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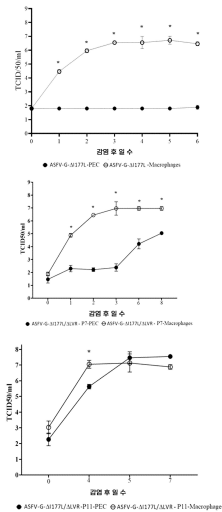
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(54) 발명의 명칭 안정적인 세포주에서 효율적인 성장을 가능하게 하는아프리카 돼지 열병 백신의 계놈 결실

(57) 요약

본 발명에서 고병원성 조지아 2007 분리주("ASFV-G")와 같은 다양한 ASFV 균주에 의해 유발되는 ASF를 예방하기 위한 재조합 아프리카돼지열병 바이러스(ASFV) 생독백신의 구성에 대한 세부 정보를 제공한다. 예시적인 백신은 안정된 세포주에서 산업적 규모로 성장할 수 있도록 다중 유전자의 결실을 포함한다.

대표도 - 도1



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명세서

청구범위

청구항 1

유전자 변형 바이러스로서, 상기 바이러스 게놈이 서열번호 1과 95% 이상 동일한 바이러스성 게놈을 포함하는 유전자 변형 바이러스.

청구항 2

제1항에 있어서, 상기 바이러스성 게놈은 서열번호 1을 포함하는, 바이러스.

청구항 3

제1항의 유전자 변형 바이러스를 포함하는 아프리카 돼지 열병 바이러스(ASFV)에 대한 백신 조성물.

청구항 4

제3항에 있어서, 상기 ASFV는 ASFV-조지아 2007 분리주(ASFV-G)인, 백신 조성물.

청구항 5

제1항의 유전자 변형 바이러스를 포함하는 약독화 생백신을 임상 ASFV 질병으로부터 돼지를 보호하는 데 효과적인 양으로 돼지에 투여하는 것을 포함하는, ASFV로부터 돼지를 보호하는 방법.

청구항 6

제5항에 있어서, 상기 ASFV는 ASFV-G인, 방법.

청구항 7

제5항에 있어서, 상기 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양은 제1항의 유전자 변형 바이러스의 10^2-10^6 HAD₅₀을 포함하는 백신인, 방법.

청구항 8

제조합 ASFV 돌연변이 바이러스로서, ORF MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF3608L, MGF360-9L, MGF360-10L, MGF360-11L의 결실 또는 이들 각각이 부분 결실된 제조합 바이러스.

청구항 9

제8항에 있어서, 상기 바이러스는 서열번호 2와 적어도 95% 동일한 게놈 단편의 결실을 포함하는, 제조합 바이러스.

청구항 10

제8항에 있어서, 상기 돌연변이 ASFV는 ASFV-조지아 분리주인, 제조합 바이러스.

청구항 11

제8항에 있어서, 상기 돌연변이 ASFV는 서열번호 1과 적어도 99% 동일한 게놈을 포함하는, 제조합 바이러스.

청구항 12

제8항의 제조합 바이러스를 포함하는 ASFV-G에 대한 백신 조성물.

청구항 13

제8항의 제조합 바이러스를 포함하는 약독화 생백신을 임상 ASFV 질병으로부터 돼지를 보호하는 데 효과적인 양

으로 돼지에 투여하는 것을 포함하는, ASFV로부터 돼지를 보호하는 방법.

청구항 14

제13항에 있어서, 상기 ASFV는 ASFV-G인, 방법.

청구항 15

제14항에 있어서, 상기 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양은 제8항의 유전자 변형 바이러스의 10^2-10^6 HAD₅₀을 포함하는 백신인, 방법.

청구항 16

배양된 안정적인 세포주에서 10^4-10^7 HAD₅₀/mL의 역가로 ASFV를 생산하는 방법으로서, 상기 배양된 안정적인 세포주에 제1항의 바이러스를 접종하는 단계; 상기 접종된 세포주를 바이러스 복제가 가능한 조건 하에서 배양하는 단계; 및 상기 바이러스를 10^4-10^7 HAD₅₀/mL의 역가로 성장시키는 단계를 포함하는, 방법.

청구항 17

제16항에 있어서, 상기 안정적인 세포주는 소 αvβ6 인테그린을 발현하도록 조작된 돼지 태아 신장 세포주인, 방법.

청구항 18

제16항에 있어서, 상기 ASFV 바이러스 게놈은 서열번호 1과 적어도 99% 동일한 바이러스 게놈을 포함하는, 방법.

발명의 설명

기술 분야

[0001] 본 발명은 고병원성(highly virulent) 조지아 2007 분리주("ASFV-G")와 같은 다양한 ASFV 균주에 의해 유발되는 ASF를 예방하기 위한 재조합 아프리카 돼지열병 바이러스(ASFV) 생 약독화 백신의 구성에 관한 세부사항을 제공한다. 예시적인 백신은 안정적인 세포주에서 효율적인 성장을 가져오는 결실(deletion)을 포함하여 산업 수준의 ASFV 백신을 생산할 수 있도록 한다.

배경 기술

[0002] 아프리카 돼지열병 바이러스(ASFV) 균주 조지아(ASFV-G)는 아스파르바이러스과(family *Asfarviridae*)에 속하는 유일한 바이러스이며, 현재 중부 유럽에서 중국과 남아시아에 이르는 인접한 넓은 지역에 영향을 미치는 팬데믹 질병의 원인균이다(Costard 외, *Philos. Trans. R. Soc. London B Biol.* (2009) 364:2683-96; O'Donnell 외, *J. Virol.*, (2015a) 89:6048-56). 이 팬데믹은 양돈 산업에 막대한 경제적 손실을 초래하고 전 세계적으로 단백질 가용성 부족을 유발하고 있다(O'Donnell 외, (2015a) 위 참조). ASFV는 복잡한 구조를 가진 매우 큰 바이러스이며, 150개 이상의 오픈 리딩 프레임(ORF)을 코딩하는 180-190 킬로베이스의 이중 가닥 DNA 게놈을 가지고 있다.

[0003] 상업적으로 이용 가능한 백신이 없기 때문에 질병 통제는 감수성(susceptible) 동물의 지리적 이동을 관리하고 감염된 동물을 도태시키는 데 전적으로 기반을 두고 있다(Costard 외, 위). 바이러스 독성과 관련된 특정 바이러스 유전자를 삭제하는 모체 독성 바이러스의 유전자 조작을 통해 약독화 바이러스 균주를 개발하여 효과적인 실험용 백신을 얻었다(O'Donnell 외, (2015a) 위, O'Donnell 외, *J. Virol.*, (2015b) 89:8556-66; O'Donnell 외, *J. Virol.*, (2017) 91:e01760-16; Borca 외, *J. Virol.*, (2020) 94:e02017-19). 자연 숙주에서 바이러스가 독성을 발현하는 과정에서 바이러스 유전자의 기능을 이해하는 것은 유전자 조작을 이용한 실험용 백신을 합리적으로 개발하는 데 있어 매우 중요한 단계이다. 이러한 실험용 약독화 생백신은 ASF를 통제하기 위한 효과적인 대응책 개발을 위한 가장 진보된 접근법이라고 할 수 있다.

[0004] 상업적으로 실행 가능한 ASF 백신을 개발하는 데 있어 주요 기술적 문제는 이러한 바이러스 균주가 돼지 대식세포의 1차 배양에서만 효율적으로 복제되어 산업 수준의 대규모 생산을 방해한다는 것이다. 이 문제를 해결하기 위해 우리는 돼지 대식세포에서의 복제 능력을 유지하면서 안정적인 세포주에서 ASFV 백신의 증식을 가능하게 하고 사육용 돼지(domestic swine)에 접종했을 때 완전히 약화시킬 수 있는 돌연변이를 제시한다.

발명의 내용

해결하려는 과제

[0005] 본 발명은, 일 실시형태에서, 바이러스 게놈이 서열번호 1과 적어도 95% 동일한 바이러스 게놈을 갖는 유전자 변형 바이러스를 제공한다. 특정 실시형태에서, 바이러스 게놈은 서열번호 1과 동일하다.

