REPLIKIN PEPTIDES IN RAPID REPLICATION OF GLIOMA CELLS AND IN INFLUENZA EPIDEMICS

CROSS REFERENCE TO OTHER APPLICATIONS

This application is an International Application , claiming priority of US applications Nos.: 10/105,232, filed March 26, 2002; 09/984,057, filed November 26, 2001, Provisional Application 60/303,396, filed July 9, 2001 and Provisional Application US 60/278,761 filed March 27, 2001, the subject matter of which is incorporated by reference hereto.

FIELD OF THE INVENTION

This invention relates to the identification and use of Replikins, a class of peptides that share structural characteristics. In particular, this invention relates to Replikins which have been identified in influenza viruses and their use in designing influenza virus vaccines.

BACKGROUND OF THE INVENTION

Influenza is an acute respiratory illness of global importance. Despite international attempts to control influenza virus outbreaks through vaccination influenza infections remain an important cause of morbidity and mortality. Worldwide influenza pandemics have occurred at irregular and previously unpredictable intervals throughout history and it is expected that they will continue to occur in the future. The impact of pandemic influenza is substantial in terms of morbidity, mortality and economic cost.

Influenza vaccines remain the most effective defense against influenza virus, but because of the ability of the virus to mutate and the availability of non-human host reservoirs it is expected that influenza will remain an emergent or re-emergent infection. Global influenza surveillance indicates that influenza viruses may vary within a country and between countries and continents during an influenza season. Virologic surveillance is of importance in monitoring antigenic shift and drift. Disease surveillance is also important in assessing the impact of epidemics. Both types of information have provided the basis of vaccine composition and the correct use of

antivirals. However, to date there has been only annual *post hoc* hematological classification of the increasing number of emerging influenza virus strains, and no specific chemical structure of the viruses has been identified as an indicator of approaching influenza epidemic or pandemic. Currently, the only basis for annual classification of influenza virus as active, inactive or prevalent in a given year is the activities of the virus hemagglutinin and neuraminidase proteins. No influenza viral chemical structure has been identified that can be used for quantitative warning of epidemics or pandemics or to design more effective and safer vaccines.

Because of the annual administration of influenza vaccines and the short period of time when a vaccine can be administered, strategies directed at improving vaccine coverage are of critical importance.

Another disease which has proved difficult to treat and for which there is no effective vaccine is malaria. Malaria causes much physical and economic hardship in tropical regions. Malaria is caused by *Plasmodium falciparum*, which has proved to be extremely resistant to treatment and to date, a vaccine for malaria remains elusive. Thus, there is a need for effective malaria vaccines and methods of treating or preventing the disease.

SUMMARY OF THE INVENTION

In one aspect of the invention there are provided isolated influenza virus peptides containing a Replikin sequence. The influenza virus peptides comprise from 7 to about 50 amino acids including (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

In another aspect of the invention there is provided a process for stimulating the immune system of a subject to produce antibodies that bind specifically to an influenza virus Replikin sequence, said process comprising administering to the subject an effective amount of a dosage of a composition comprising at least one influenza virus replikin peptide. In a preferred embodiment the composition comprises at least one peptide that is present in an emerging strain of influenza virus.

The present invention also provides antibodies that bind specifically to an influenza virus Replikin, as defined herein, as well as antibody cocktails containing a plurality of antibodies that specifically bind to influenza virus Replikins. In one3 embodiment of the invention, there are provided compositions comprising an antibody or antibodies that specifically bind to an influenza Replica and a pharmaceutically acceptable carrier.

The present invention also provides therapeutic compositions comprising one or more of isolated influenza virus peptides having from 7 to about 50 amino acids comprising

1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues, and a pharmaceutically acceptable carrier.

In another aspect of the invention there is provided an antisense nucleic acid molecule complementary to an influenza virus hemagglutinin Replikin mRNA sequence, said Replikin mRNA sequence having from 7 to about 50 amino acids comprising

(1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

In yet another aspect of the invention, there is provided a method of stimulating the immune system of a subject to produce antibodies to influenza virus comprising administering an effective amount of at least one influenza virus Replikin peptide having from 7 to about 50 amino acids comprising (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

In another aspect, there is provided a method of selecting an influenza virus peptide for inclusion in an influenza virus vaccine comprising

(1) obtaining at least one isolate of each strain of a plurality of strains of influenza virus,

(2) analyzing the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus for the presence and concentration of Replikin sequences,

(3) comparing the concentration of Replikin sequences in the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus to the concentration of Replikin sequences observed in the hemagglutinin amino acid sequence of each of the strains at at least one earlier time period to provide the concentration of Replikins for at least two time periods, said at least one earlier time period being within about six months to about three years prior to step (1),

(4) identifying the strain of influenza virus having the highest increase in concentration of Replikin sequences during the at least two time periods,

(5) selecting at least one Replikin sequence present in the strain of influenza virus peptide identified in step (4) as a peptide for inclusion in an influenza virus vaccine.

The present invention also provides a method of making an influenza virus vaccine comprising

(1) identifying a strain of influenza virus as an emerging strain,

(2) selecting at least one Replikin sequence present in the emerging strain as a peptide template for influenza virus vaccine manufacture,

(3) synthesizing peptides having the amino acid sequence of the at least one Replikin sequence selected in step (2), and

(4) combining a therapeutically effective amount of the peptides of step (4) with a pharmaceutically acceptable carrier and/or adjuvant.

In another aspect, the invention is directed to a method of identifying an emerging strain of influenza virus for diagnostic or therapeutic purposes comprising

(1) obtaining at least one isolate of each strain of a plurality of strains of influenza virus,

(2) analyzing the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus for the presence and concentration of Replikin sequences,

(3) comparing the concentration of Replikin sequences in the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus to the concentration of Replikin sequences observed in the

hemagglutinin amino acid sequence of each of the strains at at least one earlier time period to provide the concentration of Replikins for at least two time periods, said at least one earlier time period being within about six months to about three years prior to step (1), and

(4) identifying the strain of influenza virus having the highest increase in concentration of Replikin sequences during the at least two time periods.

In yet another aspect of the invention, there is provided an influenza virus vaccine comprising at least one isolated Replikin present in the hemagglutinin protein of an emerging strain of influenza virus and a pharmaceutically acceptable carrier and/or adjuvant.

Also provided by the present invention is a method of preventing or treating influenza virus infection comprising administering to a patient in need thereof a vaccine comprising at least one isolated Replikin present in the hemagglutinin protein of an emerging strain of influenza virus and a pharmaceutically acceptable carrier and/or adjuvant.

In another aspect of the invention, there are provided vaccines and methods for preventing or treating malaria. The malaria vaccines comprise at least one isolated *Plasmodium falciparum* Replikin. The present invention also provides methods for treating or preventing malaria comprising administering to a patient an effective amount of a vaccine comprising at least one isolated *Plasmodium falciparum* Replikin.

Also provided by the present invention are antibodies, antibody cocktails and compositions that comprise antibodies that specifically bind to a Replikin or Replikins present in a malaria antigen of *Plasmodium falciparum*.

As used herein, the term "peptide" refers to a compound of two or more amino acids in which the carboxyl group of one is united with an amino group of another, forming a peptide bond. The term peptide is also used to denote the amino acid sequence encoding such a compound. Thus, a peptide sequence may be a subsequence of a larger polypeptide sequence. As used herein, a Replikin peptide is a peptide having 7 to about 50 amino acids comprising (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues. Similarly, a replikin sequence is the amino acid

sequence encoding such a peptide.

The phrase "emerging strain" as used herein refers to a strain of influenza virus identified as having an increasing concentration of Replikin sequences in its hemagglutinin and/or neuraminidase protein sequence, relative to the concentration of replikins in other strains of influenza virus. The increase in concentration occurs over a period of at least about six months, and preferably over a period of at least about one year, most preferably over a period of at least about three years or more .

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph depicting the frequency of occurrence of replikins in various protein groups.

Figure 2 is a graph depicting the percentage of malignin per milligram total membrane protein during anaerobic replication of glioblastoma cells.

Figure 3 is a bar graph showing amount of antimalignin antibody produced in response to exposure to the recognin 16-mer.

Figure 4A is a photograph of a blood smear taken with ordinary and fluorescent light. Figure 4B is a photograph of a blood smear taken with ordinary and fluorescent light illustrating the presence of two leukemic cells. Figure 4C is a photograph of a dense layer of glioma cells in the presence of antimalignin antibody. Figure 4D and Figure 4E are photographs of the layer of cells in Figure 4C taken at 30 and 45 minutes following addition of antimalignin antibody.

Figure 4F is a bar graph showing the inhibition of growth of small cell lung carcinoma cells *in vitro* by antimalignin antibody.

Figure 5 is a plot of the amount of antimalignin antibody present in the serum of patients with benign or malignant breast disease pre-and post surgery.

Figure 6 is a box diagram depicting an embodiment of the invention wherein a computer is used to carry out the 3-point-recognition method of identifying replikin sequences.

Figure 7 is a graph showing the concentration of Replikins observed in hemagglutinin of influenza B and influenza A strain, H1N1, on a year by year basis from 1918 through 2001.

Figure 8 is a graph of the replikin concentration observed in hemagglutinin of influenza A strains, H2N2 and H3N2, as well as an emerging strain defined by its constituent Replikins, designated H3N2(R), on a year by year basis from 1950 to 2001.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for predicting future epidemics or pandemics of influenza virus, and vaccines and methods of designing effective vaccines against influenza virus. Identification of a new family of small peptides related to the phenomenon of rapid replication, referred to herein as Replikins, provides new targets for influenza virus detection and influenza vaccine development. Identification of this new family of peptides also provides for the detection of malaria and provides new targets for malaria vaccine development.

The first Replikin sequence to be identified was the cancer cell Replikin found in a brain cancer protein, malignin, which was demonstrated to be enriched ten-fold during rapid anaerobic replication of glioblastoma multiforme (glioma) cells. **(Figure 2)** Malignin is a 10KDa portion of the 250 KDa glycoprotein 10B, which was isolated *in vivo* and *in vitro* from membranes of glioblastoma multiforme (glioma) cells. Hydrolysis and mass spectroscopy of malignin revealed a16-mer peptide sequence, ykagvaflhkkndide **(SEQ ID NO.:4)**, which is referred to herein as the glioma Replikin and which includes the shorter peptide, kagvaflhkk **(SEQ ID NO.: 1)**, both of which apparently are absent in the normal human genome.

Table 1 illustrates how the sequence of the glioma Replikin, the 16-mer peptide sequence, ykagvaflhkkndide **(SEQ ID NO.: 4)** was determined.

Table 1

16-mer peptide sequence ykagvaflhkkndide obtained from malignin by hydrolysis and mass spectrometry

Seq	Fragment	MH+	Sequence	Method By Which Fragment Obtained			
ID NO.	Identified	(mass)		Auto-hydrolysis of malignin free in solution	Auto-hydrolysis of malignin immobilized on bromoace-tyl cellulose	Micro-waved 5 seconds	Micro-waved 30 seconds
19	1-3	381.21	()yka(g)				+
20	1-5	537.30	()ykagv(a)		+		
21	2-6	445.28	(y)kagva(f)		+		
22	2-7	592.35	(Y)kagvaf(1)			+	
23	4-11	899.55	(a)gvaf1hkk(n)				+
24	5-7	336.19	(g)vaf(1)				+
25	6-7	237.12	(v)af(1)	+			
26	6-10	615.36	(v)aflhk(k)				+
27	6-10	615.36	(v)aflhk(k)	+			
28	6-12	857.50	(v)aflhkkn(d)		+		
29	6-12	857.50	(v)afhkkn(d)	+			
30	7-8	279.17	(a)fl(h)			+	
31	10-16	861.43	(h)kkndide()		+		
32	11-14	489.27	(k)kndi(d)		+		
33	12-15	476.2-	(k)ndid(e)	+			

When the 16-mer glioma Replikin was synthesized and injected as a synthetic vaccine into rabbits, abundant antimalignin antibody was produced. (Bogoch et al., Cancer Detection and Prevention, 26(Supp. 1): 402 (2002). The concentration of antimalignin antibody in serum *in vivo* has been shown to relate quantitatively to the survival of cancer patients. (Bogoch et al., Protides of Biological Fluids, 31:739-747 (1984). *In vitro* antimalignin antibodies have been shown to be cytotoxic to cancer cells at a concentration of picograms (femtomolar) per cancer cell. (Bogoch et al., Cancer Detection and Prevention, 26(Supp. 1): 402 (2002). Studies carried out by the inventors showed that the

glioma Replikin is not represented in the normal healthy human genome. Consequently, a search for the origin and possible homologues of the Replikin sequence was undertaken by analysis of published sequences of various organisms.

By using the 16-mer glioma Replikin sequence as a template and constructing a recognition proteomic system to visually scan the amino acid sequences of proteins of several different organisms, a new class of peptides, the Replikins, was identified. The present invention provides a method for identifying nucleotide or amino acid sequences that include a Replikin sequence. The method is referred to herein as a 3-point-recognition method. By use of the "3-point recognition" method, described herein below, a new class of peptides was revealed in algae, yeast, fungi, amoebae, bacteria, plant and virus proteins having replication, transformation, or redox functions. Surprisingly, the Replikin peptides were found to be concentrated in larger 'replicating' and 'transforming' proteins (so designated by their investigators, *See* Table 2). No sequences were found to be identical to the malignin 16-mer peptide.

Table 2 illustrates several Replikin sequences that were identified by the 3-point recognition method of the invention.

Table 2:

Examples of Replikins in various organisms - prototype: Glioma Replikin* kagvaflhkk (SEQ ID No.:1)

	SEQ ID NO.		
Algae:	34	Caldophera prolifera	kaskftkh
	35	Isolepisprolifera	kaqaetgeikgh
Yeast:	36	Schizosaccharomyces pombe	ksfkypkkhk
	37	Oryza sativa	kkaygnelhk
	2	Sacch. cerevisiae replication binding protein	hsikrelgiifdk
Fungi:		Isocitrate lyase ICI l,Penicillium marneffei	kvdivthqk
	38	DNA-dependent RNA polymerase 11, Diseula dcstructiva	kleedaayhrkk
	39	Ophiostoma novo-ulm 1,RNA in Dutch elm disease	kvilplrgnikgiffkh
	40	fungus	
Amoeba:	41	Entamoeba invadens, histone H2B	klilkgdlnkh
Bacteria:	42	Pribosomal protein replication factor, Helicobacter pylori	ksvhaflk
		Replication-associated protein Staph. aureus	
	10	Mycoplasma pulmonic, chromosome replication	kkektthnk
	43	Macrophage infectivity potentiator, L. legionella	kvhffqlkk
	90	Bacillus anthracis	kihlisvkk
	91	Bacillus anthracis	hvkkekeknk
	92	Bacillus anthracis	khivkievk
	93	Bacillus anthracis	kkkkikdiygkdallh
	94	Bacillus anthracis	kwekikqh
	95	Bacillus anthracis	kklqipppiepkkddiih
	96	Bacillus anthracis	hnryasnivesayllilnew-knniqsdlikk
	97	Bacillus anthracis	havddyagylldknqsdlv-tnskk
	98	Bacillus anthracis	haerlkvqknapk
Plants:	44	Arabidopsis thaliana, prolifera	kdhdfdgdk
	45	Arabidopsis thaliana, cytoplasmic ribosomal	kmkglkqkkah
	46	Arabidopsis thaliana, DNA binding protein	kelssttqeksh
Viruses:	9	Replication associated protein A [Maize streak virus]	kekkpskdeimrdiish
	11	Bovine herpes virus 4, DNA replication protein	hkinitngqk
	12	Meleagrid herpesvirus 1, replication binding protein	hkdlyrllmk
	47	Feline immunodeficiency	hlkdyklvk
	3	Foot and Mouth Disease (O)	hkqkivapvk
	5	HIV Type 1	kcfncgkegh
	7	HIV Type 2	kcwncgkegh
	99	Small Pox Virus (Variola)	khynnitwyk
	100	Small Pox Virus (Variola)	kysqtgkeliih
	101	Small Pox Virus (Variola)	hyddvrikndivvsrck
	102	Small Pox Virus (Variola)	hrfklildski
	103	Small Pox Virus (Variola)	kerghnyyfek

