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IVS President Desk

Avian influenza A virus in Human

The current burden of influenza in India is serious. In India, it is estimated that approx. 24,000 children under 5 years of age die of Flu alone; a figure amounting ¼ th of total deaths in the world. The figures for most of the counties in South-East Asia are more or less similar and higher.

Since November 2003, more than 600 sporadic cases of human infection with highly pathogenic avian influenza (HPAI) A (H5N1) virus with high mortality have been reported, primarily by 15 countries in Asia, Africa, the Pacific, Europe and the Near East. On January 8, 2014, the first case of a human infection with H5N1 in the Americas was reported in Canada. Besides, H5N1, there are few more odd numbers of avian influenza virus has been seen to cause infection in human (H5N2, H7N9, etc.). Most human infections with avian influenza A viruses have occurred following direct or close contact with infected poultry. Illness in humans has ranged from mild to severe.

Because HPAI H5N1 viruses are evolving in unpredictable ways, it is critical to monitor the spread and circulation of these viruses among poultry and other birds, in order to understand the risk of spread to human.

Signs and symptoms of avian influenza a virus infections in humans. Signs and symptoms may depend on which avian influenza A virus caused the infection. Low pathogenic avian influenza A virus infections of humans have been associated with generally mild, non fatal illness. Highly pathogenic avian influenza A virus infections of humans have been associated with a wide range of illness. Illness has ranged from conjunctivitis only, to influenza-like illness, to severe respiratory illness with multi-organ disease, sometimes accompanied by nausea, abdominal pain, diarrhea, vomiting and sometimes neurologic changes (altered mental status, seizures). Sometimes infection with highly pathogenic avian influenza A virus infection leads to death, especially with HPAI H5N1 virus. The accuracy of clinical diagnosis of human infection with avian influenza A viruses on the basis of signs and symptoms alone is limited because symptoms from illness caused by other pathogens, including seasonal influenza A or B viruses, can overlap considerably.

Detecting avian influenza a virus infection in humans. Avian influenza A virus infection in humans cannot be diagnosed by clinical signs and symptoms alone; laboratory testing is requiredto diagnose by collecting a swab from the nose or throat of the sick person during the first few days of illness. This specimen is sent to a lab; the laboratory looks for avian influenza A virus either by using a molecular test, by trying to grow the virus, or both.

Treating avian influenza a virus infections in humans. CDC and WHO recommend oseltamivir or zanamivir, two of four prescription antiviral medications currently licensed for use in the United States, for treatment and prevention of human infection with avian influenza A viruses. Analyses of available HPAI H5N1 viruses circulating worldwide suggest that most viruses are susceptible to oseltamivir and zanamivir. However, some evidence of resistance to oseltamivir that developed has been reported in HPAI H5N1 viruses isolated from some human cases. Monitoring for antiviral resistance among avian influenza A viruses is crucial and ongoing to inform CDC and WHO antiviral treatment recommendations.

Preventing human infection with avian influenza a viruses. The best way to prevent infection with avian influenza A viruses is to avoid sources of exposure. Most human infections with avian influenza A viruses have occurred following direct or close contact with infected poultry.

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(Source of Information: WHO/CDC (Atlanta)/ NCDC, AIIMS, Delhi and Government of India Published Data.)

In this Issue

INTERNATIONAL CONFERENCE

The 8th International Geminivirus Symposium and the 6th International ssDNA Comparative Virology Workshop

The ssDNA workshop is scheduled to be held between 6 and 11 November, 2016 at New Delhi. The symposium will present an excellent platform to discuss and share the latest developments in the subject of geminiviruses and ssDNA viruses of plants, animals and human beings. The sessions will deal with topics such as Replication, Recombination, Virus-plant interactions, Virus-vector interactions, Viral Diversity, Resistance, Satellites, Emerging/Novel viruses, etc. About one hundred delegates from all over the World are expected to attend the symposium. Special sessions will be organized to give promising young scientists an opportunity for oral presentation of their research. Dedicated poster sessions will be available to enable close scientific interactions to take place between the delegates. There will also be facilities for commercial organizations to showcase their products to an international audience. Concessional rates for registration will be made available for PhD students and post-doctoral fellows. More information will soon be available in IVS website.