과제의 해결 수단

[0006] 또한, 본 발명은 아프리카 돼지 열병 바이러스(ASFV)에 대한 백신 조성물로서, 서열번호 1과 적어도 95% 동일한 바이러스 게놈을 갖는 유전자 변형 바이러스를 포함하는 백신 조성물을 제공한다. 특정 실시형태에서, ASFV는 ASFV-조지아 2007 분리주(ASFV-G)이다.

[0007] 본 발명은 추가 실시형태에서, 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양으로 서열번호 1과 적어도 95% 동일한 바이러스 게놈을 갖는 유전자 변형 바이러스를 포함하는 약독화 생백신을 돼지에게 투여하는 것을 포함하는, ASFV로부터 돼지를 보호하는 방법을 추가로 제공한다. 특정 실시형태에서, 상기 ASFV는 ASFV-G이다. 특정 실시형태에서, 백신을 접종한 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양은 서열번호 1과 적어도 95% 동일한 바이러스 게놈을 갖는 유전자 변형 바이러스의 10^2 - 10^6 HAD₅₀을 포함하는 백신이다.

[0008] 본 발명에 의해 제공되는 추가 실시형태는 ORFs MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF3608L, MGF360-9L, MGF360-10L, MGF360-11L의 결실, 또는 이들 각각의 부분 결실을 갖는 재조합 ASFV 돌연변이 바이러스이다. 특정 실시형태에서, 바이러스는 서열번호 2와 적어도 95% 동일한 게놈 단편의 결실을 포함한다. 일부 실시형태에서, 돌연변이 ASFV는 ASFV-조지아 분리주(isolate)이다. 일부 실시형태에서, 돌연변이 ASFV는 서열번호 1과 적어도 99% 동일한 게놈을 갖는다. 이러한 바이러스는 또한 ASFV-G에 대한 백신 조성물의 일부일 수 있다.

[0009] 본 발명에 의해 제공되는 추가 실시형태는 ASFV로부터 돼지를 보호하는 방법으로서, ORFs MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF3608L, MGF360-9L, MGF360-10L, MGF360-11L의 결실 또는 이들 각각의 일부 결실을 갖는 재조합을 포함하는 약독화 생백신을, 상기 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양으로, 돼지에 투여하는 것을 포함한다. 특정 실시형태에서, ASFV는 ASFV-G이다. 일부 실시형태에서, 접종된 돼지를 임상 ASFV 질병으로부터 보호하는 데 효과적인 양은 유전자 변형 바이러스의 10^2 - 10^6 HAD₅₀을 포함하는 백신이다.

[0010] 본 발명에는 배양된 안정적인 세포주에서 10^4 - 10^7 HAD₅₀/mL의 역가로 ASFV를 생산하는 방법에 대한 실시형태가 추가로 제공되며, 이는 배양된 안정적인 세포주에 서열번호 1과 적어도 95% 동일한 바이러스 게놈을 갖는 유전자 변형 바이러스를 접종하는 단계; 바이러스 복제가 가능한 조건에서 접종된 세포주를 배양하는 단계; 및 상기 바이러스를 10^4 - 10^7 HAD₅₀/mL의 역가로 성장시키는 단계를 포함한다. 일부 실시형태에서, 안정적인 세포주는 소(bovine) αvβ₆ 인테그린을 발현하도록 조작된 돼지 태아 신장 세포주이다. 특정 실시형태에서, ASFV 바이러스 게놈은 서열번호 1과 적어도 99% 동일한 바이러스 게놈을 포함한다.

[0011] 참조에 의한 통합

[0012] 본 발명에 언급된 모든 출판물, 특허 및 특허 출원은 각 개별 출판물, 특허 또는 특허 출원이 구체적이고 개별적으로 참조에 의해 통합되는 것으로 표시된 것과 동일한 범위 내에서 본 발명에 참조에 의해 통합된다.

도면의 간단한 설명

[0013] 특허 출원 파일에는 컬러로 실행된 도면이 하나 이상 포함되어 있다. 컬러 도면이 포함된 본 특허 또는 특허 출원 공보의 사본은 요청 및 필요한 수수료 지불 시 특허청에서 제공한다.

본 발명의 새로운 특징들은 청구범위에 구체적으로 기재되어 있다. 본 발명의 특징 및 장점은 다음의 상세한 설명과 첨부된 도면에 참조된다:

도 1은 안정적인 돼지(porcine) 세포주 및 일차 돼지(swine) 대식세포 배양에서 ASFV-G- Δ I177L/ Δ LVR(상단), ASFV-G- Δ I177L/ Δ LVR p7(중간), 및 ASFV-G- Δ I177L/ Δ LVR p11(하단)의 체외 성장 특성을 보여주는 그래프적으로 표현한 데이터이다. 세포 배양물은 각 바이러스로 감염(MOI= 0.01)되고 감염 후 표시된 시간에 바이러스 수율을 적정했다(titrate). 데이터는 세 가지 독립적인 실험 결과의 평균을 나타낸다. 바이러스 검출 감도는 $\geq 1.8 \log_{10}$ HAD₅₀/ml이다. 특정 시점에 두 바이러스 간 바이러스 수율의 유의미한 차이(*)는 일관된 표준 편차를 가정하지 않고 Holm-Sidak 방법($\alpha = 0.05$)을 사용하여 결정되었다. 모든 계산은 소프트웨어 GraphPad Prism 버전 8에서 수행되었다. TCID₅₀, 50% 조직 배양 감염 용량.

도 2는 두 바이러스, 즉 ASFV-G- Δ I177L/ Δ LVR과 모체 ASFV-G- Δ I177L의 계놈을 그래프적으로 비교한 것이다.

도 3은 ASFV의 세포주 적응 돌연변이를 구성하기 위한 유전자 결실 접근법의 개략도를 보여준다. Δ LVR, 또는 MGF300 1L, 2R, 4L만을 결실시키는 Δ LVR에서의 부분 결실. 표시된 도너(donor) 플라스미드는 재조합 바이러스를 생산하는 표준 방법인 상동 재조합을 위한 템플릿(template) 역할을 한다.

