Polish Vaccine Consortium — a new national player in the influenza research

The Editorial Board of *Acta Biochimica Polonica* decided to dedicate a special issue of the Journal to the influenza virus and influenza research. At first look, such decision may seem surprising. Influenza is not the main life-threatening disease in Poland at the moment. The majority of deaths in our country is attributed to cardio-vascular disorders and a growing threat to public health remains cancer. Taking into account contagious diseases, influenza — an illness in most cases followed by mild symptoms, does not seem to be as dangerous as numerous clinical infections with Streptococci or Pseudomonas sp. Spread of drug-resistant tuberculosis is also a matter of concern. With ageing population, neurological disorders (e.g. Alzheimer disease) constitute a serious burden. However, influenza is worth of attention. Efforts to get Influenza Virus (IV) spread under control constitute a model for understanding the delicate equilibrium between the public health care system and the constantly menacing pathogen. Disrupting such equilibrium may result in disastrous consequences. There are epidemiological examples of IV inducing lethal pandemics. The best known is the spread of the "Spanish" flu ravaging around 1918, responsible for deaths of about 50–100 millions of people. The outbreaks of pandemics with serious consequences are recurrent, with bursts in 1957, 1968, 1977, and recently in 2009. Today IV virus is still the most important cause of respiratory tract infections. The control of virus spread is therefore a constant matter of concern of public health system[1]. This asks for efficient monitoring of IV infections, stockpiling of appropriate drugs and, most important, for implementation of effective vaccination programs. The system that prevents the spread of disease exists, but, unfortunately, has many gaps. The evolving virus quickly escapes from modes of control implemented by novel and seemingly promising drugs[2]. Monitoring of IV is tedious and, due to technical reasons, in many cases a reliable virus identification (by PCR and sequencing) is done for only a marginal number of clinical samples[3]. But the main problem of the system is the inefficiency of the vaccination campaigns.

The main antigen inducing neutralizing antibodies is well defined; it is viral HA (hemagglutinin) polypeptide. This virus has been studied in depth[4] and since 1948 relatively efficient vaccines are available. Such standard vaccines are composed of a neutralized virus grown in embryonated hen eggs. Due to the high genetic instability of the virus, leading to constant appearance of novel strains[5], the vaccine strain has to be constantly adapted to incoming pathogen versions. Therefore WHO, monitoring the appearance of novel IV varieties, indicates each year the proper vaccine strains. Identification of the current incoming strain may be sometimes cumbersome. Massive production of the vaccine is time consuming and is always under a constant threat of misidentification of the vaccine strain. As a result, an effective vaccine often becomes available well after the outburst of an epidemic. Within the current seasonal vaccine production system epidemiologists and industry are under the threat to be outpaced by the spread of novel unexpected pathogen variants. Such consideration urges virologists towards defining a universal vaccine, equally efficient against the whole spectrum of IV strains. These efforts, seriously advanced, are taking advantage of a recent demonstration of the existence of specific Mab's directed against the conserved HA region[6]. Still, the "universal" vaccine is not at hand[7]. Therefore, many research groups are focused on development of subunit vaccines using a variety of production platforms and approaches to enhance vaccine immunogenicity[8].

