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(54) **Title:** COMPOSITION AND METHOD FOR THE TREATMENT OF VIRAL INFECTION USING CAMELID HEAVY CHAIN ANTIBODIES

(57) **Abstract:** The present invention provides a method of treating or preventing human infection with type A Influenza comprising the step of administering a therapeutically useful amount of an isolated heavy chain Camelid antibody, or an antigen binding portion thereof, to a subject in need of treatment. The invention also extends to an isolated heavy chain Camelid antibody, or an antigen binding portion thereof, for use in the prophylaxis or treatment of human infection with type A influenza. Also provided is a method of producing a polyclonal Camelid heavy chain immunoglobulin preparation which specifically binds Neuraminidase and a method of treating influenza A viral infection comprising the step of administering said immunoglobulin preparation, or an antigen-binding portion thereof, to a subject in need of therapy.

COMPOSITION AND METHOD FOR THE TREATMENT OF VIRAL INFECTION USING CAMELID HEAVY CHAIN ANTIBODIES

Field of the Invention

The present invention relates to compositions and methods for use of the same for the treatment and prophylaxis of viral infections. In particular the present invention relates to Camelid heavy chain antibodies and antigen binding portions thereof that specifically bind to the influenza Neuraminidase enzyme, and which function to inhibit Neuraminidase function. The invention further extends to the use of said Camelid antibodies in the inhibition of a broad spectrum of avian orthomyxovirus infections, more specifically type A influenza viruses of the family orthomyxoviridae, most specifically Influenza virus A, subtypes H5N1, H5N2, H7 and H9 (commonly termed "avian influenza" or "bird flu").

Background to the Invention

The Camelid family is composed of Camels, Alpacas and Llamas. Camelids have unique immune systems that allow them to generate immunoglobulins (antibodies) which inhibit enzymes; no other domestic animals can do this.

In addition to the production of classical four chain heterotetrameric immunoglobulins, comprised of a dimerisation of 2 heavy chains and 2 light chains, the Camilidae produce a further subset of antibodies known as heavy chain antibodies.

These 'heavy chain' antibodies are so named as they are comprised solely of 2 heavy chains, that is that the light chains, which are present in the classical heterotetrameric antibody, are absent in the structure of the heavy chains.

The variable domain of the Camilidae heavy chain antibody differs in both structure and function to the variable heavy domain of the classical 4 chain antibody. Unlike the variable domain present in the heavy chain of a heterotetrameric antibody, the variable domain of the Camilidae heavy chain (referred to as VHH) has no interaction with the variable light chain domain, as this is absent. Further, the CH1 constant domain is also absent from the Camelid heavy chain antibody structure.

The variable domain (VHH) of the heavy chain contains the antigen binding sites of the antibody, these being the areas of the antibody which confer its binding specificity. The binding epitope of the heavy chain antibody is thus formed solely from the variable domain as opposed to being derived from a complex of the light and heavy variable domains as is the case in the classical antibody structure.

The comparative simplicity in structure, observed specific and high affinity binding to its target (paratope) confer all the advantages associated with heterotetrameric antibodies. However, heavy chain antibodies are further known to have binding specificity to enzyme active sites, and further have the ability to mimic the enzyme's substrate. Accordingly, Camelid heavy chain antibodies present advantages over classic heterotetrameric antibodies in relation to the design, production and application of clinically valuable compounds.

Avian influenza is commonly known as "avian flu" or "bird flu". The avian influenza virus was first isolated from birds in South Africa in 1961. Wild birds are the natural host of the virus, with the virus circulating amongst birds worldwide. The virus is extremely contagious and can be deadly to birds, particularly domesticated chickens. Fifteen subtypes of the influenza virus are known to infect avians. Although primarily associated

with the occurrence of illness and infection in avians, there have been previous instances of certain subtypes of avian influenza strains "jumping" the species barrier and causing infection in humans.

Incidences of disease outbreaks include an outbreak of H5N1 in Hong Kong in 1997 causing infections in both poultry and humans; an H9N2 outbreak in China and Hong Kong in 1999, an H7N2 outbreak in Virginia in 2002, and reported case of; H7N7 in the Netherlands (2003), H9N2 in Hong Kong (2003), H7N2 in New York (2003), and H5N1 in Thailand and Vietnam (2004). In January 2003, outbreaks of highly pathogenic influenza A (H5N1) in Asia were first reported by the World Health Organization, this was followed by reported cases of H7N3 in Canada in in February 2004. Subsequently cases of H5N1 were reported in Thailand and Vietnam in June 2004, this outbreak extending to several countries in Asia. The new outbreaks of H5N1 in poultry in Asia were followed by renewed sporadic reporting of human cases of H5N1 infection in Vietnam and Thailand beginning in August and continuing into 2005.

During the 2005 outbreak, there was 1 suspected case of human to human transfer of the virus. Infections in humans coincided with devastating epidemics in poultry farms in Asian countries, with a reported mortality rate approaching 100%.

Influenza viruses are orthomyxoviruses, and fall into three types; A, B and C. Influenza A and B virus particles contain a genome of negative sense, single-stranded RNA divided into 8 linear segments. Co-infection of a single host with two different influenza viruses may result in the generation of 'reassortant' progeny viruses having a new combination of genome segments, derived from each of the parental viruses.

Influenza A viruses have been responsible for four recent pandemics of severe human respiratory illness. Influenza A viruses can be divided into subtypes according to their surface proteins, haemagglutinin (HA or H) and neuraminidase (NA or N). There are 15 known haemagglutinin subtypes and 9 neuraminidase subtypes. All haemagglutinin subtypes have been found in birds, however only three H subtypes (H1, H2 and H3) and two neuraminidase subtypes (N1 and N2) have been reported as commonly circulating in humans.

Seasonal influenza epidemics in humans are associated with amino acid changes in antigenic sites in the haemagglutinin and neuraminidase proteins, in a process termed 'antigenic drift'. Major pandemics are associated with the introduction of new haemagglutinin and neuraminidase genes from animal-derived influenza viruses, by reassortment, into the genetic background of a currently circulating human virus – called 'antigenic shift'.

H5N1 isolates from geese, ducks, and chickens from farms and poultry markets in Hong Kong during 1997 were compared with a human isolate and demonstrated to replicate in geese, pigs, rats and mice. Animal to animal transfer was not observed for mice or pigs. As pigs are receptive to avian, human and swine influenza types, they have long been thought of as a potential "mixing vessel" for antigenic shift to occur, allowing the virus to acquire human influenza-type genes permitting human to human transmission. However, more recent outbreaks have provided a clear indication that some avian influenza viruses have the potential to directly infect humans without a swine intermediate as a "mixing vessel".

H5N1 mutates rapidly and has a documented propensity to acquire genes from viruses infecting other animal species. Its ability to cause severe

disease in humans has now been documented on two occasions. In addition, laboratory studies have demonstrated that isolates from this virus have a high pathogenicity and can cause severe disease in humans. Birds that survive infection excrete virus for at least 10 days, orally and in faeces, thus facilitating further spread at live poultry markets and by migratory birds.

