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Comparison of long-term humoral memory development after immunisation against *Neisseria meningitidis* B or diphtheria toxoid

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ABSTRACT

Since genome sequence data became available there has been a marked increase in number of protein antigens that have been suggested as prospective vaccine components against *Neisseria meningitidis* B (MenB). Few studies have addressed the mechanisms by which meningococcal vaccines generate and sustain immunological memory. The goal of this study was to compare the B-cell response (antibody-secreting cells [ASC], memory B cell and IgG) evoked by a MenB vaccine (VA-MENGOC-BC®) with the B-cell response to diphtheria toxoid (DT) induced by a successful vaccine (Diphtheria–Tetanus–Pertussis [DTP]). The results showed different kinetics of specific ASC response after the primary and booster immunisations. Concerning the specific ASC kinetics, MenB vaccine induced a strong primary response, but the recall response showed a limited power over time. In contrast, DTP primary ASC response was weaker than the booster responses. We observed an increase in the relative percent of memory B cells after 1, 2 and 3 doses of MenB vaccine (mean of 0.8%, 1.3% and 1.6%, respectively) but without statistical significance. Similar frequencies were detected after boosting given at 4 months (mean of 1.3%) or 6 months (mean of 0.9%) following the third dose. DT specific memory B cell response showed a slight lower magnitude after the primary immunisation schedule (mean of 1.2% after the third dose) compared with the MenB response. However, a stronger memory B cell response was induced by booster doses of DTP vaccine at 4 months (mean of 1.9%) or 6 months (mean of 1.9%). The kinetics of specific IgG induced by both vaccines was similar, suggesting that memory B cells were responsible for the strong antibody response seen after the booster vaccination.

1. Introduction

Neisseria meningitidis is one of the leading causes of bacterial meningitis and septicaemia in children worldwide and a cause of more than 50,000 deaths a year [1]. Effective polysaccharide-based vaccines against serogroup A, C, Y and W135 meningococci are available, but there is currently no universal vaccine against serougroup B (MenB) [2]. Tailor-made wild-type OMV vaccines are used today for controlling outbreaks dominated by phenotypically highly related MenB strains belonging to the same clonal complex [3].

Killing of MenB can result from complement-mediated serum bactericidal activity or opsonophagocytosis, or a combination of both mechanisms [4]. Establishment of immunological memory is a hallmark of adaptative immune responses and the biological mechanism for the success of vaccines [5]. Immunological memory is comprised of a cellular and humoral component. Soluble antibodies maintain a first line of defence against extracellular pathogens both systemically and at mucosal surface and are recognised as serological memory [6]. The cellular components of long-term humoral memory are sustained by the existence of quiescent memory B cells and long-lived plasma cells or antibody-secreting cells (ASC) [7,8]. Understanding the mechanism by which meningococcal vaccines generate and sustain the serological and cellular immune memory is essential to improving the long-term efficacy of MenB vaccines.

Among several bacterial vaccines used in childhood immunisation, diphtheria–tetanus–pertussis vaccine (DTP) has produced a dramatic decline in the incidence of diphtheria all over the world [9,10]. After primary immunisation in the first year of life, booster

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doses are recommended at 5–6 years of age and every subsequent 10 years [10,11].

In the present study, we used this successful vaccine (DTP) and the B cell response to diphtheria toxoid (DT) as a reference for our analysis of the development of long-term humoral memory induced by the VA-MENGOC-BC®, the Cuban outer membrane vesicle vaccine against MenB.

We have previously shown the generation of a consistent MenB-ASC response in bone marrow of mice immunised with the Cuban MenB vaccine. These cells remained at significant levels up to 2 months after the third dose of the vaccine [12]. In the present study we analysed the maintenance of MenB-ASC in bone marrow for extended periods of time after immunisation as well as the development of memory B cells. The effect of booster doses on B cell response was also investigated. Similar analyses of humoral immunity to DT were done after triple DTP immunisation.

The results showed that the vaccines in study induced different kinetics of specific ASC response after the primary and booster immunisations. Based on the ASC arm of humoral memory (long-lived plasma cells), DTP vaccine induced a longer-lasting memory than MenB vaccine. Similarly, development of long-term memory B cell was stronger after DTP vaccination.

