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REVIEW

Vaccines from the Spanish Influenza as a firm foundation for new developments

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ABSTRACT

In 1914, the concept of a prophylactic vaccine, administered to a person before the disease had been contracted, was still controversial. Nevertheless, Almroth Wright tested new pneumococcus vaccines in South Africa, where the incidence of bacterial pneumonia was high amongst workers in the gold mines. He established the use of clinical trials, using around ten thousand workers, both in vaccinated and unvaccinated groups. The two groups were not matched to modern standards. Also, of course, those workers in the control unvaccinated group could not be protected: but some considered a prophylactic vaccine would exacerbate the disease. The vaccines were manufactured to contain a range of pneumococci from different clinical samples, in a serious attempt to match the microbes in the vaccine to the field bacteria. Deaths were averted by the vaccine; and side effects were noted to be minimal. Reexamination of pathology samples from the Spanish Influenza Pandemic showed quite clearly the contribution of pneumococci and streptococci to the mortality of over fifty million people in 1918–1919. The microbe causing this Pandemic was isolated in 1933, and was shown to be a true virus; this finding initiated a huge expanse and interest in influenza virus vaccines, both killed and live. A chance discovery allowed the purification of Influenza M and NP proteins then permitted the production of experimental vaccines. These vaccines were formulated to induce and B and/or T cell responses to the internal proteins. Several of these Universal Influenza Vaccines have been tested in quarantine, and have now reached Phase III trials in the community.

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At the close of the nineteenth century, no vaccine had yet been discovered or used against respiratory disease caused by viruses or bacteria. In fact, only five human vaccines had so far been introduced: those against smallpox (first used in 1798), rabies (1885), cholera (1896), typhoid (1896), and plague (1897).[1]

As an example of the blank sheet which existed in the field of respiratory disease, there was as yet, in the minds of the specialist physicians, no thought of applying vaccines as a means of prophylaxis. A standard text book on influenza, published in 1890, is silent on this point.[2] Indeed, it was not until the time of Almroth Wright, whose 'mass-experiments' were impelled by the great losses occasioned by a pneumonia amongst the workforce extracting gold ore on the Rand, that this branch of medical science can be said to have been launched.[3]

During the time of the Spanish Influenza Pandemic, from 1917 until the early 1920s,[4] bacteriologists reported that secondary infections, caused by pneumococcus, staphylococcus, and streptococcus, caused at least as many deaths as the causative microbe itself. At that point the virus had not been identified. A modern reevaluation of pathology slides of lungs of victims of the Spanish Influenza, using molecular techniques, has confirmed the pathological changes brought about by the super-infecting bacteria.[5,6]

A proposal for a combined bacterial/viral vaccine

We propose, therefore, that an entirely novel and modern vaccine composed of the same capsule carbohydrates of pneumococcus bacteria, or at least proteins (a conjugate vaccine) from the bacterial cell, could be called a 'quasi-universal influenza vaccine'; and could be formulated to contain HA and NA as a unique bacteriological/virus vaccine. In essence, such a novel vaccine could also be formulated using internally situated influenza M and NP proteins or peptides: and this could offer, at least in theory, a degree of cross protection against all influenza A viruses. The term 'Universal Influenza Vaccine' has recently come into use, in the face of potential pandemics of influenza caused by emerging Swine and Avian Influenza A viruses. At present, the formulation of the yearly epidemic influenza vaccine occupies many months. Accordingly, a pre-made bacteriological/influenza virus vaccine, as noted above, would be expected to provide significant protection against superinfection by these bacteria during the initial stages of an epidemic or pandemic.

The first prophylactic vaccines against pneumococcal pneumonia were developed in South Africa several years before the Great War

In his pioneering work conducted in the mines on the South African Rand, Sir Almroth Wright set up a programme designed to halt the depredations of pneumonia amongst the laborers whom the owners had engaged.[7] We would note here that this researcher became well-known in later years when Alexander Fleming, working in Wright's laboratory in St Mary's Hospital, London, discovered penicillin.[8] The purpose

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of the work conducted on the Rand was, in essence, economic. The mine-owners were recruiting laborers from within the Union of South Africa, and from areas closer to the tropics; but the toll of pneumonia, in terms both of sickness and of death, was high. These human losses involved the owners in considerable expense.