The direct infection of the H5N1 avian influenza virus into humans presents a high risk potential for progression to pandemic spread amongst humans. Repeated chances at replication in humans may allow this virus to become better adapted to humans and allow efficient human to human transmission. The importance of establishing a reliable treatment for not only sporadic outbreaks of H5N1 in humans, but also for use in the event that a pandemic situation arises, is clearly evident.

It is not fully known how or why H5N1 has crossed the species barrier. It is clear that upon crossing the species barrier, pathogenicity of H5N1 is high. The H5N1 virus comes in two forms, one demonstrating low pathogenicity in chickens, and the second being the highly virulent form known as "highly pathogenic avian influenza". There is mounting evidence that this strain has the unique capacity to jump the species barrier causing the severe disease, with high mortality, recently observed in humans.

At present, there is no vaccine against H5N1 for use in humans. It is clear that preventing a pandemic by way of vaccination following identification of the specific strain of avian influenza which may be responsible for the pandemic is not a reliable means of control such an influenza outbreak, largely due to the short time period between strain detection and need for immediate generation of a vaccine product.

Therefore, broad-spectrum means to control influenza infection, in the form of safe and effective anti-viral therapies, are highly desirable.

At present, there are two classes of drugs commercially available for the prevention and treatment of influenza virus infections in humans; M2 ion channel blockers and Neuraminidase inhibitors.

Amantadine and Rimantadine function by blocking the ion channel activity of the viral M2 protein, which is mainly required during virus entry in the early phase of the replication life cycle. Both treatments are highly effective in treating influenza A but cause significant side effects of the central nervous system, liver and kidneys. However, sensitive influenza strains have been observed to rapidly develop resistance in vitro and in vivo. It is also thought that some H5N1 virus isolates are resistant to M2 inhibitors.

Oseltamivir and Zanamivir block the action of neuraminidase to prevent release of newly formed virus from the infected cell and its spread within the host. Oseltamivir (oseltamivir phosphate) is a potent and selective inhibitor of influenza A and B virus neuraminidases. Zanamivir (4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid) is a recognised neuraminidase inhibitor useful in the treatment or prevention of influenza virus infection. Zanamivir is generally thought to have poor oral bioavailability due to poor dissolution in the gastrointestinal tract.

Although, Oseltamivir reduces the severity of some symptoms, it does not reduce nasal congestion or the production of excessive mucous in the respiratory tract to the same degree that an H1 histamine receptor antagonist does. Consequently, nasal congestion or the production of

excessive mucous in the respiratory tract lingers in patients having been administered Oseltamivir.

Ribavirin is a broad spectrum anti-viral agent based on a purine nucleoside analogue and is the standard treatment regimen for hepatitis C. Ribavirin is known to be active against various RNA viruses by inducing lethal mutagenesis of the viral RNA genome. Although Ribavirin has a marked anti-viral activity against a number of viruses, it is not acknowledged as a medicament for influenza infections.

In summary, there are currently no completely effective therapeutic or prophylactic treatments for humans infected with Influenza A, and in particular subtype H5N1. The potential severity and mortality linked to human infection with Influenza A (H5N1) and other subtypes such as H7 and H9 illustrates that there exists a need for an effective treatment.

The present inventors have surprisingly shown that heavy chain Camelid antibodies can be produced which act to inhibit the influenza Neuraminidase enzyme. Such antibodies have been shown to inhibit a broad spectrum of avian influenza viral strains. These enzyme inhibitory Camelid antibodies have particular utility in the inhibition of influenza A viruses in humans, without producing the side effects which result from the use of chemical inhibitors. The present inventors have successfully taken the chicken influenza vaccine, that has been proven to inhibit H5N2 virus infection in chickens, and inoculated camels with this vaccine. The subsequently generated antibodies were collected. Although the vaccine virus is of the strain H5N2, the antibodies which are produced in Camelids following the inoculation of the Camelids have surprisingly been shown to inhibit a broad spectrum of Neuraminidase enzymes.

Summary of the Invention

According to a first aspect of the present invention there is provided a method for the treatment and/or prophylaxis of a viral infection in a mammalian, the method comprising the step of administering a therapeutically effective amount of a heavy chain Camelid antibody to an individual in need of such treatment.

In one embodiment the heavy chain antibody is at least one Camelid heavy chain antibody.

As herein defined, the term "heavy chain Camelid antibody" or "Camelid heavy chain antibody" means an antibody which comprises only heavy chains, that is it is lacking in light chains commonly associated with classical heterotetrameric antibodies. Further, the heavy chain antibody lacks the CH1 constant domain.

In one embodiment the viral infection is an influenza virus infection, in particular a type A Influenza viral infection. Suitably the Influenza A virus is of the subtype H5, H7, H11 or H9. Further, the type A Influenza subtype may be of the strain H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

Suitably the Camelid heavy chain antibody has binding specificity for the neuraminidase enzyme and binds thereto. Suitably the binding of the Camelid heavy chain antibody serves to inhibit the action of the neuraminidase enzyme. The neuraminidase enzyme may be any neuraminidase subtype, particularly of subtypes N1 to N9.

In one embodiment, the Camelid heavy chain antibody comprises a polyclonal mixture of antibodies. In one embodiment the polyclonal mixture preparation of antibodies comprises antibodies of the class IgG2 and / or IgG3.

In one embodiment the Camelid (which may further be defined as a Camelidae) is a Camel, specifically Camelus bactrianus or Camelus dromderius. In further embodiments the Camelidae is a Llama such as Lama Paccos, Lama Glama or Lama Vicugna.

The Camelid antibodies are obtained from Camelids following their inoculation with a known strain of type A influenza. The strain of Influenza A administered to the Camelid should be provided in an attenuated or inactivated form such that is does not cause infection in the host, while at the same time, being identified as being foreign by the host's immune system such that a protective immune response is mounted there against. In one embodiment of the present invention the antibodies are derived from Camelids which have been inoculated with H5N2 strain of Influenza A. In particular inoculation of the Camelid with Influenza H5, an inactivated avian influenza type A, H5N2 virus coded and identified as A/Chicken/Mexico/232-CPA/94 is provided for by the present invention.

As such, in one embodiment, the Camelid heavy chain antibodies are those derived from a Camelid following the inoculation of the Camelid with Influenza H5, an inactivated avian influenza type A, H5N2 virus coded and identified as A/Chicken/Mexico/232-CPA/94. Suitably the Camelid antibodies which are obtained are polyclonal antibodies. Although the antibodies have specificity for H5N2, the inventors have surprisingly identified that the antibodies generated in the Camelid following inoculation with H5N2 are cross-reactive to other subtypes of influenza,

particularly H5N1. This principle can be extended to antibodies generated following the inoculation of Camelids with other subtypes of Influenza A, such that they can be administered in compositions and methods for the treatment of strains or subtypes of Influenza different to that of the initial inoculum.

