

and B5R are designated, respectively, M1R, A29L, A35R and B6R. (It is noted that part of the description below designates the monkeypox orthologs as L1Ro, A27Lo, A33Ro and B5Ro, for simplicity.) For the vaccines, therefore, the at least two proteins or peptides are selected from the group consisting of a protein or peptide encoded by the open reading frame of the monkeypox ortholog genes M1R, A35R, A29L, and B6R, and natural or genetically engineered orthologs of these proteins or peptides having 90% identity (hereafter referred to sometimes as the monkeypox ortholog protein vaccine), where at least one least one peptide/protein is from the M1R or A29L genes or the respective ortholog products from other poxviruses which have 90% identity in the amino acid sequence, and at least one least one peptide/protein is from the A35R or B6R gene or the respective ortholog products from other poxviruses whose gene products have 90% identity in the amino acid sequence. The proteins may be the full-length proteins, or may only include the open reading frames. The proteins may include only the ectodomains, or the immunodominant B cell epitopes. Preferably, the vaccine includes three, and most preferably, all four of the proteins/peptides or their 90% identity ortholog products. The ortholog products having 90% identity are preferably derived from an orthopoxvirus selected from the group consisting of: camelpox virus, ectromelia virus, raccoon poxvirus, skunk poxvirus, Tatera poxvirus, Uasin Gishu virus, Volepox virus, variola virus, vaccinia virus, monkeypox virus, gerbilpox and cowpox virus, or genetically engineered versions thereof. The orthologs may be genetically engineered version of the monkeypox ortholog genes M1R, A35R, A29L, and B6R genes.

**[0019]** Due to the high homology between poxviruses, and the known data regarding the cross-protection by vaccines derived from them, this protein vaccine may be protective against poxviruses including orthopoxvirus such as camelpox virus, ectromelia virus, raccoon poxvirus, skunk poxvirus, Tatera poxvirus, Uasin Gishu virus, Volepox virus, variola virus, vaccinia virus, monkeypox virus, gerbilpox and cowpox virus, or genetically engineered versions thereof. The proteins of interest are those ortholog products that correspond to the products of the M1R, A35R, A29L, and/or B6R genes, which have 90% identity. If the corresponding virus has, for instance, only 50% homology with monkeypox virus, but the ortholog products of at least two of the gene products of M1R, A35R, A29L, and B6R have at least 90% identity in the amino acid sequence, then these ortholog products are useful as a vaccine for that virus. For instance, the camelpox virus has ortholog products that have at least 90% identity with the gene products of M1R, A35R, A29L, and B6R, and those ortholog products (two or more) will be useful as vaccine components of a vaccine against camelpox. Correspondingly, and very important to this invention, the monkeypox gene products of M1R, A35R, A29L, and B6R will also be useful as a vaccine against camelpox, and the camelpox ortholog products will be useful as a vaccine against monkeypox. The key is that the ortholog products have at least 90% identity to the gene products of M1R, A35R, A29L, and B6R.

**[0020]** To that end, our invention in one embodiment contemplates a vaccine against poxviruses comprising at least two purified recombinant monkeypox virus proteins or peptides selected from the group consisting of

**[0021]** (i) a protein or peptide encoded by the open reading frame of the monkeypox ortholog M1R gene,

**[0022]** (ii) a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog

M1R gene, which protein or peptide has 90% amino acid sequence identity to a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog M1R gene;

**[0023]** (iii) a protein or peptide encoded by the open reading frame of the monkeypox ortholog A29L gene,

**[0024]** (iv) a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog A29L gene, which protein or peptide has 90% amino acid sequence identity to a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog A29L gene;

**[0025]** (v) a protein or peptide encoded by the open reading frame of the monkeypox ortholog A35R gene,

**[0026]** (vi) a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog A35R gene, which protein or peptide has 90% amino acid sequence identity to a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog A35R gene;

**[0027]** (vii) a protein or peptide encoded by the open reading frame of the monkeypox ortholog B6R gene, and

**[0028]** (viii) a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog B6R gene, which protein or peptide has 90% amino acid sequence identity to a protein or peptide encoded by the open reading frame of an ortholog of the monkeypox ortholog B6R gene;

**[0029]** wherein at least one protein or peptide is (i), (ii), (iii) or (iv) and at least one protein or peptide is (v), (vi), (vii) or (viii),

**[0030]** and an adjuvant.

**[0031]** Together with the protein vaccine, this invention contemplates other protein vaccines especially when used in conjunction with DNA vaccines. It is known that vaccination with DNA vaccines using vaccinia genes will achieve a response of cross-reaction and cross-protection against another poxvirus. Similar results were found regarding cross-reactivity using a DNA vaccine of monkeypox genes or a vaccine of monkeypox proteins, for instance in vaccinia plaque reduction neutralization tests and EEV spread inhibition assays. Since there is a high degree of cross-reactivity between vaccinia and monkeypox for all of the above-described four proteins/gene products, orthologs (especially from the variola virus which is extremely similar in sequence) having more than 90% identity will also cause immunogenic reactions in each other—and of course the orthologs will be best suited for causing immunogenic reactions in the respective poxvirus from which they are derived. Furthermore, it is preferred that the vaccines and immunogenic compositions described herein—whether DNA vaccine or protein vaccine/immunogenic composition—contain redundant IMV and EEV targets since this will increase cross-reactivity and cross-protection. Having such redundancy will provide enough or more than enough cross-reactive epitopes so as to afford vaccine protection—that is, because the proteins are so similar, the redundant nature of a DNA or protein vaccine containing three or four of the genes/gene products compensates for the possibility that the antibody to one protein won't cross-react with a particular virus. Hence, it is most preferred that the vaccines and immunogenic compositions contain all four of the genes/gene products L1R, A27L, A33R and B5R,