Wright summed up the measures which might be undertaken to reduce the impact of pneumonia amongst the labors involved: better hygiene and accommodation; earlier efforts at detection; and the quarantining of the sick. He rapidly concluded: "the place of such measures in a plan of campaign against pneumonia is at best a very subordinate one". The real impediment, he writes, is "that abiding defect of resisting power which is normal" to those recruited for the mines; and this one fact alone "dictates recourse to inoculation".[7]

Acting on this imperative, Wright conducted a total of six large-scale 'reconnoitring experiments' in inoculation.[7] They were prophylactic in their nature. Some were carried out on 'tropical' laborers, for "it is especially these who are ravaged by pneumonia". Others embraced a complete cross section of the recruits, including men from the East Coast of the country, men, "as we are informed, much more difficult to handle and at the same time more resistant to pneumonia". Each experiment involved injecting several thousand men with a given dose of vaccine, while retaining a similar number as a control group, who received no treatment whatsoever. And Wright, in his reference to 'vaccine', regards himself as entering new ground. He and his collaborators created "a very copious culture of pneumococcus"; raised this culture to a temperature of 55 degrees; and then added a small quantity of carbolic acid, to kill the pneumococcus. Of course, no-one appreciated at that time that there were multiple serotypes of pneumocci. Again, as to dosage, Wright had no preconceptions as to what he would and would not do. A dose might be 'minimal'; or it could be 100,000 times that size. We can only ask, in retrospect, whether these high doses were toxic. In different experiments, the doses varied both as to the quantity of units employed, and, in the case of a course of vaccinations, as to the time interval which elapsed between one vaccination and the next.

Whatever the volume of each dose, whatever the reliance upon one inoculation or upon a series, and whatever the naivete or otherwise, immunologically speaking, of the laborers involved, Wright judged that, overall, his results "testify … in every case to a reduction in the incidence-rate and death-rate of pneumonia in the inoculated". In fact, the immunity detectable at one month was not long-lasting. In a group of 10,626 vaccinees, and 10,508 in the control group, there were 125 and 216 cases of pneumonia respectively.

Further development work with a pneumococcal vaccine in the RAMC laboratories on the Western Front

The movement of British laboratories and their scientific infrastructure, from the homeland and across to France and Flanders, took place at the start of the Great War. It was conducted by the Royal Army Medical Corps. (The full record is to be found in the records of the Corps, which are now stored in The National Archives at Kew.) This transfer, combined with recognition that an outbreak of respiratory disease could decimate the armies, led to scientific developments in vaccine formulation and production.

In the case of the United Kingdom, the government ultimately sent half of the country's medical infrastructure abroad: thereby equipping and maintaining some seventy-five hospitals to the rear of the front line. About two hundred thousand beds could be made available, at times of greatest need, for both surgical and medical casualties. Scores of laboratories, both stationary and mobile, provided a scientific back-up, particularly for cultivating bacteria. The RAMC sought to identify infections of every description from basic pathology, and to combat these both by prophylactic measures, and by developing vaccines against the various diseases. Sir Almroth Wright proved to be a leading figure in this task. He headed an important bacteriological research laboratory in Boulogne-sur-Mer, on the north-west coast of France. In 1915, thirty-six thousand cases of influenza were reported, and a further thirty thousand in 1917: a small taste of what would come during the subsequent Pandemic.

By 1917, the pathology laboratories in France, the United Kingdom, and Australia, had formulated bacterial vaccines, for use in the British Expeditionary Force.[4]

Production of two million doses of pneumococcus vaccine in Melbourne in 1918

In 1918, WJ Penfold used a range of bacteria as a basis for an anti-influenza vaccine.[9] The theory underpinning his formulation of the vaccine is remarkably similar to the methodology of influenza virus vaccine formulated today. Both then and now, the chief aim is to match the microbe and the vaccine as precisely as possible. He describes the sources of pneumococcus from the quarantine stations in Sydney and in Melbourne hospitals. A few samples came from military laboratories, and the range took in pneumococci, diplococci, Gram positive diplococci, haemophilus influenzae. Certain of the samples were from pneumococcus cases which were taken at post mortem.

The vaccine itself was formulated to contain

- *H. influenzae* 25 millions per centilitre
- *M. catarrhalis* 25 " " "
- pneumococcus 10 " " "
- staphylococcus 10 " " "
- diplococcus 10 " " "

The organisms were collected directly from flasks and passed through 'breaking up' bottles, and then into a sterilizing bottle. Finally, the different harvests were added to eighteen-liter bottles of sterilized and tricresolised saline. This chemical had been found to be superior for retention of the properties of an immunogen, as compared to heat treatment. Three million doses of vaccine were made between October 1918 and March 1919. The authors presented data on vaccine prophylaxis amongst the staff of the Railway Authority, in Victoria, Australia, showing that the vaccine diminished pneumonias in the ratio of 8: 5. An analysis of 3,831 cases showed that the case

mortality was one-third in the vaccinated group compared to the levels seen in those who had been given the placebo.

