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(56) 선행기술조사문헌
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(73) 특허권자
씨제이바이오사이언스 주식회사
서울특별시 중구 세종대로 14, 비동 7층(남대문로5가, 그랜드센트럴타워)
(72) 발명자
그랜트, 조지
영국 에이비25 2제트엑스 에버딘 에버딘셔 콘힐 로드 라이프 사이언스즈 이노베이션 빌딩
패터슨, 안젤라 마가렛
영국 노리치 노퍽 엔알4 7제이티 유니버시티 오브 이스트 앵글리아 팩컬티 오브 메디신 앤 헬스 사이언스즈 노리치 메디컬 스쿨
(뒷면에 계속)
(74) 대리인
유미특허법인

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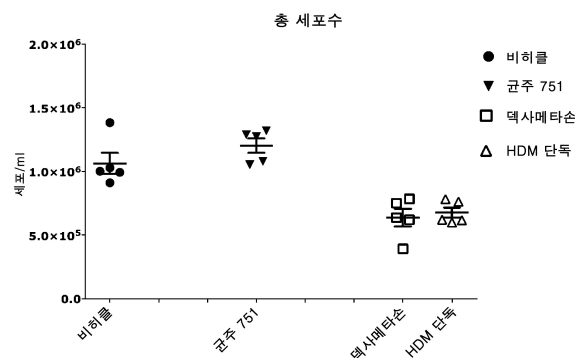
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(54) 발명의 명칭 박테리아성 균주를 함유한 조성물

(57) 요약

본 발명은 염증성 및 자가면역 질환을 치료 및 예방하기 위한 박테리아성 균주를 포함하는 조성물을 제공한다.

대표도



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(72) 발명자

멀더, 임케

영국 에이비25 2제트엑스 에버딘 에버딘서 콘힐 로
드 라이프 사이언스 이노베이션 빌딩

맥클러스키, 셔닌

영국 엘에스1 2제이제트 리즈 요크셔 본드 코트 9
써드 플로어 4디 파마 피엘씨 내

라프티스, 엠마

영국 엘에스1 2제이제트 리즈 요크셔 본드 코트 9
써드 플로어 4디 파마 피엘씨 내

명세서

청구범위

청구항 1

서열번호: 1의 16s rRNA 서열을 갖는 박테리아를 포함하는 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물로서,

상기 염증 또는 자가면역 질병은 천식; 류마티스성 관절염; 골관절염; 건선성 관절염; 유년성 특발성 관절염; 다발성 경화증; 시신경척수염(데빅(Devic) 병); 강직 척추염; 척추관절염; 건선; 전신 홍반성 낭창; 크론병; 궤양성 대장염; 소아지방변증; 만성적 폐색성 폐 질병(COPD); 포도막염; 공막염; 혈관염; 베체트(Behcet)병; 죽상 경화증; 아토피성 피부염; 폐경기증; 치주염; 알러지성 비염; 및 동종이식편 거부반응으로 이루어진 군으로부터 선택되는 질병인 것인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 2

청구항 1에 있어서, 상기 박테리아는 수탁 번호 NCIMB 42380하에 기탁된 박테리아인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 3

청구항 1에 있어서, 상기 박테리아는 폴리사카라이드 라피노스를 발효시킬 수 있는, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 4

청구항 1에 있어서, 상기 약학적 조성물은 조성물의 총 중량에 대하여 1×10^6 내지 1×10^{11} CFU/g의 박테리아 균주를 포함하는, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 5

삭제

청구항 6

삭제

청구항 7

삭제

청구항 8

청구항 1에 있어서, 상기 박테리아는 서열번호: 2의 게놈을 갖는 것인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 9

청구항 1에 있어서, 상기 약학적 조성물은 상기 박테리아 세포 또는 상기 박테리아의 생물학적으로 순수한 배양물을 포함하는 것인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 10

청구항 1에 있어서, 천식을 치료 또는 예방하는 방법에 사용하거나, 또는 천식 치료에서 호중구 또는 호산구를 감소시키는 방법에 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 11

청구항 1에 있어서, 류마티스성 관절염을 치료 또는 예방하는 방법에 사용하거나, 또는 류마티스성 관절염에서 관절 팽윤을 감소시키는 방법에 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 12

청구항 1에 있어서, 다발성 경화증을 치료 또는 예방하는 방법에 사용하거나, 또는 다발성 경화증의 질병 발생 또는 질병 중증도를 감소시키는 방법에 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 13

청구항 10에 있어서, 상기 천식은 호중구성 천식 또는 알러지성 천식인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 14

청구항 1에 있어서, 포도막염을 치료 또는 예방하는 방법에 사용하거나, 또는 포도막염에서의 망막 손상을 감소 또는 예방하는 방법에 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 15

청구항 1 내지 4, 및 8 내지 14 중 어느 한 항에 있어서, 염증 또는 자가면역 질병의 치료 또는 예방에서 IL-17 생성을 감소시키거나 Th17 세포 분화를 감소시키는 방법에 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 16

청구항 1 내지 4, 및 8 내지 14 중 어느 한 항에 있어서, 상기 조성물은 증가된 IL-17 수준 또는 Th17 세포를 갖는 환자에서 사용하기 위한, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 17

삭제

청구항 18

삭제

청구항 19

청구항 1 내지 4, 및 8 내지 14 중 어느 한 항에 있어서, 상기 조성물은 경구 투여용인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 20

청구항 1 내지 4, 및 8 내지 14 중 어느 한 항에 있어서, 상기 조성물은 하나 이상의 약학적으로 허용가능한 부형제 또는 담체를 포함하는, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 21

청구항 1 내지 4, 및 8 내지 14 중 어느 한 항에 있어서, 박테리아 균주가 동결건조된 것인, 염증 또는 자가면역 질병의 예방 또는 치료용 약학적 조성물.

청구항 22

서열번호: 1의 16s rRNA 서열을 갖는 박테리아를 포함하는 염증 또는 자가면역 질병의 예방 또는 개선용 식품 조성물로서,

상기 염증 또는 자가면역 질병은 천식; 류마티스성 관절염; 골관절염; 건선성 관절염; 유년성 특발성 관절염; 다발성 경화증; 시신경척수염(데빅(Devic) 병); 강직 척추염; 척추관절염; 건선; 전신 홍반성 낭창; 크론병; 궤양성 대장염; 소아지방변증; 만성적 폐색성 폐 질병(COPD); 포도막염; 공막염; 혈관염; 베체트(Behcet)병; 죽상

경화증; 아토피성 피부염; 폐공기증; 치주염; 알러지성 비염; 및 동종이식편 거부반응으로 이루어진 군으로부터 선택되는 질병인 것인, 염증 또는 자가면역 질병의 예방 또는 개선용 식품 조성물.

청구항 23

삭제

청구항 24

수탁 번호 NCIMB 42380하에 기탁된 박테리아의 세포.

청구항 25

청구항 24의 세포를 포함하는 염증 또는 자가면역 질병의 예방 또는 개선용 식품 조성물로서,

상기 염증 또는 자가면역 질병은 천식; 류마티스성 관절염; 골관절염; 건선성 관절염; 유년성 특발성 관절염; 다발성 경화증; 시신경척수염(데빅(Devic) 병); 강직 척추염; 척추관절염; 건선; 전신 홍반성 낭창; 크론병; 궤양성 대장염; 소아지방변증; 만성적 폐색성 폐 질병(COPD); 포도막염; 공막염; 혈관염; 베체트(Behcet)병; 죽상 경화증; 아토피성 피부염; 폐공기증; 치주염; 알러지성 비염; 및 동종이식편 거부반응으로 이루어진 군으로부터 선택되는 질병인 것인, 염증 또는 자가면역 질병의 예방 또는 개선용 식품 조성물.

청구항 26

청구항 25에 있어서, 식품적으로 허용가능한 담체 또는 부형제를 포함하는, 염증 또는 자가면역 질병의 예방 또는 개선용 식품 조성물.

청구항 27

수탁 번호 NCIMB 42380하에 기탁된 박테리아의 생물학적으로 순수한 배양물.

청구항 28

삭제

청구항 29

삭제

발명의 설명

기술 분야

[0001] 본 발명은 포유동물의 소화관으로부터 단리된 박테리아성 균주를 포함하는 조성물 분야 및 상기 조성물의 질병 치료에서의 용도에 관한 것이다.

배경 기술

[0002] 인간의 장은 *자궁에서* 무균으로 여겨지지만 태어난 직후에는 매우 다양한 모성 및 환경 미생물에 노출된다. 그 후, 미생물 콜로니와 숙주의 역동적인 기간이 발생하며, 이는 수송 방식, 환경,식이 및 숙주 유전형과 같은 요인에 의해 영향을 받으며, 이들 모두는 특히 초기 생애 동안 소화관 미생물의 구성에 영향을 미친다. 결과적으로, 미생물은 안정화되고 성체와 같이 된다[1]. 인간 장내 미생물은 본질적으로 두 개의 주요 박테리아성 분류인 박테리오테스(Bacteroidetes)와 피르미쿠테스(Firmicutes)에 속하는 500 내지 1000 가지 이상의 다른 계통형(phylogroup)을 함유한다[2]. 인간 장내 박테리아성 콜로니에서 발생하는 성공적인 공생 관계는 다양한 신진대사, 구조적, 보호 및 기타 유익한 기능을 제공한다. 콜로니화된 장내 신진대사 활동이 강화되면 소화불량 식이 성분이 부산물을 방출하여 숙주에게 중요한 영양 공급원을 제공한다. 유사하게, 장내 미생물의 면역학적 중요성은 잘 알려져 있으며, 공생 박테리아의 도입에 따라 기능적으로 재구성된 면역 체계가 손상된 무균 동물에서 예시된다[3-5].

[0003] 미생물 조성의 급격한 변화는 염증성 장 질환(IBD)과 같은 위장 장애에서 입증되었다. 예를 들어, IBD 환자에서 클로스트리디움 클러스터 XIVa 박테리아의 수치가 감소하는 반면 *대장균* 수가 증가하여 장내에서 공생균

(symbiont)과 유해균(pathobiont)의 균형이 바뀌었다고 나타났다[6-9]. 흥미롭게도, 이러한 미생물 불균형(dysbiosis)는 T 효과기(effector) 세포 집단의 불균형과 관련이 있다.

[0004] 특정 박테리아성 균주가 동물 장내에 미칠 수 있는 잠재적인 긍정적인 효과를 인식하여 다양한 질병의 치료에 다양한 균주가 제안되었다(예를 들어 [10-13] 참조). 또한 대부분 락토바실러스(*Lactobacillus*)와 비피도박테리움(*Bifidobacterium*) 균주를 포함한 특정 균주는 장에 직접 연결되지 않은 다양한 염증성 및 자가면역 질환의 치료에 사용하기 위해 제안되었다(문헌 [14] 및 [15] 참조). 그러나 다른 질병과 다른 박테리아성 균주 사이의 관계, 특정 박테리아성 균주가 장내와 전신 수준 및 특정 질병 유형에 미치는 정확한 효과에 대해서는 잘 알려져 있지 않다.

[0005] 염증 및 자가면역 질환을 치료하는 새로운 방법에 대한 요구가 있다. 장내 박테리아를 이용한 새로운 치료법을 개발할 수 있도록 장내 박테리아의 잠재적인 효과에 대한 요건도 있다.

발명의 내용

[0006] 본 발명자들은 염증 및 자가면역 질환을 치료하고 예방하기 위한 새로운 치료법을 개발하였다. 특히, 본 발명자들은 IL-17 또는 Th17 경로에 의해 매개되는 질병 및 병태를 치료 및 예방하기 위한 새로운 치료법을 개발하였다. 특히, 본 발명자들은 Th17 염증 반응을 감소시키는 데 효과적인 새로운 박테리아성 균주를 확인하였다. 실시예에 기재된 바와 같이, 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 포함하는 조성물의 경구 투여는 천식, 류마티스성 관절염 및 다발성 경화증의 마우스 모델에서 Th17 염증 반응을 포함하는 염증 반응의 중증도를 감소시킬 수 있다. 또한 실시예에 기재된 바와 같이, 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 포함하는 조성물의 경구 투여는 Th17 염증 반응과 관련될 수 있는 암 마우스 모델에서 종양 크기를 감소시킬 수 있다.

[0007] 따라서, 제1 구현예에서, 본 발명은 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태를 치료 또는 예방하는 방법에 사용하기 위해, 수탁 번호 NCIMB 42380으로 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명자들은 이러한 박테리아성 균주에 의한 처리가 IL-17을 포함하는 Th17 경로의 일부인 사이토카인의 수준을 감소시켜 Th17 염증 반응을 완화시킬 수 있고 IL-17 및 Th17 경로에 의해 매개되는 염증 및 자가면역 질환의 마우스 모델에서 임상적 이점을 제공할 수 있음을 확인했다.

[0008] 특정 구현예에서, 본 발명은 하기로 구성된 군으로부터 선택된 질병 또는 병태를 치료 또는 예방하는 방법에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다: 다발성 경화증; 관절염 예컨대 류마티스성 관절염, 골관절염, 건선성 관절염, 또는 유년성 특발성 관절염; 시신경 척수염(데빅(Devic)병); 강직 척추염; 척추관절염; 건선; 전신 홍반성 낭창; 염증성 장 질환 예컨대 크론병 또는 궤양성 대장염; 소아지방변증; 천식 예컨대 알러지성 천식 또는 호중구성 천식; 만성적 폐쇄성 폐 질환(COPD); 암 예컨대 유방암, 결장암, 폐암 또는 난소암; 포도막염; 공막염; 혈관염; 베체트병; 죽상경화증; 아토피 피부염; 폐경기증; 치주염; 알러지성 비염; 및 동종이식편 거부반응. Th17 염증 반응에 대한 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 대해 나타난 효과는 위에 열거된 것과 같은 IL-17 및 Th17 경로에 의해 매개되는 질병 및 병태에 대한 치료 효과를 제공할 수 있다.

[0009] 바람직한 구현예에서, 본 발명은 호중구성 천식 또는 알레르기성 천식과 같은 천식을 치료 또는 예방하는 방법에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명자들은 수탁 번호 NCIMB 42380하에 기탁된 박테리아로 치료하면 호중구 및 호산구가 폐로 유입되어 천식 치료 또는 예방에 도움이 될 수 있음을 확인하였다. 또한, 본 발명자들은 천식 마우스 모델에서 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 효능을 시험하고 입증하였다. 특정 구현예에서, 조성물은 호중구성 천식 또는 호산구성 천식을 치료 또는 예방하는 방법에 사용하기 위한 것이다. 호중구 및 호산구에 대한 본 발명의 조성물에 대해 나타난 효과는 이것이 호중구성 천식 및 호산구성 천식을 치료 또는 예방하는 데 특히 효과적일 수 있음을 의미한다. 실제로, 특정 구현예에서, 조성물은 천식의 치료 또는 예방에서 호중구성 염증 반응을 감소시키는 방법에 사용하기 위한 것이거나, 조성물은 천식의 치료 또는 예방에서 호산구성 염증 반응을 감소시키는 방법에 사용하기 위한 것이다. 특정 구현예에서, 본 발명은 천식의 치료에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 특히 바람직한 구현예에서, 본 발명은 천식, 특히 호중구성 천식의 치료에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 포함하는 조성물을 제공한다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아는 천식 모델에서 호중구에 특히 현저한 효과를 나타내는 것으로 나타났으며, 이 박테리아에 의한 치료는 호중구성 천식의 치료에 특히 효과적일 수 있다.

- [0010] 추가의 바람직한 구현예에서, 본 발명은 류마티스성 관절염을 치료 또는 예방하는 방법에서 사용하기 위해 수탁 번호 NCIMB 42380으로 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명자들은 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 의한 치료가 류마티스성 관절염의 마우스 모델에서 임상적 효과를 제공할 수 있고 관절 팽윤을 감소시킬 수 있음을 확인했다. 바람직한 구현예에서, 본 발명은 류마티스성 관절염의 치료에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 사용하는 조성물은 류마티스성 관절염 치료에 특히 효과적일 수 있다.
- [0011] 추가의 바람직한 구현예에서, 본 발명은 다발성 경화증을 치료 또는 예방하는 방법에서 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명자들은 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 의한 치료가 다발성 경화증 마우스 모델에서 질병 발병 및 질병의 중증도를 감소시킬 수 있음을 확인하였다. 바람직한 구현예에서, 본 발명은 다발성 경화증의 치료에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 사용하는 조성물은 다발성 경화증 치료에 특히 효과적일 수 있다.
- [0012] 추가의 바람직한 구현예에서, 본 발명은 유방암, 폐암 또는 간암과 같은 암을 치료 또는 예방하는 방법에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명자들은 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 의한 치료가 유방암, 폐암 및 간암의 마우스 모델에서 종양 성장을 감소시킬 수 있음을 밝혀냈다. 특정 구현예에서, 조성물은 암 치료에서 종양 크기를 감소시키거나 종양 성장을 예방하는 방법에 사용하기 위한 것이다.
- [0013] 추가의 바람직한 구현예에서, 본 발명은 포도막염 예를 들어 후포도막염을 치료 또는 예방하는 방법에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물은 포도막염 치료에 특히 효과적일 수 있다.
- [0014] 특정 구현예에서, 본 발명의 조성물은 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태의 치료 또는 예방에서 IL-17 생산을 감소시키거나 Th17 세포 분화를 감소시키는 방법에 사용하기 위한 것이다. 특히, 본 발명의 조성물은 천식, 류마티스성 관절염 또는 다발성 경화증 또는 천식, 류마티스성 관절염, 다발성 경화증, 암 또는 포도막염의 치료 또는 예방에서 IL-17 생산의 감소 또는 Th17 세포 분화의 감소에 사용될 수 있다. 바람직하게는, 본 발명은 천식, 류마티스성 관절염 또는 다발성 경화증의 치료 또는 예방에서 IL-17 생산을 감소시키거나 Th17 세포 분화를 감소시키는 데 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다. 본 발명은 또한 암 치료 또는 예방에서 IL-17 생산을 감소시키거나 Th17 세포 분화를 감소시키는 데 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 제공한다.
- [0015] 특정 구현예에서, 조성물은 증가된 IL-17 수준 또는 Th17 세포를 갖는 환자에 사용하기 위한 것이다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 대해 나타난 Th17 염증 반응에 대한 효과는 그러한 환자에게 특히 효과적일 수 있다.
- [0016] 본 발명의 바람직한 구현예에서, 조성물 중의 박테리아성 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아이다. 수탁 번호 NCIMB 42380하에 기탁된 생물형 박테리아성 균주는 또한 16s rRNA 서열이 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 16s rRNA 서열과 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9% 동일한 박테리아성 균주와 같은 생물형 박테리아성 균주가 또한 사용될 수 있다. 바람직하게는, 박테리아성 균주는 서열번호: 1과 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9% 동일한 16s rRNA 서열을 갖는다. 바람직하게는, 본 발명에서 사용하기 위한 박테리아성 균주는 서열번호: 1로 표시되는 16s rRNA 서열을 갖는다.
- [0017] 특정 구현예에서, 본 발명의 조성물은 경구 투여용이다. 본 발명의 균주의 경구 투여는 IL-17 또는 Th17 경로-매개 질병 및 병태의 치료에 효과적일 수 있다. 또한, 경구 투여는 환자 및 종사자에게 편리하며 장내 수송 및/또는 부분적 또는 전체 콜로니화를 허용한다.
- [0018] 특정 구현예에서, 본 발명의 조성물은 하나 이상의 약학적으로 허용가능한 부형제 또는 담체를 포함한다.
- [0019] 특정 구현예에서, 본 발명의 조성물은 동결 건조된 박테리아성 균주를 포함한다. 동결 건조는 박테리아의 수송을 허용하는 안정한 조성물을 제조하기 위한 효과적이고 편리한 기술이다.

- [0020] 특정 구현예에서, 본 발명은 상기 기재된 조성물을 포함하는 식품을 제공한다.
- [0021] 특정 구현예에서, 본 발명은 전술한 바와 같은 조성물을 포함하는 백신 조성물을 제공한다.
- [0022] 또한, 본 발명은 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함하는 조성물을 투여하는 것을 포함하는, IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태를 치료 또는 예방하는 방법을 제공한다.
- [0023] 상기 발명을 개발함에 있어서, 본 발명자들은 치료에 특히 유용한 박테리아성 균주를 동정 및 특성화하였다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아는 관절염, 천식 및 다발성 경화증과 같은 본원에 기술된 질병의 치료에 효과적인 것으로 나타났다. 따라서, 또 다른 측면에서, 본 발명은 수탁 번호 NCIMB 42380하에 기탁된 박테리아성 세포 또는 이의 유도체를 제공한다. 본 발명은 또한 이러한 세포를 포함하는 조성물 또는 이러한 세포의 생물학적으로 순수한 배양물을 제공한다. 본 발명은 또한 치료에, 특히 본원에 기재된 질병에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 세포 또는 이의 유도체를 제공한다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아는 또한 암을 치료하는 데 효과적임이 밝혀졌다.

도면의 간단한 설명

- [0024] 도 1: 집먼지 진드기-유발성 천식의 마우스 모델 - 총 BAL 액 세포 수.
- 도 2: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 총 호산구 수.
- 도 3: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 호산구의 비율.
- 도 4: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 총 대식세포 수.
- 도 5: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 대식세포의 비율.
- 도 6: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 총 호중구 수.
- 도 7: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 호중구의 비율.
- 도 8: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 총 림프구 수.
- 도 9: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 림프구 비율.
- 도 10: 중증 호중구성 천식의 마우스 모델 - 총 BAL 액 세포 수.
- 도 11: 중증 호중구성 천식의 마우스 모델 - BALF에서의 총 호산구 수.
- 도 12: 중증 호중구성 천식의 마우스 모델 - BALF에서의 호산구 비율.
- 도 13: 중증 호중구성 천식의 마우스 모델 - BALF에서의 총 대식세포 수.
- 도 14: 중증 호중구성 천식의 마우스 모델 - BALF에서의 대식세포의 비율.
- 도 15: 중증 호중구성 천식의 마우스 모델 - BALF에서의 총 호중구 수.
- 도 16: 중증 호중구성 천식의 마우스 모델 - BALF에서의 호중구의 비율.
- 도 17: 중증 호중구성 천식의 마우스 모델 - BALF에서의 총 림프구 수.
- 도 18: 중증 호중구성 천식의 마우스 모델 - BALF에서의 림프구 비율.
- 도 19: 류마티스성 관절염의 마우스 모델 - 체중, -14일 내지 0일. 데이터는 초기(-14일) 체중의 평균± SEM 백분율로 표시된다. 통계적 유의성: 비히클-처리 군과 비교했을 때 ▲ p < 0.05 및 ▲▲▲▲ p < 0.0001.
- 도 20: 류마티스성 관절염의 마우스 모델 - 체중, 0일 내지 42일. 데이터는 초기(0일) 체중의 평균± SEM 백분율로 표시된다. 비히클-처리 군과 비교했을 때 ▲ p < 0.05, ◆ p < 0.05, ▲▲▲ p < 0.001, . . . p < 0.0001 이다.
- 도 21: 류마티스성 관절염의 마우스 모델 - 임상 점수. 데이터는 평균± SEM으로 표시된다. 비히클-처리군에서 21일차와 비교했을 때 **** p < 0.0001로 나타났다. 소정의 날 비히클-처리군과 비교했을 때, ◆ 0 p < 0.05로 나타났다.
- 도 22: 류마티스성 관절염의 마우스 모델 - 콜라겐 II에 대한 비장세포 증식 반응. 배지 배경은 3H-TdR 통합에

기초하여 분당 카운트를 감한다[CII- 자극 - 배지 배경]. 모든 데이터는 평균± SEM으로 표시된다. ** 비히클 군에 비해 $p < 0.01$.

도 23: 류마티스성 관절염의 마우스 모델 - 조직 배양 상등액의 IFN γ 수준. 선은 그룹 중앙값을 나타낸다.

도 24: 류마티스성 관절염의 마우스 모델 - 조직 배양 상등액의 IL-17A 수준. 선은 그룹 중앙값을 나타낸다.

도 25: 류마티스성 관절염의 마우스 모델 - 조직 배양 상등액의 IL-10 수준. 선은 그룹 중앙값을 나타낸다.

도 26: 류마티스성 관절염의 마우스 모델 - 조직 배양 상등액의 IL-6 수준. 선은 그룹 중앙값을 나타낸다.

도 27: 조직병리학 점수 시스템.

도 28: 집먼지 진드기-유발성 천식의 마우스 모델 - 혈청에 있는 총 IgE

도 29: 집먼지 진드기-유발성 천식의 마우스 모델 - 혈청에 있는 HDM 특이적 IgG1

도 30: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 총 IgE

도 31: 집먼지 진드기-유발성 천식의 마우스 모델 - BALF에서의 HDM 특이적 IgG1

도 32: 집먼지 진드기-유발성 천식의 마우스 모델 - 조직학적 분석 - 평균 세기관지주위(Peribronchiolar) 침윤 점수

도 33: 집먼지 진드기-유발성 천식의 마우스 모델 - 조직학적 분석 - 평균 혈관주위 침윤 점수

도 34: 집먼지 진드기-유발성 천식의 마우스 모델 - 조직학적 분석 - 평균 염증 점수(세기관지주위와 혈관주위 침윤 점수의 평균)

도 35: 집먼지 진드기-유발성 천식의 마우스 모델 - 조직학적 분석 - 점액 점수

도 36: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-9 수준

도 37: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-1 α 수준

도 38: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IFN γ 수준

도 39: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-17A 수준

도 40: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-4 수준

도 41: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-5 수준

도 42: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 IL-1 β 수준

도 43: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 RANTES 수준

도 44: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 MIP-1 α 수준

도 45: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 KC 수준

도 46: 집먼지 진드기-유발성 천식의 마우스 모델 - 폐 조직의 MIP-2 수준

도 47: 중증 호중구성 천식의 마우스 모델 - 혈청내 HDM 특이적 IgG1

도 48: 중증 호중구성 천식의 마우스 모델 - 혈청내 HDM 특이적 IgG2a

도 49: 중증 호중구성 천식의 마우스 모델 - BALF내 HDM 특이적 IgG1

도 50: 중증 호중구성 천식의 마우스 모델 - BALF내 HDM 특이적 IgG2a

도 51: 중증 호중구성 천식의 마우스 모델 - 조직학적 분석 - 평균 세기관지주위 침윤 점수

도 52: 중증 호중구성 천식의 마우스 모델 - 조직학적 분석 - 평균 혈관주위 침윤 점수

도 53: 중증 호중구성 천식의 마우스 모델 - 조직학적 분석 - 평균 염증 점수(세기관지주위와 혈관주위 침윤 점수의 평균)

도 54: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 TNF α 수준

- 도 55: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IL-1a 수준
- 도 56: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IFNg 수준
- 도 57: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IL-17F 수준
- 도 58: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IL-1b 수준
- 도 59: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 RANTES 수준
- 도 60: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 MIP-2 수준
- 도 61: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 KC 수준
- 도 62: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IL-17A 수준
- 도 63: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 MIP-1a 수준
- 도 64: 중증 호중구성 천식의 마우스 모델 - 폐 조직내 IL-33 수준
- 도 65: 류마티스성 관절염의 마우스 모델 - 조직병리학 점수를 위한 가시적 템플렛. 콜라겐-유발성 관절염 실험에서 마우스 발목뼈 관절에서의 복합 점수를 보여주는 대표적인 이미지.
- 도 66: 류마티스성 관절염의 마우스 모델 - 조직병리학 : 염증 점수. 모든 데이터는 평균 $\pm$ SEM으로 표시된다. ** 비히클-처리군과 비교했을 때 $p < 0.01$.
- 도 67: 류마티스성 관절염의 마우스 모델 - 조직병리학: 연골 점수. 모든 데이터는 평균 $\pm$ SEM으로 표시된다. *** 비히클-처리군과 비교했을 때 $p < 0.001$.
- 도 68: 류마티스성 관절염의 마우스 모델 - 조직병리학: 골 점수. 모든 데이터는 평균 $\pm$ SEM으로 표시된다. *** 비히클-처리군과 비교했을 때 $p < 0.001$.
- 도 69: 류마티스성 관절염의 마우스 모델 - 조직병리학: 총 점수. 모든 데이터는 평균 $\pm$ SEM으로 표시된다. 비히클-처리군과 비교했을 때 * $p < 0.05$, *** $p < 0.001$.
- 도 70: 류마티스성 관절염의 마우스 모델 - 조직병리학: 대표적 사진. 팔호 안에 동물 ID(# n.n)와 사지(오른쪽에 R, 왼쪽에 L)가 표시된다. 왼쪽 상단 이미지(비히클): 관절주위 연조직으로 확장되는 염증 및 섬유증으로 광범위한 관절 및 뼈 파괴.
- 도 71: 다발성 경화증의 마우스 모델 - 임상 점수.
- 도 72: 다발성 경화증의 마우스 모델 - 질병 발생
- 도 73: 지방암의 마우스 모델 - 종양 부피.
- 도 74: 폐암의 마우스 모델 - 종양 부피.
- 도 75: 간암의 마우스 모델 - 간 중량.
- 도 76: 인간 세포에 MRX004 및 B. 브레베 형 균주의 부착.
- 도 77: 엑소폴리사카라이드 생산 분석.
- 도 78: MRX004에 의해 구속 및 해제된엑소폴리사카라이드 생산.
- 도 79: MRX004를 Caco-2 세포에 부착.
- 도 80: MRX004 단독(A) 및 B. 브레베 유형 균주(B)와 비교한 급속 ID 32 프로파일. 화이트 = 음성 반응(색상 변화 없음), 아래쪽 교차 해치 = 중간 양성 반응(약한 색상 변화) 및 블랙 = 양성 반응(강한 적절한 색상 변화).
- 도 81: MRX004의 API@ 50 CH 분석. 상향 교차 해치 = 음성 반응(색상 변화 없음), 하향 교차 해치 = 중간 양성 반응(약한 색상 변화), 블랙 = 양성 반응(강한 적절한 색상 변화) 및 화이트 = 의심스러운 반응(예상치 못한 색상 변화).

발명을 실시하기 위한 구체적인 내용

- [0025] **박테리아성 균주**
- [0026] 본 발명의 조성물은 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형을 포함한다. 실시예는 이러한 박테리아가 IL-17 또는 Th17 경로에 의해 매개되는 질병 및 병태를 치료 또는 예방하는 데 유용함을 입증한다. 실시예는 또한 이러한 박테리아가 암 치료 또는 예방에 유용하다는 것을 입증한다. 바람직한 박테리아성 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아이다.
- [0027] 수탁 번호 NCIMB 42380하에 기탁된 박테리아를 실시예에서 시험하였고, 본원에서는 균주 751 또는 MRX004라고도 한다. 시험된 751 균주에 대한 부분적인 16S rRNA 서열이 서열번호: 1에 제시되어 있다. 균주 751은 지티 바이올로지 리미티드(GT Biologics Ltd.)(스코틀랜드 AB25 2ZS, 애버딘 소재 라이프 사이언스 이노베이션 빌딩(Life Sciences Innovation Building))에 의해 국제 기탁 기관 NCIMB, Ltd.(스코틀랜드 AB21 9YA 애버딘 소재 퍼구슨 빌딩(Ferguson Building))에 기탁되었고, 할당된 수탁 번호는 NCIMB 42380이다. 지티 바이올로지 리미티드는 이후 포디 파마 리서치 리미티드(4D Pharma Research Limited)로 명칭을 바꾸었다.
- [0028] 균주 751에 대한 게놈 서열은 서열번호: 2에 제시되어 있다.
- [0029] 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 생물형인 박테리아성 균주는 또한 IL-17 또는 Th17 경로에 의해 매개되는 질병 및 병태를 치료 또는 예방하는 데 효과적일 것으로 기대된다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 생물형인 박테리아성 균주는 또한 암 치료 또는 예방에 효과적일 것으로 기대된다. 생물형은 생리학적 및 생화학적 특성이 동일하거나 매우 유사한 밀접히 관련된 균주이다.
- [0030] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 16s rRNA 서열과 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9% 동일한 16s rRNA 서열을 갖는다. 바람직하게는, 본 발명에 사용되는 박테리아성 균주는 서열번호: 1과 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9% 동일한 16s rRNA 서열을 갖는다. 바람직하게는, 본 발명에서 사용하기 위한 박테리아성 균주는 서열번호: 1로 표시되는 16s rRNA 서열을 갖는다.
- [0031] 대안적으로, 수탁 번호 NCIMB 42380하에 기탁되고 본 발명에 사용하기에 적합한 박테리아의 생물형인 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 대한 다른 뉴클레오티드 서열을 시퀀싱함으로써 동정될 수 있다. 예를 들어, 실질적으로 전체 게놈이 시퀀싱될 수 있고, 본 발명에 사용하기 위한 생물형 균주는 전체 게놈의 적어도 80%에 걸쳐(예컨대, 적어도 85%, 90%, 95% 또는 99%, 또는 전체 게놈에 걸쳐) 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9% 서열 동일성을 가질 수 있다. 생물형 균주를 동정하는 데 사용하기에 적합한 다른 서열은 hsp60 또는 BOX, ERIC, (GTG)₅ 또는 REP 또는 [16]과 같은 반복 서열을 포함할 수 있다. 생물형 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 상응하는 서열과 적어도 95%, 96%, 97%, 98%, 99%, 99.5% 또는 99.9%의 서열 동일성을 갖는 서열을 가질 수 있다.
- [0032] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 서열번호: 2에 대한 서열 동일성을 갖는 게놈을 갖는다. 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 서열번호: 2의 적어도 60%(예를 들어, 적어도 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% 또는 100%)에 걸쳐 서열 번호: 2에 적어도 90%의 서열 동일성(예를 들어, 적어도 92%, 94%, 95%, 96%, 97%, 98%, 99% 또는 100% 서열 동일성)을 갖는 게놈을 갖는다. 예를 들어, 본 발명에 사용하기 위한 박테리아성 균주는 서열번호: 2의 70%에 걸쳐 서열번호: 2와 90% 이상의 서열 동일성, 또는 서열번호: 2의 80%에 걸쳐 서열번호: 2와 90% 이상의 서열 동일성, 또는 서열번호: 2의 90%에 걸쳐 서열번호: 2와 90% 이상의 서열 동일성, 또는 서열번호: 2의 100%에 걸쳐 서열번호: 2와 90% 이상의 서열 동일성, 또는 서열번호: 2의 70%에 걸쳐 서열번호: 2와 95% 이상의 서열 동일성, 또는 서열번호: 2의 80%에 걸쳐 서열번호: 2와 95% 이상의 서열 동일성, 또는 서열번호: 2의 90%에 걸쳐 서열번호: 2와 95% 이상의 서열 동일성, 또는 서열번호: 2의 100%에 걸쳐 서열번호: 2와 95% 이상의 서열 동일성, 또는 서열번호: 2의 70%에 걸쳐 서열번호: 2와 98% 이상의 서열 동일성, 또는 서열번호: 2의 80%에 걸쳐 서열번호: 2와 98% 이상의 서열 동일성, 또는 서열번호: 2의 90%에 걸쳐 서열번호: 2와 98% 이상의 서열 동일성, 또는 서열번호: 2의 100%에 걸쳐 서열번호: 2와 98% 이상의 서열 동일성을 갖는 게놈을 가질 수 있다.
- [0033] 대안적으로, 수탁 번호 NCIMB 42380하에 기탁되고 본 발명에 사용하기에 적합한 박테리아의 생물형인 균주는 수탁 번호 NCIMB 42380 및 제한 단편 분석 및/또는 PCR 분석을 사용하여, 예를 들어 형광 증폭 단편 길이 다형성 (FAFLP) 및 반복적인 DNA 요소(rep)-PCR 지문 또는 단백질 프로파일링, 또는 부분적 16S 또는 23s rDNA 시퀀싱을 사용함으로써 동정될 수 있다. 바람직한 구현예에서, 이러한 기술은 수탁 번호 NCIMB 42380하에 기탁된 박테리아와 동일한 종의 균주를 동정하는 데 사용될 수 있다.

- [0034] 특정 구현예에서, 수탁 번호 NCIMB 42380하에 기탁되고 본 발명에 사용하기에 적합한 박테리아의 생물형인 균주는 증폭된 리보솜 DNA 제한 분석(ARDRA), 예를 들어 Sau3AI 제한 효소를 사용할 때(예시적인 방법 및 지침은 예를 들어 [17] 참조)에 의해 분석될 때 수탁 번호 NCIMB 42380하에 기탁된 박테리아와 동일한 패턴을 제공하는 균주이다. 대안적으로, 생물형 균주는 수탁 번호 NCIMB 42380 하에 기탁된 박테리아와 동일한 탄소화물 발효 패턴을 갖는 균주로 동정된다.
- [0035] 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 생물형이고 본 발명의 조성물 및 방법에서 유용한 박테리아성 균주는 실시예에 기재된 분석을 포함하는 임의의 적절한 방법 또는 전략을 사용하여 동정될 수 있다. 예를 들어, 본 발명에 사용하기 위한 생물형은 혐기성 YCFA에서 배양하고 및/또는 II 형 콜라겐-유발성 관절염 마우스 모델에 박테리아를 투여하고 이어서 사이토카인 수준을 평가함으로써 동정될 수 있다. 특히, 수탁 번호 NCIMB 42380 하에 기탁된 박테리아에 유사한 성장 패턴, 대사 유형 및/또는 표면 항원을 갖는 박테리아성 균주가 본 발명에서 유용할 수 있다. 생물형 균주는 NCIMB 42380 균주에 필적하는 면역 조절 활성을 가질 것이다. 특히, 생물형 균주는 천식, 관절염, 다발성 경화증 및 암 질병 모델에 필적할만한 효과를 이끌어 낼 것이고, 실시예에 나타난 효과에 대한 사이토카인 수준에 필적할만한 효과를 이끌어 낼 것이며, 이는 실시예에 기재된 배양 및 투여 프로토콜을 사용하여 확인될 수 있다.
- [0036] 본 발명의 특히 바람직한 균주는 수탁 번호 NCIMB 42380하에 기탁된 박테리아이다. 이것은 실시예에서 시험되고 질병 치료에 효과적인 것으로 입증된 예시적인 751 균주이다. 따라서, 본 발명은 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 유도체의 단리된 세포와 같은 세포를 제공한다. 본 발명은 또한 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 세포 또는 이의 유도체를 포함하는 조성물을 제공한다. 본 발명은 또한 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 생물학적으로 순수한 배양물을 제공한다. 본 발명은 또한 치료에, 특히 본원에 기재된 질병에 사용하기 위해 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 세포 또는 이의 유도체를 제공한다.
- [0037] 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 유도체는 딸 균주(자손) 또는 원본으로부터 배양된(서브클로닝된) 균주일 수 있다. 본 발명의 균주 유도체는 생물학적 활성을 제거하지 않고 예를 들어 유전자 수준에서 변형될 수 있다. 특히, 본 발명의 유도체 균주는 치료학적으로 활성이다. 유도체 균주는 NCIMB 42380 균주에 필적하는 면역 조절 활성을 가질 것이다. 특히, 유도체 균주는 천식, 관절염, 다발성 경화증 및 암 질병 모델에 필적할만한 효과를 이끌어 낼 것이고, 실시예에 나타난 효과에 대한 사이토카인 수준에 필적할만한 효과를 이끌어 낼 것이며, 이는 실시예에 기재된 배양 및 투여 프로토콜을 사용하여 확인될 수 있다. NCIMB 42380 균주의 유도체는 일반적으로 NCIMB 42380 균주의 생물형일 것이다.
- [0038] 수탁 번호 NCIMB 42380하에 기탁된 박테리아의 세포에 대한 언급은 수탁 번호 NCIMB 42380하에 기탁된 균주와 동일한 안전성 및 치료 효능 특성을 갖는 임의의 세포를 포함하며, 이러한 세포는 본 발명에 포함된다.
- [0039] 바람직한 구현예에서, 본 발명의 조성물 중의 박테리아성 균주는 성장 가능하고 장을 부분적으로 또는 전체적으로 콜로니화할 수 있다.
- [0040] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 인간의 장내 상피 세포, 특히 Caco-2 세포에 대한 접착력이 낮다. 바람직한 구현예에서, 본 발명에 사용되는 박테리아성 균주는 *비피도박테리아*, 특히 *B. 브레베*에 비해 YCFA에서 인간의 장내 상피 세포, 특히 Caco-2 세포에 대한 접착력이 낮다. 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 실시예 12에 기술된 조건 하에서 시험했을 때, 바람직하게는 0.5% 미만 또는 0.3% 미만과 같이 전체 배양물의 1% 미만의 접착력을 나타낸다.
- [0041] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 예를 들어 엑소폴리사카라이드가 박테리아성 균주의 세포의 표면에 결합되는 경우 엑소폴리사카라이드를 생산한다. 특정 구현예에서, 결합된 엑소폴리사카라이드의 생산은 본 발명에 사용하기 위한 박테리아성 균주의 점액 또는 상피 세포, 예를 들어 인간 장내 상피 세포의 표면에 대한 부착을 증가시킨다. 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 *비피도박테리아*, 특히 *B. 브레베*에 비하여 더 많은 결합된 표면 엑소폴리사카라이드를 생성한다.
- [0042] 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 *비피도박테리아*, 특히 *B. 브레베*에 비해 YCFA에서 인간 장내 상피 세포, 특히 Caco-2 세포에 대한 부착력이 낮고(예를 들어, 실시예 12에 기술된 조건 하에서 시험했을 때, 바람직하게는 0.5% 미만 또는 0.3% 미만과 같은 전체 배양물의 1% 미만의 부착력), *비피도박테리아*, 특히 *B. 브레베*와 비교하여 더 많은 결합된 표면 엑소폴리사카라이드를 생성한다.
- [0043] 특정 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 예를 들어 적절한 현탁 배지(예를

들어, API 현탁 배지)에서 37℃에서 4시간 동안 배양될 때 폴리스카라이드를 발효시킬 수 있다.

[0044] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 예를 들어 적절한 현탁 배지(예를 들어, API 현탁 배지)에서 37℃에서 4시간 동안 배양했을 때 비피도박테리아, 특히 B. 브레베에 비해 α-글루코시다아제 및/또는 β-글루코시다아제를 발효시키는 능력이 감소되었다.

[0045] 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 표 1에 열거된 하나 이상의 유전자 예를 들어 표 1의 5, 10, 20, 50 또는 모든 유전자를 포함한다. 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 예측된 ECF 수송체의 에너지 공급 모듈의 막형단 성분 BL0694 및/또는 예측된 ECF 수송체의 에너지 공급 모듈의 이중 ATPase 성분 BL0693과 같이 단일 밀줄로 강조 표시된 표 1에 열거된 하나 이상의 유전자를 포함한다. 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 말토텍스트린 글루코시다아제(EC 3.2.1.20), 추정 갈락토시다아제, 셀룰로스 신타아제(UDP-형성)(EC 2.4.1.12), 키티나아제(EC 3.2.1.14) 및 감각 상자/GGDEF 계열 단백질로부터 선택되는 1, 2, 3, 4 또는 5개의 유전자와 같은 이중 밀줄 및 굵은 체로 강조 표시된 표 1에 열거된 하나 이상의 유전자를 포함한다. 특정 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 예를 들면 오메가-3 다중 폴리불포화 지방산 합성효소 서브유닛 PfaA, I 형 폴리케티드 신타아제, 기능이 알려지지 않은 추정 글리코실 가수분해효소(DUF1680), ECF 수송체의 에너지 공급 모듈의 ATPase 성분 BioM, 양이온-수송성 ATPase E1-E2 계열, 리보스 ABC 수송 시스템 투과효소 단백질 RbsC(TC3A.1.2.1), 리보스 ABC 수송 시스템 ATP-결합 단백질 RbsA(TC3A.1.2.1), 3' 내지 5' 올리고리보뉴클레아제(orn), 악티노바실러스 단백질과 연관된 막 단백질(1944168)로부터 선택되는 1, 2, 3, 4, 5, 6, 7, 8 또는 9개의 유전자와 같이 이탤릭체로 강조 표시된 표 1에 열거된 하나 이상의 유전자를 포함한다.

[0046] 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 하기로부터 선택되는 하나 이상(예를 들어 5, 10, 15, 20, 25, 30, 40, 45, 50개 또는 모든) 유전자를 포함한다: 2-숙시닐-5-에놀피루빌-6-하이드록시-3-사이클로헥센-1-카복실산 합성효소(EC 2.2.1.9); 3'-내지-5' 올리고리보뉴클레아제(orn); 알파-갈락토시다아제(EC 3.2.1.22); ECF 일반 수송체의 에너지 공급 모듈의 ATPase 성분; 쿠에오신-관련 ECF 수송체의 에너지 공급 모듈의 ATPase 성분 STY3233; ATP-의존성 DNA 헬리카제 recG(EC 3.6.1.-); 베타-글루코시다아제(EC 3.2.1.21); 셀룰로오스 합성효소(UDP-형성)(EC 2.4.1.12); 키타나아제(EC 3.2.1.14); COG1309: 전사 조절제; D-알라닌-D-알라닌 카복시펩티다아제(EC 3.4.16.4); 예측된 ECF 수송체의 에너지 공급 모듈의 이중 ATPase 성분 BL0693; 프록토키나아제(EC 2.7.1.4); 글루코오스/만노오스:H⁺ 공동수송체 GlcP; 글리코실 전이효소(EC 2.4.1.-); GMP 합성효소 [글루타민-가수분해](EC 6.3.5.2); 인디고이딘 합성효소 indA, PfkB 키나아제 군집의 가상 당류 키나아제; 이노신-우리딘이 선호되는 뉴클레오사이드 가수분해효소(EC 3.2.2.1); LSU 리보솜 단백질 L31p @ LSU 리보솜 단백질 L31p, 아연-독립적; LSU 리보솜 단백질 L33p @ LSU 리보솜 단백질 L33p, 아연-독립적; 말토텍스트린 글루코시다아제(EC 3.2.1.20); 액티노바실러스 단백질과 관련된 막 단백질(1944168); 막-결합 용해성 뮤린 트랜스글라이코실라아제 D 전구체(EC 3.2.1.-); 메틸 전이효소(EC 2.1.1.-); NADH-의존성 부탄올 탈수소효소 A(EC 1.1.1.-); 포스포글리콜레이트 포스파타아제(EC 3.1.3.18); 포스포리보실안트라닐레이트 이성질화효소(EC 5.3.1.24); 미지 기능의 추정 글리코실 가수분해효소(DUF1680); 람노오스-함유 폴리스카라이드 전좌 퍼옥시다아제; 리보키나아제(EC 2.7.1.15); 리보스 ABC 수송 시스템, ATP-결합 단백질 RbsA(TC3A.1.2.1); 리보스 ABC 수송 시스템, ATP-결합 단백질 RbsA(TC3A.1.2.1); 리보스 ABC 수송 시스템, 고친화성 투과효소 RbsD(TC 3.A.1.2.1); 리보스 ABC 수송 시스템, 페리플라즘 리보스-결합 단백질 RbsB(TC3A.1.2.1); 리보스 ABC 수송 시스템, 투과효소 단백질 RbsC(TC 3.A.1.2.1); 리보스 ABC 수송 시스템, 투과효소 단백질 RbsC(TC 3.A.1.2.1); 솔비톨 탈수소효소(EC 1.1.1.14); SSU 리보솜 단백질 S14p(S29e) @ SSU 리보솜 단백질 S14p(S29e), 아연-독립적; 쿠에오신-조절 ECF 수송체의 기질 특이적 구성요소 STY3230; 수크로스-6-인산 가수분해효소(EC 3.2.1.B3); 테이코산 배출 ATP-결합 단백질 TagH(EC 3.6.3.40); 예측된 ECF 수송체의 에너지 공급 모듈의 막 모듈 구성요소 BL0694; 쿠에오신-조절 ECF 수송체의 에너지 공급 모듈의 막 모듈 구성요소 STY3231; HrtAB 수송체와 함께 공콜로니화된 두 구성요소 응답 조절제; I 형 제한-변형 시스템, DNA-메틸 전이효소 서브유닛 M(EC 2.1.1.72); I 형 제한-변형 시스템, 제한 서브유닛 R(EC 3.1.21.3); I 형 제한-변형 시스템, 특이성 서브유닛 S(EC 3.1.21.3); I 형 제한-변형 시스템, 특이성 서브유닛 S(EC 3.1.21.3); 자일리톨 탈수소효소(EC 1.1.1.9); 및 자일로스 ABC 수송체, 페리플라즘 자일로스-결합 단백질 XylF로부터 선택되는 하나 이상(예를 들어 5, 10, 15, 20, 25, 30, 40, 45, 50 또는 모든)의 유전자를 포함한다. 바람직한 구현예에서, 본 발명에 사용하기 위한 박테리아성 균주는 이전 문장에 열거되고 표 1에 강조 표시되지 않은 하나 이상(예를 들어 5, 10, 15, 20, 25, 30, 35 또는 모든)의 유전자를 포함한다.

[0047] **치료 용도**

- [0048] 실시예에서 입증된 바와 같이, 본 발명의 박테리아성 조성물은 Th17 염증 반응을 감소시키는 데 효과적이다. 특히, 본 발명의 조성물로 처리하면 IL-17A 수준 및 다른 Th17 경로 사이토카인의 감소 및 IL-17 및 Th17 경로에 의해 매개되는 병태의 동물 모델에서의 임상적 개선이 달성된다. 따라서, 본 발명의 조성물은 염증성 및 자가면역 질환, 특히 IL-17에 의해 매개되는 질병 또는 병태를 치료 또는 예방하는 데 유용할 수 있다. 특히, 본 발명의 조성물은 IL-17 염증 반응의 상승을 감소 또는 방지하는 데 유용할 수 있다.
- [0049] Th17 세포는 예를 들어 IL-17A, IL17-F, IL-21 및 IL22를 생성하는 T 헬퍼 세포의 하위 집합이다. Th17 세포 분화 및 IL-17 발현은 IL-23에 의해 유도될 수 있다. 이러한 사이토카인 및 다른 것들은 Th17 경로의 중요한 부분을 형성하는데, 이는(예를 들어 [18-23]에서 설명된 바와 같이) 다수의 염증 및 자가면역 질환에 기여하고 근원이 되는 잘 확립된 염증 신호 수송 경로이다. Th17 경로가 활성화되는 질병은 Th17 경로-매개 질병이다. Th17 경로-매개 질병은 Th17 세포의 분화를 줄이거나 이의 활성을 감소시키거나 Th17 경로 사이토카인의 수준을 감소시키는 등 Th17 경로를 억제함으로써 개선 또는 완화될 수 있다. Th17 경로에 의해 매개되는 질병은 IL-17A, IL-17F, IL-21, IL-22, IL-26, IL-9와 같은 Th17 세포에 의해 생성되는 사이토카인의 증가된 수준을 특징으로 할 수 있다([24]에서 검토). Th17 경로에 의해 매개되는 질병은 Stat3 또는 IL-23R과 같은 Th-17-관련 유전자의 발현 증가를 특징으로 할 수 있다. Th17 경로에 의해 매개되는 질병은 증가된 Th17 세포 수준과 관련이 있을 수 있다.
- [0050] IL-17은 염증 및 자가면역 질환 및 병태의 발병 기전에 기여하는 전염증성 사이토카인이다. 본원에 사용된 IL-17은 IL-17A, IL-17B, IL-17C, IL-17D, IL-17E 및 IL-17F를 포함하는 IL-17 계열의 임의의 구성원을 지칭할 수 있다. IL-17-매개 질병 및 조건은 IL-17의 높은 발현 및/또는 질병 또는 병태에 의해 영향을 받는 조직에서의 IL-17-양성 세포의 축적 또는 존재를 특징으로 한다. 유사하게, IL-17-매개 질병 및 병태는 높은 IL-17 수준 또는 IL-17 수준의 증가에 의해 악화되고 낮은 IL-17 수준 또는 IL-17 수준의 감소에 의해 완화되는 질병 및 병태이다. IL-17 염증 반응은 국소 또는 전신성일 수 있다.
- [0051] IL-17 또는 Th17 경로에 의해 매개될 수 있는 질병 및 병태의 예는, 다발성 경화증; 관절염 예컨대 류마티스성 관절염, 골관절염, 건선성 관절염, 또는 유년성 특발성 관절염; 시신경척수염(데빅병); 강직 척추염; 척추관절염; 건선; 전신 홍반성 낭창; 염증성 장 질환 예컨대 크론병 또는 궤양성 대장염; 소아지방변증; 천식 예컨대 알러지성 천식 또는 호중구성 천식; 만성적 폐쇄성 폐 질환(COPD); 암 예컨대 유방암, 결장암, 폐암 또는 난소암; 포도막염; 공막염; 혈관염; 베체트병; 죽상경화증; 아토피 피부염; 폐경기증; 치주염; 알러지성 비염; 및 동종이식편 거부반응을 포함한다. 바람직한 구현예에서, 본 발명의 조성물은 이들 병태 또는 질병 중 하나 이상을 치료 또는 예방하는 데 사용된다. 추가의 바람직한 구현예에서, 이들 병태 또는 질병은 IL-17 또는 Th17 경로에 의해 매개된다.
- [0052] 특정 구현예에서, 본 발명의 조성물은 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태의 치료 또는 예방에서 IL-17 생산을 감소시키거나 Th17 세포 분화를 감소시키는 방법에 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물은 염증성 또는 자가면역 질환의 치료 또는 예방에 사용하기 위한 것이며, 상기 치료 또는 예방은 Th17 염증 반응의 상승을 감소 또는 방지함으로써 달성된다. 특정 구현예에서, 본 발명의 조성물은 염증성 또는 자가면역 질환을 갖는 환자를 치료하는 데 사용하기 위한 것으로, 환자는 IL-17 수준의 상승 또는 Th17 세포의 증가 또는 Th17 염증 반응을 나타낸다. 특정 구현예에서, 환자는 만성 염증성 또는 자가면역 질환 또는 병태로 진단되었을 수 있거나, 본 발명의 조성물은 염증성 또는 자가면역 질환 또는 만성 염증성 또는 자가면역 질환 또는 병태로 진행되는 병태를 예방하는 데 사용하기 위한 것일 수 있다. 특정 구현예에서, 질병 또는 병태는 TNF- α 억제제에 의한 치료에 반응하지 않을 수 있다. 본 발명의 이러한 용도는 이전 단락에 열거된 특정 질병 또는 병태 중 어느 하나에 적용될 수 있다.
- [0053] IL-17 및 Th17 경로는 종종 만성 염증성 및 자가면역 질환과 관련되기 때문에, 본 발명의 조성물은 상기 열거된 만성 질환 또는 병태를 치료 또는 예방하는 데 특히 유용할 수 있다. 특정 구현예에서, 조성물은 만성 질환을 갖는 환자에게 사용하기 위한 것이다. 특정 구현예에서, 조성물은 만성 질환의 발병을 예방하는 데 사용하기 위한 것이다.
- [0054] 본 발명의 조성물은 IL-17 또는 Th17 경로에 의해 매개되는 질병 및 병태를 치료하고 Th17 염증 반응을 다루는 데 유용할 수 있기 때문에, 본 발명의 조성물은 만성 질환의 치료 또는 예방, 다른 치료법에 반응하지 않은 환자의 질병의 치료 또는 예방(예를 들어 TNF- α 억제제에 의한 치료) 및/또는 조직 손상 및 IL-17 및 Th17 세포와 관련된 증상의 치료 또는 예방에 특히 유용할 수 있다. 예를 들어, IL-17은 연골 및 뼈 조직에서 기질 파괴를 활성화시키는 것으로 알려져 있으며, IL17은 연골 세포 및 골아 세포에서의 기질 생성에 억제 효과를 가지

기 때문에, 본 발명의 조성물은 골 침식 또는 연골 손상을 치료 또는 예방하는 데 유용할 수 있다.

