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A proposed treatment for pathogenic enveloped viruses having high rates of mutation or replication

Kevin Roe [ID]

San Jose, California, USA

Correspondence
Kevin Roe, San Jose, CA, USA.
Email: kevin.roe@att.net

Abstract

Several enveloped viruses, particularly some RNA viruses, have high rates of mutation or replication, which can make them virulent pathogens in humans and other mammals. A proposed treatment could use synthesized proteins to mask pathogenic viral surface proteins to quickly induce an immune attack on specific enveloped viruses by using existing immune cells. One treatment could inject dual-protein ligand masks into patients' bloodstreams to mask pathogenic surface proteins used to infect mammalian cells. The mammalian immune system already uses an analogous, more complex structure called a pentraxin to neutralize some pathogens by connecting their surface proteins to immune cells. And several types of antiviral peptides have already experimentally demonstrated effectiveness in blocking various viral pathogen infections. These treatments offer advantages, especially for currently untreatable viral pathogens. Furthermore, using dual-protein ligands and the antigenic memory of some sub-populations of NK cells would also allow the creation of de-facto vaccines based on a host's NK cells, instead of vaccines utilizing CD4 and CD8 α:β T cells, which are limited by the requirement of MHC presentation of the target antigens to α:β T cells. Targeted NK cell vaccines could attack host cells latently or actively infected by intracellular pathogens, even host cells having pathogen downregulated MHC antigen presentation. Eight postulates concerning the effects of pathogen mutation, or change in phenotype from genetic recombination or rearrangement, and replication rates on pathogen vs host dominance are also listed, which should be applicable to viral and non-viral pathogens.

1 | INTRODUCTION

Viruses are so prevalent that in addition to skin, every human has barrier surfaces to minimize viral infections through the epithelium of the respiratory system, the gastrointestinal tract and the endothelium of blood vessels.[1] The 2018b.v2 report of the International Committee on Taxonomy of Viruses (ICTV) listed 1019 viral genera and 5560 viral species.[2] But only a small percentage were pathogenic to humans, and at that time 155 viral genera were known to cause human viral diseases.[3] Yet despite antiviral medicines and vaccines, pathogenic viruses infect millions of children and adults yearly worldwide.[1-4]

Kevin Roe: Retired.

Even though some viral infections can be treated or at least avoided by vaccination, for many of the untreatable viral infections, a significant fraction of the survivors frequently suffer mild-to-severe lifelong impairments. For example, the eastern equine encephalitis (EEE) virus, endemic to the Western Hemisphere, has no human vaccine or even a treatment and has been called the most deadly mosquito-borne pathogen in North America, because it can kill 35-75% of infected humans depending on the medical care received, and an estimated 35-50% of the survivors suffer severe and permanent neurological brain damage.[5,6] This makes the need for a EEE virus vaccine obvious, but the only vaccine currently available is for horses, not humans.[6]

Another untreatable and lethal virus, transmissible by bodily secretions of humans and other mammals, and even considered fully capable of a worldwide pandemic spread after mutation, is the Nipah virus, with a mortality rate ranging from 72% to 86% in the Indian subcontinent.[7-10] Peptide inhibitors against the Nipah virus have been designed and modelled to target and inhibit interacting sites on the viral attachment G receptor, the F fusion protein trimer used to fuse the viral envelope with the host cell membrane and the M matrix protein dimer used for initiating the budding of the virus; and the bonding stabilities of various antiviral peptide inhibitors were assessed with molecular dynamics (MD) simulations.[11] But at this time, there is still no vaccine and not even a treatment for humans.

The two preceding viruses illustrate the virulence of some viral pathogens after transmittal to humans, but some extremely virulent viruses can have both high rates of mutation and high rates of replication. Some DNA viruses, and particularly several RNA viruses, have high mutation rates, which can be expressed as nucleotide substitutions per nucleotide per cell infection (s/n/c); and these mutation rates typically range from 10^{-8} to 10^{-6} s/n/c for DNA viruses to 10^{-6} to 10^{-4} s/n/c for RNA viruses, although nucleotide insertions and deletions can also make a smaller contribution to the overall mutation rate.[12] And independently from mutation rates, some viral pathogens can have a high replication rate among host cells, especially after transmission to secondary hosts of other species, such as viruses that originally evolved high replication rates while they infected animals such as bats and were thus selected by the fast immune responses of bats.[13] This is also characteristic of several enveloped RNA viruses, including Nipah virus of the genus *Henipavirus*, Ebola virus of the genus *Ebolavirus*, Marburg virus of the genus *Marburgvirus* and severe acute respiratory syndrome (SARS) virus of the genus *Betacoronavirus*.[13,14] And it will obviously be quite challenging to treat virulent viruses that have both high mutation rates and high replication rates.

