本發明之細菌菌株具有與 SEQ ID NO:6 有序列一致性之染色體組。在較佳實施例中，用於本發明之細菌菌株具有在 SEQ ID NO:6 之至少 60% (例如至少 65%、70%、75%、80%、85%、95%、96%、97%、98%、99%或 100%)上與 SEQ ID NO:6 具有至少 90%序列一致性(例如至少 92%、94%、95%、96%、97%、98%、99%或 100%序列一致性)的染色體組。舉例而言，用於本發明之細菌菌株可具有在 SEQ ID

NO:6 70%上與 SEQ ID NO:6 至少 90%序列一致，或在 SEQ ID NO:6 80%上與 SEQ ID NO:6 至少 90%序列一致，或在 SEQ ID NO:6 90%上與 SEQ ID NO:6 至少 90%序列一致，或在 SEQ ID NO:6 100%上與 SEQ ID NO:6 至少 90%序列一致，或在 SEQ ID NO:6 70%上與 SEQ ID NO:6 至少 95%序列一致，或在 SEQ ID NO:6 80%上與 SEQ ID NO:6 至少 95%序列一致，或在 SEQ ID NO:6 90%上與 SEQ ID NO:6 至少 95%序列一致，或在 SEQ ID NO:6 100%上與 SEQ ID NO:6 至少 95%序列一致，或在 SEQ ID NO:6 70%上與 SEQ ID NO:6 至少 98%序列一致，或在 SEQ ID NO:6 80%上與 SEQ ID NO:6 至少 98%序列一致，或在 SEQ ID NO:6 90%上與 SEQ ID NO:6 至少 98%序列一致，或在 SEQ ID NO:6 100%上與 SEQ ID NO:6 至少 98%序列一致的染色體組。

【0111】 在某些實施例中，用於本發明之細菌菌株具有與 SEQ ID NO:7 有序列一致性之質體。在較佳實施例中，用於本發明之細菌菌株具有在 SEQ ID NO:7 之至少 60% (例如至少 65%、70%、75%、80%、85%、95%、96%、97%、98%、99%或 100%)上與 SEQ ID NO:7 具有至少 90%序列一致性(例如至少 92%、94%、95%、96%、97%、98%、99%或 100%序列一致性)的質體。舉例而言，用於本發明之細菌菌株可具有在 SEQ ID NO:7 70%上與 SEQ ID NO:7 至少 90%序列一致，或在 SEQ ID NO:7 80%上與 SEQ ID NO:7 至少 90%序列一致，或在 SEQ ID NO:7 90%上與 SEQ ID NO:7 至少 90%序列一致，或在 SEQ ID NO:7 100%上與 SEQ ID NO:7 至少 90%序列一致，或在 SEQ ID NO:7 70%上與 SEQ ID NO:7 至少 95%序列一致，或在 SEQ ID NO:7 80%上與 SEQ ID NO:7 至少 95%序列一致，或在 SEQ ID NO:7 90%上與 SEQ ID NO:7 至少 95%序列一致，或在 SEQ ID NO:7 100%上與 SEQ ID NO:7 至少 95%序列一致，或在 SEQ ID NO:7 70%上與 SEQ ID NO:7 至少 98%序列一致，或在 SEQ ID NO:7 80%上與 SEQ ID NO:7 至少 98%序列一致，或在 SEQ ID NO:7 90%上與 SEQ ID NO:7 至少 98%序列一致，或在 SEQ ID NO:7 100%上與 SEQ ID NO:7 至少 98%序列一致的質體。

【0112】 在某些實施例中，用於本發明之細菌菌株具有與 SEQ ID NO:6 有序列一致性之染色體組及與 SEQ ID NO:7 有序列一致性之質體。

【0113】 亦預期作為以登記號 42408 寄存之細菌之生物型的細菌菌株有效治療或預防由 IL-17 或 Th17 路徑介導之疾病及病狀。亦預期作為以登記號 NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之生物型的細菌菌株有效治療或預防由 IL-17 或 Th17 路徑介導之疾病及病狀。生物型為具有相同或極類似之生理及生物化學特性的密切相關菌株。

【0114】 作為以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之生物型且適合用於本發明中的菌株可藉由對以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之其他核苷酸序列進行定序來鑑別。舉例而言，基本上全基因組可定序，且用於本發明之生物型菌株可在其全基因組之至少 80%上(例如在至少 85%、90%、95%或 99%上或在其全基因組上)具有至少 95%、96%、97%、98%、99%、99.5%或 99.9%序列一致性。用於鑑別生物型菌株之其他適合序列可包括 hsp60 或重複順序，諸如 BOX、ERIC、$(GTG)_5$ 或 REP 或[17]。生物型菌株可具有與以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之對應序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%序列一致性的序列。

【0115】 或者，作為以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之生物型且適用於本發明中的菌株可藉由使用登記號 NCIMB 42408 寄存物或登記號 NCIMB 寄存物 42341 或登記號 ATCC 29148 寄存物或登記號 ATCC 29741 寄存物及限制片段分析及/或 PCR 分析，例如藉由使用螢光增強之片段長度多態現象(FAFLP)及重複 DNA 元件(rep)-PCR 指紋或蛋白質型態分析或部分 16S 或 23s rDNA 定序來鑑別。在較佳實施例中，此類技術可用於鑑別其他人糞擬桿菌或多形擬桿菌菌株。

【0116】 在某些實施例中，作為以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之生物型且適用於本發明中的菌株為當藉由增強之核蛋白體 DNA 限制分析(ARDRA)分析時，例如當使用 Sau3AI 限制酶(關於示例性方法及指導參見例如[18])時提供與以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌相同模式的菌株。或者，生物型菌株確定為具有與以登記號 NCIMB 42408、

NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌相同的碳水化合物發酵模式的菌株。

【0117】 適用於本發明之組合物及方法的其他擬桿菌菌株，諸如以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌之生物型可使用任何適當方法或策略，包括實例中描述之分析鑑別。舉例而言，用於本發明中之菌株可藉由在厭氧 YCFA 中培養及/或投與細菌至 II 型膠原蛋白誘發之關節炎小鼠模型且接著評估細胞介素含量來鑑別。詳言之，具有與以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之細菌類似的生長模式、代謝類型及/或表面抗原之細菌菌株可用於本發明。適用菌株將具有與 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 菌株可比的免疫調節活性。詳言之，生物型菌株將對氣喘、關節炎及多發性硬化疾病模型引起與實例中所示之作用可比的作用，且對細胞介素含量引起與實例中所示之作用可比的作用，其可藉由使用實例中描述之培養及投與方案來鑑別。

【0118】 本發明之一種尤其較佳之菌株為以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株。此為實例中測試之示例性 675 菌株且展示有效治療疾病。因此，本發明提供以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株或其衍生物之細胞，諸如分離細胞。本發明亦提供一種組合物，其包含以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株或其衍生物之細胞。本發明亦提供一種以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株之生物純培養物。本發明亦提供以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株或其衍生物之細胞，其用於治療中，尤其用於本文中描述之疾病。

【0119】 以登記號 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之菌株之衍生物可為子代菌株(子代)或自原始培養(次選殖)之菌株。本發明菌株之衍生物可例如在基因層面下修飾，而不消除生物活性。詳言之，本發明之衍生菌株為治療活性的。衍生菌株將具有與原始 NCIMB 42408、NCIMB 42341、ATCC 29148 或 ATCC 29741 菌株可比的免疫調節活性。詳言之，衍生物菌株將對氣喘、關節炎及多發性硬化疾病模型引起與實例中所示之作用可比的作用，且對細胞介素含量引起與實例中

所示之作用可比的作用，其可藉由使用實例中描述之培養及投與方案來鑑別。NCIMB 42408 菌株之衍生物一般將為 NCIMB 42408 菌株之生物型。NCIMB 42341、ATCC 29148 或 ATCC 29741 菌株之衍生物一般將為 NCIMB 42341、ATCC 29148 或 ATCC 29741 菌株之生物型。

【0120】 對以登記號 NCIMB 42408 寄存之人糞擬桿菌菌株之細胞的提及涵蓋具有與以登記號 NCIMB 42408 寄存之菌株相同的安全性及治療功效特性的任何細胞，且本發明涵蓋此類細胞。對以登記號 NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之多形擬桿菌菌株之細胞的提及涵蓋具有與以登記號 NCIMB 42341、ATCC 29148 或 ATCC 29741 寄存之菌株相同的安全性及治療功效特性的任何細胞，且本發明涵蓋此類細胞。

【0121】 在較佳實施例中，本發明組合物中之細菌菌株為活的且能夠部分或全部定殖於腸。

治療用途

【0122】 如實例中證明，本發明之細菌組合物有效減少 Th17 發炎反應。詳言之，用本發明之組合物治療實現 IL-17A 含量及其他 Th17 路徑細胞介素減少，及由 IL-17 及 Th17 路徑介導之病狀之動物模型中的臨床改善。因此，本發明之組合物可用於治療或預防發炎自體免疫疾病及尤其由 IL-17 介導之疾病或病狀。詳言之，本發明之組合物可用於減少或預防 IL-17 發炎反應升高。

【0123】 Th17 細胞為產生例如 IL-17A、IL17-F、IL-21 及 IL-22 之 T 輔助細胞子集。Th17 細胞分化及 IL-17 表現可由 IL-23 驅動。此等細胞介素及其他形成 Th17 路徑之重要部分，Th17 路徑為公認之發炎信號傳導路徑，其促進且引起大量發炎及自體免疫疾病(如例如[19-24]中所述)。Th17 路徑活化之疾病為 Th17 路徑介導之疾病。Th17 路徑介導之疾病可藉由抑制 Th17 路徑改善或減輕，其可經由減少 Th17 細胞分化或降低其活性或減少 Th17 路徑細胞介素之含量。藉由 Th17 路徑介導之疾病可藉由 Th17 細胞產生之細胞介素，諸如 IL-17A、IL-17F、IL-21、IL-22、IL-26、IL-9 之含量增加表徵([25]中評述)。藉由 Th17 路徑介導之疾病可藉由 Th-17 相關基因，諸如 Stat3 或 IL-23R 之表現增加表徵。藉由 Th17 路徑介導之疾病可與

增加之 Th17 細胞含量相關。

【0124】 IL-17 為一種促發炎細胞介素，其促進若干發炎及自體免疫疾病及病狀之發病機理。如本文所用，IL-17 可指 IL-17 家族之任何成員，包括 IL-17A、IL-17B、IL-17C、IL-17D、IL-17E 及 IL-17F。IL-17 介導之疾病及病狀特徵在於罹患該疾病或病狀之組織中 IL-17 之高表現及/或 IL-17 陽性細胞之累積或存在。類似地，IL-17 介導之疾病及病狀為藉由高 IL-17 含量或 IL-17 含量增加而加重且藉由低 IL-17 含量或 IL-17 含量減少而減輕的疾病及病狀。IL-17 發炎反應可為局部或全身性的。

【0125】 可藉由 IL-17 或 Th17 路徑介導之疾病及病狀之實例包括多發性硬化；關節炎，諸如類風濕性關節炎、骨關節炎、牛皮癬性關節炎或幼年特發性關節炎；視神經脊髓炎(德維克氏病)；強直性脊椎炎；脊椎關節炎；牛皮癬；全身性紅斑狼瘡；發炎性腸病，諸如克羅恩氏病或潰瘍性結腸炎；乳糜瀉；氣喘，諸如過敏性氣喘或中性粒細胞性氣喘；慢性阻塞性肺病(COPD)；癌症，諸如乳癌、結腸癌、肺癌或卵巢癌；葡萄膜炎；鞏膜炎；血管炎；白塞氏病；動脈粥樣硬化；異位性皮炎；肺氣腫；牙周炎；過敏性鼻炎；及同種異體移植排斥。在較佳實施例中，本發明之組合物用於治療或預防此等病狀或疾病中之一或多者。在其他較佳實施例中，病狀或疾病藉由 IL-17 或 Th17 路徑介導。

【0126】 在某些實施例中，本發明之組合物用於由 IL-17 或 Th17 路徑介導之疾病或病狀的治療或預防中減少 IL-17 產生或減少 Th17 細胞分化之方法中。在某些實施例中，本發明之組合物用於治療或預防發炎或自體免疫疾病，其中該治療或預防藉由減少 Th17 發炎反應或預防其升高來實現。在某些實施例中，本發明之組合物用於治療患有發炎或自體免疫疾病之患者中，其中該患者具有升高之 IL-17 含量或升高之 Th17 細胞或展現 Th17 發炎反應。在某些實施例中，患者可經診斷患有慢性發炎或自體免疫疾病或病狀，或本發明之組合物可用於預防發展成為慢性發炎或自體免疫疾病或病狀之發炎或自體免疫疾病或病狀。在某些實施例中，疾病或病狀可不對用 TNF-α 抑制劑治療起反應。本發明之此等用途可應用於前段中列出之任何特定疾病或病狀。

【0127】 IL-17 及 Th17 路徑常常與慢性發炎及自體免疫疾病相關，因此本發明之組合物可特別適用於治療或預防如上列出之慢性疾病或病狀。在某些實施例中，組合物用於患有慢性疾病之患者中。在某些實施例中，組合物用於預防慢性疾病發展。

【0128】 本發明之組合物可用於治療藉由 IL-17 或 Th17 路徑介導之疾病及病狀且對付 Th17 發炎反應，因此本發明之組合物可特別適用於治療或預防慢性疾病、治療或預防患者之對其他療法(諸如用 TNF-α 抑制劑治療)起反應之疾病，及/或治療或預防與 IL-17 及 Th17 細胞相關之組織損傷及症狀。舉例而言，已知 IL-17 活化軟骨及骨組織中基質破壞且 IL-17 對軟骨細胞及成骨細胞中基質產生具有抑制作用，因此本發明之組合物可用於治療或預防骨質侵蝕或軟骨破壞。

【0129】 在某些實施例中，用本發明之組合物治療減少 IL-17 含量、尤其 IL-17A 含量或預防其升高。在某些實施例中，用本發明之組合物治療減少 IFN-γ、IL-1β、RANTES、MIP-1α、IL-8 或 IL-6 含量或預防其升高。此等細胞介素之含量的此類減少或預防含量升高可用於治療或預防發炎及自體免疫疾病及病狀，尤其藉由 IL-17 或 Th17 路徑介導之疾病及病狀。

氣喘

【0130】 在較佳實施例中，本發明之組合物用於治療或預防氣喘。實例證明本發明之組合物減少敏化及用房塵蟎提取物激發後中性粒細胞及/或嗜酸性粒細胞募集至氣道中，因此其可用於治療或預防氣喘。氣喘為一種特徵為氣道發炎及限制之慢性疾病。氣喘發炎可藉由 IL-17 及/或 Th17 細胞介導，因此本發明之組合物可特別有效預防或治療氣喘。氣喘發炎可藉由嗜酸性粒細胞及/或中性粒細胞介導。

【0131】 在某些實施例中，氣喘為嗜酸性粒細胞或過敏性氣喘。嗜酸性粒細胞及過敏性氣喘之特徵為外周血及氣道分泌物中嗜酸性粒細胞數目增加且病理學上與基底膜區域變厚及藥理學上與皮質類固醇反應相關[26]。減少或抑制嗜酸性粒細胞募集或活化之組合物可用於治療或預防嗜酸性粒細胞及過敏性氣喘。

【0132】 在其他實施例中，本發明之組合物用於治療或預防中性粒細

胞性氣喘(或非嗜酸性粒細胞性氣喘)。高中性粒細胞數目與可對皮質類固醇治療不敏感之嚴重氣喘有關。減少或抑制中性粒細胞募集或活化之組合物可用於治療或預防中性粒細胞性氣喘。

【0133】 嗜酸性粒細胞及中性粒細胞性氣喘並非互斥病狀且一般幫助對付任一嗜酸性粒細胞及中性粒細胞反應之治療可用於治療氣喘。

【0134】 增加之 IL-17 含量及 Th17 路徑活化與嚴重氣喘有關，因此本發明之組合物可用於預防嚴重氣喘之發展或用於治療嚴重氣喘。

【0135】 在某些實施例中，本發明之組合物用於在治療或預防氣喘中降低嗜酸性粒細胞發炎反應的方法中，或用於在治療或預防氣喘中降低中性粒細胞性發炎反應的方法中。如上所指出，氣喘中高含量嗜酸性粒細胞在病理學上與基底膜區域變厚相關，因此在治療或預防氣喘中減少嗜酸性粒細胞發炎反應也許能特別地對付該疾病之此特徵。此外，與升高之嗜酸性粒細胞組合或其不存在的升高之嗜中性粒細胞與嚴重氣喘及慢性氣道變窄有關。因此，減少中性粒細胞性發炎反應可特別適用於對付嚴重氣喘。

【0136】 在某些實施例中，組合物減少過敏性氣喘中細枝氣管周浸透，或用於在過敏性氣喘之治療中減少細枝氣管周浸透。在某些實施例中，組合物減少中性粒細胞性氣喘中細枝氣管周及/或血管周浸透，或用於在過敏性中性粒細胞性氣喘之治療中減少細枝氣管周及/或血管周浸透。

【0137】 在某些實施例中，用本發明之組合物治療減少 IL-1β、IFNγ、RANTES、MIP-1α 或 IL-8 含量或預防其升高。

【0138】 在某些實施例中，本發明之組合物用於減少嗜酸性粒細胞及/或中性粒細胞性發炎反應之氣喘治療方法中。在某些實施例中，待治療之患者已經或先前已經鑑別為具有升高之嗜中性粒細胞或嗜酸性粒細胞含量，例如如經由採血或唾液分析來鑑別。

【0139】 本發明之組合物在投與新生兒或孕婦時可用於預防新生兒中氣喘發展。組合物可用於預防兒童中氣喘發展。本發明之組合物可用於治療或預防成年發作型氣喘。本發明之組合物可用於管理或減輕氣喘。本發明之組合物可特別適用於減少與由諸如房塵蟎之過敏原加重之氣喘相關的症狀。

【0140】 治療或預防氣喘可指例如減輕症狀嚴重程度或減小對患者而言成問題之惡化頻率或觸發範圍。

關節炎

【0141】 在較佳實施例中，本發明之組合物用於治療或預防類風濕性關節炎(RA)。實例證明本發明之組合物減少小鼠模型中 RA 臨床徵象，減少軟骨及骨骼破壞，且減少 IL-17 發炎反應，因此其可用於治療或預防 RA。RA 為一種主要影響關節之全身性發炎病症。RA 與引起關節腫脹、滑液增生及軟骨及骨骼破壞之發炎反應有關。IL-17 及 Th17 在 RA 中可具有關鍵作用，例如因為 IL-17 抑制軟骨細胞及成骨細胞中基質產生，且活化基質金屬蛋白酶之產生及功能，且因為 RA 疾病活動與 IL-17 含量及 Th-17 細胞數相關[27,28]，因此本發明之組合物可特別有效預防或治療 RA。

【0142】 在某些實施例中，本發明之組合物用於在 RA 治療或預防中降低 IL-17 含量或預防 IL-17 含量升高。在某些實施例中，用本發明之組合物治療減少 IL-17 含量、尤其 IL-17A 含量或預防其升高。在某些實施例中，用本發明之組合物治療減少 IFN-γ 或 IL-6 含量或預防其升高。

【0143】 在某些實施例中，用本發明之組合物治療減少關節腫脹。在某些實施例中，本發明之組合物用於關節腫脹之患者或經鑑別為處於關節腫脹風險中之患者中。在某些實施例中，本發明之組合物用於減少 RA 中關節腫脹之方法中。

【0144】 在某些實施例中，用本發明之組合物治療減少軟骨破壞或骨骼破壞。在某些實施例中，本發明之組合物用於在 RA 治療中減少或預防軟骨或骨骼破壞。在某些實施例中，組合物用於治療患有嚴重 RA 之處於軟骨或骨骼破壞風險中的患者。

【0145】 增加之 IL-17 含量及 Th17 細胞數與 RA 中軟骨及骨組織破壞有關[27,28]。已知 IL-17 活化軟骨及骨組織中基質破壞且 IL-17 對軟骨細胞及成骨細胞中基質產生具有抑制作用。因此，在某些實施例中，本發明之組合物用於在 RA 治療中預防骨質侵蝕或軟骨破壞。在某些實施例中，組合物用於治療展現骨質侵蝕或軟骨破壞之患者或經鑑別為處於骨質侵蝕或軟骨破壞風險中的患者。

【0146】 TNF-α 亦與 RA 相關，但 TNF-α 不參與該疾病後期之發病機理。相比之下，IL-17 在整個慢性疾病階段具有作用[29]。因此，在某些實施例中，本發明之組合物用於治療慢性 RA 或晚期 RA，諸如包括關節破壞及軟骨喪失之疾病。在某些實施例中，本發明之組合物用於治療先前已接受抗 TNF-α 療法之患者。在某些實施例中，尚未治療之患者不或不再對抗 TNF-α 療法起反應。

【0147】 本發明之組合物可用於調節患者之免疫系統，因此，在某些實施例中，本發明之組合物用於預防已經鑑別為處於 RA 風險中或已經診斷患有早期 RA 之患者的 RA。本發明之組合物可用於預防 RA 發展。

【0148】 本發明之組合物可用於管理或減輕 RA。本發明之組合物可特別適用於減少與關節腫脹或骨組織破壞相關之症狀。治療或預防 RA 可指例如減輕症狀嚴重程度或減小對患者而言成問題之惡化頻率或觸發範圍。

多發性硬化

【0149】 在較佳實施例中，本發明之組合物用於治療或預防多發性硬化。實例證明本發明之組合物減少多發性硬化小鼠模型(EAE 模型)中發病率及疾病嚴重程度，因此其可用於治療或預防多發性硬化。多發性硬化為一種與特別腦及脊柱中神經元髓鞘破壞相關之發炎病症。多發性硬化為一種慢性疾病，其逐步喪失活動能力且連串進展。IL-17 及 Th17 細胞在多發性硬化中可具有關鍵作用，例如因為 IL-17 含量可與多發性硬化病變相關，IL-17 可破壞血腦屏障內皮細胞緊密接頭，且 Th17 細胞可遷移至中樞神經系統中且引起神經元喪失[30,31]。因此、本發明之組合物可特別有效預防或治療多發性硬化。

【0150】 在某些實施例中，用本發明之組合物治療降低發病率或疾病嚴重程度。在某些實施例中，本發明之組合物用於降低發病率或疾病嚴重程度。在某些實施例中，用本發明之組合物治療預防運動功能衰退或改善運動功能。在某些實施例中，本發明之組合物用於預防運動功能衰退或用於改善運動功能。在某些實施例中，用本發明之組合物治療預防出現癱瘓。在某些實施例中，本發明之組合物在多發性硬化治療中用於預防癱瘓。

【0151】 本發明之組合物可用於調節患者之免疫系統，因此，在某些

實施例中，本發明之組合物用於預防已鑑別為處於多發性硬化風險中或已經診斷患有早期多發性硬化或「復發緩解型」多發性硬化之患者的多發性硬化。本發明之組合物可用於預防出現硬化症。實際上，實例展示投與本發明之組合物預防許多小鼠中出現疾病。

【0152】 本發明之組合物可用於管理或減輕多發性硬化。本發明之組合物可特別適用於減少與多發性硬化相關之症狀。治療或預防多發性硬化可指例如減輕症狀嚴重程度或減小對患者而言成問題之惡化頻率或觸發範圍。

癌症

【0153】 在較佳實施例中，本發明之組合物用於治療或預防癌症。IL-17 及 Th17 路徑在癌症發展及進展中具有重要作用，因此本發明之組合物可用於治療或預防癌症。

【0154】 雖然 IL-17 及 Th17 在癌症中之作用尚未完全瞭解，但已知 IL-17 及 Th17 細胞之許多促腫瘤作用。舉例而言，Th17 細胞及 IL-17 可促進血管生成，增加增生及腫瘤細胞存活且活化促腫瘤轉錄因子[32-34]。

【0155】 在某些實施例中，用本發明之組合物治療減少腫瘤尺寸或減少腫瘤生長。在某些實施例中，本發明之組合物用於減少腫瘤尺寸或減少腫瘤生長。本發明之組合物可有效減少腫瘤尺寸或生長。在某些實施例中，本發明之組合物用於具有實體腫瘤之患者中。在某些實施例中，本發明之組合物在癌症治療中用於減少或預防血管生成。IL-17 及 Th17 細胞在血管生成中具有重要作用。在某些實施例中，本發明之組合物用於預防轉移。

【0156】 在較佳實施例中，本發明之組合物用於治療或預防乳癌。本發明之組合物可有效治療乳癌，且 IL-17 及 Th17 細胞在乳癌中具有重要作用[35]。在某些實施例中，本發明之組合物用於在乳癌治療中減少腫瘤尺寸，減少腫瘤生長，或減少血管生成。在較佳實施例中，癌症為乳癌。在較佳實施例中，癌症為 IV 期乳癌。

【0157】 在某些實施例中，本發明之組合物用於治療或預防肺癌。本發明之組合物可有效治療肺癌，且 IL-17 及 Th17 細胞在肺癌中具有重要作用[36]。在某些實施例中，本發明之組合物用於在肺癌治療中減少腫瘤尺

寸，減少腫瘤生長，或減少血管生成。在較佳實施例中，癌症為肺癌。

【0158】 在某些實施例中，本發明之組合物用於治療或預防肝癌。本發明之組合物可有效治療肝癌，且 IL-17 及 Th17 細胞在肝癌中具有重要作用[37]。在某些實施例中，本發明之組合物用於在肝癌治療中減少腫瘤尺寸，減少腫瘤生長，或減少血管生成。在較佳實施例中，癌症為肝癌(肝細胞癌)。

【0159】 在某些實施例中，本發明之組合物用於治療或預防癌瘤。本發明之組合物可尤其有效治療癌瘤。在某些實施例中，本發明之組合物用於治療或預防非免疫原性癌。本發明之組合物可有效治療非免疫原性癌。

【0160】 在其他實施例中，本發明之組合物用於治療或預防急性淋巴母細胞性白血病(ALL)、急性骨髓性白血病、腎上腺皮質癌、基細胞癌瘤、膽管癌、膀胱癌、骨腫瘤、骨肉瘤/惡性纖維組織細胞瘤、腦幹神經膠質瘤、腦腫瘤、小腦星形細胞瘤、大腦星形細胞瘤/惡性神經膠質瘤、室管膜瘤、髓母細胞瘤、幕上原始神經外胚層腫瘤、乳癌、枝氣管腺瘤/類癌瘤、伯基特氏淋巴瘤(Burkitt's lymphoma)、類癌瘤腫瘤、子宮頸癌、慢性淋巴細胞性白血病、慢性骨髓性白血病、慢性脊髓增生病、結腸癌、皮膚 T 細胞淋巴瘤、子宮內膜癌、室管膜瘤、食道癌、尤因肉瘤(Ewing's sarcoma)、眼內黑色素瘤、視網膜母細胞瘤、膽囊癌、胃癌、胃腸道類癌腫瘤、胃腸基質腫瘤(GIST)、生殖細胞腫瘤、兒童期視覺路徑及下丘腦神經膠質瘤、霍奇金氏淋巴瘤(Hodgkin lymphoma)、黑色素瘤、胰島細胞癌瘤、卡波西氏肉瘤(Kaposi sarcoma)、腎細胞癌症、喉癌、白血病、淋巴瘤、間皮瘤、神經母細胞瘤、非霍奇金淋巴瘤、口咽癌、骨肉瘤、卵巢癌、胰臟癌、甲狀旁腺癌、咽癌、垂體腺瘤、漿細胞瘤形成、前列腺癌、腎細胞癌瘤、視網膜母細胞瘤、肉瘤、睪丸癌、甲狀腺癌或子宮癌。

【0161】 本發明之組合物在與其他治療劑組合使用時可特別有效。本發明組合物之免疫調節作用在與更直接抗癌藥劑組合時可有效。因此，在某些實施例中，本發明提供一種組合物，其包含擬桿菌屬之細菌菌株及抗癌劑。在較佳實施例中，抗癌劑為免疫檢查點抑制劑、靶向抗體免疫療法、CAR-T 細胞療法、溶瘤病毒或抑制細胞生長藥物。在較佳實施例中，組合

物包含選自由以下組成之群的抗癌藥劑：Yervoy (易普利單抗(ipilimumab)，BMS)；Keytruda (派姆單抗(pembrolizumab)，Merck)；Opdivo (納武單抗(nivolumab)，BMS)；MEDI4736 (AZ/MedImmune)；MPDL3280A (Roche/Genentech)；曲美目單抗(Tremelimumab) (AZ/MedImmune)；CT-011 (皮利珠單抗(pidilizumab)，CureTech)；BMS-986015 (利魯單抗(lirilumab)，BMS)；MEDI0680 (AZ/MedImmune)；MSB-0010718C (Merck)；PF-05082566 (Pfizer)；MEDI6469 (AZ/MedImmune)；BMS-986016 (BMS)；BMS-663513 (優瑞路單抗(urelumab)，BMS)；IMP321 (Prima Biomed)；LAG525 (Novartis)；ARGX-110 (arGEN-X)；PF-05082466 (Pfizer)；CDX-1127 (瓦利路單抗(varlilumab)；CellDex Therapeutics)；TRX-518 (GITR Inc.)；MK-4166 (Merck)；JTX-2011 (Jounce Therapeutics)；ARGX-115 (arGEN-X)；NLG-9189 (英多莫德(indoximod)，NewLink Genetics)；INCB024360 (Incyte)；IPH2201 (Innate Immotherapeutics/AZ)；NLG-919 (NewLink Genetics)；抗 VISTA (JnJ)；依帕斯塔特(Epacadostat) (INCB24360，Incyte)；F001287 (Flexus/BMS)；CP 870893 (University of Pennsylvania)；MGA271 (Macrogenix)；依瑪珠單抗(Emactuzumab) (Roche/Genentech)；加尼斯替(Galunisertib) (Eli Lilly)；優庫路單抗(Ulocuplumab) (BMS)；BKT140/BL8040 (Biokine Therapeutics)；巴維昔單抗(Bavituximab) (Peregrine Pharmaceuticals)；CC 90002 (Celgene)；852A (Pfizer)；VTX-2337 (VentiRx Pharmaceuticals)；IMO-2055 (Hybridon，Idera Pharmaceuticals)；LY2157299 (Eli Lilly)；EW-7197 (Ewha Women's University，Korea)；威羅菲尼(Vemurafenib) (Plexxikon)；達拉菲尼(Dabrafenib) (Genentech/GSK)；BMS-777607 (BMS)；BLZ945 (Memorial Sloan-Kettering Cancer Centre)；Unituxin (地土昔單抗(dinutuximab)，United Therapeutics Corporation)；Blincyto (布林莫單抗(blinatumomab)，Amgen)；Cyramza (雷莫蘆單抗(ramucirumab)，Eli Lilly)；Gazyva (歐努珠單抗(obinutuzumab)，Roche/Biogen)；Kadcyla (阿多曲妥珠單抗恩他新(ado-trastuzumab emtansine)，Roche/Genentech)；Perjeta (帕妥珠單抗(pertuzumab)，Roche/Genentech)；Adcetris (布瑞西單抗維多新(brentuximab vedotin)，

Takeda/Millennium)；Arzerra (奧法木單抗(ofatumumab)，GSK)；Vectibix (帕尼單抗(panitumumab)，Amgen)；Avastin (貝伐單抗(bevacizumab)，Roche/Genentech)；Erbitux (西妥昔單抗(cetuximab)，BMS/Merck)；Bexxar (托西莫單抗(tositumomab)-I131，GSK)；Zevalin (替伊莫單抗(ibritumomab tiuxetan)，Biogen)；Campath (阿侖單抗(alemtuzumab)，Bayer)；Mylotarg (吉妥珠單抗奧唑米星(gemtuzumab ozogamicin)，Pfizer)；Herceptin (曲妥珠單抗(trastuzumab)，Roche/Genentech)；Rituxan (利妥昔單抗(rituximab)，Genentech/Biogen)；伏洛昔單抗(volociximab) (Abbvie)；艾那維單抗(Enavatuzumab) (Abbvie)；ABT-414 (Abbvie)；埃羅妥珠單抗(Elotuzumab) (Abbvie/BMS)；ALX-0141 (Ablynx)；奧紮利單抗(Ozaralizumab) (Ablynx)；埃替單抗(Actimab)-C (Actinium)；埃替單抗-P (Actinium)；米拉珠單抗-多柔比星(Milatuzumab-dox) (Actinium)；Emab-SN-38 (Actinium)；伊土莫單抗(Naptumonmab estafenatox) (Active Biotech)；AFM13 (Affimed)；AFM11 (Affimed)；AGS-16C3F (Agensys)；AGS-16M8F (Agensys)；AGS-22ME (Agensys)；AGS-15ME (Agensys)；GS-67E (Agensys)；ALXN6000 (薩瑪利單抗(samalizumab)，Alexion)；ALT-836 (Altor Bioscience)；ALT-801 (Altor Bioscience)；ALT-803 (Altor Bioscience)；AMG780 (Amgen)；AMG 228 (Amgen)；AMG820 (Amgen)；AMG172 (Amgen)；AMG595 (Amgen)；AMG110 (Amgen)；AMG232 (阿達木單抗(adecatumumab)，Amgen)；AMG211 (Amgen/MedImmune)；BAY20-10112 (Amgen/Bayer)；瑞洛土單抗(Rilotumumab) (Amgen)；戴諾素單抗(Denosumab) (Amgen)；AMP-514 (Amgen)；MEDI575 (AZ/MedImmune)；MEDI3617 (AZ/MedImmune)；MEDI6383 (AZ/MedImmune)；MEDI551 (AZ/MedImmune)；帕莫西單抗(Moxetumomab pasudotox) (AZ/MedImmune)；MEDI565 (AZ/MedImmune)；MEDI0639 (AZ/MedImmune)；MEDI0680 (AZ/MedImmune)；MEDI562 (AZ/MedImmune)；AV-380 (AVEO)；AV203 (AVEO)；AV299 (AVEO)；BAY79-4620 (Bayer)；拉安土單抗(Anetumab ravtansine) (Bayer)；萬替珠單抗(vantictumab) (Bayer)；BAY94-9343 (Bayer)；西羅珠單抗(Sibrotuzumab) (Boehringer Ingleheim)；BI-836845 (Boehringer Ingleheim)；B-701 (BioClin)；

BIIB015 (Biogen)；歐努珠單抗(Biogen/Genentech)；BI-505 (Bioinvent)；BI-1206 (Bioinvent)；TB-403 (Bioinvent)；BT-062 (Biotest) BIL-010t (Biosceptre)；MDX-1203 (BMS)；MDX-1204 (BMS)；奈西莫單抗(Necitumumab) (BMS)；CAN-4 (Cantargia AB)；CDX-011 (Celldex)；CDX1401 (Celldex)；CDX301 (Celldex)；U3-1565 (Daiichi Sankyo)；帕曲土單抗(patritumab) (Daiichi Sankyo)；替加土單抗(tigatuzumab) (Daiichi Sankyo)；尼妥珠單抗(nimotuzumab) (Daiichi Sankyo)；DS-8895 (Daiichi Sankyo)；DS-8873 (Daiichi Sankyo)；DS-5573 (Daiichi Sankyo)；MORab-004 (Eisai)；MORab-009 (Eisai)；MORab-003 (Eisai)；MORab-066 (Eisai)；LY3012207 (Eli Lilly)；LY2875358 (Eli Lilly)；LY2812176 (Eli Lilly)；LY3012217(Eli Lilly)；LY2495655 (Eli Lilly)；LY3012212 (Eli Lilly)；LY3012211 (Eli Lilly)；LY3009806 (Eli Lilly)；西妥木單抗(cixutumumab) (Eli Lilly)；法拉維單抗(Flanvotumab) (Eli Lilly)；IMC-TR1 (Eli Lilly)；雷莫蘆單抗 Eli Lilly)；塔巴木單抗(Tabalumab) (Eli Lilly)；紮諾利單抗(Zanolimumab) (Emergent Biosolution)；FG-3019 (FibroGen)；FPA008 (Five Prime Therapeutics)；FP-1039 (Five Prime Therapeutics)；FPA144 (Five Prime Therapeutics)；卡妥索單抗(catumaxomab) (Fresenius Biotech)；IMAB362 (Ganymed)；IMAB027 (Ganymed)；HuMax-CD74 (Genmab)；HuMax-TFADC (Genmab)；GS-5745 (Gilead)；GS-6624 (Gilead)；OMP-21M18 (登西珠單抗(demcizumab)，GSK)；馬帕木單抗(mapatumumab) (GSK)；IMGN289 (ImmunoGen)；IMGN901 (ImmunoGen)；IMGN853 (ImmunoGen)；IMGN529 (ImmunoGen)；IMMU-130 (Immunomedics)；米拉珠單抗-多柔比星(Immunomedics)；IMMU-115 (Immunomedics)；IMMU-132 (Immunomedics)；IMMU-106 (Immunomedics)；IMMU-102 (Immunomedics)；依帕珠單抗(Epratuzumab) (Immunomedics)；克利瓦單抗(Clivatuzumab) (Immunomedics)；IPH41 (Innate Immunotherapeutics)；達拉土單抗(Daratumumab) (Janssen/Genmab)；CNTO-95 (Intetumumab，Janssen)；CNTO-328 (西妥昔單抗(siltuximab)，Janssen)；KB004 (KaloBios)；莫加利單抗(mogamulizumab) (Kyowa Hakko Kirrin)；KW-2871 (依美昔單抗(ecromeximab)，Life Science)；索奈珠單抗

(Sonepcizumab) (Lpath)；馬土西單抗(Margetuximab) (Macrogenics)；恩利珠單抗(Enoblituzumab) (Macrogenics)；MGD006 (Macrogenics)；MGF007 (Macrogenics)；MK-0646 (dalotuzumab，Merck)；MK-3475 (Merck)；Sym004 (Symphogen/Merck Serono)；DI17E6 (Merck Serono)；MOR208 (Morphosys)；MOR202 (Morphosys)；Xmab5574 (Morphosys)；BPC-1C (恩斯土單抗(ensituximab)，Precision Biologics)；TAS266 (Novartis)；LFA102 (Novartis)；BHQ880 (Novartis/Morphosys)；QGE031 (Novartis)；HCD122 (盧卡木單抗(lucatumumab)，Novartis)；LJM716 (Novartis)；AT355 (Novartis)；OMP-21M18 (登西珠單抗，OncoMed)；OMP52M51 (Oncomed/GSK)；OMP-59R5 (Oncomed/GSK)；萬替珠單抗(Oncomed/Bayer)；CMC-544 (奧英妥珠單抗(inotuzumab ozogamicin)，Pfizer)；PF-03446962 (Pfizer)；PF-04856884 (Pfizer)；PSMA-ADC (Progenics)；REGN1400 (Regeneron)；REGN910 (奈瓦庫單抗(nesvacumab)，Regeneron/Sanofi)；REGN421 (恩替庫單抗(enoticumab)，Regeneron/Sanofi)；RG7221、RG7356、RG7155、RG7444、RG7116、RG7458、RG7598、RG7599、RG7600、RG7636、RG7450、RG7593、RG7596、DCDS3410A、RG7414 (帕薩珠單抗(parsatuzumab))、RG7160 (伊佳珠單抗(imgatuzumab))、RG7159 (歐賓珠單抗(obintuzumab))、RG7686、RG3638 (歐奈珠單抗(onartuzumab))、RG7597 (Roche/Genentech)；SAR307746 (Sanofi)；SAR566658 (Sanofi)；SAR650984 (Sanofi)；SAR153192 (Sanofi)；SAR3419 (Sanofi)；SAR256212 (Sanofi)，SGN-LIV1A (林妥珠單抗(lintuzumab)，Seattle Genetics)；SGN-CD33A (Seattle Genetics)；SGN-75 (馬維珠單抗(vorsetuzumab mafodotin)，Seattle Genetics)；SGN-19A (Seattle Genetics)；SGN-CD70A (Seattle Genetics)；SEA-CD40 (Seattle Genetics)；替伊莫單抗(Spectrum)；MLN0264 (Takeda)；加尼妥單抗(ganitumab) (Takeda/Amgen)；CEP-37250 (Teva)；TB-403 (Thrombogenic)；VB4-845 (Viventia)；Xmab2512 (Xencor)；Xmab5574 (Xencor)；尼妥珠單抗(YM Biosciences)；卡路單抗(Carlumab) (Janssen)；NY-ESO TCR (Adaptimmune)；MAGE-A-10 TCR (Adaptimmune)；CTL019 (Novartis)；JCAR015 (Juno Therapeutics)；KTE-C19 CAR (Kite Pharma)；UCART19 (Cellectis)；BPX-401

(Bellicum Pharmaceuticals)；BPX-601 (Bellicum Pharmaceuticals)；ATTCK20 (Unum Therapeutics)；CAR-NKG2D (Celyad)；Onyx-015 (Onyx Pharmaceuticals)；H101 (Shanghai Sunwaybio)；DNX-2401 (DNAtrix)；VCN-01 (VCN Biosciences)；Colo-Ad1 (PsiOxus Therapeutics)；ProstAtak (Advantagene)；Oncos-102 (Oncos Therapeutics)；CG0070 (Cold Genesys)；Pexa-vac (JX-594，Jennerex Biotherapeutics)；GL-ONC1 (Genelux)；T-VEC (Amgen)；G207 (Medigene)；HF10 (Takara Bio)；SEPREHVIR (HSV1716，Virttu Biologics)；OrienX010 (OrienGene Biotechnology)；Reolysin (Oncolytics Biotech)；SVV-001 (Neotropix)；Cacatak (CVA21，Viralytics)；Alimta (Eli Lilly)、順鉑(cisplatin)、奧沙利鉑(oxaliplatin)、伊立替康(irinotecan)、亞葉酸(folinic acid)、甲胺喋呤(methotrexate)、環磷醯胺(cyclophosphamide)、5-氟尿嘧啶(5-fluorouracil)、Zykadia (Novartis)、Tafinlar (GSK)、Xalkori (Pfizer)、Iressa (AZ)、Gilotrif (Boehringer Ingelheim)、Tarceva (Astellas Pharma)、Halaven (Eisai Pharma)、Veliparib (Abbvie)、AZD9291 (AZ)、阿雷替尼(Alectinib) (Chugai)、LDK378 (Novartis)、蓋納特皮(Genetespib) (Synta Pharma)、Tergenpumatucel-L (NewLink Genetics)、GV1001 (Kael-GemVax)、替瓦替尼(Tivantinib) (ArQule)；Cytoxan (BMS)；Oncovin (Eli Lilly)；阿黴素 (Adriamycin) (Pfizer)；Gemzar (Eli Lilly)；Xeloda (Roche)；Ixempra (BMS)；Abraxane (Celgene)；Trelstar (Debiopharm)；Taxotere (Sanofi)；Nexavar (Bayer)；IMMU-132 (Immunomedics)；E7449 (Eisai)；Thermodox (Celsion)；Cometriq (Exellxis)；Lonsurf (Taiho Pharmaceuticals)；Camptosar (Pfizer)；UFT (Taiho Pharmaceuticals)；及 TS-1 (Taiho Pharmaceuticals)。

葡萄膜炎

【0162】 在其他較佳實施例中，本發明之組合物用於治療或預防葡萄膜炎。本發明之組合物可降低葡萄膜炎之動物模型中發病率及疾病嚴重程度，因此其可用於治療或預防葡萄膜炎。葡萄膜炎為葡萄膜發炎且可引起視網膜組織破壞。其可以不同解剖形式(前部、中間、後部或擴散)存在且由不同但相關之病因引起，包括全身性自體免疫病狀。IL-17 及 Th17 路徑主要與葡萄膜炎相關，因此本發明之組合物可特別有效預防或治療葡萄膜

炎。參考文獻[38-45]描述葡萄膜炎患者中升高血清含量之介白素-17A，IL17A 遺傳變異體與全葡萄膜炎特定相關，Th17 相關之細胞介素在實驗自體免疫葡萄膜炎之發病機理中的作用，在單相實驗自體免疫葡萄膜炎期間 Th17 細胞與調節 T 細胞之間的不平衡，葡萄膜炎及活動性亞當-白塞及沃格特-小柳-原田(Vogt-Koyanagi-Harada，VKH)疾病患者中 IL-17A 上調，用蘇金單抗(secukinumab) (抗 IL-17A 抗體)治療非感染性葡萄膜炎，及葡萄膜炎眼睛中之 Th17。

【0163】 在某些實施例中，葡萄膜炎為後葡萄膜炎。後葡萄膜炎主要存在視網膜及脈絡膜發炎，且本發明之組合物可有效減少視網膜發炎及破壞。

【0164】 在某些實施例中，用本發明之組合物治療減少視網膜損傷。在某些實施例中，本發明之組合物在葡萄膜炎治療中用於減少或預防視網膜損傷。在某些實施例中，組合物用於治療處於視網膜損傷風險中的嚴重葡萄膜炎患者。在某些實施例中，用本發明之組合物治療減少視神經盤發炎。在某些實施例中，本發明之組合物用於治療減少或預防視神經盤發炎。在某些實施例中，用本發明之組合物治療減少發炎細胞浸透視網膜組織。在某些實施例中，本發明之組合物用於減少發炎細胞浸透視網膜組織。在某些實施例中，用本發明之組合物治療維持或改善視力。在某些實施例中，本發明之組合物用於維持或改善視力。

【0165】 在某些實施例中，組合物用於治療或預防與非感染性或自體免疫疾病，諸如白塞氏病、克羅恩氏病、富克斯異色性虹膜睫狀體炎(Fuchs heterochromic iridocyclitis)、肉芽腫病伴多血管炎、HLA-B27 相關葡萄膜炎、幼年特發性關節炎、結節病、脊椎關節炎、交感性眼炎、小管間質性腎炎及葡萄膜炎症候群或沃格特-小柳-原田症候群相關之葡萄膜炎。IL-17A 已顯示與例如白塞氏病及沃格特-小柳-原田疾病相關。