RESEARCH NEWS

Oral vaccine development against viral diseases in fish using structure-based antigen


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Diseases caused by bacteria, viruses and parasites are a threat to sustainable growth of fisheries globally. Although bacterial diseases are more prevalent, viral diseases cause more economic loss worldwide. Infectious hematopoietic necrosis (IHN), infection salmon anemia (ISA), infectious pancreatic necrosis (IPN), and koi herpes virus (KHV or CyHV-3) are examples of major viral diseases that have caused catastrophic losses in finfish aquaculture. Improving animal health through vaccination is one of the corner stones in disease management in aquaculture and in other organisms (including humans). There are different types of viral vaccines including killed or attenuated live viruses, major antigen(s) expressed as recombinant protein(s), DNA vaccines and virus-like particle-based vaccines developing a vaccine that will provide broad protection against a number of widely prevalent strains of a particular virus remains a real challenge in fin fish aquaculture.

Irrespective of the type of vaccine used in aquaculture, the overwhelming delivery method is through injection. However, there are a very few commercially available oral vaccines, such as the ISA oral vaccine sold by Centrovet, Chile, or via immersion such as the KHV vaccine recently approved in the US and Canada. While oral vaccination is a “holy grail” in aquaculture, since it offers low labor and lower cost, developing such an effective vaccine is also the most difficult.

Structure based design methods have been employed to develop viral vaccines have been developed to provide broad spectrum protection in humans. We are applying similar approaches to aquaculture vaccine development. Previously we developed vaccines against viral diseases in fish (IPNV VLP vaccine, c-myc VLP) using the primary structure of the antigen.

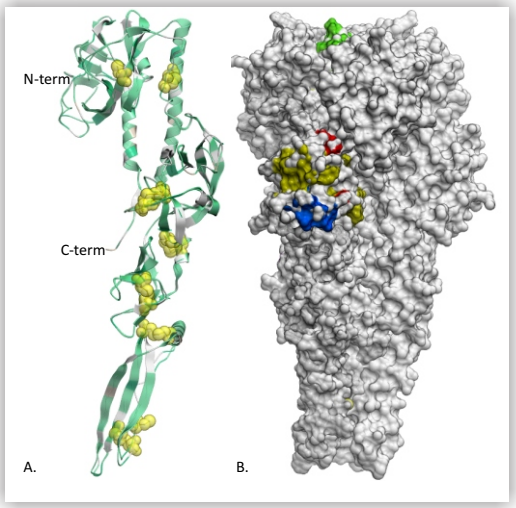

Fig.1 Infectious hematopoietic necrosis virus glycoprotein monomer molecular structural model

IHNV G-protein structure-based vaccine development at BrioBiotech. There is already a DNA vaccine was against IHNV but emergence of new IHNV strain poses a continuing threat to salmonid aquaculture because the existing vaccine may not provide adequate protection against the newly emerging strain.We generated molecular models of a monomer of the glycoprotein of an IHNV strain representing genogroup E (Fig. 1). Conserved disulfide bonds in the core of the protein are displayed as yellow spheres. Antigenic regions have been identified in the literature. A model surface representation of the trimer is on the right with these antigenic regions colored: aa 78-81 (red), 218-233 (yellow), 272-276 (blue), and 301-325 (green). All are preditedto be surface exposed by this model. Another conserved region (aa 419-444) is predicted to be buried. BrioBiotech is targeting the surface exposed regions and using bioinformatics to identify variation in these antigenic regions between strains of IHNV to better design a broadly applicable vaccine.

Using oral delivery methods and *in situ* generation of vaccines in microbial hosts used for production that can be directly included in the delivery formulation allows development of vaccines that are able to address many variants of the virus in a single formulation. BrioBiotech is dedicated in addressing this issues that fish farmers and fish industry needed badly worldwide.

Establishment of epigenetic mechanism to control geminivirus infection in tomato

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Epigenetic mechanism has emerged as a promising approach to decipher the stress tolerance in crop plants. This mechanism commences with the accumulation of small interfering RNAs (siRNAs) which in turn directs transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). Recent studies have highlighted the incidence of epigenetic regulation during virus infection in plant systems. In our lab, we have identified the existence of similar mechanism in a naturally tolerant cultivar of tomato (H-88-78-1) against *Tomato leaf curl New Delhi virus* (ToLCNDV).