Tumor Viruses:	48	.Rous sarcoma virus tyrosine-protein kinase	kklrhek
	49	v-yes, avian sarcoma	kklrhdk
	50	c-yes, colon cancer, malignant melanoma	kklrhdk
	51	v-srcC, avian sarcoma	kklrhek
	52	c-src, colon, mammary, panrcreatic cancer	kklrhek
	53	Neuroblastoma RAS viral (v-ras) oncogene	kqahelak
	54	VP1 (major capsid protein) [Polyamavirus sp.]	kthrfskh
	55	Sindbis	knlhekik
	56	El [Human papilloamavirus type 71]	khrpllqlk
	57	v-erbB from AEV and c-erb	kspnhvk
	58	v-fms (feline sarcoma)	knihlekk
	59	c-fms (acute and chronic myelomonocytic tumors)	knihlekk
	60	large t-antigen I [Polyomavirus sp.l	kphlaqslek
	61	middle t-antigen [Polyomavirus sp,l-	kqhrelkdk
	62	small t-antigen [Polyomavirus spJ,	kqhrelkdk
	63	v-abl, murine acute leukemia	kvpvlisptlkh
	64	Human T-cell lymphotropic virus typo 2	kslllevdkdish
	65	c-kit, GI tumors, small cell lung carcinoma	kagitimvkreyh
	18	Hepatitis C	hyppkpgcivpak
Trans-forming Proteins:	66	Transforming protein myb	ksgkhlgk
	67	Transforming protein myc, Burkitt lymphoma	krreqlkhk
	68	Ras-related GTP-binding protein	ksfevikvih
	69	Transforming protein ras (teratocarcinoma)	kkkhtvkk
	70	TRAF-associated NF•kB activator TANK	kaqkdhlsk
	71	RFP transforming protein	hlkrvkdlkk
	72	Transforming protein D (S.C.)	kygspkhrlik
	73	Papilloma virus type 11, transforming protein	klkhilgkarfik
	74	Protein tryosine kinasc (EC 2.7.1.ll2slk	kgdhvkhykirk
	75	Transforming protein (axl(-))	keklrdvmvdrhk
	76	Transforming protein (N-myc)	klqarqqqllkkieh
	77	Fibroblast growth factor 4 (Kaposi sarcoma)	kkgnrvsptmkvth
Cancer Cell Proteins:	78	Matrix metaloproteinase 7 (uterine)	keiplhfrk
	79	Transcription factor 7-like	kkkphikk
	80	Breast cancer antigen NY-BR-87	ktrhdplak
	81	BRCA-1-Associated Ring Domain Protein (breast)	khhpkdnlik
	82	`Autoantigen from a breast tumor'	khkrkkfrqk
	83	Glioma Replikin (this study)	kagvaflhkk
	84	Ovarian cancer antigen	khkrkkfrqk
	85	EE L leukemia	kkkskkhkdk
	86	Proto-oncogene tyrosine-protein kinase C-ABLE	hksekpalprk
	87	Adenomatosis polyposis coli	kkkkpsrlkgdnek
	88	Gastric cancer transforming protein	ktkkgnrvsptmkvth
	89	Transforming protein (K-RAS 2B),lung	khkekmskdgkkkkkksk

Identification of an amino acid sequence as a Replikin or as containing a Replikin, *i.e.*, a homologue of the glioma peptide, kagvaflhkk, requires that the three following requirements be met. The peptide sequence must have (1) at least one lysine residue located six to ten residues from another lysine residue; (2) at least one histidine residue; and (3) a composition of at least 6% lysine within an amino acid sequence of 7 to about 50 residues.

Databases were searched using the National Library of Medicine keyword "PubMed" descriptor for protein sequences containing Replikin sequences. Over 4,000 protein sequences were visually examined for homologues. Sequences of all individual proteins within each group of PubMed-classified proteins were visually scanned for peptides meeting the three above-listed requirements. An infrequent occurrence of homologues was observed in "virus peptides" as a whole (1.5%) (N=953), and in other peptides not designated as associated with malignant transformation or replication such as "brain peptides" and "neuropeptides" (together 8.5%) (N=845). However, surprisingly, homologues were significantly more frequently identified in large "replicating proteins," which were identified as having an established function in replication in bacteria, algae, and viruses. Even more surprising was the finding that Replikin homologues occurred in 100% of "tumor viruses" (N=250), in 97% of "cancer proteins" (N=401), and in 85% of "transforming viruses" (N=248). These results suggest that there are shared properties of cancer pathogenesis regardless of cell type and suggest a role of viruses in carcinogenesis, i.e., conversion of cells from a transformed albeit dormant state to a more virulent actively replicating state.

To permit classification of subtypes of Replikins, additional or "auxiliary specifications" to the basic "3-point-recognition" requirements may be added: (a) on a structural basis, such as the common occurrence of adjacent di- and polylysines in cancer cell proteins (*e.g.*, transforming protein P21B(K-RAS 2B), lung, Table 2, **SEQ ID NO.: 89**), and other adjacent di-amino acids in TOLL-like receptors, or b) on a functional basis, such as exhibiting ATPase, tyrosine kinase or redox activity as

seen in Table 2.

Whether Replikin structures are conserved or are subject to extensive natural mutation was examined by scanning the protein sequences of various isolates of foot and mouth disease virus (FMDV), where mutations in proteins of these viruses have been well documented worldwide for decades. Protein sequences of FMDV isolates were visually examined for the presence of both the entire Replikin and each of the component Replikin amino acid residues observed in a particular Replikin. For example, in the protein VP1 of FMDV type O, the Replikin **(SEQ ID NO.: 3)** "**hkqkivapvk**" was found to be conserved in 78% of the 236 isolates reported in PubMed, and each amino acid was found to be conserved in individual isolates as follows: his, 95.6%; lys, 91.8%; gln 92.3%; lys, 84.1%; ile, 90.7%; val, 91.8%; ala, 97.3%; pro, 96.2%; ala, 75.4%; and lys, 88.4%. The high rate of conservation suggests structural and functional stability of the Replikin structure. Similarly, sequence conservation was observed in different isolates of HIV for its Replikins, such as **(SEQ ID NO.: 5)** "**kcfncgkegh**" or **(SEQ ID NO.: 6)** "**kvylawvpahk**" in HIV Type 1 and **(SEQ ID NO.: 7)** "**kcwncgkegh**" in HIV Type 2 (Table 2). Other examples of conservation are seen in the constant presence of malignin in successive generations, over ten years of tissue culture of glioma cells, and by the constancy of affinity of the glioma Replikin for antimalignin antibody isolated by immunoadsorption from 8,090 human sera from the U.S., U.K., Europe and Asia (e.g., **Figure 5** and U.S. Patent #6,242,578 B 1).

As seen in **Figure 2**, during anaerobic respiration when the rate of cell replication is increased, malignin is enriched. That is, malignin is found to increase not simply in proportion to the increase in cell number and total membrane proteins, but is enriched as much as tenfold in concentration, starting with 3% at rest and reaching 30% of total membrane protein. This clear demonstration of a marked increase in Replikin concentration with glioma cell replication points to and is consistent with the presence of Replikins here sought by the 3-point recognition method and found in the proteins of various organisms which were found by mutation studies and other previous studies to be critical to replication. For example,

Replikins were identified in such proteins as "*Saccharomyces cerevisiae* replication binding protein" **(SEQ ID NO.: 2) (hsikrelgiifdk)**; the "replication associated protein A of maize streak virus" **(SEQ ID NO.: 8) (kyivcareahk)** and **(SEQ ID NO.: 9) (kekkpskdeimrdiish)**; the "replication-associated protein of *Staphylococcus aureus*" **(SEQ ID NO.: 10) (kkektthnk)**; the "DNA replication protein of bovine herpes virus 4" **(SEQ ID NO.: 11) (hkinitngqk)**; and the "Mealigrid herpes virus 1 replication binding protein" **(SEQ ID NO.: 12) (hkdlyrllmk)**. Previous studies of tomato leaf curl gemini virus show that the regulation of virus accumulation appears to involve binding of amino acids 1-160 of the "replicating protein" of that virus to leaf DNA and to other replication protein molecules during virus replication. Analysis of this sequence showed that amino acids 1-163 of this "replicating protein" contain five Replikins, namely: **(SEQ ID NO.: 13) kfrinaknyfltyph, (SEQ ID NO.: 14) knletpvnklfiricrefh, (SEQ ID NO.: 15) hpniqaaksstdvk, (SEQ ID NO.: 16) ksstdvkaymdkdgdvldh,** and **(SEQ ID NO.: 17) kasalnilrekapkdfvlqfh**.

Table 2 shows that Replikin-containing proteins also are associated frequently with redox functions, and protein synthesis or elongation, as well as with cell replication. The association with metal-based redox functions, the enrichment of the Replikin-containing glioma malignin concentration during anaerobic replication, and the cytotoxicity of antimalignin at low concentrations (picograms/cell) (Figure 4c-f), all suggest that the Replikins are related to central respiratory functions, which are perhaps less often subjected to the mutations characteristic of proteins of more superficial location or less central survival function.

Of particular interest, it was observed that at least one Replikin per 100 amino acids was found to be present in the hemagglutinin proteins of almost all of the individual strains of influenza viruses examined. The replikin sequences that were observed to occur in the hemagglutinin proteins of isolates of each of the four prevalent strains of influenza virus, influenza B, H1N1, H2N2, and H3N2, for each year that amino acid sequence data are available (1902-2001) are shown in Tables 3, 4, 5 and 6, below.

Table 3

Replikin Sequences present in hemagglutinins of Influenza B viruses in each year for which amino acid sequences were available (1902-2001).

Influenza B Replikins	**Year Detected in Influenza B strain** (Peak in Figure 7: EB1 EB2)
kshfanl**k** (SEQ ID NO. 104)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
kshfanlkgt**k** (SEQ ID NO. 105)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
kshfanlkgt**ktrgklcpk** (SEQ ID NO. 106)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hekygglnk (SEQ ID NO. 107)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hekygglnksk (SEQ ID NO. 108)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hekygglnkskpyytgehak (SEQ ID NO. 109)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hakaigncpiwvk (SEQ ID NO. 110)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hakaigncpiwvktplklangtk (SEQ ID NO. 111)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hakaigncpiwvktplklangtkyrppak (SEQ ID NO. 112)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hakaigncpiwvktplklangtkyrppakllk (SEQ ID NO. 113)	1902,19,24,38,40,43,51,59,75,76,77,89,90,93,97,98,99,00,01
hfanlkgtktrgk (SEQ ID NO. 114)	1919, 76, 89,90, 99,00,01
hfanlkgtktrgklcpk (SEQ ID NO. 115)	1919, 76, 90 00,01
hsdneiqmvklygdsk (SEQ ID NO. 116)	1919
hsdneiq**dk**mvklygdskpqk (SEQ ID NO. 117)	1919
hsdneiqmvklygdskpqk (SEQ ID NO. 118)	1919, 24, 97,98, 00
k(a/v)sil**h**evk (SEQ ID NO. 119)	1919, 40, 59, 90,93

kctgtipsakasilh (SEQ ID NO. 120)	1919, 00
kctgtipsakasilhevk (SEQ ID NO. 121)	1919, 93
kygglnkskpyytgeh (SEQ ID NO. 122)	1919
kvwcasgrskvikgslpligeadclh (SEQ ID NO. 123)	1919, 38,40,43, 59,75,76,77,89,90, 98,99,00
kpyytgehak (SEQ ID NO. 124)	1919, 38,40, 59, 89,90,93,97,98, 01
kcmgtipsakasilhevk (SEQ ID NO. 125)	1924, 43, 75,76,77, 93
hnvinaekapggpyk (SEQ ID NO. 126)	1938, 93,97, 00
hsdnetqmaklygdsk (SEQ ID NO. 127)	1938, 93,97, 00
hgvavaadlkstqeaink (SEQ ID NO. 128)	1940, 59, 00
hgvavaadlkstqeainkdtistqeaink (SEQ ID NO. 129)	1940
klygdskpqkftssangvtth (SEQ ID NO. 130)	1943, 75,76,77, 93,97, 00
hsdnetqmaklygdskpqk (SEQ ID NO. 131)	1943, 75,76,77, 93
hfanlkgtqtrgk (SEQ ID NO. 132)	1959
kprsalkckgfh (SEQ ID NO. 133)	1988
kskpyytgehakai(g/a)ncpiwvk (SEQ ID NO. 134)	2000

1. Influenza B has not been responsible for any human pandemic (global distribution).
2. Abbreviation for years: eg. "19" = 1919, "01" = 2001.
3. The first year that a given replikin appears is indicated at the beginning of the series of years in which that replikin has been found.
4. Overlapping replikin sequences are listed separately.
5. Increase in number of new replikin structures occurs in years of epidemics (underlined): eg. 1951 and 1977 and correlates with increased total replikin concentration (number of replikins per 100 amino acid residues). See Figure 7.

Table 4

H1N1 Replikin Sequences present in HINI hemagglutinins of Influenza viruses in each year for which amino acid sequences were available (1918-2000)

H1N1 Replikin H1N1 Strain	**Year Detected in Influenza** (Peak in Figure7 : P1 E1 E1.1, 1.2, 1.3 E1.4)
hp(v/i)tigecpkyv(r/k)(s/t)(t/a)k (SEQ ID NO. 135)	1918,25,28,30,31,35,47,48,51,52,55,56,57,59,63,77,79,80,81,85,87,88,89,91,92,95,96,97,98,99,00
hdsnvknly(e/g)kv(k/r)(n/s)ql(k/r)nnak (SEQ ID NO. 136)	1918, 28,30,31, 77,79,80, 88, 91, 95, 98
hdsnvknly(e/g)kv(k/r)(n/s)qlk (SEQ ID NO. 137)	1918, 28,30,31, 77,79,80, 88, 91, 95, 98
hkc(nn/dd)(a/t/e)cmesv(r/k)ngtydypkyseesklnre(e/k)idgvk (SEQ ID NO. 138)	1918, 30, 35, 77, 80, 98
hkc(nn/dd)(a/t/e)cmesv(r/k)ngtydypkyseesk (SEQ ID NO. 139)	1918, 30, 35, 77, 80, 98
hqn(e/g)qgsgyaadqkstqnai(d/n)gitnkvnsviekmntqftavgkefnklek (SEQ ID NO. 140)	1918, 28,30,31,35, 59, 79, 95
hqn(e/g)qgsgyaadqkstqnai(d/n)gitnkvnsviek (SEQ ID NO. 141)	1918, 28,30,31,35, 59, 79, 95
hqn(e/g)qgsgyaadqkstqnai(d/n)gitnk (SEQ ID NO. 142)	1918, 28,30,31,35, 59, 79, 95
kfeifpktsswpnh (SEQ ID NO. 143)	1918, 77
kg(n/s/t)sypkl(n/s)ksy(v/t)nnkgkevlvlwgvh (SEQ ID NO. 144)	1918, 35, 77, 96
ksy(v/t)nnkgkevlvlwgvh (SEQ ID NO. 145)	1918, 35, 77, 96
hkcnnecmesvkngtydypkyseesklnrekidgvk (SEQ ID NO. 146)	1928, 31, 95
hkcnnecmesvkngtydypkyseesk (SEQ ID NO. 147)	1928, 31, 95
hkcnnecmesvkngtydypk (SEQ ID NO. 148)	1928, 31, 95
hkcnnecmesvk (SEQ ID NO. 149)	1928, 31, 95
hngkssfy(k/r)nllwlt(e/g)knglypnlsksyvnnkek (SEQ ID NO. 150)	1928, 95, 00
hngkssfy(k/r)nllwlt(e/g)knglypnlsksyvnnk (SEQ ID NO. 151)	1928, 31, 95, 00
hngkssfy(k/r)nllwlt(e/g)knglypnlsk (SEQ ID NO. 152)	1928, 31, 95, 00
hngkssfy(k/r)nllwlt(e/g)k (SEQ ID NO. 153)	1928, 31, 95, 00
kssfyknllwltekng lypnlsksyvnnkekevlvlwgvh (SEQ ID NO. 154)	1928, 31, 95

