Vaxcine™: an oil-based adjuvant for influenza vaccines

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Vaccination is the method of choice for the prevention of influenza infection. However, the quantity of the antigen available, especially in the case of pandemics, often fails to meet the global demand. However, improved adjuvants can overcome this problem. Preliminary results obtained in this study revealed that one year after a single subcutaneous immunisation with influenza A H3N2 virus in an oil-based carrier, Vaxcine™, outbreed mice produced a high immunoglobulin G response that lasted for up to one year and exhibited less variation in titre compared with the response of the control group treated with alum. The haemagglutination-inhibition titres induced by Vaxcine™ were also higher than those generated by alum. These data indicate that Vaxcine™ is a good adjuvant candidate for seasonal influenza vaccines.

Key words: Vaxcine™ - adjuvant - influenza - vaccine

The start, severity and length of the influenza season can all vary widely from year to year, with the demand for the vaccine changing each month. The availability of vaccines, however, does not always coincide with the peak influenza incidence in the population (CDC 2011). This situation is worsened in the case of pandemics, during which the availability falls short of the global need (Oliverwyman 2009). One way to surmount this problem is to increase the annual number of virus particles destined for vaccine production, which are mainly obtained by virus replication in chicken eggs. However, to scale up egg production in the case of pandemics would require months, especially considering the need for certified sources of eggs and the growth characteristics of some virus isolates (Hickling & D'Hondt 2006).

We have found that the use of adjuvants can overcome this problem by broadening the immune response to influenza vaccines using a reduced amount of antigen (Miyaki et al. 2010). A good demonstration that adjuvantation can significantly reduce the amount of antigen required is provided by the work of Baras et al. (2008), who have shown that intramuscular immunisation of ferrets with 3.8 µg of haemagglutination (HA) from inactivated split H5N1 virus in an oil-in-water emulsion-based adjuvant was able to protect the ferrets against a lethal challenge with a heterologous H5N1 virus; the amount of HA used in this experiment was much smaller than the amount required for normal vaccination (15 µg). The results obtained by Hamouda et al. (2011) also demonstrated that intranasal immunisation of ferrets with the virus antigen in a soybean oil-in-water nanoemulsion induced a HA inhibition titre approximately 90-fold higher than that resulting from the standard intramuscular nonadjuvanted commercial influenza vaccine dose. Despite the fact that some oil-in-water-based adjuvants in influenza vaccines can be well tolerated by humans (Leroux-Roels et al. 2007), alum is the adjuvant used predominantly in human vaccines; however, there is still the need to identify compounds with better adjuvant properties. As mentioned previously, one approach is the use of oil-based adjuvants, whose safety and efficacy have been improved and which have been tested in clinical trials for prophylactic vaccines. In the case of influenza, it has been demonstrated that the use of the oil-based vehicle Vaxcine™ as an oral adjuvant induces a protective immune response against a lethal pandemic H5N1 challenge in mice (Prabakaran et al. 2010). It has also been shown that Vaxcine™ can increase the immune response against other pathogens when administered via the parenteral route (Domingos et al. 2008). It should be noted that Vaxcine™ differs from all other water-in-oil based carriers in that the antigen is embedded stably in the oil inside reverse micelles instead of being admixed with the oil droplets in the bulk aqueous phase. In this way, the association of antigen and adjuvant is much more intimate and longer-lived after administration. In this work, the capacity of Vaxcine™ to generate a long-lived humoral immune-response against seasonal influenza A H3N2 virus in outbreed mice was compared with that of alum.

For the Vaxcine™ formulation, the immunostimulant dioctadecyldimethylammonium bromide (DDA) was included in a mineral oil/amphiphile phase at a level of 1%. After addition of 50 µL antigen solution to 1 mL of the oil, yielding a clear microemulsion, 50 µL of oil was administered per mouse. The efficacy of Vaxcine™

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as an adjuvant for influenza was tested in female Swiss mice (6-8 weeks old) provided by the Butantan Institute Animal House. The pathogenic influenza A human H3N2 virus isolate A/SP/2/95 used in this study is the A/Beijing 353-89 strain isolated from a confirmed H3N2 case in China. This study was approved by the Ethical Committee of Butantan Institute (protocol 428/07).