【0166】 葡萄膜炎之治療或預防可指例如減輕症狀嚴重程度或預防復發。

投與模式

【0167】 較佳地，本發明之組合物待施用於胃腸道，以能夠傳遞至腸

及/或使本發明之細菌菌株部分或全部定殖於腸。一般地，雖然本發明之組合物經口投與，但其可直腸、鼻內或經由頰或舌下途徑投與。

【0168】 在某些實施例中，本發明之組合物可呈泡沫、噴霧或凝膠形式投與。

【0169】 在某些實施例中，本發明之組合物可呈栓劑，諸如直腸栓劑，例如呈可可豆油(可可脂)、合成硬脂(例如 suppocire、witepsol)、甘油基-明膠、聚乙二醇或肥皂甘油組合物之形式投與。

【0170】 在某些實施例中，本發明之組合物經由管，諸如鼻飼管、口胃管、胃管、空腸造口管(J 型管)、經皮內窺鏡胃造口術(PEG)或埠，諸如通向胃、空腸及其他適合進入埠之胸壁埠投與胃腸道。

【0171】 本發明之組合物可投與一次，或其可作為治療方案一部分連續投與。在某些實施例中，本發明之組合物將每日投與。

【0172】 在本發明之某些實施例中，根據本發明之治療伴隨有患者腸微生物叢之評估。若本發明之菌株傳遞及/或部分或全部定殖未實現，使得功效未觀測到，則可重複治療，或若傳遞及/或部分或全部定殖成功且觀測到功效，則可停止治療。

【0173】 在某些實施例中，本發明之組合物可投與懷孕動物，例如哺乳動物，諸如人類，以預防發炎或自體免疫疾病在其子女中在子宮內及/或其出生後出現。

【0174】 本發明之組合物可投與經診斷患有由 IL-17 或 Th17 路徑介導之疾病或病狀或已鑑別為處於由 IL-17 或 Th17 路徑介導之疾病或病狀風險中的患者。組合物亦可作為預防措施投與以預防健康患者中由 IL-17 或 Th17 路徑介導之疾病或病狀出現。

【0175】 本發明之組合物可投與已鑑別為具有異常腸微生物叢之患者。舉例而言，患者可具有減少或缺乏之擬桿菌屬及尤其人糞擬桿菌之定殖。

【0176】 本發明之組合物可作為食品，諸如營養增補劑投與。

【0177】 一般地，本發明之組合物用於治療人類，不過其可用於治療動物，包括單胃哺乳動物，諸如家禽、豬、貓、犬、馬或兔。本發明之組

合物可用於增強動物之生長及效能。若投與動物，則可使用經口管飼法。

組合物

【0178】 一般地，本發明之組合物包含細菌。在本發明之較佳實施例中，組合物呈凍乾形式調配。舉例而言，本發明之組合物可包含含有本發明之細菌菌株的顆粒或明膠膠囊，例如硬明膠膠囊。

【0179】 較佳地，本發明之組合物包含凍乾細菌。細菌凍乾為公知程序且相關指導可於例如參考文獻[46-48]中獲得。

【0180】 或者，本發明之組合物可包含活的活性細菌培養物。

【0181】 在較佳實施例中，本發明之組合物經囊封以能夠傳遞細菌菌株至腸。囊封保護組合物免於降解，直至經由例如用化學或物理刺激，諸如壓力、酶活性或物理性崩解(其可藉由 pH 值改變而觸發)進行破裂，在目標位置傳遞。可使用任何適當囊封法。示例性囊封技術包括截留在多孔基質內、附著或吸附在固體載體表面上、藉由絮凝或利用交聯劑而自我凝聚以及機械容納在微孔膜或微膠囊後。關於可用於製備本發明之組合物的囊封之指導可於例如參考文獻[49]及[50]中獲得。

【0182】 組合物可經口投與且可呈錠劑、膠囊或散劑形式。囊封產品為較佳，因為擬桿菌屬為厭氧菌。其他成分(諸如維生素 C)可作為除氧劑及益生基質包括以改善活體內傳遞及/或部分或全部定殖及存活。或者，本發明之益生組合物可作為食品或營養產品，諸如基於牛奶或乳清之發酵乳製品，或作為藥品經口投與。

【0183】 組合物可調配為益生菌。

【0184】 本發明之組合物包括治療有效量之本發明之細菌菌株。治療有效量之細菌菌株足以對患者發揮有益作用。治療有效量之細菌菌株可足夠傳遞至患者腸及/或部分或全部定殖於患者腸。

【0185】 例如適合於成年人之細菌日劑量可為約 1×10^{3} 至約 1×10^{11} 菌落形成單位(CFU)；例如約 1×10^{7} 至約 1×10^{10}CFU；在另一實例中，約 1×10^{6} 至約 1×10^{10} CFU。

【0186】 在某些實施例中，組合物含有相對於組合物之重量，約 1×10^{6} 至約 1×10^{11} CFU/g，例如約 1×10^{8} 至約 1×10^{10} CFU/g 之量的細菌菌株。劑量

可為例如 1 g、3 g、5 g 及 10 g。

【0187】 通常，益生菌，諸如本發明之組合物，視情況與至少一種適合益生化合物組合。益生化合物通常為不易消化之碳水化合物，諸如寡醣或多醣，或糖醇，其在上部消化道中不降解或吸收。已知之益生菌包括商業產品，諸如菊糖及反式半乳寡醣。

【0188】 在某些實施例中，本發明之益生菌組合物包括相對於組合物總重量，約 1 至約 30 重量% (例如 5 至 20 重量%)之量的益生菌化合物。碳水化合物可選自由以下組成之群：果寡糖(或 FOS)、短鏈果寡醣、菊糖、異麥芽寡糖、果膠、木寡醣(或 XOS)、幾丁寡醣(或 COS)、β-葡聚糖、阿拉伯膠改性及抗性澱粉、聚葡萄糖、D-塔格糖、阿拉伯膠纖維、角豆樹、燕麥及柑桔纖維。在一個態樣中，益生菌為短鏈果糖-寡醣(下文中簡單起見展示為 FOSs-c.c)；該等 FOSs-c.c 為不可消化之碳水化合物，一般由甜菜糖轉變獲得且包括三個葡萄糖分子鍵結之蔗糖分子。

【0189】 本發明之組合物可包含醫藥學上可接受之賦形劑或載劑。此類適合賦形劑之實例可見於參考文獻[51]中。用於治療用途之可接受之載劑或稀釋劑為醫藥技術中所熟知且描述於例如參考文獻[52]中。適合載劑之實例包括乳糖、澱粉、葡萄糖、甲基纖維素、硬脂酸鎂、甘露糖醇、山梨糖醇等等。適合稀釋劑之實例包括乙醇、甘油及水。醫藥載劑、賦形劑或稀釋劑之選擇可針對預期投藥途徑及標準醫藥實踐來選擇。醫藥組合物可包含任何適合黏合劑、潤滑劑、懸浮劑、包被劑、增溶劑作為載劑、賦形劑或稀釋劑或除載劑、賦形劑或稀釋劑之外可包含該等物質。適合黏合劑之實例包括澱粉、明膠、天然糖(諸如葡萄糖、無水乳糖、自由流動乳糖、β-乳糖、玉米甜味劑)、天然及合成樹膠(諸如阿拉伯膠、黃蓍膠)或海藻酸鈉、羧甲基纖維素及聚乙二醇。適合潤滑劑之實例包括油酸鈉、硬脂酸鈉、硬脂酸鎂、苯甲酸鈉、乙酸鈉、氯化鈉等等。防腐劑、穩定劑、染料及甚至調味劑可提供於醫藥組合物中。防腐劑之實例包括苯甲酸鈉、山梨酸及對羥基苯甲酸酯。亦可使用抗氧化劑及懸浮劑。

【0190】 本發明之組合物可調配為食品。舉例而言，除本發明之治療作用之外，食品可提供營養益處，諸如營養增補劑中。類似地，食品可經

調配以增強本發明組合物之口味，或藉由使其更類似於常見食品而非醫藥組合物，使得組合物食用起來更具吸引力。在某些實施例中，本發明之組合物調配為基於牛奶之產品。術語「基於牛奶之產品」意謂具有變化脂肪含量之任何液體或半固體基於牛奶或乳清之產品。基於牛奶之產品可為例如奶牛奶、山羊奶、綿羊奶、脫脂乳、全乳、無任何加工下奶粉與乳清重組之牛奶或加工產品，諸如酸乳酪、凝乳、凝塊、酸牛奶、酸全乳、酪乳及其他酸牛奶產品。另一重要組包括乳製飲料，諸如乳清飲料、發酵牛奶、煉乳、嬰兒或嬰孩牛奶；增香乳、霜淇淋；含牛奶之食品，諸如甜食。

【0191】 在某些實施例中，本發明之組合物含有單一細菌菌株或物種且不含有任何其他細菌菌株或物種。此類組合物可僅僅包含最低限度或生物學上不相關量之其他細菌菌株或物種。此類組合物可為基本上不含其他生物體物種之培養物。

【0192】 根據本發明使用之組合物可需要或可不需要銷售批准。

【0193】 在一些情況下，凍乾細菌菌株在投與之前復原。在一些情況下，復原係藉由使用本文中描述之稀釋劑。

【0194】 本發明之組合物可包含醫藥學上可接受之賦形劑、稀釋劑或載劑。

【0195】 在某些實施例中，本發明提供一種醫藥組合物，其包含：本發明之細菌菌株；及醫藥學上可接受之賦形劑、載劑或稀釋劑；其中細菌菌株在投與有需要之受試者時量足夠治療病症；且其中病症係選自由以下組成之群：氣喘、過敏性氣喘、中性粒細胞性氣喘、骨關節炎、牛皮癬性關節炎、幼年特發性關節炎、視神經脊髓炎(德維克氏病)、強直性脊椎炎、脊椎關節炎、全身性紅斑狼瘡、乳糜瀉、慢性阻塞性肺病(COPD)、癌症、乳癌、結腸癌、肺癌、卵巢癌、葡萄膜炎、鞏膜炎、血管炎、白塞氏病、動脈粥樣硬化、異位性皮炎、肺氣腫、牙周炎、過敏性鼻炎及同種異體移植排斥。

【0196】 在某些實施例中，本發明提供醫藥組合物，其包含：本發明之細菌菌株；及醫藥學上可接受之賦形劑、載劑或稀釋劑；其中細菌菌株之量足夠治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀。在較佳實施

例中，該疾病或病狀選自由以下組成之群：類風濕性關節炎、多發性硬化、牛皮癬、發炎性腸病、克羅恩氏病、潰瘍性結腸炎、乳糜瀉、氣喘、過敏性氣喘、中性粒細胞性氣喘、骨關節炎、牛皮癬性關節炎、幼年特發性關節炎、視神經脊髓炎(德維克氏病)、強直性脊椎炎、脊椎關節炎、全身性紅斑狼瘡、慢性阻塞性肺病(COPD)、癌症、乳癌、結腸癌、肺癌、卵巢癌、葡萄膜炎、鞏膜炎、血管炎、白塞氏病、動脈粥樣硬化、異位性皮炎、肺氣腫、牙周炎、過敏性鼻炎及同種異體移植排斥。

【0197】 在某些實施例中，本發明提供以上醫藥組合物，其中細菌菌株之量為相對於組合物之重量每公克約 1×10^{3} 至約 1×10^{11} 菌落形成單位。

【0198】 在某些實施例中，本發明提供以上醫藥組合物，其中組合物以 1 g、3 g、5 g 或 10 g 之劑量投與。

【0199】 在某些實施例中，本發明提供以上醫藥組合物，其中組合物藉由選自由口腔、直腸、皮下、鼻、頰及舌下組成之群的方法投與。

【0200】 在某些實施例中，本發明提供以上醫藥組合物，其包含選自由乳糖、澱粉、葡萄糖、甲基纖維素、硬脂酸鎂、甘露糖醇及山梨糖醇組成之群的載劑。

【0201】 在某些實施例中，本發明提供以上醫藥組合物，其包含選自由乙醇、甘油及水組成之群的稀釋劑。

【0202】 在某些實施例中，本發明提供以上醫藥組合物，其包含選自由以下組成之群的賦形劑：澱粉、明膠、葡萄糖、無水乳糖、自由流動之乳糖、β-乳糖、玉米甜味劑、阿拉伯膠、黃蓍膠、海藻酸鈉、羧甲基纖維素、聚乙二醇、油酸鈉、硬脂酸鈉、硬脂酸鎂、苯甲酸鈉、乙酸鈉及氯化鈉。

【0203】 在某些實施例中，本發明提供以上醫藥組合物，其進一步包含防腐劑、抗氧化劑及穩定劑中之至少一種。

【0204】 在某些實施例中，本發明提供以上醫藥組合物，其包含選自由苯甲酸鈉、山梨酸及對羥基苯甲酸酯組成之群的防腐劑。

【0205】 在某些實施例中，本發明提供以上醫藥組合物，其中該細菌菌株係凍乾的。

【0206】 在某些實施例中，本發明提供以上醫藥組合物，其中當組合

物儲存在密封容器中約 4℃或約 25℃下且容器置於具有 50%相對濕度之氛圍中時，在至少約 1 個月、3 個月、6 個月、1 年、1.5 年、2 年、2.5 年或 3 年時期後如以菌落形成單位量測之至少 80%細菌菌株殘留。

培養方法

【0207】 用於本發明之細菌菌株可使用如例如參考文獻[53-55]中詳述之標準微生物學技術培養。

【0208】 擬桿菌屬之細菌菌株可藉由使用諸如下文概述之方法，可提供良好生長及可靠性之方法來培養。此方法尤其可用於培養物種人糞擬桿菌之菌株。

【0209】 擬桿菌屬及尤其物種人糞擬桿菌之細菌菌株可藉由使用液體原料接種盤(或更大液體培養物)來培養，而非如一般可使用之一層冷凍原料(參見例如參考文獻[56])。若擬桿菌屬菌株之冷凍原料解凍且用作液體培養物，則成熟菌落之建立為可靠且迅速的。

【0210】 一種適於培養擬桿菌屬之細菌菌株的方法可包括：

(a) 提供細菌菌株之冷凍原料；

(b) 將冷凍原料解凍以提供液體原料；

(c) 將液體原料添加至固體或液體培養基中；以及

(d) 培育固體或液體培養基以提供細菌菌株之培養物。

【0211】 此方法尤其適用於培養人糞擬桿菌之細菌菌株。

【0212】 冷凍原料可為冷凍甘油原液。固體或液體培養基可為 YCFA 瓊脂或 YCFA 培養基。YCFA 培養基可包括(每 100 ml，近似值)：酪胰(1.0 g)、酵母提取物(0.25 g)、$NaHCO_3$ (0.4 g)、半胱胺酸(0.1 g)、K_2HPO_4 (0.045 g)、KH_2PO_4 (0.045 g)、NaCl (0.09 g)、$(NH_4)_2SO_4$ (0.09 g)、$MgSO_4 \cdot 7H_2O$ (0.009 g)、$CaCl_2$ (0.009 g)、刃天青(0.1 mg)、氯化血紅素(1 mg)、生物素(1 μg)、鈷胺素(1 μg)、對胺基苯甲酸(3 μg)、葉酸(5 μg)及吡哆胺(15 μg)。

【0213】 步驟(d)中之培育可進行至少 36 小時，諸如 48 或 72 小時。步驟(d)中之培育可在諸如厭氧罩之厭氧環境中進行。步驟(d)中提供之培養物可用於將細菌菌株之更大培養物繼代培養。此類繼代培養允許製備更大量之細菌，且可用於以商業規模提供本發明之組合物。

【0214】 步驟(b)中之解凍可在室溫下或用手加溫下進行。

【0215】 步驟(c)中添加之液體原料之量可在 300 μl 與 5 ml 之間，諸如 500 μl。原料可為 20%甘油原液。

【0216】 以上方法可用於製備醫藥組合物或食品，在此情況下該方法可包含將細菌菌株分離，視情況將細菌菌株凍乾，且將細菌菌株與一或多種醫藥學上可接受之賦形劑或載劑或一或多種食品組合的其他步驟。藉由該方法製備之此醫藥組合物或食品可用於治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀的方法中。

【0217】 培養擬桿菌屬及尤其物種人糞擬桿菌之細菌菌株的一示例性方法可包括：

1. 將 500 μl 20%甘油原液塗鋪至 YCFA 瓊脂中。
2. 盤置於厭氧罩中 48/72 小時以產生成熟菌落。
3. 將細胞在 10 ml 體積中培養，無論此來自單個菌落還是 1%液體繼代培養物。
4. 在實現成熟菌落形態後具有菌落之盤僅僅使用 2/3 天。

用於疫苗組合物中之細菌菌株

【0218】 本發明者已確定本發明之細菌菌株可用於治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀。此可能為本發明之細菌菌株作用於宿主免疫系統之結果。因此，當作為疫苗組合物投與時，本發明之組合物亦可用於預防由 IL-17 或 Th17 路徑介導之疾病或病狀。在某些此類實施例中，本發明之細菌菌株可為殺死的、滅活的或減毒的。在某些此類實施例中，組合物可包含疫苗佐劑。在某些實施例中，組合物用於經由注射，諸如經由皮下注射投與。

通則

【0219】 除非另外指明，否則本發明之實施將採用在此項技術技能內之習知化學、生物化學、分子生物學、免疫學及藥理學方法。此類技術在文獻中充分解釋。參見例如參考文獻[57]及[58-64]等。

【0220】 術語「包含」涵蓋「包括」以及「由……組成」，例如「包含」X 之組合物可僅僅由 X 組成，或可包括其他某物，例如 X + Y。

【0221】 關於數值 x 之術語「約」為視情況選用的且意謂例如 x ±10%。

【0222】 詞語「基本上」不排除「完全」，例如「基本上不含」Y 之組合物可完全不含 Y。必要時，詞語「基本上」可自本發明之定義省略。

【0223】 提及兩種核苷酸序列之間的序列一致性百分比意謂在比對時，比較兩種序列中相同的核苷酸百分比。此比對及同源性或序列一致性百分比可使用此項技術中已知之軟體程式，例如參考文獻[65]之部分 7.7.18 中描述的軟體程式確定。較佳比對藉由 Smith-Waterman 同源性搜索演算法使用仿射空位搜索(其中開放空位罰分為 12 且空位延伸罰分為 2、BLOSUM 62 矩陣)來確定。Smith-Waterman 同源性搜索演算法在參考文獻[66]中揭示。

【0224】 除非特別陳述，否則包括多個步驟之製程或方法可在方法開始或結束包括其他步驟，或可包括其他插入步驟。此外，適當時步驟可組合，省去或以替代次序進行。

【0225】 本文中描述本發明之多個實施例。應瞭解每個實施例中說明之特徵均可與其他所說明之特徵組合以提供其他實施例。詳言之，本文中強調為適合、典型或較佳之實施例可彼此組合(除非其互斥時)。

用於進行本發明之模式

實例 1-房塵蟎誘發之氣喘之小鼠模型中細菌接種物之功效

概述

【0226】 向小鼠投與包含根據本發明之細菌菌株的組合物，且隨後用房塵蟎(HDM)提取物激發以引起過敏性發炎反應。對 HDM 之發炎反應包括嗜酸性粒細胞及中性粒細胞性組分，由 IL-17 及 Th17 路徑介導，且為氣喘模型。將用本發明組合物治療之小鼠所展現的發炎反應之量值及特徵與對照組相比。發現本發明之組合物減輕發炎反應，且減少嗜酸性粒細胞及嗜中性粒細胞募集，表明其可用於治療 IL-17 及 Th17 介導之病狀，諸如嗜酸性粒細胞增多、中性粒細胞增多及氣喘。

菌株

【0227】 675：人糞擬桿菌

研究設計

【0228】 組：

1. 陰性對照組。用媒劑對照處理(經口)。

3. 用治療性細菌接種物菌株 675 處理(經口)。

7. 陽性對照組。用地塞米松處理(腹膜內)。

8. 未處理之對照組。

【0229】 每組小鼠數目 =5

【0230】 第-14 天至第 13 天：每日經口投與媒劑對照(組 1)。

【0231】 第-14 天至第 13 天：每日經口投與治療性細菌接種物(組 2-6)。

【0232】 第 0、2、4、7、9、11 天，經鼻投與於 30 ul 體積 PBS 中之 15 ug HDM (房塵蟎提取物-目錄號：XPB70D3A25，批號：231897，Greer Laboratories, Lenoir, NC, USA) (組 1-8)。

【0233】 第 0、2、4、7、9、11 天投與地塞米松(腹膜內，3 mg/kg，Sigma-Aldrich，目錄號 D1159) (組 7)。

【0234】 第 14 天處死所有動物用於分析。

【0235】 小鼠總數 =40。

終點及分析

【0236】 在第 14 天，藉由腹膜內注射致命戊巴比妥(pentabarbitol)(Streuli Pharma AG, Uznach, 目錄號：1170139A)處死動物，接著立刻進行枝氣管肺泡灌洗(BAL)。

【0237】 細胞與 BAL (枝氣管肺泡灌洗)流體分離且進行細胞分類計數(200 個細胞計數/樣品)。

材料與方法

【0238】 小鼠。自 Charles River Laboratories 購買雌性 7 週齡 BALB/c 小鼠且隨機分配至籠，每籠均 5 隻小鼠(通風籠來源於 Indulab AG, Gams, Switzerland 籠型：“The SealsafeTM - IVC 籠。產品號 1248L)。籠標記有研究號、組號及實驗開始日期。每週監測小鼠且適應設施 7 天，接著開始研究(研究第-14 天)。在研究第-14 天動物為 8 週齡。飲用水及食物可隨意取用。存在籠富集。根據地方特許牌照號 2283.1 (經 Service de la consommation et

des affaires vétérinaires du Canton de Vaud 頒予及批准)進行動物之每日護理。飲用水及食物可隨意取用且每日補充一次。存在籠富集。觀測到如瑞士官方根據 FVO (Federal Veterinary Office)關於實驗動物飼養管理、遺傳修飾動物產生及動物實驗方法之條例 455.163 所給出的動物福利規定。

【0239】 **培養細菌接種物**。在無菌工作站內，藉由將手套手溫熱使細菌之低溫小瓶解凍且約 0.7 ml 內含物注射至含有 8 ml 厭氧 YCFA 之亨蓋特管(Hungate tube)(目錄號 1020471，Glasgerätebau Ochs, Bovenden-Lenglern, Germany)中。通常每個菌株準備兩個管。接著亨蓋特管在 37℃下培育(靜止)長達 24-26 小時(菌株 675)。

【0240】 因為發現接種物菌株 675 之細菌 OD 可變，所以每天進行 3 種不同培養方法。2 個小瓶如上所述培養，且第三個樣品利用 400 ul 來自前一天培養物之等分試樣進行接種來培養。在 4 個處理日時，由於自冷凍原料之生長不好，所以利用後一方法。值得注意地，此方法使得細菌菌株 675 一直穩固生長。

【0241】 **培養媒劑對照**。將含有 8 ml 厭氧 YCFA 之亨蓋特管在 37℃下培育(靜止) 16 小時。

【0242】 **投與細菌接種物或媒劑對照**。將 400 ul 培養之細菌接種物或媒劑對照每天根據經口管飼法投與。

【0243】 **鼻內敏化**。藉由腹膜內注射每公斤 9.75 mg xylasol 及 48.75 mg ketasol (Dr. E. Graeub AG, Bern, Switzerland)使小鼠麻醉且每個鼻投與於 30 ul 體積 PBS 中之 15 ug HDM (目錄號：XPB70D3A25，批號：231897，Greer Laboratories, Lenoir, NC, USA)。

【0244】 **製備及投與陽性對照化合物地塞米松**。地塞米松 21-磷酸二鈉鹽(Sigma-Aldrich，目錄號 D1159，貨號 N° SLBD.1030V)溶解在 H_2O 中，且以 3 mg/kg 之劑量於 200 ul 體積中在以上研究方案中所指示之日經口投與動物。

【0245】 **終末程序**。在第 14 天，藉由腹膜內注射致命戊巴比妥(Streuli PharmaAG, Uznach,目錄號：1170139A)處死動物，接著立刻進行 500 ul 生理食鹽水中枝氣管肺泡灌洗(BAL)。

【0246】 **量測 BAL 中之細胞浸潤**。細胞與 BAL 流體分離且基於標準形態學及細胞化學標準進行細胞分類計數。

【0247】 **圖表及統計分析**。所有圖表均用 Graphpad Prism 第 6 版產生且應用單向 ANOVA。來自統計分析之結果具備個別資料表。誤差線表示平均標準誤差(SEM)。

結果及分析

【0248】 實驗結果展示於圖 1-9 中。

【0249】 在用細菌或媒劑處理之小鼠中未記錄到發病或死亡。兩種對照媒劑處理(陰性對照)及地塞米松處理(陽性對照)如所預期表現，其中地塞米松處理後記錄到嗜酸性粒細胞增多及中性粒細胞增多減弱。

【0250】 此實驗之最重要結果展現在圖 6 及 7 中，圖 6 及 7 報告在用 HDM 激發後在枝氣管灌洗中偵測到之嗜中性粒細胞之總數及百分比。菌株 675 相對於僅僅媒劑之對照，減少 BAL 中嗜中性粒細胞總量及嗜中性粒細胞之比例。

實例 2-嚴重中性粒細胞性氣喘之小鼠模型中細菌接種物之功效

概述

【0251】 向小鼠投與包含根據本發明之細菌菌株之組合物且隨後藉由皮下投與房塵蟎(HDM)提取物來敏化，且藉由鼻內投與 HDM 激發，以模擬嚴重中性粒細胞性氣喘之發炎反應。將用本發明組合物治療之小鼠所展現的發炎反應之量值及特徵與對照組相比。發現本發明之組合物減輕發炎反應，且尤其減少嗜中性粒細胞之募集，方式與包括投與抗 IL-17 抗體之陽性對照相當。因此，資料表明本發明之組合物可用於治療 IL-17 及 Th17 介導之病狀，諸如中性粒細胞增多及氣喘。

菌株

【0252】 675：人糞擬桿菌

研究設計

【0253】 組：

1. 陰性對照組。用媒劑對照處理(經口)。

3. 用治療性細菌接種物菌株 675 處理(經口)。

7. 陽性對照組。用抗 IL-17 處理(腹膜內)。

8. 未處理之對照組。

9: 健康小鼠(基線)。

【0254】 每組小鼠數目(組 1-8) = 5

【0255】 第-14 天至第 17 天：每日經口投與媒劑對照(組 1)。

【0256】 第-14 天至第 17 天：每日經口投與治療性細菌接種物(組 2-6)。

【0257】 第 0 天：用 CFA 中 HDM 敏化(皮下) (組 1-8)

【0258】 第 7 天：用 CFA 中 HDM 敏化(皮下) (組 1-8)

【0259】 第 13、15、17 天：經腹膜內投與抗 IL-17 中和抗體(組 7)。

【0260】 第 14、15、16、17 天：經鼻用 30 ul PBS 中 HDM 激發(組 1-8)。

【0261】 第 18 天：處死所有動物用於分析。

終點及分析：

【0262】 在第 14 天，藉由腹膜內注射致命戊巴比妥(Streuli Pharma AG, Uznach, 目錄號：1170139A)處死動物，接著立刻進行枝氣管肺泡灌洗(BAL)。細胞與 BAL 流體分離且進行細胞分類計數(200 個細胞計數/樣品)。

材料與方法

【0263】 **小鼠**。自 Charles River Laboratories 購買雌性 7 週齡 C57BL/6 小鼠且隨機分配至籠，每籠均 5 隻小鼠(通風籠來源於 Indulab AG, Gams, Switzerland 籠型：“The SealsafeTM-IVC 籠。產品號 1248L)。籠標記有研究號、組號及實驗開始日期。每週監測小鼠且適應設施 7 天，接著開始研究(研究第-14 天)。在研究第-14 天動物為 8 週齡。飲用水及食物可隨意取用。存在籠富集。根據地方特許牌照號 2283.1 (經 Service de la consommation et des affaires vétérinaires du Canton de Vaud 頒予及批准)進行動物之每日護理。飲用水及食物可隨意取用且每日補充一次。存在籠富集。觀測到如瑞士官方根據 FVO (Federal Veterinary Office)關於實驗動物飼養管理、遺傳修飾動物產生及動物實驗方法之條例 455.163 所給出的動物福利規定。

【0264】 **培養細菌接種物**。在無菌工作站內，藉由將手套手溫熱使細

菌之低溫小瓶解凍且約 0.7 ml 內含物注射至含有 8 ml 厭氧 YCFA 之亨蓋特管(目錄號 1020471，Glasgerätebau Ochs, Bovenden-Lenglern, Germany)中。通常每個菌株準備兩個管。接著亨蓋特管在 37℃下培育(靜止)長達 24-26 小時(菌株 675)。

【0265】 因為接種物菌株 675 之細菌 OD 可變，所以每天進行 3 種不同培養方法。2 個小瓶如上所述培養，且第三個樣品利用 400 ul 來自前一天培養物之等分試樣進行接種來培養。在 4 個處理日時，由於自冷凍原料之生長不好，所以利用後一方法。值得注意地，此方法使得細菌菌株 675 一直穩固生長。

【0266】 **培養媒劑對照**。含有 8 ml 厭氧 YCFA 之亨蓋特管在 37℃下培育(靜止) 16 小時。

【0267】 **投與細菌接種物或媒劑對照**。將 400 ul 培養之細菌接種物或媒劑對照每天根據經口管飼法投與。

【0268】 **HDM 敏化**。將 PBS 中 50 μg HDM (目錄號：XPB70D3A25，批號：231897, Greer Laboratories, Lenoir, NC, USA)在等體積完全弗氏佐劑(complete Freund's adjuvant，CFA Chondrex Inc. Washington, USA)中乳化，且在兩週內在對側腰窩上以 200 μl 體積皮下投與兩次。第二次免疫接種後一週，藉由腹膜內注射每公斤 9.75 mg xylasol 及 48.75 mg ketasol (Dr. E. Graeub AG, Bern, Switzerland)使小鼠麻醉，接著連續 4 日，以 30 ul 體積 PBS 中 15 μg HDM 鼻內激發。最終激發後一天進行分析。

【0269】 **陽性對照化合物抗小鼠 IL-17 抗體之製備及投與**。抗 IL-17 中和抗體來源於 Bio X Cell 且儲存在 4℃下(純系 17F3，目錄號 BE0173，Bio X Cell)，且經腹膜內以 12.5 mg/kg 之劑量在以上研究方案中指示之日投與。

【0270】 **終末程序**。在第 18 天，藉由腹膜內注射致命戊巴比妥(Streuli Pharma AG, Uznach, 目錄號：1170139A)處死動物，接著立刻進行 500 ul 生理食鹽水中枝氣管肺泡灌洗(BAL)。

【0271】 **量測 BAL 中之細胞浸潤**。細胞與 BAL 流體分離且基於標準形態學及細胞化學標準進行細胞分類計數。

【0272】 **圖表及統計分析**。所有圖表均用 Graphpad Prism 第 6 版產生且應用單向 ANOVA。來自統計分析之結果具備個別資料表。誤差線表示平均標準誤差(SEM)。

結果及分析

【0273】 實驗結果展示於圖 10-18 中。

【0274】 在用細菌或媒劑處理之小鼠中未記錄到發病或死亡。如圖 15 及 16 中所示，菌株 675 展示強烈作用及相對於陰性對照，減少之嗜中性粒細胞總數。另外，菌株 675 相對於對照，減少之嗜酸性粒細胞數目，如圖 11 及 12 中所示。

實例 3-II 型膠原蛋白誘發之關節炎小鼠模型中細菌接種物治療關節炎之功效

材料與方法

菌株

【0275】 675：人糞擬桿菌

細菌培養物

【0276】 細菌培養物根據以下生長流程在厭氧工作站(Don Whitley Scientific)中生長用於投與。

- 週一/週三/週五：將甘油原液轉移至冰且將 YCFA 盤自甘油原液劃線。如下使用盤，最多 3 天。

- 每天 PM：自盤培養物挑選每個菌株之單個菌落，轉移至 8 ml 含有 YCFA 之亨蓋特管隔夜(ON1)

- AM：取 80 ul (1%) ON1 繼代培養物於新鮮 8 ml 管中(DC1)。使用 ON1 培養物用於 AM 經口管飼法。

- PM：使用 DC1 培養物用於 PM 經口管飼法。

【0277】 細菌菌株#675 使用甘油原液生長。

【0278】 甘油原液儲存在-80℃下。每週三次，甘油原液在室溫下解凍且在 YCFA 盤上劃線。每次使用新的甘油等分試樣。使細菌在給定盤上生長多達 72 小時。

【0279】 待投與動物之溶液每日製備兩次，上午(AM)及下午(PM)處

理具有八小時時間間隔。自劃線盤挑選菌落，且轉移至含有 YCFA 培養基之管中。使細菌菌株#675 生長 16 小時，接著 AM 投與。細菌以 1%傳代培養至 YCFA 培養基中進行 PM 投與。在上午及下午處理製備後記錄每一菌株之 OD 值。

II 型膠原蛋白誘發之關節炎小鼠模型

【0280】 成年雄性 DBA/1 小鼠隨機分配至實驗組其使其適應兩週。在第 0 天，藉由皮下注射 100 微升含有 100 微克 II 型膠原蛋白(CII)於補充有 4 mg/ml 結核分枝桿菌(*Mycobacterium tuberculosis*) H37Ra 的不完全弗氏佐劑中之乳液來投與動物。在第 21 天，藉由皮下注射含有 100 μg II 型膠原蛋白於不完全弗氏佐劑中之加強乳液來投與動物。

【0281】 根據以下投與時程給與處理。自第-14 天直至第 45 天實驗結束，將動物每週稱重三次。自第 21 天直至實驗結束，每週針對關節炎之臨床徵象，包括後爪及前爪、橈腕(腕)關節及脛跗(踝)關節之腫脹對動物評分三次。

【0282】 在第 45 天，挑選小鼠且取終末血液樣品用於細胞介素分析。

【0283】 在第-14 天、第 0 天及第 45 天，收集糞便樣品用於微生物分析，立刻迅速冰凍且儲存在-80℃下。

【0284】 膠原蛋白誘發之關節炎(CIA)小鼠模型為公認的類風濕性關節炎小鼠模型[67]。用 CII 免疫接種引起包括類風濕性關節炎之若干重要病理學特徵，包括滑液增生、單核細胞浸潤及軟骨退變之發病機理。重要地，CIA 之發展藉由 Th17 細胞，經由分泌 IL-17A 介導[68]。潛於關節炎模型之免疫反應藉由使用補充有結核分枝桿菌之弗氏佐劑增強。

【0285】 在第 21 天，自每組中三個衛星動物收集脾。細胞在 II 型膠原蛋白存在或不存在下培養 72 小時。藉由 Luminex 定量培養物上清液中及終末血清中之包括 TNF-α、IL-6、IFN-γ、IL-4、IL-10 及 IL-17 之細胞介素。使用氚化胸腺嘧啶併入法定量細胞增殖。

處理組及劑量

【0286】 所有組 n = 15 (主要研究組 n = 12 且衛星組 n = 3)。

【0287】 用於生物治療劑之媒劑為酵母提取物-酪胰-脂肪酸(YCFA)

培養基。

	組	劑量	投與		疾病誘發
			途徑	方案	
1	媒劑	5 ml/kg	PO	BID：第-14天*-結束	第0天：膠原蛋白/CFA，一次，SC 第21天：膠原蛋白/IFA，一次，SC
4	生物治療劑 #675	5 ml/kg			

*PO：經口管飼法，SC：皮下注射，BID：每天兩次，CFA：完全弗氏佐劑。*除組4自第0天處理外*

體重

【0288】 自第-14天直至實驗結束，將動物每週稱重三次。將資料繪圖(平均值±SEM)。

非特定之臨床觀測結果

【0289】 自第-14天至實驗結束，每日檢查動物之非特定臨床徵象，包括姿勢異常(弓背)、異常皮毛情況(豎毛)及異常活動程度(活動減少或增加)。

臨床觀測結果

【0290】 自第21天直至第45天實驗結束，每週針對關節炎之臨床徵象，包括後爪及前爪、橈腕(腕)關節及脛跗(踝)關節之腫脹對動物評分三次。使用以下量表對每肢評分：(0)正常，(1)輕微腫脹，(2)輕度腫脹，(3)中度腫脹及(4)嚴重腫脹。藉由加入每肢評分計算臨床評分。動物之最大可能臨床評分為(16)。挑選兩個連續時刻之評分等於(12)之動物及任一時刻評分超過(12)之動物。將資料繪圖(平均值±SEM)。

細胞增殖分析

【0291】 第21天，每組挑選三個衛星動物且將脾解剖出。脾細胞在II型膠原蛋白存在或不存在下培養72小時。72小時後，細胞在氚化胸腺嘧啶存在下脈衝隔夜。藉由量測胸苷併入來定量細胞增殖。將資料繪圖(平均值±SEM)。取上清液且測試關鍵細胞介素之存在。

細胞介素分析

【0292】 藉由 Luminex 測試來自脾細胞培養物之終末上清液以定量

TNF-α、IL-6、IFN-γ、IL-4、IL-10 及 IL-17。將資料繪圖(平均值±SEM)。

微生物分析

【0293】 在第-14 天、第 0 天及第 45 天，自每個動物收集糞便樣品，立刻迅速冰凍且儲存在-80℃下。盲腸(包括內含物)立刻迅速冰凍且儲存在-80℃下。每日藉由塗鋪細菌進行細菌鑑別測試。

組織病理學

【0294】 在實驗結束時，後爪儲存在組織固定劑中。樣品轉移至脫鈣溶液中。將組織樣品加工，切片，且用蘇木精與曙紅染色。藉由對實驗設計不知情之合格組織病理學家，針對關節炎徵象，包括發炎、關節軟骨破壞及下層幹骺端骨骼之破壞，對切片評分。使用詳述評分系統(見下文)。將資料繪圖(平均值±SEM)。提供原始及分析資料以及代表性圖。

表 1：組織病理學評分系統

級別	描述
發炎	
0	正常關節
1	具有嗜中性粒細胞主宰之發炎的輕度滑液增生。關節間隙中少量嗜中性粒細胞及巨噬細胞。
2	具有涉及嗜中性粒細胞與巨噬細胞之中度至顯著發炎的滑液增生。關節間隙中嗜中性粒細胞及巨噬細胞；可能為一些壞死組織碎片。
3	具有涉及嗜中性粒細胞與巨噬細胞之顯著發炎的滑液增生。喪失滑膜細胞內層。發炎可自滑膜延伸至包括肌肉之周圍組織。關節間隙中許多嗜中性粒細胞及巨噬細胞，連同明顯的壞死組織碎片。
關節軟骨破壞	
0	正常關節
1	關節軟骨僅僅展示輕度退行性改變。可能周邊存在早期血管翳形成。
2	關節軟骨展示中度退行性改變及局灶損失。局灶上存在血管翳形成。
3	關節軟骨顯著破壞及喪失，大範圍血管翳形成。
下層幹骺端骨骼之破壞	
0	正常關節
1	下層幹骺端骨骼無變化
2	可能為幹骺端骨骼之局灶性壞死或纖維化。

3	幹骺端骨骼破壞或塌縮。大範圍發炎、壞死或纖維化，延伸至幹骺端之髓腔。

結果及分析

存活及非特異性臨床觀測結果

【0295】 一些動物因關節炎之臨床徵象的嚴重程度或因非特定臨床觀測結果之嚴重程度而在研究時程結束之前淘汰。

【0296】 組 1 中之 1 隻動物淘汰(媒劑處理之動物自供應者處到達後斷腿)。

【0297】 11 隻動物因關節炎臨床徵象之嚴重程度而淘汰：組 1 中之 5 隻動物(媒劑處理)及組 4 中之 6 隻動物(生物治療劑#675 處理)。

【0298】 5 隻動物因包括姿勢異常(弓背)、異常皮毛情況(豎毛)、異常活動程度(活動減少)之非特定臨床徵象之嚴重程度而淘汰：組 1 中 3 隻動物(媒劑處理)及組 4 中 2 隻動物(生物治療劑#675 處理)。

體重

【0299】 自第-14 天直至第 0 天記錄體重資料，且表示為初始(第-14 天)體重之百分比，藉由雙向 ANOVA，接著鄧尼特事後檢驗(Dunnett's post-test)分析，以與第-14 天進行多重比較，接著與媒劑處理組進行多重比較。資料呈現於圖 19 中。來自實驗時程結束之前淘汰之動物的資料自分析中排除。

【0300】 當與第-14 天比較時，在媒劑處理組中，在第-9 天及第-7 天，藉由經口管飼法每日投與兩次誘發顯著體重損失。

【0301】 第-11 天直至第-1 天，組 4 (未處理，直至第 0 天，接著生物治療劑#675 處理)體重顯著高於媒劑處理組中($p < 0.0001$，除了第-4 天，其中 $p < 0.05$)。

【0302】 在生物治療劑處理組中第-14 天與第-1 天之間量測的體重不與在任何給定天在媒劑處理組中量測之體重不同。

【0303】 自第 0 天直至第 28 天記錄體重資料，且表示為初始(第 0 天)體重之百分比，藉由雙向 ANOVA，接著鄧尼特事後檢驗分析，以與媒劑組中第 0 天進行多重比較，接著與媒劑處理組進行多重比較。資料呈現於圖

20 中。來自實驗時程結束之前淘汰之動物及來自衛星動物的資料自分析中排除。第 28 天、第 35 天及第 42 天資料藉由單向 ANOVA，接著鄧尼特事後檢驗進一步分析，以與媒劑處理組進行多重比較。

【0304】 當與媒劑處理組中第 0 天比較時，在第 26 天及第 28 天關節炎臨床徵象之發作與顯著體重喪失有關($p < 0.0001$)。

【0305】 當與媒劑處理組比較時，在第 3 天、第 5 天、第 10 天($p < 0.05$)及第 26 天($p < 0.001$)體重顯著高於組 4 (生物治療劑#675 處理)。

【0306】 當藉由單向 ANOVA 分析資料時，當與媒劑處理組比較時，在第 28 天體重顯著高於組 4 (生物治療劑#675 處理)($p < 0.01$)。在第 35 天或第 42 天實驗組之間差異不顯著。

臨床觀測結果

【0307】 藉由雙向 ANOVA，接著鄧尼特事後檢驗分析臨床評分資料，以在媒劑處理組中各天之間進行多重比較，接著在實驗組與媒劑處理組之間每天進行多重比較。資料呈現於圖 21 中。自實驗結束之前淘汰之動物記錄的資料自分析中排除。當動物因關節炎臨床徵象之嚴重程度而淘汰時，報告以下天之最後記錄評分且用於統計分析中。

【0308】 當與第 21 天比較時，自第 28 天直至第 45 天，在媒劑處理組中觀測到臨床評分顯著增加($p < 0.0001$)。

【0309】 當與媒劑處理組比較時，生物治療劑#675 不減少臨床評分。當與其他實驗組比較時，此組中之動物在不同時間免疫接種，此可解釋所觀測到之較高臨床評分。

細胞增殖分析

【0310】 為使有效該分析，脾細胞在作為陽性對照刺激物之可溶性抗 CD3 及抗 CD28 (抗 CD3/CD28)存在下培養以證實細胞之增殖潛能。

【0311】 在所有實驗組中均看到對抗 CD3/CD28 之強烈增殖反應，展示細胞健康、有活力且能夠對活化信號作出反應。

【0312】 為測試在 II 型膠原蛋白 (CII)存在下之增殖反應，脾細胞在 50 μg/ml CII 存在下培養。藉由雙向 ANOVA，接著斯達克事後檢驗(Sydak's post-test)，分析對 CII 之脾細胞增殖反應，以在未刺激與 CII 刺激之脾細胞

之間進行多重比較，且藉由單向 ANOVA，接著鄧尼特事後檢驗分析，以將不同實驗組與媒劑處理組中 CII 刺激之反應進行比較。資料呈現於圖 22 中。

【0313】 當與媒劑處理組中未刺激脾細胞比較時，CII 誘發 ^{3}H-胸苷併入(cpm)極顯著增加($p < 0.0001$)。

【0314】 在不同天建立用生物治療劑#675 處理之組的脾細胞分析，因此不與媒劑處理組進行比較，不過觀測到顯著減少。

組織培養上清液中細胞介素含量

【0315】 藉由 luminex 分析，在來源於抗 CD3/CD28 刺激之培養物的組織培養上清液中，量測每種細胞介素之含量。此等展示所有量測之細胞介素的穩固反應(媒劑組中平均含量如下：IL-4 = 6,406 pg/ml；IL-6 = 306 pg/ml；IL-10 = 10,987 pg/ml；IL-17A = 11,447 pg/ml；IFN-γ = 15,581 pg/ml；TNF-α = 76 pg/ml)。

【0316】 以下部分概括自 II 型膠原蛋白刺激之培養物獲得的資料。適用時，未刺激及 CII 刺激之脾細胞之上清液中細胞介素含量之間的差異統計分析使用雙向 ANOVA，接著斯達克事後檢驗進行，以進行多重比較，而單向 ANOVA、接著鄧尼特事後檢驗用於比較生物治療劑處理組與媒劑處理組之 CII 刺激之反應。在兩種情況下該等組之間的細胞介素含量無顯著差異。此可能歸因於使用之樣本尺寸小($n = 3$)。

【0317】 為更精確地呈現細胞介素之資料分佈與資料之大體佈置，此作為散佈圖呈現。

【0318】 在用 CII 刺激後組織培養上清液中 IL-4 之組平均值<5 pg/ml。此等不視為生物學上顯著的且此處不包括。用膠原蛋白刺激後組織培養上清液中 TNF-α 之組平均值低於定量限度。

IFN-γ 之上清液含量(图 23)

【0319】 與 IL-17 一起，IFN-γ 為 CIA 模型中驅動疾病之主要細胞介素。圖 23 中之散佈圖證明 CII 刺激後 IFN-γ 含量，與生物治療劑比較，媒劑處理組之組中位數更高(參見圖 27)。

IL-17A 之上清液含量(图 24)

【0320】 在媒劑處理組之 CII 刺激之培養物中 IL-17A 含量為 50

pg/ml。與媒劑處理組比較，在生物治療劑組中此細胞介素之含量似乎較低(參見圖 27)。

IL-10 之上清液含量(图 25)

【0321】 媒劑處理組中 CII 刺激及培養基對照培養物之 IL-10 含量分別為 13 pg/ml 及 2.1 pg/ml。可預期媒劑處理組之 IL-10 (其為消炎細胞介素)含量較高，因為發炎及促炎細胞介素誘發可以伴隨有消炎回饋機制。