Nevertheless, one has to stress that availability of efficient vaccine by itself does not guarantee elimination of the disease spread. To eliminate the risk of a major outbreak, the majority of target population (minimum 80%) has to be immunized. Vaccination against IV is not obligatory, and a series of social factors in many countries, including Poland, hinder vaccination campaigns. In Poland, only 5% of population is vaccinated each year against seasonal influenza; the disease is often regarded as a trivial one, the costs of imported vaccines are covered by patients themselves and, last but not least, the growing anti-vaccination movement plays a negative role. For all of these reasons, seasonal influenza spreads each year rather widely, with around 3–5milions patients infected, many hospitalized, and 15–30 deceased. Rough estimation suggests that seasonal influenza is responsible for reduction of the national income by 250–500 mln € per year. This very cautious calculation takes into account the average cost incurred by national budget resulting from leave-of-absence of influenza-attained patients. Co-lateral costs of medical consultations, hospitalization and drugs are not included. This may easily add additional 30% to the calculated costs of influenza epidemics. Trying to evaluate the economic significance of the disease, one has to stress that in 5–10% of cases influenza is followed by serious complications, including bacterial pulmonary inflammation and sometimes neurological disorders. In such cases, IV infection becomes a factor often inducing rather severe disease. Such complications results in additional economic burden on the health care system, most probably doubling the calculated rough cost of seasonal epidemics. As the price of a single dose of seasonal vaccine in Polish pharmacies varies from 5 to 8 €, the cost of effective vaccination (over 80% population) will hover around 25 mln € per year. It may however turn out that even will full social acceptance, the rational level of vaccination may be hard to attain. During 2009 swine influenza panic it turned out that the world capacity of vaccine production is simply insufficient and customers willing to buy the vaccine were facing a waiting time approaching 2 years. This means that in this case, the effectiveness of a vaccination campaign would be rather dubious, being implemented well after the peak of an epidemic.

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[1]Woźniak-Kosek *et al.,* 2014. Detection of the influenza virus yesterday and now. *Acta Biochim Polon* **61**: 465–470; Brydak & Nitsch-Osuch, 2014. Vaccination against influenza in pregnant women. *Acta Biochim Polon* **61**: 505–508; Pikuła *et al.,* 2014. Active surveillance in poultry in Poland for avian influenza subtypes H5 and H7. *Acta Biochim Polon* **61**: 459–463.

[2]Król *et al.,* 2014. Antivirals — current trends in fighting influenza. *Acta Biochim Polon* **61**: 495–504; Kocik *et al.,* 2014. Antiviral activity of novel oseltamivir derivatives against some influenza virus strains. *Acta Biochim Polon* **61**: 509–513; Nitsch-Osuch & Brydak, 2014. Influenza viruses resistant to neuraminidase inhibitors. *Acta Biochim Polon* **61**: 505–508.

[3]Miarka *et al.,* 2014. A clinical utility of a strip test for influenza A/B and comparison with detection by RT PCR. *Acta Biochim Polon* **61**: 485–487; Kocik *et al.,* 2014. Diversity of influenza-like illness etiology in Polish Armed Forces in influenza epidemic season. *Acta Biochim Polon* **61**: 489–494; Pajak & Lepek, 2014. Native nucleic acid electrophoresis as an efficient alternative for genotyping method of influenza virus. *Acta Biochim Polon* **61**: 479–483.

[4]Szewczyk *et al.,* 2014. Introduction to molecular biology of influenza A virus. *Acta Biochim Polon* **61**: 397–401; Worch, 2014. Structural biology of the influenza virus fusion peptide. *Acta Biochim Polon* **61**: 421–426.

[5]Urbaniak & Markowska-Daniel, 2014. *In vivo* reassortment of influenza viruses. *Acta Biochim Polon* **61**: 427–431.

[6]Uranowska *et al.,* 2014. Hemagglutinin stalk domain from H5N1 strain as a potentially universal antigen. *Acta Biochim Polon* **61**: 541–550; Kalenik *et al.,* 2014. Influenza prevention and treatment by passive immunization. *Acta Biochim Polon* **61**: 573–587.

[7]Kęsik-Brodecka *et al.,* A universal flu vaccine. *Acta Biochim Polon* **61**: 523–530.

[8]Redkiewicz *et al.,* 2014. Plant expression systems for production of hemagglutinin as a vaccine against influenza virus. *Acta Biochim Polon* **61**: 551–560; Saczynska, 2014. Influenza virus hemagglutinin as a vaccine antigen produced in bacteria. *Acta Biochim Polon* **61**: 561–572; Chroboczek *et al.,* 2014. Virus-like particles as vaccine. *Acta Biochim Polon* **61**: 531–539.