발명을 실시하기 위한 구체적인 내용

- [0014] 고병원성 아프리카돼지열병 바이러스(ASFV) 조지아(ASFV-G) 균주가 현재 유럽 중부부터 아시아, 남아시아에 이르는 인접한 지역에 퍼져 막대한 경제적 손실을 초래하고 있는 팬데믹의 원인균이다. 현재 상용화된 백신은 없으며, 독성이 강한 모 바이러스를 유전자 조작을 통해 약독화시킨 생균주가 가장 진보된 실험용 백신이다. 최근 ASFV-G의 계놈에서 I177L 유전자를 삭제하여 백신 후보를 개발했다(미국 특허 출원 제16/580,058호 참조). 이 돌연변이 균주는 ASFV-G의 도전에 대한 보호를 유도하는 데 매우 안전하고 매우 효과적인 것으로 나타났다. 이 돌연변이(및 기타 재조합 ASFV 백신)를 산업 생산에 적용하는 데 있어 중요한 기술적 한계는 돼지 대식세포의 1차 배양에서만 복제된다는 점이다. 여기에서는 안정적인 돼지 세포주에서 효율적으로 성장할 수 있도록 왼쪽 가변 영역(LVR)에서 10.8kb가 결실된 파생 균주(ASFV-G- Δ I177L/ Δ LVR)의 개발을 소개한다(미국 특허 제9,121,010호 참조). LVR의 결실은 계놈의 왼쪽 끝에 있는 10개의 바이러스 유전자에 영향을 미친다. 이 결실은 지속적으로 안정적인 돼지 세포주에서 30회 이상의 추가 계대(passage) 후에도 안정적으로 유지되었다. 중요한 것은 ASFV-G- Δ I177L/ Δ LVR이 모 백신과 동일한 수준의 약독화, 면역원성 특성 및 독성 ASFV-G 도전에 대한 보호 효능을 유지했다는 것이다. 합리적으로 설계된 고효능 ASF 백신 후보가 계놈 안정성을 유지하면서 안정적인 세포주에서 성장하는 것이 입증된 것은 이번이 처음이며, 산업 수준에서 ASFV 백신을 생산할 수 있게 되었다.
- [0015] 본 발명의 바람직한 실시형태들이 도시되고 여기에 설명되어 있다. 당업자에게 그러한 실시형태들은 예시적인 방법으로만 제공된다는 것은 명백할 것이다. 당업자에게는 본 발명을 벗어나지 않는 범위 내에서 수많은 변형, 변경 및 치환이 발생할 것이다. 본 명세서에 기재된 본 발명의 실시형태에 대한 다양한 대안이 본 발명의 실시예에 사용될 수 있다. 포함되는 청구범위는 본 발명의 범위를 정의하며, 이러한 청구범위 범위 내의 방법 및 구조와 그 균등물들이 이에 포함되도록 의도되었다.
- [0016] 본 명세서에서 사용되는 기술 및 과학 용어는 달리 정의되지 않는 한, 본 발명이 속하는 기술분야에서 통상의 당업자에게 통상적으로 이해되는 의미를 갖는다. 본 명세서에는 당업자에게 알려진 다양한 재료 및 방법론이 참조되어 있다. 재조합 DNA 기술의 일반적인 원리를 설명하는 표준 참고 문헌으로는 Sambrook 외, "Molecular Cloning: A Laboratory Manual", 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y., 1989; Kaufman 외, eds., "Handbook of Molecular and Cellular Methods in Biology and Medicine", CRC Press, Boca Raton, 1995; McPherson, ed., "Directed Mutagenesis: A Practical Approach", IRL Press, Oxford, 1991을 포함한다.
- [0017] 당업자에게 공지된 임의의 적절한 재료 및/또는 방법은 본 발명을 수행하는데 활용될 수 있다. 본 발명을 실시하기 위한 재료 및/또는 방법이 설명된다. 다음의 설명 및 실시예에서 참조되는 재료, 시약 등은 달리 명시되지 않는 한, 상업적 출처로부터 구할 수 있다. 본 발명은 유전자 변형 ASFV 균주를 생산하기 위한 방법을 교시하고 도구를 설명한다.
- [0018] 명세서 및 청구범위에 사용된 단수 "a", "an" 및 "the"의 사용은 문맥에서 명확하게 달리 지시하지 않는 한 복수의 참조를 포함한다.
- [0019] 본 발명에서 사용된 분리, 정제 또는 생물학적으로 순수하다는 용어는 참조된 재료의 원래 상태에서 일반적으로

수반되는 성분이 실질적으로 또는 본질적으로 없는 재료를 의미한다.

- [0020] "약"이라는 용어는 명시된 값의 플러스 또는 마이너스 10%로 정의된다. 예를 들어, 약 1.0g은 0.9g 내지 1.1g, 및 구체적으로 명시되었는지 여부에 관계없이 해당 범위 내의 모든 값을 의미한다.
- [0021] "본질적으로 다음으로 이루어진 핵산" 및 이의 문법적 변형 용어는 참조 핵산 서열과 20개 이하의 핵산 잔기가 다르며, 참조 핵산 서열의 기능도 수행하는 핵산을 의미한다. 이러한 변이체는 참조 핵산 서열보다 짧거나 긴 서열, 특정 위치에서 다른 잔기를 갖는 서열 또는 이들의 조합을 포함한다.
- [0022] "보조제(adjuvant)"라는 용어는 항원에 대한 면역 반응을 비특이적으로 향상시키는 물질 또는 비히클(vehicle)을 의미한다. 보조제는 항원이 흡착되는 미네랄(명반, 수산화 알루미늄 또는 인산염)의 현탁액; 또는 항원 용액이 광물성 오일에 유화되는 유중수(water-in-oil) 에멀전(예를 들어, 프룬드(Freund)의 불완전 보조제)을 포함할 수 있으며, 때로는 항원성을 더욱 향상시키기 위해 사멸된 마이코박테리아(프룬드의 완전 보조제)가 포함될 수도 있다. 면역 자극 올리고뉴클레오타이드도 보조제로 사용할 수 있다(예: 미국 특허 제6,194,388호; 제6,207,646호; 제6,214,806호; 제6,218,371호; 제6,239,116호; 제6,339,068호; 제6,406,705호; 및 제6,429,199호 참조). 보조제에는 동시자극 분자(costimulatory molecules)와 같은 생물학적 분자도 포함된다.
- [0023] "투여하다(administer)"/"투여(administration)"라는 용어는 효과적인 경로를 통해 대상에게 치료제와 같은 물질을 제공하는 모든 방법을 의미한다. 투여의 예시적인 경로는 주사(피하, 근육 내, 피부 내, 복강 내, 정맥 내 등), 경구, 경관 내, 설하, 직장, 경피, 비강 내, 질 및 흡입 경로를 포함하지만, 이에 제한되지 않는다.
- [0024] 본 발명에서 사용되는 "코딩 서열" 및 "코딩 영역"이라는 용어는 RNA, 단백질, 또는 RNA 또는 단백질의 일부의 합성을 위한 유전 정보를 암호화하는 뉴클레오타이드 서열 및 핵산 서열(RNA 및 DNA 모두 포함)을 의미한다.
- [0025] 본 발명에 제공된 조성물의 "유효량"이라는 용어는 유효량이 발현되는 특정 기능을 수행할 수 있는 조성물의 양을 가리킨다. 필요한 정확한 양은 관련된 조성물 및 공정과 같은 인식된 변수에 따라, 조성물마다 및 기능마다 다를 수 있다. 유효량은 하나 이상의 애플리케이션에서 전달될 수 있다. 따라서 정확한 양을 특정하는 것은 불가능하지만, 숙련된 기술자가 일상적인 실험을 통해 적절한 "유효량"을 결정할 수 있다.
- [0026] "I177L", "ASFV I177L" 및 "게놈 I177L"이라는 용어는 동의어이며, 177개의 아미노산 단백질을 암호화하고 서열 번호 3의 뉴클레오타이드 위치 175473과 176006 사이의 역가닥(reverse strand)에 위치한 ASFV-G 오픈 리딩 프레임 I177L을 가리킨다.
- [0027] 본 발명의 맥락에서, "세포주 적응 돌연변이"라는 용어는 ASFV 게놈의 변형으로 인해 ORF MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF3608L, MGF360-9L, MGF360-10L, MGF360-11L의 전체 또는 부분적인 결실을 초래하는 것을 의미한다(도 2). 특정 실시형태에서, 본 용어는 서열번호 2로 개시된 ASFV-G 게놈 서열의 결실을 가리킨다.
- [0028] 본 발명과 관련하여, "비-기능 게놈(non-functional genomic)" MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF3608L, MGF360-9L, MGF360-10L 또는 MGF360-11L이라는 용어는 ASFV의 게놈에 위치하는 변형된 유전자 또는 ORF를 지칭하며, 이러한 ASFV 유전자의 변형은 변형되지 않은 기능성 ASFV 유전자와 비교하여 유전자 산물이 전혀 없거나 생물학적으로 기능하지 않는 유전자 산물을 초래한다. 이러한 변형은 코딩 서열의 전체 또는 부분적 결실, 오픈 리딩 프레임의 중단(예: 시프트 돌연변이 삽입 또는 넌센스 코돈 삽입), 업스트림 또는 다운스트림 조절 요소의 변경(modification) 및/또는 그러한 ASFV 유전자(들)의 기능적 발현을 비활성화하거나 늦추는 기타 현재 알려져 있거나 생각할 수 있는 방법을 포함할 수 있지만 이에 국한되지 않는다.
- [0029] "ASFV-G-ΔI177LΔLVR"이라는 용어는 본 발명의 특정 실시형태, 즉 서열번호 1의 게놈 서열을 갖는 백신/바이러스를 설명하기 위해 사용된다. "ASFV-G ΔI177L"이라는 용어는 "ASFV-G-ΔI177LΔLVR"의 모 균주를 지칭하기 위해 사용되지만, "세포주 적응 돌연변이"가 결여된 균주를 지칭한다.
- [0030] '면역화(immunize)'란 백신 접종 등을 통해 대상을 전염병으로부터 보호하는 것을 의미한다.
- [0031] 본 발명의 목적상, 백분율로 표현되는 두 개의 관련 뉴클레오타이드 또는 아미노산 서열의 "서열 동일성"(백분율로 나타냄)은, 두 개의 최적으로 정렬된 서열 중에서 동일한 잔기를 갖는 위치 수(x100)를, 비교되는 위치 수로 나눈 것을 의미한다. 갭(gap), 즉 잔기가 한 서열에서 한쪽 서열에는 존재하지만 다른쪽 서열에는 없는 정렬상의 위치는, 비동일 잔기가 존재하는 위치로 간주된다. 두 염기서열의 정렬은 니들만과 운쉬 알고리즘에 의해 수행된다(Needleman and Wunsch, J Mol Biol, (1970) 48:3, 443-53). 컴퓨터 지원 시퀀스 정렬은 갭 생성 페널티