IL-6 之上清液含量(图 26)

【0322】 諸如 IL-6 及 TNF-α 之發炎細胞介素在抗 CII 培養物中通常不高含量產生。然而，其含量可能由於免疫調節而改變。CII 刺激之培養物中 IL-6 含量少，達到 10 pg/ml。雖然高於培養基對照培養物，但此等差異太小，以致於無法提供進行統計分析之基本原理。

組 4 中之組織培養上清液-生物治療劑#675 (圖 27)

【0323】 在不同天建立此組的脾細胞培養物，且因此與媒劑處理組分開。雖然直接比較可能不適當，但似乎用生物治療劑#675 處理有效降低 IFN-γ、IL-17A 及 IL-6 含量。

微生物分析

【0324】 藉由使用光譜儀量測 600 nm 下光密度來證實細菌生長。藉由比較劃線盤圖像與參考圖像，證實細菌身份。

【0325】 在改良之細菌製備方法後，如量測之高 OD 值所指示，自第-2 天及第-3 天(除了菌株#675，自第 0 天)投與一致高劑量之細菌菌株。

【0326】 在第-14 天、第 0 天及終止時收集糞便樣品且迅速冰凍。

組織病理學

【0327】 組織病理學結果展示於圖 66-71 中。如對此模型所預期，根據關節炎存在/不存在或存在之改變嚴重程度，觀測個體內及個體間變化性。

【0328】 病理性質如針對此模型所預期，滑膜及滑液囊大範圍混合型慢性活動性發炎，延伸至涉及關節周圍之軟組織(肌肉、脂肪組織、皮膚膠原蛋白)。在最嚴重受影響之關節中，存在關節軟骨退化及喪失，具有關節內碎片及發炎及關節及骨結構因纖維化及發炎而破壞。

【0329】 組織病理學改變之發生率為：媒劑-80% (16/20)；生物治療

劑#675-83% (20/24)。生物治療劑#675 有效減少在後肢關節中觀測到之組織病理學損傷且降低關節發炎評分、軟骨損傷評分、骨損傷評分及總組織病理學評分(參見圖 70)，不過未能與媒劑組進行統計學比較。

概述

【0330】 如 DBA/1 小鼠中關節炎模型中所預期，自 II 型膠原蛋白第一次投與後第 28 天觀測到臨床評分增加。生物治療劑#675 展示在此模型中有效治療關節炎。當與媒劑處理組比較時，用生物治療劑#675 處理之動物在不同時間免疫接種，此可解釋所觀測到之較高臨床評分。然而，生物治療劑#675 有效減少關節中之病理性疾病，如組織病理學分析中所證明。

【0331】 在來自所有實驗組之脾細胞培養物中均看到對 II 型膠原蛋白之增殖回憶反應。生物治療劑#675 之培養物未進行統計，因為此等培養物在不同時間建立，但觀測到相對於對照，膠原蛋白特定之反應減少。

【0332】 大部分所測試之 T 細胞細胞介素展示在媒劑處理組中 II 型膠原蛋白刺激之培養基與培養基對照之間可偵測之增加。此等增加在生物治療劑處理組中不如此明顯。此廣泛證明上述對 II 型膠原蛋白之增殖回憶反應。

【0333】 有證據證明抑制 Th1/Th17 軸，Th1/Th17 軸為此模型及人類 RA 中病原性反應。降低之細胞介素含量與減少之增殖的相關性暗示免疫調節。無證據證明此調節由 Th2 相關之 IL-4 含量增加或免疫調節細胞介素 IL-10 增加引起。

實例 4-房塵蟎誘發之氣喘之小鼠模型中細菌接種物之作用的進一步分析

【0334】 在實例 1 中測試之小鼠進行進一步分析以進一步表徵本發明之組合物對過敏性氣喘發炎反應之作用。

材料與方法

【0335】 **在第 14 天抽血及血清製備**。動物之血液樣品經由心臟刺穿收集。藉由在 14000g 下離心 5 分鐘，將血清自血液樣品分離且儲存在-20℃下。

【0336】 **在第 14 天移除器官**。左肺葉收集在福馬林中以進行改進型

組織學分析。收集右肺葉(所有剩餘葉)及移除血清以迅速冰凍及用於改進型分析。剩餘 BAL 流體迅速冰凍用於改進型分析。

血清及 BAL 流體中抗體含量之量測

【0337】 藉由 ELISA 分析，在 BAL 及血清中量測全部 IgE 及房塵蟎(HDM)特異性 IgG1 抗體產生。

肺分離及組織學分析

【0338】 左肺葉固定在福馬林中，接著埋入石蠟中，切片，且用蘇木精及曙紅及 PAS 染色。如下盲法進行隨後組織學評分：針對發炎(枝氣管周浸潤及血管周浸潤)及黏液產生，對每個樣品之 5 個隨機視場評分。發炎浸潤用以下分級系統評分：

0 - 正常

1 - 輕度發炎浸潤

2 - 中度發炎浸潤

3 - 顯著發炎浸潤

4 - 嚴重發炎浸潤

5 - 非常嚴重發炎浸潤

【0339】 在每個視場中，氣道以尺寸量測且黏液細胞數以 μM 定量。

量測肺組織中發炎介體

【0340】 分離用於定量發炎介體之右肺葉(所有剩餘葉)迅速冰凍，用於隨後藉由市售多工分析(Merck-Millipore)量測 CCL11、IFN-γ、IL-1α、IL-1β、IL-4、IL-5、IL-9、IL-17A、CXCL1、CCL3、CXCL2 及 CCL5。根據製造商之說明書進行分析。

結果及分析

【0341】 實驗結果展示於圖 28-46 中。

【0342】 支援實例 1 中描述之發現，用菌株 675 處理之小鼠之肺組織中細胞浸潤物之分析展示平均發炎評分顯著及統計上顯著之減少(參見圖 32 及 34)。

【0343】 分析 BAL 流體及血清中抗體含量(參見圖 28-31)。觀測到細菌處理對血清抗體含量無明確作用。此可反映實驗失敗，因為資料之散佈

及每一處理之誤差線大，且陽性及陰性對照似乎未如預期起作用。此外，基線血清抗體含量可已遮罩任何改變。

【0344】 類似地，觀測到細菌處理對肺組織中細胞介素含量無明確作用(參見圖 36-46)。再次，此可反映實驗失敗，因為資料之散佈及每一處理之誤差線大，且陽性及陰性對照似乎未如預期起作用。亦可能涉及之作用機制影響較早細胞介素反應，該等細胞介素反應在最終 HDM 氣道激發後第 4 天不再可偵測。歸因於所偵測之含量的變化性，當解釋當前研究中細胞介素資料時應謹慎一些。此變化性可藉由以下事實解釋：分離肺組織用於不同分析，且歸因於發炎之分佈不規則，因此一個肺葉可能不具充分代表性或可與其他小鼠中相同葉匹敵。

實例 5-嚴重中性粒細胞性氣喘之小鼠模型中細菌接種物之作用的進一步分析

【0345】 在實例 2 中測試之小鼠進行進一步分析以進一步表徵本發明之組合物對與嚴重氣喘相關之中性粒細胞性反應之作用。

材料與方法

【0346】 **在第 18 天移除器官**。左肺葉收集在福馬林中以進行改進型組織學分析。收集右肺葉(所有剩餘葉)及移除血清以迅速冰凍及用於改進型分析。剩餘 BAL 流體迅速冰凍用於改進型分析。

【0347】 **肺組織中發炎介體之量測(改進型分析)**。分離用於定量發炎介體之右肺葉(所有剩餘葉)迅速冰凍，用於隨後藉由市售多工分析(Merck-Millipore)量測 IFN-γ、IL-1α、IL-1β、CXCL1、CCL3、CXCL2、CCL5、IL-17A、TNF-α、IL-17F、IL -23 及 IL-33。根據製造商之說明書進行分析。

【0348】 **血清及 BAL 流體中抗體含量之量測(改進型分析)**。藉由 ELISA 分析，在 BAL 及血清中量測房塵蟎(HDM)特異性 IgG1 及 IgG2a 抗體產生。

【0349】 **肺分離及組織學分析(改進型分析)**。左肺葉固定在福馬林中，接著埋入石蠟中，切片，且用蘇木精及曙紅及 PAS 染色。如下盲法進行隨後組織學評分：針對發炎(枝氣管周浸潤及血管周浸潤)及黏液產生，對每個樣品之 5 個隨機視場評分。發炎浸潤用以下分級系統評分：

0 - 正常

1 - 輕度發炎浸潤

2 - 中度發炎浸潤

3 - 顯著發炎浸潤

4 - 嚴重發炎浸潤

5 - 非常嚴重發炎浸潤

結果及分析

【0350】 實驗結果展示於圖 47-64 中。

【0351】 抗體含量之進一步分析揭露細菌菌株 675 之功效亦反映在 BAL 流體及血清中 HDM 特異性 IgG1 含量降低(參見圖 47 及 49)。關於對 IgG2a 含量之作用的確定結論無法繪圖。總體而言，來自抗體分析之資料表明與對抗體同型切換之選擇性作用相對比，與總體減少之發炎反應相關的減少。

【0352】 組織學分析證明來自 BAL 流體之細胞分類計數，展示用菌株 675 處理之小鼠中細胞浸潤減少(參見圖 51-53)。

【0353】 關於細胞介素含量，如針對實例 4，資料之散佈及每一處理之誤差線大，且陽性及陰性對照似乎未如預期必要地起作用。亦可能涉及之作用機制影響較早細胞介素反應，該等細胞介素反應在最終 HDM 氣道激發後第 4 天不再可偵測。歸因於所偵測之含量的變化性，當解釋當前研究中解釋細胞介素資料時應謹慎一些。此變化性可藉由以下事實解釋：分離肺組織用於不同分析，且歸因於發炎之分佈不規則，因此一個肺葉可能不具充分代表性或可與其他小鼠中相同葉匹敵。儘管此變化性，展示菌株 675 對細胞介素含量之明確消炎作用，且陽性對照抗 IL-17 Ab 一般如預期起作用。

【0354】 在以上告誡下，圖 56、58、59、61 及 63 中之資料表明用本發明之細菌菌株處理可實現 IL-1b、IFNγ、RANTES、MIP-1a 及 KC (人類 IL-8 之小鼠直系同源物)之含量減少，此可表明與對基質或先天免疫細胞之趨化因子釋放(及因此細胞募集)之影響相關的作用機制。此等細胞介素屬於 Th17 路徑。將此資料集聯繫在一起，可得出以下明確結論：在嚴重中性粒

細胞性氣喘之此小鼠模型中菌株 675 高度有效保護小鼠避免發炎。

實例 6-多發性硬化之小鼠模型中細菌接種物之功效

概述

【0355】 向小鼠投與包含根據本發明之細菌菌株的組合物且隨後用髓磷脂少突神經膠質細胞醣蛋白將小鼠免疫接種以誘發實驗自體免疫腦脊髓炎(EAE)。EAE 為人類多發性硬化最常用之實驗模型。發現本發明之組合物對發病率及疾病嚴重程度具有顯著作用。

菌株

【0356】 675：以登記號 NCIMB 42408 寄存之細菌

研究設計

【0357】 組：

1. 陰性對照組。用媒劑對照處理(經口)。

3. 用治療性細菌接種物菌株 675 處理(經口)。

9. 陽性對照組。用地塞米松處理(腹膜內)。

10. 未處理之對照組。

【0358】 每組小鼠數目 = 10

【0359】 第-14 天至第 27 天：每日經口投與媒劑對照(組 1)。

【0360】 第-14 天至第 27 天:每日經口投與治療性細菌接種物(組 4)。

【0361】 第 0-28 天：一週投與地塞米松(腹膜內)三次(組 9)

【0362】 第 0 天：MOG35-55 (髓磷脂少突神經膠質細胞醣蛋白-2mg/ml)及 CFA (2 mg/ml MTB) 1:1 混合，產生 1 mg/ml 溶液。100 µl 肽-CFA 混合物皮下注射至每個後腿中。腹膜內投與百日咳毒素(300 ng)。

【0363】 第 1 天：腹膜內投與百日咳毒素(300 ng)。

【0364】 第 7 天-向前：量測發病率及稱重，一週三次。

終點及分析

【0365】 分析小鼠之發病率及疾病嚴重程度，一週三次。評分盲法進行。使用在 0 至 5 範圍內之臨床評分評估疾病嚴重程度，其中 5 指示小鼠死亡(參見以下臨床評分系統)。

監測

【0366】 在指示天數，將小鼠稱重且觀測疾病活動評分及發病率。

【0367】 疾病活動評分觀測結果：

0 - 與未免疫接種小鼠比較，運動功能無明顯變化。

0.5 - 尾部尖端無力。

1.0 - 尾巴無力。

1.5 - 尾巴無力及後腿抑制。

2.0 - 尾巴無力及後腿虛弱。

或-當觀測行走時存在明顯頭部傾斜徵象。平衡性差。

2.5 - 尾巴無力及後腿打滑。

- 或-存在強烈頭部傾斜，引起小鼠有時跌倒。

3.0 - 尾巴無力及後腿完全麻痹。

3.5 - 尾巴無力及後腿完全麻痹。

外加：小鼠繞籠移動，但當置於其側面上時，不能恢復常態。

後腿一起在身體一側上。

4.0 - 尾巴無力、後腿完全癱瘓及前腿部分癱瘓。

- 小鼠最低限度地繞籠移動，但似乎警覺且進食

4.5 - 後腿完全癱瘓及前腿部分癱瘓，不繞籠運動。

立刻對小鼠執行安樂死且自籠移除。

5.0 因嚴重癱瘓而對小鼠執行安樂死。

【0368】 當動物具有等於或大於 1 之疾病活動評分時，認為其具有正發病評分。

結果

【0369】 研究結果展示於圖 72 及 73 中。

【0370】 陰性對照組中疾病誘發成功，其中媒劑對照及未處理之對照展示高分。用菌株 675 處理之作用顯著且用菌株 675 處理之小鼠展現顯著降低之發病率及疾病嚴重程度。實際上，發病率及疾病嚴重程度降低可與陽性對照組相當。此等資料指示菌株 675 可用於治療或預防多發性硬化。

實例 7-穩定性測試

【0371】 含有至少一種本文中描述之細菌菌株的本文中描述之組合

物儲存在密封容器中 25℃或 4℃下且容器置於具有 30%、40%、50%、60%、70%、75%、80%、90%或 95%相對濕度之氛圍中。1 個月、2 個月、3 個月、6 個月、1 年、1.5 年、2 年、2.5 年或 3 年後，至少 50%、60%、70%、80%或 90%之細菌菌株應殘留，如以藉由標準規方案測定之菌落形成單位量測。

序列

【0372】 SEQ ID NO:1 (16S rRNA 之人糞擬桿菌基因，部分序列，菌株：M11 - AB200223)

```
   1 agagtttgat cctggctcag gatgaacgct agctacaggc ttaacacatg caagtcgagg
  61 ggcagcatga acttagcttg ctaagtttga tggcgaccgg cgcacgggtg agtaacacgt
 121 atccaacctt ccgtttactc agggatagcc tttcgaaaga aagattaata cctgatagta
 181 tggtgagatt gcatgatggc accattaaag atttattggt aaacgatggg gatgcgttcc
 241 attaggtagt aggcggggta acggcccacc tagcctgcga tggatagggg ttctgagagg
 301 aaggtccccc acattggaac tgagacacgg tccaaactcc tacgggaggc agcagtgagg
 361 aatattggtc aatgggcgag agcctgaacc agccaagtag cgtgaaggat gaaggtccta
 421 cggattgtaa acttcttttа tacgggaata aagtttccta cgtgtaggat tttgtatgta
 481 ccgtatgaat aagcatcggc taactccgtg ccagcagccg cggtaatacg gaggatgcga
 541 gcgttatccg gatttattgg gtttaaaggg agcgcagacg ggagattaag tcagttgtga
 601 aagtttgcgg ctcaaccgta aaattgcagt tgatactggt ttccttgagt gcagttgagg
 661 caggcggaat tcgtggtgta gcggtgaaat gcttagatat cacgaagaac cccgattgcg
 721 aaggcagctt gctaaactgt aactgacgtt catgctcgaa agtgtgggta tcaaacagga
 781 ttagataccc tggtagtcca cacggtaaac gatggatact cgctgttggc gatatactgt
 841 cagcggccaa gcgaaagcat taagtatccc acctggggag tacgccggca acggtgaaac
 901 tcaaaggaat tgacgggggc ccgcacaagc ggaggaacat gtggtttaat tcgatgatac
 961 gcgaggaacc ttacccgggc ttaaattgca gacgaattac gaggaaactt gtaagccgca
1021 aggcgtctgt gaaggtgctg catggttgtc gtcagctcgt gccgtgaggt gtcggcttaa
1081 gtgccataac gagcgcaacc ctcgtggtca gttactaaca ggttaagctg agggctctgg

1141 ccagactgcc atcgtaagat gtgaggaagg tggggatgac gtcaaatcag cacggccctt
1201 acgtccgggg ctacacacgt gttacaatgg gaggtacaga aggccgctac ccggcaacgg
1261 gatgccaatc cccaaaacct ctctcagttc ggactggagt ctgcaacccg actccacgaa
1321 gctggattcg ctagtaatcg cgcatcagcc acggcgcggt gaatacgttc ccgggccttg
1381 tacacaccgc ccgtcaagcc atgaaagccg ggggtacctg aagtgcgtaa ccgcaaggag
1441 cgccctaggg taaaaccggt aattggggct aagtctaaca aggtaaccaa g
```

【0373】 SEQ ID NO:2 (16S rRNA 之人糞擬桿菌基因，部分序列，菌株：M16 - AB200224)

```
   1 agagtttgat cctggctcag gatgaacgct agctacaggc ttaacacatg caagtcgagg
  61 ggcagcatga acttagcttg ctaagtttga tggcgaccgg cgcacgggtg agtaacacgt
 121 atccaacctt ccgtttactc agggatagcc tttcgaaaga aagattaata cctgatagta
 181 tggtgagatt gcatgatggc accattaaag atttattggt aaacgatggg gatgcgttcc
 241 attaggtagt aggcggggta acggcccacc tagcctgcga tggatagggg ttctgagagg
 301 aaggtccccc acattggaac tgagacacgg tccaaactcc tacgggaggc agcagtgagg
 361 aatattggtc aatgggcgag agcctgaacc agccaagtag cgtgaaggat gaaggtccta
 421 cggattgtaa acttctttta tacgggaata aagtttccta cgtgtaggat tttgtatgta
 481 ccgtatgaat aagcatcggc taactccgtg ccagcagccg cggtaatacg gaggatgcga
 541 gcgttatccg gatttattgg gtttaaaggg agcgcagacg ggagattaag tcagttgtga
 601 aagtttgcgg ctcaaccgta aaattgcagt tgatactggt ttccttgagt gcagttgagg
 661 caggcggaat tcgtggtgta gcggtgaaat gcttagatat cacgaagaac cccgattgcg
 721 aaggcagctt gctaaactgt aactgacgtt catgctcgaa agtgtgggta tcaaacagga
 781 ttagataccc tggtagtcca cacggtaaac gatggatact cgctgttggc gatatactgt
 841 cagcggccaa gcgaaagcat taagtatccc acctggggag tacgccggca acggtgaaac
 901 tcaaaggaat tgacgggggc ccgcacaagc ggaggaacat gtggtttaat tcgatgatac
 961 gcgaggaacc ttacccgggc ttaaattgca gacgaattac gaggaaactt gtaagccgca
1021 aggcgtctgt gaaggtgctg catggttgtc gtcagctcgt gccgtgaggt gtcggcttaa
1081 gtgccataac gagcgcaacc ctcgtggtca gttactaaca ggttaagctg agggctctgg
1141 ccagactgcc atcgtaagat gtgaggaagg tggggatgac gtcaaatcag cacggccctt
1201 acgtccgggg ctacacacgt gttacaatgg gaggtacaga aggccgctac ccggcaacgg
1261 gatgccaatc cccaaaacct ctctcagttc ggactggagt ctgcaacccg actccacgaa
1321 gctggattcg ctagtaatcg cgcatcagcc acggcgcggt gaatacgttc ccgggccttg
1381 tacacaccgc ccgtcaagcc atgaaagccg ggggtacctg aagtgcgtaa ccgcaaggag
1441 cgccctaggg taaaaccggt aattggggct aagtctaaca aggtaaccaa
```