In one embodiment the method further comprises the step of administering a therapeutically effective amount of an antibody preparation comprising at least one antibody with binding specificity for TNF-alpha (tumour necrosis factor alpha) in combination with said heavy chain Camelid antibodies. The anti-TNF-alpha antibody may be a polyclonal antibody, a monoclonal antibody or a binding fragment derived from a heterotetrameric antibody.

In an alternative embodiment the method comprises the further step of administering a therapeutically effective amount of an inhibitor of TNF-alpha activity, with such an inhibitor being selected from the group comprising: Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate in combination with the administration of said heavy chain Camelid antibodies.

In further embodiments the method comprise the further step of administering a therapeutically effective amount of an anti-inflammatory or immunomodulatory agents selected from the group consisting of: corticosteroids, aminosalicylates, azathioprine, methotrexate, cyclosporin, FK506, IL-10, GM-CSF, and rapamycin. Anti-TNFalpha agents and adhesion molecule antagonists may further be administered in the methods of this aspect of the invention.

According to a second aspect of the present invention there is provided an isolated heavy chain Camelid antibody or an antigen binding portion

thereof for use in the prophylaxis and/or treatment of mammalian infection with type A Influenza.

In one embodiment the Influenza A virus is of the subtype H5, H7, H11 or H9. In a further embodiment the type A Influenza subtype is of the strain H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

In one embodiment the polyclonal preparation of antibodies comprises antibodies of the class IgG2 and / or IgG3.

In one embodiment the Camelid (Camelidae) is a Camel, specifically Camelus bactrianus or Camelus dromderius.

Suitably the mammalian is a human.

In one embodiment the isolated heavy chain Camelid antibody or an antigen binding portion thereof is obtained following the inoculation of the Camelid with Influenza H5, an inactivated avian influenza type A, H5N2 virus coded and identified as A/Chicken/Mexico/232-CPA/94. Suitably the Camelid antibodies which are obtained are polyclonal antibodies.

According to a third aspect of the present invention there is provided the use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof in the preparation of a medicament for the treatment or prevention of infection with a type A Influenza virus.

In one embodiment the Influenza A virus is of the subtype H5, H7, H11 or H9. In a further embodiment the type A Influenza subtype is of the strain

H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

In one embodiment the polyclonal preparation of antibodies comprises antibodies of the class IgG2 and / or IgG3.

In one embodiment the Camelidae is a Camel, specifically Camelus bactrianus or Camelus dromderius.

In one embodiment the medicament may further comprise an antibody which binds to or inhibits the activity of TNF-alpha. In a further embodiment the medicament may further comprise an inhibitor of TNF-alpha activity such as Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate.

In one embodiment the isolated heavy chain Camelid antibody or an antigen binding portion thereof is obtained following the inoculation of the Camelid with Influenza H5, an inactivated avian influenza type A, H5N2 virus coded and identified as A/Chicken/Mexico/232-CPA/94. Suitably the Camelid antibodies which are obtained are polyclonal antibodies. Therefore the antibodies have specificity for H5N2 but are cross-reactive to other subtypes of influenza, particularly H5N1.

A yet further aspect of the present invention provides a method of treating influenza A viral infection in a subject in need thereof, comprising the step of administering to said subject a polyclonal Camelid heavy chain immunoglobulin preparation wherein said antibody or antigen-binding portion binds to Neuraminidase.

A still further aspect of the present invention provides a method for producing a polyclonal Camelid heavy chain immunoglobulin preparation which specifically binds Neuraminidase, comprising the steps of: (a) immunizing a Camelid that is capable of producing heavy chain antibodies with a strain of influenza A virus, with an immunogenic portion of an influenza A virus or with a cell or tissue expressing an influenza A virus; (b) allowing the Camelid to mount an immune response to the influenza A virus, and (c) collecting and purifying the produced antibodies.

In one embodiment the Influenza A virus is of the subtype H5, H7, H11 or H9. In a further embodiment the type A Influenza subtype is of the strain H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

In further aspects, the present invention further extends to a monoclonal Camelid heavy chain antibody which has specificity for the Neuraminidase molecule and which is capable of binding and inhibition of Neuraminidase enzyme activity. Such a monoclonal antibody can be generated, using methods commonly known to the person skilled in the art, through the selection of an antibody producing B cells of single specificity from the polyclonal antibody response generated following the immunisation of a Camelid and the resulting production of a polyclonal antibody inhibitors to Neuraminidase.

Accordingly, a further aspect of the present invention provides a monoclonal antibody which is derived from a Camelid heavy chain antibody which is specific for Neuraminidase. The neuraminidase may be of any subtype, preferably N1 to N11. Specifically the antibody serves to inhibit the function of the neuraminidase.

In a further embodiment there is provided an isolated cell line that produces such a monoclonal antibody or an antigen-binding portion thereof.

In a further embodiment there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof of the monoclonal antibody. Also provided is a vector comprising the nucleic acid molecule which encodes the monoclonal antibody. The vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule. A host cell comprising the vector or the nucleic acid molecule may also be provided.

A yet further aspect of the invention provides a method of treating a subject in need thereof with a Camelid heavy chain monoclonal antibody or an antigen-binding portion thereof that specifically binds to and/or inhibits Neuraminidase, comprising the steps of:
(a) administering an effective amount of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof; and
(b) expressing the nucleic acid molecule.

According to a still further aspect of the present invention there is provided a method of preventing or treating human infection with type A Influenza virus, the method including the step of administering a therapeutically useful amount of an isolated heavy chain Camelid antibody or an antigen binding portion thereof to a subject in need of treatment along with a therapeutically useful amount of a suitable anti-viral compound.

In one embodiment the Influenza A virus is of the subtype H5, H7 or H9. In a further embodiment the type A Influenza subtype is of the strain

H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

In one embodiment the anti-viral compound is ribavirin, amantadine, rimantadine, oseltamivir or zanamivir.

According to a yet further aspect of the present invention there is provided the use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof and an anti-viral compound in the preparation of a combined medicament for the treatment or prevention of infection with type A Influenza virus.

In one embodiment the Influenza A virus is of the subtype H5, H7 or H9. In a further embodiment the type A Influenza subtype is of the strain H5N1, H5N2, H9N2, H7N2 or H7N7. Furthermore, the type A influenza subtype may be H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

In one embodiment the anti-viral compound is ribavirin, amantadine, rimantadine, oseltamivir or zanamivir.

Alternative Avian Influenza Strains

There are 3 prominent subtypes of avian influenza virus; H5, H7 and H9. Each of these 3 viral subtypes can potentially be combined with any one of the 9 neuraminidase surface proteins, hence there is the potential for up to 9 different forms of each subtype, for example H7N1, H7N2 ... H7N9.