knllwlteknglypnlsksyvnnkekevlvlwgvh (SEQ ID NO. 155)	1928, 31, 95
knglypnlsksyvnnkekevlvlwgvh (SEQ ID NO. 156)	1928, 31, 95,96, 00
ksy(v/a)nnkekev(l/-)(v/-)lwgvh (SEQ ID NO. 157)	1928, 31, 51, 95,96, 98, 00
kesswpnhtvtk (SEQ ID NO. 158)	1928, 31, 95
het(t/n)kgvtaacpyagassfyrnllwlvkkensypklsksyvnnk (SEQ ID NO. 159)	1930, 35
het(t/n)kgvtaacpyagassfyrnllwlvkkensypklsk (SEQ ID NO. 160)	1930, 35
kfeifpktsswpnevlvlwgvh (SEQ ID NO. 161)	1930
kerswpkh (SEQ ID NO. 162)	1947, 51,52,55,56, 79, 82
klsksyvnnkekevlvlwqvh (SEQ ID NO. 163)	1947, 51
knnkekevlvlwqvh (SEQ ID NO. 164)	1947
h(k/n)(g/q)kssfy(r/k)nllwlteknq(l/s)yp(n/t)lsksyannkek (SEQ ID NO. 165)	1948 79, 89, 96
h(k/n)(g/q)kssfy(r/k)nllwltek (SEQ ID NO. 166)	1948 79, 89, 96
hakkssfyk (SEQ ID NO. 167)	1951, 57,59
hngklcrlkgk (SEQ ID NO. 168)	1951,52,55,56,57,59, 79,
hyklnn(q/g)kk (SEQ ID NO. 169)	1956, 00
hdiyrdeainnrfqiqgvkltqgyk (SEQ ID NO. 170)	1956
kgngcfeifhk (SEQ ID NO. 171)	1956
klnrliektndkyhqiek (SEQ ID NO. 172)	1956
klnrliektndkyh (SEQ ID NO. 173)	1956
kchtdkgslsttk (SEQ ID NO. 174)	1956
kinngdyaklyiwgvh (SEQ ID NO. 175)	1956
hngklcrkgiaplqlgk (SEQ ID NO. 176)	1959, 82
hetnrqvtaacpyagansffrnliwlvkkessypklsk (SEQ ID NO. 177)	1963, 81
hetnrqvtaacpyagansffrnliwlvkkessypk (SEQ ID NO. 178)	1963, 81
hpptstdqqslyqnadayifvgsskynrkfk (SEQ ID NO. 179)	1963, 81
hpptstdqqslyqnadayifvgsskynrkfkpeia (SEQ ID NO. 180)	1963, 81
hdiyrdeainnrfqiqgvkitqgyk (SEQ ID NO. 181)	1977,79, 91
hqneqgsgyaadqkstqnaidgitnkvnsviekmntqftavgk (SEQ ID NO. 182)	1977

hqneqgsgyaadqkstqnaidgitnkvnsviek (SEQ ID NO. 183)	1977		
hqneqgsgyaadqkstqnaingitnkvnsviekmntqftavgkefnklek (SEQ ID NO. 184)	1979,	91	
hngklcrlkgiaplqlglk (SEQ ID NO. 185)	1979	-	
hkcnnecnesvk (SEQ ID NO. 186)	1979		
kfeifpkasswpnh (SEQ ID NO. 187)	1981		
hdsnvknlyekvrsqlmnak (SEQ ID NO. 188)	1981		
kvnsvikkmntqfaavgkefnh (SEQ ID NO. 189)	1981		
khngklck (SEQ ID NO. 190)	1981		
kkgtsypklskythnkgkevlvlwgvh (SEQ ID NO. 191)	1981		
kgtsypklskythnkgkevlvlwgvh (SEQ ID NO. 192)	1981		
klskythnkgkevlvlwgvh (SEQ ID NO. 193)	1981		
ksythnkgkevlvlwgvh (SEQ ID NO. 194)	1981		
kgvtascshk (SEQ ID NO. 195)	1985,87		
kgvtascshkgrssfymllwltekmglypnlsk (SEQ ID NO. 196)	1985,87		
kgnsypklskyvnnkekevlvlwgih (SEQ ID NO. 197)	1988		
kefnhlek (SEQ ID NO. 198)	1988		
hpptstdqqslyqnadayvfvgsskynkkdkpeiatnpk (SEQ ID NO. 199)	1988		
hpptstdqqslyqnadayvfvgsskynkkdk (SEQ ID NO. 200)	1988		
hegkssfynllwltekegsypklknsyvnk (SEQ ID NO. 201)	1991		
hegkssfynllwltekegsypk (SEQ ID NO. 202)	1991		
hkcdnecnesvrngtydypkyseesk (SEQ ID NO. 203)	1991		
kesswpnhtvtk (SEQ ID NO. 204)	1991,92		
knllwltekmglypnlskyvnnkekeilvlwgvh (SEQ ID NO. 205)	1991,92,	96	
hngkssfy(k/n)(n/-)llwlt(e/g)(-/k)knglypnlsk (SEQ ID NO. 206)	1991,92,	96,	00
hngkssfyknllwltek (SEQ ID NO. 207)	1991,92,	96	
htvtkgvtascshngkssfyknllwlteknglypnlskyvnnkekevlvlwgvh (SEQ ID NO. 208)	1995		
htvt(k/g)gv(t/s)ascshngkssfy(k/n)(n/-)llwlt(e/g)k(-n/k)glypnlsk (SEQ ID NO. 209)	1995,		00
htvtkgvtascshngkssfyknllwltek (SEQ ID NO. 210)	1995		

kyvrstklrmvtglrnipsiqsrglfgaiagfieggwtgmidgwygyh (SEQ ID NO. 211)	1995
hqneqgsgyaadqkstqnaingitnkvnsiiekmntqftavgk (SEQ ID NO. 212)	1995
hqneqgsgyaadqkstqnaingitnkvnsiiek (SEQ ID NO. 213)	1995
hqneqgsgyaadqkstqnaingitnk (SEQ ID NO. 214)	1995
hsgarsfymllwivkkgnsypk (SEQ ID NO. 215)	1996
hsgarsfymllwivkkgnsypklnk (SEQ ID NO. 216)	1996
hsgarsfymllwivkkgnsypklnksytndk (SEQ ID NO. 217)	1996
hsgarsfymllwivkkgnsypklnksytndkgk (SEQ ID NO. 218)	1996
htvskgvttscshngk (SEQ ID NO. 219)	1996
katswpnhettk (SEQ ID NO. 220)	1996
kqvttscshnqk (SEQ ID NO. 221)	1996
kgnsypklnksytndkgkevlviwgvh (SEQ ID NO. 222)	1996
klnksytndkgkevlviwgvh (SEQ ID NO. 223)	1996
ksytndkgkevlviwgvh (SEQ ID NO. 224)	1996
hnqkssfymllwlt(e/q)knglypnlsksy(v/a)annkek (SEQ ID NO. 225)	1997,98,99
hpitigecpkyvrsak (SEQ ID NO. 226)	1997
hqneqgsgyaadqkstqnaingitnkvnsviekmntqftavgk (SEQ ID NO. 227)	1998
hqneqgsgyaadqkstqnaingitnkvnsviek (SEQ ID NO. 228)	1998
hngkssfymllwlteknglypnlsksyvnnkek (SEQ ID NO. 229)	1998

1. Influenza H1N1 was responsible for the human pandemic (global distribution) of 1918.
2. Abbreviation for years: eg. "96" = 1996.
3. The first year that a given replikin appears is indicated at the beginning of the series of years in which that replikin has been found in this work.
4. Overlapping replikin sequences are listed separately.
5. Increase in number of new replikin structures occurs in years of epidemics (underlined): eg. 1918 and 1977 and correlates with increased total replikin concentration (number of replikins per 100 amino acid residues). See Figure 7.

Table 5

Replikin Sequences present in hemagglutinins of Influenza H2N2 viruses in years 1957-2000

Influenza H2N2 Replikins	**Year Detected in Influenza H2N2 strain** (*Peak in Figure 8: P2 E2)*
khfekvkilpk (SEQ ID NO. 230)	1957,58,59,60,61,64,65,68, 78,83,84,91
khllssv**kh**fekvk (SEQ ID NO. 231)	1957,58,59,60,61, 83,84,91
ha(k/q/m)(d/n)ilekthngk (SEQ ID NO. 232)	1957,58,59,60,61,64,65,68, 78,83,84,91, 95
ha(k/q/m)(d/n)ilekthngklc(k/r) (SEQ ID NO. 233)	1957,58,59,60,61,64,65,68, 78,83,84,91, 95
hnvhpltigecpkyvksek (SEQ ID NO. 234)	1957,58,59, 65,68
hpltigecpkyvksek (SEQ ID NO. 235)	1957,58,59, 65,68,64,65,68,78,83,84,91
khllssv**kh**fekvkilpk (SEQ ID NO. 236)	1957,58,59,60,61,64,65,68, 78
krqssgimktegtlencetkcqtplgainttlpfhnv**h** (SEQ ID NO. 237)	1957, 59, 83
kgsnyp(v/i)ak(g/r)synntsgeqmliiwq(v/i)**h** (SEQ ID NO. 238)	1957,58,59, 61, 83, 91, 95
httlgqsracavsgnpsffrnmvwltekgsnypvak (SEQ ID NO. 239)	1957
khfekvk (SEQ ID NO. 240)	1957, 59, 65
kiskrgssgimktegtlencetkcqtplgainttlpf**h** (SEQ ID NO. 241)	1957, 59, 65, 91
krgssgimktegtlencetkcqtplgainttlpf**h** (SEQ ID NO. 242)	1957, 59, 65, 91
ktegtlencetkcqtplgainttlpf**h** (SEQ ID NO. 243)	1957, 59, 65, 91
kiskrgssgimktegtlencetkcqtplgainttlpf**h** (SEQ ID NO. 244)	1957, 59, 65, 91
ktegtlencetkcqtplgainttlpfhn(v/i)**h** (SEQ ID NO. 245)	1957, 59, 65, 91
kiskrgssgimktegtlencetkcqtplgainttlpf**h** (SEQ ID NO. 246)	1957, 59, 65, 91

k(e/g)snypvakgsynntsgeqmliiwgvh (SEQ ID NO. 247)	1957,	60,	65		
hpltigecpkyvksek (SEQ ID NO. 248)	1957,	60,	65		
kcqtplgaikttlpfh (SEQ ID NO. 249)	1957,		65		
hhsndqgsgyaadkestqka(f/i)dgitnkvnsviek-					
-mntqfeavgklf(n/s)nleklenlnkk (SEQ ID NO. 250)		1961,	65,68,		83,84
hsndqgsgyaadkestqka(f/i)dgitnkvnsviek-					
-mntqfeavgklf(n/s)nleklenlnkk (SEQ ID NO. 251)		1961,	65, 68,		83,84
hsndqgsgyaadkestqka(f/i)dgitnk (SEQ ID NO. 252)		1961,	65, 68,		83,84
hdsnvrnlydkvrmqlrdnak (SEQ ID NO. 253)		1964,	68,76,		84,91
hkcddecmnsvkngtydypklnrneikgvk (SEQ ID NO. 254)		1964,	65,68,76,		83,84,91
hkcddecmnsvkngtydypklnrneik (SEQ ID NO. 255)		1964,	65,68,76,		83,84,91
hkcddecmnsvkngtydypk (SEQ ID NO. 256)		1964,	65,68,76,		83,84,91
hkcddecmnsvk (SEQ ID NO. 257)		1964,	65,68,76,		83,84,91
kgsnypvakgsynntngeqiliiwgvh (SEQ ID NO. 258)			1976,78		
hsndqgsgyaadkestqkavdgitnkvnsviekmntqfeavgk (SEQ ID NO. 259)			1976,		91
krgssgimktegtlencetkcqtplgainttlpfh (SEQ ID NO. 260)			1976,78,		83,84
hpltigecpkyvksek (SEQ ID NO. 261)			1976		
hakdilekthngklck (SEQ ID NO. 262)			1976		

1. Influenza H2N2 was responsible for the human pandemic (global distribution) of 1957.
2. Abbreviation for years: eg. "58" =1958.
3. The first year that a given replikin appears is indicated at the beginning of the series of years in which that replikin has been found.in this work.
4. Overlapping replikin sequences are listed separately.
5. Increase in number of new replikin structures occurs in years of epidemics (underlined): eg. 1957 and 1965 and correlates with increased total replikin concentration (number of replikins per 100 amino acid residues). See Figure 8.

Table 6

H3N2 Replikin Sequences present in H3N2 hemagglutinins of Influenza viruses in each year for which amino acid sequences were available (1968-2000)

Influenza H3N2 Replikins	**Year Detected in Influenza H3N2 strain Influenza Replikins**					
	(Peak in Figure 8:	*P3*	*E3*			*E4)*
hdvyrdealnnrfqi**k**gvelksgy**k** (SEQ ID NO. 263)	1968,72,	75			96,97,98	
htidltdsemn**k**lfertr**k** (SEQ ID NO. 264)	1968					
kfhqie**k** (SEQ ID NO. 265)	1968,72,	75,	77		96,97,98	
ktne**k**fh(g/q)ie**k** (SEQ ID NO. 266)	1968			86	98	
klnr(v/l)ie**k**tne**k**fh (SEQ ID NO. 267)	1968,72,	75,	77		97,98	
hqie**k**efsevegriqdle**k**yvedt**k** (SEQ ID NO. 268)	1968,72,				98	
kicnnp**h**k (SEQ ID NO. 269)		1975				
klnrvi**k**ktne**k**fh (SEQ ID NO. 270)		1975				
hd(I,v)yrdealnnrfqi**k**(g/q)ve(r/**k**)s(q/g)y**k** (SEQ ID NO. 271)		1975,76,77,		86		
hqie**k**efsevegriqdle**k**yvedt**k** (SEQ ID NO. 272)		1975				
kyvedt**k**idlwsynaellvalenq**h** (SEQ ID NO. 273)		1975				
kyvkqnslklatgmrnvpe**k**qtrglfgaiagfiengwegmidgwygf**rh** (SEQ ID NO. 274)		1975				
kefsevegriqdle**k**yvedt**k**idlwsynaellvalenq**h** (SEQ ID NO. 275)		1975				2000
hqn(s/e)(e/q)g(t/s)g(q/y)aad(l/q)**k**-						
-stq(a/n)a(i/l)d(q/g)I(n/t)(g/n)**k**(l/v)n(r/s)vi(e/c)**k** (SEQ ID NO. 276)		1975				2000
hcd(g/q)f(q,r)ne**k**wdlf(v,/i)er(s/t)**k** (SEQ ID NO. 277)		1975,76,77,78,80,81,82,83,84,85,86,88,89,90,91,92,93,94,95,96,97,98				

htidltdsemn**k**k**l**fertr**k** (SEQ ID NO. 278)	1977,					
ksgstypvl**k**vtmpnndnfd**k**lyiwgv**h** (SEQ ID NO. 279)	1977					
klnwlt**k**sgntypvlnvtmpnndnfd**k**lviwgv**h** (SEQ ID NO. 280)		1982				
htidltdsemn**k**lfe**k**tr**k** (SEQ ID NO. 281)			1986			
klnrlie**k**tne**k**f**h**qte**k** (SEQ ID NO. 282)			1987			
htg**k**ssvmrsdapidfcnsecitpnqsipnd**k**pfqnvn**k**itygacp**k** (SEQ ID NO. 283)				1994		
htg**k**ssvmrsdapidfcnsecitpnqsipnd**k**pfqnvn**k** (SEQ ID NO. 284)				1994		
hpstdsdqtslyvrasgrvtvst**k**rsqqtvip**k** (SEQ ID NO. 285)				1994		
kyvedt**k**idlwsynaellvalenq**h** (SEQ ID NO. 286)					1997,98	
klfertr**k**qlrenaedmgngcf**k**iy**h** (SEQ ID NO. 287)					1998	
krrsiksffsrlnwl**h** (SEQ ID NO. 288)					1998	
hpvtigecp**k**y(v/r)**k**st**k** (SEQ ID NO. 289)						2000
kgnsyp**k**ls**k**ls**k**syii**n**k**k**k**k**evlviwgi**h** (SEQ ID NO. 290)						2000
kls**k**ls**k**s(v/y)ii**n**k**k**k**k**evlviwgi**h** (SEQ ID NO. 291)						2000
kls**k**s(v/y)ii**n**k**k**k**k**evlviwgi**h** (SEQ ID NO. 292)						2000

1. Influenza H3N2 was responsible for the human pandemic (global distribution) of 1968.
2. Abbreviation for years: eg. "77" =1977.
3. The first year that a given replikin appears is indicated at the beginning of the series of years in which that replikin has been found.
4. Overlapping replikin sequences are listed separately.
5. Increase in number of new replikin structures occurs in years of epidemics (underlined) : eg. 1975 and correlates with increased total replikin concentration (number of replikins per 100 amino acid residues). See **Figure 8.**

Both the concentration and type, i.e., composition of Replikins observed were found to relate to the occurrence of influenza pandemics and epidemics. The concentration of Replikins in influenza viruses was examined by visually scanning the hemagglutinin amino acid sequences published in the National Library of Medicine "PubMed" data base for influenza strains isolated world wide from human and animal reservoirs year by year over the past century, i.e., 1900 to 2001. These Replikin concentrations (number of Replikins per 100 amino acids, mean +/- SD) were then plotted for each strain.