2. Materials and methods

2.1. Serogroup B meningococcal strain

The Cuban vaccine strain (Cu385/83) of serotype:serosubtype:immunotype 4,7:P1.19,15:L3,7,9 was used for the preparation of outer membrane vesicles (OMV) to be used as the coating antigen for the ELISA.

2.2. Vaccine and immunisation of mice

The VA-MENGOC-BC® was obtained commercially. Diphtheria vaccine (DTP) was produced by Instituto Butantan, São Paulo, Brazil. Five- to six-week-old female Swiss mice in groups of a mean of 10 were immunised with 3 intramuscular injections of vaccine, 2 weeks apart. Each MenB vaccine injection (100 μl) contained 2 μg of OMPs, 2 μg of C polysaccharide, 400 μg of $Al(OH)_3$ and 0.01% of thimerosal as preservative [13]. DTP vaccine injection (100 μl) contained 2 μg of diphtheria toxoid, 2 μg of tetanus toxoid, 28 OP/ml of whole cells of *Bordetella pertussis*, 500 μg of $Al(OH)_3$ and 80 μg of thimerosal.

Serum samples of a group of mice was obtained before and 14 days after each injection of vaccine during the primary immunisation schedule (total of 3 injections) and at 2, 4 and 6 months after the third dose (pre-booster sera). Blood was collected 14 days after the booster dose given to different groups of mice at 2, 4 and 6 months. Serum samples were stored at −20 °C.

For ELISPOT and memory B cell assay, spleen and bone marrow cells were collected from different groups of mice before and after each immunisation following similar schedule to the one described above.

2.3. ELISPOT assay

Single cell suspensions of splenocytes or femur bone marrow were cleared of erythrocytes by a single round of 0.8% NH_4Cl treatment and suspended in RPMI (HyClone, Utah, USA) supplemented with 10% fetal bovine serum (HyClone), 5×10^{-5} M β-mercaptoethanol (Sigma, St. Louis, USA) and antibiotics (10,000 U/ml penicillin (Sigma, St. Louis, USA) and 10 mg/ml streptomycin (Proquímios, Rio de Janeiro, Brazil)). Cells were then quantified by ELISPOT technique as previously described [12]. Briefly, 96-well Maxisorp plates (Nunc, Rochester, USA) were coated either with 2 μg/ml of goat anti-mouse IgG monoclonal antibody (Kirkegaard & Perry Laboratories, MD, USA), or 4 μg/ml of OMV (Cu385 strain) or diphtheria toxoid (Instituto Butantan, São Paulo, Brazil) in 0.05 M Tris buffer, pH 9.5, overnight at 4 °C. After washing with phosphate buffer saline (PBS) 0,01 M, pH 7.2–7.4, plates were blocked with RPMI supplemented with 1% fetal bovine serum and antibiotics (150 μl/well). Spleen cells at concentrations of $\sim 1 \times 10^7$/100 μl and bone marrow cells ($\sim 1 \times 10^6$/100 μl) were added to the first well and serial 2-fold dilution was made in RPMI complete medium. Plates were incubated for 16 h at 37 °C/5% CO_2 and then washed with PBS/1% Tween 20 (T20). Secreted IgG was detected with goat anti-mouse IgG alkaline phosphatase-conjugated mAb (Kirkegaard & Perry Laboratories, MD, USA) at a dilution of 1:2000 in PBS/1% BSA/0.1% T20. ELISPOTs were developed with 1 mg/ml of 5-bromo-4-chloro-3-indolylphosphate (BCIP; Sigma) dissolved in amino-methyl-propanol buffer (Sigma). Spots were counted after 2 h by stereoscopic microscopy.

Mean values of spots were calculated from triplicates. OMV or diphtheria specific ASC levels were expressed as a percent of total IgG-secreting B-cells.

2.4. Memory B lymphocyte

An amount of 2×10^6 splenocytes/100 μl were cultured for 3 days in complete medium in the presence of *Staphylococcus aureus* cells (Cowan 1) SAC (Calbiochem, USA) diluted 1:5000 and 100 IU/ml interleukin-2 (Calbiochem, USA) [14]. Cells were then washed and quantified by ELISPOT technique described in 2.3.