Polish virologists learned a lesson from these developments. It became evident that taming a serious incoming influenza epidemic would require an appropriate mobilization to reinforce the existing system. To this aim, the Polish Vaccine Consortium was formally called into existence in 2010. The Consortium, coordinated by the Institute of Antibiotics and Biotechnology (Director — Dr. Piotr Borowicz) includes teams from the Institute of Biochemistry and Biophysics PAS, the University of Gdansk and the Institute of Animal Reproduction and Food Research PAS (Olsztyn). It also established collaboration with several leading national virologist centers in Poland, such as the National Veterinary Research Institute (PIWet) and the Military Institute of Hygiene and Epidemiology (WIHE), and with the SME Kucharczyk Ltd.

Influenza is believed to originate as a bird disease, and thus may be considered a typical zoonose circulating among wild fowl, infecting domesticated birds, pigs, horses, dogs and other mammals[9]. IV may be regarded as a potentially emerging virus that crossed the species barrier from birds to humans just around 10 000 years ago. The species adaptation is not absolute; some variants of the bird or pig virus may infect humans and vice-versa, the domesticated animals may carry the "human" type virus. The virus evolution in species-specific reservoirs is driven by adaptation to the host genome. The host's genome obviously imposes specific constrains on the variability of IV proteins. One of such well analyzed constrains resides in the species specificity of IV hemagglutinin receptors. On the surface of avian respiratory tract epithelium IV recognizes the sialic acid receptors with neuraminic acid covalently attached to carbohydrate 3 sugar by a 2,3 linkage. In mammals, the receptors differ, showing affinity for 2–6 linkage. This receptor difference in principle protects mammals from infection by the "bird" type viruses. Quite recently the two most debated publications showed that only four or five point mutations in the HA gene are sufficient to transform the "bird" type HA into one recognizing mammalian receptors[10]. Such artificial IV variants were able to spread in mammals through a respiratory route. Two of these crucial experimental mutations were novel; two others were detected in HA gene circulating in birds. The debate on such gain-of-function flu research was again stirred in June 2014 with publication from the Kawaoka's group reporting engineering de novo of IV strain analogous to the Spanish flu pathogen[11]. Such experiments are evidently calling for the risk-benefit assessment. In this context one should remember that IV propagates as a pseudo-strain, composed of a plethora of variants. It seems possible that in a dense bird population infected with cryptic IV version, a variant adapted to human type receptors may spontaneously arise. Examples of such dense bird populations, and possible incubators of virulent IV variants, are obviously industrial bird farms. In Poland, which produces over 500 mln broilers yearly, an average chicken farm hosts 20–40 000 birds during growth period of around 6 weeks. In USA, farms hosting up to 1 mln of broilers prevail. In case of latent IV infection of such a dense host population, probability of an HA gene version arising that is able to complement the human type virus seems to be a realistic threat. Indeed, gene archaeology suggests that "Spanish flu" was initiated by genetic shift introducing a modified "bird" version into the human IV reservoir. The HA receptor barrier is not absolute; in human upper respiratory tract epithelium, 2–6 type receptors prevail but in lower parts (trachea, pulmonary vesicles) the receptors are of a mixed type. Arising polymorphism in genes coding for receptors may be expected and could be followed by individual differences in affinity to foreign species of HA versions[12].

In summary, spread of a novel version of IV from bird to human virus reservoir is a probable scenario. This reinforces the understanding of the flu as a zoonose that might constantly create novel human epidemics. Clearly, for such a zoonose, the health of humans is related to health of industrial animals. Control of flu disease therefore demands the "One Health" approach, integrating human medicine and veterinary surveillance. This reasoning led PVC to concentrate first on constructions of vaccine against the bird flu of H5N1 type. The aim of PVC is to create a preventive system against the spread of novel, bird-derived IV versions into humans. This also implies a sensible monitoring of fowl and industrial birds for IV and when needed, efficient productions of appropriate vaccine versions for animals as well as humans. The fast and user-friendly monitoring proposed by PVC would be assured by a "laboratory on a chip" approach based on appropriate geno- and immuno-sensors[13]. For immunization, we opted for subunit vaccines represented by HA antigens overexpressed in baculovirus, *Escherichia coli* and *Pichia pastoris* systems. This approach eliminates the costly, time demanding and biologically unsafe standard production of IV vaccine strains in embryonated eggs. To select appropriate version of the antigen (or to assess the validity of subunit vaccine systems), leading immunity evaluations were done with DNA vaccines carrying appropriate constructs of the HA gene[14]. The selected DNA vaccine induced full immunity in chicken challenged with the homologous IV. The same construct was transformed into *Lactobacillus lactis* to create an oral vaccine[15]. When oral vaccine was applied to chickens, success was limited. Experiments with mammals are in progress. The HA constructs were over-expressed in both baculovirus and *E. coli* systems, producing highly immunogenic HA versions. Studies on the potency of a vaccine produced in *P. pastoris* are in progress[16]. In case of *E.coli,* the product positively passed the challenge experiments. *E. coli*-derived product was purified with yield of an industrial scale interest (8 000–16 000 injection from 1 liter of culture, G. Pluciennicza k, personal communication). In summary, right now the PVC has on hand the sensors as well as subunit vaccines, until now on the laboratory scale, but with yields that are of interest for industry.

