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# CORRESPONDENCE

# The γ-irradiated influenza vaccine and the prospect of producing safe vaccines in general

Vaccination is the most important control and protection strategy against pathogenic infections. However, despite our advanced knowledge in immunology, host–pathogen interactions and pathogen biology, formulating effective vaccines against many mutating viruses such as influenza continues to be a challenging task. The World Health Organization (WHO) has set up a comprehensive surveillance network, 'The WHO Global Influenza Surveillance Network', that involves 125 institutions from 96 countries and 4 collaborating centres. This massive surveillance network enables WHO to propose formulations of influenza vaccines twice annually for the subsequent influenza season and advise pharmaceutical companies of the composition of the new vaccine. In fact, such vaccines are based on the expectation that current influenza strains will still be circulating among people at the time when the vaccine becomes available for use 6 months later. Despite the relative effectiveness of this approach at protecting against closely related influenza viruses, the expected virus mutations and the associated appearance of new strains may reduce the protective efficacy of the vaccines. For example, WHO recommended the use of an A/Wisconsin/67/2005 (H3N2)-like virus as part of the trivalent inactivated vaccine for the northern hemisphere flu season in 2007–2008.[1] According to the Center for Disease Control and Prevention, Atlanta, 60% of the H3N2 influenza infections in the United States during the flu season of 2007–2008 were caused by an A/Brisbane/10/2007 (H3N2)-like virus that evolved from the A/Wisconsin/67/2005 (H3N2)-like virus and that turned out to be an antigenically distinct virus.[2] Considering the continuous antigenic variability as a consequence of virus mutations, the ability of the virus to cross species barriers and the time period (3–6 months) required to produce

closely matched vaccines, it is likely that new pandemic virus strains will arise without adequate vaccine stockpiles being available to protect large populations. Thus, we can definitively say that the currently available flu vaccines will not be sufficient nor effective enough to protect against the flu pandemics that will inevitably arise. The avian H5N1 and swine H1N1 viruses represent two recent and relevant examples. When the H5N1 virus crossed the species barrier and infected humans, the efforts of pharmaceutical companies were directed towards producing strain-matched antibody-inducing vaccines. However, vaccines based on the currently isolated H5N1 virus, a virus strain that binds to cell surface α2,3 sialic acid, will be ineffectual against H5 molecules that bind α2,6 sialic acid, the main molecular change required if human-to-human transmission is to occur and a fresh pandemic is to emerge. Thus, if such mutations did occur, the protective efficacy of currently available H5-based vaccines would be very limited. A period of 3–6 months would be needed to produce a vaccine with suitable protective potential. The rapid global spread of the influenza A (H1N1) strain, known as swine flu, in humans, has exposed all the limitations of the current available vaccines. Thus, there exists a great need for an alternative technology that can produce a more effective and cross-protective A/influenza vaccine.

Principally, vaccines capable of inducing both humoral as well as cellular immune responses are generally superior in providing protective immunity. Although the ability of antibodies to neutralize viruses is known to be affected by virus mutations, the cytotoxic T (Tc) cell response, directed against virus-infected cells, is broadly cross-reactive between influenza A strains and is important for the recovery from primary infections in combination with antibody responses.[3,4]

Furthermore, cross-recognition of avian H5N1 influenza virus-infected human target cells by human Tc-lymphocyte populations that are immune to human influenza A viruses has also been reported.[5] Accordingly, a universal influenza vaccine requires the capability of inducing cross-protective Tc cell responses. We have previously reported that γ-irradiated influenza A virus preparations do induce cross-reactive Tc cell responses against all influenza A strains,[6] and have discussed their potential for use as possible vaccine candidates to induce universal immunity for all present and arising influenza A viruses.[7] Our recent data, which show that a single intranasal administration of γ-irradiated A/PR8[H1N1] influenza virus protects mice against the lethal H5N1 virus and other heterotypic infections,[8] substantially underpins the proposition that γ-irradiated influenza vaccines may represent a universal flu vaccine product.