However, there are several known antiviral peptides that can inhibit or block viral infections, and some of them could potentially treat infections by virulent enveloped RNA viruses having high rates of mutation and replication.[15,16] There are even databases of experimentally verified antiviral peptides, typically derived from micro-organisms.[16] These antiviral peptides are members of the larger group of antimicrobial peptides that contribute to the innate immune response of many species, and they are known to act either directly or by creating an immune response.[17] Several antiviral peptides have been experimentally demonstrated to be effective against different viruses, even in serum solutions with micro-molar concentrations, and their actions typically include blocking one or more stages of a virus's infection cycle, such as host cell attachment, host cell entry, replication inside a host cell, transcription, translation, maturation or release from a host cell.[18,19]

This paper is focused on antiviral applications of specifically-designed dual-protein ligand masks that can be synthesized by conventional recombinant DNA biotechnology, and these ligand masks are theoretical and functional analogue extensions of pentraxins, which are more complex immune structures used by the mammalian immune system to neutralize some pathogens.[20]

2 | MATERIALS AND METHODS

In targeting specific viral pathogens, dual-protein ligand masks (for brevity, henceforth called dual-protein ligands) should be able to create a quick and powerful immune memory response with existing memory immune cells against some viral pathogens or virus-infected cells, without some of the practical limitations of vaccines.

Which viral pathogens or virus-infected cells are susceptible to memory T cell and memory B cells? Enveloped viruses, and some non-enveloped viruses, typically have pathogenic surface proteins, such as glycoproteins, needed for viral infection of host cells.[8,9] Some of these surface proteins cannot mutate too much without losing their functionality for infection of a host cell, so critical sections of these essential surface proteins can be targeted to block viral pathogen infections.

Most T cell activations require that an antigen (i.e. a molecular pattern that a patient's immune system recognizes as foreign to the patient) be presented by another cell, such as a dendritic cell, on a specific surface protein known as a major histocompatibility complex (MHC), in humans this is also called a human-leucocyte-associated (HLA) protein.[21] Each individual has their own genetic alleles for expressing MHC (HLA), which would make it time-consuming to match their expressed MHC in order to present antigens to activate the individual's T cells requiring the same MHC expression; this restriction is known as MHC restriction.[21,22] T cells predominantly are α:β T cells with this MHC restriction requirement

for antigen presentation to activate α:β T cells, whereas a different subset of T cells called γ:δ T cells do not require any MHC presentation of antigens.[22]

The lack of a MHC presentation requirement and the lack of MHC restriction makes it feasible to design and synthesize in advance specific dual-protein ligands with a ligand, or a ligand mimic having a sufficient molecular pattern and sufficient conformation stabilization to act as a ligand, that can activate various activating receptors of γ:δ T cell sub-populations without having to first determine and match an individual's distinct genetic alleles of MHC (HLA).[21,22] These ligands can activate the primary γ:δ T cell receptor, or natural killer group 2 member D (NKG2D) receptor, or activate γ:δ T cell natural cytotoxicity receptors including the NKp30 receptor and DNAX accessory molecule-1 (DNAM-1) receptor of various sub-populations of γ:δ T cells.[22] Such potential ligands include the endothelial protein C receptor (EPCR), human MutS homologue (hMSH2), MHC class I–related chain A (MIC-A) and UL16-binding protein (ULBP), including ULBP1 and ULBP3.[22]

Although activating γ:δ T cells is challenging, there are several distinct sub-populations of γ:δ T cells; while they are more abundant in mucosal tissues, sub-populations of γ:δ T cells are also found in several types of tissues and they are even found in blood.[22-25] Furthermore, γ:δ T cells can be transformed into memory T cells like α:β T cells; and for γ:δ T cell sub-populations residing in various tissues, certain proteins previously listed, such as EPCR, in proximity to other co-stimulating proteins, such as ICAM-1 (CD54) can activate their γ:δ T cell receptors.[23-25] Activating the γ:δ T cell receptor and in some specific cases, one or two co-stimulatory receptors, can activate γ:δ T cells, which can also ultimately induce certain types of antibody production by activated B cells.[26] So γ:δ T cells are the T cells referenced herein.

2.1 | Immune response activation

Dual-protein ligands could make specific viral pathogens targets for existing immune memory cells or innate immune cells. Dual-protein ligands could induce an immune response by mimicking the key parts of antigens that activate existing immune memory cells or innate immune cells to attack tagged viral pathogens.