With an aim of dissecting the ToLCNDV tolerance mechanism, we initially screened the available tomato germplasms for identifying naturally tolerant cultivars. This exercise successfully identified two cultivars namely 'H-88-78-1' and 'LA1777' as ToLCNDV tolerant varieties where tolerance of both the cultivars was attributed to the production of higher amount of ToLCNDV-derived siRNAs Our studies showed that diverse species (21- and 24-nucleotide) of siRNAs tend to regulate TGS and PTGS pathways in a stringent manner. While comparing the siRNA levels in tolerant cv. 'H-88-78-1' and susceptible cv. 'Punjab Chhuhara' in response to ToLCNDV infection, a higher accumulation of siRNAs corresponding to the Intergenic Region (IR) and region specific to replication associated protein (AC1) of ToLCNDV DNA-A genome was observed in the tolerant cultivar.

Recently, siRNA-mediated DNA methylation mechanism has been evidenced to be a pivotal component in epigenetic regulation of geminivirus tolerance. Therefore, the levels of DNA methylation in the region where elevated levels of siRNAs were identified i.e. specific to IR and AC1, were scanned. The result indicated the hyper-methylation of cytosines in various regions of IR and AC1. Furthermore, hyper-methylation in IR was assumed to regulate the expression of the genes responsible for virus replication, which was confirmed by the expression analysis of respective gene. More importantly, the key host methyltransferases (such as, *Domain rearranged methyltransferase*, and *Chromomethylase-3*) and components of RNA silencing pathways (*Dicer-like proteins3-4* and *Argonaute 1*) were also evidenced to be differentially expressed in cv. 'H-88-78-1' during ToLCNDV infection. Thus, establishment of DNA methylation maintaining enzymes along with RNA silencing machinery also accentuates the existence of DNA methylation-mediated tolerance in tomato against ToLCNDV. In view of this, it could be inferred that both siRNA-mediated RNA degradation and viral DNA methylation are the two modes of epigenetic regulation, which play an important role in conferring tolerance against ToLCNDV.

Our research outcomes may have significant impact not only on tomato, but also on the crops whose survival and productivity is challenged by viruses.

DNA Chip for detection of globally known viruses and viroids infecting plants

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DNA microarray is a promising new technology that allows the broad spectrum detection of plant viruses allowing parallel detection of thousands of viruses. DNA chip was designed on affymetrix platform which has probes to detect all viruses andviroids (1155) whose sequences were available in the GenBank. A set of 7-11 unique probes was designed for family, genus and virus and viroid from the sequences available in GenBank.There are 1572 probe sets totaling to 17292 unique probes for detection of viruses and viroids on the chip. Housekeeping genes of sequenced plant species areincluded as controls.This unique chip is first of its kind in India to reveal plant virome. The chip can detect both DNA and RNA viruses in a single assay. Both DNA and RNA viruses can be detected using the same chipstarting from as little as 25 ng of total plant RNA.cDNA is prepared from total RNA using random primers and viral RNA is amplified by *invitro*transcription. cDNA to the amplified RNA is then labeled and hybridized on the chip. Amount of hybridization is assessed by a laser confocal scanner and inferences are made by comparing with healthy samples. Several viruses and viroids, from different crops like chilli, grapevine, tomato, urdbeansoybean and sugarcane were detected. Viruses which do not induce symptoms could also be detected.Some of the detected viruses were not known to occur in India.This chip will be a valuable tool to identifyexotic and emerging virusesto initiate quarantine measures as wellas to prepare geographical distribution of different viruses infecting different crops in the country.

DNA chips developed at IARI, New Delhi for the detection of plant virus and viroids

Mechanisms and treatments for inflammation caused by alphaviruses

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Chikungunya is a disease without a cure, infecting millions of people around the world during the peak of an outbreak in 2006, which centred around the Indian Ocean. Although it generally has a low mortality rate, Chikungunya virus (CHIKV) belongs to a group of viruses known as alphaviruses (family *Togaviridae*) that can frequently cause rheumatic symptoms associated with arthritis in the infected patient. The acute symptoms of CHIKV infection often subside after a few days or weeks but the arthritis can linger on for much longer, causing chronic pain for the sufferer. Other such viruses include Ross River virus (RRV), the most common mosquito-borne infection in Australia, and the o'nyong'nyong virus, which infected over 2 million people in East Africa during a 1959-62 epidemic.