- [0055] 특정 구현예에서, 본 발명의 조성물로 처리하면 IL-17 수준, 특히 IL-17A 수준의 감소를 제공하거나 상승을 방지한다. 특정 구현예에서, 본 발명의 조성물을 사용한 치료는 IFN- γ , IL-1 β , RANTES, MIP-1 α , IL-8 또는 IL-6 수준의 상승을 감소시키거나 예방한다. 이러한 사이토카인의 증가된 수준의 감소 또는 예방은 염증성 및 자가면역 질환 및 병태, 특히 IL-17 또는 Th17 경로에 의해 매개되는 것들을 치료 또는 예방하는 데 유용할 수 있다.
- [0056] 특정 구현예에서, 본 발명의 조성물로 처리하는 것은 인간 세포, 예를 들어 *대장균* 및/또는 *S. 엔테리티디스*(*S. enteritidis*)와 같은 병원성 세포에 의한 인간 상피 세포에 대한 부착 또는 침입 차단을 제공한다.
- [0057] 특정 구현예에서, 본 발명의 조성물로 처리하는 것은 병원성 세포 예를 들어 *대장균* 및/또는 *S. 엔테리티디스*와 인간 상피 세포, 예를 들어 인간 장내 상피 세포의 결합을 감소 또는 방지한다.
- [0058] 특정 구현예에서, 본 발명의 조성물의 박테리아성 균주에 의한 엑소폴리사카라이드의 생성 및 방출은 병원성 중, 예를 들어 *대장균* 및/또는 *S. 엔테리티디스*에 대한 방어 효과를 가질 수 있다. 특정 구현예에서, 본 발명의 조성물의 박테리아성 균주에 의한 엑소폴리사카라이드의 생성 및 방출은 IL-17 또는 Th17 경로에 대한 박테리아의 효과를 매개할 수 있고 숙주 면역 반응에 영향을 미칠 수 있다. 특정 구현예에서, 본 발명의 조성물은 염증성 및 자가면역 질환, 특히 IL-17에 의해 매개되는 질병 또는 병태의 치료에서 엑소폴리사카라이드의 제조에 사용하기 위한 것이다.
- [0059] 특정 구현예에서, 본 발명의 조성물의 박테리아성 균주의 인간 장내 상피 세포, 특히 Caco-2 세포에 대한 낮은 부착은 IL-17 또는 Th17 경로 및 IL-17 또는 Th17 경로에 의해 매개되는 질병에 대한 본 발명의 조성물의 유의한 효과를 증가시킬 수 있다.
- [0060] 특정 구현예에서, 본 발명의 조성물로 처리하면 소장에서 라피노스의 발효가 증가된다. 실시예는 본 발명의 조성물의 박테리아성 균주가 폴리사카라이드 라피노스를 발효시키고 라피노스 발효가 증가된 맹장의 부티레이트 및 증가된 위장관 증식과 같은 숙주에 영향을 미칠 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 염증성 및 자가면역 질환, 특히 IL-17에 의해 매개되는 질병 또는 병태의 치료에서 장내 라피노스의 발효를 증가시키는 데 사용된다.
- [0061] 천식
- [0062] 바람직한 구현예에서, 본 발명의 조성물은 천식의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 집먼지 진드기 추출물에 의한 감작 및 투여 후에 기도 내로 호중구 및/또는 호산구의 모집을 감소시켜 천식의 치료 또는 예방에 유용할 수 있음을 입증한다. 천식은 염증과 기도의 제한을 특징으로 하는 만성 질환이다. 천식에서의 염증은 IL-17 및/또는 Th17 세포에 의해 매개될 수 있고, 따라서 본 발명의 조성물은 천식의 예방 또는 치료에 특히 효과적일 수 있다. 천식의 염증은 호산구 및/또는 호중구에 의해 매개될 수 있다.
- [0063] 특정 구현예에서, 천식은 호산구성 또는 알레르기성 천식이다. 호산구성 및 알레르기성 천식은 말초 혈액 및 기도 분비의 호산구 수가 증가하고 병리학적으로 기저막 부위가 두꺼워지고 약리학적으로 코르티코스테로이드 반응성과 관련이 있다[25]. 호산구 모집 또는 활성화를 감소시키거나 억제하는 조성물은 호산구성 및 알레르기성 천식을 치료 또는 예방하는 데 유용할 수 있다.
- [0064] 추가의 구현예에서, 본 발명의 조성물은 호중구성 천식(또는 비-호산구성 천식)의 치료 또는 예방에 사용하기 위한 것이다. 호중구 수가 많으면 코르티코스테로이드 치료에 민감하지 않은 심각한 천식이 동반된다. 호중구 모집 또는 활성화를 감소시키거나 억제하는 조성물은 호중구성 천식을 치료 또는 예방하는 데 유용할 수 있다.
- [0065] 호산구성 및 호중구성 천식은 상호 배타적인 조건이 아니며 호산구와 호중구 반응을 다루는 치료법은 일반적으로 천식 치료에 유용할 수 있다.
- [0066] 증가된 IL-17 수준 및 Th17 경로의 활성화는 심한 천식과 관련되기 때문에, 본 발명의 조성물은 중증 천식의 발병을 예방하거나 중증 천식 치료에 유용할 수 있다.
- [0067] 특정 구현예에서, 본 발명의 조성물은 천식의 치료 또는 예방에서 호산구성 염증 반응을 감소시키는 방법에 사용하거나, 또는 천식의 치료 또는 예방에서 호중구성 염증 반응을 감소시키는 방법에 사용하기 위한 것이다. 위에서 언급했듯이, 천식의 호산구 수치가 높으면 병리학적으로 기저막 부위가 두꺼워지므로 천식 치료 또는 예방에서 호산구성 염증 반응을 감소시키는 것이 이 질병의 특징을 구체적으로 다룰 수 있다. 또한 상승된 호중구는

호산구 증가와 함께 또는 부재시에 중증 천식 및 만성 기도 협착과 관련이 있다. 따라서 호중구성 염증 반응을 감소시키는 것은 중증 천식을 치료할 때 특히 유용할 수 있다.

[0068] 특정 구현예에서, 조성물은 알레르기성 천식에서의 기관지주위 침윤을 감소시키거나, 알레르기성 천식의 치료에서 기관지주위 침윤을 감소시키는 데 사용하기 위한 것이다. 특정 구현예에서, 조성물은 호중구성 천식에서 기관지주위 및/또는 혈관주위 침윤을 감소시키거나, 알레르기성 호중구성 천식의 치료에서 기관지주위 및/또는 혈관주위 침윤을 감소시키는 데 사용하기 위한 것이다.

[0069] 특정 구현예에서, 본 발명의 조성물을 사용한 치료는 IL-1 β IFN γ , RANTES, MIP-1 α 또는 IL-8 수준의 상승을 감소시키거나 예방한다.

[0070] 특정 구현예에서, 본 발명의 조성물은 호산구성 및/또는 호중구성 염증 반응의 감소를 초래하는 천식 치료 방법에 사용하기 위한 것이다. 특정 구현예에서, 치료될 환자는 예를 들어 혈액 샘플링 또는 객담 분석을 통해 확인된 바와 같이, 상승된 호중구 또는 호산구 수치를 갖거나 이전에 확인된 것이다.

[0071] 본 발명의 조성물은 신생아 또는 임산부에게 투여될 때 신생아에서 천식 발병을 예방하는 데 유용할 수 있다. 상기 조성물은 소아 천식의 발병을 예방하는 데 유용할 수 있다. 본 발명의 조성물은 성인-발병 천식을 치료 또는 예방하는 데 유용할 수 있다. 본 발명의 조성물은 천식을 관리하거나 경감시키는 데 유용할 수 있다. 본 발명의 조성물은 집먼지 진드기와 같은 알레르겐에 의해 악화되는 천식과 관련된 증상을 감소시키는 데 특히 유용할 수 있다.

[0072] 천식의 치료 또는 예방은 예를 들어 증상의 중증도의 완화 또는 악화 빈도의 감소 또는 환자에게 문제가 되는 유발인자의 범위를 나타낼 수 있다.

[0073] 특정 구현예에서, 본 발명의 조성물을 사용한 치료는 페닐알라닌 및/또는 히스티딘 예를 들어 장내 또는 혈장내 농도의 감소를 제공한다. 실시예는 페닐알라닌 및 히스티딘을 포함하는 아미노산의 발효에 대해 양성으로 시험된 본 발명의 조성물의 박테리아성 균주 및 페닐알라닌 및 히스티딘의 증가된 혈장 농도가 천식에서의 부작용과 관련이 있는 것으로 보고되었다. 특정 구현예에서, 본 발명의 조성물은 천식의 치료, 특히 천식과 관련된 히스타민 생산 또는 기도-과민성 반응의 치료에서 페닐알라닌 및/또는 히스티딘의 혈장 농도를 감소시키는 데 사용하기 위한 것이다.

[0074] 특정 구현예에서, 본 발명의 조성물로 처리하면 예를 들어 장내 갈락토오스 및/또는 프룩토오스의 농도가 감소한다. 실시예는 본 발명의 조성물의 박테리아성 균주가 갈락토오스 및 프룩토오스를 포함하는 탄수화물 기질을 발효시키고 고기 공급원으로부터 유래된 갈락토오스 α -1,3-갈락토오스가 아나필락시스의 알려진 알레르겐 및 원인 물질이고 프룩토오스의 섭취 수준이 증가된 천식 중증도와 상관 관계가 있음을 입증했다. 특정 구현예에서, 본 발명의 조성물은 천식의 치료, 특히 중증 천식의 치료에서 갈락토오스 및/또는 프룩토오스의 농도를 감소시키는 데 사용하기 위한 것이다.

[0075] 관절염

[0076] 바람직한 구현예에서, 본 발명의 조성물은 류마티스성 관절염(RA)의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 마우스 모델에서 RA의 임상 징후의 감소, 연골 및 뼈 손상의 감소 및 IL-17 염증 반응의 감소를 나타내기 때문에, RA의 치료 또는 예방에 유용할 수 있음을 입증했다. RA는 주로 관절에 영향을 미치는 전신성 염증 질환이다. RA는 관절의 팽윤, 활막의 과형성 및 연골과 뼈의 파괴를 가져오는 염증 반응과 관련이 있다. 예를 들어, IL-17은 연골 세포와 골아 세포에서의 매트릭스 생산을 억제하고 매트릭스 메탈로프로티나아제의 생산과 기능을 활성화시키고 RA 질병 활성은 IL-17 수준 및 Th-17 세포수와 상호 관련되기 때문에 IL-17과 Th17 세포는 RA에서 중요한 역할을 할 수 있으며[26,27], 따라서 본 발명의 조성물은 RA의 예방 또는 치료에 특히 효과적일 수 있다.

[0077] 특정 구현예에서, 본 발명의 조성물은 RA의 치료 또는 예방에서 IL-17 수준을 낮추거나 IL-17 수준의 상승을 방지하는 데 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 처리하면 IL-17 수준, 특히 IL-17A 수준의 감소를 제공하거나 상승을 방지한다. 특정 구현예에서, 본 발명의 조성물을 사용한 치료는 IFN- γ 또는 IL-6 수준의 감소를 제공하거나 상승을 방지한다.

[0078] 특정 구현예에서, 본 발명의 조성물로 치료하면 관절의 팽윤이 감소한다. 특정 구현예에서, 본 발명의 조성물은 관절이 부어 오른 환자 또는 관절이 부어질 위험이 있는 환자에게 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물은 RA에서 관절 팽윤을 감소시키는 방법에 사용하기 위한 것이다.

- [0079] 특정 구현예에서, 본 발명의 조성물로 치료하면 연골 손상 또는 골 손상이 감소된다. 특정 구현예에서, 본 발명의 조성물은 RA의 치료에서 연골 또는 골 손상을 감소 또는 예방하는 데 사용하기 위한 것이다. 특정 구현예에서, 조성물은 연골 또는 뼈 손상의 위험이 있는 중증의 RA 환자를 치료하는 데 사용하기 위한 것이다.
- [0080] 증가된 IL-17 수준과 Th17 세포수는 RA에서 연골과 뼈 파괴와 관련이 있다[26,27]. IL-17은 연골과 뼈 조직에서 기질 파괴를 활성화시키는 것으로 알려져 있으며 IL17은 연골 세포와 골아 세포에서 기질 생산에 억제 효과가 있다. 따라서, 특정 구현예에서, 본 발명의 조성물은 RA의 치료에서 골 침식 또는 연골 손상을 예방하는 데 사용하기 위한 것이다. 특정 구현예에서, 조성물은 골 침식 또는 연골 손상을 나타내는 환자 또는 골 침식 또는 연골 손상의 위험이 있는 것으로 확인된 환자를 치료하는 데 사용하기 위한 것이다.
- [0081] TNF- α 는 또한 RA와 연관되어 있지만 TNF- α 는 질병의 후기 단계의 발병 이전에 관여하지 않는다. 대조적으로, IL-17은 만성 질환의 모든 단계에서 역할을 한다[28]. 따라서, 특정 구현예에서, 본 발명의 조성물은 만성 RA 또는 연골의 관절 파괴 및 손실을 포함하는 질병과 같은 말기 RA의 치료에 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물은 이전에 항-TNF- α 요법을 받은 환자를 치료하기 위한 것이다. 특정 구현예에서, 치료될 환자는 항-TNF- α 요법에 반응하지 않거나 더 이상 반응하지 않는다.
- [0082] 본 발명의 조성물은 환자의 면역계를 조절하는 데 유용할 수 있기 때문에, 특정 구현예에서, 본 발명의 조성물은 RA의 위험이 있다고 밝혀지거나 초기 단계 RA로 진단된 환자에서 RA를 예방하는 데 사용하기 위한 것이다. 본 발명의 조성물은 RA의 발생을 예방하는 데 유용할 수 있다.
- [0083] 본 발명의 조성물은 RA를 관리하거나 경감시키는 데 유용할 수 있다. 본 발명의 조성물은 관절 팽윤 또는 골 파괴와 관련된 증상을 감소시키는 데 특히 유용할 수 있다. RA의 치료 또는 예방은 예를 들어 증상의 중증도의 완화 또는 악화 빈도의 감소 또는 환자에게 문제가 되는 유발 요인의 범위를 나타낼 수 있다.
- [0084] 다발성 경화증
- [0085] 바람직한 구현예에서, 본 발명의 조성물은 다발성 경화증의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 다발성 경화증 마우스 모델(EAE 모델)에서 질병 발병 및 질병 중증도의 감소를 달성하기 때문에, 다발성 경화증의 치료 또는 예방에 유용할 수 있음을 입증한다. 다발성 경화증은 특히 뇌와 척수에서의 신경 세포의 수초 외피에 손상을 일으키는 염증성 질환이다. 다발성 경화증은 만성 질환으로 점차적으로 무력화되고 에피소드로 진화한다. 예를 들어 IL-17 수치가 다발성 경화증 병변과 관련이 있고 IL-17이 혈액 뇌 장벽 내피 세포 결막을 파괴할 수 있고 Th17 세포가 중심 신경계로 이동하여 신경 손실을 일으킬 수 있기 때문에 IL-17 및 Th17 세포는 다발성 경화증에서 핵심적인 역할을 할 수 있다[29,30]. 따라서, 본 발명의 조성물은 다발성 경화증의 예방 또는 치료에 특히 효과적일 수 있다.
- [0086] 특정 구현예에서, 본 발명의 조성물로 치료하면 질병 발병 또는 질병 중증도가 감소한다. 특정 구현예에서, 본 발명의 조성물은 질병 발병 또는 질병 중증도를 감소시키는 데 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 치료하면 운동 기능의 저하가 방지되거나 운동 기능이 향상된다. 특정 구현예에서, 본 발명의 조성물은 운동 기능의 저하 또는 운동 기능의 개선에 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 치료하면 마비의 발병을 예방할 수 있다. 특정 구현예에서, 본 발명의 조성물은 다발성 경화증의 치료에서 마비를 예방하는 데 사용하기 위한 것이다.
- [0087] 본 발명의 조성물은 환자의 면역계를 조절하는 데 유용할 수 있으며, 따라서 특정 구현예에서, 본 발명의 조성물은 다발성 경화증의 위험이 있는 것으로 확인되었거나 초기 단계의 다발성 경화증 또는 "재발-완화형(relapsing-remitting)" 다발성 경화증으로 진단된 환자에서 다발성 경화증을 예방하는 데 사용하기 위한 것이다. 본 발명의 조성물은 경화증 발병을 예방하는데 유용할 수 있다. 실제로, 실시예는 본 발명의 조성물의 투여가 많은 마우스에서 질병의 발병을 예방함을 보여준다.
- [0088] 본 발명의 조성물은 다발성 경화증을 치료 또는 경감시키는 데 유용할 수 있다. 본 발명의 조성물은 다발성 경화증과 관련된 증상을 감소시키는 데 특히 유용할 수 있다. 다발성 경화증의 치료 또는 예방은 예를 들어 증상의 중증도의 완화 또는 악화 빈도의 감소 또는 환자에게 문제가 되는 유발 인자의 범위를 나타낼 수 있다.
- [0089] 포도막염
- [0090] 바람직한 구현예에서, 본 발명의 조성물은 포도막염의 치료 또는 예방에 사용하기 위한 것이다. 본 발명의 조성물은 포도막염의 동물 모델에서 질병 발병 및 질병 중증도의 감소를 달성할 수 있기 때문에 포도막염의 치료 또는 예방에 유용할 수 있다. 포도막염은 포도막의 염증으로 망막 조직 파괴를 일으킬 수 있다. 이것은 다른 해부

학적 형태(전방, 중급, 후방 또는 확산)로 나타날 수 있으며, 전립선자가면역 장애를 포함하여 다르지만 관련된 원인으로 인해 발생할 수 있다. IL-17 및 Th17 경로는 포도막염에 중심적으로 관여하기 때문에, 본 발명의 조성물은 포도막염의 예방 또는 치료에 특히 효과적일 수 있다. 문헌 [31-38]은 포도막염 환자에서 인터루킨-17A의 혈청 농도 증가, IL17A 유전 변이체와 관막염의 연관성, 실험적 자가면역 포도막염의 병인에서의 Th17-관련 사이토카인의 역할, 단상 실험적인 자가면역 포도막염 동안 Th17 세포와 조절 T 세포 사이의 불균형, 포도막염 및 활성 아다만티아데스-베체트(Adamantiades-Behcet) 및 보그-고야나기-하라다(Vogt-Koyanagi-Harada, VKH) 질환을 가진 환자에서 IL-17A의 상향 조절, 세쿠키누맙(secukinumab)(항 IL-17A 항체)에 의한 비-전염성 포도막염 치료, 및 포도막염 눈에서의 Th17에 대해 기술한다.

[0091] 특정 구현예에서, 포도막염은 후방 포도막염이다. 후방 포도막염은 주로 망막 및 맥락막의 염증을 나타내며, 본 발명의 조성물은 망막 염증 및 손상을 감소시키는 데 효과적일 수 있다.

[0092] 특정 구현예에서, 본 발명의 조성물로 치료하면 망막 손상이 감소한다. 특정 구현예에서, 본 발명의 조성물은 포도막염의 치료에서 망막 손상을 감소시키거나 예방하는 데 사용하기 위한 것이다. 특정 구현예에서, 조성물은 망막 손상의 위험이 있는 중증 포도막염 환자를 치료하는 데 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 치료하면 시신경 유두 염증이 감소된다. 특정 구현예에서, 본 발명의 조성물은 시신경 유두 염증을 감소 또는 예방하는 데 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 치료하면 염증 세포에 의한 망막 조직 침윤이 감소된다. 특정 구현예에서, 본 발명의 조성물은 염증 세포에 의한 망막 조직 침윤의 감소에 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물로 치료하면 시력이 유지되거나 개선된다. 특정 구현예에서, 본 발명의 조성물은 시력 유지 또는 개선에 사용하기 위한 것이다.

[0093] 특정 구현예에서, 조성물은 베체트 병, 크론 병, 푸치 이상변색 홍지모양체염, 다발 혈관염을 동반한 육아종증, HLA-B27 관련 포도막염, 청소년 특발성 관절염, 유육종증, 척추 관절염, 교감 신경 안검, 세뇨관 간질 신염 및 포도막염 증후군 또는 보그-고야나기-하라다 증후군과 같은 비-감염성 또는 자가면역 질환과 관련된 포도막염의 치료 또는 예방에 사용하기 위한 것이다. IL-17A는 예를 들어 베체트 및 보그-고야나기-하라다 질병에 관여하는 것으로 나타났다.

[0094] 포도막염의 치료 또는 예방은 예를 들어 증상의 중증도의 완화 또는 재발 방지를 의미할 수 있다.

[0095] 암 치료

[0096] 바람직한 구현예에서, 본 발명의 조성물은 암의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물의 투여가 다수의 종양 모델에서 종양 성장을 감소시킬 수 있음을 입증한다.

[0097] 특정 구현예에서, 본 발명의 조성물을 사용한 치료는 종양 크기의 감소 또는 종양 성장의 감소를 초래한다. 특정 구현예에서, 본 발명의 조성물은 종양 크기를 감소시키거나 종양 성장을 감소시키는 데 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 종양 크기 또는 성장을 감소시키는 데 효과적일 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 고형 종양 환자에게 사용하기 위한 것이다. 특정 구현예에서, 본 발명의 조성물은 암 치료에서 혈관신생을 감소 또는 예방하는 데 사용하기 위한 것이다. IL-17 및 Th17 세포는 혈관신생에 중심적인 역할을 한다. 특정 구현예에서, 본 발명의 조성물은 전이를 예방하는 데 사용하기 위한 것이다.

[0098] 특정 구현예에서, 본 발명의 조성물은 유방암의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 유방암의 치료에 효과적일 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 유방암의 치료에서 종양 크기를 감소시키거나 종양 성장을 감소시키거나 혈관신생을 감소시키는 데 사용하기 위한 것이다. 바람직한 구현예에서, 암은 유방암이다. 바람직한 구현예에서, 암은 IV 기 유방암이다.

[0099] 특정 구현예에서, 본 발명의 조성물은 폐암의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 폐암의 치료에 효과적일 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 폐암의 치료에서 종양 크기를 감소시키거나 종양 성장을 감소시키거나 혈관신생을 감소시키는 데 사용하기 위한 것이다. 바람직한 구현예에서, 암은 폐암이다.

[0100] 특정 구현예에서, 본 발명의 조성물은 간암의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 간암의 치료에 효과적일 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 간암의 치료에서 종양 크기를 감소시키거나 종양 성장을 감소시키거나 혈관신생을 감소시키는 데 사용하기 위한 것이다. 바람직한 구현예에서, 암은 간암(간세포 암종)이다.

[0101] 특정 구현예에서, 본 발명의 조성물은 암종의 치료 또는 예방에 사용하기 위한 것이다. 실시예는 본 발명의 조

성물이 다수의 암종 유형의 치료에 효과적일 수 있음을 입증한다. 특정 구현예에서, 본 발명의 조성물은 비-면역원성 암을 치료 또는 예방하는 데 사용하기 위한 것이다. 실시예는 본 발명의 조성물이 비-면역원성 암의 치료에 효과적일 수 있음을 입증한다.

[0102] IL-17 및 Th17 경로는 암 발달 및 진행에 중심적인 역할을 하며, 암에서 IL-17 및 Th17 세포의 역할은 완전히 이해되지는 않지만 IL-17 및 Th17 세포의 수많은 전구-종양 효과는 알려져 있다. 예를 들어, Th17 세포와 IL-17은 혈관신생을 촉진하고 종양 세포의 증식과 생존을 증가시키며 종양-촉진성 전사 인자를 활성화시킬 수 있다 [39-41]. 따라서, 본 발명의 조성물은 암을 치료 또는 예방하는 데 유용할 수 있다. 더욱이, 실시예는 본 발명의 조성물이 유방암, 폐암 및 간암에서 종양 부피를 감소시키는 데 효과적이며, IL-17 및 Th17 세포가 이러한 특정 유형의 암에서 중요한 역할을 한다는 것을 입증한다[42-44].

[0103] 암에 대한 본 발명의 조성물의 치료 효과는 전-염증성 메커니즘에 의해 매개될 수 있다. 염증은 암-억제 효과를 나타낼 수 있으며[45], TNF α 와 같은 전-염증성 사이토카인은 암 치료제로 연구되고 있다[46]. 본 발명의 조성물은 유사한 메커니즘을 통해 암을 치료하는 데 유용할 수 있다. 예를 들어, 본 발명의 조성물은 IFN γ -형 반응을 유도할 수 있다. IFN γ 는 종양억제 활동을 자극할 수 있는 강력한 대식세포-활성화 인자이며[47], 예를 들어 CXCL9와 CXCL10은 또한 항암 효과를 가지고 있다[48-50]. 따라서, 특정 구현예에서, 본 발명의 조성물은 암 치료에서의 염증 촉진에 사용하기 위한 것이다. 바람직한 구현예에서, 본 발명의 조성물은 암 치료에서의 Th1 염증 촉진에 사용하기 위한 것이다. Th1 세포는 IFN γ 를 생성하고 강력한 항암 효과를 갖는다[45]. 특정 구현예에서, 본 발명의 조성물은 전이되지 않은 암 또는 단계 0 또는 단계 1 암과 같은 초기 단계의 암을 치료하는 데 사용하기 위한 것이다. 염증을 촉진하는 것은 초기 단계의 암에 비해 더 효과적일 수 있다[45]. 특정 구현예에서, 본 발명의 조성물은 염증을 촉진시켜 제2 항암제의 효과를 증진시키는 데 사용하기 위한 것이다.

[0104] 추가 구현예에서, 본 발명의 조성물은 급성 림프아구성 백혈병(ALL), 급성 골수 백혈병, 부신피질 암종, 기저세포 암종, 담도암, 방광암, 골 종양, 골육종/악성 섬유질 조직구종, 뇌간 신경아교종, 뇌종양, 소뇌 별아교세포종, 뇌 별아교세포종/악성 신경아교종, 뇌실막세포종, 수모세포종, 천막상 원시 신경외배엽성 종양, 유방암, 기관지 선종/카르시노이드, 버킷 림프종, 유암종, 자궁경부암, 만성 림프구성 백혈병, 만성 골수성 백혈병, 만성 골수증식성 장애, 결장암, 피부 T-세포 림프종, 자궁내막 암, 뇌실막세포종, 식도암, 유인 육종, 안구내 흑색종, 망막모세포종, 담낭암, 위암, 위장 유암종, 위장 기질 종양(GIST), 생식세포 종양, 신경아교종, 소아기 시각적 경로 및 시상하부, 호지킨 림프종, 흑색종, 소도세포 암종, 카포시 육종, 신장 세포 암, 후두 암, 백혈병, 림프종, 중피종, 신경교세포종, 비-호지킨 림프종, 구강인두 암, 골육종, 난소암, 췌장암, 부갑상선암, 인두 암, 뇌하수체 샘종, 형질 세포 신조직형성, 전립선암, 신장 세포 암종, 망막모세포종, 육종, 고환암, 갑상선암, 또는 자궁암을 치료 또는 예방하는 데 사용하기 위한 것이다.

[0105] 본 발명의 조성물은 추가의 치료제와 조합하여 사용될 때 특히 효과적일 수 있다. 본 발명의 조성물의 면역-조절 효과는 보다 직접적인 항암제와 조합될 때 효과적일 수 있다. 따라서, 특정 구현예에서, 본 발명은 수탁 번호 NCIMB 42380하에 기탁된 박테리아 또는 이의 생물형 및 항암제를 포함하는 조성물을 제공한다. 바람직한 구현예에서, 항암제는 면역 체크포인트 억제제, 표적 항체 면역요법, CAR-T 세포 요법, 온콜리틱 바이러스 또는 세포증식 억제제이다. 바람직한 구현예에서, 상기 조성물은 하기로 이루어진 군으로부터 선택된 항암제를 포함한다: Yervoy(이필리무맙, BMS); Keytruda(렘브롤리주맙, Merck); Opdivo(니볼루맙, BMS); MEDI4736(AZ/MedImmune); MPDL3280A(Roche/Genentech); 트레멜리무맙(AZ/MedImmune); CT-011(피딜리주맙, CureTech); BMS-986015(리틸루맙, BMS); MEDI0680(AZ/MedImmune); MSB-0010718C(Merck); PF-05082566(Pfizer); MEDI6469(AZ/MedImmune); BMS-986016(BMS); BMS-663513(우렐루맙, BMS); IMP321(Prima Biomed); LAG525(Novartis); ARGX-110(argEN-X); PF-05082466(Pfizer); CDX-1127(바틸루맙, CellDex Therapeutics); TRX-518(GITR Inc.); MK-4166(Merck); JTX-2011(Jounce Therapeutics); ARGX-115(argEN-X); NLG-9189(indoximod, NewLink Genetics); INCB024360(Incyte); IPH2201(Innate Immunotherapeutics/AZ); NLG-919(NewLink Genetics); 항-VISTA(JnJ); Epacadostat(INCB24360, Incyte); F001287(Flexus/BMS); CP 870893(University of Pennsylvania); MGA271(MacroGenix); 에막투주맙(Roche/Genentech); 갈루니세르티프(Eli Lilly); 울로콕루맙(BMS); BKT140/BL8040(Biokine Therapeutics); 바비투스맙(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); 베무라페닙(Plexxikon); 다브라페닙(Genentech/GSK); BMS-777607(BMS); BLZ945(Memorial Sloan-Kettering Cancer Centre); Unituxin(디누톡시맙, United Therapeutics Corporation); Blincyto(블리나투모맙, Amgen); Cyramza(라무시루맙, Eli Lilly); Gazyva(오비누투주맙, Roche/Biogen); Kadcyra(아도-트라추주맙 엠탄신,

Roche/Genentech); Perjeta(페르투주맵, Roche/Genentech); Adcetris(브렌톡시맵 베도틴, Takeda/Millennium); Arzerra(오파투무맵, GSK); Vectibix(파니투무맵, Amgen); Avastin(베바시주맵, Roche/Genentech); Erbitux(세록시맵, BMS/Merck); Bexxar(토시투모맵-I131, GSK); Zevalin(이브리투모맵 티옥세탄, Biogen); Campath(알렘추주맵, Bayer); Mylotarg(겜투주맵 오조가미신, Pfizer); Herceptin(트라추주맵, Roche/Genentech); Rituxan(리톡시맵, Genentech/Biogen); 볼로식시맵(Abbvie); 에나바추주맵(Abbvie); ABT-414(Abbvie); 엘로투주맵(Abbvie/BMS); ALX-0141(Ablynx); 오자랄리주맵(Ablynx); 악티맵-C(Actinium); 악티맵-P(Actinium); 밀라투주맵-독스(Actinium); Emab-SN-38(Actinium); 나프투몬맵 에스타페나톡스(Active Biotech); AFM13(Affimed); AFM11(Affimed); AGS-16C3F(Agensys); AGS-16M8F(Agensys); AGS-22ME(Agensys); AGS-15ME(Agensys); GS-67E(Agensys); ALXN6000(사말리주맵, 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(아테카투무맵, Amgen); AMG211(Amgen/MedImmune); BAY20-10112(Amgen/Bayer); 킬로투무맵(Amgen); 테노수맵(Amgen); AMP-514(Amgen); MEDI575(AZ/MedImmune); MEDI3617(AZ/MedImmune); MEDI6383(AZ/MedImmune); MEDI551(AZ/MedImmune); 목세투모맵 파수도톡스(AZ/MedImmune); MEDI565(AZ/MedImmune); MEDI0639(AZ/MedImmune); MEDI0680(AZ/MedImmune); MEDI562(AZ/MedImmune); AV-380(AVEO); AV203(AVEO); AV299(AVEO); BAY79-4620(Bayer); 아네투맵 라브탄신(Bayer); 반틱투맵(Bayer); BAY94-9343(Bayer); 시브로투주맵(Boehringer Ingelheim); BI-836845(Boehringer Ingelheim); B-701(BioClin); BIIB015(Biogen); 오비누투주맵(Biogen/Genentech); BI-505(Bioinvent); BI-1206(Bioinvent); TB-403(Bioinvent); BT-062(Biotest) BIL-010t(Biosceptre); MDX-1203(BMS); MDX-1204(BMS); 넥시투무맵(BMS); CAN-4(Cantargia AB); CDX-011(Celldex); CDX1401(Celldex); CDX301(Celldex); U3-1565(Daiichi Sankyo); 파트리투맵(Daiichi Sankyo); 티가투주맵(Daiichi Sankyo); 니모투주맵(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); 식수투무맵(Eli Lilly); 플란보투맵(Eli Lilly); IMC-TR1(Eli Lilly); 라무치루맵(Eli Lilly); 타발루맵(Eli Lilly); 자놀리루맵(Emergent Biosolution); FG-3019(FibroGen); FPA008(Five Prime Therapeutics); FP-1039(Five Prime Therapeutics); FPA144(Five Prime Therapeutics); 카누막소맵(Fresenius Biotech); IMAB362(Ganymed); IMAB027(Ganymed); HuMax-CD74(Genmab); HuMax-TFADC(Genmab); GS-5745(Gilead); GS-6624(Gilead); OMP-21M18(렘시주맵, GSK); 마파투무맵(GSK); IMGN289(ImmunoGen); IMGN901(ImmunoGen); IMGN853(ImmunoGen); IMGN529(ImmunoGen); IMMU-130(Immunomedics); 밀라투주맵-독스(Immunomedics); IMMU-115(Immunomedics); IMMU-132(Immunomedics); IMMU-106(Immunomedics); IMMU-102(Immunomedics); 에프라투주맵(Immunomedics); 클리바투주맵(Immunomedics); IPH41(Innate Immunotherapeutics); 다라투무맵(Janssen/Genmab); CNTO-95(인테투무맵, Janssen); CNTO-328(실톡시맵, Janssen); KB004(KaloBios); 모가물리주맵(Kyowa Hakko Kirrin); KW-2871(에크로멕시맵, Life Science); 소넵시주맵(Lpath); 마르게톡시맵(Macrogenics); 에노블리투주맵(Macrogenics); MGD006(Macrogenics); MGF007(Macrogenics); MK-0646(달로투주맵, Merck); MK-3475(Merck); Sym004(Symphogen/Merck Serono); DI17E6(Merck Serono); MOR208(Morphosys); MOR202(Morphosys); Xmap5574(Morphosys); BPC-1C(엔시톡시맵, Precision Biologics); TAS266(Novartis); LFA102(Novartis); BHQ880(Novartis/Morphosys); QGE031(Novartis); HCD122(루카투무맵, Novartis); LJM716(Novartis); AT355(Novartis); OMP-21M18(렘시주맵, OncoMed); OMP52M51(Oncomed/GSK); OMP-59R5(Oncomed/GSK); 반틱투맵(Oncomed/Bayer); CMC-544(이노투주맵 오조가미신, Pfizer); PF-03446962(Pfizer); PF-04856884(Pfizer); PSMA-ADC(Progenics); REGN1400(Regeneron); REGN910(네스바쿠맵, Regeneron/Sanofi); REGN421(에노티쿠맵, Regeneron/Sanofi); RG7221, RG7356, RG7155, RG7444, RG7116, RG7458, RG7598, RG7599, RG7600, RG7636, RG7450, RG7593, RG7596, DCDS3410A, RG7414(파르사투주맵), RG7160(이브가투주맵), RG7159(오빈투주맵), RG7686, RG3638(오나르투주맵), RG7597(Roche/Genentech); SAR307746(Sanofi); SAR566658(Sanofi); SAR650984(Sanofi); SAR153192(Sanofi); SAR3419(Sanofi); SAR256212(Sanofi), SGN-LIV1A(린투주맵, Seattle Genetics); SGN-CD33A(Seattle Genetics); SGN-75(보르세투주맵 모포도틴, Seattle Genetics); SGN-19A(Seattle Genetics); SGN-CD70A(Seattle Genetics); SEA-CD40(Seattle Genetics); 이브리투모맵 티옥세탄(Spectrum); MLN0264(Takeda); 가니투맵(Takeda/Amgen); CEP-37250(Teva); TB-403(Thrombogenic); VB4-845(Viventia); Xmap2512(Xencor); Xmap5574(Xencor); 니모투주맵(YM Biosciences); 칼루맵(Janssen); NY-ESO TCR(Adaptimmune); MAGE-A-10 TCR(Adaptimmune); CTL019(Novartis); JCAR015(Juno Therapeutics); KTE-C19 CAR(Kite Pharma); UCART19(Collectis); 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), 시스플라틴, 옥살리플라틴, 이리노테칸, 폴린산, 메토틱세이트, 사이클로포스파미드, 5-플루오로우라실, Zykadia (Novartis), Tafinlar(GSK), Xalkori(Pfizer), Iressa(AZ), Gilotrif(Boehringer Ingelheim), Tarceva(Astellas Pharma), Halaven(Eisai Pharma), Veliparib(Abbvie), AZD9291(AZ), 알렉티닙(Chugai), LDK378(Novartis), 제네테스웍(Synta Pharma), Tergenpumatumucel-L(NewLink Genetics), GV1001(Kael-GemVax), 티반티닙(ArQule); Cytoxan(BMS); Oncovin(Eli Lilly); 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); 및 TS-1(Taiho Pharmaceuticals).

[0106] **투여 방식**

[0107] 바람직하게는, 본 발명의 조성물은 본 발명의 박테리아성 균주로 장내 수송 및/또는 부분적 또는 전체 콜로니화를 가능하게 하기 위해 위장관에 투여될 수 있다. 일반적으로, 본 발명의 조성물은 경구 투여되지만, 직장, 비내, 또는 협측 또는 설하 경로를 통해 투여될 수 있다.

[0108] 특정 구현예에서, 본 발명의 조성물은 발포체로서, 스프레이 또는 겔로서 투여될 수 있다.

[0109] 특정 구현예에서, 본 발명의 조성물은 예를 들어 테오브로마 오일(코코아 버터), 합성 경질 지방(예를 들어, 봉해제, 위캡슐), 글리세로-젤라틴, 폴리에틸렌 글리콜, 또는 비누 글리세린 조성물의 형태로 직장 좌약과 같은 좌약으로서 투여될 수 있다.

[0110] 특정 구현예에서, 본 발명의 조성물은 비위관, 위관, 위선, 공막 절개관(J 관), 경피 내시경 위장관(PEG), 또는 포트 예컨대 위장, 공장 및 기타 적합한 액세스 포트에 대한 액세스를 제공하는 흉벽 포트와 같은 관을 통해 위장관으로 투여된다.

[0111] 본 발명의 조성물은 한번 투여될 수 있거나, 치료 요법의 일부로서 순차적으로 투여될 수 있다. 특정 구현예에서, 본 발명의 조성물은 매일 투여될 수 있다.

[0112] 본 발명의 특정 구현예에서, 본 발명에 따른 치료는 환자의 장내 미생물의 평가를 동반한다. 본 발명의 균주로의 수송 및/또는 부분적 또는 전체 콜로니화가 달성되지 않아 유효성이 관찰되지 않는 경우 치료는 반복되거나 또는 수송 및/또는 부분적 또는 전체 콜로니화가 성공적으로 이루어지고 효능이 관찰되는 경우 치료는 중단될 수 있다.

[0113] 특정 구현예에서, 본 발명의 조성물은 자궁속 및/또는 태어난 후에 아이에서 발생하는 염증성 또는 자가면역 질환을 예방하기 위해 임신 동물 예를 들어 인간과 같은 포유동물에 투여될 수 있다.

[0114] 본 발명의 조성물은 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태로 진단되거나 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태의 위험이 있는 것으로 확인된 환자에게 투여될 수 있다. 상기 조성물은 또한 건강한 환자에서 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 증상의 발병을 예방하기 위한 예방적 조치로서 투여될 수 있다.

[0115] 본 발명의 조성물은 비정상적인 내장 미생물을 갖는 것으로 확인된 환자에게 투여될 수 있다. 예를 들어, 환자는 수탁 번호 NCIMB 42380하에 기탁된 박테리아에 의한 콜로니화가 감소했거나 없을 수도 있다.

[0116] 본 발명의 조성물은 영양 보충제와 같은 식품으로 투여될 수 있다.

[0117] 일반적으로, 본 발명의 조성물은 가금류, 돼지, 고양이, 개, 말 또는 토끼와 같은 단포 포유동물을 비롯한 동물을 치료하는 데 사용될 수 있지만, 인간의 치료를 위한 것이다. 본 발명의 조성물은 동물의 성장 및 성능을 향상시키는 데 유용할 수 있다. 동물에게 투여하면, 경구용 위관 영양법을 사용할 수 있다.

[0118] **조성물**

[0119] 일반적으로, 본 발명의 조성물은 박테리아를 포함한다. 본 발명의 바람직한 구현예에서, 조성물은 동결-건조된

형태로 제형화된다. 예를 들어, 본 발명의 조성물은 본 발명의 박테리아성 균주를 포함하는 과립 또는 젤라틴 캡슐 예를 들어 경질 젤라틴 캡슐을 포함할 수 있다.

[0120] 바람직하게는, 본 발명의 조성물은 동결 건조된 박테리아를 포함한다. 박테리아의 동결 건조는 잘 확립된 절차이며 관련 지침은 예를 들어 문헌[51-53]에서 얻을 수 있다.

[0121] 대안적으로, 본 발명의 조성물은 살아있는 활성 박테리아 배양물을 포함할 수 있다.

[0122] 바람직한 구현예에서, 본 발명의 조성물은 박테리아성 균주를 장내로 수송할 수 있도록 캡슐화된다. 캡슐화는 예를 들어 압력, 효소 활성 또는 pH의 변화에 의해 유발될 수 있는 물리적 붕괴와 같은 화학적 또는 물리적 자극에 의한 파열을 통해 표적 위치로 수송될 때까지 조성물이 분해되지 않도록 보호한다. 임의의 적절한 캡슐화 방법이 사용될 수 있다. 예시적인 캡슐화 기술은 다공성 매트릭스 내의 포착, 고체 담체 표면상의 부착 또는 흡착, 응집 또는 가교 결합체에 의한 자기 응집 및 미세다공성 막 또는 마이크로캡슐 후의 기계적 봉쇄를 포함한다. 본 발명의 조성물을 제조하는 데 유용한 캡슐화에 대한 지침은 예를 들어 문헌 [54] 및 [55]에서 이용가능하다.

[0123] 상기 조성물은 경구 투여될 수 있고, 정제, 캡슐 또는 분말의 형태일 수 있다. 수탁 번호 NCIMB 42380하에 기탁된 박테리아는 혐기성 균일 수 있으므로 캡슐화된 제품이 바람직하다. 기타 성분(예컨대, 비타민 C)은 생체 내에서 수송 및/또는 부분적 또는 전체 콜로니화 및 생존을 개선하기 위해 산소 제거제 및 프리바이오틱 기질로서 포함될 수 있다. 다르게는, 본 발명의 프로바이오틱 조성물은 우유 또는 유청 기반의 발효 낙농 제품과 같은 식품 또는 영양 제품 또는 의약품으로서 경구 투여될 수 있다.

[0124] 상기 조성물은 프로바이오틱으로 제형화될 수 있다.

[0125] 본 발명의 조성물은 치료 유효량의 본 발명의 박테리아성 균주를 포함한다. 치료 유효량의 박테리아성 균주는 환자에게 유익한 효과를 발휘하기에 충분하다. 박테리아성 균주의 치료학적 유효량은 환자의 장내 수송 및/또는 부분적 또는 전체 콜로니화를 초래하기에 충분할 수 있다.

[0126] 예를 들어, 성인 인간에 대한 박테리아의 적합한 일일 투여량은 약 1×10^3 내지 약 1×10^{11} 콜로니 형성 단위(CFU)일 수 있고; 예를 들어, 약 1×10^7 내지 약 1×10^{10} CFU; 다른 예에서는 약 1×10^6 내지 약 1×10^{10} CFU이다.

[0127] 특정 구현예에서, 조성물은 조성물의 중량에 대하여 약 1×10^6 내지 약 1×10^{11} CFU/g의 양으로 박테리아성 균주를 함유한다; 예를 들어, 약 1×10^8 내지 약 1×10^{10} CFU/g이다. 투여량은 예를 들어 1g, 3g, 5g 및 10g일 수 있다.

[0128] 전형적으로, 본 발명의 조성물과 같은 프로바이오틱은 임의로 적어도 하나의 적합한 프리바이오틱 화합물과 배합된다. 프리바이오틱 화합물은 일반적으로 올리고- 또는 폴리사카라이드 또는 설탕 알코올과 같은 비-소화성 탄수화물이며, 이는 상부 소화관에서 분해되거나 흡수되지 않는다. 공지의 프리바이오틱은 이눌린 및 트랜스갈락토-올리고사카라이드와 같은 시판 중인 제품을 포함한다.

[0129] 특정 구현예에서, 본 발명의 프로바이오틱 조성물은 총 중량 조성물에 대해 약 1 내지 약 30 중량%(예를 들어, 5 내지 20 중량%)의 양으로 프리바이오틱 화합물을 포함한다. 탄수화물은 프룩토-올리고사카라이드(또는 FOS), 단쇄 프룩토-올리고사카라이드, 이눌린, 이소말토-올리고사카라이드, 펙틴, 자일로-올리고사카라이드(또는 XOS), 키토산-올리고사카라이드(또는 COS), 베타-글루칸, 경질 검 개질 및 저항성 전분, 폴리덱스트로스, D-타가토스, 아카시아 섬유, 캐롭, 귀리 및 감귤류 섬유로 이루어진 군으로부터 선택될 수 있다. 하나의 측면에서, 프리바이오틱은 단쇄 프룩토오스-올리고사카라이드(이하, 간단히 FOSs-c.c로 나타냄)이다; 상기 FOSs-c.c는 일반적으로 사탕무 설탕의 전환에 의해 얻어지고 3개의 글루코오스 분자가 결합된 사카로스 분자를 포함하는 소화가 가능한 탄수화물은 아니다.

[0130] 본 발명의 조성물은 약학적으로 허용가능한 부형제 또는 담체를 포함할 수 있다. 적합한 부형제의 예는 문헌 [56]에서 확인할 수 있다. 치료적 사용을 위한 허용가능한 담체 또는 희석제는 약학 분야에 잘 알려져 있으며, 예를 들어 문헌[57]에 기재되어 있다. 적합한 담체의 예로는 락토오스, 전분, 글루코오스, 메틸 셀룰로오스, 마그네슘 스테아레이트, 만니톨, 솔비톨 등이 포함된다. 적합한 희석제의 예는 에탄올, 글리세롤 및 물을 포함한다. 약학적 담체, 부형제 또는 희석제의 선택은 의도된 투여 경로 및 표준 약제 실습과 관련하여 선택될 수 있다. 약학적 조성물은 담체, 부형제 또는 희석제로서 또는 이들 외에 임의의 적합한 결합제, 윤활제, 현탁화제,

코팅제, 가용화제를 포함할 수 있다. 적합한 결합제의 예는 전분, 젤라틴, 천연 당류 예컨대 글루코오스, 무수 락토오스, 자유-유동 락토오스, 베타-락토오스, 옥수수 감미료, 천연 및 합성 검 예컨대 아카시아, 트라가칸트 또는 나트륨 알기네이트, 카복시메틸 셀룰로오스 및 폴리에틸렌 글리콜을 포함한다. 적합한 윤활제의 예로는 올레산 나트륨, 스테아린산 나트륨, 스테아린산 마그네슘, 안식향산 나트륨, 아세트산 나트륨, 염화나트륨 등이 포함된다. 보존제, 안정화제, 염료 및 향미제조차도 약학 조성물 중에 제공될 수 있다. 보존제의 예는 나트륨 벤조에이트, 소르브산 및 p-하이드록시벤조산의 에스테르를 포함한다. 향산화제 및 현탁화제가 또한 사용될 수 있다.

[0131] 본 발명의 조성물은 식품으로서 제형화될 수 있다. 예를 들어, 식품은 영양 보충제와 같이 본 발명의 치료 효과 이외에 영양적인 이득을 제공할 수 있다. 유사하게, 식품 조성물은 본 발명의 조성물의 맛을 증강시키거나 또는 약학 조성물보다는 일반적인 식품 품목에 보다 유사함으로써 조성물을 소비하기에 더 매력적하도록 제형화될 수 있다. 특정 구현예에서, 본 발명의 조성물은 우유-기재 제품으로 제형화된다. "우유-기재 제품"이란 용어는 다양한 지방 함량을 갖는 액체 또는 반고체 우유 또는 유청 기반 제품을 의미한다. 우유-기재 제품은 예를 들어 압소 우유, 염소 우유, 양 우유, 탈지유, 전유, 분유로부터 재조합된 우유 및 임의의 비-가공 우유 또는 가공된 제품 예를 들어 요구르트, 응유, 커드(curd), 산유(sour milk), 산전유, 버터 밀크 및 다른 산유 제품일 수 있다. 또 다른 중요한 그룹에는 우유 음료, 예를 들어 유장 음료, 발효 우유, 응축 우유, 유아 또는 아기용 우유와; 맛을 낸 우유, 아이스크림; 과자와 같은 우유-함유 식품이 포함된다.

[0132] 특정 구현예에서, 본 발명의 조성물은 단일 박테리아성 균주 또는 종을 함유하고 임의의 다른 박테리아성 균주 또는 종을 함유하지 않는다. 이러한 조성물은 다른 박테리아성 균주 또는 종의 최소 또는 생물학적으로 무관한 양만을 포함할 수 있다. 이러한 조성물은 다른 유기체 종이 실질적으로 없는 배양물일 수 있다.

[0133] 본 발명에 사용하기 위한 조성물은 시판 승인을 필요로 할 수도 있고 필요로 하지 않을 수도 있다.

[0134] 일부의 경우, 동결건조 된 박테리아성 균주는 투여 전에 재구성된다. 일부의 경우, 재구성은 본원에 기재된 희석제의 사용에 의한다.

[0135] 본 발명의 조성물은 약학적으로 허용가능한 부형제, 희석제 또는 담체를 포함할 수 있다.

[0136] 특정 구현예에서, 본 발명은 본 발명의 박테리아성 균주; 및 약학적으로 허용가능한 부형제, 담체 또는 희석제를 포함하는 약학적 조성물을 제공하고; 여기서 상기 박테리아성 균주는 치료를 필요로 하는 대상체에게 투여될 때 장애를 치료하기에 충분한 양으로 존재하며, 상기 장애는 천식, 알러지성 천식, 호중구성 천식, 골관절염, 건선성 관절염, 유년성 특발성 관절염, 시신경척수염(테빅병), 강직 척추염, 척추관절염, 전신 홍반성 낭창, 체강 질병, 만성 폐색성 폐 질환(COPD), 암, 유방암, 대장암, 폐암, 난소암, 포도막염, 공막염, 혈관염, 베체트 병, 죽상경화증, 아토피성 피부염, 폐경기증, 치주염, 알러지성 비염 및 동종 이식편 거부반응으로 이루어진 군으로부터 선택된다.