γ-Irradiation is arguably one of the most powerful means of sterilization and has widely been used to ensure the safety of a variety of biological products, including human tissue allografts,[9] pharmaceuticals[10] and food.[11] In addition, γ-rays have been widely used to inactivate highly dangerous infectious agents for safe handling and biochemical analysis, such as Ebola, Marburg and Lassa viruses.[12]

Virus inactivation by γ-irradiation follows physical laws, including the concept of an 'exponential low', which means that there always exists a finite probability that an organism may survive irrespective of the irradiation dose used. The value of such a probability is called the 'sterility assurance level' (SAL).[13] SAL is in general arbitrarily determined and a value of $10^{-6}$ (one in a million chance of having live micro-organisms) is applied for the sterilization of medical products. For vaccine preparations, an

application of the SAL concept requires close consideration of two related issues: (1) the virucidal effect of γ-rays and (2) the decimal reduction value (D10), which is the radiation dose required to decrease virus titres by 1 log. The virucidal effect of γ-rays is associated with damage to the viral nucleic acid molecular structure (for example, single- or double-strand breaks, cross-linkage breaks, nucleotide damage of viral genetic material, either RNA or DNA). This damage, which renders the virion non-infectious 'or killed', is directly proportional to their genome size and is governed by an inverse relationship with the radiation dose.[14,15] It has been shown that small viruses (core size of ≤20 nm), such as Picornaviridae (genome size ~7 kb) and Retroviridae (genome size 7–10 kb), have high D10 values in the order of 0.55 and 0.35 kGy, respectively.[16] In contrast, large viruses (core size of 75–150 nm), such as Paramyxoviridae (genome size 15 kb) and Bunyaviruses (genome size 10–22 kb), have lower D10 values in the order of <0.2 kGy. This makes it possible to determine the radiation dose required to achieve a SAL for any viral preparation by establishing the initial virus titre and the D10 value.

The influenza virus is a relatively large virus with a core size between 80–120 nm and a genome size of ~12 kb, consisting of a minimum of eight single negatively stranded RNA fragments. The Manual on Radiation Sterilization of Medical and Biological Material from the International Atomic Energy Agency states that exposure to 0.65 kGy of γ-rays results in a total loss of influenza virus infectivity, but destroying the haemagglutinating activity requires an exposure of higher than 200 kGy.[17] This indicates that SAL levels are achieved in the absence of viral protein denaturation and with retention of virion integrity, which would be expected to preserve the ability of the virus to induce T-cell responses in addition to the stimulation of excellent antiviral antibody responses.

We have formulated γ-ray-inactivated influenza vaccine preparations using a radiation dose of 10 kGy and shown that these preparations are potent in eliciting cross-protective immunity against heterotypic viral challenges in mice.[8] In addition, sterile H5N1 bird flu, generated by irradiation with 50 kGy, has been used to produce neutralizing monoclonal antibodies for diagnostic purposes and with possible future therapeutic use.[18]

As atomic decay and γ-ray emission is a constant process and is inalterable, there is little that can interfere with the effectiveness of its delivery. Irradiation is a highly reliable procedure to inactive viruses, with the advantage of minimal molecular changes to viral proteins and viral structure. Although our research shows that γ-ray inactivation of influenza virus holds great promise for the formulation of a cross-protective (universal) influenza vaccine, this method may also avail itself for a less expensive alternative to produce a number of currently used vaccines, such as poliomyelitis and papillomavirus preparations, of which the latter is used as a prophylactic vaccine against uterine carcinoma. In addition, the ability of γ-ray-inactivated influenza virus to induce both a strong humoral as well as a potent Tc cell response should encourage the evaluation of this technique in the search for a vaccine against HIV. Strategies for HIV vaccines at present are focused on eliciting strong Tc cell responses. With the proviso that viral structural proteins encode the immunodominant Tc cell determinant(s), it can be expected that an efficient Tc cell response, besides anti-HIV antibody, is elicited with γ-ray-inactivated HIV preparations.

Finally, γ-ray-inactivated pathogens may yet become useful in replacing present day vaccines that have a relatively high incidence of complications, as was observed with the very successfully used smallpox vaccine, vaccinia virus. This live-virus vaccine, during its use, had a high incidence of neurological complications, such as causing encephalitis, especially in immunologically compromised vaccinees. Trials with γ-irradiated vaccinia viruses undertaken by Russian scientists during the 1950s and 1960s were discontinued because of the eradication of smallpox. However, recent bio-terrorism concerns regarding the unlawful use of variola (smallpox) may necessitate renewed smallpox vaccination programmes to be undertaken. Under such circumstances, increased vaccine safety without loss of vaccine efficacy, which can be achieved by the use of γ-irradiation for vaccine sterilization, would be highly desirable.

Mohammed Alsharifi[1] and
Arno Müllbacher[2]

[1]Microbiology and Infectious Diseases, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia and [2]Viral Immunology, The John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia
E-mail: mohammed.alsharifi@imvs.sa.gov.au

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