The strategy proposed by PVC for fighting virulent flu, based on variety of bioinformatic approaches, e.g. ISSCOR[17] and structure modeling, will hopefully result in preparedness for the instant massive production of the strain-specific vaccines, in case of an urgent need. This aim is attainable with subunit vaccine technology. A subunit vaccine that is certified against bird influenza can be easily adapted to become a human vaccine. In perspective, a vaccination of high-risk groups may be proposed to reinforce the IV species barrier. The proposed system of IV detection and subunit vaccine development is flexible, allowing for easy generation of a spectrum of influenza vaccines representing appropriate HA antigens derived from virulent IV strains. Of note, at the moment the PVC does not propose massive immunization of broilers. Economically sensitive may be a preventive vaccination of the genetic reserve, or the laying hens and chickens in the quarantine zones in case of infection burst, especially since the proposed approach meets the DIVA requirements. Further PVC studies on expressions of rational selection of vaccines are in progress. We are not

[9]Urbaniak *et al.,* 2014. Influenza A viruses of avian origin circulating in pigs and other mammals. *Acta Biochim Polon* **61**: 433–439; Smietanka & Minta, 2014. Avian influenza in Poland. *Acta Biochim Polon* **61**: 453–457.
[10]Herfest *et al.,* 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* **336**: 1534–1541; Imai *et al.,* 2012. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* **486**: 420–430.
[11]Watanabe *et al.,* 2014. Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. *Cell Host & Microbe* **15**: 692–705.
[12]Arcanjo *et al.,* 2014. Role of the host genetic variability in the influenza A virus susceptibility. *Acta Biochim Polon* **61**: 403–419.
[13]Grabowska *et al.,* 2014. Electrochemical biosensors for detection of avian influenza virus — current status and future trends. *Acta Biochim Polon* **61**: 471–478.
[14]Stachyra *et al.,* 2014. DNA vaccines against influenza. *Acta Biochim Polon* **61**: 515–522; Stachyra *et al.,* 2014. Antibody response to DNA vaccine against H5N1 in broilers immunized according to three schedule. *Acta Biochim Polon* **61**: 593–596.
[15]Radziwill-Bienkowska *et al.,* 2014. *Lactococcus lactis* IBB477 presenting adhesive and muco-adhesive properties as a candidate carrier strain for oral vaccination against influenza virus. *Acta Biochim Polon* **61**: 603–607; Szatraj *et al.,* 2014. Expression of avian influenza haemagglutinin (H5) and chicken interleukin 2 (chIL-2) under control of the *ptcB* promoter in *Lactococcus lactis. Acta Biochim Polon* **61**: 609–614.
[16]Kopera *et al.,* 2014. Expression, purification and characterisation of glycosylated influenza H5N1 hemagglutinin produced in *Pichia pastoris*. *Acta Biochim Polon* **61**: 597–602.
[17]Radomski *et al.,* 2014. Mapping of the influenza-A hemagglutinin serotypes evolution by the ISSCOR method. *Acta Biochim Polon* **61**: 441–451.

overlooking the potential of therapeutic mAbs production, testing the library for finding the ones with broad cross-immunity and for the presence of the cross-reactive mAbs against a sensible set of IV.

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