[0137] 특정 구현예에서, 본 발명은 본 발명의 박테리아성 균주; 및 약학적으로 허용가능한 부형제, 담체 또는 희석제를 포함하는 약학적 조성물을 제공하고; 여기서 박테리아성 균주는 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태를 치료 또는 예방하기에 충분한 양으로 존재한다. 바람직한 구현예에서, 상기 질병 또는 병태는 류마티스성 관절염, 다발성 경화증, 건선, 염증성 장 질환, 크론병, 궤양성 대장염, 소아지방변증, 천식, 알러지성 천식, 호중구성 천식, 골관절염, 건선성 관절염, 유년성 특발성 관절염, 시신경척수염(테빅병), 강직 척추염, 척추관절염, 전신 홍반성 낭창, 만성 폐색성 폐 질환(COPD), 암, 유방암, 결장암, 폐암, 난소암, 포도막염, 공막염, 혈관염, 베체트 병, 죽상경화증, 아토피성 피부염, 폐경기증, 치주염, 알러지성 비염, 및 동종이식편 거부반응으로 이루어진 군으로부터 선택된다.

[0138] 특정 구현예에서, 본 발명은 박테리아성 균주의 양이 조성물의 중량에 대하여 약 1×10^3 내지 약 1×10^{11} 콜로니 형성 단위/g인 약학적 조성물을 제공한다.

[0139] 특정 구현예에서, 본 발명은 상기 조성물이 1 g, 3 g, 5 g 또는 10 g의 투여량으로 투여되는 약학적 조성물을 제공한다.

[0140] 특정 구현예에서, 본 발명은 경구, 직장, 피하, 비강, 협측 및 설하로 이루어진 군으로부터 선택된 방법에 의해 조성물이 투여되는 약학적 조성물을 제공한다.

[0141] 특정 구현예에서, 본 발명은 락토오스, 전분, 글루코오스, 메틸 셀룰로오스, 마그네슘 스테아레이트, 만니톨 및 솔비톨로 이루어진 군으로부터 선택된 담체를 포함하는 약학적 조성물을 제공한다.

- [0142] 특정 구현예에서, 본 발명은 에탄올, 글리세롤 및 물로 이루어진 군으로부터 선택된 희석제를 포함하는 약학적 조성물을 제공한다.
- [0143] 특정 구현예에서, 본 발명은 전분, 젤라틴, 글루코오스, 무수 락토오스, 자유-유동 락토오스, 베타-락토오스, 옥수수 감미료, 아카시아, 트라가칸트, 알긴산 나트륨, 카복시메틸 셀룰로오스, 폴리에틸렌 글리콜, 올레산 나트륨, 스테아르 산 나트륨, 스테아르산 마그네슘, 안식향산 나트륨, 아세트산 나트륨 및 염화나트륨으로 이루어진 군으로부터 선택되는 부형제를 포함하는 약학적 조성물을 제공한다.
- [0144] 특정 구현예에서, 본 발명은 보존제, 항산화제 및 안정화제 중 적어도 하나를 추가로 포함하는 약학적 조성물을 제공한다.
- [0145] 특정 구현예에서, 본 발명은 나트륨 벤조에이트, 소르브산 및 p-하이드록시벤조산의 에스테르로 이루어진 군으로부터 선택된 보존제를 포함하는 약학적 조성물을 제공한다.
- [0146] 특정 구현예에서, 본 발명은 박테리아성 균주가 동결건조된 약학적 조성물을 제공한다.
- [0147] 특정 구현예에서, 본 발명은 조성물을 약 4°C 또는 약 25°C에서 밀봉된 용기에 저장하고 상기 용기를 50% 상대 습도를 갖는 대기에 두었을 때, 콜로니 형성 단위로 측정된 박테리아성 균주의 80% 이상은 적어도 약 1 개월, 3 개월, 6 개월, 1 년, 1.5 년, 2 년, 2.5 년 또는 3 년의 기간 후에도 존재한다.
- [0148] **배양 방법**
- [0149] 본 발명에 사용하기 위한 박테리아성 균주는 예를 들어 참고 문헌 [58-60]에 기재된 표준 미생물 기술을 사용하여 배양될 수 있다.
- [0150] 배양에 사용되는 고체 또는 액체 배지는 YCFA 한천 또는 YCFA 배지일 수 있다. YCFA 배지는 카시톤(Casitone)(1.0g), 효모 추출물(0.25g), NaHCO_3 (0.4g), 시스테인(0.1g), K_2HPO_4 (0.045g), KH_2PO_4 (0.045g), NaCl (0.09mg), $(\text{NH}_4)_2\text{SO}_4$ (0.09g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.009g), CaCl_2 (0.009g), 레사즈린(0.1mg), 헤민(1mg), 비오틴(1 μg), 코발라민(1 μg), p-아미노벤조산(3 μg), 엽산(5 μg), 및 피리독사민(15 μg)을(100 ml 당, 근사치)포함한다.
- [0151] **백신 조성물에 사용하기 위한 박테리아성 균주**
- [0152] 본 발명자들은 본 발명의 박테리아성 균주가 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 병태의 치료 또는 예방에 유용하다는 것을 확인하였다. 이는 본 발명의 박테리아성 균주가 숙주 면역계에 미치는 효과의 결과일 가능성이 있다. 따라서, 본 발명의 조성물은 또한 백신 조성물로서 투여될 때 IL-17 또는 Th17 경로에 의해 매개되는 질병 또는 증상을 예방하는데 유용할 수 있다. 이러한 특정 구현예에서, 본 발명의 박테리아성 균주는 사멸, 불활성화 또는 약화될 수 있다. 이러한 특정 구현예에서, 조성물은 백신 보조제를 포함할 수 있다. 특정 구현예에서, 조성물은 피하 주사와 같은 주사를 통한 투여용이다.
- [0153] **일반**
- [0154] 본 발명의 실시는, 달리 명시하지 않는 한, 당해 분야의 숙련 범위 내에서 화학, 생화학, 분자생물학, 면역학 및 약리학의 통상적인 방법을 사용할 것이다. 이러한 기술은 문헌에 충분히 설명되어 있다. 예를 들어 문헌 [61] 및 [62-68] 등 참조.
- [0155] 용어 "포함하는"은 "포함하는"뿐만 아니라 "이루어지는"을 포함하고, 예를 들면, X를 "포함하는" 조성물은 X만으로 이루어질 수 있거나, 예를 들어, 부가적인 것 예를 들어 X + Y를 포함할 수 있다.
- [0156] 수치 x와 관련하여 "약"이라는 용어는 선택 사항이며 예를 들어 $x \pm 10\%$ 를 의미한다.
- [0157] "실질적으로"라는 용어는 "완전히" 제외하지 않고, 예를 들어 Y가 "실질적으로 없는" 조성물은 Y를 전혀 함유하지 않을 수 있다. 필요한 경우, "실질적으로"라는 용어는 본 발명의 정의로부터 생략될 수 있다.
- [0158] 2개의 뉴클레오타이드 서열 사이의 백분율 서열 동일성에 대한 언급은, 정렬시, 뉴클레오타이드의 백분율이 2개의 서열을 비교할 때 동일하다는 것을 의미한다. 이러한 정렬 및 퍼센트 상동성 또는 서열 동일성은 당해 기술 분야에 공지된 소프트웨어 프로그램 예를 들어 문헌 [69]의 섹션 7.7.18에 기재된 소프트웨어 프로그램을 사용하여 결정할 수 있다. 바람직한 정렬은 12의 갭 오픈 패널티 및 2의 갭 연장 패널티, 62의 BLOSUM 매트릭스를 갖는 아핀 갭 탐색을 사용하는 스미쓰-워터맨(Smith-Waterman) 상동 검색 알고리즘에 의해 결정된다. 스미쓰-워

터맨 상동 검색 알고리즘은 문헌[70]에 기술되어 있다.

[0159] 특별히 언급하지 않는 한, 다수의 단계를 포함하는 공정 또는 방법은 상기 방법의 시작 또는 말미에서 추가적인 단계를 포함하거나, 또는 추가적인 개재 단계를 포함할 수 있다. 또한, 적절한 경우, 단계들은 결합되거나 생략되거나 대체 순서로 수행될 수 있다.

[0160] 본 발명의 다양한 구현예가 본원에 기술된다. 각 구현예에서 특징된 특징은 추가의 구현예를 제공하기 위해 다른 특정 특징과 결합될 수 있음을 이해할 것이다. 특히, 본원에서 적합한, 전형적 또는 바람직한 것으로 강조되는 구현예는(상호 배타적일 때를 제외하고) 서로 조합될 수 있다.

[0161] **실시예**

[0162] **실시예 1 - 집먼지 진드기 유발성 천식 마우스 모델에서의 박테리아성 접종의 효능**

[0163] 개요

[0164] 마우스에게 본 발명에 따른 박테리아성 균주를 포함하는 조성물을 투여하고 이어서 집먼지 진드기(HDM) 추출물로 알레르기성 염증 반응을 유도하였다. HDM에 대한 염증 반응은 호산구성 성분과 호중구성 성분을 포함하고 IL-17과 Th17 경로에 의해 매개되며 천식 모델이다. 본 발명의 조성물로 처리된 마우스에 의해 나타나는 염증 반응의 크기 및 특성을 대조군과 비교하였다. 본 발명의 조성물은 염증 반응을 완화시키고 호산구 및 호중구의 동원을 감소시켜 호산구, 호중구 및 천식과 같은 IL-17 매개 질병을 치료하는 데 유용할 수 있음을 나타냈다.

[0165] 균주

[0166] 751: 수탁 번호 NCIMB 42380하에 기탁된 박테리아

[0167] 실험 설계

[0168] 그룹:

[0169] 1. 음성 대조군 비히클 대조군 치료(경구 당).

[0170] 4. 치료 박테리아 접종 균주 751 치료(경구 당).

[0171] 7. 양성 대조군 텍사메타손에 의한 치료(i.p.).

[0172] 8. 치료받지 않은 대조군.

[0173] 그룹당 마우스 수 = 5

[0174] -14일 내지 13일: 경구당 비히클 대조군의 일일 투여(그룹 1).

[0175] -14일 내지 13일: 경구 당 치료 박테리아 접종물의 일일 투여(그룹 2 내지 6).

[0176] 0, 2, 4, 7, 9, 11일 15 ug HDM (비강당 30 ul PBS 중) 투여 (집먼지 진드기 추출물 - 카탈로그 번호 : XPB70D3A25, 로트 번호: 231897, 미국 노스캐롤라이나주 르노와르 소재의 그리어 래보러토리즈(Greer Laboratories))(그룹 1 내지 8).

[0177] 0, 2, 4, 7, 9, 11일 텍사메타손 투여(복강내, 3mg/kg, 시그마-알드리치(Sigma-Aldrich), 카탈로그 번호 D1159)(그룹 7).

[0178] 14일 분석을 위해 모든 동물을 희생.

[0179] 총 마우스 수 = 40.

[0180] 엔드포인트 및 분석

[0181] 14일째 펜타바비톨(우즈나흐 소재 스트레울리 파마 아게(Streuli Pharma AG), 카탈로그 번호: 1170139A)을 사용한 치사적인 복강내 주사에 의해 동물을 희생시킨 다음, 기관지 폐포 세척(BAL)하였다.

[0182] BAL(기관지 폐포 세척액) 유체로부터 세포를 분리하고 차동 세포 수(200 세포 수/샘플)를 수행하였다.

[0183] 물질 및 방법

[0184] **마우스.** 암컷 7주령의 BALB/c 마우스를 찰스 리버 래보러토리즈(Charles River Laboratories)에서 구입하여 케이지 당 5 마리씩 무작위로 케이지에 할당했다(인돌랩 아게(Indulab AG), Gams, 스위스 케이지 유형의 인공 호

흡기 케이지: "Sealsafe™ - IVC 케이지. 제품 번호 1248L). 케이지에는 실험 번호, 그룹 번호 및 실험 시작 날짜가 표시되어 있다. 마우스를 매주 모니터링하고 실험 시작 전 7일 동안 시설에 순응시켰다(실험일 -14일). 동물들은 실험일 -14일에 8 주령이었다. 음용수와 음식은 자유롭게 사용할 수 있었다. 케이지는 풍부했다. 동물의 일상 관리는 지역 허가 번호 2283.1(Service de la consommation et des affaires vétérinaires du Canton de Vaud에 의해 발행 및 승인)에 따라 수행되었다. 음용수와 음식은 매일 자유롭게 사용가능하며 하루에 한 번 새로 고침되었다. 케이지는 풍부했다. 동물 복지 규정은 실험실 축산, 유전자 조작 동물의 생산 및 동물 실험 방법에 관한 FVO(Federal Veterinary Office) 455.163 조에 의거하여 스위스 당국에 의해 주어진 바와 같이 관찰되었다.

- [0185] **박테리아 접종물의 배양.** 멸균 워크스테이션 내에서, 장갑을 낀 손으로 따뜻하게 하여 박테리아의 냉동 바이알을 해동시키고, 혐기성 YCFA 8 ml를 함유한 훈게이트(Hungate) 튜브(카탈로그 번호, 1020471, 독일 보벤텐-렐글린 소재의 글라스게뢰테바우 옥스(Glasgeratebau Ochs))에 약 0.7 ml의 내용물을 주입하였다. 통상 균주 당 2개의 튜브를 준비하였다. 그 후 훈게이트 튜브를 37℃에서 16시간 동안 배양하였다(균주 751).
- [0186] **비히클 대조군의 배양.** 8 mL의 혐기성 YCFA를 함유한 훈게이트 튜브를 37℃에서 16시간 동안(정적으로) 배양하였다.
- [0187] **박테리아 접종 또는 비히클 대조군의 관리.** 경구용 위관 영양법 당 400 uL의 배양된 박테리아 접종물 또는 비히클 대조군을 경구 위관 영양법 당 하루에 투여하였다.
- [0188] **비강내 감염.** 마우스를 i.p. 주사에 의해 kg 당 9.75 mg 자일라졸 및 48.75 mg 케사졸을 주입하여 마취시키고(스위스 베른 소재의 이 그라우베 아게(E. Graeb AG) 박사) 비강당 PBS 30 uL 부피로 15 ug의 HDM(카탈로그 번호: XPB70D3A25, 로트 번호: 231897, 미국 노스캐롤라이나주 르느와르 소재의 그리어 래보라토리즈)을 투여하였다.
- [0189] **양성 대조군 화합물 텍사메타손의 제조 및 투여.** 텍사메타손 21-포스페이트 다이소듐염(시그마-알드리치, 카탈로그 번호 D1159, 로트 번호 SLBD.1030V)을 H₂O에 용해시키고, 상기 실험 프로토콜에 표시된 날에 경구 당 200 uL의 부피로 3 mg/kg의 용량으로 동물에게 투여하였다.
- [0190] **말단 절차.** 14 일째에, 펜타바비톨(Streuli Pharma AG, Uznach, Cat : 1170139A)으로 치사되는 i.p. 주사에 의해 동물을 희생 시키고, 즉시 500 uL의 염수 중 기관지폐포 세척(BAL)을 행했다.
- [0191] **BAL 내로의 세포성 침윤의 측정.** BAL 유체로부터 세포를 분리하고, 표준 형태학적 및 세포화학적 기준에 기초하여 차동 세포 수를 측정하였다.
- [0192] **그래프 및 통계 분석.** 모든 그래프는 그래프패드 프리즘 버전(Graphpad Prism Version) 6으로 생성되었으며 일방향(one-way) ANOVA를 적용했다. 통계 분석 결과는 개별 데이터 표와 함께 제공되었다. 오차 막대는 평균의 표준 오차(SEM)를 나타낸다.
- [0193] 결과 및 분석
- [0194] 실험 결과는 도 1 내지 9에 나타내었다.
- [0195] 박테리아 또는 비히클로 치료한 마우스에서는 이환율이나 사망률이 관찰되지 않았다. 비히클 치료(음성 대조군)와 텍사메타손 치료(양성 대조군)의 두 가지 대조군은 예상대로 행동했으며, 텍사메타손 치료 후 호산구 증가와 호중구 감소증이 나타났다.
- [0196] 이 실험의 가장 중요한 결과는 도 6과 7에 나와있으며, HDM을 투여한 후 기관지 세척에서 검출된 호중구의 총수와 백분율을 보고하였다. 균주 751은 총 호중구와 BAL에서 호중구의 비율을 비히클만의 대조군에 대해 감소시켰다.
- [0197] **실시예 2 - 중증 호중구성 천식 마우스 모델에서의 박테리아 접종물의 효능**
- [0198] 개요
- [0199] 마우스에게 본 발명에 따른 박테리아성 균주를 포함하는 조성물을 투여한 후, 이어서 집먼지 진드기(HDM) 추출물의 피하 투여로 감염시키고, 여기서 HDM을 비강내 투여하여 중증 호중구성 천식의 염증 반응을 모델링하였다. 본 발명의 조성물로 처리된 마우스에 의해 나타나는 염증 반응의 크기 및 특성을 대조군과 비교하였다. 본 발명의 조성물은 항-IL-17 항체의 투여를 포함하는 양성 대조군에 필적하는 방식으로 염증 반응을 완화시키고, 특히

호중구의 동원을 감소시키는 것으로 밝혀졌다. 따라서, 데이터는 본 발명의 조성물이 호중구 및 천식과 같은 IL-17- 및 Th17- 매개된 병태의 치료에 유용할 수 있음을 나타낸다.

[0200] 균주

[0201] 751: 수탁 번호 NCIMB 42380하에 기탁된 박테리아

[0202] 실험 설계

[0203] 그룹:

[0204] 1. 음성 대조군 비히클 대조군 치료(경구 당).

[0205] 4. 치료 박테리아 접종 균주 751 치료(경구 당).

[0206] 7. 양성 대조군 항-IL-17 치료(i.p.).

[0207] 8. 치료받지 않은 대조군.

[0208] 9: 건강한 마우스(기준선).

[0209] 그룹당 마우스 수(그룹 1 내지 8) = 5

[0210] -14일 내지 약 17일: 경구당 비히클 대조군의 일일 투여(그룹 1).

[0211] -14일 내지 17일: 경구당 치료 박테리아 접종물의 일일 투여(그룹 2 내지 6).

[0212] 0일: CFA(s.c.)에서 HDM으로 감작(그룹 1 내지 8).

[0213] 7일: CFA(s.c.)에서 HDM으로 감작(그룹 1 내지 8).

[0214] 13, 15, 17일: i.p.에 따른 항 IL-17 중화 항체의 투여(그룹 7).

[0215] 14, 15, 16, 17일: 비강 당 30 ul PBS 중의 HDM으로 치료(그룹 1 내지 8).

[0216] 18일 분석을 위해 모든 동물을 희생.

[0217] 엔드포인트 및 분석:

[0218] 14일째 펜타바비톨(우즈나흐 소재 스트레울리 파마 아게(Streuli Pharma AG), 카탈로그 번호: 1170139A)을 사용한 치사적인 복강내 주사에 의해 동물을 희생시킨 다음, 기관지 폐포 세척(BAL)하였다. BAL 유체로부터 세포를 단리하고 차동 세포 카운트(200 세포 수/샘플)를 수행하였다.

[0219] 물질 및 방법.

[0220] **마우스.** 암컷 7주령의 C57BL/6 마우스를 찰스 리버 래보러토리즈에서 구입하여 케이지 당 총 5 마리씩 무작위로 케이지에 할당했다(인들랩 아게, Gams, 스위스 케이지 유형의 인공 호흡기 케이지: "The Sealsafe™ - IVC 케이지. 제품 번호 1248L). 케이지에는 실험 번호, 그룹 번호 및 실험 시작 날짜가 표시되어 있다. 마우스를 매주 모니터링하고 실험 시작 전 7일 동안 시설에 순응시켰다(실험일 -14일). 동물들은 실험일 -14일에 8주령이었다. 음용수와 음식은 자유롭게 사용할 수 있었다. 케이지는 풍부했다. 동물의 일상 관리는 지역 허가 번호 2283.1(Service de la consommation et des affaires vétérinaires du Canton de Vaud에 의해 발행 및 승인)에 따라 수행되었다. 음용수와 음식은 매일 자유롭게 사용가능하며 하루에 한 번 새로 고침되었다. 케이지는 풍부했다. 동물 복지 규정은 실험실 축산, 유전자 조작 동물의 생산 및 동물 실험 방법에 관한 FVO(Federal Veterinary Office) 455.163 조에 의거하여 스위스 당국에 의해 주어진 바와 같이 관찰되었다.

[0221] **박테리아 접종물의 배양.** 멸균 워크스테이션 내에서, 장갑을 낀 손으로 따뜻하게 하여 박테리아의 냉동 바이알을 해동시키고, 혐기성 YCFA 8 ml를 함유한 훈게이트(Hungate) 튜브(카탈로그 번호, 1020471, 독일 보벤덴-렐글린 소재의 글라스게르테바우 옥스(Glasgeratebau Ochs))에 ~0.7 ml의 내용물을 주입하였다. 통상 균주 당 2개의 튜브를 준비하였다. 그 후 훈게이트 튜브를 37℃에서 16시간 동안 배양하였다(균주 751).

[0222] **비히클 대조군의 배양.** 8 mL의 혐기성 YCFA를 함유한 훈게이트 튜브를 37℃에서 16시간 동안(정적으로) 배양하였다.

[0223] **박테리아 접종 또는 비히클 대조군의 관리.** 경구용 위관 영양법 당 400 ul의 배양된 박테리아 접종물 또는 비히

클 대조군을 경구 위관 영양법 당 하루에 투여하였다.

- [0224] **HDM 감작.** PBS 중의 HDM 50 μ g(카탈로그 번호: XPB70D3A25, 로트 번호: 231897, 미국 노스캐롤라이나주 르느와르 소재의 그리어 레보러토리즈)을 동량의 완전 프로인트 보조제(미국 워싱턴 소재의 시에프에이 콘드렉스 인코포레이티드(CFA Chondrex Inc.))에서 유화시키고 반대쪽 측면에서 2주에 걸쳐 2회 200 μ l의 부피로 피하 투여하였다. 두 번째 면역화 후 일주일 후, 마우스를 1kg 당 9.75mg의 자일라졸 및 48.75mg의 케타졸(스위스 베른 소재의 이 그라우베 아게 박사)으로 i.p. 주사하여 마취시킨 다음, 4일 연속 30 μ l의 PBS 중의 15 μ g의 HDM을 비강내 투여하였다. 마지막 투여 후 하루 동안 분석을 수행했다.
- [0225] **양성 대조군 화합물 항 마우스 IL-17 항체의 제조 및 투여.** 항-IL-17 중화 항체는 Bio X Cell에서 유도하였고, 4°C에서 보관하고(클론 17F3, 카탈로그 번호 BE0173, Bio X Cell) 상기 연구 프로토콜에 표시된 날에 12.5 mg/kg의 용량으로 i.p. 당 투여하였다.
- [0226] **말단 절차.** 18 일째에, 스텐타바비톨(Streuli Pharma AG, Uznach, Cat: 1170139A)으로 치사되는 i.p. 주사에 의해 동물을 희생 시키고, 즉시 500 μ l의 염수 중 기관지폐포 세척(BAL)을 행했다.
- [0227] **BAL 내로의 세포성 침윤의 측정.** BAL 유체로부터 세포를 단리하고, 표준 형태학적 및 세포화학적 기준에 기초하여 차동 세포 수를 측정하였다.
- [0228] **그래프 및 통계 분석.** 모든 그래프는 그래프패드 프리즘 버전(Graphpad Prism Version) 6으로 생성되었으며 일방향(one-way) ANOVA를 적용했다. 통계 분석 결과는 개별 데이터 표와 함께 제공되었다. 오차 막대는 평균의 표준 오차(SEM)를 나타낸다.
- [0229] 결과 및 분석
- [0230] 실험 결과는 도 10 내지 18에 나타내었다.
- [0231] 박테리아 또는 비히클로 치료한 마우스에서는 이환율이나 사망률이 관찰되지 않았다. 도 15 및 도 16에 도시된 바와 같이, 군주 751은 호중구성 염증 반응의 크기를 경감시키는 데 매우 효과적이었다. 실제로, 군주 751로 처리한 결과, 항-IL-17 항체로 처리한 결과와 유사한 결과가 나타났다. 또한, 도 11 및 12에 도시된 바와 같이, 군주 751은 대조군에 비해 호산구 수를 감소시켰다.
- [0232] **실시예 3 - II 형 콜라겐-유도성 관절염 마우스 모델에서 관절염을 치료하기 위한 박테리아 접종물의 효능**
- [0233] 물질 및 방법
- [0234] **균주**
- [0235] 751: 수탁 번호 NCIMB 42380하에 기탁된 박테리아
- [0236] **박테리아 배양물**
- [0237] 박테리아 배양물은 혐기성 워크스테이션(돈 휘틀리 사이언티픽(Don Whitley Scientific))에서 투여하기 위해 성장시켰다.
- [0238] 박테리아성 군주 #751을 글리세롤 스톱을 사용하여 성장시켰다. 글리세롤 스톱을 -80°C에서 보관했다. 주당 3회, 글리세롤 스톱을 실온에서 해동시키고 YCFA 플레이트 상에서 도말(streak)하였다. 각 경우에 새로운 글리세롤 분취량을 사용했다. 박테리아는 주어진 플레이트에서 최대 72시간 동안 성장하도록 했다.
- [0239] 동물에게 투여할 용액을 오전(AM) 및 오후(PM) 치료를 위해 8시간 간격으로 1일 2회 제조하였다. 박테리아성 콜로니를 도말된 플레이트로부터 골라내고 YCFA 배지를 함유하는 튜브로 옮겼다. AM 투여 전에 박테리아성 군주 #751을 16시간 동안 성장시켰다. 박테리아를 PM 투여를 위해 YCFA 배지 내에 1%로 서브-배양하였다. 오전 및 오후 치료 준비 후에 각 군주에 대한 OD 값을 기록하였다.
- [0240] **II 형 콜라겐-유도성 관절염 마우스 모델**
- [0241] 성숙한 수컷 DBA/1 마우스를 무작위로 실험 그룹에 할당하고 2주 동안 순응시켰다. 0 일째에, 4 mg/ml 결핵균 H37Ra가 첨가된 불완전 프로인트 보조제에 100 μ g의 II 형 콜라겐(CII)을 함유하는 100 μ l의 에멀전을 피하 주사하여 동물에게 투여하였다. 21 일째에, 불완전 프로인트 보조제에 II 형 콜라겐 100 μ g을 함유하는 부스터 에멀전을 피하 주사하여 동물에게 투여하였다.
- [0242] 치료는 아래 투여 일정에 따라 실시되었다. -14일부터 실험 45일까지 실험 동물은 주당 3회 무게를 달았다. 21

일부 실험이 끝날 때까지, 관절염의 임상적 징후에 대해 뒷다리 및 앞발, 라디오-손목(손목) 관절 및 경골-발목(발목) 관절의 붓기를 포함하여 주당 3회 동물을 채점했다.

[0243] 45 일째에 마우스를 도살시키고 말단 혈액 샘플을 사이토카인 분석을 위해 취하였다.

[0244] -14일째, 0일째와 45일째에, 미생물 분석을 위해 대변 샘플을 수집하고, 즉시 스냅-동결시키고 -80℃에서 보관하였다.

[0245] 콜라겐-유도성 관절염(CIA) 마우스 모델은 류마티스성 관절염에 대한 잘 확립된 마우스 모델이다[71]. CII 예방 접종은 활액 증식, 단핵 세포 침윤 및 연골 분해를 비롯해 류마티스성 관절염의 여러 중요한 병리학적 특징을 포함하는 병인생리를 일으킨다. 의미있게도, CIA의 발달은 IL-17A의 분비를 통해 Th17 세포에 의해 매개된다[72]. 관절염 모델의 기본이 되는 면역 반응은 결핵균이 보충된 프로인트 보조제의 사용으로 향상된다.

[0246] 21일째에, 각 그룹의 3 마리의 위성 동물로부터 비장을 수집하였다. 세포는 II 형 콜라겐의 존재 또는 부재하에 72시간 동안 배양되었다. TNF- α , IL-6, IFN- γ , IL-4, IL-10 및 IL-17을 포함하는 사이토카인을 배양 상등액 및 말단 혈청에서 루미넥스(Luminex)에 의해 정량화하였다. 세포 증식은 삼중체화된 티미딘 도입 방법을 사용하여 정량화하였다.

[0247] 치료 그룹 및 투여량

[0248] 모든 그룹은 n = 15이었다(주 연구 그룹은 n = 12, 위성 그룹은 n = 3)

[0249] 생물 치료제로 사용된 비히클은 효모 추출물-카시톤-지방산(YCFA) 배지였다.

	그룹	투여량	투여		질병 유도
			경로	섭생	
1	비히클	5 ml/kg	PO	BID: -14 일-끝	0 일: 콜라겐/CFA, 1 회, SC
2	생물요법 #751	5 ml/kg	PO	BID: -14 일-끝	21 일: 콜라겐/IFA, 1 회, SC

PO: 경구 위관병, SC: 피하 주사, BID: 하루에 두 번, CFA: 완전 프로인트 보조제.

[0250]

[0251] 체중

[0252] -14일부터 실험이 끝날 때까지 동물을 주당 3회 무게를 달았다. 데이터를 그래프로 나타냈다(평균 $\pm$ SEM).

[0253] 비-특이적 임상 관찰

[0254] -14일부터 실험이 끝날 때까지, 비정상적인 자세(구부린 자세), 비정상적인 코트 병태(털세움) 및 비정상적인 활동 수준(활동 감소 또는 증가)을 포함하는 비-특이적인 임상 징후가 있는지 매일 동물을 검사했다.

[0255] 임상 관찰

[0256] 21일부터 45일째 실험이 끝날 때까지, 관절염의 임상적 징후에 대해 뒷다리 및 앞발, 라디오-손목(손목) 관절 및 경골-발목(발목) 관절의 붓기를 포함하여 주당 3회 동물을 채점했다. 각각의 사지는 (0) 정상, (1) 약간의 붓기, (2) 가벼운 붓기, (3) 적당한 붓기 및 (4) 심한 붓기를 사용하여 점수를 매겼다. 각 사지 점수를 추가하여 임상 점수를 계산했다. 동물에 대한 가능한 최대 임상 점수는(16)이었다. 두 번 연속해서(12)와 동일한 점수를 가진 동물과 어떤 경우에(12)보다 큰 점수를 가진 동물이 도살되었다. 데이터를 그래프로 나타냈다(평균 $\pm$ SEM).

[0257] 세포 증식 분석

[0258] 21일째, 그룹당 3 마리의 위성 동물이 도살되고 비장이 해부되었다. 비장 세포를 II 형 콜라겐의 존재 또는 부재하에 72시간 동안 배양하였다. 72시간 후, 세포를 삼중체화된 티미딘의 존재하에 밤새 맥동시켰다. 세포 증식은 티미딘 도입을 측정함으로써 정량화하였다. 데이터를 그래프로 나타냈다(평균 $\pm$ SEM). 상등액을 채취하여

주요 사이토카인의 존재에 대해 시험하였다.

[0259] **사이토카인 분석**

[0260] TNF- α , IL-6, IFN- γ , IL-4, IL-10 및 IL-17을 루미넥스에 의해 정량하기 위해 비장 세포 배양물의 말단 상층액을 시험하였다. 데이터를 그래프로 나타냈다(평균 $\pm$ SEM).

[0261] **미생물 분석**

[0262] -14일째, 0일째와 45일째에, 각 동물로부터 대변 샘플을 수집하고, 즉시 스냅-동결시키고 -80°C에서 보관하였다. Caeca(함량 포함)는 즉시 스냅-동결되어 -80°C에 보관되었다. 박테리아 동정 시험은 박테리아를 플레이트하여 매일 수행하였다.

[0263] **조직병리학**

[0264] 실험이 끝나면, 뒷발을 조직 정착액에 보관했다. 샘플을 탈칼슘화 용액으로 옮겼다. 조직 샘플을 가공하고, 절편을 만들고, 헤마톡실린 앤드 에오신(Haematoxylin & Eosin)으로 염색하였다. 염증, 관절 연골 손상 및 근골 사이 골절의 손상을 포함하는 관절염의 징후에 대한 실험 설계에 대해 맹인인 전문 조직 병리학자가 섹션을 채점했다. 상세한 채점 시스템이 사용되었다(아래 참조). 데이터를 그래프로 나타냈다(평균 $\pm$ SEM). 원시 및 분석 데이터뿐만 아니라 대표적인 그림이 제공되었다.

[0265] **표 1: 조직병리학 점수 시스템**

등급	설명
염증	
0	정상 관절
1	호중구가 지배하는 염증을 동반한 경미한 활막 증식. 관절 공간에서 소수의 호중구 및 대식세포.
2	호중구 및 대식세포 모두를 포함하는 중등도에서 현저한 염증을 갖는 활막 증식. 관절 공간에서의 호중구 및 대식세포; 일부 피사 조직 파편일 수 있다.
3	호중구 및 대식세포 모두를 포함하는 현저한 염증을 갖는 활막 증식. 활막세포 라이닝의 손실. 염증은 활액에서 근육을 포함한 주변 조직으로 확장될 수 있다. 상당한 피사 조직 파편과 함께 관절 공간에 있는 수많은 호중구 및 대식세포.
관절 연골 손상	
0	정상 관절
1	관절 연골이 경미한 퇴행성 변화만을 보여준다. 초기 파누스(pannus) 형성이 주변에 나타날 수 있다.
2	관절 연골이 중등도의 퇴행성 변화와 국소 손실을 나타낸다. 파누스 형성이 국소적으로 존재한다.
3	광범위한 파누스 형성과 함께 관절 연골의 현저한 파괴 및 손실.
근본적인 골간단 뼈의 손상	
0	정상 관절
1	근본적인 골간단 뼈에 변화가 없다.
2	골간단 뼈의 국소 피사 또는 섬유증일 수 있다.
3	골간단 뼈의 파괴 또는 붕괴. 골간단의 골수 공간으로 확장된 광범위한 염증, 피사 또는 섬유증.

[0266]

- [0267] 결과 및 분석
- [0268] **생존 및 비특이적 임상 관찰**
- [0269] 일부 동물은 관절염의 임상 증상의 중증도 또는 비특이적 임상 관찰의 중증도로 인해 실험이 끝나기 전에 도살되었다.
- [0270] 전처리 기간(-14일 내지 0일) 동안 3 마리의 동물이 도살되거나 죽은 것으로 나타났다: 그룹 1에서 1 마리(부러진 다리가 있는 공급자로부터 비히클-처리된 동물이 도착하여 도살된 동물) 및 그룹 2에서 2 마리(생물치료제 #751-처리, 첫 번째 전처리 일의 가능한 폐 투여 및 두 번째 전처리 일의 투여 후 임상 징후).
- [0271] 관절염의 임상 징후의 중증도로 인해 8 마리의 동물이 도살되었다: 그룹 1에서 5마리(비히클 처리), 그룹 2에서 3마리(생물치료제 # 751-처리).
- [0272] 비정상적인 자세(구부린 자세), 비정상적인 코트 병태(털세움), 비정상적인 활동 수준(활동 감소) 등 비특이적인 임상 징후의 중증도로 인해 4마리의 동물이 도살되었다: 그룹 1에서 3마리(비히클-처리) 및 그룹 2에서 1 마리(생물치료제 #751-처리).
- [0273] **체중**
- [0274] -14일째부터 0일까지 기록된 체중 데이터를 초기(-14일) 체중의 백분율로 표시한 양방향 ANOVA 분석을 실시한 다음, 두넷(Dunnett) 사후 검사하여 -14일에 다중 비교한 후, 비히클 처리군과 다중 비교하였다. 데이터를 도 19에 나타내었다. 실험이 끝나기 전에 도살한 동물의 데이터는 분석에서 제외했다.
- [0275] -14일과 비교했을 때, 구강 위관 영양법에 의한 1일 2회 투여는 -9일 및 -7일에 비히클-처리군에서 유의한 체중 감소를 유발하였다.
- [0276] 생물 요법으로 치료한 그룹에서 -14일과 -1일 사이에 측정된 체중은 주어진 하루에 비히클로 치료한 그룹에서 측정된 체중과 다르지 않았다.
- [0277] 0일째부터 28일까지 기록된 체중 데이터를 초기(0일) 체중의 백분율로 표시한 양방향 ANOVA 분석을 실시한 다음, 두넷 사후 검사하여 비히클 그룹에서 0일째 다중 비교한 후, 비히클 처리군과 다중 비교하였다. 데이터를 도 20에 나타내었다. 실험이 끝나기 전에 도살한 동물 및 위성 동물의 데이터는 분석에서 제외했다. 28일, 35일 및 42일 데이터를 일방향 ANOVA에 의해 추가로 분석한 후, 비히클 처리 그룹에 대한 다중 비교를 위해 두넷 사후 검사를 수행하였다.
- [0278] 관절염의 임상적 징후의 개시는 비히클-치료 그룹에서 0일째와 비교했을 때 26일째와 28일째에 유의한 체중 감소와 관련이 있었다($p < 0.0001$).
- [0279] 35일째 또는 42일째 실험 그룹들 간에 유의한 차이는 없었다.
- [0280] **임상 관찰**
- [0281] 임상 점수 데이터는 양방향 ANOVA에 의해 분석 후 두넷 사후 검사를 통해 비히클-처리 그룹에서의 일간 다중 비교를 실시한 후, 실험 그룹과 비히클-처리 그룹 간의 다중 비교를 매일 실시하였다. 데이터를 도 21에 나타내었다. 실험이 끝나기 전에 도살한 동물로부터 기록한 데이터는 분석에서 제외했다. 관절염의 임상 징후의 중증도로 인해 동물을 도살한 경우, 마지막 기록된 점수가 다음 날에 보고되어 통계 분석에 사용되었다.
- [0282] 28일째부터 45일째까지 비히클 처리 그룹에서 21일째와 비교했을 때 유의한 임상 점수의 증가가 관찰되었다($p < 0.0001$).
- [0283] 생물치료제 #751은 31일부터 45일까지 비히클-처리 그룹과 비교했을 때 임상 점수의 감소를 유도하였으나 차이는 유의하지 않았다.
- [0284] **세포 증식 분석**
- [0285] 분석을 검증하기 위해, 세포의 증식 잠재력을 확인하기 위해 양성 대조군 자극으로서 가용성 항-CD3 및 항-CD28(항-CD3/CD28)의 존재하에 비장 세포를 배양하였다.
- [0286] 항-CD3/CD28에 대한 강력한 증식 반응은 모든 실험 그룹에서 관찰되었으며, 세포가 건강하고, 생존 가능하며, 활성화 신호에 반응할 수 있음을 보여주었다.

- [0287] II 형 콜라겐(CII)의 존재하에 증식 반응을 시험하기 위해, 비장 세포를 50 μ g/ml의 CII 존재하에서 배양하였다. CII에 대한 비장 세포 증식 반응을 양방향 ANOVA로 분석한 후 비-자극 및 CII-자극된 비장 세포와 일방향 ANOVA 간의 다중 비교에 대한 시닥(Sidak) 사후 검정 및 비히클-처리군과 다른 실험군에서의 CII-자극 반응 비교를 위한 두넷 사후 검정으로 분석하였다. 데이터를 도 22에 나타내었다.
- [0288] CII는 비히클-처리군에서 비자극된 비장 세포와 비교할 때 ^{3} H-티미딘 도입(cpm)의 매우 현저한 증가를 유도하였다($p < 0.0001$).
- [0289] 생물치료제 #751로 치료한 그룹은 비히클-처리군보다 CII-유도된 비장 세포 증식 수준이 현저히 낮았다는 것을 보여주었다.
- [0290] **조직 배양 상등액의 사이토카인 수준**
- [0291] 루미넥스 분석에 의해 항-CD3/CD28 자극 배양으로부터 유도된 조직 배양 상등액에서 각각의 사이토카인의 수준을 측정하였다. 이들은 측정된 모든 사이토카인에 대한 견고한 반응을 나타내었다(비히클 군의 평균 수준은 다음과 같다: 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).
- [0292] 다음 섹션에서는 II 형 자극 배양액에서 얻은 데이터를 요약한 것이다. 적용가능한 경우, 비자극 및 CII-자극된 비장 세포의 상등액에서의 사이토카인 수준 사이의 차이에 대한 통계 분석이 양방향 ANOVA를 사용하고 이어서 시닥의 다중 비교에 대한 사후 검정을 사용하여 수행되는 한편, 두넷의 사후 검정이 뒤따르는 일방향 ANOVA가 생물치료제로 처리된 군과 비히클 처리된 군에서의 CII 자극 반응을 비교하기 위해 사용되었다. 두 경우 모두에서 그룹들 간에 사이토카인 수준에는 유의한 차이가 없었다. 이것은 사용된 작은 샘플 크기($n = 3$) 때문일 가능성이 크다.
- [0293] 데이터의 실질적인 분포와 함께 사이토카인에 대한 데이터 분포를 보다 정확하게 나타 내기 위해, 이들은 산점도로서 제시된다.
- [0294] CII 자극 후 조직 배양 상등액에서 IL-4의 군 평균은 5pg/ml 미만이었다. 이들은 생물학적으로 유의하지 않으며 여기에 포함되지 않는다. 콜라겐 자극 후 조직 배양 상등액에서의 TNF- α 의 그룹 평균은 정량 한계 이하였다.
- [0295] **IFN- γ 의 상등액 수준(도 23)**
- [0296] IFN- γ 는 IL-17과 함께 CIA 모델에서 주요 사이토카인 유도성 질병이다. 도 23의 산란 플롯은 CII 자극 후 IFN- γ 수준을 나타내며, 생물치료제에 비해 비히클-처리군의 그룹 중앙값이 더 높다. 같은 그룹 2 대상체의 이상치 결과는 IFN- γ 및 IL-10에 대한 이 그룹의 더 높은 중앙값을 차지한다.
- [0297] **IL-17A의 상등액 수준(도 24)**
- [0298] IL-17A의 수준은 비히클 투여군에 대한 CII-자극 배양물에서 50 pg/ml이었다. 이러한 사이토카인의 수준은 비히클 치료군에 비해 생물치료제 군에서 더 낮았다.
- [0299] **IL-10의 상등액 수준(도 25)**
- [0300] 비히클 처리군에서 IL-10의 수준은 각각 CII- 자극 및 배지 조절 배양에 대해 13 pg/ml 및 2.1 pg/ml이었다. 염증 및 전-염증성 사이토카인 유도가 항-염증성 피드백 메커니즘을 수반할 수 있기 때문에, 비히클-처리군에 대한 IL-10(항-염증성 사이토카인)의 더 높은 수준이 예상될 수 있다.
- [0301] **IL-6의 상등액 수준(도 26)**
- [0302] IL-6 및 TNF- α 와 같은 염증성 사이토카인은 전형적으로 항-CII 배양물에서 높은 수준으로 생성되지 않는다. 그러나, 면역 조절의 결과로 그들의 수준이 변경될 수 있다. CII로 자극된 배양물에서 IL-6의 수준은 적당했으며 10 pg/ml에 달했다. 배지 대조군 배양물에서 보다 높았지만, 이러한 차이는 너무 작아서 통계 분석을 수행하기 위한 이론적 근거를 제공하지 못했다.
- [0303] **미생물 분석**
- [0304] 박테리아 성장은 분광광도계를 사용하여 600 nm에서의 광학 밀도를 측정함으로써 확인되었다. 박테리아 실체는 스트리킹된 판 그림과 참조 그림을 비교하여 확인되었다.
- [0305] 개선된 박테리아 제조 방법에 이어, 측정된 높은 OD 값에 의해 지시된 바와 같이, -2일 및 -3일부터 지속적으로

고농도의 박테리아성 균주가 투여되었다.

[0306] 대변 샘플을 수집하여 -14일째, 0일째 및 종료시에 급속 냉동시켰다.

[0307] **조직병리학**

[0308] 조직병리학 결과는 도 66 내지 70에 나타내었다. 이 모델에서 예상한 바와 같이, 관절염의 유무 또는 존재하는 변화의 심각성과 관련하여 개체내 및 개체간 변동성을 관찰하였다.

[0309] 조직병리학의 특징은 시노뎀(synovium)과 버사(bursa)의 넓은 혼합된 만성-활성 염증이 관절주위 연조직(근육, 아디포스 조직, 피부 콜라겐)을 포함하도록 확장되어 이 모델에서 예상한 바와 같았다. 가장 심하게 영향을 받은 관절에는 관절 연골 변성과 관절내 파편 소실 및 섬유증과 염증에 의한 염증과 관절과 골 구조의 파괴가 있었다.

[0310] 조직병리학적 변화의 빈도는: 비히클 - 80%(16/20); 생물치료제 #751 - 45%(9/20)이었다. 생물치료제 #751 치료는 비히클 처리군에 비해 마우스 뒷다리의 조직병리학 점수가 감소했다(도 66 내지 69 참조). 조직병리학 점수는 비-파라미터 데이터(크루스칼-월리스(Kruskal-Wallis) 시험)에 대한 일방향 ANOVA에 의해 분석되었고, 둔(Dunn)의 비히클-처리군에 대한 다중 비교를 위한 사후 검사를 받았다. 생물치료제 #751은 비히클-처리군에 비해 조직병리학적으로 관찰된 관절 염증 점수의 유의한 감소를 유발했다($p < 0.01$). 생물치료제 #751은 비히클-처리군과 비교했을 때 조직병리학에서 관찰된 연골 손상 점수의 유의한 감소를 유발했다($p < 0.001$). 생물치료제 #751은 비히클-처리군과 비교했을 때 조직병리학에서 관찰된 골 손상 점수의 유의한 감소를 유발했다($p < 0.001$). 생물치료제 #751은 비히클-처리군에 비해 전체 조직병리학적 점수의 유의한 감소를 유발했다($p < 0.01$).

[0311] **개요**

[0312] DBA/1 마우스의 관절염 모델에서 예상한 바와 같이 II 형 콜라겐의 최초 투여 후 28일째부터 증가된 임상 점수가 관찰되었다. 생물치료제 #751은 이 모델에서 관절염 치료에 효과적이었고 생물치료제 #751은 임상 점수의 중증도를 줄이는 데 효과적이었다. 생물치료제 #751은 조직병리학적 분석에서 입증된 바와 같이 관절의 조직병리학적 질병을 감소시키는 데에도 효과적이었다.

[0313] 콜라겐 II에 대한 증식성 회귀 반응은 모든 실험군의 비장 세포 배양물에서 관찰되었다. 콜라겐-특이적 반응은 생물치료제 #751(그룹 2)으로 치료한 후에 유의하게 감소했다.

[0314] 시험된 대부분의 T 세포 사이토카인은 콜라겐 II-자극된 배지와 비히클-처리군의 배지 대조군 간의 검출가능한 증가를 나타냈다. 이러한 증가는 생물치료제 처리군에서 명백하지 않았다. 이는 위에서 설명한 콜라겐 II에 대한 증식성 회귀 반응을 광범위하게 지지한다.

[0315] 이 모델과 인간 RA에서 병원성 반응인 Th1/Th17 축의 억제의 증거가 있었다. 사이토카인의 감소된 수준과 감소된 증식의 상관 관계는 면역 조절을 암시한다. 이러한 조절이 Th2 관련 IL-4의 증가된 수준 또는 면역 조절 사이토카인인 IL-10의 증가로부터 초래되었다는 어떠한 증거도 없었다.

[0316] **실시예 4 - 집먼지 진드기 유발성 천식의 마우스 모델에서 박테리아 접종물의 효과에 대한 추가적인 분석**

[0317] 실시예 1에서 시험된 마우스를 추가 분석하여 본 발명의 조성물의 알레르기성 천식 염증 반응에 대한 효과를 추가로 특성화하였다.

[0318] 물질 및 방법

[0319] **14일째 혈액 채취 및 혈청 제조.** 동물의 혈액 샘플을 심장 펌프를 통해 수집하였다. 혈청을 14000g에서 5분 동안 원심분리하여 혈액 샘플로부터 분리하고 -20℃에서 보관하였다.

[0320] **14일째 장기 제거.** 후속 조직병리학 분석을 위한 포르말린의 왼쪽 폐엽 수집. 오른쪽 폐엽(모든 남은 엽) 수집 및 급속 냉동 및 후속 분석을 위한 혈청 제거. 나머지 BAL 유체는 후속 분석을 위해 즉시 동결되었다.

[0321] **혈청 및 BAL 유체의 항체 수치 측정**

[0322] 총 IgE 및 집먼지 진드기(HDM) 특이적 IgG1 항체 생산은 ELISA 분석에 의해 BAL 및 혈청에서 측정되었다.

[0323] **폐의 단리 및 조직학적 분석**

[0324] 왼쪽 폐엽을 포르말린에 고정시킨 후 파라핀에 삽입하고 절편을 만들고 헤마톡실린과 에오신과 PAS로 염색하였

다. 이후의 조직학적 채점은 다음과 같이 맹검 수행되었다: 샘플 당 5개의 무작위 시야 관찰로 염증(기관지주위 침윤 및 혈관주위 침윤) 및 점액 생성에 대해 채점되었다. 염증 침윤은 다음 등급 시스템으로 채점되었다:

- [0325] 0 - 정상
- [0326] 1 - 경증 염증 침윤
- [0327] 2 - 중증 염증 침윤
- [0328] 3 - 현저한 염증 침윤
- [0329] 4 - 심한 염증 침윤
- [0330] 5 - 매우 심한 염증 침윤

[0331] 각 시야에서 기도의 크기를 측정하고 점액 세포 수를 μm 당 정량화하였다.

[0332] **폐 조직의 염증 매개체 측정**

[0333] 염증 매개체의 정량을 위해 단리된 우측 폐엽(나머지 모든 엽)은 상업적으로 이용가능한 멀티플렉스 분석(Merck-Millipore)에 의해 CCL11, IFN- γ , IL-1 α , IL-1 β , IL-4, IL-5, IL-9, IL-17A, CXCL1, CCL3, CXCL2 및 CCL5의 후속 측정을 위해 즉시 동결되었다. 분석은 제조자의 지침에 따라 수행되었다.

[0334] 결과 및 분석

[0335] 실험 결과는 도 28 내지 46에 나타내었다.

[0336] 실시예 1에 기재된 결과를 뒷받침하기 위해, 군주 751로 처리된 마우스의 폐 조직에서의 세포 침윤의 분석은 평균 염증 점수에서 현저하고 통계적으로 유의한 감소를 보였다(도 32 및 34 참조).

[0337] BAL 유체 및 혈청의 항체 수준을 분석했다(도 28 내지 31 참조). 혈청 항체 수치에 대한 박테리아 치료의 명확한 효과는 관찰되지 않았다. 이것은 실험의 실패를 반영할 수 있다. 왜냐하면 데이터의 분산 및 각 치료에 대한 오차 막대가 크고, 양성 및 음성 대조군이 예상대로 행동하지 않았기 때문이다. 또한 기준선 혈청 항체 수치가 모든 변화를 차단했을 수 있다.

[0338] 유사하게, 폐 조직의 사이토카인 수준에 대한 박테리아 처리의 명확한 효과는 관찰되지 않았다(도 36 내지 46 참조). 이것은 실험의 실패를 반영할 수 있다. 왜냐하면 데이터의 분산 및 각 치료에 대한 오차 막대가 크고, 양성 및 음성 대조군이 예상대로 행동하지 않았기 때문이다. 또한 관련된 작용 기전이 최종 HDM 기도 투여 후 4 일째 더 이상 검출할 수 없었던 초기의 사이토카인 반응에 영향을 줄 가능성이 있다. 검출된 수준의 가변성으로 인해 현재 실험에서 사이토카인 데이터를 해석할 때 일부 주의를 기울여야 한다. 이러한 가변성은 부분적으로 폐 조직이 다른 분석을 위해 분리되었다는 사실에 의해 설명될 수 있으며, 따라서 하나의 폐엽은 염증의 패치성 누적 분포로 인해 다른 마우스의 동일한 엽을 완전히 대표하거나 그에 필적할만한 것이 되지 않았을 수 있다.

[0339] **실시예 5 - 중증 호중구성 천식의 마우스 모델에서 박테리아 점종물의 효과에 대한 추가적인 분석**

[0340] 실시예 2에서 시험된 마우스를 추가 분석하여 본 발명의 조성물의 중증 천식과 관련된 호중구성 반응에 대한 효과를 추가로 특성화하였다.

[0341] 물질 및 방법

[0342] **18일째 장기 제거.** 후속 조직병리학 분석을 위한 포르말린의 왼쪽 폐엽 수집. 오른쪽 폐엽(모든 남은 엽) 수집 및 급속 냉동 및 후속 분석을 위한 혈청 제거. 나머지 BAL 유체는 후속 분석을 위해 즉시 동결되었다.