2.5. ELISA

Outer membrane proteins (OMP) coating antigen was prepared by extraction of OMVs from the wet cell pellet for 2.5 h at 50 °C with 5 ml of 0.2 M lithium chloride in a 0.1 M sodium acetate buffer (pH 5.8) per g of cells [15]. Diphtheria toxoid was provided by Instituto Butantan, São Paulo. ELISA analysis was performed, as previously described [16], with a peroxidase-conjugated anti-mouse IgG (Kirkegaard & Perry Laboratories, MD, USA). As an internal antibody standard for IgG determination, a 2-fold dilution series of a pool of positive post-vaccination sera, assigned 1000 units per milliliter (U/ml), was used in all ELISA experiments. The mean value of the observed optical density was transformed into arbitrary U/ml by a sigmoidal curve (four-parameter logistic log transformation) calculated from the values of the standard serum using an ELISA program [17].

2.6. Statistical analysis

The levels of significance of the differences between groups were examined by paired or unpaired *t* test (parametric tests). These analyses were performed with a GraphPad-Prism software, version 4.02. $P < 0.05$ was taken as significant.

3. Results

3.1. Relative concentration of ASC specific to MenB (MenB-ASC) after immunisation

Fig. 1A shows the percent of ASC specific to OMP of a B:4,7:P1.19,15 strain relative to total IgG-secreting cells detected in bone marrow and spleen of mice immunised with one, two and three doses of VA-MENGOC-BC® vaccine.

We did not detect any MenB-ASC before immunisation either in spleen or bone marrow (data not shown). After 1 or 2 vaccine injections, MenB-ASC was detected in similar frequency in spleen and bone marrow (Fig. 1A). However, the third vaccination induced

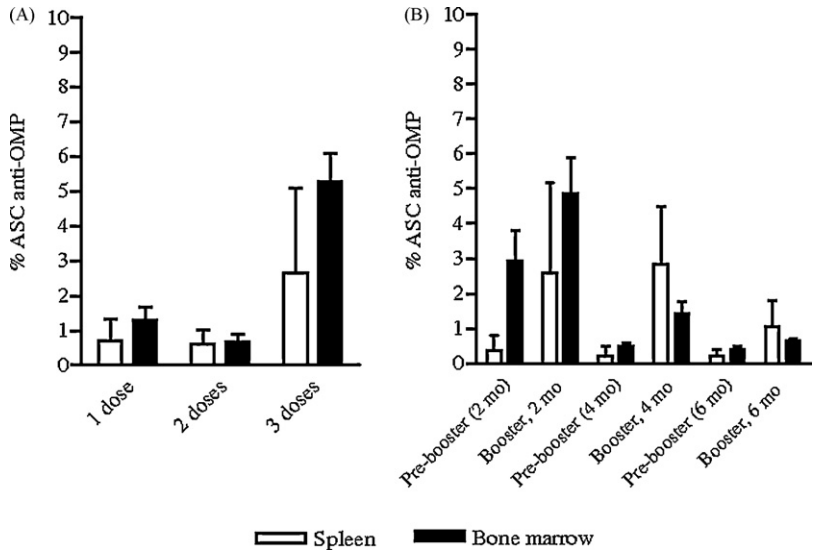

Fig. 1. ASC (ELISPOT) response to MenB:4,7:P1.19,15 strain (Cu385/83) of mice immunised with one, two or three doses of the VA-MENGOC-BC® vaccine. Cells were collected 14 days after each vaccine injection. Each time point represents an independent group of animals. Mean values of ASC frequencies as percent of total IgG-ASC detected in spleen (white bars) and bone marrow (black bars). (A) After primary immunisations, (B) before and after booster injections given at 2, 4 and 6 months post-third dose. The data show the average and standard error of a mean of 11 mice per group.

a significant increase of specific ASC, which was higher ($P = 0.06$) in bone marrow (mean of 5.3%) compared with spleen (mean of 2.6%). An important but not significant decline (about 1.8-fold) of specific ASC was observed in bone marrow 2 months (mean of 2.9%, Fig. 1B) after the third dose of vaccine. Overall, the frequency of specific ASC in bone marrow detected between 4 and 6 months was about 0.5% and similar ($P > 0.05$) to ASC measured after 1 or 2 doses of the vaccine.

