

# THE SPECTRUM OF INFLUENZA VIRUS PANDEMIC: BETWEEN AGONY AND HOPE

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## REZUMAT

Natura multicomponentă a genomului virusului influenza, îi permite acestuia remodelarea genetică și evoluția continuă a diferitelor tulpini, acest mecanism constituind de altfel baza "shift-ului" antigenic la această specie. Dezvoltarea unor noi tulpini virale în gazde secundare co-infectate cu mai mult de o tulpină, corespunde unui alt mecanism de variație antigenică a virusului, bazat în esență pe imperfecțiunea mecanismelor de asamblare genică, rezultând astfel într-o evoluție genetică rapidă. În mod natural, există o posibilitate calculată matematic ca noile tulpini virale astfel dezvoltate să dețină o virulență mai mare la om, datorită acumulării sau pierderii unor funcții specifice, efect în urma căruia rezultă o creștere a spectrului de celule permissive pentru virus și/sau o imunopatologie crescută. Ca și o consecință a acestui model, abordul combinat prin vaccinare în masă împotriva virusului influenza sezonier (tulpina umană), diagnosticul eficient al cazurilor medicale suspionate de infecție cu tulpini aviare, ambele cuplate cu administrarea de inhibitori de neuraminidază și alte metode, ar trebui să rezulte în descreșterea semnificativă a posibilității apariției pandemiilor cu virus influenza. Bazându-ne pe un raționament matematic, cea mai eficace metodă profilactică împotriva unei pandemii pare a fi vaccinarea în masă; pentru acest scop, în mod surprinzător, o compoziție completă a vaccinului în ceea ce privește diversitatea antigenică virală, ar putea să nu fie necesară. Pentru a mări gradul de "matching" între agenții patogeni și vaccinuri, prin intermediul genomicii microbiene, s-ar putea realiza o accelerare a design-ului și producerea de noi vaccinuri capabile să cuprindă elemente antigenice specifice atât tulpinilor umane, cât și aviare. În timp ce în Europa de Vest și mai ales în S.U.A. procesul de dezvoltare a unor noi strategii de vaccinare pentru utilizare generală este actualmente încetinit, datorită legislației referitoare la testarea eficacității medicamentelor, o analiză rațională a raportului risc/beneficii ar putea fi ușor justificată la nivel global.

## ABSTRACT

The multicomponent nature of the influenza virus genome enables genetic reassortment and evolution, which is the basis for shift variation. The emergence of new viral strains in secondary host species co-infected with more than one strain complements other mechanisms of influenza genetic variation, essentially caused by the imperfect proof reading mechanisms, resulting in rapid genetic evolution. Naturally, there is a mathematical possibility that emerging strains are endowed with higher virulence in humans, due to gain or loss of functions resulting in broadening of categories of permissive cells and/or increased immunopathology. A consequence of this model is that a combination approach consisting in mass vaccination against seasonal influenza virus (human strain), effective diagnosis of medical cases ascribed to avian strains coupled with use of neuraminidase inhibitors and other measures, should significantly curb the likelihood of influenza virus pandemics. Based on a mathematical rationale, the most effective prophylactic measure against a pandemic seems to be mass vaccination; to that aim, strikingly, a completely matched composition may not be necessary. To maximize the antigenic match between pathogens and vaccines, microbial genomics-based approaches may result in more rapid design and manufacturing of novel vaccine candidates encompassing antigenic elements from emerging avian / human strains. While in Western countries and in particular the U.S., the development of new vaccination strategies for widespread use is generally hampered by business, liability and drug regulation considerations, a risk/benefit ratio analysis may easily justify global interest in innovative vaccination strategies using innovative research, development and manufacturing tools that are accessible throughout the world.

## INTRODUCTION

Influenza virus is responsible for a range of health problems in human population, from seasonal flu epidemics to catastrophic pandemics caused by virus genome reassortment or shift variations.