【0374】 SEQ ID NO:3 (16S rRNA 之人糞擬桿菌基因，部分序列，菌株：M158 - AB200225)

```
   1 agagtttgat cctggctcag gatgaacgct agctacaggc ttaacacatg caagtcgagg
  61 ggcagcatga acttagcttg ctaagtttga tggcgaccgg cgcacgggtg agtaacacgt
 121 atccaacctt ccgtttactc agggatagcc tttcgaaaga aagattaata cctgatagta
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【0378】 SEQ ID NO:7 (菌株 675 質體序列)-參見電子序列表。

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【符號說明】

無

【生物材料寄存】

國內寄存資訊【請依寄存機構、日期、號碼順序註記】

(1) 食品工業發展研究所；105年4月14日；BCRC910727

(2) 食品工業發展研究所；105年9月20日；BCRC910741

國外寄存資訊【請依寄存國家、機構、日期、號碼順序註記】

(1) 英國；國家食品工業和海洋細菌中心(National Collection of Industrial, Food and Marine Bacteria；NCIMB)；103 年 12 月 3 日；NCIMB 42341

(2) 英國；國家食品工業和海洋細菌中心(National Collection of Industrial, Food and Marine Bacteria；NCIMB)；104 年 5 月 13 日；NCIMB 42408

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發明摘要

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C12N 1/20 (2006.01)
A61P 11/06 (2006.01)
A61P 19/02 (2006.01)
A61P 29/00 (2006.01)
A61P 35/00 (2006.01)
A61P 37/00 (2006.01)

【發明名稱】(中文/英文)

包含細菌菌株之組合物

COMPOSITIONS COMPRISING BACTERIAL STRAINS

【中文】

本發明提供包含細菌菌株之組合物，其用於治療及預防發炎及自體免疫疾病。

【英文】

The invention provides compositions comprising bacterial strains for treating and preventing inflammatory and autoimmune diseases.

【代表圖】

【本案指定代表圖】：第（6）圖。

【本代表圖之符號簡單說明】：

無

【本案若有化學式時，請揭示最能顯示發明特徵的化學式】：

無

申請專利範圍

1. 一種包含擬桿菌屬之細菌菌株的組合物，其用於治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀的方法中。
2. 如申請專利範圍第 1 項之組合物，其中該組合物用於治療或預防選自由以下組成之群之疾病或病狀的方法中：氣喘，諸如過敏性氣喘或中性粒細胞性氣喘；關節炎，諸如類風濕性關節炎、骨關節炎、牛皮癬性關節炎或幼年特發性關節炎；多發性硬化；視神經脊髓炎(德維克氏病(Devic's disease))；強直性脊椎炎；脊椎關節炎；牛皮癬；全身性紅斑狼瘡；發炎性腸病，諸如克羅恩氏病(Crohn's disease)或潰瘍性結腸炎；乳糜瀉；慢性阻塞性肺病(COPD)；癌症，諸如乳癌、結腸癌、肺癌或卵巢癌；葡萄膜炎；鞏膜炎；血管炎；白塞氏病(Behcet's disease)；動脈粥樣硬化；異位性皮炎；肺氣腫；牙周炎；過敏性鼻炎；及同種異體移植排斥。
3. 如申請專利範圍第 2 項之組合物，其中該組合物用於治療或預防氣喘、諸如中性粒細胞性氣喘或過敏性氣喘之方法中。
4. 如申請專利範圍第 3 項之組合物，其中該組合物用於氣喘治療中減少中性粒細胞增多或嗜酸性粒細胞增多之方法中。
5. 如申請專利範圍第 2 項之組合物，其中該組合物用於治療或預防類風濕性關節炎之方法中。
6. 如申請專利範圍第 5 項之組合物，其中該組合物用於減少類風濕性關節炎中關節腫脹之方法中。
7. 如申請專利範圍第 2 項之組合物，其中該組合物用於治療或預防多發性硬化之方法中。
8. 如申請專利範圍第 7 項之組合物，其中該組合物用於降低發病率或疾病嚴重程度之方法中。
9. 如申請專利範圍第 2 項之組合物，其中該組合物用於治療或預防癌症、諸如肺癌、乳癌或肝癌之方法中。
10. 如申請專利範圍第 9 項之組合物，其中該組合物用於減少腫瘤尺寸、減少腫瘤生長、預防轉移或預防血管生成之方法中。
11. 如申請專利範圍第 2 項之組合物，其中該組合物用於治療或預防葡萄

膜炎之方法中。

12. 如申請專利範圍第 11 項之組合物，其中該組合物用於減少或預防葡萄膜炎中視網膜損傷之方法中。
13. 如前述申請專利範圍中任一項之組合物，其中該組合物用於由 IL-17 或 Th17 路徑介導之疾病或病狀的治療或預防中減少 IL-17 產生或減少 Th17 細胞分化之方法中。
14. 如前述申請專利範圍中任一項之組合物，其中該組合物用於具有升高之 IL-17 含量或 Th17 細胞的患者中。
15. 如前述申請專利範圍中任一項之組合物，其中該細菌菌株為人糞擬桿菌(*Bacteroides coprocola*)。