In contrast to the bone marrow, the decrease of spleen MenB-ASC was a maximum of 2.8-fold, 2 months after the third dose (Fig. 1B). At this time, the frequency of MenB-ASC in spleen (mean of 0.36%) was about half the frequency detected after the first dose (0.71%, $P > 0.05$, Fig. 1A).

As shown in Fig. 1B, a booster dose administered 2 months after the third dose, induced a significant increase in specific ASC levels in spleen (mean of 2.6%; $P < 0.01$, compared with pre-booster), reaching similar frequency as detected after the third dose. Since the pre-booster ASC levels in bone marrow were still high at 2 months (mean of 2.9%) the booster dose induced a small increase in ASC levels, but reached similar frequency (mean of 4.9%) as detected after the third dose (mean of 5.3%, Fig. 1A).

In comparison with ASC levels before booster, the 4-month booster dose applied in a second group of animals induced a significant ASC response in spleen (mean of 2.8%; $P < 0.01$) but not in the bone marrow (mean of 1.4%; $P = 0.06$) (Fig. 1B). An even lower ASC response was evoked after the 6-month booster injection either for spleen (mean of 1.1%, $P < 0.05$, compared with pre-booster) or bone marrow (mean of 0.6%, $P = 0.21$, compared with pre-booster, Fig. 1B). These data indicate that a small fraction (about 0.5%) of MenB-ASC (long-lived ASC) remained in bone marrow up to 6 months post-vaccination. Despite this low frequency, bone marrow long-lived ASC may be important to maintain IgG levels in the blood. There is also a suggestion that in terms of spleen and bone marrow MenB-ASC response, booster injections of MenB vaccine have a limited effect over time after the primary immunisation.

3.2. Relative concentration of ASC specific to diphtheria toxoid (DT-ASC) after immunisation

Fig. 2A shows a weaker primary DT-ASC response when compared with the primary MenB-ASC response induced by the Cuban vaccine (Fig. 1A). After the second immunisation, the frequency of DT-ASC in spleen was higher (mean of 0.8%) compared with the bone marrow (mean of 0.2%) but without statistical significance. However, after triple immunisation, bone marrow levels of DT-ASC reached a maximum of 1% and was very close to the amount recorded in spleen (mean of 1.2%, $P = 0.45$).

About 4 months following the third dose, there were no significant changes in ASC frequency in bone marrow or spleen. Nonetheless, DT-ASC frequency in spleen showed a significant drop 6 months after the third dose, while the bone marrow ASC levels had a small decrease in the same period (Fig. 2B, pre-booster 4 and 6 months). As seen in Fig. 2A and B, a typical memory immune activation occurred after the booster injections of DTP vaccine given at 4 or 6 months after the primary schedule. Noteworthy is that after boosting the ASC, frequencies in spleen were 2–3 times higher than the frequencies in bone marrow (Fig. 2B). The booster ASC response measured in spleen 4 months (mean of 7.2%) and 6 months (mean of 8%) after the third dose, was significantly higher than the booster ASC response measured after three doses (mean 1.2.%) A significant increase in DT-ASC in bone marrow was also seen after the 4-month (mean of 2.1%) and 6-month (mean of 3%) boosting compared with the frequency of cells detected after the third dose (mean of 1%). Considering that most of the ASC induced by booster injection comes from the differentiation of memory B cells, these results suggest that DTP vaccine induced a longer-lasting memory than MenB vaccine. In addition, the booster DT-ASC response (mean of 1.8–3%) in bone marrow was higher than the ASC frequencies detected after the primary immunisation (mean of 0.2–1.0%).

Therefore, these data indicate that the MenB and DT vaccines have different mechanisms to evoke and maintain the immunological memory to the protein components of each vaccine.

3.3. Specific memory B cell response to MenB and DT

Fig. 3A shows the percent of specific memory B cells detected as specific ASC after *in vitro* stimulation of spleen memory B cells for 3 days. The results showed a small increase ($P > 0.05$) in the relative percent of memory B cells after 1, 2 and 3 doses of MenB vaccine (mean of 0.8%, 1.3% and 1.6%, respectively). There were no significant changes in the numbers of memory B cells detected at 2 months (mean of 1.1%, data not shown) and 4 months (mean