There are significant benefits in using the immune memory system to neutralize viral pathogens. One benefit is that when memory immune cells are triggered by a dual-protein ligand antigen, the antibody response of the immune memory system will produce antibody numbers much larger than the antibody numbers released by a primary immune response, or released by long-lived plasma cells resident in the bone marrow, or B cells in the respiratory and intestinal mucosal

tissue.[27] Other potential benefits of using dual-protein ligands include avoiding the need to use many different preventative antiviral vaccinations, and sufficient mass bondings to pathogenic viral surface proteins could potentially minimize T cell death (lymphopenia) from too many inflammatory stimulating signalling proteins (a 'cytokine storm') caused by some viral pathogens, such as the Ebola virus.[28]

2.2 | Strategies for dual-protein ligand masking of viral pathogen surface proteins

In summary, surface proteins are used by viral pathogens to infect mammalian cells. However, dual-protein ligands could mask and block these surface proteins before the widespread viral pathogen infection of mammalian cells.

A suggested treatment for humans uses a dual-protein ligand, that includes a first protein ligand that will mask (i.e. specifically bond to) a unique virus surface protein, or alternatively bond to a distinctive surface protein of a virus-infected cell, and a second protein ligand that matches or mimics the section of an antigen that would activate memory immune cells, wherein the second protein ligand is connected to the first protein ligand. Both the first protein and the second protein ligands can separately have their three dimensional conformations strengthened by covalent disulfide bonds (S-S bonds) formed between the sulfhydryl (-SH) groups of precisely placed cysteine amino acid residues.[29] Both the bonding between the first protein ligand to a viral surface protein, and the bonding between the second protein ligand to an immune cell, could be bondings utilizing non-covalent forces, such as electrostatic forces, van der Waals forces, hydrophobic forces, cation-pi interactions and hydrogen bonds.[30] Some component amino acid residues can produce several bonding forces; the aromatic amino acid residue tyrosine is typical and can bond by hydrogen bonding, hydrophobic forces, and through it's side chain pi-electron system have cation-pi interactions with nearby cations.[30]

In the brain or central nervous system (CNS) especially, synthesis of the second protein ligand to mimic an antigen to cause an attack on virus-infected cells by memory γ:δ T cells must also take into consideration a very small risk of excessive inflammation. There are several sub-populations of γ:δ T cells, that can be distinguished by their T cell receptor variable regions (Vγ), and they can be separated into bigger classifications, such as γ:δ T1 cells and γ:δ T17 cells.[26] However, there are some classifications of γ:δ T cells, the pro-inflammatory interleukin-17 producing γ:δ T17 cells, for example, that can stop certain infections, but they can also potentially create excessive inflammation and promote cancers and some auto-immune diseases.[31] Other alternatives for treatments involving the CNS and brain are second protein ligands that utilize the less inflammatory

γ:δ T1 cells, which are relatively scarce in blood, but more common in tissues and organs, that produce interferon-γ, which activates macrophages, but this can also cause some inflammatory interleukin-6 release.[32] Some additional ligands that could be mimicked by the second protein ligand to activate the γ:δ T cell receptors are listed later in this paper in the section that discusses the NK cell activating receptor NKG2D.

Another alternative is to synthesize a second protein that utilizes specific innate immune cells. One option is the microglia, the main resident macrophages for neurons in the CNS and brain, and they and CNS border-associated macrophages in general are among the innate immune cells that could be used.[33] Microglia are essentially macrophages, so the second protein ligand could be synthesized to bond to one of the many distinct types of receptors expressed by macrophages—including the pattern recognition receptors (PRR), such as the Toll-like receptors, the Fc antibody receptors, or the complement receptors, such as CR3, for example.[34] The receptor structures of some macrophages and other immune cells, such as the NK cell NKG2D activating receptor, are known to some extent; but any targeted immune cell receptor structure will probably require considerable research to determine enough detailed structure to enable the design of a second protein ligand having a strong bond to the targeted immune cell receptor. The NK cell NKG2D activating receptor and the structure of its ligands are discussed in more detail later in this paper.

There are several potential surface proteins on specific viral pathogens that can be targeted. For example, these surface proteins include the glycoproteins E1 and E2 on the surface of the hepatitis C virus of the *Hepacivirus* genus.[35,36] Another example is human immunodeficiency virus 1 (HIV-1), an enveloped virus of the *Lentivirus* genus with a surface protein, a trimeric glycoprotein, to induce membrane fusion for viral entry into a human host cell, and this glycoprotein is known to be targeted by antibodies.[37] Measles virus is an enveloped virus of the *Morbillivirus* genus, with a morbillivirus surface protein complex with tetrameric attachment (H) and trimeric fusion (F) glycoproteins for viral entry and infection of human host cells.[38] Eastern equine encephalitis virus is an enveloped virus of the *Alphavirus* genus with two trans-membrane envelope glycoproteins E1 and E2, where the surface protein E2 bonds to human cells for viral entry.[39,40] In summary, there are several possible target viral surface proteins for bonding to dual-protein ligands for the immune system neutralization of viral pathogens. As previously discussed, the structures of some viral surface proteins are already known to some extent; but any targeted viral surface protein will probably require considerable research to determine enough detailed structure to enable the design of a first protein ligand having a strong bond to the targeted viral surface protein.