For the experiments, groups of five mice each were immunised subcutaneously either once with 5 or 15 µg of H3N2 virus particles or three times with 3 or 15 µg of virus in 0.2 mL of phosphate buffered saline (PBS) alone or in the presence of alum (1/25) or Vaxcine™. Blood samples were collected before and at different times after immunisation for antibody measurement by enzyme linked immunosorbent assay (ELISA). The HA titre was determined as previously described (Mancini et al. 2004). The serum neutralisation activity was measured using the HA-inhibition assay (HIA). The data were analysed by Bonferroni's test using SigmaStat 3.0 software and were expressed as the arithmetic mean ± standard error. Values with $p < 0.05$ were considered statistically significant.

The results obtained in this work demonstrate that the presence of DDA in the formulation is essential to induce a high immunoglobulin G (IgG) response against influenza (Fig. 1). It was also observed that mice that received a single subcutaneous immunisation with 5 µg of H3N2 virus particles incorporated in Vaxcine™ generated, four months after immunisation, IgG1 antibodies with titres that were significantly higher than those of animals immunised with 5 µg of virus absorbed onto alum and than those of the group that received 15 µg of virus in PBS as detected by ELISA (Fig. 2). In addition, the immune response induced by Vaxcine™ was homogeneous in all animals; the same was not observed in the groups immunised either with alum or with the higher amount of virus particles, as demonstrated by the error bars. One year after immunisation, the IgG1 levels generated by animals treated with the oil vehicle were still significantly higher than those observed in the other groups (Fig. 3). Considering that the immunisation of children younger than nine years old against influenza requires more than one dose because the immune response generated by children is inferior to the one generated by adults (CVE 2006), in this study, it was determined whether the immune response after two or three immunisations with Vaxcine™ would still be higher than that in the groups immunised with alum or a five-fold higher concentration of the antigen. Accordingly, in a separate experiment, a lower dose of antigen (3 µg) was administered and the presence of antibodies capable of inhibiting HA was determined after three immunisations. The results show that the antibody levels generated by the oil vehicle were significantly higher than those induced by alum in the group that received five times more free virus particles in PBS (Fig. 4).

The data presented in this paper demonstrate that Vaxcine™ is able to increase the immune response to the influenza vaccine using a reduced amount of antigen. This response is better than that elicited by alum or by higher amounts of free antigen in PBS. In ad-

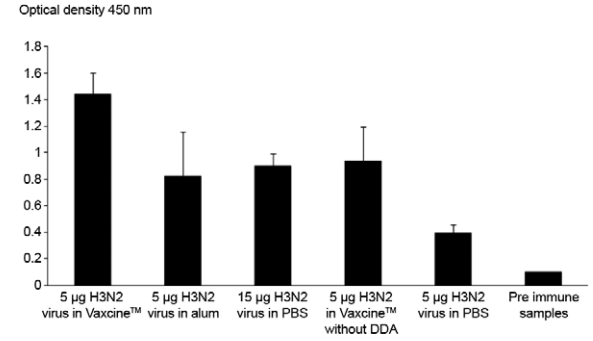

Fig. 1: antibody response against H3N2 virus. Swiss female mice (6-8 weeks old) were immunized subcutaneously with H3N2 virus either free in phosphate buffered saline (PBS), absorbed onto alum (1/25) or incorporated in Vaxcine™, with or without dioctadecyldimethylammonium bromide (DDA). Three months after immunization, blood samples were collected and diluted 1/1,280 in PBS and the presence of antibodies against H3N2 was determined by enzyme linked immunosorbent assay.