As such, a further aspect of the present invention provides for use of an isolated 2 chain Camelid antibody or an antigen binding portion thereof in

the preparation of a medicament for the prevention and treatment of human infection with type A Influenza subtype H5, H7 or H9 when each and any of the foregoing subtypes is combined with any one of the known neuraminidase surface proteins to form a specific strain of avian influenza.

Reassortment and new Influenza subtype formation

Influenza A viruses are found in many different animals, including ducks, chickens, pigs, whales, horses, and seals. However, certain subtypes of influenza A virus are specific to certain species, except for birds which are hosts to all subtypes of influenza A.

Influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species. For example, H5N1 avian influenza was responsible for a recent outbreak of bird flu in the human population, while H7N7, H9N2 and H7N2 subtypes have also been associated with transmission over the species barrier and resultant infection in humans.

Avian influenza viruses may be transmitted to humans in two main ways; (i) directly from birds or from avian virus-contaminated environments to people, (ii) through an intermediate host, such as a pig. Influenza viruses have eight separate gene segments. The segmented genome allows viruses from different species to mix and create a new influenza A virus if viruses from two different species infect the same person or animal. For example, if a pig were infected with a human influenza virus and an avian influenza virus at the same time, the viruses could reassort and produce a new virus that had most of the genes from the human virus, but a hemagglutinin and/or neuraminidase from the avian virus. The resulting new virus might then be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or

neuraminidase) not previously seen in influenza viruses that infect humans.

This type of major change in the influenza A viruses is known as antigenic shift. Antigenic shift results when a new influenza A subtype to which most people have little or no immune protection infects humans. If this new virus causes illness in people and can be transmitted easily from person to person, an influenza pandemic can occur.

It also is possible that the process of reassortment could occur in a human. For example, a person could be infected with avian influenza and a human strain of influenza at the same time. These viruses could reassort to create a new virus that had a hemagglutinin from the avian virus and other genes from the human virus. Theoretically, influenza A viruses with a hemagglutinin against which humans have little or no immunity that have reassorted with a human influenza virus are more likely to result in sustained human-to-human transmission and pandemic influenza.

The person skilled in the art would therefore be aware that the natural reassortment of influenza variants could result in a new viral subtype being produced. This new subtype may be capable of interspecies transmission.

Accordingly it is a further aspect of the present invention to provide for the use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof in the preparation of a medicament for the prevention and treatment of human infection with type A Influenza subtype which has resulted from natural reassortment of influenza variants.

In particular, the present invention extends to the use of an isolated 2 chain Camelid antibody or an antigen binding portion thereof in the preparation of a medicament for the prevention and treatment of a novel influenza subtype which has resulted from natural reassortment of human influenza and avian influenza variants.

A yet further aspect of the present invention provides a method for the treatment and / or prophylaxis of human infection with a type A Influenza subtype which has resulted from natural reassortment of influenza variants, the method including the step of administering a therapeutically useful amount of an isolated heavy chain Camelid antibody or an antigen binding portion thereof to a subject in need of treatment.

The present invention further provides an isolated antibody that specifically binds Neuraminidase, wherein at least the complementarity determining regions (CDR) sequences of said antibody are Camelid CDR sequences derived from a Camelid heavy chain antibody, or an antigen-binding portion of said antibody.

The invention also provides nucleic acid molecules encoding the heavy chain of said anti-Neuraminidase antibody or the variable region thereof or antigen-binding portion thereof.

The invention further provides vectors and host cells comprising said nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules.

Treatment / Therapy

The term 'treatment' is used herein to refer to any regime that can benefit a human or non-human animal. The treatment may be in respect of an

existing condition or may be prophylactic (preventative treatment). The term treatment means therapeutic treatment. References herein to "therapeutic" and "prophylactic" treatment is to be considered in its broadest context. The term "therapeutic" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylactic" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, therapeutic and prophylactic treatment includes amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylactic" may be considered as reducing the severity or the onset of a particular condition. "Therapeutic" may also reduce the severity of an existing condition.

Antibodies

An "antibody" is an immunoglobulin, whether natural or partly or wholly synthetically produced. The term also covers any polypeptide, protein or peptide having a binding domain that is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced.

Examples of classical 4 chain heterotetrameric antibodies are the immunoglobulin isotypes and their isotypic subclasses. Fragments of the classical antibody structure may also be provided, with said binding fragments comprising an antigen binding domain such as Fab, scFv, Fv, dAb, Fd, or a bi-specific diabody.

However, Camelid heavy chain antibodies, due to the differences in their structure, and in particular the specific absence of a light chain and a CH1 constant domain, mean that many of the fragments which result in classical 4 chain antibodies cannot be extended to Camelid heavy chain

antibodies. However, fragments and antigen binding portions of Camelid heavy chain antibodies can be produced such as chimeric antibodies and diabodies as well as polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a "bispecific" or "bifunctional" antibody (diabody) has two different binding sites.

Chimeric and bispecific antibodies can be generated from Camelid heavy chain antibodies. A chimeric antibody may be made that comprises CDRs and framework regions from different antibodies. In a preferred embodiment, the CDRs of the chimeric antibody comprises all of the CDRs of the variable region of the heavy chain of a camelid anti-Neuraminidase antibody, while the framework regions are derived from one or more different antibodies. In a more preferred embodiment, the CDRs of the chimeric antibody comprise all of the CDRs of the variable regions of the heavy chain of a Camelid anti-Neuraminidase antibody. The framework regions may be from another species and may, in a preferred embodiment, be humanized. Alternatively, the framework regions may be from another human antibody.

A bispecific antibody can be generated that binds specifically to Neuraminidase through one binding domain and to a second molecule through a second binding domain. The bispecific antibody can be produced through recombinant molecular biological techniques, or may be

physically conjugated together. In addition, a single chain antibody containing more than one VHH may be generated that binds specifically to Neuraminidase and to another molecule. Such bispecific antibodies can be generated using techniques that are well known for example see, e.g., Fanger et al., Immunol Methods 4: 72-81 (1994) and Wright and Harris, supra. and in connection with (iii) see, e.g., Traunecker et al., Int. J. Cancer (Suppl.) 7: 51-52 (1992).

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any binding member or substance having a binding domain with the required specificity. The antibody of the invention may be a monoclonal antibody, or a fragment, derivative, functional equivalent or homologue thereof. The term includes any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic.

A fragment of an antibody or of a polypeptide for use in the present invention generally means a stretch of amino acid residues of at least 5 to 7 contiguous amino acids, often at least about 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids, more preferably at least about 20 to 30 or more contiguous amino acids and most preferably at least about 30 to 40 or more consecutive amino acids.

A "derivative" of such an antibody or polypeptide, or of a fragment of an antibody means an antibody or polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion and/or substitution of one or more amino acids, preferably while providing a peptide having interferon alpha binding activity. Preferably such

derivatives involve the insertion, addition, deletion and/or substitution of 25 or fewer amino acids, more preferably of 15 or fewer, even more preferably of 10 or fewer, more preferably still of 4 or fewer and most preferably of 1 or 2 amino acids only.