The influenza virion is enveloped (encompassing a lipid bi-layer), containing a nucleoprotein-based core in close association with the eight segments of negative stranded RNA, representing the genome.<sup>1</sup> Influenza viruses display a considerable antigenic diversity – particularly at the level of hemagglutinin (HA) and neuraminidase (NA), viral proteins associated with the envelope.<sup>2</sup> Thus, a classification of influenza viruses is being done based on the antigenic sequence of HA and NA (eg. H<sub>1</sub>N<sub>1</sub> and H<sub>3</sub>N<sub>2</sub> are "human" strains whereas H<sub>5</sub>N<sub>1</sub> and H<sub>5</sub>N<sub>2</sub> are "avian" strains). In stark contrast, viral proteins such as matrix (M) or nucleoprotein (NP) are far more conserved, since they have key, sequence-dependent roles in the life cycle of the virus, such as virus-envelope, target-cell fusion and gene-expression regulation, respectively.<sup>3,4</sup> Upon infection of host cells

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such as bronchial epithelial cells, translocation of nucleoprotein-RNA complexes across endolysosomal membrane is followed by a sequence of events culminating in a very specific RNA-dependent, RNA synthesis.<sup>5</sup> The emerging positive stranded RNAs offer a blueprint for synthesis of viral proteins that is part of emerging virions, or for creation of negative RNAs that constitute the viral genome. The proof reading mechanisms overseeing viral RNA synthesis are very rudimentary, explaining the increased rate of errors translated into “drift” mutations - that, while not affecting radically the life cycle - change the antigenic structure and thus lead to seasonal epidemics.<sup>6</sup> The segmented genome of influenza virus, however, creates the possibility that co-infection with two different strains results in exchange of specific genes and creation of virions that carry new virulence and immunological features (“shift” variation).<sup>7</sup> Such events may lead to pandemics of biblical proportions when the emerging strain can easily infect human cells (resulting in inter-human transmission), replicate in a wider range of cells, induce highly pathogenic events and escape or avoid immunity due to the radically changed antigenic structure - such as the 1918 Spanish flu pandemic, that killed more people than the military operations in the first World War.

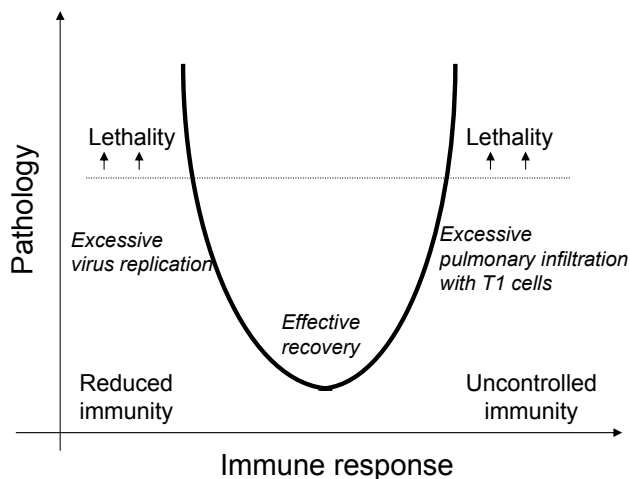
To elaborate effective measures against seasonal or cataclysmic influenza viruses, one needs to take into account a wide range of factors including but not limited to the mechanism of infection and disease, the antigenic structure of the virus, molecular diagnosis and isolation of the virus or its genes, quarantine measures, plus logistics of manufacturing and implementation of prophylactic or therapeutic tools. Herein, we outline a few essential factors that distinguish the present from the past (the state of art during the Spanish flu pandemic) and if addressed appropriately, can result in curbing of influenza virus pandemics.

## **DETERMINANTS OF DISEASE INDUCED BY INFLUENZA VIRUS INFECTION**

The common belief has been - for a long time - that influenza viruses induce disease via their direct cytopathic/cytotoxic effect on bronchial epithelial cells as a consequence of infection, viral replication and release. Higher is the virulence, higher is also the virus' capability to replicate at a fast pace - in a broader range of host cells. Surprising data accumulated during the last two decades showing milder influenza pneumonia in T cell immunodeficient mice initiated a major revision of this model.<sup>8</sup> More specifically, animals that

fail to mount a T cell mediated response to flu due to genetic deficiencies develop disease subsequent to infection with lethal titers in a delayed fashion. Wild type mice however, challenged with similar doses, develop influenza pneumonia much faster and die more rapidly. More recently, those findings have been complemented by careful analysis of cellular defense mechanisms deployed within the pulmonary tissue, in gene targeted mice lacking certain genes - such as STAT4 and 6 transcription factors, IL-4 and T-bet - that control major arms of T cell immunity.<sup>9,10</sup> In summary, such studies demonstrated a more complex determination of influenza pathology than previously thought, highlighting the dual role of T cells (protective /detrimental) that infiltrate the pulmonary parenchyma as a reaction to virus infection. First, these cells contribute to suppression of viral replication or clear virus infected cells; secondly, however, if the immune response escapes a homeostatic control, it results in detrimental effects on pulmonary function and even death. This is dramatically illustrated in IL-4 knockout mice that mount potent and effective immune responses against influenza virus but develop an aggravated form of pneumonia due to lack of down-regulation of IFN- $\gamma$  producing Th1 and especially CD8+ T cells within the pulmonary tissue.<sup>10,11</sup>