[0343] **폐 조직의 염증 매개체 측정(후속 분석).** 염증 매개체의 정량을 위해 단리된 우측 폐엽(나머지 모든 엽)은 상업적으로 이용가능한 멀티플렉스 분석(Merck-Millipore)에 의해 IFN- γ , IL-1 α , IL-1 β , CXCL1, CCL3, CXCL2, CCL5, IL-17A, TNF- α , IL-17F, IL-23 및 IL-33의 후속 측정을 위해 즉시 동결되었다. 분석은 제조자의 지침에 따라 수행되었다.

[0344] **혈청 및 BAL 유체의 항체 수치 측정(후속 분석).** 집먼지 진드기(HDM) 특이적 IgG1 및 IgG2a 항체 생산은 ELISA 분석에 의해 BAL 및 혈청에서 측정되었다.

[0345] **폐의 단리 및 조직학적 분석(후속 분석).** 왼쪽 폐엽을 포르말린에 고정시킨 후 파라핀에 삽입하고 절편을 만들고 헤마톡실린과 에오신과 PAS로 염색하였다. 이후의 조직학적 채점은 다음과 같이 맹검 수행되었다: 샘플 당 5

개의 무작위 시야 관찰로 염증(기관지주위 침윤 및 혈관주위 침윤) 및 점액 생성에 대해 채점되었다. 염증 침윤은 다음 등급 시스템으로 채점되었다:

- [0346] 0 - 정상
- [0347] 1 - 경증 염증 침윤
- [0348] 2 - 중증 염증 침윤
- [0349] 3 - 현저한 염증 침윤
- [0350] 4 - 심한 염증 침윤
- [0351] 5 - 매우 심한 염증 침윤

[0352] 결과 및 분석

[0353] 실험 결과는 도 47 내지 64에 나타내었다.

[0354] 항체 수치의 추가 분석 결과, BAL 유체 및 혈청에서 HDM-특이적 IgG1 수치가 감소됨에도 박테리아성 균주 751의 효능이 반영된 것으로 나타났다(도 47 및 49 참조). IgG2a 수준에 대한 효과에 관한 확고한 결론은 이끌어 낼 수 없다. 전반적으로, 항체 분석으로부터의 데이터는 항체 이소타입(isotype) 스위칭에 대한 선택적 효과와는 대조적으로 전반적인 감소된 염증 반응과 관련된 감소를 암시한다.

[0355] 조직학적 분석은 BAL 유체로부터의 분화된 세포 수를 지지하였으며, 이는 균주 751로 처리된 마우스에서 세포성 침윤이 감소함을 나타낸다(도 51 내지 53 참조).

[0356] 사이토카인 수준과 관련하여, 실시예 4에서와 같이, 데이터의 분산 및 각 치료에 대한 오차 막대가 크고, 양성 및 음성 대조군은 반드시 예상대로 행동하지 않은 것처럼 보인다. 또한 작용 기전이 최종 HDM 기도 투여 후 4일째 더 이상 검출할 수 없었던 초기의 사이토카인 반응에 영향을 미쳤을 수도 있다. 검출된 수준의 가변성으로 인해 현재 실험에서 사이토카인 데이터를 해석할 때 일부 주의를 기울여야 한다. 이러한 가변성은 부분적으로 폐 조직이 다른 분석을 위해 분리되었다는 사실에 의해 설명될 수 있으며, 따라서 하나의 폐엽은 염증의 패치성 누적 분포로 인해 다른 마우스의 동일한 엽을 완전히 대표하거나 그에 필적할만한 것이 되지 않았을 수 있다. 이러한 가변성에도 불구하고, 균주 751에 대한 사이토카인 수준에 대한 명확한 항염증 효과가 나타났고, 양성 대조군 항-IL-17 Ab는 일반적으로 예상대로 행동하였다.

[0357] 상기 경고들로 인해, 도 56, 58, 59, 61 및 63의 데이터는 본 발명의 박테리아성 균주, 특히 균주 751에 의한 처리가 IL-1b, IFN γ , RANTES, MIP-1a 및 KC(인간 IL-8의 마우스 오르토로그) 수준의 감소를 달성시킬 수 있으며, 이는 기질 또는 선천성 면역 세포에 의한 케모카인 방출(및 이에 따른 세포 모집)에 대한 영향과 관련된 작용 기전을 나타낼 수 있다. 이러한 사이토 카인은 Th17 경로의 일부이다. 이러한 데이터 세트를 함께 사용하면, 심각한 호중구성 천식 마우스 모델에서 염증에 대해 마우스를 보호하는 데 균주 751이 매우 효과적이라는 분명한 결론을 이끌어낼 수 있다.

[0358] **실시예 6 - 다발성 경화증 마우스 모델에서 박테리아 접종의 효능**

[0359] 개요

[0360] 마우스에게 본 발명에 따른 박테리아성 균주를 포함하는 조성물을 투여하고, 이어서 실험용 자가면역 뇌척수염(EAE)을 유도하기 위해 미엘린 회소돌기 아교세포 당단백질로 면역화시켰다. EAE는 인간 다발성 경화증에 가장 보편적으로 사용되는 실험 모델이다. 본 발명의 조성물은 질병 발생 및 질병의 중증도에 현저한 효과를 갖는 것으로 밝혀졌다.

[0361] 균주

[0362] 751: 수탁 번호 NCIMB 42380하에 기탁된 박테리아

[0363] 실험 설계

[0364] 그룹:

- [0365] 1. 음성 대조군 비히클 대조군 치료(경구 당).
- [0366] 4. 치료 박테리아 접종 균주 751 치료(경구 당).

- [0367] 9. 양성 대조군 텍사메타손에 의한 치료(i.p.).
- [0368] 10. 치료받지 않은 대조군.
- [0369] 그룹당 마우스 수 = 10
- [0370] -14일 내지 27일: 경구당 비히클 대조군의 일일 투여(그룹 1).
- [0371] -14일 내지 27일: 경구당 치료 박테리아 접종물의 일일 투여(그룹 4).
- [0372] 0 내지 28일: 주 3 회 텍사메타손(i.p.) 투여(그룹 9)
- [0373] 0일째: MOG35-55(미엘린 회소돌기아교세포 당단백질 - 2mg/ml)와 CFA(2mg/ml MTB)를 1:1로 혼합하여 1mg/ml 용액으로 만들었다. 100 μ l의 펩티드-CFA 혼합물을 각 뒷다리에 피하 주사하였다. 백일해 독소의 복강내 투여(300ng).
- [0374] 1일째: 백일해 독소의 복강내 투여(300ng).
- [0375] 7일째 이후: 1 주일에 3회 질병 발생 및 체중 측정.
- [0376] 엔드포인트 및 분석
- [0377] 마우스를 질병 발생 및 질병의 중증도에 대해 1주일에 3회 분석하였다. 채점은 맹검 수행되었다. 질병의 중증도는 0 내지 5점까지의 임상 점수를 사용하여 평가되었으며, 5점은 죽은 마우스를 나타낸다(아래 임상 채점 시스템 참조).
- [0378] 모니터링
- [0379] 지정된 날에 마우스의 무게를 측정하고 질병 활성 점수 및 질병 발생을 관찰하였다.
- [0380] 질병 활동 점수 관찰:
- [0381] 0 - 비-면역화된 마우스에 비해 운동 기능에 명백한 변화가 없다.
- [0382] 0.5 - 꼬리의 끝이 축늘어져 있다.
- [0383] 1.0 - 축늘어진 꼬리.
- [0384] 1.5 - 축늘어진 꼬리 및 뒷다리 억제.
- [0385] 2.0 - 축늘어진 꼬리 및 뒷다리 약화.
- [0386] OR - 걷기를 관찰할 때 머리 기울어짐의 명백한 징후가 있다. 균형이 좋지 않다.
- [0387] 2.5 - 축늘어진 꼬리 및 뒷다리 끌림.
- [0388] - OR - 강한 머리 기울임으로 인해 마우스가 때때로 넘어지는 경우가 있다.
- [0389] 3.0 - 축늘어진 꼬리 및 뒷다리 완전 마비.
- [0390] 3.5 - 축늘어진 꼬리 및 뒷다리 완전 마비.
- [0391] 기타: 마우스가 케이지 주위를 움직이고 있지만 측면에 놓이면 자신을 바로잡을 수 없다.
- [0392] 뒷다리가 몸의 한쪽면에 함께 있다.
- [0393] 4.0 - 축늘어진 꼬리, 완전 뒷다리 및 부분 앞다리 마비.
- [0394] - 마우스가 케이지 주위를 최소한으로 움직이지만 경계하며 급식한다.
- [0395] 4.5 - 완전 뒷다리 및 부분 앞다리 마비, 케이지 주위로의 움직임이 없다.
- [0396] 마우스를 즉시 안락사시키고 케이지에서 제거한다.
- [0397] 5.0 심한 마비로 인해 마우스를 안락사시킨다.
- [0398] 동물의 질병 활성 점수가 1 이상일 때, 질병 발병률이 양성인 것으로 간주한다.
- [0399] 결과

- [0400] 실험 결과는 도 71 및 72에 나타내었다.
- [0401] 음성 대조군에서의 질병 유도는 비히클 대조군 및 치료되지 않은 대조군에 의해 높은 점수로 성공적으로 수행되었다. 군주 751에 의한 치료 효과는 현저하였으며, 군주 751로 처리한 마우스는 질병 발생률 및 질병의 중증도가 현저하게 감소되었다. 실제로, 질병 발병 및 질병의 중증도 감소는 양성 대조군에 비견할만하다. 이러한 데이터는 군주 751이 다발성 경화증의 치료 또는 예방에 유용할 수 있음을 나타낸다.
- [0402] **실시예 7 - 암 마우스 모델에서 박테리아 접종물의 효능**
- [0403] 개요
- [0404] 이 실험은 4종의 종양 모델에서 본 발명에 따른 박테리아성 군주를 포함하는 조성물의 효능을 시험하였다.
- [0405] 물질
- [0406] **시험 물질** - 박테리아성 군주 #MRX004(군주 751).
- [0407] **참조 물질** - 항-CTLA-4 항체(클론: 9H10, 카탈로그: BE0131, 아이소타입: 시리안 햄스터(Syrian Hamster) IgG1, 바이옥셀(Bioxcell)).
- [0408] **시험 물질 및 참조 물질 비히클** - 박테리아 배양 배지(효모 추출물, 카시톤, 지방산 배지(YCFA)). 마우스에게 주사한 날마다, 항체를 PBS(참조: BE14-516F, 론자(Lonza), 프랑스)로 희석하였다.
- [0409] **치료 용량** - 박테리아: 200 μ L 중의 2×10^8 . a-CTLA-4는 10 mg/kg/inj로 주사되었다. 마우스의 가장 최근 체중에 따라 항-CTLA-4를 10 mL/kg/adm의 용량으로 투여하였다(즉, 1 마우스 20 g에 대해, 시험 물질 200 μ L 투여).
- [0410] **투여 경로** - 박테리아 접종물을 캐놀라를 통해 경구 위관 영양법(os에 따라, PO)으로 투여하였다. 캐놀라는 매일 오염 제거되었다. 항-CTLA-4는 마우스의 복강 내로 주사되었다(복강내, IP).
- [0411] **박테리아성 군주의 배양 조건** - 박테리아성 군주의 배양 조건은 다음과 같다:
- [0412] * 10 mL의 YCFA(제조된 10 mL E&O 실험실 병으로부터)의 훈게이트(Hungate) 튜브 내로의 피펫팅한다
- [0413] * 주사기 입력 및 배기 시스템을 사용하여 튜브를 밀봉하고 CO₂로 플러싱한다
- [0414] * 훈게이트 튜브를 오토클레빙한다
- [0415] * 냉각 시, 글리세롤 스톱 1 mL로 훈게이트 튜브를 접종한다
- [0416] * 튜브를 정적 37°C 배양기에 약 16시간 동안 둔다
- [0417] * 다음 날, 이 서브-배양액 1 mL를 취해 YCFA 10 mL를 접종한다(예열된 플러싱된 훈게이트 튜브를 모두 다시 한번 복제)
- [0418] * 5 내지 6시간 동안 정적 37°C 배양기에 넣는다
- [0419] **암 세포주 및 배양 조건** -
- [0420] 사용된 세포주는 아래 표에 자세히 설명되어 있다:

세포주	유형	마우스 군주	기원
EMT-6	유방암종	BALB/c	ATCC
LL/2(LLC1)	폐암종	C57BL/6	ATCC CRL1642
헤파(Hepa)1-6	간세포암종	C57BL/6	임센 이노베이션(IPSEN INNOVATION)

- [0421] .
- [0422] EMT-6 세포주는, 과형성 유방 치조 결절의 이식 후 BALB/cCRGL 마우스에서 발생한 이식가능한 뮤린(murine) 유방암으로 확립되었다[73].

[0423] LL/2(LLC1) 세포주는 원발성 루이스 폐암의 이식으로 발생한 종양이 있는 C57BL 마우스의 폐에서 확립되었다 [74].

[0424] 헤파(Hepa) 1-6 세포주는 C57/L 마우스에서 생성된 BW7756 마우스 간암의 유도체이다[75].

[0425] **세포 배양 조건** - 모든 세포주는 가슴 대기(5% CO₂, 95% 공기)에서 37℃에서 단층으로 성장시켰다. 배양 배지 및 보충제는 아래 표에 나타내었다:

세포주	배양 배지	보충제
EMT6	2mM L-글루타민(참조: BE12-702F, 론자)을 함유하는 RPMI 1640-	10% 태아 소 혈청(참조: #3302, 론자)
LL/2(LLC1)	2mM L-글루타민(참조: BE12-702F, 론자)을 함유하는 RPMI 1640-	10% 태아 소 혈청(참조: #3302, 론자)
헤파(Hepa)1-6	DMEM(참조:11960-044, 킵코(Gibco))	10% 태아 소 혈청(참조: #3302, 론자) 2mM L-글루타민 페니실린-스트렙토마이신(시그마 G-6784)

[0426]

[0427] 실험적으로 사용하기 위해, 칼슘 또는 마그네슘이 없는 행크스(Hanks) 배지에서 트립신-베르센(참조: BE17-161E, 론자(Lonza))으로 5분 처리하여 접착성 종양 세포를 배양 플라스크에서 분리하고(참조: BE10-543F, 론자(Lonza)), 완전한 배양 배지를 첨가하여 중화시켰다. 세포를 혈구계수기에서 계수하고, 생존력을 0.25% 트립판 블루 배제 분석에 의해 평가하였다.

[0428] **동물 사용 -**

[0429] 체중과 나이가 맞는 건강한 암컷 Balb/C(BALB/cByJ) 마우스를 CHARLES RIVER(L' Arbresles)에서 EMT6 모델 실험을 위해 얻었다.

[0430] 체중과 나이가 맞는 건강한 암컷 C57BL/6(C57BL16J) 마우스를 CHARLES RIVER(L' Arbresles)에서 LL/2(LLC1) 및 헤파(Hepa)1-6 모델 실험을 위해 얻었다.

[0431] 동물은 FELASA 가이드라인에 따라 SPF 건강 상태로 유지되었으며, 프랑스 및 유럽 규정에 따른 동물 주거 및 실험 절차 및 실험실 동물의 관리 및 사용에 대한 NRC 가이드를 따랐다[76,77]. 동물들은 다음과 같은 통제된 환경 조건하에 실내에서 관리되었다: 온도 22± 2℃, 습도 55± 10%, 광주기(12 시간 광도/12 시간 암흑), HEPA 여과 공기, 재순환없이 시간당 15회의 공기 교환. 동물 울타리는 기술된 바와 같이 깔짚 재료, 음식 및 물, 환경 및 사회 풍부(그룹 주거)에 의해 살균되고 적절한 공간이 제공되었다: 900 cm² 케이지(참조: 녹색, 테크니플라스트(Tecniplast)) 환기 랙, 에피시아(Epicea) 침구(SAFE), 10 kGy 조사 식단(A04-10, SAFE), 면역력이 있는 설치류를 위한 완벽한 음식 - R/M-H Extrudate, 물 병으로부터의 물.

[0432] 실험적 설계 및 처리

[0433] **항암 활성, EMT6 모델**

[0434] 치료 계획 - 첫 번째 투여 시작을 D0로 간주했다. D0에서, 생체가 없는 마우스를 Vivo manager® 소프트웨어(Biosystemes, Couternon, France)를 사용하여 개인 체중에 따라 9/8 그룹으로 무작위 배정했다. D0에서, 마우스는 비히클(배양 배지) 또는 박테리아성 균주를 받았다. D14에서, 모든 마우스는하기 기술된 바와 같이 EMT-6 종양 세포로 이식되었다. D24에서, 양성 대조군의 마우스는 항-CTLA-4 항체 치료를 받았다.

[0435] 치료 일정은 아래 표에 요약되어 있다:

그룹	동물 수	치료	투여량	경로	치료 일정
1	8	치료되지 않음	-	-	-
2	8	비히클(배지)	-	PO	Q1Dx42
3	9	박테리아성 균주 #1(MRX004)	2x10 ⁸ 박테리아	PO	Q1Dx42
4	8	항-CTLA4	10 mg/kg	IP	TWx2

[0436]

[0437] 동물 모니터링은 아래에 설명된대로 수행되었다.

[0438] 동물에서 EMT6 종양의 유도 - D14에서, 200 μ L RPMI 1640으로 1x10⁶ EMT-6 세포를 마우스의 오른쪽 옆구리에 피하 주사하여 종양을 유도했다.

[0439] 안락사 - 각 마우스는 아래 설명된대로 인간 엔드포인트에 도달하거나 투여 시작 후 최대 6 주 이후에 안락사시켰다.

[0440] **항암 활성, LL/2(LLC1) 모델**

[0441] 치료 계획 - 첫 번째 투여 시작을 D0로 간주했다. D0에서, 생체가 없는 마우스를 Vivo manager® 소프트웨어 (Biosystemes, Couteron, France)를 사용하여 개인 체중에 따라 9/8의 7개 그룹으로 무작위 배정했다. D0에서, 마우스는 비히클(배양 배지) 또는 박테리아성 균주를 받았다. D14에서, 모든 마우스는하기 기술된 바와 같이 LL/2 종양 세포로 이식되었다. D27에서, 양성 대조군의 마우스는 항-CTLA-4 항체 치료를 받았다.

[0442] 치료 일정은 아래 표에 요약되어 있다:

그룹	동물 수	치료	투여량	경로	치료 일정
1	8	치료되지 않음	-	-	-
2	9	비히클(배지)	-	PO	Q1Dx42
3	9	박테리아성 균주 #1(MRX004)	2x10 ⁸ 박테리아	PO	Q1Dx42
4	8	항-CTLA4	10 mg/kg	IP	TWx2

[0443]

[0444] 동물 모니터링은 아래에 설명된대로 수행되었다.

[0445] 동물에서 LL/2(LLC1) 종양의 유도 - D14에서, 200 μ L RPMI 1640으로 1x10⁶ LL/2(LLC1) 세포를 마우스의 오른쪽 옆구리에 피하 주사하여 종양을 유도했다.

[0446] 안락사 - 각 마우스는 아래 설명된대로 인간 엔드포인트에 도달하거나 투여 시작 후 최대 6 주 이후에 안락사시켰다.

[0447] **항암 활성, 헤파(Hepa)1-6 모델**

[0448] 치료 계획 - 첫 번째 투여 시작을 D0로 간주했다. D0에서, 생체가 없는 마우스를 Vivo manager® 소프트웨어 (Biosystemes, Couteron, France)를 사용하여 개인 체중에 따라 9개 그룹 중 7개 그룹으로 무작위 배정했다. D0에서, 마우스는 비히클(배양 배지) 또는 박테리아성 균주를 받았다. D14에서, 모든 마우스는하기 기술된 바와 같이 헤파(Hepa) 1-6 종양 세포로 이식되었다. D16에서, 양성 대조군의 마우스는 항-CTLA-4 항체 치료를 받았다.

[0449] 치료 일정은 아래 표에 요약되어 있다:

그룹	동물 수	치료	투여량	경로	치료 일정
1	9	치료되지 않음	-	-	-
2	9	비히클(배지)	-	PO	Q1Dx42
4	9	박테리아성 균주 #2(MRX004)	2×10^8 박테리아	PO	Q1Dx42
7	9	항-CTLA4	10 mg/kg	IP	TWx2

[0450]

[0451] 동물 모니터링은 아래에 설명된대로 수행되었다.

[0452] 간세포 주사에 의한 동물에서의 hepatoma(Hepa) 1-6 종양 세포의 정위적 유도 - D14에서, 50 μ L RPMI 1640 배지에서 1 백만(1×10^6)의 hepatoma(Hepa) 1-6 종양 세포를 마우스에게 비장 주사를 통해 이식하였다. 간단히 말하면, 작은 좌측 골반 대 측면 절개가 이루어졌고 비장이 외관화되었다. 비장은 멸균 거즈 패드에 노출시키고, 27-게이지 바늘로 세포 현탁액을 시각적으로 주입하였다. 세포 접종 후, 비장을 절제하였다.

[0453] 안락사 - 각 마우스는 아래 섹션에서 설명된 대로 인간 엔드포인트에 도달하거나 투여 시작 후 최대 6 주 이후에 안락사시켰다.

[0454] 안락사시 종양 부하 평가 - 종결시, 간을 수집하여 무게를 재었다.

[0455] **동물 모니터링**

[0456] 임상 모니터링 - 종양의 길이와 너비를 캘리퍼스로 일주일에 두 번 측정하였고 종양의 부피는 다음 공식에 의해 추정되었다[78]:

[0457] 종양 용적 = 폭² × 길이 / 2

[0458] 인간 엔드포인트[79]: 통증, 고통 또는 고통의 표시: 통증 자세, 통증 얼굴 가면, 행동; 정상 체중의 10%를 초과하지만 종양이 2000 mm³를 초과하지 않는 종양; 보행이나 영양을 방해하는 종양; 궤양성 종양 또는 조직 침식; 3일 연속 20% 체중 감량; 불쌍한 몸 병태, 쇠약, 악액질, 탈수; 외부 자극에 대한 자발적 반응의 부재; 급속 호흡 곤란, 빈혈, 중대한 출혈; 신경학 징후: 회전, 경련, 마비; 체온의 지속적인 감소; 복부 팽만.

[0459] 마취 - 이소플루란 가스 마취는 하기의 모든 절차에 사용되었다: 수술 또는 종양 접종, i.v. 주입, 혈액 수집. 케타민과 자일라진 마취는 정위 외과 수술에 사용되었다.

[0460] 진통제 - 카프로펜 또는 다중모델 카프로펜/부프레노르핀 진통제 프로토콜은 수술 절차의 중증도에 맞게 조정되었다. 모든 고통스런 절차에 대해 비-약학적 치료가 제공되었다. 또한, 수의사의 권고에 따라 실험(주제 치료)을 방해하지 않는 약학적 치료가 제공되었다.

[0461] 안락사 - 동물의 안락사는 가스 마취 과량 투여(이소플루란)에 이어 자궁 경관 탈구 또는 출혈로 수행되었다.

[0462] 결과

[0463] **항암 활성, EMT6 모델**

[0464] 결과는 도 73에 나타내었다. 본 발명의 박테리아성 균주로 처리하면 양성 대조군 모두에 비해 종양 부피가 명백하게 감소되었다. 양성 대조군은 또한 예상대로 종양 부피를 감소시켰다.

[0465] **항암 활성, LL/2(LLC1) 모델**

[0466] 결과는 도 74에 나타내었다. 음성 및 양성 대조군은 음성 대조군에서보다 양성 대조군으로 처리된 마우스에서 종양 부피가 더 크기 때문에 예상대로 나타나지 않았다. 그럼에도 불구하고, 본 발명의 박테리아성 균주로 처리된 마우스의 종양 부피는 양성 대조군에 필적하고, 이는 유용한 치료 효과와 일치한다.

[0467] **항암 활성, hepatoma(Hepa)1-6 모델**

- [0468] 결과는 도 75에 나타내었다. 간 중량이 다른 그룹보다 이 그룹에서 낮기 때문에 치료되지 않은 음성 대조군은 예상대로 나타나지 않았다. 그러나, 비히클만을 투여한 마우스는 항-CTLA4 항체로 치료한 마우스보다 큰 간을 갖고 있었기 때문에 비히클 음성 대조군과 양성 대조군 모두 예상대로 나타났으며, 이는 비히클 음성 대조군에서의 종양 부하가 더 크다는 것을 반영한다. 본 발명의 박테리아성 균주로 처리하면 비히클 음성 대조군의 마우스에 비해 간 중량(따라서 종양 부하)이 명백하게 감소되었다.
- [0469] 이러한 데이터는 751/MRX004 균주가 암을 치료하거나 예방하는데, 특히 유방암, 폐암 및 간암의 종양 부피를 줄이는 데 유용할 수 있음을 나타낸다.
- [0470] **실시예 8 - YCFA 배지에서 인간 세포에의 부착**
- [0471] 개요
- [0472] 균주 751 및 다수의 *비피도박테리아 브레베* 균주의 인간 세포에 대한 결합 수준을 YCFA 배지에서 3개의 상이한 시점에서 측정하였다. 인간 세포에 부착된 박테리아를 배지에 재현탁하고 배지의 광학 밀도를 분석하였다 - 광학 밀도가 높을수록 박테리아 세포의 수가 많아지므로 박테리아 세포와 인간 세포의 결합 수준이 높아진다. 751 균주는 *비피도박테리아 브레베* 참조 균주와 비교하여 인간 세포에 대한 감소된 부착을 나타내는 것으로 밝혀졌다.
- [0473] 결과 및 분석
- [0474] 실험 결과는 도 76에 나타내었다.
- [0475] 도 76에서 볼 수 있듯이, *비피도박테리아 브레베* 균주는 모든 시점에서 인간 세포에 높은 수준의 부착을 보인다. 한편, 751 균주는 인간 세포에 대한 부착 수준이 현저하게 감소했다. 따라서, 균주 751의 인간 세포에 대한 낮은 부착은 IL-17 또는 Th17 경로 및 IL-17 또는 Th17 경로에 의해 매개되는 질병에 대한 본 발명의 조성물의 유익한 효과를 증가시킬 수 있다.
- [0476] **실시예 9 - 엑소폴리사카라이드의 생성 검출 분석**
- [0477] 개요
- [0478] 본 발명의 박테리아성 균주(751) 및 다수의 *비피도박테리아 브레베* 균주에 의한 엑소폴리사카라이드(EPS) 생성 수준을 37℃에서 48시간 동안 및 30℃에서 72시간 동안 분석하였다. EPS는 박테리아 세포의 외부 표면에 결합하는 특정 박테리아에 의해 생성된 폴리사카라이드이다. 박테리아 표면의 EPS 수준은 폴리사카라이드에 결합하는 콩고 레드(Congo Red) 분석법을 사용하여 결정할 수 있다. 콩고 레드 흡광도가 높을수록 박테리아 표면의 EPS 농도가 높아진다는 것을 의미한다. 본 발명의 박테리아성 균주는 *비피도박테리아 브레베* 균주보다 더 많은 EPS를 생성하고 결합하는 것으로 밝혀졌다.
- [0479] 결과 및 분석
- [0480] 실험 결과는 도 77에 나타내었다.
- [0481] 도 77에 도시된 바와 같이, 본 발명의 박테리아성 균주는 *비피도박테리아 브레베* 균주보다 콩고 레드 흡광도가 온도와 시간-지점에서 더 많이 나타났다. 따라서, 본 발명의 균주는 보다 큰 EPS 생산 및 보다 높은 수준의 세포외 결합 EPS를 나타낸다. EPS가 박테리아를 점액 및 상피 세포에 결합시킬 수 있기 때문에, 본 발명의 박테리아성 균주는 상피 세포 및 점막 내에서 결합 부위에 대해 병원성 세포와 경쟁하는 데 유용할 수 있다. 따라서, 본 발명의 박테리아성 균주는 미생물을 조절하고 미생물과 관련된 다수의 질병을 치료하는 데 유용할 수 있다.
- [0482] **실시예 10 - 안정성 시험**
- [0483] 본원에 기재된 적어도 하나의 박테리아성 균주를 함유하는 본원에 기재된 조성물은 25℃ 또는 4℃에서 밀봉된 용기에 저장되고, 상기 용기는 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% 또는 95%의 상대 습도를 갖는 대기 중에 배치된다. 1 개월, 2 개월, 3 개월, 6 개월, 1 년, 1.5 년, 2 년, 2.5 년 또는 3 년 후, 박테리아성 균주의 적어도 50%, 60%, 70%, 80% 또는 90%는 표준 프로토콜에 의해 결정된 콜로니 형성 단위로 측정시 그대로 유지되어야 한다.
- [0484] **실시예 11 - MRX004 결합 및 방출된 엑소폴리사카라이드 생성 분석**
- [0485] EPS 추출을 위해, MRX004는 박테리아성 세포와 상등액이 원심분리에 의해 분리되었을 때 후기 지수 상에 도달할

때까지 10 ml YCFA에서 배양하였다. 세포를 PBS로 한번 세척하여 임의의 잔류 배양 배지를 제거하였다. 분비되거나 방출된 EPS(EPS-R)는 빙냉 100% 에탄올(온화하게 교반하면서 4℃에서 밤새)로 처리하여 배양 상등액으로부터 침전되었다. 캡슐 또는 결합 EPS(EPS-B)를 추출하기 위해, 세포를 0.05 M EDTA(온화하게 교반하면서 4℃에서 밤새)와 함께 배양하고, 이 처리에서 얻은 상등액을 수집하고 이어서 100% 빙냉 에탄올(온화하게 교반하면서 4℃에서 밤새)로 처리하여 EPS-B를 침전시켰다. 침전된 EPS-B 및 EPS-R을 원심분리에 의해 펠렛화하고 층류 후드에서 간단히 건조시킨 후 균일한 용액을 얻기에 충분한 멸균 초순수 물에 재현탁시켰다. 샘플을 추가로 정제하기 위해, 이들은 완충액을 3회 변화시켜 48시간 동안 1:100의 비율로 멸균 초순수 물에 대해 투석하였다. EPS-B 및 EPS-R은 글루코오스를 표준으로 사용하는 페놀-황산 방법을 사용하여 정량화되었다. 이 분석법을 사용하여, MRX004는 EPS-B(17 µg)보다 많은 양의 EPS-R(115 µg)을 생성하는 것으로 확인되었다(도 78).

[0486] **실시예 12 - MRX004와 Caco-2 세포의 부착 분석**

[0487] MRX004의 숙주 세포에의 부착은 Caco-2 장내 상피 세포와의 시험관내 공동 배양 분석을 사용하여 분석하였다. Caco-2 세포를 1 x 10⁶의 밀도로 파종 박테리아를 10 ml YCFA에서 후기 지수 상에 도달 할 때까지 배양하고, 이들이 펠렛화되었을 때, PBS로 2회 세척하고, 항생제가 없는 세포 배양 배지에 재현탁시켰다. 박테리아 밀도는 10:1(WASP 표준 프로토콜을 사용하여 YCFA 한천에 플레이팅함으로써 확인됨)의 대략 다중 감염(MOI)을 얻도록 조정되었고, MRX004를 37℃에서 2시간 동안 혐기 조건에서 Caco-2 세포와 함께 배양하였다. 이어서 배지를 제거하고 PBS로 3회 Caco-2 세포를 세척하여 미결합 박테리아를 제거하였다. 박테리아에 결합된 Caco-2 세포를 0.1% 트리톤(Triton) X-100으로 처리하여 혈관에서 용해시키고 제거하고, 50 µl 부피의 희석된 용해물을 WASP를 사용하여 YCFA 한천에 플레이팅하였다. 부착은 용해물에서 회수한 박테리아의 수를 세고 이를 총 박테리아의 백분율로 표시하여 계산했다. MRX004는 Caco-2 세포에 낮은 수준의 접촉율(전체 배양액의 0.3%)를 나타내는 것을 밝혀졌다(도 79).

[0488] **실시예 13 - 효소적 활성의 특성화**

[0489] API®(Analytical Profile Index) 시험 시스템은 박테리아 종에서 효소 활성을 분석하는 소형 생화학 시험을 포함하는 스트립으로 구성된다. MRX004(균주 751, 수탁 번호 NCIMB 42380하에 기탁된 박테리아)는 2가지 API® 시험 시스템을 사용하여 특성화하였다: 급속 ID 32A - 이 시스템은 혐기성 종을 위해 특별히 설계되었으며 알칼리성 포스파타아제 활성뿐만 아니라 탄수화물, 아미노산 및 질산 대사를 검사할 포함하고; API® 50 CH - 이 시스템은 49가지 탄수화물 공급원의 발효를 시험하며 혐기성 종 분석을 위해 API® CHL 배지와 함께 사용할 수 있다.

[0490] 급속 ID 32A 시험은 제조사의 지침에 따라 박테리아성 콜로니에 대해 수행되었다. 간단히 말하면, 박테리아를 혐기성 워크스테이션에서 37℃에서 24시간 동안 YCFA 한천에서 배양했다. 콜로니를 멸균 5 µl 점종 루프를 사용하여 플레이트에서 제거하고 맥파랜드(McFarland) 표준 번호 4와 대략 동일한 밀도가 달성될 때까지 API® 현탁액 2ml 앰플에 재현탁시켰다. 급속 ID 32A 스트립 상에 55 µl의 박테리아 현탁액을 각각의 흡판(cupule)에 첨가하고 우레아제 시험을 2 방울의 미네랄 오일로 덮었다. 스트립을 플라스틱 뚜껑으로 덮고 호기적으로 37℃에서 4시간 동안 배양하고, 그 다음에 다음과 같은 시약을 사용하여 맨 아래의 흡판을 만들었다: NIT: 각각 1 방울의 NIT1과 NIT2; IND: 1 방울의 제임스 시약; 나머지 모든 흡판: 1 방울의 패스트블루(FastBlue) 시약. 스트립을 실온에서 5분간 배양한 후, 각 흡판의 색상을 기록하고 음성, 중간 양성 또는 양성의 값을 부여하였다.

[0491] 급속 ID 32A 분석의 결과는 도 80에 나타내었다. MRX004는 여러 탄수화물 공급원, 즉 α-갈락토시다아제와 β-갈락토시다아제, α-글루코시다아제와 β-글루코시다아제, α-아라비노스, 만노오스 및 라피노오스 뿐만 아니라 아미노산 아르기닌, 프롤린, 페닐알라닌, 류신, 티로신, 글리신 및 히스티딘의 발효 양성 반응을 테스트했다. 흥미롭게도, 천식에서 이러한 아미노산의 일부에 대한 역할이 보고되었다. 예를 들어, 페닐알라닌과 히스티딘의 증가된 혈장 농도는 증가된 염증, 히스타민 생산 및기도 과-반응성을 포함하는 천식의 부작용과 관련되는 것으로 보고되었다. 또한, 아르기닌 대사는 소아 환자에서 증가된 수준의 아르기닌 대사산물인 L-오르니틴이 보고되고, 생체내 천식 모델에서 아르기닌의 투여가 염증을 약화시킴에 따라 천식 발병 기전과 관련되어 있다. 이들 보고에 따르면, MRX004에 의한 아미노산 대사가 이러한 균주의 항-천식 효과에 관여할 가능성이 있다.

[0492] 비교용 급속 ID 32A 분석은 MRX004와 4개의 *B. 브레베* 유형의 균주 사이에서 수행되었으며, 이는 Bif Ref 1(DSM 20091), Bif Ref 2(DSM 20213), Bif Ref 6(JCM 7017) 및 Bif Ref 7(UCC 2003)과 같이 도 80b에 주석이 달려 있다. 이러한 분석은 MRX004가 폴리사카라이드 라피노스를 발효시키는 유일한 균주임을 입증하였으며, 이 점을 매우 중요한데, 왜냐하면 라피노스가 엑소폴리사카라이드와 같은 박테리아 성분의 생산에 관여하고 라피노스 발효가 또한 증가된 맹장의 부티레이트, 증가된 위장관 증식 및 체중 감소와 같은 숙주에 영향을 주는 것으로 보고되었기 때문이다.

- [0493] API® 50 CH 검사는 MRX004에서 탄수화물 대사를 더 검사하기 위해 수행되었다. 제조사의 지침에 따라, 박테리아는 혐기성 워크스테이션에서 37℃에서 16 내지 18시간 동안 10 ml YCFA 브로스(broth)에서 배양되었다. 이 배양물을 10 ml API® CHL 배지로 희석하여 맥과랜드 표준 2 번과 거의 동일한 밀도를 얻었으며 이 혼합물 110 µl를 사용하여 API® 50 CH 테스트 스트립에 각 흡관을 접종하였다. 테스트 스트립은 48시간 동안 혐기성 워크스테이션에서 37℃의 가습된 배양 상자에서 배양한 다음, 각 흡관의 색상을 기록하고 음성, 중간 양성, 양성 또는 의심스러운 값을 할당했다.
- [0494] API® 50을 사용하여, MRX004는 다음과 같은 탄수화물 공급원의 이용에 대해 양성 반응을 보였다: 아미돈(전분), 아미그딘, 알부틴, 셀룰로오스, 에스쿨린, 갈락토오스, 젤티노비오스, 글루코오스, 글리코젠, 프룩토오스, 푸코스, 락토오스, 말토오스, 만노오스, 만니톨, 멜리비오스, 멜레지토스, 메틸 α-D-글루코피라노시드, N-아세틸글루코사민, 리보오스, 사카로오스(수크로오스), 살리신, 솔비톨, 트레할로스, 투라노오스 및 자일리톨. 이러한 결과는 MRX004가 두 가지 시험 시스템에서 갈락토오스, 글루코오스, 만노오스 및 라피노스의 발효를 입증한다는 점에서 급속 ID 32A 시험에서 얻은 결과와 상관 관계가 있다. 흥미롭게도, 일부 MRX004 탄수화물 기질, 즉 갈락토오스 및 프룩토오스는 문헌에 보고된 효과에 기초하여 이 균주의 작용 이전에 연루되어 있을 수 있다. 고기 공급원에서 추출한 갈락토오스 α-1,3-갈락토오스는 아나필락시스의 알려진 알레르겐 및 원인 제제이며 식이성 프룩토오스의 섭취 수준은 증가된 천식 중증도와 관련이 있다. MRX004에 대한 두 가지 API® 데이터 세트를 종합해 볼 때 이러한 균주의 신진대사가 항-천식 효과에 영향을 미칠 수 있음을 암시한다.
- [0495] **실시예 14 - 게놈 분석**
- [0496] 균주 MRX004의 게놈 함량과 *B. 브레베*의 참조 균주 1, 2, 6 및 7의 비교는 BLAST + 2.3.0 프로그램 세트의 일부로서 blastn을 사용하여 수행되었다. 분석 전반에 걸쳐 최대 E-값 컷오프 점수 10E-5가 사용되었다.
- [0497] MRX004 균주의 게놈에 존재하나, *B. 브레베* 참조 균주 1(DSM 20091), 2(DSM 20213), 6(JCM 7017) 및 7(UCC 2003)에는 존재하지 않는 333개의 유전자가 확인되었다(표 1). 표 1에 열거된 많은 유전자들이 *B. 브레베* 균주들 사이에서 초-가변성으로 관찰된다[80]. 예상대로, 가변성 영역에는 탄수화물 대사 및 수송에 관여하는 단백질질을 코딩하는 유전자, 파괴-관련 유전자, 이동성 요소, 및 단백질 또는 미지의 기능을 코딩하는 것으로 예측되는 173개의 유전자가 포함된다.
- [0498] MRX004에 존재하지만 *B. 브레베* 참조 균주 1, 2, 6 및 7에 존재하지 않는 유전자는 표 1에 열거되어 있다. 강조 표시되지 않은 유전자는 4 가지 참조 균주 중 하나 초과에는 존재하지 않는다. MRX004에 존재하지만 다수의 *B. 브레베* 참조 균주에는 존재하지 않는 다수의 유전자는 MRX004가 이들 공지된 *B. 브레베* 균주와 구별되고 및/또는 구별될 수 있음을 암시한다. 단일 밑줄로 강조 표시된 유전자는 MRX004에는 존재하지만 *B. 브레베* 참조 균주 1에는 존재하지 않는다. 이중 밑줄과 굵은 체로 강조 표시된 유전자는 MRX004에는 존재하지만 *B. 브레베* 참조 균주 2에는 존재하지 않는다. 이탤릭 체로 강조 표시된 유전자는 MRX004에는 존재하지만 *B. 브레베* 참조 균주 6에는 존재하지 않는다. blastn 분석을 위해 최대 E-값 컷오프 점수 10E-5를 사용했다.

[0499] 표 1

4DBb_0021c	다중 당류 ABC 수송체, 기질-결합 단백질
4DBb_0023	예상되는 LacI-형 전사조절인자
4DBb_0024	수크로오스-6-포스페이트 가수분해효소(EC 3.2.1.B3)
4DBb_0026c	말토덱스트린 글루코시다아제(EC 3.2.1.20)
4DBb_0036c	가상 단백질
4DBb_0038c	MSM(다중 당류 대사) 오페론 조절 단백질
4DBb_0119c	가상 단백질
4DBb_0120c	가상 단백질
4DBb_0187	가상 단백질
4DBb_0188	가상 단백질
4DBb_0203c	세포 분열 단백질 FtsL
4DBb_0204c	가상 단백질
4DBb_0205c	가상 단백질
4DBb_0206c	전사조절인자, HxlR 계열
4DBb_0207	Rrf2-연결된 NADH-플라빈 환원효소
4DBb_0208	ATP-의존성 DNA 헬리카제 RecG- 관련 단백질
4DBb_0209c	가상 단백질
4DBb_0210	추정 수송체
4DBb_0211	오메가-3 코도 불포화 지방산 합성효소 서브유닛, PfaA
4DBb_0212	I 형 폴리케타이드 합성효소
4DBb_0213c	가상 단백질
4DBb_0214c	가상 단백질
4DBb_0215	가상 단백질
4DBb_0216c	보존된 가상 단백질
4DBb_0218c	가상 단백질
4DBb_0219c	DNA-시토신 메틸트랜스퍼라제
4DBb_0220c	가상 단백질
4DBb_0221c	가상 단백질
4DBb_0222c	가상 단백질
4DBb_0223c	통합효소
4DBb_0256	가상 단백질
4DBb_0257c	LacI-형 전사조절인자
4DBb_0258	미지 기능의 추정 글리코실 가수분해효소(DUF1680)
4DBb_0284	조사 조절 인자, AraC 계열
4DBb_0285	N-아세틸-D-글루코사민 ABC 수송 시스템, 당류-결합 단백질
4DBb_0286	당류 ABC 수송체 투과효소
4DBb_0287	N-아세틸-D-글루코사민 ABC 수송 시스템, 투과효소 단백질 2
4DBb_0288	알파-갈락토시다아제(EC 3.2.1.22)
4DBb_0329c	비오틴 ECF 수송체의 에너지 공급 모듈의 ATPase 성분 BioM
4DBb_0330	주요 촉진자 수퍼계열 MFS_1

[0500]

4DBb_0368	GMP 합성효소 [글루타민-가수분해성](EC 6.3.5.2)
4DBb_0369c	Mu-유사 프로파지 단백질 gp29
4DBb_0410	추정 감락토시다아제
4DBb_0419	가상 단백질
4DBb_0421	글리코실트랜스퍼라아제 SypP
4DBb_0422	캡슐 폴리스카라이드 생합성 단백질
4DBb_0423	가상 단백질
4DBb_0424	글리코실트랜스퍼라아제
4DBb_0425	O-항원인 테이코산 리포테이코산의 유출과 관련된 막 단백질
4DBb_0426	글리코실트랜스퍼라아제(EC 2.4.1.-)
4DBb_0427	2-숙신알-5-에놀피루빌-6-하이드록시-3-사이클로헥센-1-카복실산 합성효소(EC 2.2.1.9)
4DBb_0428	가상 단백질
4DBb_0429	이동성 요소 단백질
4DBb_0430	가상 단백질
4DBb_0431c	가상 단백질
4DBb_0432c	이동성 요소 단백질
4DBb_0433c	가상 단백질
4DBb_0434c	가상 단백질
4DBb_0435	가상 단백질
4DBb_0436	가상 단백질
4DBb_0437c	가상 단백질
4DBb_0438c	이동성 요소 단백질
4DBb_0439c	가상 단백질
4DBb_0440c	이동성 요소 단백질
4DBb_0518c	PIN 도메인 단백질
4DBb_0519c	가상 단백질
4DBb_0555c	가상 단백질
4DBb_0556c	가상 단백질
4DBb_0557c	LSU 리보솜 단백질 L31p @ LSU 리보솜 단백질 L31p, 아연-독립적
4DBb_0558c	SSU 리보솜 단백질 S14p(S29e) @ SSU 리보솜 단백질 S14p(S29e), 아연-독립적
4DBb_0559c	LSU 리보솜 단백질 L33p @ LSU 리보솜 단백질 L33p, 아연-독립적
4DBb_0560c	가상 단백질
4DBb_0561	가상 단백질
4DBb_0613c	셀룰로오스 합성효소(UDP-형성)(EC 2.4.1.12)
4DBb_0614	키틀라제(EC 3.2.1.14)
4DBb_0615	감각 상자/GGDEF 계열 단백질
4DBb_0660	이동성 요소 단백질
4DBb_0662	이동성 요소 단백질
4DBb_0663c	뉴라미니다제 NanP
4DBb_0664	가상 단백질

[0501]

4DBb_0665	가상 단백질
4DBb_0666	<i>이동성 요소 단백질</i>
4DBb_0667	<i>이동성 요소 단백질</i>
4DBb_0668	이동성 요소 단백질
4DBb_0718	예측된 비오틴 조절 단백질 BioR(GntR 계열)
4DBb_0719	가상 단백질
4DBb_0720	가상 단백질
4DBb_0778	<i>가상 단백질</i>
4DBb_0789c	이동성 요소 단백질
4DBb_0790c	이동성 요소 단백질
4DBb_0837c	<i>가능한 보존된 통합 막 단백질.</i>
4DBb_0840	맥크로리드-유출 단백질
4DBb_0866	<i>양이온-수송 ATPase, EI-E2 계열</i>
4DBb_0867	가상 단백질
4DBb_0872	<i>가상 단백질</i>
4DBb_0879c	전사조절인자, Cro/CI 계열
4DBb_0880c	통합 막 단백질
4DBb_0946	MFS 일반 기질 수송체
4DBb_0947c	추정 막 단백질
4DBb_0948c	이동성 요소 단백질
4DBb_0952c	가상 단백질
4DBb_0953	가상 단백질
4DBb_0954c	가상 단백질
4DBb_0955c	가상 단백질
4DBb_0956c	예측된 투과효소
4DBb_0957	협작 보존된 가상 단백질
4DBb_0958c	보존된 가상 단백질
4DBb_0986c	가상 단백질
4DBb_0987c	<u>예측된 ECF 수송체의 에너지 공급 모듈의 중복 ATPase 성분 BL0693</u>
4DBb_0988c	<u>예측된 ECF 수송체의 에너지 공급 모듈의 막횡단 성분 BL0694</u>
4DBb_1009c	과지 홀린
4DBb_1010c	막-결합 용해질 류린 트랜스글리코실라아제 D 전구체(EC 3.2.1.-)
4DBb_1011c	가상 단백질
4DBb_1012	가상 단백질
4DBb_1013c	가상 단백질
4DBb_1014c	가상 단백질
4DBb_1015c	가상 단백질
4DBb_1016c	가상 단백질
4DBb_1017c	과지 꼬리 단백질
4DBb_1018c	과지 꼬리 길이 테이프-측정 단백질
4DBb_1019c	가상 단백질
4DBb_1020c	가상 단백질

[0502]

4DBb_1021c	가상 단백질
4DBb_1022c	가상 단백질
4DBb_1023c	가상 단백질
4DBb_1024c	가상 단백질
4DBb_1025c	가상 단백질
4DBb_1026c	가상 단백질
4DBb_1027c	가상 단백질
4DBb_1028c	가상 단백질
4DBb_1029c	가상 단백질
4DBb_1030c	파지 단백질
4DBb_1031c	파지 종단효소, 큰 서브유닛 # Pham2
4DBb_1032c	파지 종단효소, 큰 서브유닛
4DBb_1033c	가상 단백질
4DBb_1034	가상 단백질
4DBb_1035	가상 단백질
4DBb_1036c	FIG00424913: 가상 단백질
4DBb_1037c	가상 단백질
4DBb_1038c	가상 단백질
4DBb_1039c	가상 단백질
4DBb_1040c	가상 단백질
4DBb_1041c	가상 단백질
4DBb_1042c	가상 단백질
4DBb_1043c	가상 단백질
4DBb_1044c	가상 단백질
4DBb_1045c	가상 단백질
4DBb_1046c	가상 단백질
4DBb_1047c	염색체(플라스미드) 분할 단백질 ParB
4DBb_1048c	가상 단백질
4DBb_1049c	가상 단백질
4DBb_1050c	단일-가닥 DNA-결합 단백질
4DBb_1051c	가상 단백질
4DBb_1052c	가상 단백질
4DBb_1053c	가상 단백질
4DBb_1054c	가상 단백질
4DBb_1055c	가상 단백질
4DBb_1056	가상 단백질
4DBb_1057c	가상 단백질
4DBb_1058c	가상 단백질
4DBb_1059	가상 단백질
4DBb_1060	가상 단백질
4DBb_1061	가상 단백질
4DBb_1062c	가상 단백질
4DBb_1063c	가상 단백질
4DBb_1064	추정 파지 통합효소
4DBb_1113	주요 촉진자 수퍼개열의 투과효소

[0503]

4DBb_1142	가상 단백질
4DBb_1143c	가상 단백질
4DBb_1172	통합효소
4DBb_1173c	가상 단백질
4DBb_1174c	협작 보존된 가상 단백질
4DBb_1175	가상 단백질
4DBb_1176c	가상 단백질
4DBb_1177	ABC 수송체, ATP-결합 단백질
4DBb_1178	가상 단백질
4DBb_1179	가상 단백질
4DBb_1180	가상 단백질
4DBb_1181	가상 단백질
4DBb_1182	2-성분 시스템 센서 키나아제
4DBb_1183	가상 단백질
4DBb_1203c	조절 단백질, LacI
4DBb_1204c	FIG01131316: 가상 단백질
4DBb_1205c	수송 시스템 투과효소
4DBb_1206c	예측된 람노오스 올리고사카라이드 ABC 수송 시스템, 투과효소 성분 2
4DBb_1207c	세포의 용질-결합 단백질, 계열 1
4DBb_1212c	가상 단백질
4DBb_1213c	가상 단백질
4DBb_1214	이동성 요소 단백질
4DBb_1215	이동성 요소 단백질
4DBb_1219	가상 단백질
4DBb_1220c	가상 단백질,
4DBb_1221c	미지 기능 DUF262 계열의 단백질
4DBb_1222c	가상 단백질
4DBb_1223	가상 단백질
4DBb_1224	이동성 요소 단백질
4DBb_1234c	ABC-형 당류 수송 시스템, 주변세포질 성분
4DBb_1235	가상 단백질
4DBb_1328c	추정 과거 통합효소
4DBb_1329	가상 단백질
4DBb_1330	가상 단백질
4DBb_1331c	가상 단백질
4DBb_1332	가상 단백질
4DBb_1333	가상 단백질
4DBb_1334	가상 단백질
4DBb_1335c	가상 단백질
4DBb_1336c	베타-락타마제 발현의 음성적 조절인자
4DBb_1337c	가상 단백질
4DBb_1338c	가상 단백질
4DBb_1339c	가상 단백질
4DBb_1340c	가상 단백질

[0504]

4DBb_1341c	가상 단백질
4DBb_1342c	가상 단백질
4DBb_1343c	가상 단백질
4DBb_1344	가상 단백질
4DBb_1345c	파지 꼬리 섬유 단백질
4DBb_1346c	가상 단백질
4DBb_1347c	파지 작은 꼬리 단백질
4DBb_1348c	가상 단백질
4DBb_1349c	가상 단백질
4DBb_1350c	파지 단백질
4DBb_1351c	파지 단백질
4DBb_1352c	파지 단백질
4DBb_1353c	파지 단백질
4DBb_1354c	가상 단백질
4DBb_1355c	가상 단백질
4DBb_1356c	파지 주요 캡시드 단백질 #Fam0025 #Pham164
4DBb_1357c	추정 파지 프로헤드 프로테아제
4DBb_1358c	파지 포털 단백질
4DBb_1359c	gp2, 종단효소
4DBb_1360c	가상 단백질
4DBb_1361c	가상 단백질
4DBb_1362c	가상 단백질
4DBb_1363c	가상 단백질
4DBb_1364c	가상 단백질
4DBb_1365c	가상 단백질
4DBb_1366c	가상 단백질
4DBb_1367c	가상 단백질
4DBb_1368c	가상 단백질
4DBb_1369c	가상 단백질
4DBb_1370c	가상 단백질
4DBb_1371c	가상 단백질
4DBb_1372c	단일-가닥 DNA-결합 단백질
4DBb_1373c	가상 단백질
4DBb_1374c	제조합 DNA 복구 단백질 RecT(관련된 프로파지)
4DBb_1375c	파지-관련 단백질
4DBb_1376c	가상 단백질
4DBb_1377c	가상 단백질
4DBb_1378c	가상 단백질
4DBb_1379c	가상 단백질
4DBb_1380c	메틸트랜스퍼라아제(EC 2.1.1.-)
4DBb_1381c	가상 단백질
4DBb_1382c	가상 단백질
4DBb_1383c	가상 단백질
4DBb_1384	가상 단백질
4DBb_1385	가상 단백질

[0505]

4DBb_1386	가상 단백질
4DBb_1387	가상 단백질
4DBb_1388	가상 단백질
4DBb_1456	수크로오스 투과효소, 주요 촉진자 수퍼계열
4DBb_1486c	에스테라아제/리파아제
4DBb_1487c	글루코오스/만노오스; H ⁺ 공동수송체 GlcP
4DBb_1488c	2-성분 반응 조절인자 yesN
4DBb_1533c	가상 단백질
4DBb_1534c	가상 단백질
4DBb_1535c	I 형 제한-변형 시스템, 제한 서브유닛 R(EC 3.1.21.3)
4DBb_1536c	ATP-의존성 DNA 헬리카제 recG(EC 3.6.1.-)
4DBb_1537c	I 형 제한-변형 시스템, 특이성 서브유닛 S(EC 3.1.21.3)
4DBb_1538c	I 형 제한-변형 시스템, DNA-메틸트랜스퍼라아제 서브유닛 M(EC 2.1.1.72)
4DBb_1539c	가상 단백질
4DBb_1540	I 형 제한-변형 시스템, 특이성 서브유닛 S(EC 3.1.21.3)
4DBb_1541	통합효소
4DBb_1542c	I 형 제한-변형 시스템, 특이성 서브유닛 S(EC 3.1.21.3)
4DBb_1545c	리보오스 ABC 수송 시스템, 고친화성 투과효소 RbsD(TC 3.A.1.2.1)
4DBb_1546c	리보오스 ABC 수송 시스템, 주변세포질 리보오스-결합 단백질 RbsB(TC 3.A.1.2.1)
4DBb_1547c	리보오스 ABC 수송 시스템, 투과효소 단백질 RbsC(TC 3.A.1.2.1)
4DBb_1548c	리보오스 ABC 수송 시스템, ATP-결합 단백질 RbsA(TC 3.A.1.2.1)
4DBb_1550c	가상 단백질
4DBb_1551	가상 단백질
4DBb_1552c	LacI-형 전사조절인자
4DBb_1553c	테트라사이클린 저항성 단백질과 유사
4DBb_1554c	리보키나아제(EC 2.7.1.15)
4DBb_1555c	NADH-의존성 부탄올 탈수소효소 A(EC 1.1.1.-)
4DBb_1556c	포스포글리콜레이트 포스파타아제(EC 3.1.3.18)
4DBb_1557c	이노신-우리딘이 선호되는 뉴클레오타이드 가수분해효소(EC 3.2.2.1)
4DBb_1558c	프록토키나아제(EC 2.7.1.4)
4DBb_1559c	포스포리보실안트라닐레이트 이성질화효소(EC 5.3.1.24)
4DBb_1560c	쿠에오신-조절된 ECF 수송체의 에너지 공급 모듈의 ATPase 성분 STY3233
4DBb_1561c	ECF 수송체의 일반적인 에너지 공급 모듈의 ATPase 성분
4DBb_1562c	쿠에오신-조절된 ECF 수송체의 에너지 공급 모듈의 막횡단 성분 STY3231
4DBb_1563c	쿠에오신-조절된 ECF 수송체의 기질-특이적 성분 STY3230
4DBb_1564	인디코이딘 합성효소 indA, PfkB 키나아제 계열과 클러스터인 가상 당류 키나아제

[0506]

4DBb_1569	전사조절인자, TetR 계열
4DBb_1570c	에스테라아제/리파아제
4DBb_1571c	가상 단백질
4DBb_1572c	가상 단백질
4DBb_1573	COG1309: 전사조절인자
4DBb_1574c	가상 단백질
4DBb_1578c	멜리비오스 캐리어 단백질
4DBb_1579c	가상 단백질
4DBb_1580c	전사조절인자, TetR 계열
4DBb_1581	가상 단백질
4DBb_1582	HrtAB 수송체와 함께 공-국소화된 2-성분 응답 조절인자
4DBb_1583	가상 단백질
4DBb_1584c	가상 단백질
4DBb_1585	센서 히스티딘 키나아제
4DBb_1586	Mg(2+) 수송 ATPase 단백질 C
4DBb_1587	전사조절인자, AbrB 계열
4DBb_1588	가상 단백질
4DBb_1620c	3'-내지-5' 올리고리보뉴클레아제(orn)
4DBb_1769c	전사 활성인자 MltR
4DBb_1770c	자일리톨 탈수소효소(EC 1.1.1.9)
4DBb_1771c	리비톨/자일리톨/아라비톨 수송체, MFS 수퍼계열
4DBb_1773c	글리옥살라아제 계열 단백질
4DBb_1774c	리비톨/자일리톨/아라비톨 수송체, MFS 수퍼계열
4DBb_1775c	솔비톨 탈수소효소(EC 1.1.1.14)
4DBb_1926c	악티노바실러스 단백질과 관련된 막 단백질(1944168)
4DBb_1928c	글리코실 트랜스퍼라제, 그룹 2 계열 단백질
4DBb_1929c	테이코산 유출 ATP-결합 단백질 TagH(EC 3.6.3.40)
4DBb_1930c	람노오스-함유 폴리사카라이드 전와 투과효소
4DBb_1934c	가상 단백질
4DBb_1935	가능한 글리코실트랜스퍼라제
4DBb_1936c	세포벽 표면 앵커 계열 단백질
4DBb_1937c	D-알라닌-D-알라닌 카복시펩티다아제(EC 3.4.16.4)
4DBb_1965	가상 단백질
4DBb_2010c	이동성 요소 단백질
4DBb_2011	전사조절인자, LacI 계열
4DBb_2012	자일로스 ABC 수송체, 주변세포질 자일로스-결합 단백질 XylF
4DBb_2013	리보오스 ABC 수송 시스템, ATP-결합 단백질 RbsA(TC 3.A.1.2.1)
4DBb_2014	리보오스 ABC 수송 시스템, 투과효소 단백질 RbsC(TC 3.A.1.2.1)
4DBb_2015	가상 단백질
4DBb_2016	이동성 요소 단백질
4DBb_2028c	베타-글루코시다아제(EC 3.2.1.21)

[0507]

[0508]

서열

[0509] 서열번호:1(균주 751에 대한 콘센서스 16S rRNA 서열)

GGGACAGGCTCAGGATGAACGCCGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCGGGCTTTGCCT
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CGTCCAGGGCTTCACGCATGCTACAATGGCCGTTACAACGGGATGCGACAGCGCGAGCTGGAGCGGATCCCTGA
AAACCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGAATCAG
CAACGTGCGCGTGAATGCGTTCCTGGGCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGGCAGCACCCGAA
GCCGGTGGCCTAACCCCTGCGGGAGGGAGCCK

[0510]

[0511] 서열번호:2(균주 751 게놈 서열) - 전자적 서열 목록 참조.