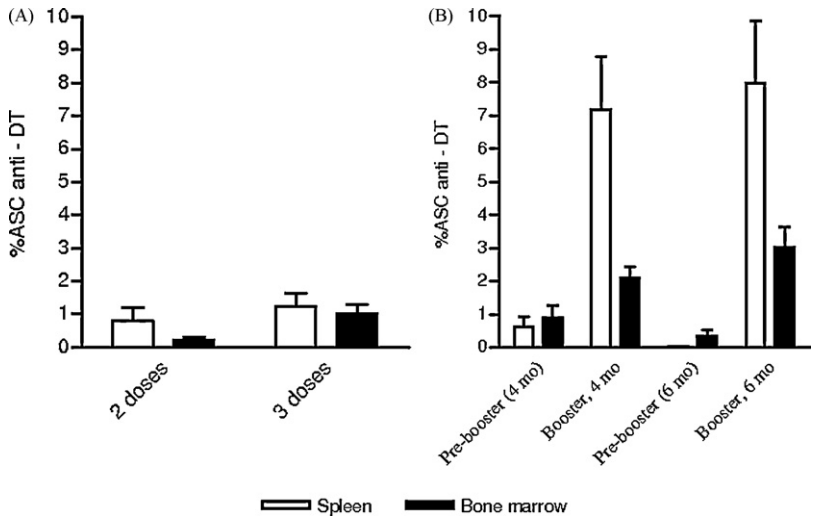

Fig. 2. ASC (ELISPOT) response to diphtheria toxoid (DT) of mice immunised with two or three doses of the DTP vaccine. Cells were collected 14 days after each vaccine injection. Each time point represents an independent group of animals. Mean values of ASC frequencies as percent of total IgG-ASC detected in spleen (white bars) and bone marrow (black bars). (A) After primary immunisations, (B) before and after booster injections given at 4 and 6 months post-third dose. The data show the average and standard error of 8–9 mice per group.

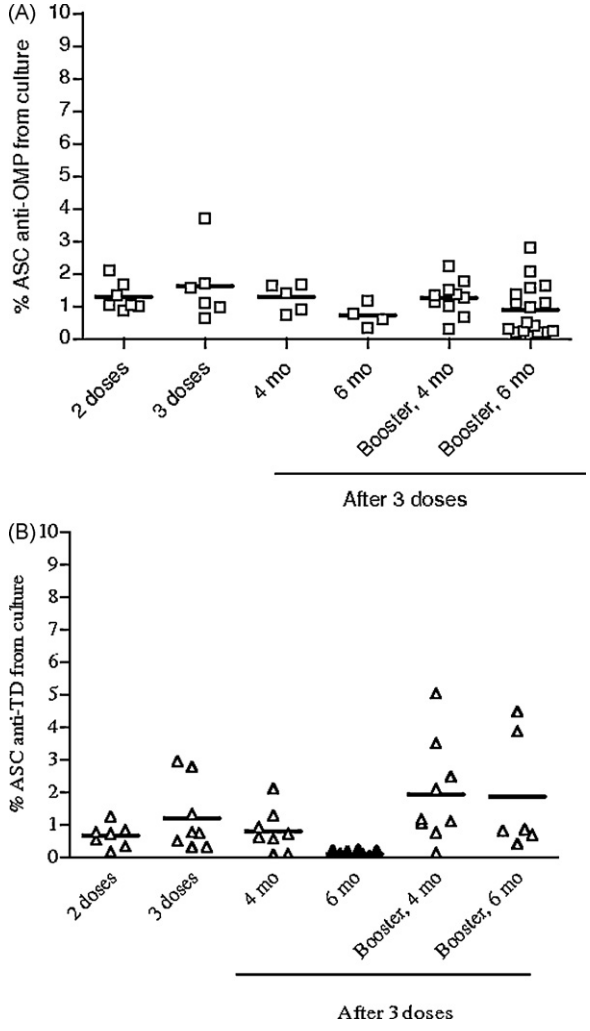

Fig. 3. Frequencies of specific ASC from culture (memory B cell) against OMV of MenB (A) and diphtheria toxoid (DT) (B). Memory B cells was detected as specific ASC after *in vitro* stimulation of spleen memory B cells for 3 days. The data show the average of 4–10 mice per group.

of 1.3%) compared with the frequency measured after the third immunisation. A decrease of memory B cells was seen 6 months (mean of 0.7%) after the last vaccine injection but without statistical significance.