Together, these results lead to the model depicted in the Figure 1: impaired or exacerbated T immunity against influenza virus may result in significant pulmonary pathogenesis, morbidity and even mortality. A decreased immunity may be due to lack of pre-existing immune memory (for example in the case of avian or porcine viruses such as H<sub>5</sub>N<sub>1</sub> or H<sub>5</sub>N<sub>2</sub>, or human strains undergoing antigenic shift variations), combined with a rapid replication rate of the virus, outpacing the initiation of the primary immune response. Increased virus load and/or replication within a broader range of host cells (within or beyond airways) may lead to functional impairment of the respiratory organ, directly. On the other hand, exacerbated T1 immunity - on the expense of other arms such as T2 and T regulatory cells - may lead to immune mediated pathology, morbidity and mortality despite relatively good control of virus replication and spread. Certain virus strains or genetic backgrounds may favor such occurrences. Finally, these two pathogenic factors are not mutually exclusive relative to each other: rapid viral replication and spread beyond the upper respiratory tract may actually result in elevated local T cell infiltration and immunopathology that although effective in curbing additional virus replication, may significantly amplify the morbidity.



**Figure 1.** Influenza virus pathology, determined by excessive virus replication and/or uncontrolled immunity.

### **CURRENT PROPHYLACTIC AND THERAPEUTIC APPROACHES AGAINST INFLUENZA**

Due to its major impact on human health - as seasonal flu or devastating pandemics such as the 1918 Spanish flu - development of diagnostics, vaccines and therapeutics effective in controlling influenza has always been of major interest. There are decades since one of the most effective medical tools in mankind's history has been developed: the seasonal influenza virus vaccination. It is essential to outline that this strategy, consisting in prophylactic induction of antibodies against surface proteins such as HA and NA by using inactivated influenza virus grown on eggs or more recently, mammalian cells, does not result in significant cross-reactive immunity that protects against drift or shift variants. This is due to the fact that the conserved immunological information is mostly located within the internal elements such as NP; that are accessible to the immune response as MHC-peptide complexes onto infected cells. A vaccine that has a broader capability to exert cross-protection must thus be able to elicit significant T cell immunity against viral proteins expressed within infected cells.

Unfortunately, that cannot be achieved with the conventional inactivated or split virus vaccines developed against seasonal influenza viruses, essentially protecting against strains already in circulation that served as a source for the vaccine. The reason is that in order to generate robust immunity against proteins expressed by infected cells, a vaccine must result in internal protein-derived, epitope expression by specialized antigen presenting cells (APCs).

To that aim, new generations of vaccines have been designed and one of them approved for human

use, recently: a live, cold-adapted influenza virus vaccine.<sup>12</sup> This consists essentially of virus adapted to replicate at a lower temperature as compared to the normal temperature of human's body - and thus, have a reduced virulence compared to wild type strains. The vaccine, designed by the US company Aviron and licensed by the US biotech giant MedImmune, is being administered as a nasal spray (FluMist®) and is designed to produce a self-limited infection of the epithelial cells within the upper respiratory tract, resulting in a broad immunity encompassing not only antibodies, but MHC class I restricted cytotoxic lymphocytes and T helper cells. The only drawback of this vaccine - undoubtedly more effective than the conventional, inactivated one - is the relatively suboptimal safety profile in children and aged individuals. This is exactly the population that needs it most during influenza seasons. As a side-note, shift variants that may result in pandemics do not exclusively affect the relatively immune suppressed subpopulations - thus, an improved vaccine that offers a degree of cross-protection may be a potent tool to mitigate flu pandemics as discussed below.