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[0515]

수탁번호

[0516]

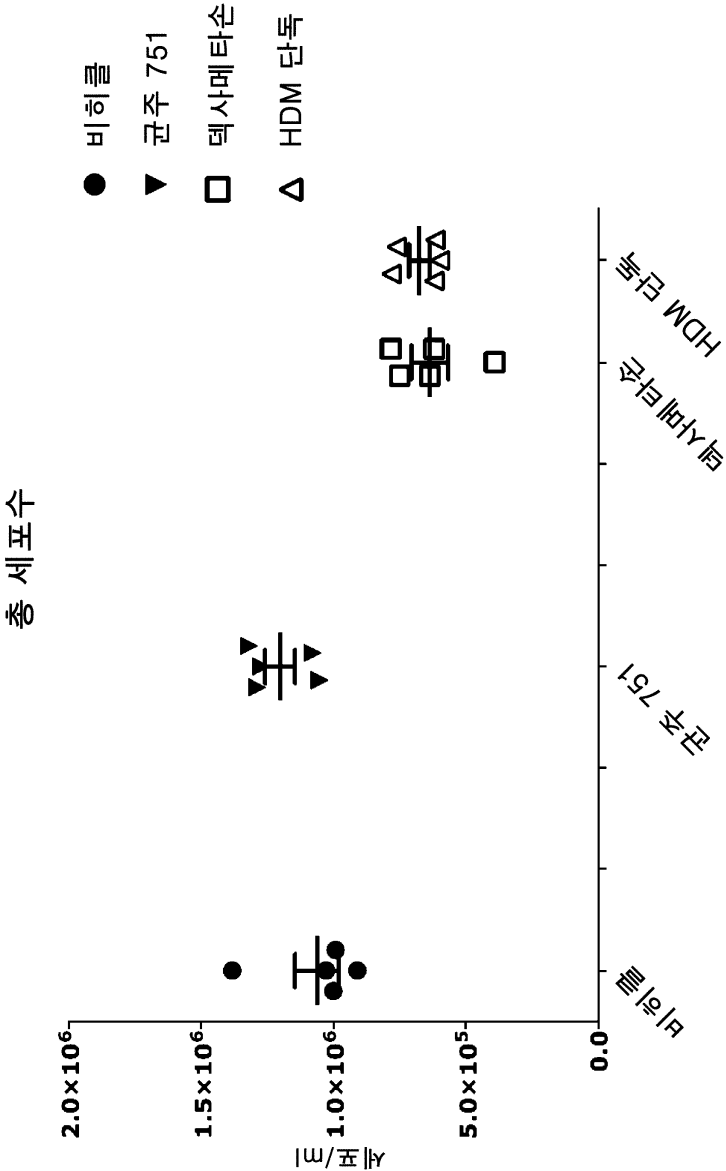
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수탁번호 : NCIMB42380

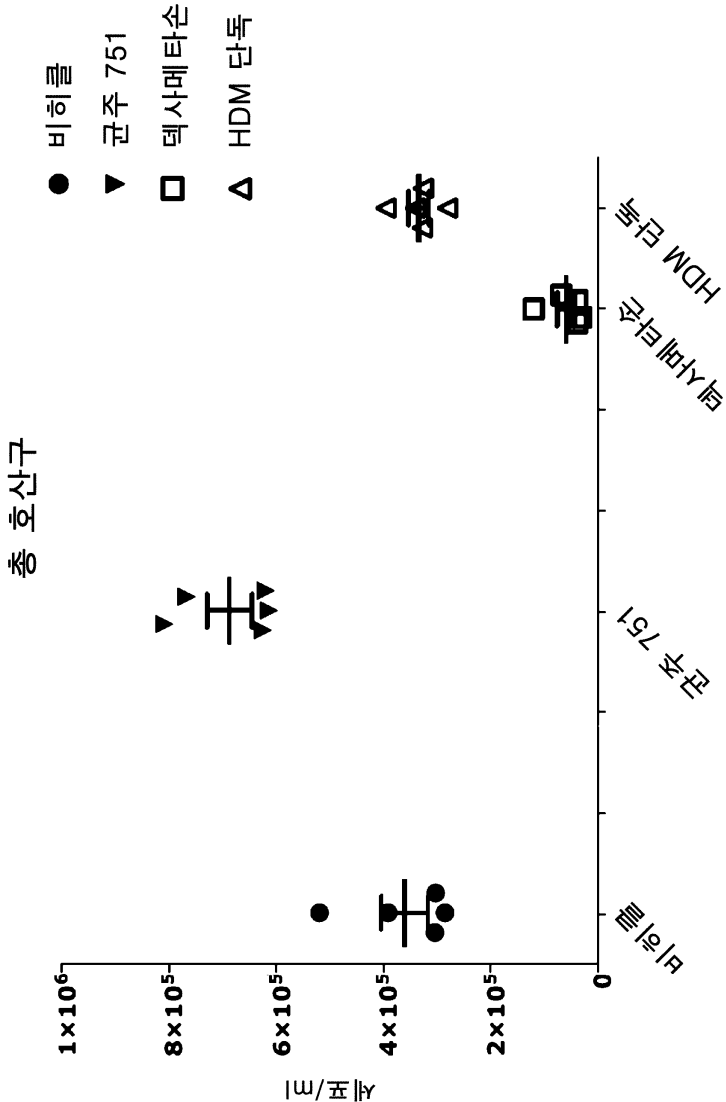
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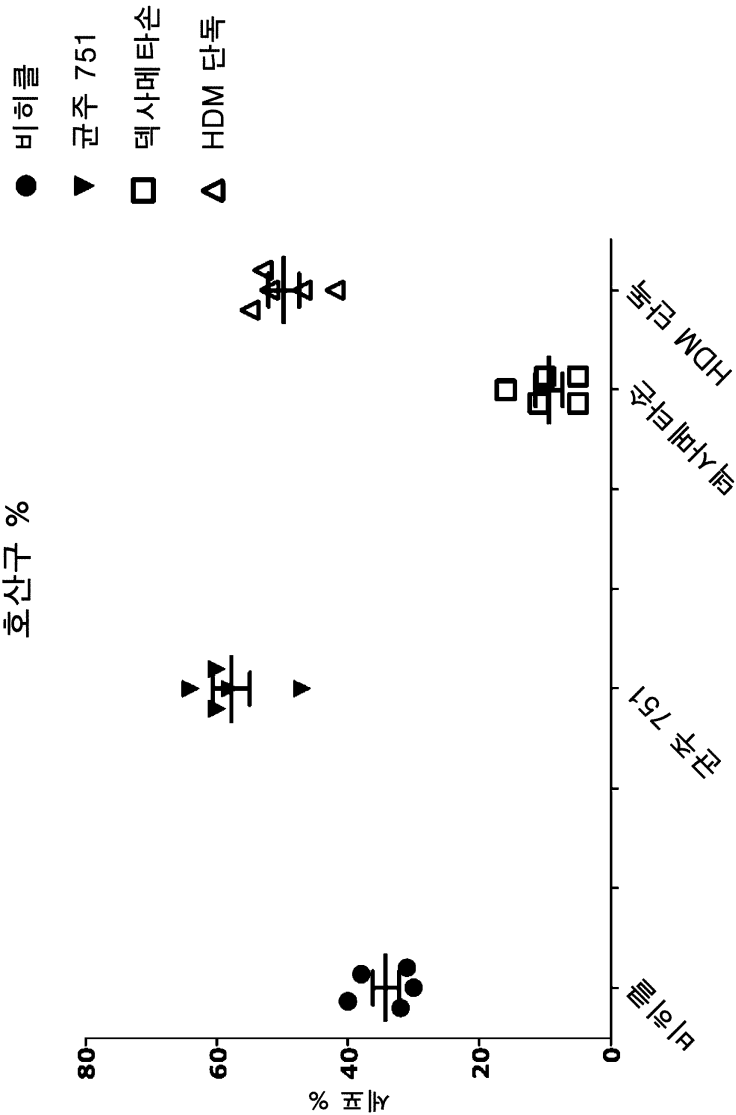
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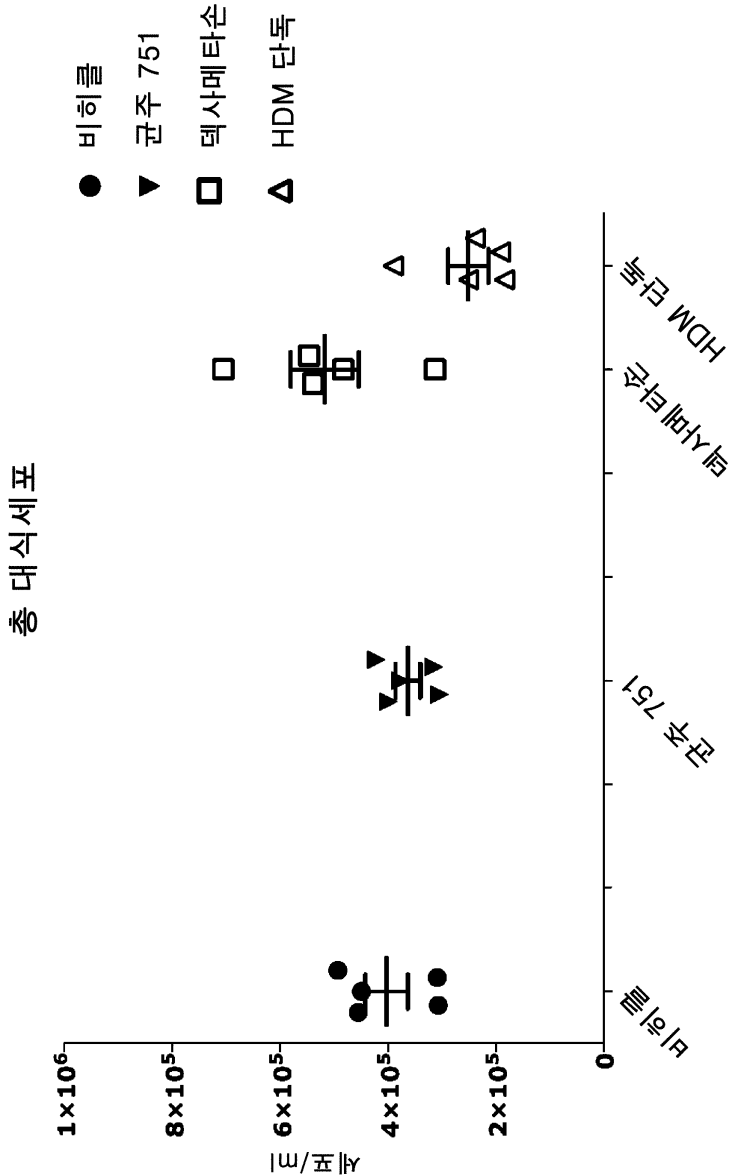
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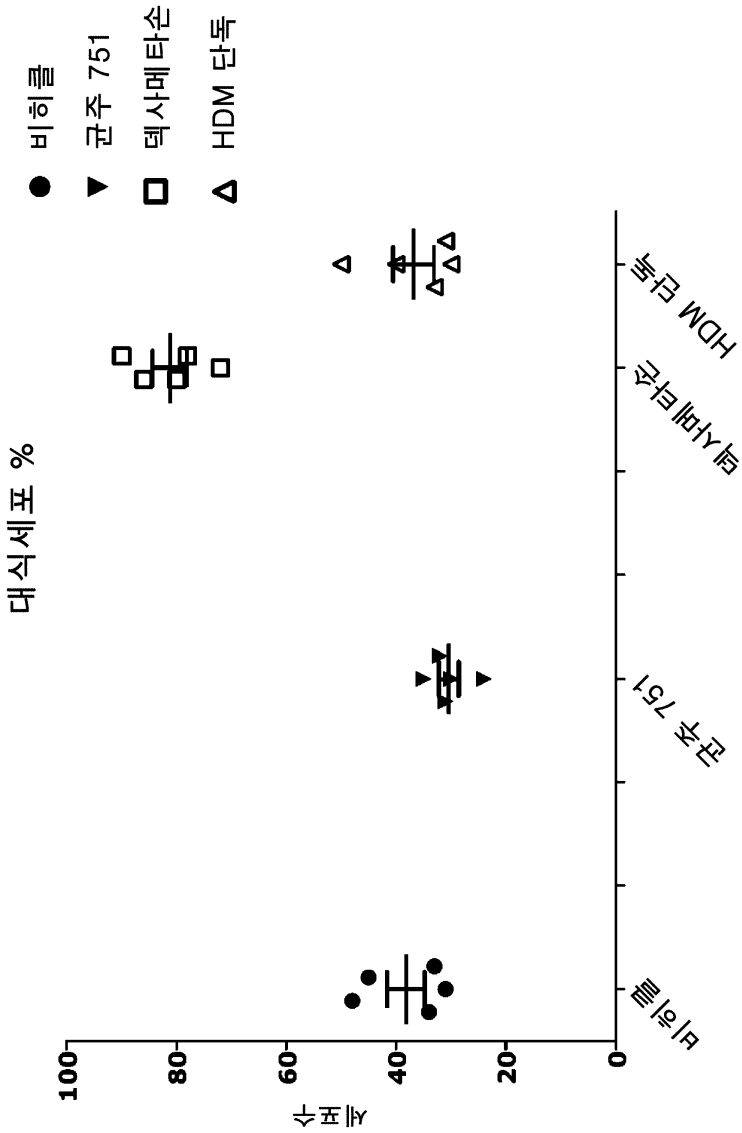
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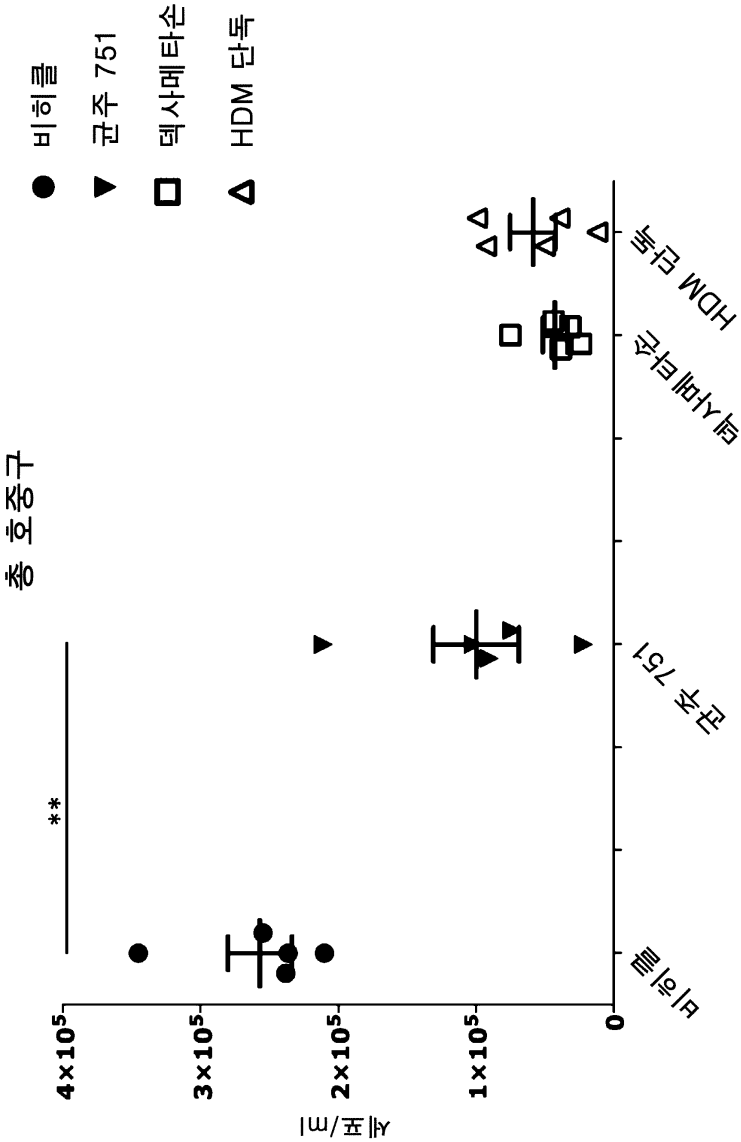
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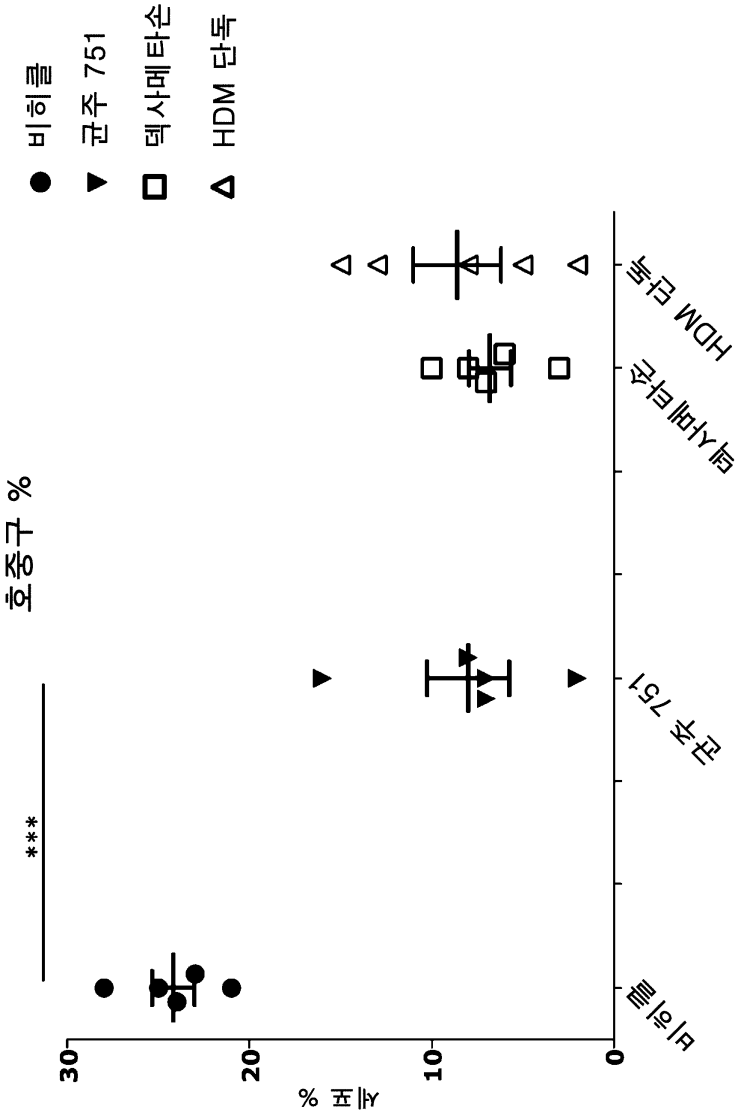
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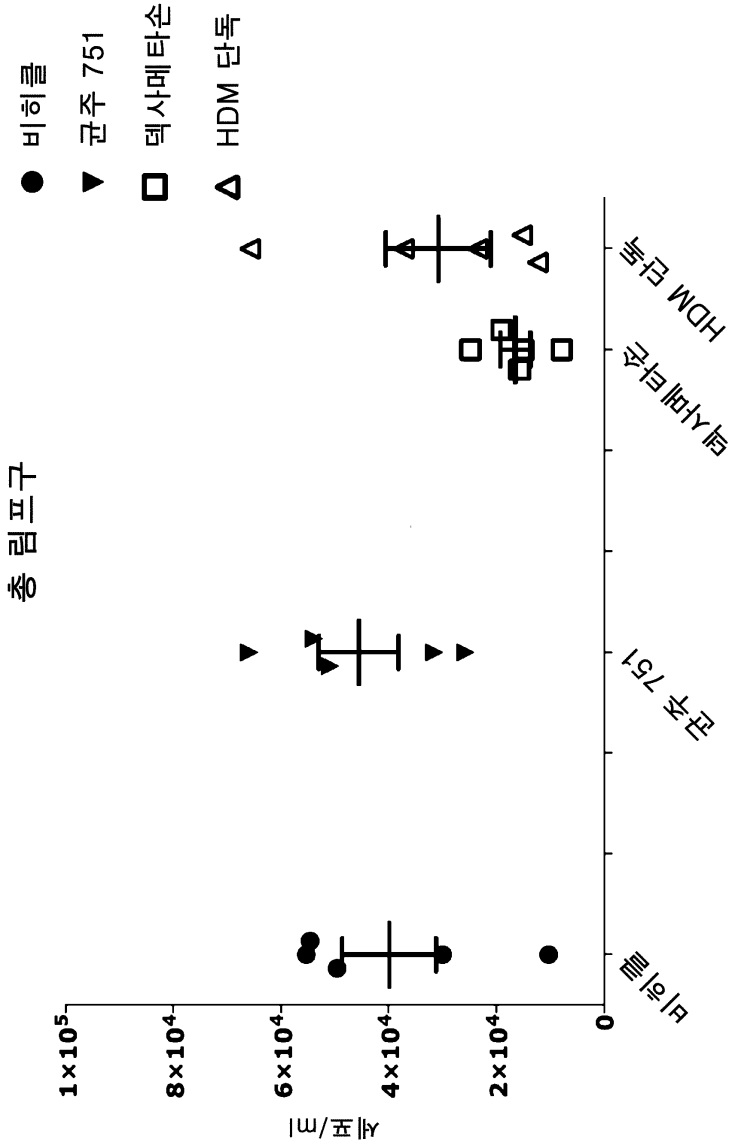
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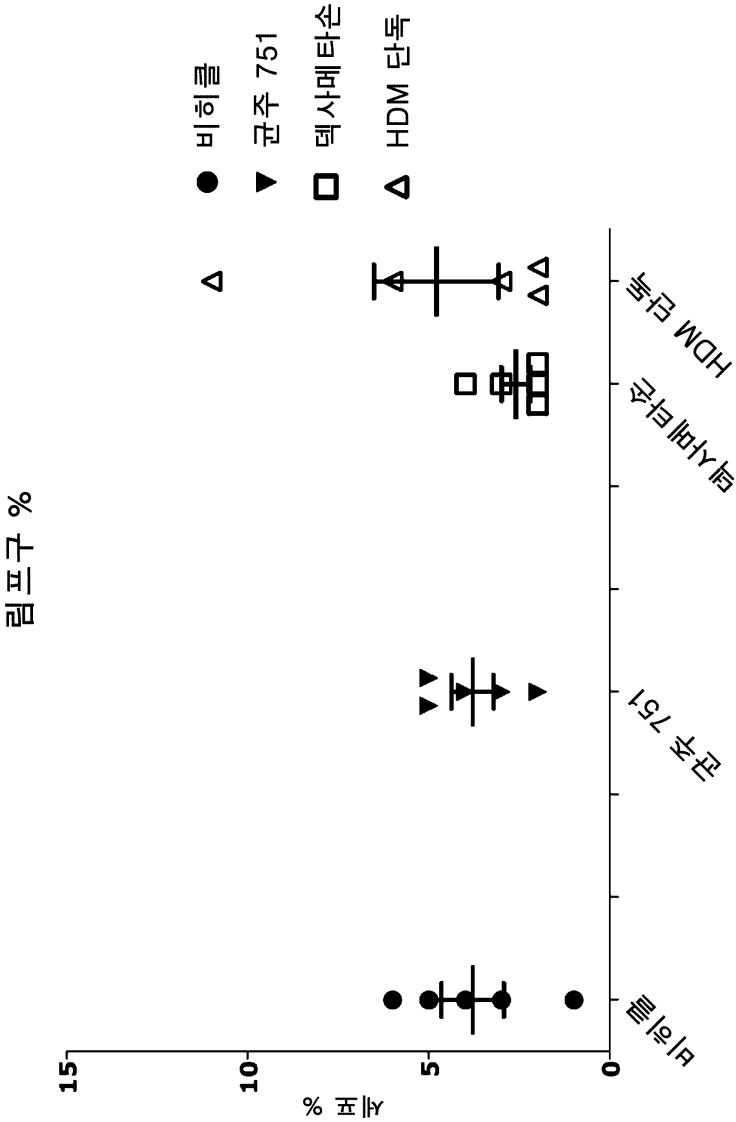
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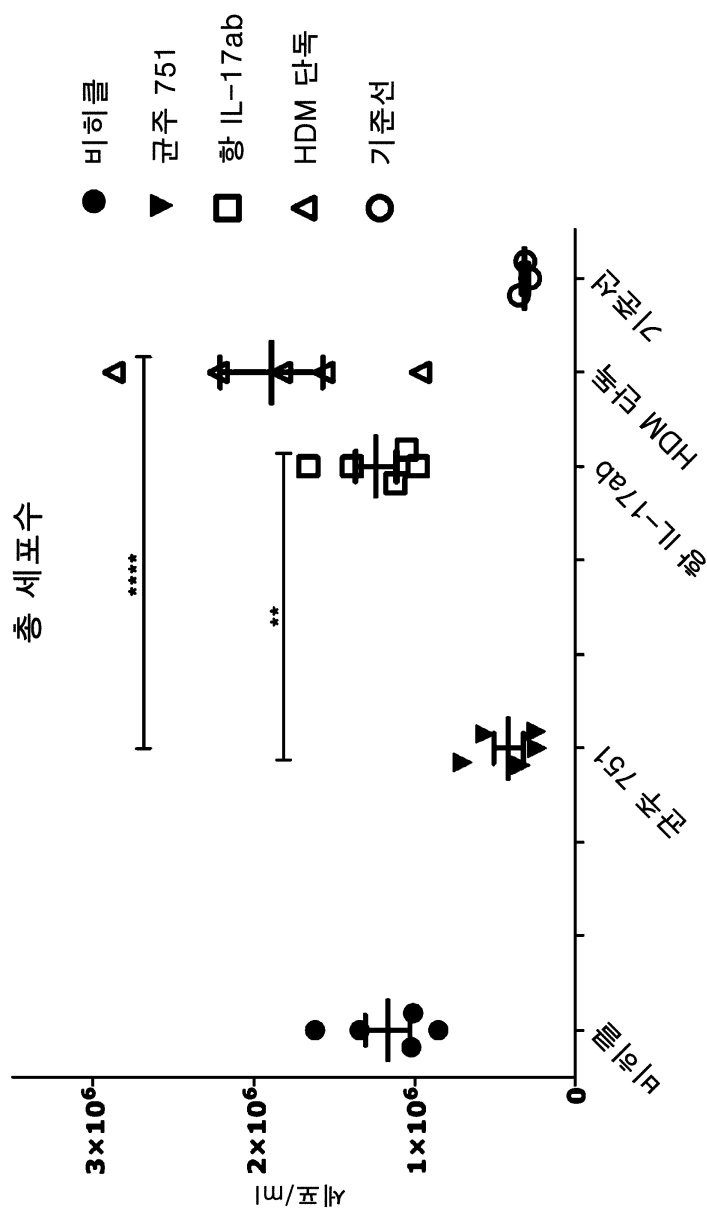
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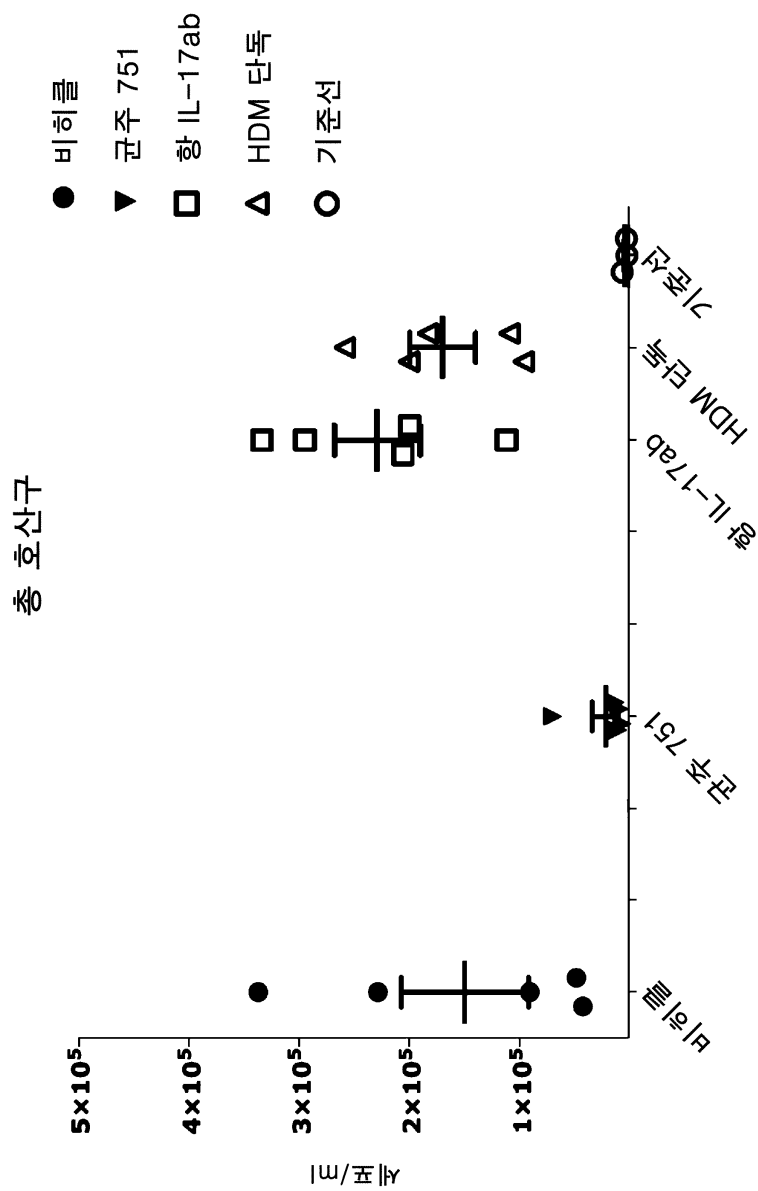
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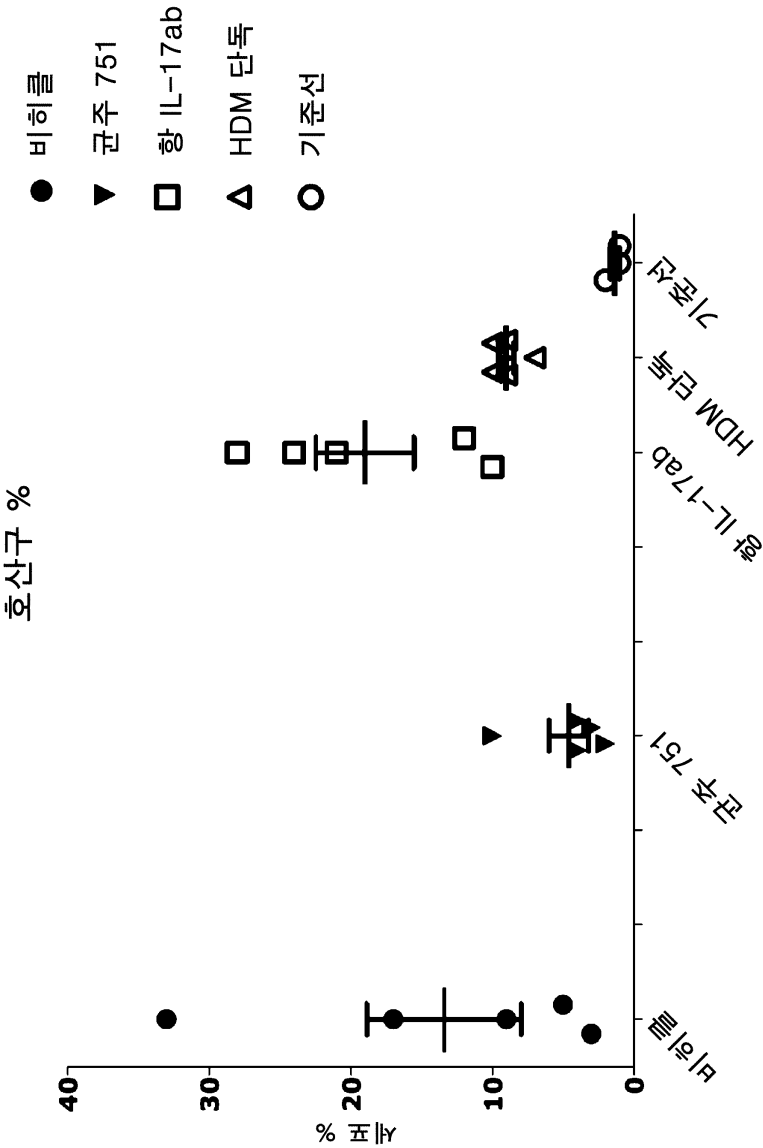
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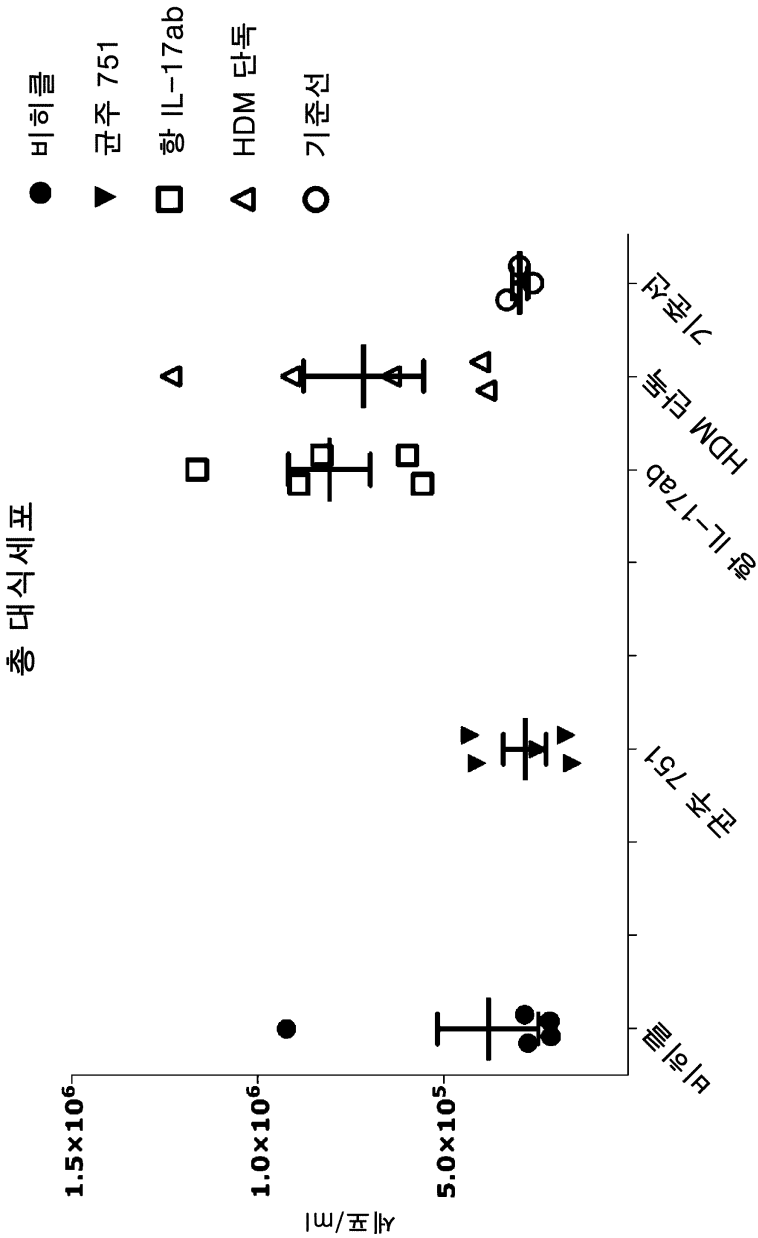
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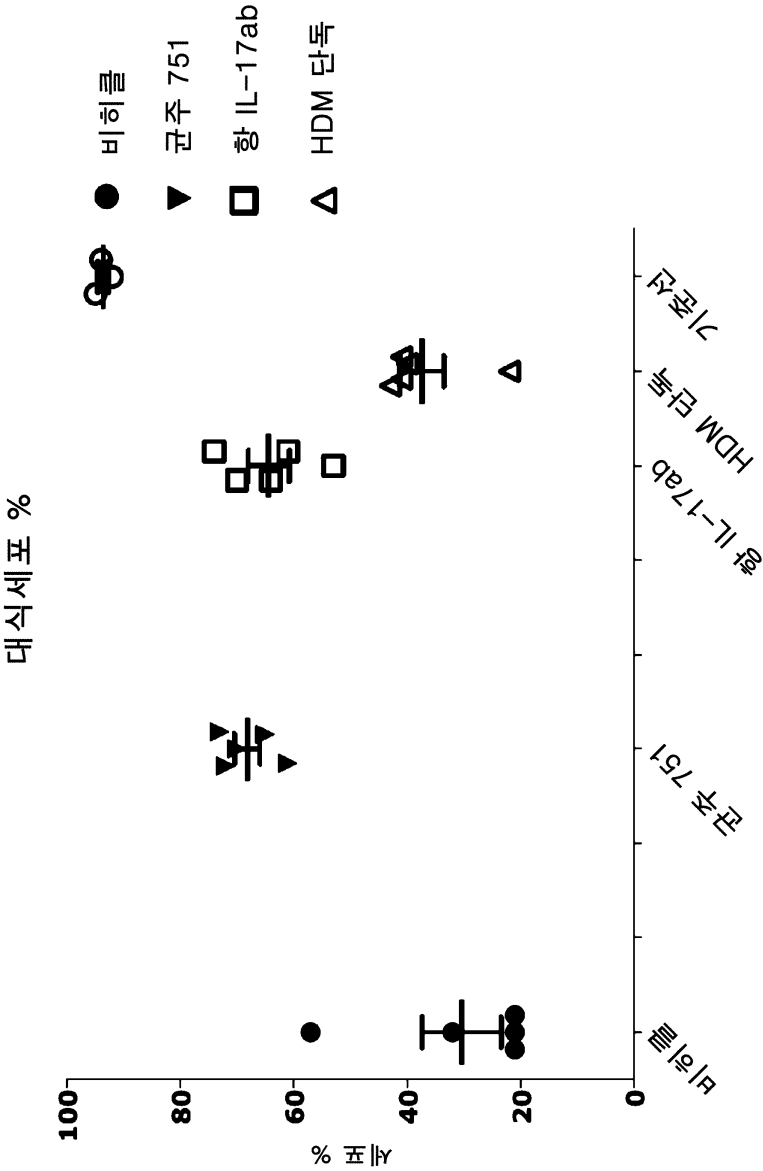
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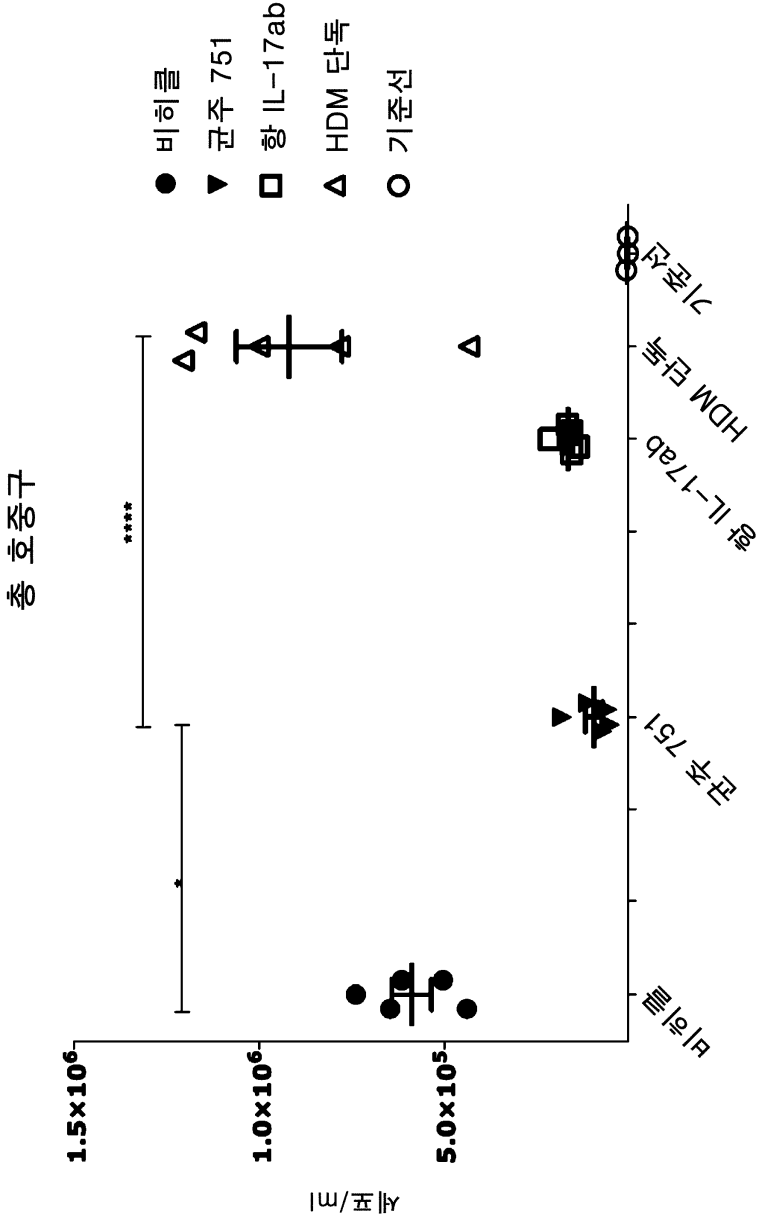
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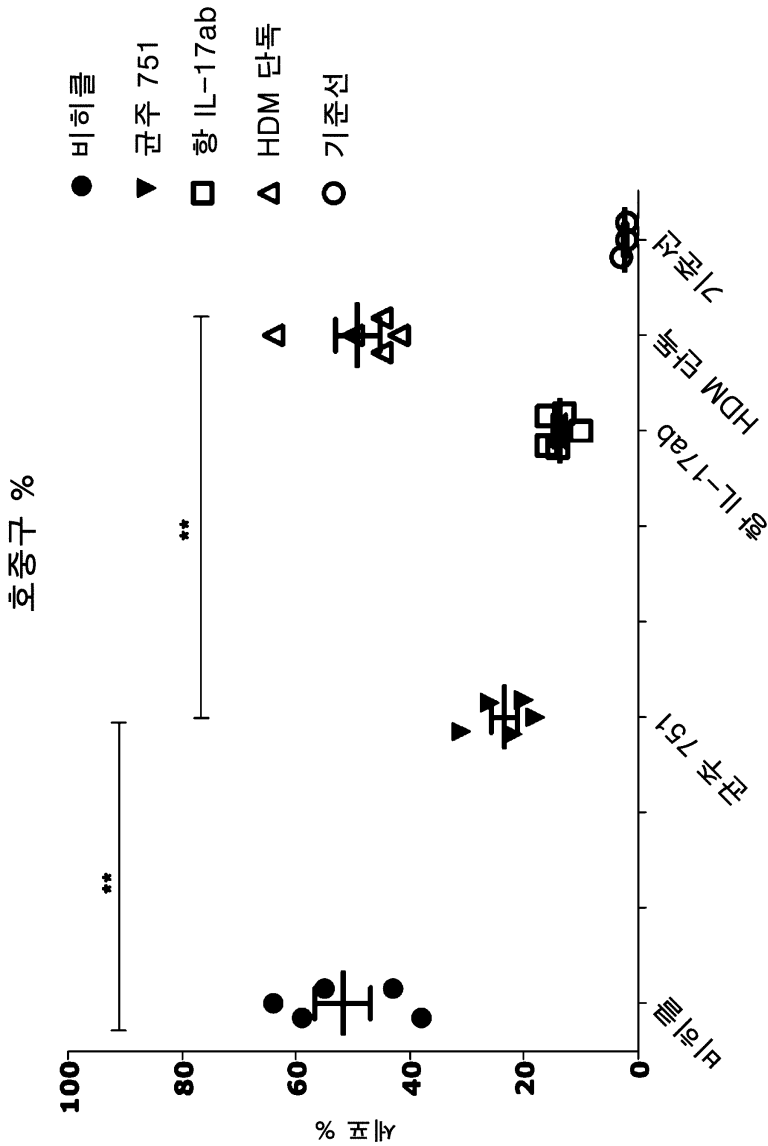
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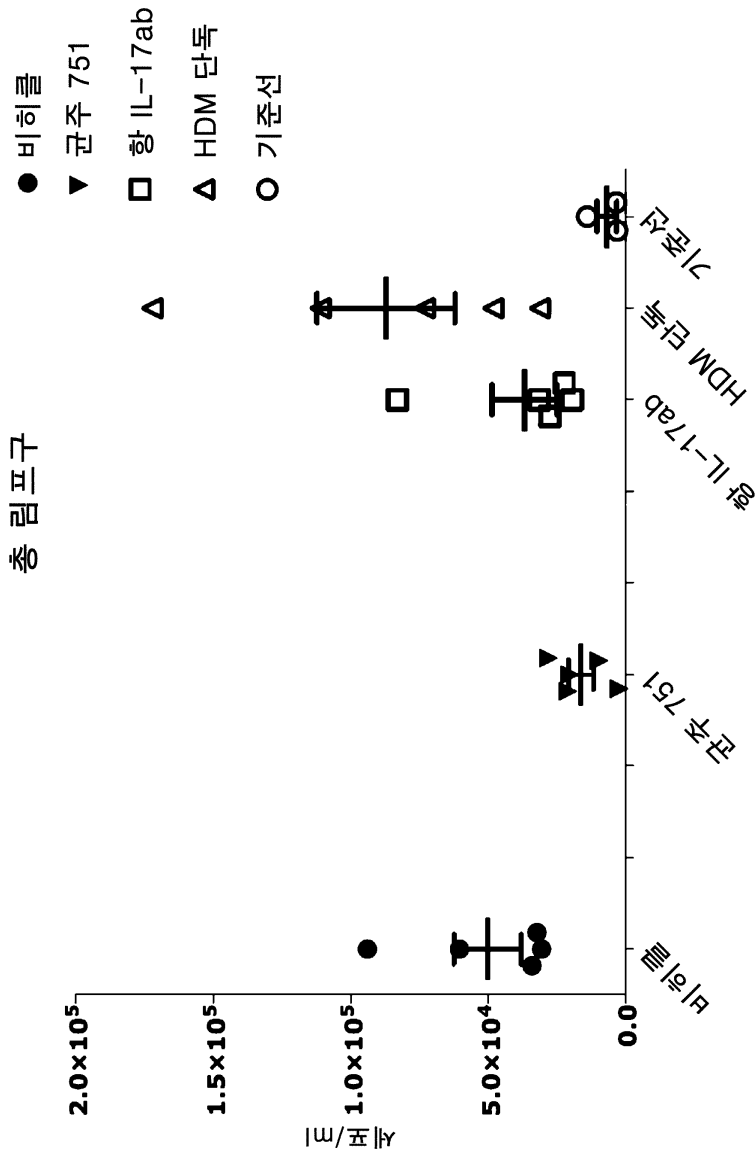
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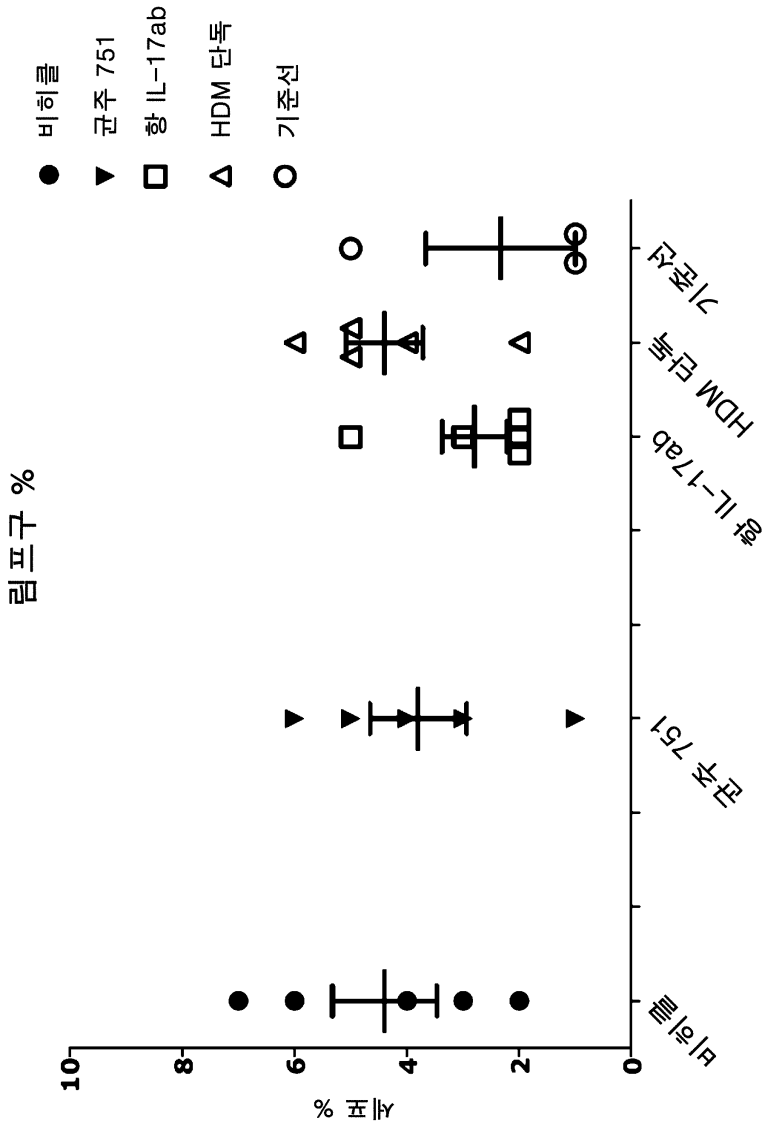
도면16