Similar to the ASC response, the booster injections of vaccine given at 4 or 6 months did not induce a significant increase of memory B cell frequency (mean of 1.3% and 0.9%). After statistical analyses of all data generated in this study, we observed a strong positive correlation between spleen ASC and memory B cells ($r = 0.81$, $P = 0.05$) but not between bone marrow ASC and memory B cells ($r = 0.58$) after the third immunisation. A significant positive correlation ($r = 0.7$, $P = 0.016$) between spleen ASC and memory B cell was seen after the 4-month booster, but not after the 6-month booster ($r = 0.29$, $P = 0.25$).

Therefore, these data indicates that an important memory B cell response was readily induced with one dose of vaccine. Nonetheless, the second and third doses of vaccine may have been important to induce a homeostatic level of about 1% of specific spleen memory B cells.

Concerning the DT specific memory B cell response, we observed a lower magnitude after the primary immunisation schedule compared with the MenB response. The frequency of DT-memory B cell after 3 doses (mean of 1.2%) was similar to the one evoked by two doses of MenB vaccine (mean of 1.3%) (Fig. 3A and B). A great ($P < 0.05$) decrease of DT-memory B cell frequency was detected 6 months after the third dose of the DT vaccine, distinguishing from the stable memory B-cell response induced by MenB vaccination (Fig. 3B). However, a significant increase of B-cell memory frequency was seen after booster immunisation at 2 months (mean of 1.0%, data not shown), 4 months (mean of 1.9%) or 6 months (mean of 1.9%) following the primary immunisation (Fig. 3B). Thus, a stronger memory B cell response was induced by booster doses of DTP vaccine compared with MenB vaccine. A significant positive correlation was observed between spleen ASC and B cell memory after the 2-month booster ($r = 0.79$, $P = 0.0189$) or 4-month booster ($r = 0.81$, $P = 0.0076$), but not after the 6-month booster injection of DTP vaccine (data not shown).

3.4. IgG response to MenB and DT

Serum IgG response of immunised mice to OMP (Cu385/83 strain) was dose-dependent (Fig. 4A). One dose of vaccine induced

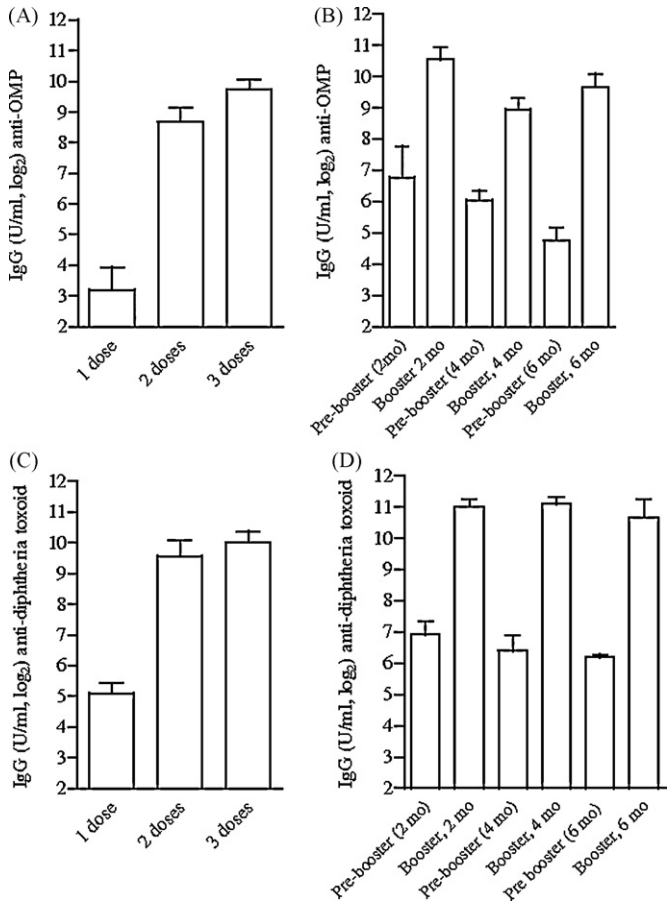

Fig. 4. Serum antibody response to MenB:4,7:P1.19,15 strain (Cu385/83) of mice immunised with VA-MENGOC-BC® vaccine (A, B) and to diphtheria toxoid of mice immunised with DTP vaccine (C, D). The data show the average and standard error of serum IgG levels (U/ml) detected by ELISA 14 days after each vaccine injection during the primary immunisation schedule (A, C), before and 14 days after booster injections given at 2, 4 and 6 months after the primary immunisation schedule (B, D). Data correspond to 5–10 mice per group.

a strong immunological memory as seen by the large increase in antibody levels after the second dose (5.6-fold increase, $P < 0.01$). The third dose did not induce a great increment in IgG anti-OMP ($\log_2$ mean of 9.7) compared with the second dose of vaccine.