16. 如前述申請專利範圍中任一項之組合物，其中該細菌菌株具有與人糞擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致的 16s rRNA 序列。
17. 如申請專利範圍第 1 項至第 15 項中任一項之組合物，其中該細菌菌株具有與 SEQ ID NO:1、2、3 或 4 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。
18. 如申請專利範圍第 17 項之組合物，其中該細菌菌株具有與 SEQ ID NO:4 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列，或其中該細菌菌株具有由 SEQ ID NO:4 表示之 16s rRNA 序列。
19. 如申請專利範圍第 1 項至第 14 項中任一項之組合物，其中該細菌菌株為多形擬桿菌(*Bacteroides thetaiotaomicron*)。
20. 如申請專利範圍第 1 項至第 14 項中任一項之組合物，其中該細菌菌株具有與多形擬桿菌之細菌菌株之 16s rRNA 序列至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致的 16s rRNA 序列。
21. 如申請專利範圍第 1 項至第 14 項中任一項之組合物，其中該細菌菌株具有與 SEQ ID NO:5 至少 95%、96%、97%、98%、99%、99.5%或 99.9%一致之 16s rRNA 序列。
22. 如申請專利範圍第 21 項之組合物，其中該細菌菌株具有由 SEQ ID NO:5 表示之 16s rRNA 序列。
23. 如申請專利範圍第 1 項至第 14 項中任一項之組合物，其中該細菌菌株

為脆弱擬桿菌(*Bacteroides fragilis*)。

24. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種人糞擬桿菌之細菌菌株，用於治療或預防類風濕性關節炎之方法中。
25. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種人糞擬桿菌之細菌菌株，用於治療或預防氣喘、諸如中性粒細胞性氣喘或過敏性氣喘之方法中。
26. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種人糞擬桿菌之細菌菌株，用於治療或預防多發性硬化之方法中。
27. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種人糞擬桿菌之細菌菌株，用於治療或預防癌症之方法中。
28. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種人糞擬桿菌之細菌菌株，用於治療或預防葡萄膜炎之方法中。
29. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種多形擬桿菌之細菌菌株，用於治療或預防類風濕性關節炎之方法中。
30. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種多形擬桿菌之細菌菌株，用於治療或預防氣喘、諸如中性粒細胞性氣喘或過敏性氣喘之方法中。
31. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種多形擬桿菌之細菌菌株，用於治療或預防多發性硬化之方法中。
32. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種多形擬桿菌之細菌菌株，用於治療或預防癌症之方法中。
33. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種多形擬桿菌之細菌菌株，用於治療或預防葡萄膜炎之方法中。
34. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種脆弱擬桿菌之細菌菌株，用於治療或預防類風濕性關節炎之方法中。
35. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種脆弱擬桿菌之細菌菌株，用於治療或預防氣喘、諸如中性粒細胞性氣喘或過敏性氣喘之方法中。
36. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種脆弱擬桿菌之細菌菌株，用於治療或預防多發性硬化之方法中。

37. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種脆弱擬桿菌之細菌菌株，用於治療或預防癌症之方法中。
38. 如申請專利範圍第 1 項之組合物，其中該組合物包含物種脆弱擬桿菌之細菌菌株，用於治療或預防葡萄膜炎之方法中。
39. 如前述申請專利範圍中任一項之組合物，其中該組合物用於經口投與。
40. 如前述申請專利範圍中任一項之組合物，其中該組合物包含一或多種醫藥學上可接受之賦形劑或載劑。
41. 如前述申請專利範圍中任一項之組合物，其中該細菌菌株係凍乾的。
42. 一種食品，其包含如前述申請專利範圍中任一項之組合物，其用於前述申請專利範圍中任一項之用途中。
43. 一種疫苗組合物，其包含如前述申請專利範圍中任一項之組合物，其用於前述申請專利範圍中任一項之用途中。
44. 一種治療或預防由 IL-17 或 Th17 路徑介導之疾病或病狀的方法，其包括向有需要之患者投與包含擬桿菌屬之細菌菌株的組合物。
45. 一種以登記號 NCIMB 42408 (BCRC910741)寄存之人糞擬桿菌菌株或其衍生物的細胞。
46. 一種組合物，其包含如申請專利範圍第 45 項之細胞。