Our team is working to find out a treatment for the arthritogenic infection. The mechanism by which the arthritis is caused is not yet understood: what is known, however, is that macrophages are of major significance in this process. Our group found macrophage migration inhibitory factor (MIF)- a protein involved in the regulation of macrophage function to play a key role in determining the severity of arthritis caused by alphaviruses. Although, macrophages form a vital component of the immune system, they can act to exacerbate symptoms of virus-induced arthritis, causing the destruction of soft tissue and increasing inflammation in the joints. This research has led the Australian investigators to the discovery that drugs which block MIF have the potential to reduce the severity of alphavirus-induced arthritis, alleviating the suffering of millions around the world.

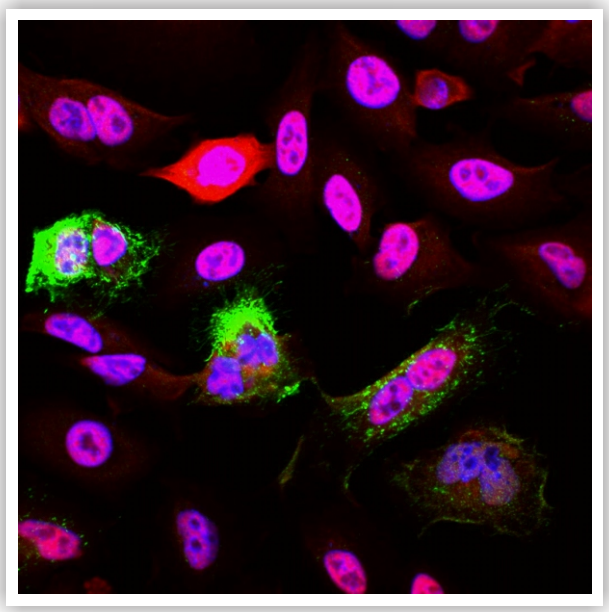

Fig. 1. Vero cells infected with alphavirus.

We have also made a significant contribution to the study of antibody-dependent enhancement (ADE), a phenomenon which sees the immune system unable to fight off infection with a variant of a virus to which it has been exposed previously. My lab was the first to discover how this signalling pathway can lead to suppression of host immunity by arboviruses. ADE commonly occurs with the dengue virus- a mosquito-borne virus that infects around 100 million people each year. Dengue virus can be separated into four serotypes, and previous infection with one serotype can hinder recovery from a later infection with a different serotype. We found that the genes which normally lead to the production of antiviral proteins are disrupted in ADE and thus key antibodies required to fight off the infection do not appear. This information not only provides an explanation for a phenomenon first observed in 1964, but also facilitates the production of effective vaccines against viral infections. The work received much publicity, featuring as a special commentary in *Trends in Immunology*.

In the search to reduce the symptoms of arthritis, our team was successful in looking at new applications for pre-existing drugs. This has made the leap from theory to clinical trial much quicker because the lengthy process of obtaining drug approval for human trials by a national health agency has already been completed. Our basic research identified the macrophage as a crucial immunopathological component in viral arthritis and, based on this discovery, we sought to test drugs that can inhibit migration of macrophages into infected joints. Our team identified bindarit as one such drug, and testing in mouse models of CHIKV and RRV arthritis, revealed that bindarit had potent therapeutic activity against viral arthritis. Bindarit is a drug produced by Angelini Pharmaceuticals and is currently in clinical development for the treatment of type 2 diabetes nephropathy and for prevention of coronary in-stent restenosis. This means that the drug already has a well-established clinical safety profile and Mahalingam is currently planning clinical trials for bindarit treatment of patients infected with CHIKV.

Collaboration has proved essential in carrying out this multidisciplinary research, and much work has been done under the flag of the Forum for European-Australian Science and Technology cooperation (FEAST). We are also involved in a large collaboration including six laboratories in Australia and India (Professors Shobha Broor and Lalit Dar, AIIMS, New Delhi) which is focusing on combating CHIKV in India. We are also involved in is Integrated Chikungunya Research (ICRES), supported by the EU. Our findings and mouse models will be incorporated into the project to eventually produce a vaccine for clinical trials against CHIKV infection in humans. The team also offers their expertise in animal models of viral infection (chikungunya, dengue, respiratory viruses) and inflammation (rheumatoid arthritis, asthma) to third parties, particularly biotechnology companies.