The concentration of Replikins was found to directly relate to the occurrence of influenza pandemics and epidemics. The concentration of Replikins found in influenza B hemagglutinin and influnza A strain, H1N1, is shown in **Figure 7**, and the concentration of Replikins found in the two other common influenza virus A strains, H2N2 and H3N2 is shown in **Figure 8** (H2N2, H3N2). The data in **Figure 8** also demonstrate an emerging new strain of influenza virus as defined by its constituent Replikins (H3N2(R)).

Each influenza A strain has been responsible for one pandemic: in 1918, 1957, and 1968, respectively. The data in **Figures 7** and **8** show that at least one replikin per 100 amino acids is present in each of the influenza hemagglutinin proteins of all isolates of the four common influenza viruses examined, suggesting a function for Replikins in the maintenance of survival levels of replication. In the 1990s, during the decline of the H3N2 strain there were no Replikins present in many isolates of H3N2, but a high concentration of new replikins appeared in H3N2 isolates, which define the emergence of the H3N2(R) strain.

Several properties of Replikin concentration are seen in **Figure 7** and **Figure 8** to be common to all four influenza virus strains: (1) Concentration is cyclic over the years, with a single cycle of rise and fall occurring over a period of two to thirty years. This rise and fall is consistent with the known waxing and waning of individual influenza virus strain predominance by hemagglutinin and neuraminidase classification. (2) Peak Replikin concentrations of each influenza virus strain previously shown to be responsible for a pandemic were observed to relate specifically and individually to each of the three years of the pandemics. For example, for the pandemic of 1918, where the influenza virus

strain, H1N1, was shown to be responsible, a peak concentration of the Replikins in H1N1 independently occurred (P1); for the pandemic of 1957, where H2N2 emerged and was shown to be responsible, a peak concentration of the Replikins in H2N2 occurred (P2); and for the pandemic of 1968, where H3N2 emerged and was shown to be the cause of the pandemic, a peak concentration of the Replikins in H3N2 occurred (P3). (3) In the years immediately following each of the above three pandemics, the specific Replikin concentration decreased markedly, perhaps reflecting the broadly distributed immunity generated in each case. Thus, this post-pandemic decline is specific for H1N1 immediately following the pandemic (P1) for which it was responsible, and is not a general property of all strains at the time. An increase of Replikin concentration in influenza B repeatedly occurred simultaneously with the decrease in Replikin concentration in H1N1, e.g., EB1 in 1951 and EB2 in 1976, both associated with influenza B epidemics having the highest mortality. (Stuart-Harris, et al., Edward Arnold Ltd. (1985). (4) A secondary peak concentration, which exceeded the primary peak increase in concentration, occurred 15 years after each of the three pandemics, and this secondary peak was accompanied by an epidemic: 15 years after the 1918 pandemic in an H1N1 'epidemic' year (E1); eight years after the 1957 pandemic in an H2N2 'epidemic' year (E2); and occurred seven years after the 1968 pandemic in an H3N2 'epidemic' year (E3). These secondary peak concentrations of specific Replikins may reflect recovery of the strain. (5) Peaks of each strain's specific Replikin concentration frequently appear to be associated with declines in Replikin concentration of one or both other strains, suggesting competition between strains for host sites. (6) There is an apparent overall tendency for the Replikin concentration of each strain to decline over a period of 35 years (H2N2) to 60 years (influenza B). This decline cannot be ascribed to the influence of vaccines because it was evident in the case of influenza B from 1901 to 1964, prior to common use of influenza vaccines. In the case of influenza B, Replikin recovery from the decline is seen to occur after 1965, but Replikin concentration declined again between 1997 and 2000 **(Figure 7)**, and this correlates with the low occurrence of influenza B in recent case isolates. H1N1 Replikin concentration peaked in 1978-1979 **(Figure 7)** together with the reappearance and prevalence of the H1N1 strain, and then peaked in 1996 coincident with

an H1N1 epidemic. **(Figure 7)**. H1N1 Replikin concentration also declined between 1997 and 2000, and the presence of H1N1 strains decreased in isolates obtained during these years. For H2N2 Replikins, recovery from a 35 year decline has not occurred **(Figure 8)**, and this correlates with the absence of H2N2 from recent isolates. For H3N2, the Replikin concentration of many isolates fell to zero during the period from 1996 to 2000, but other H3N2 isolates showed a significant, sharp increase in Replikin concentration. This indicates the emergence of a sub-strain of H3N2, which is designated herein as H3N2(R).

Figures 7 and **8** demonstrate that frequently a one to three year stepwise increase is observed before Replikin concentration reaches a peak. This stepwise increase proceeds the occurrence of an epidemic, which occurs concurrently with the Replikin peak. Thus, the stepwise increase in concentration of a particular strain is a signal that that particular strain is the most likely candidate to cause an epidemic or pandemic.

Currently, Replikin concentration in the H3N2(R) strain of influenza virus is increasing (**Figure 8**, 1997 to 2000). Three similar previous peak increases in H3N2 Replikin concentration are seen to have occurred in the H3N2-based pandemic of 1968 (Figure 8), when the strain first emerged, and in the H3N2-based epidemics of 1972 and 1975 **(Figure 8)**. Each of these pandemic and epidemics was associated with excess mortality. (Ailing, et al., Am J. Epidemiol.,113(1):30-43 (1981). The rapid ascent in concentration of the H3N2(R) subspecies of the H3N2 Replikins in 1997-2000, therefore, statistically represents an early warning of an approaching severe epidemic or pandemic. An H3N2 epidemic occurred in Russia in 2000 (**Figure 8**, E4); and the CDC report of December 2001 states that currently, H3N2 is the most frequently isolated strain of influenza virus world wide. (Morbidity and Mortality Weekly Reports (MMWR), Center for Disease Control; 50(48):1084-68 (Dec.7, 2001).

In each case of influenza virus pandemic or epidemic new Replikins emerge. There has been no observation of two of the same Replikins in a given hemagglutinin in a given isolate. To what degree the emergence of a new Replikin represents mutations versus transfer from another animal or avian pool is unknown. In some cases, each year one or more of the original Replikin structures is conserved, while at the same time, new Replikins emerge. For example, in influenza virus B hemagglutinin, five Replikins were

constantly conserved between 1919 and 2001, whereas 26 Replikins came and went during the same period (some recurred after several years absence). The disappearance and re-emergence years later of a particular Replikin structure suggests that the Replikins return from another virus host pool rather than through *de novo* mutation.

In the case of H1N1 Replikins, the two Replikins present in the P1 peak associated with the 1918 pandemic were not present in the recovery E1 peak of 1933, which contains 12 new Replikins. Constantly conserved Replikins, therefore, are the best choice for vaccines, either alone or in combination. However, even recently appearing Replikins accompanying one year's increase in concentration frequently persist and increase further for an additional one or more years, culminating in a concentration peak and an epidemic, thus providing both an early warning and time to vaccinate with synthetic Replikins (see for example, H1N1 in the early 1990's, **Figure 7**).

The data in **FIGURES 7 and 8** demonstrate a direct relationship between the presence and concentration of a particular Replikin in influenza protein sequences and the occurrence of pandemics and epidemics of influenza. Thus, analysis of the influenza virus hemagglutinin protein sequence for the presence and concentration of Replikins provides a predictor of influenza pandemics and/or epidemics, as well as a target for influenza vaccine formulation.

Composition of Replikins in Strains of Influenza Virus B: Of a total of 26 Replikins identified in this strain **(Table 3)**, the following ten Replikins are present in every influenza B isolate examined from 1902-2001. Overlapping Replikin sequences are listed separately. Lysines and histidines are in bold type to demonstrate homology consistent with the "3-point recognition."

kshfanl**k** (SEQ ID NO. 104)

kshfanl**k**gt**k** (SEQ ID NO. 105)

kshfanl**k**gt**k**trg**k**lcp**k** (SEQ ID NO. 106)

hekyggln**k** (SEQ ID NO. 107)

hekyggln**ksk** (SEQ ID NO. 108)

hekyggln**ksk**pyytge**hak** (SEQ ID NO. 10)

hakaigncpiwv**k** (SEQ ID NO. 110)

hakaigncpiwvv**kk**tpl**k**langt**k** (SEQ ID NO. 111)

hakaigncpiwv**k**tpl**k**langt**k**yrppa**k** (SEQ ID NO. 112)

hakaigncpiwv**k**tpl**k**langt**k**yrppa**k**ll**k** (SEQ ID NO. 113)

Tables 3 and 4 indicate that there appears to be much greater stability of the Replikin structures in influenza B hemagglutinins compared with H1N1 Replikins. Influenza B has not been responsible for any pandemic, and it appears not to have an animal or avian reservoirs. (Stuart-Harris et al., Edward Arnold Ltd., London (1985)).

Influenza H1N1 Replikins: Only one replikin "**h**p(v/i)tigecp**k**yv(r/**k**)(s/t)(t/a)**k**" is present in every H1N1 isolate for which sequences are available from 1918, when the strain first appeared and caused the pandemic of that year, through 2000. **(Table 4).** ("(v/i)" indicates that the amino acid v or i is present in the same position in different years.) Although H1N1 contains only one persistent replikin, H1N1 appears to be more prolific than influenza B. There are 95 different replikin structures in 82 years on H1N1 versus only 31 different Replikins in 100 years of influenza B isolates **(Table 4).** An increase in the number of new Replikin structures occurs in years of epidemics **(Tables 3, 4, 5 and 6)** and correlates with increased total Replikin concentration (**Figures 7** and **8**).

Influenza H2N2 Replikins: Influenza H2N2 was responsible for the human pandemic of 1957. Three of the 20 Replikins identified in that strain for 1957 were conserved in each of the H2N2 isolates available for examination on PubMed until 1995 **(Table 5).**

ha(**k**/q/m)(d/n)ile**k**t**h**ng**k** (SEQ ID NO. 232)

ha(**k**/q/m)(d/n)ile**k**t**h**ng**k**lc(**k**/r) (SEQ ID NO. 233)

kgsnyp(v/i)a**k**(g/r)synntsgeqmliiwq(v/i)**h** (SEQ ID No. 238)

However, in contrast to H1N1, only 13 additional Replikins have been found in H2N2 beginning in 1961. This paucity of appearance of new Replikins correlates with the

decline in the concentration of the H2N2 Replikins and the appearance of H2N2 in isolates over the years. **(Figure 8)**.

Influenza H3N2 Replikins: Influenza H3N2 was responsible for the human pandemic of 1968. Five Replikins which appeared in 1968 disappeared after 1977, but reappeared in the 1990s **(Table 6)**. The only Replikin structure which persisted for 22 years was **h**cd(g/q)f(q/r)ne**k**wdlf(v/i)er(s/t)**k**, which appeared first in 1977 and persisted through 1998. The emergence of twelve new H3N2 replikins in the mid 1990s **(Table 6)** correlates with the increase in Replikin concentration at the same time **(Figure 8)**, and with the prevalence of the H3N2 strain in recent isolates. together with the concurrent disappearance of all Replikins from some of these isolates **(Figure 8)**, this suggests the emergence of the new substrain H3N2(R).

Figures 1 and 2 show that influenza epidemics and pandemics correlate with the increased concentration of replikins in influenza virus, which is due to the reappearance of at least one replikin from one to 59 years after its disappearance. Also, in the A strain only, there is an emergence of new strain-specific Replikin compositions **(Tables 4-6)**. Increase in Replikin concentration by repetition of individual replikins within a single protein appears not to occur in influenza virus, but is seen in other organisms.

It has been believed that changes in the activity of different influenza strains are related to sequence changes in influenza hemagglutinins, which in turn are the products of substitutions effected by one of two poorly understood processes: i) antigenic drift, thought to be due to the accumulation of a series of point mutations in the hemagglutinin molecule, or ii) antigenic shift, in which the changes are so great that genetic reassortment is postulated to occur between the viruses of human and non-human hosts. First, the present data suggests that the change in activity of different influenza strains, rather than being related to non-specific sequence changes, are based upon, or relate to the increased concentration of strain-specific replikins and strain-specific increases in the replication associated with epidemics. In addition, the data were examined for a possible insight into which sequence changes are due to "drift" or "shift", and which due to conservation,

storage in reservoirs, then reappearance. The data show that the epidemic-related increase in replikin concentration is not due to the duplication of existing replikins per hemagglutinin, but is due to the reappearance of at least one replikin composition from 1 to up to 59 years after its disappearance, plus in the A strains only, the emergence of new strain-specific replikin compositions (Tables 3-6). Thus the increase in replikin concentration in the influenza B epidemics of 1951 and 1977 are not associated with the emergence of new replikin compositions in the year of the epidemic but only with the reappearance of replikin compositions which had appeared in previous years then disappeared (Table 3). In contrast, for the A strains, in addition to the reappearance of previously disappeared virus replikins, new compositions appear (e.g. in HlNl in the year of the epidemic of 1996, in addition to the reappearance of 6 earlier replikins, 10 new compositions emerged). Since the A strains only, not influenza B, have access to non-human animal and avian reservoirs, totally new compositions probably derive from non-human host reservoirs rather than from mutations of existing human replikins which appear to bear no resemblance to the new compositions other than the basic requirements of "3-point recognition" (Tables 2-5). The more prolific nature of HINl compared with B, and the fact that pandemics have been produced by the three A strains only, but not by the B strain, both may also be a function of the ability of the human A strains to receive new replikin compositions from non-human viral reservoirs.