47. 如申請專利範圍第 46 項之組合物，其包含醫藥學上可接受之載劑或賦形劑。
48. 一種以登記號 NCIMB 42408 (BCRC910741)寄存之人糞擬桿菌菌株或其衍生物的生物純培養物。
49. 一種以登記號 NCIMB 42408 (BCRC910741)寄存之人糞擬桿菌菌株或其衍生物的細胞，其用於治療中。
50. 如申請專利範圍第 49 項之細胞，其中該細胞用於如申請專利範圍第 1 項至第 14 項中任一項中定義之方法中。

圖式

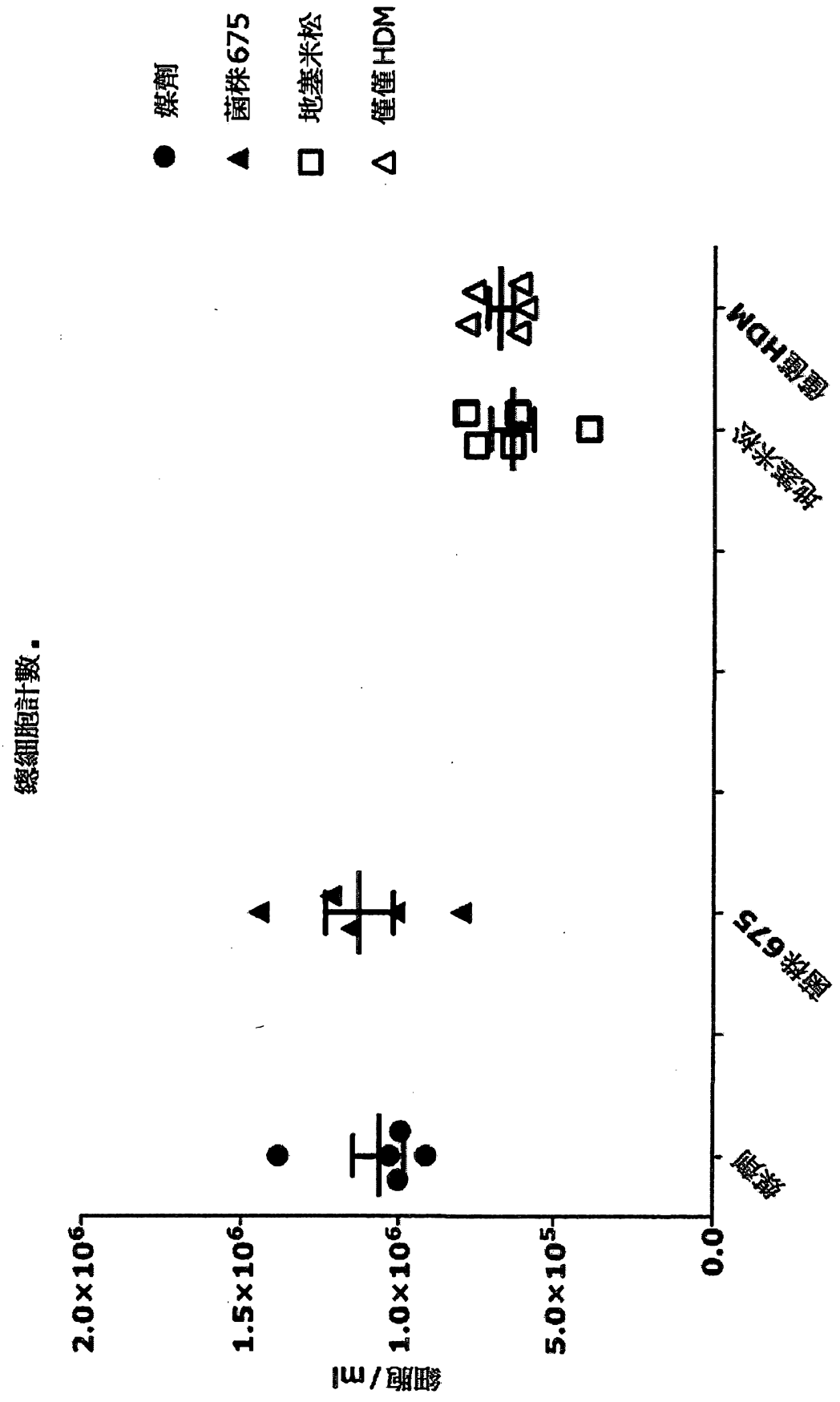

●媒劑
▲菌株675
□地塞米松
△僅僅HDM
總細胞計數。
細胞/ml
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0.0
媒劑
菌株675
地塞米松
僅僅HDM

圖 1

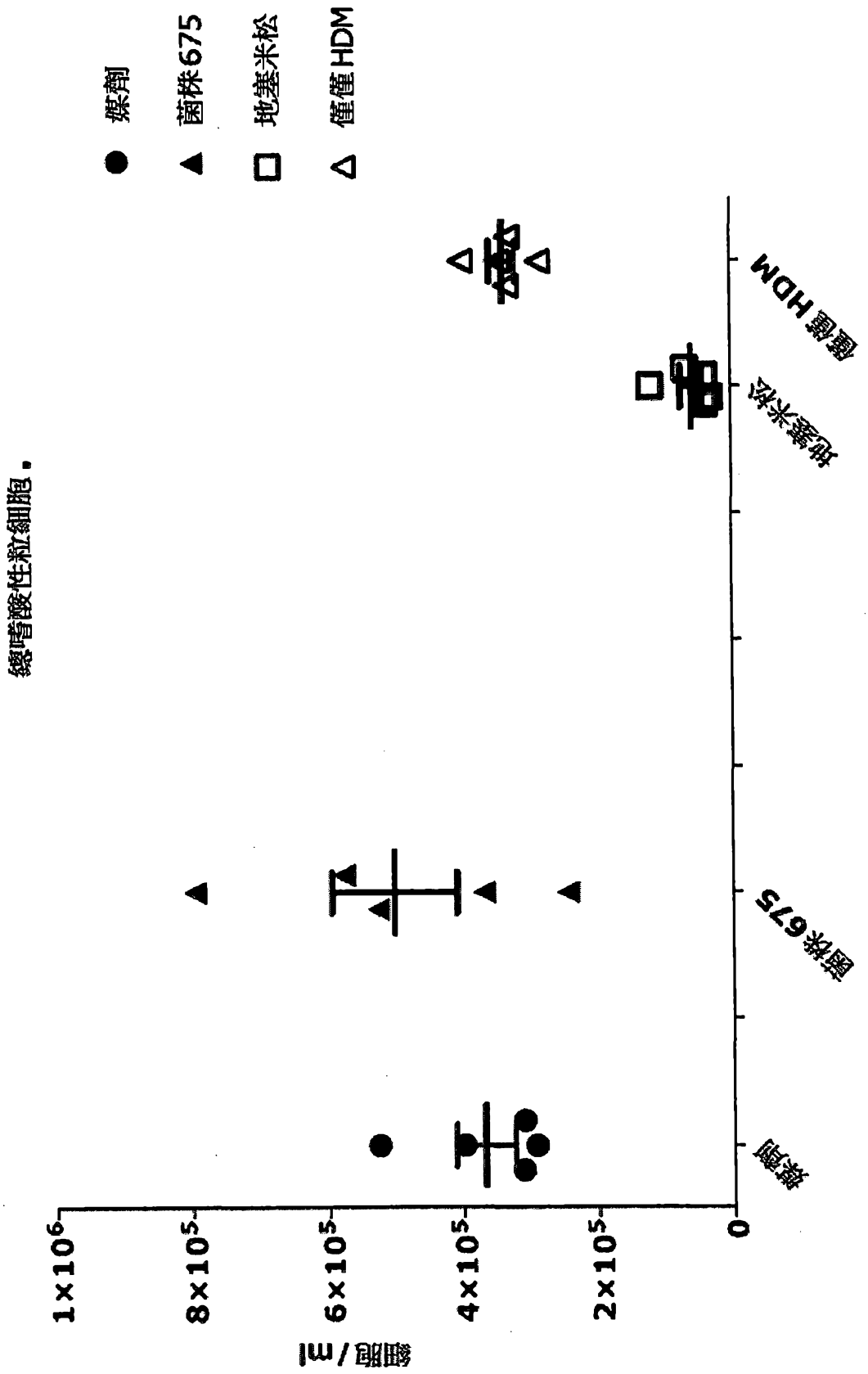

媒劑
菌株675
地塞米松
僅僅 HDM
細胞 / ml
1x10^6
8x10^5
6x10^5
4x10^5
2x10^5
0
媒劑
菌株675
地塞米松
僅僅 HDM
嗜酸性粒細胞。

圖 2

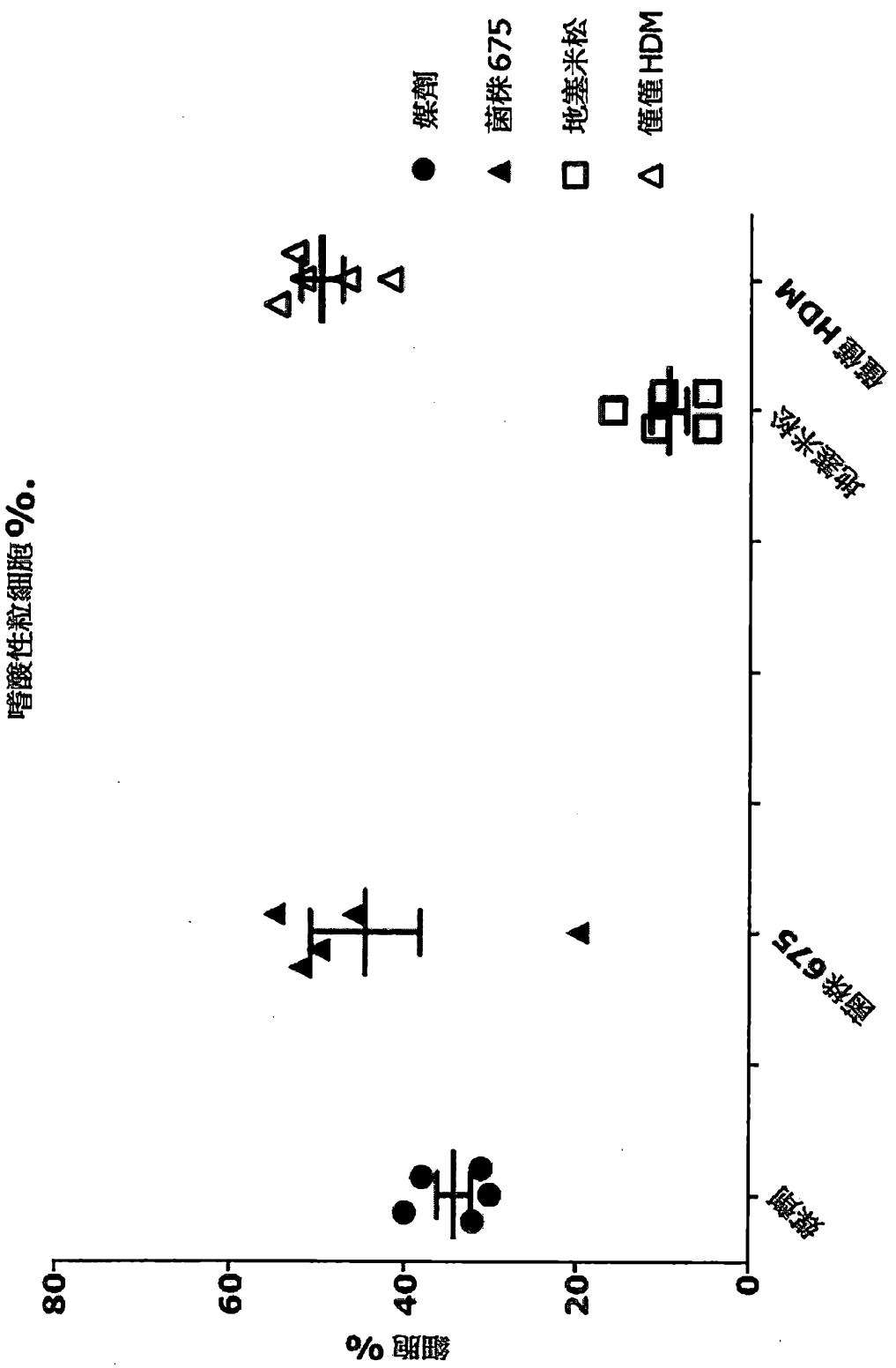


圖 3

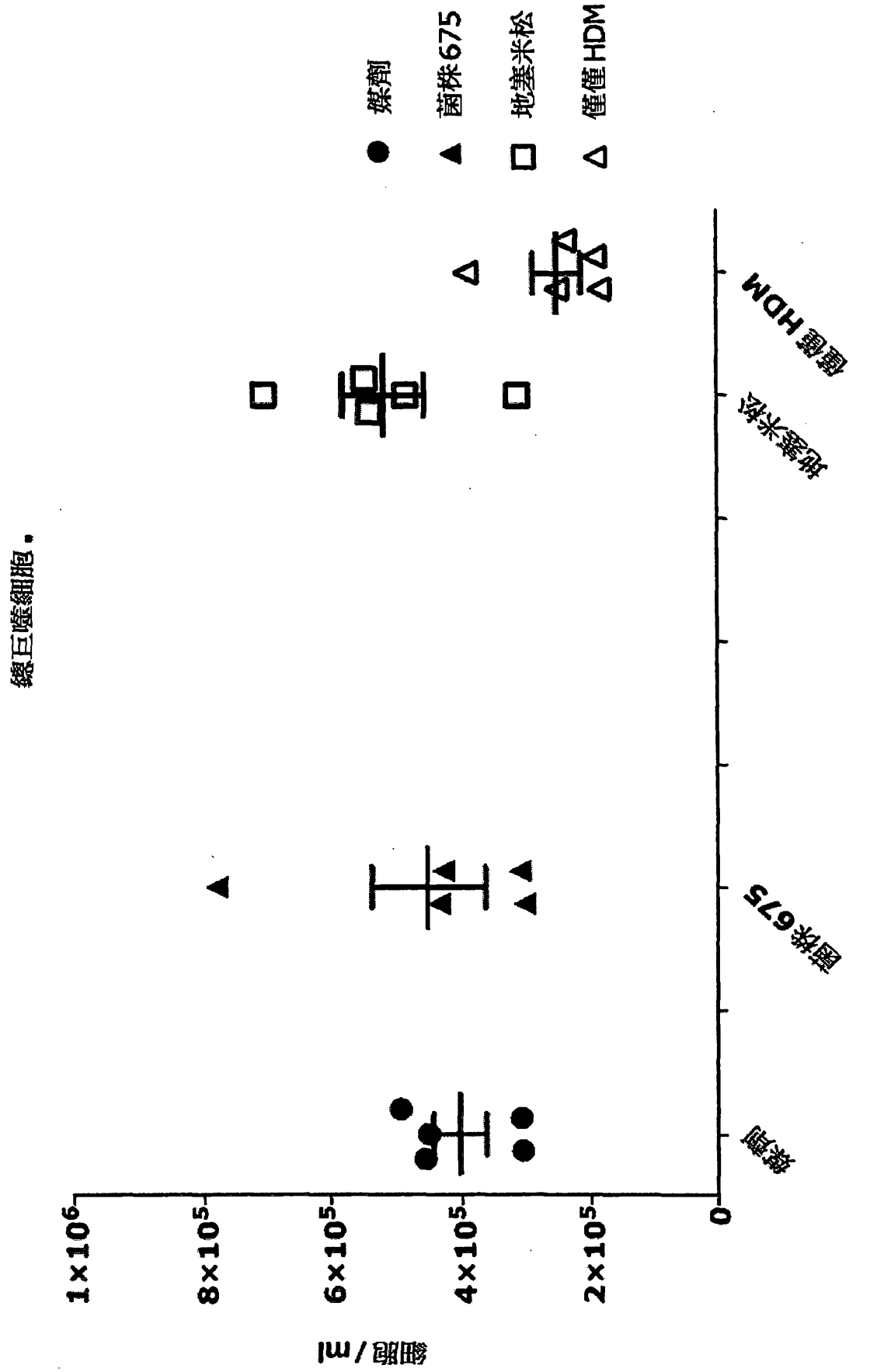

媒劑
菌株675
地塞米松
僅僅HDM
細胞 / ml
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
媒劑
菌株675
地塞米松
僅僅HDM
總巨噬細胞。

圖 4

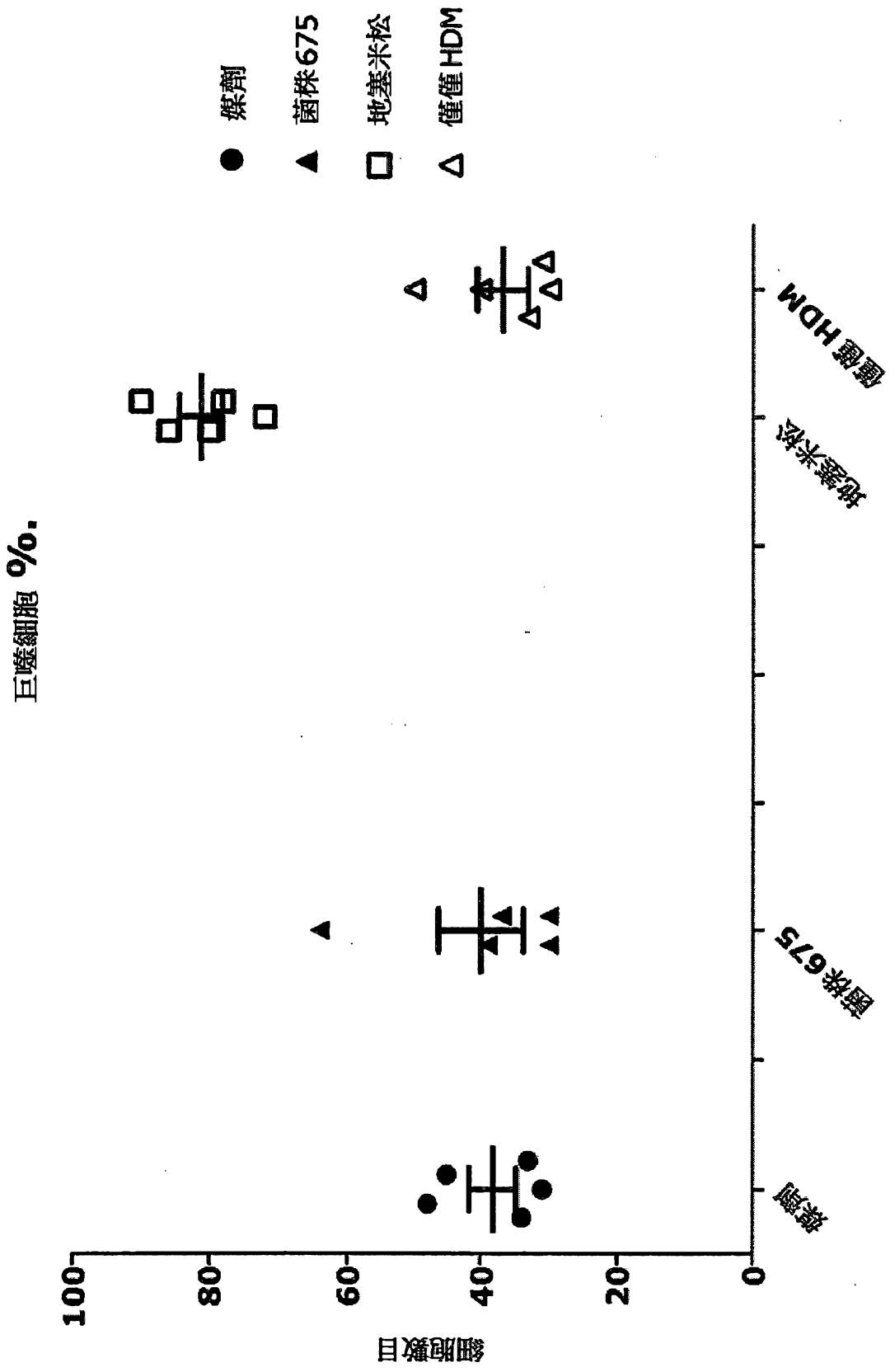

媒劑
菌株675
地塞米松
僅僅HDM
巨噬細胞 %.
細胞數目
100
80
60
40
20
0
媒劑
菌株675
地塞米松
僅僅HDM

圖 5

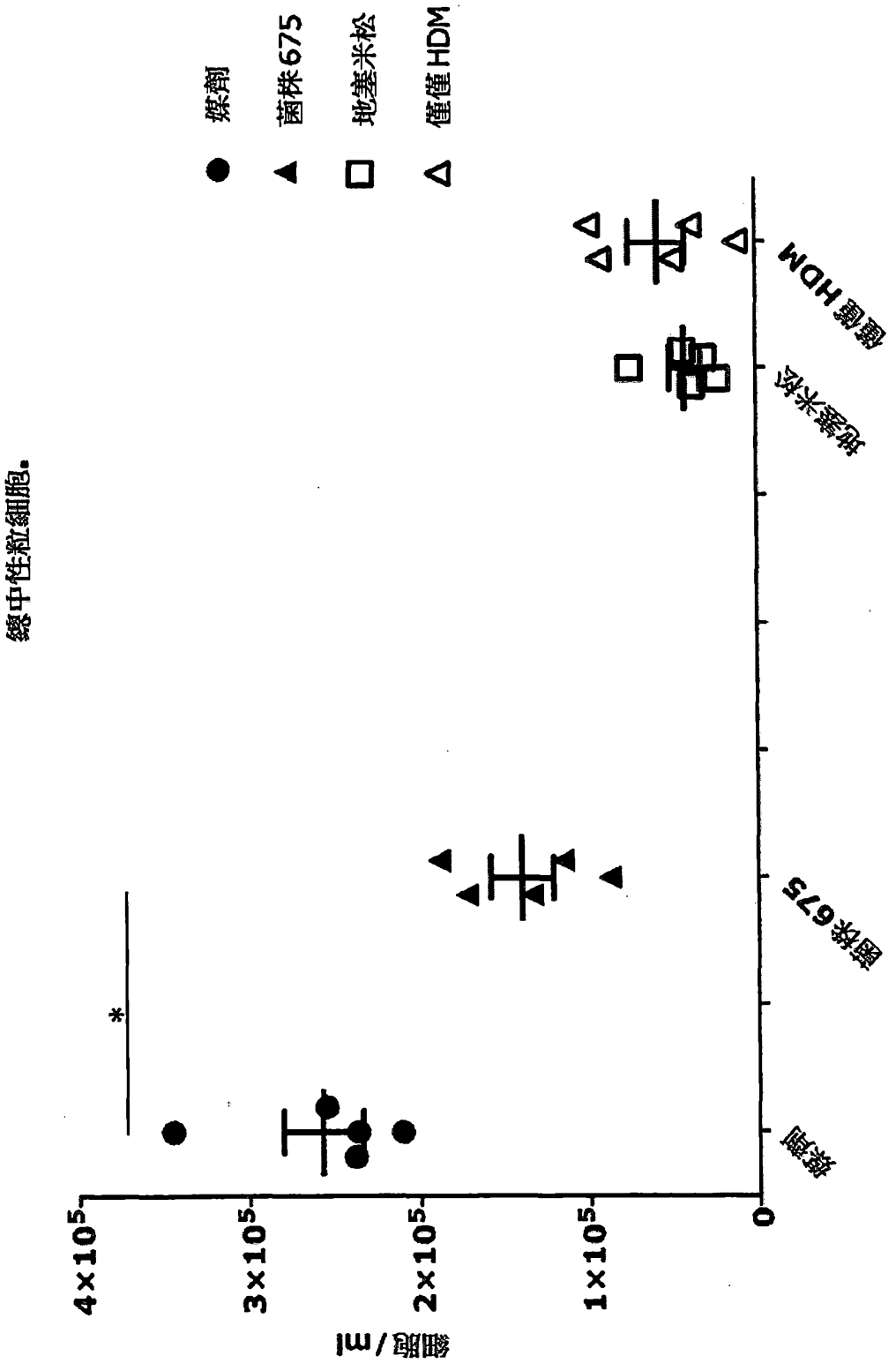

媒劑
菌株675
地塞米松
僅僅HDM
總中性粒細胞。
細胞/ml
4×10^5
3×10^5
2×10^5
1×10^5
0
*
媒劑
菌株675
地塞米松
僅僅HDM

圖 6

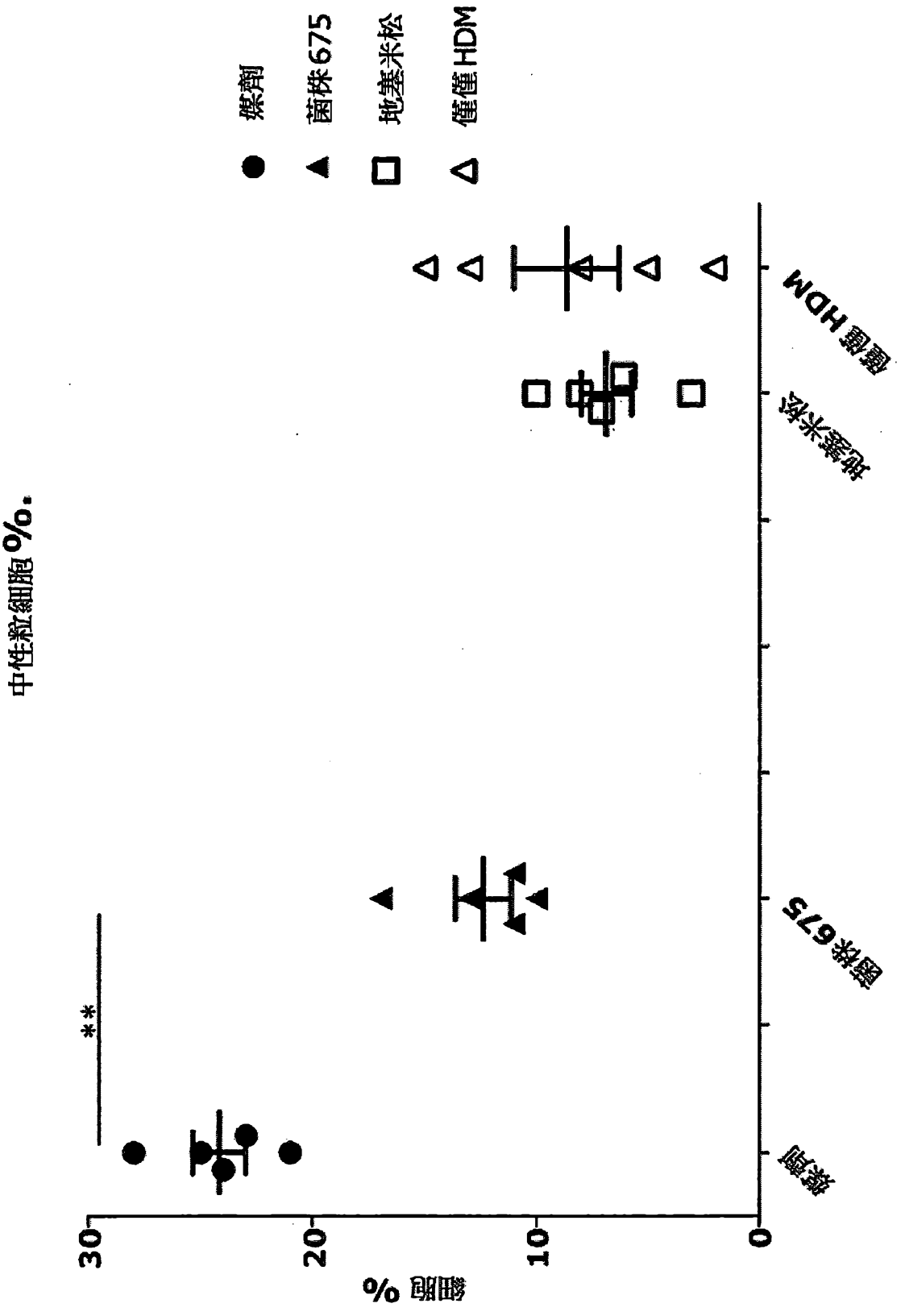


圖 7

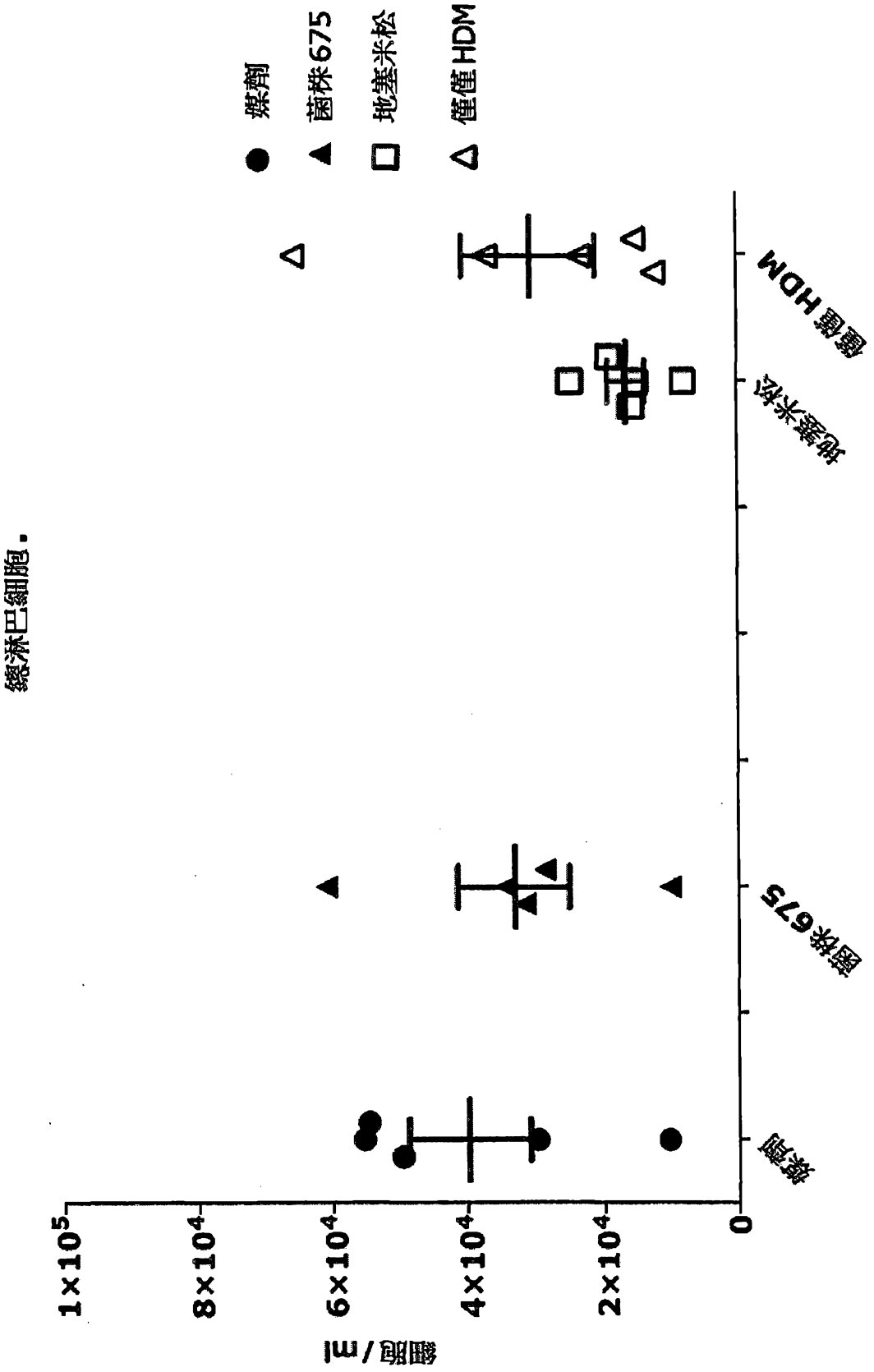

媒劑
菌株675
地塞米松
僅僅 HDM
細胞 / ml
1x10^5
8x10^4
6x10^4
4x10^4
2x10^4
0
媒劑
菌株675
地塞米松
僅僅 HDM
總淋巴細胞。

圖 8

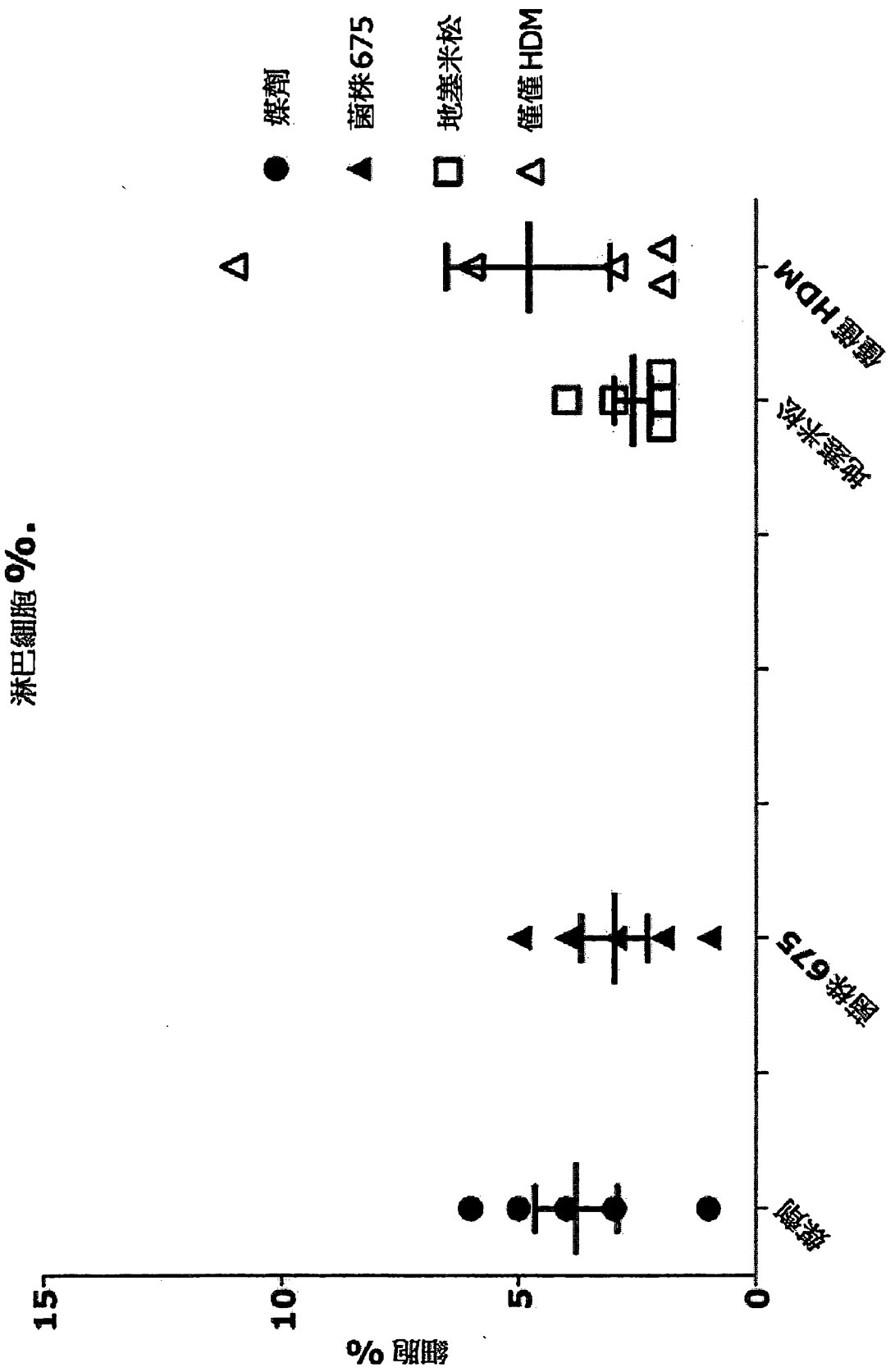


圖 9

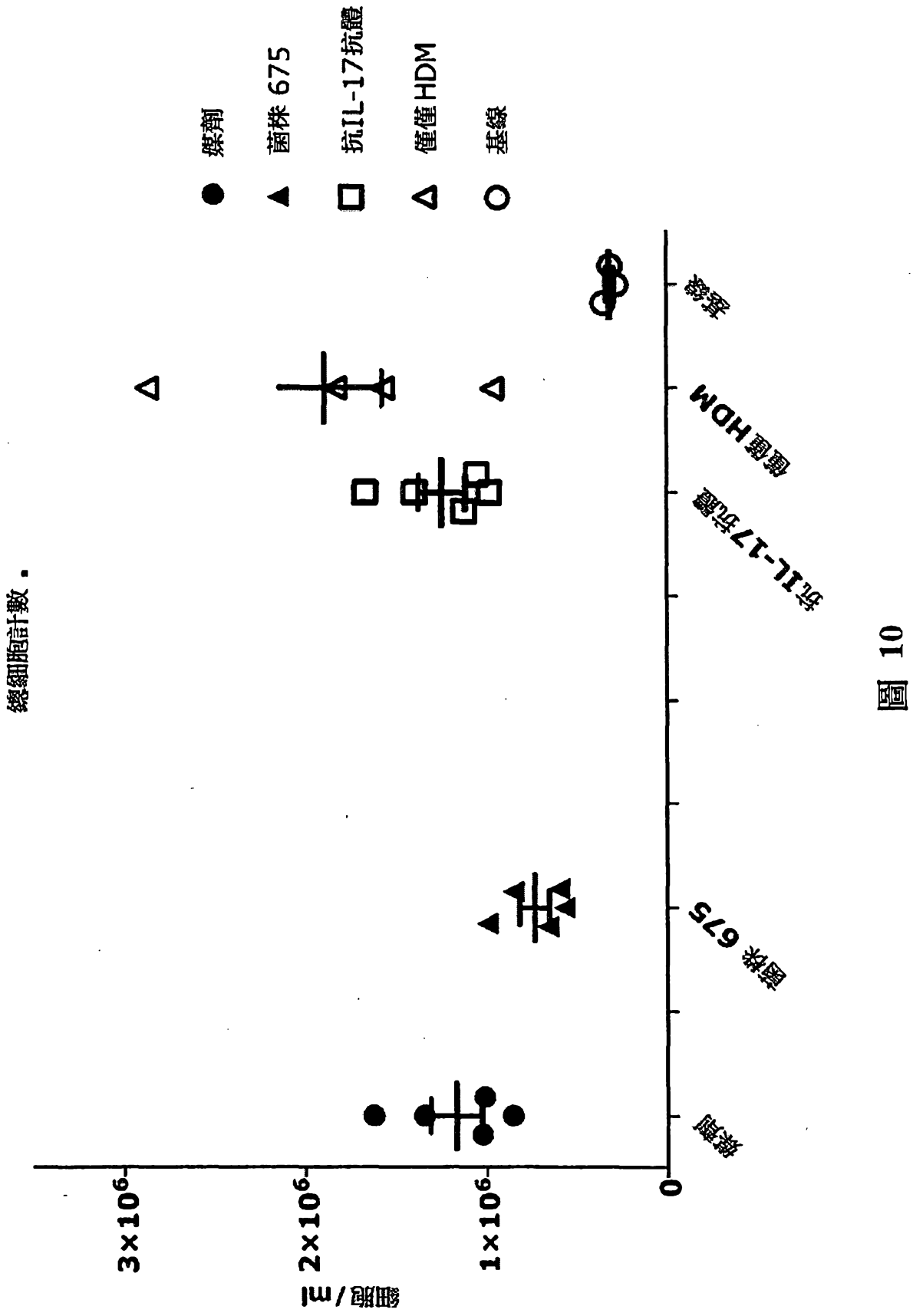

媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線
總細胞計數 •
細胞/ml
3×10^6
2×10^6
1×10^6
0
媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線

圖 10

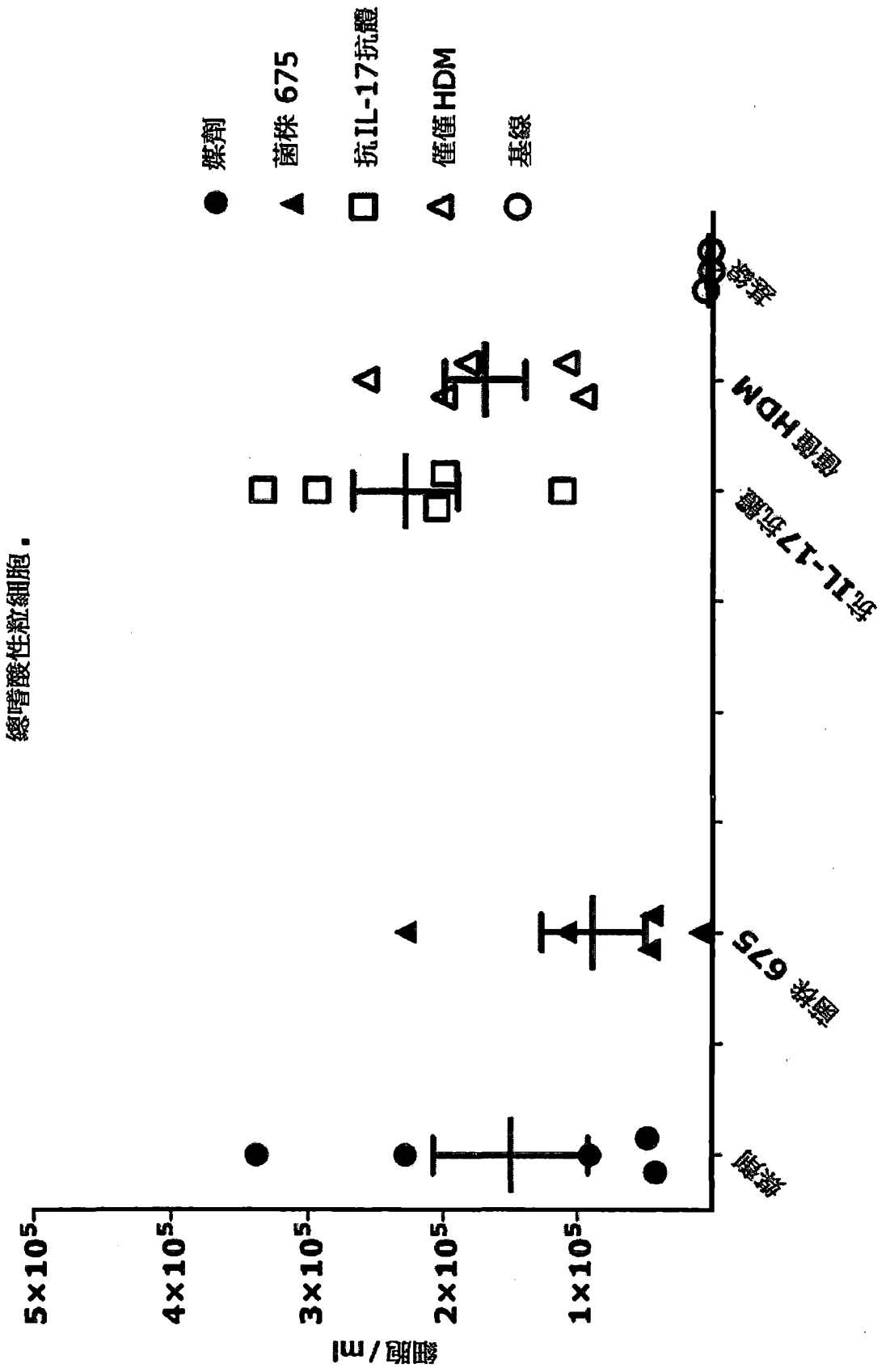

媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線
總嗜酸性粒細胞。
細胞 / ml
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線

圖 11

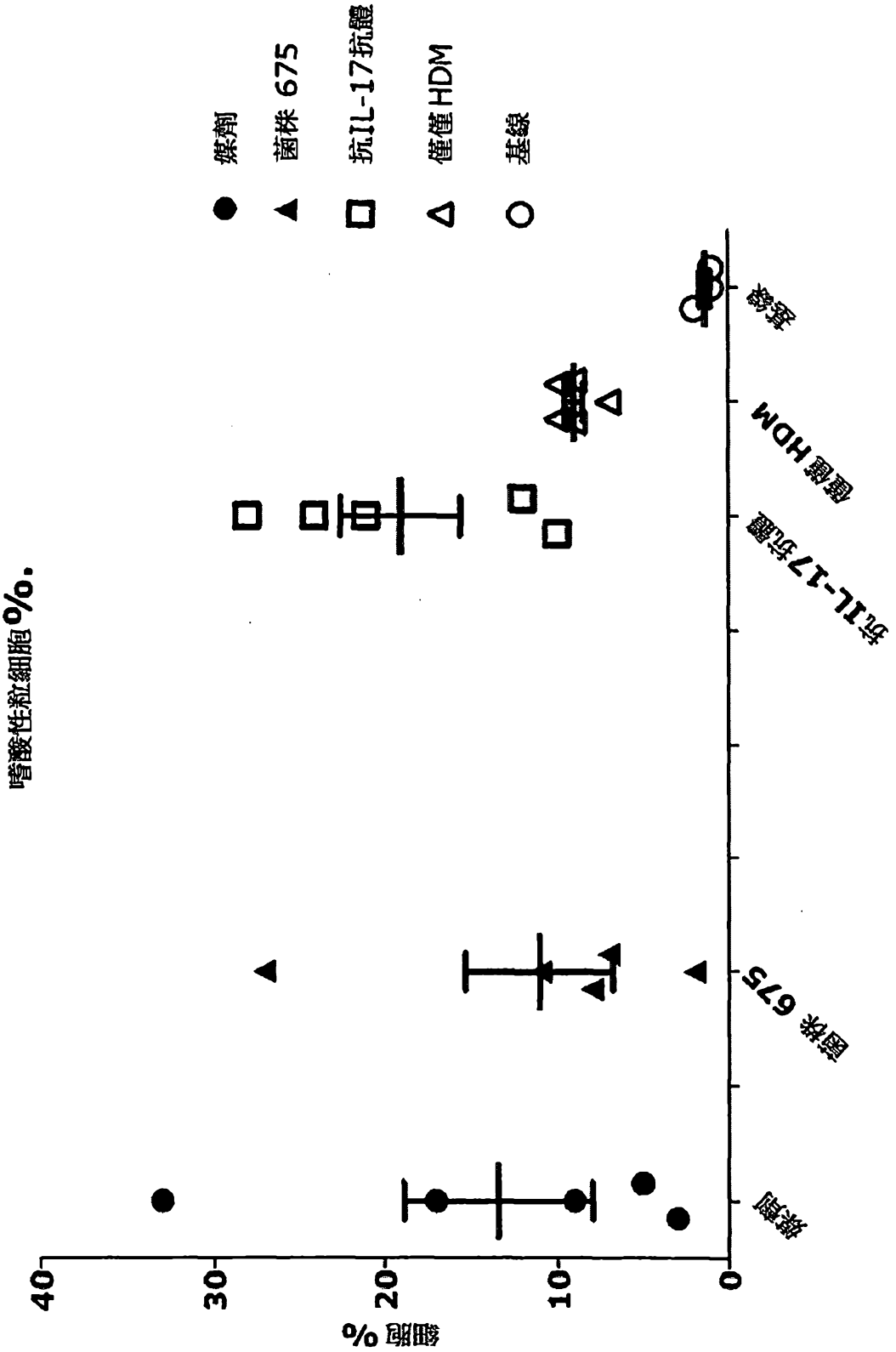

媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線
嗜酸性粒細胞%.
細胞 %
40
30
20
10
0
媒劑
菌株 675
抗IL-17抗體
僅僅 HDM
基線

圖 12

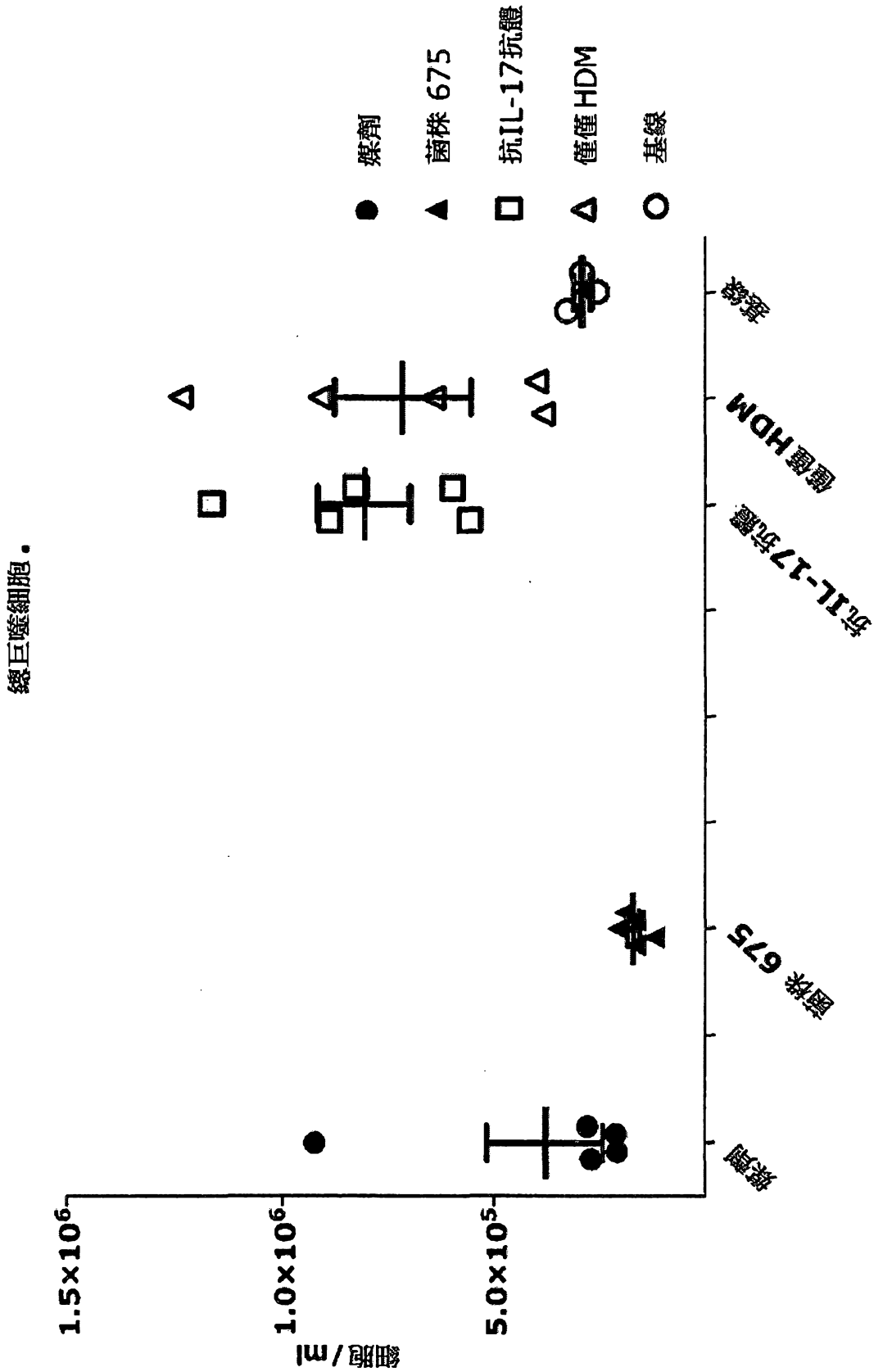

媒劑
菌株 675
抗IL-17抗體
僅僅 HDM
基線
1.5×10^6
1.0×10^6
5.0×10^5
細胞 / ml
總巨噬細胞。
媒劑
菌株 675
抗IL-17抗體
僅僅 HDM

圖 13

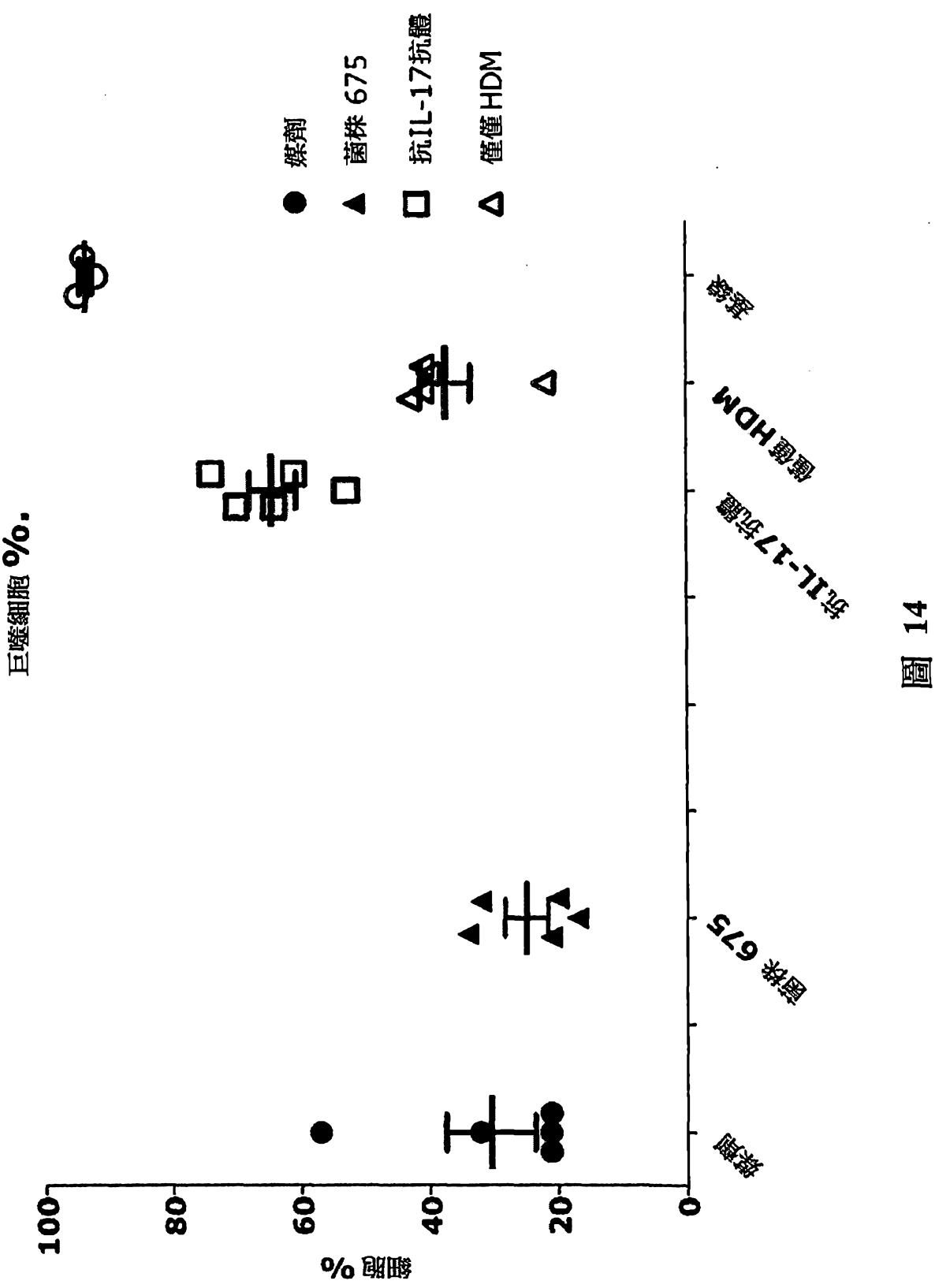

媒劑
菌株 675
抗IL-17抗體
僅僅HDM
巨噬細胞 %.
100
80
60
40
20
0
細胞 %
媒劑
菌株 675
抗IL-17抗體
僅僅HDM

圖 14

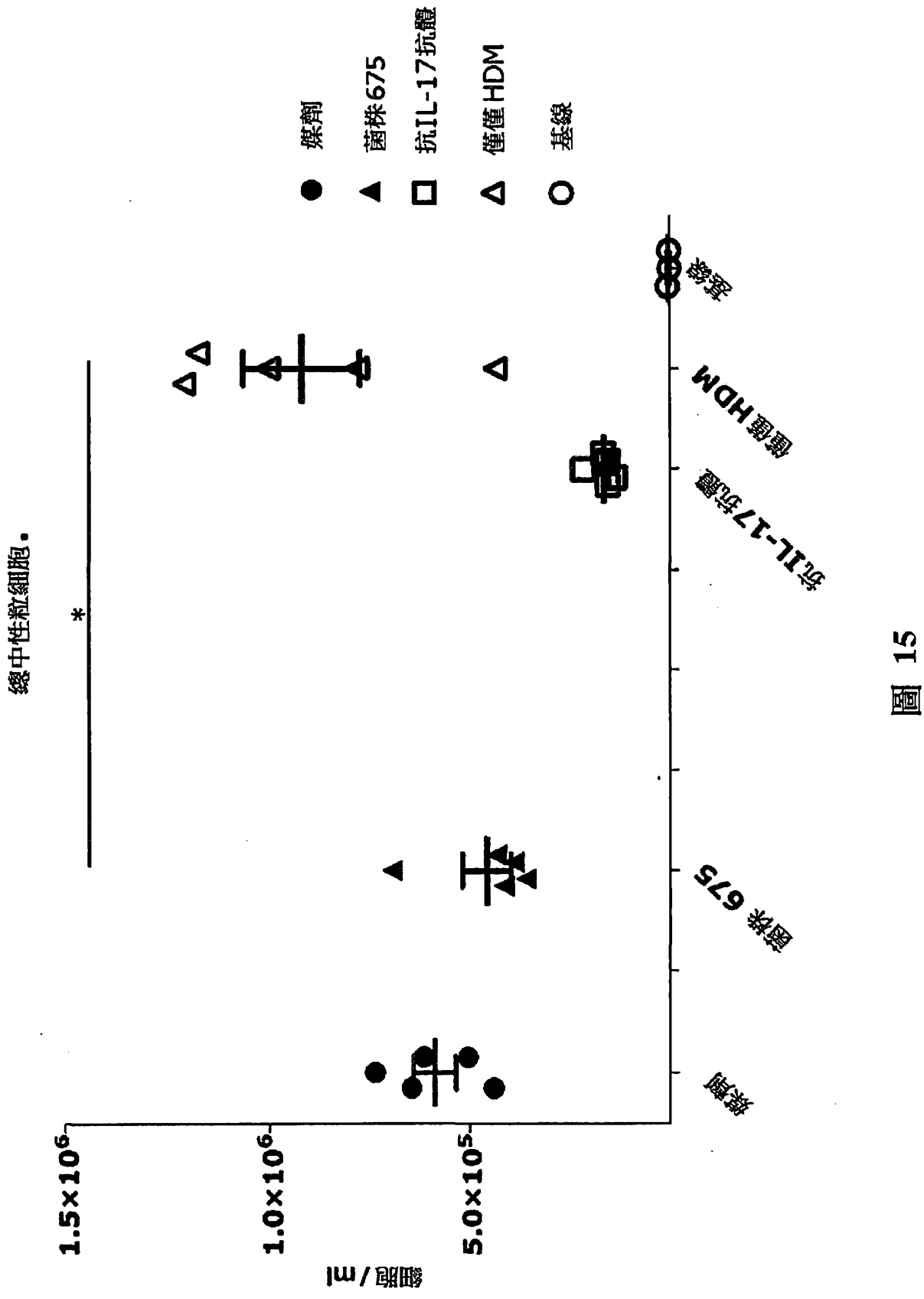

媒劑
菌株675
抗IL-17抗體
僅僅HDM
基線
總中性粒細胞。
*
細胞/ml
1.5×10^6
1.0×10^6
5.0×10^5
媒劑
菌株675
抗IL-17抗體
僅僅HDM
基線

圖 15

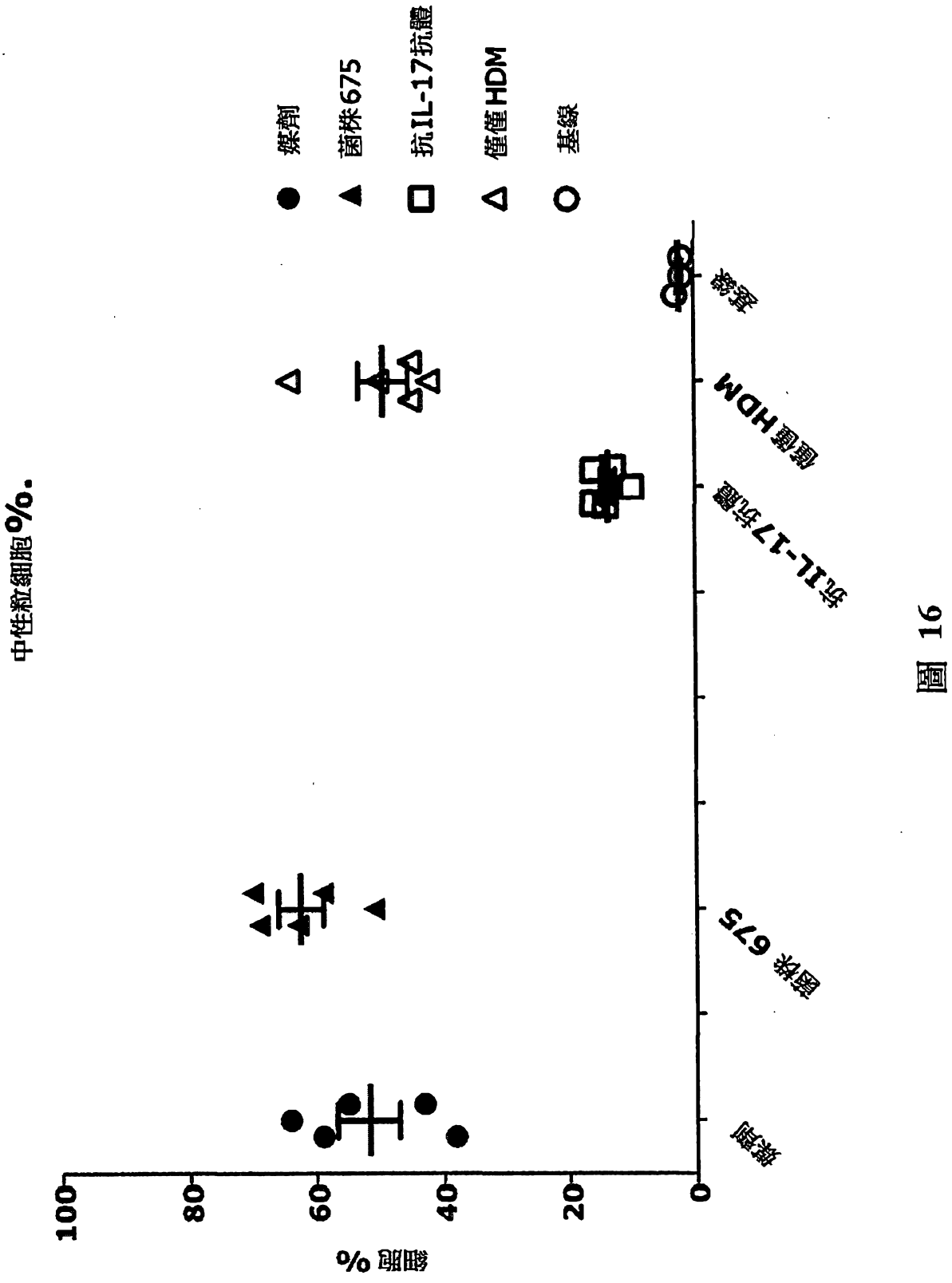

媒劑
菌株675
抗IL-17抗體
僅僅HDM
基線
中性粒細胞%.
細胞 %
100
80
60
40
20
0
媒劑
菌株 675
抗IL-17抗體
僅僅 HDM
基線

圖 16

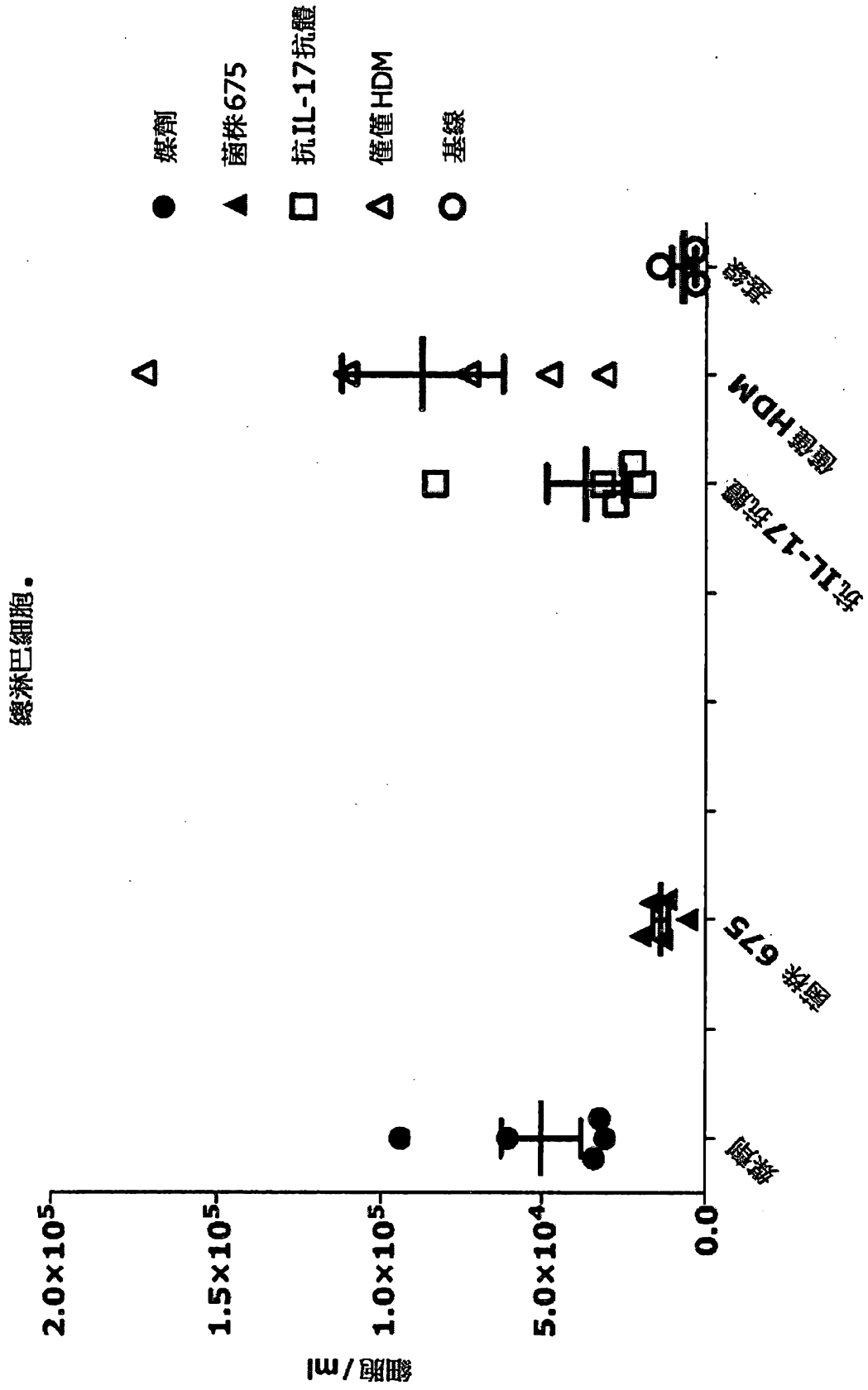

媒劑
菌株675
抗IL-17抗體
僅僅HDM
基線
總淋巴細胞。
細胞/ml
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0.0
媒劑
菌株 675
抗IL-17抗體
僅僅 HDM
幼齡

圖 17