가 50이고 갭 확장 페널티가 3인 기본 점수 매트릭스(default scoring matrix)를 사용하여 위스콘신 패키지 버전 10.1(미국 위스콘신주 매디슨, Genetics Computer Group)의 일부인 GAP와 같은 표준 소프트웨어 프로그램을 사용하여 편리하게 수행할 수 있다.

- [0032] 두 개의 폴리뉴클레오타이드 또는 폴리펩타이드와 관련하여, "높은 퍼센트로 동일한(high percent identical)" 또는 "높은 퍼센트 동일성"이라는 문구 및 그의 문법적 변이는 최대한 대응을 위해 비교 및 정렬될 때, 서열 비교 알고리즘을 사용하여 측정하거나 육안 검사에 의해 측정된, 적어도 약 80%, 동일성, 적어도 약 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% 또는 100% 뉴클레오타이드 또는 아미노산 동일성을 갖는 두 개 이상의 서열 또는 서브서열을 말한다. 일 실시형태에서, 서열은 폴리뉴클레오타이드 또는 폴리펩타이드 서열의 전체 길이에 걸쳐 높은 비율로 동일하다.
- [0033] '돼지(swine)'라는 용어는 일반적으로 수대과(Suidae family)에 속하는 모든 동물을 지칭하며 가축 및 야생 돼지, 돼지(hogs), 멧돼지(boars)를 포함한다.
- [0034] 본 발명에서 "백신"은 백신이 전달된 동물에게 보호 반응을 제공할 수 있지만 심각한 질병은 유발할 수 없는 생물학적 체제로 정의된다. 백신을 투여하면 질병에 대한 면역이 생긴다. 따라서 백신은 질병을 유발하는 병원체(예: ASFV)에 대한 항체 생성 또는 세포 면역을 자극한다. 여기서 면역은 백신을 접종하지 않은 그룹에 비해 백신 접종 후 돼지 집단에서 치사율 및 임상 증상에 대해 현저히 높은 수준의 보호가 유도되는 것으로 정의된다. 특히, 본 발명에 따른 백신은 백신 접종 동물의 대부분을 질병의 임상 증상 및 치사율의 발생으로부터 보호한다. 본 발명의 백신은 전형적으로 유전자 조작 (재조합) 돌연변이 바이러스 백신이다.
- [0035] 본 발명과 관련하여, "복제에 결함이 없다(non-deficient in its replication)"는 용어는 시험관 내 및/또는 생체 내에서 복제할 수 있고/있거나 바이러스 자손을 생산할 수 있는, 자연적으로 발생하지 않는 재조합 ASFV를 의미하지만, 그러한 복제 및/또는 바이러스 자손 생산은 변형되지 않은 모 균주에 비해 낮은 수준에서 발생할 수도 있다. 따라서, 세포 배양과 같은 시험관 내에서는 그러한 ASFV가 복제에 결함이 없지만, 포유류의 생체 내에서는 그러한 ASFV가 적어도 부분적으로 복제에 장애가 있는 경우가(예: 복제 및/또는 바이러스 자손 생산이 검출 한도 이하가 됨) 있을 수 있다.
- [0036] 본 발명에서 사용되는 "최소 용량" 또는 "최소 유효 용량"이라는 용어는 피접종자에게 독성이 없거나 최소한으로 존재하지만 원하는 결과(예: 보호 면역)를 얻을 수 있는 용량을 의미한다.
- [0037] 바이러스/백신
- [0038] 본 발명에서 제공된 새로운 돌연변이 ASFV-G 바이러스(서열번호 1)는 모체 ASFV-G 계통의 I177L 유전자 일부의 재조합 결실(미국 특허출원 제16/580,058호에 기재됨) 및 약 9kb 게놈 영역의 결실에 의해 생성된 것이다. 특정 재조합 돌연변이 ASFV-G ΔI177L의 게놈 뉴클레오타이드 서열(서열번호 1)은 본 발명에 설명되어 있으며 모체 ASFV-G를 코딩하는 게놈 뉴클레오타이드 서열과 다르다.
- [0039] ASFV-G의 예시적인 돌연변이 균주(서열번호 1)는 세포주 적응 돌연변이가 존재하는 재조합 백신의 속(genus)을 대표하며, 여기에는 결실 돌연변이, 넌센스 돌연변이, 삽입 돌연변이, 프레임 이동 돌연변이 및 돌연변이 내의 다양한 ORF의 결실, 비기능성 및 비발현을 초래하는 기타 돌연변이가 포함되지만 이에 제한되지 않는다(서열번호 2). 본원에 개시된 예시적인 바이러스는 또한 비기능적인 I177L ORF를 갖지만, 가시적인(envisoned) 기타 재조합 바이러스는, ORF들에 대한 조절 요소를 포함하는 기타 ORF 중의 돌연변이를 포함하여, 각각의 단백질의 비발현 또는 비번역을 초래한다. 이러한 변이체는 ASFV-G 야생형 게놈 배경에 있을 수 있다(서열번호 3).
- [0040] 표적 단백질의 기능적 발현을 방지하거나 표적 단백질의 발현 감소 또는 활성 감소를 방지하기 위한 변형은, DNA 또는 표적 단백질을 코딩하는 유전자의 돌연변이를 포함하고, 이것은 표적 유전자의 전체 또는 일부의 결실을 포함하고, 제한되지 않지만 표적 좌위의 오픈 리딩 프레임, 표적 좌위의 프로모터와 같은 전사 조절자, 및 오픈 리딩 프레임으로부터 5' 또는 3'에 위치한 임의의 기타 조절 핵산 서열, 오픈 리딩 프레임에 조기 정지 코돈 삽입, 및 번역의 조기 종료를 초래하는 리딩 프레임을 이동시키는 삽입 또는 결실을 포함한다. 이러한 결실 돌연변이는 당업자에게 알려진 임의의 기술을 사용하여 달성될 수 있다. 표적 단백질의 감소된 수준 또는 표적 단백질의 감소된 활성은 또한 표적 단백질을 코딩하는 DNA 또는 유전자에 점 돌연변이 또는 삽입을 통해 달성될 수 있다. 개시된 뉴클레오타이드 서열 및 유전자의 돌연변이, 삽입 및 결실 변이체는 당업자에게 잘 알려진 방법에 의해 용이하게 제조될 수 있다. 표적 단백질의 감소된 수준 및/또는 감소된 활성을 달성하기 위해 사용되는 기술에는 CRISPR/Cas, TALEN 및 Zn-핑거 뉴클레아제가 포함될 수 있다. 본원에 개시된 특정 돌연변이와 기능적으로 동등한 돌연변이, 삽입 돌연변이 및 결실 돌연변이를 만드는 것은 당업자의 숙련된 기술 내에 있다.