2.3 | Options for masking surface proteins with dual-protein ligands

One treatment option injects dual-protein ligands into the bloodstream or localized regions to mask pathogenic surface proteins used by viruses to infect mammalian cells. The dosage of dual-protein ligands necessary to treat a viral pathogen infection will vary, depending on how long the dual-protein ligands will reside inside the patient before they are removed or inactivated, depending on the concentrations of the targeted virus and the immune cell utilized, and depending on how strongly each dual-protein ligand will bond with both the targeted viral surface protein and the immune cell utilized.

The strength of bonding between ligands and proteins is quantified by an association constant (K_a), which is defined as the equilibrium molar concentration of a protein bound to a ligand, divided by the multiplication of the molar concentration of the unbound ligand and the molar concentration of the unbound protein, as seen in Equation (1).[41] Other papers use the dissociation constant (K_d), the reciprocal of the association constant K_a, as seen in Equation (2).[41]

$$K_a = \frac{[\text{molar concentration of bound ligand and protein}]}{[\text{molar conc. of unbound ligand}] \times [\text{molar conc. of unbound protein}]},$$

(1)

$$K_d = \frac{[\text{molar conc. of unbound ligand}] \times [\text{molar conc. of unbound protein}]}{[\text{molar conc. of bound ligand and protein}]}.$$

(2)

A very large affinity constant K_a between a viral surface protein and the first protein ligand indicates that even a very low serum concentration of the first protein ligand could bond to a low serum concentration of the viral surface protein. As an example, a K_a of 1×10^{10} M^{-1} (which is typically attained by a basophil FcεRI receptor bonding to the constant region of an immunoglobulin IgE antibody) indicates a very strong bonding that allows a very low serum concentration of IgE antibodies to bond to basophil FcεRI receptors, while somewhat weaker but still useful bondings could have K_a values ranging from 1×10^7 M^{-1} to 1×10^9 M^{-1}.[42] And a very strong bonding between the first protein ligand and a viral surface protein could also possibly maintain a strong overall bonding even if the viral surface protein expresses some mutations that would weaken local bonding points to the first protein ligand.

Furthermore, a large affinity constant K_a for the bonding of an immune cell receptor to the second protein ligand indicates that a very low serum concentration of the second protein ligand would bond to a very low serum concentration of immune cell receptors. Therefore, high affinity constants K_a for each bonding of the first protein ligand and the second protein ligand indicate that a very low serum concentration

of dual-protein ligands would strongly bond immune cells to targeted viral surface proteins for the neutralization of a targeted virus.

As mentioned before, a different option is utilizing cells of the innate immune system (e.g. innate lymphoid cells, macrophages or other phagocytes). As a specific example, the first protein ligand would bond to either a surface protein of a viral pathogen, or bond to a surface protein of a cell infected by the viral pathogen, and the second protein ligand would be designed to strongly bond to and activate phagocytes to consume the viral pathogen or the virus-infected cell, by designing the second protein ligand to bond to a surface protein of human macrophages or neutrophils, for instance the FcαRI receptor (CD89).[42] This option would bypass some viral defences against memory T cells or B cells and would also quickly initiate an attack by innate immune system cells, in this instance by phagocytic cells.

2.4 | How could the treatments be used?

An injection of dual-protein ligands into the bloodstream of patients will be easier than trying to introduce dual-protein ligands from inside the gastrointestinal tract, whether by using oral capsules or therapeutic bacteria.[43,44] And injections into the bloodstream, or injections into localized regions, can be used separately or in a combination. A combination of both treatment approaches is probably better for treating viral pathogen infections that also include the brain or CNS. In such cases, a lumbar puncture into the patient's spine can safely inject the dual-protein ligands into the cerebrospinal fluid of the patient, and this is a standard procedure to deliver antibiotics directly into the brain and CNS, while avoiding the blood-brain barrier, a serious impediment to introducing most medicines into the brain by blood circulation.[45]

These proposed treatments raise the question of whether there is any risk of creating dangerous immune system reactions by injections of dual-protein ligands. This risk is small, because as discussed in an earlier paper, by themselves most pure proteins and peptides rarely induce an immune response.[46] This is one motivation to combine a chemical adjuvant with certain vaccinations to make their antigens sufficiently immunogenic to induce a strong immune response, and influence the antibody titre and isotype and increase cell-mediated responses.[47] However, since memory T cells and memory B cells already are activated effector cells, dual-protein ligand injections should be able to activate these targeted memory cells without requiring any adjuvants combined with the injections. But including certain cytokines can be helpful; cytokine therapies using type I interferons and interleukin-2 have been approved for some cancer treatments; and certain cytokines will help NK cell receptor activation, which will be discussed in more detail later.[48]

2.5 | These treatments can potentially have relatively low cost

It should be possible to synthesize dual-protein ligands in advance in the quantities needed to stop viral pathogens at relatively low cost. The modification of bacterial genomes, such as the genome of *Escherichia coli*, using restriction enzymes can induce expression of specific and desirable peptides and proteins, and this is a very mature and long commercialized technology.[49] Thus, recombinant DNA techniques, applied to one of the commercially available strains of bacteria, could be one option utilized to synthesize dual-protein ligands.