The term "antibody" includes antibodies which have been "humanised". Methods for making humanised antibodies are known in the art. Methods are described, for example, in Winter, U.S. Patent No. 5,225,539. A humanised antibody may be a modified antibody having the hypervariable region or the variable region of a Camelid monoclonal antibody and the constant region of a human antibody. Thus the binding member may comprise a human constant region.

The variable region other than the hypervariable region may also be derived from the variable region of a human antibody and/or may also be derived from a monoclonal antibody. In such case, the entire variable region may be derived from a Camelid antibody and the antibody is said to be chimerised. Methods for making chimerised antibodies are known in the art. Such methods include, for example, those described in U.S. patents by Boss (Celltech) and by Cabilly (Genentech). See U.S. Patent Nos. 4,816,397 and 4,816,567, respectively.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other

changes, which may or may not alter the binding specificity of antibodies produced.

Production of Antibodies

The antibodies or antibody fragments of and for use in the present invention may be generated wholly or partly by chemical synthesis. The antibodies can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, (1984), in M. Bodanzsky and A. Bodanzsky, (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry.

Another convenient way of producing antibodies or antibody fragments suitable for use in the present invention is to express nucleic acid encoding them, by use of nucleic acid in an expression system. Thus the present invention further provides the use of an isolated nucleic acid encoding antibodies or antibody fragments which bind to influenza neuraminidase.

Nucleic acid for use in accordance with the present invention may comprise DNA or RNA and may be wholly or partially synthetic. In a preferred aspect, nucleic acid for use in the invention codes for antibodies or antibody fragments of the invention as defined above. The skilled person will be able to determine substitutions, deletions and/or additions to such nucleic acids which will still provide an antibody or antibody fragment of the present invention.

Nucleic acid sequences encoding antibodies or antibody fragments for use with the present invention can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook et al.(1989), and Ausubel et al, (1992)), given the nucleic acid sequences and clones available. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. DNA encoding antibody fragments may be generated and used in any suitable way known to those of skill in the art, including by taking encoding DNA, identifying suitable restriction enzyme recognition sites either side of the portion to be expressed, and cutting out said portion from the DNA. The portion may then be operably linked to a suitable promoter in a standard commercially available expression system. Another recombinant approach is to amplify the relevant portion of the DNA with suitable PCR primers. Modifications to the sequences can be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide or to take account of codon preferences in the host cells used to express the nucleic acid.

The nucleic acid may be comprised as constructs in the form of a plasmid, vector, transcription or expression cassette which comprises at least one nucleic acid as described above. The construct may be comprised within a recombinant host cell which comprises one or more constructs as above. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression the antibody or antibody fragments may be isolated and/or purified using any suitable technique, then used as appropriate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NS0 mouse myeloma cells. A common, preferred bacterial host is E. coli.

The expression of antibodies and antibody fragments in prokaryotic cells such as E. coli is well established in the art. For a review, see for example Plückthun (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a binding member, see for recent review, for example Reff, (1993); Trill et al., (1995).

Alternatively, antibodies or antibody fragments for use in the invention may be produced in transgenic organisms, for example mammals, Camelids, avians, fish, insects or plants using methods known in the art. In such transgenic methods, nucleic acid encoding the binding member(s) may be introduced to the cell or embryo by methods including but not limited to direct injection, electroporation, nuclear transfer techniques or by use of vectors, e.g. viral vectors such as lentiviral vectors.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Sambrook et al., (1989). Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid

constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Ausubel et al. eds., (1992).

The nucleic acid may be introduced into a host cell by any suitable means. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous or foreign to the cell.

Administration

Antibodies of and for use in the present invention may be administered alone but will preferably be administered as a pharmaceutical composition, which will generally comprise a suitable pharmaceutical excipient, diluent or carrier selected dependent on the intended route of administration.

Antibodies of and for use in the present invention may be administered to a patient in need of treatment via any suitable route.

It should be noted however, that the present inventors have surprisingly identified that the Camelid antibodies of the invention may be administered orally without the conditions of the stomach or gastro-intestinal tract adversely affecting the ability of the antibody to effect its use as defined herein.

Some suitable routes of administration include (but are not limited to) oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration or administration via oral or nasal inhalation.

In preferred embodiments, the composition is deliverable as an injectable composition, is administered orally, is administered to the lungs as an aerosol via oral or nasal inhalation.

For administration via the oral or nasal inhalation routes, preferably the active ingredient will be in a suitable pharmaceutical formulation and may be delivered using a mechanical form including, but not restricted to an inhaler or nebuliser device.

Further, where the oral or nasal inhalation routes are used, administration is by a SPAG (small particulate aerosol generator) may be used.

Intravenous administration

For intravenous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Oral administration

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

The composition may also be administered via microspheres, liposomes, other microparticulate delivery systems or sustained release formulations placed in certain tissues including blood. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shared articles, e.g. suppositories or microcapsules. Implantable or microcapsular sustained release matrices include polylactides (US Patent No. 3, 773, 919; EP-A-0058481) copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al, Biopolymers 22(1): 547-556, 1985), poly (2-hydroxyethyl-methacrylate) or ethylene vinyl acetate

(Langer et al, J. Biomed. Mater. Res. 15: 167-277, 1981, and Langer, Chem. Tech. 12:98-105, 1982).

Examples of the techniques and protocols mentioned above and other techniques and protocols which may be used in accordance with the invention can be found in Remington's Pharmaceutical Sciences, 18th edition, Gennaro, A.R., Lippincott Williams & Wilkins; 20th edition (December 15, 2000) ISBN 0-912734-04-3 and Pharmaceutical Dosage Forms and Drug Delivery Systems; Ansel, H.C. et al. 7th Edition ISBN 0-683305-72-7 the entire disclosures of which is herein incorporated by reference.

Pharmaceutical Compositions

As described above, the present invention extends to a pharmaceutical composition for the treatment of Influenza virus A, subtypes H5N1, H7 and H9 (commonly termed "avian influenza" or "bird flu") in humans, wherein the composition comprises at least one isolated Camelid heavy chain antibody. Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention may comprise, in addition to active ingredient (i.e. one or more isolated heavy chain Camelid antibody), a pharmaceutically acceptable excipient, carrier, buffer stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be, for example, oral, intravenous, intranasal or via oral or nasal inhalation.

The formulation may be a liquid, for example, a physiologic salt solution containing non-phosphate buffer at pH 6.8-7.6, or a lyophilised or freeze dried powder.