Efforts to develop non-infectious anti-influenza virus vaccines that are able to generate broad, cross-reactive immunity against emerging but yet to be isolated strains, have been considerable. For example, the pharmaceutical company Merck & Co designed and analyzed a DNA-based vaccine encompassing a single, conserved influenza virus protein - NP - and demonstrated in preclinical testing induction of heterologous, T cell immunity, suppressing a distinct viral strain.<sup>13</sup> However, the potency of the vaccine was quite limited - and subsequent efforts to improve on the efficacy of monovalent DNA resulted in strategies that are more amenable to immunotherapy of chronic infections or cancer, rather than mass vaccination.<sup>14</sup> Nevertheless, subsequent efforts were aimed at evaluating multivalent DNA vaccines encompassing immunogenic elements from surface (HA) and internal (NP) proteins, as detailed below, but their promising efficacy profile in preclinical models is yet to translate into clinical efficacy.<sup>15</sup>

Finally, anti-viral agents such as Oseltamivir/Tamiflu® developed by Roche - which is essentially a neuraminidase inhibitor - complements current and emerging vaccination approaches. That is since Oseltamivir - as a therapeutic rather than a vaccine - interferes with the maturation and release of virus from infected cells. It is widely believed that the molecular target site of Oseltamivir onto NA is widely conserved in different strains of influenza viruses including the dreaded H<sub>5</sub>N<sub>1</sub> - making this drug a

potentially valuable tool in the control of a pandemic (see below). However, it is important to stress out that oseltamivir and analogues do not prevent or obliterate infection with influenza virus but potentially curb its replication and spread in vivo, most likely enabling a recovery from disease mediated by the endogenous immune response.<sup>16</sup>

## KEY ELEMENTS FOR THE CONTROL OF INFLUENZA VIRUS PANDEMIC

Herein we bring arguments toward prophylactic vaccination as being a potentially key tool in preventing a flu pandemic. Contrary to widespread belief, even partially effective vaccination may have a profoundly positive impact in laying the foundation for an effective control of an incipient pandemic. To exemplify this aspect, prophylactic, mass vaccination triggering immunity against a set of epitopes shared by multiple strains, may not confer complete protection against infection, but enable the endogenous immunity to curb the replication of the virus within the respiratory tract. This may lead to a decreased production of infectious virions and reduced inter-subject transmission rate.

To illustrate how important may be this aspect in effective prevention of pandemic, we modeled two scenarios (Figure 2): in the first case, the transmission rate is approximately 3 (represented by  $R_0$ , which is the number of newly infected people by a single subject), believed to be roughly similar to that of the Spanish flu strain responsible for the most lethal pandemic in modern history. Assuming an incubation interval of 2.5 days until infectivity, within five weeks from the mutation conferring inter-human transmission, a population of approximately 6 million people would have already been infected should there be no quarantine measures. If we assume mass prophylactic vaccination, only partially effective in protecting against infection with an avian or porcine strain that acquired inter-human spreading properties, the transmission rate would still be significant but lesser than 3.

If we hypothesize a new  $R_0$  rate of 2 in a setting of a partially matched vaccine, within the same interval (five weeks) from the mutation, the number of infected subjects would be lesser than 40,000 - two orders of magnitude fewer than in the first scenario. That would allow effective control of the emerging pandemic, by implementation of quarantine in conjunction with anti-viral agents such as neuraminidase inhibitors (oseltamivir) - further curbing the spreading rate to non-critical values.

$\sum_{X=0}^n R_0^X = N$		<p><math>N</math> = total number infected within the interval <math>T</math></p> <p><math>n = T/t</math>, where <math>t</math> is the incubation interval (approx. 2.5 days)</p> <p><math>R_0</math> = spreading coefficient</p>
<p><b>Scenario 1:</b></p> <p>Highly virulent strain on a setting of no immune memory within the target population</p> <p><math>R_0 = 3</math></p>	<p><b>Scenario 2:</b></p> <p>Highly virulent strain on a setting of mass immunity with a partially effective vaccine</p> <p><math>R_0 = 2</math></p>	
<p><math>N</math> (within 5 weeks) &gt; 6,000,000</p> <p><b>Out of Control</b></p>		<p><math>N</math> (within 5 weeks) &lt; 40,000</p> <p><b>Manageable</b></p>

**Figure 2.** Prophylactic vaccination campaign with even a partially matched composition can have a significant impact on the progression of an outbreak with a highly virulent influenza strain.