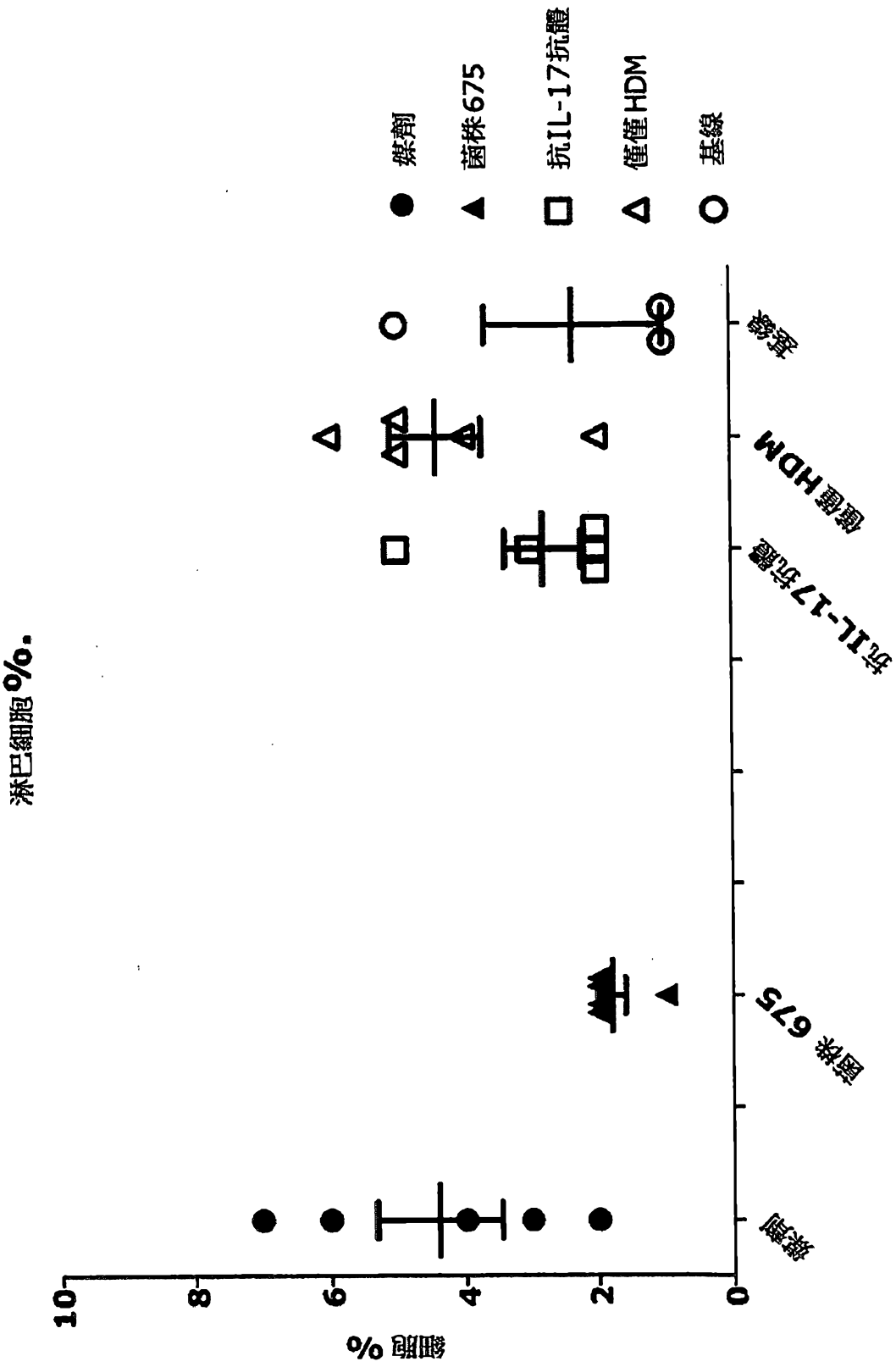

媒劑
菌株675
抗IL-17抗體
僅僅HDM
基線
嗜酸 %
10
8
6
4
2
0
媒劑
菌株 675
抗IL-17抗體
僅僅HDM
基線
淋巴細胞%.

圖 18

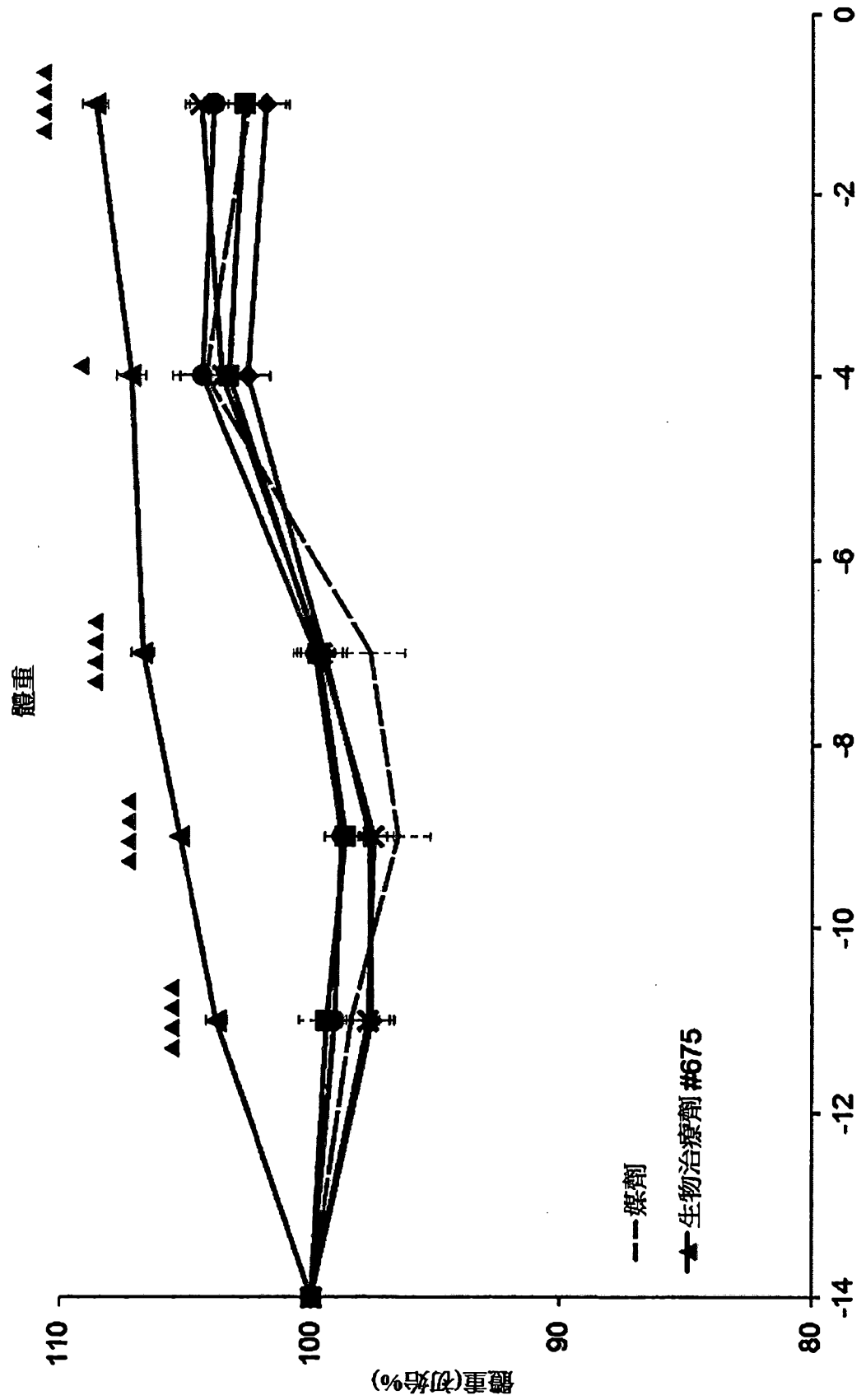


圖 19

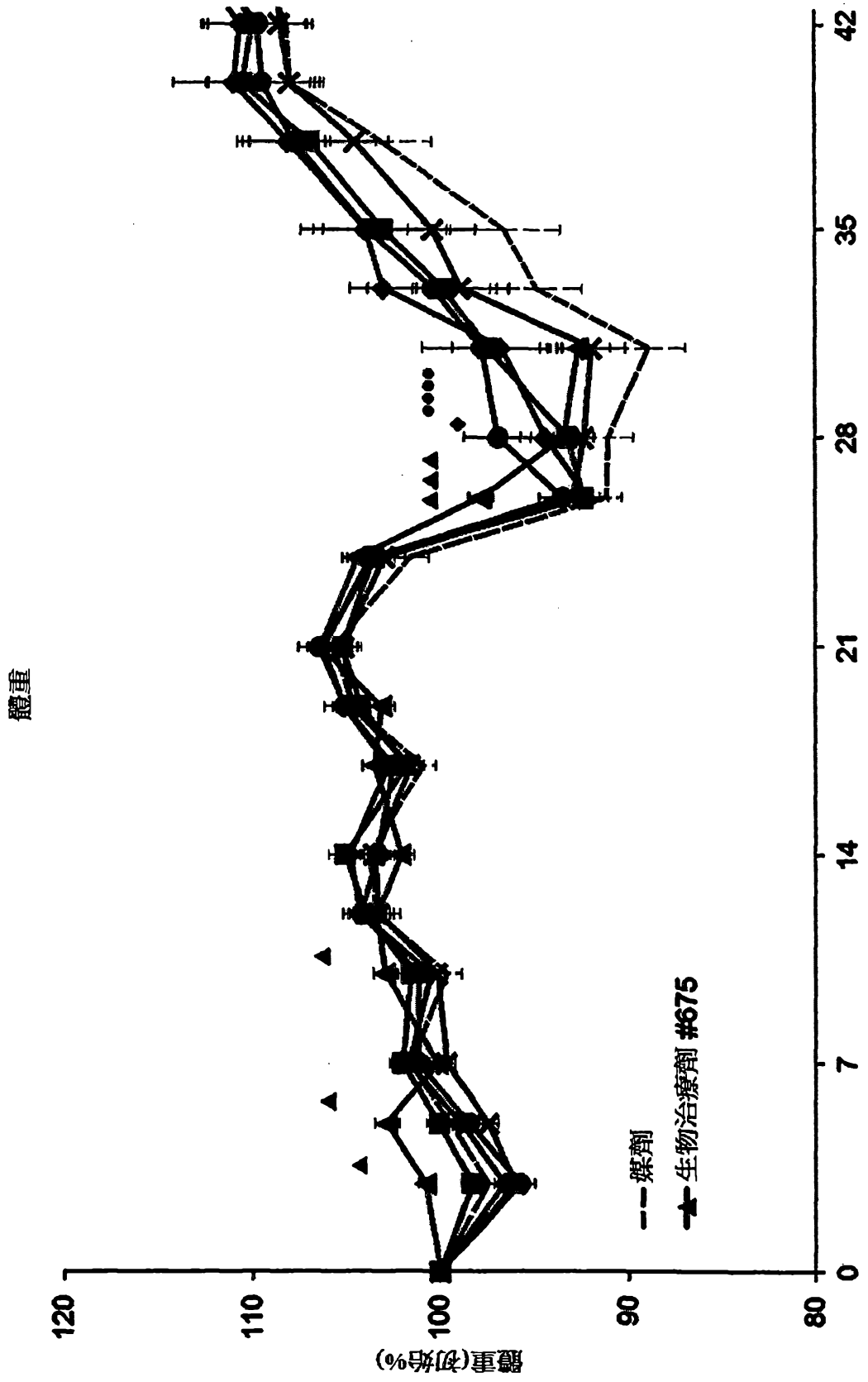


圖 20

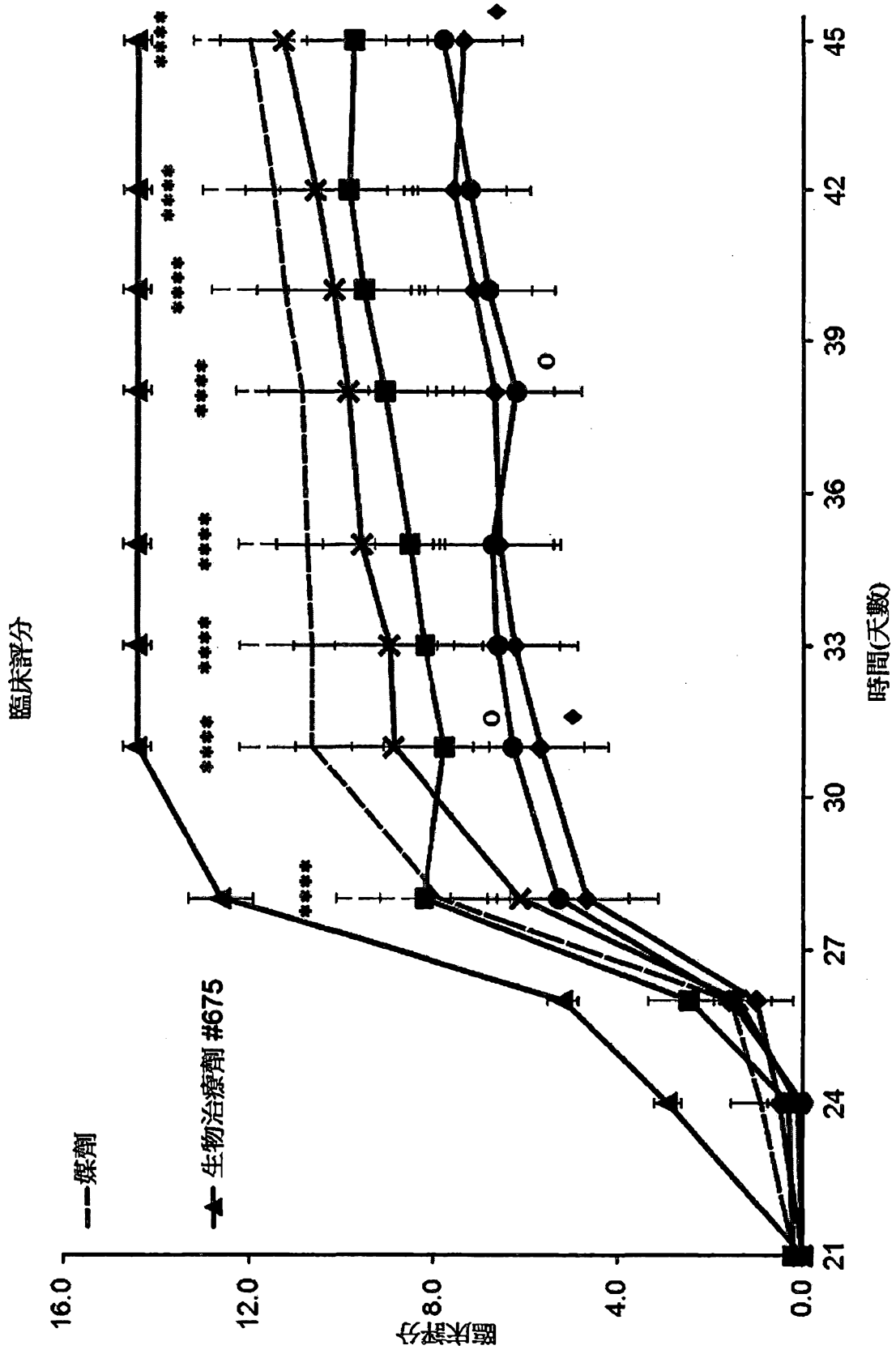

臨床評分
16.0
12.0
8.0
4.0
0.0
21
24
27
30
33
36
39
42
45
時間(天數)
臨床評分
—煤劑
生物治療劑 #675

圖 21

對II型膠原蛋白之脾細胞增殖
3H-胸苷併入(cpm)。減去培養基背景。
18000
12000
6000
0
媒劑
生物治療劑
#675

圖 22

IFN-γ含量(pg/ml)
120
60
0
培養基
II型膠原蛋白

圖 23

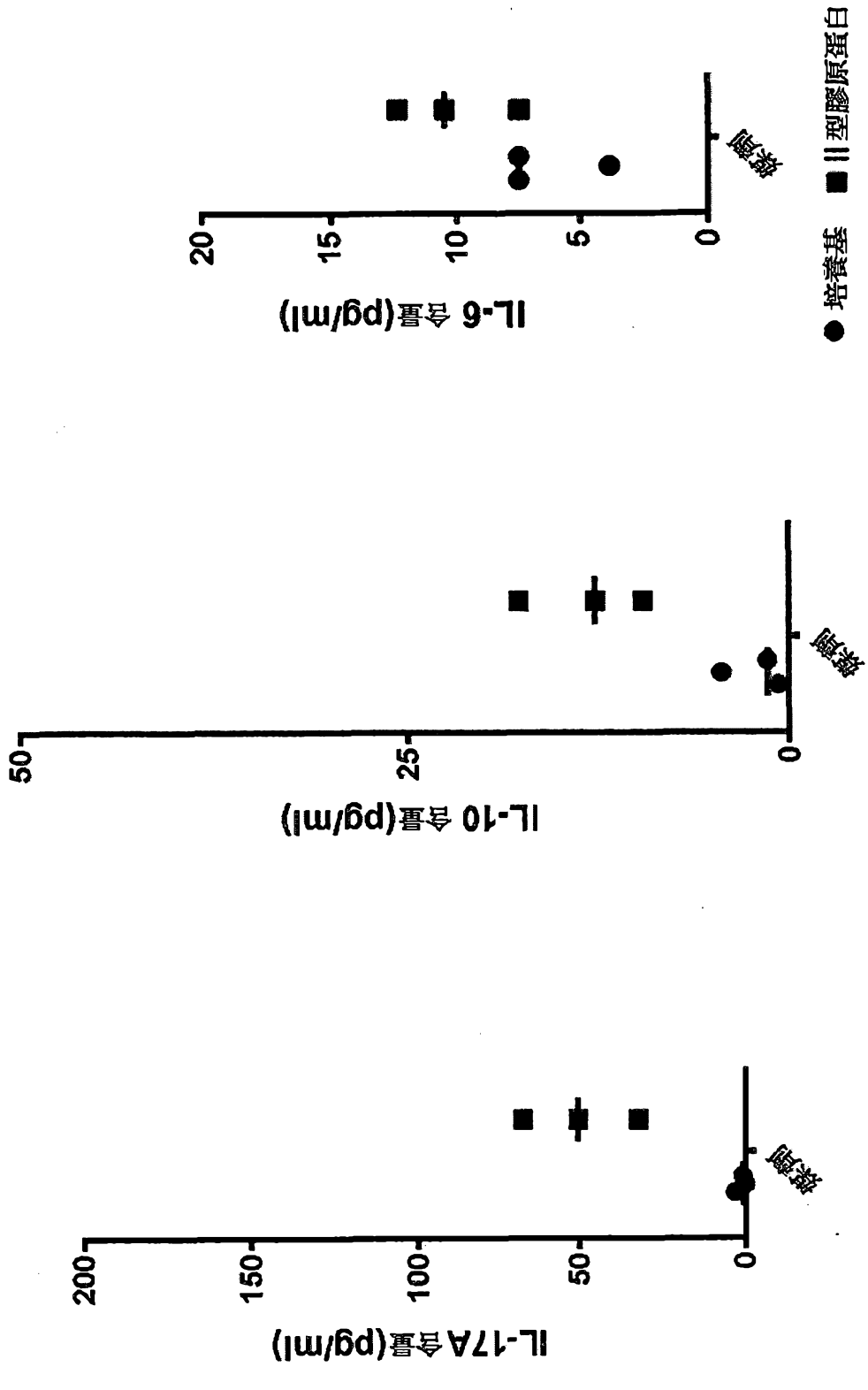

IL-17A含量(pg/ml)
200
150
100
50
0
陰性
● 培養基 ■ II型膠原蛋白

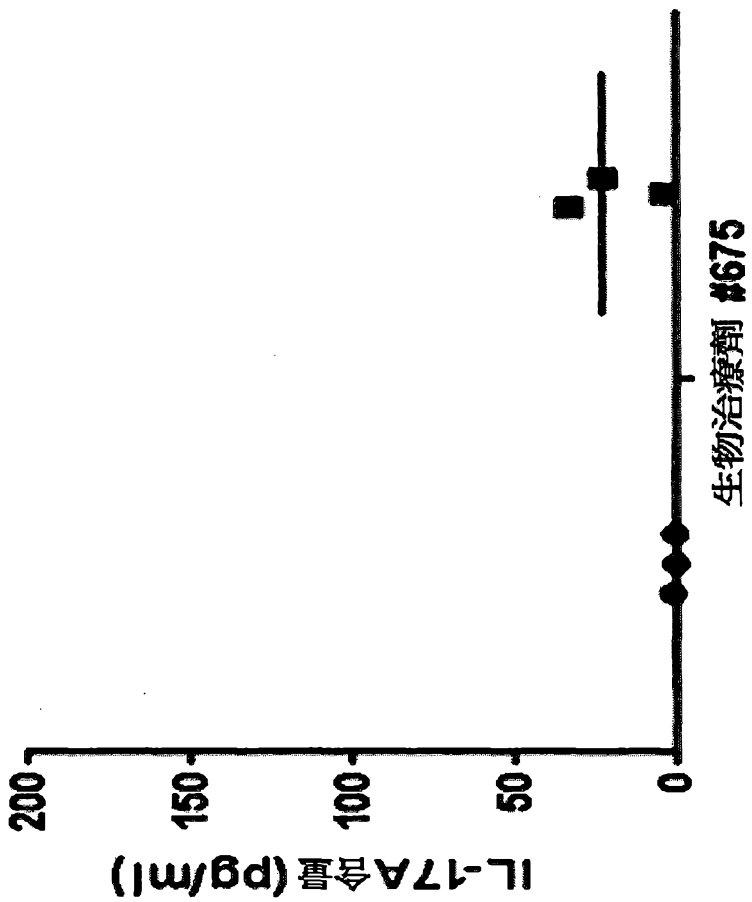
圖 24

IL-10 含量(pg/ml)
50
25
0
陰性
● 培養基 ■ II型膠原蛋白

圖 25

IL-6 含量(pg/ml)
20
15
10
5
0
陰性
● 培養基 ■ II型膠原蛋白

圖 26


IL-17A含量 (pg/ml)
200
150
100
50
0
生物治療劑 #675
● 培養基
■ II型膠原蛋白

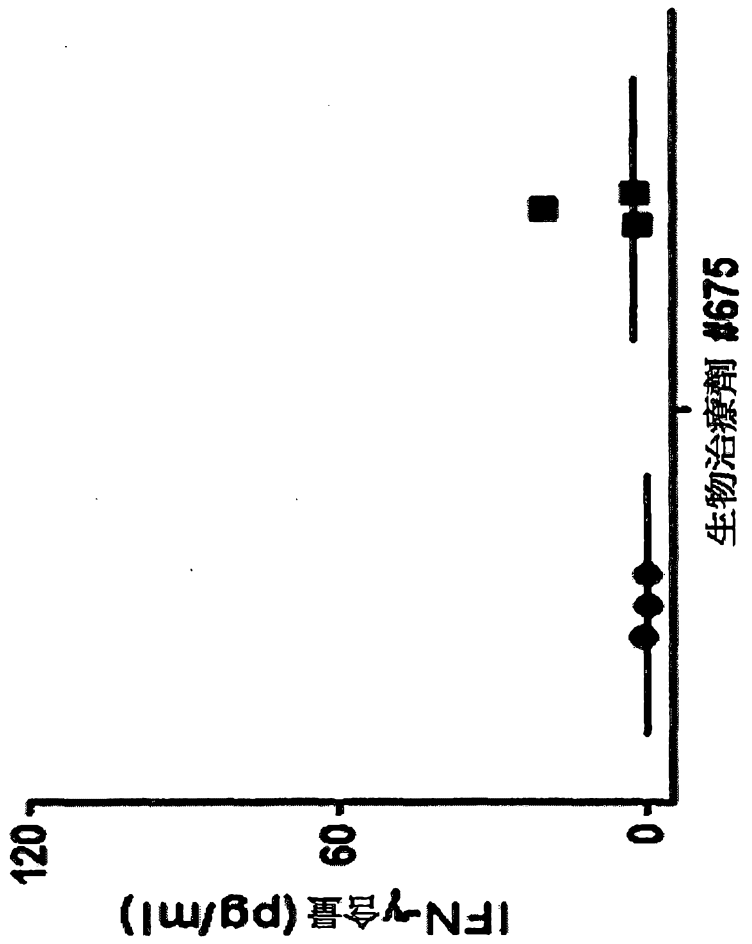

IFN-γ含量 (pg/ml)
120
60
0
生物治療劑 #675
● 培養基
■ 型膠原蛋白

圖 27

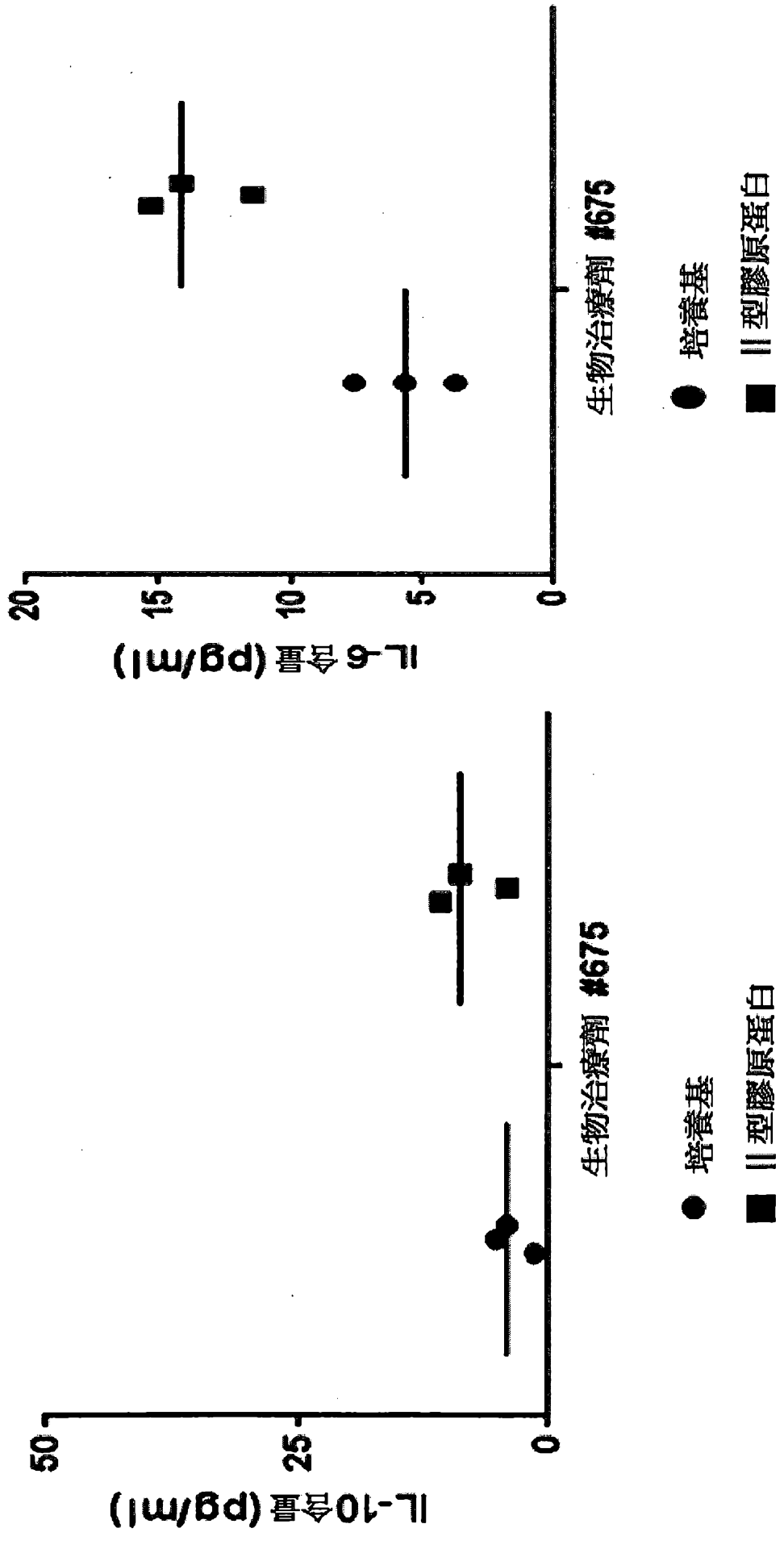

IL-10含量 (pg/ml)
50
25
0
生物治療劑 #675
培養基
II型膠原蛋白
IL-6 含量 (pg/ml)
20
15
10
5
0
生物治療劑 #675
培養基
II型膠原蛋白

圖 27(續)

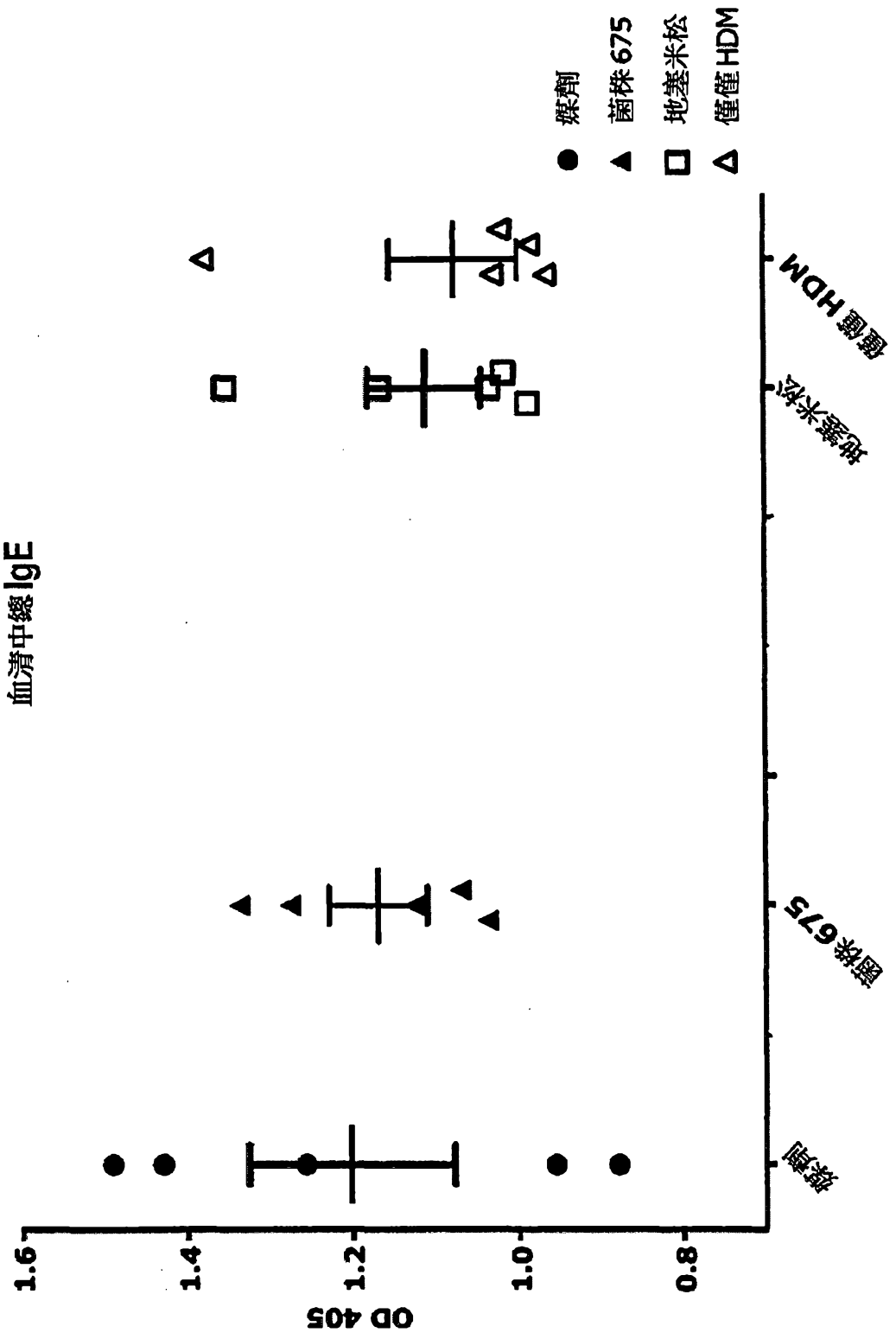

媒劑
菌株675
地塞米松
塵蟎HDM
血清中總IgE
OD 405
1.6
1.4
1.2
1.0
0.8
媒劑
菌株675
地塞米松
塵蟎HDM

圖 28

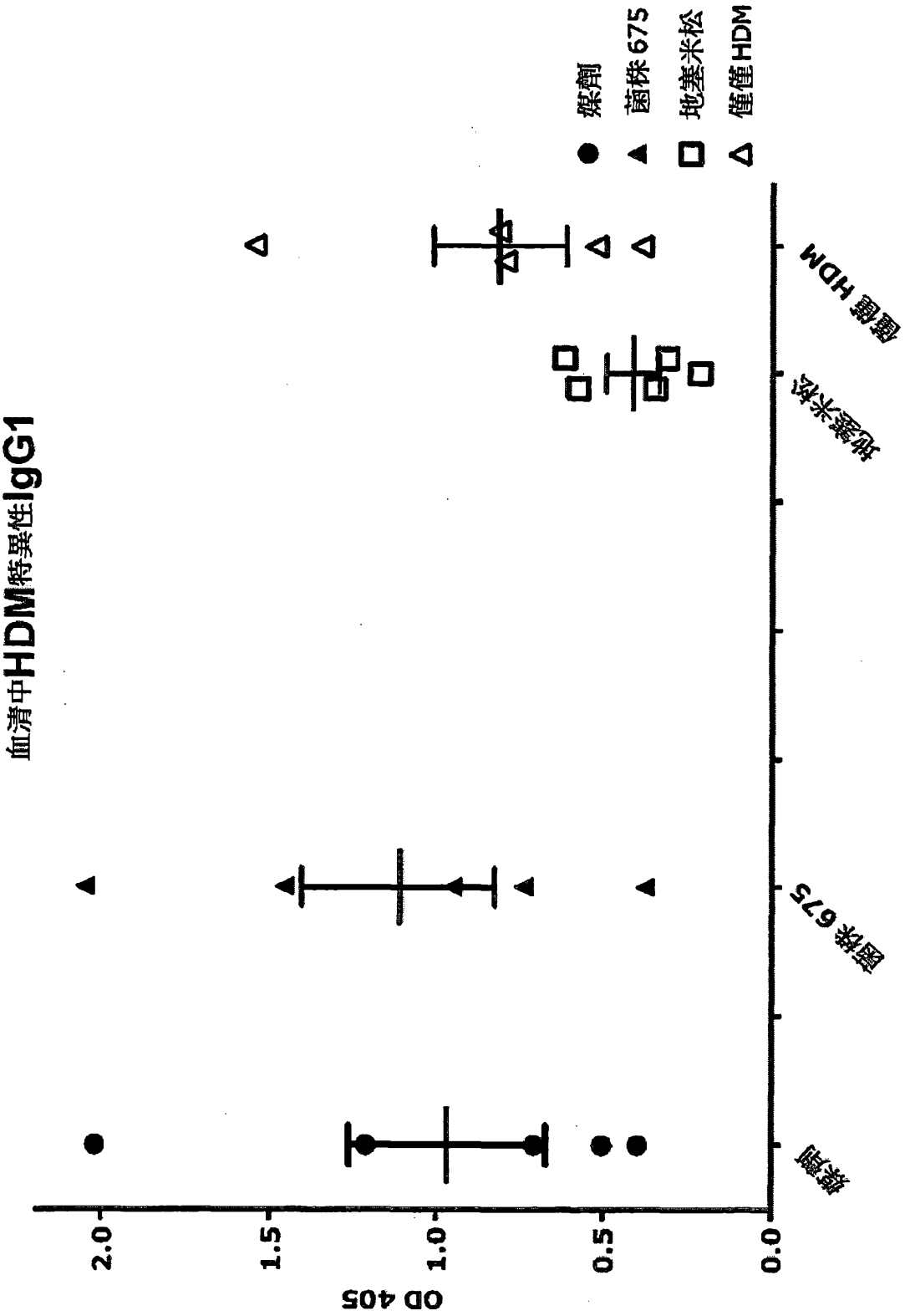

血清中HDM特異性IgG1
OD 405
2.0
1.5
1.0
0.5
0.0
媒劑
菌株 675
地塞米松
僅僅 HDM
媒劑
菌株 675
地塞米松
僅僅 HDM

圖 29

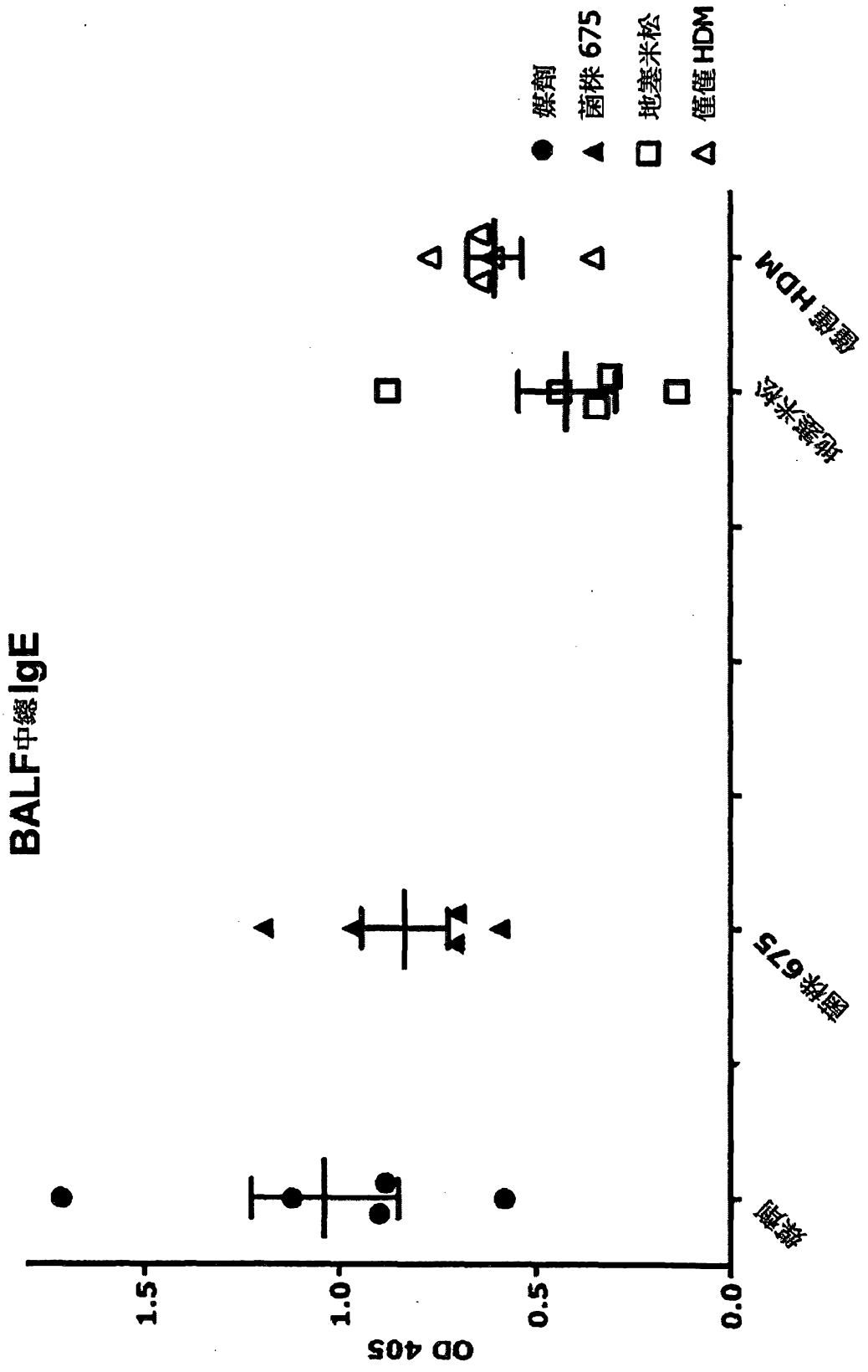

BALF中總IgE
OD 405
1.5
1.0
0.5
0.0
媒劑
菌株675
地塞米松
塵蟎HDM
媒劑
菌株 675
地塞米松
塵蟎 HDM

圖 30

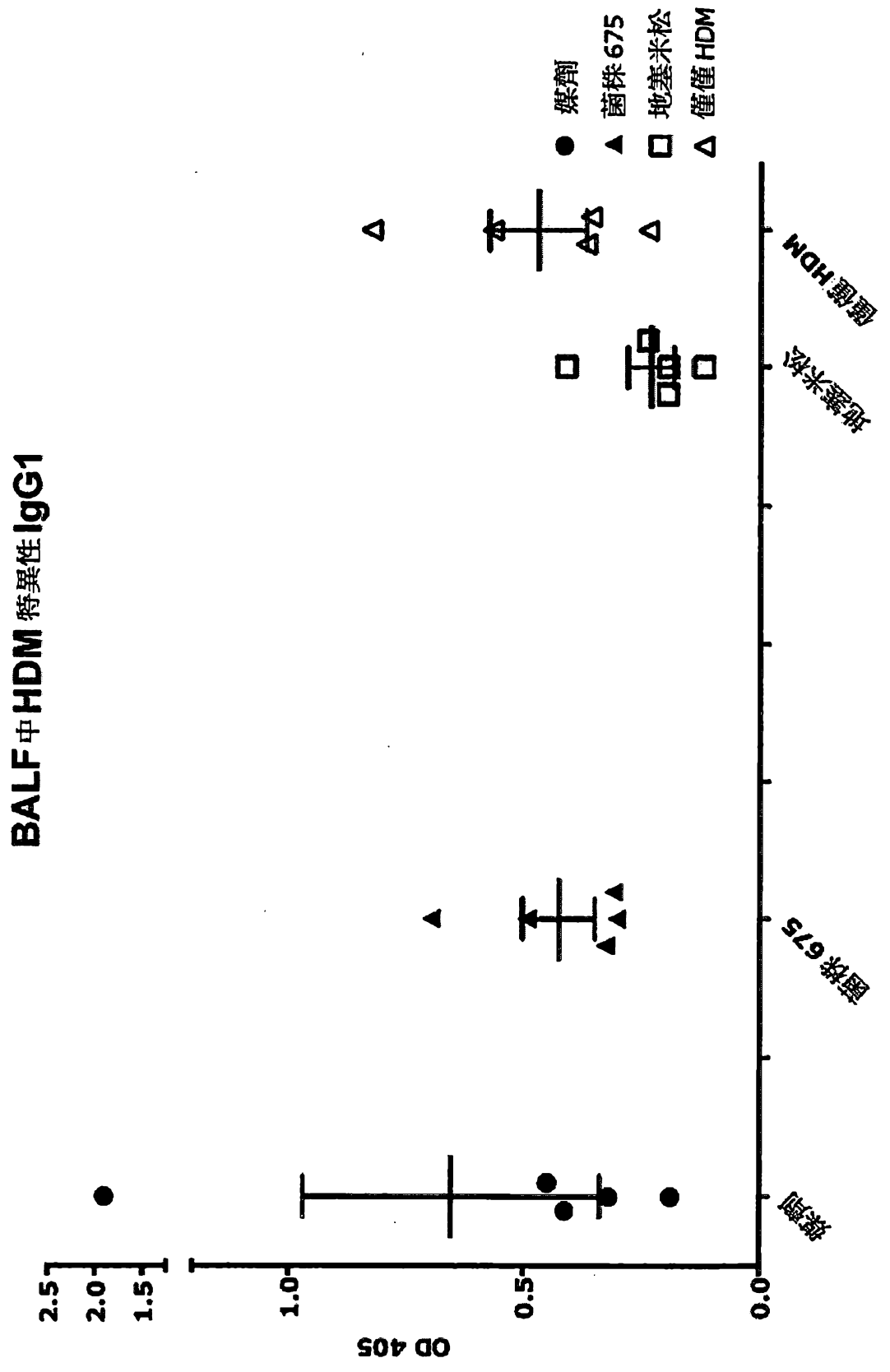

BALF中HDM特異性IgG1
OD 405
2.5
2.0
1.5
1.0
0.5
0.0
媒劑
菌株675
地塞米松
僅僅HDM
媒劑
菌株675
地塞米松
僅僅HDM

圖 31

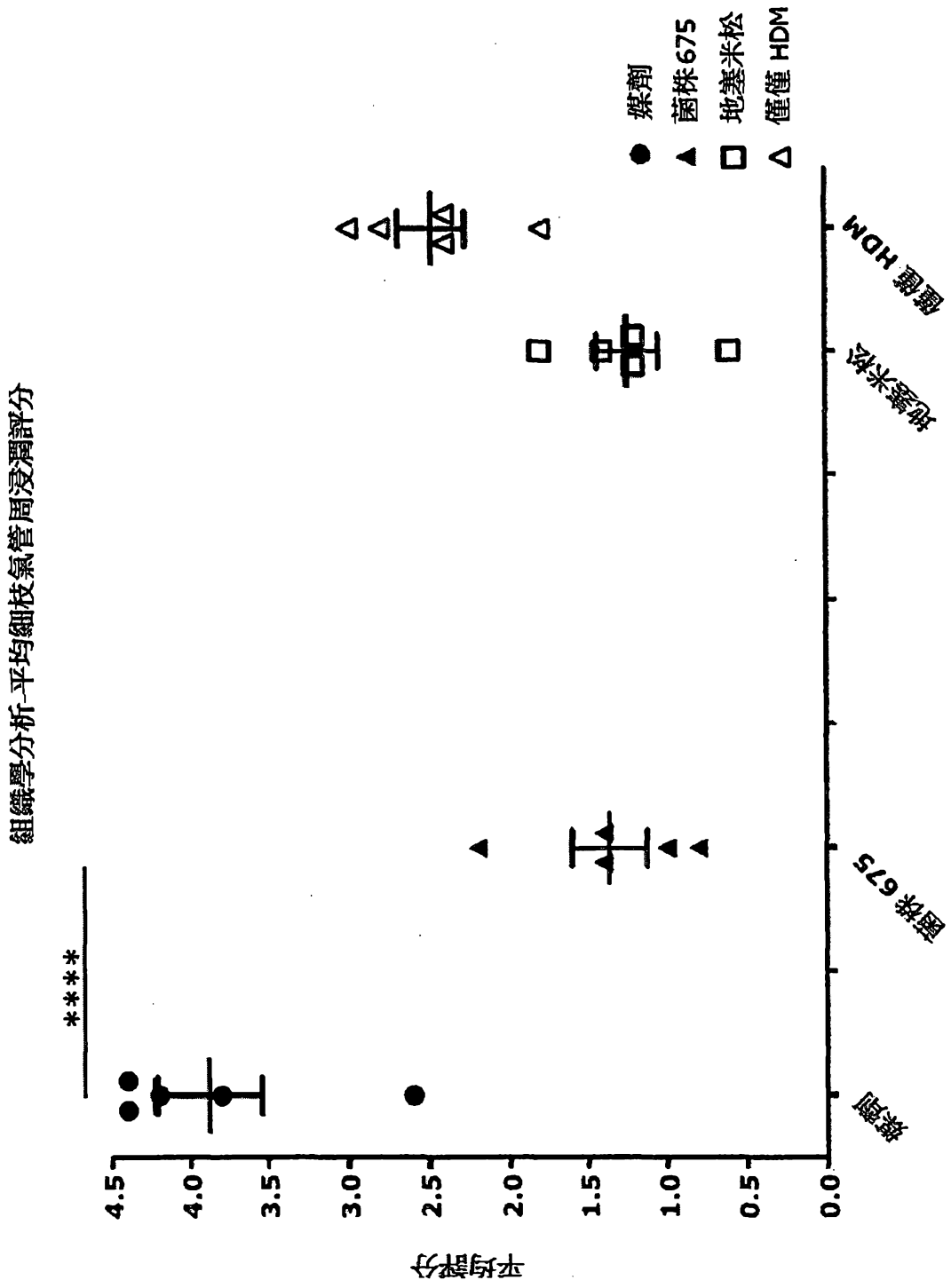

組織學分析-平均細枝氣管周浸潤評分
****
平均評分
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
媒劑
菌株675
地塞米松
僅僅 HDM
媒劑
菌株675
地塞米松
僅僅 HDM

圖 32

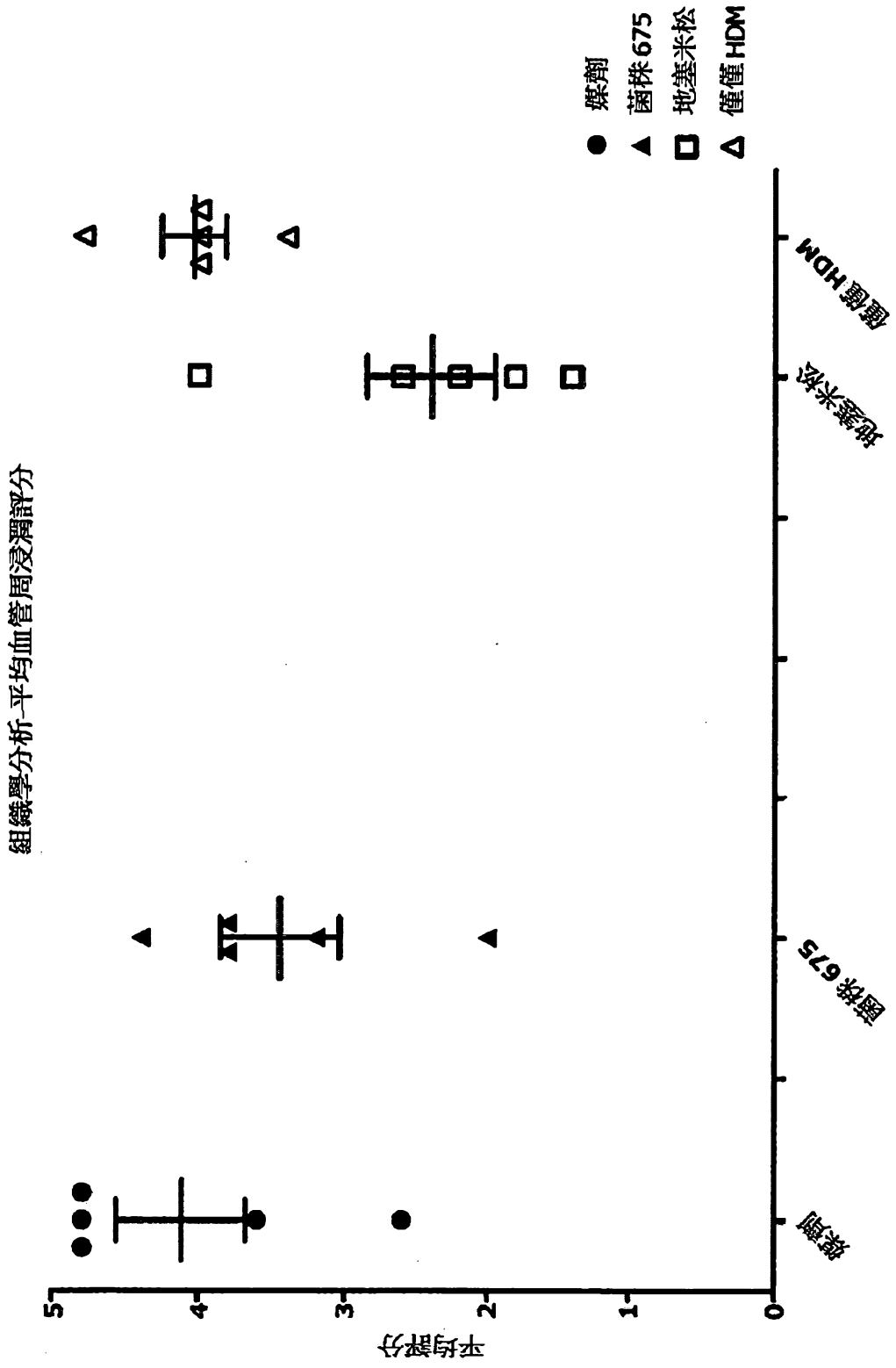

組織學分析-平均血管周浸潤評分
平均評分
0
1
2
3
4
5
媒劑
菌株675
地塞米松
僅僅HDM
媒劑
菌株675
地塞米松
僅僅HDM

圖 33

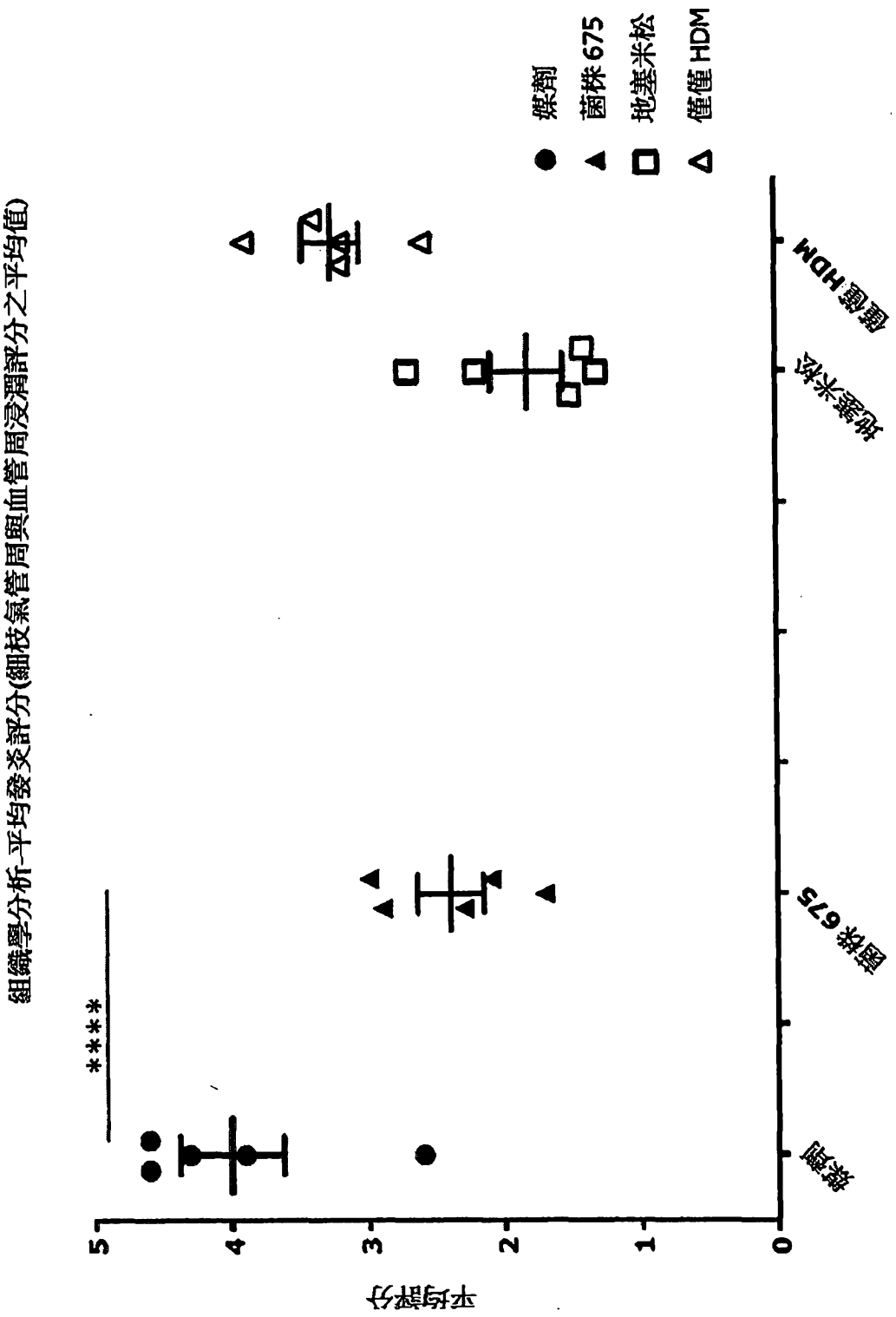

組織學分析-平均發炎評分(細枝氣管周與血管周浸潤評分之平均值)
****
平均評分
0
1
2
3
4
5
媒劑
菌株675
地塞米松
僅僅HDM
媒劑
菌株675
地塞米松
僅僅HDM

圖 34

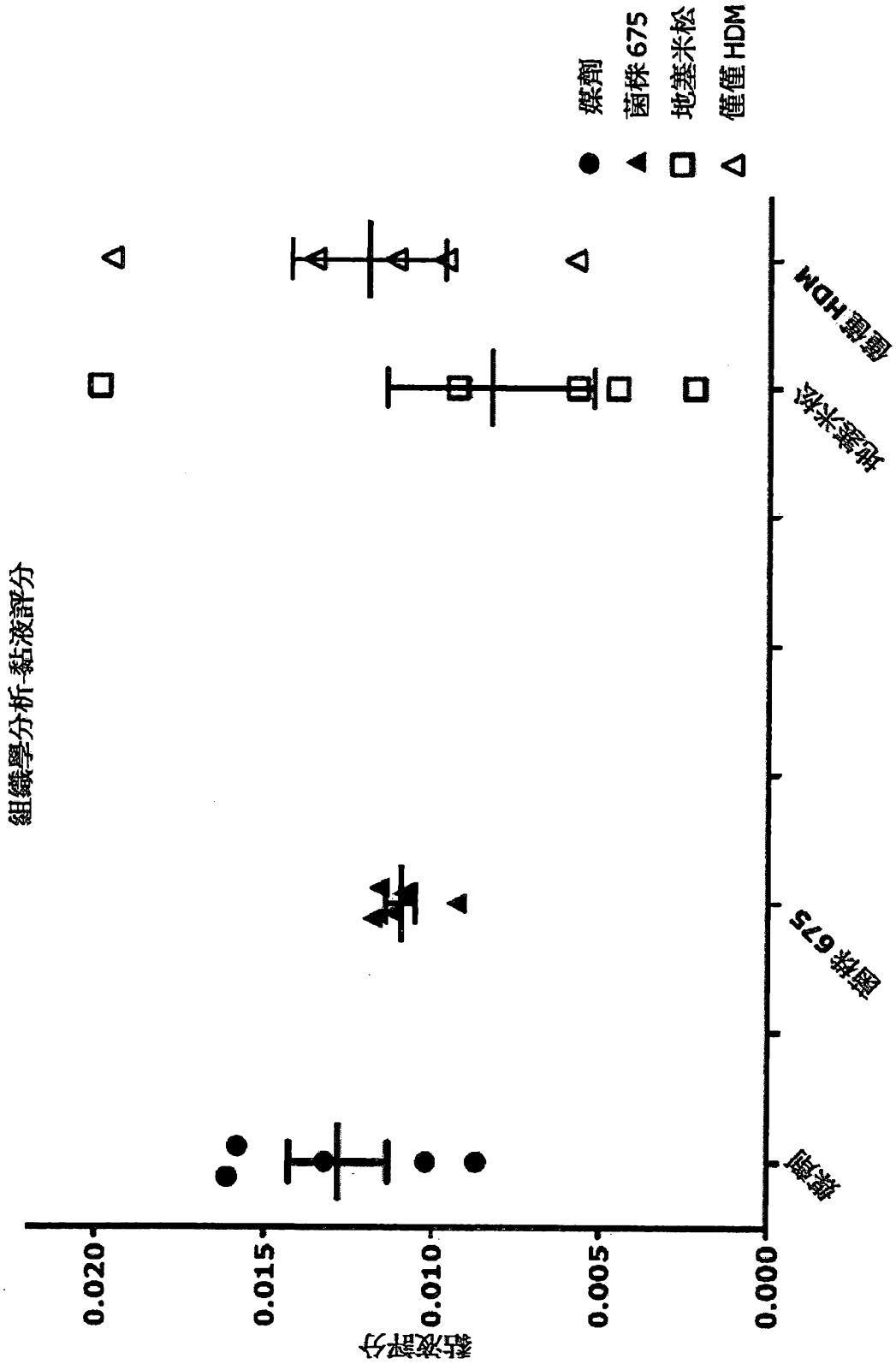

組織學分析-黏液評分
黏液評分
0.020
0.015
0.010
0.005
0.000
媒劑
菌株675
地塞米松
僅僅HDM
媒劑
菌株675
地塞米松
僅僅HDM

圖 35

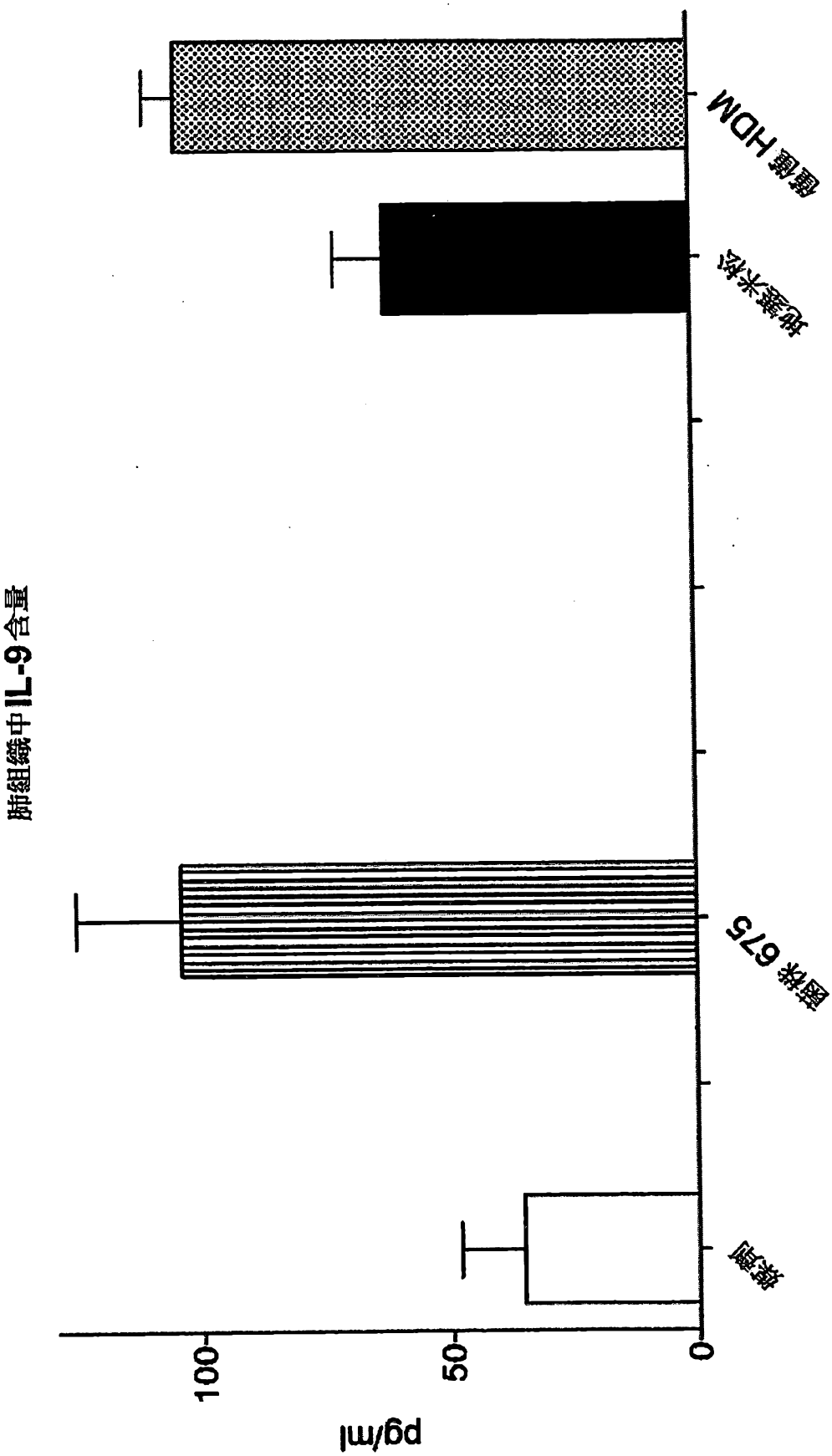


圖 36

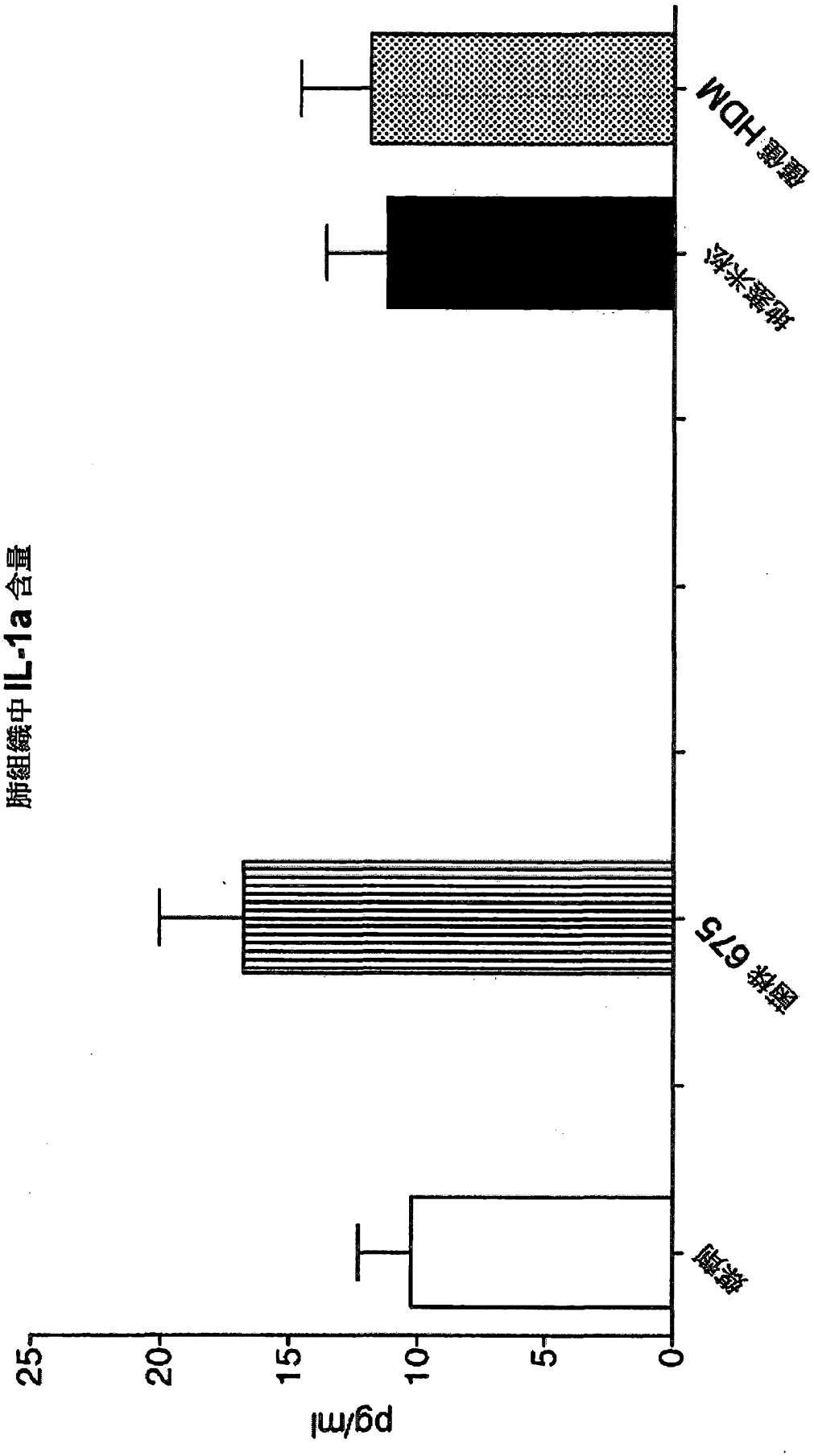


圖 37

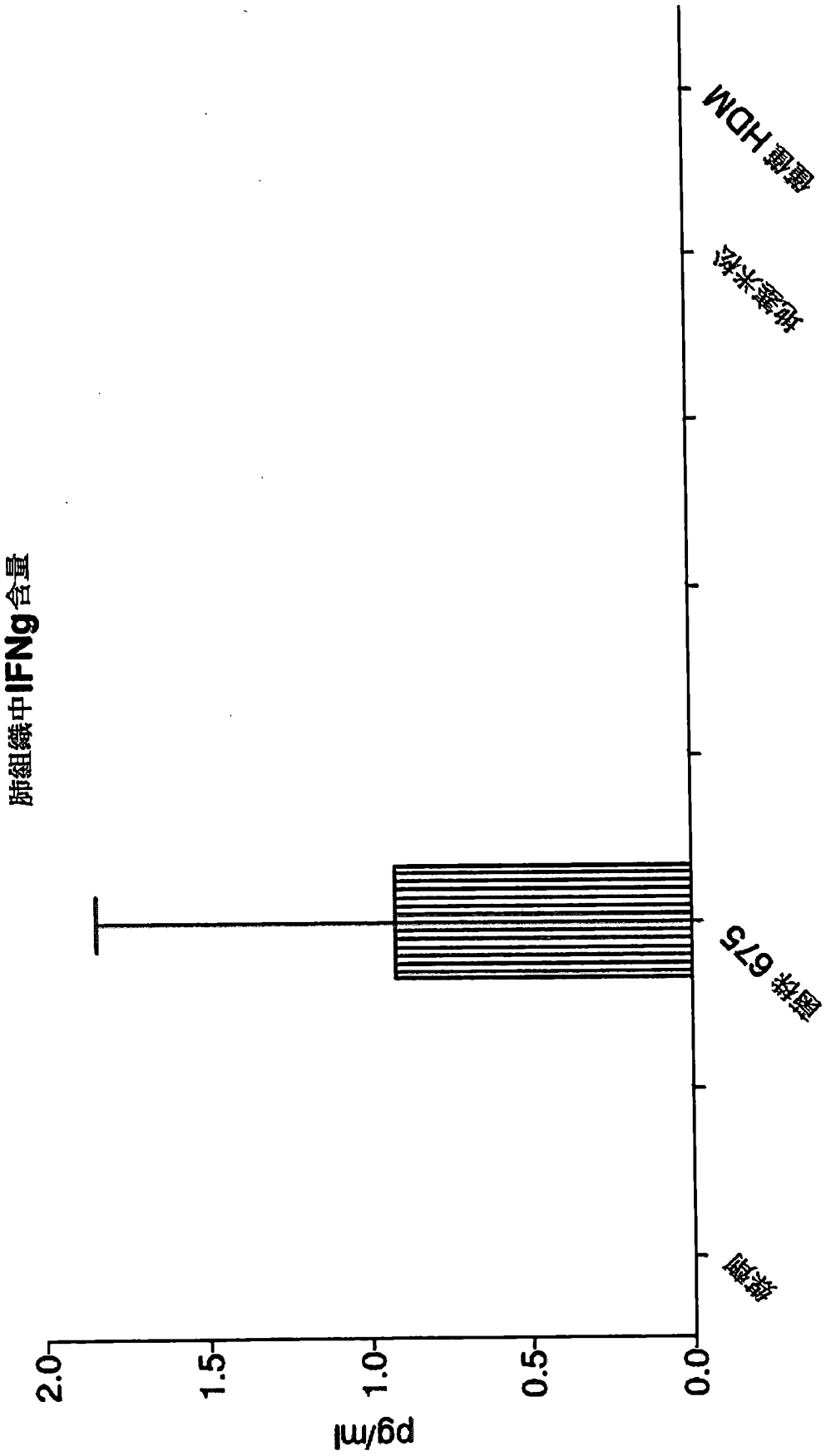


圖 38

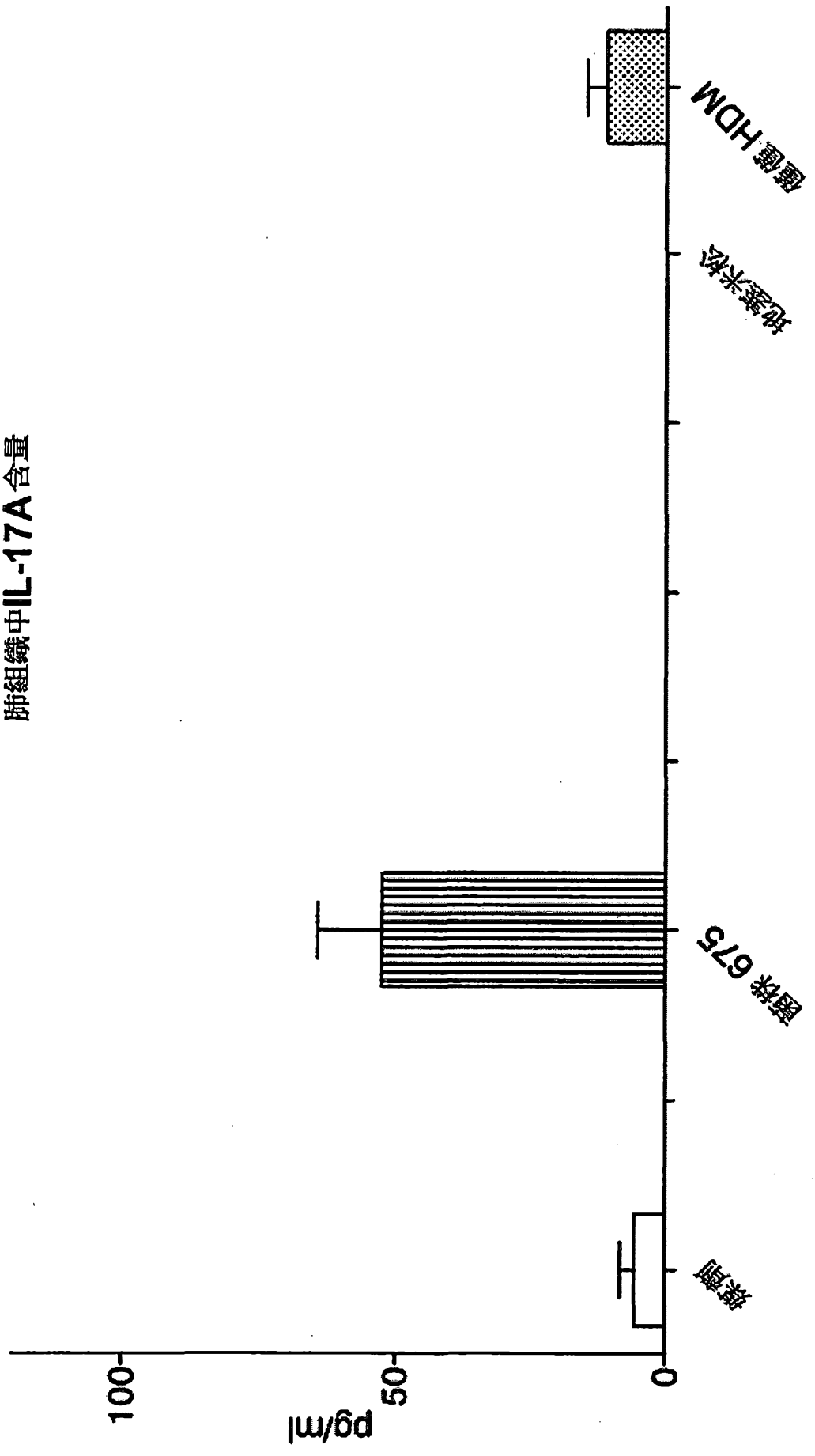


圖 39

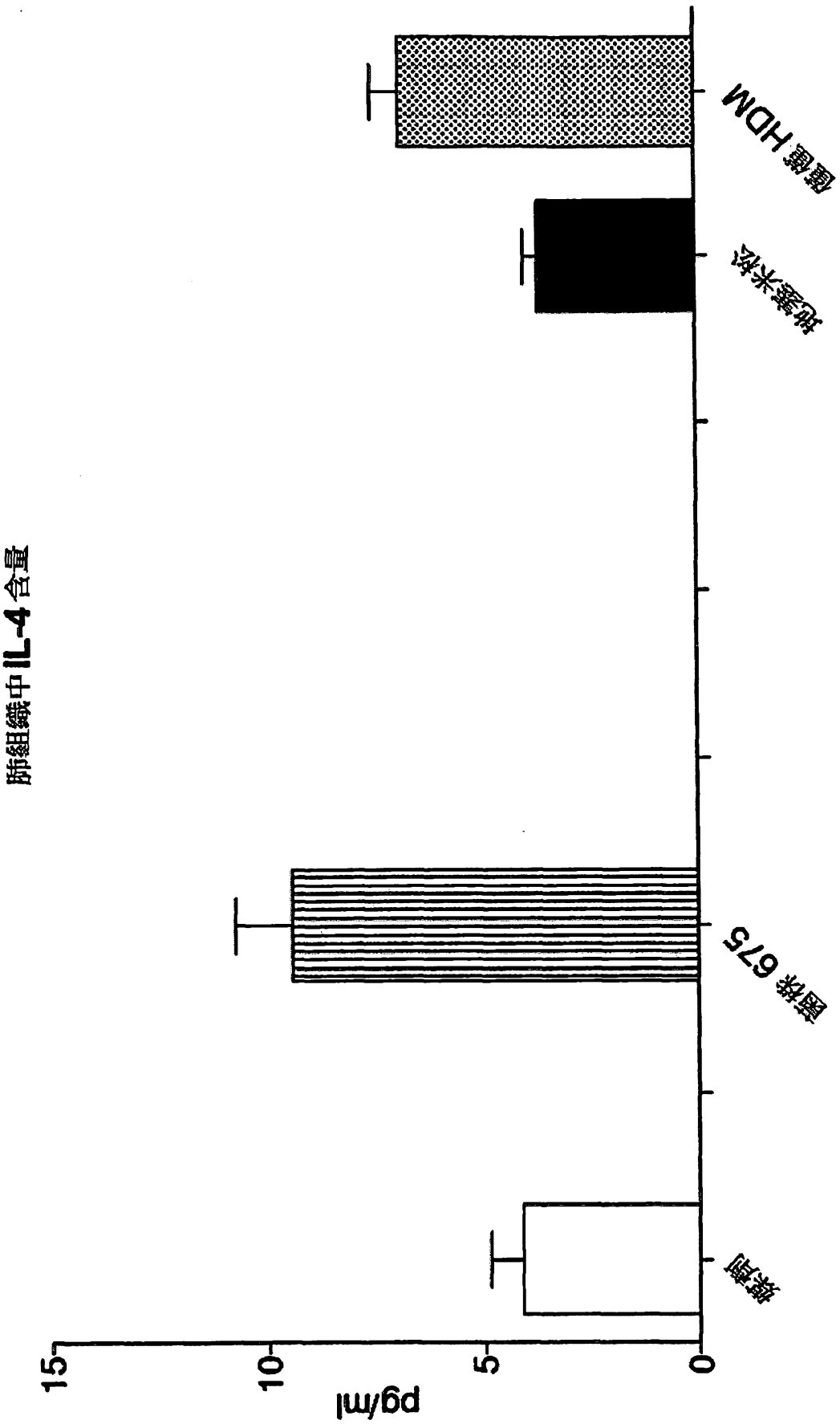


圖 40

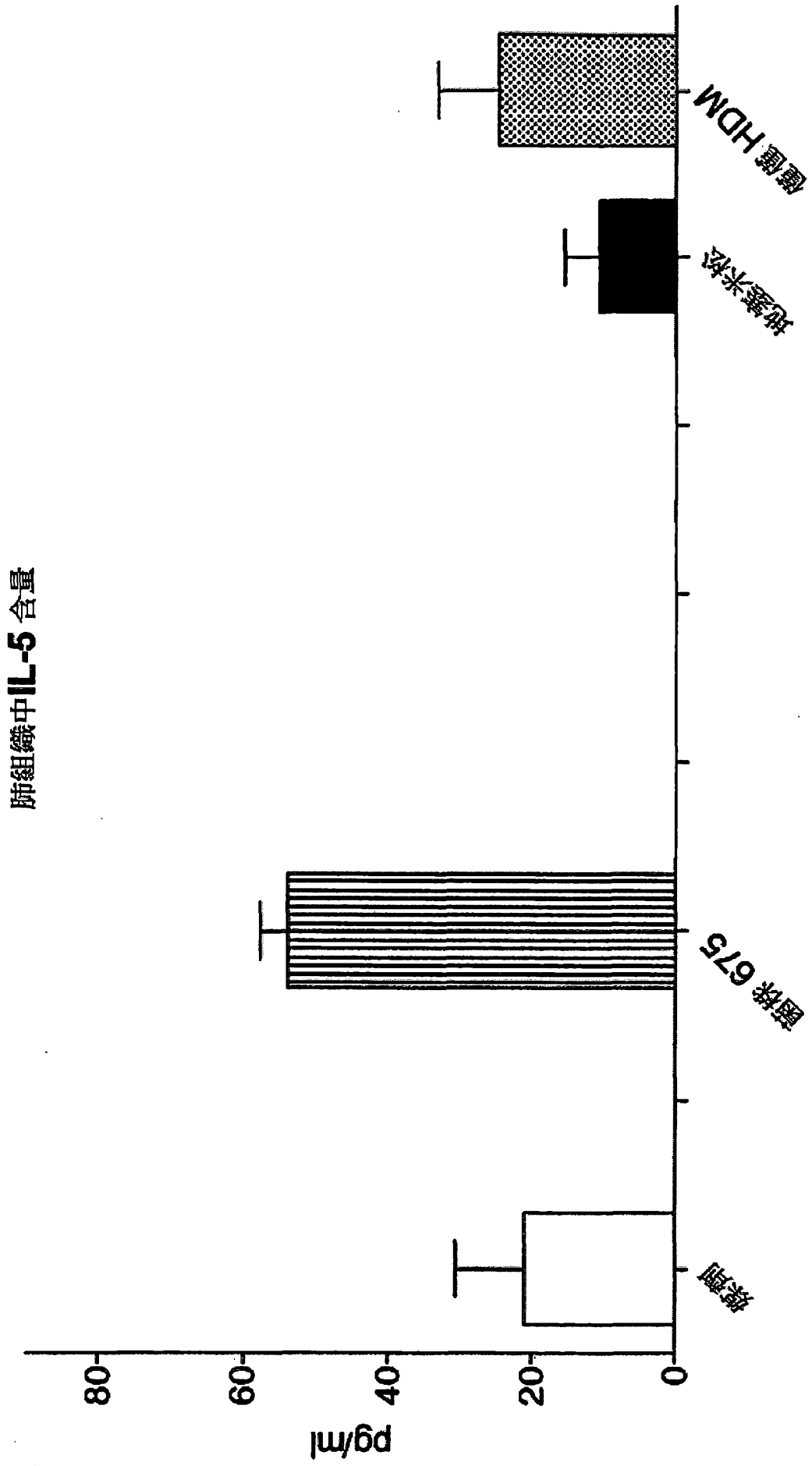


圖 41

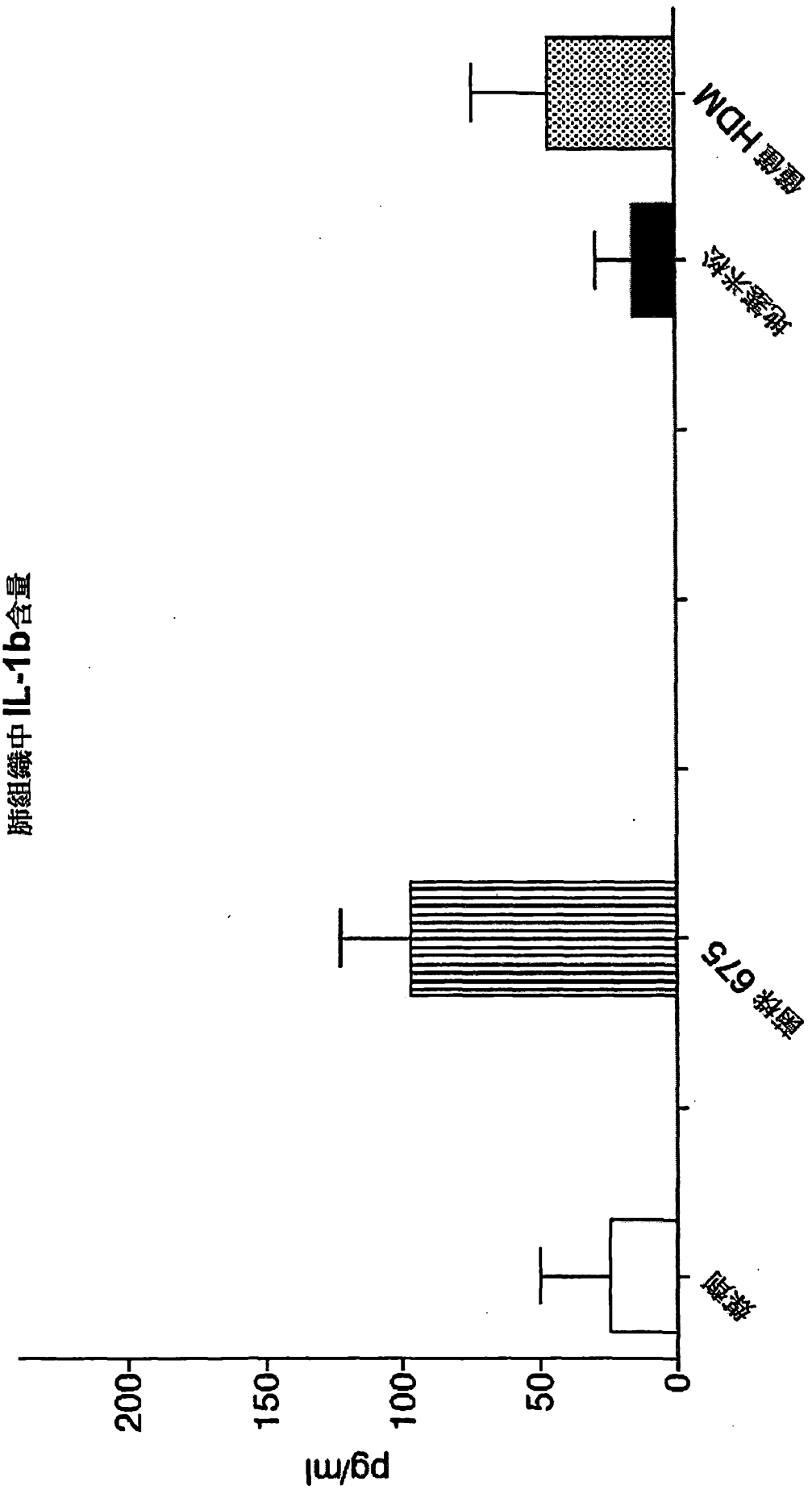


圖 42

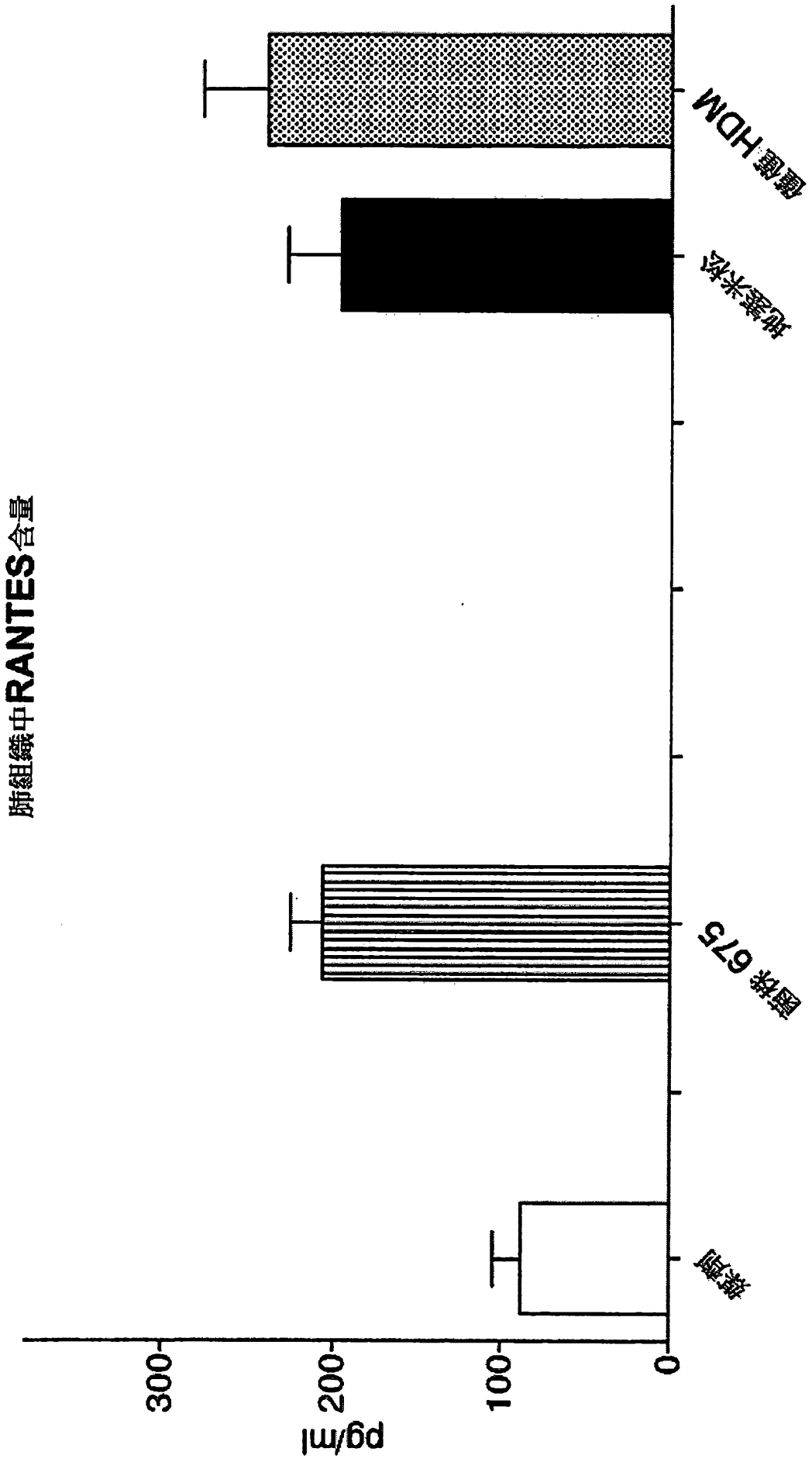