The emergence of the novel promoter variant strains of HIV-1 in India and other global regions

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Human immunodeficiency virus 1 (HIV-1) (genus Lentivirus, family Retroviridae) there are many genetic families that are referred to as subtypes (or clades) A to K. Of the at least 9 primary genetic subtypes, subtype C commands a special position as this family alone is responsible for nearly half of the global HIV infections. Over the past decades subtype C prevalence has been progressively on the rise from 25-48% as of today. In India, more than 95% of the infections of HIV-1 are attributed to subtype C. This subtype is also the major one in China (although as a recombinant), eastern and southern African countries and presently emerging in southern Brazil.

The African primates and their natural Simian immunideficiency virus (SIVs) evolved together over several thousands of years shaping the evolutionary trajectory of each other. As a consequence, the natural SIV is infectious, but not pathogenic for its own host. Reciprocating this favor from the parasite, the natural primate host will not be hostile to own natural SIV. Although this association is not a symbiosis, where both the species are benefited be each other, at least they do not harm each other. We therefore can call this association 'a peaceful coexistence'. Of note, the SIV strain may not be pathogenic for its natural host, but the virus will destroy a different primate species if injected experimentally (Figure-1). In other words, the virus hasn't really become non-pathogenic, it only attenuated itself to tolerate its natural primate host. Extending this observation further, can we say that HIV-1 is likely to undergo a similar evolutionary adaptation to become progressively less pathogenic to the human beings? This is the real question.

A school of thought proposes that subtype C of the diverse subtypes of HIV-1 has taken a step in the direction of making itself less pathogenic to the human beings. Could this be one reason for the successful global expansion of subtype C over the years? The work emerging from our laboratory in the recent years supports this proposition. Most of the viral proteins, such as Tat and envelope of subtype C appear to be toned down versions of toxicity as compared to their counterparts from other viral subtypes. The viral promoter alone appears to be superior in subtype C as compared to other subtypes. While the promoter in subtype C alone contains 3 NF-kappaB binding motifs, in most of the other viral subtypes there are only 2 such motifs. The number of the NF-kappaB sites typically correlates to the overall strength of the promoter. Could the presence of a stronger viral promoter be one of the reasons underlying the global success of subtype C? When other HIV-1 subtypes contain 2 NF-kappaB sites, why should subtype C alone contain 3 such elements in its promoter? As we were striving to find a logical answer to the above question, we found something more appalling in India. The emergence and rapid expansion of novel HIV-1 strains containing 4 NF-kappa B binding sites.

A decade ago, in 2000-2003, we found at least two different variant viral strains emerging in small numbers. One of the two types contained 4 NF-kappaB sites when the native viral strains contain only three such elements. The other type contains 2 RBEIII binding sites (like NF-kappaB, RBEIII is a different and important transcription factor), while the native viral strains contain only one RBEIII element (Mahesh Bachu et al, AIDS Research and Human Retroviruses, 28, 1262-8, 2012). These new viral strains were found at a prevalence of only 1-2% at that time. A decade later, we received a big jolt to find that these two

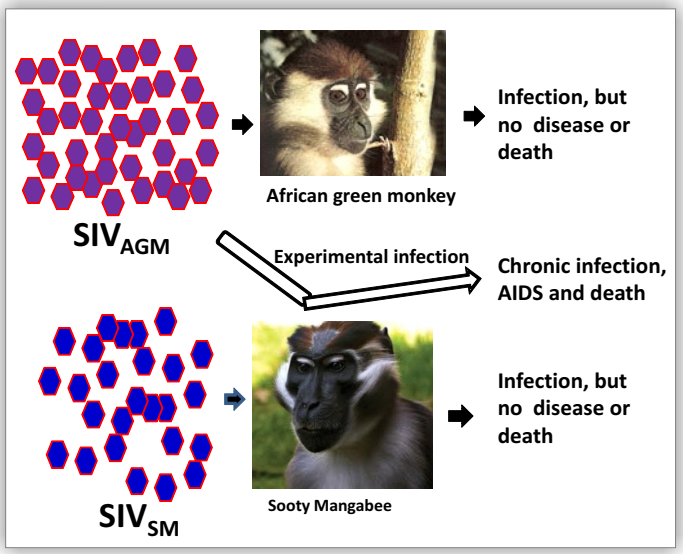

Fig. 1. SIV$_{AGM}$ and SIV$_{SM}$ are viruses like HIV that infect in the wild their own natural primate hosts the African Green Monkey and the Sooty Mangabee, respectively. Infection by the natural viruses does not cause disease or death in the natural hosts. However, if a virus is experimentally introduced into a wrong host, there will be disease progression that ultimately leads to the death of the wrong host.