Some replikins have appeared in only one year, disappeared, and not reappeared to date (Tables 3-6). Other replikins disappear for .from one to up to 81 years, when the identical replikin sequence reappears. Key replikin 'k' and 'h' amino acids, and the spaces between them, are conserved during the constant presence of particular replikins over many years, as shown in Tables 23-6for the following strain-specific replikins: ten of influenza B, the single replikin of HlN1, and the single replikin of H2N3, as well as for the reappearance of identical replikins after an absence. Despite the marked replacement or substitution activity of other amino acids both inside the replikin structure and outside it in the rest of the hemagglutinin sequences, influenza replikin histidine (h) appears never to be, and lysine (k) is rarely replaced. Examples of this conservation are seen in the H1N1 replikin"**h**p(v/i)tigecp**k**yv(r/**k**)(s/t)(t/a)**k**," (SEQ ID NO. 135) constant between 1918 and

2000, in the H3N2 replikin "**h**cd(g/q)f(q,r)ne**k**wdlf(v/i)er(s/t)**k**" (SEQ ID NO. 277) constant between 1975 and 1998 and in the H3N2 replikin "**h**qn(s/e)(e/q)g(t/s)g(q/y)aad(l/q)kstq(a/n)a(i/l)d(q/g)I(n/t)(g/n)**k**,(l/v)n(r/s)vi(e/c)**k**" (SEQ ID NO. 276) which first appeared in 1975, disappeared for 25 years, and then reappeared in 2000. While many amino acids were substituted, the basic replikin structure of 2 lysines, 6 to 10 residues apart, one histidine, a minimum of 6% lysine in not more than approximately 50 amino acids, was conserved.

Totally random substitution would not permit the persistence of these H1N1 and H3N2 replikins, nor from 1902 to 2001 in influenza B the persistence of 10 replikin structures, nor the reappearance in 1993 of a 1919 18mer replikin after an absence of 74 years. Rather than a random type of substitution, the constancy suggests an orderly controlled process, or in the least, protection of the key replikin residues so that they are fixed or bound in some way: lysines, perhaps bound to nucleic acids, and histidines, perhaps bound to respiratory redox enzymes. The mechanisms which control this conservation are at present unknown.

Whether the conservation of replikin structures is unique to influenza or occurs in other virus replikins was examined in foot and mouth disease virus (FMDV) isolates, where extensive mutations in proteins of this virus have been well-documented worldwide over decades. In the protein VP1 of FMDV type 0, the replikin "**h**k**q**kivapv**k**" (SEQ ID NO. 3) was found to be conserved in 78% of the 236 isolates reported in PubMed, and each amino acid was found to be conserved in individual isolates as follows: **h**,95.6%; **k**,91.8%; q,92.3%; **k**,84.1%; i,90.7%; v,91.8%; a..97.3%; p,96.2%; a,75.4%; k,88.4%. Similarly, conservation was observed in different isolates of HIV for its replikins such as "**k**cfncg**k**eg**h**" (SEQ ID NO. 5) or "**k**vylawvpa**hk**" (SEQ ID NO. 6) in HIV Type 1 and "**k**cwncg**k**eg**h**" (SEQ ID NO. 7) in HIV Type 2[16]. The high rate of conservation observed in FMVD and HIV replikins suggests that conservation observed in influenza replikins is a general property of viral replikins.

Data on anti-Replikin antibodies also support Replikin class unity. An anti-Replikin antibody response has been quantified by immunoadsorption of serum antimalignin antibody to immobilized malignin (see Methods in U.S. Patent #5,866,690).

The abundant production of antimalignin antibody by administration to rabbits of the synthetic version of the 16-mer peptide whose sequence was derived from malignin, absent carbohydrate or other groups, has established rigorously that this peptide alone is an epitope, that is, it is a sufficient basis for this immune response **(Figure 3)**. The 16-mer peptide produced both IgM and IgG forms of the antibody. Antimalignin antibody was found to be increased in concentration in serum in 37% of 79 cases in the U.S. and Asia of hepatitis B and C, early, in the first five years of infection, long before the usual observance of liver cancer, which develops about fifteen to twenty-five years after infection. Relevant to both infectious hepatitis and HIV infections, transformed cells may be one form of safe haven for the virus: prolonging cell life and avoiding virus eviction, so that the virus remains inaccessible to anti-viral treatment.

Because administration of Replikins stimulates the immune system to produce antibodies having a cytotoxic effect, peptide vaccines based on the particular influenza virus Replikin or group of Replikins observed to be most concentrated over a given time period provide protection against the particular strain of influenza most likely to cause an outbreak in a given influenza season., e.g., an emerging strain or re-emerging strain For example, analysis of the influenza virus hemagglutinin amino acid sequence on a yearly or bi-yearly basis, provides data which are useful in formulating a specifically targeted influenza vaccine for that year. It is understood that such analysis may be conducted on a region-by-region basis or at any desired time period, so that strains emerging in different areas throughout the world can be detected and specifically targeted vaccines for each region can be formulated.

Currently, vaccine formulations are changed twice yearly at international WHO and CDC meetings. Vaccine formulations are based on serological evidence of the most current preponderance of influenza virus strain in a given region of the world. However, prior to the present invention there has been no correlation of influenza virus strain specific amino acid sequence changes with occurrence of influenza epidemics or pandemics.

The observations of specific Replikins and their concentration in influenza virus proteins provides the first specific quantitative early chemical correlates of influenza

pandemics and epidemics and provides for production and timely administration of influenza vaccines tailored specifically to treat the prevalent emerging or re-emerging strain of influenza virus in a particular region of the world. By analyzing the protein sequences of isolates of strains of influenza virus, such as the hemagglutinin protein sequence, for the presence, concentration and/or conservation of Replikins, influenza virus pandemics and epidemics can be predicted. Furthermore, the severity of such outbreaks of influenza can be significantly lessened by administering an influenza peptide vaccine based on the Replikin sequences found to be most abundant or shown to be on the rise in virus isolates over a given time period, such as about one to about three years.

An influenza peptide vaccine of the invention may include a single Replikin peptide sequence or may include a plurality of Replikin sequences observed in influenza virus strains. Preferably, the peptide vaccine is based on Replikin sequence(s) shown to be increasing in concentration over a given time period and conserved for at least that period of time. However, a vaccine may include a conserved Replikin peptide(s) in combination with a new Replikin(s) peptide or may be based on new Replikin peptide sequences. The Replikin peptides can be synthesized by any method, including chemical synthesis or recombinant gene technology, and may include non-Replikin sequences, although vaccines based on peptides containing only Replikin sequences are preferred. Preferably, vaccine compositions of the invention also contain a pharmaceutically acceptable carrier and/or adjuvant.

The influenza vaccines of the present invention can be administered alone or in combination with antiviral drugs, such as ganciclovir; interferon; interleukin; M2 inhibitors, such as, amantadine, rimantadine; neuraminidase inhibitors, such as zanamivir and oseltamivir; and the like, as well as with combinations of antiviral drugs.

Analysis of the primary structure of a *Plasmodium farciparum* malaria antigen located at the merozoite surface and/or within the parasitophorous vacuole revealed that this organism, like influenza virus, also contains numerous Replikins. However, there are several differences between the observation of Replikins in *Plasmodium falciparum* and influenza virus isolates. For example, *Plasmodium falciparum* contains several partial Replikins, referred to herein as "Replikin decoys." These decoy structures contain an

abundance of lysine residues, but lack the histidine required of Replikin structures. It is believed that the decoy structure maximizes the chances that an anti-malarial antibody or other agent will bind to the relatively less important structure containing the lysines, i.e., the Replikin decoys, rather than binding to histidine, which is present in Replikin structure, such as replikins in respiratory enzymes, which could result in destruction of the trypanosome.

Another difference seen in *Plasmodium falciparum* is a frequent repetition of individual Replikin structures within a single protein, which was not observed with influenza virus. Repitition may occur by (a) sharing of lysine residues between Replikins, and (b) by repetition of a portion of a Replikin sequence within another Replikin sequence.

A third significant difference between Replikin structures observed in influenza virus isolates and *Plasmodium falciparum* is a marked overlapping of Replikin structures throughout malarial proteins, e.g., there are nine overlapping replikins in the 39 amino acid sequence of SEQ ID NO. 393 (Replikin concentration = 23.1/100 amino acids); and 15 overlapping replikins in the 41 amino acids of SEQ ID NO. 467 (Replikin concentration = 36.6/100 amino acids). Both of these overlapping Replikin structures occur in blood stage trophozoites and schizonts. In contrast, influenza virus Replikins are more scattered throughout the protein and the maximum Replikin concentration is about 7.5/100 amino acids **(Figure 7)**; and tomato leaf curl gemini virus, which was also observed to have overlapping replikins has only about 3.1/100 amino acids.

This mechanism of lysine multiples is also seen in the Replikins of cancer proteins such as in gastric cancer transforming protein, ktkkgnrvsptmkvth (SEQ ID NO. 88), and in transforming protein P21B (K-RAS 2B) of lung, khkekmskdgkkkkkks (SEQ ID NO. 89).

The relationship of higher Replikin concentration to rapid replication is also confirmed by analysis of HIV isolates. It was found that the slow-growing low titer strain of HIV (NSI, "Bru", which is prevalent in early stage HIV infection has a Replikin concentration of 1.1 (+/- 1.6) Replikins per 100 amino acids, whereas the rapidly-growing high titer strain of HIV (S1, "Lai"), which is prevalent in late stage HIV infection has a Replikin concentration of 6.8 (+/- 2.7) Replikins per 100 amino acid residues.

The high concentration of overlapping Replikins in malaria, influenza virus and cancer cells is consistent with the legendary high and rapid replicating ability of malaria organisms. The multitude of overlapping Replikins in malaria also provides an opportunity for the organism to flood and confuse the immune system of its host and thereby maximize the chance that the wrong antibody will be made and perpetuated, leaving key malaria antigens unharmed.

As in the case of influenza virus, for example, peptide vaccines based on the Replikin structure(s) found in the malaria organism can provide an effective means of preventing and/or treating malaria. Vaccination against malaria can be achieved by administering a composition containing one or a mixture of Replikin structures observed in *Plasmodium falciparum*. Furthermore, antibodies to malaria Replikins can be generated and administered for passive immunity or malaria detection purposes.

Table 7 provides a list of several *Plasmodium falciparum* Replikin sequences. It should be noted that this list is not meant to be complete. Different isolates of the organism may contain other Replikin structures.

Table 7

Malaria replikins

a) Primary structure of a Plasmodium falciparum malaria antigen located at the merozoite surface and within the parasitophorous vacuole

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a) i) DECOYS:

(C-Terminal)

keeeekekekekekeekekeekekeekekekeekekekeekeeekk (SEQ ID NO. 293), or

keeeekekekekekeekekeekekeekekekeekekekeekeeekkek (SEQ ID NO. 294), or

keeeekekekekekeekekeekekeekekekeekekekeekeekeeekk (SEQ ID NO. 295), or

keeeekekek (SEQ ID NO. 296)

ii) REPLIKINS:

Hkklikalkkniesiqnkk (SEQ ID NO. 297)

hkklikalkkniesiqnkm (SEQ ID NO. 298)

hkklikalkk (SEQ ID NO. 299)

hkklikalk (SEQ ID NO. 300)

katysfvntkkkiislksqghkk (SEQ ID NO. 301)

katysfvntkkkiislksqghk (SEQ ID NO. 302)

katysfvntkkkiislksqgh (SEQ ID NO. 303)

htyvkgkkapsdpqca dikeeckellkek (SEQ ID NO. 304)

kiislksqghk (SEQ ID NO. 305)

kkkkfeplkngnvsetiklih (SEQ ID NO. 306)

kkkfeplkngnvsetiklih (SEQ ID NO. 307)

kkfeplkngnvsetiklih (SEQ ID NO. 308)

kngnvsetiklih (SEQ ID NO. 309)

klihlgnkdkk (SEQ ID NO. 310)

kvkkigvtlkkfeplkngnvsetiklihlgnkdkkh (SEQ ID NO. 311)

hliyknksynplllscvkkmnmlkenvdyiqnqnlfkelmnqkatysfvntkkkiislk (SEQ ID NO. 312)

hliyknksynplllscvkkmnmlkenvdyiqnqnlfkelmnqkatysfvntk (SEQ ID NO. 313)

hliyknksynplllscvkkmnmlkenvdyiqnqnlfkelmnqk (SEQ ID NO. 314)

hliyknksynplllscvkkmnmlkenvdyiqknqnlfk (SEQ ID NO. 315)

hliyknksynplllscvkkmnmlk (SEQ ID NO. 316)

ksannsanngkknnaeemknlvnflqshkklikalkkniesiqnkkh (SEQ ID NO. 317)

kknnaeemknlvnflqshkklikalkkniesiqnkkh (SEQ ID NO. 318)

knlvnflqshkklikalkkniesiqnkkh (SEQ ID NO. 319)

kklikalkkniesiqnkkh (SEQ ID NO. 320)

klikalkkniesiqnkkh (SEQ ID NO. 321)

kkniesiqnkkh (SEQ ID NO. 322)

kniesiqnkkh (SEQ ID NO. 323)

knnaeemknlvnflqsh (SEQ ID NO. 324)

kklikalkkniesiqnkkqghkk (SEQ ID NO. 325)

kknnaeemknlvnflqshk (SEQ ID NO. 326)

knnaeemknlvnflqsh (SEQ ID NO. 327)

klikalkkniesiqnkkqghkk (SEQ ID NO. 328)

kvkkigvtlkkfeplkngnvsetiklih (SEQ ID NO. 329)

kngnvsetiklih (SEQ ID NO. 330)

klihlgnkdkk (SEQ ID NO. 331)

ksannsanngkknnaeemknlvnflqsh (SEQ ID NO. 332)

kknnaeemknlvnflqsh (SEQ ID NO. 333)

kklikalkkniesiqnkkh (SEQ ID NO. 334)

kalkkniesiqnkkh (SEQ ID NO. 335)

kkniesiqnkkh (SEQ ID NO. 336)

kelmnqkatysfvntkkkiislksqgh (SEQ ID NO. 337)

ksqghkk (SEQ ID NO. 338)

kkkiislksqgh (SEQ ID NO. 339)

kkiislksqgh (SEQ ID NO. 340)

kkniesiqnkkh (SEQ ID NO. 341)

kniesiqnkkh (SEQ ID NO. 342)

htyvkgkkapsdpqcadikeeckellkek (SEQ ID NO. 343)

htyvkgkkapsdpqcadikeeckellk (SEQ ID NO. 344)

b)"liver stage antigen-3" gene="LSA-3" Replikins

henvlsaalentqseeekkevidvieevk (SEQ ID NO. 345)

kenvvttilekveettaesvttfsnileeiqentitndtieekleelh (SEQ ID NO. 346)

hylqqmkekfskek (SEQ ID NO. 347)

hylqqmkekfskeknnnvievtnkaekkgnvqvtnktekttk (SEQ ID NO. 348)

hylqqmkekfskeknnnvievtnkaekkgnvqvtnktekttkvdknnk (SEQ ID NO. 349)

hylqqmkekfskeknnnvievtnkaekkgnvqvtnktekttkvdknnkvpkkrrtqk (SEQ ID NO. 350)

hylqqmkekfskeknnnvievtnkaekkgnvqvtnktekttkvdknnkvpkkrrtqksk (SEQ ID NO. 351)

hvdevmkyvqkidkevdkevskalesknd vtnvlkqnqdffskvknfvkkyk (SEQ ID NO. 352)

hvdevmkyvqkidkevdkevskalesknd vtnvlkqnqdffskvknfvkk (SEQ ID NO. 353)

hvdevmkyvqkidkevdkevskalesknd vtnvlkqnqdffsk (SEQ ID NO. 354)

hvdevmkyvqkidkevdkevskalesknd vtnvlk (SEQ ID NO. 355)

hvdevmkyvqkidkevdkevskalesk (SEQ ID NO. 356)

hvdevmkyvqkidkevdkevsk (SEQ ID NO. 357)