But pre-occupation with war *per se*, with the terms of the peace, and with the collapse of the dynasties of Central Europe, diverted political will away from public health strategies.

The modern era of formulation of inactivated Influenza virus vaccines or live attenuated counterparts

Following the discovery that influenza was caused by a true virus,[10] rather than a bacterium such as *H. Influenzae*, scientific groups around the world began to formulate virus vaccines. In the USSR, a group in Leningrad headed by A. Smorodintsev explored the use of live attenuated virus, by passage at low temperatures to select mutants.[11] These viruses had mutations in most genes, and from a safety point of view, replicated preferentially in the upper airways of the respiratory tract. The group started a forty-year clinical programme of clinical studies; and the vaccine is now formulated and manufactured in collaboration with pharma groups in India. A parallel group in Michigan, in the United States, headed by HF Massab, also began to work on the live virus, weakened by cold adaptation; and this formulation of ca and ts mutations scattered across all eight genes, was clinically tested in the United States.[12] The resulting vaccine is marketed in the United Kingdom and the United States as Flumist. TA Reichert, a vaccinologist with a mathematical bent, noted that the Japanese killed-vaccine given to children in the 1980s also appeared to protect their grandparents from serious illness and death.[13] Nowadays the vaccine is used to protect both children and their grandparents in the United States and the United Kingdom.

The 1960s was an important era for the development of new virus vaccines, against diseases such as measles, mumps, and rubella.[14] A few years earlier, two polio vaccines, one killed and one live attenuated, had been developed and used in clinical practice. Jonas Salk, who was the inventor of the formalin-inactivated polio vaccine, had used the same technology to kill influenza virus; and published early data to show the effectiveness of the vaccine in the armed forces. At that stage, the conclusion was that the protective effects of the killed influenza vaccine was caused by IgG immunity to the HA and NA surface proteins of the influenza virion, with a component of local IgA immunity.

A chance discovery leads to the concept of a Universal Influenza Vaccine

Studies in France and the United States in the 1960s, which were purely academic in their nature, showed that mice infected with a A/PR/8/34 H1N1 virus were later able to resist infection with a hundred lethal doses of a competely different virus sub-type such as Influenza A (H3N2).[15,16] A conclusion was tentatively reached: to the effect that protection was mediated not by HA or NA, but by the internal proteins N and NP, which are shared by all influenza A viruses. This single discovery initiated a chain of investigations, many of which are being followed up today. These studies were initiated in ferrets and mice immunized with purified N and MP proteins. There are now at least fourteen experimental Universal Influenza Vaccines, formulated with different adjuvants, different physical presentations, and peptides of NP or M or HA stem or NA. However, the ensuing experiments in animal models, using these internal structural proteins, showed that they were less effective than vaccines containing HA. (Oxford JS, unpublished data.)

This early work depended on a simple method discovered by WG Laver in Canberra using cellulose acetate strips.[17] Purified and detergent-disrupted influenza was streaked into the middle of the strip, and an electric current applied. Chance determined the direction of movement of the HA, NA, M, or NP proteins, and therefore a virus with a good separation of M and NP enabled these proteins to elute in pure form. The M and NP could then be used as a single imunogen in a vaccine. Our own experiments showed a poor immune response to these proteins;[18,19] but later reformulation,[20] and the discovery of a small protein called M2e, produced the first positive results of what would later be described as a Universal Influenza Vaccine.[21]

Two experimental Universal Influenza Vaccines have been tested in a quarantine unit using a live virus challege

We have used a quarantine unit in London (hVivo/Open Orphan) to test two experimental vaccines against influenza. One vaccine candidate was given to sixteen volunteers; twenty-one days later, vaccinated and placebo volunteers were challenged with Influenza A/Wisconsin/67/2005.[22] Nineteen days post-vaccination, the vaccine group, but not the placebo group, developed specific IFN gamma responses, eight-fold higher as compared to the control. Volunteers with such specific cellular responses also had reductions in virus titers and symptom scores. It was concluded that high levels of cellular immunity to influenza, in the absence of an anti-body response, can mediate reductions in influenza virus shedding and symptom scores. This experimental vaccine has now reached clinical testing at Stage 2 in the community.