Figure 1 shows a dual-protein ligand with a first protein ligand and a covalently peptide bonded second protein ligand. Here, the first protein ligand is bonding to a viral pathogen, or a viral pathogen–infected cell surface protein, and the second protein ligand is bonding to and activating cell surface
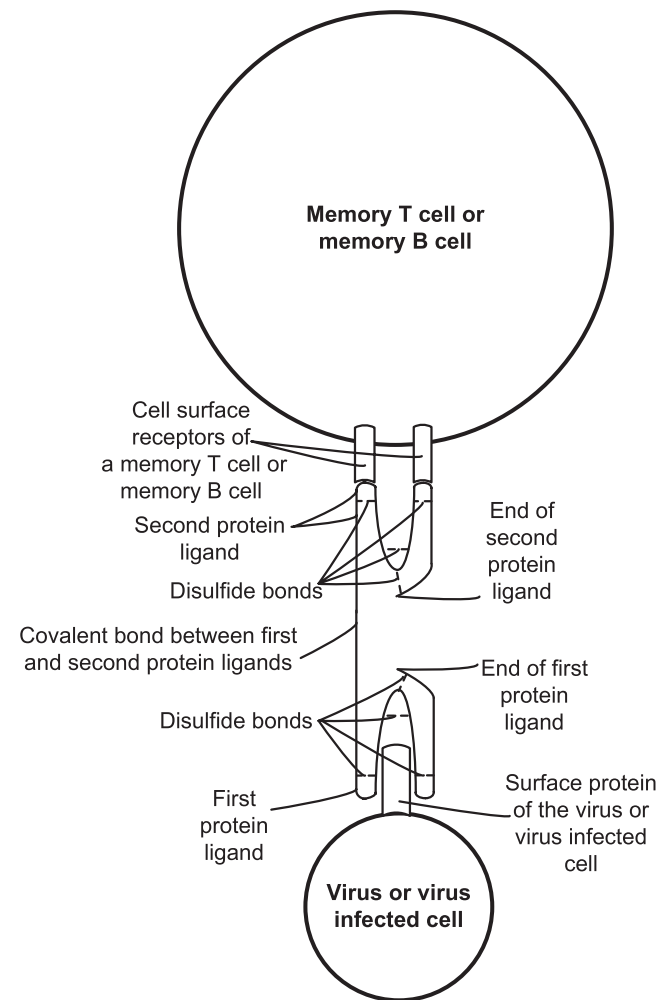
FIGURE 1 Drawing of a dual-protein ligand (including a first protein ligand and a second protein ligand). The first protein ligand is masking and bonding to a targeted virus or virus-infected cell surface protein, and the second protein ligand is activating and bonding to cell surface receptors of an immune memory cell

receptors of an immune memory T or B cell. Four disulfide bonds are shown as dotted lines bonding the side chains of cysteine amino acid residues in each protein ligand to stabilize the first protein ligand and the second protein ligand in conformations for stronger bonding to surface proteins and cell surface receptors. Thus, the viral pathogen surface or viral pathogen–infected cell, masked by the dual-protein ligand, presents a mimic of an antigen that will activate the cell surface receptors of an immune memory cell. This activates an immune memory response against any viral pathogen, or viral pathogen–infected cell, having such a dual-protein ligand mask.

Figure 2 shows a dual-protein ligand with a first protein ligand and a covalently peptide bonded second protein ligand. Here, the first protein ligand is masking and bonding to a viral pathogen, or a viral pathogen–infected cell, surface protein and the second attached protein ligand is activating and bonding to cell surface receptors of an innate immune cell, such as an innate lymphoid cell (ILC), or a phagocytic cell, such as a macrophage. This figure shows two receptors needed to activate an innate immune cell, but a more preferable case would only require one receptor to activate an innate immune cell, which is possible and discussed later. As shown in the previous figure, four disulfide bonds are shown as dotted lines linking the side chains of cysteine amino acid residues in each protein ligand, to stabilize the first protein ligand and the second protein ligand in conformations for stronger bonding to surface proteins and cell surface receptors.