hvdevmkyvqkidkevdk (SEQ ID NO. 358)

hvdevmkyvqkidk (SEQ ID NO. 359)

kdevidlivqkekriekvkakkkklekkveegvsglkkh (SEQ ID NO. 360)

kvkakkkklekkveegvsglkkh (SEQ ID NO. 361)

kakkkklekkveegvsglkkh (SEQ ID NO. 362)

kkkklekkveegvsglkkh (SEQ ID NO. 363)

kkklekkveegvsglkkh (SEQ ID NO. 364)

kklekkveegvsglkkh (SEQ ID NO. 365)

klekkveegvsglkkh (SEQ ID NO. 366)

kkveegvsglkkh (SEQ ID NO. 367)

kveegvsglkkh (SEQ ID NO.368)

hveqnvyvdvdvpamkdqflgilneagglkemffnledvfksesdvitveeikdepvqk (SEQ ID NO. 369)

hikgleeddleevddlkgsildmlkgdmelgdmdkesledvttklgerveslk (SEQ ID NO. 370)

hikgleeddleevddlkgsildmlkgdmelgdmdkesledvttk (SEQ ID NO. 371)

hikgleeddleevddlkgsildmlkgdmelgdmdk (SEQ ID NO. 372)

hikgleeddleevddlkgsildmlk (SEQ ID NO. 373)

hiisgdadvlssalgmdeeqmktrkkaqrpk (SEQ ID NO. 374)

hditttldevvelkdveedkiek (SEQ ID NO. 375)

kkleevhelk (SEQ ID NO. 376)

kleevhelk (SEQ ID NO. 377)

ktietdileekkkeiekdh (SEQ ID NO. 378)

kkeiekdhfek (SEQ ID NO. 379)

kdhfek (SEQ ID NO. 380)

kfeeeaeeikh (SEQ ID NO. 381)

c) 28 KDA ookinete surface antigen precursor Replikins:

kdgdtkctlecaqgkkcikhksdhnhksdhnhksdpnhkkknnnnnk (SEQ ID NO. 382)

kdgdtkctlecaqgkkcikhksdhnhksdhnhksdpnhkk (SEQ ID NO. 383)

kdgdtkctlecaqgkkcikhksdhnhksdhnhksdpnhk (SEQ ID NO. 384)

kdgdtkctlecaqgkkcikhksdhnhksdhnhk (SEQ ID NO. 385)

kdgdtkctlecaqgkkcikhksdhnhk (SEQ ID NO. 386)

kdgdtkctlecaqgkkcikhk (SEQ ID NO. 387)

kdgdtkctlecaqgkk (SEQ ID NO. 388)

kdgdtkctlecaqgk (SEQ ID NO. 389)

kciqaecnykecgeqkcvwdgih (SEQ ID NO. 390)

kecgeqkcvwdgih (SEQ ID NO. 391)

hieckcnndyvltnryecepknkctsledtnk (SEQ ID NO. 392)

d) Blood stage trophozoites and schizonts Replikins:

ksdhnhksdhnhksdhnhksdhnhksdpnhkkknnnnnk (SEQ ID NO. 393)

ksdhnhksdhnhksdhnhksdpnhkkknnnnnk (SEQ ID NO. 394)

ksdhnhksdhnhksdpnhkkknnnnnk (SEQ ID NO. 395)

ksdhnhksdpnhkkknnnnnk (SEQ ID NO. 396)

kkknnnnnkdnksdpnhk (SEQ ID NO. 397)

kknnnnnkdnksdpnhk (SEQ ID NO. 398)

knnnnnkdnksdpnhk (SEQ ID NO. 399)

kdnksdpnhk (SEQ ID NO. 400)

ksdpnhk (SEQ ID NO. 401)

hslyalqqneeyqkvknekdqneikkikqlieknk (SEQ ID NO. 402)

hslyalqqneeyqkvknekdqneikkik (SEQ ID NO. 403)

hslyalqqneeyqkvknekdqneikk (SEQ ID NO. 404)

hslyalqqneeyqkvknekdqneik (SEQ ID NO. 405)

hklenleemdk (SEQ ID NO. 406)

khfddntneqk (SEQ ID NO. 407)

kkeddekh (SEQ ID NO. 408)

keennkkeddekh (SEQ ID NO. 409)

ktssgilnkeennkkeddekh (SEQ ID NO. 410)

knihikk (SEQ ID NO. 411)

hikkkegidigyk (SEQ ID NO. 412)

kkmwtcklwdnkgneitknih (SEQ ID NO. 413)

kkgiqwnllkkmwtcklwdnkgneitknih (SEQ ID NO. 414)

kekkdsnenrkkkqkedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 415)

kkdsnenrkkkqkedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 416)

kdsnenrkkkqkedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 417)

kkqkedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 418)

kqkedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 419)

kedkknpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 420)

knpnklkkieytnkithffkaknnkqqnnvth (SEQ ID NO. 421)

kkieytnkithffkaknnkqqnnvth (SEQ ID NO. 422)

kieytnkithffkaknnkqqnnvth (SEQ ID NO. 423)

kithffkaknnkqqnnvth (SEQ ID NO. 424)

hknnedikndnskdikndnskdikndnskdikndnnedikndnskdik (SEQ ID NO. 425)

hknnedikndnskdikndnskdikndnskdikndnnedikndnsk (SEQ ID NO. 426)

hknnedikndnskdikndnskdikndnskdikndnnedik (SEQ ID NO. 427)

hknnedikndnskdikndnskdikndnskdik (SEQ ID NO. 428)

hknnedikndnskdikndnskdikndnsk (SEQ ID NO. 429)

hknnedikndnskdikndnskdik (SEQ ID NO. 430)

hknnedikndnskdikndnsk (SEQ ID NO. 431)

hknnedikndnskdik (SEQ ID NO. 432)

hknnedik (SEQ ID NO. 433)

kkyddlqnkynilnklknsleekneelkkyh (SEQ ID NO. 434)

kyddlqnkynilnklknsleekneelkkyh (SEQ ID NO. 435)

kynilnklknsleekneelkkyh (SEQ ID NO. 436)

klknsleekneelkkyh (SEQ ID NO. 437)

knsleekneelkkyh (SEQ ID NO. 438)

kneelkkyh (SEQ ID NO. 439)

hmgnnqdinenvynikpqefkeeeeedismvntkk (SEQ ID NO. 440)

knsnelkrindnffklh (SEQ ID NO. 441)

kpclykkckisqclykkckisqvwwcmpvkdtfntyernnvlnskienniekiph (SEQ ID NO. 442)

hinneytnknpkncllykneernyndnnikdyinsmnfkk (SEQ ID NO. 443)

hinneytnknpkncllykneernyndnnikdyinsmnfk (SEQ ID NO. 444)

hinneytnknpkncllyk (SEQ ID NO. 445)

knktnqskgvkgeyekkketngh (SEQ ID NO. 446)

ktnqskgvkgeyekkketngh (SEQ ID NO. 447)

kgvkgeyekkketngh (SEQ ID NO. 448)

kgeyekkketngh (SEQ ID NO. 449)

ksgmytnegnkscecsykkkssssnkvh (SEQ ID NO. 450)

kscecsykkkssssnkvh (SEQ ID NO. 451)

kkkssssnkvh (SEQ ID NO. 452)

kkssssnkvh (SEQ ID NO. 453)

kssssnkvh (SEQ ID NO. 454)

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himlksgmytnegnkscecsykkk (SEQ ID NO. 456)

himlksgmytnegnkscecsykk (SEQ ID NO. 457)

himlksgmytnegnkscecsyk (SEQ ID NO. 458)

kplaklrkrektqinktkyergdviidnteiqkiiirdyhetlnvhkldh (SEQ ID NO. 459)

krektqinktkyergdviidnteiqkiiirdyhetlnvhkldh (SEQ ID NO. 460)

ktqinktkyergdviidnteiqkiiirdyhetlnvhkldh (SEQ ID NO. 461)

kplaklrkrektqinktkyergdviidnteiqkiiirdyhetlnvh (SEQ ID NO. 462)

kplaklrkrektqinktkyergdviidnteiqkiiirdyh (SEQ ID NO. 463)

klrkrektqinktkyergdviidnteiqkiiirdyh (SEQ ID NO. 464)

krektqinktkyergdviidnteiqkiiirdyh (SEQ ID NO. 465)

ktqinktkyergdviidnteiqkiiirdyh (SEQ ID NO. 466)

kkdkekkkdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 467)

kdkekkkdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 468)

kekkkdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 469)

kkkdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 470)

kkdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 471)

kdsnenrkkkqkedkknpndnklkkieytnkith (SEQ ID NO. 472)

kkkqkedkknpndnklkkieytnkith (SEQ ID NO. 473)

kkqkedkknpndnklkkieytnkith (SEQ ID NO. 474)

kqkedkknpndnklkkieytnkith (SEQ ID NO. 475)

kedkknpndnklkkieytnkith (SEQ ID NO. 476)

kknpndnklkkieytnkith (SEQ ID NO. 477)

knpndnklkkieytnkith (SEQ ID NO. 478)

klkkieytnkith (SEQ ID NO. 479)

kkieytnkith (SEQ ID NO. 480)

kieytnkith (SEQ ID NO. 481)

hgqikiedvnnenfnneqmknkyndeekmdiskskslksdflek (SEQ ID NO. 482)

hgqikiedvnnenfnneqmknkyndeekmdiskskslk (SEQ ID NO. 483)

hgqikiedvnnenfnneqmknkyndeekmdisksk (SEQ ID NO. 484)

hgqikiedvnnenfnneqmknkyndeekmdisk (SEQ ID NO. 485)

kkyddlqnkynilnklknsleekneelkkyh (SEQ ID NO. 486)

kyddlqnkynilnklknsleekneelkkyh (SEQ ID NO. 487)

kynilnklknsleekneelkkyh (SEQ ID NO. 488)

klknsleekneelkkyh (SEQ ID NO. 489)

knsleekneelkkyh (SEQ ID NO. 490)

kneelkkyh (SEQ ID NO. 491)

hmgnnqdinenvynikpqefkeeeeedismvntkkcddiqenik (SEQ ID NO. 492)

ktnlyniynnknddkdnildnenreglylcdvmknsnelkrindnffklh (SEQ ID NO. 493)

knsnelkrindnffklh (SEQ ID NO. 494)

krindnffklh (SEQ ID NO. 495)

hinneytnknpkncllykneernyndnnikdyinsmnfkk (SEQ ID NO. 496)

hinneytnknpkncllykneernyndnnikdyinsmnfk (SEQ ID NO. 497)

hinneytnknpkncllyk (SEQ ID NO. 498)

kpclykkckisqvwwcmpvkdtfntyernnvlnskienniekiph (SEQ ID NO. 499)

kckisqvwwcmpvkdtfntyernnvlnskienniekiph (SEQ ID NO. 500)

kienniekiph (SEQ ID NO. 501)

knktngskgvkgeyekkketngh (SEQ ID NO. 502)

ktngskgvkgeyekkketngh (SEQ ID NO. 503)

kgvkgeyekkketngh (SEQ ID NO. 504)

kgeyekkketngh (SEQ ID NO. 505)

ktiekinkskswffeeldeidkplaklrkrektqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 506)

kinkskswffeeldeidkplaklrkrektqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 507)

kplaklrkrektqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 508)

himlksqmytnegnkscecsykkkssssnkvh (SEQ ID NO. 509)

klrkrektqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 510)

krektqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 511)

ktqinktkyergdviidnteiqkiirdyh (SEQ ID NO. 512)

kplaklrkrektqinktkyergdviidnteiqkiirdyhtlnvhkldh (SEQ ID NO. 513)

klrkrektqinktkyergdviidnteiqkiirdyhtlnvhkldh (SEQ ID NO. 514)

krektqinktkyergdviidnteiqkiirdyhtlnvhkldh(SEQ ID NO. 515)

ktqinktkyergdviidnteiqkiirdyhtlnvhkldh (SEQ ID NO. 516)

kplaklrkrektqinktkyergdviidnteiqkiirdyhtlnvh (SEQ ID NO. 517)

klrkrektqinktkyergdviidnteiqkiirdyhtlnvh (SEQ ID NO. 518)

krektqinktkyergdviidnteiqkiirdyhtlnvh (SEQ ID NO. 519)

ktqinktkyergdviidnteiqkiirdyhtlnvh (SEQ ID NO. 520)

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ksqmytnegnkscecsykkkssssnkvh (SEQ ID NO. 522)

kscecsykkkssssnkvh (SEQ ID NO. 523)

kkkssssnkvh (SEQ ID NO. 524)

kkssssnkvh (SEQ ID NO. 525)

kssssnkvh (SEQ ID NO. 526)

himlksqmytnegnkscecsykkkssssnk (SEQ ID NO. 527)

himlksqmytnegnkscecsykkk (SEQ ID NO. 528)

himlksqmytnegnkscecsykk (SEQ ID NO. 529)

himlksqmytnegnkscecsyk (SEQ ID NO. 530)

hnnhniqiykdkrinfmnphkvmyhdnmsknertek (SEQ ID NO. 531)

hnnhniqiykdkrinfmnphkvmyhdnmsk (SEQ ID NO. 532)

hnnhniqiykdkrinfmnphk (SEQ ID NO. 533)

hkvmyhdnmsknertek (SEQ ID NO. 534)

hkvmyhdnmsk (SEQ ID NO. 535)

Synthetic Replikin vaccines, based on Replikins such as the glioma Replikin **(SEQ ID NO.: 1) "kagvaflhkk"** or the hepatitis C Replikin **(SEQ ID NO.: 18) "hyppkpgcivpak"**, or HIV Replikins such as **(SEQ ID NO.: 5) "kcfncgkegh"** or **(SEQ ID NO.: 6) "kvylawvpahk"** or preferably, an influenza vaccine based on conserved and/or emerging or re-emerging Replikin(s) over a given time period may be used to augment antibody concentration in order to lyse the respective virus infected cells and release virus extracellularly where chemical treatment can then be effective. Similarly, a malaria vaccine, based on Replikins observed in *Plasmodium falciparum* malaria antigens on the merozoite surface or within the parasitophorous vacuole, for example, can be used to generate cytotoxic antibodies to malaria. Recognin and/or Replikin peptides may be administered to a subject to induce the immune system of the subject to produce anti-Replikin antibodies. Generally, a 0.5 to about 2 mg dosage, preferably a 1 mg dosage of each peptide is administered to the subject to induce an immune response. Subsequent dosages may be administered if desired.

In another embodiment of the invention, isolated Replikin peptides may be used to generate antibodies, which may be used, for example to provide passive immunity in an individual. Passive immunity to the strain of influenza identified by the method of the invention to be the most likely cause of future influenza infections may be obtained by administering antibodies to Replikin sequences of the identified strain of influenza virus to patients in need. Similarly, passive immunity to malaria may be obtained by administering antibodies to *Plasmodium falciparum* Replikin(s).

Various procedures known in the art may be used for the production of antibodies to Replikin sequences. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies that are linked to a cytotoxic agent may also be generated. Antibodies may also be administered in combination with an antiviral agent.

Furthermore, combinations of antibodies to different Replikins may be administered as an antibody cocktail.

For the production of antibodies various host animals may be immunized by injection with a Replikin peptide or a combination of Replikin peptides, including but not limited to rabbits, mice, rats, and larger mammals. Various adjuvants may be used to enhance the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels, such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, key limpet hemocyanin, dintrophenol, and potentially useful human adjuvants such as BCG and *Corynebacterium parvum*.

Monoclonal antibodies to Replikins may be prepared by using any technique that provides for the production of antibody molecules. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72), and the EBV hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, Proc. Nat. Acad. Sci USA, 81:6851-6855) or other techniques may be used. Alternatively, techniques described for the production of single chain antibodies (US 4,946,778) can be adapted to produce Replikin-specific single chain antibodies.