A significant unknown in this equation is however, whether the currently licensed inactivated vaccines confer any partial - even modest - cross-protection. The likelihood that this would be the case with the live, cold attenuated virus vaccine (FluMist<sup>®</sup>) is significantly higher as compared to the killed vaccine, based on the mechanism of action, involving induction of T cell responses against viral proteins expressed by infected cells. Since its administration to immune depressed recipients such as infants, children and aged individuals is in question due to the safety profile, mass vaccination with FluMist<sup>®</sup> or equivalents of adult population would still have a decisive effect in curbing a pandemic (“herd effect”). On the other hand, in the worst case scenario, with no available vaccine that can provide cross protection against incoming highly virulent strains, seasonal vaccination against strains in circulation may only minimize the likelihood of genetic reassortment and shift variation simply because the likelihood of co-infection with multiple strains would be diminished. However, should that occur and if the emerging strain acquires inter-human transmission capability, there would be nothing in the way of a pandemic except quarantine and potentially, oseltamivir or equivalents that are still under development. More precisely, using the same model from Figure 2 and assuming an interval of 4 weeks from the first event to the implementation of stringent quarantine and additional measures, with no prior immune memory, there would be roughly 600,000 infected subjects to deal with - well above the number of infected people in a setting of even a modest prophylactic vaccine and probably beyond reasonable management.

Of course, another mechanism (non-mutually exclusive) to control an outbreak would be to rapidly implement measures of quarantine and/or deploy large scale anti-viral treatment: if we assume a prompt deployment that would result in curbing the spread coefficient from 3 to 1 and if the implementation is made no later than 3 weeks after the first patient, then the number of infected subjects within 5 weeks would be in the range of 30-35,000 (significantly less than 6 million or so). However, if the implementation of such drastic measures is not carried out within 4 weeks, assuming an  $R_0$  of 3, the total number of infected patients within 5 weeks from the initial event would be in excess of 1.5 million – a situation that would likely get out of control.

This illustrates how important is to achieve and maintain in the general population a permanent – even if not perfectly matched – immunity level, precluding the need for unrealistic, desperate quarantine and anti-viral treatment measures that may not be effectively deployed in timely fashion, within the affected areas. Only preexisting immunity may curb  $R_0$  during the initial phase of the outbreak, before reliable confirmation of a highly virulent strain is being confirmed.

Thus, every effort should be made to develop safe and at least partially effective vaccines encompassing antigenic elements that are most likely to be present in highly virulent strains. By then, mass seasonal vaccination with the vaccines is an indirect prophylactic strategy with a value that still needs assessment, with the live attenuated vaccine having the highest likelihood of success rather than the inactivated version, globally available.

## RESEARCH AND DEVELOPMENT OF NEW ANTI-INFLUENZA VACCINATION STRATEGIES: MICROBIAL GENOMICS AS AN EMERGING TOOL

To the aim of ensuring presence of immunity against key conserved epitopes of influenza virus, it is critical that new strategies receive increased attention since the currently available seasonal influenza virus vaccine (inactivated) fails to induce significant cross-reactive immunity despite the fact that some formulations contain internal proteins.

One direction is the development of generic vaccines that are able to elicit immunity against conserved epitopes while being devoid of replication capability within the body and thus, have a more favorable safety profile. Plasmid vectors - circular dsDNA encompassing a potent viral promoter driving

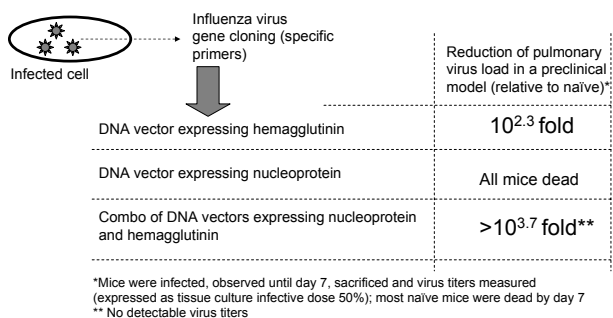
the expression of an antigen in situ - enter APC, persist transiently in an episomal state and are then eliminated through the process of intracellular turnover of nucleic acids. In line with the model depicted in the Figure 3 and in contrast to the inactivated seasonal vaccines, such vectors, instead of generating only antibody and Th responses, are capable of eliciting MHC class I-restricted, cross-reactive immunity since the vaccine antigen is synthesized within APC.