- [0041] 본 발명에서 기술된 접근법은 자연 발생 및 재조합 균주를 포함하여 다양한 ASFV 분리주(예: 아시아, 유럽 또는 아프리카에서 순환하는 분리주)에 사용될 수 있다. 이러한 접근법은 제한되지 않지만, 형광 단백질, 발색 기질과 함께 사용할 수 있는 베타-글루쿠로니다아제 또는 베타-갈락토시다아제와 같은 효소, 및 약물 선택 메이커와 같이 정제를 통해 재조합 바이러스를 선택할 수 있는 다양한 선택 마커를 사용하는 등 당업자에게 알려진 방법론에 따라 다양할 수 있다. 이러한 접근법은 본 발명에서 설명된 더 큰 돌연변이 내의 개별 ORF에 대한 돌연변이를 생성하는 데에도 사용할 수 있다. 예를 들어, MGF360-4L, MGF360-6L, X69R, MGF300-1L, MGF300-2R, MGF300-4L, MGF360L, MGF360-9L, MGF360-10L 또는 MGF360-11L에서 선택된 임의의 단일 ORF뿐만 아니라, 이러한 ORF의 발현 및 번역을 조절하는 조절 요소로 인해 해당 ORF에 의해 생성되는 비기능성 단백질이 생성될 수 있다. 본 발명의 모든 용도에 대해, 이러한 ORF에 사용되는 명명법은 숙련된 당업자에 의해 이러한 ASFV ORF의 표준 명칭을 활용하는 것으로 인식될 것이다.
- [0042] 다른 ASFV 균주 및 유전자형의 돌연변이도 본 발명에 포함된다. 서열번호 2와 적어도 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 또는 99%의 동일성을 나타내는 핵산 서열의 합성 돌연변이를 포함하는 ASFV 균주가 본 발명에 포함된다. 또한, 서열번호 1과 95%, 96%, 97%, 98%, 99% 이상으로 동일성을 갖는 전체 계놈을 포함하는 ASFV 균주도 본 발명에 포함된다.
- [0043] 본 발명은 세포주 적응 돌연변이와 다른 재조합 돌연변이의 조합을 더 고려한다. 따라서, 본원에 개시된 바와 같이 변형될 수 있는 것은 야생형 바이러스뿐만 아니라, 다른 유전자 또는 계놈 영역에서 자연적으로 발생하지 않는 돌연변이를 포함하는 균주도 포함된다(예를 들어, 미국 특허 제9,814,771호 참조).
- [0044] 본 발명은 합리적으로 설계된 살아있는 약독화 ASFV-G 돌연변이를 면역원 조성물에 통합하여 ASFV-G로 도전받을 때 임상 ASF 질병으로부터 돼지와 같은 동물을 보호하는 데 효과적인 백신을 생산할 수 있음을 제공한다. 따라서, 본 발명의 한 가지 목적은 이성적으로 설계된 약독화 생백신을 효과적인 양으로 투여하여 동물을 ASFV-G로부터 보호하는 방법을 제공하는 것이다. 다른 실시형태에서, 본 발명은 동물, 바람직하게는 수대(Suidae)과(예를 들어, 집돼지(*Sus scrofa domesticus*), 야생 돼지(*Sus scrofa scrofa*), 흑멧돼지(warhogs)(*Potamochoerus porcus*), 멧돼지(bushpigs)(*Potamochoerus larvatus*), 거대한 숲돼지(*Hylochoerus meinertzhageni*) 및 야생 돼지)에서 보호 면역 반응을 유도하는 방법을 제공하고, 그러한 방법은 전형적으로 본원에 기술된 하나 이상의 ASFV 면역원 조성물 및 백신을 그러한 동물에게 투여하는 것을 포함할 것이다.
- [0045] 본 발명에 개시된 면역원성 조성물의 면역원성 유효량은 다중 파라미터에 따라 달라질 수 있다. 그러나, 일반적으로, 근육 내 적용을 위한 투여 단위당 유효량은 약 10^2 50% 혈액흡수 용량("HAD₅₀") 내지 10^6 HAD₅₀일 수 있다. 본 발명의 방법론을 실행하는 데 하나, 둘 또는 그 이상의 투여 단위가 활용될 수 있다. 투여 단위는 당업자에 의해 원하는 부피 또는 질량에 맞도록 쉽게 수정될 수 있다. 투여 단위 파라미터에 관계없이, 본 발명에 개시된 면역원성 조성물은 면역 반응을 생성하는 데 효과적인 양으로 투여될 수 있다.
- [0046] 본 발명에 개시된 백신의 활성 성분의 투여 수준은 당업자에 의해 대상 또는 용도별로 원하는 결과를 달성하기 위해 변화될 수 있다. 따라서, 선택된 투여 수준은 제형, 다른 치료법과의 조합, 기존 질환의 중증도, 및 보조제의 존재 또는 부재를 포함하지만 이에 국한되지 않는 다양한 요인에 따라 달라질 수 있다. 바람직한 실시형태에서, 면역원성 조성물의 최소 용량이 투여된다. 최소 용량의 결정은 당업자의 능력 범위 내에 있다.
- [0047] 본 발명의 백신은 상업적으로 이용 가능한 약독화 생 ASFV 백신에 사용되는 통상적인 방법으로 제조될 수 있다. 특정 실시형태에서, 감수성 기질에 본원에 개시된 ASFV 돌연변이를 접종하고, 바이러스가 원하는 역가까지 복제될 때까지 증식시킨 후, ASFV 함유 물질을 수확한다. 그 후, 수확된 물질은 면역원성 특성을 갖는 백신 제제로 제형화될 수 있다. 본 발명에 제공된 재조합 바이러스의 복제를 지원할 수 있는 모든 기질은 돼지 말초혈액 대식세포의 1차 배양 또는 감염된 돼지의 혈액을 포함하여 본 발명에서 사용될 수 있다.
- [0048] 안정적인 세포주에서의 바이러스 복제 및 생산
- [0049] 본 발명의 세포주 적응 돌연변이를 포함하는 본원에서 제공되는 백신은 돌연변이가 없는 바이러스에 비해 안정적인 세포주에서 더 빠른 속도로 복제 및 성장한다. ASFV-G-I177L은 돼지 대식세포에서 역가가 4~5배 증가(3~5일 이상)할 수 있지만, 시험된 안정적인 세포주에서는 성장하지 못했다. 적응 돌연변이를 백신에 추가하면 대식세포에서 모 백신과 비슷한 비율로 성장하여 백신의 역가를 4~5배(3~6일 이상) 증가시킬 수 있었다.
- [0050] 제형 및 투여
- [0051] 본 발명에서 제공되는 백신은 상기에 정의된 재조합 바이러스 중 하나를 생체 형태(live form)로 포함하고, 이

러한 조성물에 통상적으로 사용되는 약학적으로 허용되는 담체 또는 희석제를 포함한다. 담체에는 안정화제, 보존제 및 완충제가 포함된다. 적합한 안정화제에는 예를 들어 SPGA(자당, 인산염, 글루타메이트 및 알부민), 탄수화물(소르비톨, 만니톨, 전분, 자당, 텍스트란, 글루타메이트 및 포도당), 단백질(건조 우유, 혈청, 알부민, 카제인) 또는 이들의 분해 산물이 포함된다. 적합한 완충제에는 예를 들어 알칼리 금속 인산염이 포함된다. 활용할 수 있는 보존제에는 티메로살, 메르티올레이트 및 겐타마이신이 포함되지만 이에 국한되지는 않는다. 희석제에는 물, 수성 완충제(예: 완충 식염수), 알코올 및 폴리올(예: 글리세롤)이 포함된다.

[0052] 일부 예에서, 본 발명의 백신은 또한 면역원 조성물에 의해 유도되는 수혜자의 면역 반응을 향상시키는 면역원 조성물 제형에 포함된 임의의 물질을 포함하는 하나 이상의 보조제를 포함하거나 포함할 수 있다. 경우에 따라, 그러한 보조제는 재조합 바이러스에 포함된 다른 성분의 단백질을 포함할 수 있다. 다른 보조제는 면역원성 조성물의 추가 성분으로 포함될 수 있으며, 알루미늄 염(명반), 오일 에멀전, 사포닌, 면역 자극 복합체(ISCOM), 리포솜, 미세 입자, 비이온성 블록 공중합체, 유도체화 다당류, 사이토카인 및 다양한 박테리아 유도체 등의 분류를 포함할 수 있다. 당업자에게 공지된 모든 관련 보조제는 본원에 개시된 발명을 실시하는 데 활용될 수 있다. 보조제의 선택에 영향을 미치는 요인에는 동물 종, 특정 병원체, 항원, 면역 접종 경로 및 필요한 면역 유형이 포함되며 당업자에 의해 쉽게 결정될 수 있다.