promoter variant strains grew to 20-35% in India (Mahesh Bachu et al, Journal of Biological Chemistry, 287, 44714-35, 2012). The new viruses have been expanding at a rapid pace replacing the native viral strains. The emergence of the new promoter-variant viral strains is not unique to India, but can be seen in other countries where subtype C is prevalent such as South Africa and China. A recent publication from Brazil too confirmed the emergence of the variant viral strains in that country (Boullosa et al, Viruses, 6, 2495-2504, 2014).

Using a wide range of molecular strategies, we proved that the novel viral strains containing 4 NF-kappaB sites (the 4-kB strains) in the promoter dominate the genetically

similar viral strains that have only 3 such motifs (the 3-kB strains). Importantly, the 4-kB strains make more daughter viruses from the target cells as compared to the 3-kB strains. Additionally, people infected with the 4-kB strains contained more number of viral particles in their blood than those infected with the 3-kB strains. We generated substantial experimental evidence and proved that only subtype C, but not other HIV-1 subtypes, has a potential to acquire the additional NF-kappaB motif. Don't forget that subtype C played this trick once before to acquire a third NF-kappaB site. Subtype C repeats that trick once again to acquire a fourth NF-kappaB site. We presently have some experimental evidence to explain how subtype C plays this trick at the molecular level.

Our findings raise many important questions. A stronger viral promoter may positively contribute to the successful expansion of the variant viral strains. This proposition, however, appears to be counterintuitive because the consequential stronger viral gene expression should also elicit a stronger immune response from the host that may be counterproductive to viral survival. Furthermore, establishment of viral latency (absence of viral gene expression in the infected host cell) is critical for HIV. How a virus with a strong promoter can establish and maintain viral latency must be examined. Additionally, if the acquisition of a stronger viral promoter is necessary for the evolutionary success, why other subtypes of HIV-1 do not use the same trick for their own success? Moreover, where is the natural limit for the gaining of the strength of the viral promoter? We are presently addressing some of these questions.

What are the implications for disease management? First of all, our data prove that subtype C possesses biological characteristics that make it unique. In other words, variations seen at the genetic level are associated with biological differences of the viral subtypes. Second, we do not know if the new viral strains of subtype C are likely to alter the landscape of the HIV demographics in India and other places in the coming years. In the recent past, the rate of viral expansion has slowed or even declined in several global regions, including India. Our study suggests that the new viral strains of HIV-1 are more infectious. It remains to be determined how the rates of viral prevalence are going to be affected as a consequence of the emerging viral strains in India and elsewhere. Third, it is possible that the advantages gained by subtype C by developing a stronger viral promoter are transmitted to other HIV-1 subtypes through genetic recombination. Lastly, we do not know if the new HIV-1 strains are likely to promote faster disease progression.

Our working hypothesis to understand this, we are planning additional studies. In collaboration with four different institutions (YRG CARE, Chennai; St. John's Hospital, Bengaluru; National AIDS Research Institute, Pune and All India Institute of Medical Sciences, New Delhi), we will monitor 100 subjects at each site (50 subjects each with 3- or 4-kB viral infections) for disease progression over a period of 2 years. This work supported by the Department of Bio-Technology is to begin soon.

Novel adjuvant technology for effective and stable vaccines

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Adjuvants are compounds that enhance the specific immune response against co-inoculated antigens. The role of adjuvants was noticed in 1920s from observations of Ramon *et al.* who noted that horses that developed an abscess at the inoculation site of diphtheria toxoid generated higher specific antibody titers.

With possible threat of safety issues due to reactogenic crude antigen, inclusion of poorly immunogenic purified, subunit or recombinant antigens in modern age vaccine is becoming more and more frequent. It is thus becoming more important that a proper adjuvant should be used in a vaccine formulation in order to get the best possible outcome in terms of immune response without compromising on the safety part.

Different adjuvant technologies have been developed. Mainly used technologies are (1) oil emulsions (oil in water, water in oil or double emulsions) (2) Mineral salts (suchasaluminium hydroxide) (3) Immunostimulant polymers (4) Chemical particles (such as liposomes and nanoparticles) (5) adjuvants from biological origin (such as saponins, TLR ligands, cytokines). Keeping this fact in mind that there is no universal adjuvant, the proper adjuvant selection should be the first step to optimize the vaccine thus it is very important to make a proper adjuvant choice depending on the antigen used in a vaccine formulation in order to have the best balance between safety, efficacy and stability to get the best possible outcome of vaccine.