圖 43

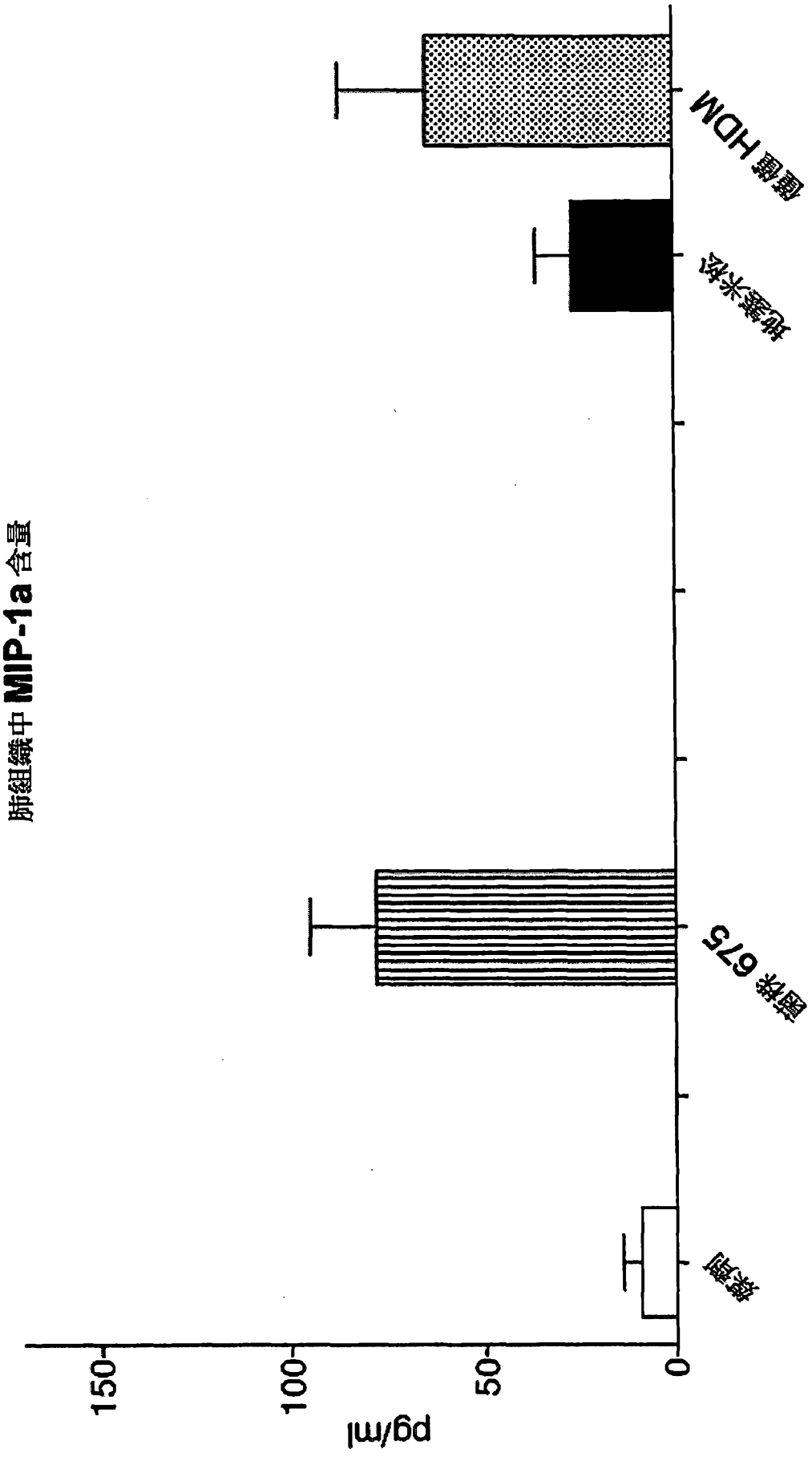


圖 44

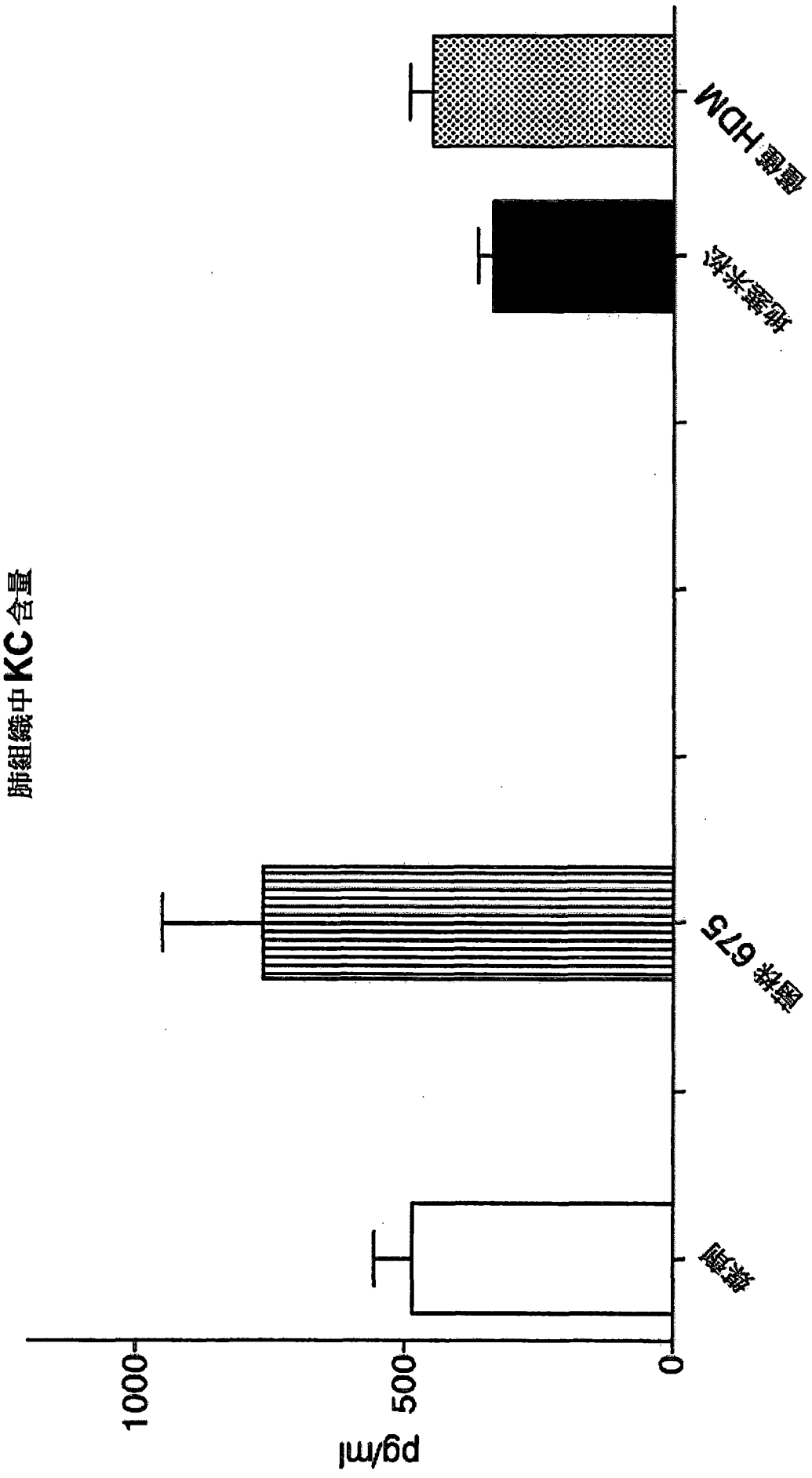


圖 45

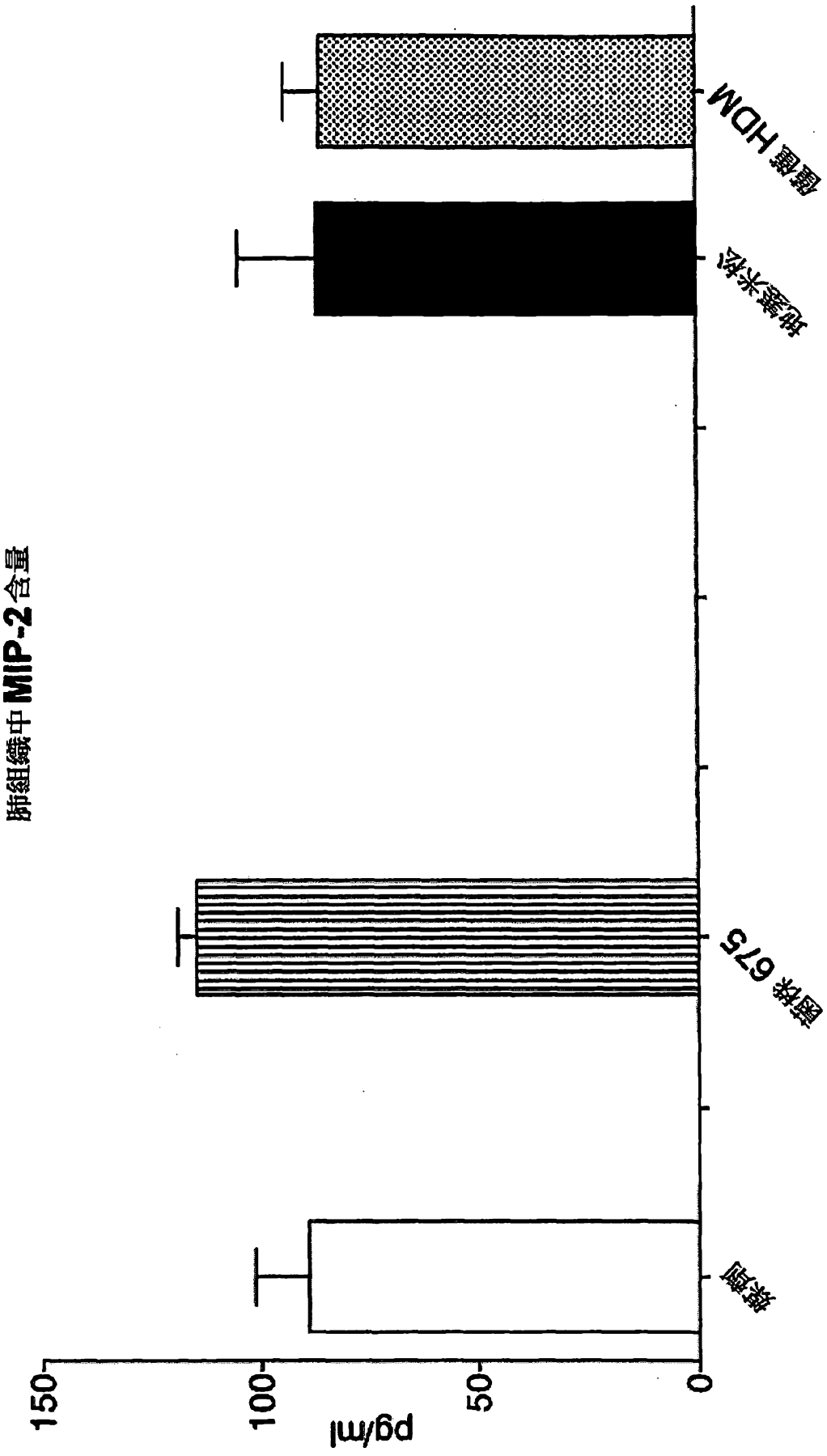


圖 46

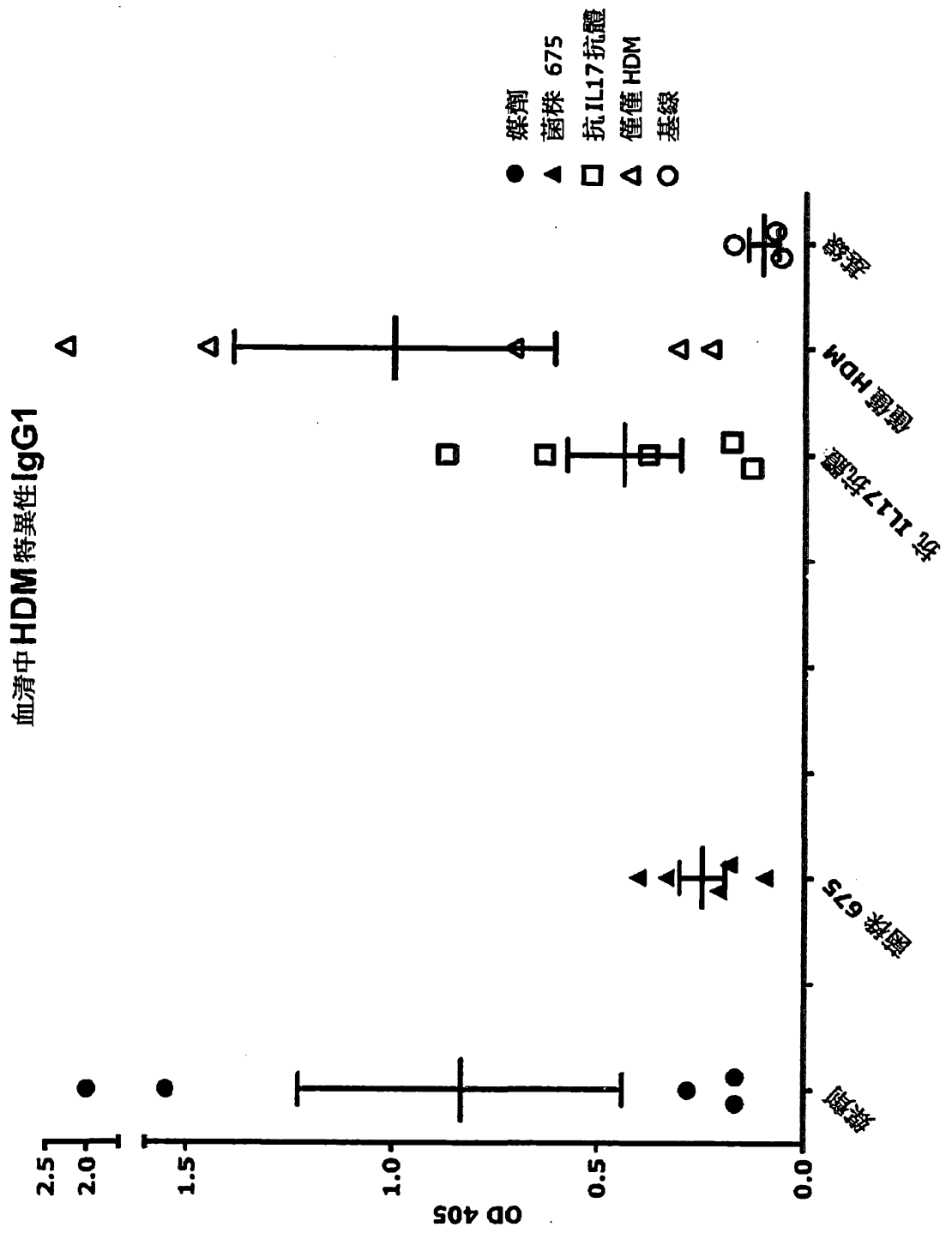

血清中HDM特異性IgG1
OD 405
2.5
2.0
1.5
1.0
0.5
0.0
媒劑
菌株 675
抗 IL17 抗體
僅僅 HDM
基線
媒劑
菌株 675
抗 IL17 抗體
僅僅 HDM
基線

圖 47

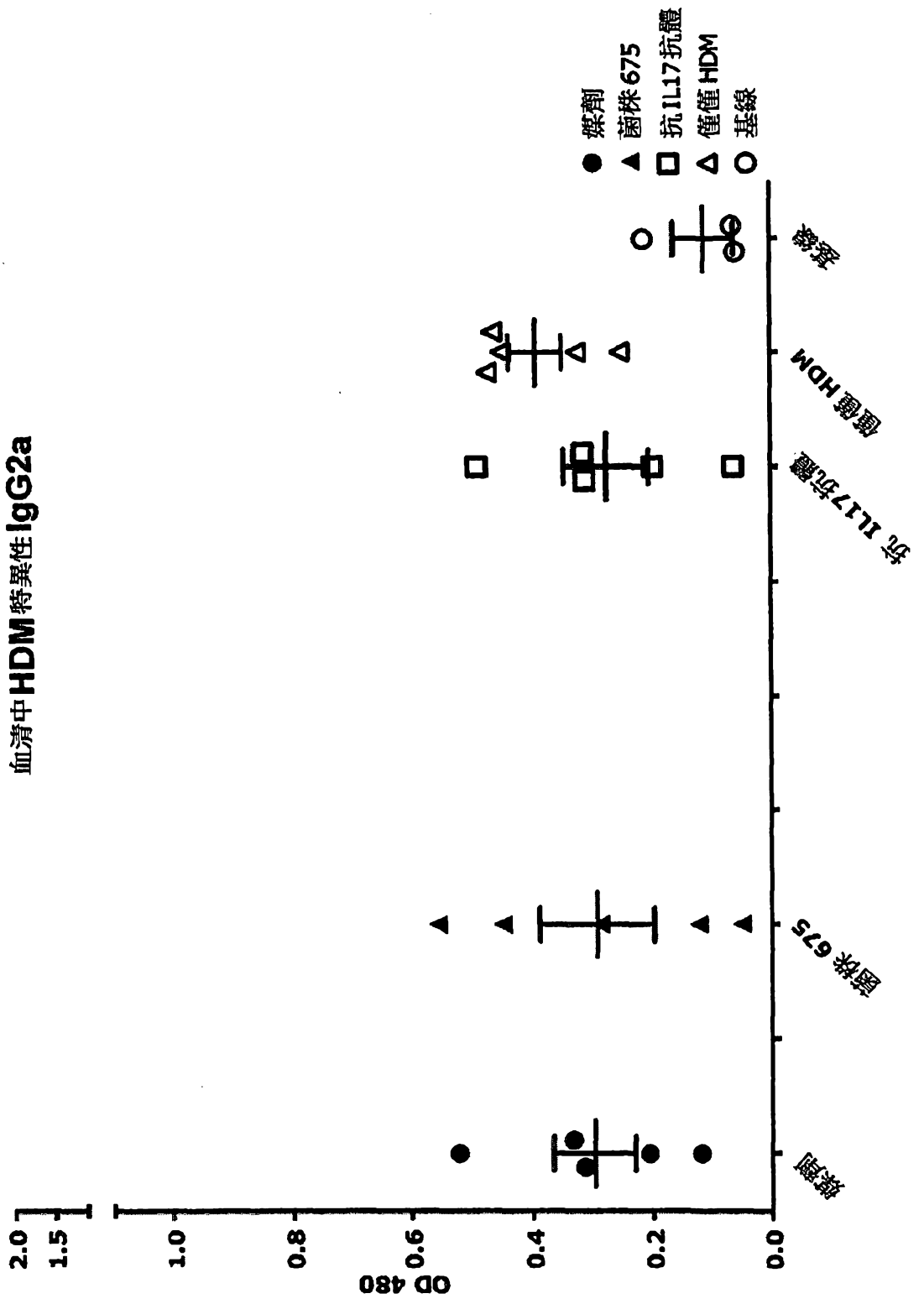

血清中HDM特異性IgG2a
OD 480
2.0
1.5
1.0
0.8
0.6
0.4
0.2
0.0
媒劑
菌株675
抗IL17抗體
僅僅HDM
基線
媒劑
菌株675
抗 IL17抗體
僅僅 HDM
基線

圖 48

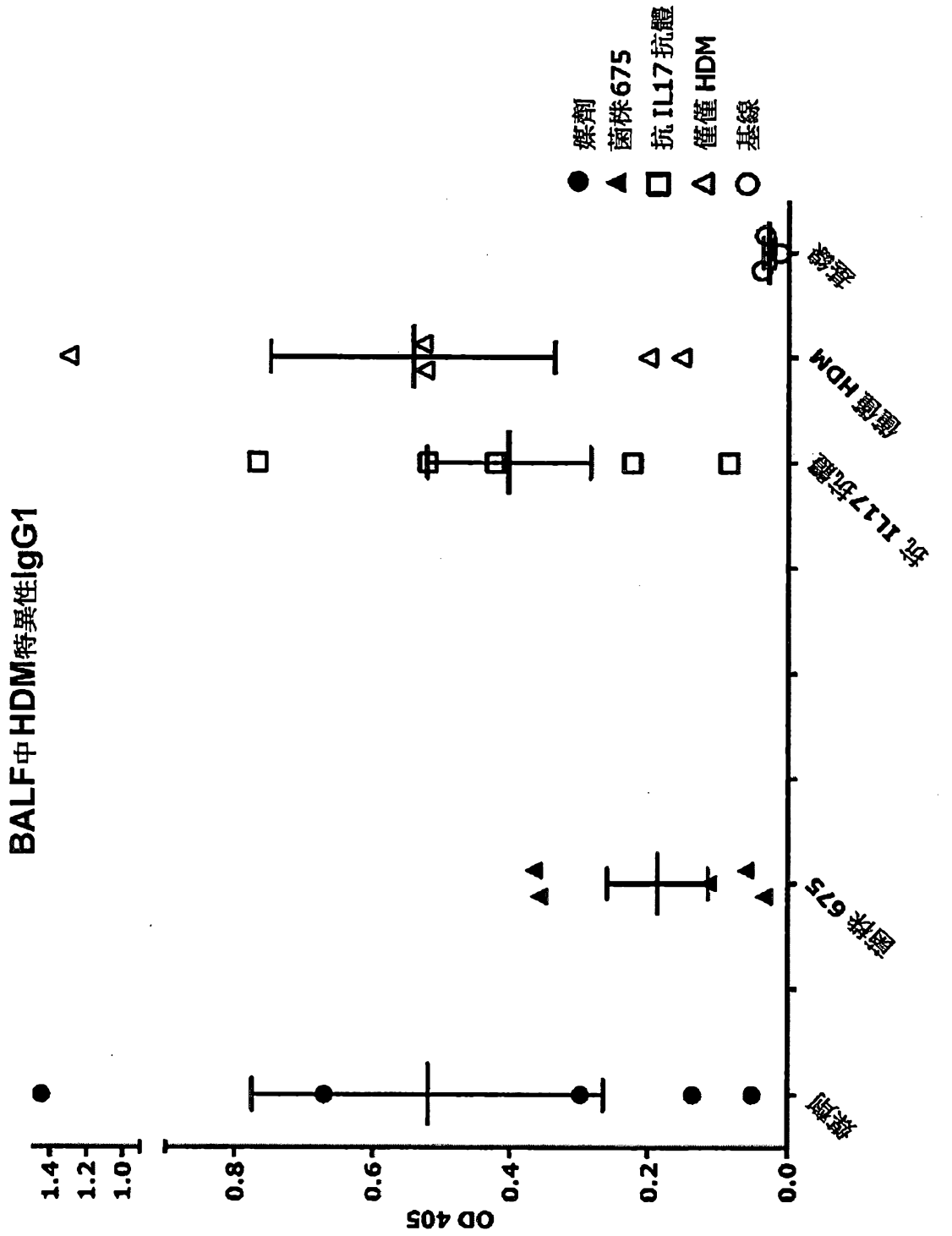

BALF中HDM特異性IgG1
OD 405
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
媒劑
菌株675
抗 IL17抗體
僅僅 HDM
基線
媒劑
菌株 675
抗 IL17抗體
僅僅 HDM
基線

圖 49

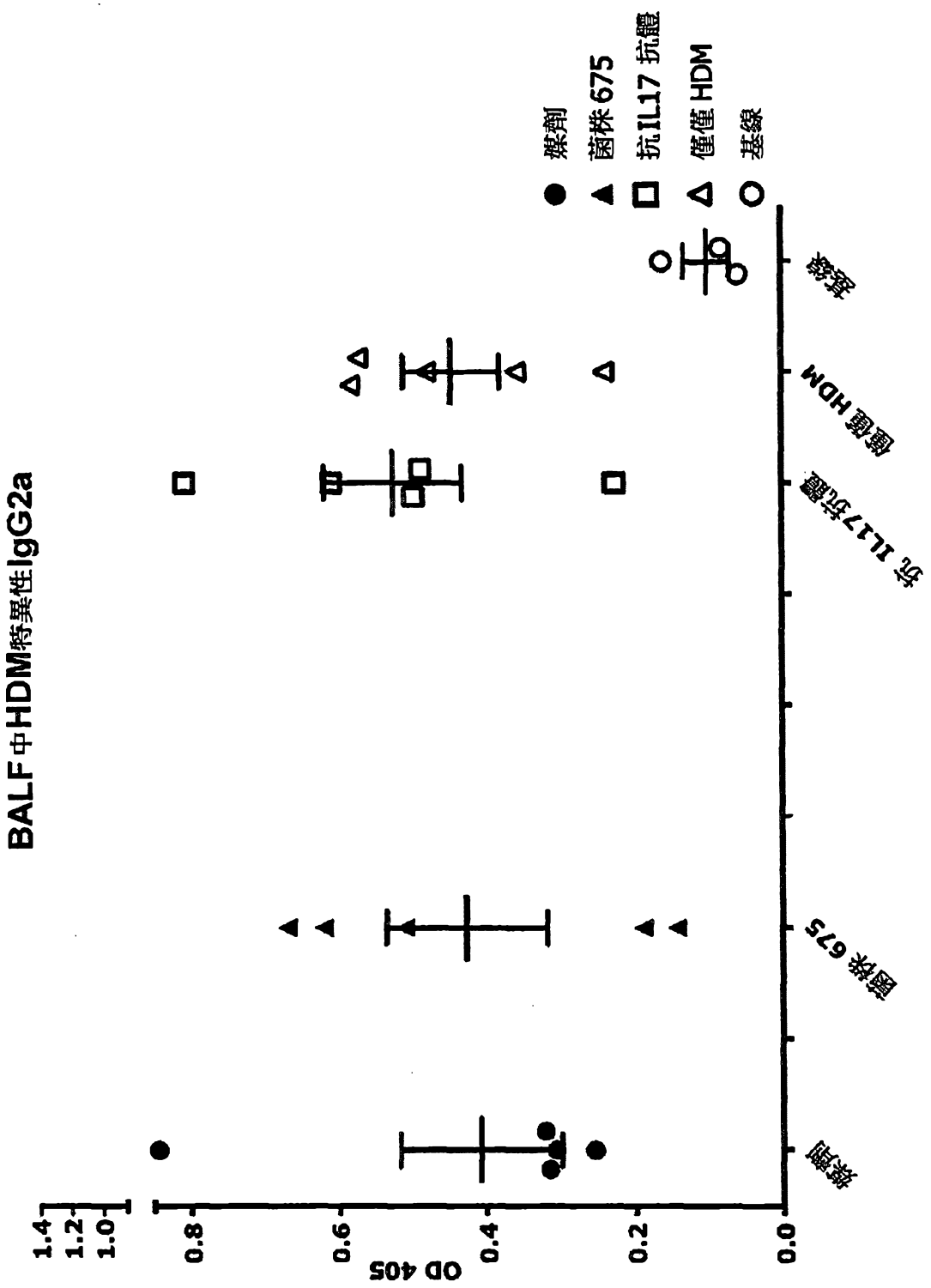

BALF中HDM特異性IgG2a
OD 405
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
媒劑
菌株675
抗IL17抗體
僅僅HDM
基線
媒劑
菌株675
抗 IL17抗體
僅僅 HDM
基線

圖 50

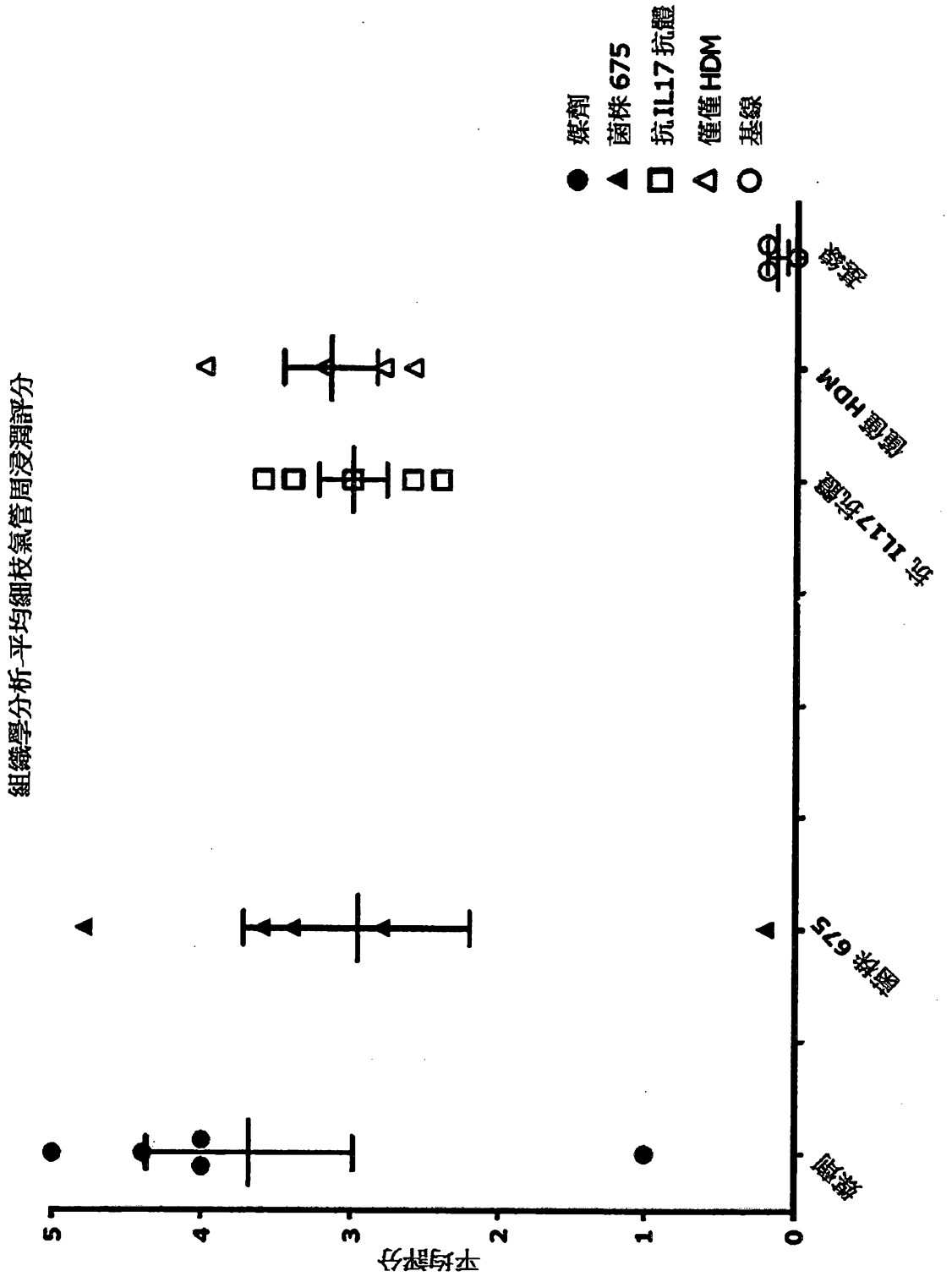

組織學分析-平均細枝氣管周浸潤評分
媒劑
菌株675
抗IL17抗體
僅僅HDM
基線
平均評分
0
1
2
3
4
5
媒劑
菌株675
抗 IL17抗體
僅僅 HDM
基線

圖 51

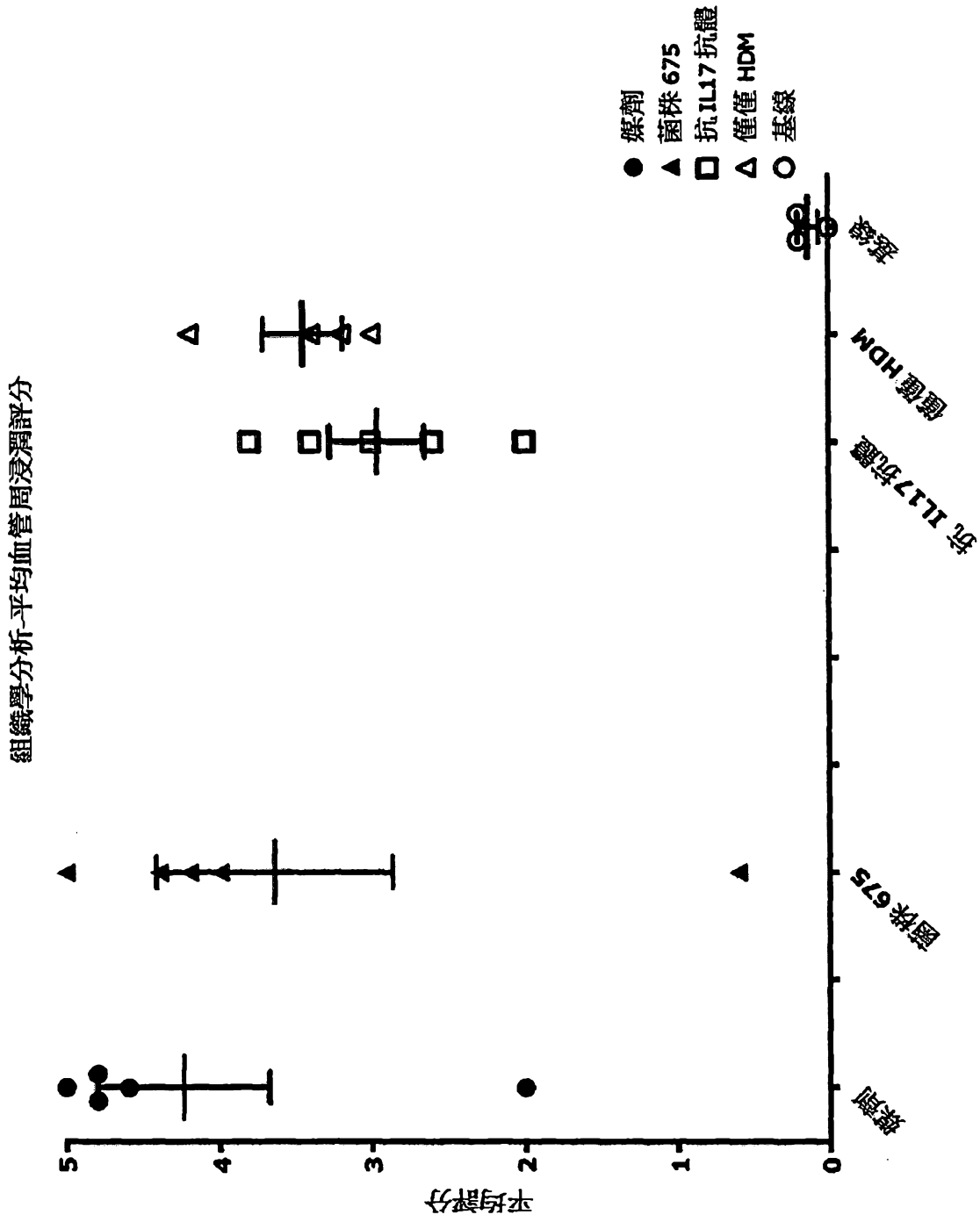

組織學分析-平均血管周浸潤評分
平均評分
0
1
2
3
4
5
媒劑
菌株 675
抗 IL17 抗體
僅僅 HDM
基線
● 媒劑
▲ 菌株 675
□ 抗 IL17 抗體
△ 僅僅 HDM
○ 基線

圖 52

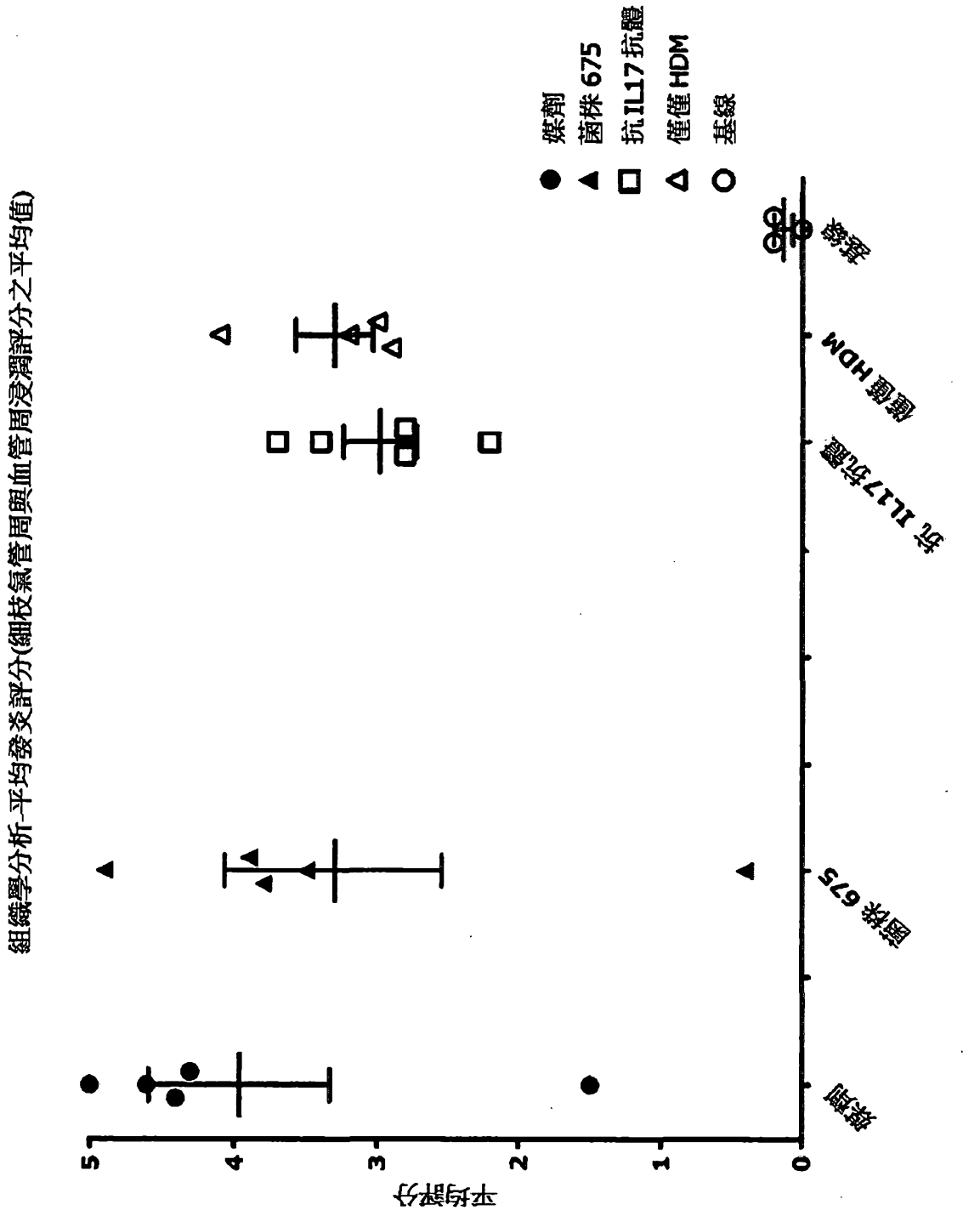

組織學分析-平均發炎評分(細枝氣管周與血管周浸潤評分之平均值)
媒劑
菌株 675
抗 IL17 抗體
僅僅 HDM
基線
平均評分
0
1
2
3
4
5
媒劑
菌株 675
抗 IL17 抗體
僅僅 HDM
基線

圖 53

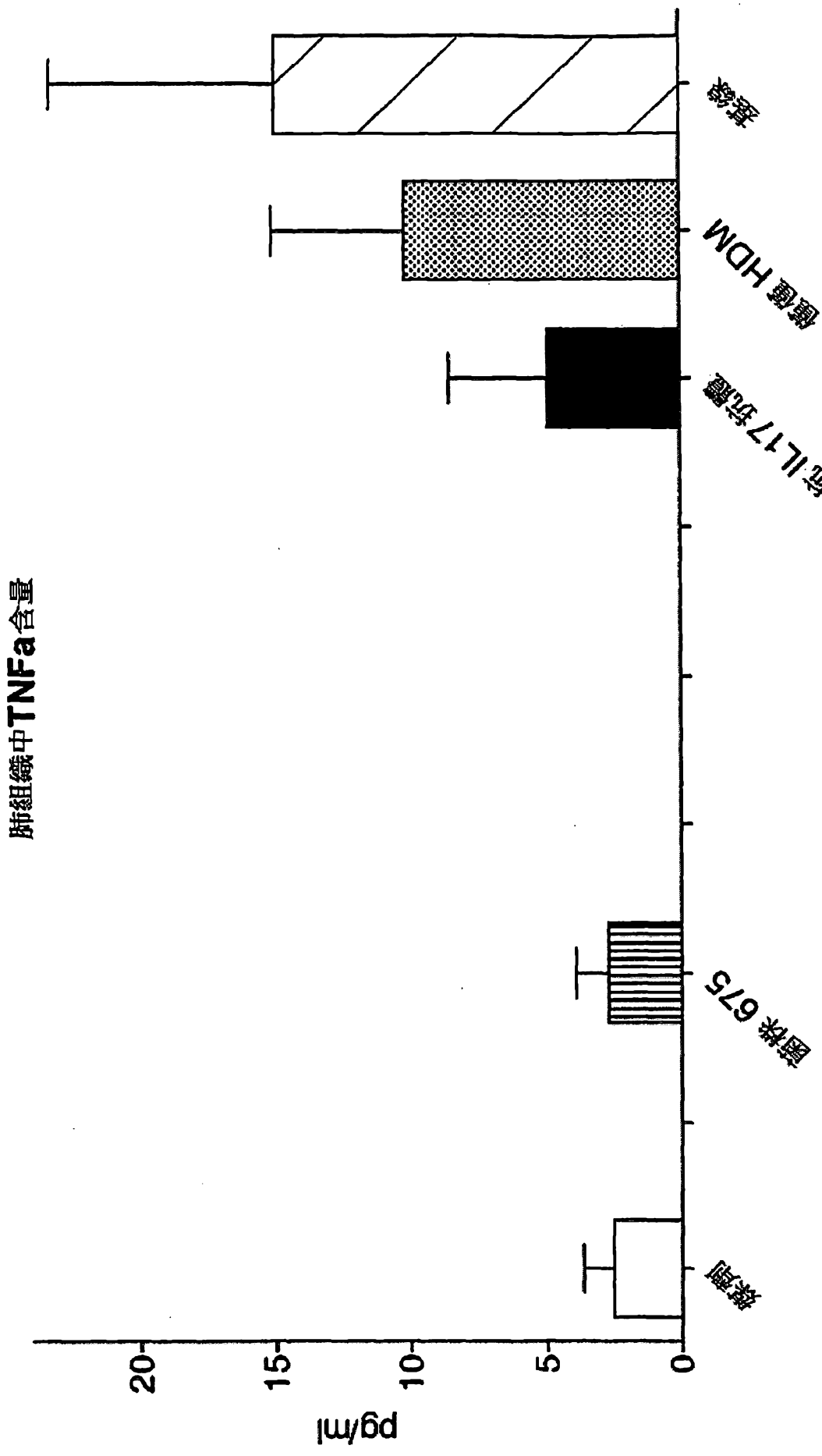


圖 54

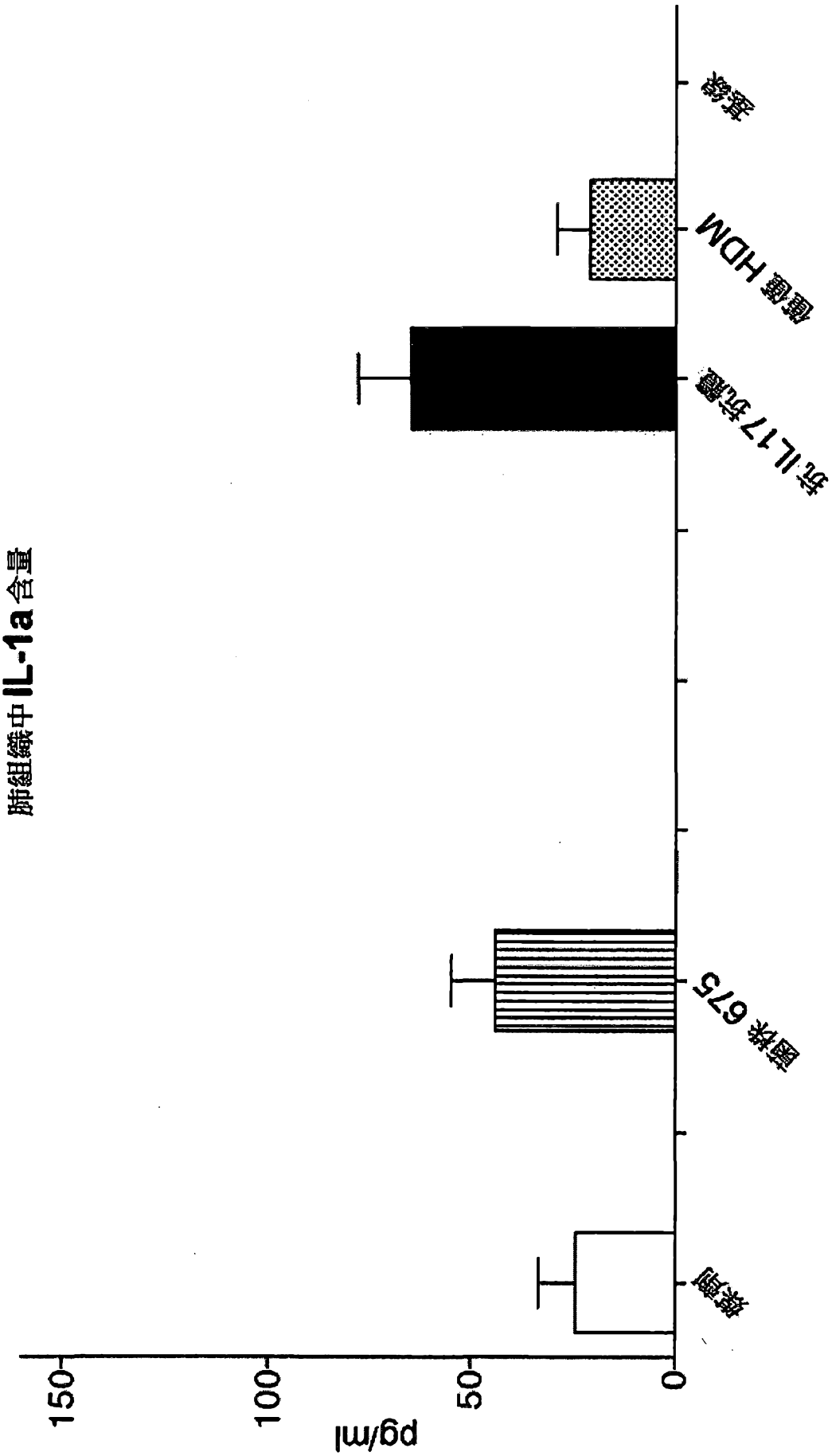


圖 55

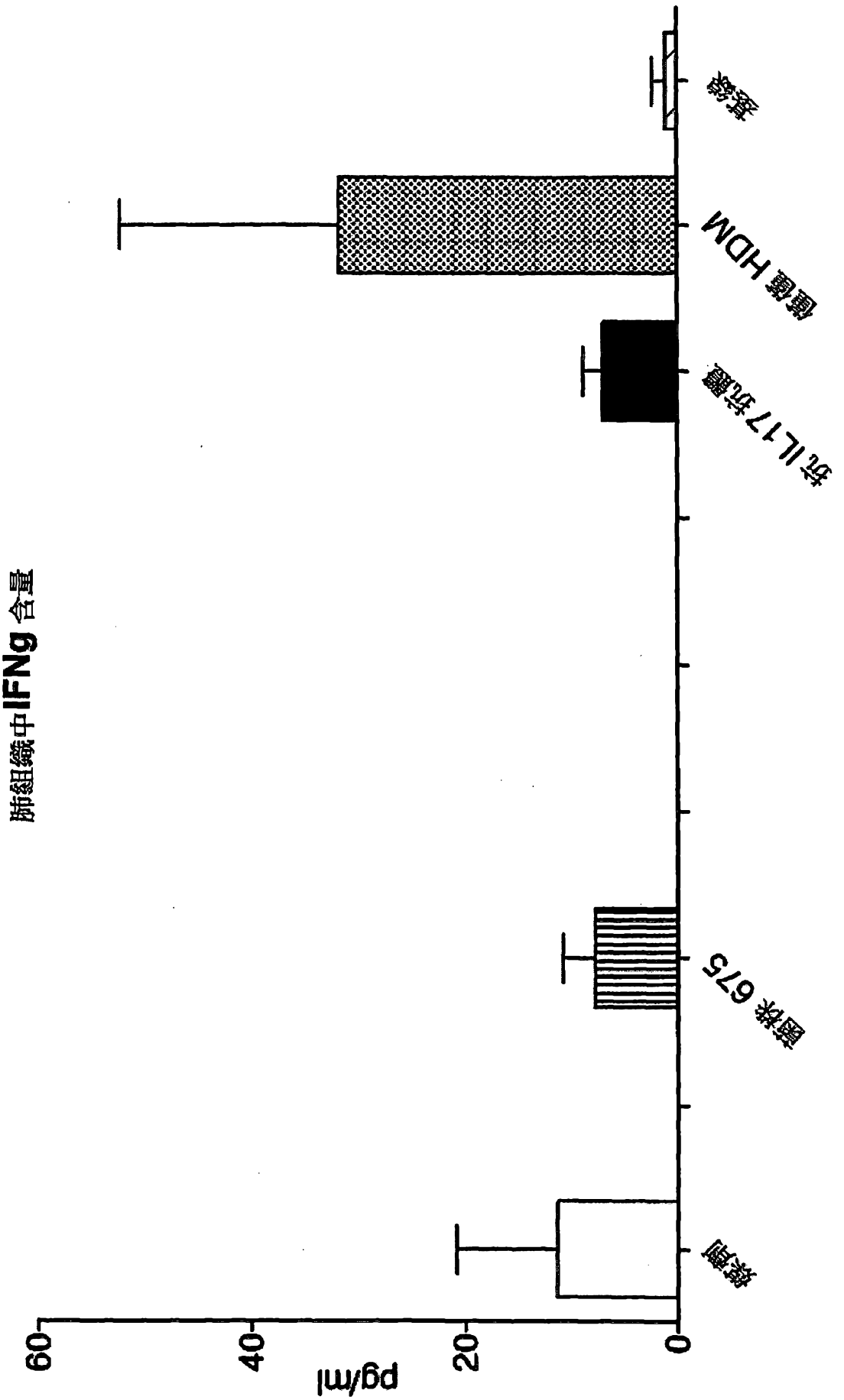


圖 56

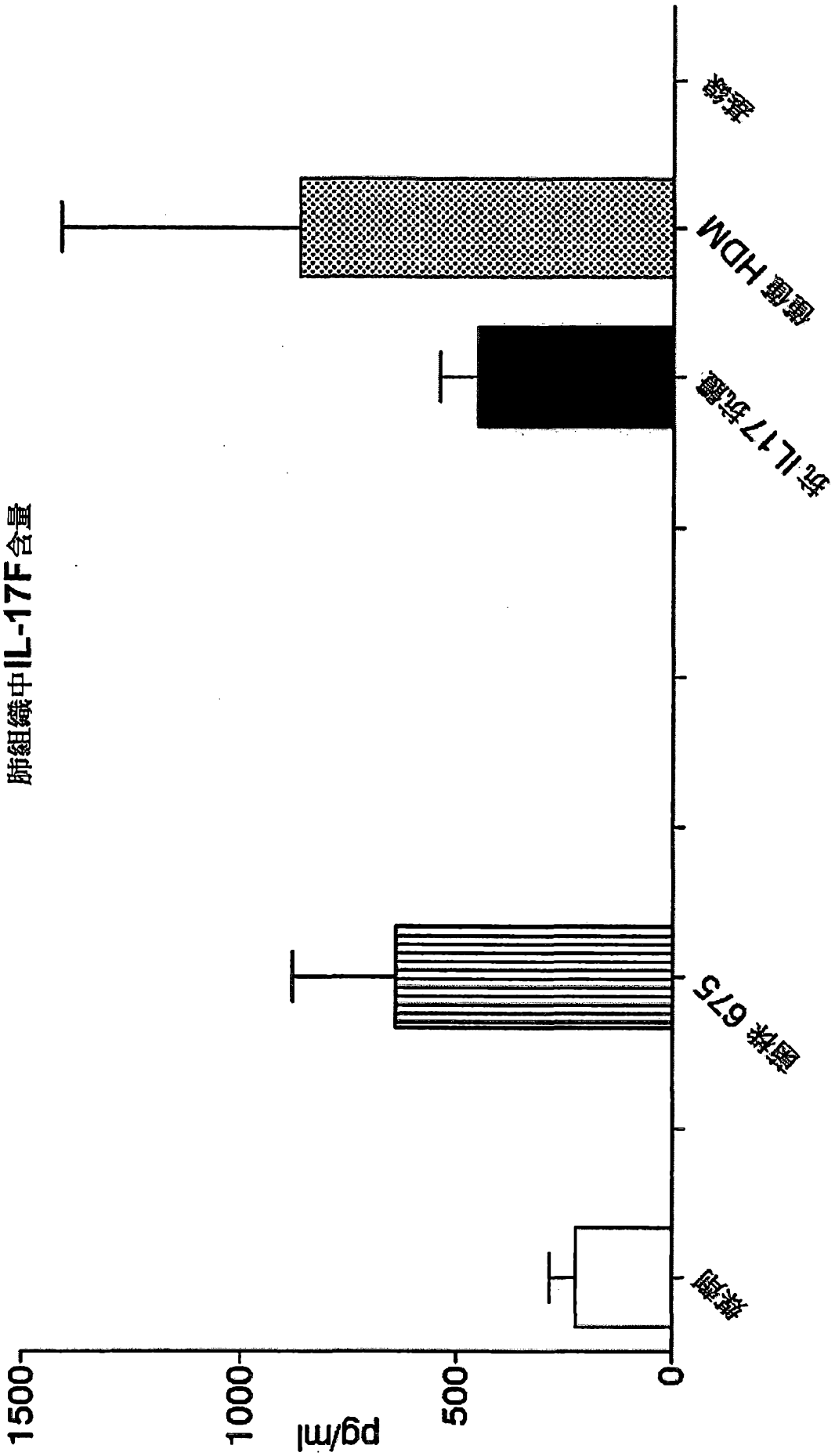


圖 57

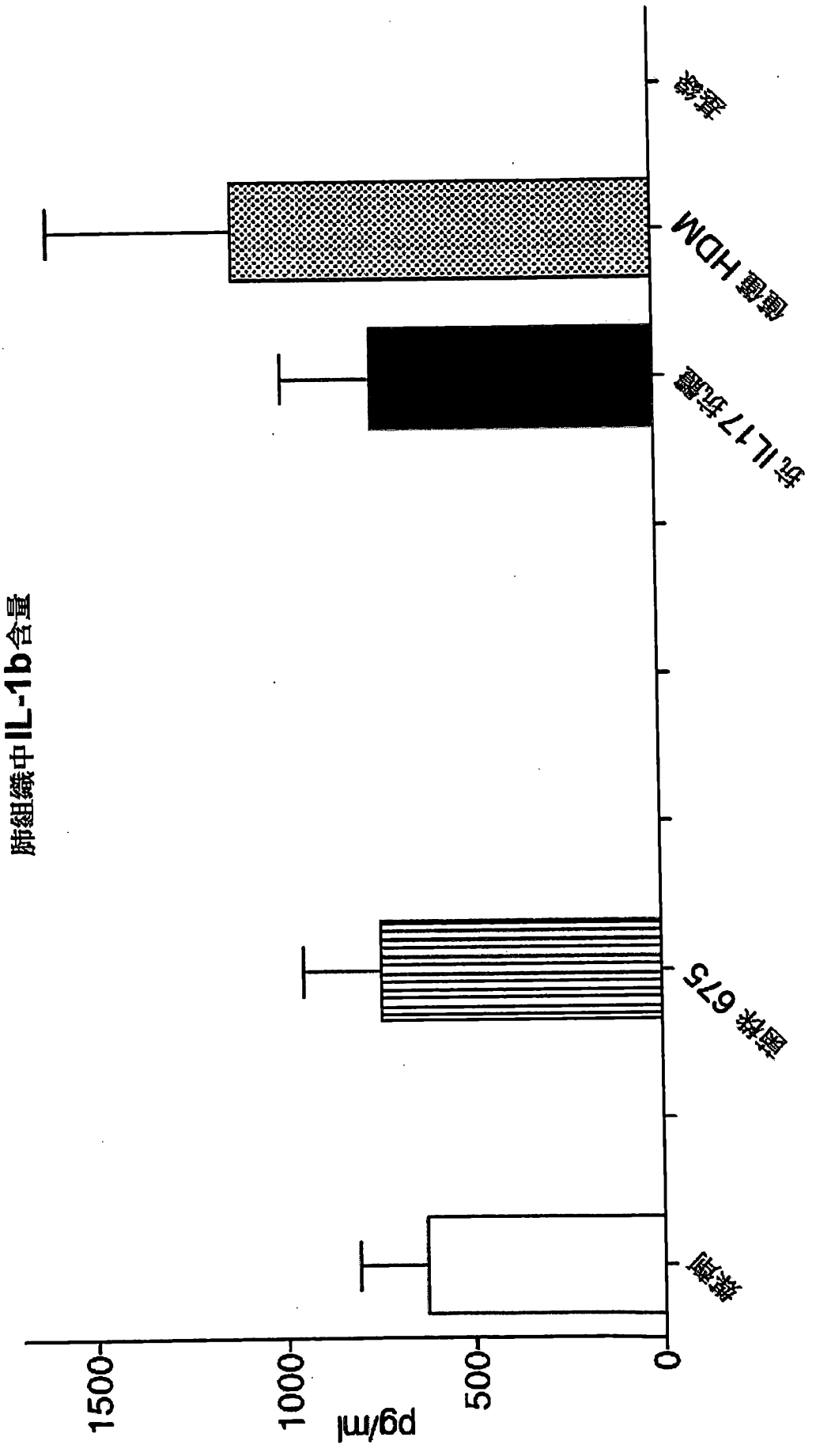


圖 58

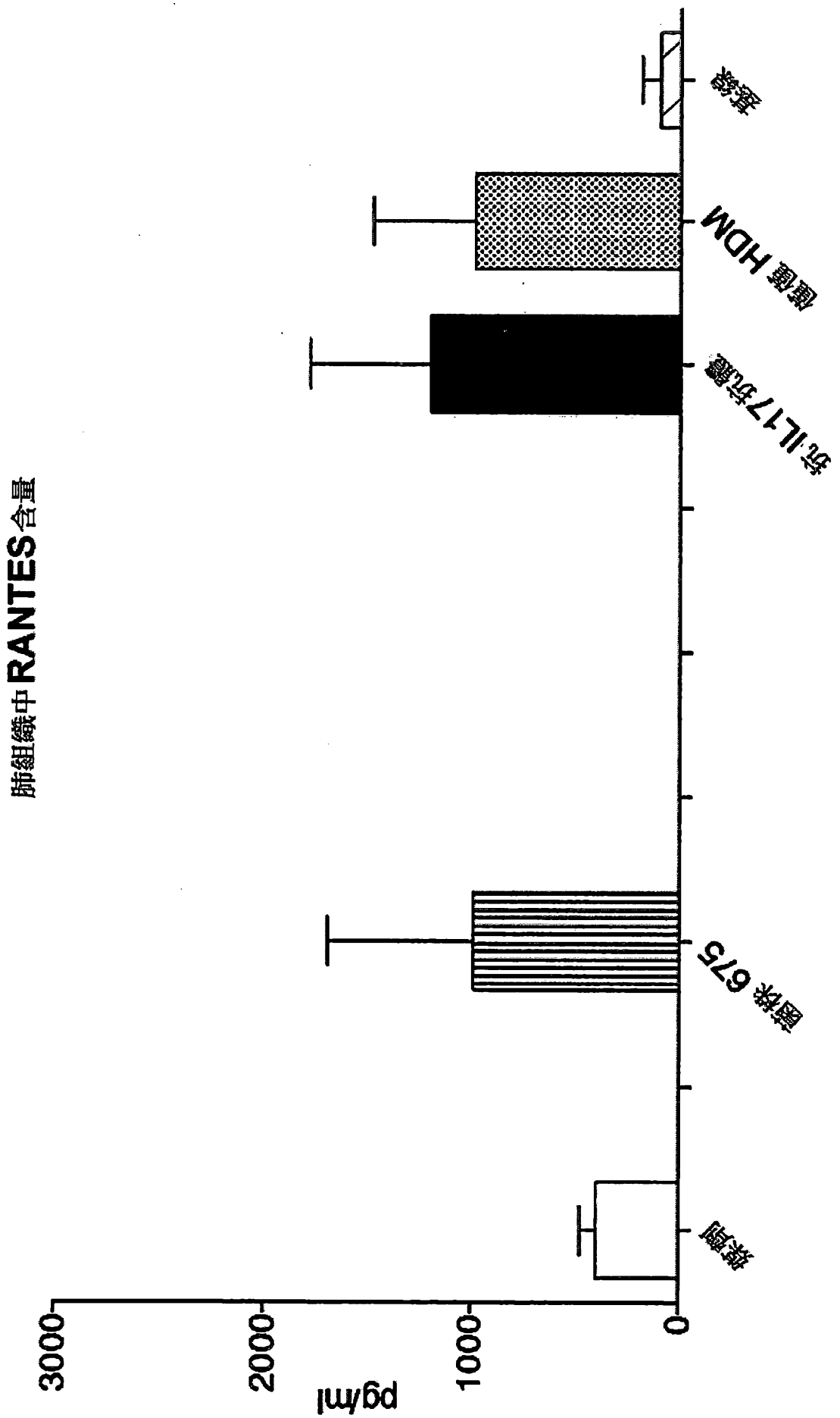

肺組織中RANTES含量
pg/ml
3000
2000
1000
0
無菌
屋塵 675
屋塵 HDM
抗IL17抗體
無菌

圖 59

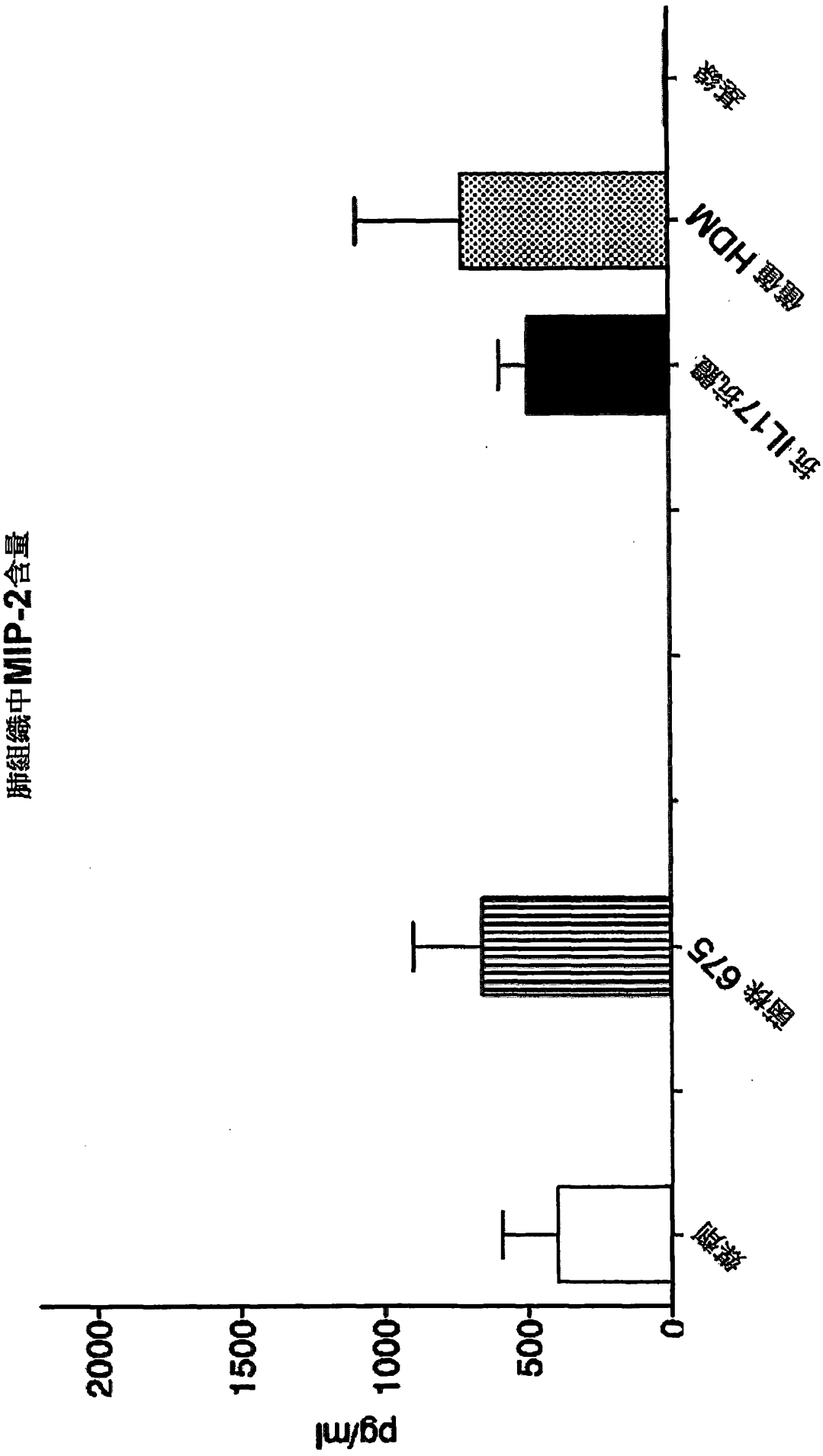

肺組織中MIP-2含量
pg/ml
2000
1500
1000
500
0
載體
載體 HDM
抗 IL17抗體
類固醇 675

圖 60

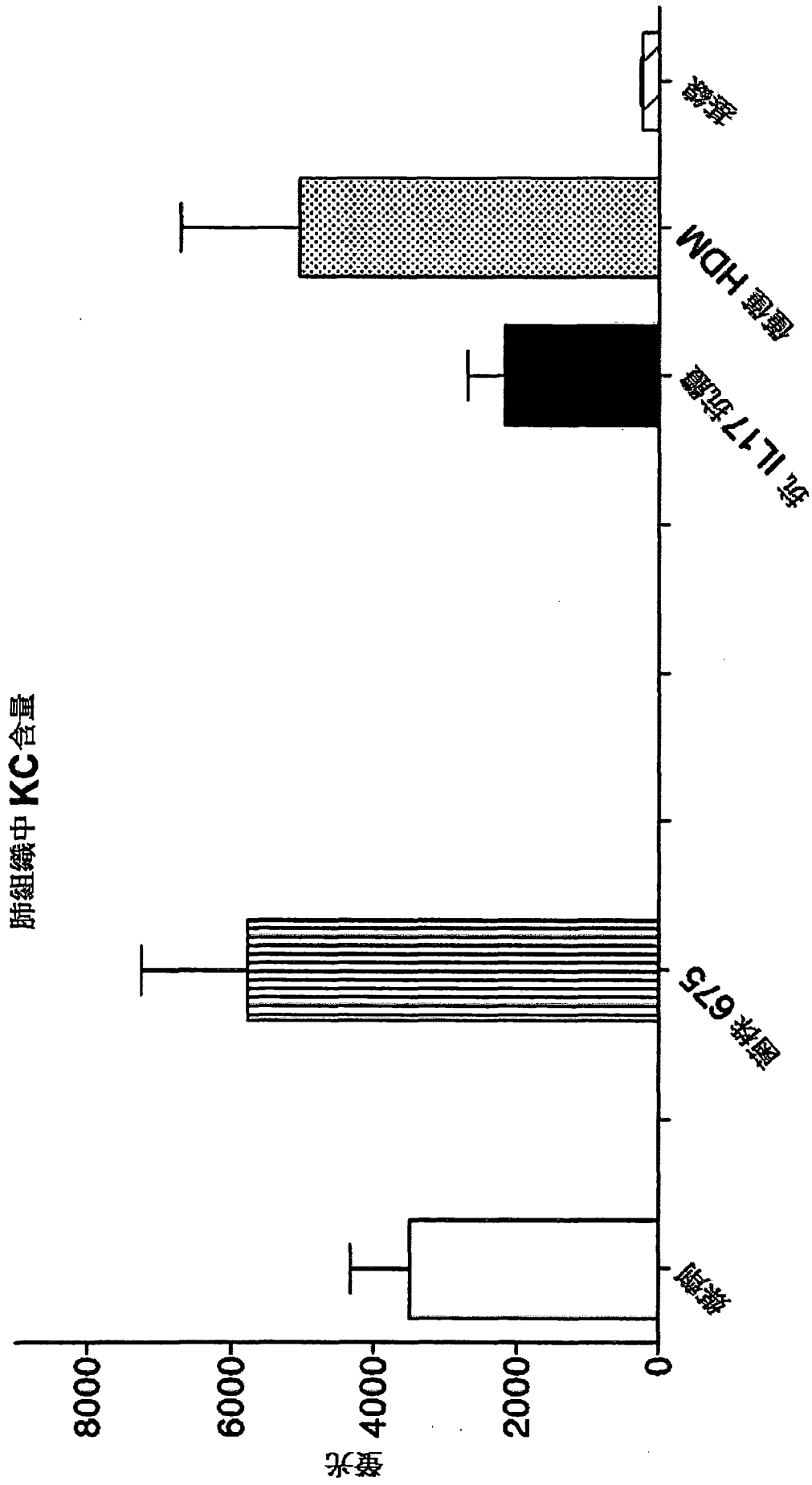


圖 61

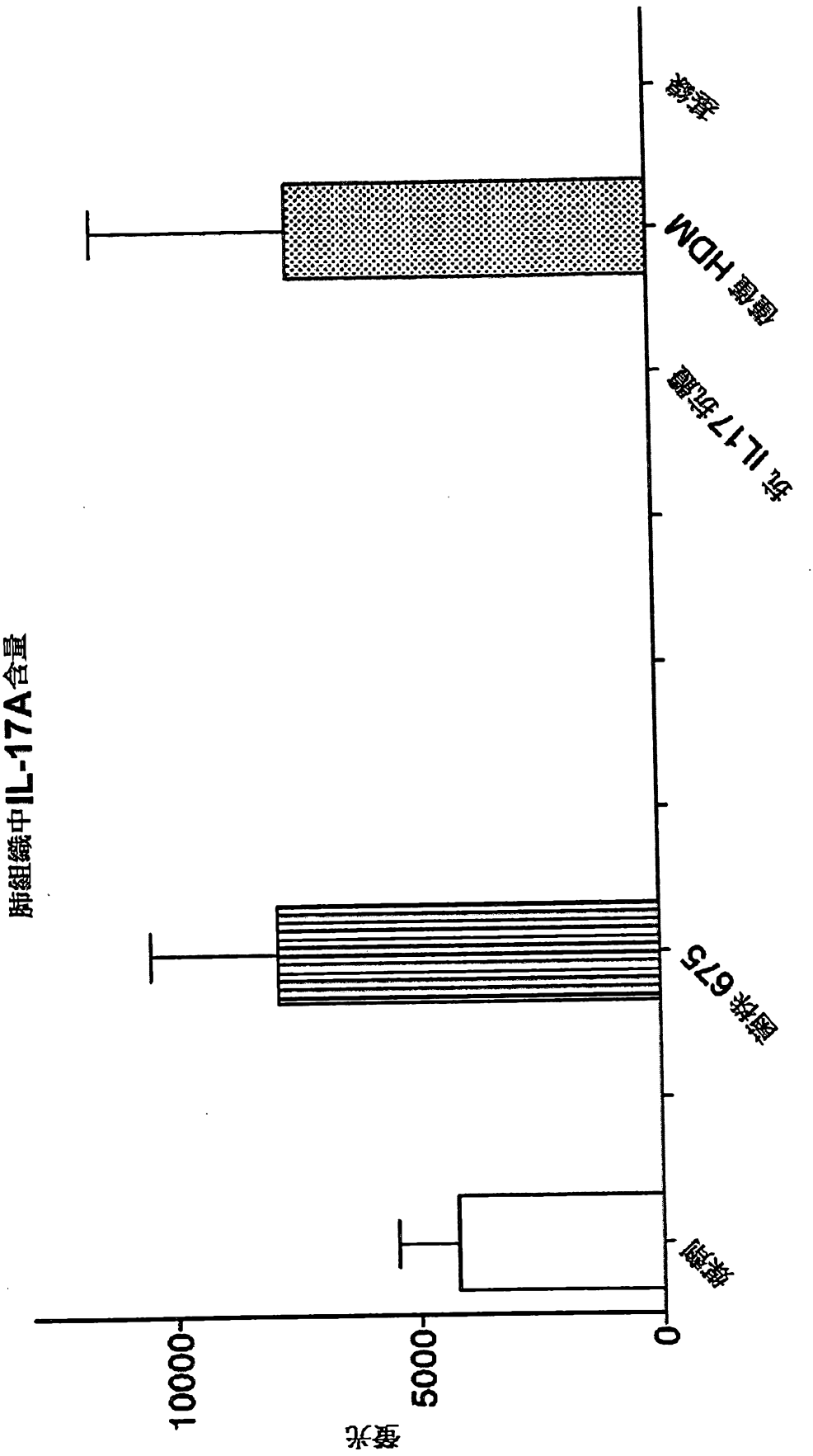


圖 62

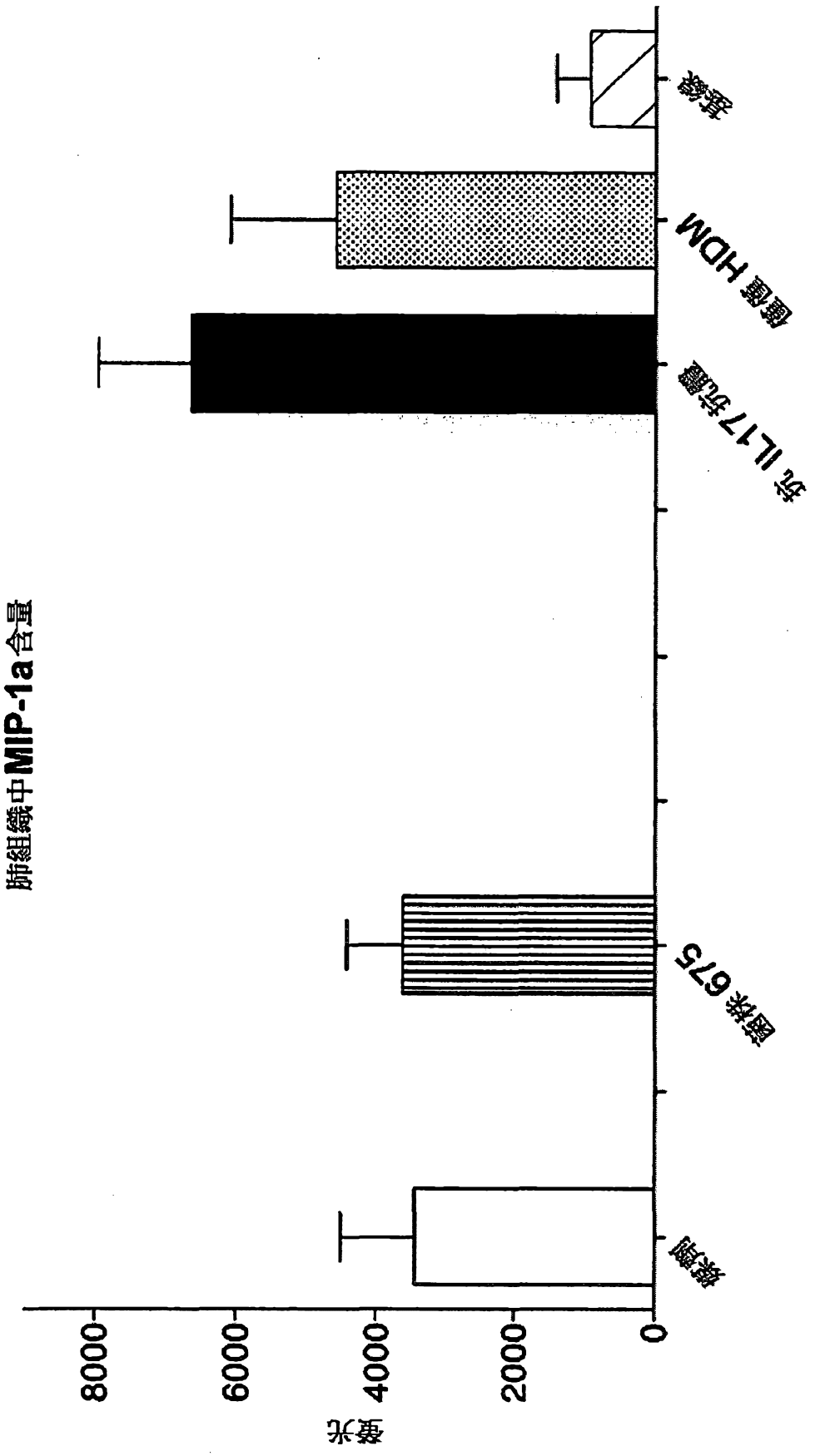

肺組織中MIP-1a含量
螢光
8000
6000
4000
2000
0
磷酸鹽緩衝液
圖案675
塵蟎HDM
卵IL17抗體
載體

圖 63

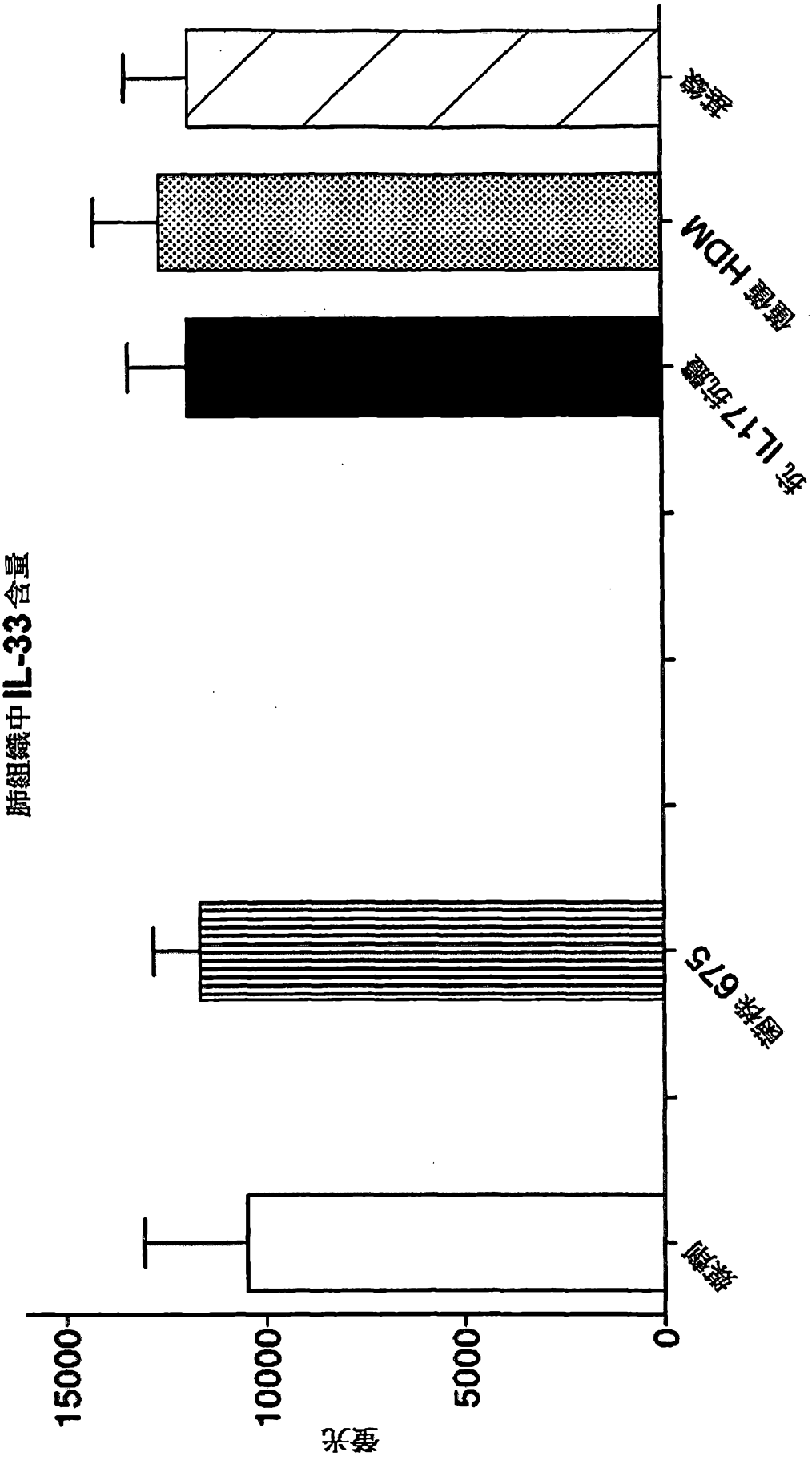


圖 64

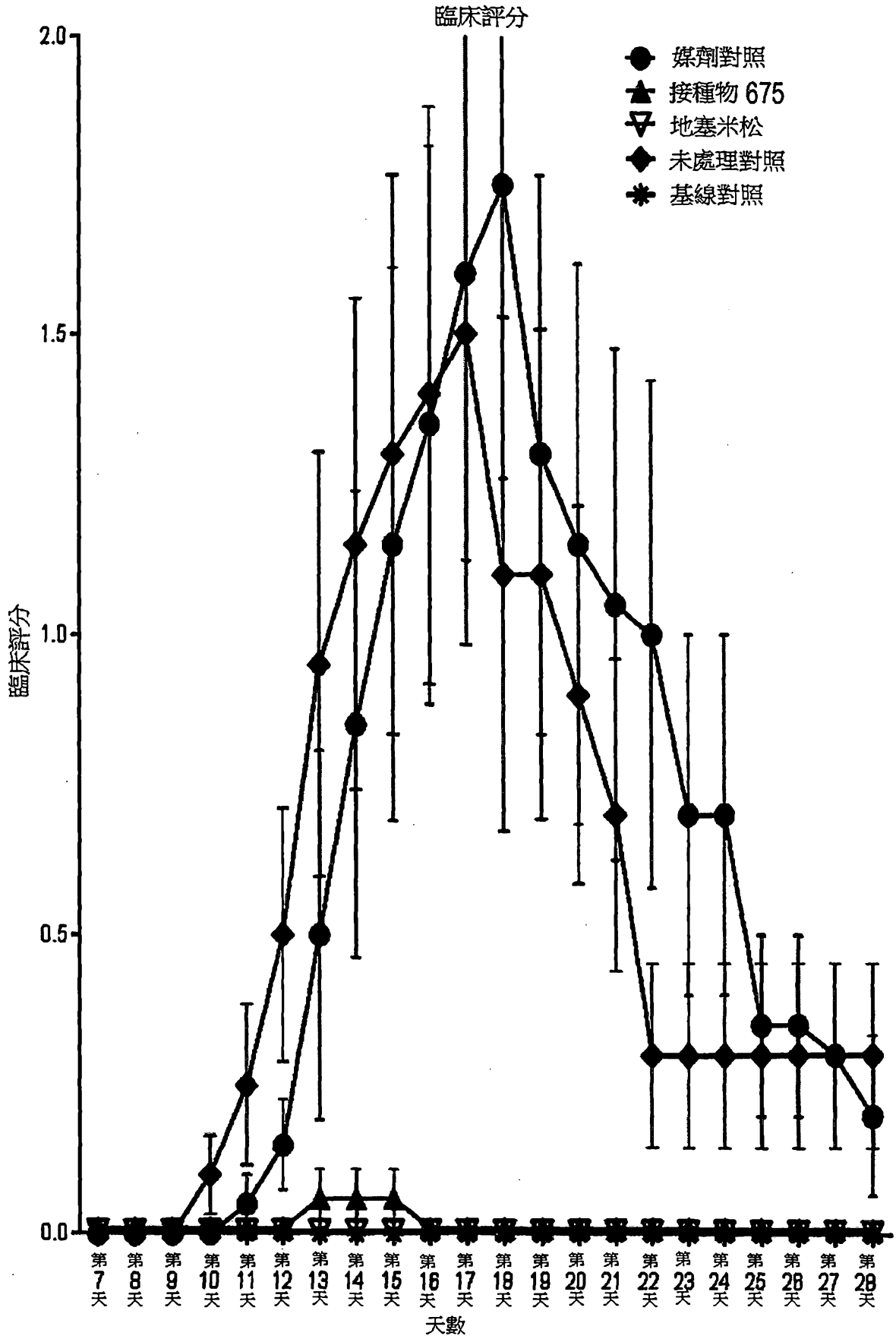

臨床評分
媒劑對照
接種物 675
地塞米松
未處理對照
基線對照
2.0
1.5
1.0
0.5
0.0
臨床評分
第7天
第8天
第9天
第10天
第11天
第12天
第13天
第14天
第15天
第16天
第17天
第18天
第19天
第20天
第21天
第22天
第23天
第24天
第25天
第26天
第27天
第28天
天數

圖 72

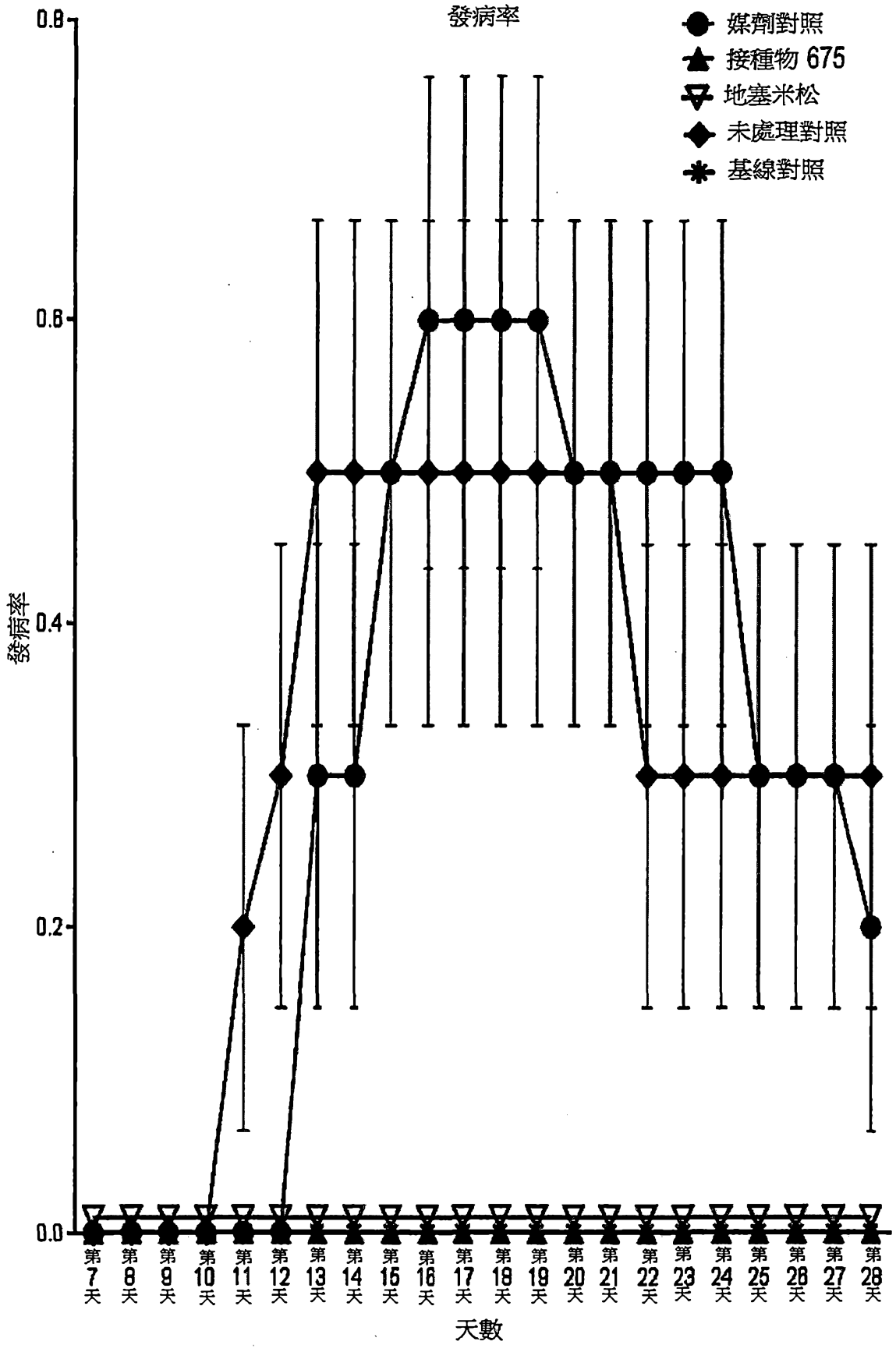

發病率
媒劑對照
接種物 675
地塞米松
未處理對照
基線對照
0.8
0.6
0.4
0.2
0.0
發病率
第 7 天
第 8 天
第 9 天
第 10 天
第 11 天
第 12 天
第 13 天
第 14 天
第 15 天
第 16 天
第 17 天
第 18 天
第 19 天
第 20 天
第 21 天
第 22 天
第 23 天
第 24 天
第 25 天
第 26 天
第 27 天
第 28 天
天數

圖 73