[0053] 본 발명의 면역원 조성물은 또한 재조합 바이러스에 더하여 담체를 포함할 수 있다. 본 명세서에 제공된 면역원 조성물의 실시예 이용되는 담체는 당업자에게 공지된 임의의 것일 수 있으며, 액체, 고체, 반고체 또는 겔일 수 있다. 제형의 유형은 항원의 투여 경로에 따라 변경될 수 있다. 담체는 수용체에게 무독성인 것이 바람직하다. 당업자는 가금류와 같은 수용 동물에 적용하기 위해 이러한 담체를 쉽게 선택할 수 있다.

[0054] 여기에 제공된 백신은 악성 ASFV 균주의 도전으로부터 동물을 보호하는데 효과적인 양으로 근육 내, 피하, 비강 내 또는 주사로 투여될 수 있다. 백신은 경구, 직접 경구 접종, 식수 또는 미끼 전달 시스템을 통해 투여할 수 있다. 재조합 바이러스의 유효량은 당업자가 고려하는 파라미터에 따라 달라질 수 있다. 유효량은 당업자가 필요에 따라 공지된 방법 또는 본 실시예에 제공된 지침에 따라 실험적으로 결정할 수 있다.

[0055] 본 발명을 개략적으로 설명하였으므로, 본 발명을 더욱 상세히 설명하기 위해 본 명세서에 포함된 특정 실시예를 참조함으로써 본 발명이 더욱 잘 이해될 것이며, 청구범위에 의해 정의된 본 발명의 범위를 한정하려는 의도가 아니다.

[0056] 실시예

[0057] 실시예 1

[0058] 세포 배양 및 바이러스

[0059] 돼지 대식세포의 1차 배양은 이전에 설명된 절차에 따라 돼지 혈액에서 준비했다(O'Donnell 외, (2015b), 위 참조) 바이러스 적정(titration)을 위한 96-웰 플레이트에서의 대식세포 배양 준비도 이전에 설명된 대로 수행했다(O'Donnell 외, (2015b), 위 참조).

[0060] 돼지 상피 세포(PEC)는 소 $\alpha v \beta 6$ 인테그린을 발현하도록 조작된 돼지 태아 신장 세포주인 모체 LFPK $\alpha v \beta 6$ 세포주(미국 특허 제9,121,010호)로부터 60회 이상 계대한 후 추출한 세포 서브클론이다. 세포 배양은 5% CO₂ 하에서 37°C에서 10% 열 불활성화 태아 소 혈청(HI-FBS; Thermo Fisher Scientific, Waltham, MA)과 1% 항생제-항진균제(Thermo Fisher Scientific)가 포함된 DMEM 배지(Life Technologies, Grand Island, NY)를 사용하여 계대 배양했다(passaged in culture).

[0061] ASFV-G- Δ I177L Δ LVR(서열번호 1)은 아래에 설명된 대로 약독화 생백신 후보 균주 ASFV-G- Δ I177L(미국 특허 출원 제16/580,058호)을 순차적으로 계대한 후 생성되었다.

[0062] 일차 돼지 대식세포와 안정적인 돼지 세포 배양에서 ASFV-G- Δ I177L(모 균주)과 ASFV-G- Δ I177L Δ LVR(서열번호 1) 간의 비교 성장 곡선을 수행했다. 24-웰 플레이트에서 미리 형성된 단일 층을 제조하고 0.01의 MOI로 감염시켰다(일차 돼지 대식세포 배양에서 이전에 결정된 HAD₅₀에 기반). 5% CO₂에서 37°C에서 1시간 동안 흡착한 후 접종액을 제거하고 세포를 PBS로 두 번 행겼다. 그런 다음 단일 층을 대식세포 배지로 행구고 5% CO₂에서 37°C에서 2시간, 24시간, 48시간, 72시간, 96시간 동안 배양했다. 적절한 시점에 pos0 감염된 세포를 -70°C 이하에서 동결하고, 해동한 용해물을 사용하여 일차 돼지 대식세포 배양에서 HAD₅₀/ml로 역가를 측정했다. 분석 내 변동성을 피하기 위해 모든 샘플을 동시에 실행했다.

- [0063] 바이러스 적정(virus titration)은 96웰 플레이트에서 일차 돼지 대식세포 배양에 대해 수행되었다. 바이러스 회석 및 배양은 대식세포 배지를 사용하여 수행되었다. 바이러스의 존재 여부는 혈구 흡착(HA)으로 평가하고 바이러스 역가는 리드와 켈치 방법(Reed & Muench, Am. J. Hygiene, (1938), 27:493-497)으로 계산했다. 동물 켈린지 실험에 사용된 ASFV 조지아(ASFV-G)는 조지아 공화국 트빌리시에 있는 농업부 연구소(Laboratory of the Ministry of Agriculture, LMA)의 니노 베프흐바체(Nino Vepkhvadze)가 친절하게 제공한 현장 분리주이다. 감염된 세포에서 ASFV DNA를 추출하고 앞서 설명한 대로 정량화했다. 바이러스 게놈의 전체 길이 염기서열은 앞서 설명한 대로 Illumina NextSeq 500 시퀀서를 사용하여 측정했다(Borca et al, Sci. Rep., (2018), 8:3154).
- [0064] **실시예 2**
- [0065] 세포주 돌연변이 및 적응(adaptation)
- [0066] 상업적으로 이용 가능한 돼지 유래 세포주를 포함하여 우리 실험실에서 테스트한 30개 이상의 세포주에서 ASFV-G ΔI177L이 유의하게 복제되지 않았다. 대부분의 경우, ASFV-G ΔI177L 게놈에 존재하는 재조합 RFP의 발현으로 인한 형광의 세포 내 발현 존재로 입증되는 초기 복제 단계만 감지되었으며 바이러스 수율은 미미하거나 전혀 검출되지 않았다(데이터는 표시되지 않음).
- [0067] 안정적인 돼지 세포주 세포에서 6회 연속 계대 후 ASFV-G ΔI177L은 명확한 세포 병증 효과가 발생하는 것으로 나타났다. ASFV-G-ΔI177L의 첫 번째 계대는 10의 MOI를 사용하여 수행되었다. 6회 계대에서 ASFV-G-ΔI177L 수율은 약 10^5 HAD₅₀/ml의 역가에 도달했다. 그 후의 연속적인 통과에서는 약 10^7 HAD₅₀/ml의 바이러스 수율에 도달했다(도 1).
- [0068] 차세대 염기서열 분석(NGS)을 통해 6번째 계대 후 얻은 바이러스에서 ASFV-G-ΔI177L(ASFV-G-ΔI177LΔLVR)의 계대에 따른 게놈 변화를 평가했다. 모체 바이러스와 비교했을 때, 바이러스 게놈의 16818번 내지 27660번 위치에서 결실이 발생하여 10842bp의 결실이 발생했다. 이 게놈 변형으로 인해 다음 유전자가 완전히 삭제되었다: MGF360-6L, X69R, MGF300-1L, MGF300-1L, MGF300-2R, MGF300-4L, MGF360-8L, MGF360-9L 및 MGF360-10L. 또한, 게놈 변형은 MGF360-4L 유전자의 N- 말단 부분과 MGF360-11L 유전자의 C- 말단도 결실시킨다(도 2). 이 결실은 새로운 하이브리드 단백질인 MGF360-4I/11L을 생성한다. 역코딩 가닥에 존재하는 이 결과물인 ORF는 592nt의 MGF-360-4L이 결합된 839nt의 MGF-360-11L를 갖는다. 최종 ORF는 새로운 476 아미노산 단백질을 암호화하는 1432nt로 구성된다(서열번호 4).
- [0069] 20번째와 30번째 계대 후 얻은 바이러스 집단에서 ASFV-G-ΔI177LΔLVR의 게놈 안정성을 추가로 평가했다. 놀랍게도 NGS 분석 결과, 6번째 계대 후 얻은 바이러스의 게놈과 비교했을 때 주요한 추가 게놈 변화가 없는 것으로 나타났다. 이 결과는 ASFV-G-ΔI177LΔLVR의 바이러스 게놈이 안정적인 돼지 세포주 세포에서 지속적인 계대를 통해 안정적으로 유지된다는 것을 나타낸다.
- [0070] 일차 돼지 대식세포에서 ASFV-G-ΔI177LΔLVR 복제
- [0071] aptASFV-G-ΔI177L 개발의 목표는 백신 균주로 사용하는 것이다. 따라서 돼지 감염 시 ASFV가 표적으로 삼는 일차 세포인 돼지 대식세포에서 복제하는 능력을 평가하는 것이 중요하다. 그런 다음 일차 돼지 대식세포 배양에서 ASFV-G-ΔI177LΔLVR의 시험관 내 성장 특성을 다단계 성장 곡선을 통해 평가했다. 6번째와 12번째 계대인, 두 가지 다른 계대에서 ASFV-G-ΔI177LΔLVR의 PEC를 테스트했다. 세포 배양은 0.01의 다중 감염(MOI)으로 감염되었고, 샘플은 감염 후 2시간과 이후 6~8일 동안 24시간마다 수집되었다. 그 결과, 두 균주 모두 모체 ASFV-G-ΔI177L와 비교하여 유사한 성장 동역학을 나타냈다(도 1). 따라서 안정적인 돼지 세포주 세포에서 성장하는 ASFV-G-ΔI177LΔLVR의 능력은 일차 돼지 대식세포 배양에서 시험관 내 복제하는 ASFV-G-ΔI177LΔLVR의 능력에 영향을 미치지 않는다.
- [0072] **실시예 3**
- [0073] 동물 실험
- [0074] 동물 실험은 미국 농무부 및 미국 국토안보부의 PIADC 기관 동물 관리 및 사용 위원회(프로토콜 번호 225.04-16-R, 09-07-16)가 승인한 프로토콜에 따라 플럼 아일랜드 동물 질병 센터(Plum Island Animal Disease Center, PIADC)의 동물 시설에서 생물 안전 수준 3-농업(3-AG) 조건으로 수행되었다.
- [0075] ASFV-G-ΔI177LΔLVR의 게놈 변화가 모 바이러스 ASFV-G-ΔI177L의 약화 표현형에 어떤 변화를 가져왔는지 평가하기 위해 80~90 파운드 돼지 그룹에 고용량인 106 혈구 흡수 용량(HAD50)으로 근육 내(IM) 접종하고, 28일 동안