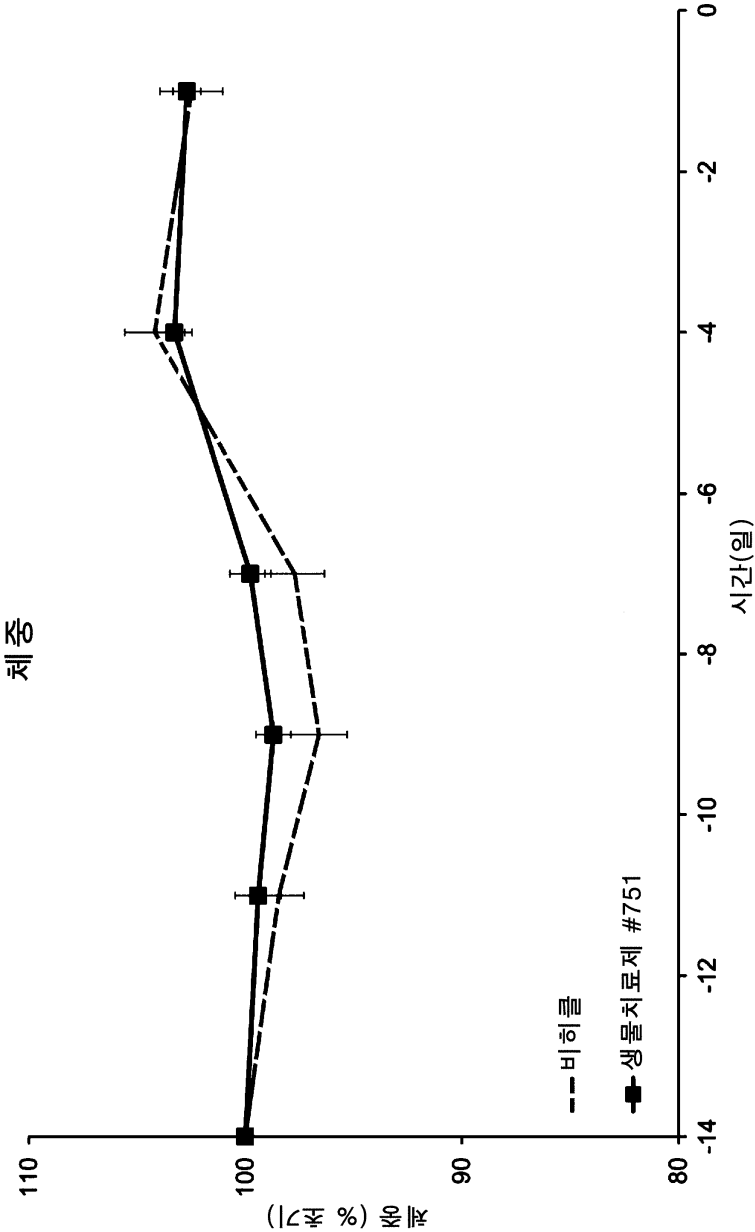
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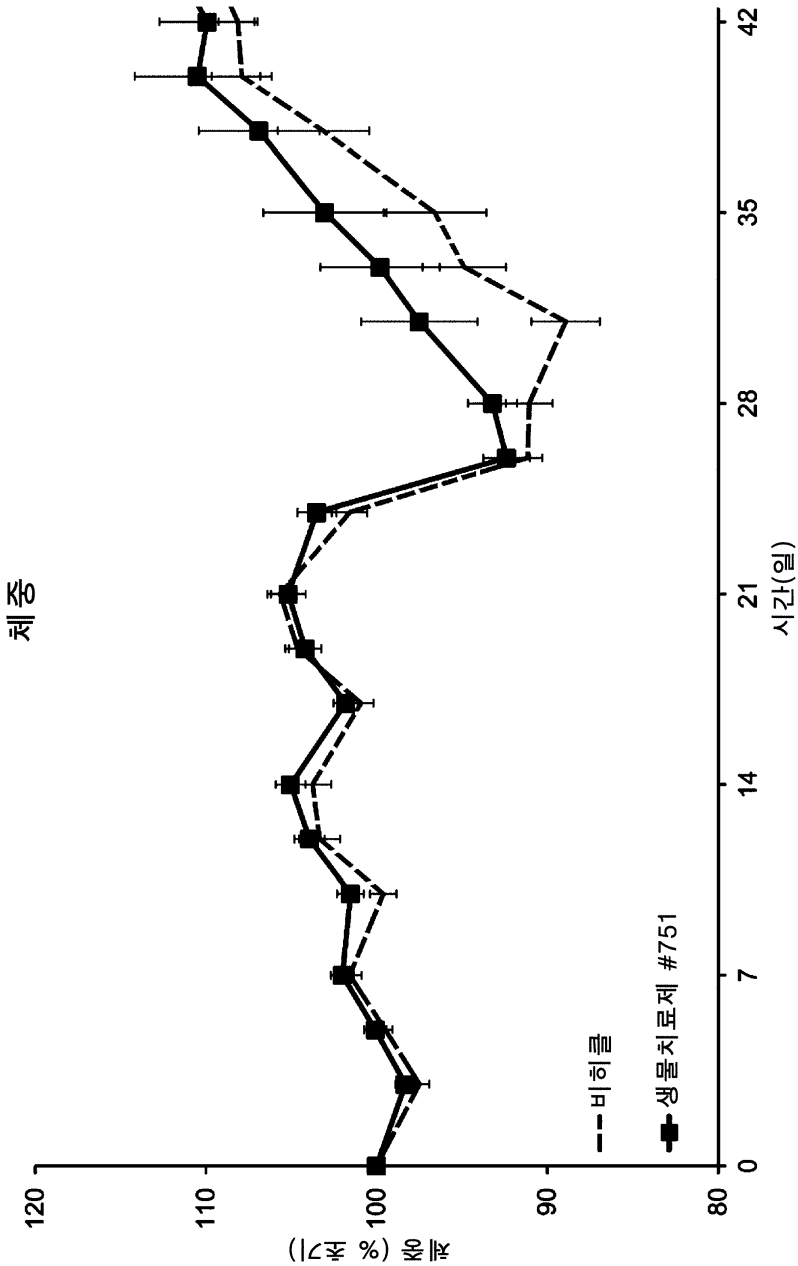
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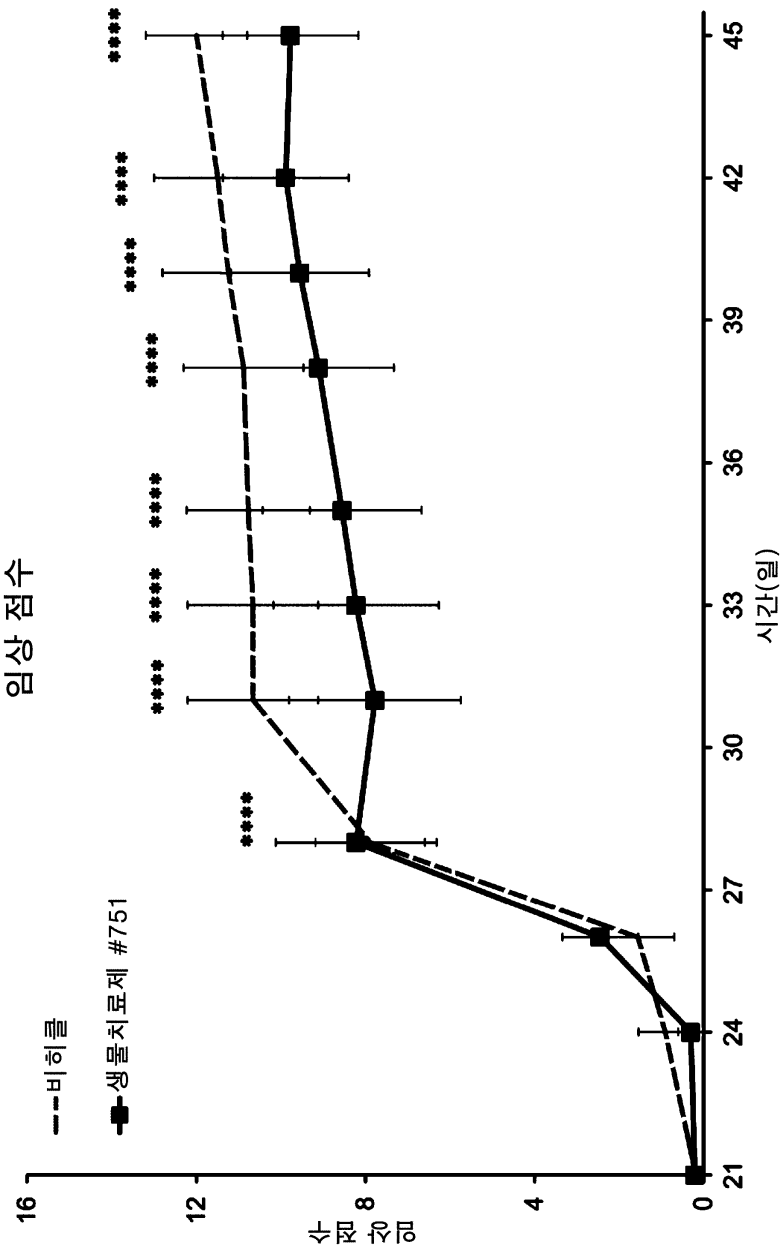
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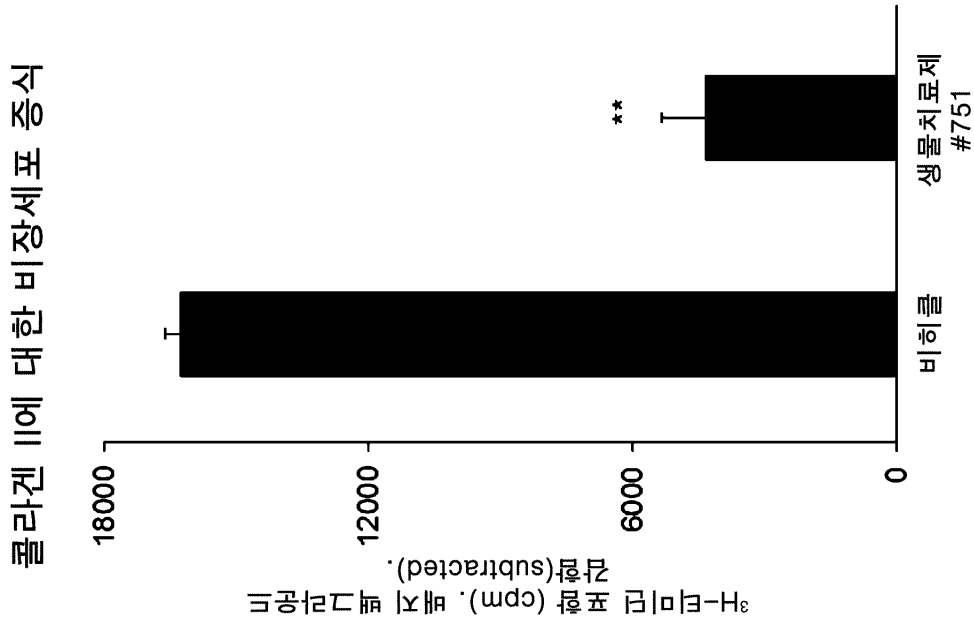
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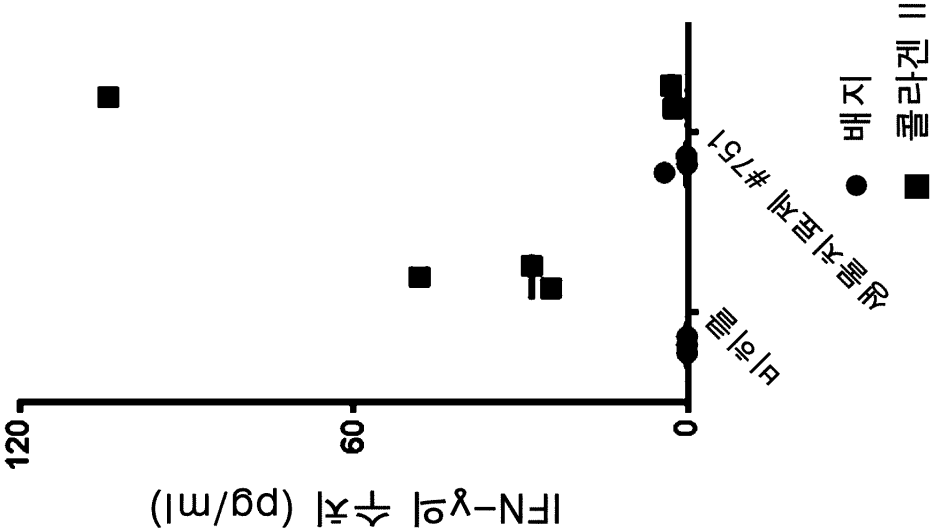
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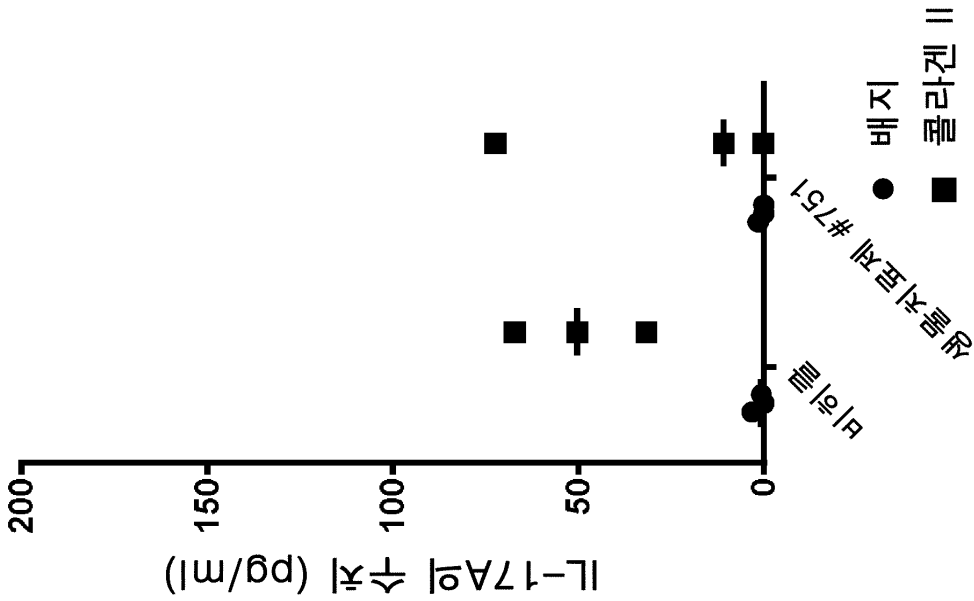
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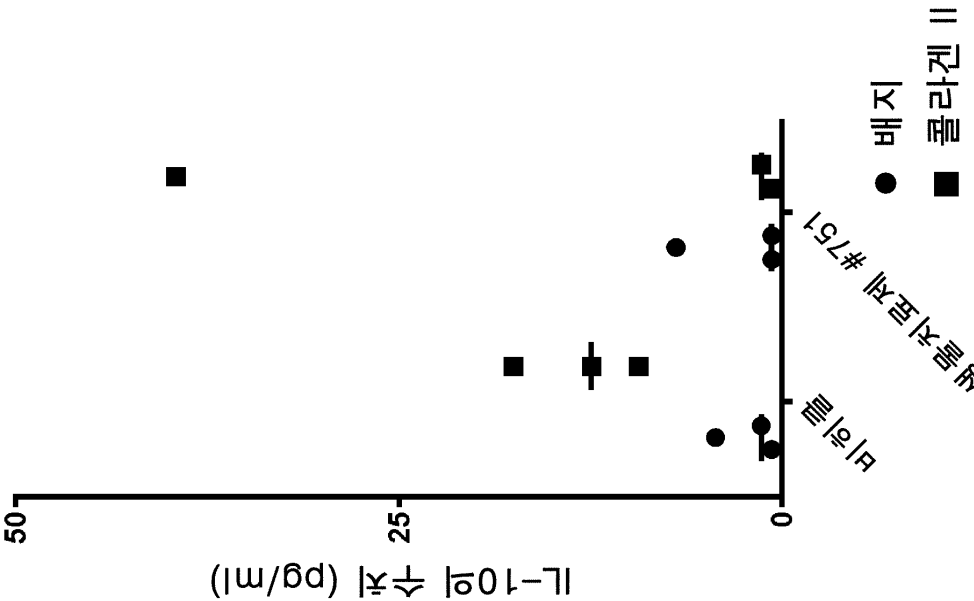
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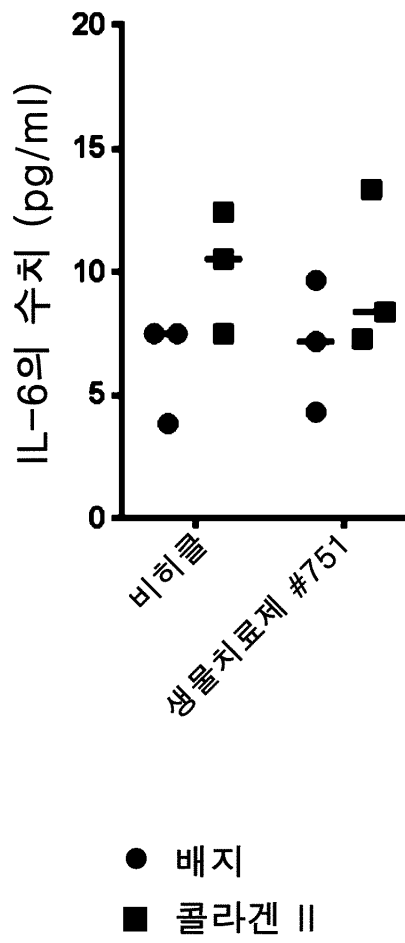
도면24



도면25



도면26



도면27

조직병리학 점수 시스템

등급 설명

염증

0	정상 관절
1	호중구가 지배하는 염증을 동반한 경미한 활막 증식. 관절 공간에서 소수의 호중구 및 대식세포.
2	호중구 및 대식세포 모두를 포함하는 중등도에서 현저한 염증을 갖는 활막 증식. 관절 공간에서의 호중구 및 대식세포; 일부 과사 조직 파편일 수 있다.
3	호중구 및 대식세포 모두를 포함하는 현저한 염증을 갖는 활막 증식. 활막세포 라이닝의 손실. 염증은 활액에서 근육을 포함한 주변 조직으로 확장될 수 있다. 상당한 과사 조직 파편과 함께 관절 공간에 있는 수많은 호중구 및 대식세포.

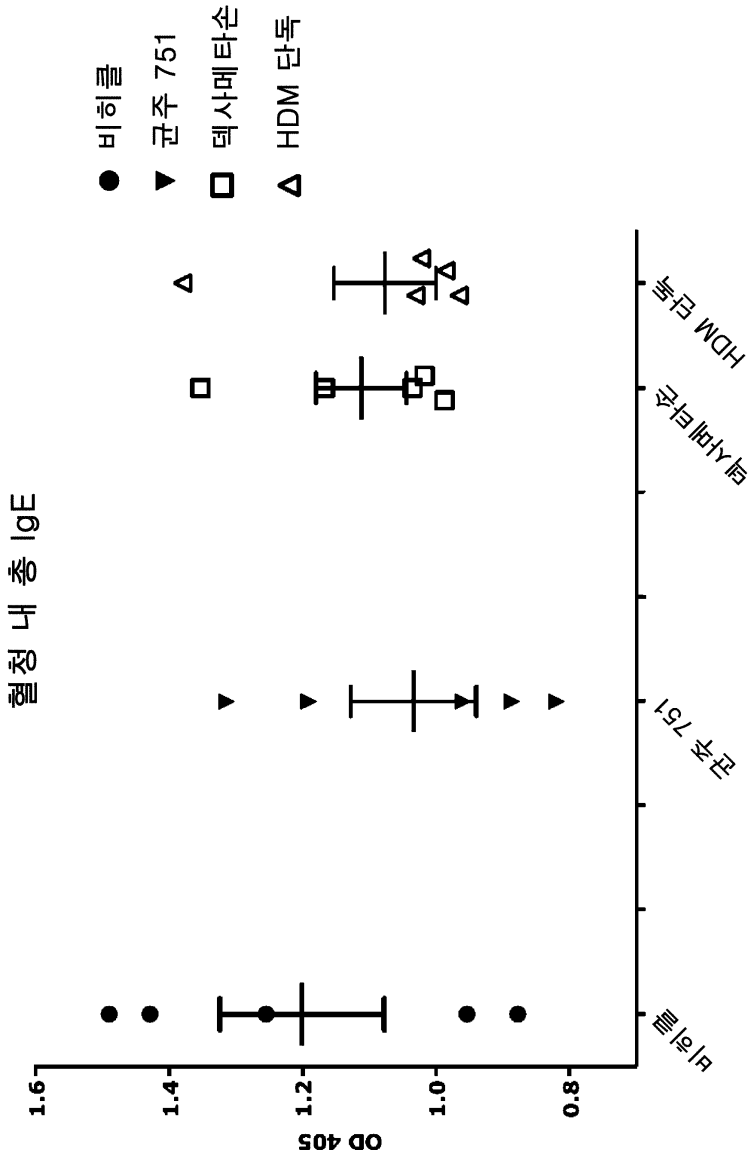
관절 연골 손상

0	정상 관절
1	관절 연골이 경미한 퇴행성 변화만을 보여준다. 조기 파누스(pannus) 형성이 주변에 나타날 수 있다.
2	관절 연골이 중등도의 퇴행성 변화와 국소 손실을 나타낸다. 파누스 형성이 국소적으로 존재한다.
3	광범위한 파누스 형성과 함께 관절 연골의 현저한 파괴 및 손실.

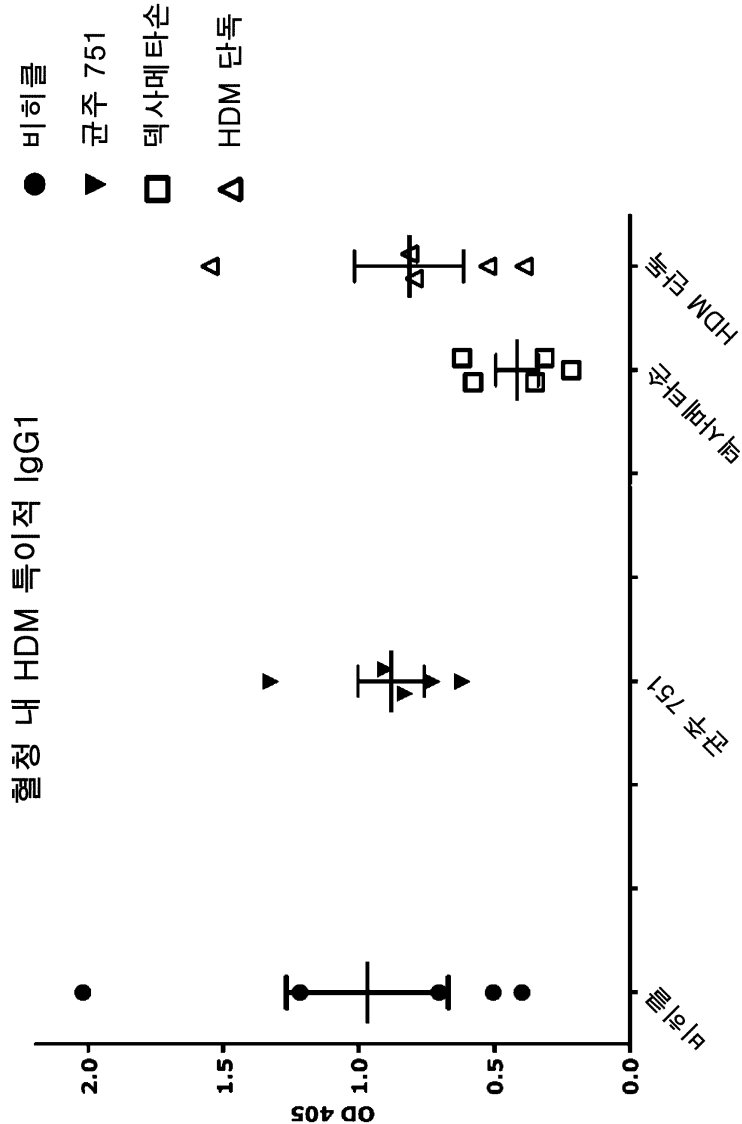
근본적인 골간단 뼈의 손상

0	정상 관절
1	근본적인 골간단 뼈에 변화가 없다.
2	골간단 뼈의 국소 과사 또는 섬유증일 수 있다.
3	골간단 뼈의 파괴 또는 붕괴. 골간단의 골수 공간으로 확장된 광범위한 염증, 과사 또는 섬유증.

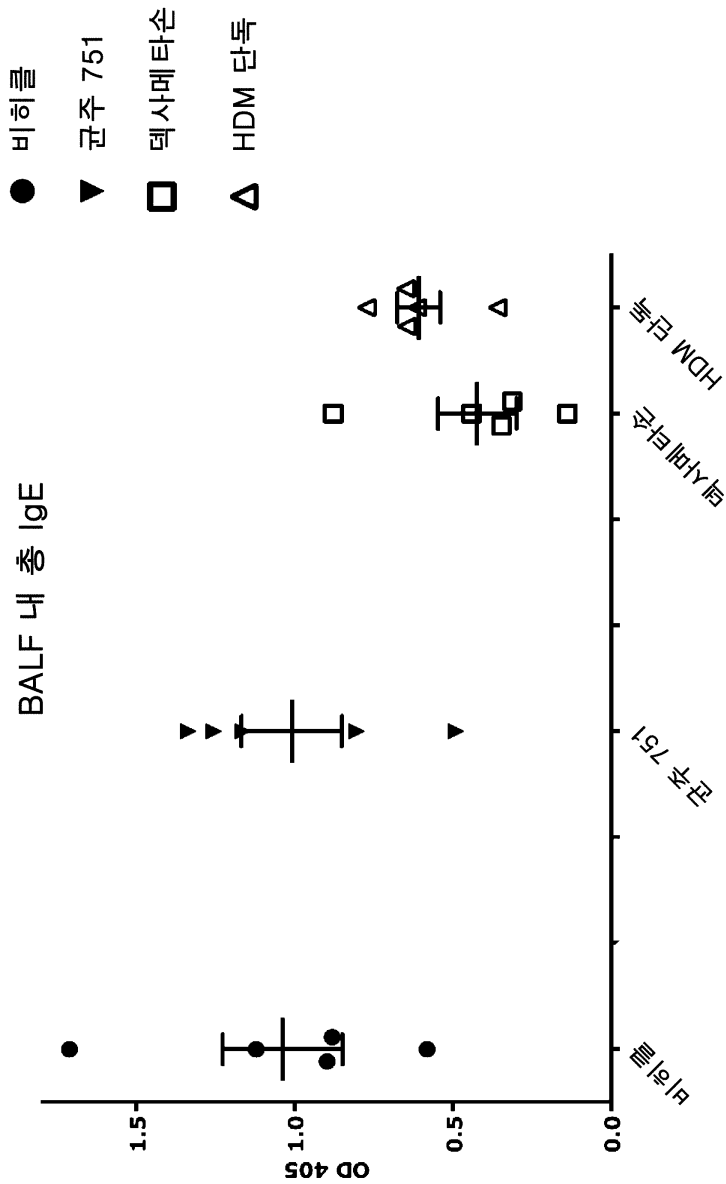
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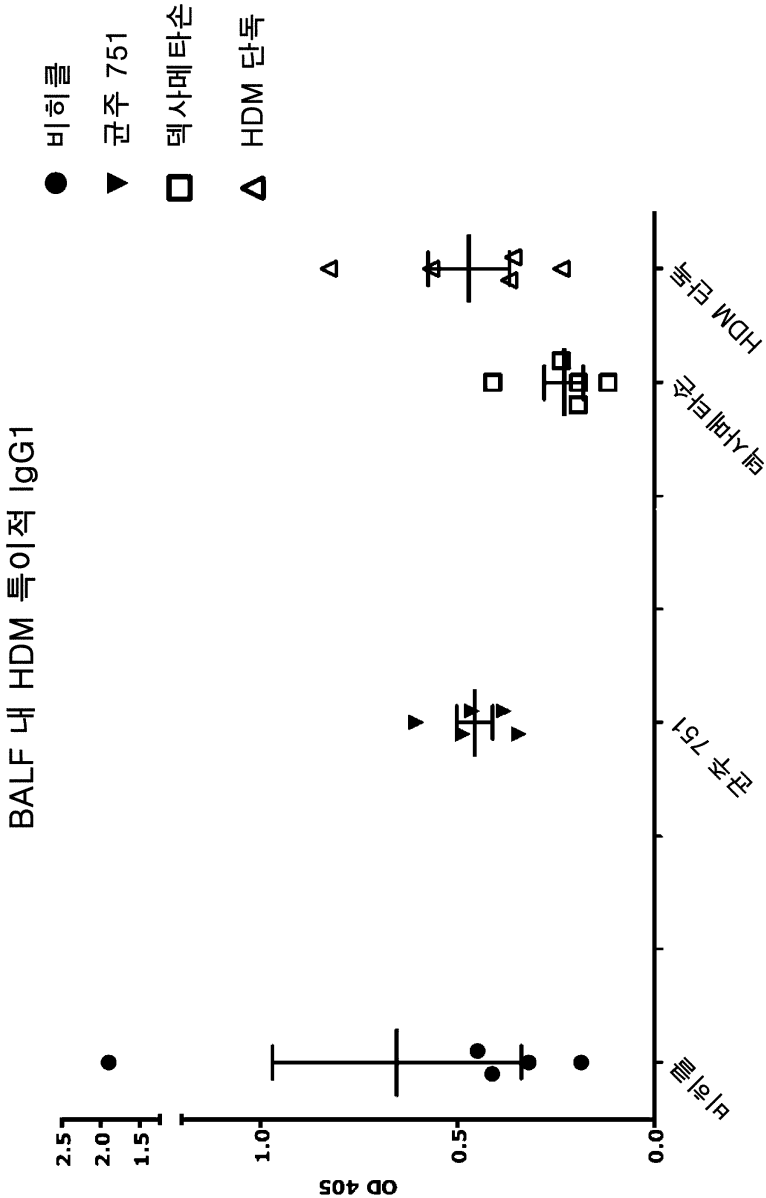
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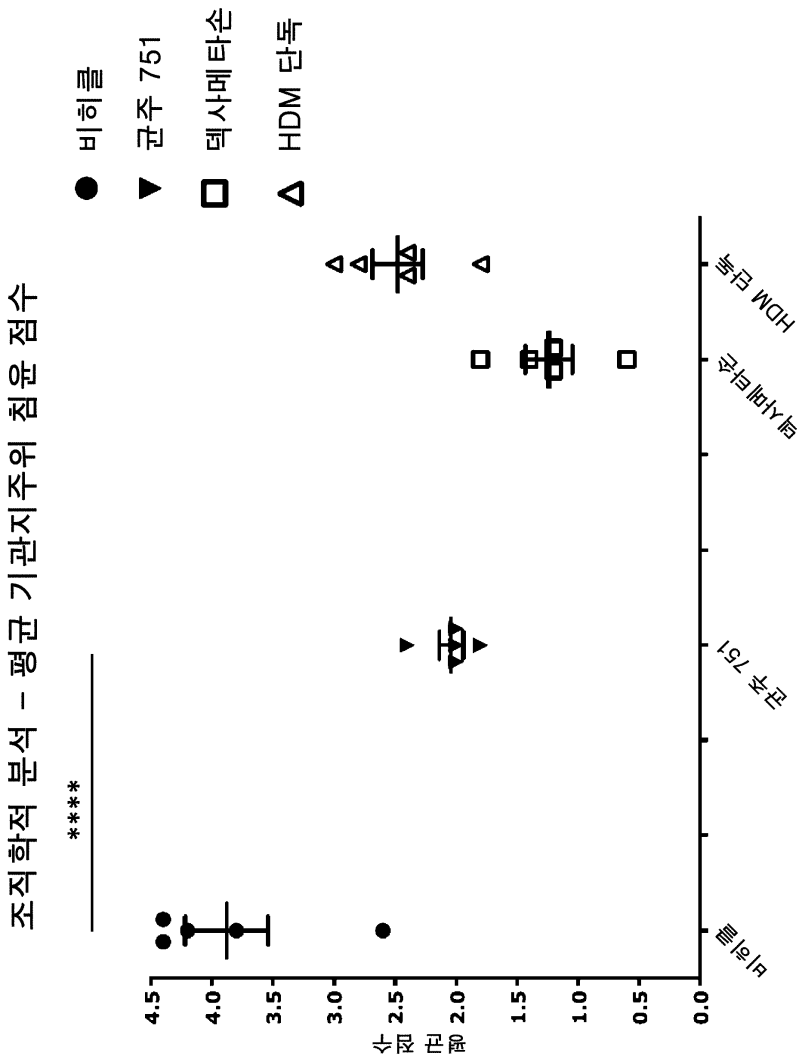
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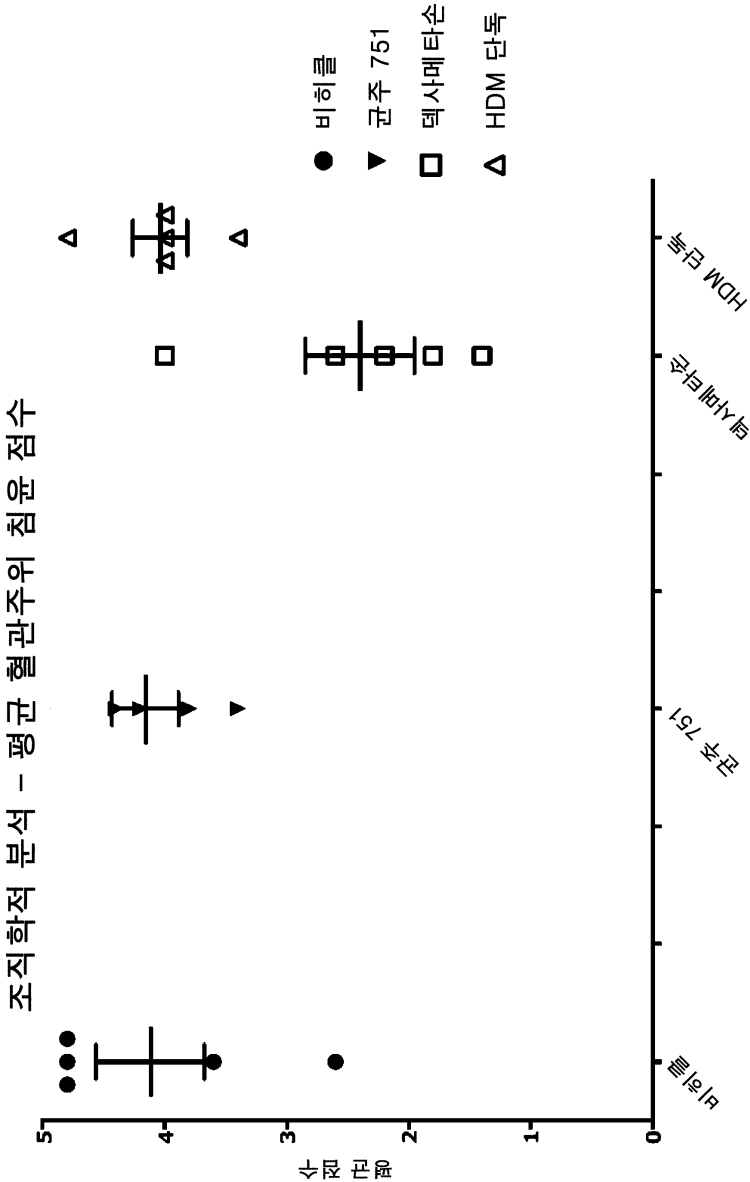
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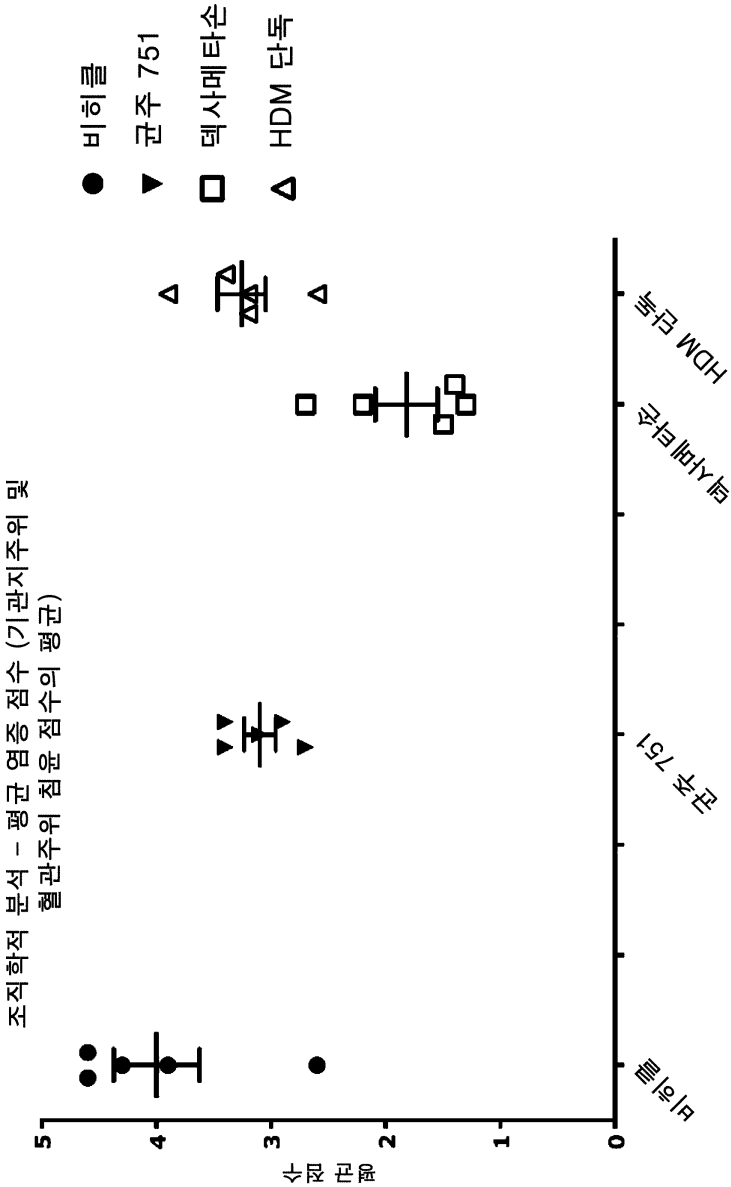
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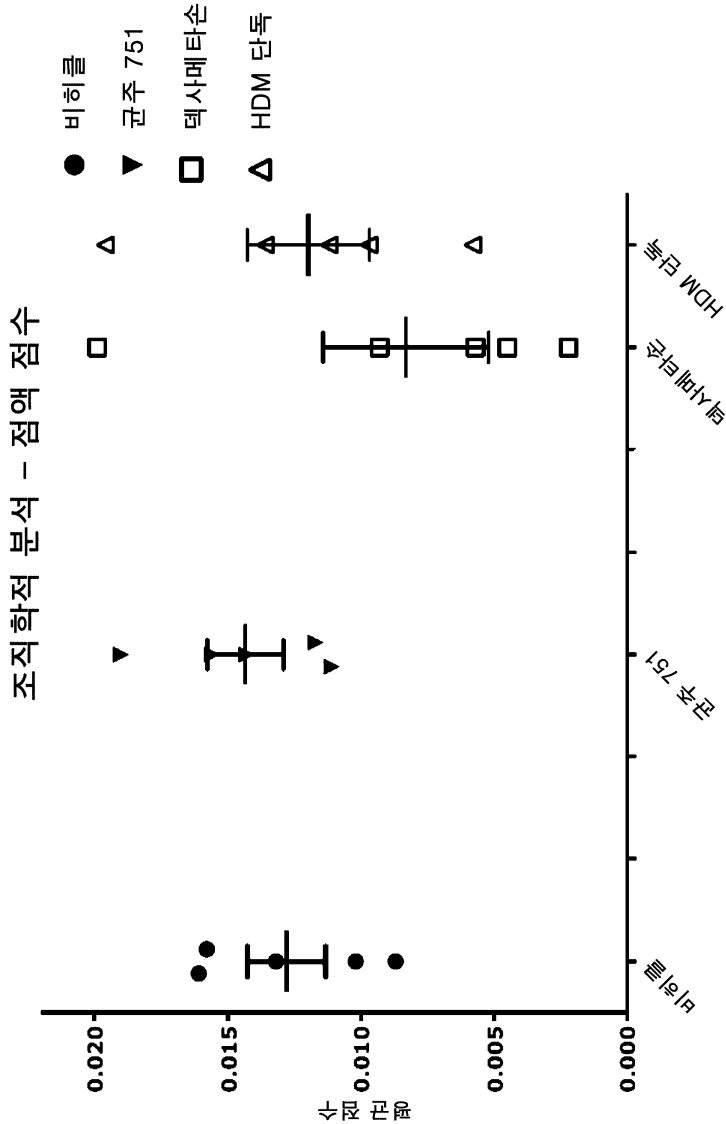
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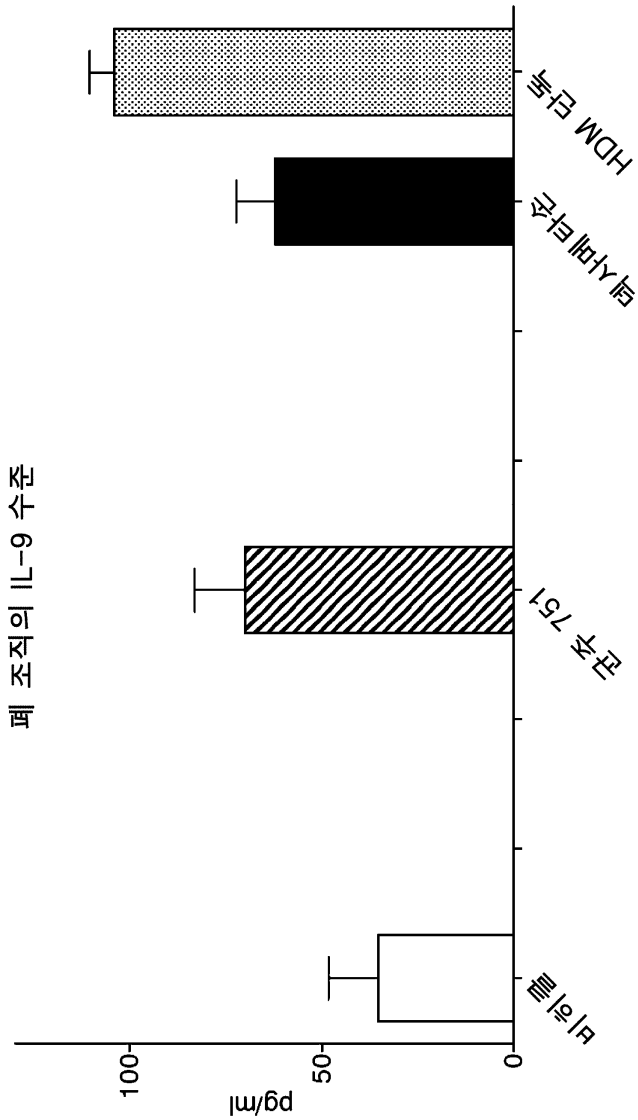
도면34