For viral pathogen–infected cells, other activation options include a natural killer (NK) cell, which releases interferon-γ, perforin and granzymes that can induce the death of viral pathogen–infected cells. NK cells can be tissue-resident, or circulate in the blood, and they are the longest known antiviral ILCs, but there are also tissue-resident antiviral ILC1 cells that only release interferon-γ.[50] Usually, an NK cell must have two receptors activated before the NK cell induces other cells to die; however, a sub-population of NK cells can induce a cell death when a single receptor FcγRIIIA (CD16) is activated.[51] For example, if the second protein ligand mimics the Fc constant region of an immunoglobulin IgG1 antibody, the virus-infected cell masked with the dual-protein ligand can activate an NK cell to induce the death of the virus-infected cell—this is an indirect application of antibody-dependent cell-mediated cytotoxicity (ADCC).[50,51]

Furthermore, NK cells have now demonstrated an even greater versatility in stopping viral infections, because recent experiments have verified that some sub-populations of NK cells have a memory for certain antigens, and that memory is not exclusive to memory T cells and memory B cells as was previously believed.[52] NK cells have shown antigenic memory for previous infections by human cytomegalovirus, hantavirus, influenza, herpes simplex and vaccinia viruses, which suggests a dual-protein ligand with a second protein
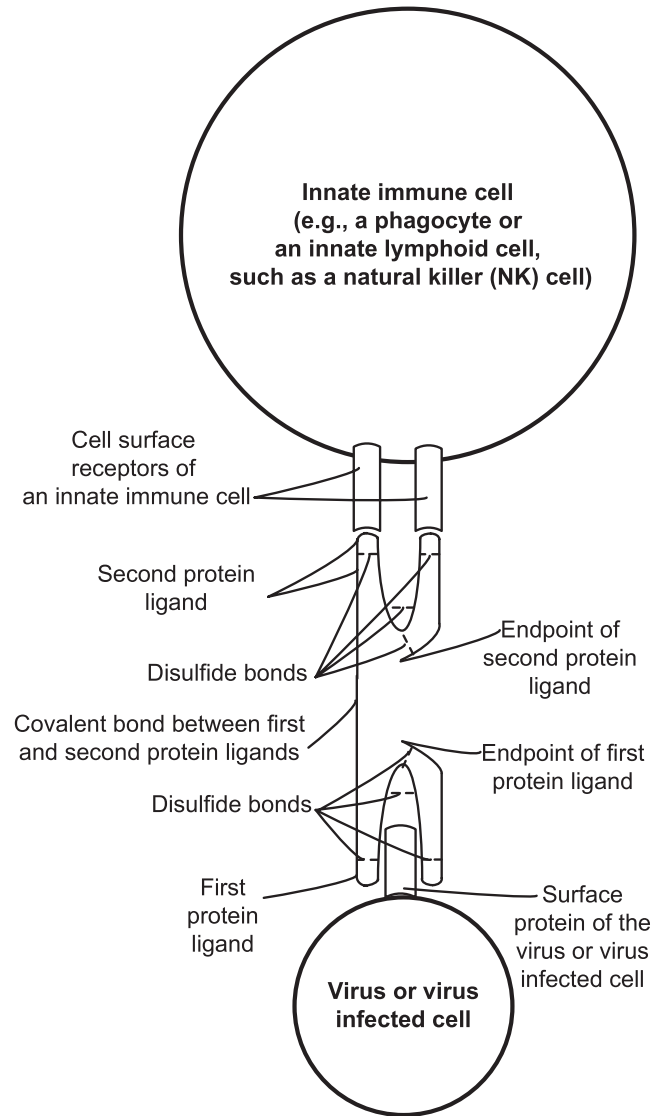

FIGURE 2 Drawing of a dual-protein ligand (including a first protein ligand and a second protein ligand). The first protein ligand is masking and bonding to a targeted virus or virus-infected cell surface protein, and the second attached protein ligand is activating and bonding to cell surface receptors of an innate immune system cell (a phagocyte or an innate lymphoid cell such as a natural killer [NK] cell)

ligand mask synthesized to mimic one of these already recognized antigens could also induce attacks by NK cells on mammalian cells infected with a different viral pathogen.[52] Using dual-protein ligands and the antigenic memory of sub-populations of NK cells would allow the creation of de-facto vaccines utilizing the host's NK cells, instead of vaccines utilizing CD4 and CD8 α:β T cells, which are limited by the requirement of MHC presentation of the target antigens to α:β T cells.[21,22] Targeted NK cell vaccines could attack host cells latently or actively infected by intracellular pathogens, even host cells having pathogen downregulated MHC antigen presentation for pathogen immunoevasion, such as immunoevasion by HIV, etc.[53]