안 동물의 임상 경과를 관찰했다. 접종한 5마리의 동물은 ASF 관련 징후가 나타나지 않았으며, 전체 관찰 기간 동안 임상적으로 정상 상태를 유지하였으며, 이는 ASFV-G-ΔI177LΔLVR이 완전히 약화된 상태임을 나타낸다(표 1). 따라서 ASFV-G-ΔI177LΔLVR은 모 바이러스의 완전한 약화 표현형을 유지한다.

[0076] 표 1. 다양한 용량의 ASFV-G-ΔI177LΔLVR 감염 후 돼지 생존율 및 발열 반응

표 1

바이러스 및 용량 (HAD ₅₀) ⁽¹⁾	생존자 수/ 총계	평균 사망 시간 (일 ± SD)	발열		
			발병까지의 일수 (일 ± SD)	지속 일수 (days ± SD)	일일 최대온도 (°F ± SD)
ASFV-G-ΔI177LΔLVR 10 ⁶	5/5	-	-	-	103 (0.64)
ASFV-G-ΔI177LΔLVR 10 ²	5/5	-	-	-	103.8 (1.04)
ASFV-G-ΔI177LΔLVR 10 ⁴	5/5	-	-	-	103.2 (1.68)
ASFV-G-ΔI177LΔLVR 10 ⁶	5/5	-	-	-	102.9 (0.46)

[0077]

[0078] 모체 ASFV-G의 도전에 대한 ASFV-G-ΔI177L의 보호 효능

[0079] ASFV-G-ΔI177LΔLVR 감염이 고병원성 모체 바이러스 ASFV-G의 도전에 대한 보호를 유도하는 능력을 평가하기 위해, ASFV-G-ΔI177LΔLVR에 감염된 모든 동물에게 28일 후 10² HAD₅₀의 ASFV-G를 IM 경로를 통해 도전했다. 추가로 5마리의 미감염(naive) 동물 그룹을 모의 접종 대조군(mock)으로 삼았다. 모든 모의 접종 동물은 접종 후 3~4일(dpc)이 지나면서 질병 관련 징후를 보이기 시작했고, 이후 몇 시간 동안 질병의 중증도가 급격히 증가하여 5dpc 경에 안락사되었다(표 2). 반면, ASFV-G-ΔI177LΔLVR에 감염된 동물 그룹은 21일 관찰 기간 동안 질병의 심각한 징후를 보이지 않고 임상적으로 건강한 상태를 유지했다. 따라서 ASFV-G-ΔI177LΔLVR로 처리한 동물은 독성이 강한 모체 바이러스에 감염되었을 때 임상적 질병으로부터 보호된다.

[0080] 모체 독성 ASFV-G에 대해 돼지를 보호하는 ASFV-G-ΔI177LΔLVR의 효과를 정량화하기 위해, 세 그룹의 돼지에 각각 10², 10⁴ 또는 10⁶ HAD₅₀의 ASFV-G-ΔI177LΔLVR을 면역 접종했다. 모든 동물은 모체 독성 ASFV-G를 10² HAD₅₀으로 IM 접종하기 전 28일 동안 임상적으로 정상 상태를 유지했다. 21일 동안 동물을 관찰했다. 모든 동물은 접종 후 ASF 임상 관련 징후를 보이지 않고 임상적으로 정상 상태를 유지했다(표 2). 따라서 ASFV-G-ΔI177LΔLVR 효능은 ASFV-G-ΔI177L(미국 특허출원 제16/580,058호)에 대해 보고된 효능과 유사하다.

[0081] 표 2. 모체 ASFV-G 바이러스에 감염된 ASFV-G-ΔI177LΔLVR 감염 동물의 돼지 생존율 및 발열 반응

표 2

바이러스 및 용량 (HAD ₅₀)	생존자 수/ 총계	평균 사망 시간 (일 ± SD)	발열		
			발병까지의 일수 (days ± SD)	지속 일수 (days ± SD)	일일 최대온도 (°F ± SD)
ASFV-G-ΔI177LΔLVR 10 ⁶	5/5	-	-	-	103 (0.59)
ASFV-G-ΔI177LΔLVR 10 ²	5/5	-	-	-	103 (0.29)
ASFV-G-ΔI177LΔLVR 10 ⁴	5/5	-	-	-	103 (0.28)
ASFV-G-ΔI177LΔLVR 10 ⁶	5/5	-	-	-	102.7 (0.44)
ΔI177L (10 ²)	5/5	-	-	-	102.8 (0.52)
Mock	10/10	-	-	-	

[0082]