Dose

The isolated antibody or antibodies of the invention or the pharmaceutical composition which may be dived there from is preferably administered to an individual in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is ultimately within the responsibility and at the discretion of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

The optimal dose can be determined by physicians based on a number of parameters including, for example, age, sex, weight, severity of the condition being treated, the active ingredient being administered and the route of administration.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.025 to 50 mg/kg, more preferably 0.1 to 50 mg/kg, more preferably 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. In some embodiments, a formulation contains 5 mg/mL of antibody in a buffer of 20 mM sodium acetate, pH 5.5, 140 mM NaCl, and 0.2 mg/mL polysorbate 80. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage

ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

A yet further aspect of the present invention advantageously provides an isolated heavy chain Camelid antibody or an antigen binding portion thereof for the effective clearance of avian influenza from humans, said effective clearance resulting when said antibodies are administered at a clinically effective concentration.

According to a further aspect of the present invention there is provided an assay method for determining the efficacy of a composition in the treatment of Influenza virus A, subtypes H5N1, H7 and H9 (commonly termed "avian influenza" or "bird flu") in humans, wherein the composition comprises an isolated heavy chain Camelid antibody or an antigen binding portion thereof.

In a yet further aspect of the present invention, there is provided an assay method for determining the efficacy of a candidate agent in the treatment of Influenza virus A, subtypes H5N1, H7, H9 and H11 (commonly termed "avian influenza" or "bird flu") in humans, wherein the assay method includes the steps of; (i) incubating virus infected cells in the presence of the candidate agent, and (ii) determining the degree of inhibition of the cytopathic effect of the virus on the cells.

In preferred embodiments of this aspect of the invention, the method further includes the step of comparing the degree of inhibition obtained using the candidate agent with the degree of inhibition obtainable with incubation with an isolated heavy chain Camelid antibody or an antigen binding portion thereof.

Preferred assays for use in the assay methods of the invention include cytopathic endpoint assays and plaque reduction assays.

Another aspect of the present invention provides kits comprising an anti-Neuraminidase Camelid heavy chain antibody or binding fragment derived there from or a composition comprising such an antibody. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit can also include instructions for use in a diagnostic or therapeutic method.

In a yet further aspect of the invention there is provided an in-vitro assay method for the determination of the level of neuraminidase in an individual, said assay method comprising the steps of:

- providing a sample from a subject, said sample preferably being derived from nasal discharge or other suitable discharge,
- exposing said sample to Camelid heavy chain antibodies which bind to or inhibit neuraminidase activity,
- exposing the sample to signal generating molecules capable of binding to the Camelid heavy chain antibodies present in the sample,
- removing signal generating molecules not bound to heavy chain antibodies, and
- quantifying the amount of bound signal generating molecules to determine the percentage level of neuraminidase present in the sample.

In one embodiment the quantification of the amount of bound Camelid heavy chain antibody in said sample is determined through a comparison of the signal generated by said assay against a pre-determined standard.

In a further embodiment, the assay method is a rapid assay method, which may be performed at the point of care, thus providing the advantage that a

result can be provided immediately and further that said sample from the individual does not have to be taken, stored and transported to a further location for testing. The use of a rapid antibody based assay test will be well known to the person skilled in the art, who will be aware that said test can take the form of a dipstick test or the like.

Preferred features and embodiments of each aspect of the invention are as for each of the other aspects mutatis mutandis unless the context demands otherwise.

Throughout the specification, unless the context demands otherwise, the terms 'comprise' or 'include', or variations such as 'comprises' or 'comprising', 'includes' or 'including' will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference.

Detailed Description of the Invention

The present invention will now be described with reference to the following examples which are provided for the purpose of illustration and are not intended to be construed as being limiting on the present invention.

EXAMPLES

Materials and Methods

The antigen used for the Camelidae inoculation programme was NobilisTM Influenza H5, an inactivated avian influenza type A, H5N2 virus coded and identified as A/Chicken/Mexico/232-CPA/94, it is a water and oil emulsion vaccine manufactured by Intervet International, Boxmeer, the Netherlands.

Camel Immunisation Protocols

After collection of pre-immune sera, four camels (C. dromedaries) were injected subcutaneously (s.c.) at days 0, 7, 14, 21, 36, 50, and 65 with increasing amounts of 150, 200, 250, 300, 350, 500 µg and 1 mg of the NobilisTM Influenza H5 vaccine (H5N2) respectively. The animals received the first H5N2 vaccine together with 1 ml of complete Freund's adjuvant as an additional immune booster. Subsequent sc injections of the NobilisTM H5N2 vaccine were administrated with 1ml sc incomplete Freund's adjuvant to enhance immune response. The immune response of the inoculated camels was monitored by Elisa analysis of anti H5N2 IgG's. These antibody titers rose rapidly after the 25th day reaching a

maximum on day 65. These high titers were maintained by boosting at 30 day intervals with 1 mg of the antigen. Blood was withdrawn on day 65 from the jugular vein prior to each inoculation injection for examination of its ability to inhibit viral Neuraminidase enzymes.

Serum was obtained by centrifugation of blood at 4000 rpm for 10 minutes. This was stored at –20°C until further use.

Isolation of immunoglobulin concentrate from the Camelidae blood

Isolation of immunoglobulin may be achieved by utilizing the following procedure. Anticoagulant is added to whole blood after it is extracted from the jugular vein of the animal. The blood is centrifuged to separate the plasma. Any anticoagulant may be used for this purpose, including sodium citrate and heparin. Calcium is then added to the plasma to promote clotting, the conversion of fibrinogen to fibrin; however other methods known to the skilled person are also acceptable. This mixture is then centrifuged to remove the fibrin portion. Once the fibrin is removed from plasma resulting in serum, the serum can be used as a principal source of IgG. Alternatively, this portion of the clotting mechanism may be inactivated through the use of various anticoagulants.

The defibrinated plasma was next treated with an amount of salt compound or polymer sufficient to precipitate the albumin or globulin fraction of the plasma. Examples of phosphate compounds which may be used for this purpose include all polyphosphates, including sodium hexametaphosphate and potassium polyphosphate. The globulin may also be precipitated through the addition of polyethylene glycol or ammonium sulfate.

Following the addition of the phosphate compound, the pH of the plasma solution is lowered to stabilize the albumin precipitate. The pH should not be lowered below 3.5, as this will cause the proteins in the plasma to become damaged. Any type of acid can be used for this purpose, so long as it is compatible with the plasma solution. Examples of suitable acids are HCl, acetic acid, H_2SO_4, citric acid, and H_2PO_4. The acid is added in an amount sufficient to lower the pH of the plasma to the designated range. Generally, this amount will range from a ratio of about 1:4 to 1:2 acid to plasma. The plasma is then centrifuged to separate the globulin fraction from the albumin fraction.

Affinity Purification

Affinity purification against the H5N2 vaccine antigen (NOBILIS TM). Pooled sera from the four immunized camels were fractionated, by adsorption on protein G then protein A, into heavy chain and conventional IgG antibodies. Three different types of IgG were obtained. The conventional IgG antibodies (IgG1) were found in the second eluted fraction (pH 2.7) of the protein G column. This IgG consists, under reducing condition, of heavy chains with an apparent molecular weight of 55 kDa and light chains with an apparent molecular weight of 30 kDa that bind to protein A and protein G under non-reducing condition. The apparent 160 kDa molecular weight matches the heterotetrameric IgG composed of two heavy (H) and two light (L) chains.