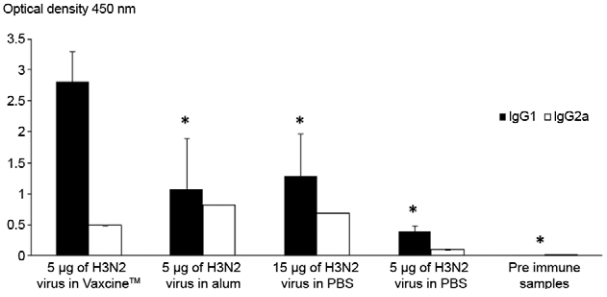

Fig. 2: antibody response against H3N2 virus. Swiss female mice (6-8 weeks old) were immunized subcutaneously with H3N2 virus either free in phosphate buffered saline (PBS) or absorbed onto alum (1/25) or incorporated in Vaxcine™. Four months after immunization blood samples were collected and diluted 1/1,280 in PBS and the presence of antibodies against H3N2 was determined by enzyme linked immunosorbent assay. Asterisks mean statistically significant ($p < 0.05$) difference between experimental (Vaxcine™) and control groups.

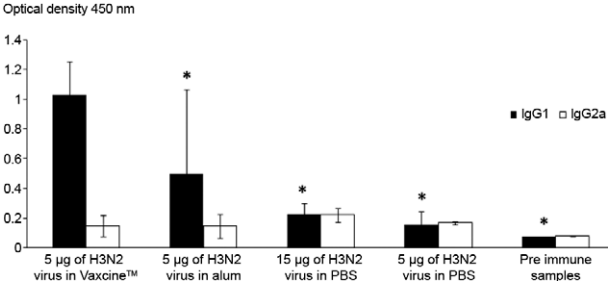

Fig. 3: antibody response against H3N2 virus. Swiss female mice (6-8 weeks old) were immunized subcutaneously with H3N2 virus either free in phosphate buffered saline (PBS) or absorbed onto alum (1/25) or incorporated in Vaxcine™. One year after immunization blood samples were collected and diluted 1/1,280 in PBS and the presence of antibodies against H3N2 was determined by enzyme linked immunosorbent assay. Asterisks mean statistically significant ($p < 0.05$) difference between experimental (Vaxcine™) and control groups.

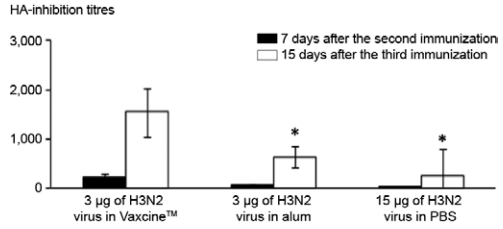

Fig. 4: haemagglutination (HA)-inhibition titres of antibodies against H3N2 virus. Swiss female mice (6-8 weeks old) were immunized subcutaneously three times with H3N2 virus either free in phosphate buffered saline (PBS) or absorbed onto alum (1/25) or incorporated in Vaxcine™. Seven days after the second immunization and 15 days after the third serum samples were collected to determine the HA-inhibition titre of antibodies against H3N2 virus particles. Asterisks mean statistically significant ($p < 0.05$) difference between experimental (Vaxcine™) and control groups.

dition, in contrast to alum, which provided variable results, Vaxcine™ induced a homogeneous immune response in all immunised animals. More importantly, these results were obtained in outbreed mice, suggesting that Vaxcine™ can induce a high and homogeneous inhibitory antibody response in animals with different genetic backgrounds. In addition, contrary to other approaches that use recombinant technology, Vaxcine™ is a simple and cheap oil-based formulation that can accommodate a wide range of antigens and immunostimulants and contains components that have all been previously tested in humans.

In summary, the data presented in this paper suggest that Vaxcine™ is an excellent candidate for use as an adjuvant in the formulation of influenza vaccines due its ability to induce a long-lasting humoral immune response against influenza in animals with different genetic backgrounds. In addition, this formulation is cheap, simple and easy to manufacture. Moreover, as previously shown, Vaxcine™ has the potential to be used as an antigen delivery system for oral influenza vaccines.

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