[0083] 실시예 4

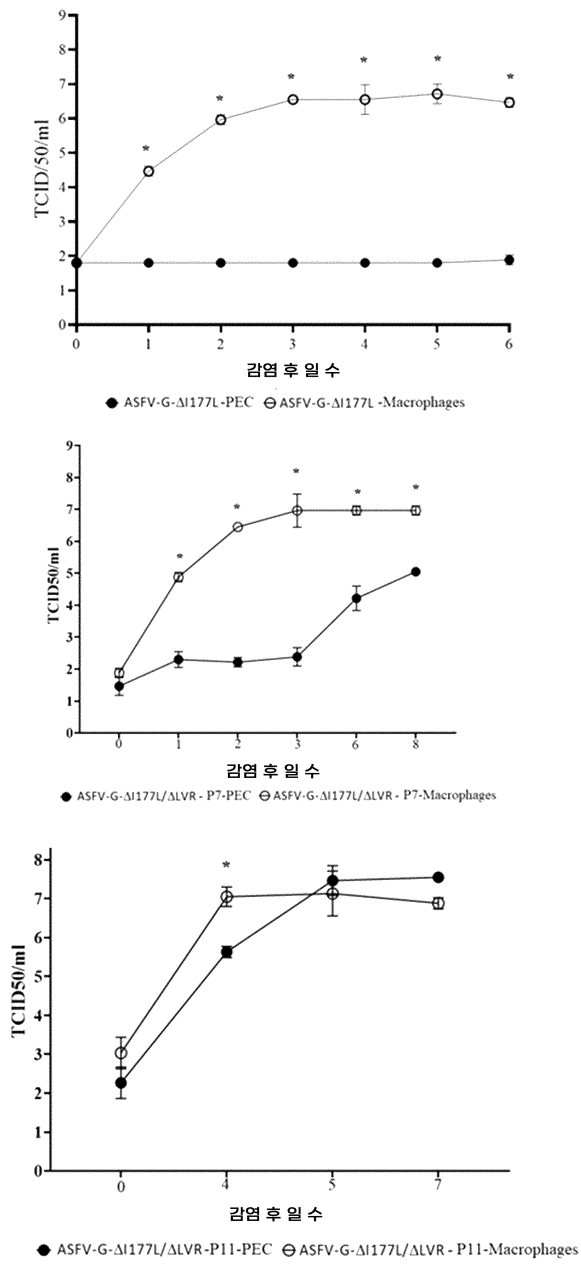
[0084] 재조합 바이러스 구성

[0085] 하나 이상의 유전자가 결여된 재조합 ASFV는 우리 및 다른 실험실에서 생산되었다. 도너 플라스미드는 결실을 목표로 하는 영역의 양쪽에 동일한 DNA(전형적으로 1000bp)의 측면 팔(flanking arm)을 포함하도록 만들어지고, 이 두 팔 사이에는 재조합 ASFV를 선택할 수 있게 하는 리포터 카세트(즉, GFP, RFP)가 있다. 이 플라스미드를 바이러스에 감염된 세포에 도입하면 도너 플라스미드와 ASFV DNA를 교환할 수 있다. 이것은 특히 상동 DNA 팔에서 발생한다. 이러한 예는 개별 유전자 I177L, 9GL, UK를 삭제하거나(O'Donnell et al, (2015b), 위; O'Donnell et al, (2017), 위; Borca et al, (2020), 위), 여러 MGF 유전자를 삭제하는 것이다(O'Donnell et al, (2015a), 위). 도 3은 이러한 방법의 개략도를 보여 주는데, 4A에서 ASFV-G 게놈에서 ΔLVR을 정확히 삭제하고 ΔLVR 영역에서 MGF3001L, 2R, 4 유전자 3 개만 삭제하는 부분적 결실을 실시한다. 동일한 방법론을 적용하여 세포주 적응 변이 영역(서열번호 2) 또는 게놈의 다른 부분에 있는 임의의 유전자 또는 임의의 유전자 그룹을 삭제할 수 있다. 재조합 바이러스의 선택 및 스크리닝은 본 명세서에 기술된 바와 같이, 또는 당업자에게 알려진 임의의 수단에 의해 수행될 수 있다.

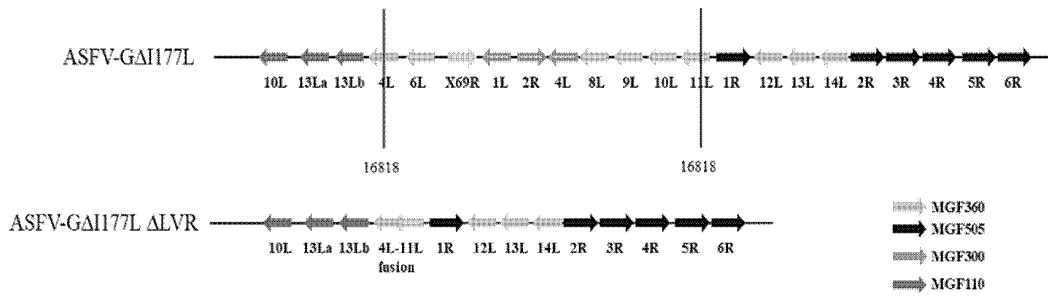
[0086] 본 발명은 예시된 실시예의 상세한 내용을 참조하여 설명되었지만, 이러한 상세한 내용은 첨부된 청구범위에 정의된 본 발명의 범위를 한정하기 위한 것이 아니다. 배타적 재산권 또는 특권이 청구되는 본 개시의 실시예는 다음과 같이 정의된다.

도면

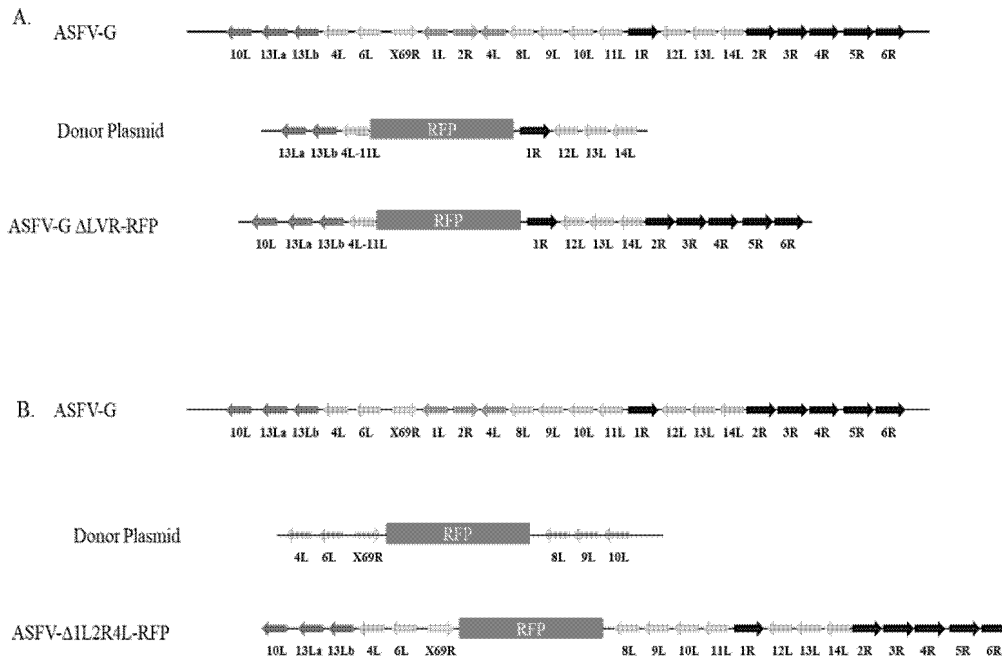
도면1



도면2



도면3



서열 목록

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Trp His Asn Gly Pro Ile Met Leu His Ile Arg Arg Asn Lys Leu Phe

 35 40 45

Ile Arg Ser Thr Cys Phe Ser Gln Gly Ile Glu Leu Asn Ile Gly Leu

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 Ala Cys Ala Arg Pro Gly Asn Lys Leu Gly Ile Tyr Tyr Cys Phe Ala
 340 345 350

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 Cys Ile Glu Gly Ser Tyr Leu Ser Asn Val Tyr Phe Cys Ile Gly Leu
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 Gly Ala Asn Pro Tyr Thr Lys Lys Ile Gln Glu Ile Ile Lys Gln Lys
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 Pro His Ser Val Leu Gln Asn Lys Ile Leu Asp Pro Ser Asp Val His
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 Lys Met Ile Ser Thr Tyr Lys Asn Thr Glu Ser Phe Tyr Pro Phe Ser
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