Vaccine specificity	Composition	Advantages	Drawbacks
Surface antigens (HA, NA)	Inactivated, whole virus; Purified antigens	Safe, long track record, potent against same strain	Narrow immunity, (homologous strain)
Internal, conserved antigens (with or without surface antigens)	Live attenuated virus; Recombinant DNA	Broad immunity (CTL along with Th and B cells)	Safety and potency to be ascertained

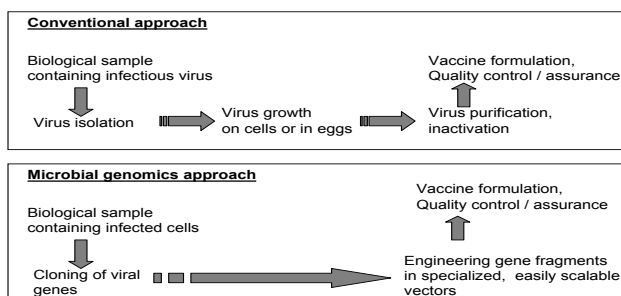
**Figure 3.** The Achille's heel of influenza virus is represented by "internal" or conserved elements (such as NP, M proteins) that are essential for the biological/replication cycle of the virus. The current problem is under-availability of vaccines with an appropriate mechanism of action.

Based on the somewhat disappointing results of testing mono-antigen plasmid vaccines, a key element in building their potency was consideration of multiple, key antigens (multivalent compositions).<sup>17</sup> The hypothesis was that co-induction of broad spectrum responses against multiple epitopes from various antigens, may not only increase the overall efficacy but result in more pronounced cross-protective potential. Using an  $H_1N_1$  strain of influenza that is lethal in wild type mice (A/WSN/ $H_1N_1$  32), we tested a bivalent plasmid DNA vaccine encompassing a surface antigen of a drift variant (HA of A/PR<sub>8</sub>/ $H_1N_1$  34) and a highly conserved internal antigen (NP), respectively.<sup>15</sup> Mice co-immunized with the two vectors were able to control more effectively the replication of this lethal strain (WSN), besides the homologous strain PR<sub>8</sub>, resulting in decreased morbidity - as opposed to animals immunized with either one of the plasmids, separately. (Fig. 4). More recent testing of such vaccines in newborn primates showed that they are safe and effective in eliciting immunity.<sup>18</sup> However, the mechanism of action of such vectors may not be compatible with generation of sterile protection against infection with heterologous strains. Nevertheless, as concluded from the mathematical analysis from above – even partial cross-protection would offer a significant advantage in preventing or controlling a pandemic.



**Figure 4.** Proof of principle - a bivalent DNA vector-based vaccine encompassing a surface antigen (hemagglutinin) and an internal protein (nucleoprotein) interferes with replication of a highly virulent influenza virus in a preclinical model. Combinatorial approaches integrating immune elements from multiple antigens enhance the potency of the vaccine.

The revolution of microbial genomics created an unprecedented opportunity to design vaccines tailored to emerging, highly virulent strains. That can be approached only in conjunction with vaccine technologies permitting rapid development and manufacturing - such as the DNA-based ones – in order to permit prompt deployment in face of a pandemic. In brief, highly virulent strains that acquired inter-human spread capability can be used as a source for gene cloning. (Fig. 5) Influenza virus gene fragments corresponding to outer and inner antigens can be then incorporated in one or multiple gene vectors (using molecular biology tools), resulting in multivalent vaccine candidates ready for potency testing in standard assays and quality control. The manufacturing process would involve bacterial (*E. Coli*) fermentation only, with the final product being recombinant DNA rather than proteins or the whole virus. This path obviates the need to produce, manipulate and scale-up virus, an extremely cumbersome and time consuming process associated with the current approaches.



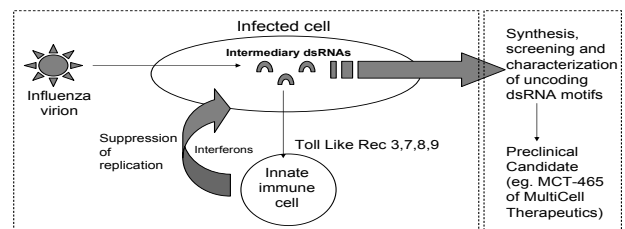
**Figure 5.** Changing the paradigm - microbial genomics as a strategy to engineer and produce novel anti influenza virus vaccines.