2.6 | The dual-protein ligands may require only hundreds of amino acid residues

Another question concerns the number of amino acid residues required to synthesize a dual-protein ligand. The most preferable dual-protein ligand would activate cytotoxic immune cells, such as NK cells, by bonding to a single activating receptor. In fact, some human NK cell sub-populations can be activated solely by certain protein ligands bonding to the NK cell activating receptor NKG2D in the presence of interleukin-2 or interleukin-15.[54] Furthermore, protein ligands expressed from cells in cellular stress, such as the human MIC-A, MIC-B and ULBP family of ligands, each have isoforms less than 300 amino acid residues long, and these ligands can activate the NK cell receptor NKG2D.[54-57] MIC-A and MIC-B can also activate receptors for certain γ:δ T cell sub-populations.[58] If bonding to viral pathogen surface proteins can also be implemented with first protein ligands having an equivalent number of amino acid residues, this implies that entire dual-protein ligands could be synthesized with several hundred amino acid residues, in some cases with as little as 600 amino acid residues, possibly including a short section between the first protein ligand to the second protein ligand to provide more flexibility. Each protein ligand only needs to be long enough to provide a strong bond with its respective target, and each protein ligand may need conformal stabilization provided by disulfide bonds between precisely placed cysteine amino acid residues within each protein ligand.

One last question concerns the therapeutic duration of the dual-protein ligands in the bloodstream of a mammal. One significant factor determining the duration of a dual-protein ligand will be the presence and concentrations of pathogen and mammalian proteolytic enzymes, also known as proteases or peptidases, having nucleophilic active sites, that typically cleave peptide bonds by hydrolysis, such as at the carbonyl (C=O) of the peptide bond.[59] Any peptide segment of the dual-protein ligand is a potential peptidase target, but the peptide segment in the linkage of the two protein ligands of a dual-protein ligand will be particularly accessible to peptidases. Fortunately, many peptidases also have known inhibitors, and carefully chosen specific inhibitors for the most inconvenient peptidases could also be injected with the dual-protein ligands to prevent or slow peptidase attacks, preferably without causing major disruption to the normal physiological functions of mammalian peptidases. The most appropriate inhibitor for a peptidase can be found in an extensive database of peptidases, their substrates and their inhibitors, such as the MEROPS database.[60] As of September 2017, there were 5267 peptidase identifiers and 868 inhibitor identifiers listed in the MEROPS 12.0 database, along with the peptidase's substrate.[60]

2.7 | Eight postulates concerning the effects of pathogen mutation, change in phenotype by genetic recombination or rearrangement, and replication rates on pathogen vs host dominance

Eight postulates concerning the effects of pathogen mutation, pathogen change in phenotype by genetic recombination or rearrangement, and pathogen replication rates on pathogen vs host dominance are listed below and should be applicable not only to viral pathogens, but also applicable to bacterial, fungal, and protozoan pathogens. The term 'pathogen strain' as used below is defined as a sub-species or sub-type of a pathogen species. The term 'dominate' as used below is defined as survive, or at least maintain an infection, where a pathogen strain possibly, but not necessarily, could kill its host; whereas when a host 'dominates' a pathogen strain, it may, or may not, be able to eliminate the pathogen strain. The term 'mutation' as used below is defined as random changes in one or more genetic nucleotides, by substitution, deletion or insertion of nucleotides, etc. The term 'beneficial mutation' as used below is defined as a mutation that helps the pathogen strain, against the host or against other pathogen strains. A 'beneficial change in phenotype' as used below is also defined as helping the pathogen. The term 'genotype' as used below is define as the entire genome of a pathogen strain, and the term 'phenotype' as used below is defined as the characteristics of a pathogen strain, expressed as the interaction of a pathogen strain's genotype with its age, its various conditions of activity or latency, or its environment. It should be noted that a pathogen's phenotype can also change as a result of genetic recombination or rearrangement (called 'genetic recombination' for brevity henceforth), either randomly or by programming, recombining or rearranging groups of nucleotides within a single pathogen strain or from multiple pathogen strains; such as a change in viral phenotype after random genetic recombination during co-infection of a host cell by two different viral strains.[61] One specific example of a viral change in phenotype would be the antigenic shift in one or more antigenic determinants of an influenza virus resulting from the genetic recombination of various bird and mammalian influenza virus strains.[61]

1. A beneficial mutation or beneficial change in phenotype by genetic recombination that gives a pathogen strain an advantage over the immune system of its host, or over other strains of the pathogen, will enable that pathogen strain to eventually become the predominant strain in the host.

A beneficial mutation for a pathogen strain could be a higher rate of replication, an improvement in its infectivity or the transmission of the pathogen strain, an improvement in

reducing environmental, resource or metabolic requirements for the survival, replication or spread of the pathogen strain, an improvement in evading or overcoming the immune defence of a host by changing one or more antigenic determinants, and so forth. And as previously discussed, a beneficial change in phenotype by genetic recombination of a pathogen strain can possibly result in an antigenic shift in one or more antigenic determinants that were essential to the host's immune system for recognition and defence against the pathogen strain. This postulate could also apply to a host species, as well as a host individual.