Two months after the third dose of vaccine there was a significant decline ($P < 0.01$) in antibody levels ($\log_2$ mean of 6.8, Fig. 4B) and a gradual decline in the subsequent periods ($\log_2$ mean of 6.1 and 4.8 at 4 and 6 months post 3 doses, respectively, Fig. 4B).

A booster dose, injected at 2, 4 or 6 months after the primary immunisation schedule, induced about a 4-fold increase ($P < 0.05$) in specific IgG levels ($\log_2$ mean of 10.5, 8.9 and 9.6, respectively, Fig. 4B), reaching similar levels to that one observed 14 days after the third dose.

DTP vaccine induced the same pattern of IgG response to DT (Fig. 4C and D) as described above for MenB. There were no great differences in DT-IgG levels if the booster dose was applied at 2, 4 or 6 months after the third immunisation (Fig. 4D).

For both vaccine there were no significant correlations between IgG levels and ASC or memory B cell frequencies.

4. Discussion

Despite important progress in MenB vaccine research over the last few years [2,3], only OMV vaccines have been used to disrupt clonal outbreaks [18]. Since the 1960s, the induction of bactericidal antibodies, mainly to homologous strains, has been the immunological surrogate for protection against meningococcal disease [19]. In general, the capacity of MenB vaccines to generate immunological memory has been studied by measuring antibodies after a booster vaccination [20]. Further understanding of the mechanism by which MenB vaccines generate and sustain antibody response is likely to lead to both a more rational choice of vaccine formulations and/or schedules to provide more sustained protection, since immunised individuals may eventually become infected with MenB [21,22]. The contribution of B cells to immunological memory encompasses two distinct populations of cells that are generated during primary immune responses, long-lived plasma cells (in this study referred as ASC), which continue to secrete high levels of immunoglobulin for protracted periods of time well after antigen clearance, and memory B cells, which can rapidly proliferate and differentiate into plasma cells following recurrent exposure to the initial immunising antigen, thereby simultaneously increasing the precursor frequency of antigen-specific memory B cells and enriching the pool of antigen-specific immunoglobulin [6,8].

The data presented here confirmed our previous results [12], demonstrating that in comparison with spleen, bone marrow was the anatomical site which showed higher relative frequency of MenB-ASC after the primary immunisation schedule. Nonetheless, an important reduction in bone marrow MenB-ASC response was detected 2 months later continuing at 4 and 6 months after the third dose. At these times there were no differences between specific ASC response in spleen and bone marrow and the responses were similar to that one detected after 1 or 2 doses of the vaccine. These data differ from a report using a viral infection model which showed that after clearing the infection and for the life of the mouse, the bone marrow contains >90% of virus-specific plasma cells [8].

We then analysed the effect on ASC levels of a booster injection of VA-MENGOC-BC® given at different times after the primary immunisation. For spleen specific ASC levels, booster doses injected up to 4 months after the third dose of vaccine induced a response similar to the one evoked by triple immunisation, indicating the activation of specific memory B cells and consequent differentiation into ASC. In contrast, an inverse correlation was seen between time of boosting and ASC levels in bone marrow, suggesting differences in bone marrow homing capacity of ASC generated after boosting. It is noteworthy that mice did not mount a significant ASC response following the 6-month booster injection, either in bone marrow or in spleen. The reasons for that are not known but it may be related to the vaccine antigens since a typical booster response could be detected after DTP booster injections, even at 6 months after the primary immunisation.