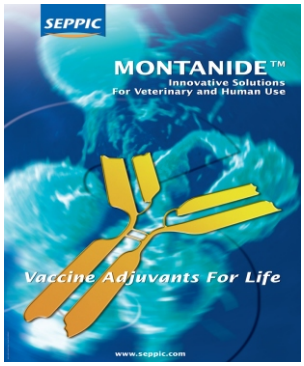
Seppic a subsidiary of Air Liquid Group Company has brought onto the market a unique range of vaccine adjuvants and has managed to sustain as a world leader in vaccine adjuvant business over past 60 years. Seppic has developed unique adjuvant technology and takes in to account several parameters critically affecting the vaccine performance in order to select the right adjuvant for each vaccine application. Seppic's MONTANIDE ™ range of adjuvants has been very successful worldwide in the treatment of every possible species against a pool of viral, bacterial and parasitic diseases.

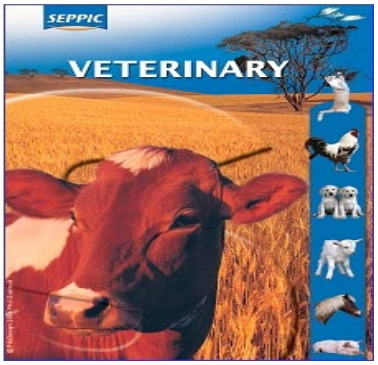
With the advancement of molecular mechanism of disease pathogenesis, it is a proven fact that in addition to the humoral immunity we need to have more and more cell mediated immune response in order to have effective vaccine outcome and better protection coverage against diseases.

Presently, there are many adjuvants which can induce a cellular response at laboratory level but a very few can be used at the industrial level. Seppic offers a unique innovative range of commercial adjuvants which are ready to use products and requires no or very less sophistication for blending at a very large commercial scale for vaccine formulation. With the advancement in adjuvant science and continued research, Seppic could successfully launch new generation adjuvants which can selectively enhance the cellular immune response without compromising with humoral immune response which is found to be intact and at the same level. These innovative range of new generation products includes, MONTANIDE ISA 61 VG a water in oil (W/O) for Cattle and Sheep, MONTANIDE ISA 201 VG a water in oil in water (W/O/W) for Cattle and Swine and MONTANIDE ISA 71 VG a water in oil (W/O) for Poultry.

In addition to the wide range of adjuvants available for animals, Seppic has also launched its range of vaccine adjuvants for humans with a very high safety profile suitable for human injections. It is being widely used in more than 150 clinical trials from phase I to phase III in all over the world in the domain of therapeutic vaccines, mainly against cancer, HIV, malaria and autoimmune diseases. The major human adjuvants are MONTANIDE ISA 51 VG which is recently approved in a commercial vaccine against lung cancer in humans and MONTANIDE ISA 720 VG which is intended against several infectious disease vaccine applications in human. Both these adjuvants are DMF available, multicompendial product and can be seen for regulated market vaccine business.

SEPPIC: World leader of ready to use adjuvants for veterinary and human vaccines





New vaccine for highly pathogenic Avian Influenza (H5N1)

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For the control of avian influenza in poultry, India follows a policy of elimination of infected and susceptible avian population at the face of outbreak and does not recommend vaccination. However, as part of preparedness for emergency situations wherein biosecurity measures alone cannot control avian influenza, readiness with a suitable vaccine against highly pathogenic H5N1 influenza virus is required. With the advent of reverse genetic systems for AI virus, custom made inactivated AI vaccines are possible using infectious clones systems. These viruses can be tailored to have same HA type but different NA type from field strain to allow DIVA (differentiation of infected from vaccinated animals) and be rendered safe by altering the hemagglutinin (HA) cleavage site.