2. A pathogen strain that can beneficially mutate or beneficially change its phenotype by genetic recombination against the host's immune defences faster than its host can improve the effectiveness of its immune defences will eventually dominate its host.

Examples of how the effectiveness of host defences could be improved include an improved blocking of the infectivity of the pathogen, or an improvement in targeting, accessing or in neutralizing the pathogen, such as provided by adaptive immune system immunoglobulin antibody isotype switching or somatic hypermutation and affinity selection of antibodies against the pathogen, producing more effective cytokines, activating other more effective innate or adaptive immune cells, and so forth.[27,62] Introduction of medicines or treatments are an alternative equivalent means to improve the effectiveness of the host defences. This postulate could also apply to a host species, as well as a host individual.

3. A pathogen strain that can replicate faster than a host can replicate an effective immune defence will eventually dominate its host.

A host can replicate effective defences in one or more ways, such as by increasing the number of effective antibodies, increasing the number of innate or adaptive immune cells, increasing the number of effective cytokines, increasing the number of activated immune cells, and so forth. Introduction of medicines or treatments are an alternative equivalent means to replicate host defences faster. This postulate could also apply to a host species, as well as a host individual.

4. A pathogen strain that can beneficially mutate or beneficially change its phenotype by genetic recombination against a first host's immune defences faster than its first host can improve the effectiveness of its immune defences will dominate its first host, and after transmission to a second host it will be enabled to more virulently dominate a second host individual or species having a slower or weaker immune defence, which otherwise provides an equivalent host environment for the pathogen strain.

5. A pathogen strain that can replicate faster than a first host can replicate an effective immune defence against the pathogen strain will dominate its first host, and after transmission to a second host it will be enabled to more virulently dominate a second host individual or species having a slower or weaker immune defence, which otherwise provides an equivalent host environment for the pathogen strain.

6. A host that can improve the effectiveness of its immune defences faster than any pathogen strain can beneficially mutate or beneficially change its phenotype by genetic recombination against the host's immune defences can eventually dominate the pathogen.

This postulate could also apply to a host species, as well as a host individual.

7. A host that can replicate an effective immune defence faster than any pathogen strain can replicate can eventually dominate the pathogen.

This postulate could also apply to a host species, as well as a host individual.

8. A host that dominates, but does not completely eliminate, a pathogen strain will enable the pathogen strain to possibly beneficially mutate its genotype or beneficially change its phenotype by genetic recombination, and enable the pathogen strain to evade the host's immune system, typically starting from the host locations with the weakest immune defences, in order to regain dominance over the host.

These eight postulates summarize the effects of pathogen mutation, pathogen change in phenotype by genetic recombination or rearrangement, and pathogen replication rates on pathogen vs host dominance and their scope is limited to pathogen vs host dominance, but these postulates should be applicable to viral pathogens, and also applicable to bacterial, fungal, and protozoan pathogens. The host could be a mammal, but could possibly be non-mammalian, or even outside of the animal kingdom, since plants also struggle for survival and dominance over several types of pathogens.

3 | CONCLUSIONS

Targeted dual-protein ligands could mask viral surface proteins to quickly treat some untreatable virus infections by using already existing immune cells. One treatment uses

injection of the dual-protein ligands into the blood of patients, and another treatment injects the dual-protein ligands into less accessible viral pathogen infections, such as the brain or central nervous system, to bond to a viral surface protein used to infect mammalian cells. These treatments could have advantages, especially for enveloped RNA viruses having high rates of mutation or replication, although the initial development and implementation of these new treatment approaches will require substantial resources. Furthermore, using dual-protein ligands and the antigenic memory of some sub-populations of NK cells would also allow the creation of defacto vaccines based on a host's NK cells, instead of vaccines based on α:β T cells, which are limited by the requirement of MHC presentation of the target antigens to α:β T cells. Targeted NK cell vaccines could attack host cells latently or actively infected by intracellular pathogens, even host cells having less MHC antigen presentation capabilities, such as neurons. Eight postulates concerning the effects of pathogen mutation, or change in phenotype from genetic recombination or rearrangement, and replication rates on pathogen vs host dominance have also been listed, which should be applicable to viral and non-viral pathogens.

CONFLICT OF INTEREST

The author has no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

The author attests that he conceived the paper, wrote the paper and approved the final version of the manuscript, and attests that he meets all of the ICMJE criteria for authorship.

ETHICAL APPROVAL

The author confirms that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is an article with no original research data.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Kevin Roe https://orcid.org/0000-0002-4843-9835

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