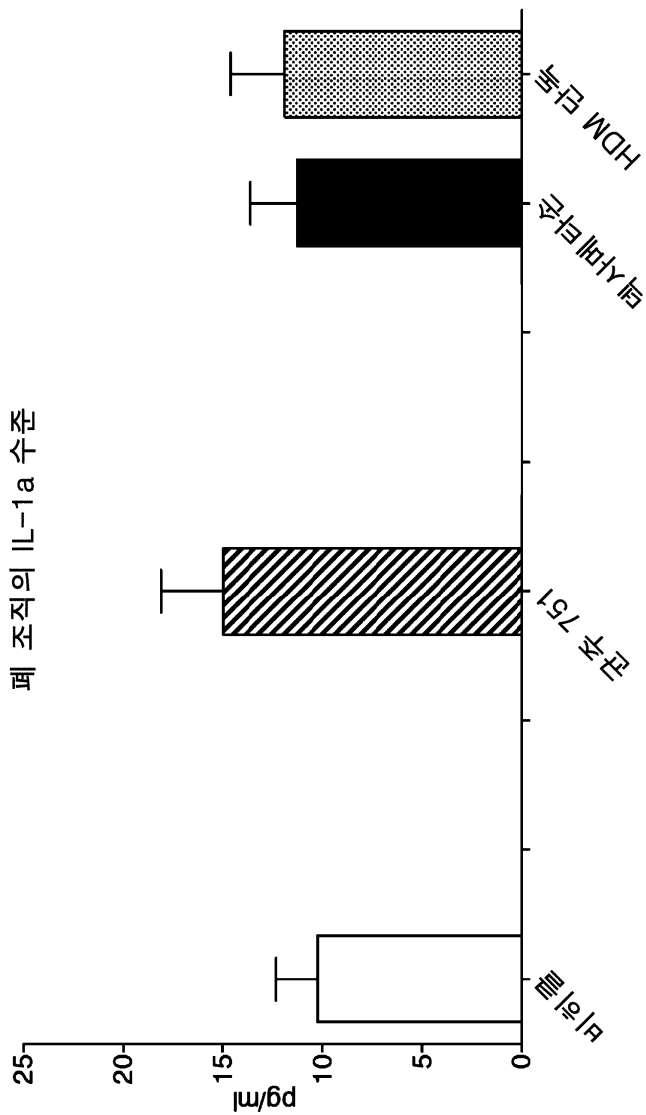
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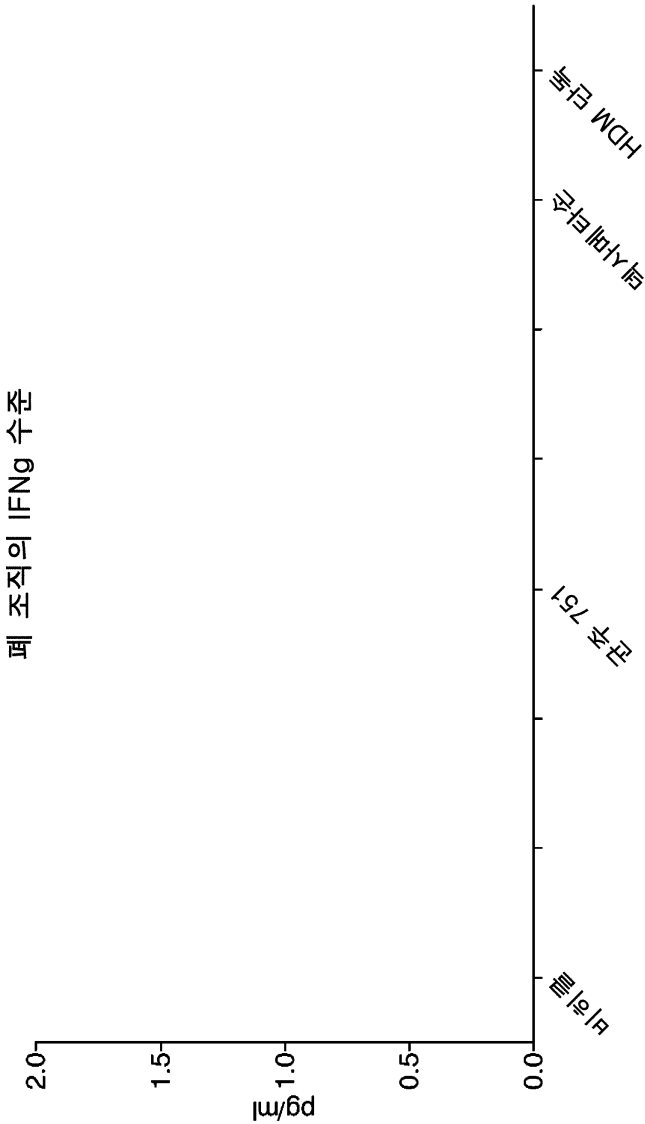
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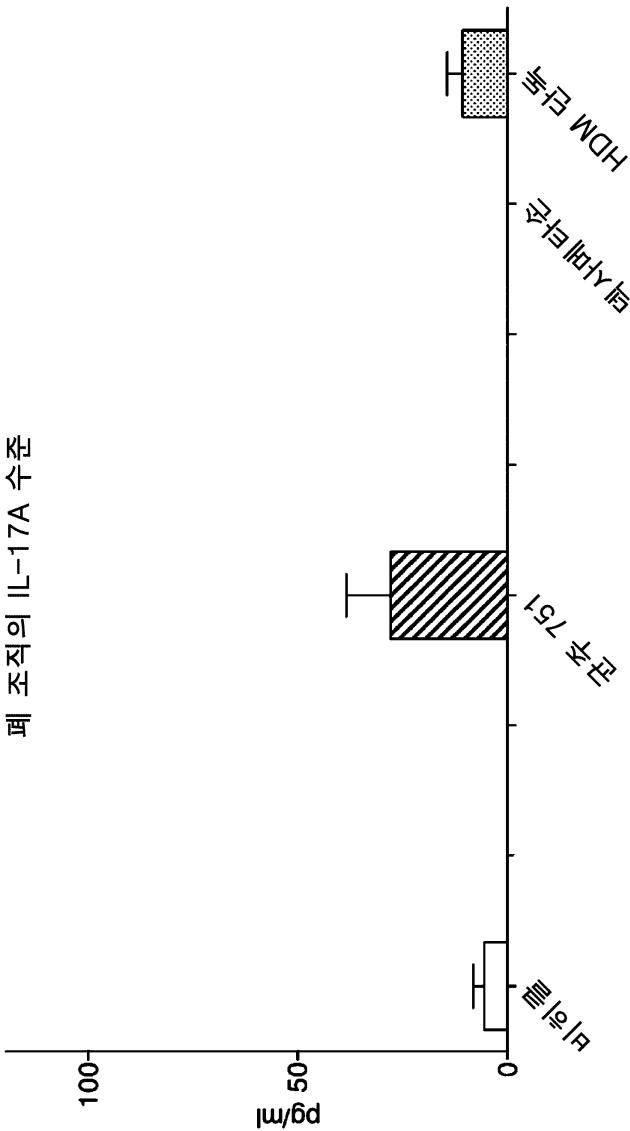
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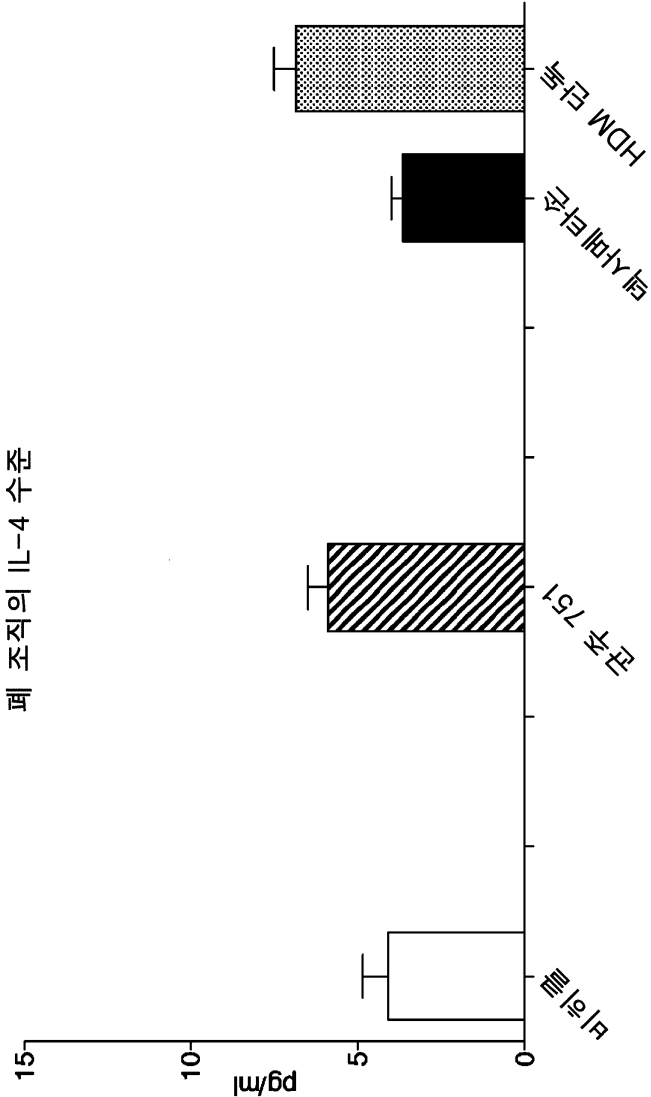
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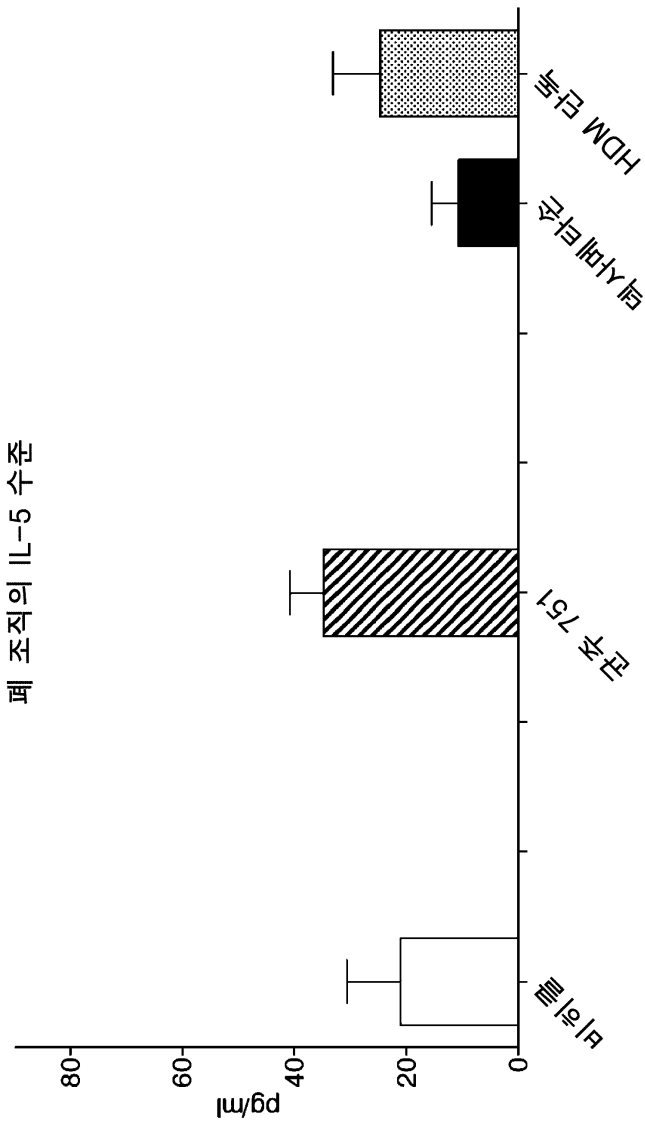
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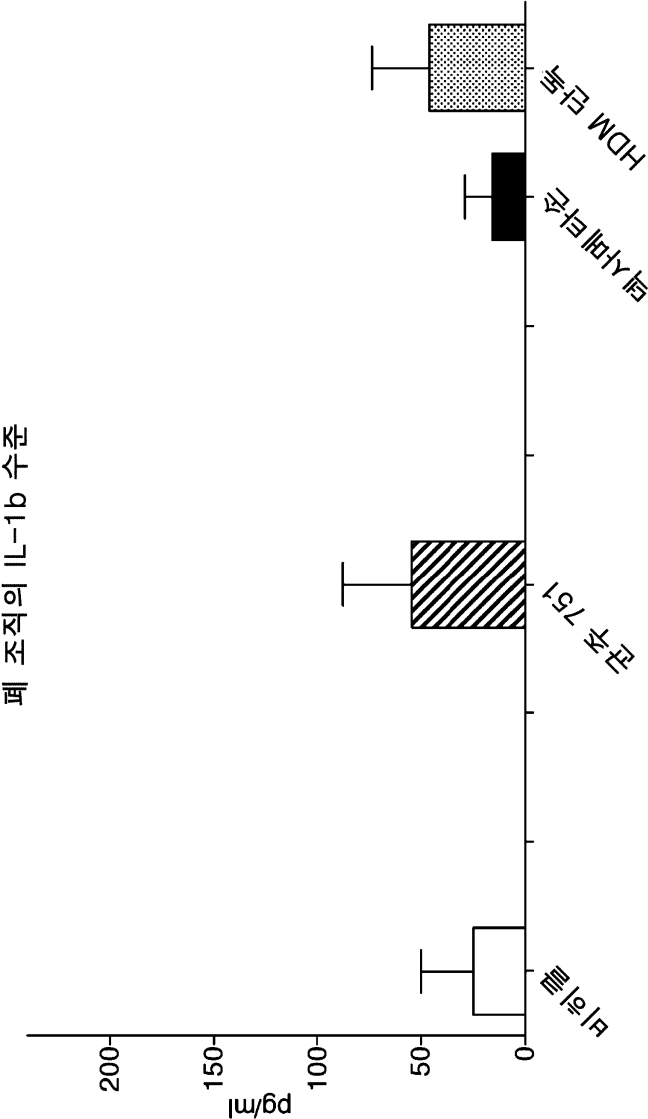
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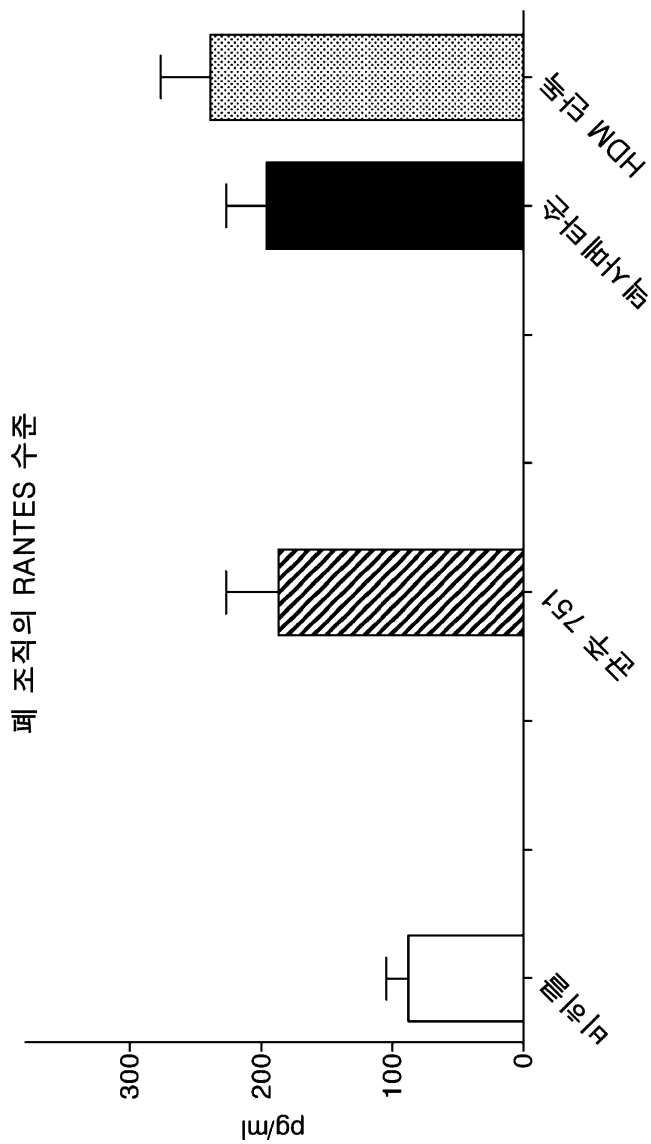
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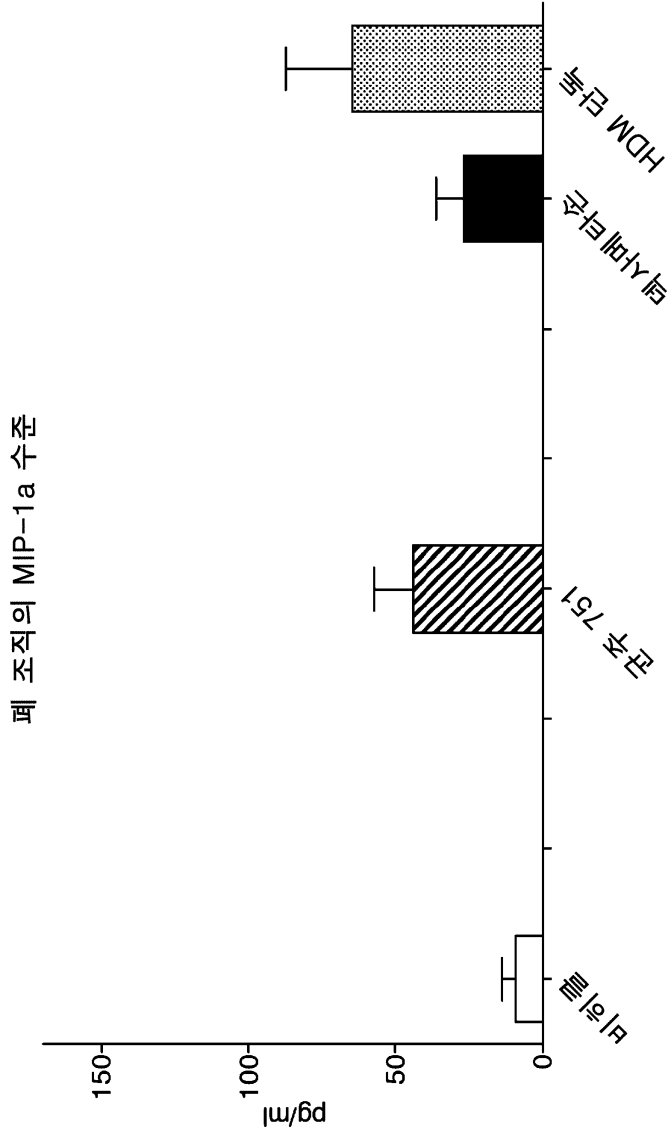
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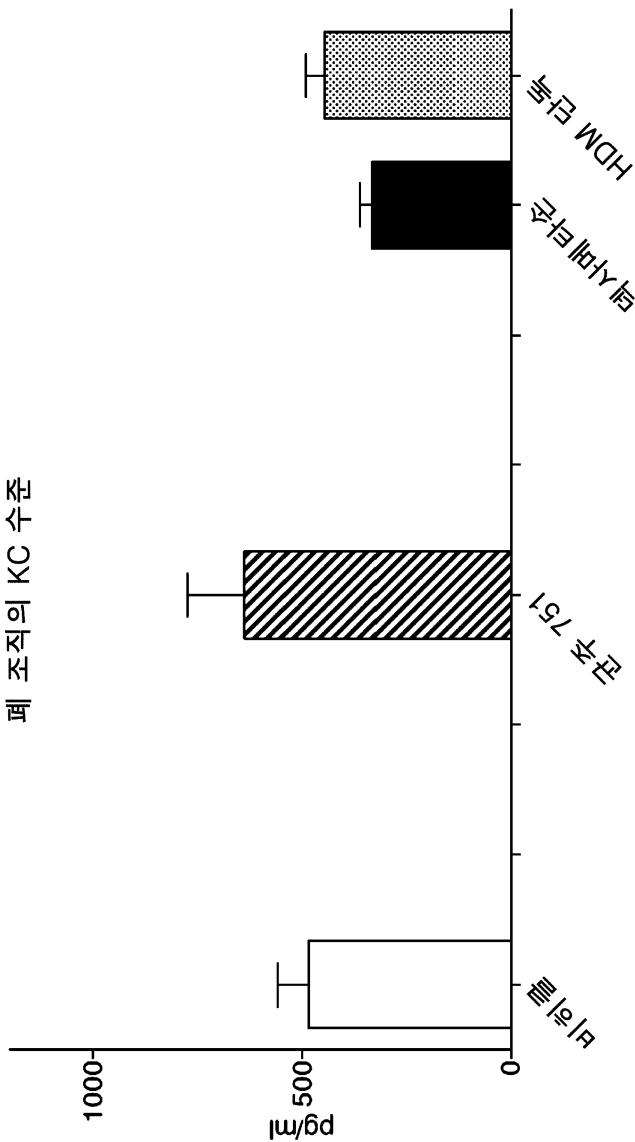
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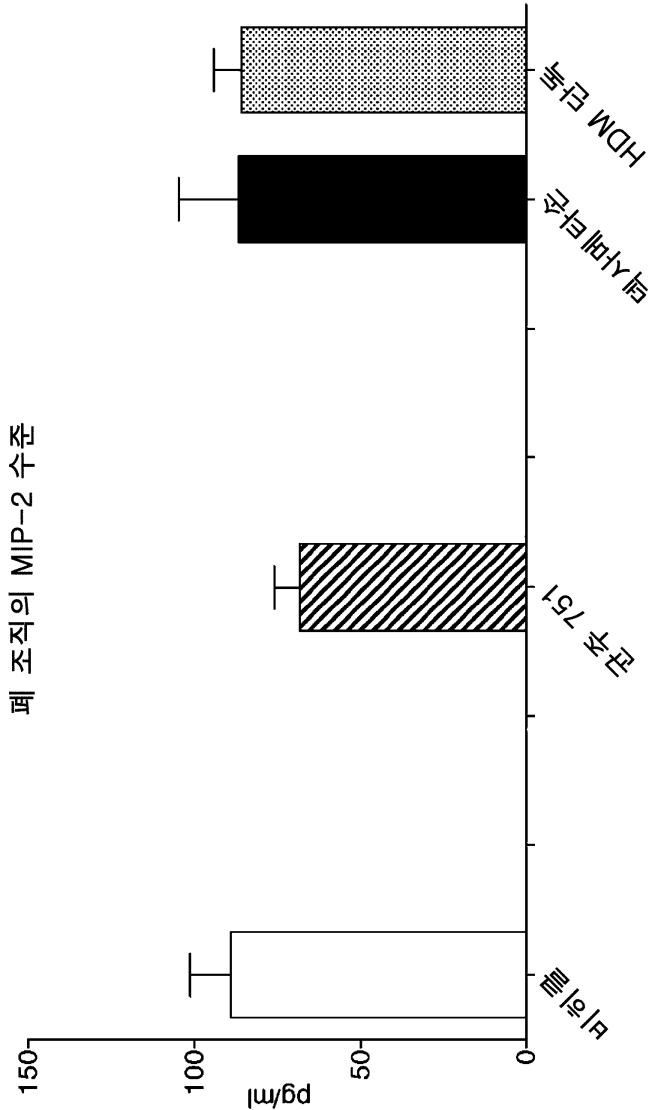
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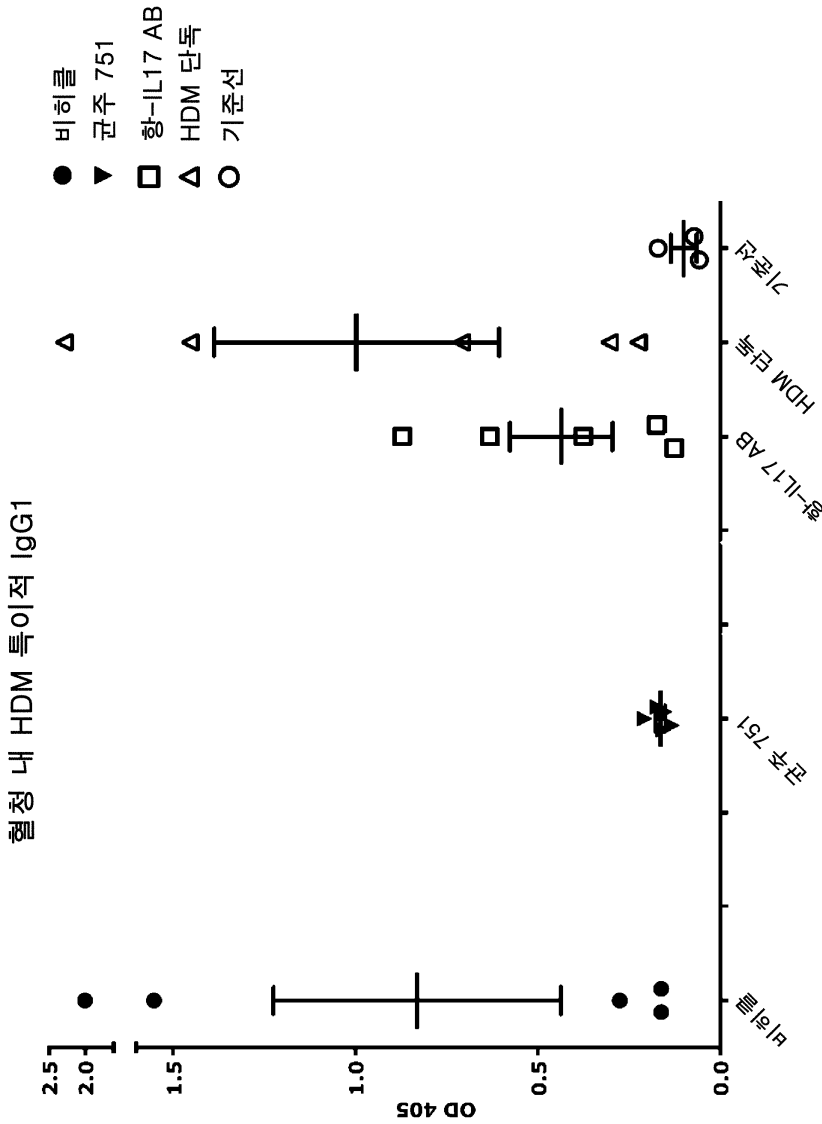
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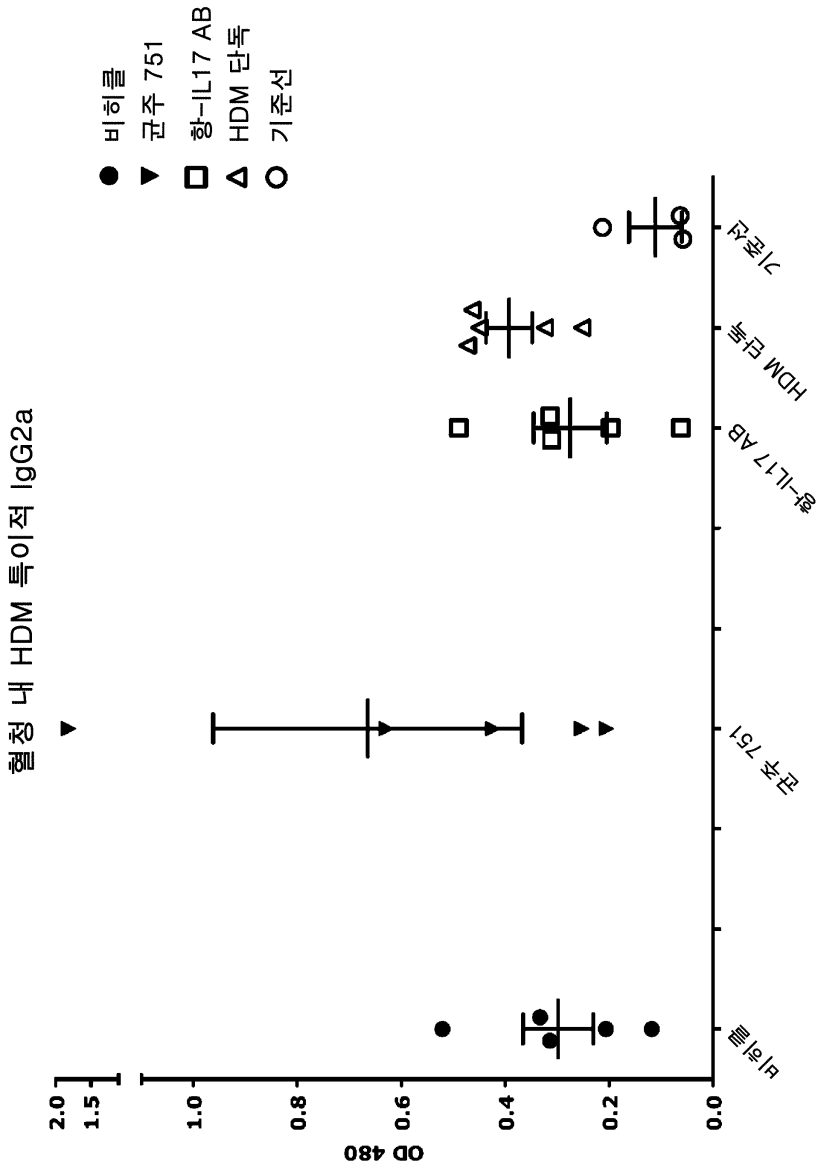
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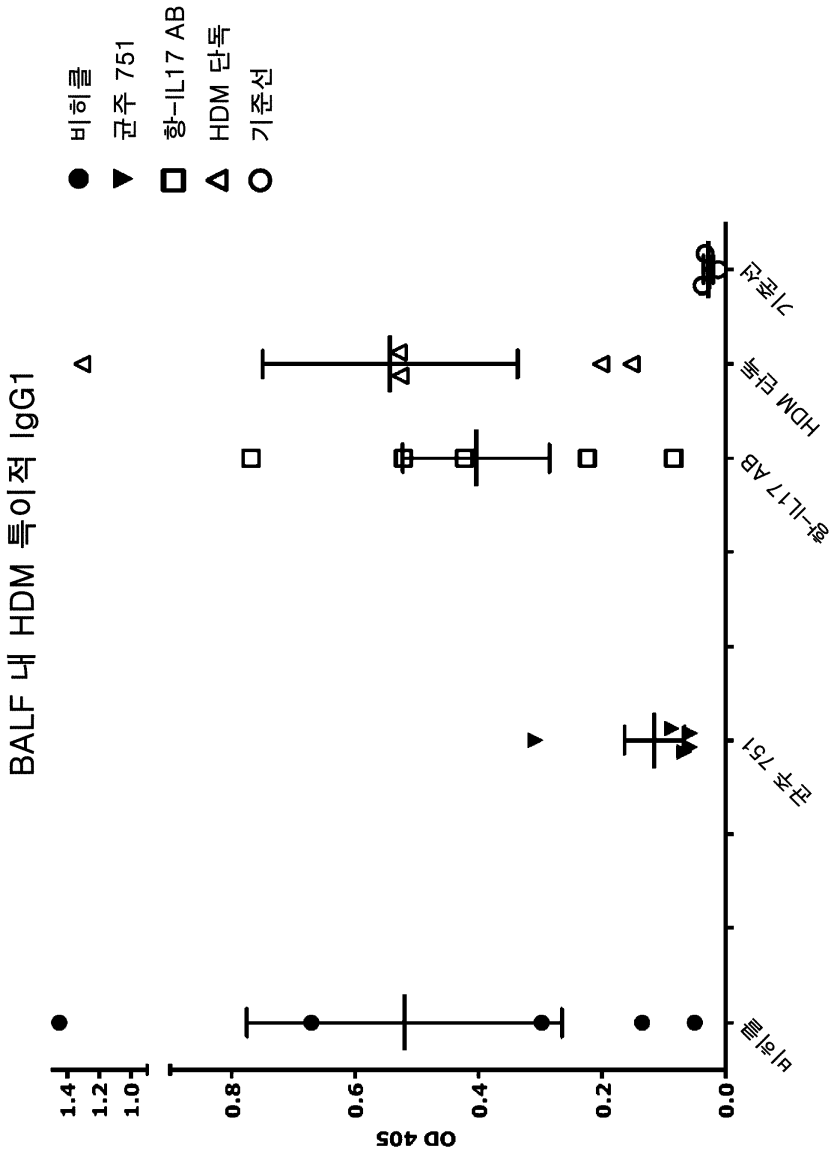
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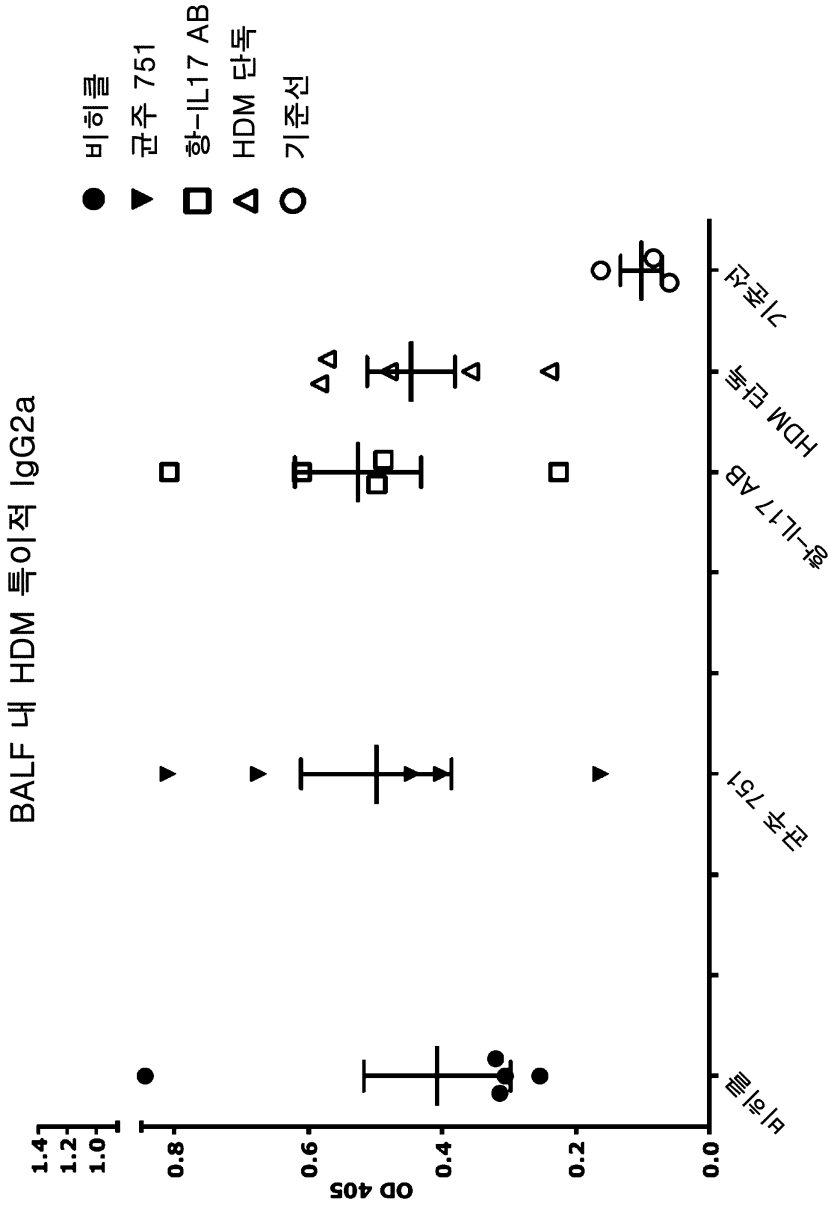
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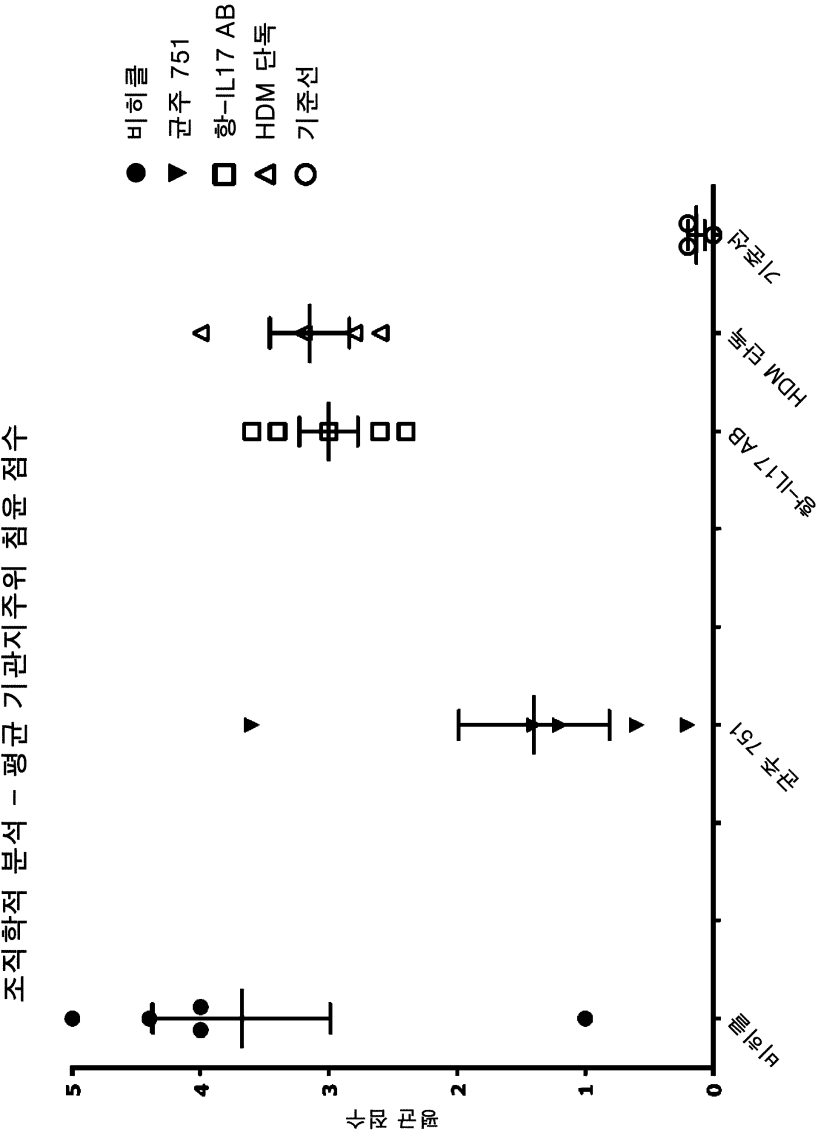
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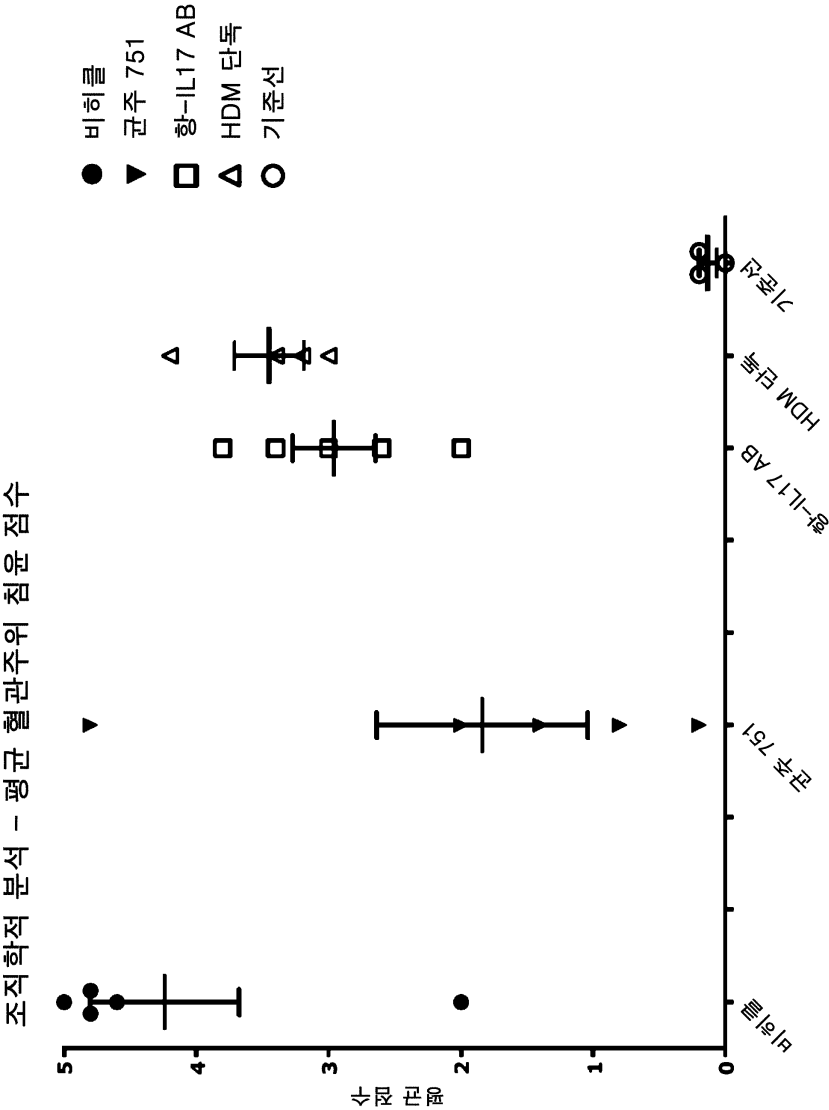
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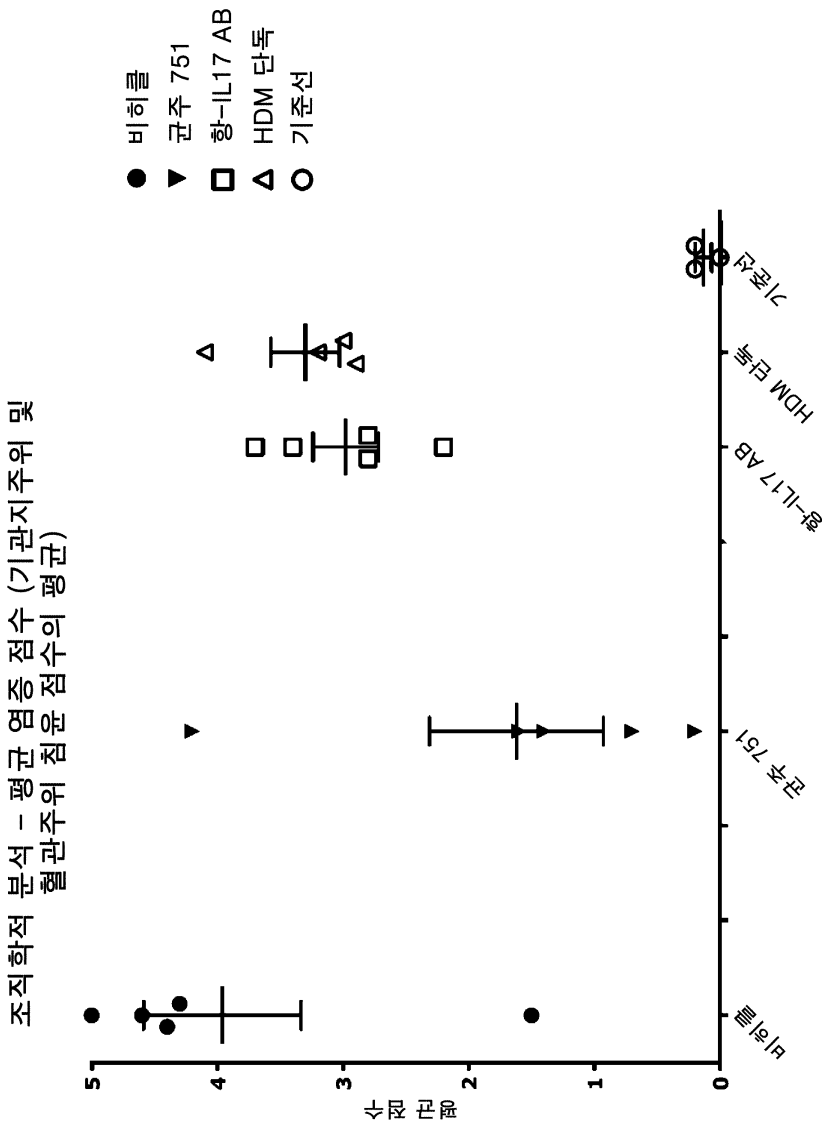
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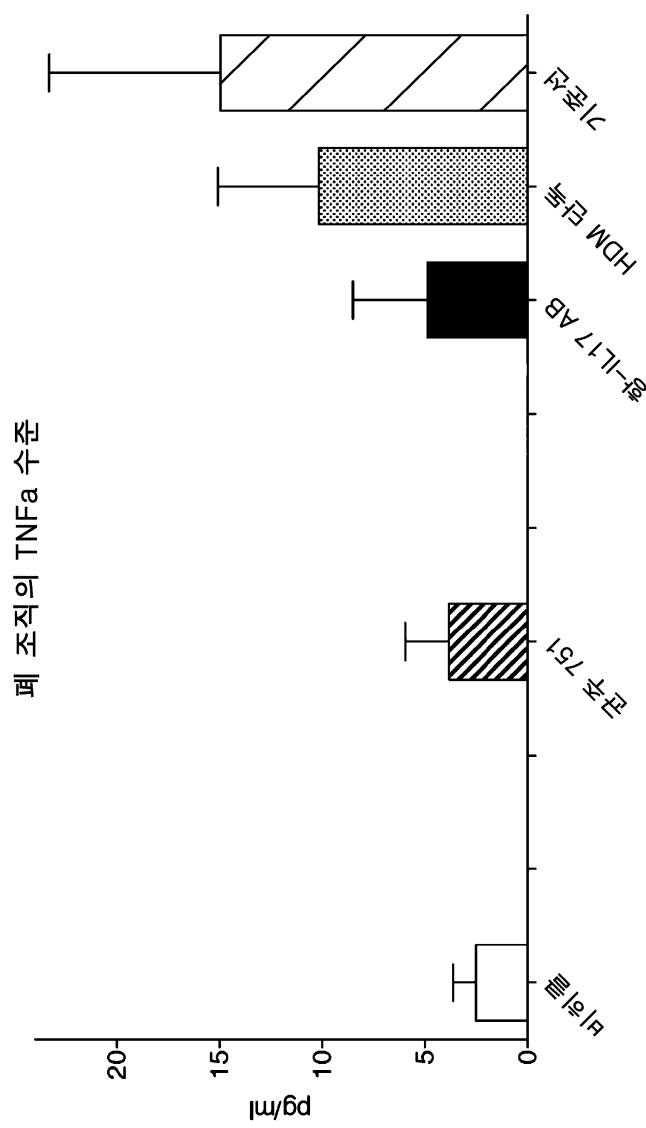
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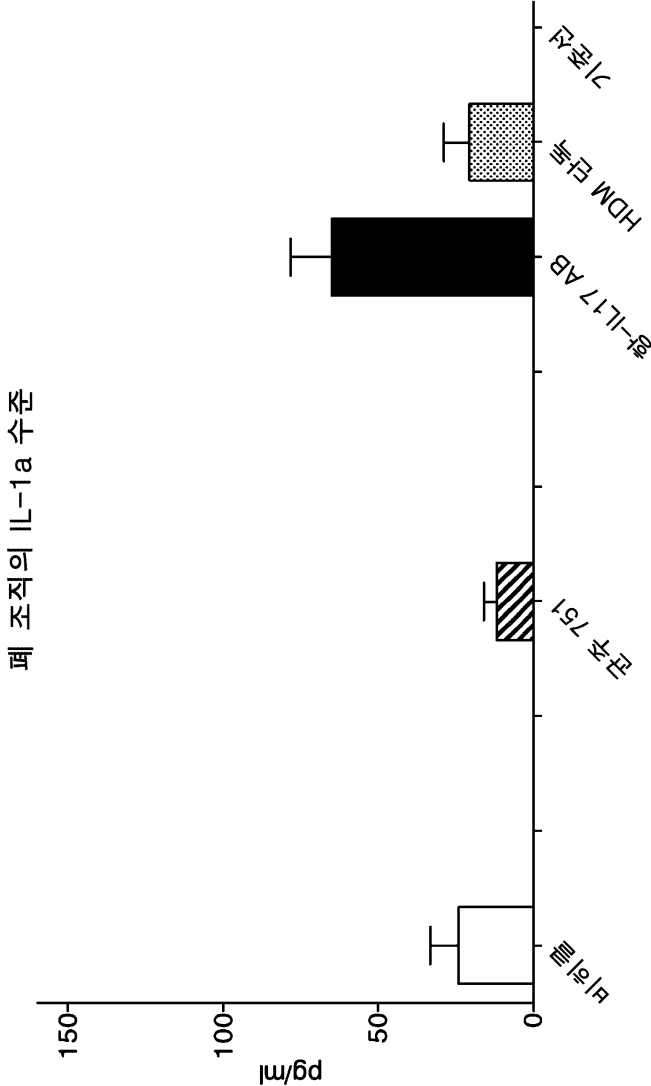
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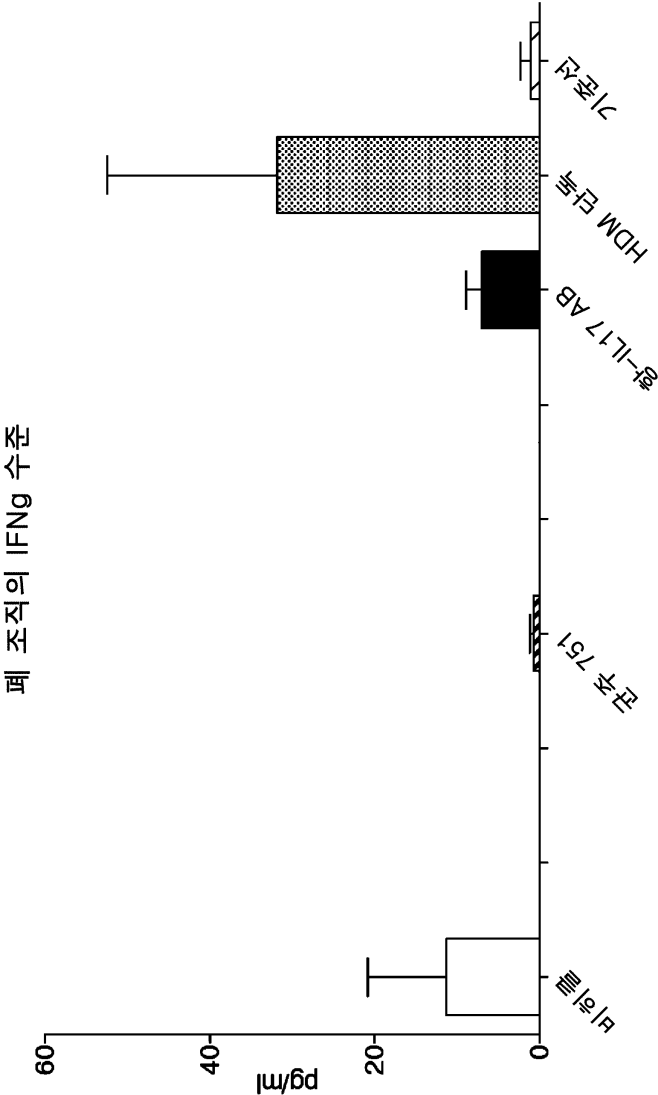
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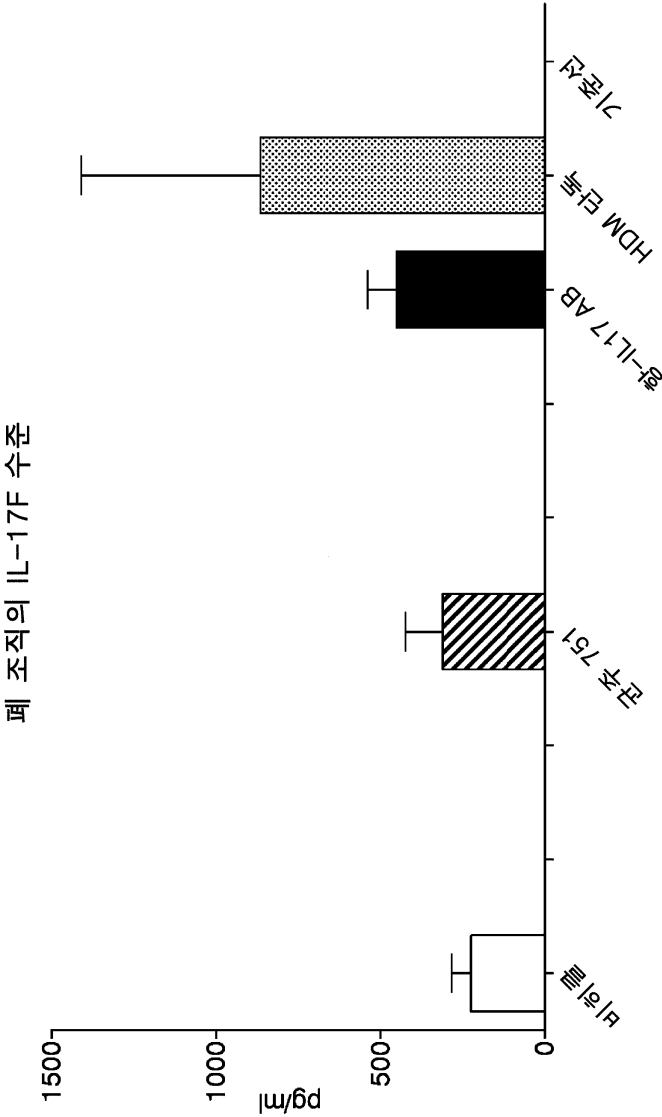
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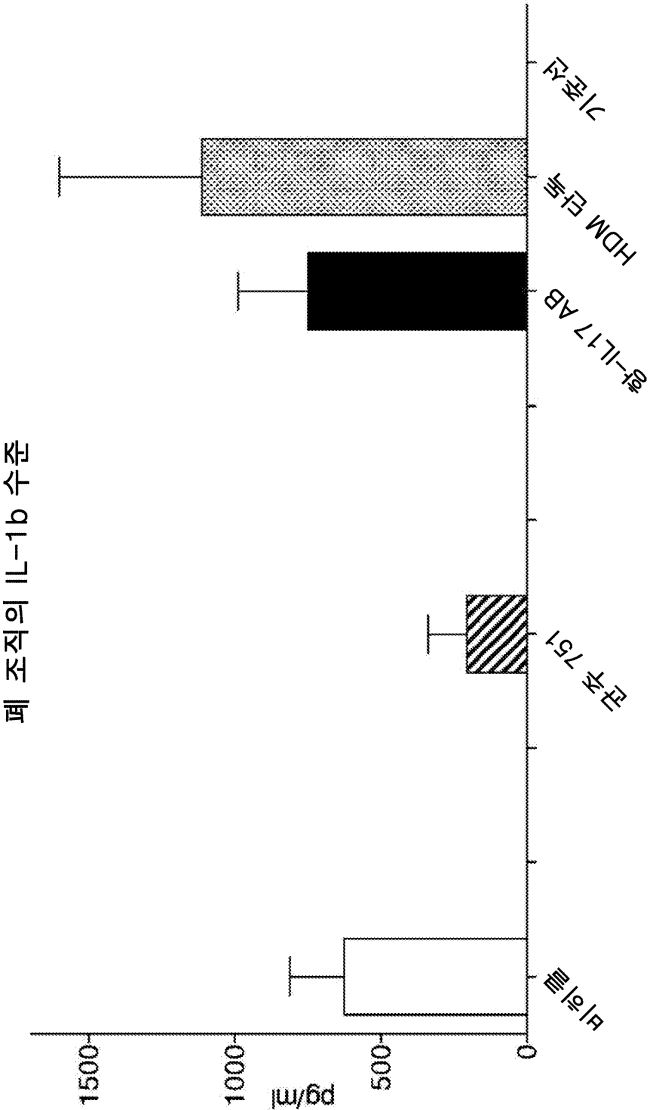
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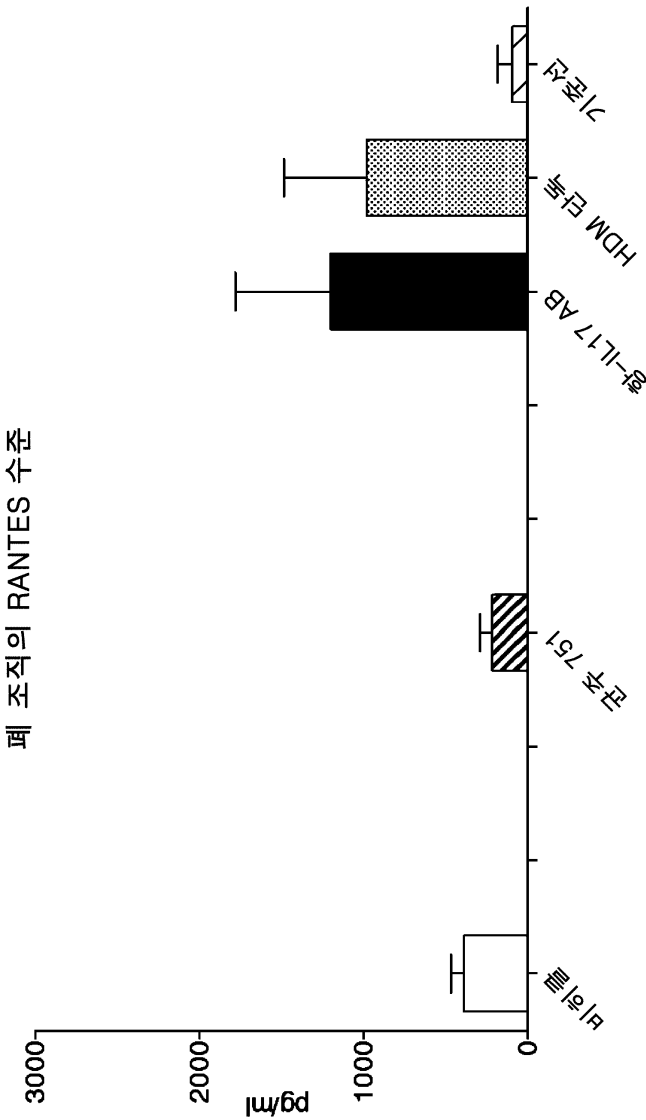
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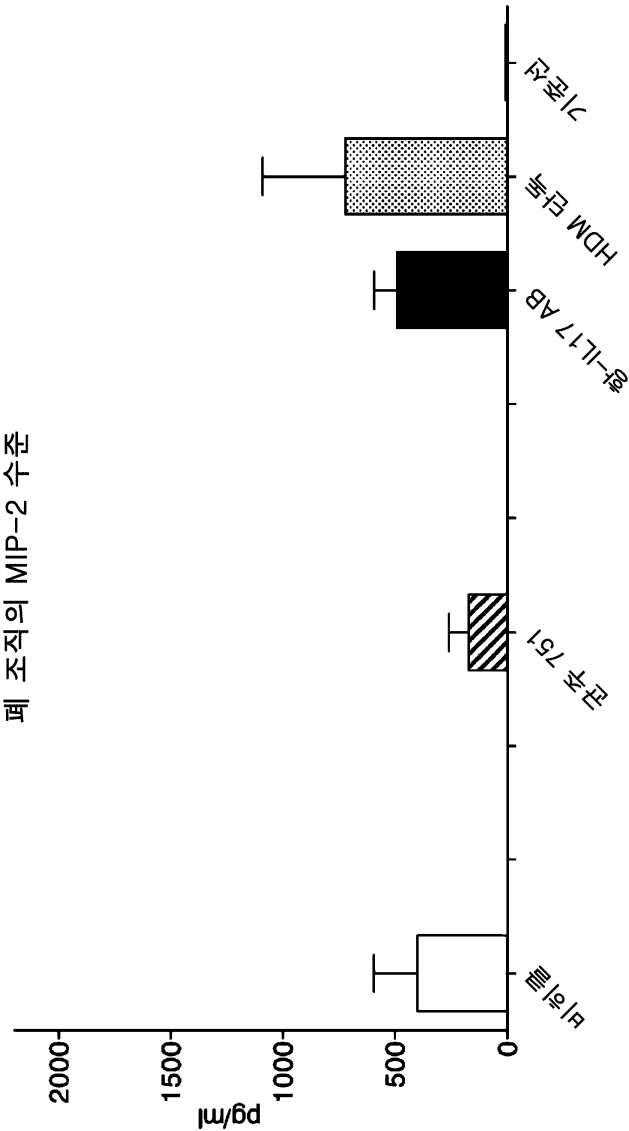
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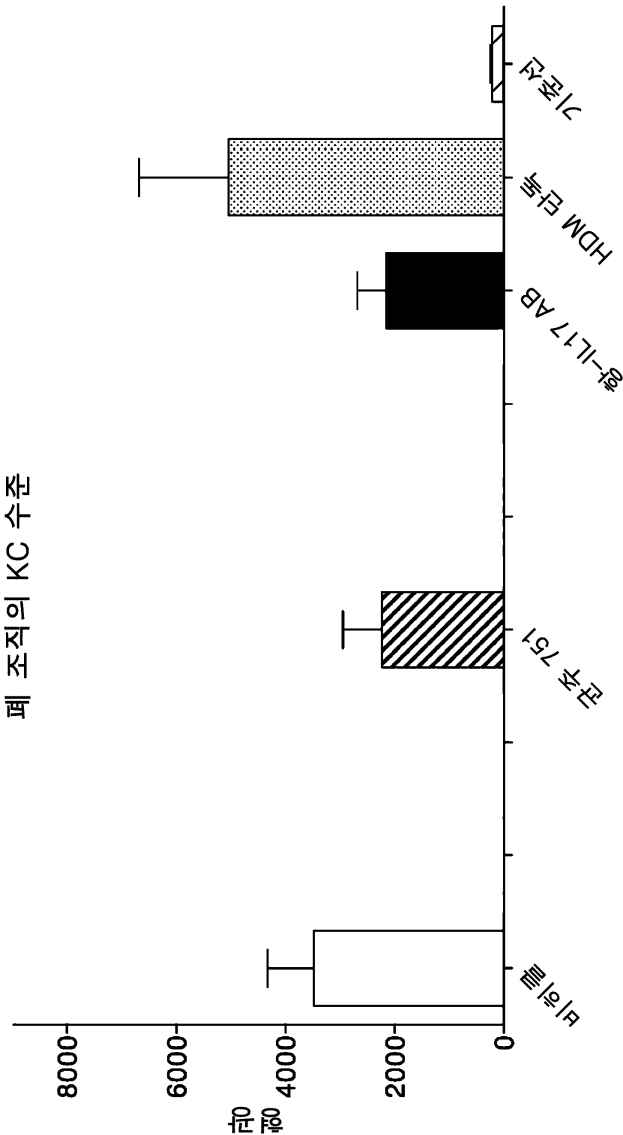
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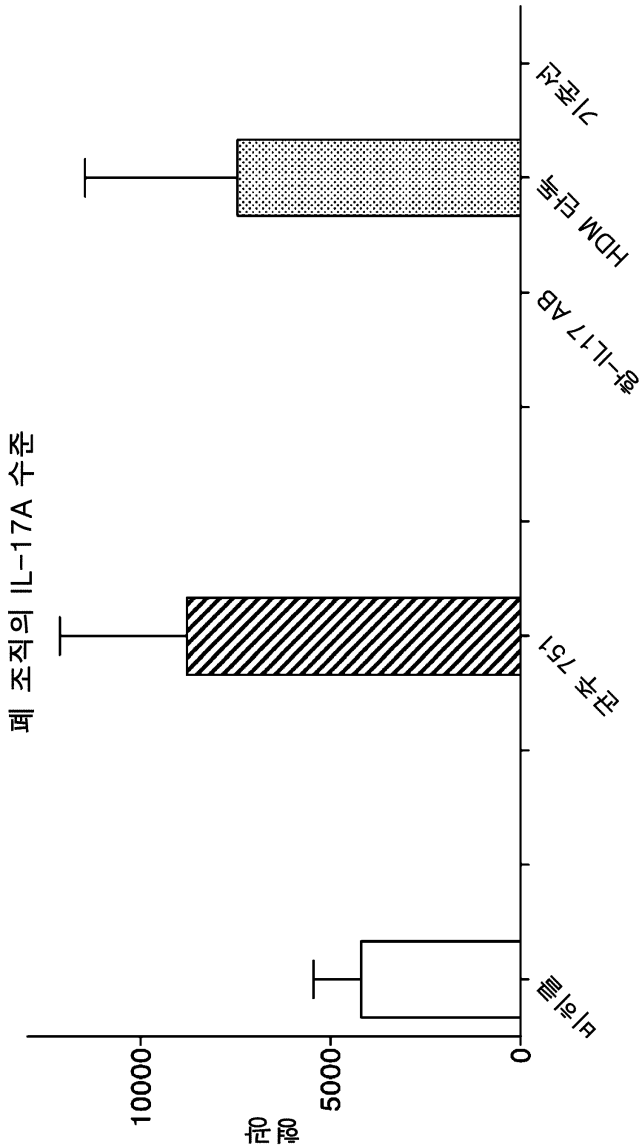
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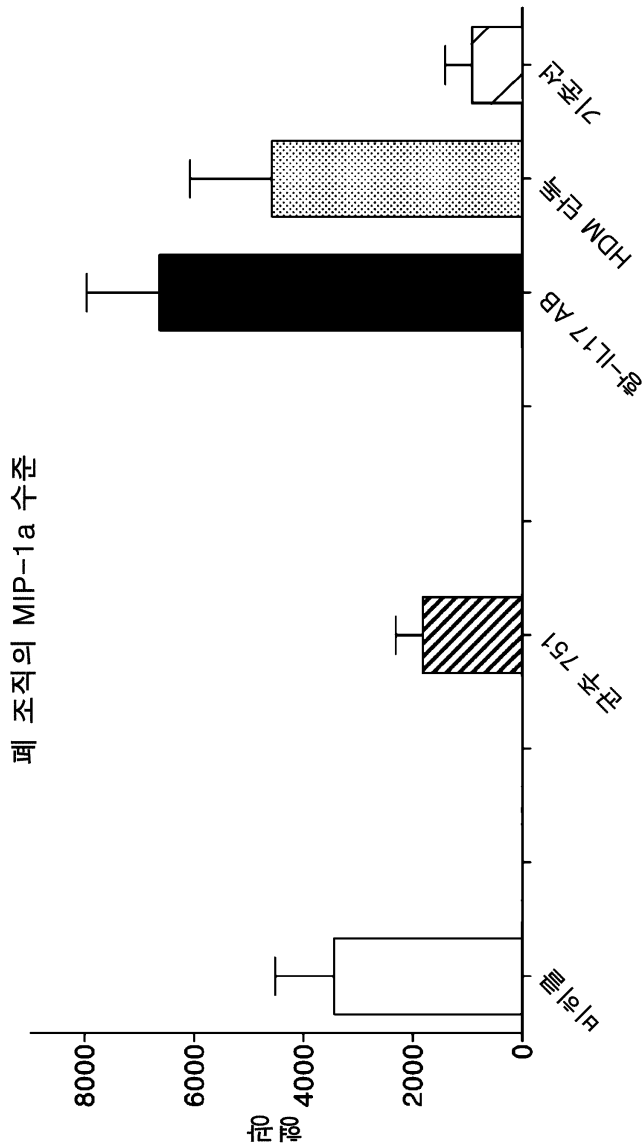
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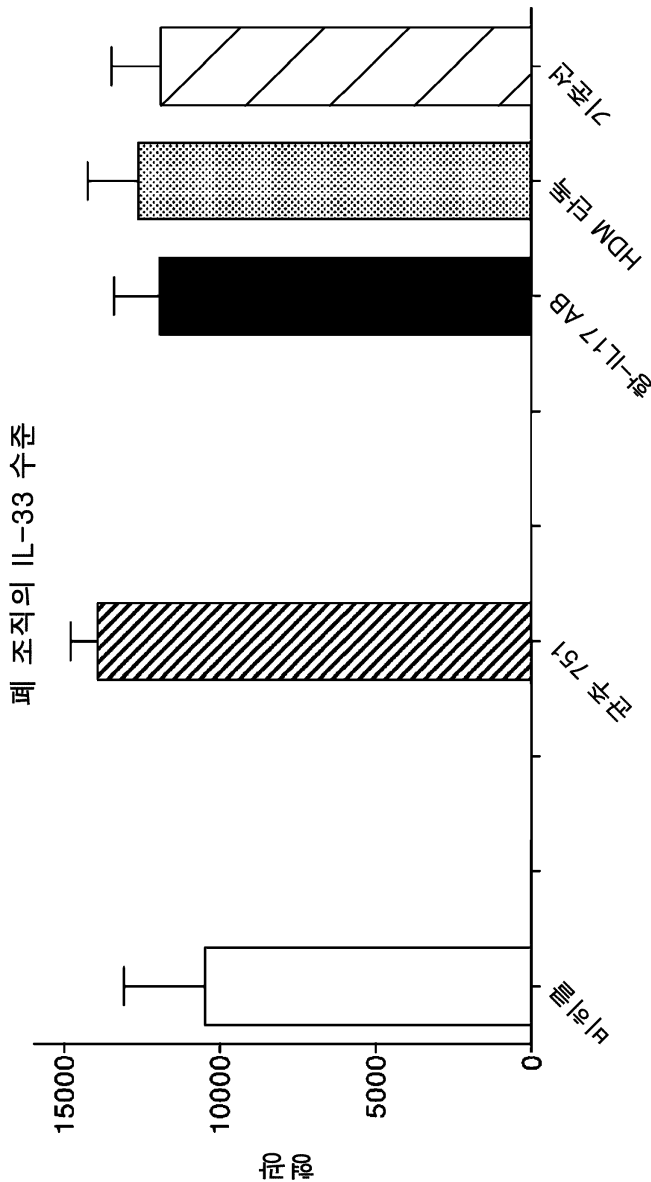
도면62