Particularly useful antibodies of the invention are those that specifically bind to Replikin sequences contained in peptides and/or polypeptides of influenza virus. For example, antibodies to any of peptides observed to be present in an emerging or re-emerging strain of influenza virus and combinations of such antibodies are useful in the treatment and/or prevention of influenza. Similarly, antibodies to any replikins present on malaria antigens and combinations of such antibodies are useful in the prevention and treatment of malaria.

Antibody fragments which contain binding sites for a Replikin may be generated by known techniques. For example, such fragments include but are not limited to $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecules and the

Fab fragments that can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be generated (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

The fact that antimalignin antibody is increased in concentration in human malignancy regardless of cancer cell type **(Figure 5)**, and that this antibody binds to malignant cells regardless of cell type now may be explained by the presence of the Replikin structures herein found to be present in most malignancies **(Figure 1** and Table 2). Population studies have shown that antimalignin antibody increases in concentration in healthy adults with age, and more so in high-risk families, as the frequency of cancer increases. An additional two-fold or greater antibody increase which occurs in early malignancy has been independently confirmed with a sensitivity of 97% in breast cancers 1-10 mm in size. Shown to localize preferentially in malignant cells *in vivo,* histochemically the antibody does not bind to normal cells but selectively binds to **(Figure 4a,b)** and is highly cytotoxic to transformed cells *in vitro* **(Figure 4c-f)**. Since in these examples the same antibody is bound by several cell types, that is, brain glioma, hematopoietic cells (leukemia), and small cell carcinoma of lung, malignant Replikin class unity is again demonstrated.

Antimalignin does not increase with benign proliferation, but specifically increases only with malignant transformation and replication in breast *in vivo* and returns from elevated to normal values upon elimination of malignant cells (Figure 5). Antimalignin antibody concentration has been shown to relate quantitatively to the survival of cancer patients, that is, the more antibody, the longer the survival. Taken together, these results suggest that anti-Replikin antibodies may be a part of a mechanism of control of cell transformation and replication. Augmentation of this immune response may be useful in the control of replication, either actively with synthetic Replikins as vaccines, or passively by the administration of anti-Replikin antibodies, or by the introduction of non-immune based organic agents, such as for example, carbohydrates, lipids and the like, which are similarly designed to target the Replikin specifically. For organisms such as diatom plankton, foot and mouth disease virus, tomato leaf curl gemini

virus, hepatitis B and C, HIV, influenza virus and malignant cells, identified constituent Replikins are useful as vaccines, and also may be usefully targeted for diagnostic purposes. Blood collected for transfusions, for example, may be screened for contamination of organisms, such as HIV, by screening for the presence of Replikins shown to be specific for the contamination organism. Also, screening for Replikin structures specific for a particular pathological organism, e.g., anthrax., leads to diagnostic detection of the organism in body tissue or in the environment.

The Replikin sequence structure is associated with the function of replication. Thus, whether the Replikins of this invention are used for targeting sequences that contain Replikins for the purpose of diagnostic identification, promoting replication, or inhibiting or attacking replication, for example, the structure-function relationship of the Replikin is fundamental. Thus, while the structure of the Replikin may be a part of a larger protein sequence, which may have been previously identified, it is preferable to utilize only the specific Replikin structure when seeking to induce antibodies that will recognize and attach to the Replikin fragment and thereby cause destruction of the cell. Even though the larger protein sequence may be known in the art as having a "replication associated function," vaccines using the larger protein often have failed or proven ineffective, even though they contain one or more Replikin sequences.

Although the present inventors do not wish to be held to a single theory, the studies herein suggest that the prior art vaccines are ineffective because they are based on the use of the larger protein sequence. The larger protein sequence invariably has one or more epitopes (independent antigenic sequences that can induce specific antibody formation); Replikin structures usually comprise one of these potential epitopes. The presence of other epitopes within the larger protein may interfere with adequate formation of antibodies to the Replikin, by "flooding" the immune system with irrelevant antigenic stimuli which may preempt the Replikin antigens, *See*, e.g., Webster, R.G., J. Immunol., 97(2):177-183 (1966); and Webster et al., J. Infect. Dis., 134:48-58, 1976; Klenerman et al, Nature 394:421-422 (1998) for a discussion of the well-known phenomenon "original antigenic sin"). The formation of an antibody to a non-Replikin epitope may allow binding to the cell, but not necessarily lead to cell destruction. The presence of structural

"decoys" on the C-termini of malaria proteins is another aspect of this ability of other epitopes to interfere with binding of effective anti-Replikin antibodies, since the decoy epitopes have many lysine residues, but no histidine residues. Thus, decoy epitopes may bind anti-Replikin antibodies, but keep the antibodies away from histidine -bound respiratory enzymes.

It is well known in the art that in the course of antibody production against a "foreign" protein, the protein is first hydrolyzed into smaller fragments. Usually fragments containing from about six to ten amino acids are selected for antibody formation. Thus, if hydrolysis of a protein does not result in Replikin-containing fragments, anti-Replikin antibodies will not be produced. In this regard, it is interesting that Replikins contain lysine residues located six to ten amino acids apart, since lysine residues are known to bind to membranes.

Furthermore, Replikin sequences contain at least one histidine residue. Histidine is frequently involved in binding to redox centers. Thus, an antibody that specifically recognizes a Replikin sequence has a better chance of inactivating or destroying the cell in which the Replikin is located, as seen with anti-malignin antibody, which is perhaps the most cytotoxic antibody yet described, being active at picograms per cell.

One of the reasons that vaccines directed towards a particular protein antigen of a disease causing agent have not been fully effective in providing protection against the disease (such as foot and mouth vaccine which has been developed against the VP1 protein or large segments of the VP1 protein) is that antibody to the Replikins have not been produced. That is, either epitopes other than Replikins present in the larger protein fragments may interfere according to the phenomenon of "original antigenic sin", and/or because the hydrolysis of larger protein sequences into smaller sequences for processing to produce antibodies results in loss of integrity of any Replikin structure that is present, e.g., the Replikin is cut in two and/or the histidine residue is lost in the hydrolytic processing. The present studies suggest that for an effective vaccine to be produced, the Replikin sequences, and no other epitope, should be used as the vaccine. For example, a vaccine of the invention can be generated using any one of the Replikin peptides identified by the three point recognition system. Particularly preferred peptides for an influenza vaccine

include peptides that have been demonstrated to be conserved over a period of one or more years, preferably about three years or more, and/or which are present in a strain of influenza virus shown to have the highest increase in concentration of Replikins relative to Replikin concentration in other influenza virus strains, e.g., an emerging strain. The increase in Replikin concentration preferably occurs over a period of at least about six months to one year, preferably at least about two years or more, and most preferably about three years or more. Among the preferred Replikin peptides for use in an influenza virus vaccine are those replikins observed to "re-emerge" after an absence from the hemagglutinin amino acid sequence for one or more years.

The Replikin peptides of the invention, alone or in various combinations are administered to a subject, preferably by i.v. or intramuscular injection, in order to stimulate the immune system of the subject to produce antibodies to the peptide. Generally the dosage of peptides is in the range of from about 0.1 μg to about 10 mg, preferably about 10 μg to about 1 mg, and most preferably about 50 μg to about 500 ug. The skilled practitioner can readily determine the dosage and number of dosages needed to produce an effective immune response.

Replikin DNA or RNA may have a number of uses for the diagnosis of diseases resulting from infection with a virus, bacterium or other Replikin encoding agent. For example, Replikin nucleotide sequences may be used in hybridization assays of biopsied tissue or blood, e.g., Southern or Northern analysis, including *in situ* hybridization assays, to diagnose the presence of a particular organism.

Also within the scope of the invention are oligoribonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes that function to inhibit the translation of Replikin- or recognin-containing mRNA. Both antisense RNA and DNA molecules and ribozymes may be prepared by any method known in the art. The antisense molecules can be incorporated into a wide variety of vectors for delivery to a subject. The skilled practitioner can readily determine the best route of delivery, although generally i.v. or i.m. delivery is routine. The dosage amount is also readily ascertainable.

Particularly preferred antisense nucleic acid molecules are those that are complementary to a Replikin sequence contained in a mRNA encoding an influenza virus

polypeptide, wherein the Replikin sequence comprises from 7 to about 50 amino acids including (1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues. More preferred are antisense nucleic acid molecules that are complementary to a Replikin present in the coding strand of the gene or to the mRNA encoding the influenza virus hemagglutinin protein, wherein the antisense nucleic acid molecule is complementary to a nucleotide sequence encoding a Replikin that has been demonstrated to be conserved over a period of six months to one or more years and/or which are present in a strain of influenza virus shown to have an increase in concentration of Replikins relative to Replikin concentration in other influenza virus strains. The increase in Replikin concentration preferably occurs over a period of at least six months, preferably about one year, most preferably about two or three years or more.

In another embodiment of the invention, immune serum containing antibodies to one or more Replikins obtained from an individual exposed to one or more Replikins may be used to induce passive immunity in another individual or animal. Immune serum may be administered *via* i.v. to a subject in need of treatment. Passive immunity also can be achieved by injecting a recipient with preformed antibodies to one or more Replikins. Passive immunization may be used to provide immediate protection to individuals who have been exposed to an infectious organism. Administration of immune serum or preformed antibodies is routine and the skilled practitioner can readily ascertain the amount of serum or antibodies needed to achieve the desired effect.

In another aspect of the invention, Replikin structures are used to increase the replication of of organisms. The present invention demonstrates that in influenza virus, for example, increased replication associated with epidemics is associated with increased concentration of Replikins. The increase is due to 1) the reappearance of particular replikin structures, which were present in previous years, but which then disappeared for one or more years; and/or 2) by the appearance of new replikin compositions. In addition, in malaria Replikins, repetition of the same Replikin in a single protein occurs. Thus, the present invention provides methods and compositions for increasing the replication of organisms. For example, the production of crops which are critical to feeding large

populations throughout the world, such as rice, for example, can be improved by increasing the concentration (number of Replikins/100 amino acid residues) of any particular strain of the food crop.

As an example, in the *Oryza sativa* strain of rice, catalase isolated from immature seeds was observed to contain three different Replikins within the 491 amino acid sequence of the protein. Thus, by using recombinant gene cloning techniques well known in the art, the concentration of Replikin structures in an organism, such as a food crop plant, can be increased, which will promote increased replication of the organism.

The present invention also provides methods for identifying Replikin sequences in an amino acid or nucleic acid sequence. Visual scanning of over four thousand sequences was performed in developing the present 3-point-recognition methods. However, data banks comprising nucleotide and/or amino acid sequences can also be scanned by computer for the presence of sequences meeting the 3 point recognition requirements.

The three point recognition method may also be modified to identify other useful compounds of covalently linked organic molecules, including other covalently linked amino acids, nucleotides, carbohydrates, lipids or combinations thereof. In this embodiment of the invention a sequence is screened for subsequences containing three or more desired structural characteristics. In the case of screening compounds composed of covalently linked amino acids, lipids or carbohydrates the subsequence of 7 to about 50 covalently linked units should contain (1) at least one first amino acid, carbohydrate or lipid residue located seven to ten residues from a second of the first amino acid, carbohydrate or lipid residue; (2) encoding at least one second amino acid, lipid or carbohydrate residue; and (3) at least 6% of the first amino acid, carbohydrate or lipid residue. In the case of screening nucleotide sequences, the subsequence of about 21 to about 150 nucleotides should contain (1) at least one codon encoding a first amino acid located within eighteen to thirty nucleotides from a second codon encoding the first amino acid residue; (2) at least one second amino acid residue; and (3) encodes at least 6% of said first amino acid residue.

According to another embodiment of the invention, the methods described herein may be performed by a computer. **Figure 6** is a block diagram of a computer available for

use with the foregoing embodiments of the present invention. The computer may include a processor, an input/output device and a memory storing executable program instructions representing the 3-point-recognition methods of the foregoing embodiments. The memory may include a static memory, volatile memory and/or a nonvolatile memory. The static memory conventionally may be a read only memory ("ROM") provided on a magnetic, or an electrical or optical storage medium. The volatile memory conventionally may be a random access memory ("RAM") and may be integrated as a cache within the processor or provided externally from the processor as a separate integrated circuit. The non-volatile memory may be an electrical, magnetic or optical storage medium.

From a proteomic point of view the construction of a "3-point recognition" template based on the new glioma peptide sequence led directly to identification of a biology-wide class of proteins having related structures and functions. The operation of the 3-point-recognition method resembles identification by the use of a "keyword"search; but instead of using the exact spelling of the keyword "kagvaflhkk" **(SEQ ID NO.: 1)** as in a typical sequence homology search, or in the nucleotide specification of an amino acid, an abstraction of the keyword delimited by the "3-point-recognition" parameters is used. This delimited abstraction, although derived from a single relatively short amino acid sequence leads to identification of a class of proteins with structures that are defined by the same specifications. That particular functions, in this case transformation and replication, in addition to structures, turn out also to be shared by members of the exposed class suggests that these structures and functions are related. Thus, from this newly identified short peptide sequence, a molecular recognition 'language' has been formulated, which previously has not been described. Further, the sharing of immunological specificity by diverse members of the class, as here demonstrated for the cancer Replikins, suggests that B cells and their product antibodies recognize Replikins by means of a similar recognition language. Since "3-point-recognition" is a proteomic method that specifies a particular class of proteins, using three or more different recognition points for other peptides similarly should provide useful information concerning other proteins classes. Further, the "3-point- recognition" method is applicable to other recognins, for example to the TOLL 'innate' recognition of lipopolyssacharides of organisms.

Several embodiments of the present invention are specifically illustrated and described herein. However, it will be appreciated that modifications and variations of the present invention are encompassed by the above teachings and within the purview of the appended claims without departing from the spirit and intended scope of the invention.

EXAMPLE 1

PROCESS FOR EXTRACTION, ISOLATION AND IDENTIFICATION OF REPLIKINS AND THE USE OF REPLIKINS TO TARGET, LABEL OR DESTROY REPLIKIN-CONTAINING ORGANISMS

a) Algae

The following algae were collected from Bermuda water sites and either extracted on the same day or frozen at -20 degrees C and extracted the next day. The algae were homogenized in a cold room (at 0 to 5 degrees C) in 1 gram aliquots in neutral buffer, for example 100 cc. of 0.005M phosphate buffer solution, pH 7 ("phosphate buffer") for 15 minutes in a Waring blender, centrifuged at 3000 rpm, and the supernatant concentrated by perevaporation and dialyzed against phosphate buffer in the cold to produce a volume of approximately 15 ml. The volume of this extract solution was noted and an aliquot taken for protein analysis, and the remainder was fractionated to obtain the protein fraction having a pK range between 1 and 4. The preferred method of fractionation is chromatography as follows:

The extract solution is fractionated in the cold room (4 degrees C) on a DEAE cellulose (Cellex-D) column 2.5x11.0 cm, which has been equilibrated with 0.005M phosphate buffer. Stepwise eluting solvent changes are made with the following solutions:

Solution 1- 4.04 g. NaH2P04 and 0.5g NaH2P04 are dissolved in 15 litres of distilled water (0.005 molar, pH 7);

Solution 2 - 8.57 g. NaH2P04 is dissolved in 2,480 ml. of distilled water;

Solution 3 - 17.1 g. of NaH2P04 is dissolved in 2480 ml of distilled water (0.05 molar, pH 4.7);

Solution 4 - 59.65 g. of NaH2P04 is dissolved in 2470 ml distilled water (0.175 molar);

Solution 5 - 101.6 g. of NaH2P04 is dissolved in 2455 ml distilled water

(pH 4.3);

Solution 6 - 340.2 g. of NaH2P04 is dissolved in 2465 of distilled water (1.0 molar, pX-i 4.1);

Solution 7 - 283.63 g. of 80% phosphoric acid (H3P04) is made up in 2460 ml of distilled water (1.0 molar, pH 1.0).