The 100kDa apparent molecular weight corresponds to heavy chain antibodies (HC-Abs). The first eluted fraction at pH 3.5 through the same protein G affinity column corresponds to the heavy chain subclass IgG3 with apparent heavy chain molecular weight of 43 kDa. Heavy chain antibodies with molecular weight of 46 kDa (IgG2) were purified from protein G non adsorbed fraction through elution at pH 2.7 on protein A

column. This IgG2 purified fraction, that failed to interact with protein G, was also composed of homodimeric H-chains. Relative amounts of each camel IgG subtype isolated from affinity chromatography were evaluated. 0.97mgs of IgG1, 0.51 mgs of IgG2 and 0.8 mgs of IgG3 were purified per ml of camel serum collected on day 65 of the inoculation scheme.

Antiviral Activity

The antiviral activity of the affinity purified Camelidae antibodies, generated in response to the inoculation with the NOBILIS TM Avian influenza vaccine, coded CC07, was monitored using a Neuraminidase A activity assay. To avoid immunoprecipitation pure VHH was generated. This is the N-terminal variable region of these Camelidae heavy chain antibodies which contain a minimum sized antigen binding domain. These VHH's are readily obtained by the limited proteolytic digestion of the IgG (IgG2 & IgG3) fractions with endo-GluV8 protease.

VHH fragments isolated from the serum were concentrated and varying amounts of this pool of antibody fragments, range zero to 350µg/ml were used to determine their ability to inhibit Neuraminidase activity. NA activity was determined by using 2'-(4-methylumbelliferyl)- -D-N-acetylneuraminic acid (MUN; Sigma Chemical Co., St. Louis, Mo.) as a substrate. Viruses used in the NA assay were grown in embryonated chicken eggs and were obtained from the allantoic fluid after centrifugation at 2,000 × g for 10 minutes. The NA activity of each virus was determined before it was used in NA inhibition tests. Briefly, 10 µl of each of a series of twofold virus dilutions was mixed with 10 µl of enzyme buffer [33 mM 2-(N-morpholino) ethanesulfonic acid (MES), pH 6.5, and 4 mM CaCl2] and 30 µl of substrate in enzyme buffer to give a final MUN concentration of 100 µM. The reaction mixtures were incubated on a shaker at 37°C for 30 minutes. The reactions were then stopped by addition of 150 µl of

0.014 N NaOH in 83% ethanol to each well. The fluorescence of the released 4-methylumbelliferone was quantified in a Fluoroskan II spectrophotometer (excitation wavelength, 355 nm; emission wavelength, 460 nm).

NA inhibition was assayed by determining the camel antibody concentration required to reduce NA activity to 50% of control NA activity (IC50). Fourfold dilutions ranging from 10µg to 300µg of CC07 were made, and 10 µl of each dilution was incubated with 10 µl of virus-containing allantoic fluid at a standard amount of NA activity (100 to 150 relative fluorescence units). The mixture was shaken at 37°C for 30 minutes to allow interaction of antibody and the virus. The enzymatic reaction was initiated by adding 30 µl of substrate in enzyme buffer at a final concentration of 100 µM. The reaction was stopped after 1 hour of incubation at 37°C. Standard curves were constructed by plotting the percentage of fluorescence inhibition relative to the activity of controls against the logs of inhibitor concentrations. The IC_{50}s, as shown in Table A were obtained from the graphs by extrapolation, and the means were calculated on the basis of three independent experiments.

a) MUN (final concentration of 100 µM) was used as the substrate. Values are means of at least three independent determinations.

b) CC07 is a Camelidae Antibody VHH fragment affinity purified to the A/chicken/mexico232-CPA/94 viral strain.

All documents referred to in this specification are herein incorporated by reference. Various modifications and variations to the described embodiments of the inventions will be apparent to those skilled in the art without departing from the scope of the invention. Although the invention

has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes of carrying out the invention which are obvious to those skilled in the art are intended to be covered by the present invention.

TABLE A - Inhibition of the NA activity of avian influenza A viruses by Camelidae Antibody CC07[b]

NA subtype	Virus	Camelidae Antibody CC07 Mean IC50 (µg/ml)
N1	A/teal/Hong Kong/W312/97 (H6N1)	97 µg/ml
N2	A/chicken/NY/13307-3/95 (H7N2)	83 µg/ml
N2	A/chicken/Mexico/232-CPA/94 (H5N2)	71 µg/ml
N3	A/duck/Singapore/3/97 (H5N3)	106 µg/ml
N4	A/red knot/DE/254/94 (H8N4)	105 µg/ml
N5	A/M duck/Astrakhan/263/82 (H14N5)	132 µg/ml
N6	A/duck/Czechoslovakia/56 (H4N6)	81 µg/ml
N7	A/chicken/Jena/1816/87 (H7N7)	142 µg/ml
N8	A/duck/Hong Kong/Y264/97 (H4N8)	91 µg/ml
N9	A/duck/Hong Kong/P50/97 (H11N9)	84 µg/ml

Claims

1. A method for the treatment and/or prophylaxis of a viral infection in a mammal, the method comprising the step of administering a therapeutically effective amount of a heavy chain Camelid antibody to an individual in need of such treatment.

2. A method according to claim 1 wherein the viral infection is an Influenza A virus.

3. A method according to claim 1 or claim 2 wherein the Influenza A virus is of the subtype H5, H7, H11 or H9.

4. A method according to any one of claims 1 to 3 wherein the Influenza A virus is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

5. A method as claimed in any one of claims 1 to 4 wherein the method comprises the further step of administering a therapeutically effective amount of an antibody composition specific for TNF-alpha in combination with said heavy chain Camelid antibodies.

6. A method as claim in any one of claims 1 to 5 wherein the method comprises the further step of administering a therapeutically effective amount of an inhibitor of TNF-alpha activity, with such an inhibitor being selected from the group comprising: Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate.

7. A method according to any one of claims 1 to 6 wherein said Camelid heavy chain antibodies are of the subclass IgG2.

8. A method according to any one of claims 1 to 6 wherein said Camelid heavy chain antibodies are of the subclass IgG3.

9. A method according to any one of claims 1 to 8 wherein the Camelid is Camelus bactrianus or Camelus dromderius.

10. An isolated heavy chain Camelid antibody or an antigen binding portion thereof for use in the prophylaxis or treatment of human infection with Influenza A virus.

11. Use according to claim 10 wherein the Influenza A virus is of the subtype H5, H7, H11 or H9.

12. Use according to claim 10 or claim 11 wherein the Influenza A virus is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

13. Use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof in the preparation of a medicament for the treatment or prevention of infection with a Influenza A virus.