To further streamline the whole process, components against conserved elements can be generated separately (generic components) while genes corresponding to emerging strains can be added whenever available or necessary (by a mixing

and matching/tailoring process), leveraging the multicomponent nature of this strategy.

Finally, use of a category of vaccine does not necessarily preclude co-use of a different type - for example seasonal influenza vaccination may be complemented by vaccination against conserved antigens using a different strategy, as long as there is no generation of anti-vector immunity. That would further contribute to the increase of memory immunity in the human population - a key aspect in preventing a pandemic. Finally, the strategy of controlling an outbreak within the animal realm (for example the Russian H<sub>5</sub>N<sub>1</sub> vaccine applicable to poultry) may further diminish the likelihood of co-infection with different strains in a different host (humans) and genetic reassortment leading to more virulent strains.

An innovative way to factor in microbial genomics in developing anti-influenza virus agents stemmed from the key observation that the immune response against this RNA virus is multifaceted, incorporating rapid recognition by the innate immunity of in situ generated dsRNA that binds to specialized TLRs within the endolysosomal compartment of APC.<sup>19</sup> Screening of synthetic dsRNA motifs for their impact on innate and adaptive immunity, resulted in discovery of molecules that can rapidly mobilize an anti-viral response within the respiratory tract by triggering cytokine (IFN) and chemokine production. Importantly, such synthetic dsRNAs were able to suppress the replication of a lethal influenza virus strain in preclinical models; currently, selected candidates undergo preclinical testing in mouse and avian influenza virus models - an effort led by MultiCell Immunotherapeutics (San Diego - CA).<sup>19</sup> (Fig. 6)



**Figure 6.** A different facet of microbial genomics and a resulting approach to curb influenza virus replication: synthetic polynucleotide based immune stimulation (SPIS).

The potential applicability of synthetic polynucleotide immune stimulating approaches (SPIS) is dual: first to improve on the efficacy and cross-reactivity of current seasonal vaccines; and secondly, more importantly, to offer a means to curb transmission within population of highly virulent

strains in face of a pandemic, by raising the activity of innate immunity and diminishing the virus load, morbidity and inter-human spread of new strains. A safe and effective generic immune stimulant would thus nicely complement the use of oseltamivir and constitute a potent tool to obliterate a flu pandemic at its inception (by further reducing the  $R_0$  coefficient - as modeled in Figure 2).

## **CONCLUSIONS: POINTS TO CONSIDER ON A SHORT AND LONG TIMEFRAME**

In contrast to the 1918 Spanish flu pandemic and even the more recent but the much less catastrophic one in 1968 - we have a broader range of tools to deal with a potential pandemic even if that would originate from an  $H_5N_1$  avian strain.

In the absence of effective vaccines against new strains of flu that enter the human population and may acquire human to human spreading capability, a series of measures need to be implemented and reinforced in face of evidences for zoonotic influenza outbreaks. A partitioning of farm animals, poultry and shielding access to wild-life are of course good measures; but equally important is to restrict the exposure of relatively immune depressed subjects such as infants, children and elderly. To minimize the likelihood of influenza gene reassortment and emergence of virulent strains with inter-human transmission capability, mass vaccination against seasonal influenza should be encouraged and expanded to subpopulations less susceptible to seasonal disease (such as immune competent adults). A more advanced vaccine (FluMist<sup>®</sup>), already available in U.S. to adults, capable to trigger immunity against conserved antigens, may be more useful in that regard due to its mechanism of action and resulting, broad immunity. Once animal to human transmission occurred, it is important to ensure - in addition to more stringent quarantine measures - that anti-virals (Oseltamivir) are being rapidly deployed and provided to subjects that may already be in the incubation phase. Suspicions of inter-human transmission based on epidemiologic data, should be met of course by measures that are more stringent - including larger scale deployment of oseltamivir accompanying strict quarantine.

