

smallpox (~30%). However, in contrast to smallpox, monkeypox cannot be eradicated. The virus has an unknown animal reservoir and the existence of more virulent strains is plausible. The 2003 U.S. human monkeypox outbreak (5) was the first to be seen outside Africa; however, cases have continued to occur in central Africa in the decades following the cessation of smallpox vaccination.

[0011] The live vaccine (Dryvax) that has eradicated smallpox worldwide poses serious side effects in a subset of people with acquired or congenital defects in the immune system. Moreover, the live virus vaccine is infectious and can be transmitted from the vaccinee to close contacts, including children and persons with weakened immune systems. Thus, vaccination was halted in the late nineteen seventies as it was perceived that the risks of vaccination outweighed its benefits, in the absence of a known smallpox threat. Recent sociopolitical changes worldwide however have raised concerns about the possibility of a deliberate introduction of smallpox in humans. As Dryvax is a replicating vaccine strain and a mass vaccination with it could result in serious adverse effects for high-risk individuals (29), efforts were devoted to the development of safer attenuated smallpox vaccines. MVA and NYVAC, both VACV derivatives, have been shown to be safe in immune-compromised macaques (14,30) and both can protect immune-competent macaques from lethal monkeypox virus challenge (11) (unpublished results).

[0012] There is evidence that the humoral response to vaccination is a necessary and sufficient component of smallpox vaccine-mediated protective immunity. Antibodies play a pivotal role in protection from monkeypox (14); therefore, live poxvirus vectors may not be needed if a subunit vaccine can elicit antibodies that protect macaques against monkeypox. The inventors obtained the proof of principle that indeed it is possible to induce protective antibody responses using recombinant DNA and proteins. Interestingly, DNA alone when delivered by needle injection, or proteins alone did not confer acceptable protection, whereas the combination of DNA and proteins did.

[0013] The inventors have developed poxvirus vaccines and immunogenic compositions useful in connection with poxviruses including vaccinia virus, variola virus (smallpox), monkeypox virus and other orthopoxviruses, and virtually any poxvirus having 90% amino acid sequence identity for any of the L1R, A33R, B5R or A27L gene product at the amino acid level. In particular, it is preferable that a vaccine against infection by vaccinia virus include two, three or more of the peptide products of the VACV genes L1R, A33R, A27L and B5R; a vaccine against infection by monkeypox virus include two, three or more of the monkeypox virus orthologs of the peptide products of the VACV genes L1R, A33R, A27L and B5R; a vaccine against infection by variola virus include two, three or more of the variola virus orthologs of the peptide products of the VACV genes L1R, A33R, A27L and B5R, etc. Thus, in one embodiment it is preferred that a vaccine for a poxvirus has two, three or more of the particular poxvirus orthologs of the peptide products of the VACV genes L1R, A33R, A27L and B5R, as long as the poxvirus shares 90% amino acid sequence identity of any of the L1R, A33R, A27L or B5R gene products.

[0014] However, due to the high homology between poxviruses, and orthopoxviruses in particular, where the poxvirus has orthologs of the L1R, A33R, A27L or B5R genes (or as described below, orthologs to the corresponding monkeypox virus M1R, A35R, A29L or B6R gene), and those orthologs

produce proteins/peptides that share 90% identity with the amino acid sequence of the gene products of two, three or more of the L1R, A33R, A27L or B5R genes (or as described below, gene products of the monkeypox virus M1R, A35R, A29L or B6R gene), those poxvirus ortholog gene products may be used as vaccine components for other poxviruses—as long as those other poxviruses themselves have orthologs that produce proteins/peptides that share 90% identity with the amino acid sequence of the gene products of two, three or more of the L1R, A33R, A27L or B5R genes (or as described below, gene products of the monkeypox virus M1R, A35R, A29L or B6R gene).

[0015] In this invention, the term “ortholog” denotes the well-known meaning of this term. In this art, orthologs are genes in different species which evolved from a common ancestral gene. Due to their separation following a speciation event, orthologs may diverge, but usually have similarity at the sequence and structure levels; furthermore, orthologs usually have identical functions. Orthology is a type of homology. In this application, the term ortholog is used to include the ortholog gene (DNA or RNA) or the peptide/protein product of the ortholog. Sometimes the peptide/protein product of the ortholog is referred to as “ortholog product” or simply “ortholog”. The meaning is evident from the context (e.g., a protein vaccine or immunogenic composition will contain peptides or proteins that may be referred to as orthologs—that is, products of an ortholog gene—of another poxvirus; a nucleic acid vaccine will contain nucleic acids that may be referred to as orthologs of another poxvirus—that is, an ortholog gene).

[0016] These vaccines and immunogenic compositions are based on recombinant vaccinia proteins or peptides that, when administered to a person or mammal, confer protection from poxviruses. (By “peptides” it is meant an amino acid sequence that is less than the full-length protein sequence.) The inventors have further discovered that a combination of recombinant vaccine modalities (nucleic acid plus proteins) was superior to either nucleic acid or proteins alone—for instance, a prime-boost regimen of a DNA vaccine prime followed by a protein vaccine conferred protection against severe monkeypox infection of rhesus macaques. Protection from disease correlated with the titers of binding antibodies to all proteins and to the extent of virus-specific CD4⁺ T cell responses elicited by vaccination. (See data below.)

[0017] Thus, what is described here, in one embodiment, is a protein-based replacement vaccine and vaccination methodology to effectively protect against variola virus (smallpox), monkeypox virus, other poxviruses having 90% amino acid identity, and engineered poxviruses without any of the drawbacks associated with live-virus vaccines. This is especially relevant to immunocompromised persons who cannot be vaccinated with live vaccinia virus. It also represents an improvement over DNA vaccines alone, in terms of being simpler and more convenient, and often more effective.

[0018] One embodiment entails a protein vaccine against poxviruses. As shown in the details following, the inventors have demonstrated here the first reported protection in non-human primates, using the four proteins discussed below. However, to be effective, this vaccine comprises at least two purified recombinant monkeypox virus proteins or peptides—where at least one is specific to an IMV immunogen (ortholog product of L1R or A27L) and at least one is specific to an EEV immunogen (ortholog product of A33R or B5R). In the art, the monkeypox virus orthologs of L1R, A27L, A33R