14. Use according to claim 13 wherein the Influenza A virus is of the subtype H5, H7 or H9.

15. Use according to claim 13 or claim 14 wherein the Influenza A virus is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

16. The use of Camelid heavy chain monoclonal or polyclonal anti-idiotypic immunoglobulins generated against influènza A Camelidae inhibitory antibodies in the preparation of a vaccine formulation, containing Camelid heavy chain antibodies of different specificities, for immunising individuals against influenza A virus.

17. Use according to claim 16 wherein the Influenza A virus is of the subtype H5, H7 or H9.

18. Use according to claim 16 or claim 17 wherein the Influenza A virus is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

19. Use according to any one of claims 16 to 18 wherein one or more immunologic adjuvants are admixed to the polyclonal immunoglobulin preparation.

20. A method of treating influenza A viral infection in a subject in need thereof, comprising the step of administering to said subject a polyclonal Camelid heavy chain immunoglobulin preparation wherein said immunoglobulin or antigen-binding portion thereof binds to Neuraminidase.

21. A method for producing a polyclonal Camelid heavy chain immunoglobulin preparation which specifically binds Neuraminidase, comprising the steps of:
(a) immunising a Camelid that is capable of producing heavy chain antibodies with a strain of influenza A virus, with an immunogenic portion of an influenza A virus or a with cell or tissue expressing an influenza A virus; and

(b) allowing the Camelid to mount an immune response to the influenza A virus.

22. A method according to claim 21 wherein the Influenza A virus is of the subtype H5, H7 or H9.

23. A method according to claim 21 or claim 22 wherein the Influenza A virus is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

24. An antibody composition for the treatment of type A influenza comprising a monoclonal antibody which is derived from a Camelid heavy chain antibody which is specific for Neuraminidase.

25. An antibody composition as claimed in claim 24 wherein the antibody is selected from the group comprising immunoglobulin of the subclass IgG2 or IgG3.

26. A method of treating a subject in need thereof with a Camelid heavy chain monoclonal antibody or an antigen-binding portion thereof that specifically binds to Neuraminidase, comprising the steps of:
(a) administering an effective amount of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof; and
(b) expressing the nucleic acid molecule.

27. A method of preventing or treating human infection with type A Influenza virus, the method including the step of administering a therapeutically useful amount of an isolated heavy chain Camelid antibody or an antigen binding portion thereof to a subject in need of treatment

along with a therapeutically useful amount of a suitable anti-viral compound.

28. A method according to claim 27 wherein the Influenza A virus is of the subtype H5, H7 or H9.

29. A method according to claim 27 or claim 28 wherein the type A Influenza subtype is of the strain H5N1, H5N2, H9N2, H7N2, H7N7, H6N1, H5N3, H8N4, H14N5, H4N6, H7N7, H4N4 or H11N9.

30. A method according to any one of claims 27 to 29 wherein the anti-viral compound is ribavirin, amantadine, rimantadine, oseltamivir or zanamivir.

31. A method as claimed in any one of claims 27 to 30 wherein the method comprises the further step of administering an antibody composition specific for TNF-alpha in combination with said isolated heavy chain Camelid antibodies.

32. A method as claim in any one of claims 27 to 31 wherein the method comprises the further step of administering an inhibitor of TNF-alpha activity, with such an inhibitor being selected from the group comprising: Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate.

33. The use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof and an anti-viral compound in the preparation of a combined medicament for the treatment or prevention of infection with type A Influenza virus.

34. Use as claimed in claim 33 wherein the medicament further comprises an antibody with specificity to TNF-alpha.

35. Use as claimed in claim 33 wherein the medicament further comprises an inhibitor of TNF-alpha activity selected from the group comprising; Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate.

36. The use of an isolated heavy chain Camelid antibody or an antigen binding portion thereof in the preparation of a medicament for the prevention and treatment of human infection with type A Influenza subtype which has resulted from natural reassortment of influenza variants.

37. A method for the treatment and / or prophylaxis of human infection with a type A Influenza subtype which has resulted from natural reassortment of influenza variants, the method including the step of administering a therapeutically useful amount of an isolated heavy chain Camelid antibody or an antigen binding portion thereof which has binding specificity or the ability to inhibit the function of the Neuraminidase enzyme to a subject in need of treatment.

38. A pharmaceutical composition comprising an effective amount of a Camelid heavy chain antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier.

39. A pharmaceutical composition as claimed in claim 38 further comprising an inhibitor of TNF-alpha activity selected from the group comprising; Thaliomide, Butyrate Hydroxy Toluene (BHT) or Quinine Sulphate.

40. A pharmaceutical composition as claimed in claim 38 further comprising an antibody which binds to or inhibits the activity of TNF-alpha.

41. A method for producing a human monoclonal antibody that specifically binds and / or inhibits Neuraminidase, comprising the steps of:
(a) immunizing a Camelid that is capable of producing heavy chain antibodies with a strain of influenza A, with an immunogenic portion of an influenza virus or with a cell or tissue expressing an influenza A virus; and
(b) allowing the Camelid to mount an immune response to the influenza A virus.

42. An in-vitro assay method for the determination of neuraminidase levels in a subject, said assay method comprising the steps of:
- providing a sample from a subject, said sample preferably being derived from nasal discharge or other suitable discharge,
- exposing said sample to Camelid heavy chain antibodies which bind to or inhibit neuraminidase activity,
- exposing the sample to signal generating molecules capable of binding to the Camelid heavy chain antibodies present in the sample,
- removing signal generating molecules not bound to heavy chain antibodies, and
- quantifying the amount of bound signal generating molecules to determine the percentage level of neuraminidase present in the sample.

43. An assay method as claimed in claim 42 wherein the quantification of the amount of bound Camelid heavy chain antibody in said sample is determined through a comparison of the signal generated by said assay against a pre-determined standard.

44. A diagnostic kit comprising an antibody according to claim 24 and means for detecting binding by that antibody.

45. A diagnostic kit according to claim 44 wherein said means for detecting binding comprises a detectable label that is linked to said antibody.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IE2006/000126

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/65057 A (UNILEVER PLC [GB]; UNILEVER NV [NL]; LEVER HINDUSTAN LTD [IN]) 2 November 2000 (2000-11-02) page 4, line 26 - page 8, line 31	1,10,38
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X	WO 94/04678 A (CASTERMAN CECILE [BE]; HAMERS RAYMOND [BE]) 3 March 1994 (1994-03-03) page 8, line 11 - page 29, line 32	1,7-9
Y		2-6, 11-37, 39-45

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 2007	18/04/2007

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/085945 A (UNIV ERASMUS [NL]; GROSVELD FRANK [NL]) 31 October 2002 (2002-10-31) page 6, line 16 - page 36, line 30	1,7,8,38
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International application No
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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RIECHMANN L ET AL: "Single domain antibodies: comparison of camel VH and camelised human VH domains" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 231, no. 1-2, 10 December 1999 (1999-12-10), pages 25-38, XP004187632 ISSN: 0022-1759 page 35, column 1, line 36 - column 2, line 19	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IE2006/000126

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-9, 20, 26-32 and 37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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