도면63

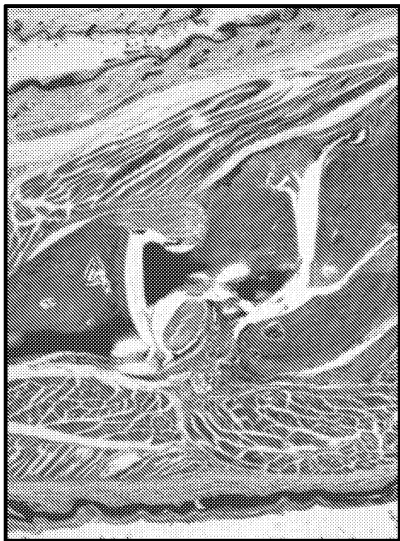


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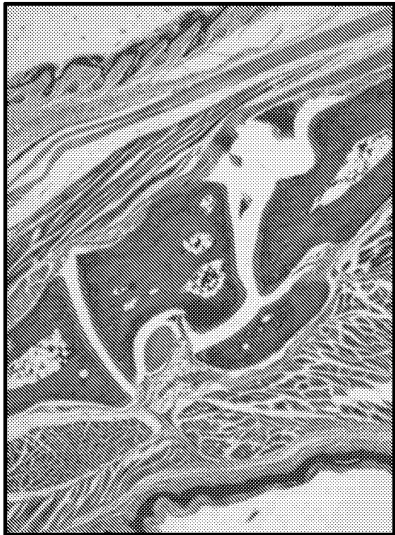


도면65a

조직병리학 채점을 위한 시각적 템플릿



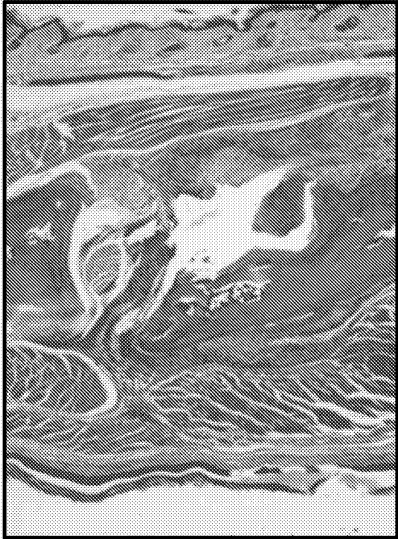
도면 1



도면 0

도면65b

(제 4)

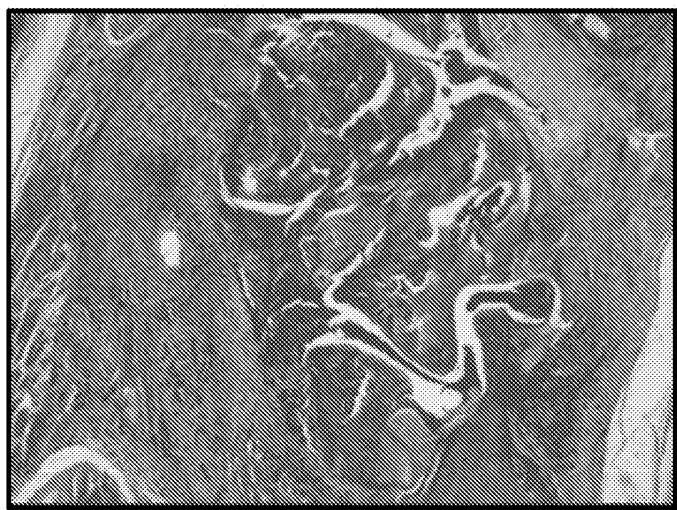


4
미크로



7
미크로

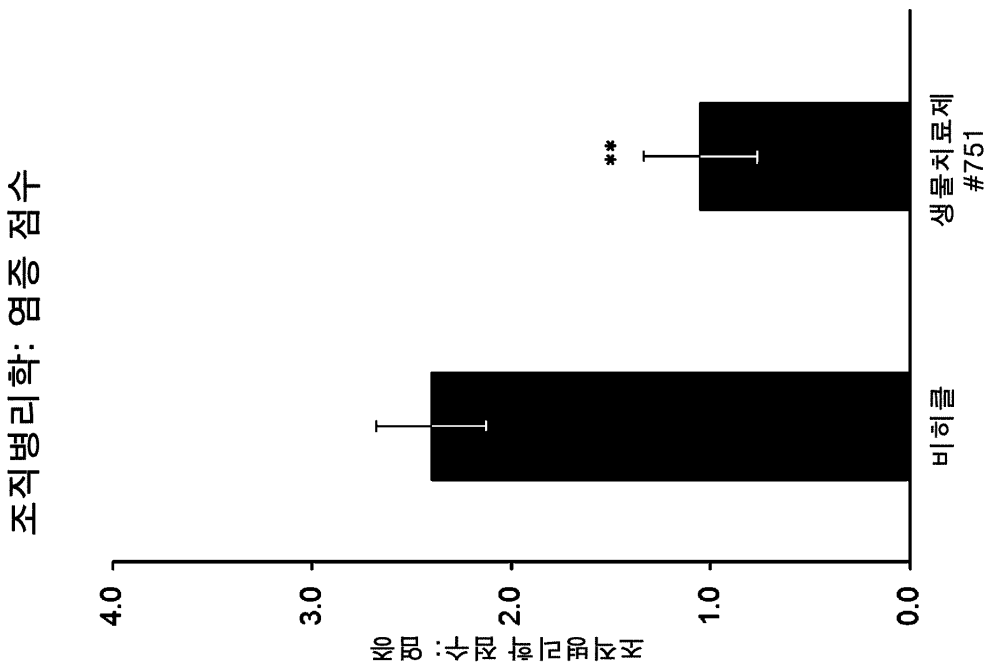
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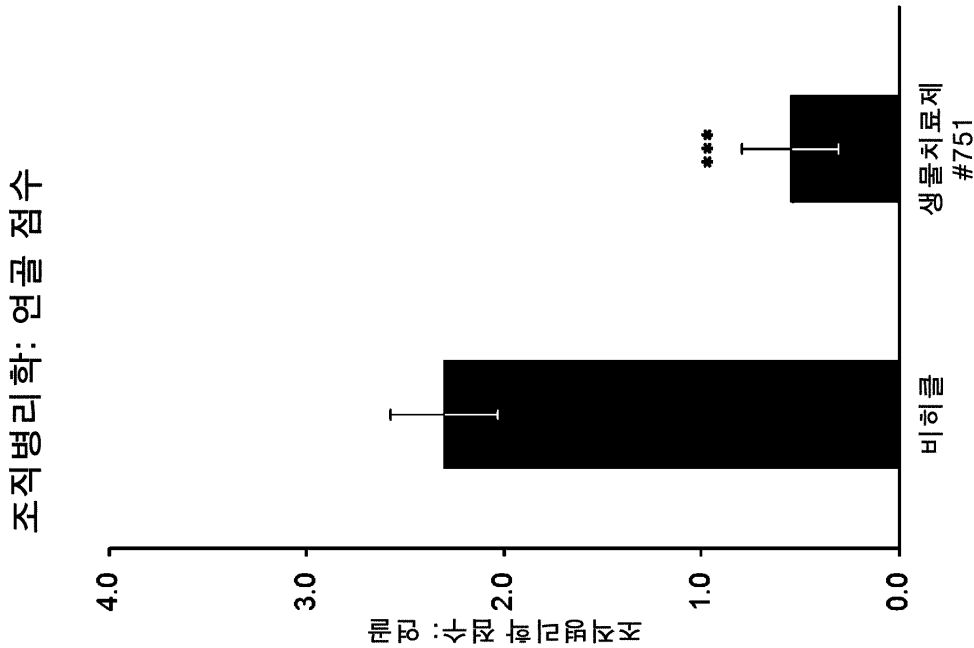
등급 9

(계속)

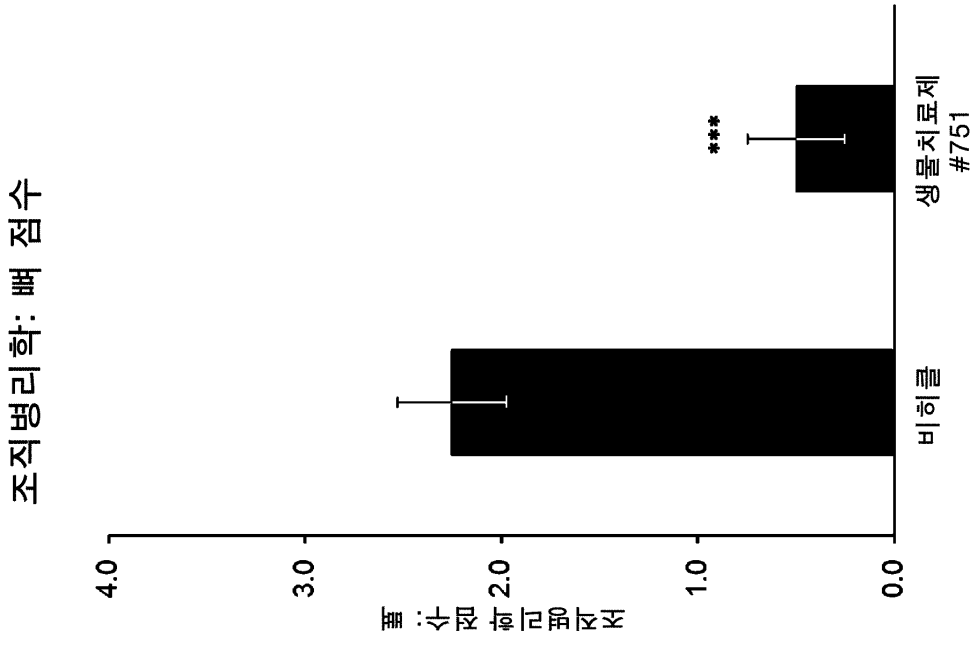
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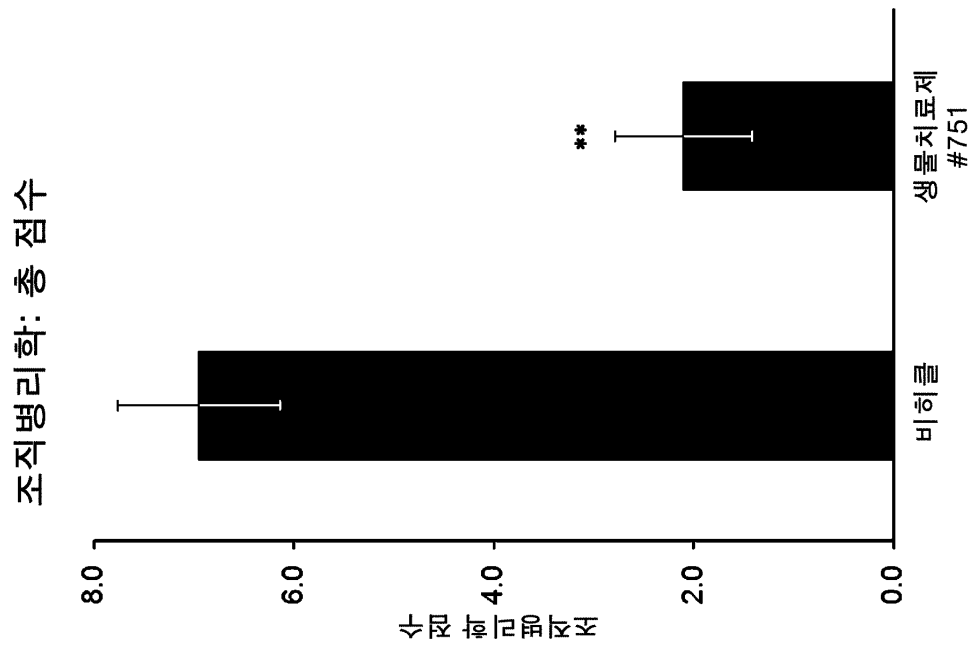
도면67



도면68



도면69



도면70

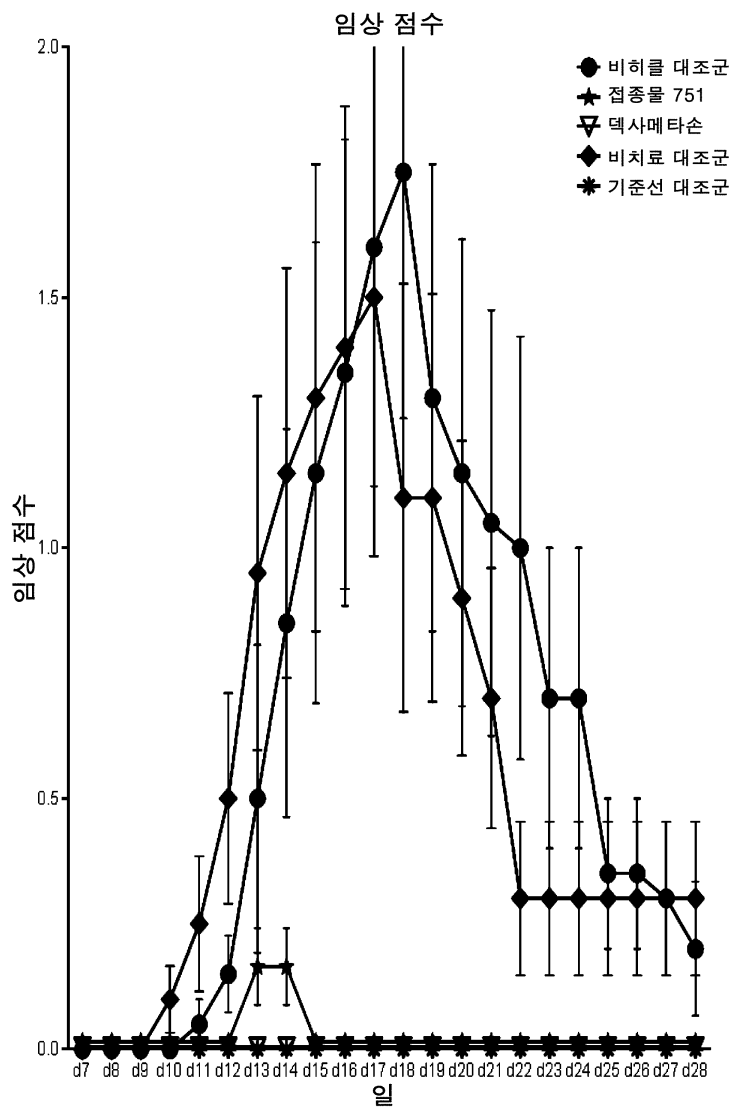


관주 #751 (#2.7R, 등급 0)

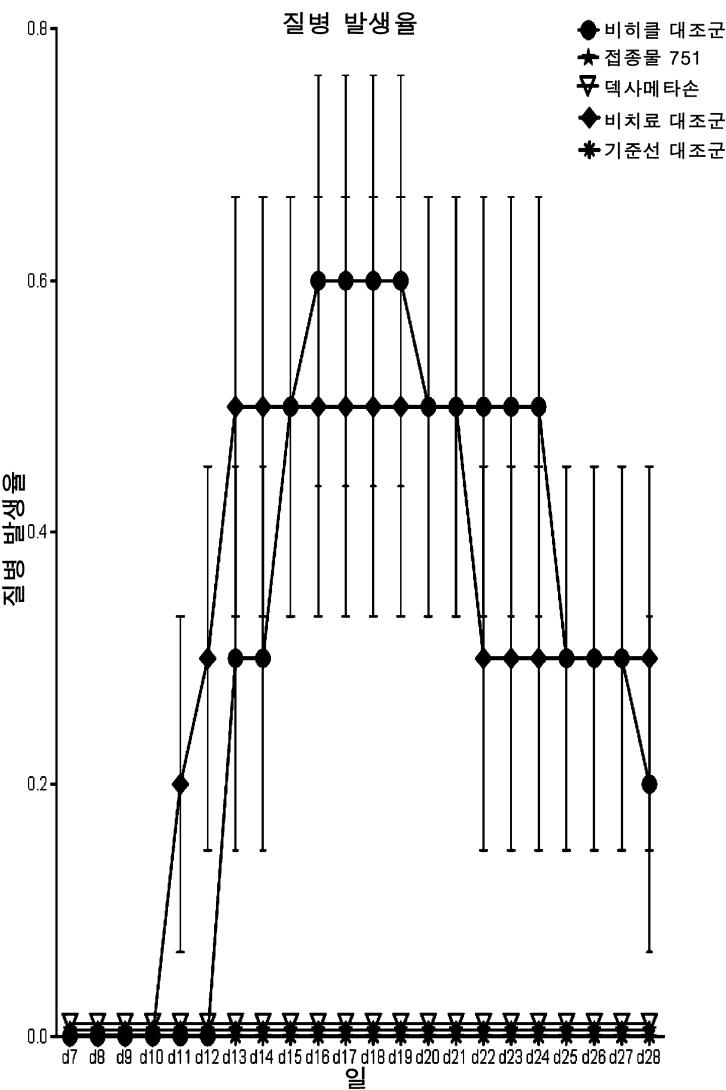


비히클 (#1.1 1R, 등급 9)

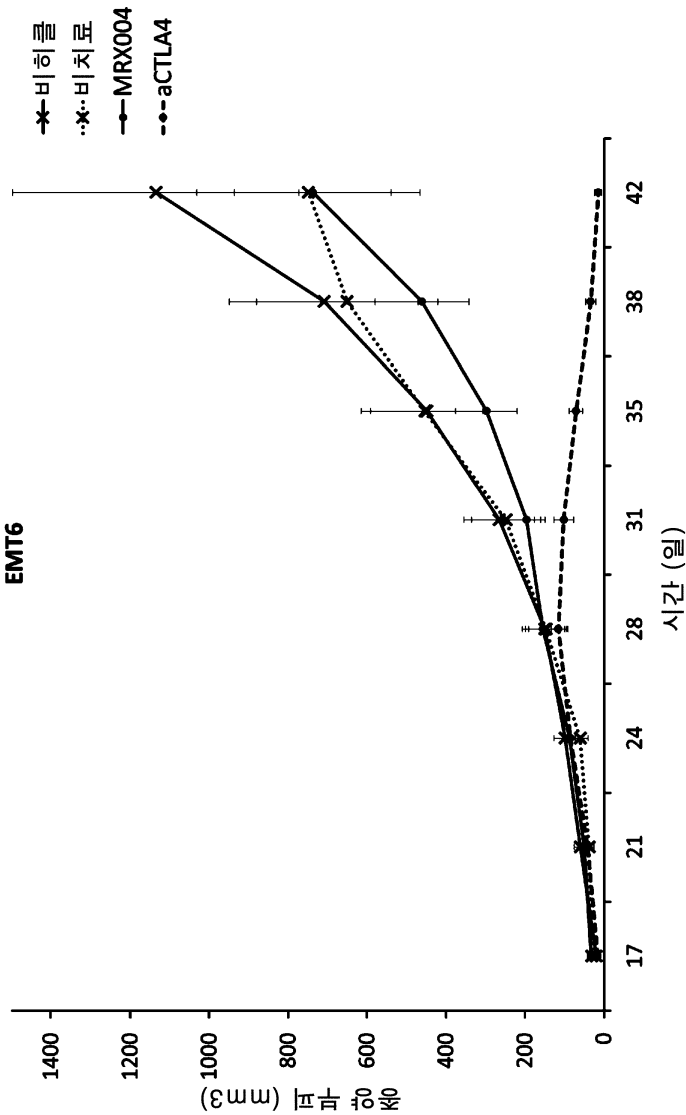
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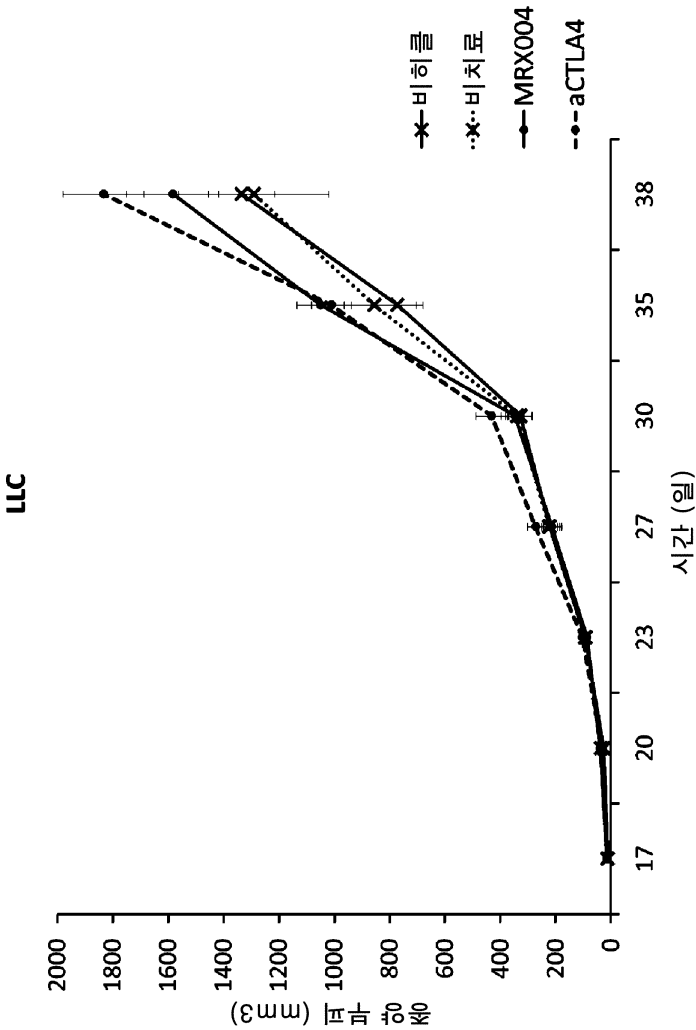
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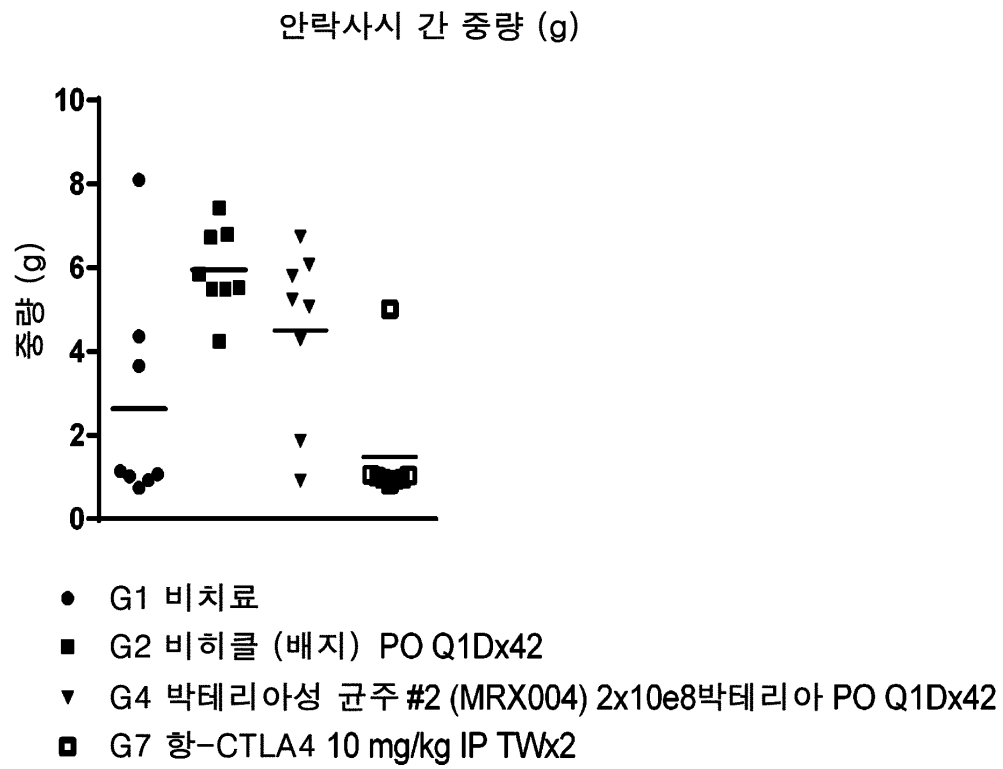
도면73



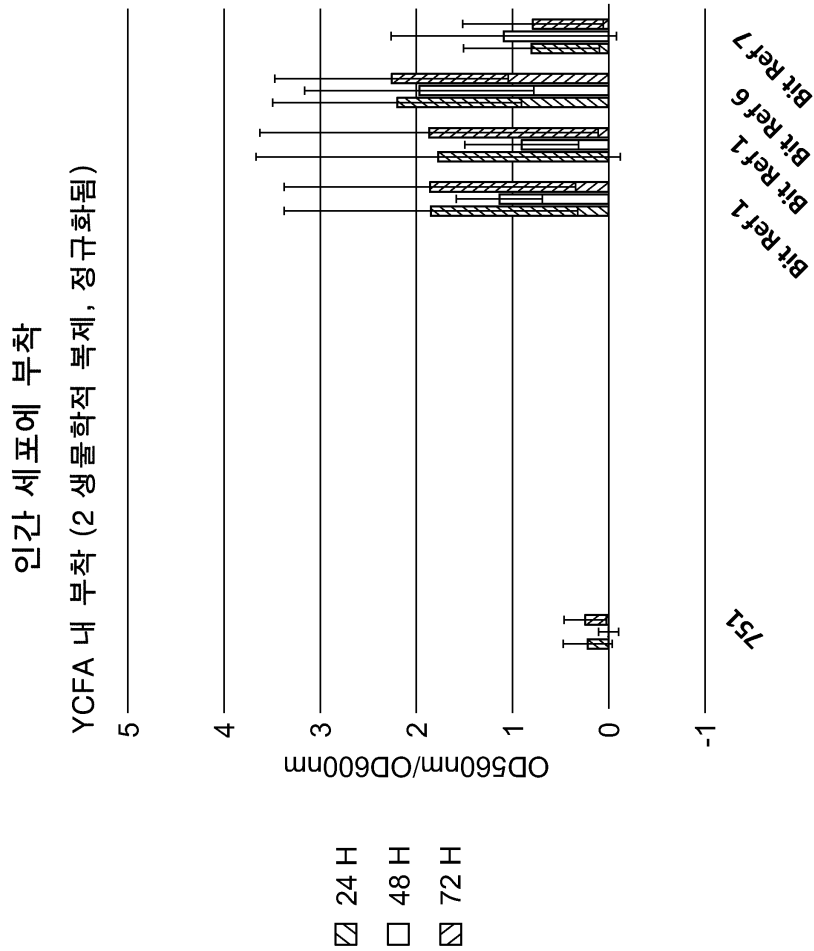
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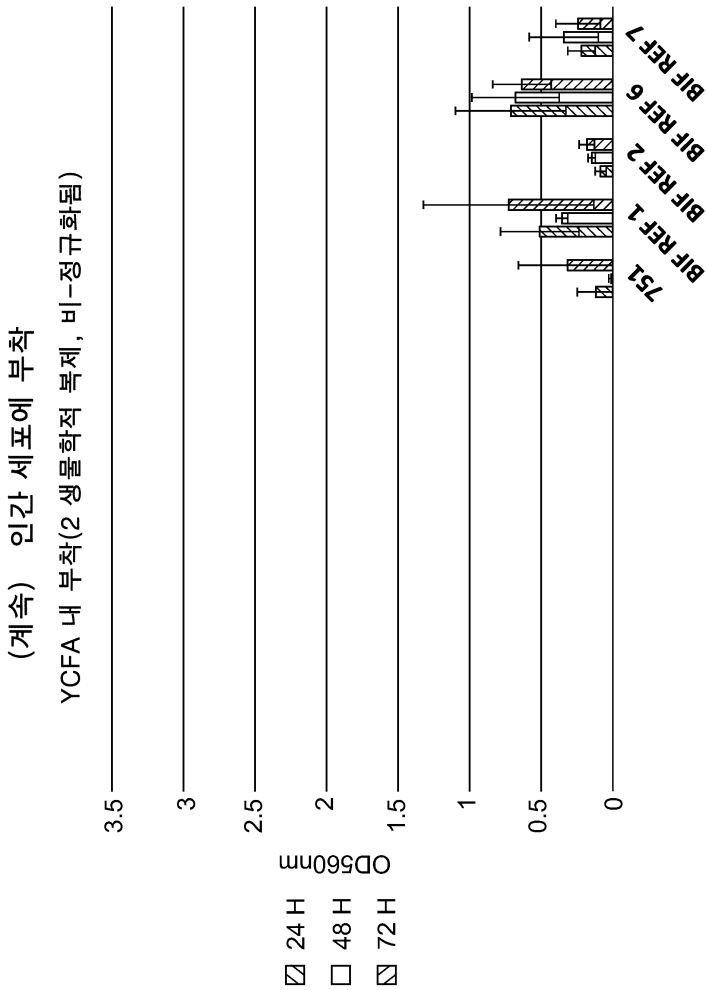
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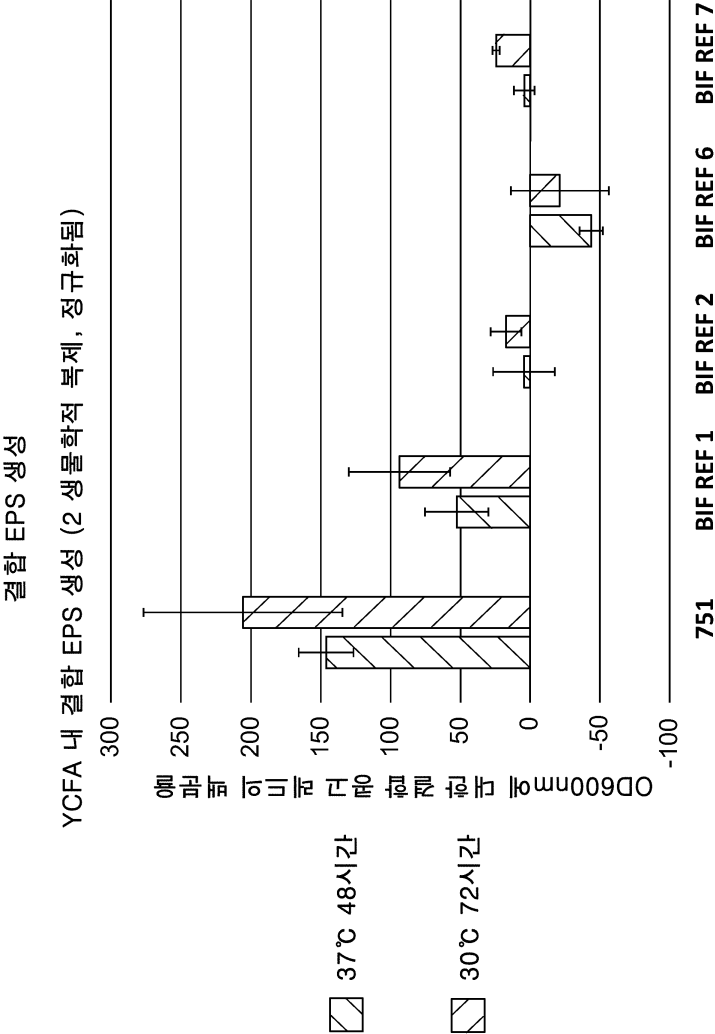
도면76a



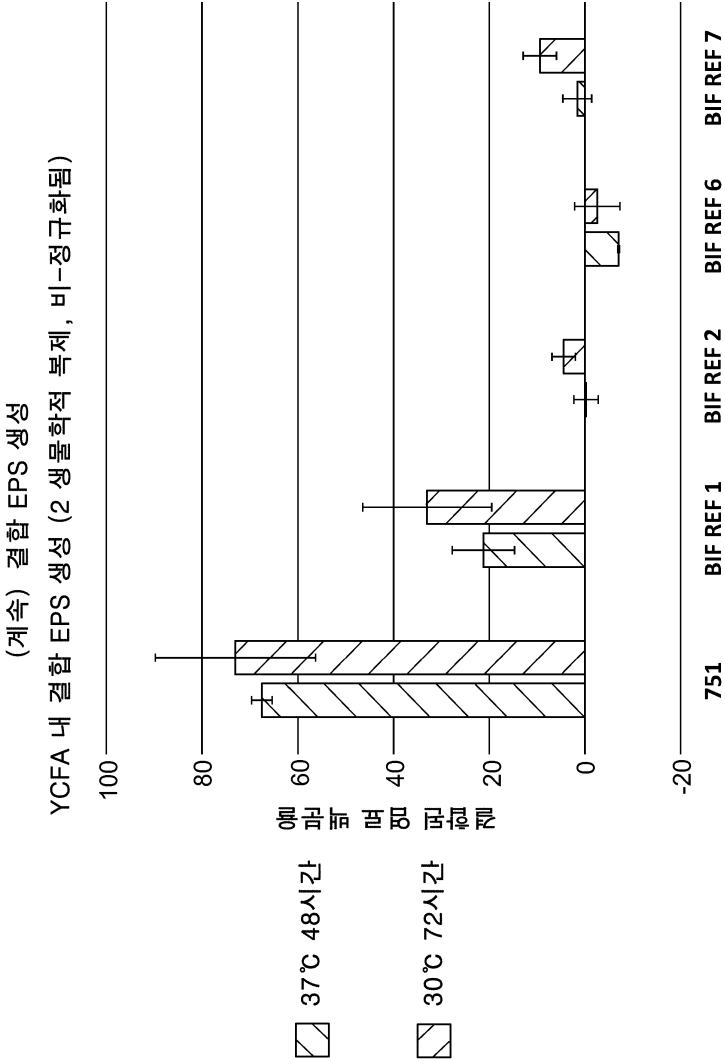
도면76b



도면77a

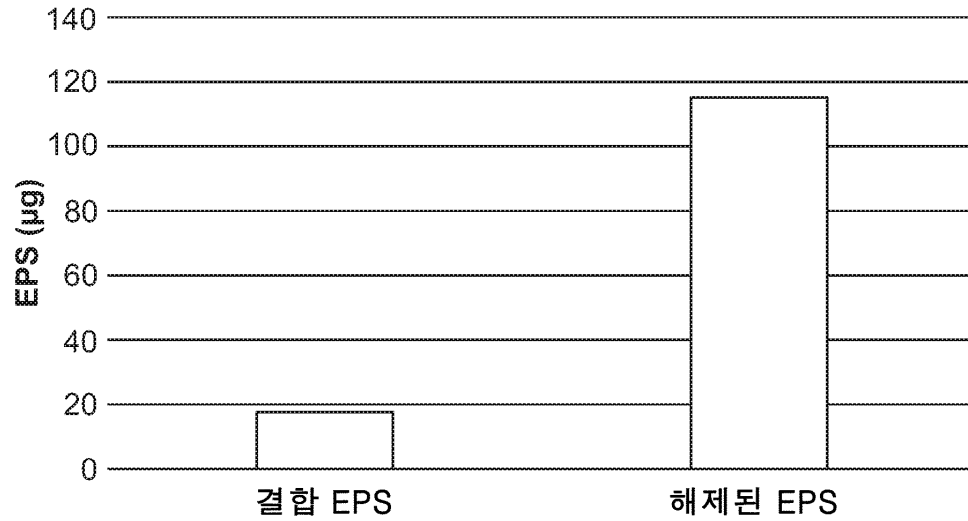


도면77b



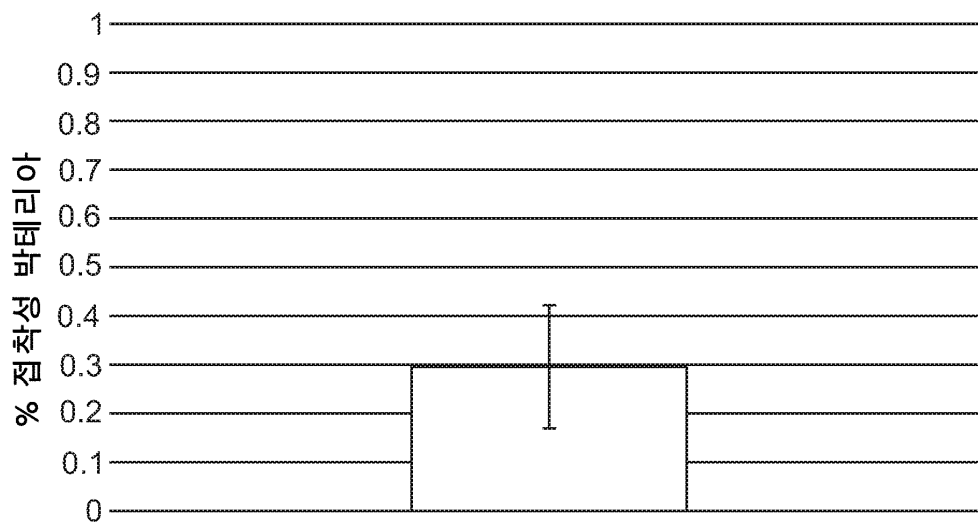
도면78

결합 및 해제
MRX004에 의한 엑소폴리사카라이드 생성

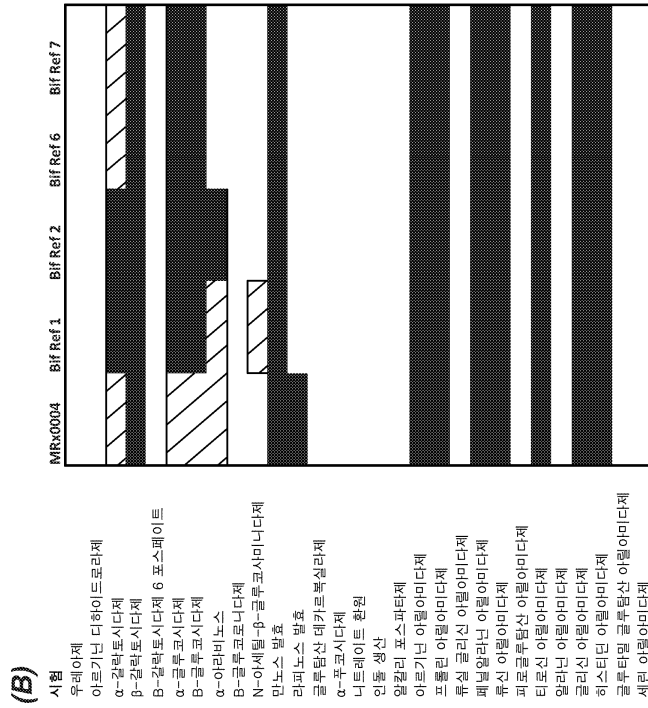
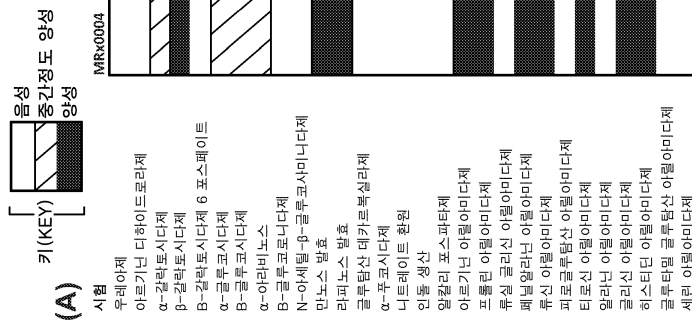


도면79

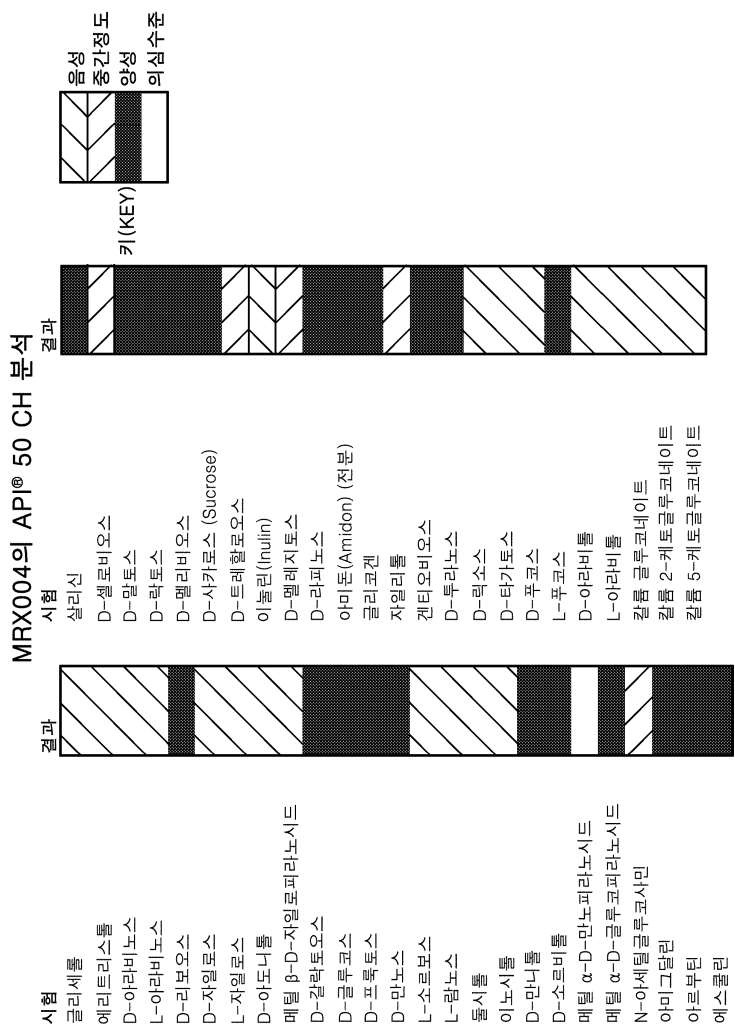
MRX004의 Caco-2 세포에 대한 부착



급속 ID 32 A 프로파일 - MRX004 대 비피도박테리아 브레베 형 균주



도면81



서열목록

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- <120> COMPOSITIONS COMPRISING BACTERIAL STRAINS
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- <150> GB 1510467.2
- <151> 2015-06-15
- <150> GB 1520501.6
- <151> 2015-11-20
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