The primary and booster cellular response induced by the DTP vaccine was quite different from the ASC response induced by the MenB vaccine. The primary ASC response to DT was much lower, either in spleen or bone marrow, than the primary cellular response to MenB. In contrast, the booster response induced by DTP vaccine at 4 or 6 months (but not at 2 months) was much higher than the MenB vaccine induced cellular response. We do not know the cause for these differences, but it is important to consider that the MenB vaccine is composed basically of outer membrane proteins and residual lipooligosaccharide in the form of OMV. DTP vaccine used in this study is formulated with secreted proteins and whole cells of *Bordetella pertussis*, a Gram-negative bacterium. Lipopolysaccharide is a potent activator of B cells and exhibits a prosurvival activity in both immature and mature B cells through NF-κB activation and prevention of mitochondrial translocation of Bax, a pro-apoptotic protein of the Bcl2 family [23]. A question remains if the distinct presentations of this molecule as a component of the two vaccines in study could have distinct effects *in vivo*.

The relative memory B cell response anti-MenB detected in spleen reached its peak (mean of 1.6%) after the third immunisation. It had a small decrease thereafter but remained at about 1%

even after the booster doses. Although we did not find a important increment in spleen and bone marrow cellular response (memory B cell and/or ASC) anti-MenB after the booster dose administered 6 months following the third injection of vaccine, the antibody response was markedly increased and reached similar levels to those detected after the third dose. These data suggest that spleen memory B cells, which remained at constant levels during the whole study, were responsible for the strong antibody response seen after the booster vaccination. Accordingly, Blink et al. [24] showed that the numbers of circulating memory B cells remained constant from early on in the immune response, although the affinity of their antibodies increased. It is noteworthy that we did not study lymph nodes from vaccinated animals, raising questions about the possibility of other secondary lymphoid organs constituting an additional reservoir for long-lived memory B cells.

A human study showed that a fourth dose of the Norwegian MenB vaccine given 10 months after the primary immunisation (3 injections) induced a higher bactericidal antibody response than the one seen after the primary immunisation. The subsequent decline of antibody levels was slower than after the third dose, resulting in a longer-lasting response. By using an animal model, in the present study, we observed that the two vaccines in analysis induced different ASC response after the primary immunisation and also after boosting. However, less pronounced differences were seen for the generation and maintenance of memory B cells and the IgG response. We do not know the exact implications of these findings for human vaccinology but since the efficacy of OMV vaccines is still unsatisfactory, additional studies should be conducted in humans.

Concerning DTP vaccine, we highlight the increase in memory B cell response with the booster doses administered as late as 6 months after the primary immunisation. This was seen directly by measuring B cell memory frequencies and indirectly through the ASC levels detected in spleen after boosting. The mean of B cell memory frequencies after the booster doses (given at 4 and 6 months) was about 1.9% for DTP vaccine and 1.1% for MenB vaccine. Together with the DT-ASC response in spleen which showed higher frequencies after boosting compared with the response induced by the third dose, these data suggest that the spleen is a major reservoir for long-lived memory B cells specific for DT. In addition, the booster DT-ASC response in bone marrow was higher than the ASC frequencies detected after the primary immunisation, indicating a gradual increase in the frequency of ASC migrating from spleen to bone marrow. Although we could not compare the amount of specific IgG induced by each vaccine, we observed that the kinetic of DT-IgG response was similar to the MenB-IgG.

This study showed no significant correlations between IgG concentrations and ASC or memory B cells, suggesting that antibody studies should not be the only marker of vaccine efficacy.

In conclusion, data of this study suggest that spleen memory B cells were an important mechanism of long-term immunological memory induced by the Cuban vaccine. It seems that these cells reached a threshold value after one dose of vaccine which was maintained throughout the study and could be activated as long as 6 months after the primary immunisation generating a significant antibody response. Although in small frequency, bone marrow long-lived ASC might be important for the maintenance of IgG levels after primary immunisation. Concerning the DTP vaccine, our data indicated that specific ASC and memory B cell in spleen strongly contributed to the cellular memory response to the DT. Bone marrow DT-ASC was also present as a mechanism of long-term immune memory induced by DTP vaccination.

Altogether, these data suggested that spleen long-lived memory B cells is an important mechanism of humoral memory induced by vaccination against meningococci and diphtheria toxoid. It remains unknown which factors control the magnitude and duration of the ASC and LBm responses and the relationship between these cells. Further studies should be designed to evaluate the effect of antigen composition of MenB vaccine on the mechanisms of development of immune memory cells.

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