The extract solution, in 6 to 10 ml volume, is passed onto the column and overlayed with Solution 1, and a reservoir of 300 ml of Solution 1 is attached and allowed to drip by gravity onto the column. Three ml aliquots of eluant are collected and analyzed for protein content at OD 280 until all of the protein to be removed with Solution 1 has been removed from the column. Solution 2 is then applied to the column, followed in succession by Solutions 3, 4, 5, 6 aid 7 until all of the protein which can, be removed with each Solution is removed from the column. The eluates from Solution 7 are combined, dialyzed against phosphate buffer, the protein content determined of both dialysand and dialyzate, and both analyzed by gel electrophoresis. One or two bands of peptide or protein of molecular weight between 3,000 and 25,000 Daltons are obtained in Solution 7. For example the algae *Caulerpa mexicana*, *Laurencia obtura*, *Cladophexa prolifera*, *Sargassum natans*, *Caulerpa verticillata*, *Halimeda tuna*, and *Penicillos capitatus*, after extraction and treatment as above, all demonstrated in Solution 7 eluates sharp peptide bands in this molecular weight region with no contaminants. These Solution 7 proteins or their eluted bands are hydrolyzed, and the amino acid composition determined. The peptides so obtained, which have a lysine composition of 6% or greater are Replikin precursors. These Replikin peptide precursors are then determined for amino acid sequence and the replikins are determined by hydrolysis and mass spectrometry as detailed in U.S. patent 6,242,578 B1. Those which fulfill the criteria defined by the " 3-point-recognition" method are identified as Replikins. This procedure can also be applied to obtain yeast, bacterial and any plant Replikins.

b) Virus

Using the same extraction and column chromatography separation methods as above in a) for algae, Replikens in virus-infected cells are isolated and identified.

c) Tumor cells *in vivo* and *in vitro* tissue culture

Using the same extraction and column chromatography separation methods as above in a) for algae, Replikins in tumor cells are isolated and identified. For example, Replikin precursors of Astrocytin isolated from malignant brain tumors, Malignin (Aglyco lOB) isolated from glioblastoma tumor cells in tissue cu0lture, MCF7 mammary carcinoma cells in tissue culture, and P_3J Lymphoma cells in tissue culture each treated as above in a) yielded Replikin precursors with lysine content of 9.1%, 6.7%, 6.7%, and 6.5% respectively. Hydrolysis and mass spectrometry of Aglyco lOB as described in Example 10 U.S. 6,242,578 B1 produced the amino acid sequence, ykagvaflhkkndiide the 16-mer Replikin.

EXAMPLE 2:

As an example of diagnostic use of Replikins: Aglyco lOB or the 16-mer Repliken may be used as antigen to capture and quantify the amount of its corresponding antibody present in serum for diagnostic purposes are as shown in Figures 2,3,4 and 7 of U.S. 6,242,578 B1.

As an example of the production of agents to attach to Replikins for labeling, nutritional or destructive purposes: Injection of the 16-mer Replikin into rabbits to produce the specific antibody to the 16-mer Replikin is shown in Example 6 and Figures 9A and 9B of U.S. 6,242,578 B1.

As an example of the use of agents to label Replikins: The use of antibodies to the 16-mer Replikin to label specific cells which contain this Replikin is shown in Figure 5 and Example 6 of U.S. 6,242,578 B1.

As an example of the use of agents to destroy Replikins: The use of antibodies to the 16-mer Replikin to inhibit or destroy specific cells which contain this Replikin is shown in Figure 6 of U.S. 6,242,578 B1.

EXAMPLE 3

Analysis of sequence data of isolates of influenza virus hemagglutinin protein or neuraminidase protein for the presence and concentration of Replikins is carried out by

visual scanning of sequences or through use of a computer program based on the 3-point recognition system described herein. Isolates of influenza virus are obtained and the amino acid sequence of the influenza hemagglutinin and/or neuraminidase protein is obtained by any art known method, such as by sequencing the hemagglutinin or neuraminidase gene and deriving the protein sequence therefrom. Sequences are scanned for the presence of new Replikins, conservation of Replikins over time and concentration of Replikins in each isolate. Comparison of the Replikin sequences and concentrations to the amino acid sequences obtained from isolates at an earlier time, such as about six months to about three years earlier, provides data that are used to predict the emergence of strains that are most likely to be the cause of influenza in upcoming flu seasons, and that form the basis for seasonal influenza peptide vaccines or nucleic acid based vaccines. Observation of an increase in concentration, particularly a stepwise increase in concentration of Replikins in a given strain of influenza virus for a period of about six months to about three years or more is a predictor of emergence of the strain as a likely cause of influenza epidemic or pandemic in the future.

Peptide vaccines or nucleic acid-based vaccines based on the Replikins observed in the emerging strain are generated. An emerging strain is identified as the strain of influenza virus having the highest increase in concentration of replikin sequences within the hemagglutinin and/or neuraminidase sequence during the time period. Preferably, the peptide or nucleic acid vaccine is based on or includes any Replikin sequences that are observed to be conserved in the emerging strain. Conserved replikins are preferably those Replikin sequences which are present in the hemagglutinin or neuraminidase protein sequence for about two years and preferably longer. The vaccines may include any combination of Replikin sequences identified in the emerging strain.

For vaccine production, the Replikin peptide or peptides identified as useful for an effective vaccine are synthesized by any method, including chemical synthesis and molecular biology techniques, including cloning, expression in a host cell and purification therefrom. The peptides are preferably admixed with a pharmaceutically acceptable carrier in an amount determined to induce a therapeutic antibody reaction thereto. Generally, the dosage is about 0.1 μg to about 10 mg.

The influenza vaccine is preferably administered to a patient in need thereof prior to the onset of "flu season." Influenza flu season generally occurs in late October and lasts through late April. However, the vaccine may be administered at any time during the year. Preferably, the influenza vaccine is administered once yearly, and is based on Replikin sequences observed to be present, and preferably conserved in the emerging strain of influenza virus. Another preferred Replikin for inclusion in an influenza vaccine is a Replikin demonstarated to have re-emerged in a strain of influenza after an absence of one or more years.

EXAMPLE 4

Analysis of sequence data of isolates of *Plasmodium falciparum* antigens for the presence and concentration of Replikins is carried out by visual scanning of sequences or through use of a computer program based on the 3-point recognition system described herein. Isolates of *Plasmodium falciparum* are obtained and the amino acid sequence of the protein is obtained by any art known method, such as by sequencing the gene and deriving the protein sequence therefrom. Sequences are scanned for the presence of Replikins, conservation of Replikins over time and concentration of Replikins in each isolate. This information provides data that are used to form the basis for anti-malarial peptide vaccines or nucleic acid based vaccines.

Peptide vaccines or nucleic acid-based vaccines based on the Replikins observed in the malaria causing organism are generated. Preferably, the peptide or nucleic acid vaccine is based on or includes any Replikin sequences that are observed to be present on a surface antigen of the organism. The vaccines may include any combination of Replikin sequences identified in the malaria causing strain.

For vaccine production, the Replikin peptide or peptides identified as useful for an effective vaccine are synthesized by any method, including chemical synthesis and molecular biology techniques, including cloning, expression in a host cell and purification therefrom. The peptides are preferably admixed with a pharmaceutically acceptable carrier

in an amount determined to induce a therapeutic antibody reaction thereto. Generally, the dosage is about 0.1 μg to about 10 mg.

Then malaria vaccine is preferably administered to a patient in need thereof at any time during the year, and particularly prior to travel to a tropical environment.

What is claimed is:

Claim 1. An isolated influenza virus peptide having from 7 to about 50 amino acids comprising

(1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 2. The peptide of claim 1 wherein the peptide is present in an emerging strain of influenza virus.

Claim 3. An antibody that specifically binds to an influenza virus peptide sequence having from 7 to about 50 amino acids comprising (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 4. The antibody of claim 3, wherein said antibody specifically binds to an influenza virus peptide present in an emerging strain of influenza virus.

Claim 5. An antibody cocktail comprising a plurality of antibodies, wherein each of said antibodies specifically binds to an influenza virus peptide sequence having from 7 to about 50 amino acids comprising (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 6. The antibody cocktail of claim 5 wherein the plurality of antibodies each independently and specifically binds to a Replikin peptide sequence present in an emerging strain of influenza virus.

Claim 7. A composition comprising the antibody of claim 3 or claim 4 and a pharmaceutically acceptable carrier and/or adjuvant.

Claim 8. A composition comprising the antibody cocktail of claim 5 or 6 and a pharmaceutically acceptable carrier and/or adjuvant.

Claim 9. A therapeutic composition comprising the isolated influenza virus peptide of claim 1 and a pharmaceutically acceptable carrier and/or adjuvant.

Claim 10. The therapeutic composition of claim 9 wherein the peptide is conserved in a strain of influenza virus for at least two consecutive years including the current year.

Claim 11. A therapeutic composition comprising a plurality of isolated influenza virus peptides each having from 7 to about 50 amino acids comprising

(1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues,

and a pharmaceutically acceptable carrier.

Claim 12. The therapeutic composition of claim 11 wherein at least one of the plurality of influenza virus peptides is conserved in the influenza virus hemagglutinin amino acid sequence for at least two consecutive years including the current year.

Claim 13. An antisense nucleic acid molecule complementary to an influenza virus hemagglutinin Replikin mRNA sequence, said Replikin mRNA sequence having from 7 to about 50 amino acids comprising

(1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 14. An antisense nucleic acid molecule complementary to the coding strand of the gene or the mRNA encoding influenza virus hemagglutinin, wherein said antisense nucleic acid molecule is complementary to a nucleotide sequence present in an emerging strain of influenza virus.

Claim 15. A method of stimulating the immune system of a subject to produce antibodies to influenza virus comprising administering an effective amount of at least one isolated influenza virus Replikin peptide having from 7 to about 50 amino acids comprising (1) at least one lysine residue located six to ten amino acid residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 16. The method of claim 15 wherein the at least one Replikin peptide is present in influenza virus hemagglutinin protein.

Claim 17. The method of claim 15 wherein at least one of the at least one Replikin peptide is conserved in an emerging strain of influenza virus for at least two consecutive years, including the current year.

Claim 18. A method of selecting an influenza virus peptide for inclusion in an influenza virus vaccine comprising

(1) obtaining at least one isolate of each strain of a plurality of strains of influenza virus,

(2) analyzing the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus for the presence and concentration of Replikin sequences,

(3) comparing the concentration of Replikin sequences in the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus to the concentration of Replikin sequences observed in the hemagglutinin amino acid sequence of each of the strains at at least one earlier time period to provide the concentration of Replikins for at least two time periods, said at least one earlier time period being within about six months to about three years prior to step (1),

(4) identifying the strain of influenza virus having the highest increase in concentration of Replikin sequences during the at least two time periods,

(5) selecting at least one Replikin sequence present in the strain of influenza virus peptide identified in step (4) as an isolated peptide for inclusion in an influenza virus vaccine.

Claim 19. The method of claim 18 wherein the Replikin sequence is conserved for at least about two consecutive years within the strain of influenza virus having the highest increase in Replikin sequence concentration during the at least two time periods.

Claim 20. The method of claim 18 wherein in step (5) a plurality of Replikin sequences is selected.

Claim 21. A method of making an influenza virus vaccine comprising

(1) identifying a strain of influenza virus as an emerging strain,

(2) selecting at least one Replikin sequence present in the emerging strain as a peptide template for influenza virus vaccine manufacture,

(3) synthesizing peptides having the amino acid sequence of the at least one Replikin sequence selected in step (2), and

(4) combining a therapeutically effective amount of the peptides of step (4) with a pharmaceutically acceptable carrier and/or adjuvant.

Claim 22. The method of claim 21 wherein the at least one Replikin selected in step (2) is conserved in the influenza virus hemagglutinin sequence for at least two consecutive years, including the current year.

Claim 23. A method of identifying an emerging strain of influenza virus comprising

(1) obtaining at least one isolate of each strain of a plurality of strains of influenza virus,

(2) analyzing the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus for the presence and concentration of Replikin sequences,

(3) comparing the concentration of Replikin sequences in the hemagglutinin amino acid sequence of the at least one isolate of each strain of the plurality of strains of influenza virus to the concentration of Replikin sequences observed in the hemagglutinin amino acid sequence of each of the strains at at least one earlier time period to provide the concentration of Replikins for at least two time periods, said at least one earlier time period being within about six months to about three years prior to step (1), and

(4) identifying the strain of influenza virus having the highest increase in concentration of Replikin sequences during the at least two time periods.

Claim 24. An influenza virus vaccine comprising at least one isolated Replikin present in the hemagglutinin protein of an emerging strain of influenza virus and a pharmaceutically acceptable carrier and/or adjuvant.

Claim 25. The influenza virus vaccine of claim 24 wherein the vaccine comprises a plurality of isolated Replikins.

Claim 26. The vaccine of claim 24 wherein the at least one isolated Replikin is conserved for at least two consecutive years in the emerging strain.

Claim 27. A method of preventing or treating influenza virus infection comprising administering to a patient in need thereof a vaccine comprising at least one isolated Replikin present in the hemagglutinin protein of an emerging strain of influenza virus and a pharmaceutically acceptable carrier and/or adjuvant.

Claim 28. The method of claim 27 wherein the vaccine is administered prior to the onset of flu season.

Claim 29. The method of claim 27 further comprising administering an antiviral agent.

Claim 30. the method of claim 27 wherein the antiviral agent is gancyclovir.

Claim 31. The method of claim 27 wherein the vaccine comprises a Replikin that has been conserved in the emerging strain for at least about one year.

Claim 32. A malaria vaccine comprising at least one isolated *Plasmodium falciparum* replikin and a pharmaceutically acceptable carrier.

Claim 33. The vaccine of claim 32 comprising a plurality of *Plasmodium falciparum* replikins.

Claim 34. The vaccine of claim 33 comprising overlapping Replikins.

Claim 35. The vaccine of claim 33 wherein the Replikin is present on the merozoite surface or within the parasitophorous vacuole of the *Plasmodium falciparum.*

Claim 36. An isolated *Plasmodium falciparum* peptide comprising (1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 37. The peptide of claim 36 comprising overlapping replikins.

Claim 38. An antibody that specifically binds to a Plasmodium falciparum peptide sequence having (1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 39. An antibody cocktail comprising a plurality of antibodies, wherein each of the plurality of antibodies specifically binds to a *Plasmodium falciparum* peptide sequence having (1) at least one lysine residue located six to ten residues from a second lysine residue; (2) at least one histidine residue; and (3) at least 6% lysine residues.

Claim 40. A composition comprising the antibody of claim 38 or antibody cocktail of claim 39 and a pharmaceutical composition.

Claim 41. A method of stimulating the immune system of a subject to produce antibodies to *Plasmodium falciparum* comprising administering to the subject an effective amount of at least one *Plasmodium falciparum* Replikin and a pharmaceutically acceptable carrier.

Claim 42. The method of claim 41 wherein the replikin is located on the merozoite surface or within the parasitophorous vacuole of the *Plasmodium falciparum*.

Claim 43. A method of preventing or treating malaria comprising administering to a patient a therapeutically effective amount of a vaccine comprising at least one *Plasmodium falciparum* Replikin and a pharmaceutically acceptable carrier.

Claim 44. A method of detecting the presence of a contaminating organism in a body sample or environmental sample comprising

1) isolating nucleic acids from the body sample or environmental sample;

2) screening the nucleic acids for the presence of a replikin structure; and

3) correlating the presence of a Replikin structure with the presence of the contaminating organism.

Claim 45. The method of claim 44 wherein the body sample is blood.

Claim 46. A method for increasing the replication rate of an organism comprising transforming a gene encoding an enzyme having a replication function in the organism with at least one Replikin structure.

Claim 47. The method of claim 46 wherein the organism is a food plant.

Claim 48. The method of claim 46 wherein the organism is a rice plant.