The same quarantine unit was used to test a second experimental universal vaccine. Lillie et al. immunized volunteers with a vaccine based on an attenuated pox virus.[23] These volunteers were infected in quarantine with Influenza A/Wisconsin/2055(H3N2). Two of the eleven vaccinees, and five of the eleven control volunteers developed laboratory confirmed influenza (symptoms plus virus shedding). There was also a significant reduction in the number of days of virus shedding in the vaccinated group.

Neither of these two vaccines has yet reached Phase 3 testing in the community. However, a peptide vaccine developed by Biondvax is being tested on twelve thousand participants in Eastern Europe; the results are yet to be published.

Discussion

At the time of the initial clinical trials with pneumococcus vaccine, as conducted by Wright on the South African Rand, the concept of a prophylactic vaccine was entirely new. A concern was raised that such a vaccine could exacerbate levels of infection. Indeed, forty years later, an experimental Respiratory Syncytial Vaccine seriously exacerbated infection

in children immunized and naturally infected with the virus.[14] Nevertheless, Wright proceeded with his clinical trials, despite the organizational problems arising from the fluid nature of the labor force, and despite the lack of backing from the wider scientific community. It is a measure of contemporary indifference that the many pages occupied by his findings, in successive editions of *The Lancet*, failed to excite any real response from the scientific and medical community. This outcome may well have been dispiriting for him and his collaborators. Today, his activities seem unusually far-seeing, in that the numbers which Wright used in the trials, up to ten thousand at a time, are those commonly employed today, in investigating the field efficacy of current influenza vaccines.

Today's control groups are carefully matched for age, pre-existing health; something which was far more difficult for Wright to carry through. Later studies of pneumococcus vaccines, during the Spanish Influenza Pandemic, were able to more closely match vaccinated and non-vaccinated volunteers. For example, studies of the vaccine in the New Zealand armed forces, by Eyre et al.,[24] ensured that the volunteers were matched for age, general health, and freedom from TB. In these experiments, 16,104 soldiers were immunized, and the mortality in severe and complicated cases was 8%, compared to 23% in the 5,700 uninoculated men.

In the course of sixty years, the development of live attenuated influenza vaccines is seen as rather slow. The concern has been whether ts or ca mutants would revert to wild type and cause disease. For example, this problem of reversion to pathogenic, wild-type virus has been noted with live attenuated polio vaccines. Therefore the present use of live influenza vaccines is closely monitored to detect the emergence of unwanted mutants.

Of course, the important question is whether vaccinologists can sidestep the rapid yearly antigenic change of influenza HA and NA, and develop a T or B cell vaccine which would protect against all forms of the disease. Such a vaccine might operate against the shared internally situated M and NP proteins, or even against the stalk of HA, which is less prone to mutate.

Are these new vaccines as efficacious as typical sub-unit HA/NA vaccines? At present, we have too little data from the field trials to give a coherent answer this question. Overall, however, the experimental quarantine data shows these newly-developed vaccines to be less effective than one would expect a typical HA/NA vaccine to be. Meanwhile, two such vaccines are being tested in field trials in the European Union amongst at-risk persons immunized with licensed vaccines: the aim being to observe whether increased efficacy can be detected with these adjuncts.

There is an underlying scientific argument that a live attenuated virus vaccine may exert a broader protective effect. Analysis of quarantine volunteers, to compare attack rates and clinical disease rates, in persons with high levels of T-cell reactivity to M and NP peptides, have shown that they have lower virology attack rates, and reduced clinical titers, when infected with influenza.

But to return to the pivotal animal data, which we noted above,[15,16] it is possible that mice recognize an epitope in influenza which is obscured to the human immune response. Alternatively, it is possible that, given the high attack rates of influenza among adults, many have acquired an immunity which protects them against a new pandemic strain. Without this acquired level of protection, a newly emerging virus might prove very much more fatal.

We have attempted to re-analyze the data laid down by our predecessors – men and women who called themselves pathologists, rather than scientists, immunologists, or bacteriologists. The level of their dedication is not to be gainsaid. Dr A. Graeme Gibson died of influenza whilst working on a filter-passing virus in an army laboratory in 1918. Equally, Dr Alexander Fleming toiled as an army pathologist, throughout the Great War, long before he discovered penicillin. They, and their colleagues, worked within earshot of the guns; and in the spring of 1918, many were forced to flee under fire. Nonetheless, the research work proceeded, and a sense of duty passed on. The team at St Bartholomew's Hospital in London, who, at long last, identified the influenza virus, had been schooled and trained by workers such as these.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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