We developed a DIVA marker H5N2 inactivated vaccine using the rgH5N2 virus (8+2 reassortant) generated. The H5-HA gene donor virus was selected from available H5N1 (clade 2.2) viruses through antigenic cartography. The HA-H5 gene amplified from the selected donor virus A/chicken/West Bengal/80995/2008(H5N1), was mutated to replace the basic amino acid cleavage site (RRRKKR*GLF) with IETR*GLF by site directed mutagenesis. A reassortant rgH5N2 virus was generated using the 12-plasmid based reverse genetics system with the mutated H5-HA and N2-NA gene from H9N2 field isolate (A/chicken/Uttar Pradesh/2543/2004). The rgH5N2 vaccine candidate virus was found to grow up to 2^{10} HA titre in SPF chicken embryos. The non-pathogenic phenotype and safety of the rgH5N2 vaccine candidate virus was validated by intravenous pathogenicity test and intranasal challenge test. The protective efficacy of the inactivated rgH5N2 vaccine was shown to be 100% against high dose challenge with HPAI virus (10^9 EID_{50}/ bird) in vaccinated chickens with single vaccination (0.5 ml/bird) on 28th day post-vaccination (Fig.1). The vaccinated birds showed high level of anti-H5 antibody response estimated by HI test (>2^{10}). Challenge virus shedding via oro-pharynx of the vaccinated chickens, estimated by real time RT-PCR, was found to be at minimal detectable level on 1st and 3rd day post challenge. In another experiment, a single dose vaccinated chickens showed high HI titres (>2^{10}) which was maintained without booster vaccination at least till 5 months. The work has established India's self-sufficiency to generate re-assortant influenza viruses through use of reverse genetics for making DIVA marker avian influenza vaccines and to carry out other important studies on avian influenza virus. This work was conducted under National Fellow project funded by Education Division of ICAR.

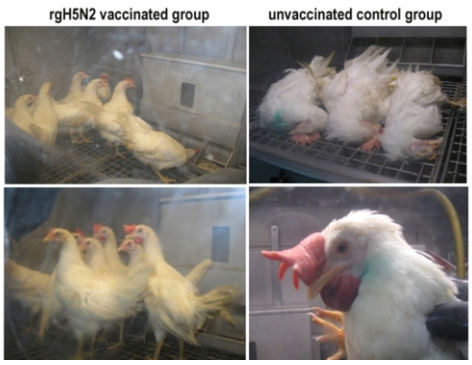

Fig.1. The SPF chicken administered with inactivated rgH5N2 vaccine remained healthy, whereas the control group died within 24-96 h of challenge of the virus.

Ebola epidemic 2014: An international concern

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Currently various countries in Africa, including Liberia, Sierra Leone, and Guinea, are facing disaster due to Ebola virus disease (EVD) with at least 17,800 cumulative cases and 6,331 deaths. EVD is a serious fatal illness caused by the *Ebola virus* of family *Filoviridae*. The first evidence of the Ebola was dated in 1976, in two places simultaneously, one in Nzara, Sudan, and the other in Yambuku, Democratic Republic of Congo. The latter one occurred in the village near the Ebola River, thus the name given to the virus. Since then there are the reported cases of the chronological outbreaks of the Ebola in the various African countries. Recent outbreak in 2014 is supposed to be the largest outbreak.

The current Ebola outbreak is generally due to the human to human contact transmission of the virus. The initial phase of the Ebola disease includes the flu like symptoms such as fatigue, fever, headaches, joint, muscle, and abdominal pain. The initial phase is followed by the bleeding phase, which is characterized by internal and subcutaneous bleeding. As there are no initial typical clinical symptoms for the EVD, it is difficult to distinguish the disease from the other infectious disease such as malaria, typhoid fever and meningitis. The disease is diagnosed by cell culture, PCR and ELISA. Genome or gene sequence is used for the efficient identification of the virus and also strain identification.

No vaccine/specific treatment for the EVD are approved by the Food and Drug Administration (FDA, USA) till now. Treatment of the Ebola is symptomatic and early supportive care with rehydration can increase the survival rate. Several treatment measures such as packed red blood cells, platelets or fresh frozen plasma, regulators of coagulation have also been tried including heparin in an effort to prevent intravascular blood clotting and clotting factors to reduce bleeding are applied. However, there is no evidence that these methods are significant for the treatment. A wide variety of investigational drugs are being tested for the treatment against the Ebola, but all of them are yet in the level of clinical trial in animals or further. The outbreak of the 2014 has proposed a great risk. The prime focus must be given on the development of reliable diagnostics, vaccine and therapeutic drugs. If optimum precautions are not taken then this outbreak may result into another historic epidemic of mankind.

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