In regard to future research and development of new prophylactics and therapeutics, based on the epidemiological and mathematical considerations resulting from the need to control the spreading index ( $R_0$ ), the following directions should be pursued. First, until a microbial genomics- based approach to rapidly design, manufacture and deploy matched vaccines for

any emerging virulent strain is available, generation of new vaccines that incorporate immunization against highly conserved flu antigens is desirable and expected to have a highly beneficial effect. That involves, however, a paradigm shift - ie changing the vaccination vectors - since the conventional inactivated vaccine is unable to achieve this aim. The advantage of such new vaccines, if based on a combinatorial approach (eg. encompassing a seasonal and a conserved element) would be protection against both seasonal flu and enabling control of emerging pandemics. Secondly, to complement therapeutic measures such as anti-virals (Oseltamivir/Tamiflu<sup>®</sup>), to be utilized in areas where virulent strains emerge and in order to prevent rapid spread of virus, immune stimulators interfering with viral replication should be investigated and developed.

The overall picture is that despite the globalization and increased exposure to zoonotic agents resulting in a higher likelihood of emergence of new and virulent influenza virus strains, the mankind benefits now from approaches to tackle and develop increasingly more effective means against the threat of flu pandemic.

## **REFERENCES**

1. Palese P. Influenza: old and new threats. *Nat Med.* 2004;10(12):S82-7.
2. Thomas PG, Keating R, Hulse-Post DJ, et al. Cell-mediated protection in influenza infection. *Emerg Infect Dis.* 2006;12(1):48-54.
3. Mikulasova A, Vareckova E, Fodor E. Transcription and replication of the influenza A virus genome. *Acta Virol.* 2000;44(5):273-82.
4. Fiers W, De Filette M, Birkett A, et al. A "universal" human influenza A vaccine. *Virus Res.* 2004;103(1-2):173-6.
5. Neumann G, Brownlee GG, Fodor E, et al. Orthomyxovirus replication, transcription, and polyadenylation. *Curr Top Microbiol Immunol.* 2004;283:121-43.
6. Hay AJ, Gregory V, Douglas AR, et al. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci.* 2001;356(1416):1861-70.
7. Webster RG, Hulse DJ. Microbial adaptation and change: avian influenza. *Rev Sci Tech.* 2004;23(2):453-65.
8. Wells MA, Albrecht P, Ennis FA. Recovery from a viral respiratory infection. I. Influenza pneumonia in normal and T-deficient mice. *J Immunol.* 1981;126(3):1036-41.
9. Bot A, Rodrigo E, Wolfe T, et al. Infection-triggered regulatory mechanisms override the role of STAT 4 in control of the immune response to influenza virus antigens. *J Virol.* 2003;77(10):5794-800.
10. Bot A, Holz A, Christen U, et al. Local IL-4 expression in the lung reduces pulmonary influenza-virus-specific secondary cytotoxic T cell responses. *Virology.* 2000;269(1):66-77.
11. Bot A, Smith KA, von Herrath M. Molecular and cellular control of T1/T2 immunity at the interface between antimicrobial defense and immune pathology. *DNA Cell Biol.* 2004;23(6):341-50.
12. Arvin AM, Greenberg HB. New viral vaccines. *Virology.* 2006; 344(1):240-9.
13. Montgomery DL, Shiver JW, Leander KR, et al. Heterologous and homologous protection against influenza A by DNA vaccination: Optimization of DNA vectors. *DNA Cell Biol.* 1993;12(9):777-83.
14. Bot A, Smith K, Liu X, et al. Potent Immunity achieved by targeted, sequential administration of recombinant DNA vectors and anchor modified epitope peptides. *J. Immunother.* 2005;6:653.

15. Bot A, Bot S, Bona C. Enhanced protection against influenza virus of mice immunized as newborns with a mixture of plasmids expressing hemagglutinin and nucleoprotein. *Vaccine*. 1998;16(17):1675-82.
16. Jefferson T, Demicheli V, Rivetti D, et al. Antivirals for influenza in healthy adults: systematic review. *Lancet*. 2006;367(9507):303-13.
17. Wareing MD, Tannock GA. Influenza update: vaccine development and clinical trials. *Curr Opin Pulm Med*. 2002;8(3):209-13.
18. Bot A, Shearer M, Bot S, et al. Induction of immunological memory in baboons primed with DNA vaccine as neonates. *Vaccine*. 2001;19(15-16):1960-7.
19. Wang L, Smith D, Bot S, et al. Noncoding RNA danger motifs bridge innate and adaptive immunity and are potent adjuvants for vaccination. *J Clin Invest*. 2002;110(8):1175-84.